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PREDICTION OF BIOAVAILABILITY OF SELECTED BISPHOSPHONATES USING *IN SILICO* METHODS TOWARDS CATEGORIZATION INTO BIOPHARMACEUTICAL CLASSIFICATION SYSTEM

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Abstract: The physicochemical properties relevant to biological activity of selected bisphosphonates such as clodronate disodium salt, etidronate disodium salt, pamidronate disodium salt, alendronate sodium salt, ibandronate sodium salt, risedronate sodium salt and zoledronate disodium salt were determined using *in silico* methods. The main aim of our research was to investigate and propose molecular determinants that affect bioavailability of above mentioned compounds. These determinants are: stabilization energy (ΔE), free energy of solvation (ΔG_{solv}), electrostatic potential, dipole moment, as well as partition and distribution coefficients estimated by the log P and log D values. Presented values indicate that selected bisphosphonates are characterized by high solubility and low permeability. The calculated parameters describing both solubility and permeability through biological membranes seem to be a good bioavailability indicators of bisphosphonates examined and can be a useful tool to include into Biopharmaceutical Classification System (BCS) development.

Keywords: bisphosphonates, Biopharmaceutical Classification System, molecular modeling, bioavailability, solubility, permeability

Bisphosphonates are a group of drugs, which are commonly used to treat diseases that result from increased bone resorption. Bisphosphonates are used in postmenopausal, senile and steroid-induced osteoporosis as well as for the treatment of Paget's bone disease and bone metastases (1, 2). From the chemical point of view bisphosphonates are synthetic analogues of inorganic pyrophosphate. In the chemical structure of bisphosphonates oxygen atom present in pyrophosphates (P-O-P) is replaced by carbon atom, whereas two side chains bound to this carbon atom determine diversity of bisphosphonates structures (1-4).

The first-generation bisphosphonates, such as clodronate and etidronate, do not contain nitrogen atom. A second generation of compounds, characterized by an amino terminal group, includes pamidronate and alendronate (with a basic aminoalkyl group) and ibandronate (with a tertiary amino group). Risedronate and zoledronate, a third-generation bisphosphonates, have a cyclic side chain. Bisphosphonates containing nitrogen have a more powerful antiresorptive activity compared to non-nitrogen bisphosphonates, therefore, the second- and third-generation bisphosphonates are significantly more potent than their first-generation predecessors (5).

Bisphosphonates are characterized by a very low bioavailability, which is falling below 1% for second and third-generation bisphosphonates and a few percent for the first-generation bisphosphonates (6-10). For alendronate oral bioavailability in women is 0.64% for 5 to 70 mg doses taken after all night fasting and two hours before breakfast. In case of men the oral bioavailability is 0.59% after 10 mg dose administered under the same conditions (11). There are two main reasons for poor absorption of bisphosphonates. First, is a very low lipophilicity which prevents transcellular transport across the epithelial barriers. This is caused by phosphonate groups present in bisphosphonates chemical structure which penetrate the lipid layer of cell membranes with great difficulty. Second, is the fact that all bisphosphonates are expected to be completely ionized and negatively charged at physiological pH (6-8) (12). Additionally, absorption of bisphospho-

