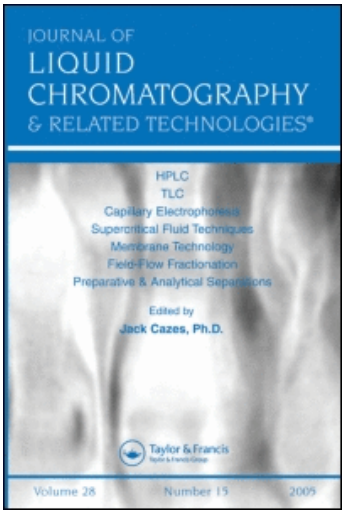

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Lipophilicity of 5'-Carbonates of Lamivudine with Antiretroviral Activity. Correlation Between Different Methods

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Lipophilicity of 5′-Carbonates of Lamivudine with Antiretroviral Activity. Correlation Between Different Methods

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Abstract: This study reports on the lipophilicity of novel 5′-carbonates of lamivudine with anti-HIV activity. Reversed-phase thin layer chromatography (RP-TLC), reversed-phase high performance liquid chromatography (RP-HPLC), conventional shake flask (log P_{oct}), and the theoretical CLOGP methods were used, in order to establish if the linear relationships between the conventional and chromatographic methods permit an extrapolation procedure. The nature of the organic modifiers used in the chromatographic techniques did not substantially affect the measurement of lipophilicity, since a good correlation between experimental log P_{oct} and extrapolated RP-TLC and RP-HPLC values (R_{Mw} and log k'_w, respectively) supported the validity of the extrapolation technique.

Keywords: Lipophilicity, 5′-Carbonates of lamivudine, RP-TLC, RP-HPLC, log P_{oct}, CLOGP

INTRODUCTION

Lipophilicity is a molecular property of solute solvent interactions, generally characterized in terms of partition coefficients. In order to quantify lipophilicity, the commonly accepted parameter is the logarithm of the partition coefficient, log P, where P is the partition coefficient of the neutral species in

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equilibrium between two immiscible solvents. Octanol is the most frequently used organic solvent, and the octanol-water partition coefficient is one of the parameters which influence the biological activity of a substance. When determining the partition coefficient by direct measurement using the shake flask equilibration method, problems arise such as poor reproducibility, time consuming experiments, and the fact that a reasonable quantity of the pure compound is needed. The alternative indirect methods are the chromatographic ones, reversed-phase thin layer chromatography (RP-TLC) and reversed-phase high performance liquid chromatography (RP-HPLC).[1–3] To replace log P_{oct} with chromatographic lipophilicity parameters, the partitioning process in reversed-phase chromatography should mimic, as closely as possible, that occurring in the standard octanol-water partitioning system. In this way, a linear relationship between the retention parameters and the concentration of the organic modifier (φ) in the aqueous mobile phase has to be established for a successful chromatographic measurement of lipophilicity.[4,5]

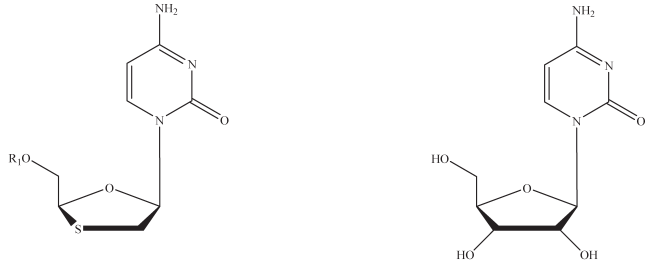
The purpose of the present study is to investigate the chromatographic behavior of a newly synthesized series of lamivudine derivatives with proven anti-HIV activity,[6] by using RP-TLC and RP-HPLC, in order to establish if the linear relationships between $R_M = f(\varphi)$, and $\log k' = f(\varphi)$, allow the extrapolation procedure.[1,2] Also, a comparison with the experimental shake flask log P_{oct} and those values calculated using the CLOGP computer program[7] is included.

EXPERIMENTAL

Chemicals

Cytidine (1-β-D-ribofuranosylcytosine, Cyt, 1) was obtained from Sigma Co. Lamivudine (2′,3′-dideoxytiacytidine, 3TC, 2) was a generous gift from Filaxis (Argentina). The novel compounds 3TC-Metha (2′,3′-dideoxy-3′-thiacytidin-5′-yl *O*-methyl carbonate, 3), 3TC-Etha (2′,3′-dideoxy-3′-thiacytidin-5′-yl *O*-ethyl carbonate, 4), 3TC-nPro (2′,3′-dideoxy-3′-thiacytidin-5′-yl *O*-propyl carbonate 5), 3TC-2Pro (2′,3′-dideoxy-3′-thiacytidin-5′-yl *O*-2propyl carbonate, 6), 3TC-Buta (2′,3′-dideoxy-3′-thiacytidin-5′-yl *O*-butyl carbonate, 7), 3TC-Penta (2′,3′-dideoxy-3′-thiacytidin-5′-yl *O*-pentyl carbonate, 8), 3TC-Hexa (2′,3′-dideoxy-3′-thiacytidin-5′-yl *O*-hexyl carbonate, 9) and 3TC-Octa (2′,3′-dideoxy-3′-thiacytidin-5′-yl *O*-octyl carbonate, 10) were prepared under our previously developed methodology.[8] The structures of assayed compounds are shown in Figure 1.

