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MOLECULAR PROPERTIES IMPACT ON BIOAVAILABILITY OF SECOND GENERATION TRIAZOLES ANTIFUNGAL AGENTS

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Abstract: The bioavailability of active compounds depends on their two main features: solubility and permeability. The experimental determination of these factors is rather cumbersome. The free enthalpies of salvation ΔG in water and chloroform, and the electrostatic potential surface around examined molecules were *ab initio* calculated by HF method for voriconazole, posaconazole and ravuconazole. These quantities are assumed to be the new determinants correctly describing both solubility and the affinity of biologically active compounds to lipophilic tendency to cross cellular membranes. The values of ΔG were compared to the theoretically and experimentally determined partition coefficients. The calculated values of ΔG and electrostatic potentials appeared to be consistent with these partition coefficients. It leads to conclusion that these theoretically derived parameters ΔG and electrostatic potential could be useful tools for fast and precise classification of chemical substances within the Biopharmaceutics Classification System (BCS).

Keywords: bioavailability, triazoles, antifungals, solubility, permeability, BCS

In the antifungal therapy a few groups of compound are used of which azoles are the most common. The azole antifungals include two classes of compounds, imidazoles and triazoles, which share the same mechanism of action. They selectively inhibit CYP450 14α-demethylase (CYP51, lanosterol demethylase).

The nitrogen (N-4) atom in triazole ring binds the heme iron atom of the active site of CYP51 [1]. Lanosterol 14α-demethylase is an enzyme, which catalyzes oxidative removal of the 14α-methyl group from lanosterol in ergosterol biosynthesis pathway. This is the crucial point of biosynthesis, therefore, CYP51 is a good molecular target for antifungal agents. The main function of ergosterol is regulation of fluidity and permeability of the fungal membranes as well as regulation of activity and distribution of membrane-bound enzymes. Inhibition of sterol 14α-demethylase results in the lack of ergosterol and induces the block of fungal growth. Furthermore the toxic sterol precursors like squalene, zymosterol, lanosterol, 4,14-dimethyl-zymosterol and 24-methylenedihydrolanosterol are being accumulated and make the membranes of fungal cells unstable (1-3).

The agents from second generation of triazoles: voriconazole, posaconazole and ravuconazole (Fig. 1) were examinated. The attributes of these drugs are good bioavailability after oral administration, high effectiveness in treatment of deep-seated mycosis, broad activity spectrum and negligible side effects. Voriconazole has the highest oral bioavailability - approximately 96%. Good penetration to cerebrospinal fluid and brain tissue it owes to its lipophilic properties (3, 4). Voriconazole shows antifungicidal activity against *Aspergillus* species and fungistatic activity against *Candida, Fusarium* and *Scedosporium* species. Therefore, its main therapeutic indications are treatment of invasive aspergillosis, fluconazole-resistant serious invasive *Candida* infections and grave fungal infections caused by *Scedosporium* spp. and *Fusarium* spp.(3, 4). In contrast, the oral bioavailability of posaconazole is elevated by ingestion of a high-fat meal and this drug has higher distribution volume than voriconazole (5).

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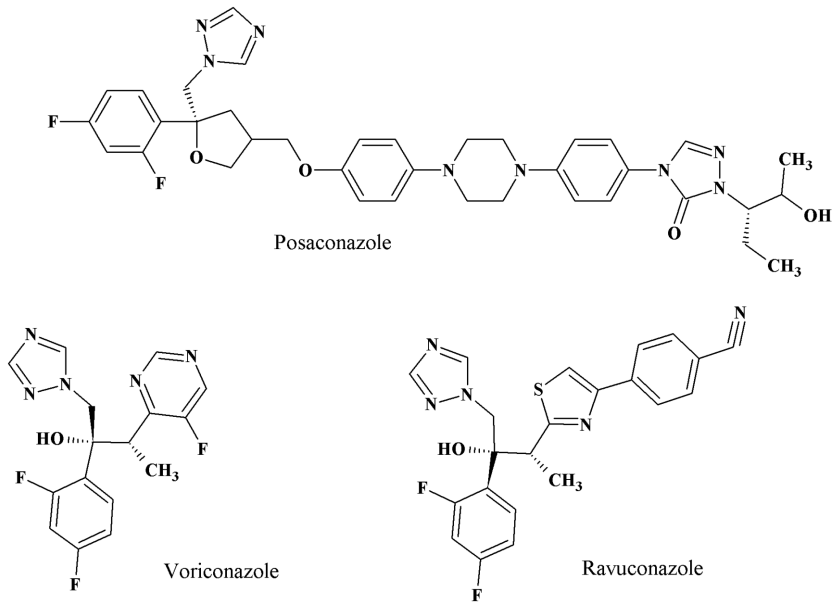

Figure 1. The examined second generation antifungal triazoles

Pharmacokinetics of posaconazole depends, to large extent, on its hydrophobic properties. The antifungal activity of posaconazole is similar to voriconazole, although it has also activity against *Zygomycetes, Coccidioides* species and is effective in treatment of chromoblastomycosis. Moreover, one of its main applications is prophylaxis of invasive aspergillosis and candidosis in immunocompromised patients (5, 6). The pharmacokinetics of ravuconazole, which is currently in phase II/III clinical trials, is similar to posaconazole because of its strong lipophilic properties. Ravuconazole shows fungicidal activity similar to voriconazole, although it has not activity against *Fusarium* and *Scedosporium* species (7-9).

