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Cytisine basicity, solvation, log *P*, and log *D* theoretical determination as tool for bioavailability prediction

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ABSTRACT

Cytisine, an $\alpha_4\beta_2$ nicotinic receptor partial agonist, is a plant alkaloid widely used as a smoking cessation agent. Despite long history of use, knowledge on pharmacokinetics of cytisine still demands an extension. This work is aimed at theoretical determination of physicochemical parameters that affect the bioavailability of cytisine. The acidic dissociation constant, Gibbs free energy of solvation in water and *n*-octanol as well as *n*-octanol/water partition coefficient and *n*-octanol/water distribution coefficient of cytisine were calculated as quantities corresponding to its solubility and permeability. Cytisine structure was optimized with several quantum chemical methods—*ab initio*: HF and MP2, and DFT functionals (B3LYP, B3LYP-D3, CAM-B3LYP, M06-2X, TPSS, VSXC) with 6-311++G(d,p) basis set. Solvation of cytisine in water and *n*-octanol was determined with the SMD continuum model. It was shown that lipophilicity of cytisine depends on the pH of an environment. Protonated cytisine, the most populated state under acidic conditions, is characterized by enhanced hydrophilicity. Then neutral cytisine, dominating in a basic environment, demonstrates more lipophilic character. It appears that cytisine is very well soluble in the gastrointestinal (GI) tract fluids. Then the distribution of cytisine ought to occur very rapidly. However, permeability of cytisine through the mucous membrane of the GI tract may be limited, leading to the diminished bioavailability.

1. Introduction

(−)-Cytisine (−)-1,[(1R,5S)-1,2,3,4,5,6-hexahydro-1,5-methano-8*H*-pyrido[1,2a] [1,5] diazocin-8-one is a quinolizidine alkaloid found in plants belonging to *Leguminosae* (*Fabaceae*) family and seeds of *Laburnum anagyroides* (*Cytisus Laburnum*) [1,2]. Recently, cytisine is a reborn compound used in smoking cessation [3,4]. It is forseen that within 5 years it will take over the market of nicotine cessation aid in highly developed countries [5].

Cytisine has long history of use, however knowledge on its pharmacokinetics is still incomplete. To date, several studies on the pharmacokinetic profile of cytisine have been published. In mice, the absorption rate after an oral administration of cytisine has reached the 42% level [6]. In rabbits, the bioavailability of cytisine was determined to be 32.18%, suggesting erratic absorption or first pass metabolism [7].

The cytisine structure has been widely discussed in literature. It was shown that in solid state, cytisine prefers the chair conformation with the equatorially positioned hydrogen atom in the amine group of piperidine [8,9]. Two isomers-axial and equatorial of the chair conformation of cytisine were found in aqueous solution [10–12] and in gas phase [13,14]. The experimental investigations did not reveal the existence of the boat conformations of cytisine in gas, liquid and solid environments. However, a human organism is much more complicated system than those thus it is not out of the question that cytisine conformation conversion may occur in certain conditions such as during interaction with some macromolecules. Therefore, we have decided to consider the boat conformations of cytisine in our calculations.

Here, the aim was to investigate molecular physicochemical determinants of pharmacokinetic properties of cytisine such as basicity, solvation energy, log *P*, and log *D*. Those are crucial to predict bioavailability without the need of testing chemical compounds in humans.

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2. Computational methods

Cytisine structure was optimized with several quantum chemical methods—*ab initio*: non-correlated—HF (Hartree–Fock method), correlated—MP2 (Möller-Plesset Second Order Perturbation Theory [15]) and DFT functionals (B3LYP [16], B3LYP-D3 [17], CAM-B3LYP [18], M06-2X [19], TPSS [20], VSXC [21]) with 6-311++G(d,p) basis set. Thermodynamic properties of cytisine were evaluated under standard conditions (298.15 K, 1 atm). No imaginary frequencies were detected what proves that the conformers correspond to the true energy minima on potential energy surface (PES). Solvation of cytisine in water and *n*-octanol was performed using the SMD continuum model [22]. All of the calculations were carried out with Gaussian 09 package [23].

