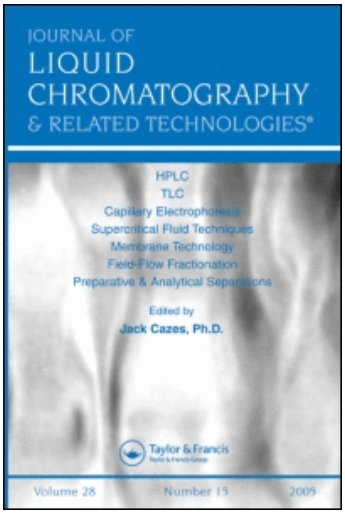

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A LIPOPHILICITY STUDY FOR SOME 2-HYDRAZINOTHIAZOLIC DERIVATIVES WITH ANTIFUNGAL ACTIVITY BY REVERSED PHASE THIN LAYER CHROMATOGRAPHY

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ABSTRACT

The lipophilicity of 15 2-hydrazinothiazolic derivatives was studied by reversed phase thin layer chromatography on silica-C8 plates with methanol-water mobile phase. The studied compounds have shown antifungal activity against *Lepidium sativum*, and the corresponding inhibition values that measure their mitodepresive activity are given.

The R_{Mw} values were obtained by extrapolation to 100% water as the mobile phase and are a measure of the compounds' lipophilicity. The partition coefficient between n-octanol and water, Log $P_{o/w}$, was calculated using two different procedures, Rekker's revised fragmental constant system and ACD/Log P software. Linear correlations have been obtained between the R_{Mw} values and the calculated Log P values. No linear correlation was found between the inhibition values, I, and R_{Mw} or calculated Log P. A good linear correlation ($r>0.99$) was obtained between the extrapolated R_{Mw} values and the slope, a_1, of the linear relationships $R_M = f(\varphi)$, where φ is the concentration of methanol in the mobile phase, showing that the compounds have a similar chromatographic behavior. The replacement of R_{Mw} values with the isocratic hydrophobic index, φ_0, does not improve the linearity of the correlations with the calculated Log P values, although the extrapolation to 100% water as the mobile phase was performed from high concentrations of methanol.

INTRODUCTION

The lipophilicity of substances can be expressed by the partition coefficient between 1-octanol and water, $P_{o/w}$.[1-3] The Log $P_{o/w}$ values can be measured experimentally by the shake-flask method or can be calculated by using different calculation procedures. The direct measurement of Log $P_{o/w}$ values by equilibration between 1-octanol and water faces some difficulties, such as the necessary high purity of the substance that must be available in an adequate quantity. In addition, this method is time consuming and can be applied only in a limited range on the lipophilicity scale. The difficulties can be overcome by using reversed phase liquid chromatography (RPLC), and these methods have been applied for some years.[4-7] The chromatographic methods show distinct advantages, such as speed of determination, better reproducibility, and the need for only small amounts of sample that need not be of high purity. Among liquid chromatographic methods, reversed phase thin-layer chromatography (RP-TLC) is an alternative technique that can correlate the lipophilicity of compounds with the retention parameters.[8,9] Martin and Synge[10] and Consden et al.[11] derived a relationship between the partition coefficient *P* and R_f values in partition chromatography. Bate-Smith and Westall[12] introduced the term $R_M = \log (1/R_f - 1)$. Boyce and Milborrow[13] suggested the use of this value in order to avoid the practical difficulties that often arise in the direct determination of the partition coefficient. The R_M value measured by RP-TLC has been used as a reliable alternative to the classical Log P in order to express the lipophilic character of a substance.[14-16]

The aim of the present study was to find a linear correlation between the R_M values obtained for some 2-hydrazinothiazolic derivatives by RP-TLC and the calculated Log P values. Methanol-water was used as a binary mobile phase and silica gel-C_8 as the nonpolar stationary phase. The Log P values were calculated by using Rekker's revised fragmental constant system or ACD/Log P software.

The calculated Log P values and the experimental R_M values were compared with the inhibition values (I%), which measure the antifungal activity of the studied substances.

