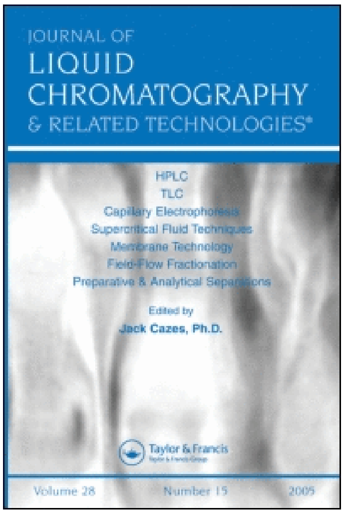

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K. Valkó[a]; I. Fellegvári[a]; A. Katti[bc]; L. Ötvös[a]
[a] Central Research Institute for Chemistry, Hungarian Academy of Sciences, Budapest, Hungary [b] Department of Chemical Engineering, Yale University, New Haven, Connecticut [c] Analytical Chemistry Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, U.S.A.

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CORRELATION OF HPLC RETENTION DATA OF TRIAZINES WITH THE INHIBITION OF DHFR FROM L1210 CELLS: DEVELOPMENT OF NEW CHROMATOGRAPHIC ADSORPTION PARAMETER IN QSAR

K. Valkó[1], I. Fellegvári[1], A. Katti[2]*,
and L. Ötvös[1]

[1] Central Research Institute for Chemistry
Hungarian Academy of Sciences
P. O. Box 17
Budapest, Hungary
[2] Yale University
Department of Chemical Engineering
New Haven, Connecticut

ABSTRACT

The use of chromatographic retention measurements for quantitative structure-activity relationships (QSAR) was investigated by the development of an adjusted logarithmic retention factor. Retention data of 15 triazine derivatives, which inhibit dihydrofolate reductase were measured by HPLC on octyl-silica and silica gel columns. From these data two parameters were calculated. First, the intercept of the plot of the logarithmic retention factor versus acetonitrile concentration was calculated from the reversed-phase retention measurements (log k'o) and, second, the difference between the logarithmic retention factor at

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*Present address: Oak Ridge National Laboratory, Analytical Chemistry Division, Oak Ridge, Tennessee, U.S.A.

high ammonium salt concentration and low ammonium salt concentration in the mobile phase was calculated from retention measurements on the silica gel column. The first parameter represents hydrophobic properties and the second parameter characterizes adsorption properties of the triazine derivatives. The results show a correlation between the two above mentioned parameters and the inhibition of L1210/71 cell growth.

INTRODUCTION

Quantitative relationships between the chemical structure, which can be characterized by hydrophobic, electronic and steric parameters, and biological activity is an important aspect of drug design (1). Since chromatographic retention involves hydrophobic, electronic and steric effects, HPLC has also been used as a tool for estimation of some of these parameters (2). The logarithm of the appropriate chromatographic retention data are proportional to the logarithm of the distribution coefficient of the solute in the mobile and the stationary phases and in such a way are proportional to the free-energy change of the solute - stationary phase interactions modified by the mobile phase (3). The linear free-energy related (LFER) parameters thus obtained can replace hydrophobic, electronic and steric parameters in QSAR equations (4). Recently, the use of reversed-phase chromatography for the measurement of hydrophobicity by HPLC has been reviewed (5). The aim of the present paper is to extend the use of HPLC also to the estimation of the electronic effects in QSAR studies.

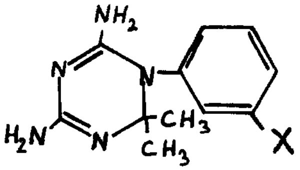

Fig.1. The general structure of the investigated triazine derivatives.

Retention data for 15 triazine derivatives having the general structure shown in Fig. 1. for which the QSAR was known (6) were measured by reversed-phase and normal phase chromatography. A correlation between chromatographic retention and inhibition of L1210/R71 cell growth was investigated and it was compared with the correlation of biological activity data and hydrophobic parameter (π) and molar refractivity (MR) of the X substituent published earlier (6).

