Journal of Pharmaceutical and Biomedical Analysis 48 (2008) 678–684

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Physico-chemical characterization of a novel group of dopamine D_3/D_2 receptor ligands, potential atypical antipsychotic agents

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ARTICLE INFO

Article history:
Received 21 March 2008
Received in revised form 24 June 2008
Accepted 24 June 2008
Available online 6 July 2008

Keywords:
Ionization
Lipophilicity
$\Delta \log P$
log BB
Antipsychotics

ABSTRACT

The fundamental physico-chemical properties such as ionization and lipophilicity of twelve alkyl–aryl-piperidine and aryl-piperazine derivatives have been determined. Compounds are members of a recently identified, new class of potent dopamine D_3/D_2 receptor ligands as potential atypical antipsychotic agents and were used in the development of a promising drug candidate (RGH-188) being present currently in clinical phase II investigations. The ionization constant (pK_a) and the partition coefficient in octanol/water ($\log P_{oct}$) and cyclohexane/water systems ($\log P_{ch}$) were measured by validated analytical methods. Based on the highly precise physico-chemical data the structure–property relationships (SPR) were studied. The effect of the polar and apolar heteroatoms as well as polar and apolar surface areas on the partition in the two solvent systems was investigated by linear regression and multivariate linear regression analyses. Brain/blood concentration ratios (BB values) as a function of time were determined by HPLC analyses on plasma and brain homogenates of Wistar rats. A linear relationship has been found between $\Delta \log P$ values ($\log P_{oct} - \log P_{ch}$) and experimental log BB values, verifying that physico-chemical data can predict pharmacokinetic behaviour.

1. Introduction

The physico-chemical properties, such as solubility, ionization (i.e., acidity, basicity), lipophilicity, permeability, H-bond donor/acceptor capacity, etc., have long been recognized as predictors of pharmacokinetic (ADME) parameters and have, therefore, been increasingly used in early stage of drug development [1–5].

The acid/base character sets the charge of a molecule in solution at a particular pH. It can be described by the dissociation constant (pK_a). The knowledge of the charge state is necessary for the understanding of absorption, transport and receptor binding of drugs at the molecular level. Lipophilicity is another molecular property of immense importance in medicinal chemistry. The logarithm of octanol/water partition coefficient ($\log P_{oct}$) is the most extensively used parameter to quantitate lipophilicity. The $\log P_{oct}$ value has been found to be a good predictor of the passive transport of drugs through the lipoidal membranes of the human body.

A further molecular property, the capability to penetrate through the blood–brain barrier (BBB) has fundamental importance in the drug design either the drug is intended to achieve high CNS or peripheral presence. The endothelial cells of the brain capillaries create the BBB, which is a specific physical barrier and a complex biochemical interface containing many physiological functions. Unfortunately, the *in vivo* experimental determination of brain/blood concentration ratio (Eq. (1)) is time-consuming, expensive and difficult. An additional problem is that in early screening of new chemical entities the synthesized material is not enough to carry out *in vivo* studies. *In vitro* biological models such as tissue culture monolayers may provide valuable additional data on transport processes in either direction beyond passive diffusion data; however, these methods are time-consuming, labor-intensive and have low throughput. Thus there is considerable interest in developing reliable, simple non-biological models for the prediction of blood–brain barrier permeation.

$$\mathrm{BB} = \frac{\text{concentration in brain}}{\text{concentration in blood}} \quad (1)$$

Lots of effort has been made to replace the animal experiments with *in vitro* or *in silico* methods for prediction of BBB penetration [6]. The recently developed PAMPA (parallel artificial membrane permeability assay) method using porcine brain lipids [7] still needs standardization and validation. Evidently, it would be useful if the log BB values could be predicted by an easy-to-measure physico-chemical parameter, like $\log P$. Young et al. [8] investigated 20

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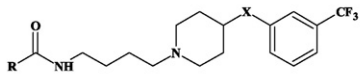
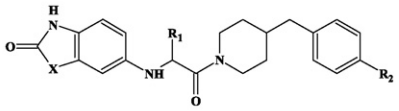
doi:10.1016/j.jpba.2008.06.021

