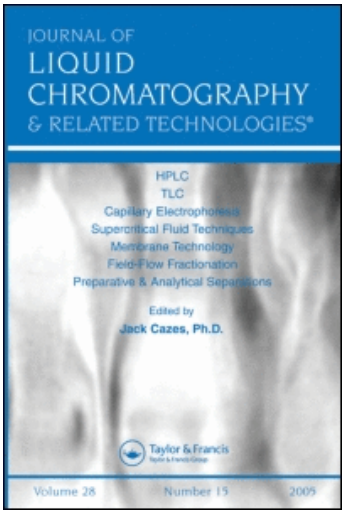

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Comparative Study of Hydrophobicity Parameters of Novel 5′-Carbamates of Zidovudine

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Abstract: The lipophilic character of a series of 5′-carbamates of zidovudina has been studied. The lipophilicity was measured by means of reversed-phase thin layer chromatography (RP-TLC) and reversed-phase high performance liquid chromatography (RP-HPLC) techniques giving the corresponding R_{Mw} and log k'_w parameters, respectively. These values were compared with those obtained by the classical shake flask methodology. RP-TLC assays were performed on the basis of thermodynamically true R_M values, and buffer pH's 7.4 and 12.03 were chosen to determine the influence of the pH on the lipophilicity of the studied compounds. In addition, the influence of the organic modifier was studied in RP-TLC, showing the superiority of methanol as compared with acetone as the organic modifier, and physicochemical reasons have been discussed. Based on RP-TLC results, RP-HPLC studies were carried out using methanol and buffer pH 7.4 as mobile phase. Chromatographic data (R_{Mw} and log k'_w) proved to be reliable parameters for describing the lipophilic properties of the test compounds.

Keywords: 5′-Carbamates of zidovudina, Lipophilicity, Lipophilic chromatographic parameters

INTRODUCTION

Lipophilicity is one of the inherent properties of chemical compounds, affecting their biological activities. It plays a determinant role by passing

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compounds through a biological system, and it may also influence the formation of a complex between a compound and a receptor or a biomacromolecule at the site of action.[1] All these processes involving partition between lipid membranes and aqueous extra and intracellular fluids, are believed to be affected by drug lipophilicity. Also, the affinity of a drug towards nonpolar binding sites of blood proteins or a receptor facing an aqueous environment depends on drug lipophilicity.[1,2] Adaptation of chromatographic procedures to the experimental determination of lipophilic properties resulted in the development of reversed-phase chromatography, in which the hydrophilic, polar stationary phase is replaced by a hydrophobic, non-polar one. Thus, chromatographic approaches, such as reversed-phase thin layer chromatography (RP-TLC) and reversed-phase high performance liquid chromatography (RP-HPLC) are very important experimental alternatives to octanol/water partitioning (log P_{oct}).[2–4]

The purpose of the present study is to investigate the chromatographic behavior of novel 5′-carbamates of zidovudina,[5] by RP-TLC and RP-HPLC, in order to establish if the linear relationships between R_M (or log k′) vs. concentration of organic modifier allows the extrapolation procedure. Moreover, the influence of the organic modifier (methanol and acetone), as well as that of the pH, were studied in the RP-TLC determination. The best conditions founded for RP-TLC were employed in RP-HPLC analyses. Then, the reliability of the R_{M_w} and log k'_w values as lipophilic parameters for describing these carbamate derivatives of AZT, was determined by the relationship of the log P values obtained from the shake flask method (log P_{oct}) with those of chromatographic methods (log $P_{RP\text{-}TLC}$ and log $P_{RP\text{-}HPLC}$).

EXPERIMENTAL

Chemicals

Thymidine (Thym, 1) and Zidovudine (3′-azido-3′-deoxythymidine, AZT, 2) were generous gifts of Filaxis (Buenos Aires, Argentina). The novel compounds, AZT-Ethy (3′-azido-3′-deoxythymidin-5′-yl-*N*-[ethylpyperazine] carbamate, 3), AZT-Pyp (3′-azido-3′-deoxythymidin-5′-yl-*N*-[pyperazine] carbamate, 4), AZT-Py (3′-azido-3′-deoxythymidin-5′-yl-*N*-[pyridine] carbamate, 5), AZT-Tos (3′-azido-3′-deoxythymidine-5′-tosylate, 6), AZT-Cycl (3′-azido-O^2, 5′ ciclothymidine, 7), and AZT-Ac (3′-azido-3′-deoxythymidin-5′-yl-carboxylic acid, 8) were prepared under the methodology reported previously.[5] The structures of assayed compounds are shown in Figure 1.

