ELSEVIER

Journal of Chromatography A, 723 (1996) 135–143

JOURNAL OF CHROMATOGRAPHY A

Study of lipophilic character of serotonergic ligands

Gian Luigi Biagi[a,*], Anna Maria Barbaro[a], Andrea Sapone[a], Pier Andrea Borea[b], Katia Varani[b], Maurizio Recanatini[c]

[a]*Dipartimento di Farmacologia, Università di Bologna, via Irnerio 48, 40126 Bologna, Italy*
[b]*Istituto di Farmacologia, Università di Ferrara, Ferrara, Italy*
[c]*Dipartimento di Scienze Farmaceutiche, Università di Bologna, Bologna, Italy*

First received 25 April 1995; revised manuscript received 21 July 1995; accepted 21 July 1995

Abstract

The R_M values of a series of serotonergic derivatives were measured using a reversed-phase TLC system with acetone, acetonitrile or methanol as the organic modifier of the mobile phase. As regards the basic aspects of the chromatographic technique, the series of compounds studied here behave in quite the same way as the previously investigated series of chemicals, i.e. a very good correlation is found between the experimental and extrapolated R_M values as well as between the extrapolated R_M values from different organic solvent systems; the relationship between intercepts and slopes of the TLC equations in series of congeneric compounds; the influence of the organic modifier on the slopes of the TLC equations. The R_M values from the above system were shown to be correlated with those obtained with a C_{18} high-performance TLC. Finally, both chromatographic parameters were compared with calculated octanol–water log P values.

Keywords: Lipophilicity; Partition coefficients; Quantitative structure–activity relationships; Serotonin; Serotonergic compounds

1. Introduction

Serotonin (5-hydroxytriptamine, 5-HT) is an endogenous compound involved in several physiological and pathological events. It was recognized to mediate vasoconstriction and peripheral nervous system stimulation. Appetite, learning and memory, thermoregulation, sleep, behaviour such as sex, aggression and feeding, anxiety and depression are some of the processes which have been linked with the neurotransmitter serotonin. Specific receptors (which can be classified into at least three, possibly up to seven classes) mediate the pathophysiological events triggered [1,2]. They comprise the $5HT_1$, $5HT_2$ and $5HT_3$ classes, as well as the "uncloned" $5HT_4$ receptor. The $5HT_5$, $5HT_6$ and $5HT_7$ receptor genes have been also cloned recently, even though their mode of operation is still not fully characterized [3].

For many years our laboratory has been using a reversed-phase TLC technique for measuring R_M values, as an expression of the lipophilic character of molecules, which is a well known physicochemical factor affecting biological activities. In view of a quantitative structure–activi-

* Corresponding author.

0021-9673/96/$15.00
SSDI 0021-9673(95)00809-8

ty relationships (QSAR) study dealing with 5-HT receptor binding, the main purpose of the present work was to measure the R_M values of a series of typical 5-HT receptor ligands belonging to different chemical classes. The R_M values were obtained both from reversed-phase silicone thin-layer chromatography (silicone RP-TLC) and C_{18} high-performance TLC (C_{18} RP-HPTLC), and were compared with calculated octanol–water log P values.

2. Experimental

2.1. Chemicals

Serotonergic ligands were purchased from RBI (Natick, MA, USA). All other chemicals and solvents were of analytical-reagent grade.

2.2. Determination of R_M values by silicone RP-TLC

The details of the reversed-phase chromatographic technique were described previously [4]. The non-polar stationary phase was a layer of silica gel GF254 impregnated with silicone DC200 (350 cS) from Applied Science Labs. (State College, PA, USA). In order to obtain a better control of the pH of the stationary phase, the slurry of silica gel GF254 was obtained with 0.36 M sodium hydroxide solution. The mobile phase was glycine buffer at pH 9.0 alone or mixed with various amounts of acetone, methanol or acetonitrile (4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 45, 50, 55, 60, 65, 70, 75, 80%). The test compounds were dissolved in water, methanol or dilute hydrochloric acid (1–2 mg/ml) and 1 μl of solution was spotted randomly on the plates. The developed plates were dried and the spots detected under UV light (254 nm). The R_M values were calculated by means of equation $R_M = \log[(1/R_F) - 1]$. For each R_F measurement at least 4–6 replications were carried out. Averaged standard deviations were calculated for the R_F measurements of most of the investigated compounds. They resulted to be in the range of those calculated for many series of chemicals in our laboratory over the last 30 years, i.e. from 0.01 to 0.03.

