Journal of Chromatography B, 878 (2010) 61–67

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

HPLC-based lipophilicity of pyrrolyl-acetic acid ARIs: Relationships with biological activity

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ARTICLE INFO

Article history:
Received 9 June 2009
Accepted 12 November 2009
Available online 17 November 2009

Keywords:
Pyrrolyl-acetic acid derivatives
Lipophilicity
RP-HPLC
n-Octanol as mobile phase additive
Aldose reductase inhibitory activity

ABSTRACT

Reversed phase HPLC was used to assess the lipophilicity of a series pyrrolyl-acetic acid derivatives with aldose reductase inhibitory activity. The pH conditions were adjusted at 3.0 to investigate the behavior of the neutral species and at pH 7.4, at which the ionized form predominates, using phosphate and MOPS buffer. Retention was monitored in absence and in presence of different amounts of n-octanol in the mobile phase in order to explore the chromatographic conditions which best reproduce the octanol–water partition or distribution coefficients. The effect of n-octanol in retention was systematically studied and its role in lipophilicity assessment was evaluated. Nevertheless rather moderate regression equations were obtained, which deviated significantly from the ideal 1:1 correlation. No significant effect of buffer was observed. The appropriateness of retention factors to be used in correlation with aldose reductase inhibitory activity was further evaluated and compared to the efficiency of the corresponding octanol–water log *P* values.

1. Introduction

Lipophilicity, expressed by the logarithm of n-octanol–water partition coefficient log *P* or distribution coefficient log *D*, if ionized molecular species are present, constitutes a physicochemical property of paramount importance in the design of new drug molecules [1,2]. The difficulties associated with the direct partitioning experiments in combination with the requirement for rapid lipophilicity screening of compound libraries led to the development of a large arsenal of calculation systems [3–8]. However the complex nature of lipophilicity as the outcome of inter- and intra-molecular interactions is far from being precisely encoded in the various algorithms and the reliability of the available software depends on the chemical structure and the inherent conception of the method [9]. Thus for new chemotypes synthesized as potential drug candidates calculated log *P* values may be misleading in modeling their biological activity or in estimating their permeability potential [10,11]. Thus, the necessity for measured lipophilicity is still an issue. In this aspect chromatographic techniques and in particular reversed phase HPLC offers a friendly alternative with many practical advantages such as speed, accuracy, repeatability, broader dynamic range (especially to high lipophilicity areas), high automatization ability, insensitivity to impurities/degradation products and reduced sample handling/sample sizes [12–15]. The most widely used stationary phases for lipophilicity assessment by HPLC is C-18 silanized silica gel, either end-capped (BDS column) or polar embedded (ABZ$^+$, Discovery-RP-Amide-C16 column) in order to reduce silanophilic effects which may interfere as secondary interactions to the partition mechanism. Polar embedded columns contain an amide functional group which provides electrostatical shielding to the silanol sites, while a high degree of orientation of the alkyl chains is achieved [16].

Standardization of the chromatographic conditions, for lipophilicity determination of basic and neutral drugs has been suggested utilizing either an ABZ or a BDS column as stationary phase, methanol as organic modifier and n-decylamine and n-octanol as mobile phase additives [17,18]. Morpholine-propanesulfonic acid (MOPS) was used as buffer to control the pH. It is considered that this buffer due to its zwitterionic structure interferes neither with

Abbreviations: log k_w/PB, extrapolated retention factors derived using phosphate buffer, no addition of n-octanol; S/PB, the corresponding slope according to Eq. (2); log k_w0.05/PB, extrapolated retention factors derived using phosphate buffer, and 0.05% n-octanol in the volume of methanol; S0.05/PB, the corresponding slope according to Eq. (2); log k_w0.25/PB, extrapolated retention factors derived using phosphate buffer and 0.25% n-octanol in the volume of methanol; S0.25/PB, the corresponding slope according to Eq. (2); log k_w/MOPS, extrapolated retention factors derived using MOPS, no addition of n-octanol; S/MOPS, the corresponding slope according to Eq. (2); log k_w0.05/MOPS, extrapolated retention factors derived using MOPS and 0.05% n-octanol in the volume of methanol; S0.05/MOPS, the corresponding slope according to Eq. (2); log k_w0.25/MOPS, extrapolated retention factors derived using MOPS and 0.25% n-octanol in the volume of methanol; S0.25/MOPS, the corresponding slope according to Eq. (2).

