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Journal of Chromatography B, 857 (2007) 181–187
JOURNAL OF CHROMATOGRAPHY B
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Investigation of the lipophilic behaviour of some thiazolidinediones Relationships with PPAR-γ activity

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Received 10 February 2007; accepted 7 July 2007
Available online 13 July 2007

Abstract

Various lipophilicity aspects of five well-known PPAR-γ ligands, belonging to the thiazolidinedione (TZD) class, ciglitazone (CSZ), troglitazone (TGZ), netoglitazone (NGZ) and the ampholytic pioglitazone (PGZ) and rosiglitazone (RGZ), have been explored. The compounds were found to be highly lipophilic as assessed by direct octanol–water partitioning experiments and further confirmed by reversed phase HPLC measurements under different conditions. Immobilised artificial membrane (IAM) chromatographic indices were also determined as an alternative expression of lipophilicity. They were found to show less diversity forming two clusters. Experimental log *D*/log *P* values were compared to those predicted by three widely used calculation systems. For the two ampholytic TZDs, the lipophilicity and retention/pH profiles were established over a broad pH range and compared to the corresponding calculated profiles. Lipophilicity indices derived under the different conditions were further compared to biological activity, concerning in vitro transactivation (pEC_{50}) and binding affinity (pK_i) data, taken from literature. The most active TZD (RGZ) in both transactivation and binding assay proved to be the less lipophilic analogue. An equation relating pEC_{50} data to experimental log $D_{7.4}$ or reversed-phase log k_w values could be established, while pK_i data did not lead to satisfactory correlation.

Keywords: Thiazolidinediones; PPAR-γ ligands; Lipophilicity; *n*-Octanol/water partitioning; RP-HPLC; IAM chromatography

1. Introduction

Thiazolidinediones (TZDs) represent a new class of oral anti-diabetic agents currently used for the treatment of type II diabetes mellitus [1]. Beyond their anti-diabetic therapeutic benefits, TZDs have recently exhibited a wide spectrum of actions, including anti-inflammatory [2] and anti-neoplastic properties [3]. TZDs mostly exert their effects by selectively binding to and activating the nuclear peroxisome-proliferator activated receptor-γ (PPAR-γ) [4], while receptor independent actions have not been excluded [5]. In general, the members of TZD class posses a few essential pharmacophore elements which comprise an acidic group linked to a central flat ring and a large lipophilic substructure [6]. To date, a large number of TZD derivatives, synthesized through various structural modifications of these essential features, have been tested for PPAR-γ functional activity. However, experimental studies regarding their lipophilicity profile, which could contribute to the optimization and the understanding of their action, are still missing in literature. The fact that PPAR-γ is dramatically highly expressed in adipose tissue supports an essential role of lipophilicity for its ligands. The minimum hydrophobicity concept, formulated by Hansch et al. 20 years ago is considered as a general guideline in Drug Design [7], while for drugs intended for oral administration Lipinski's rule of 5 suggests an upper limit for lipophilicity [8]. In this aspect, the question arises how much lipophilicity should be incorporated in the ligands so that they satisfy the receptor and receptor environment requirements and comply with principles nowadays generally accepted.

The most widely used index of lipophilicity is the *n*-octanol/water partition or distribution coefficient (log *P* or log *D*) [9,10]. This choice offers a quite representative simulation of drug partitioning into bio-membranes [11]. Due, however, to difficulties involved in direct *n*-octanol/water partitioning experiments, reversed-phase liquid chromatography (RP-HPLC) has alternatively been applied for drug lipophilicity assessment. This

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doi:10.1016/j.jchromb.2007.07.013

technique offers several practical advantages compared to the traditional shake-flask method, including speed, reproducibility, broader dynamic range and insensitivity to impurities or degradation products [12,13]. Extrapolated RP-HPLC indices ($\log k_w$) are usually calibrated towards the *n*-octanol/water system and appropriate conditions are chosen, so that the best parallelism with $\log P/\log D$ is achieved [13–16]. In addition, a rich arsenal of calculation procedures has been developed for rapid estimation of $\log P$ [17–19]. For complex structures, however, their performance is often disputable. Predictions may be even less accurate in the case of $\log D$ for molecules containing ionizable groups. On the other hand, *n*-octanol/water system and RP-HPLC reflect non specific interactions with cell membranes, but they fail to predict specific ones [20]. For this purpose, the development of immobilised artificial membrane (IAM) chromatography has unfolded new perspectives in the application of HPLC for rapid evaluation of drug partitioning into bio-membranes, since it is thought to mimic the phospholipid bilayers more closely. In fact, IAM columns contain phospatidylcholine incorporated on a silica-propylamine backbone, simulating better the ion-pairing and hydrogen bonding interactions [21–23].