2.3. Determination of R_M values by C_{18} RP-HPTLC

The HPTLC determinations were carried out on Whatman $KC_{18}F$ plates as previously described [5]. Solvent mixtures of acetone–glycine buffer at pH 9.0 were used as mobile phase. The acetone concentration ranged from 30 to 70%. The solutes were detected under UV light.

2.4. Octanol–water partition coefficients

The octanol–water partition coefficients were obtained from the QSAR program [6].

3. Results

3.1. R_M values from silicone RP-TLC

The R_M values of the compounds in Fig. 1 were measured with a mobile phase of glycine buffer pH 9.0 alone or mixed with various amounts of acetone, methanol or acetonitrile. In fact most of the compounds listed in Table 1, because of their lipophilicity, did not migrate when only the aqueous buffer was used as mobile phase. As a consequence, an organic modifier had to be added to the mobile phase. The chromatographic work showed that for each compound there existed a linear relationship between the R_M values and a certain concentration range of organic solvent in the mobile phase. The equations of the straight lines are reported in Table 1, where their intercepts ($R_M = a$) represent the theoretical R_M values at 0% organic solvent in the mobile phase. In Table 1 are also reported the ranges of organic solvent concentrations used for the calculation of these equations; the number of investigated modifier percentages for most of the compounds varied from 4 to 17, depending on the more or less wide range of the linear relationship. In the case of

