European Journal of Pharmaceutical Sciences 47 (2012) 921–925

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmaceutical Sciences

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

Thyroxine lipophilicity is dominated by its zwitterionic microspecies

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ARTICLE INFO

Article history:
Received 3 August 2012
Received in revised form 17 September 2012
Accepted 18 September 2012
Available online 28 September 2012

Keywords:
Zwitterion lipophilicity
Microscopic partition coefficient
Microscopic protonation constant
Thyroid hormones
Thyroxine
Deductive method

ABSTRACT

Species-specific partition coefficients were determined for a triprotic molecule for the first time. Thyroxine, the vitally important thyroid hormone which exists in solution in the forms of eight microspecies due to its phenolate, amino and carboxylate basic sites, was studied by combined methods of microspeciation and lipophilicity. Partition of the individual microspecies was mimicked by model compounds of the closest possible similarity, then correction factors were determined and introduced. The non-charged microspecies is only 2.40 times as lipophilic as its zwitterionic protonation isomer, showing that for thyroxine the iodinated aromatic rings are the structural elements that determine the lipophilicity of this molecule, and the protonation state of the other substituents plays only a minor role. The overwhelming dominance of the zwitterionic form, however, ensures that its contribution to the overall lipophilicity exceeds 14,500 times that of the non-charged one. This fact is so far the sharpest counter-example of the widespread belief that passive diffusion into lipophilic media is predominated by the non-charged species. The lipophilicity profile of thyroxine is expressed, calculated and depicted in terms of species-specific lipophilicities over the entire pH range.

1. Introduction

Thyroxine (3,5,3′,5′-tetraiodothyronine, T4), liothyronine (3,5,3′-triiodo L-thyronine, T3) and "reverse" liothyronine (3,3′,5′-triiodo L-thyronine, rT3), the thyroid hormones are formed in the human thyroid gland by iodination and coupling reactions of tyrosine (Chemburkar et al., 2010). T3 and rT3 are produced during the peripheral metabolism primarily when T4 is converted to T3 or rT3 (Nagao et al., 2011). The thyroid hormones are general enhancers of cellular metabolism. They are also crucial for the normal development of the central nervous system (CNS) in infants, the skeletal growth in children, and also for the normal function of multiple organ systems in adults (Cody, 1980).

Thyroid hormones are very lipophilic substances, they are therefore able to cross the plasma membrane of target cells even by passive diffusion. Nevertheless, many active iodothyronine transporters have been identified (e.g. the OATP1C1, OATP14 organic anion transporter and the amino acid transporter LAT-1), and it is now accepted that the cellular uptake is effected by energy dependent, carrier-mediated processes as well (Hennemann et al., 2001).

Lipophilicity is a molecular property of immense importance in pharmacy, bio-, and medicinal chemistry. Its applications include apparently diverse fields such as drug design for targeted delivery and development of chromatographic separations. The ability of drugs to diffuse passively through biological membranes has long been known to be largely influenced by their lipophilicity (Fujita, 1990). The pH-partition hypothesis postulates that absorption of ionizable drugs takes mainly place in compartment(s) where the local pH ensures the maximum concentration of the non-charged form relative to the ionized form(s) (Avdeef, 2002). In addition, lipophilicity is a tool to unravel biologically relevant intramolecular interactions and intermolecular forces of recognition (Testa et al., 1996; Liu et al., 2011).

In order to quantitate lipophilicity, the commonly accepted parameter is log *P*, the logarithm of the partition coefficient. It is the concentration ratio of a solute in a single electrical state, being in equilibrium between two immiscible solvents. Octanol is the most often used organic solvent, and the octanol–water partition coefficient is the most widely used descriptor of lipophilicity in QSAR studies (Hansch, 1994). When more than one electrical species are present in solution, the observed ratio of concentrations is the distribution coefficient (*D*), a pH-dependent, overall parameter, composed of the intrinsic lipophilicity of the various electrical species present (p_i), and their mole fractions in the aqueous phase (x_i).

$$D = \sum (x_i p_i) \quad (1)$$

The variation of log *D* as a function of the aqueous pH is the lipophilicity profile. It is a *sine qua non* condition to understand the pharmacokinetic, toxicokinetic and even pharmacodynamic properties (Pagliara et al., 1997).