Piperidinederivatives

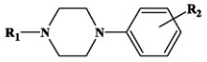


Compound	X	R
1	O	
2	NH	
3	O	



Compound	X	R_1	R_2
4	NH	H	F
5	O	=O	H

Piperazinederivatives



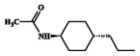
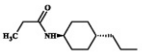
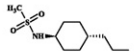
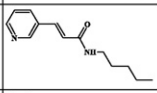
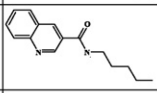
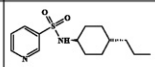
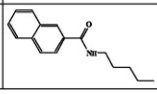
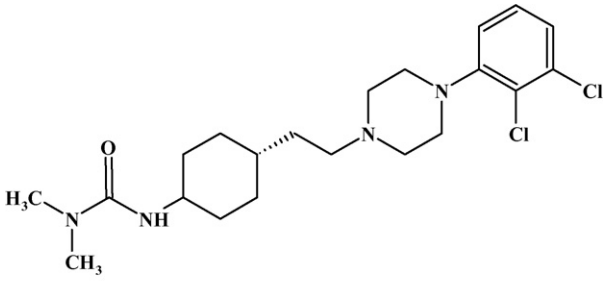
Compound	R_1	R_2
6		2 -Cl 3 -Cl
7		2 -Cl 3 -Cl
8		2 -Cl 3 -Cl
9		3 -CN 5 -CF_3
10		3 -CN 5 -CF_3
11		3 -CN 5 -CF_3
12		2 -OCH_3

Fig. 1. Structure of the molecules.

centrally acting H_2 receptor antagonists and reported poor correlation between log BB and $\log P_{oct}$ ($r = 0.436$). Better correlation was observed with cyclohexane/water partition coefficient ($\log P_{ch}$) ($r = 0.732$) and with the $\Delta \log P$ parameter of Seiler ($r = 0.831$), calculated as a difference between $\log P_{oct}$ and $\log P_{ch}$ [9]. The $\Delta \log P$ value is considered a measure of the hydrogen-bonding ability of a compound. The larger the $\Delta \log P$ value, the more capable the molecule to form hydrogen-bonding. Consequently, the more hydrophilic the molecule will be, and can, therefore, better distribute toward blood than brain, since blood is known to be more water-like and less lipophilic than brain [10]. However, others have found conflicting results in case of hydroxypyridinones where $\Delta \log P$ produced the weakest correlation and $\log P_{ch}$ proved to be the better predictor of brain penetration [11].

In our previous work the complex physico-chemical profiling of sertraline, a centrally acting antidepressant drug was studied, allowing interpretation of its excellent pharmacokinetics. The almost complete absorption is governed by the high lipophilicity (i.e., $\log P_{oct} = 4.30$) but the good brain penetration (and the high log BB coefficient) can be explained in terms of $\Delta \log P = 0$ value, i.e., the lack of polar interactions [12].

In this paper we report a study on physico-chemical profiling of a recently synthesized set of compounds discovered for antipsychotic activity. The molecules containing aryl-piperidine or aryl-piperazine moiety (Fig. 1) act on dopamine D_3/D_2 receptors. There are several antipsychotic drugs in the therapy containing similar moieties, for example, aripiprazole, ziprasidone and risperidone, which display $5\text{-}TH_{2A} > D_2$ antagonist activity. The hypothesis of this development was that (i) a moderate degree of D_2 antagonism is a prerequisite to antipsychotic activity (ii) D_3 antagonism may carry favourable effects such as cognitive enhancement and lack of catalepsy and (iii) in order to achieve simultaneous behavioural manifestation of D_2 and D_3 receptor antagonism the antipsychotic drug candidate should be more potent on D_3 than on D_2 receptors [13]. Several compounds were evaluated in a 3D-QSAR study using CoMFA based on their affinity to D3 receptors. The synthesis, the receptor affinity and the pharmacokinetic properties of some representatives were reported earlier [14]. RGH-188 was found as the most potent dopamine D_3/D_2 receptor antagonist/partial agonist in the series. The compound fulfils the above requirements, displayed high *in vitro* affinity at cloned human D_3 receptors ($K_i = 0.085$ nM) with about six-fold less affinity for D_2 receptors. It potently inhibited apomorphine-induced climbing in mice ($ED_{50} = 0.27$ mg/kg). RGH-188 did not produce catalepsy in rats up to 100-fold dose of its ED_{50} value [13]. Owing to its good pharmacologic features (beneficial cognitive effects) and favourable side effect profile (low risk of EPS liability), RGH-188 is considered to be a promising novel antipsychotic drug candidate, developed in cooperation between Gedeon Richter Plc. (Budapest, Hungary) and Forest Laboratories, Inc. (New York, USA) and being present currently under clinical phase II trials [15].



RGH-188

The objective of the present study was to determine the most important physico-chemical properties of **1**–**12** compounds from the antipsychotic D_3/D_2 project resulting finally RGH-188, including the aqueous dissociation constant (pK_a), the partition coefficient ($\log P$) in both octanol/water and cyclohexane/water solvent systems using validated methods. Based on the obtained, highly precise experimental data our purpose was to reveal the structure–property relationships (SPR), and to predict the pharmacokinetic properties, and to investigate the validation of log BB vs.

