

DETERMINATION OF *IN VITRO* METABOLISM OF A NEW NON-IMIDAZOLE HISTAMINE H₃ RECEPTOR ANTAGONIST 1-[3-(4-TERT-BUTYLPHENOXY)PROPYL]PIPERIDINE

AGATA KRYCZYK-POPRAWA^{1*}, MAŁGORZATA SZAFARZ², ANETA PALAK²,
DOROTA ŁĄŻEWSKA³, KAMIL KUŚ⁴, WŁODZIMIERZ OPOKA¹
and JOANNA SZYMURA-OLEKSIK²

¹Department of Inorganic and Analytical Chemistry, Faculty of Pharmacy,
Jagiellonian University Medical College, Krakow, Poland

²Department of Pharmacokinetics and Physical Pharmacy, Faculty of Pharmacy,
Jagiellonian University Medical College, Krakow, Poland

³Department of Technology and Biotechnology of Drugs, Faculty of Pharmacy,
Jagiellonian University Medical College, Medyczna 9, 30-688 Krakow, Poland

⁴Jagiellonian University, Jagiellonian Centre for Experimental Therapeutics,
Bobrzynskiego 14, 30-348 Krakow, Poland

Abstract: Early understanding of the pharmacokinetics of drug candidates is essential during the evaluation of drug development process. DL76 1-[3-(4-tert-butylphenoxy)propyl]piperidine is a novel promising non-imidazole H₃ receptor antagonist. The presented study was undertaken to compare with each other the predicted hepatic clearance values of DL76 calculated, using two most common mathematical models “well-stirred” and “parallel tube”, based on the data obtained in *in vitro* experiments. The metabolic intrinsic clearance of DL76 equaled to 0.4848 mL/min/mg protein was scaled up and the values of hepatic clearance estimated from “well-stirred” and “parallel tube” models were 55.47 mL/min/kg and 58.80 mL/min/kg, respectively. They were further compared with pharmacokinetic parameters calculated based on the concentration-time profile obtained following intravenous administration of DL76 to rats at the dose of 6 mg/kg. The estimated systemic serum clearance value of 144.5 mL/h/kg and blood clearance value of 81.17 mL/min/kg indicate the potential extrahepatic metabolism of the investigated compound. This study demonstrates the utility of rat liver microsomes for the prediction of DL76 hepatic clearance.

Keywords: 1-[3-(4-tert-butylphenoxy)propyl]piperidin, well-stirred model, parallel tube model, non-imidazole, histamine H₃ receptor antagonist

Histamine plays a variety of physiological roles in the central nervous system and peripheral tissues through the four known G protein-coupled receptors H₁, H₂, H₃, and H₄. The histamine H₃ receptor (H₃R), discovered in 1983 by Arrang and co-workers, is a presynaptic autoreceptor within the Class A of GPCR family, which also functions as a heteroreceptor modulating levels of others, beside histamine, neurotransmitters such as dopamine, acetylcholine, norepinephrine, serotonin, GABA, and glutamate (1). This clearly justifies the great interest H₃R represents in the possible treatment of various neurological dysfunctions and psychiatric diseases such as schizophrenia, attention-deficit

hyperactivity disorder (ADHD), dementia, epilepsy, obesity, narcolepsy and pain (2-6). Early generations of H₃ receptor ligands were based on structures containing the imidazole moiety, as analogs of histamine, leading in many cases to the potential drug–drug interactions through inhibition of hepatic cytochrome P₄₅₀ enzymes as well as low oral bioavailability or rapid metabolism and poor CNS penetration (7). In 1998 Ganellin et al. described active compounds with piperidine and pyrrolidine moiety as a good replacement for the imidazole ring (8). Now, this class of compounds is rapidly developing with unexpectedly large structural variations. As many H₃R antagonists/inverse agonists have