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http://dx.doi.org/10.1016/j.ejps.2012.09.009

The lipophilicity of ionizable drugs and solutes has been underrepresented in the literature, due mainly to the lack of reliable methods to determine the partition coefficients of the ionic forms. This is especially true for ionization/protonation isomers, such as the zwitterionic and non-charged forms of amphoteric compounds.

In order to gain insight into the partition microequilibria of amphoteric drugs at the species-specific level, we have recently elaborated a method and studied on three systems (Mazák et al., 2011; Mazák and Noszál, 2012a,b). The partition properties of the compound in question and its microspecies-mimicking synthetic derivatives were investigated on niflumic acid, a highly lipophilic non-steroidal anti-inflammatory drug. We reported, for the first time for any compound, experimental microscopic partition coefficients for the two protonation isomers (Mazák et al., 2011). Subsequently we reported the complete set of experimental microscopic partition coefficients of morphine, the best known opiate alkaloid. The lipophilicity profile of morphine was expressed, calculated and depicted in terms of species-specific lipophilicities over the entire pH range (Mazák and Noszál, 2012a). Finally we reported the complete set of experimental microscopic partition coefficients of the amphoteric eburnane alkaloid *cis*- and *trans*-apovincaminic acids, providing the first experimental proof for the predominant contribution of zwitterionic species to the overall lipophilicity (Mazák and Noszál, 2012b).

As expected, the non-charged form was much more lipophilic than its zwitterionic protonation isomer in each case. Although niflumic acid is a dominantly zwitterionic compound, having 16 times as many zwitterionic than non-charged microspecies in aqueous solution, because of the orders of magnitude larger lipophilicity of the non-charged form, its contribution to the overall lipophilicity is around 25 times more important than that of the zwitterionic protonation isomer.

The non-charged microspecies of the cis-apovincaminic acid is 30,900 times as lipophilic as its zwitterionic protonation isomer, while the analogous ratio for the trans-epimer is around 15,800. Due to the overwhelming dominance of the zwitterionic form, however, its contribution to the overall lipophilicity exceeds eight and five times that of the non-charged one for the two epimers, respectively.

A recent study on the microscopic acid–base properties of these triprotic thyroid hormones showed that in aqueous solutions they exist in their differently ionized forms (Tóth et al., 2012), and the concentration of the non-charged, most lipophilic form is several magnitudes smaller for each molecule.

Because of the very poor aqueous solubility of thyroxine, only one experimental study (Comer and Box, 2008) attempted to characterize the lipophilicity of the molecule in the octanol–water system. The reported log *P* 3.21 value, however, is still to be clarified, whether it refers to the lipophilicity of the non-charged microspecies or all the neutral forms collectively.

In this study we characterize the lipophilicity of the microspecies of thyroxine (T4), and quantitate their contribution to the overall lipophilicity. Experimental microscopic partition coefficients for triprotic molecules have not been reported before.

2. Materials and methods

2.1. Materials

L-Thyroxine sodium salt was obtained from Sigma–Aldrich Co. All other reagents were of analytical grade (Reanal). All solutions were prepared from freshly boiled distilled water.

2.2. Synthesis of derivatives with reduced number of basic site(s)

The carboxymethyl (C-methyl) ester of T4 was synthesized according to literature applying direct esterification of D-tyrosine (Ishigami et al., 2009). The O-methyl ether of T4 was synthesized according to literature involving methylation with diazomethane and subsequent alkaline hydrolysis of the O-methyl-carboxymethyl ester (Loeser et al., 1938). The structure of the synthesized compounds was confirmed by ^{1}H NMR spectra in DMSO-d_6, using a Varian Inova 600 MHz NMR spectrometer.

2.3. Partition coefficient measurements by the stir-flask method

The distribution coefficients were calculated from the absorbance of the molecules before and after partitioning at several octanol/water phase ratios (Mazák et al., 2003). We usually had to use the octanol phase for absorbance measurements because of the poor water solubility of these molecules. The total concentration of the measured molecules was 1.1×10^{-4} mol/dm^3 before partitioning. The partitioning was performed in a room thermostated to 25 °C. The absorbance measurements were done in another room, at ambient temperature. For the pH control buffers composed of phosphate and citrate; and standardized HCl and NaOH solutions were used with an ionic strength of 0.15 M. For pH-measurement a Metrohm 6.0234.110 combined glass electrode, calibrated by aqueous NIST standard buffer solutions, was used in the pH range 4–8.