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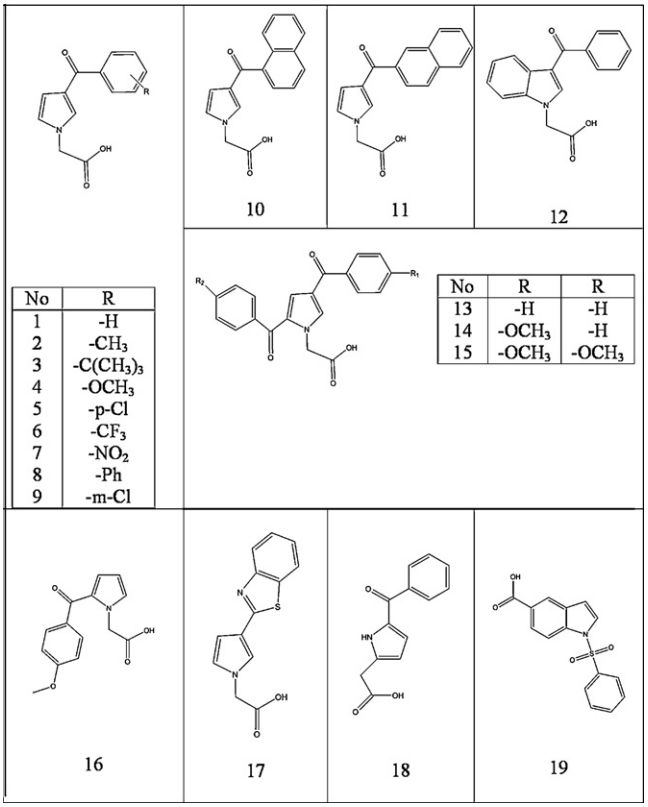
doi:10.1016/j.jchromb.2009.11.020

the stationary phase nor with the solutes, so the formation of ion pairs does not affect the partition mechanism [19]. Under these conditions 1:1 correlation between extrapolated retention factors $\log k_w$ and octanol–water $\log D$ are obtained. Less investigated are the chromatographic conditions for acidic compounds. Liu et al., using a Discovery-RP-Amide-C-16, reported 1:1 correlation between $\log P$ and $\log k_w$ for neutral and acidic drugs in their unionized state. In that study the pH was adjusted by phosphate buffer. The addition of n-octanol in the methanol fraction of the mobile phase proved to be a critical factor in this case as well [20]. The role of n-octanol was further investigated in a systematic study concerning structurally diverse neutral and acidic compounds at both unionized state and at pH 7.4 [21]. The authors (one belonging to our group) reported that the influence of n-octanol is more pronounced for hydrophilic compounds, despite the fact that in water-rich mobile phase used in this case, its total concentration is lower. Moreover they found that at low pH the use of n-octanol saturated buffer produces $\log k_w$ values which show 1:1 correlation with $\log P$. At pH 7.4 the presence of 0.25% n-octanol is necessary since anions are strongly retained on silica-based stationary phases probably due to the presence of trace metal impurities [22]. Under this condition 1:1 correlation between $\log k_w$ and $\log D_{7.4}$ for weakly acidic compounds was produced while for fully ionized acids a highly significant regression equation was also obtained, although with a large negative intercept and a slope lower than unity [21]. Nevertheless a model derived from structurally diverse compounds may not be applicable within a congeneric series of compounds sharing a new scaffold as is often the case in medicinal chemistry.

In a previous study we reported on the consistency of commonly used calculation systems to predict the $\log P$ and $\log D_{7.4}$ values of a series of pyrrolyl-acetic acid derivatives, inhibitors of aldose reductase, and the better performance of measured $\log P$ to correlate with biological activity [23]. In the present study we extended our investigation on the same series of compounds using reversed phase HPLC to assess their lipophilicity at low pH (neutral species) and at pH 7.4. In the light of the considerations concerning the standardization of the chromatographic conditions to reproduce the octanol–water system in the case of acidic compounds, retention was monitored utilizing different mobile phases in respect to buffer constitution and the addition of n-octanol. The relationships of $\log k_w$ values with $\log P$ and $\log D_{7.4}$ were established. The appropriateness of $\log k_w$ to be used in correlation with aldose reductase inhibitory activity was further evaluated.

2. Materials and methods

All compounds were synthesised and tested in vitro for aldose reductase inhibitory activity (ARI) as reported in refs. [24–27]. Their chemical structures are presented in Fig. 1. They contain at position 3 of the pyrrole nucleus a keto group serving as a bridge to a substituted phenyl of naphthalene ring. In compound 17 the keto group has been replaced by the bioisosteric thiazole moiety [24]. Compound 12 contains an indole ring instead of pyrrole. In compound 16 the keto group is at position 2 of the pyrrole nucleus. Compound 18 is a pyrrol-2-yl-acetic acid derivative possessing free hydrogen on the pyrrole nitrogen. The percentage of ARI activity (%Inh) refers to a concentration equal to 10^{-6} M of the compounds tested against aldose reductase isolated from rat lenses. %Inh was converted to logit values according to equation: logit = log(%Inh/(100 − %Inh)). This numerical transformation enables proper QSAR computations while it does not affect the reliability level of the data [28]. The logit values are presented in Table S1 in Supplementary Material.