EXPERIMENTAL

The studied 2-hydrazinothyazolic derivatives are compounds which have shown antifungal activity, determined by the phytobiologic test measuring the inhibition against *Lepidium sativum*.[17-19] The compounds were synthesised at the Organic Chemistry Department of the University of Medicine and Pharmacy (Cluj-Napoca, Romania) and are shown in Fig. 1.

RP-TLC was performed on chemically bonded silica-C_8 plates with 254 nm fluorescence indicator, 10 x 10 cm (Merck, Darmstadt, Germany). The mobile phases were methanol-water mixtures with methanol concentrations ranging from 90% to 75% (v/v) in increments of 5%.

Samples were prepared as solutions in methanol (0.1 mg/mL) and were applied onto the plate, 5-10 μL/spot, 1.5 cm from the bottom edge. The sample spots were applied with calibrated micropipets, dried in a gentle air stream, and developed in normal chromatographic chambers, previously equilibrated for 30 min. The mobile phase migration distance was 8 cm in all cases. Each sample was applied in triplicate, and the mean R_f value was used in calculations. After development, the plates were dried at room temperature and were evaluated in 254 nm UV light.

The R_f values were measured by densitometry with a Shimadzu CS-9000 dual-wavelength flying spot scanner, operated in the reflection mode at 254 nm.

RESULTS AND DISCUSSION

The R_f values obtained for compounds 1-15 (Fig. 1) and the corresponding R_M values are shown in Table 1. The linear correlations between the R_M values and the organic modifier concentration in the mobile phase, φ (v/v), for the studied compounds together with the statistical parameters, calculated for 95%