3. Results

3.1. Acid–base equilibria

Thyroxine contains two acidic and one basic site, thus it exists in solutions in eight microscopic protonation forms (microspecies), and 12 microconstants are needed to describe its protonation microequilibria (Bjerrum, 1923; Noszál, 1990).

The protonation scheme of thyroxine is depicted in Fig. 1.

A recent study of our research group (Tóth et al., 2012) has presented the microconstants and the logarithmic distribution

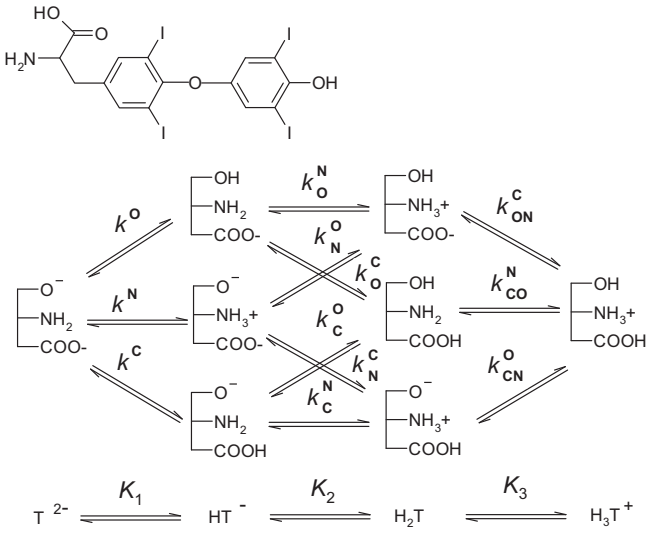

Fig. 1. The micro- and macro-speciation scheme of thyroxine, where microconstants with superscript O, N and C belong to the phenolate, amino and carboxylate site, respectively, and K_1, K_2 and K_3 are stepwise macroconstants (log K_1 = 8.60, log K_2 = 6.59, log K_3 = 2.01 from Tóth et al., 2012). The superscript on the microconstant indicates the protonating site, while the subscript (if any) stands for the site already protonated.

diagram for all the microspecies of thyroxine. This distribution diagram (Fig. 2) shows that the dominant species in highly acidic solutions is the triprotonated cationic, in less acidic solutions the diprotonated (OH,NH_3^+,COO^-) zwitterionic, at the pH of the blood the monoprotonated (O^-,NH_3^+,COO^-) anionic, and in more alkaline solutions the completely deprotonated, dianionic form, respectively.

3.2. Lipophilicity

Out of the eight microspecies first we selected those that can have a significant contribution toward the overall lipophilicity of the molecule. The monoprotonated ($O^-,NH_2,COOH$) anionic form can well be ignored because of its low relative concentration. The relative concentration of the diprotonated ($O^-,NH_3^+,COOH$) and ($OH,NH_2,COOH$) forms is practically equal, but the former is zwitterionic, whereas the latter is the only non-charged form of the molecule, with an obvious much higher expected microscopic lipophilicity. Thus we only have to consider six microspecies that can have a significant contribution to the distribution coefficient of the molecule.

As shown in Eq. (1), the distribution coefficient takes into account the intrinsic lipophilicity of these six microspecies, and their mole fractions in the aqueous phase.

The aqueous mole fractions include protonation microconstants and hydrogen ion activities, expressing thus the pH-dependence of the D distribution coefficient. For example,

$$x_{(O^-,NH_2,COO^-)} = \frac{1}{1 + K_1 a_{H^+} + K_1K_2(a_{H^+})^2 + K_1K_2K_3(a_{H^+})^3} \quad (2)$$

$$x_{(OH,NH_2,COOH)} = \frac{k^O k_O^C (a_{H^+})^2}{1 + K_1 a_{H^+} + K_1K_2(a_{H^+})^2 + K_1K_2K_3(a_{H^+})^3} \quad (3)$$

In order to obtain species-specific log p values for the six microspecies, we applied our recently developed method to determine the microscopic partition coefficients (Mazák et al., 2011; Mazák and Noszál, 2012a,b). We used the C-methyl ester and the O-methyl ether to mimic the non-charged ($OH,NH_2,COOH$), and the (OH,NH_2,COO^-) form, respectively. Then we determined and introduced correction factors to minimize effects of chemical derivatization. The effect of methylation in both model compounds was taken into account by comparing their lipophilicity to that of thyroxine in their uniformly cationic ionization state, which overwhelmingly exists in sufficiently acidic solutions.