In the present study, we exploited the above mentioned alternatives in order to investigate in detail the lipophilicity of five well-known TZDs, ciglitazone (CSZ), troglitazone (TGZ), netoglitazone (NGZ), pioglitazone (PGZ), rosiglitazone (RGZ). For this purpose the distribution coefficients ($\log D$) in *n*-octanol/water system were measured and compared with $\log D$ values estimated by three widely used calculation systems. RP-HPLC lipophilicity indices determined under different conditions were evaluated for their ability to reproduce the *n*-octanol/water $\log D$ values. In addition, the behavior of TZDs in IAM chromatography was assessed. Lipophilicity measures obtained from the different systems were further used in relation to literature biological data.

2. Materials and methods

2.1. Materials

Ciglitazone-CGZ, troglitazone-TGZ, netoglitazone-NGZ, pioglitazone-PGZ and rosiglitazone-RGZ were purchased from Cayman Chemical Company, Michigan, USA. Their structures are presented in Fig. 1.

Octanol was extra pure purchased by Panreac Quimica, Spain. Methanol and acetonitrile were HPLC grade and were purchased from Lab-Scan Science Ltd., Ireland. Sodium hydrogen phosphate, potassium dihydrogen phosphate, potassium chloride, sodium chloride and 3-morpholinepropanesulfonic acid (MOPS) were purchased from Merck, Darmstadt, Germany. Water was deionised and further purified by means of a Milli-Q Plus Water purification system, Millipore Ltd.

2.2. Octanol/water partitioning experiments

Octanol/water distribution coefficient values were measured by the shake-flask method, using a standard procedure as described in [24]. Briefly, the protocol is as follows:

TZDs were first diluted in DMSO at a concentration of 5 mg/ml (stock solution). Aqueous TZD solutions were then prepared by the addition of appropriate volume of stock solutions in order for a concentration approximately 10^{-5} M to be obtained. The final concentration of DMSO in the aqueous solution did not exceed 0.2%. The pH of the aqueous solutions was pre-

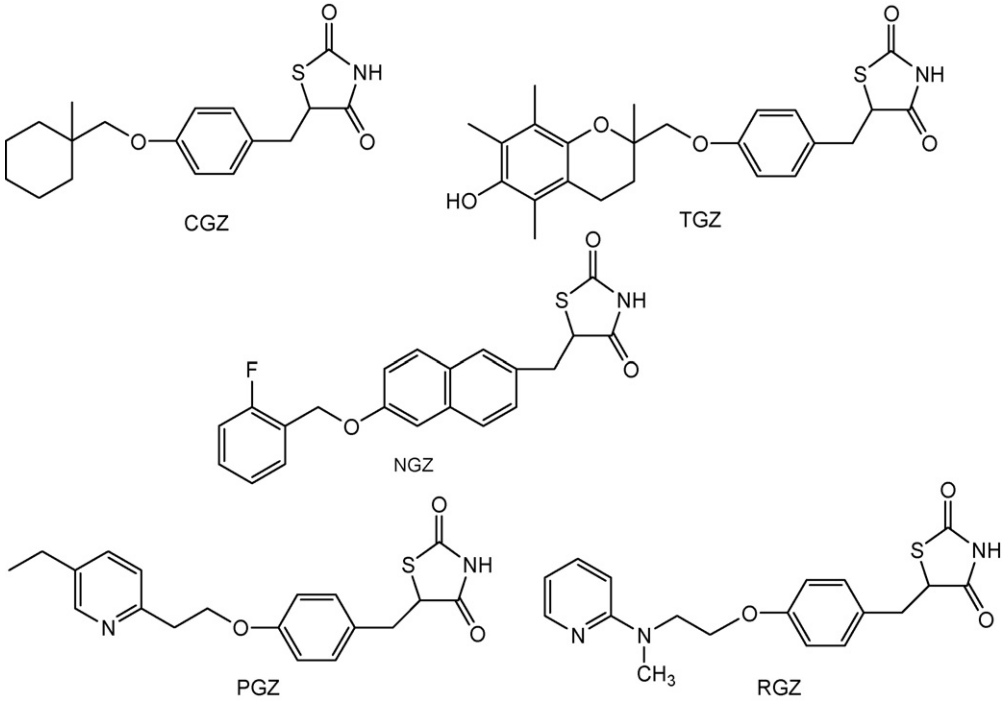

Fig. 1. Structure of TZDs.

