Physico-chemical profiling of centrally acting molecules for prediction of pharmacokinetic properties

Theses of doctoral (Ph.D.) dissertation

Katalin Deák

Semmelweis University

Doctoral School of Pharmaceutical and Pharmacological Sciences

Supervisor:	Prof. Dr. Krisztina Takács-Novák, D. Sc.
Opponents:	Prof. Dr. Éva Szökő, D. Sc.
	Dr. István Greiner, C. Sc.
Head of Examination Committee:	Prof. Dr. Imre Klebovich, D. Sc.
Members of Examination Committee:	Dr. Pál Perjési, C. Sc.
	Prof. Dr. István Hermecz, D. Sc.

Semmelweis University, Department of Pharmaceutical Chemistry

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1 Introduction

The drug research has had a great development in the last twenty years. The new technologies introduced into drug research significantly increased the chance of lead finding. Together this, the R&D costs enhanced, but the number of newly registered molecules has not changed. Therefore the demand has increased for filtering out drug like molecules in the earliest stage of drug discovery. In consequence of the new strategy in drug research in 90's, beside finding and optimizing biologically active molecules, development of drug like properties has become important task.

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.

The acid/base character sets the charge of a molecule in solution at a particular pH. It can be described by the protonation constant (log$K$). The knowledge of the charge state is necessary for 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 (logP_{oct}) is the most extensively used parameter to quantitate lipophilicity. The logP_{oct} value has been found to be a good predictor of the passive transport of drugs through the lipoid membranes of the human body.

The spread of the neurodegenerative diseases shows increasing tendency. Because of growing demand the neuropharmacological market has become the largest segment of pharmaceutical industry. Currently the delivery of the molecules into the brain through the blood-brain barrier is the key challenge in the industrial and academical laboratories.

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 are a specific physical barrier and a complex biochemical interface containing many physiological functions. Unfortunately, the *in vivo* experimental determination of brain/blood concentration ratio (BB = concentration in brain / concentration in blood) is time-consuming, expensive and difficult. An additional problem is that in early screening of new chemical entities, the synthesized material is not available 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, labour-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.

During my doctoral work, within the framework of the research cooperation between Department of Pharmaceutical Chemistry at Semmelweis University and Department of Pharmacology and Drug Safety in Gedeon Richter Plc., the relationship between the physico-chemical parameters and pharmacokinetic properties of centrally acting compounds was investigated.

2 Objectives

1.) Explanation of unusual pharmacokinetic properties by physico-chemical parameters

We determined the most important physico-chemical parameters of sertraline, a potent antidepressive drug which has excellent brain penetration property. Then, these highly precise experimental data are used to find a better insight into the pharmacokinetic processes of sertraline.

2.) Determination of physico-chemical properties of a recently synthesized set of compounds discovered for antipsychotic activity to predict pharmacokinetic properties

The most important physico-chemical properties such as ionization and lipophilicity of 15 molecules synthesized by Gedeon Richter Plc. containing aryl-piperidine and aryl-piperazine moiety discovered for antipsychotic activity were determined. Based on the obtained, highly precise experimental data our purpose was to reveal the structure-property relationships (SPR), and to predict the pharmacokinetic properties. Our further aim was to understand the main molecular factors, which determine the partition processes of this series of compounds.

3.) Model development for the prediction of brain penetration

Our further aim was to set up a model for the prediction of the passive transport of the molecules through the blood-brain barrier by the simple physico-chemical parameters. The $\Delta\log P$ values (calculated as a difference between octanol/water and cyclohexane/water log*P* value) and the logBB values were correlated.

3 Methods

3.1 Determination of the protonation constants (log*K*)

Due to the poor water solubility of the molecules the protonation constants (log*K*) were determined by either potentiometric *co-solvent* method or UV-pH titration. For the potentiometric and UV-pH titrations PCA 101 and GLpKa automated log*K* and log*P* analysers (Sirius Analytical Instr. Ltd., Forest Row, UK) were used.

3.1.1 Electrode calibration

The four-parameter procedure was used for electrode standardization in both aqueous and semi-aqueous media. The operational pH reading was related to p_cH values by the standard multiparametric equation ($pH = \alpha + S\ p_cH + j_H\ [H^+] + j_{OH}\ K_w / [H^+]$, 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).