Analytical grade *n*-octanol, acetone (Me_2CO), and methanol (MeOH), were purchased from Merck Co and HPLC grade methanol from Sintorgan. The water for HPLC was purified using a Milli-Q water-purification system (Millipore)® and mobile phases were filtered through a Millipore® Type FH filter (0.45 μm pore size) and vacuum degassed, as well as all solutions

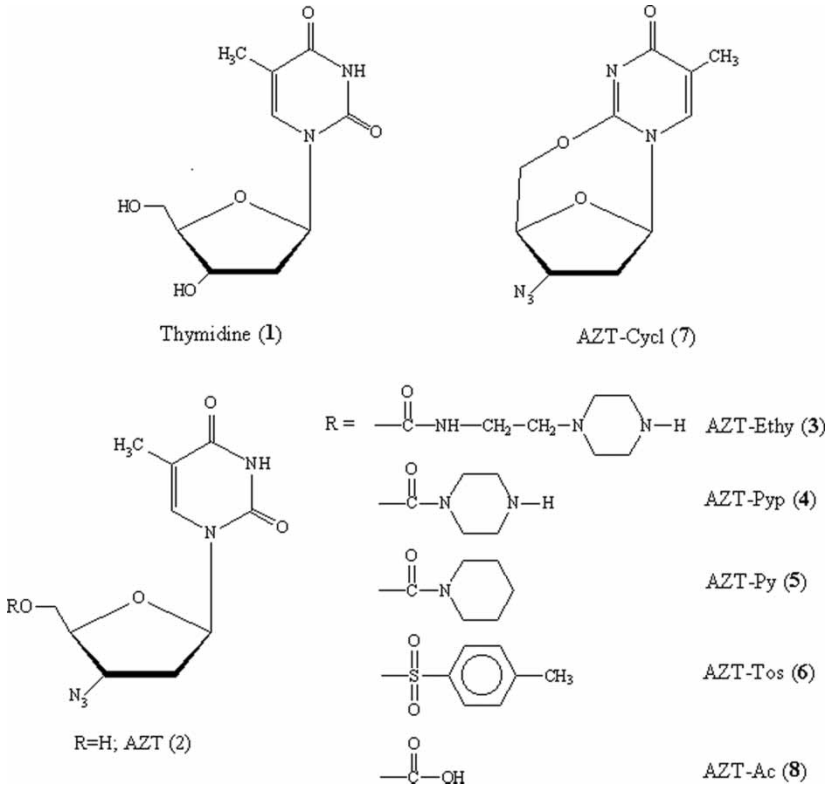

Figure 1. Chemical structures of studied compounds.

used for HPLC. Buffer pH 7.40 (66 mM) was prepared with potassium phosphate monobasic and sodium phosphate dibasic dihydrate (potassium phosphate monobasic 19.7%, sodium phosphate dibasic dihydrate, 80.3%) in Milli-Q water, and buffer pH 12.03 (52 mM) was prepared with sodium phosphate dibasic dihydrate and sodium hydroxide (sodium phosphate dibasic dihydrate 48.1%, sodium hydroxide 51.9%). All other chemicals were of analytical-reagent grade and used as delivered.

R_M Assay

Precoated thin layer chromatographic plates (RP-18 HPTLC F_{254}, 5 × 10 cm), purchased from Merck (Darmstadt, Germany), were used for the measurements of the chromatographic hydrophobic constant (R_M), since they have a considerable advantage of high stability, allowing their use over a large range of organic modifier contents. Compounds 1, 2, and 8 were dissolved in acetone, 3, 4, and 7 in methanol, and 5 and 6 in chloroform, reaching

a final concentration in the corresponding solvent of 1 mg/mL. In order to determine the thermodynamically true position of the front, potassium iodide (KI) was used.[4,6] A 50 μL aliquot of the test solutions of each compound was applied to the plates in random positions. Methanol-buffer mixtures were used as mobile phase with methanol contents between 30 and 80% (v/v) or acetone-buffer mixtures with modifier contents between 20 and 80% (v/v), in 5 or 10% increments. Finally, plates were dried at 40°C in an oven and developed with UV radiation. The thermodynamically true R_M values were calculated according to $R_M = \log [(1/Rf) - 1]$ equation,[2,4,6] where Rf is the ratio of the migration distance of the analyte to that of the marker (KI) front distance from the start point.