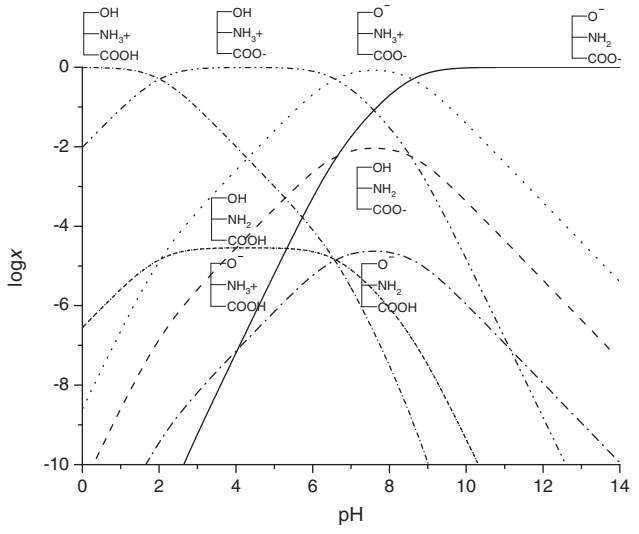

Fig. 2. The logarithmic distribution diagram for all the microspecies of thyroxine.

We chose a standardized HCl solution of 0.15 M (pH 0.82), as the majority of log K and log P values are determined at this physiological ionic strength. The results are listed in Table 1, including results obtained with a standardized NaOH solution of 0.15 M (pH 13.18). Table 1 also lists some additional log D values that are needed for the calculation of species-specific lipophilicities.

Due to the predominance of the cation at low pH, the reported log D values in Table 1 characterize overwhelmingly the lipophilicity of the cationic species. For analogous reason, log D values in the highly alkaline solution characterize the lipophilicity of the dianionic form of thyroxine, and the monoanionic form of its O-methyl ether. The log D value determined close to the isoelectric point of the C-methyl ester (pH 6.75) characterizes the lipophilicity of the non-charged ($OH,NH_2,COOCH_3$) form. The two additional log D values of thyroxine are measured at pH values where the relative concentration of the non-charged ($OH,NH_2,COOH$), and the anionic (OH,NH_2,COO^-) form is close to its maximum. However, these values cannot *a priori* be regarded equal to the microscopic log p values of these microspecies, since other forms also contribute to the overall lipophilicity at these pH values.

Based on our data in Table 2, the lipophilicity of the cationic form of thyroxine is close to its C-methyl ester, the difference is only 0.01 in log D units. The O-methyl ether is slightly more lipophilic, with an analogous difference of 0.21 in log D units.

These differences are valid only for the physiological ionic strength, as the lipophilicity of ionic species depends on the type and concentration of the background electrolyte (Johnson and Westall, 1990; Escher and Schwarzenbach, 1996; Takács-Novák and Szász, 1999; Reymond et al., 2001; Bouchard et al., 2001).

It is plausible that the methylation-derived differences in the lipophilicity of the cationic forms will be very close to the differences between the non-charged forms of thyroxine and its C-methyl ester, and between the anionic forms of thyroxine and its O-methyl ether, respectively. These differences can serve as appropriate correction factors.

Using the experimentally determined partition coefficients of the model compounds, and taking into account the correction factors, we calculated the logarithm of the microscopic partition coefficients (log p) for various microspecies of thyroxine. For its non-charged ($OH,NH_2,COOH$) form log p is 2.98 = 2.99 − 0.01 (log P of the non-charged form of its C-methyl ester − correction factor); whereas for the anionic (OH,NH_2,COO^-) form log p is 2.26 = 2.47 − 0.21 (log P of the anionic form of its O-methyl ether − correction factor).

