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1,2,3,4-Tetrahydroisoquinoline derivatives; lipophilicity evaluation vs. 5-HT_{1A} receptor affinity

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Retention parameters (log k′) of 1,2,3,4-tetrahydroisoquinoline derivatives – a new class of 5-HT_{1A} receptor ligands – were determined on the basis of reversed-phase HPLC experiments. Good correlations were found between the log k′ values and the calculated log P_c, van der Waals volume of the R substituent (V_R) as well as the 5-HT_{1A} receptor affinity (K_i) of the investigated compounds. It was demonstrated that hydrophobic forces played a pivotal role in stabilizing the ligand-receptor bioactive complex for that group of compounds.

1. Introduction

It is generally accepted that one of the most characteristic features of the 5-HT_{1A} receptor binding sites is the presence of a region acommodating bulky substituents [1]. The nature of interactions in this region is complex, but it has been established that hydrophobic forces play a major role in stabilization of the ligand-receptor bioactive complex [2–4]. We used the recently synthesized new class of 5-HT_{1A} receptor ligand (THIQ) derivatives **1a–c–10a–c** [5] in order to determine how strongly interactions of that type could influence the binding of those compounds to the receptor. The hydrocarbon character of R substituents in **1–10** resulted almost exclusively in hydrophobic interactions. Moreover, separation of R from the basicity center allowed us to evaluate directly the contribution of hydrophobic forces to the global affinity of the compounds.

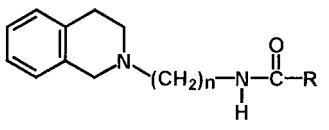
Partition chromatography is one of the methods for evaluation of lipophilicity, log P, of compounds. The chromatographic index log k′, obtained from HPLC procedures, can be directly applied as a measure of log P (eqs. 1 and 2), which has been shown in a number of examples [6, 7].

$$\log P = a \log k' + b \quad (1)$$

$$\log k' = (t_r - t_0)/t_0 \quad (2)$$

where t_r and t_0 are retention times of the compound and the solvent front, respectively.

Chromatographic parameters of the three series (**a**, **b** and **c**) of derivatives and their relationship with the calculated log P_c, van der Waals volume of R substituent V_R, and the affinity K_i of the compounds are discussed in present paper.



	a	b	c
n =	2	3	4

No.	1	2	3	4	5	6
R:	$-CH_3$					

No.	7	8	9	10
R:		$-CH_2-$		

2. Investigations, results and discussion

Log k′ values were calculated from retention parameters which were measured using a methanol-aqueous buffer at different organic modifier concentrations – up to 50% in most cases. Excellent linearity of regression lines was obtained, and the correlation coefficients were 0.99 (with a few exceptions), but never lower than 0.97. In order to remove the influence of the organic solvent, extrapolated log k'_w values were calculated (Fig. 1, Table 1).

A controversy often arises over the application of the measured log k′ instead of log k'_w values [7] in such correlations, hence we probed log k'_{60} and log k'_w in relation to the calculated log P parameters (Table 2, eqs. 3–10). Statistically high correlations were obtained in each case, which proves that for a series of compounds in which only one element is changed, the directly measured k′ values can be used as descriptors of their lipophilic properties.

When the van der Waals volume of a substituent R (V_R) increased, higher log k′ values were obtained. Correlations between those two parameters were determined, and again linear relationships were obtained for both log k'_{60} and log k'_w (Table 2, eqs. 11–18). These results are consistent with the findings reported in the literature [8, 9] and show that V_R reliably reflects changes in the hydrophobic properties for such a closely related series of compounds.

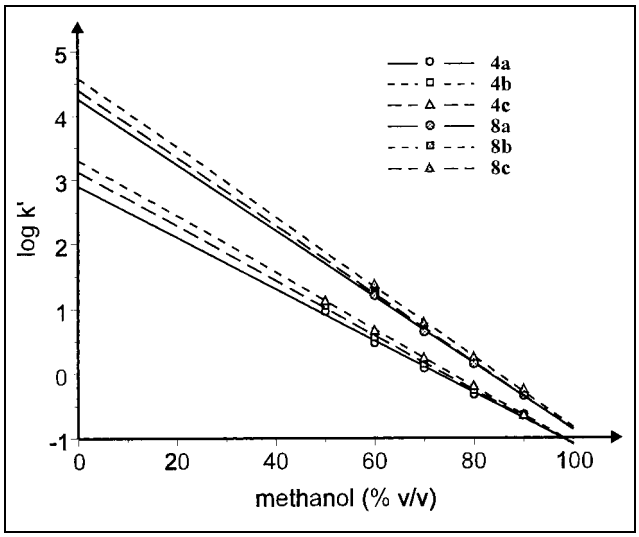


Fig. 1: Relationship between log k′ and volume fraction of methanol (φ) for compounds **4a–c** and **8a–c**. Example of correlation for **4b**: $\log k' = -0.42\varphi + 3.12$ ($r = 0.999$)

