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LIPOPHILIC CHARACTER OF PYRIMIDINIC NUCLEOSIDE DERIVATIVES: CORRELATION BETWEEN SHAKE FLASK, CHROMATOGRAPHIC (RP-TLC AND RP-HPLC) AND THEORETICAL METHODS

S. A. Teijeiro^a; G. N. Moroni^a; M. I. Motura^a; M. C. Briñón^a

^a Departamento de Farmacia, Universidad Nacional de Córdoba, Córdoba, Argentina

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PYRIMIDINIC NUCLEOSIDE DERIVATIVES:
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S. A. Teijeiro, G. N. Moroni, M. I. Motura, M. C. Briñón*

Departamento de Farmacia
Facultad de Ciencias Químicas
Ciudad Universitaria
Universidad Nacional de Córdoba
5000 Córdoba, Argentina

ABSTRACT

Chromatographic R_{Mw} and $\log k'_w$ parameters have been used as alternatives to the shake flask n-octanol/water partition coefficient method to study the lipophilic character of several known pyrimidinic nucleoside derivatives and two novel ones. The R_M and $\log k'$ values were measured by means of reversed-phase thin-layer chromatography (RP-TLC) and reversed-phase high performance liquid chromatography (RP-HPLC) respectively. Good linear relationships were observed between $\log P$ values obtained from the classical shake flask method ($\log P_{o/w}$) and each one of those obtained by chromatographic techniques ($\log P_{RP-TLC}$ and $\log P_{RP-HPLC}$) and by theoretical calculations using the CLOGP program ($\log P_{CLOGP}$). The R_{Mw} and $\log k'_w$ values have proven to be reliable parameters for describing the lipophilic properties of this family of compounds.

INTRODUCTION

The lipophilicity of a drug expressed by the octanol/water partition coefficient ($P_{o/w}$), considerably affects a number of pharmacokinetic parameters, since a drug normally has to pass biological membranes by passive diffusion to reach its target. The partition coefficient determined by the shake flask method¹ established as the reference system and used as its logarithm form ($\log P_{o/w}$) is the most common index of lipophilicity correlating molecular structure and biological response.^{2,3} Because this method has a number of disadvantages⁴ reversed-phase chromatographic methods, such as, thin layer chromatography (RP-TLC)⁵⁻¹⁰ and high performance liquid chromatography (RP-HPLC),¹¹⁻¹⁴ have been satisfactorily developed as reliable and reproducible methods to express drug lipophilicity.

Pyrimidinic nucleoside derivatives are a very important family of biological active compounds of antiviral and antitumoral activity.^{15,16} The lipophilicity of these compounds is in many cases a major factor ruling their potency. Regarding efficient anti HIV-1 agents, we have recently developed two novel pyrimidinic nucleosides with antiviral activity similar to that of AZT.¹⁷ In view of a QSAR study, the aim of this work was to study the lipophilic character of these two compounds and some known related pyrimidinic nucleoside derivatives by RP-TLC and RP-HPLC techniques. In addition, the reliability of the R_{Mw} and $\log k'_w$ values as lipophilicity parameters for describing this family of compounds was determined by the correlation of the $\log P$ values obtained from the shake flask method ($\log P_{o/w}$) with those of chromatographic methods ($\log P_{RP-TLC}$ and $\log P_{RP-HPLC}$) and theoretical calculations using the CLOGP program ($\log P_{CLOGP}$).

EXPERIMENTAL

Chemicals

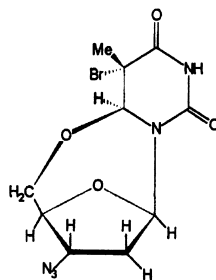
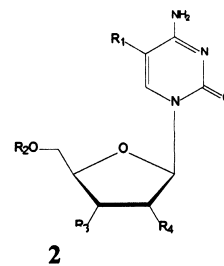
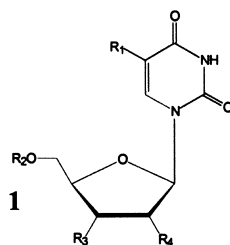
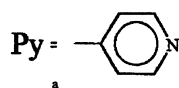
Thymidine (Thym, **1a**) and Zidovudine (3'-azido-3'-deoxythymidine, AZT, **1c**) were generous gifts of Filaxis (Paraná, Entre Ríos, Argentina). Uridine (Uri, **1f**) and Cytidine (Cyt, **2a**) were obtained from Sigma Co. 3'-azido-3'-deoxy-5'-O-isonicotinoylthymidine (Iso-AZT, **1e**) and (-)-*trans*-(5S,6S)-5-bromo-6,5'-epoxy-5,6-dihydro-3'-azido-3'-deoxythymidine (Br-AZT, **3**), were newly synthesized drugs with anti HIV-1 activity.¹⁷ 5'-O-acetylthymidine (Ac-Thym, **1b**), 5'-O-acetyl-3'-azido-3'-deoxythymidine (Ac-AZT, **1d**), and 5'-O-acetylcytidine (Ac-Cyt, **2b**), three known compounds,¹⁸ have been prepared according to our improved method herein described.