No	R
1	-H
2	-CH_3
3	-$C(CH_3)_3$
4	-OCH_3
5	-p-Cl
6	-CF_3
7	-NO_2
8	-Ph
9	-m-Cl

No	R	R
13	-H	-H
14	-OCH_3	-H
15	-OCH_3	-OCH_3

Fig. 1. Chemical structures of the investigated compounds.

1-Octanol was extra pure purchased by Pancreac Quimica, Spain. Methanol was HPLC grade and purchased from Lab-Scan Analytical Sciences Ltd., Ireland. Sodium hydrogen phosphate, potassium dihydrogen phosphate and morpholine-propanesulfonic acid were purchased from Merck, Darmstadt, Germany. Water was deionized and further purified by means of a Milli-Q Plus water purification system (Millipore Co, USA).

2.1. Chromatographic conditions

The HPLC isocratic pumping system consisted of a GBC Model 1126 pump and a Rheodyne Model 7725i GBC injector with a 20 μl loop, which were coupled to a GBC Model LC1210 UV–Vis detector, operated at 254 nm. Data acquisition and recording of peak areas was performed using WinChrom Chromatography software package version 2.1 implemented in the chromatographic system. The stationary phase consisted of a Supelcosil ABZ$^+$ column (15 mm × 4.6 mm i.d., 5 μm particle size). The mobile phase consisted of different mixtures of methanol/buffer in the range 70/10% in presence of different amounts of n-octanol and in absence of n-octanol. The pH was controlled at 3.0 and 7.4 using phosphate buffer (PB). For pH 7.4, at which the compounds are ionized morpholine-propanesulfonic acid (MOPS) was used in combination with the different amounts of n-octanol. For pH 3.0, MOPS was used only in combination of 0.25% n-octanol. The amount of n-octanol was added in the volume of methanol. In this case n-octanol saturated buffer was used to prepare the mobile phase.

In total, the following methanol-buffer mixtures were used in the mobile phase:

(1) pH 3.0
   (a) methanol/phosphate buffer
   (b) methanol + 0.05% n-octanol/phosphate buffer

(c) methanol + 0.25% n-octanol/phosphate buffer
(d) methanol + 0.25% n-octanol/MOPS
(2) pH 7.4
(a) methanol/phosphate buffer
(b) methanol + 0.05% n-octanol/phosphate buffer
(c) methanol + 0.25% n-octanol/phosphate buffer
(d) methanol/MOPS
(e) methanol + 0.05% n-octanol/MOPS
(f) methanol + 0.25% n-octanol/MOPS

Retention times t_r were measured at least from three separate injections and converted to the logarithm of retention factors via equation:

$$\log k = \log\left(\frac{t_r - t_o}{t_o}\right) \quad (1)$$

t_o being the retention time of methanol.

2.2. Lipophilicity and molecular properties

Octanol–water partition $\log P$ and distribution coefficients $\log D_{7.4}$ for compounds **1**–**18** were taken from ref. [23]. For compound **19**, $\log P$ and $\log D_{7.4}$ were measured in the present work by the shaking flask method according to the procedure used in ref. [23].

Hammett electronic constants σ for the substituents on the phenyl ring and Abraham's hydrogen bond basicity (B) parameter were also taken from ref. [23].

All parameters are included in Table S1 Supplementary Material.

3. Results and discussion

The retention factors corresponding to 100% water ($\log k_w$) were derived from the linear part of the $\log k/\varphi$ relationship, using at least five isocratic $\log k$ values, according to the following equation:

$$\log k = -S\phi + \log k_w \quad (2)$$

φ is the volume fraction of methanol and S is the slope of the regression curve.

The $\log k_w$ values for the different mobile phase conditions are presented in Tables S2 and S3 in Supplementary Material, along with the corresponding slopes S. Correlation coefficients were higher than $r > 0.995$. Standard error of $\log k_w$ values was lower than ±0.13 and that of the corresponding slopes S lower than ±0.20. As expected, lower $\log k_w$ values were obtained upon addition of n-octanol, accompanied by a considerable decrease in the corresponding slopes. The effect of n-octanol was found to be more pronounced in the case of highly retained (highly lipophilic) compounds in their neutral state (compounds 3, 5, 6, 14 and 15), in contrast to previous findings which report a greater effect in the case of hydrophilic compounds [20,21].