Log k′ Assay

The high performance liquid chromatographic (HPLC) measurements were performed on a Spectra System P2000 chromatograph, using an UV detector at $\lambda = 267$ nm for 1–6 and 8 and $\lambda = 250$ nm for 7 equipped with a Phenomenex® column, Hypersil ODS 5 μ particle diameter, 250 mm length, 4.6 mm internal diameter, packed with a C_{18} (octadecyl silane) chemically bonded non-polar stationary phase. Data were acquired by means of a Peak Simple Chromatography Data System.® Methanol-buffer pH 7.40 was used as a mobile phase with methanol content between 30% and 75% (v/v) in 5 or 10% increments, at a flow-rate of 1 mL/min. Compounds 1–4, 7, and 8 were dissolved with methanol, and 5 and 6 with methanol and 0.1 mL of dimethylsulfoxide (DMSO) as cosolvent. The solutions were injected with a Rheodyne 7725 into the column by a 20 μL loop. Experiments were performed at room temperature. From the equation $k' = (t_R - t_0)/t_0$, the capacity factors (k′) were calculated, where t_R is the retention time of the solute and t_0 the hold-up time defined as the retention time of a non-retained compound (MeOH).[4,6,7]

Conventional Shake-Flask Methods (Log P_{oct})

Partition coefficients of 1–8 were measured by means of the shake-flask method, using *n*-octanol as lipidic phase and buffer pH 7.40 as the polar phase; each phase was previously saturated with the other one. The concentration of samples in both of the *n*-octanol and buffer phases was determined by UV spectrophotometric analyses (Shimadzu UV-260), applying the equations previously reported.[4,6] The absorbance for 1–6 and 8 were measured at $\lambda = 267.0$ nm and for 7 at $\lambda = 250.0$ nm. Taking into account that pKa values for the acid group of 8 is 5.61 and for the pyperazinil moiety of 3 and 4 are 9.03 and 6.52, respectively, they present a different grade of ionization at the work buffer solution pH's. For this reason, to

calculate true log P values, eq. 1 was employed for compound 8 and eq. 2 for compounds 3 and 4,[8] where P'_{oct} and P_{oct} are the apparent and true partition coefficients, respectively.

$$for\ acids \quad P_{oct} = P'_{oct}[1 + 10^{(pH-pKa)}] \tag{1}$$

$$for\ bases \quad P_{oct} = P'_{oct}[1 + 10^{(pKa-pH)}] \tag{2}$$

Statistics

All statistical procedures were run with Sigma Plot 8.0 for Windows programs, and Statistica for Windows R 4.5. Deviations are given as 95% confidence intervals.

RESULTS AND DISCUSSION

Reversed-Phase Thin Layer Chromatography (RP-TLC)

The chromatographic value R_M is widely used to describe the lipophilic property of a molecule.[2,8] The equations, as well as organic modifier and ranges of organic solvent concentrations used for their calculations are reported in Table 1.

From the regression analyses, it can be seen that the studied compounds exhibited a good linear correlation between the organic modifier content of the mobile phase and the corresponding R_M value. R_M data for AZT-Ethy and AZT-Pyp using methanol 70–80% and acetone 55–80% as modifier concentrations were omitted from the regression analysis because of their large deviations. These deviations from linearity for 3 (Figure 2a) and 4 (Figure 2b), probably are due to the so-called silanophilic effect, which has been widely described by different authors.[2,9,10] This effect is based on polar interactions between free silanol moieties of the RP material and polar moieties of the test molecules, where the silanol groups are only partially etherified with octadecanol for stereochemical reasons.[9]

In solvent mixtures with high buffer content, water molecules quantitatively protect silanol groups and the chromatographic process is based almost exclusively on partitioning (reversed-phase behavior). As modifier content increases, the possibility of polar interactions of the silanol groups increases (normal-phase behavior). For this reason, deviations from linearity were observed when water content decreased.

