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Chromatographic separation and molecular modelling of triazines with respect to their inhibition of the growth of L1210/R71 cells

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ABSTRACT

The potential anti-cancer activity of triazines was characterized by the inhibition of the growth of L1210/R71 cells. The retention times for fifteen triazine derivatives were measured by high-performance liquid chromatography on octyl silica and silica gel columns. The slope and intercept values of the plot of the logarithmic capacity factor *versus* acetonitrile concentration were calculated from the reversed-phase retention measurements. The adsorption properties of the compounds were characterized by the retention data obtained on silica gel columns using high and low concentrations of ammonium salts in the hydro-organic mobile phase. The non-polar, non-polar unsaturated and polar surface areas, the surface energies, the dipole moments and the Van der Waals radii of the molecules were calculated from their chemical structures after energy minimization on the basis of molecular mechanics. Correlation analysis of these parameters showed that the inhibitory effect is dependent on the polar and non-polar surface areas of the molecules. The reversed-phase slope showed a significant correlation with the difference between the accessible and the total non-polar surface areas of the compounds, whereas the intercept values correlated with the non-polar accessible surface area. The adsorption properties of the triazines on silica gel cannot be described by the molecular parameters investigated here.

INTRODUCTION

Triazine derivatives are potential anti-cancer agents as they can inhibit the dihydrofolate reductase enzyme [1]. The inhibitory effect of 62 triazine derivatives on the growth of L1210/R71 cells has been studied with respect to the hydrophobic parameter (π) and the molar refractivity (MR) index of the substituents [2]. The general structure of the molecules is shown in Fig. 1. The quantitative structure–activity relationship (QSAR) study of Hansch and Fujita [3] suggested the presence of a hydrophobic pocket on the enzyme surface into which the R substituents can be fitted. The theoretical basis of using chromatographic retention data in drug design has been discussed previously [4]. For QSAR purposes the chromatographic behaviour of fifteen derivatives selected from a total of 62 was investigated by high-performance liquid chromatography on reversed-phase and silica gel stationary phases [5]. The measured reversed-phase retention data could be used to characterize the hydrophobicity of the compounds instead of the π values. Although the retention data obtained on the silica gel stationary phase did not correlate with the size of the molecules (*i.e.*, to the MR index of the R substituents), they

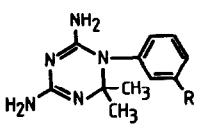

Fig. 1. General structure of the triazine derivatives investigated.

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could be used to explain the variance of the inhibition of leukaemia cell growth [5].

The investgation of the three-dimensional structure of molecules and their fit to receptor sites is another approach to drug design. Many methods have been devised for the determination of molecular structures. It is now possible to determine molecular structures accurately by computational methods, typically by *ab initio* calculations for small molecules and by molecular mechanics [6] for large molecules.

This work investigated the relationship between molecular parameters calculated on the basis of molecular mechanics and the chromatographic retention and biological activity of triazines.

EXPERIMENTAL

The triazines used in this study were synthesized by the method of Selassie *et al.* [2] and were a kind gift of Professor C. Hansch (Pomona College, Claremont, CA, USA). The structure of the compounds is given in Table I. The data for the inhibition of the growth of L1210/R71 cells given in Table I were obtained from Selassie *et al.* [1].

The experimental conditions for the determination of the chromatographic retention data have been described in detail by Valkó *et al.* [5]. Table I gives the slope and intercept (log k'_0) values of the compounds obtained by plotting the log k' values against the acetonitrile concentration of the mobile phase in reversed-phase chromatography. The log k'_n values were measured as the difference between the log k'_a and log k'_b values, which were obtained on the same silica stationary phase using 95% (v/v) methanol and 5% (v/v) of a 0.25% (w/v) and a 1% (w/v) aqueous ammonium chloride solution [5], respectively. The retention data obtained on silica gel columns [5] are also given in Table I.