Analytical grade *n*-octanol was purchased from Riedel-de Haën; acetone (Me_2CO) and methanol (MeOH) from Cicarelli, and HPLC grade methanol from Sintorgan. The water for HPLC was purified using a Milli-Q water purification system (Millipore)® and mobile phases, as well as all solutions used



1, Cytidine

Compound	R
2, 3TC	$R_1 = H$
3, 3TC-Metha	$R_1 = C(O)OCH_3$
4, 3TC-Etha	$R_1 = C(O)OCH_2CH_3$
5, 3TC-nPro	$R_1 = C(O)O(CH_2)_2CH_3$
6, 3TC-2Pro	$R_1 = C(O)OCH(CH_3)_2$
7, 3TC-Buta	$R_1 = C(O)O(CH_2)_3CH_3$
8, 3TC-Penta	$R_1 = C(O)O(CH_2)_4CH_3$
9, 3TC-Hexa	$R_1 = C(O)O(CH_2)_5CH_3$
10, 3TC-Octa	$R_1 = C(O)O(CH_2)_7CH_3$

Figure 1. Chemical structure of lamivudine derivatives studied.

for HPLC, were filtered through a Millipore® Type FH filter (0.45 μm pore size) and then vacuum degassed. Buffer pH 7.40 (12.5 mM) was prepared with potassium phosphate monobasic and sodium phosphate dibasic dihydrate (potassium phosphate monobasic 15.78%, sodium phosphate dibasic dihydrate 84.22%) in Milli-Q water. All other chemicals were of analytical reagent grade and used as delivered.

Reversed-Phase Thin Layer Chromatography

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 the considerable advantage of high stability, thus allowing their use over a large range of organic modifier contents. All compounds (1–10) were dissolved in methanol, reaching a final concentration of 1 mg/mL in the corresponding solvent. In order to determine the thermodynamically true solvent front position, potassium iodide (KI) was used.[8–11] A 50 μL aliquot of the test solution of each compound was applied to the plates in random positions. Methanol-buffer pH 7.40 and acetone-buffer pH 7.40 mixtures were used as

mobile phases, with modifier contents being between 40 and 80% (v/v) in 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 the equation $R_M = \log\ [(1/R_f) - 1]$,[9–11,13,14] where R_f is the ratio of the migration distance of the analyte to that of the marker (KI) front distance from the start point.

Reversed-Phase HPLC

The high performance liquid chromatographic (HPLC) measurements of **1–10** were performed on an Agilent Series 1100 chromatograph, using a UV detector at $\lambda = 272$ nm equipped with a Phenomenex® column, Hypersil ODS 10 μ particle diameter of 250 mm in length, and 4.6 mm internal diameter, packed with a C_{18} (octadecyl silane) chemically bonded non-polar stationary phase. Data were produced by means of a Peak Simple Chromatography Data System.® Methanol-buffer pH 7.4 mixtures were used as mobile phases, with a methanol content between 50% and 90% (v/v) in 10% increments, at a flow rate of 1 mL/min. The solutions 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 $k' = (tr - t_0)/t_0$, where tr is the retention time of the solute and t_0 is the hold up time defined as the retention time of a non-retained compound.[9–11,13,14]

Shake flask Octanol-Water Partition Coefficients

Partition coefficients of **1–10** were measured by means of the shake flask method[15] using *n*-octanol as the non-polar phase and buffer pH 7.40 as the polar phase, with each phase being previously saturated with the other one. The concentration of samples in both the *n*-octanol and the buffer phases was determined by UV spectrophotometric analyses (Shimadzu UV-260), and applying the following equations which have been previously reported,[9,10]

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

where A_w^i, A_o^i, and A_w^f, A_o^f represent the absorbance at 272.0 nm for each compound in the aqueous phase (w) and the organic phase (o), before (i) and after (f) distribution, respectively.

Statistics

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

RESULTS AND DISCUSSION

In this paper, the lipophilicity of carbonate derivatives of lamivudine (**2**–**10**) shown in Figure 1 were analyzed by reversed-phase thin layer chromatography (RP-TLC), reversed-phase high performance liquid chromatography (RP-HPLC), the conventional shake flask ($\log P_{oct}$), and the theoretical CLOGP methods. The methodology was similar to that of chromatographic work previously carried out in our laboratory, which used RP-TLC and RP-HPLC analyses for different nucleoside derivatives of AZT, and provided the corresponding lipophilic parameters.[9–11]

Reversed-Phase Thin Layer Chromatography (RP-TLC)

The chromatographic value, R_M, was determined using acetone-buffer pH 7.4 and methanol-buffer pH 7.4 as mobile phases. The R_M values decreased linearly with increasing organic modifier content of the mobile phase. Table 1 shows parameters obtained from the linear relationships between R_M values and acetone and also with methanol organic modifier concentrations, including the values of the intercept ($a \pm st$), slope ($b \pm st$), standard error (s), and correlation coefficient (r) obtained from the corresponding linear relationship, for all compounds studied (1–10).

From the regression analyses (Figures 2 and 3), it can be seen that 1–10 exhibited a good linear correlation between the R_M values and the range of the organic modifier content of the mobile phases (40–80). The RP-TLC experiments were not performed for organic modifier values of below 40% in the mobile phase in order to avoid experimental errors when measuring low R_F values. These results have shown that each compound of the carbonate lamivudine series presents a linear relationship between the R_M value and the organic solvent concentration in the mobile phase (acetone and methanol). These features confirm that the separation mechanism is a partition of the analyte between the mobile and stationary phases.