3.1.2 Potentiometric co-solvent method

6.0 - 10.0 ml of 0.75 - 1.32 mM semi-aqueous solutions of the samples containing 22.8 - 63.97 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 12.0). The titrations were performed at 25.0 ± 0.1 ^oC, 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 protonation constants in the mixed solvent ($\log_s K$) were calculated from the difference (Bjerrum) plot. The Yasuda-Shedlovsky procedure was applied to estimate the aqueous log$K$ value.

3.1.3 UV-pH titration

In case of compound **RG-13**, a 1.0 mM stock solution of the sample was prepared in methanol. 500 µl aliquot of the stock solution was diluted to 15.0 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 1.8 using 0.5 M HCl and then titrated with 0.5 M KOH to pH 10.0. 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 log*K* values of samples were calculated by Target Factor Analysis.

3.2. Determination of partition coefficients

The partition of the molecules was studied in octanol/water and alkane/water systems determining the logP_{oct} and logP_{ch} values by potentiometric and shake-flask methods, respectively.

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

8.0 - 10.0 ml of 0.73 - 1.38 mM aqueous solutions of samples were titrated under the same conditions as in log*K* 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 logP_{oct} value of the compound. 3 - 7 parallel titrations were carried out. From the octanol containing titrations the apparent protonation constants (log$_o$*K*) and then the log*P* values were estimated and refined by a weighted non-linear least-squares procedure where the log*K* values were used as unrefined contributions.

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

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. In some cases 1 - 10 % methanol was used in the aqueous medium in order to increase the solubility of the compounds. The phase ratio applied was varied from 30.0 ml water - 0.1 ml cyclohexane to 5.0 ml water - 20.0 ml cyclohexane, depending on the expected

logP_{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 hour, at 25.0 ± 0.1 ^{o}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 partition). From the concentration decrease the logP_{ch} was calculated. 3-12 parallel measurements were performed.

3.3. Determination of logBB values

12 male Wistar rats (~ 200g, 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 h, 1 h, 2 h and 5 h post dose. Blood samples of the animals were collected into heparinised tubes and centrifuged at 1000 g for 20 minutes. The harvested plasma was stored at -20 °C until analysis. Whole brain samples of the animals were homogenised in 2.5 fold volume of water by using Ultra-turrax T25 homogenizer and the resultant homogenates were stored at -20 °C until analysis.

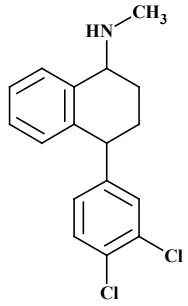
HPLC with UV detection was used for the quantitative determination of each compound in the plasma and brain, against calibration curves from 6 matrix-spiked standards. Generally 0.5 ml of sample (plasma or brain homogenate) was extracted by 5 ml of terc-buthyl-methylether or chlorobutane, the organic 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}).

4 Results and conclusion

4.1 Physico-chemical profiling of sertraline

Sertraline belongs to the selective serotonin reuptake inhibitor (SSRI) antidepressants. The molecule is a tetrahydro-naphthalenamine derivate, containing an aliphatic secondary amino group as basic site.

Sertraline has good pharmacokinetic properties. It has excellent brain penetration property. The brain concentration in rats has been found 40 times higher than in plasma.



The protonation constant (log$K$), the partition coefficient in octanol/water (logP_{oct}) and cyclohexane/water (logP_{ch}) systems and Δlog$P$ value (calculated as the difference between logP_{oct} and logP_{ch}) of sertraline were determined (Table 1).

Table 1 Physico-chemical parameters of sertraline

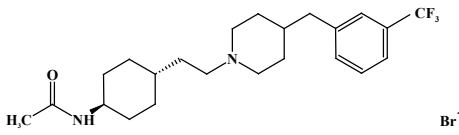
log$K$ ± SD	9.16 ± 0.02
logP_{oct} ± SD	4.30 ± 0.01
logP_{ch} ± SD	4.30 ± 0.12
Δlog$P$ = logP_{oct} - logP_{ch}	0.00

The favourable pharmacokinetic properties of sertraline are confirmed by its physico-chemical parameters. The 9.16 log*K* value indicates that the molecule is present mainly in the ionized (BH^+) form in different compartments of the human body. The predominant ionized state in the GI tract is generally unfavourable to the absorption, which may be, however counterbalanced by the high lipophilicity of sertraline. $\Delta \log P = 0$ value of sertraline indicates the lack of H-bond formation and explains well its high brain concentration.