The three-dimensional structure of the compounds was determined based on energy minimization. After setting up the geometries of the molecules with the smallest molecular mechanics (mmx) energy, the non-polar (nopol), non-polar unsaturated and polar surface areas and surface energies were calculated. The water solvation shell was also considered in the calculations of the accessible polar (polac) and non-polar (npac) surface areas. The dipole moment values and Van der Waals radii of the molecules were also calculated.

The correlations of these parameters were investigated by stepwise linear regression analysis using the

TABLE I

INHIBITORY ACTIVITY [LOG(1/*C*)] AND CHROMATOGRAPHIC RETENTION DATA OF TRIAZINES OBTAINED FROM SELASSIE *et al.* [1] AND VALKÓ *et al.* [5], RESPECTIVELY

The serial numbers of the compounds are the same as in ref. 1. The log k'_0 and the slope values refer to the reversed-phase chromatographic retention and log k'_n refers to the retention data obtained on silica gel. All of the data are from ref. 5.

Compound No.	R	Log(1/*C*)	Slope	Log k'_0	Log k'_n
1	H	4.49	−0.00643	0.831	0.431
2	SO_2NH_2	3.57	−0.00388	0.385	0.569
3	$CONH_2$	3.52	−0.00270	0.188	0.490
5	OH	4.41	−0.00507	0.589	0.364
12	CN	4.89	−0.00691	0.814	0.297
14	CH_2CH_3	5.27	−0.01225	1.436	0.350
20	OCH_3	4.42	−0.00734	0.970	0.388
21	OCH_2CH_3	4.96	−0.01075	1.285	0.352
23	$O(CH_2)_3CH_3$	5.09	−0.01215	1.672	0.419
25	$O(CH_2)_5CH_3$	5.62	−0.02107	2.458	0.256
40	$CH_2OC_6H_5$	5.12	−0.01778	1.897	0.412
42	$CH_2OC_6H_4$-3′-CN	4.67	−0.01253	1.546	0.387
43	$CH_2OC_6H_4$-3′-OCH_3	5.20	−0.01237	1.601	0.351
44	$CH_2OC_6H_4$-3′-CH_2OH	4.76	−0.01672	1.502	0.322
49	$CH_2OC_6H_4$-3′-C_6H_5	5.62	−0.02538	2.707	0.402

TABLE II

CALCULATED MOLECULAR PARAMETERS FOR THE TRIAZINES ON THE BASE OF MOLECULAR MODELLING

Compound No.	nopol	npac	δnp\npc[a]	polac	tscal
1	229.8	216.8	13.0	121.8	−3.2
2	184.1	173.4	10.7	235.5	−14.6
3	179.6	168.9	10.7	216.8	−12.8
5	194.4	183.1	11.3	164.7	−8.0
12	197.9	186.5	11.4	167.5	−8.4
14	293.8	276.7	17.1	124.7	−2.3
20	270.3	253.9	16.3	134.9	−3.5
21	313.2	295.0	18.2	129.6	−2.0
23	371.2	349.8	21.4	129.1	−0.5
25	431.6	406.9	24.7	127.7	1.2
40	340.4	321.2	19.2	121.9	0.5
42	304.3	287.1	17.2	167.1	−4.8
43	379.4	357.3	22.1	131.1	0.3
44	344.3	324.7	19.6	160.1	−2.8
49	402.5	379.6	22.9	126.8	2.4

[a] δnp\npc = difference between the non-polar and non-polar accessible surface areas.

Drugidea program system developed for drug design (Chemicro, Budapest, Hungary). All the calculations were performed on an IBM AT compatible personal computer.

RESULTS AND DISCUSSION

The chemical structure, inhibitory activity and chromatographic retention data of the investigated triazine derivatives are given in Table I. The calculated molecular parameters are presented in Table II. The correlation coefficients between each pair of investigated parameters are given as a correlation matrix in Table III.

The reversed-phase retention data which showed a significant correlation with the hydrophobicity of the compounds (r = 0.982 from Valkó *et al.* [5]) could also be described as a function of the calculated molecular parameters. The log k'_0 values showed the best correlation with the non-polar accessible surface area of the molecules, as formulated in eqn. 1:

$$\log k'_0 = 8.53\ (\pm 0.84) \cdot 10^{-3} \cdot \text{npac} - 1.05 \quad (1)$$

where n = 15, r = 0.942, s = 0.249 and F = 102.9 stand for the number of compounds, the correlation coefficient, the standard error of the estimate and the Fisher test value, respectively.