The intercepts of the equations reported in Table 1 represent the theoretical R_M values at 0% acetone or methanol. These extrapolated R_M values at 0% of organic modifier could be considered as a measure of the partitioning of the compounds between an aqueous buffer mobile phase and the non-polar stationary phase, in a standard system where

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

Compound	a ± (s.t)[a]	b ± (s.t)[a]	r	n	Org. modif. (%)
$R_M = a + b$ (%organic modifier): Methanol					
1, Cytidine	−0.262 ± (0.052)	−0.004 ± (0.008)	0.994	5	40–80
2, 3TC	0.269 ± (0.146)	−0.009 ± (0.002)	0.990	5	40–80
3, 3TC-Metha	0.771 ± (0.239)	−0.015 ± (0.004)	0.991	5	40–80
4, 3TC-Etha	1.115 ± (0.248)	−0.019 ± (0.004)	0.993	5	40–80
5, 3TC-nPro	1.927 ± (0.394)	−0.028 ± (0.006)	0.993	5	40–80
6, 3TC-2Pro	1.635 ± (0.346)	−0.025 ± (0.006)	0.993	5	40–80
7,3TC-Buta	2.377 ± (0.401)	−0.032 ± (0.006)	0.994	5	40–80
8, 3TC-Penta	2.648 ± (0.484)	−0.034 ± (0.008)	0.992	5	40–80
9, 3TC-Hexa	2.849 ± (0.471)	−0.034 ± (0.008)	0.995	5	40–80
10, 3TC-Octa	3.279 ± (0.393)	−0.035 ± (0.006)	0.995	5	40–80
$R_M = a + b$ (% organic modifier): Acetone					
1, Cytidine	−0.129 ± (0.134)	−0.012 ± (0.002)	0.995	5	40-80
2, 3TC	−0.105 ± (0.088)	−0.008 ± (0.001)	0.995	5	40–80
3, 3TC-Metha	0.507 ± (0.224)	−0.015 ± (0.004)	0.991	5	40–80
4, 3TC-Etha	1.007 ± (0.286)	−0.022 ± (0.005)	0.993	5	40–80
5, 3TC-nPro	1.254 ± (0.307)	−0.021 ± (0.005)	0.991	5	40–80
6, 3TC-2Pro	0.869 ± (0.274)	−0.017 ± (0.004)	0.990	5	40–80
7,3TC-Buta	2.591 ± (0.406)	−0.035 ± (0.006)	0.995	5	40–80
8, 3TC-Penta	2.908 ± (0.362)	−0.037 ± (0.006)	0.996	5	40–80
9, 3TC-Hexa	3.154 ± (0.484)	−0.038 ± (0.008)	0.994	5	40–80
10, 3TC-Octa	3.466 ± (0.058)	−0.038 ± (0.009)	0.991	5	40–80
$\log k' = a+b$ (% organic modifier): Methanol					
1, Cytidine	0.760 ± (0.384)	−0.022 ± (0.005)	0.991	5	40–80
2, 3TC	1.002 ± (0.201)	−0.025 ± (0.003)	0.998	5	40–80
3, 3TC-Metha	1.054 ± (0.229)	−0.020 ± (0.003)	0.996	5	40–80
4, 3TC-Etha	1.093 ± (0.196)	−0.020 ± (0.003)	0.997	5	40–80
5, 3TC-nPro	1.156 ± (0.188)	−0.019 ± (0.002)	0.997	5	40–80
6, 3TC-2Pro	1.154 ± (0.396)	−0.018 ± (0.005)	0.996	5	40–80
7,3TC-Buta	1.225 ± (0.181)	−0.017 ± (0.002)	0.997	5	40–80
8, 3TC-Penta	1.300 ± (0.141)	−0.015 ± (0.002)	0.998	5	40–80
9, 3TC-Hexa	1.304 ± (0.209)	−0.015 ± (0.003)	0.994	5	40–80
10, 3TC-Octa	1.323 ± (0.266)	−0.014 ± (0.004)	0.990	5	40–80

[*]s: standard deviation; t: student t-test for n-2 liberty grades.

all the compounds could be compared according to their lipophilic character. These results, summarized in Table 2, made it possible to compare the assayed compounds on the basis of their intrinsic lipophilicity.

Inspection of Table 2 shows that although some differences were obtained in the R_{Mw} values of 2–10, which were determined with acetone and methanol

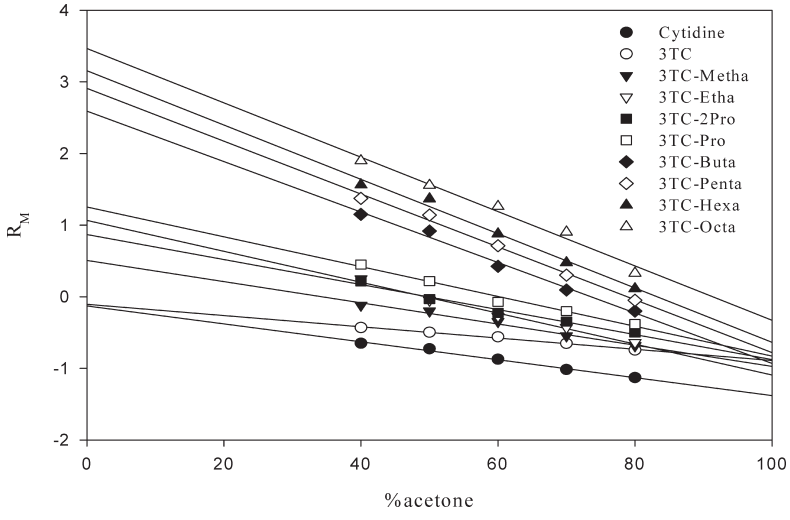


Figure 2. Relationships between R_M values and acetone in the mobile phase for the selected lamivudine derivatives.

as the organic modifier, a good correlation between R_{MwMeOH} and $R_{MwMe2CO}$ was observed (Equation (1)).

$$R_{MwMeOH} = 0.755(\pm 0.076)R_{MwMe2CO} + 0.556(\pm 0.161)$$
$$n = 9; \quad r = 0.967; \quad s = 0.278; \quad F = 99.53 \tag{1}$$