In most cases good linearity in the $\log k/\varphi$ relationship was observed in a wide range of methanol fraction under the different chromatographic conditions. However for certain compounds a plateau was produced at upper methanol fractions in absence of n-octanol at both pH and upon addition of 0.05% n-octanol at pH 3.0. At pH 7.4 a downward curvature was observed for some compounds in presence of n-octanol at low percentage of methanol. Compounds exhibiting curvatures are marked with asterisks in Tables S2 and S3.

3.1. The effect of n-octanol concentration in isocratic retention

Generally, addition of n-octanol leads to lower $\log k_w$ values. In standard procedures a concentration of 0.25% of n-octanol in methanol fraction is proposed [17,18]. However as reported in ref. [21] for hydrophilic acidic compounds in their neutral state the decrease in the final retention outcome may occur simply by using n-octanol saturated buffer as the aqueous component of the mobile phase. Less investigated is the effect of n-octanol on isocratic retention factors. In the present study we monitored the effect of n-octanol in retention throughout the entire $\log k/\varphi$ profile using the standard percentage of 0.25% in methanol fraction and 0.05%. It should be noted that addition of 0.05% n-octanol in methanol fraction produces less variation in the total n-octanol concentration within the different mobile phases. We found that the decrease of $\log k_w$ values in presence of n-octanol is actually due to the significantly lower slopes S in the corresponding $\log k/\varphi$ profile. Considering that the slopes S reflect solute–mobile phase interactions the presence of n-octanol seems to facilitate the formation of a cavity to accommodate the solute. Nevertheless, in certain cases a reverse effect in isocratic $\log k$ values was observed with higher retention factors in presence of n-octanol. This behavior seems to depend on compound structure, concentration of n-octanol in the mobile phase as well as on the pH and buffer composition, being more evident for rather bulky compounds at pH 3.0 and 0.05% n-octanol. At pH 7.4 a reverse effect was observed for few compounds in presence of MOPS buffer. In Figs. 2–4 the $\log k/\varphi$ profiles for representative compounds **10**, **14** and **19** under the different chro-

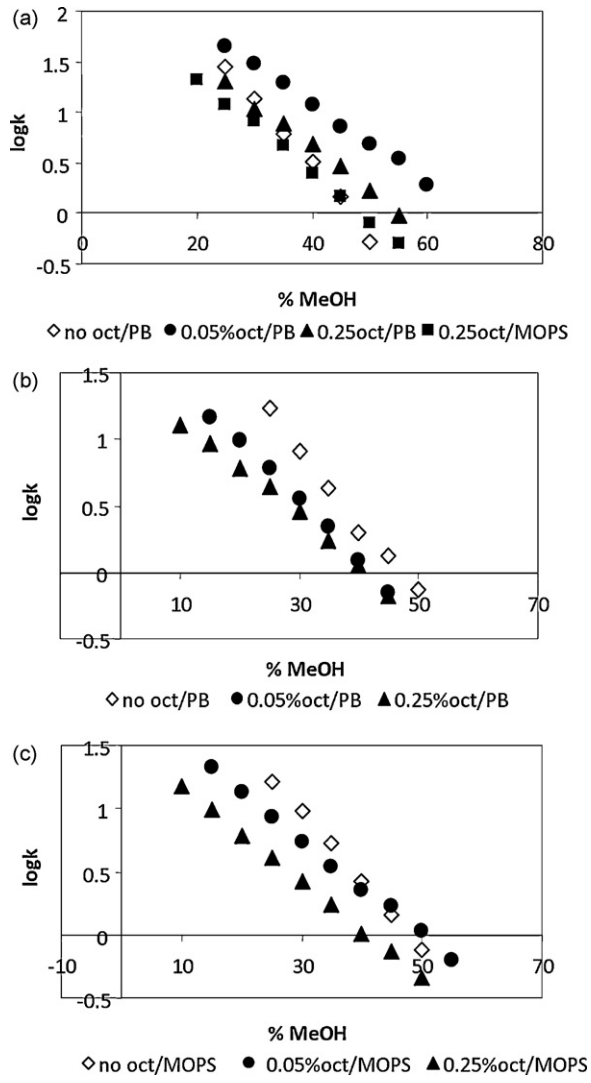


Fig. 2. Retention profile of compound **10** (a) pH 3.0, (b) pH 7.4 (PB) and (c) pH 7.4 (MOPS).

