

## Estimation of lipohydrophilic properties of molecules with potential $\beta_3$ -agonistic activity

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### Introduction

Lipophilicity is considered to be a very important molecular descriptor. It plays a crucial role in determining pharmacokinetic ADMET-properties (absorption, distribution, metabolism, excretion, and toxicity) and the pharmacodynamic profile, which correlates well with their bioactivity in the end<sup>1</sup>). Successful drug development requires efficient delivery to target sites as the drug must be able to reach a specific biophase by crossing several biomembranes by passive and/or active transport. A predominant factor influencing the pharmacokinetic behaviour is lipophilicity. For example, commonly used  $\beta_3$ -blockers, which are structurally similar to  $\beta_3$ -adrenergic receptor agonists, may be divided into lipophilic and hydrophilic drugs, or are in an intermediate position<sup>2</sup>): sotalol ( $\log P_{\text{oct}} = -0.79$ ), atenolol ( $\log P_{\text{oct}} = 0.23$ ), nadolol ( $\log P_{\text{oct}} = 0.71$ ), practolol ( $\log P_{\text{oct}} = 0.76$ ), pindolol ( $\log P_{\text{oct}} = 1.75$ ), acebutolol ( $\log P_{\text{oct}} = 1.87$ ), timolol ( $\log P_{\text{oct}} = 2.10$ ), metoprolol ( $\log P_{\text{oct}} = 2.15$ ), alprenolol ( $\log P_{\text{oct}} = 2.61$ ) and propranolol ( $\log P_{\text{oct}} = 3.65$ ). Lipophilicity of compounds can influence oral absorption, diffusion through biological barriers (e.g. placenta or blood/brain), degree of metabolism/renal elimination, plasma half-life, receptor selectivity, or tissue concentration<sup>3</sup>). Highly lipophilic drugs are insoluble in aqueous media and bind strongly to plasma proteins, which results in a low free blood concentration, and are distributed only into lipid bilayers. On the other hand, highly polar compounds cannot be absorbed through the gut wall because of lower membrane solubility. Keeping the optimal lipophilicity range can lead to an improvement of therapeutic efficacy and side-effect profiles of new drugs<sup>4</sup>).

### Experimental methods

To describe the transfer of a substance from the aquatic environment into an organism and its bioaccumulation potential, the partition coefficient of a substance between water and a lipophilic solvent (octan-1-ol, cyclohexane, heptane) was determined.

Partition coefficient ( $P$ ) is defined as the ratio of the equilibrium concentrations ( $C_i$ ) of a dissolved substance in a two-phase system consisting of two immiscible solvents<sup>5</sup>):

$$P = C_{\text{LS}}/C_{\text{W}}, \quad [1]$$

where  $C_{\text{LS}}$  is the concentration of a compound in the lipophilic phase and  $C_{\text{W}}$  is the concentration of a compound in the aqueous phase. Partition coefficient is usually given in the form of its log arithm to the base ten ( $\log P$ ).

### Studied compounds

Chemical structure and the basic physicochemical parameters of the studied substances *BL-14S2-BL-44S2* (chemically 3-{4-[(alkoxycarbonyl)amino]phenoxy}-*N*-{2-[4-(aminosulfonyl)phenyl]ethyl}-2-hydroxypropan-1-ylammonium chlorides) are shown in Table 1.

### Devices

An analytical balance Chyo JL-180 (Chyo Balance Corporation, Japan), a mechanical shaker, a UV spectrophotometer (Shimadzu, UV-1800, Japan), a pH-meter (Hanna Instruments, Slovak Republic).

### Chemicals

The aqueous phase was represented in all cases by phosphate buffer prepared from a water solution of disodium hydrogen phosphate, p.a. (CentralChem, Slovak Republic) with the concentration  $c = 0.2 \text{ mol} \cdot \text{l}^{-1}$  and a water solution of citric acid, p.a. with the concentration  $c = 0.1 \text{ mol} \cdot \text{l}^{-1}$ . Measurements were performed under equilibrium conditions at  $\text{pH} = 7.4$ . The lipophilic phase was represented by high purity analytical grade octan-1-ol (Merck, Germany), cyclohexane (CentralChem, Slovak Republic) and heptane (CentralChem, Slovak Republic).