2.1. Calculation of the pK_a in water

For the protonated base deprotonation reaction in water is as follows:

$$BH^{+}_{(w)} \rightleftarrows B_{(w)} + H^{+}_{(w)} \tag{1}$$

The Gibbs free energy is related to dissociation constant as follows:

$$\Delta G_{(w)} = -RT\ln K_a = -2.303RT\log K_a \tag{2}$$

Then, the pK_a, defined as the negative logarithm of the dissociation constant, is given by:

$$pK_a = \frac{\Delta G_{(w)}}{2.303RT} \tag{3}$$

where $\Delta G_{(w)}$ is the Gibbs free energy of the deprotonation reaction in the aqueous solution, R is the gas constant, T is the temperature.

The Gibbs free energy of Reaction (1) is a difference between the Gibbs free energy of products (a neutral base and a proton) and Gibbs free energy of a substrate (a protonated base).

$$\Delta G_{(w)} = G_{(w)}(B) + G_{(w)}\left(H^{+}\right) - G_{(w)}\left(BH^{+}\right) \tag{4}$$

The Gibbs free energy of a proton in water $G_{(w)}\left(H^{+}\right)$ was computed as a sum of the Gibbs free energy of a proton in gas, Gibbs free energy of solvation of a proton in water and Gibbs free energy of change in standard state from 1 atm to 1 M (1.89 kcal/mol) [24]

$$G_{(w)}\left(H^{+}\right) = G_{(g)}\left(H^{+}\right) + \Delta G_s\left(H^{+}\right) + \Delta G^{1atm\rightarrow IM} \tag{5}$$

The gas-phase standard Gibbs free energy of a proton is −6.287 kcal/mol at 298.15 K, derived from the equation:

$$G_{(g)}\left(H^{+}\right) = H_{(g)}\left(H^{+}\right) - TS_{(g)}\left(H^{+}\right) \tag{6}$$

where $H_{(g)}\left(H^{+}\right) = \frac{5}{2}RT = 1.48 kcal/mol$ and $S_{(g)}\left(H^{+}\right) = 26.05\frac{cal}{mol\,K}$

The Gibbs free energy of solvation of a proton in water $\Delta G_s(H^{+})$ is −265.9 kcal/mol [25].

The protonated to neutral forms concentration ratio at the specific pH of an environment can be derived from the definition of the acidic dissociation constant (pK_a):

$$pK_a = -\log\frac{[B]\left[H^{+}\right]}{\left[BH^{+}\right]} = -\log\frac{[B]}{\left[BH^{+}\right]} - \log\left[H^{+}\right]$$

$$-\log\frac{[B]}{\left[BH^{+}\right]} = pK_a - pH \tag{7}$$

$$\frac{\left[BH^{+}\right]}{[B]} = 10^{pK_a - PH}$$

2.2. Calculation of the Gibbs free energy of solvation

The Gibbs free energy of solvation is defined as a difference between the Gibbs free energy in a solvent and Gibbs free energy in the gas-phase.

$$\Delta G_s = \Delta G_{(solv)} - \Delta G_{(g)} \tag{8}$$

2.3. Calculation of the log P and log D

Considering a system containing two immiscible-aqueous and oil phases with a solute B, the Gibbs free energy of a transfer of the solute B from water to oil is a difference between the Gibbs free energy of the solute B in oil and Gibbs free energy of the solute B in water.

$$B_{(w)} \rightleftarrows B_{(o)} \tag{9}$$

$$\Delta G_{(o/w)} = \Delta G\left(B_{(o)}\right) - \Delta G\left(B_{(w)}\right) \tag{10}$$

The equilibrium constant of this process can be expressed as:

$$K_{(o/w)} = \frac{\left[B_{(o)}\right]}{\left[B_{(w)}\right]} \tag{11}$$

It can be noticed that $K_{o/w}$ is equal to the partition coefficient P.

$$P = \frac{\left[B_{(o)}\right]}{\left[B_{(w)}\right]} \tag{12}$$

Using the equilibrium constant and Gibbs free energy relationship, the partition coefficient, expressed in the logarithm, can be computed as follows:

$$\Delta G = -2.303RT\log K \tag{13}$$

$$\log P = -\frac{\Delta G_{(o/w)}}{2.303RT} \tag{14}$$

The distribution coefficient D is similar to the partition coefficient P but apart from neutral species it also takes charged forms into account.