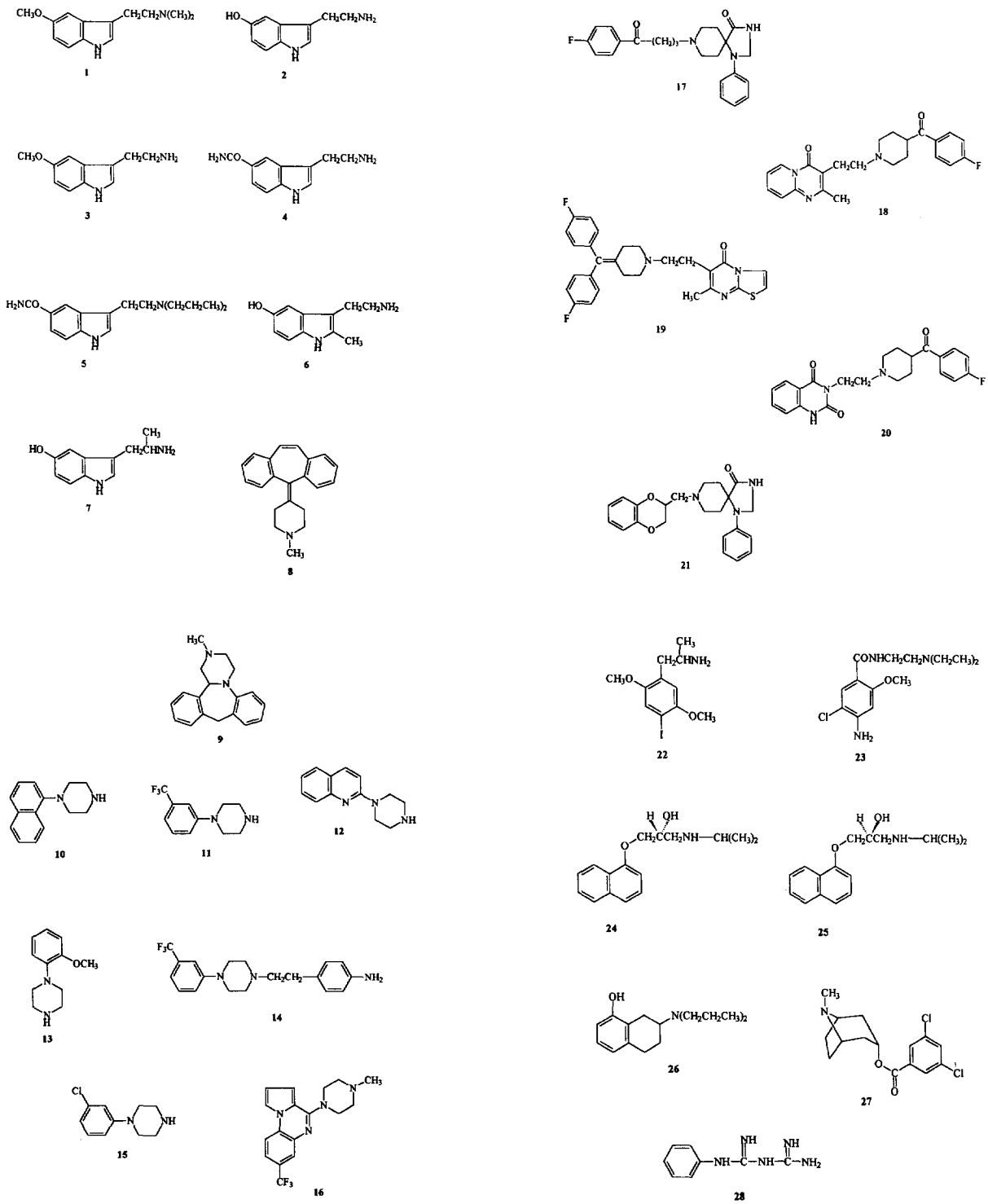

Fig. 1. Structures of the compounds investigated.

Table 1
TLC equations of serotonergic ligands at pH 9.0 in acetone, acetonitrile and methanol system

Compound no.	$R_{M\ exptl}$	$R_M = a + b$ (% organic modifier)											
		Acetone				Acetonitrile				Methanol			
		a	*b*	*r*	Range	*a*	*b*	*r*	Range	*a*	*b*	*r*	Range
1		1.63	−0.030	0.999	32–40	1.62	−0.035	0.949	20–40	1.84	−0.022	0.992	36–60
2	0.77	0.73	−0.014	0.855	0–16	0.74	−0.009	0.935	0–32	0.75	−0.007	0.953	0–16
3	1.06	1.10	−0.016	0.971	0–20	1.10	−0.010	0.996	0–12	1.08	−0.008	0.946	0–75
4	0.78	0.79	−0.014	0.970	0–16	0.78	−0.020	0.890	0–16	0.83	−0.016	0.955	0–16
5		1.78	−0.031	0.985	32–55	1.41	−0.030	0.934	8–40	1.64	−0.020	0.965	20–80
6	0.97	0.99	−0.030	0.977	0–12	0.93	−0.022	0.990	0–12	0.90	−0.011	0.940	0–16
7	0.91	0.93	−0.032	0.982	0–16	0.88	−0.027	0.998	0–8	0.91	−0.011	0.920	0–20
8		3.01	−0.069	0.991	40–70	3.10	−0.060	0.936	45–60	3.26	−0.042	0.974	50–80
9		2.60	−0.044	0.995	28–65	2.22	−0.042	0.862	28–50	2.73	−0.036	0.985	40–75
10		2.23	−0.043	0.952	40–55	2.23	−0.044	0.998	55–70	2.54	−0.028	0.981	40–90
11		2.21	−0.040	0.922	36–60	1.82	−0.035	0.967	12–40	2.18	−0.025	0.990	36–80
12		2.21	−0.043	0.962	32–60	1.70	−0.034	0.932	16–36	2.13	−0.029	0.990	24–60
13		1.45	−0.034	0.994	20–40	1.45	−0.026	0.956	16–40	1.70	−0.022	0.985	20–60
14		3.01	−0.069	0.993	28–65	2.56	−0.060	0.959	20–40	2.67	−0.039	0.988	40–80
15		1.73	−0.038	0.998	20–65	1.74	−0.033	0.939	12–75	1.91	−0.022	0.986	24–80
16		2.89	−0.060	0.996	40–70	2.62	−0.052	0.855	28–40	3.18	−0.041	0.983	45–75
17		2.54	−0.052	0.992	24–65	2.38	−0.056	0.952	16–36	2.67	−0.039	0.987	36–75
18		2.08	−0.048	0.988	32–50	2.51	−0.048	0.972	24–40	2.46	−0.037	0.971	36–80
19		2.89	−0.064	0.985	40–70	2.54	−0.056	0.966	32–40	2.90	−0.034	0.994	55–80
20		2.35	−0.056	0.996	20–45	2.52	−0.055	0.982	20–40	2.36	−0.037	0.984	36–80
21		2.47	−0.050	0.991	40–70	2.64	−0.048	0.929	20–36	2.73	−0.039	0.978	45–80
22		2.16	−0.052	0.995	36–70	2.17	−0.045	0.955	20–40	2.35	−0.029	0.987	40–80
23		1.84	−0.033	0.996	32–60	1.84	−0.036	0.939	12–40	2.00	−0.024	0.947	32–80
24		2.24	−0.049	0.993	36–70	2.14	−0.043	0.936	16–36	2.10	−0.025	0.990	40–80
25		2.39	−0.056	0.997	40–60	2.34	−0.052	0.931	20–36	2.42	−0.033	0.983	40–80
26		2.16	−0.056	0.990	32–70	2.22	−0.049	0.957	20–40	2.33	−0.030	0.990	36–80
27		2.46	−0.024	0.999	50–65	2.20	−0.020	0.954	50–80	2.12	−0.016	0.920	70–80
28	0.90	0.88	−0.021	0.981	0–12	0.93	−0.022	0.829	16–30	0.83	−0.019	0.843	0–8
Mean slope ($x \pm$ S.E.)		−0.042 (±0.003)				−0.038 (±0.003)				−0.026 (±0.002)			
E_o		0.56				0.65				0.95			
$1/E_o$		1.78				1.54				1.05			

