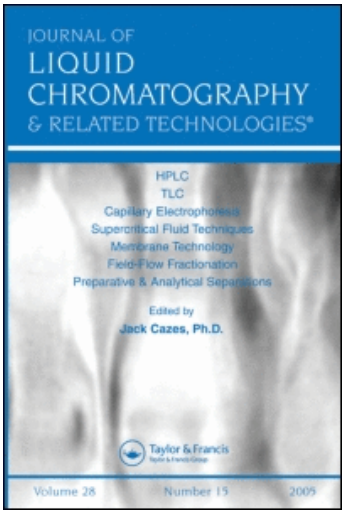

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LIPOPHILIC CHARACTER OF NOVEL AMINO ACID DERIVATIVES OF ZIDOVUDINE WITH ANTI HIV ACTIVITY

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ABSTRACT

The lipophilic properties of a novel series of zidovudine amino acid derivatives were measured using chromatographic techniques, reversed-phase thin layer chromatography (RP-TLC) and reversed-phase high performance liquid chromatography (RP-HPLC), as well as the classic shake flask (log $P_{o/w}$) and theoretical CLOGP methods. These novel derivatives, obtained by association of zidovudine (AZT) with the essential amino acids leucine (AZT-Leu), isoleucine (AZT-iLeu), phenylalanine (AZT-Phe), valine (AZT-Val), proline (AZT-Prol) and tryptophane (AZT-Tryp), exhibited an increased log $P_{o/w}$ as compared with the parent compound as follows: AZT-iLeu > AZT-Leu > AZT-Tryp > AZT-Val > AZT-Phe > AZT-Prol > AZT > Thym. All assays were performed using a buffer, pH 2, as mobile phase, at which the mentioned compounds were completely as their non-ionized forms. In addition, good linear

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relationships were observed between log P values determined by the shake flask method (log $P_{o/w}$), and those obtained by chromatographic techniques (log $P_{RP\text{-}TLC}$ and log $P_{RP\text{-}HPLC}$) and from theoretical calculations using the CLOGP program (log P_{CLOGP}). These results demonstrate the applicability of the chromatographic methods to describe the lipophilic properties of this family of compounds.

Key Words: Zidovudine derivatives; Lipophilic parameters; Anti HIV drugs

INTRODUCTION

The pharmacokinetics of drugs, as well as their capabilities of access to the receptor sites, are strongly dependent upon their interaction with biological membranes. Molecular hydrophobicity is assumed to be one of the driving forces of passive diffusion through biological membranes determined by the *n*-octanol/water partition coefficient, which is widely employed for structure-activity studies (i.e., the logarithm of partition coefficient, log *P*). Alternatively, other lipophilic parameters used in drug design include theoretical calculations (CLOGP), chromatographic capacity factors determined by reversed-phase high performance liquid chromatography (RP-HPLC) and chromatographic hydrophobic constants determined by reversed-phase thin layer chromatography (RP-TLC).[1]

Although the partition coefficient obtained by the shake flash method,[2] considered as a reference system and frequently used as its logarithm form log $P_{o/w}$, is the most common index of lipophilicity correlating molecular structure and biological response;[3,4] however, its applicability in new drug design is limited by several drawbacks.[5] Therefore, some alternative methods to obtain related lipophilic parameters have been established. These methods encompass reversed-phase chromatography, including thin layer chromatography (RP-TLC),[6–11] and high performance liquid chromatography (RP-HPLC).[12–15] The parameters obtained from these last methods are the Chromatographic Hydrophobic Constant (R_M) and the Capacity Factor (log k′), respectively.

Regarding efficient anti HIV-1 agents, we have recently developed novel zidovudine derivatives exhibiting interesting anti HIV and bactericidal activity, as well as a diminished cytotoxicity compared to AZT.[16–18] In this way, the purpose of this paper was to establish a relationship between log $P_{o/w}$ values of some novel zidovudine derivatives obtained by molecular association with essential amino acids and measured by the conventional shake flask method, with the chromatographic parameters obtained by RP-HPLC and RP-TLC. Also, a comparison with the log $P_{o/w}$, calculated using a CLOGP computer program, is included.[19]

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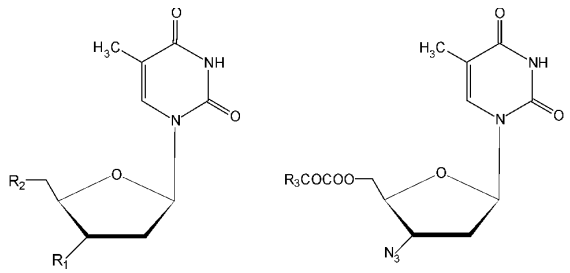
EXPERIMENTAL

Chemicals

Thymidine (Thym, 1) and Zidovudine (3′-azido-3′-deoxythymidine, AZT, 2a) were generous gifts of Filaxis (Buenos Aires, Argentina). Compounds AZT-Leu (3′-azido-3′-deoxy-5′-O-oxalyl-N-leucinethymidine, 3), AZT-iLeu (3′-azido-3′-deoxy-5′-O-oxalyl-N-isoleucinethymidine, 4), AZT-Phe (3′-azido-3′-deoxy-5′-O-oxalyl-N-phenylalaninethymidine, 5), AZT-Val (3′-azido-3′-deoxy-5′-O-oxalyl-N-valinethymidine, 6), as well as the novel ones: AZT-Tryp (3′-azido-3′-deoxy-5′-O-oxalyl-N-tryptophanthymidine, 7) and AZT-Prol (3′-azido-3′-deoxy-5′-O-oxalyl-N-prolinethymidine, 8) were prepared as previously reported methodology.[18] The structures of assayed compounds are shown in Table 1.