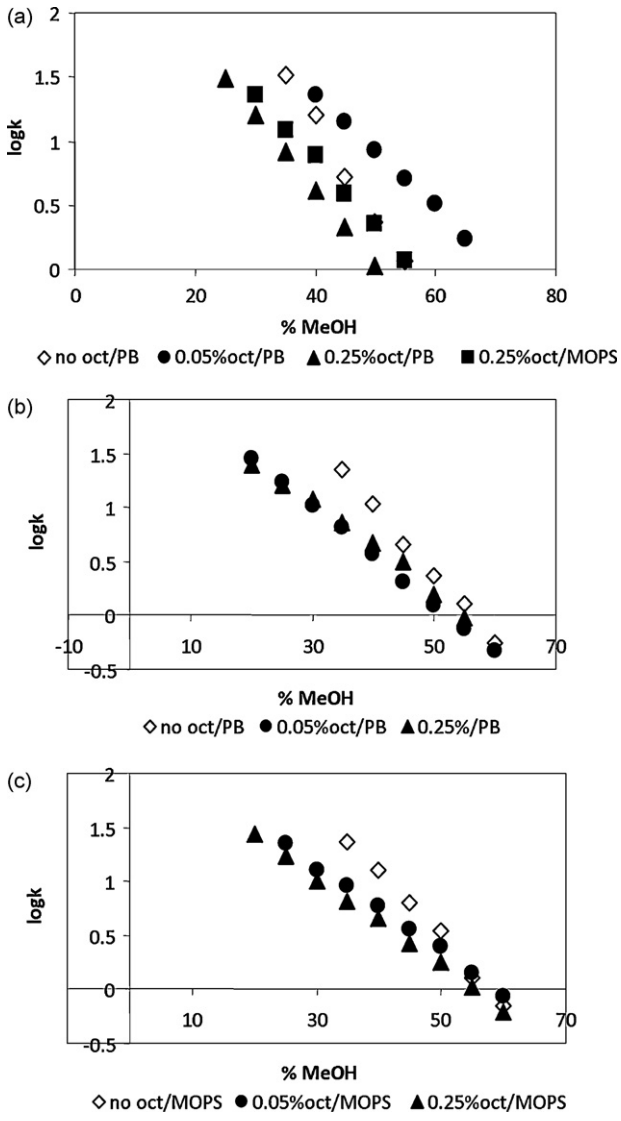


Fig. 3. Retention profile of compound **14** (a) pH 3.0, (b) pH 7.4 (PB) and (c) pH 7.4 (MOPS).

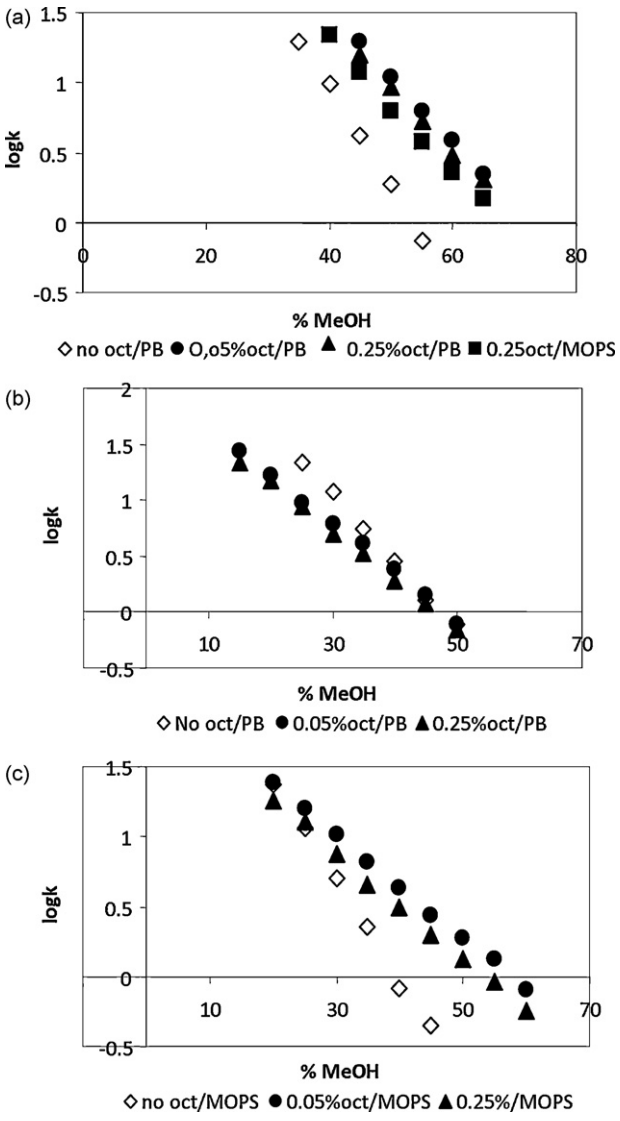


Fig. 4. Retention profile of compound **19** (a) pH 3.0, (b) pH 7.4 (PB) and (c) pH 7.4 (MOPS).

matographic conditions are presented. The above findings suggest that the effect of n-octanol should be considered to be related to both solute/stationary phase and solute/mobile phase interactions, being more intricate than so far considered [20,21].

