



Impact of biorelevant media on pharmacologically important properties of potential neuroprotectors based on 1,2,4-thiadiazole



Tatyana Volkova^{a,*}, Ekaterina Chibunova^a, Oleg Silyukov^a, Alexey Proshin^b, Irina Terekhova^a

^a Institute of Solution Chemistry, Russian Academy of Sciences, 153045 Ivanovo, Russia

^b Institute of Physiologically Active Compounds, Russian Academy of Sciences, 142432 Chernogolovka, Russia

ARTICLE INFO

Article history:

Received 10 July 2017

Received in revised form 22 September 2017

Accepted 22 September 2017

Available online 23 September 2017

Keywords:

1,2,4-Thiadiazole
Biorelevant medium
Dissolution
Distribution
Permeability

ABSTRACT

Pharmacologically important properties of two structurally related biologically active 1,2,4-thiadiazole derivatives were investigated in phosphate buffer and biorelevant media FaSSIF simulating the intestinal fluid. Solubility and distribution coefficients of thiadiazoles were found to be substantially higher in FaSSIF than in blank buffer. On the contrary, permeability coefficients decreased and dissolution rate was not changed in FaSSIF. Mechanism of FaSSIF components action on thiadiazoles behavior was revealed using ¹H NMR and UV-spectroscopy.

© 2017 Elsevier B.V. All rights reserved.

1. Introduction

Understanding of drug compound behavior in the gastro-intestinal (GI) tract where it is released from the formulation, dissolved in biological fluids and passed through the intestine membranes is a key task of biomedicine and biopharmaceutics. *In vivo* and *in situ* study of these processes is laborious and expensive; therefore, the *in vitro* experiments carrying out under the conditions close to the human physiological state can be efficient. To this end, the biorelevant media containing the main components of human GI tract such as bile salts, lecithin, pepsin and *etc.* have been developed to simulate the fasted and fed states of GI tract [1]. No wonder that the components of the biorelevant media are expected to have considerable influence on the solubility, dissolution and permeability of the orally administrated drugs [2–4]. In particular, micelles of surfactants existing in the intestinal fluids may affect the overall bio-availability by enhancing solubility and modulating membrane permeability [5]. As it is known, the solubility-permeability interplay should be taken into account when drug formulations are examined in the solutions with different solubilizing additives [3,6–8]. Distribution and permeability coefficients being pharmacologically important parameters also depend on the behavior of drug molecules in different buffers. Therefore, the influence of gastrointestinal fluids should be considered and it is reasonable to investigate the pharmacologically important properties of oral drugs in biorelevant fluids in order to evaluate their overall impact on drug absorption. Application of the biorelevant

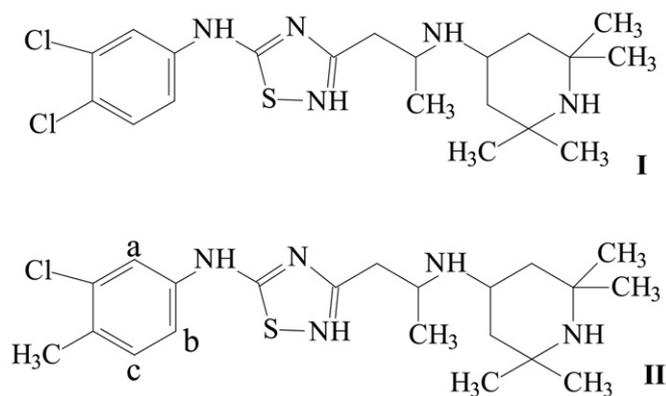
media in drug testing is expected to give more reliable and meaningful results. Undoubtedly, it is useful for newly synthesized drug candidates since the cost of the *in vivo* experiments can be minimized by excluding the compounds with poor properties on early stages of drug design.

Many drugs containing thiadiazole nucleus are available in the market due to a wide variety of biological activities to the specific targets of different pathologies [9]. As it was reported by Kushwaha et al. [10], the strong aromaticity of the thiadiazole ring system leads to great *in vivo* stability and generally, a lack of toxicity. Moreover, when diverse functional groups interacting with biological receptors are attached to this ring, the compounds possessing outstanding properties are obtained.

The objects of present study are {2-[5-(3,4-dichlorophenylamino)-1,2,4-thiadiazol-3-yl]-1-methylethyl}-(2,2,6,6-tetramethylpiperidine-4-yl)-amine (I) and {2-[5-(3-chloro-4-methylphenylamino)-1,2,4-thiadiazol-3-yl]-1-methylethyl}-(2,2,6,6-tetramethylpiperidine-4-yl)-amine (II), the structure of which are shown in Scheme 1. These compounds belong to an essentially new group of compounds in a series of 1,2,4-thiadiazole derivatives, which can be the positive modulators of AMPA-receptors [11,12]. Due to pharmacological importance of these compounds investigation of the effect of the biorelevant dissolution medium on their transport properties and evaluating the interactions of the thiadiazoles under study with the medium components seems to be useful.

The 1,2,4-thiadiazole related compounds I and II were selected in this work in order to assess their absorption behavior in the intestine. The aims of the present study were: (1) to determine the main physico-chemical parameters (solubility, dissolution rate, distribution and permeability coefficients) of 1,2,4-thiadiazole derivatives in biorelevant

* Corresponding author at: 1 Akademicheskaya str., 153045 Ivanovo, Russia.
E-mail address: vtv@isc-ras.ru (T. Volkova).



