Acta Poloniae Pharmaceutica - Drug Research, Vol. 59 No. 5 pp. 341-346, 2002 ISSN 0001-6837
Polish Pharmaceutical Society

LIPOPHILICITY PARAMETER FROM HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ON AN IMMOBILIZED ARTIFICIAL MEMBRANE COLUMN AND ITS RELATIONSHIPS TO BIOACTIVITY OF THE GROUP OF 2,4-DIHYDROXYTHIOBENZANILIDES

KRZYSZTOF JÓŹWIAK[a], HALINA SZUMIŁO[a] and BOGUSŁAW SENCZYNA[b]

[a] Department of Inorganic and Analytical Chemistry, Medical University of Lublin, 6 Staszica Str., 20-081 Lublin
[b] Department of Chemistry, University of Agriculture, 15 Akademicka Str., 20-950 Lublin, Poland

Abstract: Several groups of 2,4-dihydroxythiobenzanilide derivatives are extensively studied in our laboratories as potential antifungal or antibacterial agents. Previous papers showed that their biological activities are closely correlated with the lipophilicity determined in HPLC experiments using on octadecylsilyl stationary phase (RP-18). In the present paper we attempted to measure chromatographic indices of 2,4-dihydroxythiobenzanilides, using a silica-based immobilized artificial membrane (IAM) column especially designed to evaluating biological lipophilicity. The theoretical (extrapolated) retention in pure water the log $k_{w(IAM)}$ parameter for 23 derivatives was determined during poly-cratic experiments. Statistical analysis exhibited good correlations between the log $k_{w(IAM)}$ parameter and the biological data concerning bacteriostatic activity of these compounds. Retention on the IAM phase can be used as predictor of biological membrane permeability and biological lipophilicity of 2,4-dihydroxythiobenzanilides in spite of the acidic properties of this group of compounds.

Keywords: HPLC, immobilized artificial membrane (IAM), thiobenzanilides, QSAR.

The pharmacokinetics of drugs as well as the access capability to the receptor site are strongly dependent on their interaction with biological membranes. The affinity to biomembranes is assumed to be the driving force of passive diffusion in living organisms. Immobilized artificial membranes (IAM) are chromatographic surfaces prepared by covalently immobilizing cell membrane phospholipids. These stationary phases were especially designed to evaluating biological lipophilicity (1-3). They have lately become phases of great importance in quantitative structure-activity relationship analyses (4-6). Since it closely mimics phospholipidic surfaces of biomembranes, it is used for predicting membrane permeability and biological lipophilicity of potential drugs. Such investigations dealt mainly with neutral or basic molecules; previous works indicated that the affinity of acidic molecules to phospholipids is affected not only by the lipophilicity but also by ionization of analytes (5).

A few groups of 2,4-dihydroxythiobenzanilide derivatives are extensively studied in our laboratories. They express broad spectrum of physicochemical properties as well as biological activities (7). Previous papers showed that their bacteriostatic and fungostatic activities are closely correlated with the lipophilicity of the particular derivative determined in HPLC experiments using octadecylsilyl stationary phase (8,9). In the present paper we attempted to measure chromatographic parameters of 2,4-dihydroxythiobenzanilides, using a silica-based immobilized artificial membrane (IAM) column, and then to relate these parameters to biological activity of the compounds.

EXPERIMENTAL

23 Analogues of 2,4-dihydroxythiobenzanilide with various modifications in the anilide ring (Figure 1) (chemistry: see reference (10)) were chosen for chromatographic experiments. HPLC was performed on a Knauer-WellChrom apparatus with a spectrophotometric detector (λ = 320 nm). 20 µl of 0.2% methanol solutions of a particular derivative were injected into the IAM column (IAM.PC.C10/C3, length: 150 mm, internal diameter: 4.6 mm from Regis Technologies Inc. Illinois). The retention times of compounds were determined under isocratic conditions in binary mobile phases (flow rate = 1 ml/min). Several acetonitrile - aqueous buffer solutions with different compositions were used for the elution (acetonitrile: Lichrosolv Gradient Grade, E. Merck, Germany). To avoid