viously controlled by 20 mM MOPS buffer at 7.4 and 9.0 for CGZ, TGZ, NGZ and at a broad range from 2.5 to 11 for the ampholytes PGZ and RGZ. Both *n*-octanol and aqueous phases were mutually saturated before use. The volume ratio of the two phases was chosen, so that adequate amount of the solute remained in the aqueous phase after equilibration. In fact, due to the high lipophilicity of CGZ, TGZ and NGZ, volume ratios Vaq/Voct were 480/1 and 600/1 for pH 9.0 and 7.4, respectively. For the less lipophilic PGZ and RGZ, the volume ratio was in the range 20/1–100/1, depending on the pH. An equilibration time of 2 h was chosen, since further shaking until 5 h did not affect the partitioning. Centrifugation followed for 15 min at 2500 rpm. The aqueous phase, before and after equilibration, was analyzed by HPLC, using 75/25% methanol/20 mM MOPS mixtures (pH 7.0) for PGZ and RGZ and 80/20% for the remaining TZDs. Details for HPLC system are described in Section 2.3.

Distribution coefficient D was calculated according to Eq. (1):

$$D = \frac{Ao - A1}{A1}\frac{Vaq}{Voct} \quad (1)$$

Ao and A_1 being the peak area before and after equilibration and V_{aq}, V_{oct} the volumes of aqueous and *n*-octanol phase, respectively. Each determination was performed at least in triplicate and mean log D values at pH 7.4 and 9.0 are reported in Table 2. Standard deviations were within ±0.03.

2.3. HPLC system

The HPLC isocratic pumping system consisted of a GBC Model 1126 pump and a Rheodyne Model 7725i injector with a 20 μl loop, which were coupled to a GBC Model LC1210 UV–vis detector operated at appropriate wavelength, 228 nm for CGZ and TGZ, 267 nm for PGZ and 254 nm for RGZ and NGZ. Data acquisition was performed using WinChrom chromatography software package version 2.1.

2.3.1. Determination of reversed phase chromatographic indices

As stationary phase, three different columns were used, an endcapped Hypersil BDS C-18 (250 mm × 4.6 mm i.d., 5 μm particle size), a polar embedded Supelcosil ABZ+ Plus (150 mm × 4.6 mm i.d., 5 μm particle size) and a polar endcapped Supelcosil Aquasil (150 mm × 4.6 mm i.d., 5 μm particle size). The mobile phase consisted of methanol/buffer mixtures at varying proportions of methanol from 35 to 80%. The pH was controlled by 20 mM MOPS buffer at 7.4 for all columns and in the range 2.5–7.4 for BDS C-18. Alternative chromatographic conditions at pH 7.4 were further applied by adding *n*-octanol in the mobile phase at a concentration of 0.25% in respect to the fraction of methanol. In this case, *n*-octanol saturated buffer was used. The eluent flow rate was 1 ml/min. The mobile phase was filtered through a 0.45 μm nylon membrane before use. Retention times t_r were measured at least from three separate injections and converted to the logarithm of retention factor log k through Eq. (2):

$$\log k = \log\left[\frac{tr - to}{to}\right] \quad (2)$$

t_0 represents the retention time of methanol.

2.3.2. IAM chromatography

The stationary phase was an IAM.PC.DD2 column, Regis Technology, Morton Grove, IL, USA. The mobile phase consisted of different mixtures of acetonitrile/0.01 M Phosphate Buffered Saline (PBS) in the pH range 2.5–7.4. Acetonitrile was added in the mobile phase at concentrations ranging from 10 to 35%. The eluent flow rate was 1, 2, or 3 ml/min. The chromatographic procedure was the same as described in Section 2.3.1. IAM retention factors log k^{IAM} were determined from retention times via Eq. (2). Potassium dichromate was used for the measurement of t_o.

2.4. Log P, log D and pK_a estimation

Prolog D module implemented in Pallas 3.1.2.1 (CompuDrug Chemistry Ltd.) and AB/logD module implemented in ADME boxes 4.0 (PharmaAlgorithms Inc.) were applied for calculation of log P and log D values. C log P (Biobyte 4.0) was used as reference systems to calculate log P. pK_a was calculated separately by ACD Labs software (Advanced Chemistry Development Inc.) and combined with C log P to generate C log D according to Eq. (3):

$$\text{Log } D = \log\ P - \log(1 + 10^{\text{pH}-\text{p}K_a}) \quad (3)$$

Eq. (3) could not be applied for estimation of log $D_{7.4}$ for PGZ with close overlapping pK_a values and RGZ with $pk_{a_{acidic}} < pk_{a_{basic}}$, since zwitterionic species should be expected to contribute to lipophilicity at pH 7.4.