The chemical structures of the tested compounds are listed in Table 1. Analytical grade n-octanol, acetone, acetic anhydride, methansulfonic acid and ethyl ether were purchased from Merck Co. and HPLC grade methanol from

Table 1

Structural Formulae of Tested Drug Molecules

Compounds		R ₁	R ₂	R ₃	R ₄
1a	Thym	CH ₃	H	OH	H
1b	Ac-Thym	CH ₃	COCH ₃	OH	H
1c	AZT	CH ₃	H	N ₃	H
1d	Ac-AZT	CH ₃	COCH ₃	N ₃	H
1e	Iso-AZT	CH ₃	OCPy ^a	N ₃	H
1f	Uri	H	H	OH	OH
2a	Cyt	H	H	OH	OH
2b	Ac-Cyt	H	COCH ₃	OH	OH



Sintorgan. Water was obtained from a Milli-Q water system. All solutions used for HPLC were filtered through membrane filters of 0.45 μm pore size from Rainin Instrument Co. Inc. All other chemical compounds and solvents, if not indicated otherwise, were analytical grade.

General Synthetic Procedure for Acetyl Derivatives

The process was straightforward for acetyl compounds (Ac-Thym, **1b**; Ac-AZT, **1d** and Ac-Cyt, **2b**). A solution of 1.5 mmol of the desired compound, (Thym, AZT or Cyt) in acetic anhydride (6 mL) with methansulfonic acid (0.05 mL), was stirred in a water bath at 25°C for 5 minutes, and then 20 mL of cool ethyl ether was added to the reaction mixture and phases were separated. The ether solution was washed with 10 mL of a cool solution of Na₂CO₃/NaHCO₃ (pH 10), dried over Na₂SO₄, and the solvent removed in vacuum. In each case, the corresponding product was purified by column chromatography (Silica gel 60 from Merck) eluted with a polarity gradient solvent mixture yielding: Ac-Thym, 70%; Ac-AZT, 96%; and Ac-Cyt, 30%. Spectroscopic data and melting points are in accordance with those of the literature.¹⁸

R_M Assay

Precoated TLC plates (RP-18 HPTLC F_{254S}, 10 x 10 cm), purchased from Merck (Darmstadt, Germany) were used, since they have a considerable advantage of high stability, allowing their use over large ranges of varying modifier/water contents. A 0.3 µL volume of a solution of the test compounds in acetone (1 mg/mL) was randomly spotted on the plates in order to avoid any systematic error. The starting points of the test compounds were positioned 10 mm from the bottom edge of the plate and at least 25 mm from the side of the plate with 5 mm in between.

For determining the thermodynamically true position of the front,¹⁹ 50 mg potassium iodide dissolved in 10 mL of a water-ethanol mixture (25:75, v/v) was used and 0.3 µL of this solution was applied to the plate in the middle and at the two side ends. Acetone-water mixtures were used as a developing solvent with acetone content between 0% and 80% (v/v) in 10% increments. Then the plates were dried at 40°C in an oven and developed with UV radiation. The thermodynamically true R_M values were calculated according to equation $R_M = \log [(1/R_f) - 1]$,⁵ where R_f is the ratio of the distance run by the analyte from the start point to the front marker (KI).

Log k' Assay

The HPLC measurements were assayed on a Konik chromatograph, using an UV detector at $\lambda = 265$ nm. Chromatography was performed by means of a Shimadzu L.C. column shim-pack CLC-ODS(N)PN 228-17873-91(15 cm) QTY:2, packed with a C₁₈ (octadecyl silane) chemically bonded non-polar stationary phase. Methanol-water mixtures of 30-80 methanol concentration were used as the mobile phase at a flow-rate of 1 mL/min. The solutions in methanol were injected into the column by a 20 µL loop. Experiments were performed at room temperature.

The capacity factor k' , was determined from the equation $k' = (t_R - t_0)/t_0$, where t_R is the retention time of the solute and t_0 is the hold-up time¹¹⁻¹⁴ defined as the retention time of a non-retained compound (MeOH).