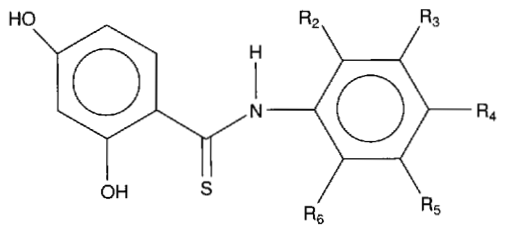

Figure 1. The chemical structures of investigated 2,4–dihydroxythiobenzanilides.
R_2 to R_6 = *–H, –F, –Cl, –I, –OH, –CF₃, –CN, –COOH, –CH₃, –OCH₃* etc.

ionization process of investigated compounds during chromatographic experiments we utilized phosphate buffer (concentration: 50 mmol/l) with pH adjusted to 3.0; this was the only value which gave reproducible and measurable results. The void volume was determined by injection of pure methanol.

Biological data concerning bacteriostatic activity of 2,4–dihydroxythiobenzanilides were presented elsewhere (Jóźwiak et al., 1999). The investigated analogues were tested against various microorganisms from the Gram positive bacteria group. Minimal Inhibitory Concentrations (MIC) [mmol/l] were determined by standard dilution methods.

Statistical analysis of the data was carried out with STATISTICA for Windows (Polish version), StatSoft, Inc.

RESULTS AND DISCUSSION

The capacity factors of investigated compounds were measured at several different concentrations of acetonitrile in the mobile phase (volume fraction (φ) in the range 0.3 – 0.65). Carrying out the experiments in such, so–called *poly–cratic* (11) manner, one can obtain not only the retention indexes for the particular eluent composition but also the parameters calculated from regression analysis of the relation log $k = f(\varphi)$ according to equation (12):

Table 1. Retention (log$k$ values) of particular derivatives obtained for different compositions of mobile phase; $\log k_{w(IAM)}$ parameter calculated in regression analysis and the correlation coefficient (r).

		volume fraction of acetonitrile (φ)								$\log k_w$	r
	derivative	0.30	0.35	0.40	0.45	0.50	0.55	0.60	0.65	(IAM)	
I	not substituted	1.155	0.863	0.588	0.374	0.194	0.009	–0.148		2.366	0.993
II	2,4–di–CH_3		1.103	0.764	0.564	0.334	–0.007	–0.136		2.809	0.995
III	2,4–di–F		0.953	0.655	0.437	0.242	0.048	–0.147		2.404	0.996
IV	4–I			1.183	0.937	0.735	0.524	0.345	0.216	2.704	0.996
V	3–CF_3			0.965	0.707	0.492	0.273	0.087	–0.099	2.625	0.998
VI	4–OCH_3	1.177	0.823	0.51	0.326	0.148	–0.070	–0.262		2.457	0.991
VII	3–OH	1.044	0.734	0.472	0.308	0.151	–0.074	–0.187		2.159	0.991
VIII	4–CO–NH_2–CH_2COOH	0.736	0.430	0.163	–0.015	–0.198	–0.387	–0.520		1.882	0.992
IX	4–N(C_2H_5)–C_2H_4OH	0.807	0.598	0.343	0.219	0.062	–0.208	–0.323		1.912	0.996
X	2,5–di–Cl			1.207	0.984	0.783	0.587	0.405	0.172	2.821	0.999
XI	2–Cl,4–CH_3			1.124	0.874	0.666	0.461	0.283	0.131	2.673	0.997
XII	2–F	1.229	0.896	0.588	0.400	0.208	–0.031	–0.163		2.507	0.991
XIII	3–COOH,4–OH			0.981	0.762	0.704	0.510	0.423	0.239	2.080	0.992
XIV	4–F	1.262	0.924	0.628	0.414	0.238	0.005	–0.147		2.549	0.992
XV	4–CN		1.039	0.726	0.520	0.325	0.164	–0.008		2.393	0.992
XVI	2–CH_3,5–F		1.067	0.741	0.532	0.322	0.052	–0.109		2.648	0.996
XVII	4–CO–C_2H_5		1.108	0.777	0.575	0.371	0.198	–0.025		2.566	0.995
XVIII	2,3–di–Cl			1.095	0.859	0.662	0.464	0.181	0.040	2.804	0.998
XIX	3–Cl,4–F			1.045	0.789	0.587	0.385	0.216	0.069	2.555	0.996
XX	4–t–butyl			1.219	0.914	0.673	0.441	0.236	0.057	3.013	0.996
XXI	2–$COOCH_3$	1.175	0.839	0.558	0.339	0.161	–0.042	–0.210		2.432	0.992
XXII	2–CH_3,3–Cl			0.947	0.719	0.517	0.214	0.055	–0.095	2.645	0.995
XXIII	2–CH_3,4–OH	0.964	0.648	0.377	0.208	–0.041	–0.195	–0.325		2.153	0.991