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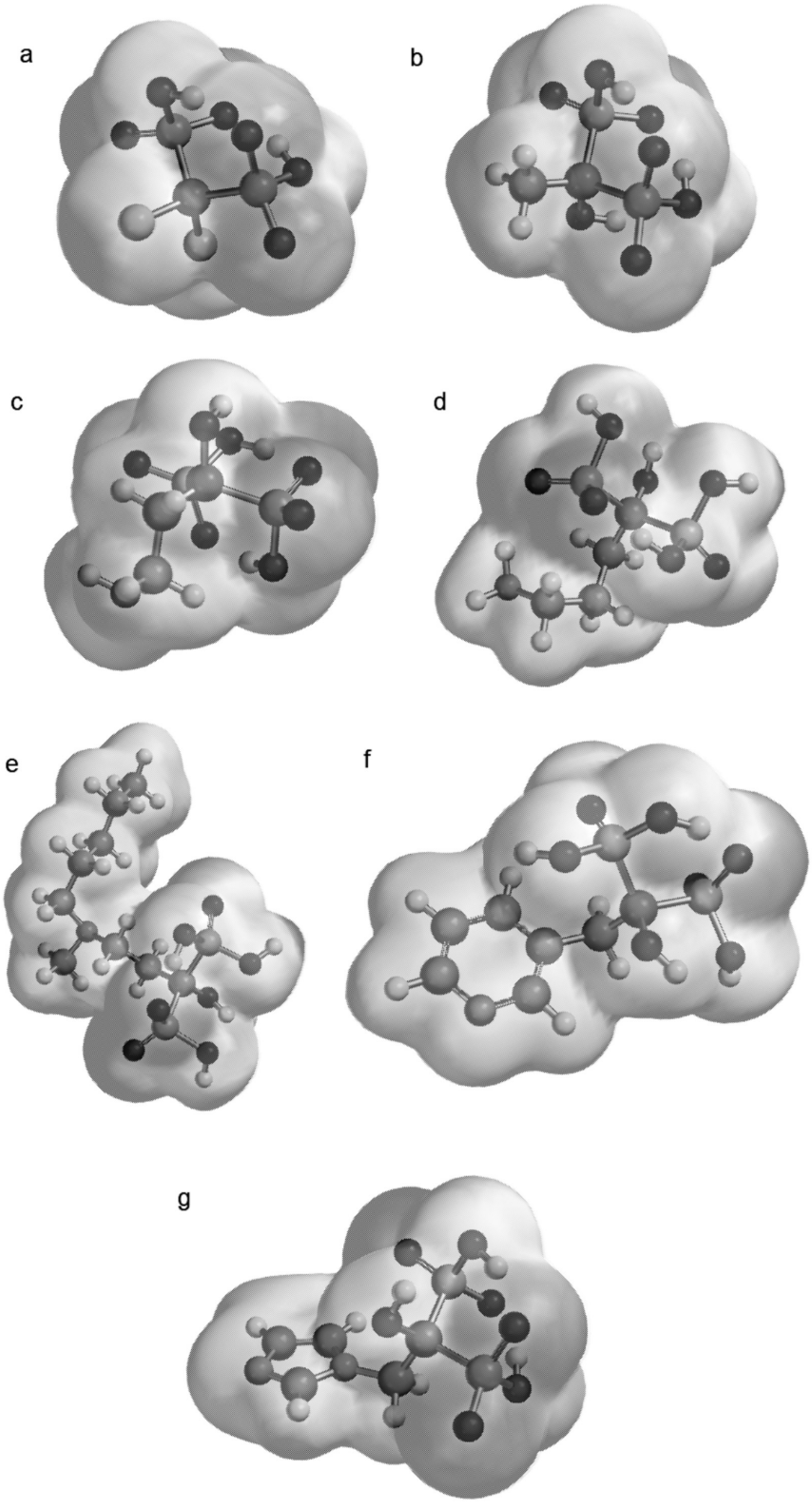

Figure 1. The lowest energy structures with electrostatic potential distribution at isodensity surfaces of (a) clodronate disodium salt, (b) etidronate disodium salt, (c) pamidronate disodium salt, (d) alendronate sodium salt, (e) ibandronate sodium salt, (f) risedronate sodium salt and (g) zoledronate disodium salt molecules

Table 1. The values of ΔE, ΔG_{solv}, electrostatic potential and dipole moment.

