1st International Conference
on Chemo and BioInformatics
ICCBIKG 2021

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BOOK OF PROCEEDINGS

October 26-27th, 2021,
Kragujevac, Serbia

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THE MOLECULAR BASIS OF DRUG-PLASMA PROTEIN INTERACTION FOR CNS ACTIVE COMPOUNDS

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Abstract

The human serum albumin (HSA) is well known for its extraordinary binding capacity for both endogenous and exogenous compounds, including a wide range of drugs. The goal of our investigation was to evaluate the distribution process for 15 CNS active compounds. The drug-plasma protein interaction was evaluated under simulative physiological conditions on the HSA-based stationary phase by using the mixture of Sørensen phosphate buffer (pH 7.40) and acetonitrile modifier as a mobile phase (84:16 *v*/*v*). The retention parameters (*k*) were used to approximate the % of protein-binding by calculating the *P*(%) values. The results obtained through this study demonstrated that the constitutional properties (e.g. number of total bonds, atoms, carbon atoms) and lipophilicity have a strong positive impact on the HSA-binding affinity. The coefficient of diffusion has a negative impact, while the atoms and sites available for the CYP450 oxidation showed the most significant correlation (r = 0.92). This study provides a basis for further *in vitro* chromatographical investigations of drug-HSA interaction for CNS active compounds. The correlation between obtained retention data and the availability to enzymes oxidation indicates the application of the tested system in the assessment of the metabolic degradation profile of CNS related drugs.

Key words: human serum albumin, binding affinity, CNS compounds, molecular characterization

1. Introduction

The characterization of molecular basis between drug and plasma proteins is routinely performed as part of the drug discovery process. Only the unbound drug is available to act at physiological sites of action. The plasma proteins and their interactions with drugs can have a strong influence on the pharmacokinetic and pharmacological properties of the compound such as adsorption, distribution, metabolism and excretion [1-3]. To simulate the drug-HSA binding interaction in biological system, the reversed-phase (RP) chromatographic mode was applied. The retention behaviour of 15 CNS active compounds was investigated on HSA-based stationary phase, and by using the mixture of a phosphate buffer (pH 7.4) and organic modifier as a mobile phase. In order to select the molecular basis of investigated drug-plasma protein interaction, the correlation between obtained retention characteristics an molecular properties of compounds was defined.

2. Experimental

2.1 Chemicals and reagents

The set of investigated compounds were provided by from Sigma - Aldrich, St. Louis, MO, USA (clozapine, citalopram hydrobromide, maprotiline hydrochloride, mianserin hydroc hloride, mirtazapine, prazepam, selegiline hydrochloride, rasagiline mesylate, ropinirole hydro chloride, viloxazine hydrochloride); Merck, Darmstadt, Germany (fluoxetine hydrochloride); Hemofarm, Vrsac, Serbia (aripiprazole, olanzapine); and Pfizer, NY, US (haloperidol). The ch emical structures of the investigated compounds are given on the Fig.1.

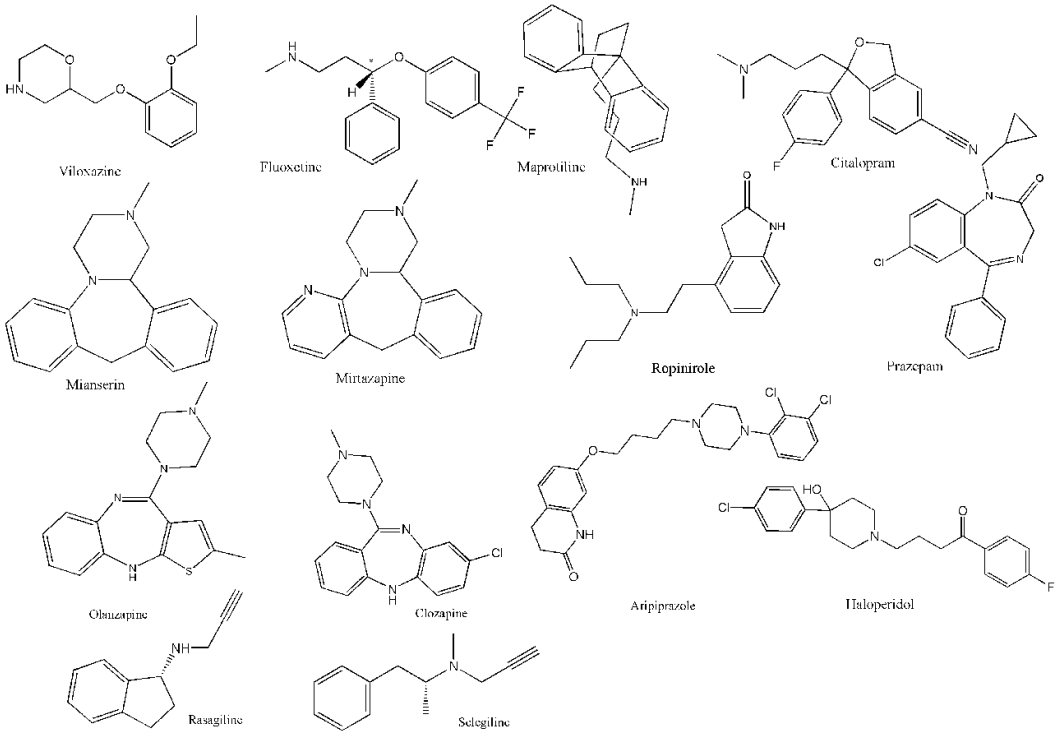

Fig. 1. The chemical structures of the investigated compounds.

