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SHORT COMMUNICATION

DOCKING OF THIOPURINE DERIVATIVES TO HUMAN SERUM ALBUMIN AND BINDING SITE ANALYSIS WITH MOLEGRO VIRTUAL DOCKER

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The replacement of the oxygen by sulfur atom at 6-position of guanine and hypoxanthine produced the compounds which are antimetabolites of nucleic acid purines (1). These thiopurine analogues have anticancer (6-mercaptopurine, 6-thioguanine) and immunosuppressive (azathioprine) activities. 6-Mercaptopurine (6-MP) is used for remission induction and in the maintenance therapy of acute lymphatic leukemia, and usually in combination with other drugs. 6-MP is also used to treat autoimmune diseases, such as inflammatory bowel diseases and rheumatoid arthritis (2–4).

Human serum albumin (HSA) is a major protein component of blood plasma and due to its endogenous and exogenous ligand binding properties, plays an important role in the transporting and distribution of numerous pharmaceutical compounds, mainly as a complex, to various organs and tissues within human organism. HSA consists of three homologous domains (I, II and III) and each domain is formed by two subdomains (A and B) (5). The X-ray analysis of different ligand–HSA complexes showed the existence of two binding sites in the II and III domains, which have cavities formed mostly of hydrophobic and positively charged residues and in which a very wide range of compounds may be accomodated (5, 6). The principal binding regions of albumin are located in subdomain IIA (the warfarin–azapropazone binding site, site I) and in subdomain IIIA (indole–benzodiazepine binding site, site II) (7, 8). Albumin may bind the acidic, basic and neutral molecules of drugs. The subdomain IIIA of albumin exhibits the primary binding activity, whereas subdomain IIA seems to be more specialized. Bulky heterocyclic compounds with a negative charge bind primarily to site I, whereas site II is preferred by aromatic carboxylates and anesthetics. Most drugs bind with the amino acids of target binding site in a reversible manner by means of weak chemical bonds. The several types of intermolecular bonding interaction such as hydrophobic, electrostatic, ionic and hydrogen bonds differ in their bond strengths. The number and types of these interactions depend on the structure of the drug and functional groups that are present in the drug (9).

The study of the interactions between albumin and drugs are important in pharmacology and clinical medicine, and also in research and design of new compounds. Numerous analytical methods including, among others, equilibrium dialysis, various spectroscopic, chromatographic and electrophoretic methods (10–13) are conventionally and most commonly used for investigation of the HSA–drug binding process. Additionally, the computational simulation by molecular docking procedure may be used to have more information about the specificity of the binding site and for the prediction of ligand-protein interactions. The *in silico* method will be used as an alternative and complement to *in vitro* methods and may give details of these molecular interactions, which would not be possible in solution studies (14–16). The studies by molecular docking are important, not only from a theoretical viewpoint, to explain the relationship between the structure of ligand and the function of protein but also in terms of practical applications, as they allow interpretation of the transporting process and therapeutic effectiveness of drugs.

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The previous results of computational docking study demonstrated that 6-MP may bind to the active site I located in subdomain IIA of the HSA structure (17). In order to obtain more information on how important is chemical structure of 6-MP for its interaction with albumin, a series of the sulfur purine derivatives was docked to site I of HSA and the results for this group of compounds were compared with those for 6-MP with HSA.

EXPERIMENTAL

Protein and ligand structures

The X-ray structure of HSA (PDB ID: 1AO6) (18) was downloaded from Protein Data Bank (PDB). The chemistry of the protein was corrected for missing hydrogen consistent with protonation states at pH 7.4, the His, Arg and Lys were in protonation state, while Asp and Glu were deprotonated; the protein was treated as a rigid body and the polar hydrogen atoms of the protein were added. The crystallographic water molecules were removed from the protein. The two dimensional (2D) structures of ligands were obtained using the ChemBioDraw computer program (19). 2D to three dimensional (3D) representations were converted by the use of ChemBio3D (20) software and then, these were energetically minimized by using method implemented in the same software, and saved as MDL moleFile (*.mol). The chemical 2D structures of all ligands are shown in Figure 1.

