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LIPOPHILICITY OF THIOBARBITURATES DETERMINED BY TLC

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Abstract: Lipophilicty of 29 thiobarbituric acid derivatives were assessed by reversed-phase thin-layer chromatography using methanol : water mixtures as a mobile phase. A linear relationship was found between R_M values and methanol concentrations in the mobile phase. The retention parameter, R_{M0}, was related to theoretical partition coefficients calculated by means of different theoretical procedures (Alog*P*s, IAlog*P*, miLog*P*, logP_{Kowwin}, xlog*P*).

Keywords: thiobarbituric acid derivatives, lipophilicity, theoretical partition coefficient

Lipophilicity is a term mainly employed by medicinal chemists to describe transport process of compound in biological systems and is the most frequently used parameter in QSAR analysis. It is predominant descriptor of pharmacodynamic, pharmacokinetic and toxic aspects of drug activity. The lipophilic character of compounds has been defined in many ways. The most applied one is a partition coefficient, *P*, or its decimal logarithm, log*P*, which represents the tendency of a molecule to partition itself between organic and aqueous phase. The traditional "shake-flash" partition method between n-octanol and water is often substituted by chromatographic approaches (RP-HPLC and RP-TLC methods). These methods are based on the assumed linear relationship between log*P* and the logarithm of chromatographic capacity factor data (log *k* and R_M) (1).

Many authors described the application of the RP-TLC to determine the lipophilicity of different chemical compounds or drugs (2-8). They reported a good linear correlation between R_M extrapolated to zero organic modifier content and the lipophilicity parameters determined by other methods: calculated log*P* values (2,4,5,7,8), from RP-HPLC measurements (3) and from the shake flask method (3,6). Raviolo et al. (3) have demonstrated the superiority of methanol as compared with acetone as the organic modifier for application in RP-TLC method. In our previous paper (5) we described studies on lipophilicity of 5,5-disubstituted derivatives of barbituric acid obtained by the RP-TLC method. We demonstrated that R_{M0} can be better correlated with selected biological activity of barbiturates then log*P* (calculated and experimental).

The aim of this work was to evaluate the lipophilicity of the series of thiobarbituric acid derivatives by the RP-TLC method and to compare the retention parameter, R_{M0}, with theoretical partition coefficients calculated using different theoretical procedures.

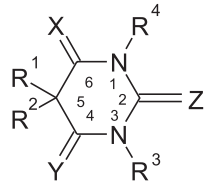
EXPERIMENTAL

The structures of studied thiobarbituric acid derivatives are listed in Table 1.
Compounds **1 – 23** and **28** (9) were synthesized in our lab according to the published procedure (10) (synthesis of compounds **1 – 10** and **14** to be published, see Table 2). Compounds **24** – **27** and **29** were commercial or analytical samples and were kindly provided by Abbott Laboratories and Bayer, respectively. Methanol (HPLC grade) was purchased from Merck (Darmstadt, Germany).

Thin layer chromatography was performed on TLC aluminium sheets 20 × 20 cm RP-18 F_{254S} (Merck, Darmstadt, Germany). Mixtures of methanol-water were used as the mobile phases with methanol content ranging from 55 to 90% (v/v) in 5% increments.

The methanol solutions (1%, w/v) of the investigated compounds were applied on the start line with a Hamilton syringe (10 μL). The chromatograms were developed on 12 cm distance at 22 ± 1°C. After development and drying, the spots were visualized with the UV_{254} light. The R_F values were

Table 1. Structure of the investigated thiobarbituric acids derivatives.