$$D = \frac{\left[B_{(o)}\right] + \left[BH^{+}_{(o)}\right]}{\left[B_{(w)}\right] + \left[BH^{+}_{(w)}\right]} \tag{15}$$

$$P_0 = \frac{\left[B_{(o)}\right]}{\left[B_{(w)}\right]}$$

$$P_1 = \frac{\left[BH^{+}_{(o)}\right]}{\left[BH^{+}_{(w)}\right]}$$

$$\left[BH^{+}_{(w)}\right] = \left[B_{(w)}\right] \times 10^{pK_a - pH}$$

$$D = \frac{P_0 \times \left[B_{(w)}\right] + P_1 \times \left[BH^{+}_{(w)}\right]}{\left[B_{(w)}\right] + \left[BH^{+}_{(w)}\right]} = \frac{P_0 \times \left[B_{(w)}\right] + P_1 \times \left[B_{(w)}\right] \times 10^{pK_a - pH}}{\left[B_{(w)}\right] + \left[B_{(w)}\right] \times 10^{pK_a - pH}} = \frac{P_0 + P_1 \times 10^{pK_a - pH}}{1 + 10^{pK_a - pH}} \tag{16}$$

where P_0— the partition coefficient for neutral species, P_1— the partition coefficient for protonated species, pK_a— the acidic dissociation constant in water

From the distribution coefficient D, the absorption from the hydrophilic to lipophilic phase $A_{(o/w)}$ can be calculated in Eq. (17).

$$A_{(o/w)} = \frac{\left[B_{(o)}\right] + \left[BH^{+}{}_{(o)}\right]}{\left[B_{(o)}\right] + \left[BH^{+}{}_{(o)}\right] + \left[B_{(w)}\right] + \left[BH^{+}{}_{(w)}\right]} \times 100\% \quad (17)$$

3. Results and discussion

3.1. Calculation of Boltzmann populations

(−)-Cytisine may exist in two isomers-axial and equatorial. It was shown that the energetic barrier between them is quite low [26] then the both isomers were considered in our study. The each isomer may take on chair/boat conformations. Therefore the four distinct structures of cytisine were involved in calculations (Fig. 1). All of the investigated parameters are based on the Gibbs free energy. They were computed for the each conformer separately and then weighted with the Boltzmann populations (18) of the corresponding structures to give their total average values (19).

$$p_i = \frac{n_i}{\sum_{i=1}^{n} n_i} = \frac{e - \frac{\Delta G_i}{RT}}{\sum_{i=1}^{n} e - \frac{\Delta G_i}{RT}} n \quad (18)$$

$$\Delta G = -RT\ln\left(\sum\nolimits_i^n e^{-\frac{\Delta G_i}{RT}}\right) + RT\sum\nolimits_i^n p_i \ln(p_i) \quad (19)$$

The Boltzmann distribution of the four cytisine structures in water calculated with several quantum chemical methods is shown in Fig. 2. The chair type conformation tends to be more preferable than boat conformation in all of the used theoretical models. All of the applied methods, except VSXC, predicted the axial isomer to be more populated than the equatorial isomer. For the boat type conformers, axial isomerism is more favorable than equatorial isomerism for all of the used models.

3.2. Calculation of the pK$_a$

Cytisine contains three heteroatoms–pyridone nitrogen and oxygen linked in the amide group, and piperidine nitrogen. It was demonstrated that two of them, the pyridone oxygen atom and piperidine nitrogen atom are potential basic sites. In the gas-phase, the preferable protonation site is oxygen, whereas in water the piperidine nitrogen atom is more favorable [27]. In our study, we investigated the basicity of cytisine in water so piperidine nitrogen was protonated. The acidic dissociation constant (pK_a) of cytisine was determined experimentally to be 7.92 [28] or 7.8 in a recent study [29]. We tried to find the model that predicts the pK_a of cytisine in accordance with the experimental examinations. Then the such method could be considered as well-working for cytisine thus may provide reasonable results of calculation of other properties of cytisine. We tested a wide spectrum of quantum chemical methods. The results of the theoretically determined pK_a of cytisine are presented in Fig. 3. For majority of the applied methods, the pK_a of the individual conformers of cytisine increases as follows: chair axial < boat axial < chair equatorial < boat equatorial. The most deviations from this trend is observed for Hartree–Fock method and can be related to neglecting of electron correlation effects. The best agreement of the computed pK_a with experimental pK_a was achieved by the MP2 method. Thus it was further used to calculate the Gibbs free energy of solvation in water and n-octanol, as well as n-octanol/water partition coefficient and n-octanol/water distribution coefficient of cytisine.