In order to obtain a parameter that allowed us to analyze the results independently from the composition of the mobile phase, the R_{Mw} values (R_M corresponding to 0% of organic modifier) for 1–8 that correspond to the intercept of the regression lines were determined (Table 2).[2,4,6,10] These results made it possible to compare the assayed compounds on the basis of their intrinsic lipo-

Table 1. RP-TLC and RP-HPLC equations of zidovudine derivatives at pH 7.4

Compound	a ± (s.t)	b ± (s.t)	r	n	% Org. modif.
	$R_M = a + b$ (% organic modifier): Methanol				
Thymidine (1)	0.294 ± (0.144)	−0.017 ± (0.002)	0.994	6	30–80
AZT (2)	1.140 ± (0.094)	−0.022 ± (0.001)	0.999	6	30–80
AZT-Ethy (3)	1.753 ± (0.179)	−0.015 ± (0.003)	0.997	4	30–60
AZT-Pyp (4)	0.855 ± (0.143)	−0.011 ± (0.003)	0.996	4	30–60
AZT-Py (5)	2.862 ± (0.238)	−0.037 ± (0.003)	0.998	5	40–80
AZT-Tos (6)	2.845 ± (0.423)	−0.037 ± (0.006)	0.995	5	40–80
AZT-Cycl (7)	0.791 ± (0.107)	−0.016 ± (0.001)	0.997	6	30–80
AZT-Ac (8)	2.862 ± (0.272)	−0.041 ± (0.004)	0.998	5	40–80
	$R_M = a + b$ (% organic modifier): Acetone				
Thymidine (1)	−0.395 ± (0.093)	−0.013 ± (0.003)	0.993	7	20–80
AZT (2)	0.634 ± (0.097)	−0.020 ± (0.017)	0.995	9	20–80
AZT-Ethy (3)	1.705 ± (0.286)	−0.034 ± (0.006)	0.993	5	20–50
AZT-Pyp (4)	0.510 ± (0.202)	−0.016 ± (0.005)	0.985	5	20–50
AZT-Py (5)	1.923 ± (0.176)	−0.032 ± (0.002)	0.995	8	30–80
AZT-Tos (6)	2.621 ± (0.401)	−0.041 ± (0.006)	0.996	5	40–80
AZT-Cycl (7)	0.144 ± (0.077)	−0.011 ± (0.003)	0.993	7	20–80
AZT-Ac (8)	2.148 ± (0.272)	−0.040 ± (0.005)	0.992	8	30–80
	$\log k' = a + b$ (% organic modifier): Methanol				
Thymidine (1)	−0.279 ± (0.397)	−0.163 ± (0.008)	0.960	5	35–55
AZT (2)	0.892 ± (0.325)	−0.022 ± (0.005)	0.981	6	30–70
AZT-Ethy (3)	1.396 ± (0.439)	−0.031 ± (0.008)	0.982	6	30–70
AZT-Pyp (4)	1.386 ± (0.169)	−0.030 ± (0.003)	0.997	6	30–70
AZT-Py (5)	2.667 ± (0.717)	−0.038 ± (0.013)	0.987	5	50–75
AZT-Tos (6)	2.872 ± (1.483)	−0.039 ± (0.022)	0.953	5	55–75
AZT-Cycl (7)	0.484 ± (0.261)	−0.023 ± (0.005)	0.992	5	30–60
AZT-Ac (8)	2.818 ± (0.597)	−0.044 ± (0.008)	0.988	6	50–75

philicity. Thus, the R_{Mw} values can be considered as a measure of the partitioning of 1–8 between silicone oil and an aqueous buffer.

Influence of the Modifier on R_M

Inspection of Table 2 shows that R_{Mw} values of some compounds determined with acetone as modifier are lower than those measured with the methanol system, although a good correlation between R_{MwMeOH} and $R_{MwMe2CO}$ (eq. 3) was obtained.