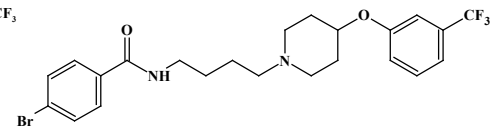
4.2 Physico-chemical profiling of RG-molecules

The most important physico-chemical properties (Table 2) of 15 piperazine and piperidine derivatives from the antipsychotic D_3/D_2 project resulting finally RGH-188 (cariprazine), which is being present currently under clinical phase II trials were determined.

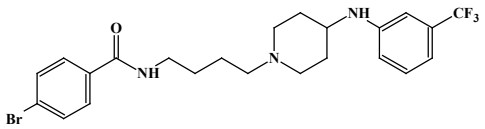
Piperidine derivatives



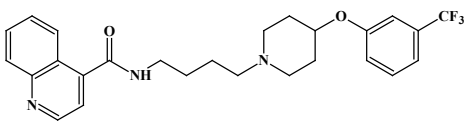
RG-1



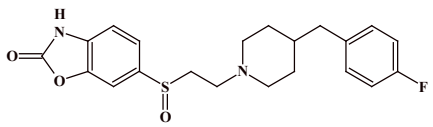
RG-2



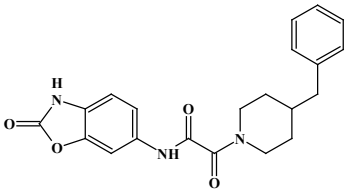
RG-3



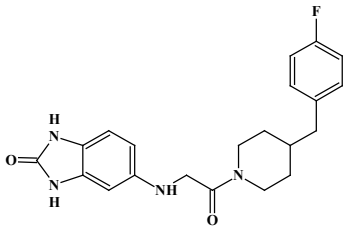
RG-4



RG-5

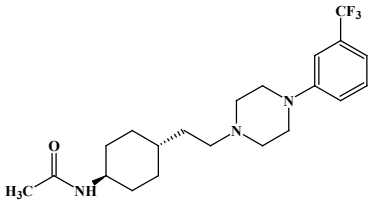


RG-6

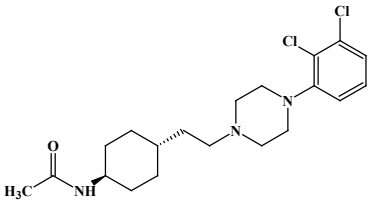


RG-7

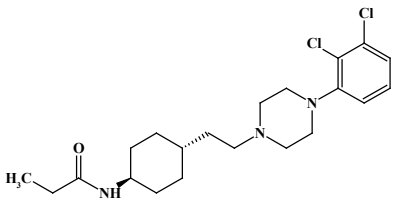
Piperazine derivatives



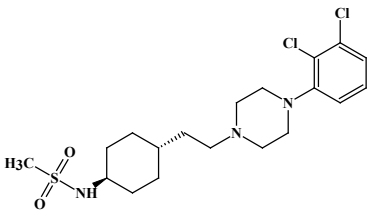
RG-8



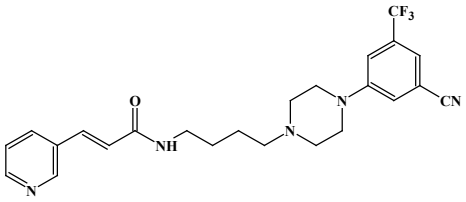
RG-9



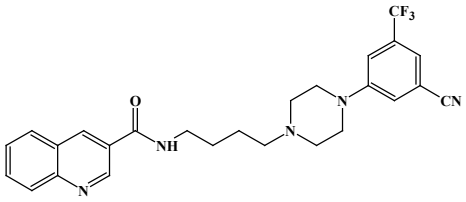
RG-10



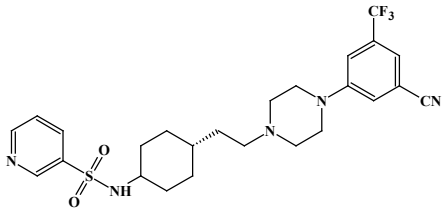
RG-11



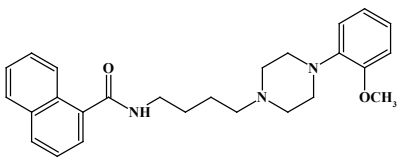
RG-12



RG-13



RG-14



RG-15

Table 2 Physico-chemical parameters of **RG** molecules

Compound	log$K$ ± SD	logP_{oct} ± SD	logP_{ch} ± SD	Δlog$P$
1	9.81 ± 0.02	4.43 ± 0.01	2.21 ± 0.08	2.22
2	8.64 ± 0.01	4.28 ± 0.01	3.04 ± 0.07	1.24
3	8.78 ± 0.01	3.76 ± 0.01	2.27 ± 0.03	1.49
4	8.53 ± 0.04 3.22 ± 0.03	4.22 ± 0.01	1.59 ± 0.05	2.63
5	7.79 ± 0.03 7.18 ± 0.01	3.08 ± 0.02	-1.15 ± 0.14	4.23
6	8.14 ± 0.01	3.78 ± 0.01	-0.57 ± 0.05	4.35
7	3.64 ± 0.02	3.21 ± 0.01	-1.17 ± 0.2	4.38
8	7.63 ± 0.03	3.71 ± 0.01	0.85 ± 0.07	2.86
9	8.00 ± 0.02	4.18 ± 0.01	1.50 ± 0.02	2.68
10	7.99 ± 0.01	4.29 ± 0.01	2.13 ± 0.06	2.16
11	8.06 ± 0.02	4.03 ± 0.01	1.92 ± 0.06	2.11
12	7.27 ± 0.04 3.95 ± 0.02	3.62 ± 0.01	-0.62 ± 0.05	4.24
13	7.50 ± 0.14 3.23 ± 0.02	4.55 ± 0.02	0.82 ± 0.05	3.73
14	10.60 ± 0.04 7.83 ± 0.03	4.61 ± 0.01	1.00 ± 0.02	3.61
15	7.93 ± 0.01	3.69 ± 0.01	1.24 ± 0.03	2.45

The relationship between the obtained physico-chemical parameters and the structure of the molecules was investigated.

The examined compounds differ considerably in acid-base properties representing a variety of proton-binding sites in both the type and the strength. There are monovalent bases (**RG-1** – **RG-3**, **RG-7** – **RG-11**, **RG-15**), bivalent

bases (**RG-4**, **RG-12**, **RG-13**), an acid (**RG-6**) and ampholyte (**RG-5**, **RG-14**) molecules. Based on the diverse acid-base properties, the molecules are present in different protonation state in the compartments of human body.