In this way, Biagi et al. studied the RP-TLC lipophilicity behavior for different series of compounds, concluding that R_{Mw} values were not dependent on the nature of the organic modifier.[16–19] However, Nasal et al. have observed that the retention values depend on the organic modifier in an aqueous eluent.[5] This can be explained taking into account the fact that the physicochemical properties of acetone are distinctly different from those of water, whereas methanol is very similar. Accordingly, methanol has to be viewed as the most commonly used modifier for the chromatographic determination of lipophilicity. For this reason, Braumann[20] recommends methanol as the best modifier, due to its physicochemical similarity to water. Hence, it provides the strongest hydrogen bond donor and acceptor properties of all the modifiers used, so its addition to an aqueous phase over a wide range of volume fractions has only a moderate effect on the ordering of water molecules. These considerations are substantiated by our present investigations, since some differences between $R_{MwMe2CO}$ and R_{MwMeOH} values have been found (Table 2), as we also observed for other zidovudine derivative series.[20]

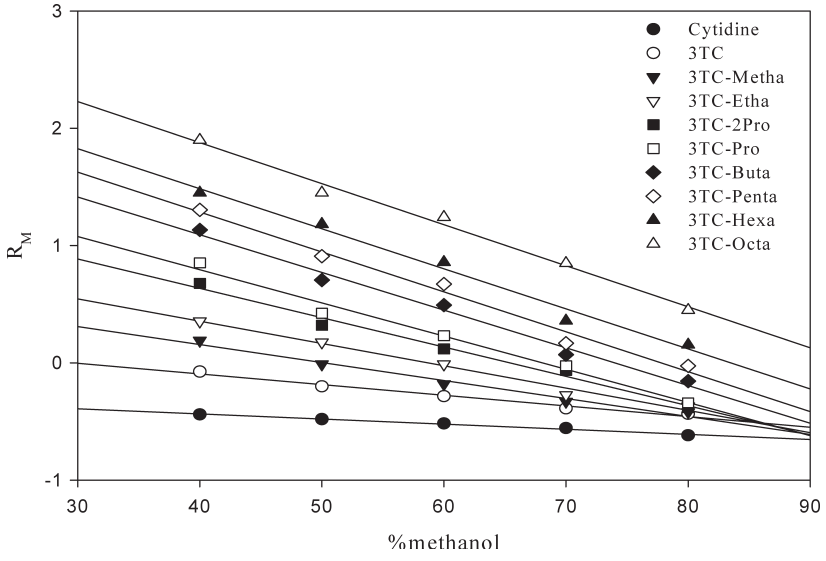


Figure 3. Relationships between R_M values and methanol in the mobile phase for the selected lamivudine derivatives.

Reversed-Phase HPLC (RP-HPLC)

Since no considerable differences were found in RP-TLC between the use of acetone or methanol as the organic modifier, and also taking into account Brauman's recommendations, only methanol was used as the organic phase in RP-HPLC analysis.

The correlation between the log k′ values and the composition of the mobile phase was established, and showed a linear relationship, as can be seen in Table 1 and in Figure 4. Table 2 summarizes the extrapolated log k′ at 0% methanol (log k'_w).

From Table 1 it is possible to note that the slope is negative in all cases, being related to the hydrophobic surface of the molecule which interacts with the non-polar stationary phase.[21,22]

Relationship Between R_{Mw} or Log k'_w with Log P_{oct} Values

Assuming the extra thermodynamic linear free energy relationship, one may expect the standard log P_{oct} data to be linearly related to the partition chromatographic parameters, R_{Mw} and log k'_w, obtained from RP-TLC and RP-HPLC, respectively.

The above considerations seem to point to the reliability of the R_M or log k′ values as a measure of the partitioning between an aqueous mobile phase and a

Table 2. Lipophilic parameters and log P values of novel lamivudine derivatives obtained from different methods

					Chromatographic log P values						Theorical log P values	
Compound	$R_{MwMe2CO}$	R_{MwMeOH}	Log k'_w	Log P_{oct}	RP-TLC[a]	Δ[b]	RP-TLC[c]	Δ[d]	RP-HPLC[e]	Δ[f]	CLOGP[g]	Δ[h]
2, 3TC	−0.105	0.269	1.002	−0.910	−1.035	0.125	−1.181	0.271	−1.229	0.319	−0.930	0.020
3, 3TC-Metha	0.507	0.771	1.054	−0.810	−0.568	−0.242	−0.693	−0.117	−0.775	−0.035	−0.859	0.049
4, 3TC-Etha	1.067	1.114	1.093	−0.310	−0.140	−0.170	−0.360	0.050	−0.436	0.126	−0.330	0.020
5, 3TC-nPro	1.254	1.927	1.156	0.200	0.003	0.198	0.429	−0.229	0.114	0.086	0.199	0.001
6, 3TC-2Pro	0.869	1.635	1.154	−0.150	−0.291	0.141	0.145	−0.295	0.097	−0.247	−0.021	−0.129
7, 3TC-Buta	2.590	2.377	1.225	0.860	1.023	−0.163	0.866	−0.006	0.714	0.146	0.728	0.132
8, 3TC-Penta	2.908	2.649	1.300	1.230	1.265	−0.035	1.129	0.100	1.361	−0.131	1.257	−0.027
9, 3TC-Hexa	3.154	2.849	1.304	1.570	1.453	0.117	1.324	0.246	1.397	0.173	1.786	−0.216
10, 3TC-Octa	3.466	3.278	1.322	1.720	1.691	0.027	1.741	−0.021	1.561	0.159	2.844	−1.124

[a] $\log P_{RP\text{-}TLCMe2CO}$ values obtained from Eq. (8).
[b] $\Delta = \log P_{oct} - \log P_{RP\text{-}TLCMe2CO}$.
[c] $\log P_{RP\text{-}TLCMeOH}$ values obtained from Eq. (9).
[d] $\Delta = \log P_{oct} - \log P_{RP\text{-}TLCMeOH}$.
[e] $\log P_{RP\text{-}HPLC}$ values obtained from Eq. (10).
[f] $\Delta = \log P_{oct} - \log P_{RP\text{-}HPLC}$.
[g] $\log P_{CLOGP}$ values obtained from CLOGP program.
[h] $\Delta = \log P_{oct} - \log P_{CLOGP}$.