3.2. Interrelationship between chromatographic data

An overview of the interrelationships between the chromatographic data is provided in Tables 1 and 2, which present their inter-correlation matrix at pH 3.0 and 7.4, respectively. Best correlation coefficients are marked in bold. Abbreviations used in Tables 1 and 2 are explained in the list of abbreviations. At pH 3.0 the correlation between the different $\log k_w$ values is significantly affected by the presence or absence of n-octanol. The degree of linearity between $\log k_w$ values and the corresponding slopes S is considered as an indicative measure of the uniformity in the retention mechanism [15]. Moreover S is related to the specific

Table 1
Correlation matrix between chromatographic data measured at pH 3.0, log P and logit values.

	$\log k_w$/PB	S/PB	$\log k_w$ 0.05/PB	S0.05/PB	$\log k_w$ 0.25/PB	S0.25/PB	$\log k_w$ 0.25/MOPS	S0.25/MOPS	log P	logit
$\log k_w$/PB	1									
S/PB	0.89	1								
$\log k_w$0.05/PB	0.88	0.80	1							
S0.05 (PB)	0.87	0.87	0.88	1						
$\log k_w$0.25/PB	0.85	0.79	**0.97**	0.89	1					
S0.25/PB	0.82	0.70	0.78	0.77	0.82	1				
$\log k_w$0.25/MOPS	0.85	0.79	**0.97**	0.91	**0.995**	0.81	1			
S0.25/MOPS	0.59	0.54	0.48	0.56	0.51	0.70	0.51	1		
log P	0.75	0.65	**0.88**	0.80	**0.87**	0.70	**0.88**	0.50	1	
logit	0.46	0.33	0.60	0.49	0.48	0.40	0.49	0.18	0.59	1

Table 2
Correlation matrix between chromatographic data measured at pH 7.4, log $D_{7.4}$ and logit values.

	log k_w/PB	S/PB	log k_w MOPS	S MOPS	log k_w 0.05 (PB)	S0.05 (PB)	log k_w 0.05 MOPS	S 0.05 MOPS	log k_w 0.25 (PB)	S 0.25 (PB)	log k_w 0.25 MOPS	S 0.25 MOPS	log $D_{7.4}$	logit
log k_w/PB	1													
S/PB	0.87	1												
log k_w/MOPS	**0.98**	0.81	1											
S/MOPS	0.83	0.74	0.83	1										
log k_w0.05/PB	**0.94**	0.83	0.94	0.76	1									
S 0.05/PB	0.83	0.82	0.83	0.74	**0.91**	1								
log k_w0.05/MOPS	**0.94**	0.77	0.96	0.77	**0.97**	0.83	1							
S 0.05/MOPS	0.59	0.44	0.60	0.44	0.63	0.40	0.69	1						
log k_w0.25/PB	**0.95**	0.77	0.95	0.84	**0.94**	0.86	0.93	0.54	1					
S 0.25/PB	0.92	0.79	0.91	0.85	0.94	0.91	0.92	0.49	**0.96**	1				
log k_w0.25/MOPS	**0.95**	0.76	**0.96**	0.83	**0.94**	0.85	**0.95**	0.55	**0.996**	**0.95**	1			
S 0.25/MOPS	0.90	0.80	0.90	0.76	0.94	0.87	**0.93**	0.54	0.90	**0.95**	**0.91**	1		
log $D_{7.4}$	0.87	0.67	0.85	0.73	0.80	0.66	0.86	0.61	0.86	0.84	0.86	0.80	1	
logit	0.54	0.62	0.50	0.52	0.42	0.41	0.37	0.15	0.46	0.33	0.45	0.31	0.39	1

hydrophobic surface area of the solutes, being a hydrophobicity parameter per se [28]. In general moderate correlation between log k_w and S, as well as between the different S values, was observed. In contrast at pH 7.4 good interrelations between the different log k_w values are observed, while the log k_w/S relationships are satisfactory in the case of phosphate buffer. However in the case of MOPS, log k_w correlate well with the corresponding slopes S only in presence of 0.25% n-octanol. Inter-correlation between the slopes S is observed under strictly similar conditions.

In Table 3 representative regression equations reflecting the log k_w interrelation are presented (Eqs. (3a), (3b), (4a)–(4c)). 1: 1 correlation was observed between log k_w values determined using different buffer and the same n-octanol concentration at both pH values, an indication that ion pair formation between ionized compounds and phosphate buffer cations is negligible.