$\Delta\log P$ correlation. Our further aim was to understand the main molecular factors, which determine the partition processes of this series of compounds.

2. Experimental

2.1. Materials

Samples of **1**–**12** were synthesized in Gedeon Richter Plc. (Budapest, Hungary) according to methods described in Hungarian [16–18] and US [19] patents and published partly in Ref. [14]. The purity of the samples was above 95%. The distilled water was of pharmacopeial grade [20] and all other reagents of analytical grade were purchased from commercial suppliers. The Britton-Robinson buffer (acetic, phosphoric and boric acids, each at 0.04 M, treated with 0.2 M NaOH) was used as the aqueous phase of different pH in lipophilicity measurements.

2.2. Apparatus

For the potentiometric titrations PCA 101 and GLpKa automated pK_a and log P analyzers (Sirius Analytical Instr. Ltd., Forest Row, UK), for the spectrophotometric measurements Jasco V-550 spectrometer (Easton, USA), for pH measurements Radiometer MeterLab PHM200 pH meter (Lyon, France), for separation of partitioning phases Hermle Z 382 K centrifuge (Wehingen, Germany), for shaking Lauda M20S thermostated shaker (Lauda-Königshofen, Germany) and for bioanalysis HP 1050 and HP 1100 HPLC (Hewlett-Packard, Palo Alto, USA) were used.

2.3. Softwares

The STATISTICA 7.1 mathematical and statistical software package was used for the calculation of the linear regression equations. Polar surface areas were calculated by ChemDesk, a web-based in-house toolkit of Gedeon Richter Plc. Its PSA module calculates polar surface as the sum of solvent accessible surface of polar atoms including attached hydrogens on the 3D geometry obtained by Concord.

2.4. Methods

2.4.1. Electrode calibration

The four-parameter procedure was used for electrode standardization in both aqueous and semi-aqueous media [21,22]. HCl solutions of known concentration, containing 0–62.71 wt% methanol were titrated with standardized KOH at 25.0 ± 0.1 °C, at 0.15 M ionic strength using KCl, under N_2 atmosphere, in the pH interval 1.8–12.2. The operational pH reading was related to p_cH values by the standard multiparametric equation:

$$pH = \alpha + Sp_cH + j_H[H^+] + j_{OH}K_w/[H^+] \quad (2)$$

where α corresponds to the negative logarithm of the activity coefficient of $[H^+]$ at working temperature and ionic strength, S is the ratio between the electrode slope and the Nernst slope. The j_H and j_{OH} terms correct the electrode junction effects at low and high pH, respectively. The parameters were determined by a weighted non-linear least squares procedure.

2.4.2. Determination of the dissociation constants

Due to the poor water solubility of the molecules the dissociation constants (pK_a) were determined by either potentiometric *co-solvent* method [23] or UV-pH titration [24].

2.4.2.1. Potentiometric co-solvent method. 6–10 ml of 0.67–1.32 mM semi-aqueous solutions of the samples containing 26.0–62.71 wt% methanol were pre-acidified to pH 1.8–3.0 with 0.5 M HCl, and were then titrated with 0.5 M KOH to appropriate high pH (maximum 12.2). The titrations were performed at 25.0 ± 0.1 °C, under nitrogen atmosphere, at I = 0.15 M ionic strength using KCl. Measurements were carried out in three different methanol–water mixtures. For each molecule a minimum of three but typically six separate titrations were performed. The apparent ionization constants in the mixed solvent (p_sK_a) were calculated from the difference (Bjerrum) plot. The Yasuda-Shedlovsky (YS) procedure was applied to estimate the aqueous pK_a value.

2.4.2.2. UV-pH titration. In case of compound **10**, a 1.0 mM stock solution of the sample was prepared in methanol. 500 μl aliquot of the stock solution was diluted to 15 ml with 0.15 M KCl solution to produce the required sample concentration. In each experiment, the pH of the sample solution was adjusted to pH 2 using 0.5 M HCl and then titrated with 0.5 M KOH to pH 10. Spectral data were recorded in the region of 200–700 nm after each pH measurement. The titrations were performed at 25.0 ± 0.1 °C, under nitrogen atmosphere, at I = 0.15 M ionic strength using KCl. Three parallel measurements were carried out and the pK_a values of samples were calculated by Target Factor Analysis.

2.4.3. Determination of the partition coefficient in the octanol/water system

The logarithm of octanol/water partition coefficient ($\log P_{oct}$) was determined by the dual-phase potentiometric titration [25,26].