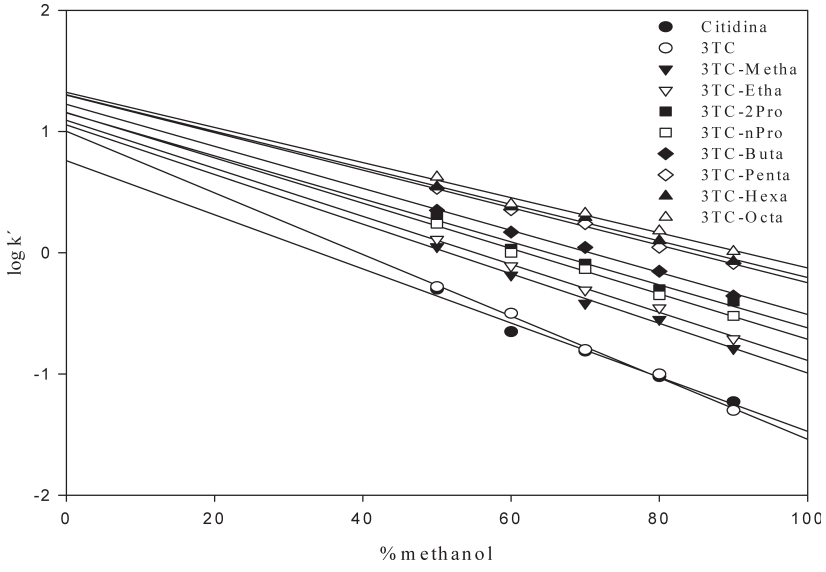


Figure 4. Relationships between log k′ values and methanol in the mobile phase for the selected lamivudine derivatives.

non-aqueous stationary phase. Thus, resulting linear dependences were observed in the correlations between $R_{MwMe2CO}$ and R_{MwMeOH} with log P_{oct} obtained by the shake flask method, which have been represented in Figures 5 and 6.

In a similar way, good correlations can be noted between the log k'_w and log P_{oct} values of the newly synthesized series of 5′-carbonates of lamivudine (Figure 7).

Cytidine (points shown in Figures 5–7 as ■ shaped points), was omitted from the regression analyses because of its large deviation, due to the different structural features possessed by this nucleoside.

Relationship Between Log k'_w with R_{Mw}

Other interesting linear correlations were observed between log k'_w and R_{Mw} (Equations (2) and (3), Figures 8 and 9).

$$\log k'_w = 0.087(\pm 0.018)R_{MwMe2CO} + 1.026(\pm 0.039)$$
$$n = 9; \quad r = 0.974; \quad s = 0.028; \quad F = 130.99 \qquad (2)$$
$$\log k'_w = 0.114(\pm 0.016)R_{MwMeOH} + 0.966(\pm 0.034)$$
$$n = 9; \quad r = 0.988; \quad s = 0.019; \quad F = 289.17 \qquad (3)$$

These results show a very good correlation between log k'_w and the R_{Mw} values obtained from two different chromatographic systems. It is interesting

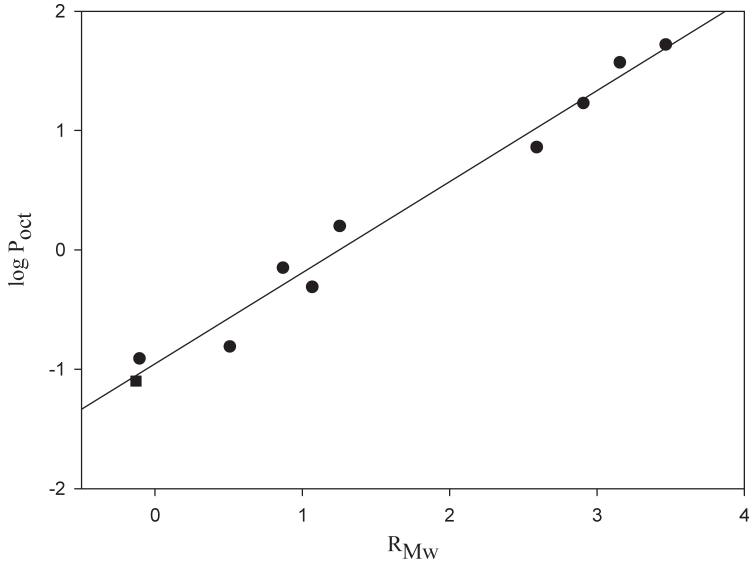


Figure 5. Relationship between log P_{oct} and $R_{MwMe2CO}$ values. ■ Cytidine.

to point out, that the correlations described by Equations (2) and (3) hold over a wide range of lipophilicity. This justifies our confidence in the use of chromatographic data as lipophilic parameters.[5,16–20] Also, cytidine was omitted in Figures 8 and 9 due to its large deviation.