3.3. Relationships with lipophilicity

Comparison between log P, log $D_{7.4}$ and log k_w values (Tables S1–S3) reveals that chromatographic indices are considerably higher than octanol–water partition or distribution coefficients. The addition of n-octanol should be considered as a favorable condition, since it leads to lower log k_w values, thus reducing the differences between them and log P or log $D_{7.4}$, which however remain large, especially for the data measured at pH 7.4. In Table 4 the regression equations between log k_w and log P are presented (Eqs. (5a)–(5d)). Although correlation coefficients remain moderate also in presence of n-octanol the slopes in the regression equations are shifted close to 1, implying an analogous balance of forces between retention and octanol–water partitioning. The negative intercepts reflect the differences in magnitude between the chromatographic indices and log P. Table 5 includes representative regression equations derived from log $D_{7.4}$/log k_w correlation (Eqs. (6a)–(6f)). The quality of the equations is not improved in presence of n-octanol (Eqs. (6c)–(6f)) however as in the case of equations Eqs. (5b)–(5d) the slopes are shifted close to 1, suggesting similar energetics between the two processes. The negative intercept in Eqs. (6c)–(6f) is however considerably larger than that in Eqs. (5b)–(5d). The percentage of n-octanol in the mobile phase had no further effect on the relationships with log P or log $D_{7.4}$, in agreement with our previous findings that lower amounts of n-octanol are sufficient to improve the octanol-like performance of the chromatographic system [21]. No significant influence of buffer was observed throughout.

3.4. The use of log k_w in relationships with biological activity

In our previous publication concerning the same data set we reported on the poor performance of log P calculation systems to detect any trend between lipophilicity and aldose reductase activity, whereas experimentally determined log P values proved more useful and, in combination with hydrogen bond basicity B or electronic parameter σ, they led to satisfactory regression equations with biological activity [23]. Analogous results were obtained by replacing log P with retention factors, log $k_{w(PB)[0.05\%]}$ offering the best performance. In Fig. 5 the plot of logit values versus both log $k_{w(PB)[0.05\%]}$ (filled squares) and log P (empty squares) is presented. Compound **18** with free hydrogen on the pyrrole nitrogen shows a distinct deviating behavior in both plots. Compounds **3** and **12**, lying below the logit/log P trendline, fit reasonably in the logit/log $k_{w(PB)[0.05\%]}$ trendline. On the other hand, the logit/log P trendline accommodates the methoxy derivative 4, which appears as an outlier in the logit/log $k_{w(PB)[0.05\%]}$ plot. Exclusion of com-

Table 3
Interrelationships between $\log k_w$ values according to the equation: $\log k_{w(I)} = a + b\log k_{w(II)}$ ($n = 19$).

(I)		(II)	a	b	r	s
pH 3.0						
$\log k_w$0.25/PB	(3a)	$\log k_w$PB	0.41 (±0.30)	0.58 (±0.09)	0.849	0.254
$\log k_w$0.25/MOPS	(3b)	$\log k_w$0.25/PB	**0.06**(±0.06)	**0.96**(±0.02)	0.995	0.046
pH 7.4						
$\log k_w$0.25/PB	(4a)	$\log k_w$/PB	−0.27 (±0.15)	0.72 (±0.06)	0.953	0.151
$\log k_w$0.25/MOPS	(4b)	$\log k_w$/MOPS	−0.36 (±0.14)	0.75 (±0.05)	0.960	0.141
$\log k_w$0.25/MOPS	(4c)	$\log k_w$0.25/PB	**0.01**(±0.04)	**1.00**(±0.02)	0.996	0.044

Table 4
Representative regression equations correlating $\log P$ and $\log k_w$ values. $\log P = a + b\log k_w$ ($n = 19$).

$\log k_w$		a	b	r	s
$\log k_w$/PB	(5a)	−0.27 (±0.51)	0.70 (±0.15)	0.753	0.429
$\log k_w$0.05/PB	(5b)	−0.83 (±0.38)	1.13 (±0.15)	0.881	0.308
$\log k_w$0.25/PB	(5c)	−0.71 (±0.39)	1.18 (±0.16)	0.871	0.320
$\log k_w$0.25/MOPS	(5d)	−0.80 (±0.38)	1.23 (±0.16)	0.880	0.309

Table 5
Representative regression equations correlating $\log k_{w(I)}$ and $\log D_{7.4}$ values. $\log D_{7.4} = a + b\log k_w$ ($n = 19$).