MATERIALS

The triazines used in this study were synthesized by Selassie et. al. (7). HPLC grade acetonitrile and methanol were purchased from Merck (Darmstadt, F. R. G.), HEPES (N-2-hydroxyethyl-piperazine-N'-ethansulfonic acid) was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.), and analytical grade sodium nitrate and ammonium chloride was purchased from Reanal Fine Chemical Works (Budapest, Hungary).

The data for inhibition of cell growth in L1210/R71 cells (log 1/C), values and the molar refractivity (MR) values for the substituents were obtained from ref. 6 and are summarized in Table 1.

Equipment

The chromatograph used for reversed phase chromatography was assembled from an Altex Model 110A pump (Altex Inc., Berkeley, California, U. S. A.), a Model 7010 Rheodyne Injector (Rheodyne Inc., Cotati, California, U. S. A.), and a Model LC-55 Perkin Elmer UV variable wavelength detector. Measurements with the silica gel column were carried out by using a Model OE-308 variable wavelength UV detector at 240 nm and Liquopump Model 312 pump (LaborMIM, Budapest, Hungary) with Rheodyne Model 7125 injector with a 20 µl sample loop (Rheodyne, Cotati, California, U. S. A.).

METHODS

Procedures

For retention measurements by reversed phase chromatography a 4.6 x 150 mm RP-8 cartridge column (Perkin Elmer, Norwalk, CT, U.S.A.) was used. The mobile phase was a mixture of an aqueous solution containing 25 mM HEPES and 0.1% TEA , pH 7.7, and 20 to 70 % (v/v) acetonitrile at a flow rate of 1.0 ml/min. The samples were prepared by dissolving 5 mg of the

Table 1. Inhibitory effect (log 1/C), MR and $\tilde{\pi}$ values of the triazines investigated. From ref. 6.

No.	X	log 1/C	MR	$\tilde{\pi}$
1.	H	4.49	0.10	0.00
2.	SO_2NH_2	3.57	1.23	-1.82
3.	$CONH_2$	3.52	0.98	-1.49
5.	OH	4.41	0.29	-0.67
12.	CN	4.89	0.63	-0.57
14.	CH_2CH_3	5.27	1.03	1.03
20.	OCH_3	4.42	0.79	-0.02
21.	OCH_2CH_3	4.96	1.25	0.38
23.	$O(CH_2)3CH_3$	5.09	2.17	1.59
25.	$O(CH_2)5CH_3$	5.62	3.07	2.67
40.	$CH_2OC_6H_5$	5.12	3.17	1.66
42.	$CH_2OC_6H_4$-3'-CN	4.67	3.70	1.09
43.	$CH_2OC_6H_4$-3'-OCH_3	5.20	3.86	1.64
44.	$CH_2OC_6H_4$-3'-CH_2OH	4.76	3.79	0.63
49.	CH_2-O-C_6H_4-3'-C_6H_5	5.62	4.61	3.69

The serial numbers of the compounds are the same as in ref. 6.

respective triazines in 1 ml of the actual mobile phase. The column void volume was taken as the retention volume of sodium nitrate and retention factors, k', were calculated in the usual way. The log k' values were found to be linear with the acetonitrile concentration in the mobile phase for each compound and the slopes and intercepts of such plots are listed in Table 2.

Retention measurements on silica gel were carried out by using a 4.0 x 250 Nevisorb 10 µm silica gel column obtained from Bioseparation Technique Ltd. (Budapest, Hungary). Two mobile phases were used. Eluent A was a mixture of 95% (v/v) methanol and 5% of an aqueous ammonium chloride solution containig 0.25% w/v. Eluent B was a mixture of 95% (v/v) methanol and 5% of an aqueous solution containig 1% (w/v) ammonium chloride. Thus, the salt concentration in the eluent B was 4 times higher then in eluent A. The flow rate was 1.0 ml/min and the dead time was determined from the solvent peak. The samples were prepared by dissolving 5 mg of the respective triazines in 1 ml of methanol.

All measurements were repeated 3 times, consecutively and averaged. The processing of the chromatographic data and the statistical calculations were carried out on an IBM PC microcomputer using the Labsware program package (Compudrug Ltd., Budapest, Hungary).