* Corresponding author: e-mail: agata.kryczyk@uj.edu.pl

failed in the first or second phase of clinical research, further lead optimization and pharmacokinetic screening along with an increased understanding of the role of H₃Rs in human CNS diseases are necessary as significant steps in early-stage H₃R antagonist/inverse agonist drug discovery (7). Compound DL76 [1-[3-(4-*tert*-butylphenoxy)propyl] piperidine (Fig. 1) is the new promising non-imidazole H₃ receptor antagonist showing high affinity towards the H₃ receptor determined by the assay with human H₃ receptors stably expressed in CHO-K1 cells ($hK_1 = 22 \pm 3$ nM) and the ED₅₀ equal to 2.8 ± 0.4 mg/kg in a central histamine H₃ receptor assay *in vivo* after p.o. administration to mice (9). In a rat model of vascular dementia, based on a permanent bilateral occlusion of the common carotid arteries (BCCAO), treatment with DL76 (6 mg/kg) appeared to exert a considerable influence on measured memory function, especially improving working memory, rather than reference memory (10). One of the most important processes affecting fate of the drug in a body is elimination, and liver is considered to be the major organ of importance influencing drug disposition and especially its elimination in both unchanged form and through metabolites. The knowledge of processes taking place in this organ, as well as their quantitative approach, can be crucial to understanding the pharmacokinetic profile of a new drug. Therefore, the lack of information regarding DL76 metabolic profile prompted us to conduct a preliminary pharmacokinetic study in rats in order to provide a basis for its further development. The biotransformation of DL76 using rat liver microsomes was investigated for prediction of *in vitro* hepatic clearance. These data were further used to simulate *in vivo* hepatic clearance based on two theoretical models of hepatic elimination: "well-mixed" and "parallel tube".

MATERIALS AND METHODS

Chemicals and materials

The 1-[3-(4-*tert*-butylphenoxy)propyl]piperidine as a monobasic oxalate was supplied from the Department of Technology and Biotechnology of Drugs (Faculty of Pharmacy, Jagiellonian University Medical College, Krakow, Poland). Pentoxifylline, 3,7-dimethyl-1-(5-oxohexyl)-3,7-dihydro-1*H*-purine-2,6-dione (PTX), used as an internal standard was obtained from Sigma (St. Louis, MO, USA). HPLC grade acetonitrile, water, formic acid, and ethyl acetate were purchased from Merck (Darmstadt, Germany). β -nicotinamide adenine dinucleotide 2'-phosphate in reduced form (NADPH),

β -nicotinamide adenine dinucleotide 2'-phosphate (NADP⁺), D-glucose-6-phosphate, glucose-6-phosphate dehydrogenase, TRIS, Folin and Ciocalteu's phenol reagent were obtained from Sigma Aldrich (St. Louis, MO, USA). All other chemicals and reagents for determining microsomal protein content and conducting *in vitro* assay were of analytical grade and commercially available.

Animals

The livers used for obtaining the microsomal fractions were collected from Male Wistar rats (Charles River, Germany), weighing between 280 and 300 g. Rats were kept under conditions of constant temperature (21-25°C) and relative humidity of approximately 40-65% with standard light/dark cycle and were given free access to standard rodent food and water *ad libitum*. The animals did not receive any drugs, and prior to the experiment, they were fasted for 24 hours and then weighed. The study was approved by the Institutional Animal Care and Ethics Committee of the Jagiellonian University Medical College, Krakow, Poland.

Preparation of rat liver microsomes

Rat liver microsomes were prepared using a differential centrifugation method. Animals were sacrificed by decapitation without anesthetics, the livers were removed rapidly, washed immediately with ice-cold 20 mM Tris/KCl buffer (pH 7.4), weighed and homogenized with three volumes of buffer. The homogenate was centrifuged at 11500 x g for 20 min at 4°C, and the supernatant (S9 fraction) was transferred to the fresh centrifuge tubes and then centrifuged again at 100 000 g for 60 min at 4°C. The pellets were then suspended in 0.15 M KCl, homogenized and centrifuged at 100 000 x g for 1 h at 4°C. The pellets from final centrifugation were suspended in ice-cold 20 mM Tris buffer containing 0.25 M sucrose (pH 7.4) and stored at -80°C until use. All operations were carried out on the ice, and the buffer solutions were made on the day of the experiment and used after cooling to approx. 4°C.