$\log k_w$		a	b	r	s
$\log k_w$/PB	(6a)	−2.70 (±0.28)	0.75 (±0.10)	0.867	0.287
$\log k_w$/MOPS	(6b)	−2.75 (±0.30)	0.76 (±0.11)	0.854	0.301
$\log k_w$0.05/PB	(6c)	−2.49 (±0.33)	0.94 (±0.17)	0.801	0.346
$\log k_w$0.05/MOPS	(6d)	−2.68 (±0.29)	1.04 (±0.15)	0.858	0.296
$\log k_w$0.25/PB	(6e)	−2.33 (±0.23)	0.99 (±0.14)	0.864	0.291
$\log k_w$0.25/MOPS	(6f)	−2.33 (±0.24)	0.98 (±0.14)	0.861	0.294

pounds **4** and **18** led to Eq. (7)

$$\text{logit} = 1.17(\pm0.17)\log k_{w(PB)[0.05\%]} - 2.70(\pm0.44) \quad (7)$$

$n = 16$, $r = 0.872$, $r^2cv = 0.673$, $s = 0.314$, $F = 18.47$. In Eq. (7) r^2cv corresponds to the cross-validated correlation coefficient obtained by the leave-one out procedure.

Introduction of hydrogen bond basicity B or the electronic parameter σ as an additional term in the regression equations, in analogy with the previously reported analysis [23], led to Eqs. (8) and (9) with slightly improved statistics. The absolute values of the Student's test $|t|$ of B and σ and the cross-validated correlation coefficients r^2cv are also reported.

$$\text{logit} = 0.88(\pm0.18)\log k_{w(PB)[0.05\%]} + 0.73(\pm0.32)B - 2.90(\pm0.39) \quad (8)$$

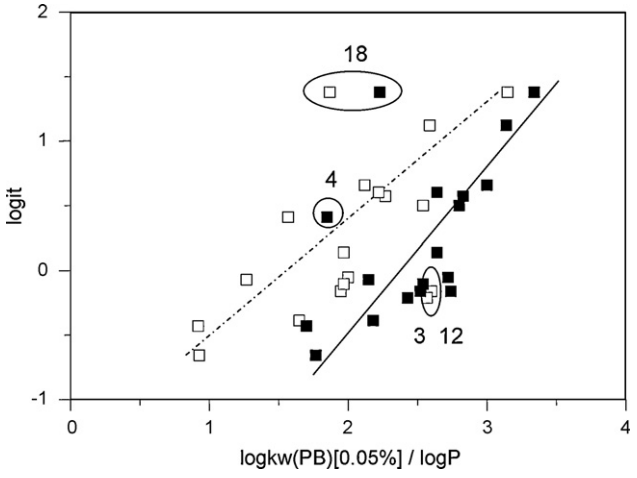


Fig. 5. Plot of logit values versus $\log k_{w(PB)[0.05\%]}$ (filled squares) and $\log P$ (empty squares). Encircled are the outliers in both cases.

$n = 16$, $r = 0.872$, $r^2cv = 0.763$, $s = 0.256$, $F = 31.53$, $t|B = 2.28$.

$$\text{logit} = 0.98(\pm0.17)\log k_{w(PB)[0.05\%]} - 0.45(\pm0.22)\sigma - 2.35(\pm0.42) \quad (9)$$

$n = 16$, $r = 0.872$, $r^2cv = 0.689$, $s = 0.27$, $F = 28.6$, $t|(\sigma) = 1.94$.

4. Conclusions

The intricate effect of n-octanol in the retention behavior of a congeneric series of pyrrolyl-acetic acid derivatives was demonstrated as a modulation in both solute/stationary phase and solute–mobile phase interactions, being more evident in the case of highly retained analogs. The role of low amounts of n-octanol as mobile phase additive in lipophilicity assessment was evaluated in producing similar energetics between retention and octanol–water partitioning. However, moderate correlation between $\log P$ or $\log D_{7.4}$ and $\log k_w$ values was obtained also in presence of n-octanol, while regression equations corresponding to 1:1 correlation could not been established. This contradiction with previously results, reported for the neutral species of acidic compounds, indicates that global models derived from structurally diverse compounds may not always be applied within congeneric series, based on a new scaffold. Retention factors of the neutral species determined at 0.05% n-octanol were found to be suitable to replace the octanol–water $\log P$ parameter in relationships with biological activity. Considering friendliness and speed as additional advantages, HPLC might be regarded as the methodology of choice to express lipophilicity in biological activity modeling.

Acknowledgements

This work was partly supported by the grant 'Kapodistrias', E.L.K.E. 70/4/5876, University of Athens, Greece and by the COST Action B35 (2006–2010).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jchromb.2009.11.020.

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