Acetonitrile (J.T. Baker, Deventer, Netherlands) of HPLC grade and deionized water (TKA water purification system, Niederelbert, Germany) were used throughout this study. Potassium dihydrogen phosphate and disodium hydrogen phosphate were obtained from Merck (Darmstadt, Germany).

2.2 Chromatographic conditions

The HPLC analysis was performed at 25 °C using the Agilent Technologies 1200 HPLC system (Santa Clara, CA, USA) consisting of the UV detector. The flow rate was set to 0.5 mL min^{-1}, and the UV detection was carried out at 254 nm. The retention behaviour of selected compounds were examined on CHIRALPAK®HSA column 15 cm x 10 mm I.D. packed with human serum albumine chemically bound to silica particles size of 5 μm (DAICEL CORPORATION, France). The mobile phases consisted of Sørensen phosphate buffer (pH 7.0; 0.01 M) : acetonitrile modifier = 84 : 16 (*v*/*v*). Retention time (t_r) of each compound was used to calculate the retention factor (k) value, $k = (t_r - t_0)/t_0$, where t_0 is the column dead time measured as the time of the first baseline perturbation. The obtained k values were used to calculate the % of protein binding (P(%)) according to the following equation:

$$P(\%) = 100 \cdot k / (k + 1) \quad (1)$$

The obtained results are graphically presented in Figure 2.

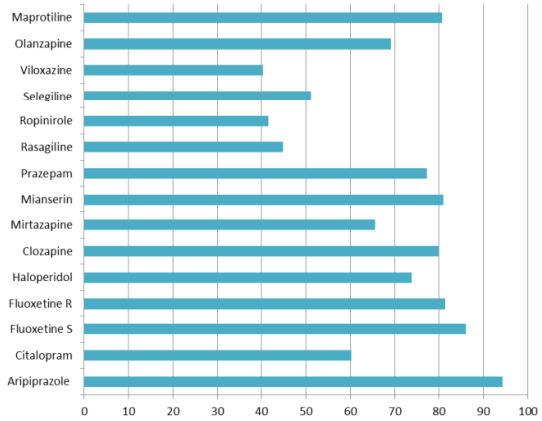

Fig. 2. The values of obtained parameters (*P*(%)) that reflect the nature of drug-plasma protein interaction.

2.3 Aproximation of the main molecular properties

Selection of the dominant molecules/cations and the dominant tautomeric forms at pH 7.0 was performed for a set of 15 structures using the Marvin Sketch 6.1.0., Chem Axon program. The selected forms were pre-optimized with the semiempirical PM3 method, d then refined by using the more precise HartreeFock/3-21G method using the Gaussian09 software included in the ChemBio3D Ultra 13.0. program. For all the optimized molecular structures, the molecular descriptors were calculated using ADMET Predictor 9.5 programmes.

3. Results and disscutions

According to the results given in the Fig.2, aripiprazole, maprotiline, fluoxetine and mianserin showed a high interaction with the HSA-stationary phase (*P*(%) > 80%), so a low concentration of unbound form in the blood is expected. Contrary, for rasagiline, ropinirole, selegiline and viloxazine, there is a moderate degree of interaction (*P*(%) 40 - 50 %). For the rest of the investigated compounds, the interaction with HSA is moderate to high (50 – 80%). The simple correlation between obtained *P*(%) and calculated molecular properties showed that number of sites in the molecule available for CYP450 enzyme oxidation have the main impact on HSA-binding affinity ($r = 0.92$):

$$P(\%) = - 23.24 + 3.70 \cdot \text{N\_CYPSites} \quad (2)$$

$r = 0.92$, adj$R^2 = 0.84$, $p = 1.57\text{E-}06$

It is known that interactions between drugs and plasma proteins influence the main pharmacokinetic properties including the distribution and metabolism [3-4]. Thus, the strong correlation between *P*(%) and availability for CYP450 enzyme oxidation confirms applicability of tested chromatographic conditions in assessment of the distribution process, and also for metabolism characterization. The graphical representation of the obtained correlations (*r*) is given on the Figure 3.

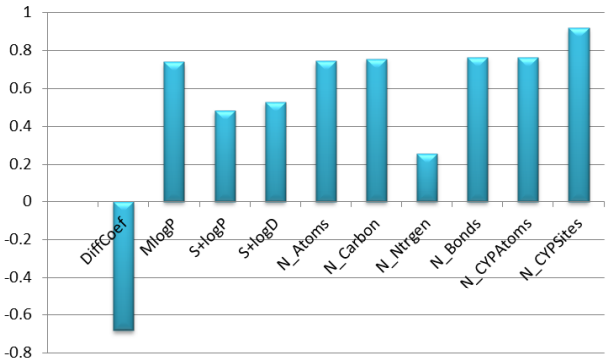

Fig. 3. The graphical representation of the correlation between *P*(%) *vs.* calculated molecular properties of the investigated compounds.

On the other hand, the constitutional properties such as number of total atoms (N_Atoms), bonds (N_Bonds), and number of carbon atoms (N_Carbon) showed an important impact on the *P*(%) value. The positive influence was also presented for lipophilic characteristics (MlogP), while the coefficient of diffusion (DiffCoef) has a negative influence on *P*(%) value.

4. Conclusions

The obtained results provide a basis for further *in vitro* chromatographic investigations of drug-HSA interaction, and their application in the assessment of the metabolic degradation profile of CNS related drugs.

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