HPLC grade methanol was purchased from the J. T. Baker Company. Water was obtained from a Milli-Q water system. All solutions used for HPLC assays were filtered through filter membranes of 0.45 μm pore size from Millipore Co. Inc. All other chemical compounds and solvents, if not indicated, were of analytical grade. Buffer, pH 2, was prepared with citric acid and sodium

Table 1 Chemical Structure of Studied Zidovudine Derivatives



Compound	R
1, Thym	$R_1 = R_2 = OH$
2a, AZT	$R_1 = N_3$; $R_2 = OH$
2b, AZT-Ox	$R_3 = Cl$
3, AZT-Leu	$R_3 = NH{-}CH(COOH)CH{-}CH(CH_3)_2$
4, AZT-iLeu	$R_3 = NH{-}CH(COOH)CH(CH_3)CH_2C$
5, AZT-Phe	$R_3 = NH{-}CH(COOH)CH_2C_6H_5$
6, AZT-Val	$R_3 = NH{-}CH(COOH)CH(CH_3)_2$
7, AZT-Tryp	$R_3 = NH{-}CH(COOH)CH_2C_8H_6N$
8, AZT-Prol	$R_3 = NC_4H_7COOH$

monoacid phosphate (citric acid: 91%, sodium monoacid phosphate: 9%) in Milli Q water.

General Synthetic Procedure for AZT Derivatives

The general procedure for the novel compounds 7 and 8, was straightforward, as previously reported for 3–6,[18] that is to say by the association of the 5′-OH of zidovudine, 2a, with the essential amino acids (tryptophan and proline). The first step was the reaction of 2a with oxalyl chloride, obtaining 3′-azido-3′-deoxy-5′-O-oxalylthymidine chloride (AZT-Ox, 2b) in quantitative yields as previously reported.[18] Then, to a suspension of sodium amino acid salt (3 mmol) in dry THF (15 mL), a solution of 2b (1.5 mmol) in dry THF (5 mL) was added drop wise while stirring at room temperature for 2 h revealing, by TLC assay (ethyl acetate-acetone-glacial acetic acid, 10 : 6 : 0.1), the absence of 2b. The remaining amino acid salt was removed by filtration and the solution was then concentrated to dryness, under reduced pressure, at temperatures below 40°C to obtain the desired compounds (7 and 8) with yields of 85–95%. Products were precipitated from acetyl acetate (minimum volume) by addition of cool hexane. The solid residue was washed with a cool solution of HCl (0.5 M) and dried under reduced pressure. Spectroscopic data are in accordance with the proposed structures.

3′-Azido-3′-deoxy-5′-O-oxalyl-N-prolinethymidine (AZT-Pro, 7). ^{1}H NMR (DMSO-d_6) δ 1.78 [s, 3 H, CH_3], 1.76 [m, $J = 6.94$, 2 H, CH_2-5″], 1.89 [m, J = 6.94, 2 H, CH_2-6″], 2.29 [m, $J = 6.94$, 1 H, H-2′$_b$], 2.36 [m, $J = 6.58$, 1 H, H-2′$_a$], 3.60 [dd, $J = 4.68$, 2H, CH_2-7″], 3.60 [t, $J = 6.94$, 1 H, NHCH(COOH)CH_2], 4.12 [m, $J = 4.02$, 1 H, H-4′], 4.42 [dd, $J = 10.60$, 2 H, H-5′], 4.51 [m, $J = 4.02$, 1 H, H-3′], 6.18 [t, $J = 6.58$, 1 H, H-1′], 7.52 [s, 1 H, H-6], 6.87 [s, 1 H, COOH], 11.32 [s, 1 H, NH-*base*]; ^{13}C NMR (DMSO-d_6), 11.93 [CH$_3$-*base*], 30.39 [−NCHCH$_2$$CH_2CH_2$−], 30.82 [−NCH$CH_2CH_2CH_2$−], 35.67 [$CH_2$-2′], 47.61 [−NCHCH$_2CH_2$$CH_2$−], 58.66 [−N$CHCH_2CH_2CH_2$−], 60.52 [$C$H-3′], 65.29 [$CH_2$-5′], 80.39 [$C$H-4′], 83.49 [$C$H-1′], 110.14 [$C$-5], 135.69 [$C$H-6], 150.41 [$C$O-2], 158.66 [AZT-OC(O)$C$(O)-], 160.54 [AZT-O$C$(O)C(O)-], 163.62 [$C$O-4], 173.18 [$C$OOH]. $\lambda_{max.}$(water)/nm 206.4 and 265.0; $\nu_{max.}$ (KBr/cm^{-1}) 3338.0 (NH-base); 3068.2 (OH acid); 2110.9 (N_3); 1673.0 (CO).

3′-Azido-3′-deoxy-5′-O-oxalyl-N-tryptophanethymidine (AZT-Tryp, 8). ^{1}H NMR (DMSO-d_6) δ 1.78 [s, 3 H, CH_3], 2.28 [m, $J = 6.94$, 1 H, H-2′$_b$], 2.37 [m, $J = 6.58$, 1 H, H-2′$_a$], 3.25 [t, $J = 4.38$, 2 H, CH_2-5″], 4.11 [m, $J = 4.02$, 1 H, H-4′], 4.36 [dd, $J = 8.77$, 2 H, H-5′], 4.43 [t, $J = 4.38$, 1 H, NHCH(COOH)CH_2−], 4.51 [m, $J = 4.38$, 1 H, H-3′], 6.18 [t, $J = 6.58$, 1 H, H-1′], 7.52 [s, 1 H, H-6], 6.87 [s, 1 H, COOH], 11.32 [s, 1 H, NH-*base*]; ^{13}C NMR (DMSO-d_6), 11.93 [CH$_3$-*base*], 30.39 [−NCHCH$_2$$CH_2CH_2$−], 30.82 [−NCH$CH_2CH_2$-CH$_2$−],

35.67 [*C*H_2-2′], 47.61 [−NCHCH$_2$CH$_2$*C*H$_2$−], 58.66 [−N*C*HCH$_2$CH$_2$CH$_2$−], 60.52 [*C*H-3′], 65.29 [*C*H_2-5′], 80.39 [*C*H-4′], 83.49 [*C*H-1′], 110.14 [*C*-5], 135.69 [*C*H-6], 150.41 [*C*O-2], 158.66 [AZT-OC(O)*C*(O)−], 160.54 [AZT-O*C*(O)C(O)−], 163.62 [*C*O-4], 173.18 [*C*OOH]. $\lambda_{max.}$(water)/nm 217.8 and 268.2; $\nu_{max.}$(KBr/cm^{-1}) 3339.2 (NH-base); 3058.0 (OH acid); 2116.0 (N_3); 1698.4 (CO); 1545.7 (NH-amino acid).