8–10 ml of 0.81–1.38 mM aqueous solutions of samples were titrated under the same conditions as in pK_a determinations but with the presence of a partitioning solvent, water-saturated octanol. The phase ratio applied was varied from 16.0 ml water −0.1 ml octanol to 10.0 ml water −0.6 ml octanol, depending on the expected $\log P_{oct}$ value of the compound. 3–7 parallel titrations were carried out. From the octanol-containing titrations the apparent ionization constants (p_oK_a) and then the log P values were estimated and refined by a weighted non-linear least squares procedure where the pK_a values were used as unrefined contributions.

2.4.4. Determination of the partition coefficient in the cyclohexane/water system

The partition coefficient in the cyclohexane/water system ($\log P_{ch}$) was determined by the traditional shake-flask method [27–29].

Before measurements, the aqueous and organic phases were saturated with each other by shaking. The phases were then allowed to separate on standing and they were then filtered. Britton-Robinson buffer solutions were used as the aqueous phase for the pH range 3.64–10. 0–10% methanol was used in the aqueous medium for increasing the solubility of the compounds. Since methanol has very low solubility in cyclohexane, the used content of methanol does not influence the partition. The effect of methanol on the partition has been previously studied experimentally by us in case of some compounds. The same log P results were obtained with and without methanol. The phase ratio applied was varied from 20.0 ml water −0.25 ml cyclohexane to 5.0 ml water −20.0 ml cyclohexane, depending on the expected $\log P_{ch}$ value of the compound. The sample was dissolved in the aqueous phase, the concentration was measured by spectrophotometry at the wavelength of the absorption maximum of the compound before the partition, then cyclohexane was added to the solution and the phases were equilibrated by shaking for 1 h, at 25.0 ± 0.1 °C. After separation of the phases in a centrifuge at 2000 rpm for 10 min, the concentration of the solute was determined in the aqueous phase again (after parti-

Table 1
pK_a values obtained by Yasuda-Shedlovsky extrapolation

Compound	wt% methanol	$p_sK_a + \log[H_2O] = a/\varepsilon + b$		R^2	n	p$K_a \pm$ S.D.
		a	b			
1	37.8–53.9	−172.6	12.591	0.9921	6	8.64 ± 0.01
2	37.7–53.4	−155.1	12.509	0.9937	6	8.78 ± 0.01
3	26.0–45.3	−203.1	7.555	0.9940	4	3.22 ± 0.03
		−167.2	12.406	0.9630		8.53 ± 0.04
4	38.14–54.99	−149.4	7.112	0.9958	6	3.64 ± 0.02
5	36.81–60.74	12.5	10.048	0.9810	6	8.14 ± 0.01
6	38.2–54.4	−179.7	12.036	0.9940	6	8.00 ± 0.02
7	37.8–53.5	−170.3	11.909	0.9919	6	7.99 ± 0.01
8	45.6–54.4	−213.9	12.540	0.9910	6	8.06 ± 0.02
9	30.97–50.79	−250.1	8.895	0.9967	6	3.95 ± 0.02
		−191.3	11.468	0.9795		7.27 ± 0.04
10	36.52–62.71				3	3.23 ± 0.02[a]
		−237.0	12.282	0.9710	3	7.50 ± 0.14
11	34.95–53.27	−269.6	13.028	0.9906	6	7.83 ± 0.03
		34.3	11.948	0.9795		10.60 ± 0.04
12	34.4–46.7	−167.0	11.809	0.9955	6	7.93 ± 0.01

[a] Determined by UV-pH titration in aqueous medium.

tion). From the concentration decrease the log P_{ch} was calculated. 4–11 parallel measurements were performed.

2.4.5. Determination of log BB values

Animal maintenance and research were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals. All the procedures carried out on animals had been approved by the local ethical committee.

12 male Wistar rats (∼200 g, Toxicoop) were administered the investigated compound per os by gavage at the dose of 10 mg/kg. Three animals were bled by decapitation per time points of 0.25, 1, 2 and 5 h post-dose. Blood samples of the animals were collected into heparinized tubes and centrifuged at 1000 × *g* for 20 min. The harvested plasma was stored at −20 °C until analysis. Whole brain samples of the animals were homogenized in 2.5-fold volume of water by using Ultra-turrax T25 homogenizer and the resultant homogenates were stored at −20 °C until analysis.

HPLC with UV detection was used for the quantitative determination of each compound in the plasma and brain, against calibration curves from 6 matrix-spiked standards. Generally 0.5 ml of sample (plasma or brain homogenate) was extracted by 5 ml of *tert*-butyl methyl ether or chlorobutane, the organic phase evaporated to dryness under N_2 stream. The reconstituted residue was measured by HPLC at conditions appropriately adjusted for the investigated compound. Area under the tissue-concentration vs. time curve (AUC) was calculated by the trapezoidal rule both for plasma and brain. The log BB value was calculated as $\log(AUC_{brain}/AUC_{plasma})$.