compounds 2,3,4,6,7 and 28 a reliable R_M value could be measured even at 0% organic modifier. Therefore in Table 1 both the experimental and extrapolated R_M values are reported for these six compounds.

The present chromatographic data agree with the basic aspects of the TLC equations, already discussed in previous papers [7–9].

First of all, Eqs. 1–3 show good correlations between experimental and extrapolated R_M values in each RP-TLC system:

For acetone

$$R_{M\ exptl} = 0.107(\pm 0.102) + 0.869(\pm 0.112)R_{M\ extrap} \quad (1)$$

($n = 6$; $r = 0.968$; $s = 0.034$; $F = 60.255$)

For acetonitrile

$$R_{M exptl} = 0.146(\pm 0.096) + 0.843(\pm 0.107)R_{M\ extrap} \quad (2)$$

($n = 6$; $r = 0.969$; $s = 0.030$; $F = 62.563$)

For methanol

$$R_{M\ exptl} = 0.106(\pm 0.186) + 0.897(\pm 0.209)R_{M\ extrap} \quad (3)$$

($n = 6$; $r = 0.906$; $s = 0.053$; $F = 18.341$)

This is in agreement with our recent equation correlating experimental and extrapolated R_M values for 240 data points in different RP-TLC systems [7].

A second basic aspect is illustrated by Eqs. 4–6, which show that quite similar extrapolated R_M values could be obtained, whether the organic modifier was acetone, methanol or acetonitrile [7].

$$R_{M\ acetonitrile} = 0.107(\pm 0.121) + 0.903(\pm 0.057)R_{M\ acetone} \quad (4)$$

($n = 28$; $r = 0.951$; $s = 0.208$; $F = 246.80$)

$$R_{M\ methanol} = 0.021(\pm 0.108) + 1.021(\pm 0.051)R_{M\ acetone} \quad (5)$$

($n = 28$; $r = 0.969$; $s = 0.185$; $F = 398.81$)

$$R_{M\ acetonitrile} = 0.111(\pm 0.094) + 0.873(\pm 0.043)R_{M\ methanol} \quad (6)$$

($n = 28$; $r = 0.970$; $s = 0.164$; $F = 411.49$)

The analysis of the residuals in Eqs. 4–6 shows that in each equation only 1 or 2 points tend to deviate from the correlation. However, this does not affect the substantial overlapping R_M values from different organic modifiers, as already shown for many series of compounds [7].