Table 1: Volume of substituents (R), calculated partition coefficients (log P_c[a]), binding constants to 5-HT_{1A} receptors (K_i), log k'_{60} and extrapolated log k'_w values of the investigated compounds

Compd.	Volume of R ($Å^3$)	log P_c[a]	5-HT_{1A} $K_i \pm$ SEM (nM)	log k'_{60}	log k'_w
1a	26.37	0.48	4400 ± 100	0.021	1.825
2a	53.44	1.22	4350 ± 100	0.281	2.755
3a	60.51	1.66	2400 ± 80	0.352	2.872
4a	68.65	1.72	1800 ± 70	0.467	2.898
5a	77.67	2.04	2050 ± 50	0.595	3.125
6a	84.12	2.29	450 ± 20	0.654	3.522
7a	100.27	2.79	40 ± 2	0.848	3.136
8a	122.94	2.94	81 ± 3	1.204	4.261
9a	123.74	3.12	54 ± 4	1.188	4.372
10a	142.14	3.37	15 ± 0.2	1.471	4.612
1b	26.37	0.84	11200 ± 800	0.085	2.660
2b	53.44	1.57	1850 ± 50	0.316	2.752
3b	60.51	2.01	1270 ± 50	0.402	2.562
4b	68.65	2.07	875 ± 50	0.555	3.120
5b	77.67	2.39	870 ± 40	0.691	3.456
6b	84.12	2.65	580 ± 40	0.718	3.447
7b	100.27	3.15	36 ± 3	0.949	3.441
8b	122.94	3.3	89 ± 5	1.247	4.398
9b	123.74	3.47	64 ± 15	1.261	4.497
10b	142.14	3.72	68 ± 1	1.665	5.139
1c	26.37	1.35	5700 ± 400	0.215	2.299
2c	53.44	2.08	900 ± 60	0.446	3.023
3c	60.51	2.52	600 ± 70	0.517	2.927
4c	68.65	2.58	170 ± 14	0.654	3.297
5c	77.67	2.9	920 ± 30	0.701	3.306
6c	84.12	3.16	92 ± 9	0.835	3.615
7c	100.27	3.66	25.5 ± 1.5	1.008	3.624
8c	122.94	3.81	77 ± 11	1.350	4.578
9c	123.74	3.98	2.4 ± 0.05	1.325	4.459
10c	142.14	4.23	0.95 ± 0.04	1.544	4.822

[a] log P_c were calculated using a Prolog P 5.1 expert system (CompuDrug Ltd., Hungary)

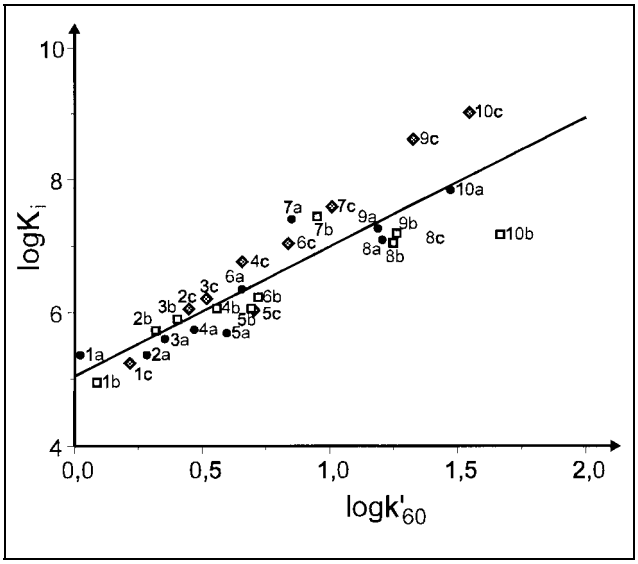


Fig. 2: Correlation between log k'_{60} and binding constants to 5-HT_{1A} receptors (K_i) for all the investigated compounds (eq. 19)

Since the compounds under discussion were synthesized as tools for probing hydrophobic properties of 5-HT_{1A} receptor binding sites, in the last step we correlated the observed pK_i values, obtained from radioligand binding assays, and the capacity parameters log k' (Table 2, eqs. 19–26). In all the three series tested, a significant correlation was obtained for both the log k'_{60} and log k'_w values. Additionally, a statistically significant correlation was obtained for the entire set of compounds (Table 2, eqs. 17 and 21; Fig. 2), which means that hydrophobic interactions dominated the biological activity of THIQ derivatives. These results not only support the well-known phenomenon that the lipophilic pocket at 5-HT_{1A} receptor binding sites can accommodate bulky substituents, but

