Research Article

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Structural analyses of the interactions between the thyme active ingredients and human serum albumin

Kekik aktif maddeler ile insan serum albüminleri arasındaki etkileşimlerin yapısal analizleri

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Abstract

Objective: Therapeutic effects of thyme and the mechanism underlying the function of its active ingredients are the areas of active investigation. In this regard, understanding the potential interactions between the active ingredients of the thyme leaf and the serum albumin would bring about new insight on the bio-distribution, circulatory half-life and consequently their pharmacodynamics and pharmacokinetic properties.

Methods: The 3D structures of carvacrol, linalool, p-cymene and thymol molecules as the thyme active ingredients and the 3D structure of albumin were harnessed from the structural databases. Then, these structures were prepared for molecular docking analyses by Autodock vina software. Ultimately, the binding energies between docked albumin and thyme active ingredients were calculated and their interactions were predicted.

Results: Our results indicated that all active ingredients of thyme can interact with albumin molecule at drug binding site 3 and fatty acid binding site 5. The structural properties of the ingredients effect their interaction sites and binding energies.

Conclusion: It could be concluded that albumin, as the most abundant protein of the serum, could act as the bio-distributor of thyme active ingredients. This property would be of great significance to exert the desired therapeutic effects.

Keywords: Bioinformatics; Thyme; Albumin; Bio-distribution; Active ingredients.

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Özet

Amaç: Kekik ve aktif maddelerinin rol aldığı fonksiyonlarının altında yatan mekanizmanın teröptik etkileri güncel bir araştırma konusudur. Bu bağlamda, kekik yaprakı aktif maddeleri ile serum albümleri arasındaki potansiyel etkileşimleri anlamak, biyolojik dağılım, dolaşım yarım ömrü ve dolaysıyla bunların farmakodinamik ve farmakokinetik özellikleri hakkında yeni bir fikir doğuracaktır.

Yöntem: Kekik aktif maddeleri olarak karvakrol, linalol, p-simen ve timol molekülerinin 3B yapıları ve albüminin 3B yapısı yapışal veri tabanından elde edildi. Sonra, bu
The statistics shows that approximately 40% of the discovered drugs fail to pass developmental stage [1]. Therefore, extensive study of their therapeutic properties is a pivotal and yet highly expensive and time-consuming prerequisite for drug development and marketing. In this regard, performing an absorption, distribution, metabolism, toxicity and excretion (ADMET) study could provide a wealth of useful information from a drug development perspective [2]. Conducting structural and molecular modeling investigations is lately taken in to account as an alternative to conventional methods to understand the ADMET properties of the drug candidates. These studies are based on physical and chemical characteristics of drugs [3,4] including their solubility in water [3,5] and digestive status [6].

Apart from the route of administration, a drug should work its way into plasma to be distributed in the body with the blood circulation. The existence of various interfering molecules (including the proteins), as blood contents, could influence drug properties like the transmission quality, the amount of free drug and the catabolism rate [7]. The blood contains a class of molecules which are responsible for transmission of hydrophobic and insoluble molecules including albumin, alpha-1-acid glycoprotein, lipoproteins and pre-albumin [7]. Amongst, albumin is the most abundant serum protein (with 2.5–4 g/dL concentration) which acts as a carrier of blood molecules like free fatty acids, hormones and some of the drugs [8–11]. Moreover, albumin is used as a source of amino acid deposition and it plays a role in generation and maintenance of osmotic pressure [8]. Since it is a general and nonspecific transporter, a wide spectrum of molecules including the administered drugs could bind to and transferred by albumin. However, general and nonspecific function of albumin as a transporter should not be interpreted as nonspecific binding of all compounds to the albumin. It means that albumin does not exclusively binds to specific ligand and various molecules can bind albumin upon their encounter. Albumin binding could increase the drug circulatory half-life as well.