Table 1
pK_a values of TZD members calculated according to the different software or estimated from log k_w/pH profile

TZDs	ACD		HPLC (BDS)		HPLC (IAM)	
	Acidic	Basic	Acidic	r	Acidic	r
CGZ	6.36 (±0.50)	–	6.77 (±0.03)	0.997	6.37 (±0.06)	0.991
TGZ	6.35 (±0.50)	–	6.78 (±0.04	0.993	6.69 (±0.04)	0.993
NGZ	6.28 (±0.50)	–	6.59 (±0.05	0.993	6.34 (±0.04)	0.995
PGZ	6.35 (±0.50)	5.56 (±0.29)	[a]		[a]	
RGZ	6.35 (±0.50)	6.48 (±0.12)	[a]		[a]	

[a] Estimation not possible.

Table 2
Experimental and calculated log D and log P values of TZD members

TZDs	Exp.			AB/log D			Prolog D			C log P		
	Log P	Log $D_{7.4}$	Log $D_{9.0}$	Log P	Log $D_{7.4}$	Log $D_{9.0}$	Log P	Log $D_{7.4}$	Log $D_{9.0}$	Log P	Log $D_{7.4}$	Log $D_{9.0}$[a]
CGZ	5.71[b]	4.63	3.49	4.09	3.14	1.60	4.55	4.06	2.66	5.07	3.99[c]	2.43
TGZ	5.30[b]	4.20	3.28	3.34	2.99	1.47	5.06	4.67	3.29	5.17	4.07[c]	2.52
NGZ	5.24[b]	4.09	3.19	4.45	3.50	1.95	4.56	3.97	2.49	4.99	3.84[c]	2.27
PGZ	3.31	3.14	2.32	2.72	1.98	0.44	3.73	3.45	2.07	3.53	[a]	1.19
RGZ	2.78	2.63	1.98	1.59	1.35	−0.08	3.57	3.24	1.75	3.02	[a]	1.17

[a] Correction was not possible because of the overlapping pK_a values.
[b] Log P values were calculated from experimental log $D_{7.4}$ according to Eq. (3) using ACD pK_a values.
[c] Log $D_{7.4}$ and log $D_{9.0}$ were calculated from log P according to Eq. (3) using ACD pK_a values.

The pK_a of the acidic TZDs was estimated also from the log k_w/pH profile on the basis of Eq. (4) by non linear fitting.

$$\text{Log}\, k_w = \log\, k_{w(\text{neutral})} - \log(1 + 10^{\text{pH}-\text{p}K_a}) \quad (4)$$

The ACD pK_a values and those generated from retention/pH profile are presented in Table 1.

2.5. Statistical analysis

Regression analysis and non linear fitting were performed using SPSS v.11.0 for Windows.

3. Results and discussion

3.1. Octanol/water partitioning—comparison between experimental and calculated values

Taken into consideration the high log P values estimated by the different calculation systems, especially for the acidic TZDs, partitioning experiments were first conducted at pH 9.0 at which log D values were lower due to extended ionization. The determination of distribution coefficients log D at physiological pH 7.4 became feasible by the use of high aqueous phase/octanol volume ratios (see Section 2). The determination of log P values related to the neutral species was impossible for acidic CGZ, TGZ and NGZ. They were calculated from experimental log $D_{7.4}$ according to Eq. (3) using the pK_a value calculated by ACD software (Table 1). For CGZ, the log $D_{7.4}$ value was found close to the upper limit of accuracy for the shake flask method. It should be noted that for CGZ, Lipinski et al. reported log P values related to its unionized form equal to 3.57 and 5.15 measured by shake flask method and potentiometric titration, respectively [25]. Our findings are in better agreement with the latter value.

Experimental log $D_{9.0}$, log $D_{7.4}$ and the thereupon derived log P values are included in Table 2, along with the corresponding calculated log D/log P values. Experimental log $D_{7.4}$ values were closer to those generated by Prolog D and C log D, whereas they were considerable higher than the estimates provided by AB/log D. For all calculation systems, deviations were significantly larger regarding log $D_{9.0}$ values.