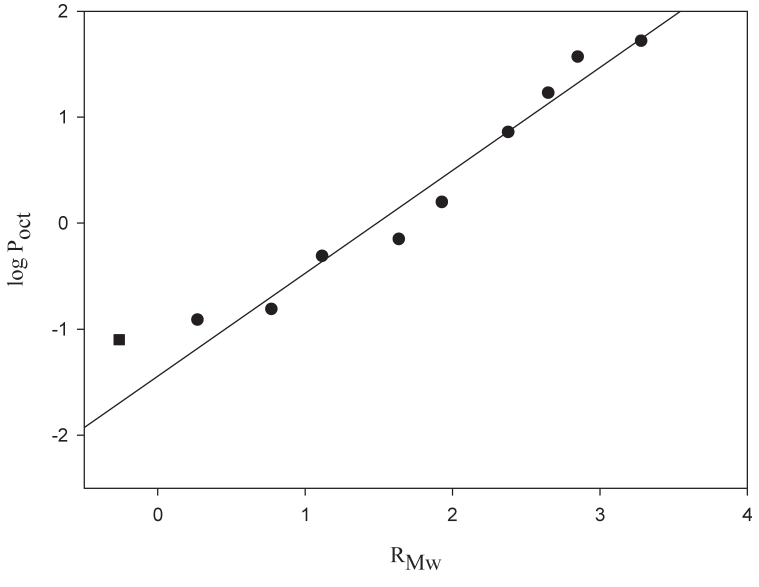


Figure 6. Relationship between log P_{oct} and R_{MwMeOH} values. ■ Cytidine.

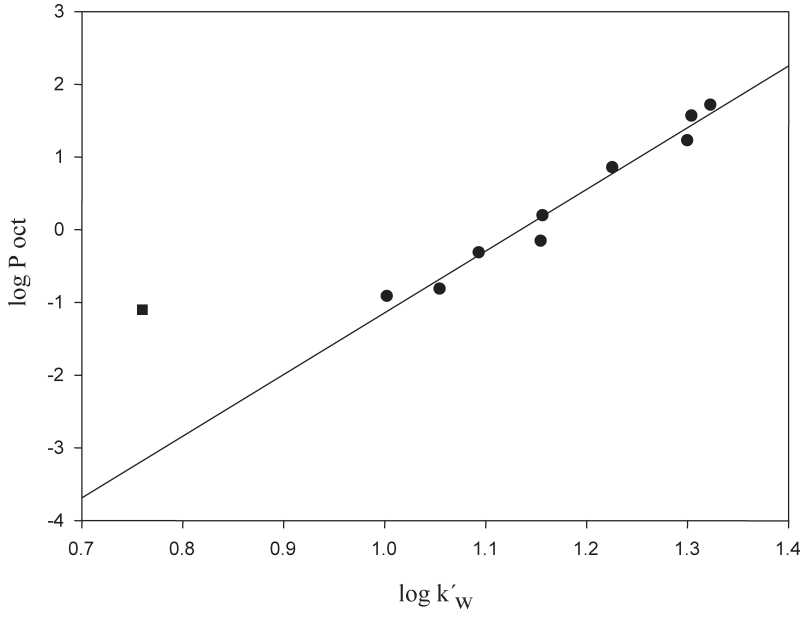


Figure 7. Relationship between log P_{oct} and log k'_w values. ■ Cytidine.

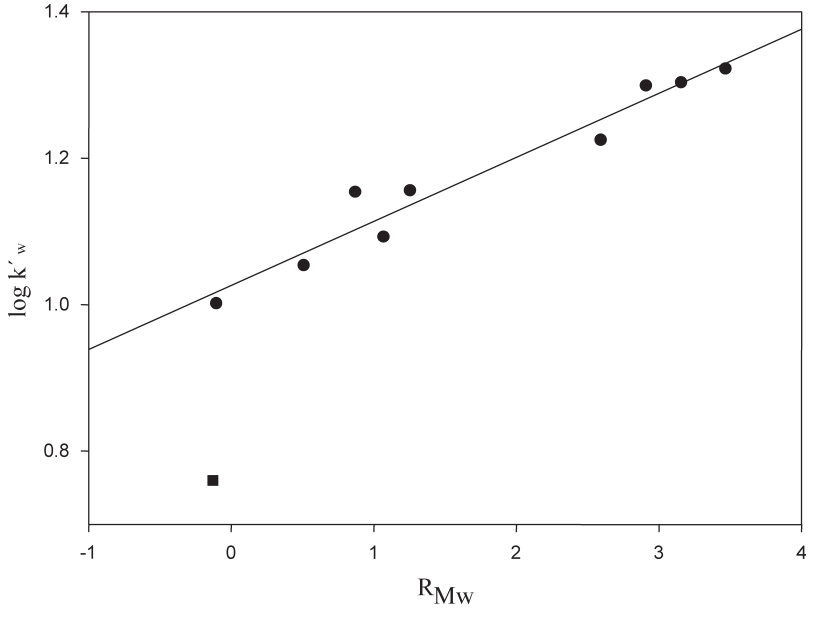


Figure 8. Relationship between log k'_w and $R_{MwMe2CO}$ values. ■ Cytidine.

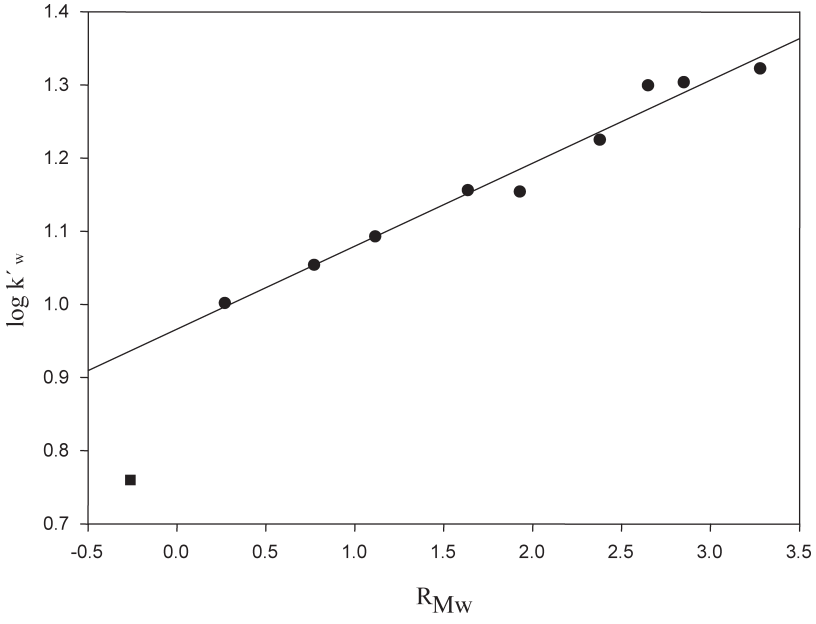


Figure 9. Relationship between log k'_w and R_{MwMeOH} values. ■ Cytidine.