### Estimation of partition coefficient $\log P_2$

In the present study, the generally accepted and well-known shake-flask method for obtaining the  $\log P$  values in three mediums was used. The first one was octan-1-ol/phosphate buffer ( $\log P_{\text{O}}$ ), second cyclohexane/phosphate buffer ( $\log P_{\text{C}}$ ) and the third was heptane/phosphate buffer ( $\log P_{\text{H}}$ ). The authors prepared 50 ml of basic solutions of the studied compounds *BL-14S2-BL-44S2* in phosphate buffer at  $\text{pH} = 7.4$  with the concentration  $c = 5.10^{-5} \text{ mol} \cdot \text{l}^{-1}$  and measured their absorbance  $A_{\lambda}$ . To 10 ml of the solution, 0.5 ml of the lipophilic medium represented by octan-1-ol, cyclohexane and heptane, respectively, was added. This system was

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shaken for 1h, the phases of the solvent system were mutually saturated and after that settled for 1 h, then the lipophilic and aqueous phases were separated. The absorbance  $A_2$  of the aqueous phase was measured. The values of absorbance were in both cases measured at the wavelength  $\lambda = 227$  nm. The data of  $\log P_o$ ,  $\log P_c$ ,  $\log P_H$  for each compound were calculated from the equations 2–4<sup>6)</sup>:

$$P_{\text{exp}} = (1000 g) - (a \times c_{\text{H}_2\text{O}} \times M_r) / b \times c_{\text{H}_2\text{O}} \times M_r \quad [2]$$

$$c_{\text{H}_2\text{O}} = A_2 \quad [3]$$

$$\varepsilon = A_1 / c, \quad [4]$$

where  $g$  is the weight of the studied compound in grams in 10 ml of measured solution,  $a$  is the number of millilitres of the aqueous phase,  $b$  is the number of millilitres of the lipophilic phase,  $M_r$  is the relative molecular weight of the compound under study,  $c_{\text{H}_2\text{O}}$  is the amount of the inspected compound in the aqueous phase after shaking,  $c$  is the concentration of the measured solution expressed in  $\text{mol} \cdot \text{l}^{-1}$ .

## Results and discussion

This short research paper was focused on the estimation of lipophilicity of four newly synthesized substances potentially active as  $\beta_3$ -adrenergic receptor agonists. Solubility in lipids refers to the ability of a compound to dissolve in fats, oils, lipids and non-polar solvents<sup>7)</sup>. The

experimentally estimated partition coefficient ( $\log P$ ) is routinely used as an assessment of lipid solubility *in vivo* and it is a key event of molecular desolvation in the transfer from aqueous phases to the cell membrane and protein bindings. The values of  $\log P$  were experimentally determined in three systems, whereby the aqueous phase was always composed of phosphate buffer with  $\text{pH} = 7,4$  and the lipophilic phase was formed of octan-1-ol, cyclohexane and heptane. Octan-1-ol represents a simple model of the cell phospholipidic membrane. The  $\log P$  data acquired from the systems with cyclohexane and heptane signify the margin of penetration through the *stratum corneum* and blood-brain barrier. Octan-1-ol has eight atoms of carbon which are responsible for its lipophilic properties and on the other hand, it has also hydroxyl functionality which is accountable for its hydrophilic attribute. Cyclohexane is a lipophilic solvent composed of a planar six-membered cycle; therefore the molecules with benzene rings should easily incorporate into this system. Heptane is also a lipophilic solvent but it is composed of linear aliphatic chains. Compounds with aromatic scaffold cannot incorporate into heptane molecules so easily<sup>8)</sup>.