R_M Assay

Based on the high stability over a wide range of mobile phases observed in a previous work,[20] RP-18 HPTLC F_{254S}, 5 × 10 cm precoated TLC plates, purchased from Merck (Darmstadt, Germany) were used for the determination of the chromatographic hydrophobic constant. Drugs 1, 2a, 3–8 were dissolved in an appropriate volume of methanol, in which they appeared to be stable to hydrolysis, reaching a final concentration of 1 mg/mL. A 50 μL aliquot of these solutions was spotted onto the plates according to the following distances: 10 mm from the bottom edge of the plate and at least 10 mm from the side of the plate, with 5 mm in between spots.

In order to determine the thermodynamically true solvent front position,[20,21] a solution of potassium iodide was used as marker, and was prepared by dissolving 50 mg of drug in 10 mL of a water–ethanol mixture (25 : 75, v/v). A 50 μL aliquot of the marker solution was spotted onto the plate in several positions to determine the thermodynamically true solvent front line. Mixtures of acetone–buffer, pH 2, were used as developing solvent with an acetone content between 40% and 80% (v/v) in 10% increments. Finally, plates were dried at 40°C in an oven and developed with UV radiation. In order to obtain the chromatographic hydrophobic constant values from the measured distances, we used the following equation,[6]

$$R_M = 8 \log[(1/Rf) - 1]$$

log k′ Assay

The HPLC measurements were assayed on a Spectra System P2000 chromatograph, using a UV detector at $\lambda = 267$ nm equipped with an Alltech, Allsphere column, ODS-1 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 2, mixtures were used as mobile phase, with methanol content between 40% and 80% (v/v) at a flow-rate of 1 mL/min. 1, 2a, 3-8 drug solutions were prepared following the same procedure as indicated for the R_M determinations and were injected into the column by means of a 20 μL

loop. Experiments were performed at room temperature. From the equation $k' = (t_R - t_0)/t_0$,[12–15] (being t_R the retention time of the solute and t_0 the hold-up time defined as the retention time of a non-retained compound (MeOH)), the capacity factors (k') were calculated.

Shake Flask Octanol-Water Partition Coefficients

The n-octanol-buffer, pH 2, partition coefficients of 3–8 were measured by means of the shake flask method,[4] applying the equations previously reported:[20]

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

where A_w^i, A_o^i and A_w^f, A_o^f represent the absorbance at 267.0 nm for each compound in the aqueous phase (w) and the organic phase (o), before (i) and after (f) distribution, respectively.

In all cases, the absorbance values were assayed with a Shimadzu UV-260 (UV/visible recording spectrophotometer).

Statistics

All statistical procedures were run with 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.[22,23] Equations (1–8) describe the linear relationships between R_M values and acetone modifier concentrations for compounds 1, 2a, 3–8, whose graphic representations are shown in Figure 1.

$$R_M(\text{Thym, 1}) = -0.016\ (\pm 0.001)\ \%Me_2CO - 0.303\ (\pm 0.065)$$
$$n = 5;\ r = 0.999;\ s = 0.010;\ F = 2342.95 \qquad (1)$$

$$R_M(\text{AZT, 2a}) = -0.021\ (\pm 0.003)\ \%Me_2CO + 0.724\ (\pm 0.246)$$
$$n = 5;\ r = 0.994;\ s = 0.040;\ F = 276.10 \qquad (2)$$

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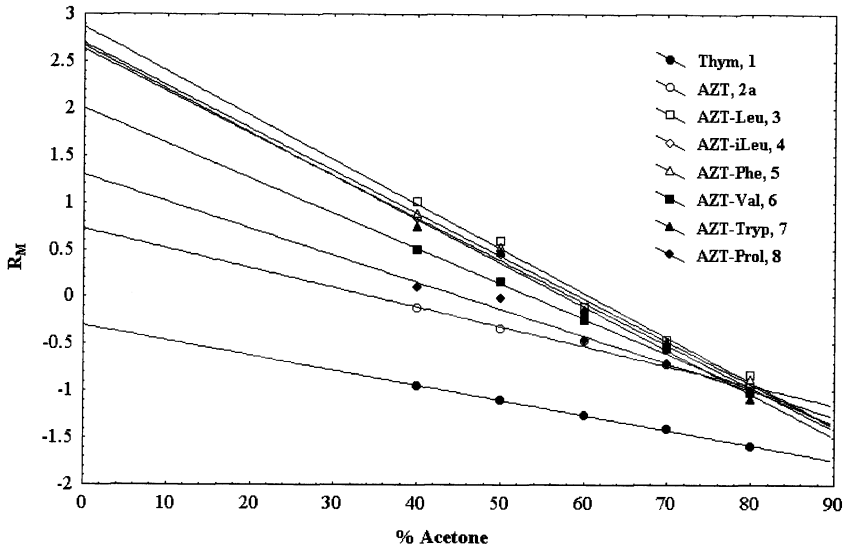

Figure 1. Relationship between R_M values and acetone concentrations in the mobile phase for the selected derivatives.