Contemporary, the medicinal plants and traditional medicine are experiencing a growing attention with an affectivity comparable to chemical counterparts. Determining the existing compounds within the edible parts of the medicinal plants is considered to be the key to understand the therapeutic properties of them [1]. Thyme is one the native Iranian medicinal plants which is of significant financial importance. This plant is repeatedly advised in traditional medicine. A wide range of therapeutic effects is proposed for this plant including the effects on nervous system disorders, digestion, dermatology diseases and its anti-allergic and anti-spasmodic effects [2]. Hudia et al. has applied Capillary GC/MS analysis based on polar and non-polar columns to evaluation of the volatile oils hydro-distilled from thyme. Their study has introduced the carvacrol, linalool, p-cymene and thymol as the main active ingredients of thyme [12]. Various studies have reported anti-oxidant [13], anti-microbial [14], anti-inflammatory [15], carminative [16], anti-parasite [13,17], anti-fungal [18] and anti-nociceptive [19] effects for thyme active ingredients. These effects which are mostly irrelevant provide the researchers with a staggering opportunity to scrutinize the intricacies of the thyme therapeutic effects and the compounds which are involved. Based on prior investigations, most of the therapeutic effects of the thyme are caused by these compounds as the active ingredients. It should be noted that, these active ingredients, like other drug components, should transmit into the blood circulation for sufficient distribution throughout the body. Once in the blood, these active ingredients inevitably encounter with albumin. Bioinformatics have insinuates itself into every corner of biology including vaccine design [20–22], structural studies [23–25] and drug-protein and protein-protein interactions [26,27]. Utilizing the bioinformatics tools would circumvent the costly and arduous analyses of protein-ligand interactions and the mechanisms of protein functions [28–30] and help to decrease the inevitable ethical concerns of empirical and clinical studies.

The interactions between albumin and various other molecules have been investigated with both experimental and computational approaches [31–36]. These studies have elucidated the intricacies of albumin interactions with different molecules. Using similar in silico approaches, we
aim to delineate the potential interactions between the active ingredients of the thyme leaf and the serum albumin. Performing various experiments, our study tries to indicate if albumin can act as a bio-distributor for thyme active ingredient. This study would bring about new insights about the mechanism in which thyme exerts its therapeutic effects. To this end, various state-of-the-art bioinformatics tool employed to analyze the possible interactions and their possible consequences. Our results indicate that all of the active ingredients of the thyme interact with the serum albumin with proper binding energies.

**Methods**

### Retrieving albumin 3D structure

The RCSB PDB database at http://www.rcsb.org/pdb/home/home.do was employed to find a resolved 3D structure for albumin. In order to find a human albumin structure with highest resolution, the search results were sorted according to their X-ray diffraction resolution.

### Edition of PDB file and cavity detection

The selected PDB file for albumin structure should be edited to omit the unnecessary water and ligand molecules. The ConTEXT software (v0.98.6) was used to delete unwanted molecules. Following the file edition, the resulting PDB file was fed as the input file for cavity detection using the Molegro Virtual Docker software.

### Retrieving 3D structures of thyme active ingredients

The 3D structures of active ingredients for thyme were obtained from ZINC database at http://zinc.docking.org/. The 3D structures were saved in PDB file format. Lipinski-type properties of each active ingredient were analyzed using the I-Lab 2 server at https://ilab.acdlabs.com/iLab2/.

### Molecular docking

The AutoDock Vina molecular docking software was used to analyze the possible orientations for the interactions between the albumin and thyme active ingredients. Autodock vina software is a new program for molecular docking and virtual screening analysis which brings about approximately two orders of magnitude speed-up compared to Autodock 4. The energy minimization run and conversion to PBDQT format was executed on all imported compounds by Open Babel tool. The albumin 3D structure was prepared for docking analysis adding hydrogen atoms and merging all nonpolar hydrogens. The binding energy for the interactions between the albumin and thyme active ingredients was calculated by the Autodock vina software.

### 2D interaction plot

The schematic diagrams of detailed protein-ligand interactions were produced using LigPlus program (v.1.4.5). This program shows the potential interactions between the ligand molecule and albumin residues located at its vicinity.