Molecular docking

Molecular docking procedure was performed using the Molegro Virtual Docker (MVD) program

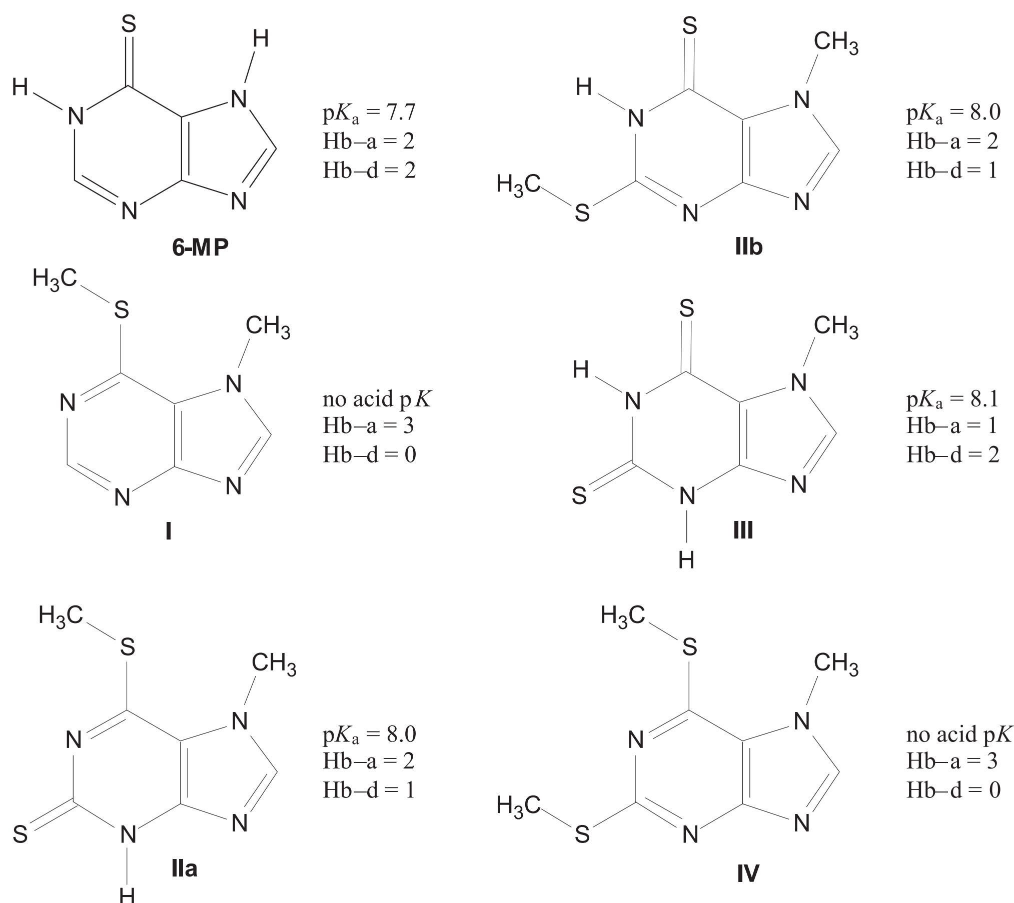

Figure 1. Chemical structures of the investigated thiopurines, their acid dissociation constants (pK_a) values and number of potential hydrogen bond donors (Hb–d) and acceptor (Hb–a) groups (pK_a values and number of hydrogen bond donors and acceptor were calculated with the use of the computer program (27). Structures of compounds were taken from data published in (28)