For a more thorough investigation of the effect of ionization on lipophilicity and the corresponding performance of the calculation systems, the log D/pH profile of PGZ and RGZ over a broad pH range was established. For both ampholytes, a bell shape curve was obtained with a maximum at pH 6.5 and a fairly constant lipophilicity in the pH range 6–7.4 (Fig. 2). In the same Figure, the calculated log D/pH profiles as generated by Prolog D and AB/log D are depicted. The Prolog D/pH profiles agree better with the experimental profiles.

3.2. Reversed phase chromatographic indices as measures of lipophilicity

The practical advantage of chromatographic lipophilicity indices becomes more evident in the case of highly lipophilic compounds since they may be used to confirm the results of the shake-flask method. In the present study, three different reversed phase columns were investigated for their efficiency to provide lipophilicity indices for the TZDs which reproduce log D values in the most satisfactory way. BDS and ABZ are the usual columns of choice for the lipophilicity assessment. We also used Aquasil, which due to its lower hydrophobicity may constitute

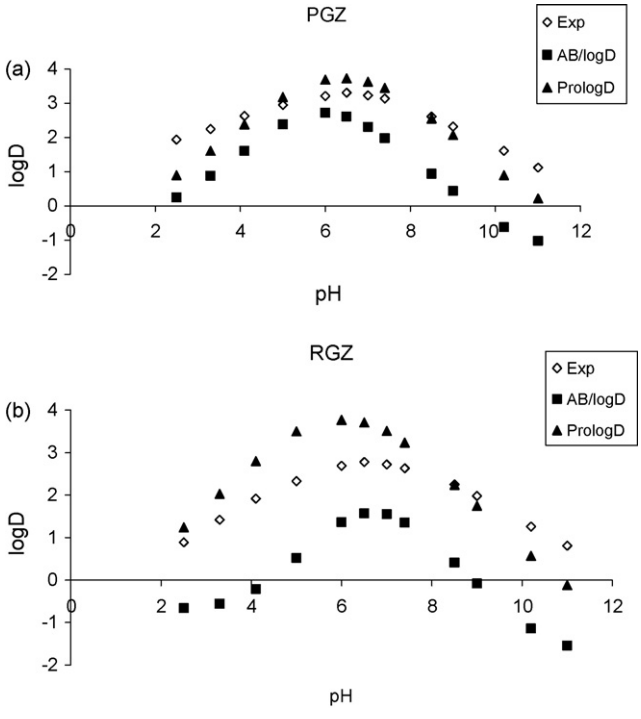

Fig. 2. Experimental and calculated log D/pH profiles of (a) PGZ and (b) RGZ.

a better choice for the highly lipophilic TZDs. A number of relevant studies have suggested the addition of *n*-octanol in the mobile phase as a crucial factor in order for a better simulation of *n*-octanol/water partitioning to be achieved [14–16]. These studies concerned basic and neutral drugs (in theses cases *n*-decylamine should also be added as a masking agent), as well as acidic drugs at low pH at which they exist in their neutral form. No relevant studies so far have dealt systematically with ampholytes or acidic compounds at pH 7.4. In this aspect, we investigated the retention of TZDs at pH 7.4 both in the presence and absence of *n*-octanol using MOPS to control the pH. MOPS is usually the buffer of choice for lipophilicity assessment by reversed-phase HPLC, since, due to its zwitterionic nature, it is considered not to interfere with the solutes and/or the stationary phase [12,14].

In all cases, extrapolated retention factors $\log k_w$ corresponding to 100% aqueous phase were used as lipophilicity indices. They were derived according to Eq. (5) using at least five isocratic $\log k$ values determined at different concentrations of methanol.

$$\log\ k = -S\varphi + \log\ k_w \quad (5)$$

φ being the methanol fraction. The slope S and the intercept $\log k_w$ were generated by linear regression analysis. Correlation coefficients were higher than 0.997. Standard deviations of the extrapolated $\log k_w$ values were within ±0.03.

The $\log k_w$ values, presented in Table 3, confirm the high lipophilicity of the TZDs under study. In presence of *n*-octanol, lower $\log k_w$ values were obtained in all cases. Concerning the stationary phase, retention on Aquasil column was weaker. It should be noted that under all different chromatographic conditions, CGZ was found to possess a considerably higher $\log k_w$ value than the two other acidic TZDs, in agreement with the results of the shake flask method.

The slopes S of Eq. (5), usually correlate with the extrapolated retention factors $\log k_w$. The good linearity between S and $\log k_w$ may be considered as an indicative measure of the uniformity in the retention mechanism [24]. With respect to TZDs, a good correlation was obtained independently of the columns used. The corresponding Eqs. (6)–(11) are presented in Table 4. The presence of *n*-octanol improved the linear correlation between S and $\log k_w$.