$$R_{MwMeOH} = 0.945(\pm 0.265)R_{MwMe2CO} + 0.578(\pm 0.408) \qquad (3)$$

$$n = 8;\ r = 0.963;\ s = 0.309$$

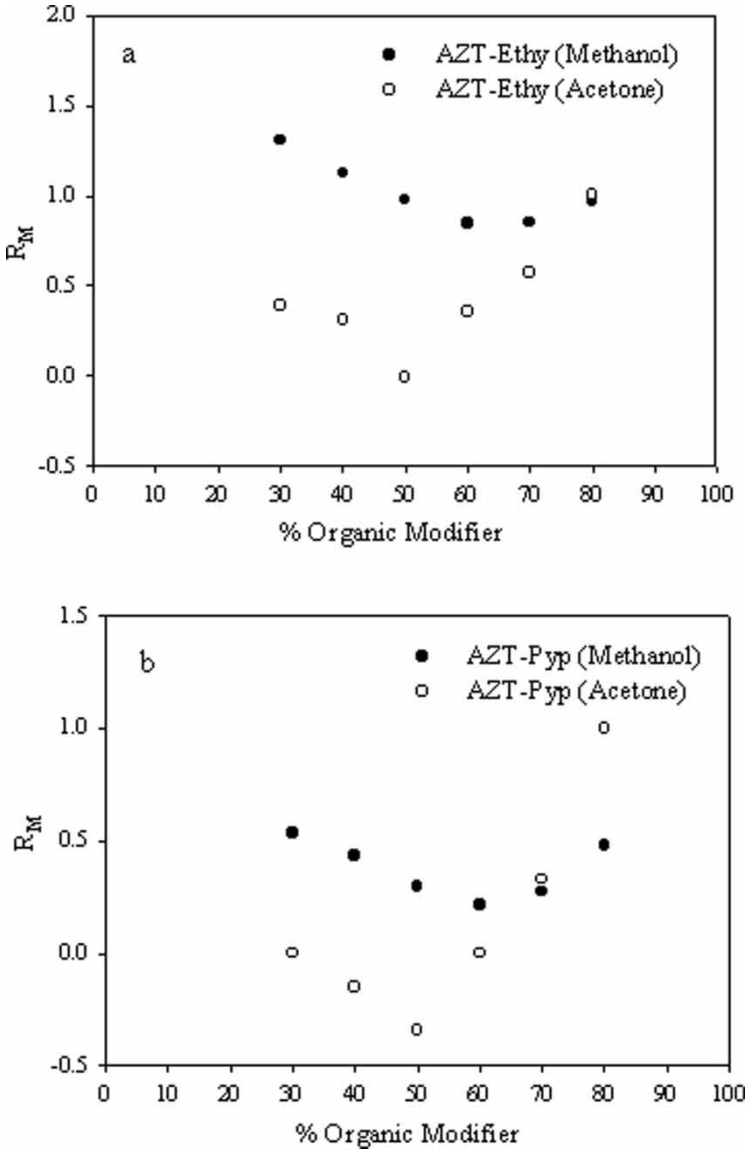

Figure 2. Silanophilic effect on R_M using methanol and acetone as organic modifier. a) AZT-Ethy; b) AZT-Pyp.

Braumann[10] has comprehensively described the pronounced influence of the modifier on the quality of chromatographic data, since the distribution of the test compound into the octadecyl phase of the RP-18 phase depends significantly on physicochemical properties of the modifier, such as dipole

Table 2. Lipophilic Parameters and log P values of novel zidovudina derivatives obtained from different methods

Compounds	R_{MwMeOH}	$R_{MwMe2CO}$	Log k'_w	Log P_{oct}	Chromatographic log P values			
					RP-TLC[a]	Δ[b]	RP-HPLC[c]	Δ[d]
Thymidine (1)	0.294	−0.395	−0.028	−1.125	−1.066	−0.059	−1.139	0.014
AZT (2)	1.140	0.634	0.892	0.063	−0.119	0.182	−0.202	0.265
AZT-Ethy (3)	1.753	1.705	1.398	0.777[e]	0.567	0.210	0.314	0.463
AZT-Pyp (4)	0.855	0.510	1.386	−0.481[e]	−0.438	−0.043	0.301	−0.782
AZT-Py (5)	2.862	1.923	2.667	1.548	1.807	−0.259	1.607	−0.058
AZT-Tos (6)	2.845	2.621	2.872	1.585	1.788	−0.203	1.815	−0.230
AZT-Cycl (7)	0.791	0.144	0.484	−0.659	−0.510	−0.149	−0.618	−0.041
AZT-Ac (8)	2.862	2.148	2.818	2.128[f]	1.808	0.320	1.761	0.367

[a] $\log P_{RP\text{-}TLC}$ values obtained from eq. 4.
[b] $\Delta = \log P_{oct} - \log P_{RP\text{-}TLC}$.
[c] $\log P_{RP\text{-}HPLC}$ values obtained from eq. 12.
[d] $\Delta = \log P_{oct} - \log P_{RP\text{-}HPLC}$.
[e] $P_{oct} = P'_{oct}\,[1 + 10^{(pKa-pH)}]$.
[f] $P_{oct} = P'_{oct}\,[1 + 10^{(pH-pKa)}]$.

moment or hydrogen-donating and hydrogen-accepting properties. Thus, the properties of methanol are very similar to those of water, whereas acetone is different from there, leading to some differences between R_{MwMeOH} and R_{MwMe_2CO} values (Table 2).