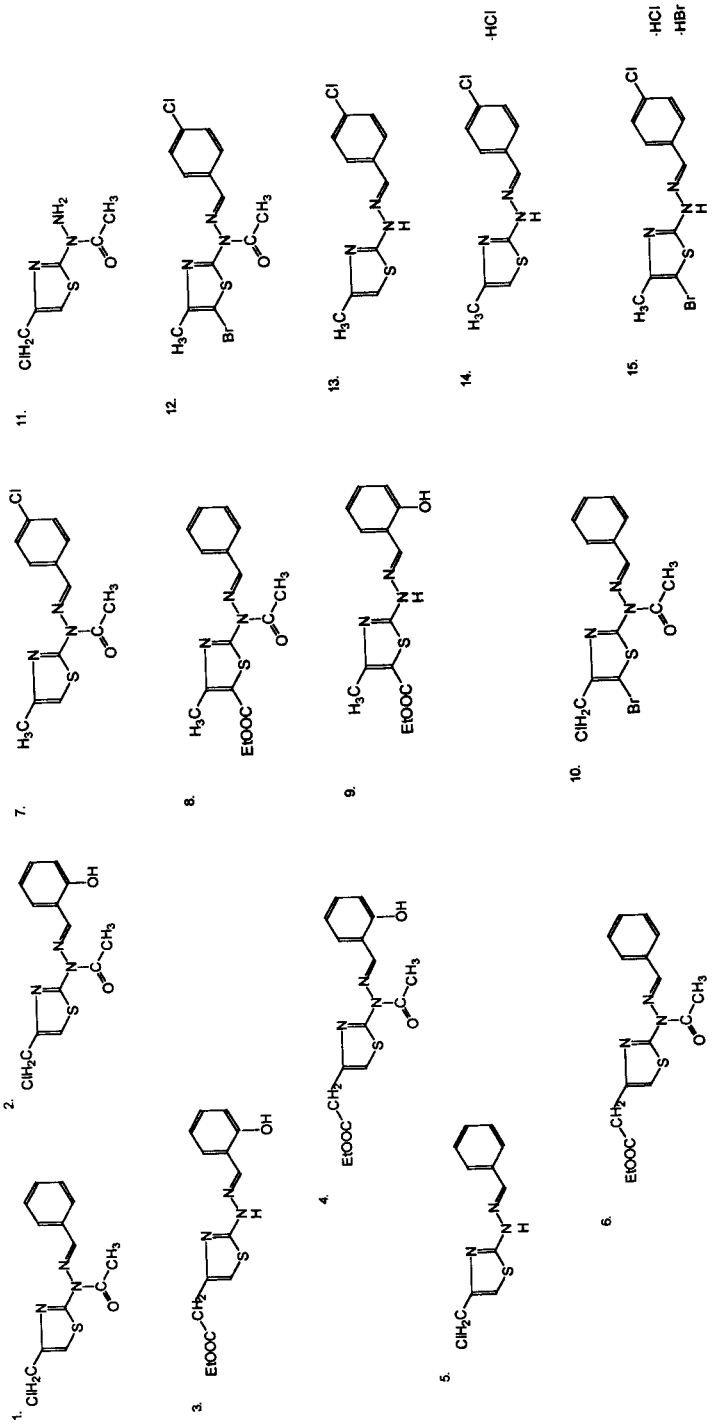

Figure 1. The structure of the studied 2-hydrazinothiazolic derivatives.

Table 1

The Dependence of R_f Data[a] and R_M[b] for 15 Hydrazinothiazolic Derivatives as a Function of Organic Modifier Concentration, φ[c]

Cpd.	90:10 (v/v)	85:15 (v/v)	80:20 (v/v)	75:25 (v/v)	70:30 (v/v)	65:35 (v/v)
1	0.61	0.48	0.41	0.30	0.21	0.13
	-0.194	0.035	0.158	0.368	0.575	0.825
2	0.70	0.56	0.49	0.38	0.28	0.18
	-0.368	-0.105	0.017	0.213	0.410	0.658
3	0.68	0.54	0.48	0.35	0.25	0.14
	-0.327	-0.070	0.035	0.269	0.477	0.788
4	0.72	0.59	0.53	0.40	0.30	0.19
	-0.410	-0.158	-0.052	0.176	0.368	0.630
5	0.61	0.47	0.38	0.26	0.18	0.09
	-0.194	0.052	0.213	0.454	0.658	1.004
6	0.67	0.54	0.46	0.34	0.23	0.14
	-0.307	-0.070	0.069	0.288	0.525	0.788
7	0.62	0.49	0.40	0.29	0.19	0.12
	-0.213	0.017	0.176	0.389	0.630	0.865
8	0.51	0.35	0.26	0.16	0.10	0.04
	-0.017	0.269	0.454	0.720	0.954	1.380
9	0.47	0.32	0.25	0.14	0.08	0.04
	0.052	0.327	0.477	0.788	1.061	1.380
10	0.49	0.33	0.24	0.13	0.07	0.04
	0.017	0.307	0.500	0.825	1.123	1.380
11	0.80	0.72	0.72	0.63	0.56	0.49
	-0.602	-0.410	-0.410	-0.231	-0.105	0.017
12	0.43	0.27	0.19	0.10	0.05	0.03
	0.122	0.432	0.630	0.954	1.279	1.510
13	0.44	0.30	0.24	0.14	0.08	0.03
	0.105	0.368	0.500	0.788	1.061	1.510
14	0.44	0.31	0.25	0.15	0.09	0.03
	0.105	0.347	0.477	0.753	1.004	1.510
15	0.31	0.18	0.14	0.07	0.03	0.01
	0.347	0.658	0.788	1.123	1.510	1.996

[a] First value of each row. [b] Second data. [c] Volumetric percentage, v/v.