The different sets of $\log k_w$ values were then correlated with $\log D_{7.4}$. Regression Eqs. (12)–(17) are presented in Table 5. In this case, *n*-octanol was found not to be advantageous as denoted by Eqs. (15)–(17). In contrast, 1:1 correlation with

Table 3
RP and IAM chromatographic indices of TZD members at pH 7.4

TZDs	BDS		ABZ		Aquasil		IAM
	Log k_w	Log k_w^{oct}	Log k_w	Log k_w^{oct}	Log k_w	Log k_w^{oct}	Log k_w
CGZ	4.89	4.59	4.85	4.36	4.27	3.93	3.72
TGZ	4.34	3.86	4.69	4.00	3.89	3.34	3.69
NGZ	4.23	3.96	4.54	4.08	3.95	3.41	3.51
PGZ	3.33	2.55	3.17	2.41	3.18	2.54	2.51
RGZ	2.81	2.29	2.66	2.16	2.82	2.13	2.35

Table 4
Correlations between $\log k_{w7.4}/S$

No.	Equations	Log $k_{w7.4} = aS + b$		
	Columns	a	b	R^2
6	BDS	0.70	2.26	0.931
7	ABZ	0.63	2.43	0.978
8	Aquasil	0.61	2.75	0.953
9	BDS^{oct}	0.79	1.69	0.980
10	ABZ^{oct}	0.76	1.78	0.994
11	Aquasil^{oct}	0.73	1.97	0.982

highly significant statistics was obtained between $\log D_{7.4}$ and $\log k_{w7.4}^{BDS}$ in absence of *n*-octanol. The latter conditions were further used to establish the $\log k_w^{BDS}$/pH profiles over the entire pH range, permitted by the limitation of the column. In Fig. 3, the $\log k_w^{BDS}$/pH profiles of the acidic TZDs are illustrated. The good agreement of $\log k_w^{BDS}$ values, at low pH, with $\log P$ (Table 2) further supports the lack of secondary (silanophilic) interactions in retention under the above conditions. The $\log k_w^{BDS}$/pH profiles of the amplolytes are presented in Fig. 4 along with the relevant part of the experimental $\log D$/pH profiles for reasons of comparison. A maximum in retention at pH 6.5 and a fairly constant lipophilicity in the pH range 6.0–7.4 were observed in agreement with the corresponding $\log D$/pH curve. However, the slopes at the acidic pH region were considerably lower compared to the corresponding slopes of the $\log D$/pH profiles. The enhanced retention at low pH, responsible for the shallow curve, may be attributed to the silanophilic interactions due to the presence of the protonated basic groups of PGZ and RGZ.

3.3. IAM chromatography

Extrapolated retention factors $\log k_w^{IAM}$ were obtained from at least five isocratic $\log k$ values determined at different concentrations of acetonitrile, according to Eq. (5). Correlation coefficients were higher than 0.996 and standard deviations of extrapolated $\log k_w^{IAM}$ values were within ±0.03. $\log k_w^{IAM}$ were determined throughout the entire pH range, permitted by the limitations of the column. PBS was used to control the pH, since it simulates better physiological conditions and is the buffer of choice in IAM chromatography. For reasons of comparison with the different reversed phase $\log k_w$ values, $\log k_w^{IAM}$ at pH 7.4 are included in Table 3. Interestingly, $\log k_w^{IAM}$ indices did not pos-

Table 5
Correlations between $\log D_{7.4}/\log k_{w7.4}$

No.	Equations	Log $D_{7.4} = a\log k_{w7.4} + b$		
	Columns	a	b	R^2
12	BDS	0.98	−0.12	0.997
13	ABZ	0.82	0.48	0.977
14	Aquasil	1.37	−1.23	0.993
15	BDS^{oct}	0.83	0.89	0.976
16	ABZ^{oct}	0.78	1.07	0.962
17	Aquasil^{oct}	1.13	0.26	0.987