Scheme 1. Structures of 1,2,4-thiadiazoles under study.

medium and corresponding blank buffer; (2) to reveal the effect of the biorelevant medium composition on the investigated parameters; (3) to characterize the interactions between the drugs and dissolution medium components in order to obtain more meaningful results which would be helpful for promotion of the considered thiadiazoles to the market.

2. Material and methods

2.1. Materials

1,2,4-Thiadiazole derivatives were synthesized in the Institute of Physiologically Active Compounds of the Russian Academy of Sciences. Synthetic approaches and scheme was described by us before [13].

2.1.1. {2-[5-(3,4-Dichlorophenylamino)-1,2,4-thiadiazol-3-yl]-1-methylethyl}-(2,2,6,6-tetramethylpiperidine-4-yl)-amine

White powder. Yield 67%. Calcd, %: C 54.29, H 6.61, N 15.83 C₂₀H₂₉Cl₂N₅S. Found, %: C 54.67, H 6.66, N 15.48. ¹H NMR [200 MHz] δ: 0.79 (2H, dt, *J* = 12.0, 21.2 Hz, C(3)HH, C(5)HH), 1.08 and 1.12 (both s, 6H, 2 CH₃), 1.15 (3H, d, *J* = 6.6 Hz, CH₂CHCH₃), 1.84 (2H, dd, *J* = 3.3, 12.6 Hz, C(3)HH, C(5)HH), 2.84 (1H, dd, *J* = 6.0, 14.2 Hz, CHHCHMe), 2.92 (1H, dd, *J* = 4.0, 14.2 Hz, CHHCHMe), 3.00 (1H, m, C(4)H), 3.42 (1H, m, CH₂CHMe), 7.17 (1H, dd, *J* = 2.2, 8.6 Hz, H_{arom}), 7.45 (1H, d, *J* = 8.6 Hz, H_{arom}), 7.51 (1H, d, *J* = 2.2 Hz, H_{arom}). Purity ≥98%.

2.1.2. {2-[5-(3-Chloro-4-methylphenylamino)-1,2,4-thiadiazol-3-yl]-1-methylethyl}-(2,2,6,6-tetramethylpiperidine-4-yl)-amine

White powder. Yield 77%. Calcd, %: C 59.77, H 7.64, N 16.59 C₂₁H₃₂ClN₅S. Found, %: 59.87, H 7.34, N 16.44. ¹H NMR [200 MHz] δ: 0.65 (1H, t, *J* = 12.0 Hz, CHH), 0.75 (1H, t, *J* = 12.0 Hz, CHH), 1.00 (6H, s, (CH₃)₂), 1.06 (3H, d, *J* = 6.3 Hz, CCH₃), 1.10 (6H, s, (CH₃)₂), 1.71 (2H, dd, *J* = 3.5, 12.3 Hz, 2CH), 2.31 (3H, s, Ph-CH₃), 2.71 (2H, dd, *J* = 6.3, 14.2 Hz, CH₂), 2.89 (1H, tt, *J* = 3.5, 11.6 Hz, NCH), 3.32 (1H, m, NCH), 7.17 (1H, d, *J* = 8.4 Hz, H_{arom}), 7.29 (1H, dd, *J* = 2.2, 8.4 Hz, H_{arom}), 7.71 (1H, d, *J* = 2.2 Hz, H_{arom}). Purity ≥98%.

Dibasic sodium phosphate (Aldrich), sodium hydroxide (Aldrich), sodium chloride (Aldrich), lecithin (Fisher Chemicals), sodium taurocholate (TCI) and 1-octanol (Aldrich) were used as received. Phosphate buffer and FaSSIF (Fasted state simulation intestinal fluid) were used in the experiments. Table 1 shows the composition of FaSSIF medium, which was prepared according to the procedure proposed by Galia et al. [14].

2.2. Equilibrium solubility in FaSSIF and corresponding blank buffer

Solubility measurements were carried out at 298.15 K by the shake-flask method. An excess amount of thiadiazoles was added to the

Table 1
Composition of the media to simulate the contents of the small intestine in the fasted state [14].

Buffer component	FaSSIF	Blank buffer
Sodium taurocholate (mM)	3	–
Lecithin (mM)	0.75	–
Dibasic sodium phosphate (mM)	28.65	28.65
Sodium hydroxide (mM)	8.7	8.7
Sodium chloride NaCl (mM)	105.85	105.85
pH	6.5	6.5

dissolution medium. The solutions were shaken in thermostatically controlled chamber during 72 h. After equilibration solutions were kept in thermostat during 3 h to avoid supersaturating and then centrifuged (Biofuge pico, Thermo Electron LED GmbH, Germany) at 372g for 20 min at 298.15 K. The pH control of the saturated solutions was performed using Mettler Toledo Five Easy pH-meter. Concentration of thiadiazoles in solutions was determined spectrophotometrically (spectrophotometer Shimadzu UV-1800, Japan). The experimental results are reported as an average value of three replicated experiments with an accuracy of 2–4%.

2.3. In vitro dissolution study

The dissolution of the investigated thiadiazoles was performed using dissolution tester (Labindia, India) and basket method. Tablets were prepared by direct compression using laboratory hydraulic press PGR-10. The thiadiazole content in all tablets was constant and it was equal to 6 (±0.002) mg. Experiments were carried out in blank buffer (pH 6.5) and FaSSIF (pH 6.5) at constant temperature of 37 °C and baskets rotation speed of 70 rpm. Aliquots each of 5 mL were withdrawn from the dissolution medium (450 mL) at the predetermined time intervals and replaced with the same amount of fresh buffer. The samples were analyzed spectrophotometrically (spectrophotometer Shimadzu UV-1800, Japan). The experimental results are reported as an average value of three replicated experiments with an accuracy of 2–8%.