Another feature of our chromatographic technique is described by the plots in Fig. 2. As already pointed out [7–9], for series of congeneric compounds, the relationship between intercepts and slopes of the TLC equations can be described by a straight line. In all three chromatographic systems only compound 27 seems to deviate from linearity, breaking down the congenerity of the group. As a consequence, Eqs. 7–9 were calculated without this derivative.

For acetone

$$\text{Intercept} = 0.230(\pm 0.149) - 41.147(\pm 3.301)\text{slope} \quad (7)$$

($n = 27$; $r = 0.928$; $s = 0.267$; $F = 155.41$)

For acetonitrile

$$\text{Intercept} = 0.208(\pm 0.123) - 43.381(\pm 2.979)\text{slope} \quad (8)$$

($n = 27$; $r = 0.946$; $s = 0.222$; $F = 212.08$)

For methanol

$$\text{Intercept} = 0.244(\pm 0.142) - 67.293(\pm 4.930)\text{slope} \quad (9)$$

($n = 27$; $r = 0.939$; $s = 0.263$; $F = 186.28$)

Finally, it can be shown that the slopes of the TLC equations in a given solvent system are related to the eluting power of the organic modifier, as expressed by the reciprocal of its solvent strength parameter ($1/E_o$). In particular, in agreement again with previous papers [8,9], the ratios between the mean slopes in two different solvent systems are close to the ratios between the $1/E_o$ values for the corresponding solvents. The slopes of the TLC equations for the present series of derivatives were averaged and are reported in Table 1, which also lists the $1/E_o$ values for each solvent. When considering the mean ratios, those referred to acetone–acetonitrile, acetone–methanol and acetonitrile–methanol, i.e., 1.10, 1.61 and 1.46, are close to the ratios between the corresponding $1/E_o$ values, i.e., 1.15, 1.70 and 1.47, respectively.

The same kind of conclusion can be reached by means of the b values of the equations 7–9 correlating intercepts and slopes of the TLC equations. In fact the ratios between the b values of these equations are close to the ratios between the corresponding E_o values as listed in Table 1. In particular, the ratios referring to acetone–acetonitrile, acetone–methanol and acetonitrile–methanol, i.e., 0.95, 0.61 and 0.64, can be compared with the corresponding E_o ratios, i.e., 0.86, 0.59 and 0.68, respectively. In a previous paper [8], it was shown why in this case the E_o values are used instead of their reciprocals, $1/E_o$.

3.2. R_M values in C_{18} RP-HPTLC

The chromatographic work carried out at pH 9.0 with Whatman $KC_{18}F$ plates showed the usual linear relationship between R_M values and