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

The "congenerity" of substances can be expressed as the linearity between the extrapolated parameter log k'_w (or R_{Mw}) and the slope.[1,16–18] This is due to the complex nature of the factors that contribute to retentions in reverse phase chromatographic methods, which are drastically different from those in the transfer of solutes between an isotropic aqueous and an organic solvent. In this way, Figures 10a–c show good linear relationships between the slopes and the intercepts (R_{Mw} and log k'_w) of the RP-TLC and RP-HPLC equations.

$$\text{Intercept}_{\text{RP-TLCMe2CO}} = -111.513(\pm 13.874)\,\text{slope}_{\text{RP-TLCMe2CO}} - 1.110(\pm 0.386)\ \ n = 9;\quad r = 0.990;\quad s = 0.191;\quad F = 359.28 \tag{4}$$

$$\text{Intercept}_{\text{RP-TLCMeOH}} = -105.809(\pm 19.328)\,\text{slope}_{\text{RP-TLCMeOH}} - 0.854(\pm 0.527)\ \ n = 9;\quad r = 0.980;\quad s = 0.217;\quad F = 168.94 \tag{5}$$

$$\text{Intercept}_{\text{RP-HPLCMeOH}} = 32.628(\pm 9.796)\,\text{slope}_{\text{RP-HPLCMeOH}} + 1.776(\pm 0.182)\ \ n = 9;\quad r = 0.948;\quad s = 0.039;\quad F = 61.22 \tag{6}$$

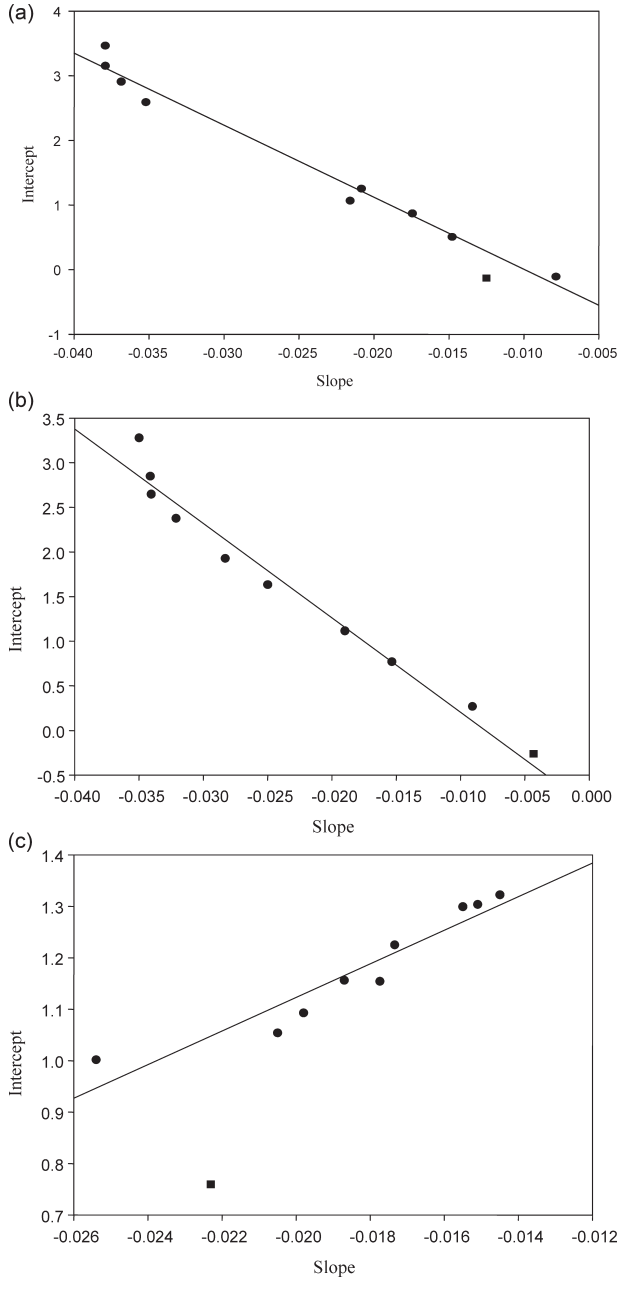


Figure 10. Relationships between slopes and intercepts. a) RP-TLC_{Me2CO} for test compounds as described by Equation (4). b) RP-TLC_{MeOH} for test compounds as described by Equation (5). c) RP-HPLC for test compounds as described by Equation (6). ■ Cytidine.

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 the chromatographic equations.[9,16] The physicochemical parameters R_{Mw}, log k'_w and the intercepts of linear regression of Equations (4–6), can be considered to be measures of the partitioning of compounds between a polar mobile phase (buffer) and a non-polar stationary phase (octadecanol in RP-TLC and octadedecilsilane in RP-HPLC).[9,16]

The slopes indicate the rate at which the solubility of the compounds increases in the mobile phase as the percentage 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 therefore having a higher slope) exhibits a higher R_{Mw} and greater log k'_w values. Conversely, a more hydrophilic compound with a lower slope and less sensitivity to a decrease in the mobile phase polarity, will have a lower R_{Mw} and smaller log k'_w values. These features account for the linear correlations between the intercepts and slopes, as observed in Equations (4–6). A strong slope vs intercept relationship can only be found when dealing with strictly congeneric compounds. The deviation from linearity of cytidine was also attributed to the different structural features possessed by this nucleoside.