2.4. In vitro permeability study

Permeability study was conducted in Franz diffusion cell (PermeGear, Inc., PA, USA) with a volume of 5 mL. The dialysis membrane Spectra/Por® 2 (MWCO 12000–14,000 Da) was used. The membrane was pretreated with distilled water for 30 min and dried under air before use. The membrane was then mounted between donor chamber and receptor chamber with an effective surface area of 0.64 cm². The donor compartment (bottom chamber) was filled with 5 mL of thiadiazole suspension in blank phosphate buffer (pH 6.5) or FaSSIF. The system was maintained at 25.0 ± 0.1 °C and the suspension in the donor compartment was stirred vigorously. The receptor compartment (upper chamber) was filled with 1.0 mL of phosphate buffer. Thus, in all cases a reverse dialysis set up was employed [15]. An aliquot of 0.5 mL of the receptor solution was withdrawn from the receptor chamber at predetermined time intervals (30 min) and replaced with fresh phosphate buffer. After that it was analyzed spectrophotometrically (spectrophotometer Shimadzu UV-1800, Japan).

The fractional amount of permeated drug (*dQ*, expressed in number of moles) was calculated over the time interval (*dt*, expressed in seconds). The flux (*J*) slope of the linear regression of the cumulative plot of the drug through the barrier normalized by the surface area (*A*) was calculated according to the equation:

$$J = \frac{dQ}{A \times dt} \quad (1)$$

The apparent permeability coefficient (*P_{app}*) was calculated by normalizing the flux (*J*) measured over the concentration of the drug in

the donor compartment (C_0) as described:

$$P_{app} = \frac{J}{C_0} \quad (2)$$

Permeability study of compounds **I** and **II** was carried out at least in triplicate. The result permeability coefficient value of **I** was determined from the average of the experimental values with an accuracy of 2–8%.

2.5. Distribution coefficient determination

The distribution coefficients of compounds **I** and **II** in 1-octanol/buffer system were measured at 25 °C by standard shake-flask method described in literature papers [16–19]. The FaSSIF and corresponding blank buffer at pH 6.5 were used as aqueous phase. 1-Octanol and buffer were mutually saturated at 25 °C for 24 h and then left to separate. After separation of the two phases the stock solution of thiazole in buffer saturated 1-octanol phase was prepared. The total concentration of thiazole before partitioning was $1.14 \cdot 10^{-3}$ M. The buffer saturated octanol phase with the dissolved substance and octanol saturated buffer phase were placed in a glass vials and mixed during 24 h at 25 °C. After 24 h, the phases were left to separate, and the absorbance of the molecules before and after partitioning at several octanol/buffer phase ratios was detected by UV-spectroscopy (spectrophotometer Cary-50, USA). The distribution coefficients (D) were calculated from the absorbance of the molecules before and after partitioning according to the following equation:

$$D = \frac{(C_0 - C_{oct})V_{oct}}{C_{oct}V_{buf}} \quad (3)$$

where C_0 and C_{oct} are the compound concentrations in 1-octanol phase before and after partition experiment, respectively; V_{oct} and V_{buf} are the volume of 1-octanol and aqueous phase. Each experiment was performed in four replicates. The result distribution coefficient value of **I** was an average of the experimental values with an accuracy of 2–4%.

3. Results and discussion

It is well known that the main properties determining the bioavailability of drugs, such as solubility, distribution, and permeability are dependent on the ionization state of the compound and pH of the dissolution medium. Compounds **I** and **II** have several ionizable groups in the structure and they are considered as tribasic compounds.

The dissociation constants were calculated using ACD/ChemSketch computer program. Application of ACD/ChemSketch for prediction of pK_a values of drugs was demonstrated by Schonherr et al. [20]. A good accordance between the experimentally measured and calculated pK_a of examined drugs has been observed. In our case, the following pK_a values were derived: pK_{a1} was equal to 1.08 and 1.50 for **I** and **II**, respectively; pK_{a2} was equal to 7.33 and 7.34 for **I** and **II**, respectively; pK_{a3} – 10.95 (similar for **I** and **II**). Depending on pH, compounds **I** and **II** exist in solution in the following forms: neutral molecule (L), monocation (LH^+), dication (LH_2^{2+}), and trication (LH_3^{3+}). The monocation (~20%) and dication (~80%) present in buffer solutions (pH 6.5) under study.

The equilibrium solubility of compounds **I** and **II** measured by the shake-flask method in FaSSIF and corresponding blank buffer (pH 6.5) is given in Fig. 1. As one can see, solubility of 1,2,4-thiazole derivatives is higher in FaSSIF than in the blank buffer. A ratio of the solubility enhancement $\frac{S_{FaSSIF}}{S_{blank}}$ is approximately equal to 2.5 for both compounds. The FaSSIF has complicate composition (Table 1) and contains physiologically relevant surfactants as the main components, which make this medium similar to the digestive juices. The observed solubilizing effect of FaSSIF is caused by the possible interactions of thiazoles with the micelles of taurocholate and lecithin which are present in the simulated intestinal fluid (Table 1).