Eqn. 1 indicates that the larger the non-polar accessible surface area of the molecule, the higher the reversed-phase retention of the compounds.

The dependence of the retention of the compounds on the organic phase concentration of the mobile phase in reversed-phase chromatography can be expressed by the slope of the straight line obtained by plotting the log k' values against the concentration of the organic phase. As was emphasized previously [7], the slope values do not necessarily show a good correlation with the intercept (log k'_0) values for structurally unrelated compounds and they can be expressed by the contact hydrophobic surface area of the molecules.

For the triazine derivatives investigated, the log k'_0 and slope values showed a high correlation (r = 0.85), proving that they are similar with respect to their reversed-phase retention behaviour. Similar to results obtained previously [8], where structurally

TABLE III

CORRELATION COEFFICIENTS FOR THE PARAMETERS LISTED IN TABLES I AND II

	Log(1/C)	Slope	Log k'_0	Log k'_n	nopol	npac	δnp\npc	polac	tscal
Log(1/C)	1.00	−0.73	0.88	−0.75	0.84	0.84	0.83	−0.75	0.84
Slope	–	1.00	−0.85	0.37	−0.76	−0.76	−0.88	0.41	−0.65
Log k'_0	–	–	1.00	−0.49	0.94	0.94	0.93	−0.68	0.88
Log k'_n	–	–	–	1.00	−0.49	−0.49	−0.49	0.59	−0.57
nopol	–	–	–	–	1.00	0.99	0.99	−0.67	0.90
npac	–	–	–	–	–	1.00	0.99	−0.67	0.90
δnp\npc	–	–	–	–	–	–	1.00	−0.68	0.89
polac	–	–	–	–	–	–	–	1.00	−0.92
tscal	–	–	–	–	–	–	–	–	1.00

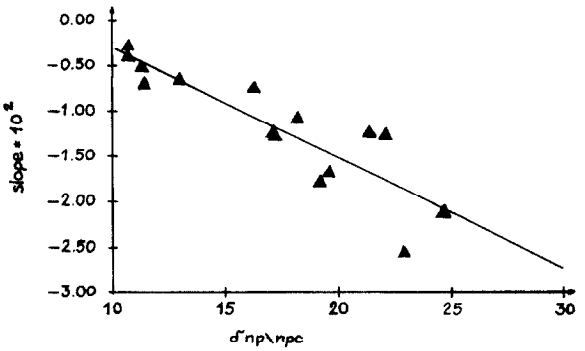

Fig. 2. Plot of the slope values against the difference between the non-polar and non-polar accessible surface areas (δnp\npc) of the molecules according to eqn. 3.

unrelated compounds were investigated, the variance of the slope values can be described by the difference of the non-polar and non-polar accessible surface areas of the molecules. For the triazine derivatives, eqn. 2 was obtained:

$$\text{slope} = 5.75\ (\pm 2.21) \cdot 10^{-3} \cdot \text{nopol} - 6.17\ (\pm 2.34) \cdot 10^{-3} \cdot \text{npac} + 6.65 \cdot 10^{-3} \quad (2)$$

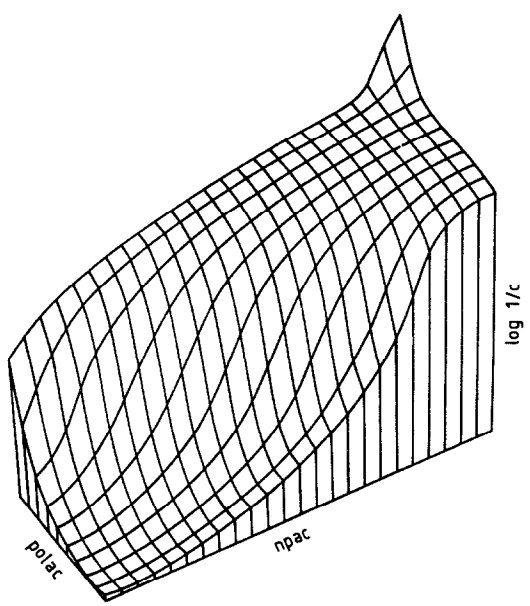

Fig. 3. Three-dimensional plot of eqn. 4, which represents the correlation between the inhibition activity log(1/C) and the polar (polac) and non-polar (npac) accessible surface areas of the molecules.