$$R_M(\text{AZT-Leu, 3}) = -0.047\,(\pm 0.009)\,\%Me_2CO + 2.869\,(\pm 0.655)$$
$$n = 5;\ r = 0.992;\ s = 0.106;\ F = 199.79 \quad (3)$$

$$R_M(\text{AZT-iLeu, 4}) = -0.045\ (\pm 0.006)\ \%Me_2CO + 2.632\ (\pm 0.388)$$
$$n = 5;\ r = 0.997;\ s = 0.063;\ F = 515.16 \quad (4)$$

$$R_M(\text{AZT-Phe, 5}) = -0.045\ (\pm 0.009)\ \%Me_2CO + 2.697\ (\pm 0.572)$$
$$n = 5;\ r = 0.994;\ s = 0.092;\ F = 240.70 \quad (5)$$

$$R_M(\text{AZT-Val, 6}) = -0.037\ (\pm 0.003)\ \%Me_2CO + 2.005\ (\pm 0.254)$$
$$n = 5;\ r = 0.998;\ s = 0.041;\ F = 830.27 \quad (6)$$

$$R_M(\text{AZT-Tryp, 7}) = -0.046\ (\pm 0.009)\ \%Me_2CO + 2.669\ (\pm 0.648)$$
$$n = 5;\ r = 0.992;\ s = 0.104;\ F = 195.99 \quad (7)$$

$$R_M(\text{AZT-Prol}, 8) = -0.029\ (\pm 0.006)\ \%Me_2CO + 1.298\ (\pm 0.477)$$
$$n = 5;\ r = 0989;\ s = 0.077;\ F = 138.62 \quad (8)$$

From the regression analyses, it can be concluded that the studied compounds exhibited a good linear correlation between the acetone content of the mobile phase and the corresponding R_M value. At 80% of organic modifier, the more polar compounds, especially Thym, 1, migrated with the solvent front, showing a very slight retention in the stationary phase; so the acetone content could not be increased. On the other hand, 40% of organic modifier was established as the minimal concentration of acetone to avoid errors in the measurement of the Rf, since the more lipophilic compounds 3, 4, 5, and 7 remained at the starting line.

From Figure 1 and negative slope values of Eqs. (1–8), it was possible to deduce that the more lipophilic compounds are the most affected by the variation of mobile phase.

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 acetone) for 1, 2a, 3–8 that correspond to the intercept of the regression lines were determined.[10] These results made it possible to compare the assayed compounds on the basis of their intrinsic lipophilicity. The procedure was feasible because a thermodynamic marker (KI) was used to determine the solvent front in the R_M determination.[20,21]

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

The linear dependence observed in the correlation between the R_{Mw} and the log $P_{o/w}$ obtained by the shake flask method can be considered as a Collander type equation (Eq. (9), Figure 2).

$$\log P_{o/w} = 0.745\ (\pm 0.211)\ R_{Mw} - 0.812\ (\pm 0.399)$$
$$n = 6;\ r = 0.980;\ s = 0.205;\ F = 96.24 \quad (9)$$

R_{Mw} data for AZT-Phe, 5, and AZT-Tryp, 7 (points shown in Figure 2 as □ shaped points), were omitted from the regression analysis because of their large deviation. It is noteworthy that both excluded compounds from the regression analysis showed a lower log $P_{o/w}$ value than the expected one. It is known that the log $P_{o/w}$ of a drug is influenced by certain parameters of the molecule,[22] among them the area of the molecule and some others derived from the electronic distribution, such as dipolar moment and hydrogen bonding capacity.[22] In this way, the correlation of R_{Mw} and log $P_{o/w}$ values of compounds 1, 2a, 3–8 with

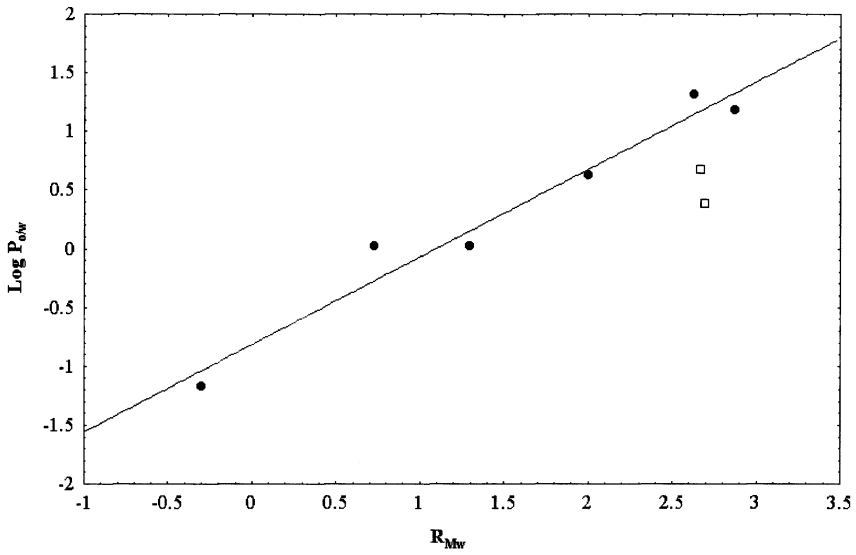

Figure 2. Relationship between R_{Mw} and log $P_{o/w}$ values as described by Eq. (9).

their molecular area (M.A.), determined for the conformation of minimal energy by means of the software WinMopac V.2.0 and Chem3D Pro V5.0, leads to the following equations:

$$\log P_{o/w} = 0.008\ (\pm 0.005)\ \text{M.A.} - 2.217\ (\pm 2.168)$$
$$n = 8;\ r = 0.775;\ s = 0.536;\ F = 9.02 \quad (10)$$

$$R_{Mw} = 0.013\ (\pm 0.005)\ \text{M.A.} - 2.684\ (\pm 1.779)$$
$$n = 8;\ r = 0.933;\ s = 0.439;\ F = 40.49 \quad (11)$$