3.3. Calculation of the Gibbs free energy of solvation

A parameter derived from quantum chemical calculations and related to solubility is the Gibbs free energy of solvation. A negative value of the Gibbs free energy of solvation indicates that interactions between solute and solvent are energetically favorable. The more negative the Gibbs free energy of solvation, the higher solubility.

The Gibbs free energy of solvation in water (as a hydrophilic phase) and n-octanol (as a lipophilic phase) of all of the cytisine structures was calculated. The Gibbs free energy of solvation of the neutral and charged forms are quite different thus the overall Gibbs free energy of solvation of cytisine depends on their relative populations. Then the protonated to neutral species concentrations ratio is controlled by the pK_a and pH of an environment. In Fig. 4, the Gibbs free energy of solvation in water and n-octanol of the each individual cytisine conformer and Gibbs free energy of solvation in water and n-octanol averaged over all the cytisine structures weighted with the Boltzmann populations are shown. The profile of solvation in water and n-octanol in the pH scale is similar for all of the cytisine structures. At higher values of the pH, neutral cytisine is more populated and slightly more soluble in the lipophilic than hydrophilic phase. The Gibbs free energy of solvation in water and n-octanol of the individual cytisine structures increases as follows: boat equatorial < chair equatorial < chair axial < boat axial. With a decrease in the pH, the neutral to protonated molecules concentrations ratio falls and cytisine is increasingly more soluble in water than n-octanol. At the lower pH, the Gibbs free energy of solvation in water and n-octanol rises as follows: chair axial < chair equatorial < boat axial < boat equatorial. Based on the calculations of the Gibbs free energy of solvation of cytisine in water with regard to the pH, it could be argued that cytisine should be well soluble in the aqueous environment of the gastrointestinal tract. After an oral administration of cytisine, the dissolution phase ought to be fast and not limiting its absorption.

3.4. Calculation of the log P and log D

The n-octanol/water partition coefficient ($\log P$) and n-octanol/water distribution coefficient ($\log D$) are parameters describing equilibrium of the transfer of a solute from a hydrophilic to lipophilic phase, therefore can be used in estimation of permeability through the GI tract mucous membrane. A pharmacokinetic quantity that can be derived from the distribution coefficient, is the absorption.

The partition coefficient ($\log P$) becomes equal to the distribution coefficient ($\log D$) when the protonated to neutral species ratio goes to zero. It is observed in a very basic environment where the most cytisine molecules are in a neutral state. The theoretical values of the n-octanol/water partition coefficient ($\log P$) and n-octanol/water distribution coefficient ($\log D$) of the cytisine structures are shown in Fig. 5. Lipophilicity of the cytisine conformations, measured by the $\log P$, increases as follows: boat axial < chair axial < chair equatorial < boat equatorial. For all of the cytisine conformations, the $\log P$ values are positive indicating higher solubility in the oil phase than in the aqueous solution. However, lowering the pH of an environment, protonated molecules of cytisine are increasingly more contributing to the overall population. In the pH 4–9 range, there is significant linear decrease in the $\log D$. Equilibrium in the concentration of cytisine in the hydrophilic and lipophilic phases is achieved at the pH 7.85. In a very acidic environment (pH 1–4), the total average $\log D$ of cytisine is ca −2.76. Then cytisine demonstrates highly hydrophilic character. For the individual conformers, the $\log D$ increases as in the row: chair axial < chair equatorial < boat axial < boat equatorial at the lower pH values. In majority of the pH scale, cytisine displays