Table 2. The slope and the intercept (log k' o) values of the HPLC log k' – organic phase concentration relationships.

Compound	slope	intercept	R
1.	-0.06430	0.8307	0.994
2.	-0.00388	0.3854	0.994
3.	-0.00270	0.1878	0.999
5.	-0.00507	0.5887	0.998
12.	-0.00691	0.8143	0.998
14.	-0.01225	1.4360	0.996
20.	-0.00734	0.9703	0.992
21.	-0.01075	1.2849	0.993
23.	-0.01215	1.6720	0.992
25.	-0.02107	2.4579	0.985
40.	-0.01778	1.8969	0.994
42.	-0.01253	1.5458	0.996
43.	-0.01237	1.6009	0.999
44.	-0.01672	1.5019	0.992
49.	-0.02538	2.7072	0.987

RESULTS AND DISCUSSION

In a previous study (6) the variance of the inhibitory effect (log 1/C) cell growth in L1210/R71 cells by 62 triazines was explained by the hydrophobic (π) and steric (MR) parameters of the substituents.

Out of that group of compounds 15 representative triazines listed in Table 1 were chosen for the HPLC retention data measurements. The correlation between the log 1/C values and the π and MR values for the 15 compounds was almost the same as measured by the similar regression coefficients and other statistical parameters as for the larger set of compounds as shown by the equations 1 and 2.

$$\log 1/C = 0.48(\pm 0.07)\pi - 0.13(\pm 0.07)\,MR + 4.73 \quad (1)$$

$n=15 \quad r=0.927 \quad s=0.254 \quad F=36.8$

$$\log 1/C = 0.42(\pm 0.05)\pi - 0.15(\pm 0.05)\,MR + 4.83 \quad (2)$$

$n=62 \quad r=0.941 \quad s=0.220 \quad F=39.9$

where $\underline{n}$ stands for the number of compounds, $\underline{r}$ is the multiple correlation coefficient, $\underline{s}$ is the standard error of the estimate, $\underline{F}$ is the Fisher-test value which is significant at 0.001% level. The correlation between the measured and calculated log 1/C values according to equation 1 is shown in Fig. 2.

In order to replace the calculated π and MR values of the substituents, these parameters were evaluated from HPLC data. Chromatography on reversed-phase and silica gel columns was employed to measure the hydrophobic character and the electronic characteristics of the compounds, respectively.

From the reversed-phase HPLC measurements, the slope and the intercept values were calculated from a

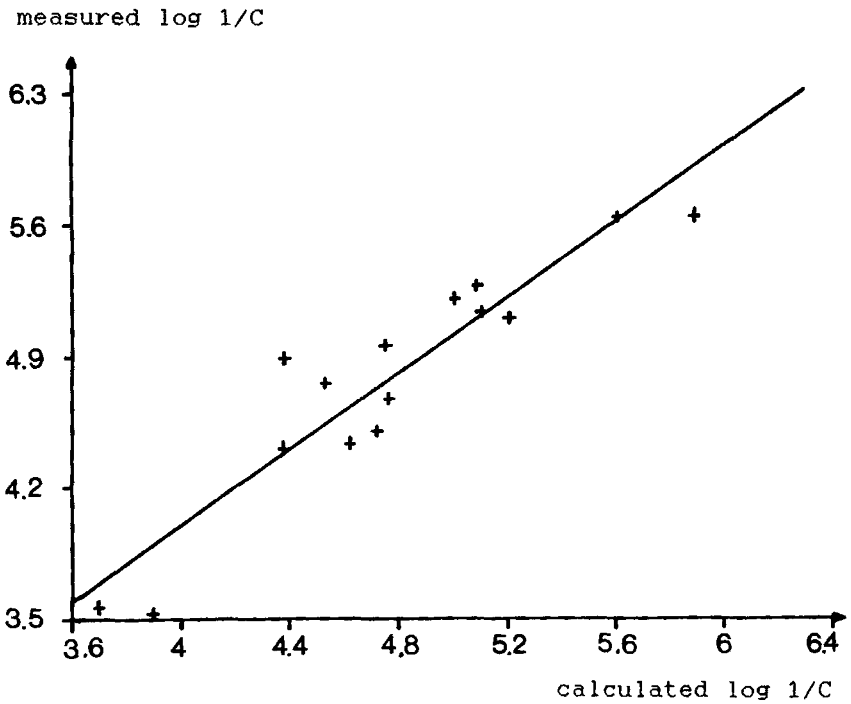

FIGURE 2. The measured and the calculated inhibition data (log 1/C) according to eqn. 1.