Relationship Between R_{Mw} and log P_{oct}

The linear dependence observed in the correlation between the R_{MwMeOH} and $R_{MwMe2CO}$ with log P_{oct} obtained by the shake flask method, can be considered as a Collander type equation (eqs. 4 and 5).

$$\text{Log}\, P_{oct} = 1.119\ (\pm 0.201)\ R_{MwMeOH} - 1.395\ (\pm 0.391) \qquad (4)$$
$$n = 8;\ r = 0.984;\ s = 0.230$$

$$\text{Log}\, P_{oct} = 1.071\ (\pm 0.314)\ R_{MwMe_2CO} - 0.764\ (\pm 0.483) \qquad (5)$$
$$n = 8;\ r = 0.960;\ s = 0.366$$

Based on the extra thermodynamic linear-energy relationship one may expect the standard log P_{oct} data to be linearly related to the partition chromatographic parameter R_{Mw}. The slope's Collander equations are a measure of the solvent system sensitivity to changes in the hydrophobicity of solutes relative to *n*-octanol.[10] Taking into account that slope values of eqs. 4 and 5 are not significantly different from 1.0, an isodiscriminative behavior would be expected for 1–8. However, the correlation of the chromatographic data with log P_{oct} are slightly more significant in the case of the methanol system as compared with the acetone one, since methanol provides both strong hydrogen-donating and hydrogen-accepting capabilities. Thus, (*i*) the stationary phase-mobile phase interface contains hydrogen-bonding activity owing to adsorbed methanol molecules and (*ii*) the water molecule network can incorporate a fairly large amount of methanol, maintaining, in principle, the highly ordered array of water molecules that is the driving force for the hydrophobic effect, which is the force governing the liquid-liquid partitioning process. For acetone, the reduced hydrogen-bonding capability of the eluent contributes to a solvophobic effect, with different energetic process operating in the *n*-octanol-water and in the methanol-water systems. Finally, the participation of the residual silanol groups in the retention process seems to be more pronounced in acetone than in methanol, as has been demonstrated in Figures 2a,b.

Influence of Buffer pH on R_{Mw}

In order to validate, the hypothesis that the silanophilic effect, but not the lipophilic distribution depend on solvent pH,[2,9,11] we selected compounds 3 and 4, which exhibit pKa values of 6.52 and 9.03, respectively in their pyper-

azine moieties. Since adequate properties exhibited for methanol, equations 6–9 describe the linear relationships for 3 and 4 between R_M values and methanol as organic modifier, at pH 7.40 (where 3 and 4 are in 97.7% and 11.6% as dissociate form, respectively) and at pH 12.03 (where 3 and 4 are in 99.90% and 99.99 % as neutral form, respectively). As can be seen in Figures 3a,b linear relationships were observed until 60% of organic modifier, from that point R_M data for AZT-Ethy and AZT-Pyp were omitted