Table 2

Linear Correlation $R_M = a_0 + a_1\varphi$*

Cpd.	$a_0 = R_{Mw}$	a_1	s_{a0}	s_{a1}	s	F	r
1	3.361 ± 0.367	-0.039 ± 0.005	0.132	0.002	0.035	544.0	0.996
2	3.180 ± 0.376	-0.039 ± 0.005	0.136	0.002	0.036	509.4	0.996
3	3.495 ± 0.568	-0.043 ± 0.015	0.205	0.003	0.055	262.9	0.992
4	3.195 ± 0.424	-0.040 ± 0.005	0.153	0.001	0.041	417.7	0.995
5	3.929 ± 0.520	-0.046 0.007	0.187	0.002	0.050	365.7	0.994
6	3.528 ± 0.391	-0.043 ± 0.005	0.141	0.002	0.038	560.4	0.996
7	3.606 ± 0.274	-0.042 ±0.003	0.099	0.001	0.026	1127.7	0.998
8	4.747 ± 0.699	-0.053 ± 0.009	0.252	0.003	0.067	271.1	0.993
9	4.734 ± 0.523	-0.052 ± 0.007	0.188	0.002	0.051	469.2	0.996
10	4.938 ± 0.336	-0.055 ± 0.004	0.121	0.001	0.032	1243.1	0.998
11	1.565 ± 0.445	-0.024 ± 0.006	0.160	0.002	0.043	135.2	0.985
12	5.163 ± 0.372	-0.056 ± 0.005	0.134	0.002	0.036	1061.3	0.998
13	4.881 ± 0.973	-0.054 ± 0.012	0.350	0.004	0.094	142.5	0.986
14	4.805 ± 1.175	-0.053 ±0.015	0.423	0.005	0.113	95.3	0.980
15	6.002 ± 1.206	-0.064 ±0.015	0.434	0.005	0.116	130.5	0.985

* φ is the organic modifier concentration in the mobile phase (v/v); $a_0 = R_{Mw}$ is the intercept, a_1 is the slope, s_{a0} and s_{a1} are standard errors for the intercept and slope, s is the fit standard error, F is the parameter for the F-test, and r is the correlation coefficient for 95% confidence limits.

confidence limits, are shown in Table 2. These correlations have a good linearity, usually with r>0.99. Taking into account the presumption that the linearity of the relationships $R_M = f(\varphi)$ is maintained even at low methanol concentration in the mobile phase, the extrapolated R_M values to 100% water as the mobile phase are reliable.

The Log P values were calculated by using two methods: the ACD/Log P software (Advanced Chemistry Inc., Toronto, Canada) and Rekker's revised fragmental constant system.[20,21] Neither method takes into account that substances 14 and 15 are salts. Compounds 2, 3, 4, and 9 exist preferably in the ionic or zwitterionic form(s), so the calculated Log P_{ACD} values, probably, cannot be verified experimentally. The Log P_{ACD} values have been calculated for the uncharged molecule. The Log P_{ACD} values for compounds 7, 8, and 9 have been calculated with a high degree of uncertainty because their structures contain aromatic interactions not accounted for by the current ACD/Log P algorithm. The interactions between the hydrazinothiazolium fragment and the aromatic hydroxyl, or between the hydrazinothiazolium fragment and the aromatic chlorine, were approximated. The interactions through the aromatic system were assumed to be zero (compound 8), and the interactions through the aromatic system between the aromatic bromide and the hydrazinothiazolium fragment were approximated to zero.

The Rekker revised fragmental constant system calculates the Log P_{Rekker} values by the addition of the corresponding fragmental constants and a number of so-called "magic constants," $C_M = 0.219$. These C_M values were included in the Log P_{Rekker} data following the Rekker system rules for corrections: 4 C_M for polar groups separated by an aliphatic carbon, 2 or 3 C_M for polar groups separated by two aliphatic carbons, 1 C_M for intramolecular hydrogen bonding, and 2 C_M for extended conjugation.

The calculated Log P_{ACD} and Log P_{Rekker} values are presented in Table 3 together with the extrapolated R_M values to 100% water as the mobile phase, R_{Mw}, and arranged in an increasing lipophilicity scale. It can be seen, from Table 3, that the calculated Log P values (Log P_{Rekker} or Log P_{ACD}) are arranged in the same order in the lipophilicity scale. Comparing the R_{Mw} values with the calculated Log P values, some differences can be observed, but these are in the experimental error range. Compound 11 is the most hydrophilic and compound 15 is the most lipophilic as can be seen from the scale presented in Table 3. The close R_{Mw} values for compounds 13 and 14 confirm that the salt form of the substance does not have a significant influence on the chromatographic partition, and it should not be taken into account when calculating Log P values. From this point of view, the lipophilicity of compounds 13 and 14 should be similar. However, the biologic activity (Table 4) is very different for these substances.