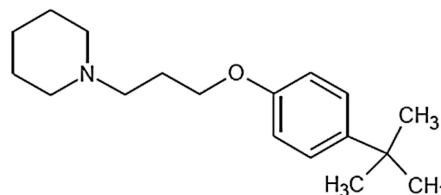


Figure 1. Chemical structure of DL76

The microsomal protein concentration was measured colorimetrically applying the Lowry method and bovine serum albumin was used as standard (11). The average enzyme protein concentration determined in the microsomal suspension was 9.54 mg/mL.

Microsomal incubations

The DL76 incubation process with the biological material was carried out in Eppendorf tubes. The incubation medium at the volume of 0.5 mL consisted of potassium phosphate buffer 0.1 mM (pH 7.4), 10 mM MgCl₂, 1 mM NADPH and microsomal protein in a final concentration of 1 mg/mL. The incubations were initiated by the addition of 25 µL of the substrate, generating an absolute DL76 concentrations of 250, 500, 1000, 2000 or 4000 ng/mL. The mixtures were incubated at 37°C in the water bath with slight agitation (100 rpm) and reactions were terminated by cooling on an ice and an addition of 1 mL of acetonitrile followed by vortex-mixing. After centrifugation at 13000 g for 15 min, the upper organic layer was pipetted out into another clean centrifuge tube. Samples were evaporated till dryness under a stream of nitrogen at 37°C, the residue was dissolved in 100 µL of acetonitrile/water (50/50, v/v) with IS (PTX at the concentration of 2.5 µg/mL) and used for LC/MS/MS analysis. The incubation mixture without NADPH was used as the blank control.

In order to select the appropriate incubation medium, the tested compound was incubated with: NADPH or NADPH generating system (1 mM NADP, 10 mM glucose-6-phosphate, 3 U/mL glucose-6 dehydrogenase phosphate). The reaction was carried out for 5 min at a concentration of enzyme proteins equal to 1 mg/mL. The medium containing NADPH was chosen for further studies due to the greater efficiency of the metabolic reaction. In order to determine the optimal incubation time, the DL76 solution was incubated at a fixed concentration of 500 ng/mL with a microsomal fraction at the concentration of 1 mg/mL. Incubation was carried out for: 2, 5, 10, 20, 30, 45 and 60 minutes. The highest decrease in DL76 concentration was observed between 2 and 5 min, therefore these two times were chosen for further experiments.

In vivo study and blood serum partitioning

Male Wistar rats, 13–15 weeks of age and weighing between 200 and 220 g were used for this study. DL76 compound dissolved in 0.9% sterile isotonic saline at a dose of 6 mg/kg was adminis-

tered intravenously. Rats were anesthetized with thiopental at a dose of 70 mg/kg and blood samples were collected after decapitation to centrifuge tubes at 5, 10, 30, 60, 120 and 240 min after dosing (n = 5). Serum samples were harvested by centrifuging at 1500 g for 10 min at room temperature and stored at -30°C until bioanalysis.

Blood to serum partitioning coefficient for DL76 was calculated from the concentrations of investigated drug in blood and serum. Compound DL76 was added to blood at the ratio of 1 : 10 to give a final concentration of 600 ng/mL. Samples were incubated at 37°C for 2 h. Aliquots of blood were removed at 0.5 and 2 h and the remaining blood was centrifuged to obtain serum. Samples were analyzed using LC-MS/MS method.