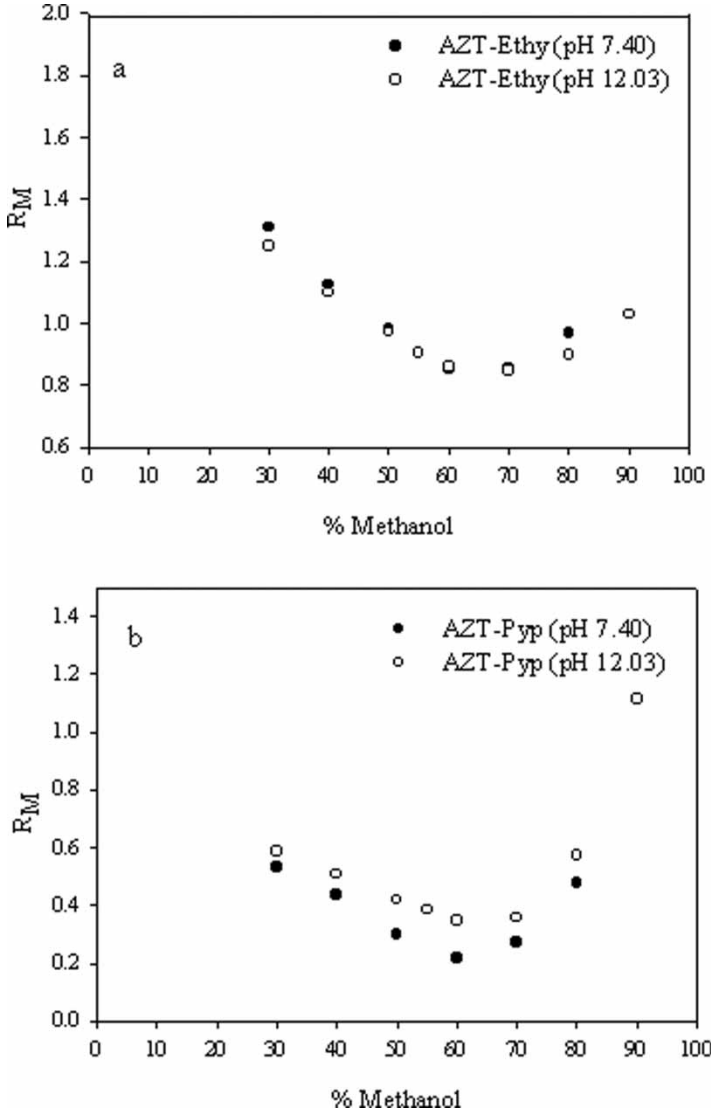

Figure 3. Influence of buffer pH on R_{Mw}. a) AZT-Ethy; b) AZT-Pyp.

from the regression analysis because of their large deviations. The similar R_{Mw} values for 3 and 4, show that at low methanol contents R_M depends only on the lipophilic distribution. In other words, the influence of buffer pH on the R_M is negligible at low organic modifier content in the mobile phase. If the concentration of the modifier increases, polar adsorption becomes more pronounced and the influence of the pH on R_M becomes strong in the case of basic solutes.

$$\text{AZT-Ethy: } R_{MpH7.40} = -0.015\ (\pm 0.003)\%\text{MeOH} + 1.753(\pm 0.179) \quad (6)$$
$$n = 4;\ r = 0.997;\ s = 0.020$$
$$R_{MpH12.03} = -0.013\ (\pm 0.001)\%\text{MeOH} + 1.635\ (\pm 0.079) \quad (7)$$
$$n = 5;\ r = 0.998;\ s = 0.013$$
$$\text{AZT-Pyp: } R_{MpH7.40} = -0.011\ (\pm 0.046)\%\text{MeOH} + 0.855\ (\pm 0.142) \quad (8)$$
$$n = 4;\ r = 0.996;\ s = 0.016$$
$$R_{MpH12.03} = -0.008\ (\pm 0.004)\%\text{MeOH} + 0.827\ (\pm 0.027) \quad (9)$$
$$n = 5;\ r = 0.999;\ s = 0.004$$

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

The correlation between the log k′ values and the composition of the mobile phase was established finding a linear relationship, as can be seen in Table 1. Surprisingly, in RP-HPLC the silanophilic effect was not observed for these compounds in spite of having been reported by other authors.[10,12] Table 2 summarized the extrapolated log k′ at 0% of methanol (log k'_w).

Relationship Between Log k'_w and R_{Mw}

Another interesting linear correlation was observed between log k'$_w$ and R_{Mw} (eqs. 10 and 11, Figures 4 a,b), showing a good correlation between the extrapolated parameters comparing the RP-HPLC and the RP-TLC measurements.

$$\text{Log } k'_w = 1.017(\pm 0.278)R_{MwMeOH} - 0.143(\pm 0.539) \quad (10)$$
$$n = 8;\ r = 0.965;\ s = 0.317$$
$$\text{Log } k'_w = 0.976(\pm 0.343)R_{MwMe2CO} + 0.428(\pm 0.538) \quad (11)$$
$$n = 8;\ r = 0.943;\ s = 0.400$$

Relationship Between Log k'_w and log P_{oct}

Equation 12 pointed out the excellent correlation between log P_{oct} and log k'_w of the studied compounds. Hence, the 1.019 slope value shows that 1–8 exhibit