Compound no.	R^1	R^2	R^3	R^4	X	Y	Z
1	methyl	methyl	H	H	O	O	S
2	methyl	methyl	H	H	O	S	O
3	methyl	methyl	H	H	O	S	S
4	methyl	methyl	H	H	S	S	O
5	methyl	methyl	H	H	S	S	S
6	ethyl	ethyl	H	H	O	O	S
7	ethyl	ethyl	H	H	O	S	O
8	ethyl	ethyl	H	H	O	S	S
9	ethyl	ethyl	H	H	S	S	O
10	ethyl	ethyl	H	H	S	S	S
11	ethyl	phenyl	H	H	O	O	S
12	ethyl	phenyl	H	H	O	S	O
13	ethyl	phenyl	H	H	O	S	S
14	ethyl	phenyl	H	H	S	S	O
15	ethyl	phenyl	H	H	S	S	S
16	ethyl	phenyl	H	methyl	O	O	S
17	ethyl	phenyl	H	methyl	O	S	O
18	ethyl	phenyl	H	methyl	O	S	S
19	ethyl	phenyl	H	methyl	S	S	S
20	ethyl	phenyl	methyl	methyl	O	O	S
21	ethyl	phenyl	methyl	methyl	O	S	O
22	ethyl	phenyl	methyl	methyl	O	S	S
23	ethyl	phenyl	methyl	methyl	S	S	O
24[a]	ethyl	1-methylbutyl	H	H	O	O	S
25[b]	ethyl	2-methyl-2-propenyl	H	H	O	O	S
26[c]	allyl	2-cyclohexenyl	H	H	O	O	S
27[d]	allyl	1-methylbutyl	H	H	O	O	S
28	ethyl	phenyl	H	ethyl	O	O	S
29	allyl	isobutyl	H	H	O	O	S

Drug name:
[a] Thiopental, [b] Mosidol, [c] Kemithal, [d] Surital, [e] Baytinal

run in duplicate and the mean values were used for calculation of R_M parameters according to the expression (1):

$$R_M = \log\left[\left(\frac{1}{R_F} - 1\right)\right] \quad (1)$$

The R_M values were extrapolated to the zero methanol concentration (R_{M0}) using the expression (11, 12):

$$R_M = R_{M0} + bC \quad (2)$$

where C is the concentration of methanol (in %, v/v) in the mobile phase and b is the change in the R_M value due to the 1% increase of methanol content in the mobile phase.

The lipophilicity of the investigated compounds, expressed by partition coefficient log$P$ was also calculated theoretically using the follow-

Table 2. Properties of thiobarbituric acids derivatives (compounds **1 – 10**, **14**).

Compound no.	M.p., color	Method of preparation	NMR [ppm] (in DMSO)	MS (%) *m/z*	IR [cm^{-1}] (in KBr) ν C=O	Analysis [%] found (calc.)
1	223-224°C pale yellow (lit. 205°C (21))	C	1.33 (s, 6H,C<u>H</u>$_3$-) 12.18 (s, 2H, <u>H</u>N^{1,3}<)	172.0(100) [M^+]	1719	N – 16.00 (16.27) S – 18.50 (18.62) C – 42.03 (41.85) H – 4.68 (4.68)
2	212-213°C yellow (lit. 201°C (22))	A	1.51 (s, 6H,C<u>H</u>$_3$-) 11.44 (s, 1H, <u>H</u>N^1<) 12.68 (s, 1H, <u>H</u>N^3<)	172.0(100) [M^+]	1749, 1704	N – 16.07 (16.27) S – 18.56 (18.62) C – 42.07 (41.85) H – 4.77 (4.68)
3	226-227°C yellow	C	1.49 (s, 6H,C<u>H</u>$_3$-) 12.51 (s, 1H, <u>H</u>N^1<) 13.46 (s, 1H, <u>H</u>N^3<)	188.0(100) [M^+]	1699	N – 14.66 (14.88) S – 33.98 (34.06) C – 38.45 (38.28) H – 4.10 (4.28)
4	184-186°C yellow (lit. 126°C (22))	A 12.95	1.73 (s, 6H,C<u>H</u>$_3$-) (s, 2H, <u>H</u>N^{1,3}<)	188.0(100) [M^+]	1729, 1696	N – 14.67 (14.88) S – 33.98 (34.06) C – 38.61 (38.28) H – 4.00 (4.28)
5	263-265°C orange (lit. 134°C (22))	A	1.70 (s, 6H,C<u>H</u>$_3$-) 13.77 (s, 2H, <u>H</u>N^{1,3}<)	203.9(77.75) [M^+]		N – 13.76 (13.71) S – 47.19 (47.08) C – 35.21 (35.27) H – 3.78 (3.95)
6	174-175°C pale yellow (lit. 174-175°C (23))	A	0.72 (t, 6H,C<u>H</u>$_3$-CH$_2$-) 1.82 (q, 4H, CH$_3$-C<u>H</u>$_2$-) 12.60 (s, 2H, <u>H</u>N^{1,3}<)	200.0(87.49) [M^+]	1739, 1722, 1673	
7	198-200°C yellow (lit. 192-193°C (24))	A	0.70 (t, 6H, C<u>H</u>$_3$-CH$_2$-) 2.01 (m, 4H, CH$_3$-C<u>H</u>$_2$-) 11.84 (s, 1H, <u>H</u>N^1<) 13.00 (s, 1H, <u>H</u>N^3<)	200.0(38.57) [M^+]	1762, 1699 1671	