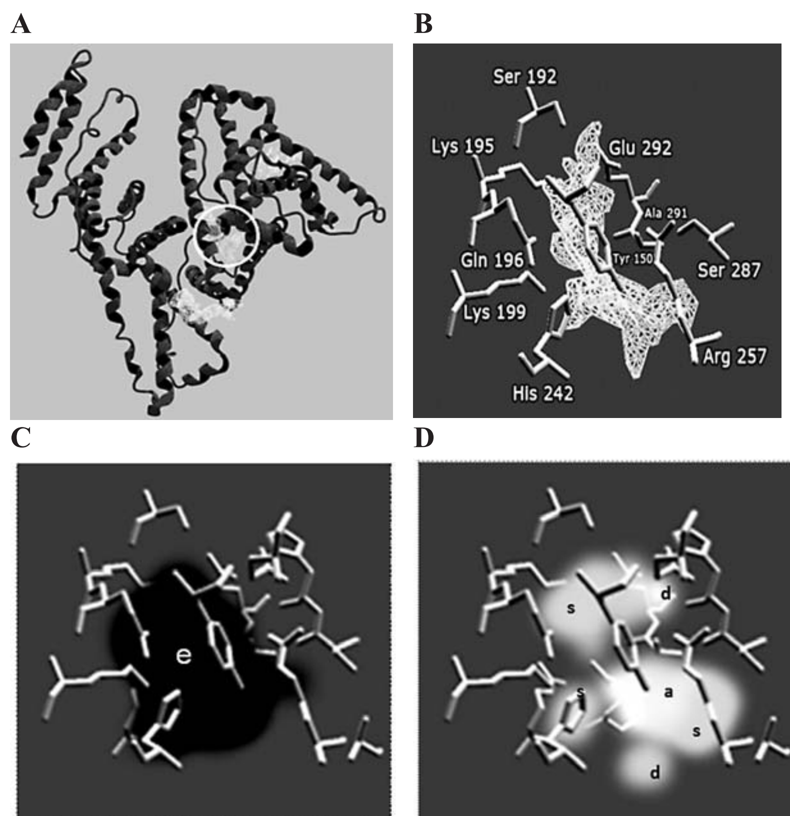

Figure 2. **A** – Ribbon model of HSA (HSA from PDB ID: 1AO6) with detected binding site for 6-MP in subdomain IIA (the site is marked with a white circle). Images **B**, **C** and **D** present the enlarged area corresponding to the 6-MP binding site in subdomain IIa; **B** – the close-up of detected site with a hydrophobic (Ala), hydrophilic uncharged (Ser, Gln, Tyr) and hydrophilic positively (Arg, Lys, His) or negatively (Glu) charged amino acid residues, according to the hydropathy index (29), capable of interacting with 6-MP (only residues around 6 Å of the docked 6-MP were taken into account); **C** – e-region shows the electrostatic potential of albumin in the binding site; **D** – s-region shows the spot where it is favorable to place non-polar atoms of the ligand, a-region shows the spot where it is favorable to place a ligand atom capable of accepting a hydrogen bond i.e., spot near donor in the protein, d-region shows the spots for heavy atoms in the ligand that are able to donate a hydrogen to hydrogen bond

(16, 21). The identification of the cavity with the potential binding site for ligands in subdomain IIA in HSA crystal structure was performed automatically using the grid-based cavity prediction algorithm. The residues close to cavity were minimized. During the minimization only torsion angles in the side chains were modified, all other properties (including bond lengths and backbone atom positions) were held fixed. The 3D structures of ligands were imported to MVD as *.mol.

During the docking simulation the backbone was kept rigid, but the torsional angles in the side chains of amino acids close to the detected cavity were allowed to change. The following steps were applied during the docking simulation: the ligands were docked with the softened potentials. At this point the receptor was kept rigid at its default conformation. After each ligand was docked, the side chains chosen for minimization were minimized with respect to the found pose. After repositioning the side chains, the ligand was energy-minimized. The repositioning of the side chains and minimization of the ligand were performed using the standard non-softened potentials. All flexible torsions in the ligand were set rigid during docking because the complexity of the docking search can be significantly reduced if the number of torsions that are set flexible during the docking run is lowered.

First, to obtain the 6-MP–HSA complexes, the 6-MP molecule as neutral and monoanionic forms, were docked individually to the cavity. For each complex, 10 independent runs were conducted, each of these runs was returning to a single final solution (pose). The resulting conformations were clustered and only the negative lowest-energy representation from each cluster was returned when the docking