Analytical methods

The quantitative measurements of DL76 in rat liver microsomes were made using LC-MS/MS analytical method. LC-MS/MS system consisted of high-performance liquid chromatograph Agilent 1100 (Agilent Technologies, Waldbronn, Germany) and an Applied Biosystems MDS Sciex (Concord, Ontario, Canada) API 2000 triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) interface. The positive ion mode was used for ion generation and the system operated in the selected reactions monitoring mode (SRM) monitoring the transitions of the protonated molecular ions m/z 276 to 98 for DL76 and 279 to 181 for the internal standard (PTX). Chromatographic separation was carried out on an XBridgeTMC18 (2.1 x 30 mm, 3.5 µm, Waters, Ireland) analytical column. The mobile phase consisted of acetonitrile/water with an addition of 0.1% of formic acid, was set at the flow rate of 300 µL/min and the gradient elution was used. Samples were prepared by the liquid-liquid extraction technique. A 100 µL of rat serum containing an unknown concentration of DL76 was briefly mixed with the 10 µL of IS (PTX at the concentration of 2.5 µg/mL) and then 1 mL of ethyl acetate was added. The mixture was shaken on a mechanical shaker for 20 min and centrifuged at 1500 g for 15 min. The organic layer (0.5 mL) was transferred into conical tubes and evaporated to dryness. The dry residue was reconstituted with 100 µL of acetonitrile/water (50/50, v/v) mixture and an aliquot of 10 µL was injected onto the LC-MS/MS system. The quality control samples were prepared at different concentrations along the calibration range (LLOQ at 5 ng/mL, low at 40 ng/mL, medium at 400 ng/mL and high at 1800 ng/mL of DL76).

In vitro calculations

Enzymatic metabolic parameters were calculated from *in vitro* metabolism study with rat liver microsomes based on the substrate disappearance rate. K_M and V_{max} values were estimated by linear regression analysis of Lineweaver-Burk plots. *In vitro* intrinsic clearance was computed as V_{max}/K_M . The intrinsic clearance value expressed per milligram of microsomal protein calculated from the *in vitro* metabolism experiments was scaled up per kilogram of body weight taking account of the microsomal protein content per gram of liver – 61 mg/g (12) and average liver weight per kilogram of body weight – 32.67 g/kg.

In vitro-In vivo scaling using the “well-stirred” and “parallel-tube” models

The hepatic clearance was predicted from intrinsic clearance using the equations for both the “well-stirred” (Eq. 1) and “parallel-tube” (Eq. 2) liver models (13):

$$Cl_H = Q_H \cdot E_H = Q_H \cdot \frac{Cl_{int} \cdot f_u}{Q_H + Cl_{int} \cdot f_u} \quad (1)$$

$$Cl_H = Q_H \cdot E_H = Q_H \cdot (1 - e^{-Cl_{int} \cdot f_u / Q_H}) \quad (2)$$

where:

Q_H – the hepatic blood flow

E_H – the extraction ratio

Cl_{int} – the intrinsic clearance

f_u – the unbound fraction of DL76 in plasma

Pharmacokinetic analysis

Pharmacokinetic parameters were calculated by employing a non-compartmental approach from the average concentration values, using Phoenix WinNonlin software (Pharsight, USA). The area

under the mean serum concentration versus time curve extrapolated to infinity ($AUC_{0 \rightarrow \infty}$) was estimated using the log/linear trapezoidal rule and the extrapolated area calculated as C_{last}/λ_z . AUMC was estimated by calculation of total area under the first-moment curve by combining the trapezoid calculation of $AUMC_{0 \rightarrow \infty}$ and extrapolated area determined according to the Eq. (5), where C_{last} is a last measured concentration at the last sampling time (t_{last}).

$$AUMC_{0 \rightarrow \infty} = \frac{t_{last} \cdot C_{last}}{\lambda_z} + \frac{C_{last}}{\lambda_z^2} \quad (3)$$

The mean residence time (MRT) was calculated from $AUMC_{0 \rightarrow \infty}/AUC_{0 \rightarrow \infty}$. The terminal rate constant (λ_z) was calculated by log-linear regression of the investigated compound concentration data in the terminal phase (at least three latest sampling time points were used) and the half-life ($t_{1/2}$) was calculated as $0.693/\lambda_z$. The systemic serum clearance (Cl_S) was estimated from the administered dose divided by $AUC_{0 \rightarrow \infty}$.