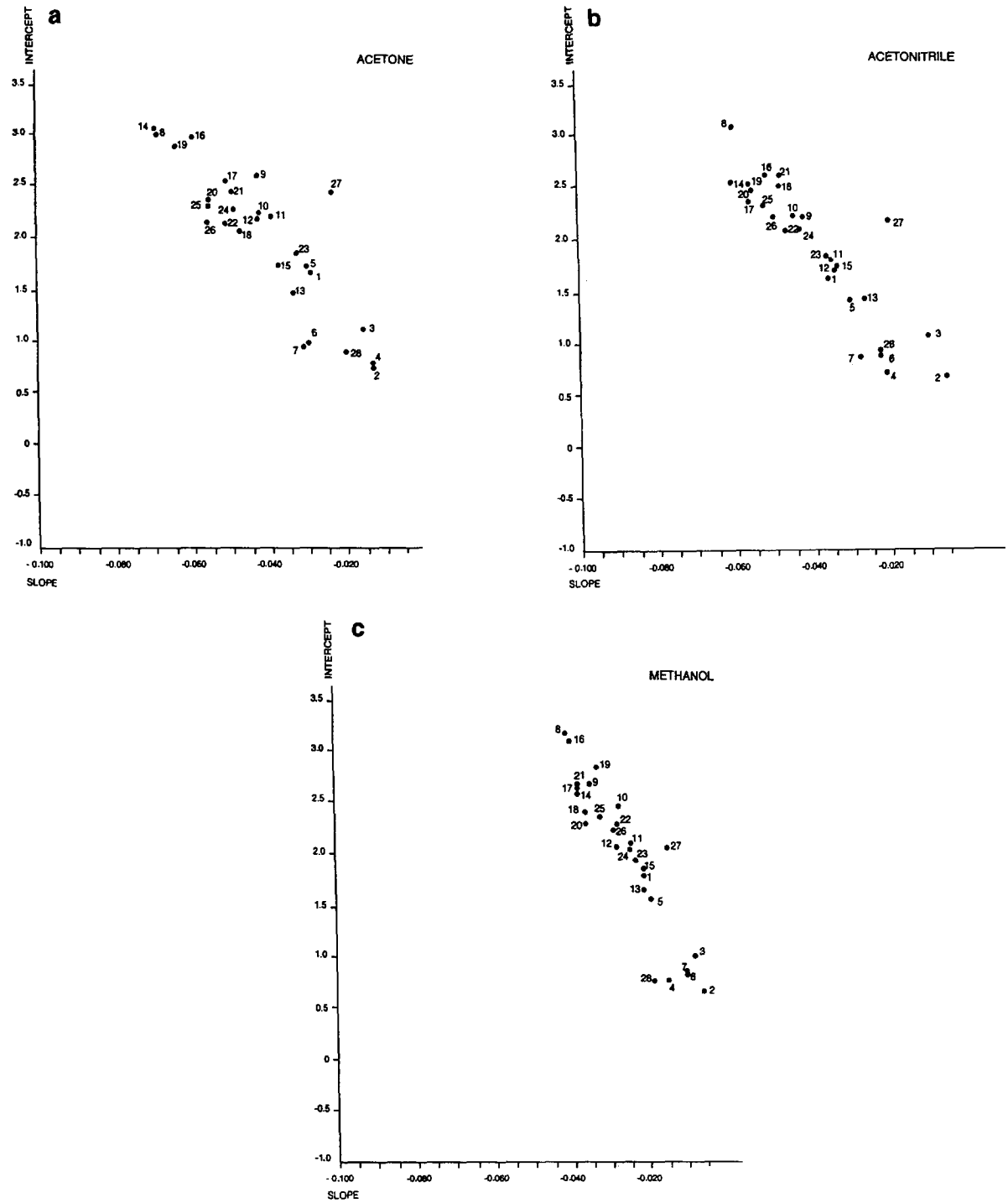

Fig. 2. Relationships between intercepts and slopes of the TLC equations in different solvent systems: (a) acetone, (b) acetonitrile and (c) methanol.

acetone concentration for each compound. The equations of the straight lines yielded the extrapolated $R_{M\ C18}$ values at 0% acetone (Table 2). Since Eqs. 4–6 show that the R_M values from the acetone, acetonitrile and methanol systems (Table 1) are quite similar, these were averaged and are reported in Table 2 as $R_{M\ mean}$. The relationship between the $R_{M\ mean}$ and R_{MC18} values is described by the following equation:

$$R_{M\ mean} = 0.629(\pm 0.090) + 0.927(\pm 0.055)R_{M\ C18} \quad (10)$$

($n = 28$; $r = 0.957$; $s = 0.205$; $F = 280.24$)

The slope of Eq. 10, close to 1, shows that the sensitivity to lipophilicity changes is fairly the same in both chromatographic systems. On the other hand, the intercept points out that a given compound seems to be more lipophilic when measured in the silicone RP-TLC system than in the C_{18} RP-HPTLC system.

3.3. Octanol–water partition coefficients

The QSAR program produced the calculated log P (CLOGP) values listed in Table 2. The relationships between chromatographic parameters and CLOGP values are described by Eqs. 11 and 12.

Table 2
Chromatographic parameters, partition coefficients and ionization constants of serotonergic drugs