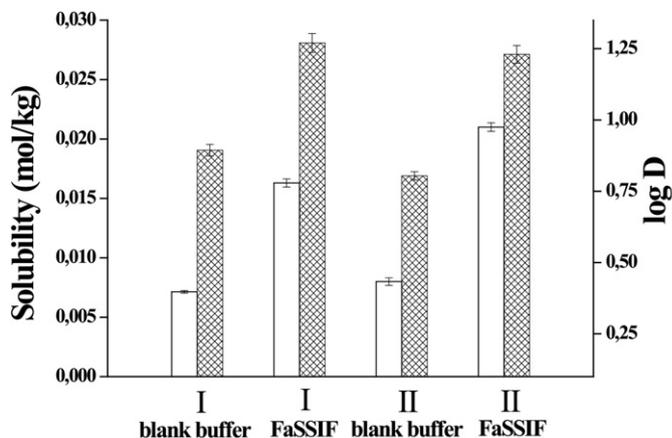


Fig. 1. Solubility (empty columns) and distribution coefficients in 1-octanol/buffer system (shaded columns) of 1,2,4-thiazole derivatives at 25 °C.

It is evident from Fig. 1 that solubility of **II** is only slightly higher in comparison with **I**. Thus, **I** has lower affinity to the polar solvent as compared with **II**. This is in agreement with the obtained distribution coefficients (D) of **I** and **II** in 1-octanol/FaSSIF and 1-octanol/blank buffer systems. Values of $\log D$ are shown in Fig. 1. As one can see, the ratio $\log D_I > \log D_{II}$ is maintained for the blank buffer and FaSSIF. Taking into account these values, one can conclude that lipophilicity of thiazole **I** is higher than **II**. As consequence, the aqueous solubility of **I** is lower (Fig. 1).

It should be noted that buffer composition affects the $\log D$ values. The revealed tendency $\log D_{FaSSIF} > \log D_{blank\ buffer}$ shows that lipophilicity of both thiazoles under consideration increases in FaSSIF. This can be explained by the possible electrostatic interactions existing between the protonated thiazoles and negatively charged micelles of taurocholate and lecithin. Thus, thiazole molecules become less polar in FaSSIF. It favors their transfer into octanol phase and, as consequence, results in the $\log D_{FaSSIF}$ increase (Fig. 1). It should be mentioned herein that lipophilicity plays an important role in the drug delivery especially in the case of compounds with potential neuroprotective action.

In order to elucidate the localization and binding mode of the investigated compounds to the micelles present in FaSSIF medium, the UV-spectroscopy was employed. It is known that absorption spectra of most compounds are sensitive to changes in the environment and especially in the dielectric constant of the environment [21]. The change in absorption maxima is considered to be a function of the dielectric constant. The micelles contain both hydrophilic and hydrophobic regions. Therefore, it is accepted, if the compound is solubilized in the inner core (non-polar) of the micelle, its UV-spectra will be similar to its UV-spectra in a non-polar solvent [22]. On the contrary, the similarity of the absorption spectra in polar solvent and micellar solution points out the location of the compound in the outer (polar) region of the micelle. To this end, the absorption spectra were recorded in hexane (non-polar solvent), blank buffer pH 6.5 (polar solvent) and FaSSIF. As an example, absorption spectra of compound **I** is shown in Fig. 2. The absorption maximum of **I** was detected at 283, 287 and 290 nm in hexane, blank buffer and FaSSIF, respectively. It was noticed that absorption maximum is shifted towards longer wavelengths in FaSSIF and considerable difference (7 nm) between UV-spectra in hexane and FaSSIF was observed. On the other hand, the difference between absorption spectra in blank buffer and FaSSIF is not so pronounced (3 nm). Thus, the UV-spectra in blank buffer and FaSSIF are closer. These results are consistent with the assumption that compound **I** is more likely to be located in the polar region of the micelles.

To give more insight into binding of thiazoles with taurocholate micelles, the 1H NMR study was undertaken. For this purpose, the 1H NMR spectra of 1,2,4-thiazole derivatives were recorded in the presence of variable amounts of sodium taurocholate. It should be

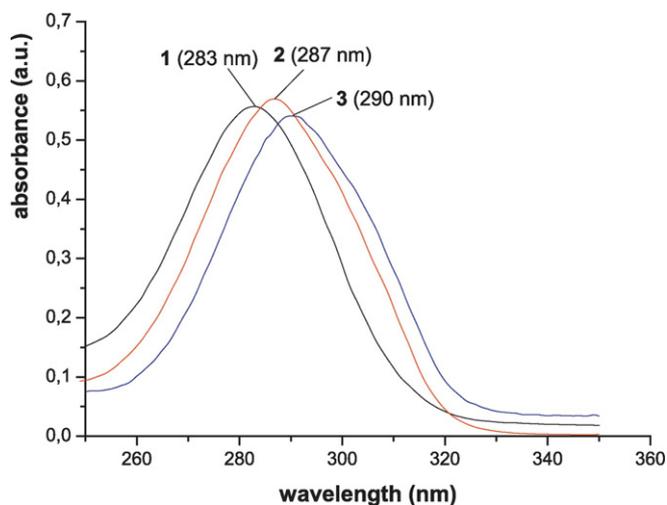


Fig. 2. Absorption spectra of 1,2,4-thiadiazole derivatives in different media (1 – hexane; 2 – blank buffer pH 6.5; 3 – FaSSIF).