In order to determine p of the zwitterionic (OH,NH_3^+,COO^-) form, Eq. (1) has to be rearranged as follows:

$$p^{(OH,NH_3^+,COO^-)} = (D_{(pH)} - x_{(OH,NH_2,COOH)}p^{(OH,NH_2,COOH)} - x_{(OH,NH_3^+,COOH)}p^{(OH,NH_3^+,COOH)})/x_{(OH,NH_3^+,COO^-)} \quad (4)$$

Thus, in the knowledge of distribution coefficients near the isoelectric point (pH 4.31), and the lipophilicity contribution of the two other important microspecies, p of the zwitterionic (OH,NH_3^+,COO^-) form can be calculated.

In order to determine p of the anionic (O^-, NH_3^+, COO^-) form, Eq. (1) has to be rearranged as follows

$$p^{(O^-,NH_3^+,COO^-)} = (D_{(pH)} - x_{(OH,NH_3^+,COO^-)}p^{(OH,NH_3^+,COO^-)} - x_{(OH,NH_2,COO^-)}p^{(OH,NH_2,COO^-)})/x_{(O^-,NH_3^+,COO^-)} \quad (5)$$

Thus, in the knowledge of distribution coefficients at pH 7.61, and the lipophilicity contribution of the two other important microspecies, p of the anionic (O^-,NH_3^+,COO^-) form can be calculated.

Table 2 shows all the experimentally determined microscopic partition coefficients of thyroxine.

Table 1
Log *D* values of the investigated compounds in the octanol/water system. Standard deviations are in parentheses.

Compound	pH 0.82	pH 4.31	pH 6.75	pH 7.61	pH 13.18
Thyroxine	3.04(0.02)	2.60(0.02)		2.28(0.03)	−1.89(0.03)
C-methylthyroxine	3.05(0.05)		2.99(0.06)		
O-methylthyroxine	3.25(0.06)				2.47(0.05)

Table 2
The logarithm of microscopic partition coefficients of thyroxine in the octanol/water system at 0.15 M ionic strength.

Microspecies		Log *P*
Dianionic	(O^-,NH_2,COO^-)	−1.89
Anionic	(OH,NH_2,COO^-)	2.26
Anionic	(O^-,NH_3^+,COO^-)	2.27
Non-charged	$(OH,NH_2,COOH)$	2.98
Zwitterionic	(OH,NH_3^+,COO^-)	2.60
Cationic	$(OH,NH_3^+,COOH)$	3.04

4. Discussion

4.1. Acid–base equilibria

Owing to the significantly different basicities of the three protonation sites, the k^N, k_N^O and k_{ON}^C microconstants indicate the major protonation pathway and they are practically equal to the K_1, K_2 and K_3 macroconstants, respectively.

Irrespective of the pH of the solution, there are 34,700 times as many zwitterionic microspecies of thyroxine, than non-charged ones. When comparing the anionic forms, there are 91 times as many (O^-,NH_3^+,COO^-) than (OH,NH_2,COO^-) forms.

In the pH range 2.02–6.58 thyroxine mainly exists in the zwitterionic (OH,NH_3^+,COO^-) form, whereas in the pH range 6.60–8.59 in the monoprotonated (O^-, NH_3^+,COO^-) form.

4.2. Lipophilicity

The non-charged microspecies of thyroxine is only 2.40 times as lipophilic as its zwitterionic protonation isomer. The lipophilicity of the two anionic protonation isomers is practically the same. These small differences show that for thyroxine the iodinated aromatic rings are the structural elements that mainly determine the lipophilicity of this molecule, and the protonation state of the other substituents plays a minor role. This phenomenon is also underlined by the fact that in the same cationic state the lipophilicity of thyroxine is practically equal to its C-methyl ester. Methylation on the phenolic hydroxyl group increases the lipophilicity more significantly, as this change directly influences the lipophore aromatic center, as opposed to the C-methyl ester formation.

Using the mole fractions and microscopic partition coefficients of the non-charged and zwitterionic species, their pH-independent contribution ratio to the distribution coefficient can also be quantified. This $x_{(OH,NH_3^+,COO^-)}p^{(OH,NH_3^+,COO^-)}/x_{(OH,NH_2,COOH)}p^{(OH,NH_2,COOH)}$ value is 14,500. Thus the contribution of the zwitterionic microspecies is much more important than that of the non-charged form to passive membrane-penetration and other lipophilicity-related processes.