Table 2. cont.

Compound no.	M.p., color	Method of preparation	NMR [ppm] (in DMSO)	MS (%) *m/z*	IR [cm^{-1}] (in KBr) ν C=O	Analysis [%] found (calc.)
8	206-207°C yellow (lit. 205-206°C (25))	A	0.72 (t, 6H, CH_3-CH_2-) 2.02 (m, 4H, CH_3-CH_2-) 12.82 (bs, 1H, HN^{1}<) 13.79 (bs, 1H, HN^{3}<)	216.0(71.24) [M$^+$]	1692, 1675	
9	161-163°C yellow (lit. 163-164°C (26))	B	0.72 (t, 6H, CH_3-CH_2-) 2.30 (q, 4H, CH_3-CH_2-) 13.26 (s, 2H, HN^{1,3}<)	216.0(75.90) [M$^+$]	1741, 1720	
10	194-195°C orange (lit. 194-195°C (25))	A	0.75 (t, 6H, CH_3-CH_2-) 2.30 (q, 4H, CH_3-CH_2-) 14.03 (bs, 1H, HN^{1,3}<)	232.0(100) [M$^+$]		
14	200-201°C yellow	B	0.88 (t, 3H, CH_3-CH_2-) 2.86 (q, 2H, CH_3-CH_2-) 7.22-7.34 (m, 5H, C_6H_5-) 13.41 (s, 1H, HN^{1,3}<)	172.0(100) [M$^+$]	1749, 1704	N – 16.00 (16.27) S – 18.50 (18.62) C – 42.03 (41.85) H – 4.68 (4.68)

A – thionation with Lawesson's reagent
B – desulfurization using NO^+ ($NaNO_2$)
C – desulfurization in ethanol solution

ing methods: Pallas 3.2 (13), Alog*P*s (14, 15), IAlog*P* (14, 16), miLog*P* (17), logP_{Kowwin} (14, 18) and xlog*P* (14, 19). The parameter R_{M0} was correlated with these values according to the expression (1, 20):

$$R_{M0} = A + B\log P \quad (3)$$

Calculations were done using the Statistica PL 6.0 computer program.

RESULTS AND DISCUSSION

The relative lipophilicity of 29 thiobarbituric acids derivatives, expressed by the chromatographic value of R_{M0}, was estimated by RP-TLC on RP-18 plates with mixtures of methanol and water as the mobile phases. The R_M values of the compounds decreased linearly with increasing concentration of methanol in the mobile phase. Examples of dependence of R_M for compounds **7**, **8**, and **10** on methanol concentration in the mobile phase are presented in Figure 1. Parameters of linear correlation between R_M values of the investigated compounds and methanol content in the mobile phase are listed in Table 3.