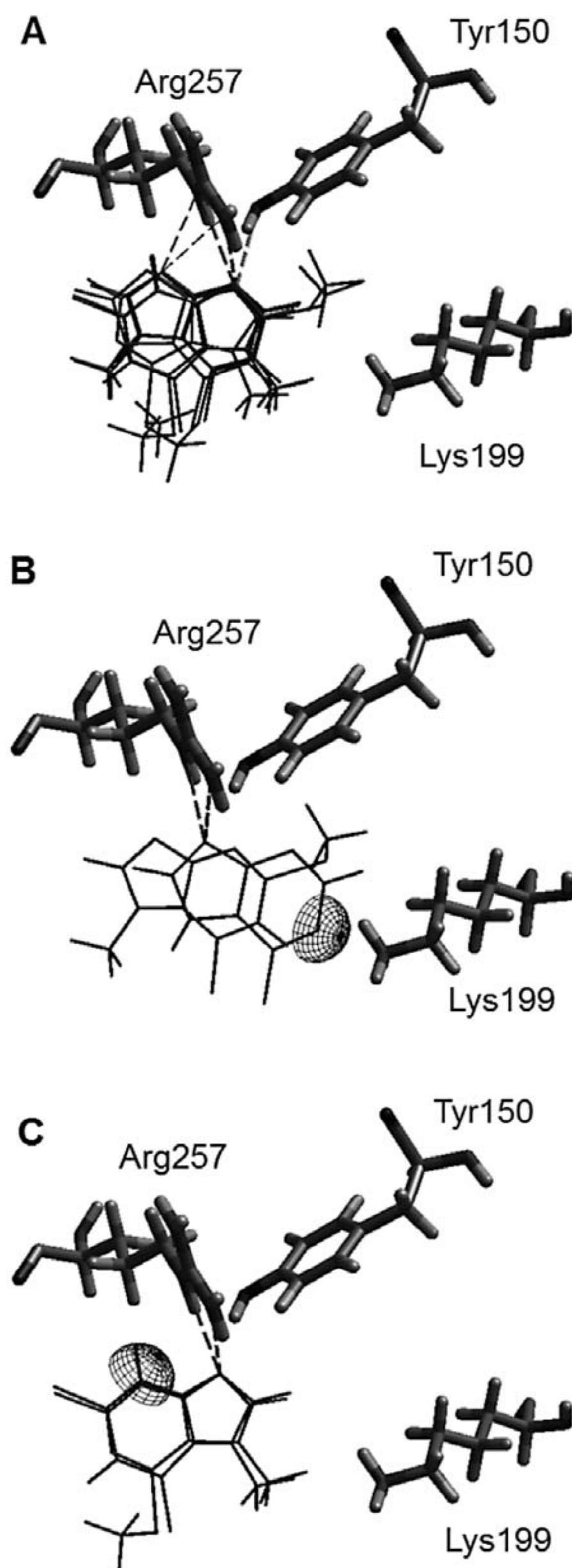

Figure 3. Overlaped docking poses of a neutral forms of compounds I–IV and 6-MP (**A**), anionic forms of IIb and 6-MP (**B**), anionic forms of IIa and III (**C**). The amino acid residues are shown in stick representation, the poses of compounds I–IV and 6-MP are shown in thin stick. Hydrogen bonding interactions are shown in dashed lines, electrostatic interaction is visualized as partial sphere oriented in the direction of the interaction

run was completed; the similar poses were removed keeping the best-scoring one. The cluster of ten poses was sorted in order of the MolDock Score. In order to increase the accuracy of the ranked order of the poses, the weighted reranking scores (Rerank Score) were used to evaluate the poses. For analysis, one pose with the lowest value of Rerank Score was selected as the best solution for each complex. Next, the same procedure was followed in order to obtain the thiopurine–HSA complexes. The compounds I and IV (basic compounds) were docked as neutral forms. The compounds IIa, IIb and III as well as 6-MP are acidic compounds. Their dissociation constants, p*K*, are about 8.0 (Fig. 1) and in aqueous solution at pH 7.4 may exist as a mixture of the neutral and monoanionic forms. Therefore, the structures of IIa, IIb and III were docked as neutral and anionic forms.

Before docking of the ligands into the HSA structure, the MVD docking protocols were validated using the two crystal structures of RWF–HSA and PhB–HSA complexes as previously described (17).

RESULTS AND DISCUSSION

The 6-MP is a purine derivative, containing four nitrogen atoms and double-bonded sulfur atom as a side group in the 6 position of the pyrimidine ring (C(6)=S). The overall purine has the potential to interact with binding site through hydrophobic interaction, while the individual N atoms present in the structure could interact by hydrogen or ionic interaction. In the molecule of 6-MP the N(3) and N(9) atoms have one lone pair of electrons and can act as an acceptor for hydrogen bonds, and N(1)H and N(7)H groups can act as a donor for hydrogen bonds, and N(1)H can act as an ionic center if it is deprotonated. Although sulfur atom is electronegative, it is a weak H–bond acceptor. Its lone pairs are in third–shell orbitals that are larger and more diffuse. This means that the orbitals concerned interact less efficiently with the small 1s orbitals of hydrogen atoms (9, 22, 23).

The binding region in subdomain IIA of HSA (namely Sudlow`s site I) is formed as a pocket with the inside wall being formed by amino acids hydrophobic side chains (Ala, Leu, Phe, Trp, Val, Ile). The interior of the pocket is predominantly apolar but contains two cluster of polar residues, an inner one towards the bottom of the pocket (Tyr150, His242, Arg257) and an outer cluster at the pocket composed by positively charged residues (Lys195, Lys199, Arg218 and Arg222) (24, 25).