The blood clearance (Cl_B) was calculated according to the following relationship:

$$Cl_B \cdot C_B = Cl_S \cdot C_S \quad (4)$$

where C_B and C_S are concentrations in blood and serum, respectively and Cl_S is a systemic serum clearance.

RESULTS

The developed LC-MS/MS method previously described for the quantification of DL76 in rat serum was suitable for the determination of DL76 in the presence of its metabolite. (14). The calibration curve for DL76 compound was generated by weight-

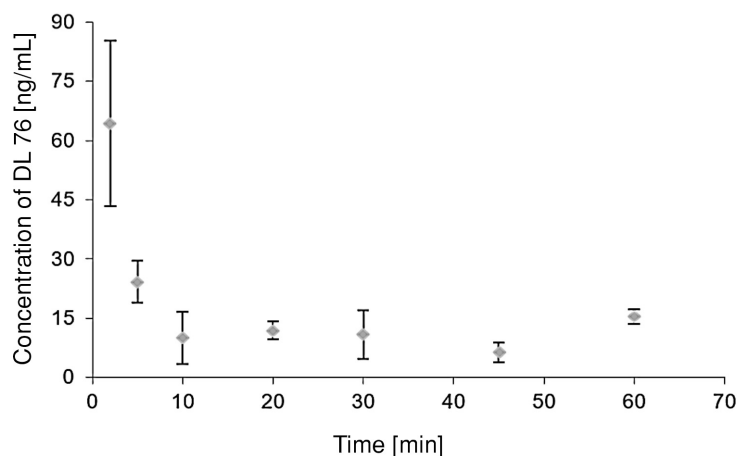


Figure 2. The effect of incubation time on DL76 concentration

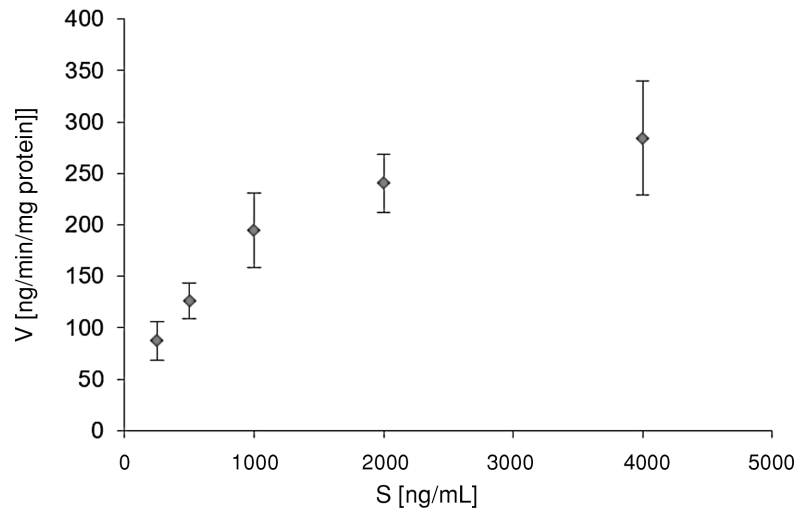


Figure 3. Kinetic plot for the metabolism of DL76 in rat hepatic microsomes

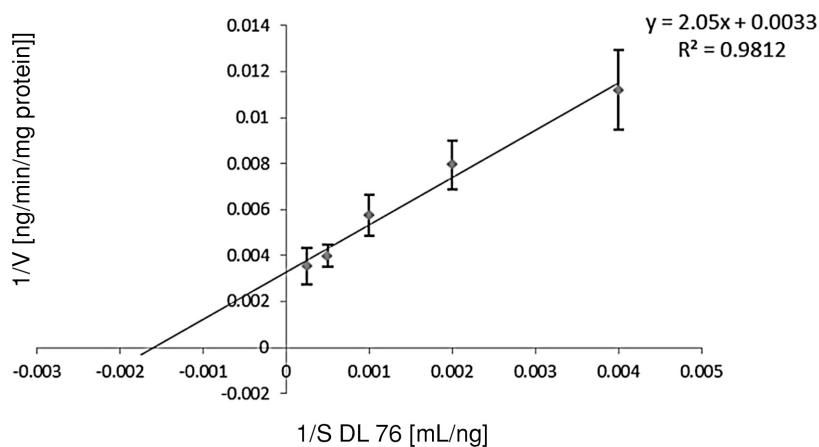


Figure 4. Lineweaver-Burk Plot of DL76

ed ($1/x$) linear regression analysis and was linear in the range from 5 to 2000 ng/mL. The exemplary linear regression equation was $y = 0.0054x + 0.0104$ for which the determination coefficient was equal to 0.9984. The limit of detection in the SRM mode was found to be 1 ng/mL and the limit of quantification was 5 ng/mL. The precision and accuracy for both intra- and inter-day determination of DL76 ranged from 2.65 to 17.03% and from 94.17 to 10.63%.

The highest decrease in DL76 concentration was observed between 2 and 5 min, therefore these two times were chosen for further experiments (Fig. 2). The elimination rate of DL76 was quantified at the substrate concentrations from 250 to 4000 ng/mL to represent the enzyme kinetics. Rates of

DL76 metabolism (V_0) were calculated from the slope of the initial linear decline at each respective substrate concentration. The plot of initial reaction rate (ng/min/mg protein) against substrate concentration (ng/mL) was hyperbolic and could be described well by the classical Michaelis-Menten kinetics as shown in Figure 3. Then the Lineweaver-Burk plot was used to calculate enzyme kinetic parameters V_{max} and K_m ($R^2 = 0.9812$, $P < 0.05$) (Fig. 4). Pharmacokinetic parameters determined for the biotransformation of the tested compound at substrate concentrations 250-4000 ng/mL were: $V_{max} = 303.03$ ng/min/mg protein, $K_M = 625$ ng/mL.

The value of the intrinsic clearance for the reaction of DL76 metabolism was 0.4848