least square fit of a plot of the logarithmic retention factor versus per cent organic solvent content of the mobile phase for each triazines studied, shown in Table 2. A good correlation is seen as indicated by the correlation coefficients. A high correlation, r=0.974, was found between the slope and the intercept values indicating that these two parameters are not independent. Therefore the log k' values obtained at any mobile phase compositions correlate to the hydrophobic parameter π

The correlation coefficients for the $\tilde{\pi}$- slope and $\tilde{\pi}$-intercept relationships were -0.936 and 0.982. Therefore, the intercept was used as a parameter equivalent to the $\tilde{\pi}$ values in the ensuing QSAR studies. The normal phase measurements were used to model electronic/adsorptive interactions. The triazines were retained very strongly by silica gel even with highly polar mobile phases such as a mixture of 95% methanol and 5% water. The retention was attenuated by using ammonium chloride in the eluent according to Gazdag et. al. (8, 9) who have found that ammonium salts in the mobile phase reduced the retention of quaternary ammonium compounds. At high salt concentrations, however, electronic interactions are suppressed so that retention occures predominantly by hydrophobic interactions. In order to obtain data which reflects the electronic character of triazines, we took the difference log k'n between the logarithmic capacity factors measured at low log k'a and high log k'b salt concentrations in the eluent according to equation 3.

$$\log k'_n = \log k'_a - \log k'_b \qquad (3)$$

The so obtained log k'n values which are listed in Table 3 are regarded as a measure of electronic/adsoptive properties of triazines.

The relationships between the chromatographic and other parameters were investigated and a summary of the

Table 3. The retention data of triazine derivatives obtained on silica stationary phase.

Compound	log k'a	log k'b	log k'n
1.	0.159	-0.273	0.431
2.	0.080	-0.489	0.569
3.	0.193	-0.297	0.490
5.	0.031	-0.334	0.364
12.	0.100	-0.197	0.297
14.	0.133	-0.217	0.350
20.	0.172	-0.216	0.388
21.	0.059	-0.293	0.352
23.	-0.019	-0.439	0.419
25.	-0.033	-0.289	0.256
40.	0.083	-0.329	0.412
42.	0.073	-0.314	0.387
43.	0.072	-0.279	0.351
44.	0.044	-0.278	0.322
49.	0.043	-0.359	0.402

correlation coefficients is presented in Table 4. Whereas, the log k'n values do not correlate with the MR or π values ($r = -0.22$, $r = -0.476$, respectively), they show a correlation with the log 1/C values ($r = -0.720$).

Table 4. The correlation coefficients of the variables summarized in a correlation matrix.

	log1/C	π	MR	logk'o	logk'n
log1/C	-	0.903	0.542	0.894	-0.720
π	0.903	-	0.755	0.982	-0.476
MR	0.542	0.755	-	0.787	-0.220
log k'o	0.894	0.982	0.787	-	-0.489
log k'n	-0.720	-0.476	-0.220	-0.489	-

Developing a QSAR equation using log k'o and log k'n values as the two independent variables instead of the π and MR values, equation 4 was obtained.

$$\log 1/C = 0.62(\pm 0.09)\ \log k'o - 3.06(\pm 0.84)\ \log k'n + +5.13 \qquad (4)$$

n= 15 r=0.951 s=0.210 F=56.3

The results are also illustrated by plotting the measured against the calculated log 1/C values according to eqn. 4 in Fig. 3. The correlation is slightly better than those obtained with the correlation based on the π and MR values and shown in Table 4 and equation 1. Additionally, the intercorrelation between the independent variables log k'o and log k'n was lower (r= -0.426) for the HPLC parameters than that between the π and MR values (r =