Our microscopic partition coefficients in Table 2 allow to calculate and depict the contribution of the six important microspecies to the distribution coefficient of thyroxine at any pH value (Fig. 3). The broad black line is the overall lipophilicity profile of the molecule, the sum of the contributions of its six microspecies.

The figure shows that in the pH range 2.46–6.91 the contribution of the zwitterionic (OH,NH_3^+,COO^-), whereas in the pH range 6.93–12.75 that of the monoprotonated (O^-,NH_3^+,COO^-) form is dominant, respectively. At lower or higher pH values the cationic and dianionic forms prevail the distribution coefficient, respectively. The pH-independent contribution ratio of the protonation isomer pairs (OH,NH_3^+,COO^-) versus $(OH,NH_2,COOH)$ and (O^-, NH_3^+,COO^-) versus (OH,NH_2,COO^-) is visualized by the parallel lines on the graphs.

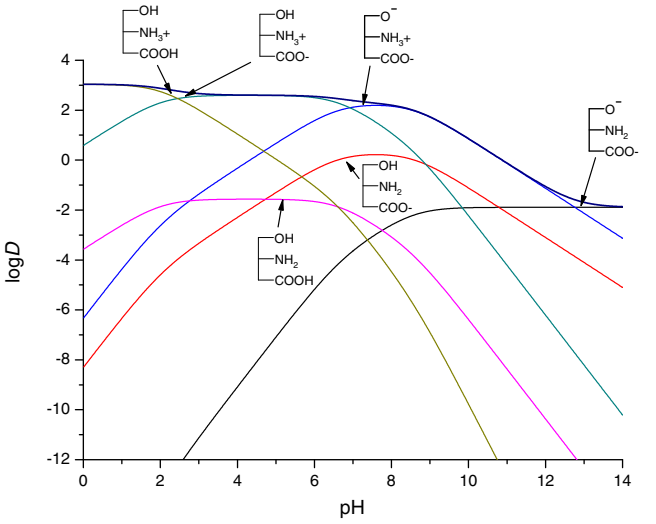

Fig. 3. Contribution of the six microspecies of thyroxine its lipophilicity profile (with broad line).

Our study presents for the first time experimental microscopic partition coefficients of a triprotic molecule – at least for the significant microspecies. Thyroxine is a further example of a case where the contribution of the zwitterionic species to the overall lipophilicity is more important than that of the non-charged one.

Acknowledgment

This work was supported by TÁMOP 4.2.1.B-09/1/KMR and the National Research Fund of Hungary, OTKA T 73804.

References

Avdeef, A., 2002. Physicochemical profiling. Curr. Top. Med. Chem. 1, 277–351.
Bjerrum, N., 1923. Dissociation constants of polybasic acids and their application to the calculation of molecular dimensions. Z. Phys. Chem. 106, 219–242.
Bouchard, G., Carrupt, P.A., Testa, B., Gobry, V., Girault, H.H., 2001. The apparent lipophilicity of quaternary ammonium ions is influenced by Galvani potential difference, not ion-pairing: a cyclic voltammetry study. Pharm. Res. 18, 702–708.
Chemburkar, S.R., Deming, K.C., Reddy, R.E., 2010. Chemistry of thyroxine: an historical perspective and recent progress on its synthesis. Tetrahedron 66, 1955–1962.
Cody, V., 1980. Thyroid hormone interactions: molecular conformation, protein binding, and hormone action. Endocr. Rev. 1, 140–166.
Comer, J.E.A., Box, K.J., 2008. Using measured p*K*(a), log *p* and solubility to investigate supersaturation and predict BCS class. Curr. Drug Metab. 9, 869–878.
Escher, B.I., Schwarzenbach, R.P., 1996. Partitioning of substituted phenols in liposome–water, biomembrane–water, and octanol–water systems. Environ. Sci. Technol. 30, 260–270.
Fujita, T., 1990. The extrathermodynamic approach to drug design. In: Ramsen, C.A., Hansch, C., Sammes, P.G., Taylor, J.B. (Eds.), Comprehensive Medicinal