mL/min/mg protein which was further converted using the physiological parameters to 981.18 mL/min/kg body weight of the rat. Subsequently, the values of hepatic clearance were determined based on the data obtained from *in vitro* experiments. The two most commonly used mathematical models describing drug elimination by the liver: “well-stirred” and “parallel tube” were used to predict *in vivo* hepatic clearance of DL76. The flow rate value adopted at physiological level was 58.8 mL/min/kg (15). The average value of f_u equal to 0.4 was assumed based on the previously published albumin binding characteristics of DL76 compound (16). Given the above data, the values of hepatic clearance estimated from “well-stirred” and “parallel tube” models were 55.47 mL/min/kg and 58.80 mL/min/kg, respectively.

Pharmacokinetic parameters calculated based on the concentration-time profile after i.v. administration of DL76 compound to rats at the dose of 6 mg/kg are presented in Table 1. Blood to serum partitioning coefficient equaled to 1.78 and was used to calculate the blood clearance. The systemic serum clearance (CL_s) was 144.5 mL/min/kg and the blood clearance (CL_B) estimated according to the Eq. 6 was 81.17 mL/min/kg.

A comparative summary of the hepatic clearance values of DL76 obtained *in vitro*, *ex vivo* and *in vivo* including or excluding plasma protein binding is presented in Table 2.

The presented study was undertaken to determine the hepatic clearance of a new non-imidazole histamine H_3 receptor antagonist, compound DL76 (1-[3-(4-tert-butylphenoxy) propyl]piperidine). The determination of intrinsic clearance for drug candidates in the early discovery stage is a general practice in both academia and the pharmaceutical industry (17). The application of *in vitro* metabolism data using rat liver microsomes is the most common way to predict the value of hepatic clearance in rat. The *in vitro* CL_{int} may be derived traditionally from the metabolite formation or based on the substrate depletion method (15, 18, 19). For the purpose of this study, the latter approach was utilized. To achieve appropriate estimates of *in vitro* kinetic parameters it was necessary to optimize the incubation procedure of DL76 with the microsomal fraction. The following conditions of the incubation process were carefully chosen: the way of NADPH supplementation for oxidative transformation either by direct addition of NADPH or via an NADPH-regenerating system, the time of incubation and the concentration of microsomal protein.