The high correlation observed in Eq. (11) suggests that the main factor affecting the RP-TLC retention is the drug molecular area. However, the lower one for Eq. (10) leads us to consider that the molecular area is not the only parameter involved in log $P_{o/w}$ parameters. Consequently, the electronic distribution of these molecules could be more important in the shake flask partition phenomena than in the RP-TLC method. It is also remarkable that both excluded compounds have aromatic side chains in their esterifying amino acids, this fact being consistent with the difference in the electronic distribution of the amino acid moiety suggested. This type of deviation between the R_{Mw} and the log P value has previously been reported by Biagi et al., for harmaline and harmalol molecule drugs.[24]

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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 Eqs. (12–19) and as plotted in Figure 3.

$$\log k'(\text{Thym, 1}) = -0.007\ (\pm 0.001)\ \%\text{MeOH} - 0.150\ (\pm 0.075)$$
$$n = 5;\ r = 0.996;\ s = 0.012;\ F = 356.93 \quad (12)$$

$$\log k'(\text{AZT, 2a}) = -0.020\ (\pm 0.002)\ \%\text{MeOH} + 0.959\ (\pm 0.140)$$
$$n = 4;\ r = 0.999;\ s = 0.013;\ F = 1152.2 \quad (13)$$

$$\log k'(\text{AZT-Leu, 3}) = -0.038\ (\pm 0.008)\ \%\text{MeOH} + 2.540\ (\pm 0.495)$$
$$n = 5;\ r = 0.987;\ s = 0.080;\ F = 229.39 \quad (14)$$

$$\log k'(\text{AZT-iLeu, 4}) = -0.043\ (\pm 0.003)\ \%\text{MeOH} + 2.783\ (\pm 0.155)$$
$$n = 4;\ r = 0.999;\ s = 0.014;\ F = 4472.41 \quad (15)$$

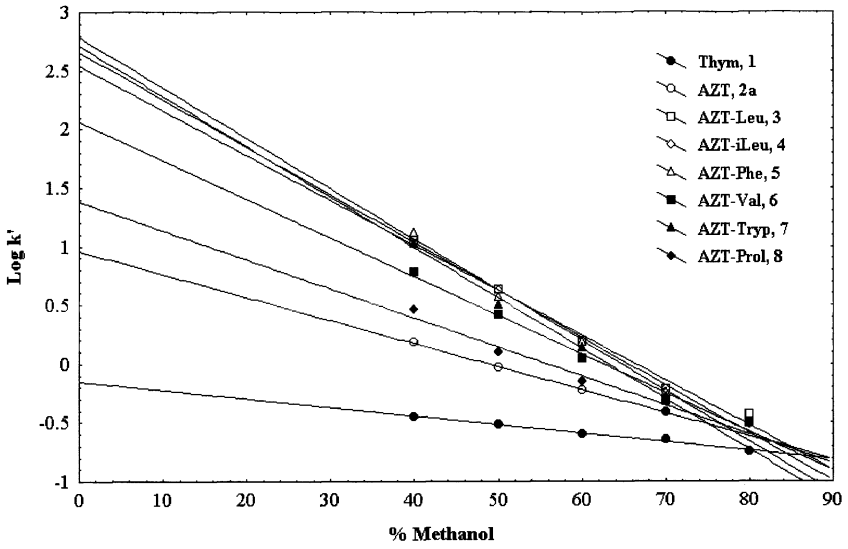

Figure 3. Relationship between log k′ values and methanol concentrations in the mobile phase for the selected derivatives.

$$\log k'(\text{AZT-Phe, 5}) = -0.041\ (\pm 0.009)\ \%\text{MeOH} + 2.658\ (\pm 0.529)$$
$$n = 5;\ r = 0.993;\ s = 0.085;\ F = 225.65 \quad (16)$$

$$\log k'(\text{AZT-Val, 6}) = -0.033\ (\pm 0.007)\ \%\text{MeOH} + 2.062\ (\pm 0.420)$$
$$n = 5;\ r = 0.994;\ s = 0.069;\ F = 235.96 \quad (17)$$

$$\log k'(\text{AZT-Tryp, 7}) = -0.043\ (\pm 0.010)\ \%\text{MeOH} + 2.709\ (\pm 0.542)$$
$$n = 4;\ r = 0.997;\ s = 0.050;\ F = 365.48 \quad (18)$$

$$\log k'(\text{AZT-Prol, 8}) = -0.025\ (\pm 0.008)\ \%\text{MeOH} + 1.381\ (\pm 0.516)$$
$$n = 5;\ r = 0.983;\ s = 0.083;\ F = 88.10 \quad (19)$$

Relationship Between $\log k'_w$ and $\log P_{o/w}$

As previously discussed for R_{Mw} data, the relationship between the $\log k'$ value obtained by extrapolation to a 0% methanol content in the mobile phase ($\log k'_w$) with the $\log P_{o/w}$ resulted in a good linear correlation as shown in Eq. (20) (Figure 4). $\log k'_w$ for AZT-Phe, 5, and AZT-Tryp, 7 (points shown in Figure 4 as □ shaped points), were again omitted from the regression analysis, because of their large deviation.

$$\log P_{o/w} = 0.816\ (\pm 0.193)\ \log k'_w - 0.955\ (\pm 0.362)$$
$$n = 6;\ r = 0.986;\ s = 0.172;\ F = 138.28 \quad (20)$$

From the results of Eqs. (9) and (20), it can be seen that the slope values are close to the unit. This finding suggests that, for this family of compounds, the affinity for the stationary apolar phase is almost the same as that for the mobile apolar phase.