Table 3

Increasing Lipophilicity Scale for the Calculated Log P_{ACD} and Log P_{Rekker} and, Respectively, for the Extrapolated R_M Values to 100% Water as the Mobile Phase, R_{Mw}

Cpd.	Log P_{ACD}	Log P_{Rekker}	Cpd.	R_{Mw}
11	-0.09 ± 0.62	0.263	11	1.565 ± 0.445
2	2.48 ± 0.65	2.232	4	3.195 ± 0.424
4	2.49 ± 0.65	2.309	2	3.180 ± 0.038
1	2.51 ± 0.64	2.443	1	3.361 ± 0.367
6	2.52 ± 0.64	2.429	3	3.495 ± 0.568
3	2.91 ± 0.61	2.630	6	3.528 ± 0.391
5	2.93 ± 0.60	2.764	7	3.606 ±0.274
8	3.21 ± 0.89	2.868	5	3.929 ± 0.520
10	3.29 ± 0.67	3.592	9	4.734 ± 0.523
7	3.46 ± 0.64	3.319	8	4.747 ± 0.699
9	3.60 ± 0.62	3.069	14	4.805 ± 1.175
13	3.88 ± 0.60	3.640	13	4.881 ± 0.973
14	3.88 ± 0.60	3.640	10	4.938 ± 0.336
12	4.23 ± 0.67	4.468	12	5.163 ± 0.372
15	4.65 ± 0.63	4.789	15	6.002 ± 1.206

Table 4

Inhibition Values, I (%), for the Fifteen 2-Hydrazinothiazolic Derivatives

Cpd.:	1	2	3	4	5	6	7	8
I (%):	41	47	87.2	84	53	84.3	84	53.6
Cpd.:	9	10	11	12	13	14	15	
I (%):	39.5	66	99	86	80	98	97	

The calculated Log P values were correlated with the extrapolated R_{Mw} values in order to find a linear relationship (Equations 1 and 2).

$$\text{Log } P_{ACD} = -0.727\ (\pm 0.930) + 0.930\ (\pm 0.221)\ R_{Mw} \quad (1)$$
$$s_{a0} = 0.430,\ s_{a1} = 0.102,\ s = 0.421,\ F = 82.8,\ r = 0.930,\ n = 15$$

$$\text{Log } P_{Rekker} = -0.725\ (\pm 0.853) + 0.907\ (\pm 0.202)\ R_{Mw} \quad (2)$$
$$s_{a0} = 0.395,\ s_{a1} = 0.094,\ s = 0.386,\ F = 93.6,\ r = 0.937,\ n = 15$$

where s_{a0}, s_{a1} are standard errors for the intercept and slope, s is the fit standard error, F = the statistic parameter for the F-test, r is the correlation coefficient, and n is the number of compounds (all statistical data were calculated for 95% confidence limits).

The inhibition values against *Lepidium sativum* for the 2-hydrazinothiazolic derivatives, I(%), are presented in Table 4.[18,19] The I values measure the mitodepresive activity of these compounds. There is no linear correlation between the extrapolated R_M values to 100% water as the mobile phase, R_{Mw}, or the calculated Log P values (Log P_{Rekker} or Log P_{ACD}), and the inhibition, I. The calculated Log P values (Log P_{Rekker} and Log P_{ACD}) are identical for compounds 13 and 14. However, the antifungal activity measured by the inhibition value, I, for these compounds is different, showing that the salt forms of the substances influence the biological activity.