### Results

#### Albumin 3D structure

The PDB structure under the RCSB PDB ID of 1N5U was selected as the 3D structure for albumin. The X-ray diffraction resolution of 1.9 Å convinced us to use this structure as the albumin 3D structure.

#### PDB file edition and cavity search

All of the non-protein molecules including the water molecules and ligand molecules were omitted from the 1N5U structure. Figure 1 illustrates the 1N5U structure before and after file edition. As the Figure 2 illustrates, four main

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**Figure 1:** Prepared structure of albumin. (A) is the albumin structure after edition while (B) is 1N5U PDB file without any edition. The red spheres are the water molecules and the green molecules are the ligand molecules interacting with albumin.
Thyme active ingredients

The 3D structure of four main thyme active ingredients was obtained from ZINC database. The ZINC IDs of these compounds are listed in Table 1. Moreover, the Lipinski-type properties of each active ingredients are listed in Table 1. These information would be a useful analyzing the possible interactions between these molecules and their binding protein partners within the serum.

Molecular docking results

Molecular docking results indicated the most probable orientation for interaction between the albumin and thyme active ingredients. Figure 3 indicates the predicted possible orientation for each compound. Table 2 listed the binding energies between the interacting molecules. Lower energies indicate more stable and more strong interactions between the albumin and the ligands.

Results for 2D interaction plots

The interactions between the albumin and carvacrol, linalool, p-cymene and thymol molecules are depicted in Figure 4. As illustrated each molecule have several interactions with the residues of the albumin molecule. Most of the interactions are of hydrophobic nature.

Discussion

The intrinsic properties of drugs are extremely important in pharmaceutical studies. The costly and time consuming nature of novel drug discovery and development methods is partially rooted in this fact [1]. However, due to incidence of serious side effects or unsatisfactory therapeutic efficiency, a great deal of meticulously designed drugs fails to proceed as widely prescribed drugs before or even after marketing [37]. In this regard, investigating the ADMTE properties of the drug candidates considered to be imperative. The binding partners of therapeutic agents within the serum could significantly contribute to their bio-distribution as an ADMTE property. Impaired bio-distribution of drug candidates is one of the challenges ahead of developing therapeutic agents. In the present study, our analyses in search of a serumic binding partner for the active ingredients of thyme have attained promising results. The obtained results have revealed that the active ingredients of thyme can bind the serum albumin with suitable affinity compared to similar studies [38, 39]. Given the hydrophobic nature of these ingredients the hydrophobic interactions should be the dominant forces keeping them in engagement with albumin. Upon their entry into blood, albumin can act as a transporter for thyme active ingredients. Since the reversible binding to a wide variety of ligands is one of the most outstanding property of the albumin [40], it can play a pivotal role in decimation of thyme active ingredients. Moreover, due to its size and interaction with the FcRn mediated recycling pathway, albumin has an extraordinarily extended circulatory half-life. This inherent property of albumin could be harnessed to increase the circulatory half-life of drug molecules. Given these circumstances, implementation of half-life extension strategies like albumin binding has garnered a lot of attention. Based on our results, the drug molecules like thyme active ingredients which interact with albumin protein could have an improved circulatory half-life as a consequence of albumin binding [41].

The interaction between the drug and the albumin molecule could block the binding site of the drug with endogenous and exogenous molecules. This phenomena in turn could bring about novel properties or side effects which could result in altered pharmacodynamics and pharmacokinetic properties of the drug [9, 42].
are various studies that the structural properties and the interactions of the albumin with other plasma proteins and different endogenous and exogenous molecules are investigated [43, 44]. The albumin binding is not necessarily associated with undesirable consequences. On the contrary, albumin could act as a drug delivery agent to alleviate the availability of the drug molecules for the target cells. However, strong binding of the drug molecule to the albumin with high binding energy would end with reduced amounts of free drug, while weak binding would end with reduced drug half-life and ineffective distribution. The albumin structure has been proposed to be comprised of three main domains. Sudlow et al. have primarily suggested that albumin contains two drug binding sites which were marked as drug binding site 1 and 2 [6], while further structural analyses have introduced drug binding site 3, multi-metal binding site and five fatty acid binding sites [5, 45–48]. Our results indicate that p-cymene and linalool bind to drug binding site 3, while the thymol and carvacrol molecules bind to the fatty acid binding site 5 of the albumin structure. Although according to their Lipinski-type properties some of these molecules have hydrogen bond donor and acceptor atoms, none of the active ingredients formed hydrogen bonds. This could be due to lack of suitable hydrogen bond donor or acceptor at vicinity of the interaction site. Due to their vicinity to the tryptophan 214 within the drug binding site 1 of the albumin molecule, p-cymene and linalool binding is anticipated to change the structural properties of albumin and its florescence. The studies conducted by Kanakiks