Taking into account the results of the lipophilicity presented in Table 3 the following regularities could be found:

1. the lipophilicity of thiobarbituric acids increases with increasing number of sulfur atoms introduced into barbiturate ring (compounds **1 – 5**, **6 – 10**, **11 – 15**, and **16 – 23**) and 2S-derivatives are less lipophilic than 4S-derivatives. The lipophilicity increases according the following order:

2-thioderivatives < 4-thioderivatives < 2,4-dithioderivatives < 4,6-dithioderivatives < 2,4,6-trithioderivatives.

2. elongation of aliphatic chain of C5 substituent and its branching caused also the increase of lipophilicity (compounds **1 – 5**, **6 – 10**, **11 – 15**, and **24 – 29**).

3. introduction of phenyl ring at the C5 atom yielded further increase of the lipophilicity (compounds **6 – 10** and **11 – 15**).

4. alkyl-substitution at the nitrogen atom in the barbiturate ring caused an increase of lipophilicity, and compounds which are substituted at two nitrogen atoms are more

Table 3. Parameters of linear correlation between R_M values of tiobarbiturates and methanol content in the mobile phase acc. to Eq. (2).

Compound no.	R_{M0}	b	n	Correlation coefficient r	Coefficient of determination r^2	Standard error of estimation s	F-test of significance[a] (F)
1	0.892 (± 0.101)	-0.020 (± 0.001)	7	0.988	0.976	0.038	204
2	1.406 (± 0.070)	-0.024 (± 0.001)	7	0.996	0.991	0.026	576
3	1.625 (± 0.089)	-0.024 (± 0.001)	8	0.992	0.985	0.039	391
4	2.239 (± 0.087)	-0.029 (± 0.001)	8	0.995	0.990	0.039	599
5	2.455 (± 0.110)	-0.030 (± 0.001)	6	0.996	0.991	0.029	454
6	1.239 (± 0.058)	-0.020 (± 0.001)	8	0.995	0.990	0.026	616
7	1.924 (± 0.047)	-0.026 (± 0.001)	8	0.998	0.996	0.021	1674
8	2.314 (± 0.062)	-0.029 (± 0.001)	8	0.997	0.995	0.027	1202
9	2.936 (± 0.116)	-0.035 (± 0.002)	8	0.994	0.988	0.051	495
10	3.507 (± 0.120)	-0.041 (± 0.002)	8	0.995	0.991	0.053	632
11	1.887 (± 0.090)	-0.026 (± 0.001)	8	0.994	0.987	0.040	463
12	2.139 (± 0.102)	-0.029 (± 0.001)	8	0.993	0.986	0.045	428
13	2.273 (± 0.063)	-0.029 (± 0.001)	7	0.998	0.996	0.022	1197
14	2.715 (± 0.082)	-0.034 (± 0.001)	8	0.997	0.993	0.036	915
15	3.757 (± 0.223)	-0.046 (± 0.003)	8	0.987	0.974	0.098	226
16	2.934 (± 0.156)	-0.036 (± 0.002)	8	0.990	0.980	0.069	291
17	2.825 (± 0.129)	-0.036 (± 0.002)	8	0.993	0.986	0.057	427
18	3.920 (± 0.136)	-0.047 (± 0.002)	7	0.996	0.992	0.051	590
19	5.126 (± 0.192)	-0.057 (± 0.002)	7	0.995	0.990	0.067	510
20	4.324 (± 0.148)	-0.048 (± 0.002)	7	0.996	0.992	0.052	617
21	3.717 (± 0.134)	-0.042 (± 0.002)	6	0.997	0.993	0.036	606
22	5.692 (± 0.172)	-0.060 (± 0.002)	6	0.997	0.995	0.046	745
23	4.835 (± 0.280)	-0.052 (± 0.004)	6	0.991	0.981	0.075	213
24	2.685 (± 0.072)	-0.034 (± 0.001)	8	0.998	0.995	0.032	1228
25	1.860 (± 0.048)	-0.026 (± 0.001)	8	0.998	0.996	0.021	1604
26	2.404 (± 0.036)	-0.031 (± 0.001)	7	0.999	0.999	0.013	4016
27	2.971 (± 0.072)	-0.037 (± 0.001)	8	0.998	0.996	0.032	1424
28	3.726 (± 0.136)	-0.044 (± 0.002)	8	0.995	0.989	0.060	560
29	2.121 (± 0.056)	-0.028 (± 0.001)	7	0.998	0.997	0.020	1462

[a] for all cases the probability p was lower than 0.0001

Table 4. Theoretical partition coefficients for the investigated thiobarbiturates.