The isocratic hydrophobic index, φ_0, was introduced in RPLC as an alternative chromatographic parameter that can measure the lipophilicity of the substances.[22] The φ_0 values were calculated for RP-TLC for the studied compounds in the same manner that was applied in RPLC by using Equation 3, where I is the intercept and S the slope of the linear relationships between the R_M values and the methanol concentration in the mobile phase (Tables 1 and 2):

$$\varphi_0 = -\ I/S \quad (3)$$

It is claimed in the literature that the isocratic hydrophobic index can overcome the errors that appear when extrapolating the chromatographic retention parameters, logk or R_M values to 100% water as the eluent, especially when the extrapolation is performed from high concentrations of the organic modifier in the mobile phase. The intercept and the slope values used for obtaining φ_0 values are a_0 and a_1 values from Table 2. In order to obtain reliable φ_0 values, the calculations should be performed on the linear part of the correlation $R_M = f(\varphi)$, where the retention time is approximately double of the dead time, or, in other words, the R_f values are half the R_f corresponding to the solvent front. These conditions are important, especially when the substances are not very lipophilic and the retention parameters can be measured even at low concentrations of organic modifier in the mobile phase.

The linear correlations between the $LogP_{Rekker}$ or $LogP_{ACD}$ and φ_0 values are shown in Equations 4 and 5:

$$\text{Log } P_{ACD} = 13.857\ (\pm 2.076) - 917.796\ (\pm 175.731)\ \varphi_0 \quad (4)$$
$$s_{a0} = 0.961,\ s_{a1} = 81.357,\ s = 0.348,\ F = 127.3,\ r = 0.953,\ n = 15$$

$$\text{Log } P_{Rekker} = 12.964\ (\pm 2.707) - 849.823\ (\pm 229.194)\ \varphi_0 \quad (5)$$
$$s_{a0} = 1.253,\ s_{a1} = 106.108,\ s = 0.454,\ F = 64.1,\ r = 0.911,\ n = 15$$

where s_{a0}, s_{a1} are standard errors for intercept and slope, respectively, s is the fit standard error, F is the statistic parameter for the F-test, r is the correlation coefficient for 95% confidence limits, and n is the number of compounds. It can be seen from Equations 4 and 5 that the replacement of R_{Mw} values with the isocratic hydrophobic index, φ_0, does not improve the linearity of the correlations with the calculated Log P values, although the extrapolation to 100% water as mobile phase was done from high concentrations of methanol.

It was previously shown in the literature that between the intercept, a_0, and the slope, a_1, of the linear relationship $R_M = a_0 + a_1\varphi$ (where φ is the concentration of the organic modifier in the mobile phase), usually a linear correlation is established.[14] The intercept value a_0 has been named R_{Mw}, that is the R_M value obtained for 100% water as the mobile phase. The linearity of the relationship between R_{Mw} and a_1 shows that the chromatographic behavior of compounds is similar. The a_1 value is proportional to the hydrophobic area of compound, which is with the area of that part of the molecule that interacts with the non-polar stationary phase. The corresponding relationship for compounds 1-15 is shown in Equation 6, where the statistical parameters have the same significance as for Equations 4-5:

$$R_{Mw} = -0.011(\pm 0.002) - 0.009(\pm 0.0004)\ a_1 \quad (6)$$
$$s_{a0} = 0.001,\ s_{a1} = 0.0002,\ s = 0.001,\ F = 2347.56,\ r = 0.997,\ n = 15$$

Table 5

Isocratic Hydrophobic Index, φ_0, Calculated for Compounds 1-15*

Cpd.:	1	2	3	4	5	6	7	8
φ_0:	0.0116	0.0123	0.0123	0.0125	0.0117	0.0122	0.0116	0.0112
Cpd.:	9	10	11	12	13	14	15	
φ_0:	0.0110	0.0111	0.0153	0.0108	0.0111	0.0110	0.0107	

* Equation 6, Table 2.

The high correlation coefficient obtained in Equation 6 shows that all studied compounds have similar partition characteristics in the chromatographic system. The interactions between the compounds and the residual silanol groups on the C_8-silica surface are expected to be stronger than for a C_{18} non-polar stationary phase.

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