3. Results and discussion

3.1. Ionization

The examined compounds (**1**–**12**) differ considerably in acid–base properties representing a variety of proton-binding sites in both the type and the strength. There are *monovalent* bases (**1**, **2**, **4**, **6**–**8**, **12**) with piperidine (**1**, **2**), piperazine (**6**–**8**, **12**) and aniline nitrogen atom(s) (**4**). Compounds **3**, **9**, **10** are *bivalent* bases having two proton-binding sites: piperidine N and quinoline N (**3**); piperazine N and pyridine N (**9**) or quinoline N atom (**10**). Compound **5** has weak acidic character due to the imino-group of benzoxazolone ring, while compound **11** is the only ampholyte molecule within the series. In its structure the piperazine N is the basic, the sulfonamide moiety is the weak acidic functional group.

The ionization ability of the molecules is characterized here by the dissociation constant (pK_a) value(s) measured by the *co-solvent* potentiometric technique in methanol–water mixtures. Data are shown in Table 1.

Generally, potentiometry in aqueous medium is the method of choice for the pK_a determination for molecules of solubility higher than 0.8 mM concentration in the whole pH interval of the titration. Our compounds do not achieve this solubility criterion, therefore, the apparent dissociation constants, p_sK_a values were measured in three different methanol–water mixtures and the pK_a value was obtained by Yasuda-Shedlovsky (YS) extrapolation to zero methanol content. In Fig. 2 the YS plot of compound **6** indicates that experiments had to be carried out in methanol-rich region, thus long-distance extrapolation was the choice to obtain the aqueous pK_a values. However, as our previous validation study on *co-solvent* method has proved, using the right experimental protocol high precision of the extrapolated data can be obtained. The standard deviation is less than 0.05 log unit [23]. Table 1 summarizes the methanol weight percent range used for the titration, the statistical parameters of YS equations including the determination coefficient (R^2) and the extrapolated aqueous pK_a values.

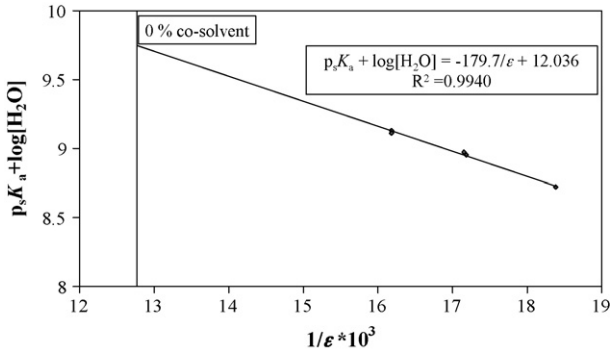


Fig. 2. Yasuda-Shedlovsky extrapolation of compound **6**.

Table 2
The percentage concentration of the species in stomach (pH 1.5), gastrointestinal tract (jejunum pH 6.5) and plasma (pH 7.4)

Compound	Percentage concentration					
	Stomach pH 1.5		Small intestine (jejunum) pH 6.5		Plasma pH 7.4	
	B[a]	BH^{+}[a]	B[a]	BH^{+}[a]	B[a]	BH^{+}[a]
1	0	100	0.72	99.28	5.44	94.56
2	0	100	0.52	99.48	4.00	96.00
4	0.72	99.28	99.86	0.14	99.98	0.02
6	0	100	3.07	96.93	20.08	79.92
7	0	100	3.13	96.87	20.45	79.55
8	0	100	2.68	97.32	17.95	82.05
12	0	100	3.58	96.42	22.79	77.21

Compound	Percentage concentration								
	Stomach pH 1.5			Small intestine (jejunum) pH 6.5			Plasma pH 7.4		
	B[b]	BH^{+}[b]	BH_2^{2+}[b]	B[b]	BH^{+}[b]	BH_2^{2+}[b]	B[b]	BH^{+}[b]	BH_2^{2+}[b]
3	0	1.87	98.13	0.92	99.02	0.05	6.9	93.10	0
9	0	0.35	99.64	14.48	85.28	0.24	57.42	42.66	0.02
10	0	1.83	98.17	9.09	90.86	0.05	44.27	55.73	0

Compound	Percentage concentration					
	Stomach pH 1.5		Small intestine (jejunum) pH 6.5		Plasma pH 7.4	
	A^{-}[c]	AH[c]	A^{-}[c]	AH[c]	A^{-}[c]	AH[c]
5	0	100	2.24	97.76	15.40	84.60

Compound	Percentage concentration								
	Stomach pH 1.5			Small intestine (jejunum) pH 6.5			Plasma pH 7.4		
	X^{-}[d]	XH[d]	XH_2^{+}[d]	X^{-}[d]	XH[d]	XH_2^{+}[d]	X^{-}[d]	XH[d]	XH_2^{+}[d]
11	0	0	100	0	4.47	95.53	0.02	27.08	72.90

[a] Monovalent bases.
[b] Bivalent bases.
[c] Acid.
[d] Ampholyte.