Compound	ΔE in water [kJ/mol]	ΔE in diethyl ether [kJ/mol]	ΔG_{solv} in water [kJ/mol]	ΔG_{solv} in diethyl ether [kJ/mol]	Electrostatic potential range [kJ/mol]	Dipole moment in vacuum [D]	Dipole moment in water [D]	Dipole moment in diethyl ether [D]
Clodronate disodium salt	-815.88	-567.35	-820.75	-568.37	-3543.97 to -2133.21	2.39	3.57	2.91
Etidronate disodium salt	-850.19	-584.09	-854.83	-585.00	-3679.79 to -1975.31	1.61	2.21	1.79
Pamidronate disodium salt	-826.34	-564.00	-836.95	-567.38	-3663.97 to -1560.09	2.59	5.06	3.42
Alendronate sodium salt	-264.01	-189.54	-275.10	-188.09	-2183.04 to 276.14	2.34	2.79	2.40
Ibandronate sodium salt	-265.27	-205.02	-277.43	-206.22	-2270.87 to 177.19	6.18	7.01	7.33
Risedronate sodium salt	-282.42	-204.60	-297.36	-206.05	-2429.77 to 227.78	7.53	12.01	7.65
Zoledronate disodium salt	-792.87	-560.66	-804.13	-561.99	-3500.46 to -1274.45	4.39	7.56	5.49

nates decreases when they are taken with nourishment, especially rich in calcium or other divalent cations, which bind with bisphosphonates molecules (13, 14).

The main aim of our investigations was to examine and propose bioavailability determinants of selected bisphosphonates (clodronate disodium salt, etidronate disodium salt, pamidronate disodium salt, alendronate sodium salt, ibandronate sodium salt, risedronate sodium salt, zoledronate disodium salt). This knowledge can be used to categorize above mentioned drugs within the Biopharmaceutical Classification System (BCS) (15-17). The BCS orders drug substances into one of four classes based on their water solubility and intestinal membrane permeability. Those two parameters influence both the rate of absorption and bioavailability of pharmacologically active compounds. Biopharmaceutical Classification System is a useful tool in a process of the generic drug development and evaluation because it replaces certain bioequivalence studies with accurate *in vitro* dissolution tests. It has been also used by numerous drug regulatory agencies to set bioavailability/bioequivalence standards for immediate-release oral drug approval (18-20).

Methods

Quantum-chemical calculations of selected bisphosphonates bioavailability determinants were performed during *in silico* studies.

For each compound, geometry optimization was the first stage of calculation. Further, for each optimized molecule, the free energy of solvation (ΔG_{solv}) both in water and diethyl ether medium was determined. Additionally, the electrostatic potential distribution at isodensity surfaces, and dipole moment for each studied molecule were calculated.

Calculations were carried out using DFT/B3LYP method and the 6-31+G* basis set implemented in Spartan '08 package (21).

The n-octanol/water partition coefficient estimated by the log P was predicted using two most popular applications: ALOGPS 2.1 and KOWWIN (22-24).

The distribution coefficient (under pH value equal: 1.7, 4.6, 6.5, 7.4 and 8), estimated by the log D values was calculated using the method based on semiempirical algorithms (Sparc v 4.5 program) (25).

RESULTS AND DISCUSSION

Values of ΔE, ΔG_{solv} (both in water and diethyl ether), electrostatic potential and dipole moment calculated for clodronate disodium salt, etidronate disodium salt, pamidronate disodium salt, alendronate sodium salt, ibandronate sodium salt, risedronate sodium salt and zoledronate disodium salt are presented in Table 1.

Diethyl ether was chosen as a lipophilic solvent in calculation of a solvation free enthalpy since all developed theoretical models of solvation implemented to available professional software are not parameterized for n-octanol. Yet, diethyl ether has similar dielectric properties (dipole moment 1.29 D and dielectric constant 4.35) to those ones for n-octanol (1.64 D and 10.34). Such approach is quite reasonable since the all solvation models in calculations of solvation free enthalpy include only electrostatic interaction but not hydrogen bonding.

Presented values of stabilization energy (ΔE) are negative both in water and diethyl ether medium, however, they indicate that studied compounds should be better soluble in water than in diethyl ether.

Table 2. The values of log D and log P.