The logP_{oct} values vary between 3.08 - 4.61. By the high lipophilicity of the molecules good penetration properties through the lipoid membrane can be predicted. In cyclohexane/water system log$P$ values are lower than in octanol/water system; the range of logP_{ch} values is -1.17 through 3.04. The variance is caused by different solvation properties of octanol and cyclohexane.

Simple parameters such as number of heteroatoms, surface and Abraham's descriptors, which influence the partition in different organic solvent/water systems were studied.

4.3 Model for predicting the penetration through the blood-brain barrier

Based on the literature data the penetration through the blood-brain barrier can be mostly predicted by the $\Delta\log P$ value. However different data also can be found. Hence we examined the correlation between $\Delta\log P$ and logBB values in case of RG-molecules with similar structures. Linear correlation have been found ($\log BB = -0.408\ \Delta\log P + 1.726$, $n = 12$, $R = 0.971$, $F = 162.03$, $s = 0.125$). The validity of the model was proven by sertraline having not similar structure with RG-compounds. Calculating with the $\Delta\log P = 0$ value of sertraline we can predict logBB value in good agreement with the experimental value. Hereby the model can be suitable for prediction of brain penetration in case of diverse molecule.

4.4 Practical aspects of the results

The knowledge of physico-chemical parameters allows of predicting pharmacokinetic properties and screening drug like molecules in early stage of drug discovery.

This study provides a better insight into the relation between physico-chemical properties and pharmacokinetic data of sertraline and can be used as basic information in the development of new antidepressive drugs.

The investigation of the physico-chemical properties of RG-molecules developed for antipsychotic activity can contribute to developing further CNS active compounds with favourable pharmacokinetic properties.

The $\Delta \log P$ value is a simple and good predictor of logBB and it can be applied in drug development for early stage selection of compounds intended for brain penetration.

5 References

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Citable abstract

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