It is noteworthy that AZT-Phe, 5 and AZT-Tryp, 7 exhibited the same behavior in both RP-TLC and RP-HPLC chromatographic systems, showing a higher lipophilicity than the one observed by the shake flask method. This kind of behavior has previously been described for mesitylene (1,3,5-trimethylbenzene), where its $\log P_{o/w}$ value was lower than the predicted by the RP-HPLC method.[22]

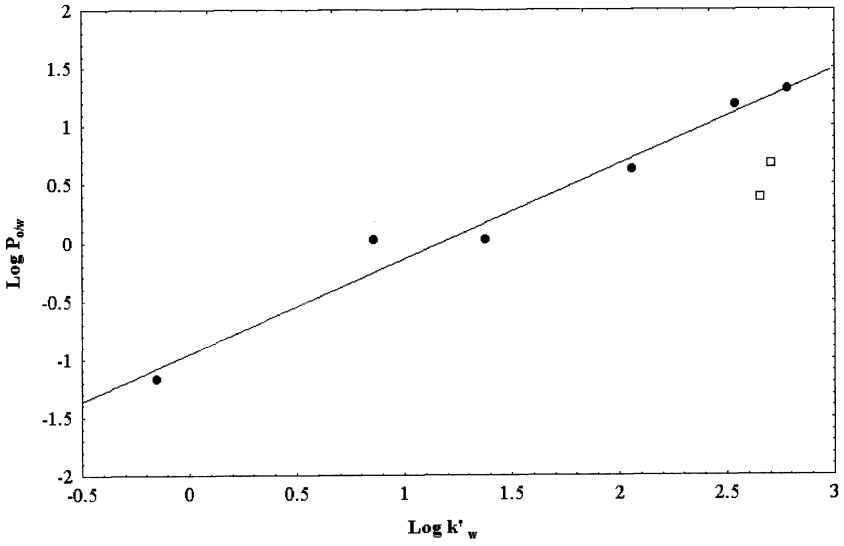

Figure 4. Relationship between log $P_{o/w}$ and log k'_w values as described by Eq. (20).

In the same way as R_M analysis, Eq. (21) correlates log k'_w values with the molecular area:

$$\log k'_w = 0.012\ (\pm 0.004)\ \text{M.A.} - 2.437\ (\pm 1.534)$$
$$n = 8;\ r = 0.944;\ s = 0.379;\ F = 49.17 \qquad (21)$$

Remarkably, the dependence with the molecular area was almost the same for both chromatographic systems since the slopes and the intercepts were statistically equal. From the above data, it is possible to conclude that both chromatographic methods could be mainly described by the molecular area, while the log $P_{o/w}$ also needs to include other parameters (e.g., electronic parameters) to obtain an acceptable correlation.

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

Figure 5a shows a good linear relationship between the slopes and the intercepts (R_{Mw}) of the RP-TLC Eqs. (1–8), as described by Eq. (22).

$$\text{Intercept} = -92.649\ (\pm 13.303)\ \text{slope} - 1.488\ (\pm 0.450)$$
$$n = 8;\ r = 0.990;\ s = 0.177;\ F = 291.07 \qquad (22)$$

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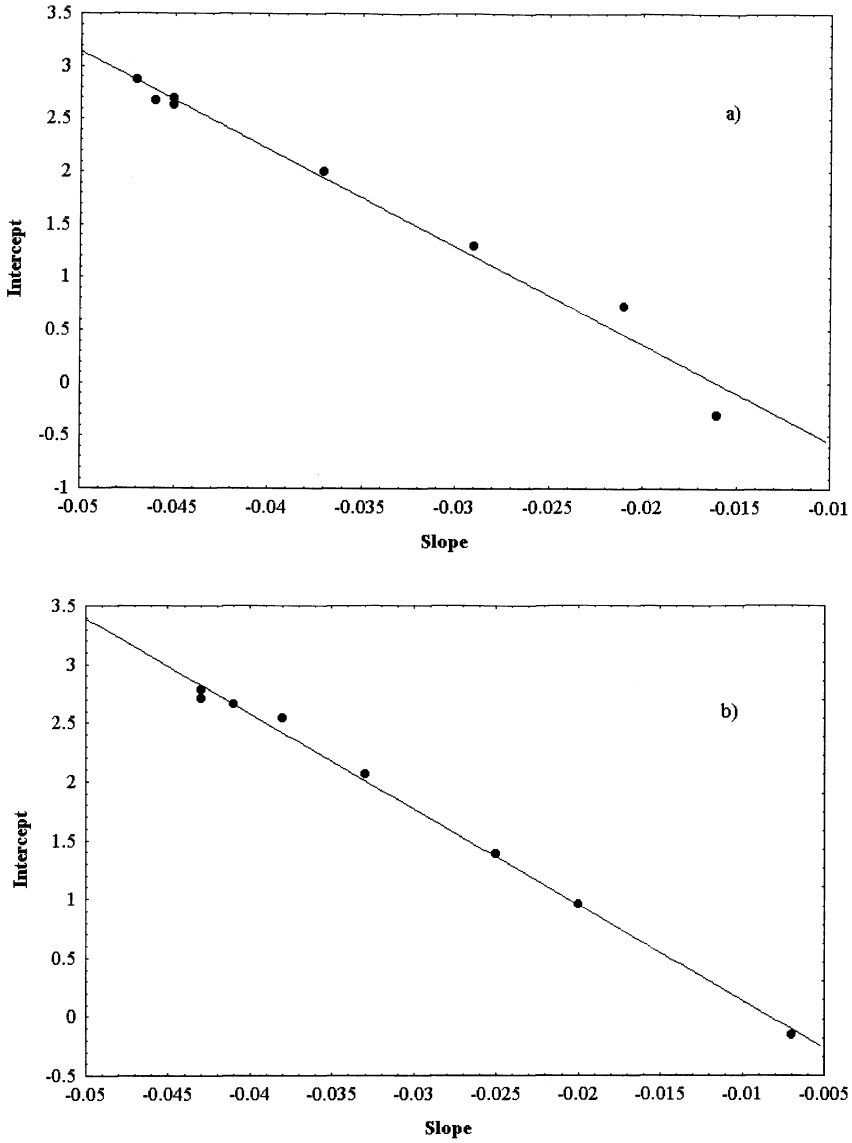

Figure 5. Relationship between slopes and intercepts. a) RP-TLC as described by Equation 22; b) RP-HPLC as described by Equation 23.