The molecular docking simulation using the MVD program was previously employed to investi-

Table 1. Interaction energies between HSA and compounds I–IV and 6-MP at the binding site I on HSA

Ligand	Interaction energy (arbitrary unit)				
	E-Total	Steric	H-Bond	Electro	E-Intra
Neutral form					
6-MP	–63.8	–59.5	–8.0	0.0	3.7
I	–64.2	–64.7	–7.8	0.0	8.3
IIa	–80.0	–78.6	–7.3	0.0	5.9
IIb	–73.4	–72.6	–7.7	0.0	6.9
III	–73.4	–72.8	–7.3	0.0	6.7
IV	–78.3	–77.0	–7.3	0.0	6.0
Anionic form					
6-MP	–69.8	–47.0	–8.7	–17.8	3.7
IIa	–77.6	–66.1	–4.3	–13.2	6.0
IIb	–81.3	–66.9	–9.0	–12.3	6.9
III	–74.6	–63.0	–6.3	–12.0	6.7

The energy is not normalized in chemical units and should only be used to compare the results within one results set. E-Total is the total energy (the sum of internal ligand energies, protein interaction energies and soft penalties), H-Bond is the hydrogen bonding energy between protein and ligand. Steric is the steric interaction energy between protein and ligand. Electro is the sum of short-range ($r < 4.5$ Å) and long-range ($r > 4.5$ Å) electrostatic protein–ligand interaction energy, E-Intra is the total internal MolDock Score energy of the pose (21)

gation of the binding mode of 6-MP with HSA and the location of the 6-MP binding site in the HSA structure and structural characteristics of this site were described (17). The binding site for 6-MP found to be in subdomain IIA is presented in Figure 2A. Hydrophobic, hydrophilic as well as positively charged residues present in this site (Fig. 2B) were able to participate in electrostatic interaction (Fig. 2C), hydrogen bonding and steric interaction (Fig. 2D) with 6-MP.

To obtain the complexes of compounds I–IV and 6-MP with albumin, docking of these thio derivatives into binding cavity of HSA was performed. The final solutions of this procedure are presented in Table 1 and in Figure 3. Total binding energy between selected conformation of each ligand and HSA, and components of this energy determined from the MVD results are listed in Table 1. The results were interpreted on the assumption that the more negative are the values of predicted binding energy, the more thermodynamically favorable is binding energy (26). The estimated E-Total binding energy for all the ligands was strongly dependent on steric interactions. Hydrogen bonds and electrostatic interaction energies accounted for markedly smaller proportion of total binding energy. It can be noticed in case of all the thiopurine derivative–HSA complexes that the values of E-Total energy were larger than those for 6-MP and resulted mainly from differences between steric energy. It was also observed, that binding energy between 6-MP and compounds I–IV and HSA depends on the compounds' ionization state, and was stronger for anionic form than for unionized molecules.

The best poses of 6-MP and compounds I–IV docked to site I are shown in Figure 3. Comparing the interactions between HSA and compounds I–IV with those for the 6-MP, it was found that the investigated compounds had the same amino acids residues involved in the interaction as 6-MP. The preferred poses conformation of neutral forms of compounds I–IV were positioned to form the hydrogen bonds with Arg257 and Tyr150 (Fig. 3A) and only N(3) and N(9) atoms of 6-MP and molecules of compounds I–IV were involved in hydrogen bond interactions (as acceptors). The sulfur atom was not found to form hydrogen bonding. However, the negatively charged N(1) atom of anionic forms of 6-MP and compound IIb was bonded by a salt bridge to positively charged ε-amino group of Lys199 (Fig. 3B), while the negatively charged N(3) atom of anionic forms of compound IIa and III was bonded by a salt bridge to positively charged guanidinium group of Arg257 (Fig. 3C).

The results of molecular docking showed that despite differences in molecular structures, the investigated neutral compounds with 2- or 2,6-purinethione structure (weak acids) and with methylthio substituents in the 2- or 2,6-positions of purine nucleus (weak bases) (Fig. 1) did not differ in hydrogen bonding interaction pattern. However, for compounds with the purinethiones structure, the positions of double bonded sulfur atom seems to be important for the mode of ionic bonding interaction with HSA.

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