mentioned that the ^1H NMR spectra were not analyzed in the region of 0–4 ppm since the located herein signals of the protons of the piperidine ring and its methyl side groups as well as the signals of the protons of the hydrocarbon chain connecting the thiadiazole and piperidine rings in the molecules of **I** and **II** are overlapped with the signals of taurocholate protons [23,24]. Therefore, the signals of the protons of benzene ring (Ha, Hb and Hc) located in the downfield region (7–8 ppm) of spectrum were only considered. Fig. 3 shows the experimental dependences of the chemical shifts of Ha, Hb and Hc protons on taurocholate concentration. As one can see, the chemical shifts decrease with concentration rise up to 3 mmol/kg and after that they dramatically increase. The observed variation of the chemical shifts was due to the change of surrounding environment of 1,2,4-thiadiazole derivatives. The bending point at concentration of 3 mmol/kg can be considered as CMC of taurocholate in the solutions of **I** and **II**. This value is in accordance with CMC of sodium taurocholate, which has been reported in literature to be 3–6 mM [25,26]. Thus, CMC of taurocholate is not shifted in the solutions of 1,2,4-thiadiazole derivatives and the micelles are present in FaSSIF. The observed significant change of the chemical shifts of the aromatic protons of the compounds **I** and **II** (Fig. 3) can be caused by incorporation of the benzene ring into hydrophobic region of the taurocholate micelles. In this case, piperidine ring is placed outside and interacts with the micelle exterior.

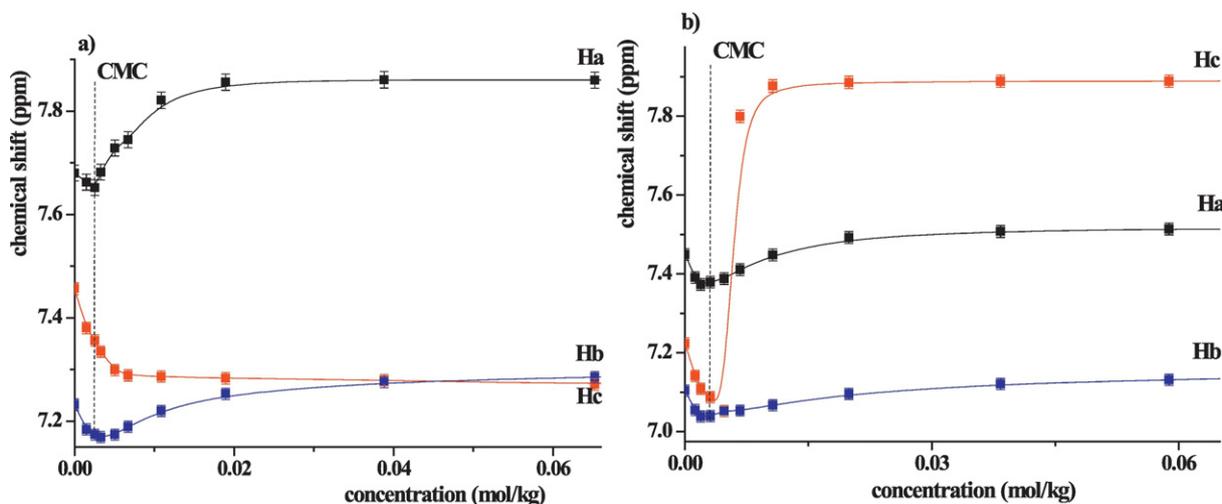


Fig. 3. Dependences of chemical shift changes of protons of compounds **I** (a) and **II** (b) on taurocholate concentration.

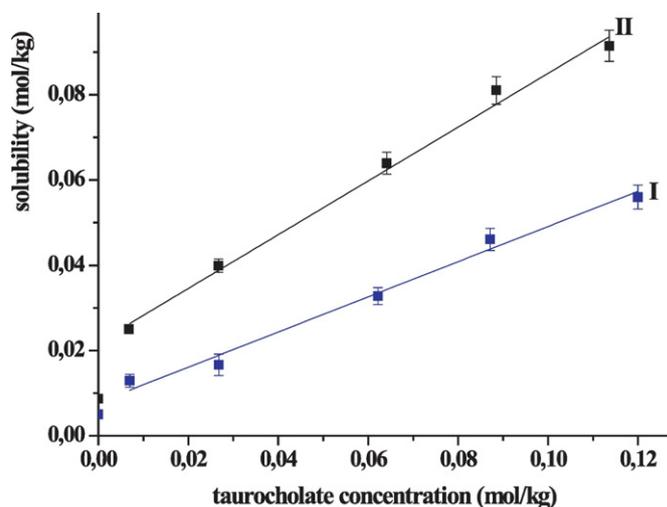


Fig. 4. Dependences of solubility of 1,2,4-thiadiazoles on concentration of sodium taurocholate.

The equation for the equilibrium constant of binding of the micelles with 1,2,4-thiadiazole derivatives can be written:

$$K = \frac{[\text{thiadiazole} \cdot \text{micelle}]}{[\text{micelle}] \cdot [\text{thiadiazole}]} \quad (4)$$

where $[\text{thiadiazole}]$ and $[\text{thiadiazole} \cdot \text{micelle}]$ are the concentration of free and micelle associated thiadiazole; $[\text{micelle}]$ is concentration of the micelles which can be determined from the total concentration (c) and CMC of taurocholate and aggregation number (N) equal to 5 [27]:

$$[\text{micelle}] = (c - \text{CMC})/N \quad (5)$$

The K values calculated from the chemical shifts of Ha, Hb and Hc protons were equal to 528 ± 44 and 596 ± 55 kg/mol for compounds **I** and **II**, respectively. These values are relatively high. For comparison, equilibrium constants for binding of SDS micelles with dipeptides and tripeptides were in the range of $60\text{--}69 \text{ M}^{-1}$ [28] and $17\text{--}24 \text{ M}^{-1}$ [29], respectively. High stability of the taurocholate micelles with **I** and **II** can be determined by electrostatic interactions occurring between the positively charged groups of 1,2,4-thiadiazole derivatives and the negatively charged external surface of the micelle.