Chemistry. The Rational Design, Mechanistic Study and Therapeutic Applications of Chemical Compounds, vol. 4. Pergamon, Oxford, pp. 497–560.
Hansch, C., 1994. On the future of QSAR. In: Wermuth, C.G., Koga, N., König, H. (Eds.), Medicinal Chemistry for the 21st Century. Metcalf Blackwell, Oxford, pp. 281–293.
Hennemann, G., Docter, R., Friesema, E.C., de Jong, M., Krenning, E.P., Visser, T.J., 2001. Plasma membrane transport of thyroid hormones and its role in thyroid hormone metabolism and bioavailability. Endocr. Rev. 22, 451–476.
Ishigami, K., Katsuta, R., Shibata, C., Hayakawa, Y., Watanabe, H., Kitahara, T., 2009. Synthesis and structure revision of tyroscherin, and bioactivities of its stereoisomers against IGF-1-dependent tumor cells. Tetrahedron 65, 3629–3638.
Johnson, C.A., Westall, J.C., 1990. Effect of pH and KCl concentration on the octanol-water distribution of methylanilines. Environ. Sci. Technol. 24, 1869–1875.
Liu, X.L., Testa, B., Fahr, A., 2011. Lipophilicity and its relationship with passive drug permeation. Pharm. Res. 28, 962–977.
Loeser, A., Ruland, H., Trikojus, V.M., 1938. Darstellung, Eigenschaften und biologische Wirkungen yon Derivaten (Athern) des Thyroxins, Dijodthyronins und Dijodtyrosins. Naunyn-Schmiedebergs Archiv für Experimentelle Pathologie und Pharmakologie 189, 664–665.
Mazák, K., Noszál, B., 2012a. Lipophilicity of morphine microspecies and their contribution to the lipophilicity profile. Eur. J. Pham. Sci. 45, 205–210.
Mazák, K., Noszál, B., 2012b. Zwitterions can be predominant in membrane penetration of drugs: an experimental proof. J. Med. Chem. 55, 6942–6947.
Mazák, K., Vámos, J., Nemes, A., Rácz, Á., Noszál, B., 2003. Lipophilicity of vinpocetine and related compounds characterized by reversed-phase thin-layer chromatography. J. Chromatogr. A. 996, 195–203.
Mazák, K., Kökösi, J., Noszál, B., 2011. Lipophilicity of zwitterions and related species: a new insight. Eur. J. Pham. Sci. 44, 68–73.
Nagao, H., Imazu, T., Hayashi, H., Takahashi, K., Minato, K., 2011. Influence of thyroidectomy on thyroxine metabolism and turnover rate in rats. J. Endocrinol. 210, 117–123.
Noszál, B., 1990. Acid-base properties of bioligands. In: Burger, K. (Ed.), Biocoordination Chemistry: Coordination Equilibria in Biologically Active Systems. Ellis Horwood, Chichester, pp. 18–55.
Pagliara, A., Carrupt, P.A., Caron, G., Gaillard, P., Testa, B., 1997. Lipophilicity profiles of ampholytes. Chem. Rev. 97, 3385–3400.
Reymond, F., Gobry, V., Bouchard, G., Girault, H., 2001. Electrochemical aspects of drug partitioning. In: Testa, B., van de Waterbeemd, H., Folkers, G., Guy, R. (Eds.), Pharmacokinetic Optimization in Drug Research. Wiley-VCH Publishers, Zürich, pp. 327–349.
Takács-Novák, K., Szász, Gy., 1999. Ion-pair partition of quaternary ammonium drugs: the influence of counter ions of different lipophilicity, size and flexibility. Pharm. Res. 16, 1633–1638.
Testa, B., Carrupt, P.A., Gaillard, P., Tsai, R.S., 1996. Intramolecular interactions encoded in lipophilicity: their nature and significance. In: Pliska, V., Testa, B., van de Waterbeems, H. (Eds.), Lipophilicity in Drug Action and Toxicology. VCH Publishers, Weinheim, pp. 49–71.
Tóth, G., Hosztafi, S., Kovács, Zs., Noszál, B., 2012. The site-specific basicity of thyroid hormones and their precursors as regulators of their biological functions. J. Pham. Biomed. Anal. 61, 156–164.