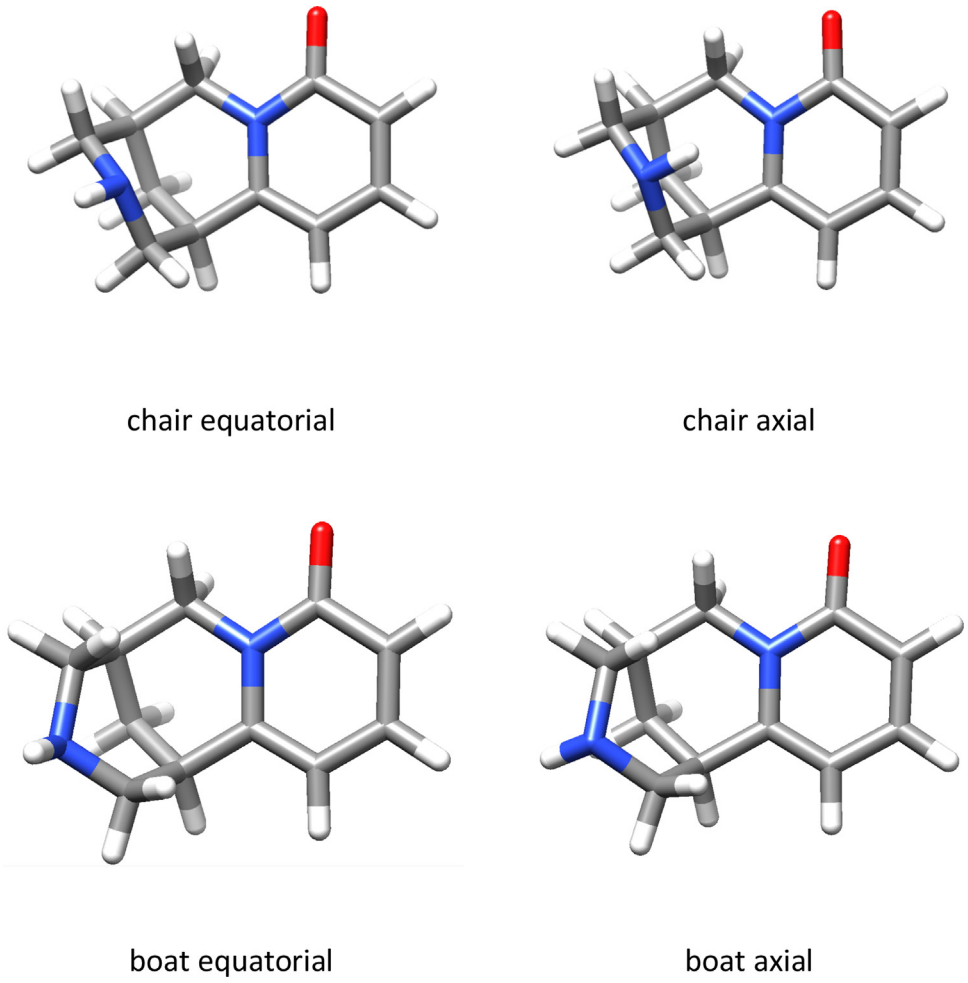

Fig. 1. The cytisine structures.

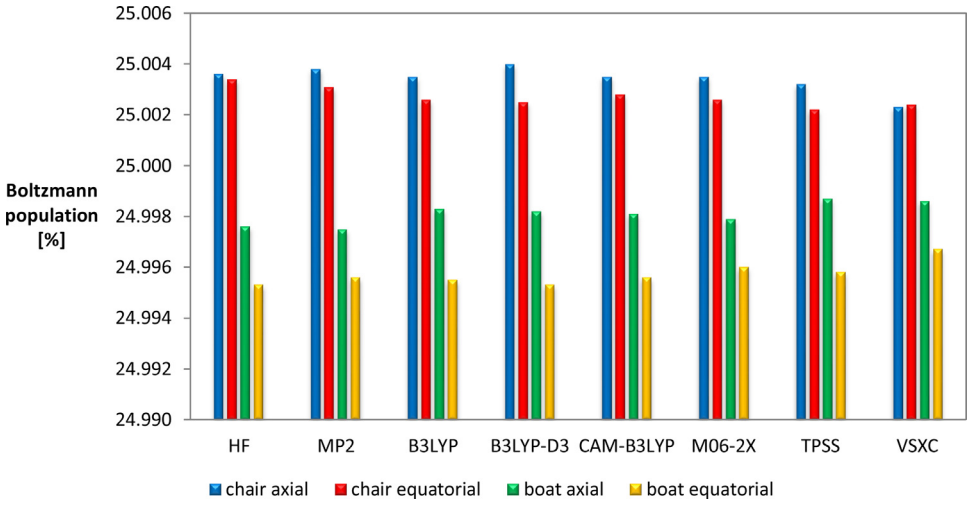

Fig. 2. The theoretical Boltzmann populations of the cytisine structures calculated with various quantum chemical methods.

more hydrophilic than lipophilic properties. Based on the calculations, it can be concluded that in an acidic environment of the gastric fluids, cytisine should be rather hardly absorbed through the gastric mucous membrane in the passive diffusion process (Fig. 6). Permeability of cytisine is probably enhanced in a more basic environment (pH > 6) such as the intestinal fluids or the CNS. The calculated absorption of cytisine suggests that it should be poorly transferred across cellular membranes. However, apart from the passive transcellular way, there are also paracellular passive diffusion and mechanism of the active transport. The paracellular way is often exploited by hydrophilic drugs for crossing the mucous membrane of the GI tract. However, a transport through