Table 2: Linear correlations (y = a + b × x) of HPLC parameters (log k'_{60} and log k'_w) with volume of substituent (V_R), logarithms of theoretically calculated partition coefficients (P_c) and binding constants to 5-HT_{1A} receptors (K_i) for different series of compounds

Eq.	Correlation	Series of compounds	a	b	s	r	F	N
3	log $P_c = f(\log k'_{60})$	**1a–c–10a–c**	1.01	1.98	0.34	0.938	203.91	30
4	log $P_c = f(\log k'_{60})$	**1a–10a**	0.81	1.92	0.24	0.969	121.19	10
5	log $P_c = f(\log k'_{60})$	**1b–10b**	1.10	1.80	0.26	0.963	101.01	10
6	log $P_c = f(\log k'_{60})$	**1c–10c**	1.28	2.03	0.24	0.970	128.10	10
7	log $P_c = f(\log k'_w)$	**1a–c–10a–c**	−1.06	1.04	0.44	0.895	112.14	30
8	log $P_c = f(\log k'_w)$	**1a–10a**	−1.17	1.00	0.33	0.939	60.06	10
9	log $P_c = f(\log k'_w)$	**1b–10b**	−0.81	0.94	0.45	0.887	29.67	10
10	log $P_c = f(\log k'_w)$	**1c–10c**	−0.86	1.08	0.31	0.949	72.84	10
11	$V_R = f(\log k'_{60})$	**1a–c–10a–c**	26.29	75.99	6,28	0.984	867.87	30
12	$V_R = f(\log k'_{60})$	**1a–10a**	30.86	77.83	3.07	0.997	1241.35	10
13	$V_R = f(\log k'_{60})$	**1b–10b**	28.21	73.23	4.77	0.992	509.49	10
14	$V_R = f(\log k'_{60})$	**1c–10c**	15.13	82.44	3.38	0.9996	1022.25	10
15	$V_R = f(\log k'_w)$	**1a–c–10a–c**	−55.64	40.54	10.36	0.957	301.71	30
16	$V_R = f(\log k'_w)$	**1a–10a**	−49.42	40.57	9.72	0.967	116.68	10
17	$V_R = f(\log k'_w)$	**1b–10b**	−54.28	39.54	12.30	0.947	69.96	10
18	$V_R = f(\log k'_w)$	**1c–10c**	−73.14	44.26	6.47	0.986	273.58	10
19	log $K_i = f(\log k'_{60})$	**1a–c–10a–c**	5.04	1.95	0.47	0.885	100.95	30
20	log $K_i = f(\log k'_{60})$	**1a–10a**	5.02	1.90	0.36	0.935	55.21	10
21	log $K_i = f(\log k'_{60})$	**1b–10b**	5.22	1.46	0.38	0.896	32.51	10
22	log $K_i = f(\log k'_{60})$	**1c–10c**	4.83	2.48	0.50	0.916	41.97	10
23	log $K_i = f(\log k'_w)$	**1a–c–10a–c**	3.13	0.98	0.59	0.814	55.00	30
24	log $K_i = f(\log k'_w)$	**1a–10a**	3.31	0.92	0.55	0.838	18.92	10
25	log $K_i = f(\log k'_w)$	**1b–10b**	3.79	0.73	0.52	0.788	13.12	10
26	log $K_i = f(\log k'_w)$	**1c–10c**	2.24	1.32	0.56	0.895	32.26	10

they also show that hydrophobic interactions are strong enough to make the compounds potent 5-HT_{1A} receptor ligands.

3. Experimental

3.1. Synthesis

Synthetic procedures for the tested compounds are described elsewhere [5].

3.2. High performance liquid chromatography (HPLC)

The HPLC system consisted of a Waters 600E solvent delivery system fitted with a Purospher RP18e (125 × 3 mm) column (Merck) and coupled with a Waters 991J photodiode array detector. The column was maintained at room temperature, and the flow rate was 1.0 ml/min. Methanol-phosphate buffer (0.025M, ph 7) mixtures were used as mobile phases, a methanol content ranging from 50 to 90% (v/v) in 10% of increments. Concentrations of 5 mM were used, and the injection volume was 5 μl per sample. The isocratic capacity factor, log k′, was defined as $\log[(t_r - t_0)/t_0]$.

3.3. Radioligand binding assays

Radioligand binding experiments were conducted in the hippocampus of the rat brain for 5-HT_{1A} receptors and in the cortex for 5-HT_{2A} receptors according to published procedures [10]. The radioligand used were [^{3}H]-8-OH-DPAT (190 Ci/mmol, Amersham), and [^{3}H]-ketanserin (60 Ci/mmol, NEN Chemicals) for 5-HT_{1A} and 5-HT_{2A} receptors, respectively. The K_i values were determined on the basis of at least three competition binding experiments in which compounds **1**–**10** in concentrations of $10^{-10} - 10^{-3}$ M (run in triplicate), were used.

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