The quantitative relationship between intrinsic clearance, blood flow and unbound fraction of the investigated compound can only be understood with the use of a conceptual model of the liver and several models and approaches have been proposed. In the present study, the predictive ability of two mathematical models of hepatic clearance was verified,

Table 1. Pharmacokinetic indices of DL76 following an i.v. administration at a dose of 6 mg/kg to rats (n = 5); data are presented as median values \pm SD.

Pharmacokinetic parameter	C_0 [ng/mL]	$t_{0.5kz}$ [min]	CL_s [mL/min/kg]	$AUC_{0 \rightarrow \infty}$ [ng·h/mL]	V_{ss} [L/kg]	MRT [min]
Parameter value	652.7	141	144.5	684.78	26.2	181.8

Table 2. Comparison between average values of hepatic clearance of DL76 obtained *in vitro*, *ex vivo* and *in vivo*.

Well-stirred model		
All protein binding ignored	$Cl_H = Q_H \cdot \frac{Cl_{int}}{Q_H + Cl_{int}}$	55.47 mL/min/kg
Including plasma protein binding (f_u)	$Cl_H = Q_H \cdot \frac{Cl_{int} \cdot f_u}{Q_H + Cl_{int} \cdot f_u}$	51.11 mL/min/kg
Parallel tube model		
All protein binding ignored	$Cl_H = Q_H \cdot (1 - e^{-Cl_{int}/Q_H})$	58.80 mL/min/kg
Including plasma protein binding (f_u)	$Cl_H = Q_H \cdot (1 - e^{-Cl_{int} \cdot f_u / Q_H})$	58.73 mL/min/kg
Serum clearance		144.5 mL/min/kg
Blood clearance		81.17 mL/min/kg

since the predictions based on these assumptions represent the two opposite extremes of all the available models. The “well-stirred model” views the liver as a well-stirred compartment with concentration of unbound drug in the liver in equilibrium with that in the blood, while the “parallel tube model” regards the liver as a series of parallel tubes with enzymes evenly distributed and the concentration of unbound drug declining along the tube length (20). Pharmacokinetic dissimilarities among these models are mainly due to the differences in assumptions made on the anatomical structure of the liver and the extent of blood mixing within this organ but both of them incorporate scaling factors affecting the hepatic clearance of compounds such as protein binding, hepatic blood flow, and enzymatic activity. Accurate prediction of hepatic clearance requires the appropriate use of protein binding phenomena, both the nonspecific binding of the compound to the microsomal milieu and binding to plasma proteins. The inclusion of nonspecific microsomal binding is often discussed as a potential correction factor but currently available data also showed that its use in the hepatic clearance calculation does not seem to substantially improve *in vitro-in vivo* correlation (18). The percentage of DL76 bound to plasma protein used for calculation in the presented manuscript was assumed to be equal to 60% and the nonspecific binding to microsomes was omitted (16). The aforementioned data have been obtained using equilibrium dialysis over the concentration range of 0.32 nM – 317.18 μ M and at a physiological bovine serum albumin (BSA) concentration of 602 μ M (16). In the presented study the hepatic blood flow rate value assumed at physiological level was equal to 58.8 mL/min/kg, but there are large discrepancies between the values reported in literature (from 55 to 80 mL/min/kg), which may also have an impact on the accuracy of the *in vitro/in vivo* extrapolation (21, 22).