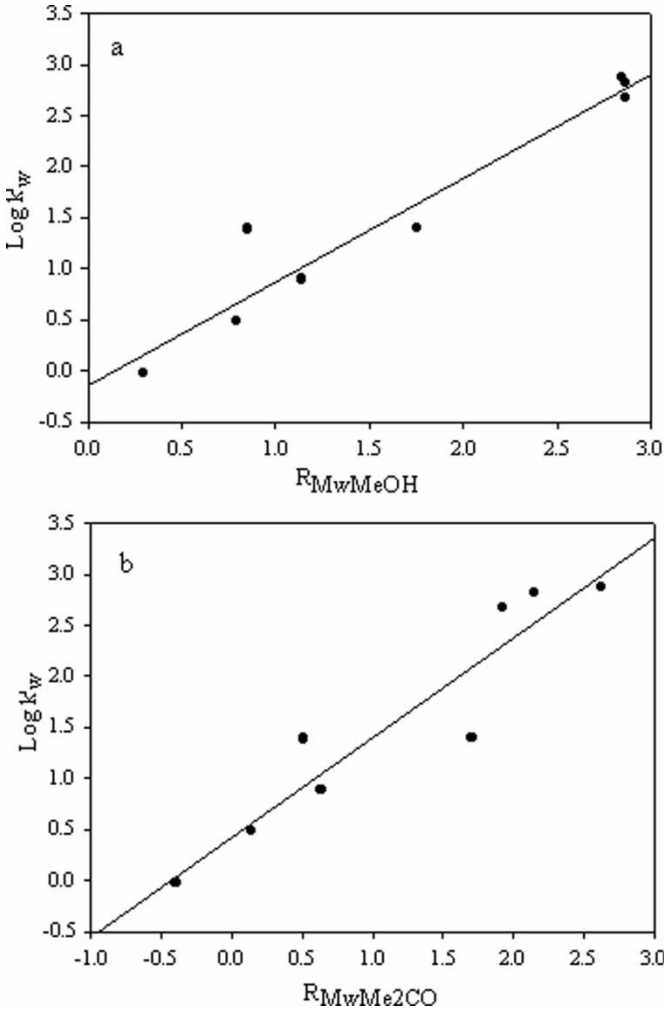

Figure 4. Relationship between log k'_w and R_{Mw} values. a) Using methanol as organic modifier in RP-TLC, as described by Eq. 10; b) using acetone as organic modifier in RP-TLC, as described by Eq. 11.

the same affinity for stationary phase (C18) than for water phase, which was also observed by RP-TLC, since the slope of eqs. 4 and 5 are almost equal to 1.

$$\text{Log } P_{oct} = 1.019\ (\pm 0.352) \log k'_w - 1.111\ (\pm 0.271) \qquad (12)$$
$$n = 8;\ r = 0.945;\ s = 0.426$$

Correlation Between Different Techniques

In order to analyze the applicability of the different techniques employed in the present work, the log P_{oct} values obtained by the shake-flask method and those from eqs. 4 and 12 (log $P_{RP\text{-}TLC}$ and log $P_{RP\text{-}HPLC}$) derived from the RP-TLC and RP-HPLC techniques, respectively, were analyzed. In this way, eqs. 13 and 14 were obtained by linear regression analysis, whose values for all studied compounds are summarized in Table 2. In addition, the corresponding deviations (Δ log P) between log P_{oct} (shake flask methods) and the log $P_{RP\text{-}TLC}$ and log $P_{RP\text{-}HPLC}$ (chromatographic techniques) are shown (Table 2).

$$\text{Log } P_{oct} = 0.969\ (\pm 0.173)\ \log P_{RP-TLC} + 0.015\ (\pm 0.210) \qquad (13)$$
$$n = 8;\ r = 0.984;\ s = 0.225$$
$$\text{Log } P_{oct} = 0.892\ (\pm 0.308)\ \log P_{RP-HPLC} + 0.052\ (\pm 0.379) \qquad (14)$$
$$n = 8;\ r = 0.945;\ s = 0.402$$

CONCLUSIONS

Inspection of eqs. 13 and 14, lead us to conclude that the chromatographic methods (RP-TLC and RP-HPLC) have demonstrated the applicability of these techniques to determine the lipophilicity of these novel 5′-carbamates of zidovudina.[3–8]

It is important to point out that *n*-octanol-water partition coefficients of ionizable compounds as 3 and 4 depends strictly on pKa values of each compound and the pH of the buffer phase. Hence, a reliable determination of the log P needs an accurate measurement of both log P'_{oct} and pKa. On the other hand, chromatography bases lipophilicity determinations are independent of pKa/pH.[9]

As it can be seen in Table 2, compounds 5, 6, and 8 are the most lipophilic compounds, whereas 4 and 7 are more hydrophilic than its precursor (AZT). In this way, 5, 6, and 8 are excellent candidates to pass biological membranes by passive diffusion to reach the target.

No information has been reported in this paper about lipophilicity values obtained through the CLOGP program,[13] since the partition coefficient for this family of compounds could not be correctly estimated. These features were observed not only for other pyrimidinic nucleosides derivatives,[4,6] but also for different families of compounds.[14,15]

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