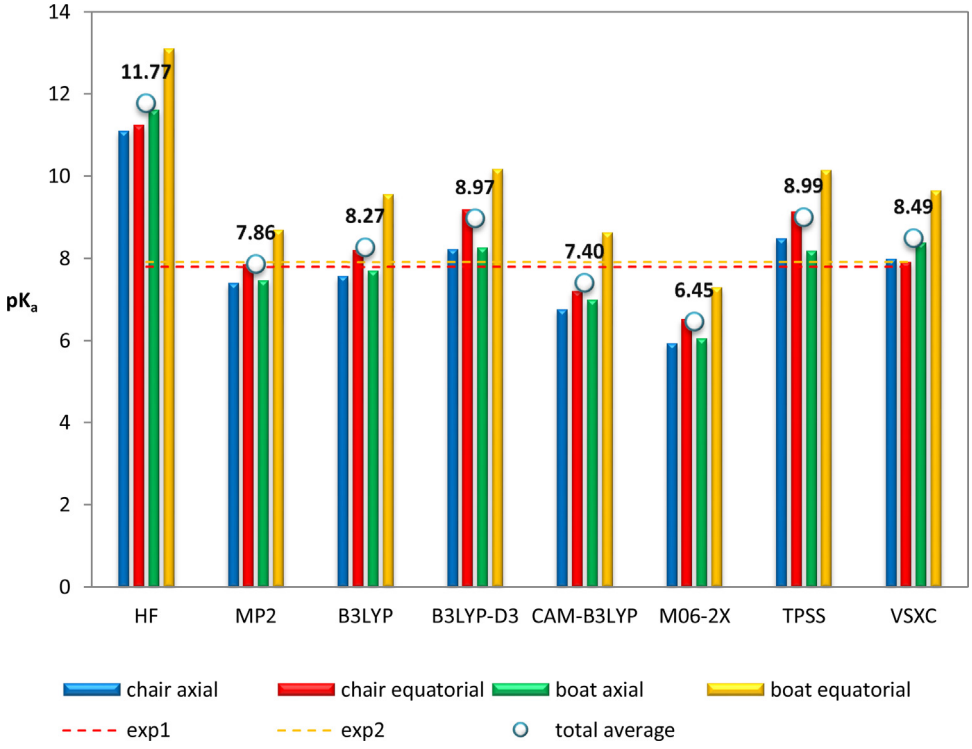

Fig. 3. The theoretical values of the pK_a of cytisine calculated with various quantum chemical methods.