In parallel with the *in vitro* assessment of the metabolic stability, the pharmacokinetics of new chemical entities is often examined in order to identify the major route(s) of elimination in laboratory animals from *in vitro-in vivo* correlation analysis. The systemic blood clearance of DL76 following i.v. administration of this compound to rats was high (81.17 mL/min/kg). This value compared with the calculated hepatic clearance and considering the physiological rate of hepatic blood flow in rats equal to 58.8 mL/min/kg may indicate potential extrahepatic metabolism of the tested compound, which could theoretically contribute to the discrepancies between hepatic and systemic clearances of DL76 (23, 24, 25). Presented results may be a source of

useful information for scientists searching for new chemical entities in this group of compounds.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Lebois E.P., Jones C.K., Lindsley C.W.: *Curr. Top. Med. Chem.* 11, 648 (2011).
2. Sadek B., Schwed J.S., Subramanian D., Weizel L., Walter M. et al.: *Eur. J. Med. Chem.* 22, 269 (2014).
3. Miyazaki S., Onodera K., Imaizumi M., Timmerman H.: *Life Sci.* 61, 355 (1997).
4. Hancock A.A.: *Curr. Opin. Investig. Drugs* 4, 1190 (2003).
5. Ito C.: *Drug News Perspect.* 17, 383 (2004).
6. Pillot C., Héron A., Schwartz J.C., Arrang J.M.: *Eur. J. Neurosci.* 7, 307 (2003).
7. Nikolic K., Filipic S., Agbaba D., Stark H.: *CNS Neurosci. Ther.* 20, 613 (2014).
8. Ganellin C.R., Leurquin F., Piripitsi A., Arrang J.M., Garbarg M. et al.: *Arch. Pharm. (Weinheim)* 331, 395 (1998).
9. Lazewska D., Ligneau X., Schwartz J.C., Schunack W., Stark H., Kiec-Kononowicz K.: *Bioorg. Med. Chem.* 14, 3522 (2006).
10. Stasiak A., Mussur M., Unzeta M., Lazewska D., Kiec-Kononowicz K., Fogel W.A.: *J. Physiol. Pharmacol.* 62, 549 (2011).
11. Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J.: *J. Biol. Chem.* 193, 265 (1951).
12. Smith R., Jones R.D., Ballard P.G., Griffiths H.H.: *Xenobiotica* 38, 1386 (2008).
13. Pang K.S., Rowland M.: *J. Pharmacokinet. Biopharm.* 5, 625 (1977).
14. Szafarz M., Szymura-Oleksiak J., Lazewska D., Kiec-Kononowicz K.: *Chromatographia* 73, 913 (2011).
15. Naritomi Y., Terashita S., Kimura S., Suzuki A., Kagayama A., Sugiyama Y.: *Drug Metab. Dispos.* 29, 1316 (2001).
16. Szymura-Oleksiak J., Kryczyk A., Szafarz M., Jawień W., Łazewska D., Kieć-Kononowicz K.: *Acta Pol. Pharm.* 69, 1043 (2012).
17. Jones H.M., Houston J.B.: *Drug Metab. Dispos.* 32, 973 (2004).
18. Mohutsky M.A., Chien J.Y., Ring B.J., Wrighton S.A.: *Pharm. Res.* 23, 654 (2006).
19. Obach R.S., Reed-Hagen A.E.: *Drug Metab. Dispos.* 30, 831 (2002).

20. Pang K.S., Rowland M.: J. Pharmacokinet. Biopharm. 5, 625 (1977).
21. Murata M., Tamai I., Sai Y., Nagata O., Kato H., Sugiyama Y., Tsuji A.: Drug Metab. Dispos. 26, 1113 (1998).
22. Oliver R.E., Jones A.F., Rowland M.: J. Pharmacokinet. Pharmacodyn. 28, 27 (2001).
23. Davies B., Morris T.: Pharm. Res. 10, 1093 (1993).
24. Hanada K., Ikemi Y., Kukita K., Mihara K., Ogata H.: Drug Metab. Dispos. 36, 2037 (2008).
25. Szafarz M., Kryczyk A., Lazewska D., Kieć-Kononowicz K., Wyska E.: Xenobiotica 45, 912 (2015).

Received: 21.02.2019