The hydrophilic-lipophilic balance is a very important feature for oral absorption and membrane permeability of triazole antifungal agents.

The main aim of our studies was to verify hypothesis stating that properties like: free enthalpy of solvation (ΔG_{solv}) in water and organic solvents, and the electrostatic potential surface around molecule in water can be good criteria in an estimation of the bioavailability of antifungal agents. This work is a continuation of our previous studies (10), but both investigations comprise new approach. The ΔG_{solv} value and the electrostatic potential range along with the theoretical and the experimental logarithm P values were used to predict an aqueous solubility and the intestinal permeability of examined compounds.

METHOD

The ΔG_{solv} in water and chloroform values were calculated. At first, the lowest energy conformers of each antifungal agent were found with the use of the molecular mechanics (force field MMFF) and Monte Carlo method (Spartan (11)). In the next stage, all conformers were used as starting point structures in *ab initio* calculations (Gaussian 03 (12)), which were carried out at the Hartree-Fock level. Then, the optimized structures of voriconazole, posaconazole and rawuconazole were used to determine ΔG_{solv} in water and chloroform - IEFPCM model (Gaussian 03) (13-15) and the electrostatic potential surface around molecule in water (Fig. 2). We chose chloroform instead of 1-octanol, because of the lack of ε for this solvent in Gaussian 03 parameterization ($c_{chloroform} = 4.9$; $\varepsilon_{n\text{-octanol}} = 5.1$).

The next stage of our studies was experimental determination of octanol/water partition coefficient for voriconazole and posaconazole. The logarithm P value was determined with the use of the shake-flask method – two not mixed solvents: water and n-octanol are the important feature of this

Table 1. The ΔG_{solv} and the electrostatic potential values.

Compounds	HF method ΔG_{solv} [kcal/mol]		Electrostatic potential range [kcal/mol]
	water	chloroform	
Voriconazole	-1.49	-1.83	-83.64 - 52.24
Posaconazole	-3.37	-2.93	-58.18 - 34.05
Ravuconazole	-2.65	-2.35	-60.58 - 34.71

Table 2. The experimental and the theoretical log P values.

Compounds	Experimental logP	ACD/logP	Ghose-Crippen
Voriconazole	1.98	0.93	2.28
Posaconazole	2.59	2.25	4.12
Ravuconazole	-	3.89	5.42

methodology. At last, after measuring concentration of examined compounds in both solvents by UV/VIS spectroscopy, the logarithm P was calculated as logarithm from the ratio of examined substance concentration in n-octanol to its concentration in water.

Finally, the theoretical logP was calculated with the use of ACD/logP algorithm (ACD/Chem Sketch 12.0) (16, 17) and Ghose-Crippen algorithm (Spartan) (18, 19). The first one is classified as the most common fragment constant methods and the second one in based on atomic contributions approach (20).

RESULTS AND DISCUSSION

The calculated ΔG_{solv} values in water and chloroform as well as the electrostatic potential range are presented in Table 1.

There we particularly focused on the identification of molecular determinants which are likely to influence bioavailability of three triazole antifungal agents: posaconazole, ravuconazole and voriconazole. The solvation free enthalpy is a representative measure of the affinity of solvents to studied compounds and consequently, the strength of solute-solvent interaction in water and chloroform. The electrostatic potential value gives additional information which explains solute - water (polar solvent) interactions and polarity of examined compounds. The experimental and theoretical logarithm P reveals lipophilicity of active substances (Table 2).

The results for voriconazole point to good solubility in water, but still better in organic solvent - chloroform. The wider electrostatic potential range implies a strong interaction of the molecule with polar solvent – water. The logarithm P value reflects hydrophobic - hydrophilic properties relation of the solute. All the theoretical and the experimental data point at voriconazole as a very effective triazole antifungal agent and confirm its high bioavailability, which may also explain its wide activity spectrum and effectiveness in fungal infections treatment.

The lower energy of solvation in water than in chloroform for ravuconazole and posaconazole suggests a little better solubility of these compounds in water than in n-octanol. The electrostatic potential values show stronger solute-solvent interactions of ravuconazole with polar solvent than those of posaconazole. Both the theoretical and experimental log P values reveal lipophilic attributes of these compounds. All results indicate a good bioavailability of ravuconazole and posaconazole.

The calculated ΔG_{solv} values seems to be a good predictor of solubility of compounds in polar and organic solvents and their intestinal permeability. Calculation of the free enthalpy of solvation could be a new standard for bioavailability determination – both for drugs which are under investigation or those which are at the molecular modeling stage of development.

The aqueous solubility and intestinal permeability are two factors which are used as discriminative criteria for the BCS – The

Biopharmaceutics Classification System (20, 21). The electrostatic and thermodynamic properties of active compounds could be successfully used as new indicators of drugs bioavailability. The free enthalpy of solvation in water and organic solvents could be a useful tool for the fast active compounds classification into four BCS classes. The same holds for the electrostatic potential range. The theoretical and experimental logarithm P determination could improve efficiency of drug bioavailability predictions.

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