The pK_a data in Table 1 allow interpretation of the structure–property relationships (SPR) between the basicity and the chemical structure of the molecules. In compounds **1**–**5**, the central element of the structure is a piperidine ring, the N atom of which is the proton-binding site in **1**–**3**, while in compounds **4**, **5** this site has no any measurable basicity being part of a carboxamide bond. The pK_a values of **1**–**3** are more than one log unit below than expected (*N*-methyl-piperidine pK_a: 10.08 [30]). This shows electron-withdrawing effect of the 3-CF_3-phenyl group, through the –O– or –NH– linkage, despite the 4σ bond distance.

In case of the piperazine derivatives (**6**–**12**) the basicity of the non-anilinic N atom was determined, because no protonation of the other (aniline type, adjacent to the aromatic ring) nitrogen was observed down until pH 1.8. The compounds have little structural difference that could significantly influence their protonation (variation of Cl, CF_3, CN, OCH_3 substituents on phenyl ring) so they exhibit rather similar basicity around pK_a 8, except for the two diprotic compounds that have lower basic character.

The positive slope of YS equation (Table 1) at compounds **5** and **11** (here for the second ionization step) is due to the acidic function in these molecules (lactam and sulfonamide, respectively).

Further on we used the pK_a values to calculate the percentage concentration of differently protonated species at three relevant pH values in the body. Data are summarized in Table 2. The pH-partition hypothesis considers the non-ionized, neutral species (at base: B, at acid: AH, at amphoteric molecule: XH form) to be favourable for the absorption using passive transport through the lipoid membranes. Monovalent bases (**1**, **2**, **6**–**8**, **12**) are mainly present in ionized (BH^{+}) form in different compartments, except the weak base compound **4**, whose neutral form (B) is the dominant one in the jejunum and in the plasma. The bivalent bases exist also overwhelmingly in ionized forms in the GI tract: BH_2^{2+} species in the stomach, BH^{+} in the jejunum. The weak acid compound **5** is exceptional within the series because its neutral form (AH) is dominant in all three compartments of the human body.

3.2. Lipophilicity

The lipophilicity of the compounds is characterized here in terms of the logarithm of partition coefficient (log *P*), determined in two solvent systems. The $\log P_{oct}$ values were measured by dual-phase potentiometric titration. This method, being fast, precise and automated, is considered the "*gold standard*" of log *P* determination [31], and it can be used for log *P* measurements even for compounds of high lipophilicity.

Since high $\log P_{oct}$ values were expected, therefore, small amount of octanol was applied during the titration. Fig. 3 shows the shift of the Bjerrum curve in the course of titration with the presence of octanol for compound **7**. From the octanol-containing titrations the apparent ionization constants (p_oK_a) and then the log *P* value were calculated using the equation below,

$$P = \frac{10^{pK_a - p_oK_a} - 1}{r} \quad (3)$$

where r is the volume ratio of the organic and aqueous phases.

Albeit pH-metry can be applied for log *P* determination in solvent systems other than octanol/water (o/w) (e.g., 1,2 dichloroethane, chloroform, etc.), we have not found it ideal for

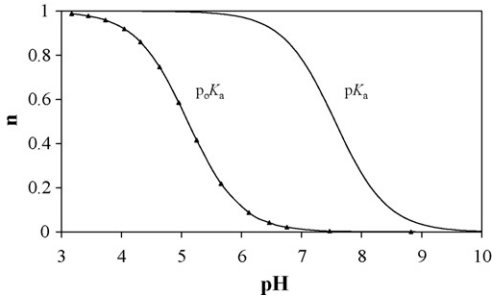


Fig. 3. Bjerrum curves of compound **7**.

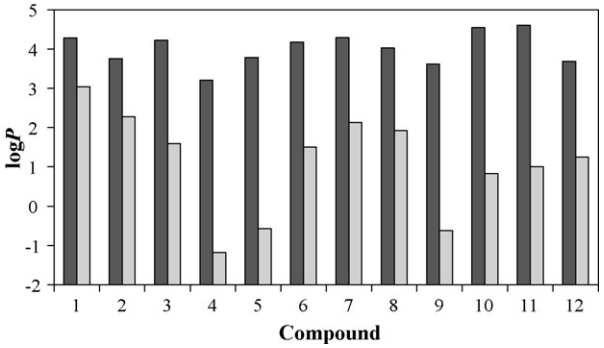


Fig. 4. $\log P_{oct}$ and $\log P_{ch}$ values of the molecules (where black and grey columns denote $\log P_{oct}$, and $\log P_{ch}$ values, respectively).