The evaluated compounds *BL-14S2-BL-44S2* are structurally based on the aryloxyaminopropanol pharmacophore bearing a benzene sulfonamide fragment in the basic part of the molecule differing from each other in the alkoxy-carbonylamino moiety. The presence of two aromatic rings is responsible for their lipophilic character. Furthermore, with elongation of the alkoxy-carbonylamino fragment attached to the aromate, lipophilicity enhances. On the other hand, the presence of quaternary ammonium,

Table 1. General characterization of investigated molecules *BL-14S2-BL-A4S2*

	<b>R</b>	<b>Formula</b>	<b><math>M_r</math></b>	<b>m.p. (°C)</b>
<i>BL-14S2</i>	CH <sub>3</sub>	C <sub>19</sub> H <sub>26</sub> ClN <sub>3</sub> O <sub>6</sub> S	459.944	219–222
<i>BL-24S2</i>	C <sub>2</sub> H <sub>5</sub>	C <sub>20</sub> H <sub>28</sub> ClN <sub>3</sub> O <sub>6</sub> S	473.971	210–214
<i>BL-34S2</i>	C <sub>3</sub> H <sub>7</sub>	C <sub>21</sub> H <sub>30</sub> ClN <sub>3</sub> O <sub>6</sub> S	487.997	220–223
<i>BL-44S2</i>	C <sub>4</sub> H <sub>9</sub>	C <sub>22</sub> H <sub>32</sub> ClN <sub>3</sub> O <sub>6</sub> S	502.024	222–224

Table 2. Values of absorbances for calculation  $\log P_o$ ,  $\log P_c$ ,  $\log P_H$  for studied compounds *BL-14S2-BL-44S2*

<b>Entry</b>	<b><math>A_1</math></b>	<b><math>A_{2o}</math></b>	<b><math>A_{2c}</math></b>	<b><math>A_{2H}</math></b>
<i>BL-14S2</i>	1.130	0.803	1.128	1.129
<i>BL-24S2</i>	1.097	0.516	1.096	1.096
<i>BL-34S2</i>	1.117	0.706	1.116	1.118
<i>BL-44S2</i>	0.869	0.253	0.806	0.812

$A_1$  – absorbance measured before shaking,  $A_{2o}$  – absorbance measured after shaking for the system octan-1-ol/phosphate buffer,  $A_{2c}$  – absorbance measured after shaking for the system cyclohexane/phosphate buffer,  $A_{2H}$  – absorbance measured after shaking for the system heptane/phosphate buffer

Table 3. Experimentally estimated values of partition coefficients for inspected compounds *BL-14S2-BL-A4S2* in octan-1-ol / phosphate buffer ( $\log P_o$ ), cyclohexane / phosphate buffer ( $\log P_c$ ) and heptane / phosphate buffer ( $\log P_H$ )

<b>Entry</b>	<b><math>\log P_o</math></b>	<b><math>\log P_c</math></b>	<b><math>\log P_H</math></b>
<i>BL-14S2</i>	0.90	–	–
<i>BL-24S2</i>	1.07	–	–
<i>BL-34S2</i>	1.35	–	–
<i>BL-44S2</i>	1.70	0.22	0.17

hydroxyl and sulfonamide groups is responsible for their hydrophilicity. Whereas, cyclohexane and heptane are highly lipophilic solvents, only the substance *BL-44S2* with a butoxycarbonylamino moiety was able to penetrate into them. The partition coefficient in the medium cyclohexane/phosphate buffer ( $\log P_c$ ) system was of the value of 0.22 and was higher than the partition coefficient estimated in heptane/phosphate buffer, which was of the value of 0.17, in consequence of a different structural character of the molecules of the solvents mentioned above. Into the non-polar environment of octan-1-ol all of the substances were able to penetrate and the values of  $\log P_o$  ranged from 0.90 to 1.70 (Table 3), whereby with an elongation of the alkoxy carbonylamino fragment, the  $\log P_o$  values increased constantly.

### Conclusions

In conclusion, following the obtained results it can be assumed that the currently investigated compounds *BL-14S2*–*BL-44S2* which could be classified as potential  $\beta_3$ -adrenergic agonists, are not highly lipophilic and according to that, they are unlikely to cross the blood-brain barrier and cause some serious undesirable side effects on the CNS. It could be hypothesized that they would not be extensively metabolized, have not poor absorption, solubility, low bioavailability and a shorter half-life compared to their lipophilic analogues.

This work was supported by the following Grant projects: FaF UK/29/2015, UK/346/2015, FaF UK/28/2015, FaF UK/44/2015, FaF

UK/63/2015; Comenius University in Bratislava Science Park supported by the Research and Development Operational Programme funded by the ERDF – Grant number: ITMS 26240220086.

**Conflicts of interest:** none.

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