Compound no.	logP_{Pallas}	Alog$P$s	IAlog$P$	miLog$P$	logP_{Kowwin}	xlog$P$
1	0.19	0.93	0.45	0.61	0.49	-0.45
2	0.07	0.83	0.69	-0.08	-0.46	-0.45
3	0.60	1.65	1.53	0.47	-0.15	-0.20
4	0.25	1.71	1.93	-0.22	-0.74	-0.20
5	0.89	2.15	3.30	0.32	-0.49	0.05
6	1.74	1.72	1.49	1.28	1.47	0.68
7	0.78	1.78	1.76	0.59	0.52	0.68
8	1.12	2.42	2.49	1.13	0.83	0.93
9	0.94	2.47	2.83	0.45	0.25	0.94
10	1.30	2.83	3.96	0.99	0.49	1.19
11	2.27	2.34	2.64	1.34	2.20	1.57
12	1.59	2.47	2.85	0.98	1.25	1.57
13	1.69	3.12	3.19	1.52	1.56	1.82
14	1.68	3.19	3.44	1.16	0.97	1.82
15	1.70	3.55	3.25	1.70	1.22	2.07
16	1.89	2.50	2.57	1.58	2.41	1.71
17	1.89	2.64	2.89	1.78	1.46	1.71
18	1.85	3.16	3.41	1.76	1.77	1.96
19	1.91	3.59	4.71	1.95	1.43	2.21
20	2.09	2.54	2.39	1.83	2.62	1.85
21	2.10	2.77	2.44	1.47	1.67	1.85
22	2.16	3.03	2.29	2.01	1.98	2.10
23	2.16	3.17	2.46	1.65	1.39	2.10
24	2.37	3.05	2.96	2.59	2.87	2.33
25	1.64	1.72	1.88	1.86	2.38	0.95
26	2.17	2.89	2.57	1.99	3.39	2.24
27	2.23	3.11	3.18	2.62	3.23	2.53
28	2.38	2.99	2.82	1.96	2.90	2.13
29	2.37	2.54	2.60	2.09	2.74	1.96

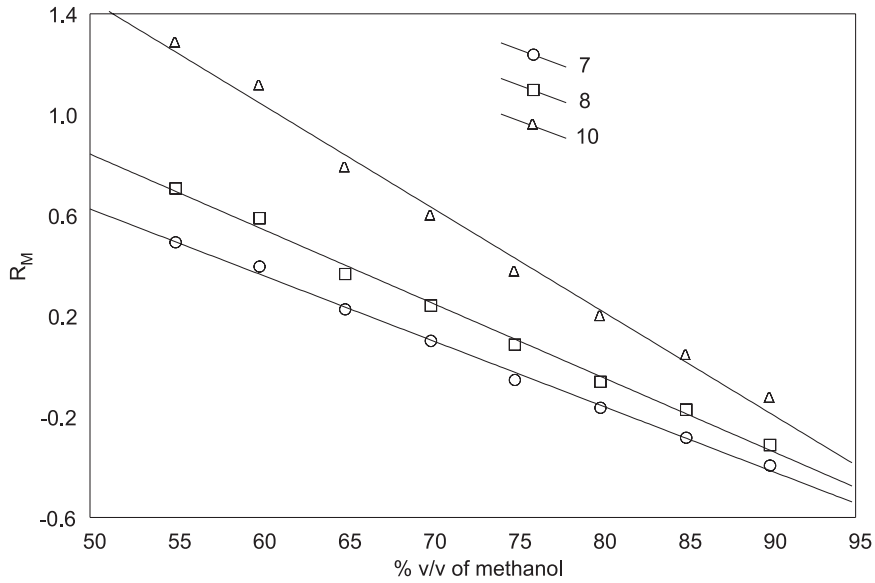


Figure 1. Dependence of R_M for compounds **7**, **8** and **10** on methanol concentration in the mobile phase.