the cyclohexane/water (ch/w) system, due to the high volatility of the organic solvent. Thus, the $\log P_{ch}$ values of the compounds were determined by the traditional shake-flask method. Because of the poor water solubility of the non-ionized form the direct measurements of $\log P_{ch}$ was hindered, the apparent partition coefficients ($\log P_{app,ch}$) were measured at pH values where ionization improved the solubility and the true $\log P_{ch}$ (referring to the non-ionized form) was calculated by means of the following equations:

Bases:

$$\log P = \log P_{app} + \log(1 + 10^{pK_a - pH}) \quad (4)$$

Acids:

$$\log P = \log P_{app} + \log(1 + 10^{pH - pK_a}) \quad (5)$$

Ampholyte molecules:

$$\log P = \log P_{app} + \log(1 + 10^{pH - pK_{a2}} + 10^{pK_{a1} - pH}) \quad (6)$$

Table 3 contains the $\log P_{oct}$, and $\log P_{ch}$ values of the compounds. As expected, there is significant difference between partition coefficients obtained in the two solvent systems. While octanol is an amphiprotic apolar solvent, it is capable to take part not only in hydrophobic, but also in H-bond donor and acceptor interactions with the solute, the alkane-type cyclohexane forms no polar interactions, its solvation is based on dispersion interactions only. The $\log P_{oct}$ values have here always been found higher than the $\log P_{ch}$ values. The $\log P_{oct}$ values vary between 3.21 and 4.61, while the range of $\log P_{ch}$ values is −1.17 through 3.04. From these $\log P$ ranges it is obvious that partition of **1**–**12** compounds spans in ch/w system in order of magnitudes wider range than in o/w system. In other words the ch/w system is more distinctive than o/w (Fig. 4).

The following structure–lipophilicity relationships (SPR) can be drawn on the basis of the $\log P$ data in the two solvent systems. (i) Replacement of an etheric O atom for –NH– moiety (**1** → **2**) decreases the $\log P$ value in both systems (o/w: −0.52; ch/w: −0.77). (ii) Introduction of a $-CH_2-$ to the carboxamide moiety (**6** → **7**) causes $\log P$ increase in both systems (0.11 in o/w and 0.63 in ch/w). These effects on partitioning show the same direction but different extent in the two systems. However, the alteration of an acetamide group to methylsulfonamide (**6** → **8**) has opposite impact (−0.15 in o/w and 0.42 in ch/w). It calls our attention to the lack of understanding about the molecular factors governing the partition process particularly in ch/w system.

Table 3
$\log P_{oct}$, $\log P_{ch}$, $\Delta \log P$, and log BB values of the compounds

Compound	$\log P_{oct} \pm$ S.D.	$\log P_{ch} \pm$ S.D.	$\Delta \log P$	log BB
1	4.28 ± 0.01	3.04 ± 0.07	1.24	1.04
2	3.76 ± 0.01	2.27 ± 0.03	1.49	1.18
3	4.22 ± 0.01	1.59 ± 0.05	2.63	0.48
4	3.21 ± 0.01	−1.17 ± 0.2	4.38	0
5	3.78 ± 0.01	−0.57 ± 0.05	4.35	0
6	4.18 ± 0.01	1.50 ± 0.02	2.68	1.08
7	4.29 ± 0.01	2.13 ± 0.06	2.16	0.90
8	4.03 ± 0.01	1.92 ± 0.06	2.11	1.00
9	3.62 ± 0.01	−0.62 ± 0.05	4.24	0
10	4.55 ± 0.02	0.82 ± 0.05	3.73	0
11	4.61 ± 0.01	1.00 ± 0.02	3.61	0
12	3.69 ± 0.01	1.24 ± 0.03	2.45	0.78

Proceeding in considerations, we examined the cavity theory of solution [32], size-related and interaction-related molecular factors using linear regression (LR) and multivariate linear regression analyses (MLR).