Shake Flask Octanol-Water Partition Coefficients

The n-octanol-water partition coefficients of **1a-1e** and **2b** were measured by means of the shake-flask method,³ applying the following equations,

$$P = \left(\frac{A_w^i - A_w^f}{A_w^f} \right) \quad P = \left(\frac{A_o^f}{A_o^i - A_o^f} \right)$$

where, A_w^i , A_o^i and A_w^f , A_o^f represent the absorbance at 267.0 nm of each compound in the aqueous phase (w) and the organic phase (o), before (i) and after (f) distribution respectively. The partition coefficient of **3** was calculated by means of the equation:

$$P = \left(\frac{D_o^f}{D_o^i - D_o^f} \right) \left(\frac{V_w}{V_o} \right)$$

where D_o^i and D_o^f are the second derivative absorbance at 245.0 nm in the n-octanol phase before (i) and after (f) partitioning respectively; V_w is the volume of the aqueous phase and V_o is the volume of the octanolic phase.

In all cases, the absorbance values were assayed with a Shimadzu UV-260 (UV/visible recording spectrophotometer). The log $P_{o/w}$ value of Uri (**1f**) was taken from the STARLIST file of the CLOGP database.²⁰ All log P values are summarized in Table 2.

Statistics

All statistical procedures were run with ORIGIN for Windows and STATISTIX (V.92) programs. Deviations are given as 95% confidence intervals.

RESULTS AND DISCUSSION

Reversed-Phase Thin Layer Chromatography (RP-TLC)

The chromatographic value, R_M , so-called *Chromatographic Hydrophobic Constant*, is a well known expression of the lipophilic nature of a molecule.^{21,22} Figure 1 describes the relationship between R_M values of selected pyrimidinic

Table 2
Lipophilic Parameters and Log P Values of Pyrimidinic Nucleoside Derivatives Obtained From Different Methods

Comp	R _{MW}	Log k' _w	Log P _{ow}	Chromatographic Log P Values			Δ ^d	Theoretical Log P Values	
				RP-TLC ^a	Δ ^b	RP-HPLC ^c			CLOGP ^e
1a	-0.252	0.131	-1.17	-1.34	0.17	-1.099	-0.071	-1.937	0.767
1b	1.236	0.583	-0.17	0.21	-0.38	-0.249	0.079	-1.035	0.865
1c	0.738	0.667	0.05	-0.31	0.36	-0.091	0.141	-0.197	0.247
1d	1.470	0.922	0.36	0.45	-0.09	0.389	-0.029	0.705	-0.345
1e	1.864	1.139	0.82	0.86	-0.04	0.798	0.022	1.085	-0.265
1f	-0.741	-0.292	-1.98	-1.85	-0.13	-1.896	-0.084	-2.825	0.845
2a	0.291	-0.440	-2.13	---	---	-2.174	0.044	-3.082	0.952
2b	-0.161	---	-1.33	---	0.08	---	---	-2.179	0.849
3	1.929	1.356	1.11	0.93	0.18	1.206	-0.096	0.719	0.391

^a Log P_{RP-TLC} values obtained from equation 10. ^b Δ = Log P_{ow} - log P_{RP-TLC}. ^c Log P_{RP-HPLC} values obtained from equation 19.

^d Δ = Log P_{ow} - log P_{RP-HPLC}. ^e Log P_{CLOGP} values obtained from CLOGP program. ^f Δ = log P_{ow} - log P_{CLOGP}.

nucleoside derivatives and the composition of the mobile phase in a reversed-phase thin layer chromatographic system (RP-TLC). As it can be seen, all the studied members fitted the respective straight lines, showing a good correlation between R_M values and the organic solvent concentrations in the mobile phase.

At a higher acetone concentration (80%), Thym (**1a**) and Uri (**1f**) tend to move with the solvent front, whereas at lower acetone concentrations (10% and 20%), very lipophilic drugs such as Iso-AZT (**1e**) and Br-AZT (**3**) virtually remain at the starting line.

In both cases their R_M values could not be determined. The linear regression curves of Figure 1 are described by equations 1-9, where %Me₂CO indicates acetone concentration in the mobile phase:

$$R_M(\text{Thym, } \mathbf{1a}) = -0.014 (\pm 0.001) \% \text{Me}_2\text{CO} - 0.252 (\pm 0.036)$$