Likewise, Figure 5b shows an optimal correlation between the slopes and intercepts ($\log k'_w$) of Eqs. (12–19) (RP-HPLC), as expressed by Eq. (23).

$$\text{Intercept} = -81.310\ (\pm 5.476)\ \text{slope} - 0.673\ (\pm 0.183)$$
$$n = 8;\ r = 0.998;\ s = 0.077;\ F = 1323.02 \quad (23)$$

It has been previously reported that one of the basic features of the chromatographic assays of lipophilicity is the relationship between the slopes and intercepts of TLC equations.[10] The physicochemical parameters R_{Mw}, $\log k'_w$, and the intercepts of linear regression Eqs. (1–8) and (12–19), respectively, can be considered as a sign of the partitioning process between a polar mobile phase (buffer) and a non-polar stationary phase (octadecanol in RP-TLC and octadedecylsilane in RP-HPLC).[10]

The slopes indicate the rate at which the solubility of the compounds increases in the mobile phase as the percent of organic solvent increases. 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 higher R_{Mw} and $\log k'_w$ values. Conversely, a more hydrophilic compound with lower slope and sensibility to a decrease in mobile phase polarity will have lower R_{Mw} and $\log k'_w$ values. These features account for the linear correlations between intercepts and slopes, as observed in Eqs. (22) and (23).

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

Here, the correlation between $\log k'_w$ and R_{Mw}, Eq. (24) (Figure 6) was also established.

$$\log k'_w = 0.921\ (\pm 0.114)\ R_{Mw} + 0.174\ (\pm 0.242)$$
$$n = 8;\ r = 0.992;\ s = 0.142;\ F = 389.72 \quad (24)$$

From the above equation, it was possible to deduce that both chromatographic methods assayed generated consistent results, making it possible to use either the RP-TLC or RP-HPLC method to obtain lipophilic parameters derived from chromatographic data.

log P Calculations

In Quantitative Structure Activity Relationship (QSAR) studies, it is significant to predict the log P values of not yet synthesized molecules. In this way, and for comparative purposes, log P values for compounds 1, 2a, 3–8 were calculated by the CLOGP program.[19a]

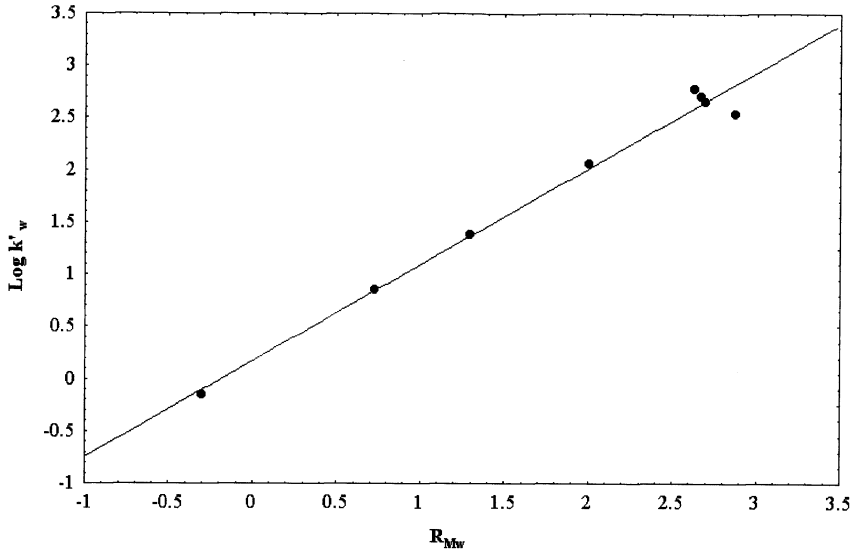

Figure 6. Relationship between $\log k'_w$ and R_{Mw} values as described by Eq. (24).

According to the results shown in Table 2, we can deduce that, although this calculation method appeared to be useful in the qualitative determination of the relative lipophilicity among this group of compounds, it was not possible to obtain an acceptable correlation between the CLOGP and the experimentally determined log P value. These observations derive from the absence of some molecular fragments and correction factors included in the software calculation (mainly the oxalyl fragment), a fact that was evidenced in the program calculation report.

Correlation Between Different Techniques

In order to analyze the applicability of the different techniques employed in the present work, the log P values obtained by the shake-flask method and those from Eqs. (9) and (20) ($\log P_{RP\text{-}TLC}$, $\log P_{RP\text{-}HPLC}$) derived from the RP-TLC and RP-HPLC techniques, respectively, as well as with CLOGP, were correlated. In this way, Eqs. (25–27) were obtained by a linear regression analysis; plots are shown in Figure 7.

$$\log P_{o/w} = 0.999\ (\pm 0.283)\ \log P_{RP\text{-}TLC} + 0.0001\ (\pm 0.251)$$
$$n = 6;\ r = 0.980;\ s = 0.205;\ F = 96.26 \qquad (25)$$

Table 2. Lipophilic Parameters and log P Values of Novel Amino Acid Zidovudine Derivatives Obtained from Different Methods