Log P Calculations

In drug design, and in the quantitative structure-activity relationship (QSAR), it is useful to predict the log P of unknown drug molecules. For comparative purposes, log P values for carbonate lamivudine derivatives were calculated by the CLOGP program (Table 2).[7] Equation (7) shows that the calculated CLOGP values for lamivudine derivatives are a reliable index for these compounds. However, the CLOGP program does not correctly estimate the partition coefficient of **1** ($\log P = -2.130$)[23] and **10** ($\log P = 1.720$) due to their high hydrophilicity and lipophilicity, respectively.

$$\begin{aligned} \text{CLOGP} &= 1.045(\pm 0.107)\log P_{oct} + 0.009(\pm 0.096) \\ & n = 8; \quad r = 0.994; \quad s = 0.108; \quad F = 566.32 \end{aligned} \tag{7}$$

Correlation Between Different Techniques

Table 2 shows the log P values obtained from the RP-TLC and RP-HPLC techniques, as well as those for the shake flask and the CLOGP program methods. In addition, the corresponding deviations (Δlog P) between the conventional shake flask method and the other different techniques studied ($\log P_{RP\text{-}TLCMe2CO}$, $\log P_{RP\text{-}TLCMeOH}$, $\log P_{RP\text{-}HPLC}$ and $\log P_{CLOGP}$) are also shown. Figure 11 shows the log P relationship between the conventional shake flask method and the chromatographic and theoretical ones. The

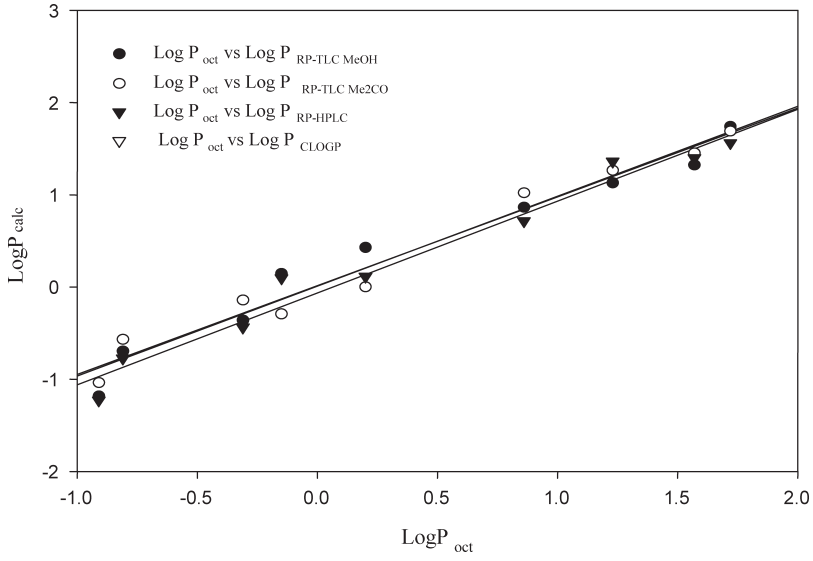


Figure 11. Relationship between log P_{oct} values obtained by the shake-flask method and log P values obtained from different techniques.

linear regressions are described by Equations (8–11), which can be considered as Collander type equations.

$$\log P_{oct} = 0.975(\pm 0.141)\log P_{RP\text{-}TLCMe2CO} + 0.009\,(\pm 0.143)$$
$$n = 9; \quad r = 0.987; \quad s = 0.169; \quad F = 268.54 \tag{8}$$

$$\log P_{oct} = 0.963(\pm 0.169)\log P_{RP\text{-}TLCMeOH} + 0.014\,(\pm 0.173)$$
$$n = 9; \quad r = 0.981; \quad s = 0.203; \quad F = 180.28 \tag{9}$$

$$\log P_{oct} = 0.996(\pm 0.155)\log P_{RP\text{-}HPLC} - 0.065(\pm 0.158)$$
$$n = 9; \quad r = 0.985; \quad s = 0.185; \quad F = 230.88 \tag{10}$$

$$\log P_{oct} = 0.999(\pm 0.103)\log P_{CLOGP} + 0.004(\pm 0.092)$$
$$n = 8; \quad r = 0.995; \quad s = 0.103; \quad F = 566.14 \tag{11}$$

CONCLUSIONS

All these novel lamivudine derivatives were shown to be considerably more lipophilic than the parent 3TC, in the following order: 3TC-Octa > 3TC-Hexa > 3TC-Penta > 3TC-Buta > 3TC-nPro > 3TC-2Pro > 3TC-Etha > 3TC-Metha > 3TC, thus making them potential pharmacological agents in the treatment of AIDS and related diseases, due to their exhibited anti-HIV-1 activity.[6] It is clear that the chain length of the substituent of the

5′-position influences the lipophilicity, since the addition of a methylene group reduces the side chain polarity, thereby increasing the lipophilicity.

The correlation between the extrapolated R_M values obtained using different organic solvents is another important factor, supporting the reliability of using the extrapolated R_M values as an expression of the partitioning between an aqueous mobile phase and the silicone oil of the stationary phase. In fact, the nature of the organic modifier does not substantially affect the extrapolated R_M values. Although, the RP-TLC and RP-HPLC experiments were carried out using from 40% and from 50% of organic modifier, respectively, it is assumed that the linearity of the resulting relationships is maintained, even at low concentrations of the organic modifier in the mobile phase.

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

The partition coefficients calculated by the CLOGP program are in good agreement with the measured n-octanol/water partition coefficients, except in the case of cytidine and 3TC-Octa, probably due to their very hydrophilic and lipophilic characteristics, respectively.

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