$$n = 6; r = -0.991; s = 0.038; F = 216.05 \quad (1)$$

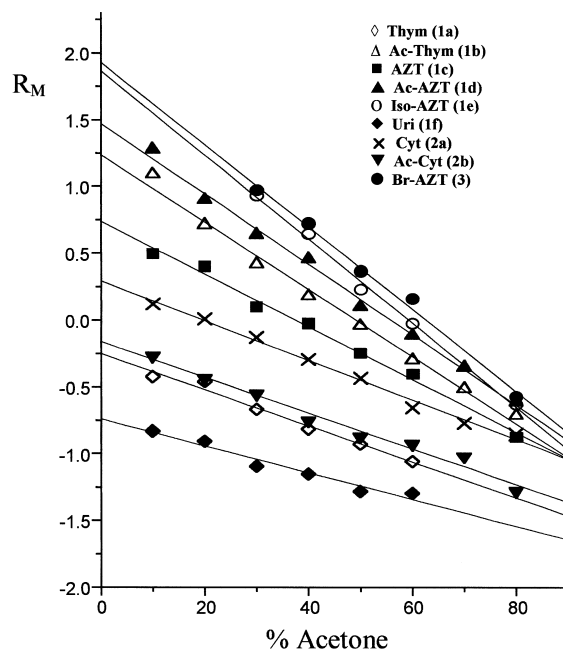


Figure 1. relationship between R_M values and acetone concentrations in the mobile phase for the selected pyrimidinic nucleoside derivatives.

$$R_M(\text{Ac-Thym, } \mathbf{1b}) = - 0.025 (\pm 0.001) \% \text{Me}_2\text{CO} + 1.236 (\pm 0.049)$$

$$n = 8; r = - 0.996; s = 0.062; F = 685.67 \quad (2)$$

$$R_M(\text{AZT, } \mathbf{1c}) = - 0.020 (\pm 0.001) \% \text{Me}_2\text{CO} + 0.738 (\pm 0.039)$$

$$n = 7; r = - 0.996; s = 0.049; F = 576.57 \quad (3)$$

$$R_M(\text{Ac-AZT, } \mathbf{1d}) = - 0.026 (\pm 0.001) \% \text{Me}_2\text{CO} + 1.47 (\pm 0.039)$$

$$n = 8; r = - 0.998; s = 0.050; F = 1172.73 \quad (4)$$

$$R_M(\text{Iso-AZT, } \mathbf{1e}) = - 0.031 (\pm 0.001) \% \text{Me}_2\text{CO} + 1.864 (\pm 0.063)$$

$$n = 5; r = - 0.998; s = 0.044; F = 759.67 \quad (5)$$

$$R_M(\text{Uri, } \mathbf{1f}) = - 0.010 (\pm 0.001) \% \text{Me}_2\text{CO} - 0.741 (\pm 0.042)$$

$$n = 6; r = - 0.978; s = 0.045; F = 88.04 \quad (6)$$

$$R_M(\text{Cyt, } \mathbf{2a}) = - 0.015 (\pm 0.001) \% \text{Me}_2\text{CO} + 0.291 (\pm 0.027)$$

$$n = 8; r = - 0.996; s = 0.034; F = 796.44 \quad (7)$$

$$R_M(\text{Ac-Cyt, } \mathbf{2b}) = - 0.013 (\pm 0.001) \% \text{Me}_2\text{CO} - 0.161 (\pm 0.040)$$

$$n = 8; r = - 0.990; s = 0.051; F = 290.06 \quad (8)$$

$$R_M(\text{Br-AZT, } \mathbf{3}) = - 0.031 (\pm 0.002) \% \text{Me}_2\text{CO} + 1.929 (\pm 0.080)$$

$$n = 5; r = - 0.997; s = 0.056; F = 441.84 \quad (9)$$

The plots of Figure 1 and the negative slopes of eqns. 1-9 mean that with increasing acetone concentrations the R_M values of more lipophilic compounds decrease faster than those of less lipophilic derivatives, which confirms that lipophilic compounds are more sensitive to polarity variations.

The intercepts of the regression lines of eqns 1-9 represent the theoretical R_M values at 0% organic solvent in the mobile phase, R_{Mw} , (Table 2). It is a very important parameter in drug design, since it is a constant for each drug molecule regardless of the quality and quantity of organic solvent in the mobile phase. Thus, all compounds can be compared on the bases of their lipophilicity.⁹ This is possible because we have determined thermodynamically true R_M values¹⁹ and the intercepts of RP-TLC equation can be regarded as a measure of

the partitioning of the compounds between a non-polar stationary phase (octadecanol) and an aqueous polar mobile phase (at 0% organic solvent).⁹

Relationship Between R_{Mw} and $\log P_{o/w}$

Correlation between the conventional $\log P_{o/w}$ and R_{Mw} data yielded a Collander type equation 10 ($\log P_{RP-TLC}$), which is represented in Figure 2. R_{Mw} for cytidine (**2a**) was omitted from eqn. 10 and Figure 2 because of its large deviation, which might suggest that **2a** could interact with the stationary phase in the RP-TLC system.