Table 5. Linear correlation (acc. to Eq. (3)) of thin layer chromatographic parameter (R_{M0}) with logarithms of theoretically calculated partition coefficients for the investigated tiobarbiturates.

Equation no.	Correlation	A	B	n	Correlation coefficient r	Coefficient of determination r^2	Standard error of estimation s	F-test of significance (F)	Probability p
4	R_{M0} = f(logP_{Pallas})	1.453 (± 0.479)	0.876 (± 0.278)	29	0.519	0.269	1.021	10	< 0.0039
5	R_{M0} = f(Alog*P*s)	-0.245 (± 0.556)	1.229 (± 0.213)	29	0.743	0.552	0.799	33	< 0.0001
6	R_{M0} = f(IAlog*P*)	0.877 (± 0.566)	0.760 (± 0.207)	29	0.577	0.333	0.976	13	< 0.0011
7	R_{M0} = f(milog*P*)	1.820 (± 0.417)	0.753 (± 0.271)	29	0.471	0.222	1.054	8	< 0.0099
8	R_{M0} = f(logP_{Kowwin})	2.428 (± 0.346)	0.289 (± 0.190)	29	0.281	0.079	1.146	2	< 0.0039
9	R_{M0} = f(xlog*P*)	1.701 (± 0.314)	0.836 (± 0.193)	29	0.640	0.409	0.918	19	< 0.0002

lipophilic than those with one substituent (compounds **11** – **15**, **16** – **19**, and **20** – **23**).

The values of log*P* calculated by use of different theoretical procedures (logP_{Pallas}, Alog*P*s, IAlog*P*, miLog*P*, logP_{Kowwin}, and xlog*P*) are presented in Table 4. The lipophilicity parameter R_{M0} was compared with the predicted values of log*P*. The parameters of linear correlation are listed in Table 5. The calculated values of parameters for the linear correlation between each of the predicted partition coefficients are presented in Table 6. The data collected in Table 5 and 6 indicate that the correlations between the values of these parameters were not statistically significant. The best linear correlation between the R_{M0} values and the theoretical partition coefficients was found for that calculated using Alog*P*s method (r = 0.743, r^2 = 0.552).

The correlation improved when compound **22** was excluded (the residual value is 2.213, the standardized residual is 2.768 and the studentized residual is 3.340).

$$R_{M0} = -0.110\ (\pm 0.476) + 1.143\ (\pm 0.184)\ \text{Alog}P\text{s} \quad (4)$$

n = 28; r = 0.774; r^2 = 0.599; s = 0.682; F = 39, p < 0.0001

The remaining theoretical partition coefficients show low correlation with the R_{M0} values.

CONCLUSIONS

The RP-TLC method was used to investigate lipophilicity of thiobarbituric acid derivatives. Good correlation between the retention parameters obtained by RP-TLC and the concentration of methanol in the mobile phase was obtained for the studied compounds. Statistically, highly significant correlation was found between R_{M0} values and the log*P* predicted using Alog*P*s method.

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Table 6. Correlation coefficients of linear dependences between particular parameters of lipophilicity.

	R_{M0}	logP_{Pallas}	Alog$P$s	IAlog$P$	miLog$P$	logP_{Kowwin}	xlog$P$
R_{M0}	1						
logP_{Pallas}	0.519	1					
Alog$P$s	0.743	0.744	1				
IAlog$P$	0.577	0.516	0.854	1			
miLog$P$	0.471	0.905	0.687	0.438	1		
logP_{Kowwin}	0.281	0.881	0.506	0.252	0.917	1	
xlog$P$	0.640	0.925	0.893	0.657	0.884	0.808	1

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