$$\log k = \log k_{w} - S_{\varphi}$$

$\log k_w$ is the theoretical retention in the eluent without acetonitrile (pure buffer). This parameter is especially frequently employed in QSAR analyses as a chromatographic lipophilicity parameter.

Table 1 presents the retention parameters (log k) of particular derivatives for different concentrations of acetonitrile in mobile phase (φ). Table 1 includes also the log $k_{w(IAM)}$ parameter calculated for each compound and the correlation coefficient (r) of the linear regression analysis of the relation $\log k = f(\varphi)$.

Generally, 2,4–dihydroxythiobenzanilides have a strong affinity to the immobilized artificial membrane chromatographic phase. At least 0.3 volume fraction of acetonitrile in the eluent is required to determine retention parameters in the optimal accuracy range. However, due to very high correlation coefficients of regression, the extrapolated theoretical retention parameter for pure buffer, log $k_{w(IAM)}$, was determined very precisely.

The log $k_{w(IAM)}$ lipophilicity scale obtained during the presented chromatographic analysis of 2,4–dihydroxythiobenzanilides using the IAM column distinguishes from thot determined earlier on a octadecylsilyl stationary phase. Table 2 presents the correlations between the log $k_{w(IAM)}$ and the parameters obtained on the octadecylsilyl chromatographic column in *poly-cratic* experiments using two different eluent systems: containing methanol (log $k_{w(MeOH)}$) or acetonitrile (log $k_{w(MeCN)}$) (8).

The log $k_{w(IAM)}$ values are moderately cor-

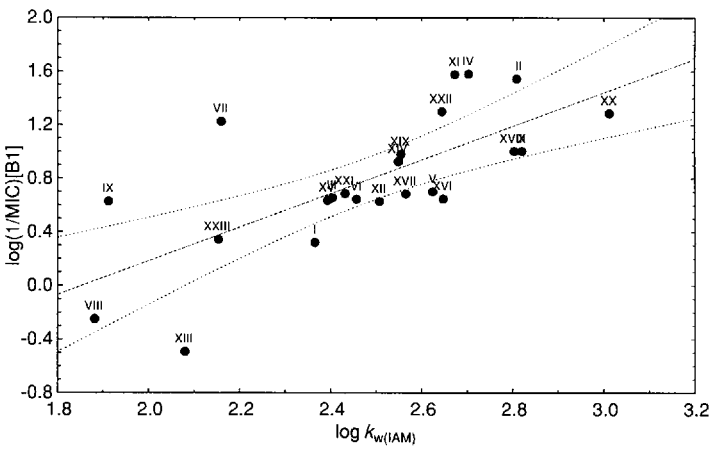

Figure 2. Correlation between log $k_{w(IAM)}$ parameter and bacteriostatic activity of tested 2,4–dihydroxythiobenzanilide derivatives against *Staphylococcus aureus ATCC 25923*[B1].