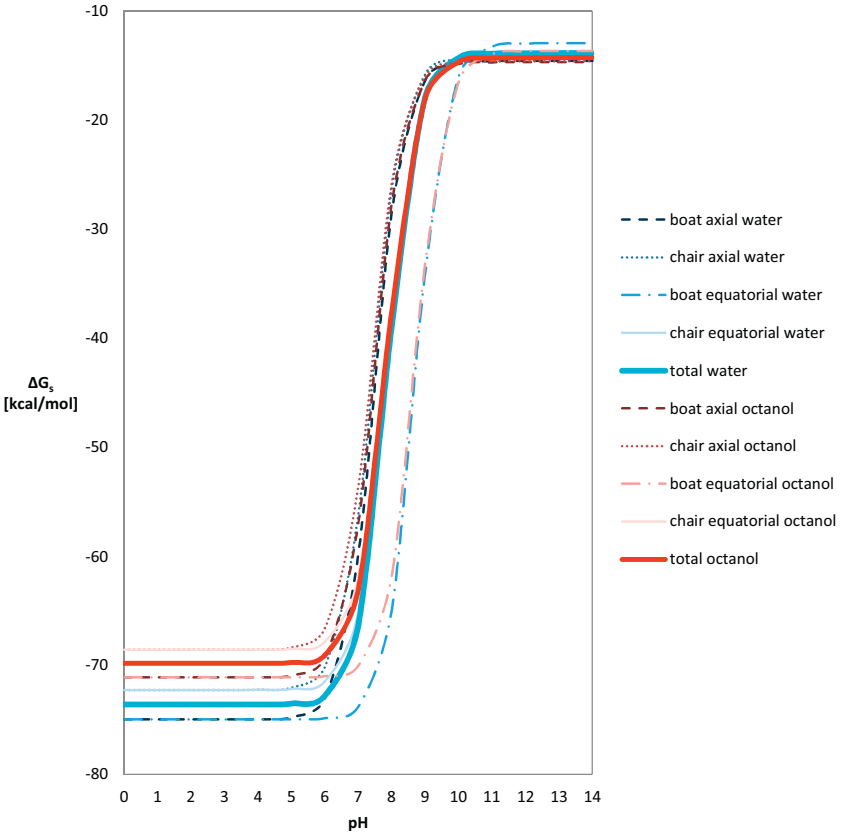

Fig. 4. The Gibbs free energy of solvation in water and in *n*-octanol of cytisine at the full MP2/6-311++g(d,p) level of theory.

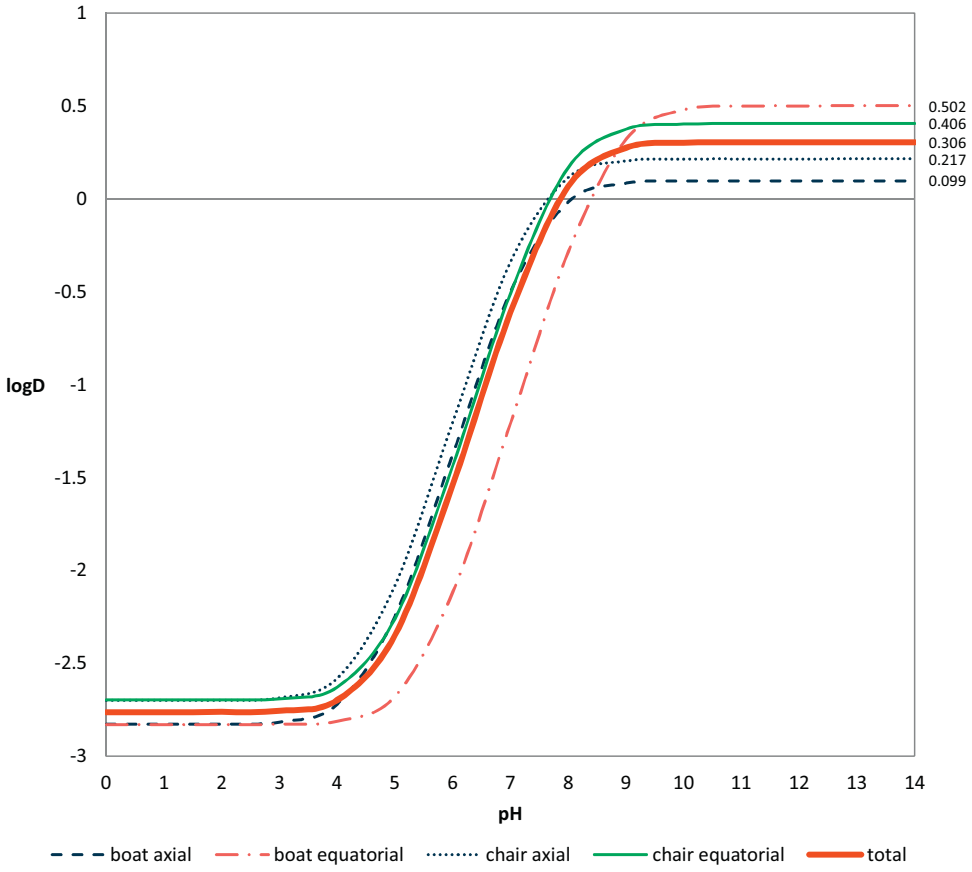

Fig. 5. The theoretical n-octanol/water partition coefficient (log *P*) and *n*-octanol/water distribution coefficient (log *D*) of cytisine at the full MP2/6-311++g(d,p) level of theory.