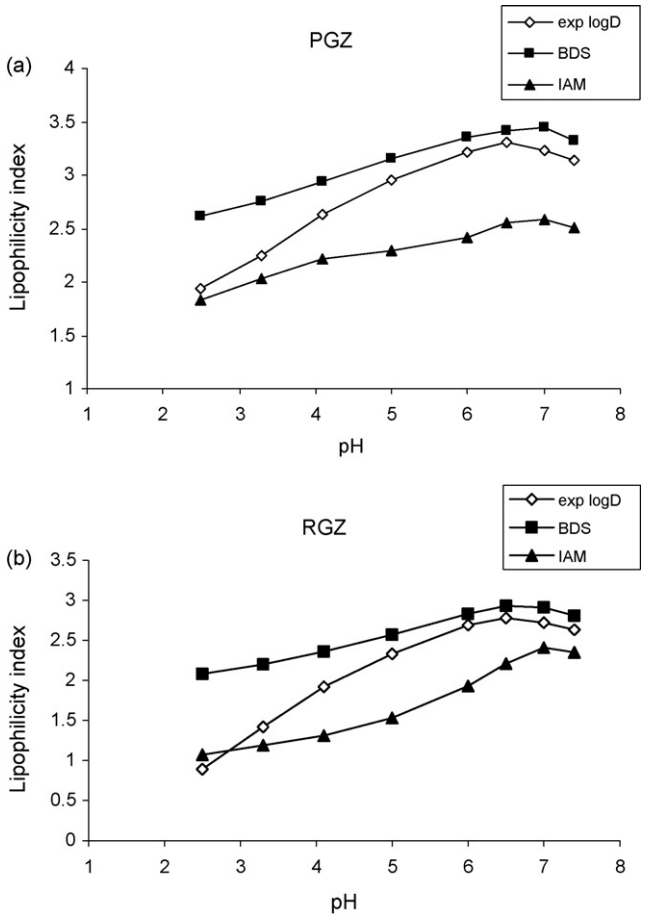

Fig. 3. Experimental lipophilicity indices as a function of pH (a) PGZ and (b) RGZ.

sess enough diversity forming two clusters, one including the ampholytes, PGZ and RGZ, associated with weaker retention and the other including the strongly retained acidic TZDs.

Generally, log k_w^{IAM} indices correlate well with *n*-octanol–water distribution coefficients, unless electrostatic interactions are present [23,26]. The log k_w^{IAM}/pH profiles of the ampholytes, PGZ and RGZ, are included in Fig. 3 with the corresponding log k_w^{BDS}/pH and the analogous part of the experimental log *D*/pH profiles. For both PGZ and RGZ, the slope of the descending part in their log k_w^{IAM}/pH profiles was lower than the slope of the log *D*/pH profile and almost parallel with the slope of the corresponding log k_w^{BDS}/pH profile. This behavior should be attributed to the presence of the protonated basic groups, which undergo electrostatic interactions with the phosphate anions of the phosphatidylcholine, incorporated in the IAM stationary phase [23], while silanophilic interactions should not be excluded. The log k_w^{IAM}/pH profiles of the acidic TZDs are illustrated in Fig. 4 together with the corresponding log k_w^{BDS}/pH. In all three cases log k_w^{IAM} indices follow a lower curve practically parallel to the log k_w^{BDS} curve, indicating that in IAM retention, secondary (electrostatic) interactions are not involved upon ionization of the acidic center.

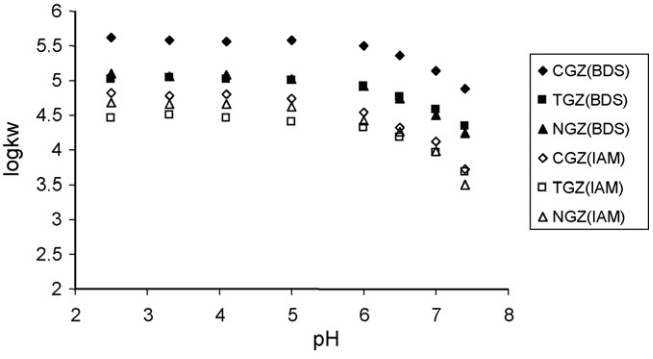

Fig. 4. Retention/pH profiles of the acidic TZDs.

It should be noted that systematic studies concerning the investigation of IAM retention as a function of pH are not found in literature.

3.4. Estimation of pKa from the retention/pH profiles

The log k_w^{BDS}/pH profiles of the acidic TZDs were used to generate the apparent pK_a values according to Eq. (4) [27] (see also Section 2). The pK_a(app) values derived by this procedure are 0.4 log units higher than the pK_a estimated by ACD, but within the prediction error provided by the software (Table 1). It should be taken into consideration that the presence of organic modifier in the mobile phase affects its pH as well as the pK_a of the solutes. In the case of acidic compounds, the pK_a is shifted to higher values [28,29]. These effects are minimized in extrapolated retention factors, which theoretically correspond to 100% aqueous mobile phase. However, the influence of organic modifier on pK_a of solutes may still be present affecting the final retention outcome [28,29].