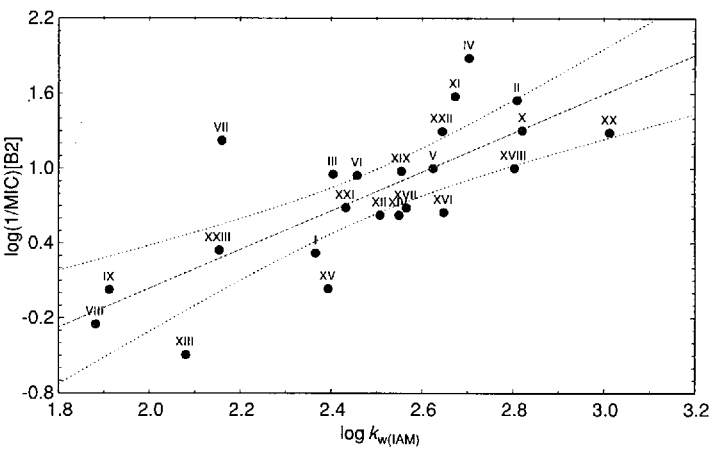

Figure 3. Correlation between log $k_{w(IAM)}$ parameter and bacteriostatic activity of tested 2,4–dihydroxythiobenzanilide derivatives against *Staphylococcus aureus 209p*[B2].

Table 2. Correlations between $\log k_{w(IAM)}$ and $\log k_{w(MeOH)}$ or $\log k_{w(MeCN)}$ parameters of investigated compounds.

$\log k_{w(IAM)} = 0.41(\pm0.07)\log k_{w(MeOH)} + 1.15(\pm0.24)$ $r = 0.781$ $s = 0.187$ $F = 32.818$ $n = 23$
$\log k_{w(IAM)} = 0.89(\pm0.11)\log k_{w(MeCN)} + 0.46(\pm0.25)$ $r = 0.874$ $s = 0.145$ $F = 67.801$ $n = 23$

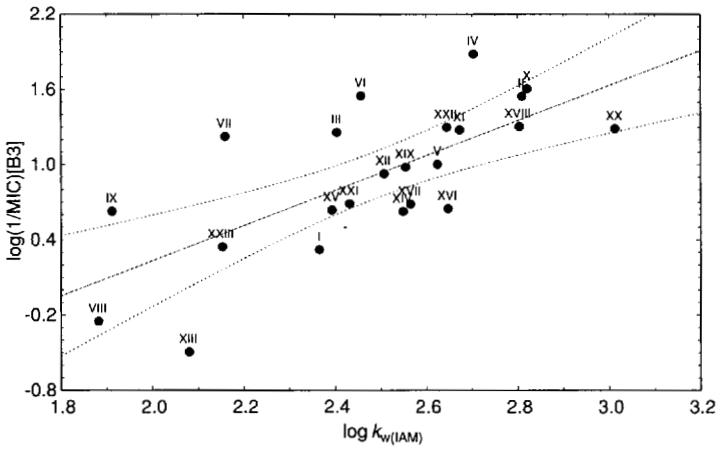

Figure 4. Correlation between log $k_{w(IAM)}$ parameter and bacteriostatic activity of tested 2,4–dihydroxythiobenzanilide derivatives against *Staphylococcus epidermidis*[B3].