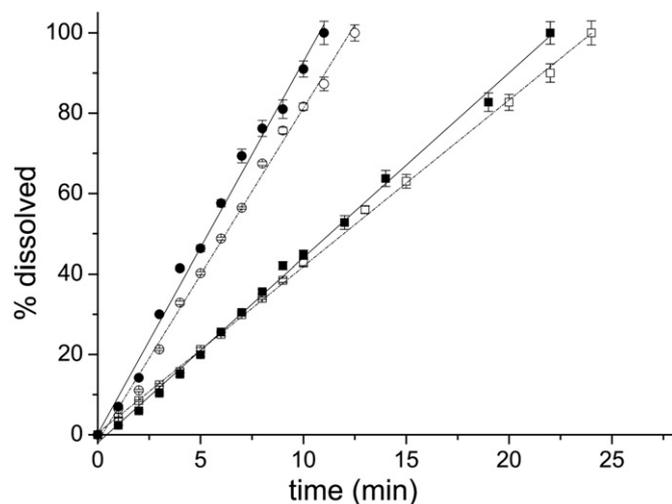


Fig. 5. Dissolution profiles of I and II in FaSSIF (■ and ●, respectively) and corresponding blank buffer pH 6.5 (□ and ○, respectively) at 37 °C.

Interactions of 1,2,4-thiadiazole derivatives with taurocholate micelles results in the improvement of their solubility. Solubility diagrams, representing the dependences of solubility of 1,2,4-thiadiazole derivatives on the concentration of taurocholate micelles, are given in Fig. 4. As one can see, solubilizing action of the taurocholate is more pronounced for thiadiazole II. The linear increase of the solubility of 1,2,4-thiadiazole derivatives with the rise of the concentration of taurocholate micelles can be described by the following equation [30,31]:

$$\frac{S}{S_0} = 1 + KC_{micelle} \quad (6)$$

where K is the binding constant; S and S_0 are solubility of 1,2,4-thiadiazole derivatives in a solution with and without taurocholate, respectively; $C_{micelle}$ is the concentration of taurocholate micelles. Binding constants were calculated from the slope of the solubility diagrams (Fig. 4). Values of K were found to be 480 ± 45 kg/mol and 566 ± 38 kg/mol for the binding of taurocholate micelles with compounds I and II, respectively. A good agreement between K obtained from ^1H NMR and solubility data is observed. The comparative analysis of the binding constants shows that affinity of II to the taurocholate micelles is slightly higher. This can be caused by the availability of the $-\text{CH}_3$ side group in the structure of compound II. In this case, the attraction of II to the hydrophobic region of the micelles will be stronger in comparison with I having two $-\text{Cl}$ substituents.

It is evident that drugs may have different affinities to the components of the biorelevant media, hence, different dissolution rates of the drugs in various media can be expected. To this end, dissolution of the tablets prepared from compounds I and II was investigated both in FaSSIF and corresponding blank buffer pH 6.5 at 37 °C. Results of this kinetic study are presented in Fig. 5. It is easy to see that a rapid dissolution of I and II in both media is observed. According to Pharmacopoeia [32], dissolution of >80% of active substance during 45 min is considered as immediate process. Fig. 5 clearly illustrates that 100% of

the substance is dissolved during 30 min indicating an excellent dissolution behavior of both compounds.

The dissolution profiles are linear and can be described by zero-order kinetic model [33]:

$$F = kt \quad (7)$$

where F is the fraction of TDZ dissolved up to time t ; k is the apparent dissolution rate constant. The results of the regression analysis of dissolution profiles are given in Table 2. It is not difficult to see that zero-order kinetic model fits well the dissolution profiles with coefficients $R^2 > 0.992$ (Table 2). It should be mentioned that pharmaceutical dosage forms following zero-order dissolution are considered as ideal for rapid pharmacological action. As it follows from Table 2, the dissolution rate of compound II is 2 times higher than that of compound I. However, the influence of buffer composition on the dissolution behavior is not so significant.

Permeability of the investigated substances through the synthetic cellulose membrane MWCO 12–14 kDa was measured using the Franz diffusion cell. The apparent permeability coefficients (P_{app}) calculated using Eq. (2) are given in Table 2. The investigated substances are structural analogues and, therefore, it is not surprising that their permeability coefficients do not differ significantly. A slightly higher P_{app} value of thiadiazole II in blank buffer may be due to lower molecular weight of this compound and, as a consequence, its increased mobility. The same regularity has been observed in our previous study concerning the permeability of 1,2,4-thiadiazole derivatives across the Permeapad™ barrier in buffer (pH 7.4) [13]. As follows from Table 2, permeability coefficients of 1,2,4-thiadiazoles under study decrease in FaSSIF. In this biorelevant medium composed of taurocholate and lecithin micelles, compounds I and II exist in the equilibrium between free and micelle-incorporated states. The fraction of the thiadiazole molecules bound to the micelles will migrate at a slower speed than that, which is unbound, owing to the larger size of the micelles [4]. Due to this fact, the mobility of the compound fractioned into micelles decreases lowering the rate of the permeation. In FaSSIF, the difference between P_{app} of compounds I and II is not so pronounced as in the corresponding blank buffer. On the one hand, II has lower molecular weight and higher mobility compared with I. On the other hand, results of ^1H NMR and solubility studies confirmed stronger binding of II with the micelles. These two factors compensate each other and it results in insignificant difference between P_{app} of compounds I and II (Table 2).