$$\log P_{o/w} = 1.042 (\pm 0.091) R_{Mw} - 1.081 (\pm 0.111)$$

$$n = 8; r = 0.978; s = 0.248; F = 131.57 \quad (10)$$

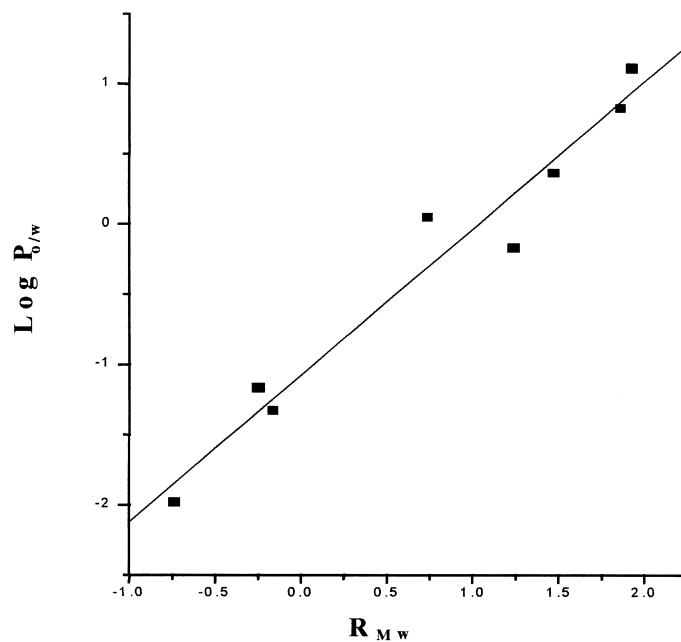


Figure 2. Relationship between R_{Mw} and $\log P_{o/w}$ values, as described by eqn. 10.

Reversed-Phase High Performance Liquid Chromatography (RP-HPLC)

In a similar way to RP-TLC, linear relationship between $\log k'$ values (RP-HPLC) and composition of the mobile phase can be seen in Figure 3. Their corresponding regression lines are described by equations 11-18, where %MeOH indicates the methanol concentration in the mobile phase.

$$\text{Log } k'(\text{Thym, } \mathbf{1a}) = -0.007 (\pm 0.0004) \% \text{MeOH} + 0.131 (\pm 0.020)$$

$$n = 6; r = -0.995; s = 0.015; F = 394.73 \quad (11)$$

$$\text{Log } k'(\text{Ac-Thym, } \mathbf{1b}) = -0.012 (\pm 0.0004) \% \text{MeOH} + 0.583 (\pm 0.021)$$

$$n = 6; r = -0.998; s = 0.0152; F = 998.63 \quad (12)$$

$$\text{Log } k'(\text{AZT, } \mathbf{1c}) = -0.013 (\pm 0.0004) \% \text{MeOH} + 0.667 (\pm 0.011)$$

$$n = 6; r = -0.9995; s = 0.0079; F = 4391.68 \quad (13)$$

$$\text{Log } k'(\text{Ac-AZT, } \mathbf{1d}) = -0.014 (\pm 0.001) \% \text{MeOH} + 0.922 (\pm 0.039)$$

$$n = 6; r = -0.995; s = 0.028; F = 435.58 \quad (14)$$

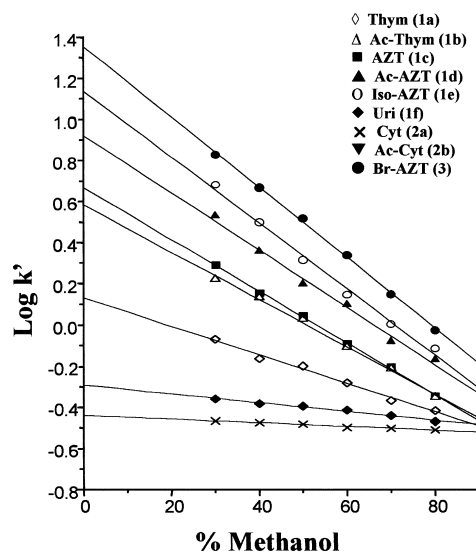


Figure 3. Relationship between $\text{Log } k'$ values and methanol concentrations in the mobile phase for the selected pyrimidinic nucleoside derivatives.