Compound	Name	$R_{M\,mean}$	$R_{M\ C18}$	CLOGP	pK_a	log $D^a_{9.0}$
1	5-Methoxy-N,N-dimetyhyltryptamine	1.70	0.93	1.85		
2	Serotonin	0.74	0.24	0.61	9.97	−0.41
3	5-Methoxytryptamine	1.09	0.74	1.29		
4	5-Carboxamidotryptamine	0.80	0.45	−0.02		
5	N,N-Dipropyl-5-carboxamidotryptamine	1.61	0.94	2.41		
6	2-Methylserotonin	0.94	0.22	1.06		
7	α-Methylserotonin	0.91	0.18	0.92		
8	Cyproheptadine	3.12	2.23	4.92	8.87	4.68
9	Mianserin	2.52	1.89	4.41		
10	1-(1-Naphthyl)piperazine	2.33	1.82	2.99		
11	1-(3-Trifluoromethylphenyl)piperazine	2.07	1.69	3.00	8.85	2.76
12	Quipazine	2.01	1.64	2.25	8.82	2.03
13	1-(2-Methoxyphenyl)piperazine	1.53	0.75	1.84	9.37	1.31
14	*p*-Aminophenylethyl-*m*-trifluoromethlphenyl piperazine	2.75	2.13	4.57		
15	1-(3-Chlorophenyl)piperazine	1.79	1.18	2.70	8.85	2.47
16	CGS-12066B	2.90	2.34	4.56		
17	Spiperone	2.53	2.05	3.21	9.09	2.87
18	Pirenperone	2.34	2.00	2.23		
19	Ritanserin	2.78	2.51	4.87		
20	Ketanserin	2.41	1.91	3.02		
21	Spiroxatrine	2.61	2.34	3.14		
22	± -1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane	2.23	1.55	2.51		
23	Metoclopramide	1.89	1.13	1.87	9.36	1.35
24	(−)-Propranolol	2.16	1.62	2.75	9.60	2.06
25	(+)-Propranolol	2.38	1.63	2.75	9.60	2.06
26	(±)-8-Hydroxydipropylaminotetralin	2.24	1.83	3.57		
27	MDL 72222	2.26	2.20	3.93		
28	1-Phenylbiguanide	0.88	0.73	−0.75		

[a] $D = P/1 + 10^{pK_a - pH}$.

$$R_{M\ mean} = 0.844(\pm 0.107) + 0.440(\pm 0.036)\text{CLOGP} \quad (11)$$

($n = 28$; $r = 0.922$; $s = 0.272$; $F = 148.02$)

$$R_{M\ C18} = 0.352(\pm 0.141) + 0.428(\pm 0.048)\text{CLOGP} \quad (12)$$

($n = 28$; $r = 0.870$; $s = 0.357$; $F = 80.887$)

The similar slopes and different intercepts of Eqs. 11 and 12 are a consequence of the relationship described by Eq. 10.

As regards the CLOGP values, one has to remember that they are defined as octanol–water partition coefficients of the neutral form of the molecules. In order to estimate the effect of ionization on the partitioning at pH 9.0, the pK_a values of some compounds of the series listed in the QSAR database were used [6]. For ten derivatives the distribution coefficient at pH 9.0 ($\log D_{9.0}$) was calculated and the values are reported in Table 2. The relationships between chromatographic parameters and calculated $\log D$ values are described by Eqs. 13 and 14,

$$R_{M\ mean} = 1.074(\pm 0.157) + 0.448(\pm 0.064) \log D_{9.0} \quad (13)$$

($n = 10$; $r = 0.927$; $s = 0.250$; $F = 49.085$)

$$R_{M\ C18} = 0.539(\pm 0.174) + 0.414(\pm 0.071) \log D_{9.0} \quad (14)$$

($n = 10$; $r = 0.900$; $s = 0.277$; $F = 34.172$)

which do not show any significant improvement over Eqs. 11 and 12, respectively. This is likely to be due to the fact that the pK_a values for the considered derivatives are similar.

4. Discussion

The reliability of the R_M values as lipophilicity parameters has been established in a number of papers [7–11]. Recently, some basic aspects of the chromatographic technique were pointed out, i.e. the very good correlation between experimental and extrapolated R_M values as well as the correlations between extrapolated R_M values from different organic solvent systems; the relationship between intercepts and slopes of the TLC equations in series of congeneric compounds; and, finally, the influence of the organic modifier on the slopes of the TLC equations [7–9].