First, to seek the impact of heteroatoms on the solvation, two simple parameters the HET_{apolar} (number of Cl + Br + F + S heteroatoms/number of carbon atoms) and HET_{polar} (number of N + O heteroatoms/number of carbon atoms) were correlated with $\log P$. No linear relation was found between $\log P_{oct}$ and HET_{apolar} ($r = 0.485$, $n = 12$), and only poor relation could be found between $\log P_{ch}$ and HET_{polar} ($r = 0.768$, $n = 12$). Next, the apolar (APSA) and polar surface area (PSA) were studied as cavity-related parameters on the solvation. APSA showed correlation neither with $\log P_{oct}$ nor with $\log P_{ch}$. Better correlation was obtained with PSA in case of ch/w system ($\log P_{ch} = -0.019$ PSA + 2.851; $n = 12$, $r = 0.848$, $s = 0.716$, $F_{(1.1)} = 25.604$). No correlation was observed between PSA and $\log P_{oct}$ ($r = 0.301$, $n = 12$). The MLR analysis using both cavity-related and interaction-related parameters resulted in the equations below.

$$\log P_{oct} = 3.825 - 0.001\,\mathrm{PSA} + 2.929\,HET_{apolar} \quad n = 12,\ r = 0.513,\ s = 0.393,\ F_{(2.9)} = 1.604 \quad (7)$$

$$\log P_{ch} = 2.931 - 0.019\,\mathrm{PSA} - 0.519\,HET_{polar} \quad n = 12,\ r = 0.848,\ s = 0.755,\ F_{(2.9)} = 11.527 \quad (8)$$

3.3. The $\Delta \log P$ value and its correlation with log BB

The $\Delta \log P$ values were obtained as the difference of the partition coefficient ($\log P_{oct} - \log P_{ch}$) and then they were correlated with the experimental log BB values (Table 3). We have found a significant linear relationship:

$$\log BB = -0.422\,\Delta \log P + 1.772 \quad n = 12,\ r = 0.926\ s = 0.200,\ F_{(1.1)} = 60.420 \quad (9)$$

Fig. 5 indicates one heavy outlier (marked with circle). Leaving out this point (compound **6**) the statistical analysis parameters improve to $r = 0.962$, $F_{(1.9)} = 110.32$.

Previously we determined the $\Delta \log P$ value of sertraline, a drug, known for its extremely good brain penetration. Eq. (9) predicts its

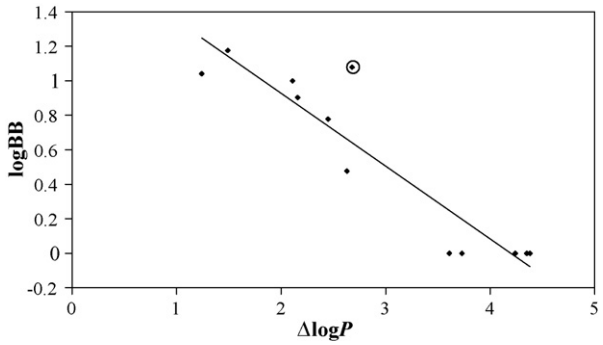


Fig. 5. The correlation between $\Delta \log P$ and log BB values.

log BB value: 1.77, which is in good agreement with the experimental 1.60 value [12].

4. Conclusions

In this study the ionization and the lipophilicity of compounds **1**–**12** as members of a novel class of potent dopamine D_3/D_2 receptor ligands were described in terms of thermodynamic physico-chemical constants (p*K*a, $\log P_{oct}$ and $\log P_{ch}$).

Based on physico-chemical data, the following compound-specific and compartment-selective pharmacokinetic behavior could be predicted and were eventually verified by the overall log BB values:

(i) Absorption from the stomach can be expected only for the weak acid, compound **5** being present in completely non-ionized and lipophilic form at acidic pH of this compartment.

(ii) The predominant ionized state of the other compounds (mono- and bivalent bases and an ampholyte molecule) in the GI tract is generally unfavorable to the absorption, which may be, however, counterbalanced by the high lipophilicity of compounds ($\log P_{oct}$ values). Even the least lipophilic compound (**4**) dissolves 1600 times better in lipids than in water, while the most lipophilic derivative (**11**) exhibits about 40,000 times higher affinity to apolar than polar phase. In cases of such high lipophilicity, the presence of a minute portion of non-ionized form (e.g., ~3% of B form at compounds **6**, **7**, **8**) can be sufficient for complete absorption. It is to be noted, however, that highly lipophilic compounds often exhibit poor oral bioavailability due to their very low aqueous solubility and their absorption is often dissolution-limited.

(iii) For drugs of CNS activity other transport process of key importance is BBB penetration. If the molecule is sufficiently lipophilic and does not contain too many polar substituents, it may have good brain penetration. The $\Delta \log P$/log BB correlation shows that in the examined series of D_3/D_2 receptor ligands, compounds with $\Delta \log P$ 1–3 have good chance to excess the brain, while derivatives with $\Delta \log P$ higher than 3.5 the BBB penetration is hindered. Our data support that $\Delta \log P$ is a simple and good predictor of log BB and it can be applied in drug development for early stage selection of compounds intended for brain penetration. A $\Delta \log P \leq 2$ predicts good penetration ability to the brain.

Acknowledgement

The authors wish to thank Dr László Molnár for the calculation of PSA.

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