$$\text{Log } k'(\text{Iso-AZT, } \mathbf{1e}) = -0.016 (\pm 0.001) \% \text{MeOH} + 1.139 (\pm 0.036)$$

$$n = 6; r = -0.997; s = 0.026; F = 644.94 \quad (15)$$

$$\text{Log } k'(\text{Uri, } \mathbf{1f}) = -0.002 (\pm 0.0004) \% \text{MeOH} - 0.292 (\pm 0.009)$$

$$n = 6; r = -0.989; s = 0.007; F = 178.29 \quad (16)$$

$$\text{Log } k'(\text{Cyt, } \mathbf{2a}) = -0.001 (\pm 0.0001) \% \text{MeOH} - 0.440 (\pm 0.004)$$

$$n = 6; r = -0.991; s = 0.003; F = 207.44 \quad (17)$$

$$\text{Log } k'(\text{Br-AZT, } \mathbf{3}) = -0.017 (\pm 0.0004) \% \text{MeOH} + 1.356 (\pm 0.020)$$

$$n = 6; r = -0.999; s = 0.014; F = 2522.64 \quad (18)$$

The extrapolated logs k' at 0% methanol ($\log k'_w$) are summarized in Table 2. Equation 19 (Figure 4) pointed out the excellent correlation between $\log P_{\text{RP-HPLC}}$ and $\log k'_w$ of the studied compounds.

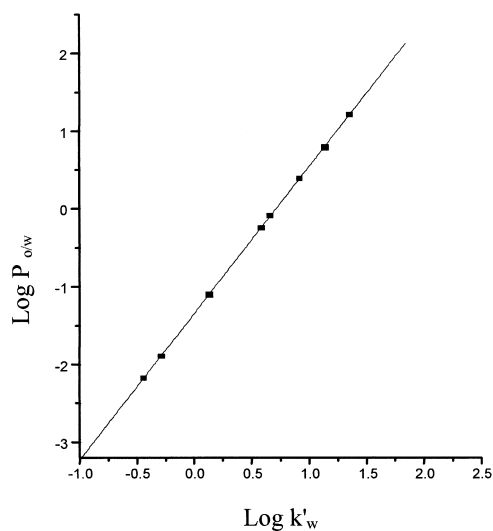


Figure 4. Relationship between $\log P_{\text{RP-HPLC}}$ and $\log k'_w$ values, as described by eqn. 19.

$$\text{Log } P_{o/w} = 1.882 (\pm 0.053) \log k'_w - 1.346 (\pm 0.042)$$

$$n = 8; r = 0.998; s = 0.092; F = 1260.14 \quad (19)$$

The 1.871 slope value shows that when conversion of the stationary phase into the mobile one is performed, nucleosides seem to exhibit approximately half the affinity for stationary phase (C18) than for water phase.

This feature has not been observed by RP-TLC since the slope for eqn. 10 is almost equal to 1.

Relationship Between Slopes and Intercepts (RP-TLC and RP-HPLC)

The plot of Figure 5a shows a good linear relationship between the slopes and the intercepts (R_{Mw}) of the 1-9 RP-TLC equations, as described by eqn. 20.

$$\text{Intercept} = - 119.504 (\pm 6.756) \text{ slope} - 1.750 (\pm 0.148)$$

$$n = 9; r = - 0.989; s = 0.15; F = 314.32 \quad (20)$$

Likewise, Figure 5b shows an optimal correlation between the slopes and intercepts ($\log k'_w$) of equations 11-18 (RP-HPLC), as expressed by eqn. 21.

$$\text{Intercept} = - 104.553 (\pm 5.883) \text{ slope} - 0.562 (\pm 0.069)$$

$$n = 8; r = - 0.991; s = 0.097; F = 315.83 \quad (21)$$

Biagi et al.⁹ have pointed out the relationship between slopes and intercepts of the TLC equations as one of the basic features of the chromatographic assays of lipophilicity. The physicochemical parameters R_{Mw} and $\log k'_w$, the intercepts of linear regression equations 1-9 and 11-18 respectively, can be considered as a sign of the partitioning process between a polar mobile phase (water) and a non-polar stationary phase (octadecanol in RP-TLC and octadecylsilane in RP-HPLC).

The slopes indicate, as already mentioned, the rate at which the solubility of the compounds increase in the mobile phase as percent of organic solvent increase. Hence, it is reasonable that the most lipophilic compound (more sensitive to a decrease in the polarity of the mobile phase and thereby with a higher slope) exhibits a higher R_{Mw} and $\log k'_w$. Conversely, a more hydrophilic compound with lower slope and sensibility to a decrease in mobile phase polarity will have less R_{Mw} and $\log k'_w$. This accounts for the linear correlations between intercepts and slopes.