As regards the present series of serotonergic compounds, Eqs. 1–9 show that these derivatives behave in quite the same way as the previously investigated series of compounds. In particular, the deviation of only compound 27 from the relationship between intercepts and slopes described by Eqs. 7–9 shows that the series is mostly congeneric from the chromatographic point of view. This is not readily apparent when considering the variety of structures in Fig. 1. The deviation from congenerity of compound 27 could be a consequence of its large hydrophobic surface area, which is characterized by two bulky and apolar moieties such as tropane and dichlorophenyl groups. In a previous paper, we proposed to relate the chromatographic congenerity of the compounds of a series to their available hydrophobic surface [7]. The occurrence of the two mentioned structural elements, strongly affecting the size and shape of the hydrophobic surface, might cause compound 27 not to fit the relationship; this compound might belong to another series with respect to all the other derivatives.

In previous reports the use of a C_{18} RP-HPTLC system to measure the same chromatographic index was described [12,13]. For the present series of serotonergic derivatives the two chromatographic systems seem to be quite similar in detecting variations in lipophilicity (Eq. 10).

The relationships described by Eqs. 11 and 12 show the good correlation between experimental chromatographic parameters and theoretical CLOGP values. Considering the ionizable nature of all the compounds of Fig. 1, one could have expected a different result. The good correlations of Eqs. 11 and 12 can be explained only by assuming that ionization affects all the derivatives in almost the same way. In fact the pK_a values of Table 2 are rather close and inspection

of the structures in Fig. 1 allows to assume a similar behaviour for the remaining compounds.

However, the correlations in the two chromatographic systems are closer when dealing with the distribution coefficients (corrected for ionization, log D) than with the partition coefficients (uncorrected CLOGP). This points to a higher sensitivity of the C_{18} stationary phase to changes of pK_a and consequently of the ionized fraction of the compounds. An explanation of this behaviour might be the presence of free silanol groups in the silicone system, allowing polar interactions to take place between the solute and the stationary phase. This is not likely to happen with the octadecyl phase.

In conclusion, the present data show that despite the large variety of chemical structures, the investigated serotonergic compounds show a similar chromatographic behaviour in two different TLC systems. The two series of R_M values are in turn well correlated with the calculated log P values, and this, besides confirming the reliability of R_M values, points out their suitability in checking calculated log P values.

References

[1] R.A. Glennon, J. Med. Chem., 30 (1987) 1.
[2] E. Zifa and G. Fillion, Pharmacol. Rev., 44 (1992) 402.
[3] D. Hoyer, D.E. Clarke, J.R. Fozard, P. Hartig, G.R. Martin, E.J. Mylecharane, P.R. Saxena and P.P.A. Humphrey, Pharmacol. Rev., 46 (1994) 158.
[4] G.L. Biagi, A.M. Barbaro, M.F. Gamba and M.C. Guerra, J. Chromatogr., 41 (1969) 371.
[5] M.C. Pietrogrande, P.A. Borea and G.L. Biagi, J. Chromatogr., 447 (1988) 404.
[6] QSAR Program Ver. 1.87, BioByte Corp., Claremont, CA, 1994.
[7] G.L. Biagi, A.M. Barbaro, A. Sapone and M. Recanatini, J. Chromatogr. A, 662 (1994) 341.
[8] G.L. Biagi, A.M. Barbaro and M. Recanatini, J. Chromatogr. A, 678 (1994) 127.
[9] G.L. Biagi, A.M. Barbaro, A. Sapone and M. Recanatini, J, Chromatogr. A, 669 (1994) 246.
[10] G.L. Biagi, M. Recanatini, A.M. Barbaro, M.C. Guerra, A. Sapone, P.A. Borea and M.C. Pietrogrande, in C. Silipo and A. Vittoria (Editors), QSAR: Rational Approaches to the Design of Bioactive Compounds, Elsevier, Amsterdam, 1991, p. 83.
[11] R. Kaliszan, Quantitative Structure–Chromatographic Retention Relationships, Wiley, New York, 1987.
[12] G.L. Biagi, M.C. Pietrogrande, A.M. Barbaro, M.C. Guerra, P.A. Borea and G. Cantelli-Forti, J. Chromatogr., 469 (1989) 121.
[13] G.L. Biagi, M.C. Guerra, A.M. Barbaro, S. Barbieri, M. Recanatini, P.A. Borea and M.C. Pietrogrande, J. Chromatogr., 498 (1990) 179.