4. Conclusions

In this work, the impact of biorelevant medium FaSSIF on the pharmacologically important properties of 1,2,4-thiadiazole derivatives proposed for the prevention of Alzheimer's disease was investigated. It was demonstrated that solubility, distribution and permeability coefficients of 1,2,4-thiadiazole derivatives under consideration are sensitive to FaSSIF composition, while influence of buffer components on the dissolution rate of these compounds is not so pronounced. The revealed effect of FaSSIF is determined by the binding of thiadiazole molecule with the micelles of taurocholate and lecithin. Interactions of 1,2,4-thiadiazole derivatives with the components of FaSSIF medium were studied by means of ^1H NMR spectroscopy. It was found that benzene

Table 2

Parameters of dissolution profiles of compounds I and II in FaSSIF and blank buffer (pH 6.5) at 37 °C.

Dissolution medium	Zero-order dissolution model				$P_{app} \cdot 10^5$, cm/s	
	k		R^2		I	II
	I	II	I	II		
Blank buffer (pH 6.5)	4.14 ± 0.03	8.29 ± 0.15	0.999	0.996	1.60 ± 0.08	2.17 ± 0.12
FaSSIF (pH 6.5)	4.60 ± 0.06	9.26 ± 0.25	0.997	0.992	0.79 ± 0.17	0.84 ± 0.14

ring of 1,2,4-thiadiazole derivative molecule is immersed into micelle core while piperidine ring exists in the exterior surface of the micelle. At the same time, piperidine ring is placed outside and interacts with exterior of the micelle. Thiadiazole-micelle binding constants were calculated and discussed. Testing a dissolution of 1,2,4-thiadiazole derivatives in biorelevant medium can provide information on the real behavior of these compounds in intestinal fluids.

Acknowledgment

This work was supported by Russian Science Foundation (project № 15-13-10017). Authors thank the “The upper Volga region Centre of physicochemical research” (Ivanovo, Russian Federation) for equipment provided for NMR experiments.