As commented in Section 3.3 secondary (electrostatic) interactions seem not to be involved in IAM retention of the acidic TZDs. Thus apparent pK_a values could be derived also from the log k_w^{IAM}/pH profiles. In this case lower pK_a(app) values were obtained, in particular for CGZ and NGZ, which coincide with ACD pK_a values. The reason for this difference may be the fact that the isocratic log *k* values used for the extrapolation procedure were determined at lower concentration of organic modifier. In this aspect pK_a values derived from log k_w^{IAM}/pH profile may be closer to the true pK_a values.

3.5. Relationship between lipophilicity and biological data

TZDs usually elicit a response through binding to and activating PPAR-γ. In the present study, an attempt was made to correlate the different lipophilicity measures at physiological pH 7.4 with the functional activity for PPAR-γ expressed as pEC_{50} transactivation data (Table 6) [30–32]. It should be noted that no correlation was found between any set of calculated log $D_{7.4}$ values and pEC_{50}. Using experimental log $D_{7.4}$ a negative linear correlation with satisfactory statistics was obtained (Eq. (18)), indicating an increase in activity with decrease in lipophilicity.

$$pEC_{50} = -0.64(\pm 0.16)\ \log\ D_{7.4} + 8.60(\pm 0.62) \quad (18)$$

$n = 5$, $r = 0.913$, $s = 0.270$, $F = 15.08$, p-value $= 0.0111$, p-value (log $D_{7.4}$) $= 0.0302$.

Table 6
Transactivation pEC_{50} and binding affinity pK_i data of TZD members obtained from references [30–32]

TZDs	pEC_{50}	pK_i
CGZ	5.52	5.51
TGZ	6.11	6.52
NGZ	6.12	5.98
PGZ	6.26	5.91
RGZ	7.12	7.33

The correlation was slightly improved, when log $k_{w7.4}^{BDS}$/pH values were used (Eq. (19)).

$$pEC_{50} = -0.65(\pm 0.16)\ \log k_{w7.4}^{DBS} + 8.75(\pm 0.62) \quad (19)$$

$n=5$, $r=0.922$, $s=0.256$, $F=17.09$, p-value $=0.0088$, p-value $(\log D_{7.4}) = 0.0257$.

Correlation of pEC_{50} with all other reversed phase log k_w values determined in this study led to analogous results (equations not shown), while with IAM chromatographic indices Eq. (20) with moderate statistics was obtained. It should be noted, however, that the low diversity of log k_w^{IAM} renders Eq. (20) rather disputable.

$$pEC_{50} = -0.69(\pm 0.29)\ \log k_w^{IAM} + 8.40(\pm 0.94) \quad (20)$$

$n=5$, $r=0.805$, $s=0.394$, $F=5.53$, $p=0.0631$, $p(\log k_w^{IAM}) = 0.1001$).

F and p values of Eq. (20) are significant in the 90% confidence level.

For cell-based assays, membrane permeation may be the rate limiting step. Thus, the highly lipophilic CGZ may stick on the membrane, while the less lipophilic RGZ has the ability to penetrate readily into the cell.

Lipophilicity was further compared to TZD binding affinity to PPAR-γ expressed as pK_i values [30–32]. Also in this case, high affinity was found to be associated with lower lipophilicity. However, correlation of pK_i with log $D_{7.4}$ and log $k_{w7.4}^{BDS}$ was inferior ($r=0.701$, $r=0.713$, respectively, equations not shown). Poor correlation was obtained with IAM chromatographic indices ($r=0.541$, equation not shown).

4. Conclusions

The different protocols used in the present study confirmed the high lipophilicity of the investigated TZDs. The use of a BDS column in lipophilicity assessment by HPLC proved more appropriate, while addition of *n*-octanol in the mobile phase was not justified. IAM chromatographic indices constituted a lower lipophilicity scale, while they did not possess enough diversity, creating two clusters for the ampholytes and the acidic TZDs, respectively. The most active TZD (RGZ) in both transactivation and binding assays proved to be the less lipophilic analogue, with log $D_{7.4} = 2.63$. In the light of Hansch's minimum hydrophobicity concept [7], future design should be oriented to structures with analogous or even lower lipophilicity. Moreover, the fairly constant lipophilicity of RGZ around physiological pH, tolerating micro pH changes in the biological environment, may be considered as an additional favourable characteristic.

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