				Chromatographic log P Values				Theoretical log P Values	
Compound	R_{Mw}	log k'_w	log $P_{o/w}$	RP-TLC[a]	Δ[b]	RP-HPLC[c]	Δ[d]	CLOGP[e]	Δ[f]
Thym **(1)**	–0.303	–0.150	–1.170	–1.038	–0.132	–1.077	–0.093	–1.937	0.767
AZT (**2a**)	0.724	0.959	0.020	–0.273	0.293	–0.172	0.192	–0.197	0.217
AZT-Leu **(3)**	2.869	2.540	1.183	1.325	–0.142	1.118	0.065	1.836	–0.653
AZT-iLeu **(4)**	2.632	2.783	1.310	1.149	0.161	1.316	–0.006	1.836	–0.526
AZT-Phen **(5)**	2.697	2.658	0.382	1.197	–0.815	1.214	–0.832	1.797	–1.415
AZT-Val **(6)**	2.005	2.062	0.630	0.682	–0.052	0.728	–0.098	1.307	–0.677
AZT-Try **(7)**	2.669	2.709	0.670	1.176	–0.506	1.256	–0.586	1.787	–1.117
AZT-Prol **(8)**	1.298	1.381	0.028	0.155	–0.127	0.172	–0.144	1.360	–1.332

[a]log $P_{RP\text{-}TLC}$ values obtained from Eq. (9). [b] $\Delta = \log P_{o/w} - \log P_{RP\text{-}TLC}$. [c]log $P_{RP\text{-}HPLC}$ values obtained from Eq. (20). [d]$\Delta = \log P_{o/w} - \log P_{RP\text{-}HPLC}$. [e]log P_{CLOGP} values obtained from CLOGP program.[20a] [f]$\Delta = \log P_{o/w} - \log P_{CLOGP}$.

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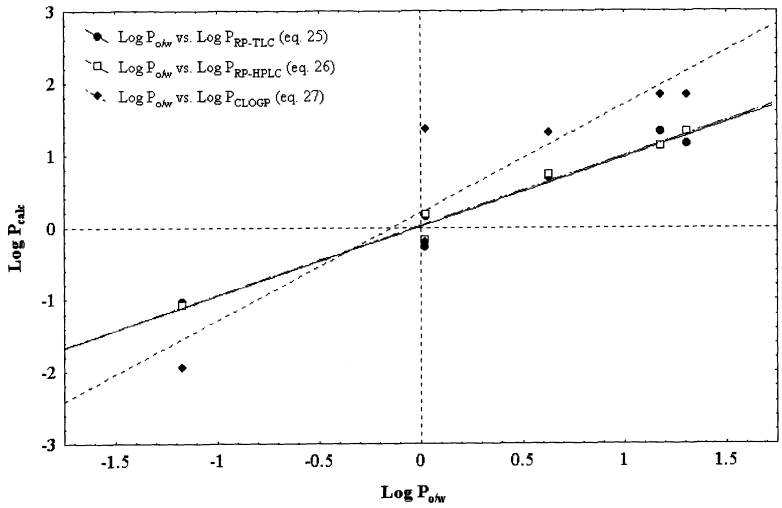

Figure 7. Relationship between log $P_{o/w}$ values obtained by shake flask methods and log P values obtained from different techniques.

$$\log P_{o/w} = 1.016\ (\pm 0.194)\ \log P_{RP-HPLC} - 0.0193\ (\pm 0.172)$$
$$n = 6;\ r = 0.991;\ s = 0.140;\ F = 212.21 \quad (26)$$

$$\log P_{o/w} = 0.565\ (\pm 0.336)\ \log P_{CLOGP} - 0.063\ (\pm 0.516)$$
$$n = 6;\ r = 0.919;\ s = 0.405;\ F = 24.74 \quad (27)$$

From the analysis of the above equations, it is clear that both chromatographic methods (RP-TLC and RP-HPLC) represent a more accurate lipophilicity measure compared to the CLOGP method, based on the fact that in Eqs. (25) and (26), the slope is $\cong$ 1 and the intercept is $\cong$ 0. Also, the log P values obtained by the chromatographic methods exhibited a higher correlation with those obtained by the shake flask method than with those obtained by the predictive method (CLOGP).

CONCLUSIONS

Based on the good correlations obtained, the chromatographic methods (RP-TLC and RP-HPLC) have proven to be reliable and accurate techniques to describe the lipophilic character of this nucleoside family of compounds. As

previously stated, the lipophilic properties measured by chromatographic methods could be explained mainly by the molecular area of the tested compounds.

From the analysis of Table 2, it can be established that the chromatographic methods yield lower deviations related to the experimental log $P_{o/w}$ than the theoretical ones, resulting the RP-HPLC method to be slightly more accurate than the RP-TLC for this set of compounds.

Although CLOGP is a useful and simple method to predict the lipophilicity rank for many compounds, it appeared to be of limited applicability for this nucleoside family, needing a correction equation in order to obtain a predicted value closer to the experimental one. For these novel studied compounds, the lipophilicity calculated by the CLOGP program was always overestimated. This fact could be due to the absence of certain fragments of the molecule in the CLOGP program database. In addition, the CLOGP program does not correctly estimate the partition coefficient for nucleoside derivatives, generally, because it does not take into account the interaction between the pyimidine ring and the sugar moiety, which were also reported by other authors.[25]

Furthermore, for compounds possessing aromatic moieties in the esterified amino acid, a deviation from the correlation of the other studied compounds was observed. Possibly, this effect is due to the electronic distribution on the aromatic amino acids, since we determined that the molecular area was not the cause for the deviation. Based on the previous statement, it could be assumed that log $P_{o/w}$ represents a better model for streams transport of drugs, such as in the circulatory system, while the chromatographic data may describe the binding onto surfaces, such as a receptor site.[22]

All these novel zidovudine derivatives showed to be significatively more lipophilic than the parent AZT, making them potential pharmacological agents in the treatment of AIDS and related diseases, according to their exhibited anti HIV-1 activity.[17]

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