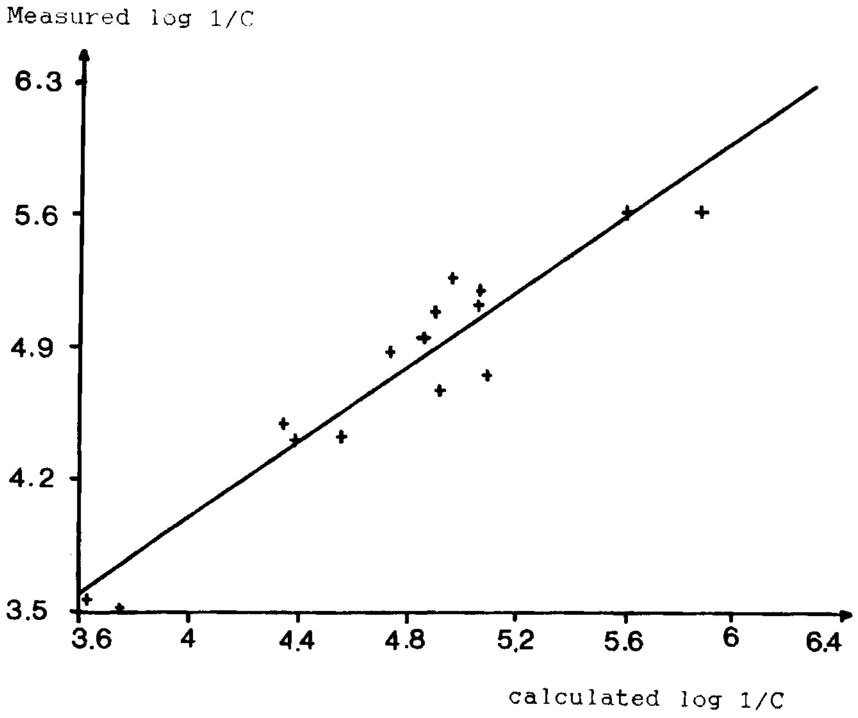

FIGURE 3. The measured and the calculated inhibition data (log 1/C) according to euq. 4.

0.755). The poor correlation between the MR values and log k'n indicates that log k'n is not a measure of steric properties. The log k'a or log k'b parameters did not correlate to any other parameters and did not improve the correlation to the log 1/C values as well.

As MR values play an important role in the QSAR equation such as eqn. 1, MR values were introduced as a third independent variable into eqn. 4 to obtaine the relationship described by eqn. 5.

$$\log 1/C = 0.85(\pm 0.13)\ \log k'_o - 2.54(\pm 0.78)\ \log k' - -0.12(+0.06)\ MR + 4.88 \qquad (5)$$

$$n=15 \quad r=0.966 \quad s=0.184 \quad F=50.4$$

Equation 5 represents a further improvement in the QSAR of the 15 triazines and shows that chromatographic data can adequately serve to express the pertinent hydrophobic, electronic and steric properties. The retention data from reversed-phase HPLC provide easily measurable parameters for characterizing the hydrophobic properties of the triazines. On the other hand the log k'n values from HPLC with silica gel column can be used to express the electronic/adsorptive properties of the compounds investigated. The log k'n values measure the change in the interaction of eluits with the polar stationary phase caused by the changing salt concentration. Although the use of ammonium ions in the eluent was necessitated by practical considerations the log k'n values can be considered as measures of the competition of the triazines and ammonium ions for the adsorptive sites. The good correlations suggest that the binding of the triazine derivatives at the sites of biological action parallels their adsorption at the silica surface.

Although the chromatographic measurements were carried out only with 15 derivatives, it is believed that these compounds are a representative sample. This is evidenced by the similarity in the QSAR relationships derived from using π and MR parameters in eqn 1.

The new chromatographic retention parameter k'n has been found to be useful for such QSAR studies and this finding gives further support to the use of HPLC retention data in the investigation of quantitative structure - activity relationships.

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