References

- [1] D. Horter, J.B. Dressman, Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract, *Adv. Drug Deliv. Rev.* 46 (2001) 75–87.
- [2] N. Fotaki, M. Vertzoni, Biorelevant dissolution methods and their applications in *in vitro-in vivo* correlations for oral formulations, *Open Drug Delivery J.* 4 (2010) 2–13.
- [3] K. Kleberg, J. Jacobsen, A. Müllertz, Characterizing the behaviour of poorly water soluble drugs in the intestine: application of biorelevant media for solubility, dissolution and transport studies, *JPP* 62 (2010) 1656–1668.
- [4] J.H. Fagerberg, O. Tsinman, N. Sun, K. Tsinman, A. Avdeef, C.A.S. Bergström, Dissolution rate and apparent solubility of poorly soluble drugs in biorelevant dissolution media, *Mol. Pharm.* 7 (2010) 1419–1430.
- [5] K. Yano, Y. Masaoka, M. Kataoka, S. Sakuma, S. Yamashita, Mechanisms of membrane transport of poorly soluble drugs: role of micelles in oral absorption processes, *J. Pharm. Sci.* 99 (2010) 1336–1345.
- [6] J.M. Miller, A. Beig, R.A. Carr, G.K. Webster, A. Dahan, The solubility–permeability interplay when using cosolvents for solubilization: revising the way we use solubility-enabling formulations, *Mol. Pharm.* 9 (2012) 581–590.
- [7] G. Ottaviani, D.J. Gosling, C. Patissier, S. Rodde, L. Zhou, B. Falle, What is modulating solubility in simulated intestinal fluids? *Eur. J. Pharm. Sci.* 41 (2010) 452–457.
- [8] A. Dahan, J. Miller, A. Hoffman, G. Amidon, G. Amidon, The solubility–permeability interplay in using cyclodextrins as pharmaceutical solubilizers: mechanistic modeling and application to progesterone, *J. Pharm. Sci.* 99 (2010) 2739–2749.
- [9] A. Castro, T. Castano, A. Encinas, W. Porcal, C. Gil, Advances in the synthesis and recent therapeutic applications of 1,2,4-thiadiazole heterocycles, *Bioorg. Med. Chem.* 14 (2006) 1644–1652.
- [10] N. Kushwaha, S.K.S. Kushwaha, A.K. Rai, Biological activities of thiadiazole derivatives: a review, *Int. J. ChemTech Res.* 4 (2012) 517–531.
- [11] A.N. Proshin, I.V. Serkov, L.N. Petrova, S.O. Bachurin, 5-Amino-3-(2-aminopropyl)-1,2,4-thiadiazoles as the basis of hybrid multifunctional compounds, *Russ. Chem. Bull. Int. Ed.* 63 (2014) 1148–1152.
- [12] S.O. Bachurin, E.V. Bovina, A.A. Ustyugov, Drugs in clinical trials for Alzheimer's disease: the major trends, *Med. Res. Rev.* (2017) <https://doi.org/10.1002/med.21434>.
- [13] T.V. Volkova, I.V. Terekhova, O.I. Silyukov, A.N. Proshin, A. Bauer-Brandl, G.L. Perlovich, Towards the rational design of novel drugs based on solubility, partitioning/distribution, biomimetic permeability and biological activity exemplified by 1,2,4-thiadiazole derivatives, *Med. Chem. Commun.* 8 (2017) 162–175.
- [14] E. Galia, E. Nicolaidis, D. Hörter, R. Löbenberg, C. Reppas, J.B. Dressman, Evaluation of various dissolution media for predicting *in vivo* performance of class I and II drugs, *Pharm. Res.* 15 (1998) 698–705.
- [15] M. di Cagno, H.A. Bibi, A. Bauer-Brandl, New biomimetic barrier Permeapad™ for efficient investigation of passive permeability of drug, *Eur. J. Pharm. Sci.* 73 (2015) 29–34.
- [16] A. Andrés, M. Rosés, C. Ràfols, E. Bosch, S. Espinosa, V. Segarra, J.M. Huerta, Setup and validation of shake-flask procedures for the determination of partition coefficients (logD) from low drug amounts, *Eur. J. Pharm. Sci.* 76 (2015) 181–191.
- [17] OECD 107 Method, OECD Guideline for the Testing of Chemicals Adopted by the Council on 27th July 1995 Partition Coefficient (n-Octanol/Water): Shake Flask Method, <http://www.oecd.org/chemicalsafety/risk-assessment/1948169.pdf> 1995.
- [18] A.J. Leo, C. Hansch, D. Elkins, Partition coefficients and their uses, *Chem. Rev.* 71 (1972) 525–616.
- [19] T.V. Volkova, S.V. Blokhina, A.V. Sharapova, M.V. Ol'khovich, G.L. Perlovich, The solubility of ethionamide and structural analogues in buffer solutions, octanol and hexane at several temperatures, *Thermochim. Acta* 640 (2016) 1–7.
- [20] D. Schönherr, U. Wollatz, D. Haznar-Garbacz, U. Hanke, K.J. Box, R. Taylor, R. Ruiz, S. Beato, D. Becker, W. Weitschies, Characterisation of selected active agents regarding pKa values, solubility concentrations and pH profiles by SiriusT3, *Eur. J. Pharm. Biopharm.* 92 (2015) 155–170.
- [21] S. Riegelman, N.A. Allawala, M.K. Hrenoff, L.A. Strait, The ultraviolet absorption spectrum as a criterion of the type of solubilization, *J. Colloid Sci.* 13 (1958) 208–217.
- [22] A. Aburub, D.S. Risley, D. Mishra, A critical evaluation of fasted state simulating gastric fluid (FaSSGF) that contains sodium lauryl sulfate and proposal of a modified recipe, *Int. J. Pharm.* 347 (2008) 16–22.
- [23] M. Chislov, O. Silyukov, R. Kumeev, A. Proshin, G. Perlovich, I. Terekhova, Complex formation of cyclodextrins with some pharmacologically active 1,2,4-thiadiazole derivatives. Thermodynamics and binding mode, *J. Therm. Anal. Calorim.* 127 (2017) 1797–1805.
- [24] H. Ishikawa, T. Nakashima, K. Inaba, H. Mitsuyoshi, Y. Nakajima, Y. Sakamoto, T. Okanoue, K. Kashima, Y. Seo, Proton magnetic resonance assay of total and taurine-conjugated bile acids in bile, *J. Lipid Res.* 40 (1999) 1920–1924.
- [25] N. Funasaki, M. Nomura, S. Ishikawa, S. Neya, Hydrophobic self-association of sodium taurochenodeoxycholate and tauroursodeoxycholate, *J. Phys. Chem. B* 104 (2000) 7745–7751.
- [26] S.M. Meyerhoffer, L.B. McGown, Critical micelle concentration behavior of sodium taurocholate in water, *Lang. Des.* 6 (1990) 187–191.
- [27] L.B. McGown, K. Nithipatikom, Sodium taurocholate micelles in fluorometric analysis, *J. Res. Natl. Bur. Stand.* 93 (1988) 443–444.
- [28] K.R. Deaton, E.A. Feyen, H.J. Nkulabi, K.F. Morris, Pulsed-field gradient NMR study of sodium dodecyl sulfate micelle–peptide association, *Magn. Reson. Chem.* 39 (2001) 276–282.
- [29] L. Orfi, M. Lin, C.K. Larive, Measurement of SDS micelle–peptide association using ¹H NMR chemical shift analysis and pulsed-field gradient NMR spectroscopy, *Anal. Chem.* 70 (1998) 1339–1345.
- [30] D. Attwood, A.T. Florence, *Surfactant Systems. Their Chemistry, Pharmacy and Biology*, 1st ed. Chapman and Hall, London, 1983.
- [31] S.D. Bhattamishra, R.K. Padhy, Estimation of ibuprofen solubilization in cationic and anionic surfactant media: application of micelle binding model, *Indian J. Chem. Technol.* 16 (2009) 426–430.
- [32] U.S. Pharmacopeia, *The United States Pharmacopeial Convention*, Rockville, MD, 28th edition, 2004.
- [33] P. Costa, J.M.S. Lobo, Modeling and comparison of dissolution profiles, *Eur. J. Pharm. Sci.* 13 (2001) 123–133.