where $n = 15$, $r = 0.936$, $s = 0.0025$ and $F = 42.1$.

Although the two independent variables in eqn. 2 show a high cross-correlation (Table III), neither alone shows as high a correlation to the slope values as they show together. Therefore the correlation of the difference between the two variables, δnp\npc, (δnp\npc = nopol − npac) and the slope values was also investigated. The relationship between the slope values and the δnp\npc values can be described by eqn. 3:

$$\text{slope} = 1.21\ (\pm 0.18) \cdot 10^{-3} \cdot \delta\text{np\npc} + 9.122 \cdot 10^{-3} \quad (3)$$

where $n = 15$, $r = 0.878$, $s = 0.0032$ and $F = 43.8$.

This relationship is illustrated in Fig. 2.

An possible explanation of eqn. 3 is that it describes the slope as a function of the difference between the non-polar surface area of the molecules and the accessible non-polar surface area by the hydrophobic surface of the stationary phase and by the organic solvent molecules.

The log k'_n values, which are the differences between the logarithmic capacity ratio measured at low (log k'_a) and high (log k'_b) salt concentrations in the eluent are significant in the correlation of triazines with the inhibition of cell growth [5]. The log k'_n values cannot be described by any combination of the investigated molecular parameters. As was suggested previously [5], the log k'_n values reflect the electronic interactions of the compounds because at high salt concentrations the retention occurs predominantly by hydrophobic interactions and at low salt concentrations both the electronic and the hydrophobic interactions affect the retention behaviour.

A significant linear regression equation can be derived for the inhibitory effect of the compounds on L1210/R71 cell growth [log(1/C)] and the investigated molecular parameters:

$$\log(1/C) = 3.994\ (\pm 1.265) \cdot 10^{-3} \cdot \text{npac} - 8.794\ (\pm 2.836) \cdot 10^{-3} \cdot \text{polac} + 4.985 \quad (4)$$

where $n = 15$, $r = 0.913$, $s = 0.276$ and $F = 30.1$.

Fig. 3 shows the three-dimensional plot of eqn. 4. A relatively wide range of polar and non-polar surfaces provides a medium inhibitory activity for the compounds and only a narrow range of properties can be attributed to a strong inhibitory effect.

The inhibitory effect of the compounds on the

growth of L1210/R71 cells can also be mathematically described by the total surface energy (tscal) of the compounds and the electronic and adsorptive properties (log k'_n) obtained from adsorption chromatography:

$$\log(1/C) = 0.0913\ (\pm 0.0149)\ \text{tscal} - 2.514\ (\pm 0.983)\ \log k'_n + 6.100 \quad (5)$$

where $n = 15$, $r = 0.940$, $s = 0.231$ and $F = 45.3$.

The statistical characteristics of eqn. 5 indicate the importance of the electronic and adsorptive properties of the compounds which cannot be correlated with the calculated molecular parameters. It underlines the need to understand better the chromatographic retention mechanisms on silica stationary phases.

In conclusion, this work has illustrated the applicability of molecular modelling to the understanding of the chromatographic retention behaviour and the anti-cancer activity data of triazines. The reversed-phase retention data are related to the non-polar surface areas of the molecules.

The adsorption chromatographic retention behaviour of the compounds cannot be described by the molecular parameters investigated because none of these parameters characterizes the electronic or electrostatic properties of the compounds.

The inhibitory effect of the triazines on the growth of leukaemia cells is correlated to their accessible polar and non-polar surface areas.

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