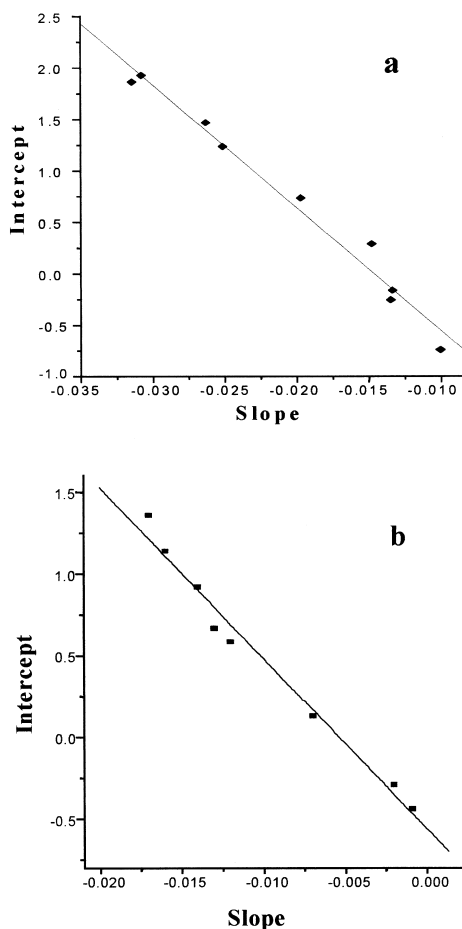


Figure 5. Relationship between slopes and intercepts. **a)** RP-TLC equations 1-9 for test compounds as described by eqn 20. **b)** RP-HPLC equations 11-18 for test compounds as described by eqn 21.

Relationship Between $\log k'_w$ and R_{Mw}

Another interesting linear correlation was observed between $\log k'_w$ and R_{Mw} (eqn. 22, Figure 6).

$$\log k'_w = 0.536 (\pm 0.059) R_{Mw} + 0.165 (\pm 0.077)$$

$$n = 7; r = 0.971; s = 0.149; F = 83.64 \quad (22)$$

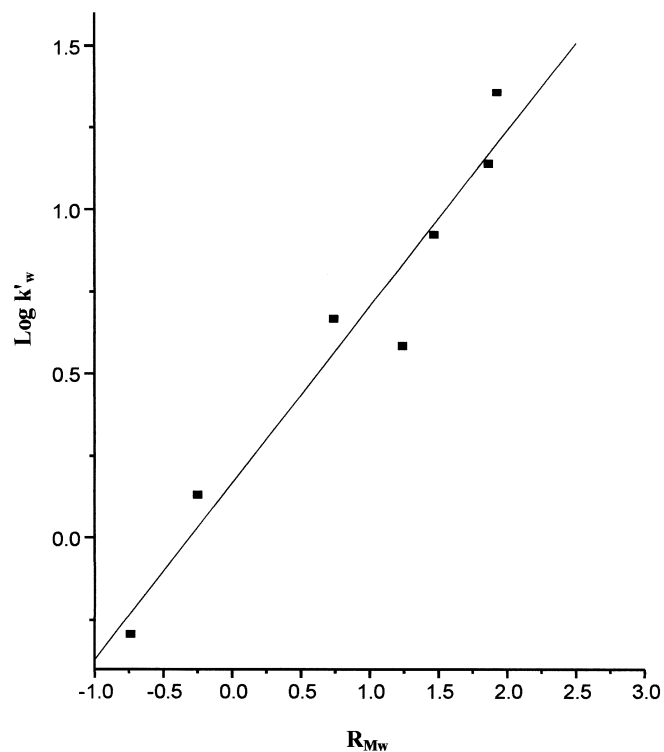


Figure 6. Relationship between $\log k'_w$ and R_{Mw} values, as described by eqn. 22.

This last correlation not only verifies the self consistency of RP-TLC and RP-HPLC methods but also justifies the confidence in the use of chromatographic data as lipophilic parameters.

Log P Calculations

In drug design and in Quantitative Structure Activity Relationship (QSAR), it is significant to predict the $\log P$ of unknown drug molecules. In this way and for comparative purposes, $\log P$ values for pyrimidinic nucleoside derivatives were calculated by CLOGP program^{20a} (Table 2).

Correlations Between Different Techniques

Table 2 shows the log P values obtained from RP-TLC and RP-HPLC techniques (eqns. 10 and 19 respectively), as well as for the CLOGP program and shake flask method. In addition, the corresponding deviations ($\Delta \log P$) between the conventional shake flask method and the other different studied techniques ($\log P_{\text{RP-TLC}}$, $\log P_{\text{RP-HPLC}}$ and $\log P_{\text{CLOGP}}$) are shown. Figure 7 shows log P relationship between the conventional shake-flask method and the chromatographic and theoretical ones. The linear regression curves are described by equations 23-25.