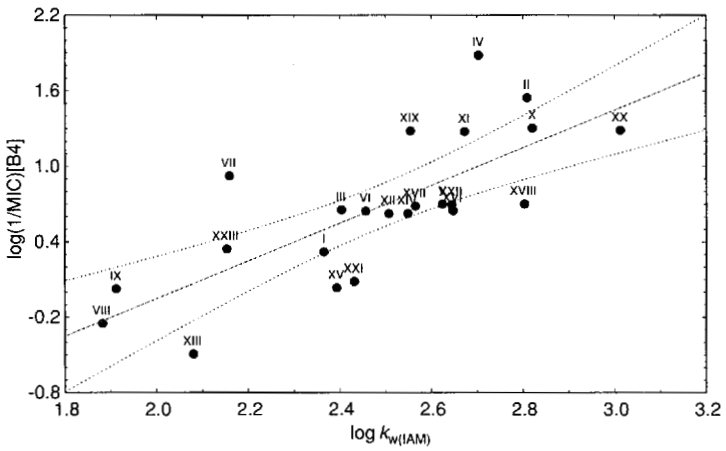

Figure 5. Correlation between log $k_{w(IAM)}$ parameter and bacteriostatic activity of tested 2,4–dihydroxythiobenzanilide derivatives against *Sarcina lutea*[B4].

related with similar parameters derived from the octadecylsilyl chromatographic column. A better correlation between log $k_{w(IAM)}$ and log $k_{w(MeCN)}$ is observed (in both cases acetonotrile was used as a modifier of the mobile phase), whereas correlation between log $k_{w(IAM)}$ and log $k_{w(MeOH)}$ is markedly lower. Thus, log $k_{w(IAM)}$ can be regarded as another, alternative lipophilicity parameter of 2,4–dihydroxythiobenzanilides for QSAR analysis.

The retention on the IAM column is assumed to be related to the permeation ratio through biological bilayer. Statistical analysis exhibited a relatively high correlation between log $k_{w(IAM)}$ parameter and bacteriostatic activity of the investigated compounds determined earlier (8). Figures 2,3,4,5 and 6 present correlations between the log $k_{w(IAM)}$ parameter and the log(1/MIC) values determined for the 23 tested compounds against the following

Table 3. Correlations between bacteriostatic activity and $\log k_{w(IAM)}$ parameter of investigated compounds.

$\log(1/MIC)(B1) = 1.26(\pm0.28)\log k_{w(IAM)} - 2.33(\pm0.69)$ $r = 0.705$ $s = 0.378$ $F = 20.803$ $n = 23$
$\log(1/MIC)(B2) = 1.56(\pm0.29)\log k_{w(IAM)} - 3.07(\pm0.73)$ $r = 0.757$ $s = 0.400$ $F = 28.231$ $n = 23$
$\log(1/MIC)(B3) = 1.40(\pm0.31)\log k_{w(IAM)} - 2.57(\pm0.77)$ $r = 0.702$ $s = 0.424$ $F = 20.423$ $n = 23$
$\log(1/MIC)(B4) = 1.50(\pm0.29)\log k_{w(IAM)} - 3.05(\pm0.71)$ $r = 0.754$ $s = 0.391$ $F = 27.596$ $n = 23$
$\log(1/MIC)(B5) = 1.10(\pm0.25)\log k_{w(IAM)} - 2.65(\pm0.62)$ $r = 0.696$ $s = 0.339$ $F = 19.718$ $n = 23$

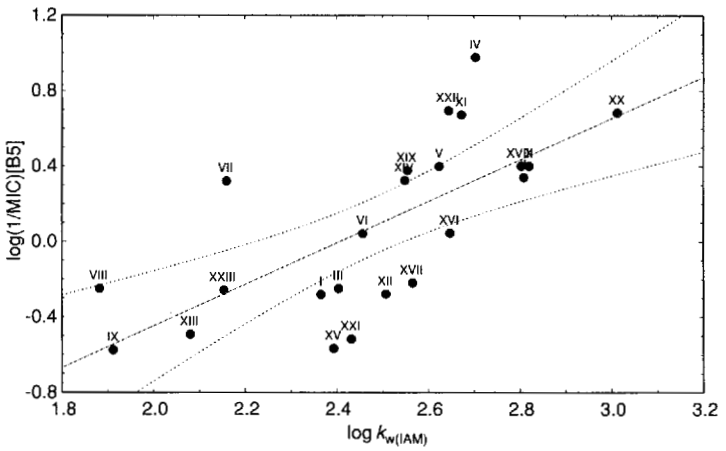

Figure 6. Correlation between log $k_{w(IAM)}$ parameter and bacteriostatic activity of tested 2,4–dihydroxythiobenzanilide derivatives against *Bacillus subtilis*[B5].