Compound	log D					log P[a]	log P[b]
	pH = 1.7	pH = 4.6	pH = 6.5	pH = 7.4	pH = 8		
Clodronate	-8.01	-8.64	-9.59	-10.04	-10.19	0,81	-4,36
Etidronate	-4.82	-8.10	-9.60	-9.67	-9.82	-0,39	-5,84
Pamidronate	-8.54	-9.38	-9.73	-10.21	-10.48	-1,18	-6,82
Alendronate	-8.26	-8.98	-9.17	-9.33	-9.61	-1,25	-4,49
Ibandronate	-5.38	-6.23	-6.58	-7.06	-7.33	0,42	-2,34
Risedronate	-6.68	-7.58	-8.00	-8.65	-9.12	-0,59	-3,48
Zoledronate	-7.62	-8.29	-8.50	-8.76	-9.18	-0,93	-1,03

[a]ALOGPS; [b]KOWWIN

The free energy of solvation is the direct description of the solute-solvent interaction. The calculated values of ΔG_{solv} reflect better solubility in water in comparison with diethyl ether.

The ΔE and ΔG_{solv} values for studied compounds reveal that the clodronate disodium salt, etidronate disodium salt, pamidronate disodium salt and zoledronate disodium salt have a stronger affinity to water than the other bisphosphonates molecules. The ΔG_{solv} value is also a measure of lipophilicity and indicates that bisphosphonates fall into third class of BCS. From an experiment it is hard to estimate lipophilicity because compounds stay in equilibrium in which all hydrophilic states are included into global log P value. The ΔG_{solv} calculated for isolated molecules yields fairly more discreet results relevant to properties of the molecule itself.

The map of electrostatic potential provides additional information on distribution of solvent molecules in a solvation zone (Fig. 1). Ranges of electrostatic potential define polarity of compounds. Wider range of electrostatic potential indicates stronger interaction of the molecule with water medium, due to increased dipole moment (Table 1). The range of negative electrostatic potential distribution at isodensity surfaces of clodronate disodium salt, etidronate disodium salt, pamidronate disodium salt and zoledronate disodium salt molecules indicate a high ability to form hydrogen bonds necessary to effect the substances interaction with polar water solvent.

The partition coefficient parameter (Table 2) describes the lipophilic properties of studied compounds. Negative values of log P indicate that they are readily soluble in water and potentially cannot diffuse across cell membranes. Similar to ΔE, ΔG_{solv} and electrostatic potential rates, most predicted values of log P indicate that the selected compounds have an affinity for both polar and nonpolar solvent but the affinity is stronger in the case of the polar solvent. The opposite situation is predicted by ALOGPS for clodronate and ibandronate. However, it has to be taken into account that values of log P are predicted using Associative Neural Network that was first trained with standard set of compounds. That is why for classes of compounds that are not similar to those from training set log P prediction is characterized with low accuracy and high error rates (24). This makes log P less effective in predicting bioavailability than other parameters described in this paper.

The partition coefficient (log P) applies to neutral species, whereas the distribution coefficient (log D) is used for the analysis of structure-activity relationships of ionizable compounds. D is the ratio of the equilibrium concentration of compound in an organic phase to the total concentration of un-ionized and ionized species in the aqueous phase at a given pH. The values of log D are given in Table 2. These values may indicate low bioavailability which is dependent on the pH changes in the gastrointestinal tract.

Calculated parameters reveal that selected bisphosphonates are characterized by high solubility and low permeability through the cell membranes.

The physicochemical properties like stabilization energy, free energy of solvation, electrostatic potential as well as dipole moment of studied molecules describing both solubility and permeability through biological membranes seem to be good bioavailability indicators and can be useful tools to Biopharmaceutical Classification System (BCS) development.

Partition and distribution coefficients are also useful in determination of bioavailability but their prediction using standard tools has very low accuracy. In order to make the prediction accuracy better, creation of custom library containing compounds similar to one being tested is needed (24). Without such preparation values of log P and log D can be only treated as high level estimates, which indicate that stabilization energy (ΔE), free energy of solvation (ΔG_{solv}), electrostatic potential and dipole moment better serve as tool for BCS categorization. Results based on such approach indicate that bisphosphonates fall into III class of Biopharmaceutical Classification System.

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