$$\begin{aligned} \log P_{\text{o/w}} &= 1.001 (\pm 0.087) \log P_{\text{RP-TLC}} - 0.001 (\pm 0.091) \\ n &= 8; r = 0.978; s = 0.248; F = 131.78 \end{aligned} \quad (23)$$

$$\begin{aligned} \log P_{\text{o/w}} &= 1.000 (\pm 0.028) \log P_{\text{RP-HPLC}} + 0.001 (\pm 0.034) \\ n &= 8; r = 0.998; s = 0.092; F = 1258.44 \end{aligned} \quad (24)$$

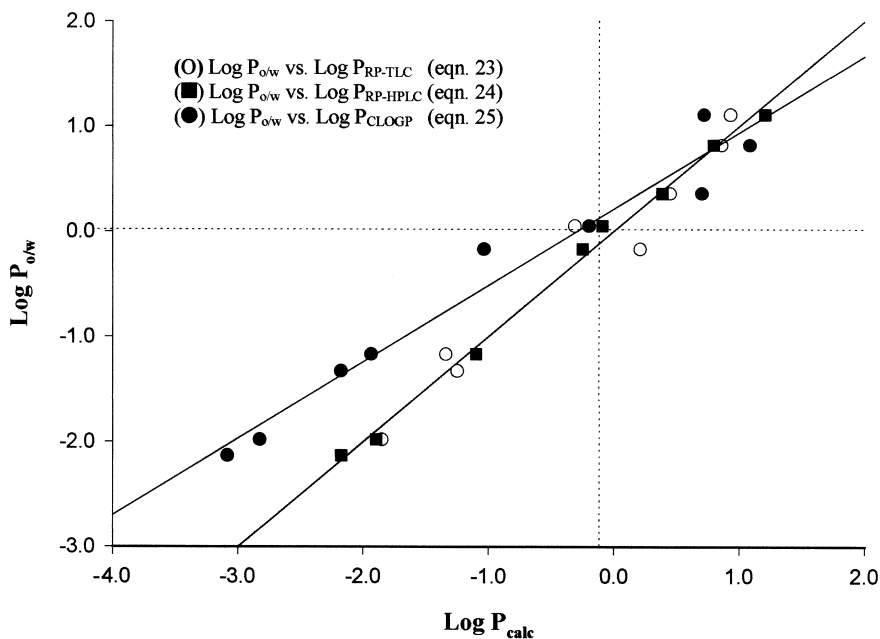


Figure 7. Relationship between $\log P_{\text{o/w}}$ values obtained by shake flask methods and $\log P$ values obtained from different techniques.

$$\text{Log } P_{o/w} = 0.727 (\pm 0.057) \log P_{\text{CLOGP}} - 0.214 (\pm 0.103)$$

$$n = 9; r = 0.979; s = 0.261; F = 161.74 \quad (25)$$

Equations 23-25 show that the RP-TLC, as well as, the RP-HPLC methods, both of them with slope $\cong 1$ and intercept $\cong 0$, provide more accurate lipophilicity measures for pyrimidinic nucleosides than those obtained from the CLOGP theoretical method (slope = 0.727, intercept = 0.214), as we can see in Figure 7.

CONCLUSIONS

Reversed phase chromatographic methods (RP-TLC and RP-HPLC) have proven to be reliable and accurate methods to describe the lipophilicity nature of pyrimidinic nucleoside derivatives.

From Table 2 it may be inferred that chromatographic methods yield lower deviations than theoretical ones, while RP-HPLC technique offers better results for this set of compounds.

Although CLOGP is a good predictor of lipophilicity rank for pyrimidinic nucleoside derivatives according to the good correlation shown in eqn. 25, and may be used for QSAR studies, it is not advisable to achieve absolute values of log P. This finding, which is pointed out through the higher $\Delta \log P$ values obtained between $\log P_{o/w}$ and $\log P_{\text{CLOGP}}$, is due to the fact that the CLOGP program does not correctly estimate the partition coefficient for nucleoside derivatives, since they do not take into account the interactions between the pyrimidinic ring and the sugar moiety, which were also reported by other authors.^{7,23}

In addition, it can be seen that **3** was the most lipophilic studied compound, whereas cytidine (**2a**) appeared to be the most hydrophilic one and that the two novel derivatives, Iso-AZT (**1e**, log P = 0.8) and Br-AZT (**3**, log P = 1.11) are more lipophilic than their precursor drug (AZT, log P = 0.05). Since the partition coefficient is an expression of the lipophilic character of a molecule and serves as an index of the capacity to pass membranes by passive diffusion^{3a} and that Iso-AZT and Br-AZT display biological activity similar to AZT,¹⁷ these new agents might be used as potential pharmacological agents in the treatment of AIDS and related diseases.

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