Gram–positive strains of microorganisms: *Staphylococcus aureus ATCC 25923*[B1], *Staphylococcus aureus 209p*[B2], *Staphylococcus epidermidis*[B3], *Sarcina lutea*[B4] and *Bacillus subtilis*[B5], respectively. Table 3 represents the equations of these correlations.

Experimental values of retention (log k) on the IAM column (determined at particular concentrations of acetonitrile) are also frequently employed to model the affinity of compounds to biological bilayer. However, in case of the investigated 2,4–dihydroxythiobenzanilides, the log $k_{w(IAM)}$ values are better correlated with the tested bioactivity than any set of experimental log k values. The log $k_{w(IAM)}$ parameter (as a theoretical retention in the eluent without acetonitrile) is a more efficient predictor of biological lipophilicity than experimental retentions because it reduces the influence of organic modifier in the mobile phase. Acetonitrile is necessary in the mobile phase in order to obtain measurable elution, however, it introduces unfavorable intermolecular interactions during experiments, which can distort the determination of the affinity of compounds to the IAM phase.

The presented correlations between log $k_{w(IAM)}$ and bacteriostatic activity of the investigated 2,4–dihydroxythiobenzanilides are higher than the respective correlations between log k_w parameters obtained using the octadecylsilyl stationary phase presented earlier (8).

Since the immobilized artificial membrane stationary phase mimics the surface of biological bilayer, the parameters derived from retention on IAM columns can be utilized as the predictors of drug–membrane permeability in quantitative struc-

ture–activity relationships analyses, in spite of acidic properties of this group of compounds.

Acknowledgement

We greatly appreciate receiving the IAM column from the laboratory of Prof. Roman Kaliszan, Medical University of Gdańsk, Poland.

REFERENCES

1. Pidgeon C., Ong S., Liu H., Qiu X., Pidgeon M., Dantzig A.H., Munroe J., Hornback W. J., Kasher J. S., Glunz L., Szczerba T.: J. Med. Chem. 38, 590 (1995).
2. Ong S., Liu H., Qiu X., Bhat G., Pidgeon C.: Anal. Chem. 67, 755 (1995).
3. Liu H., Ong S., Glunz L., Pidgeon C.: Anal. Chem. 67, 3550 (1995).
4. Nasal A., Sznitowska M., Buciński A., Kaliszan R.: J. Chromatogr. A 692, 83 (1995).
5. Barbato F., La Rotonda M. I., Quaglia F.: J. Pharm. Sci. 86, 225 (1997).
6. Genty M., Gonzalez G., Clere C., Desangle–Gouty V., Legendre J. Y.: Eur. J. Pharm. Sci. 12, 223 (2001).
7. Matysiak J., Niewiadomy A., Mącik–Niewiadomy G.: Eur. J. Pharm. Sci. 10, 119 (2000)
8. Jóźwiak K., Szumiło H., Senczyna B., Niewiadomy A.: SAR QSAR Environ. Res. 10, 509 (1999).
9. Jóźwiak K., Szumiło H., Senczyna B., Niewiadomy A.: Chromatographia 52, 159 (2000).
10. Matysiak J., Niewiadomy A., Mącik–Niewiadomy G., Korniłłowicz T.: Eur. J. Med. Chem. 35, 393 (2000).
11. Kaliszan, R.: Structure and Retention in Chromatography; A Chemometric Approach., Harwood Academic Publishers, Amsterdam B.V. (1997).
12. Snyder L. R., Dolan J. W., Grant J. R.: J. Chromatogr. 165, 3 (1979).

Received: 21.02.2002