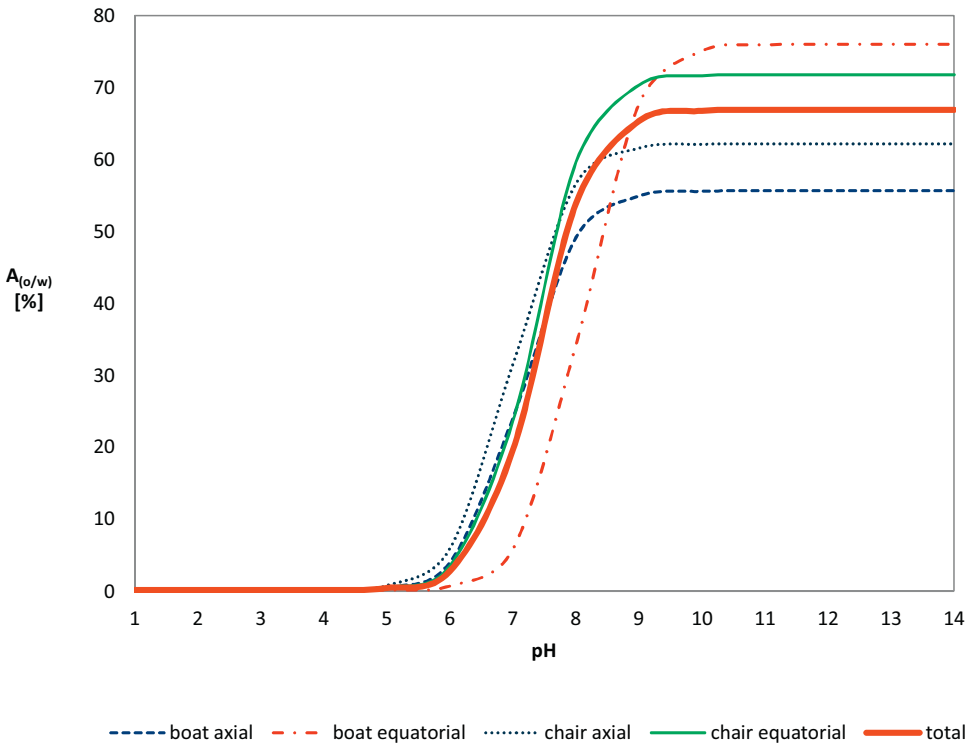

Fig. 6. The theoretical absorption of cytisine from the hydrophilic to lipophilic phase at the full MP2/6-311++g(d,p) level of theory.

this way is slower than through the transcellular way. The mechanism of the active transport should also be considered as a possible way of permeation of cytisine through the gastrointestinal mucous membrane.

It should be mentioned that the log *P* and log *D* are not dependent from values of the Gibbs free energy of solvation in water and Gibbs free energy of solvation in *n*-octanol but rather a difference between them. Thus the error in calculation of the absolute Gibbs free energy of solvation should not affect the log *P* and log *D*. The acidic dissociation constant (pK_a) computed at the SMD/MP2/6-311++G(d,p) level of theory is in perfect agreement with the experiment therefore it should not diminish the quality of the log *D* prediction. Elements that could contribute to erroneous results of the log *P* and log *D* are the implicit model of solvation that neglects specific interactions between a solute and a solvent as well as imitation of the lipophilic phase by *n*-octanol.

4. Conclusions

Here, we aimed to determine the physicochemical parameters that affect the bioavailability of cytisine. We have used a set of quantum chemical methods, including *ab initio*—Hartree–Fock and MP2 as well as some DFT functionals with the SMD solvation model. Based on the excellent agreement of the theoretically computed pK_a with the experimental pK_a of cytisine, the MP2 method was chosen to calculate other properties of cytisine. These were: the Gibbs free energy of solvation in water and *n*-octanol, *n*-octanol/water partition coefficient (log *P*) and *n*-octanol/water distribution coefficient (log *D*) as corresponding to solubility and permeability, respectively. All of these quantities were computed as averages weighted with the Boltzmann populations of the four cytisine conformations under standard conditions. It was demonstrated that they change significantly with the pH. In a basic environment, cytisine is more soluble in *n*-octanol than in water what is reflected in the more negative Gibbs free energy of solvation and positive value of the log *D*. Then the transfer of cytisine from the lipophilic to hydrophilic phase is thermodynamically driven. With increase in the acidity of an environment, cytisine is increasingly more soluble in water than in *n*-octanol. It can lead to the diminished transport of cytisine from the aqueous to oil phase. It appears that cytisine is likely to be very well solvated in fluids of the GI tract but poorly absorbed through the GI tract mucous membrane in the passive diffusion process. Based on our studies, it could be stated that cytisine should be categorized in the III class of the Biofarmaceutics Classification System (BCS). Knowledge of the bioavailability of cytisine and its physicochemical basis could be a factor stimulating the registration of cytisine as a smoking cessation agent with no cumbersome procedures. Furthermore, the new derivatives of cytisine demonstrating enhanced pharmacokinetic properties may be designed.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jmgm.2015.11.003.

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