| Common name | ZINC ID and 2D structure | Molecular weight (Da) | Hydrogen bond donors | Hydrogen bond acceptors | Topological polar surface area | Rotatable bonds |
|-------------|-------------------------|----------------------|----------------------|-------------------------|-----------------------------|----------------|
| p-Cymene    | ZINC00968246            | 134.22               | 0                    | 0                       | 0                           | 1              |
| Linalool    | ZINC01529819            | 154.25               | 1                    | 1                       | 20.23                       | 4              |
| Thymol      | ZINC00967597            | 150.22               | 1                    | 1                       | 20.23                       | 1              |
| Carvacrol   | ZINC00967563            | 150.22               | 1                    | 1                       | 20.23                       | 1              |
et al. [49] and Xie et al. [50] have demonstrated that binding to tryptophan 214 of the albumin molecule or its proximity would decrease albumin florescence. Moreover, structural composition of the albumin molecule would be changed regarding its alpha helix and beta sheet content [51, 52]. Since the linalool molecule is in direct interaction with tryptophan 214, the changes are expected to be more tangible.

The albumin drug binding site 3 is capable of accommodating various ligands. Thus, diverse compounds like p-cymene and linalool could be found within this hydrophobic site. The bound p-cymene and linalool would affect the binding properties of other probable ligands and consequently would change their pharmacokinetics and pharmacodynamics. High structural similarity between the thymol and carvacrol and the presence of a polar oxygen in their respective molecules, makes
them to share similar binding sites (fatty acid binding site 5). The oxygen position within the structure of these compounds does not exert a drastic change in their location. However, the existence of this oxygen atom distinguishes them from the p-cymene and linalool. In comparison to drug binding site 3 the fatty acid binding site 5 is more hydrophilic and the compounds harboring an oxygen atom favors fatty acid binding site 5 for interaction. This property could be in part rooted in the electronegative nature of oxygen atom which draws the electron cloud of the aromatic ring towards itself and consequently result in higher polarity and lower hydrophobicity. It should be noted that the albumin binding sites have the capacity to accommodate only one molecule of each compound.

The albumin binding is one of the imperative characteristics of therapeutic components which enter the blood stream. The drug could be divided into two fractions within the context of blood proteins. The unbound fraction is the free drug which actually exerts the respective functions of the drug, while the bound fraction is the portion in interaction with blood proteins. The bound portion acts as a reservoir or depot to maintain the equilibrium between the bound and unbound portions. The unbound portion is susceptible to be metabolized and/or excreted from the body. Therefore, the bound portion plays a pivotal role to slowly release the drug as the unbound form [8, 41, 43, 53]. It is apparent that numerous studies have been conducted to study the binding properties of the albumin to various compounds [43]. In this regard, the Sudlow’s site I and Sudlow’s site

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Figure 4: 2d Interaction plots.
The ligand molecules (carvacrol, linalool, p-cymene and thymol) are placed in the center of each plot, while the interacting residues of the albumin molecule are around the ligand. All of the residues formed hydrophobic interactions.
II are among the most important sites where the albumin binding to various drug molecules takes place. Warfarin (an anti-coagulant drug) and propionic acid derivatives like ibuprofen and ketoprofen (non-steroidal anti-inflammatory agents) are respectively deemed as stereotypical ligands of Sudlow’s site I and Sudlow’s site II (reviewed in [54]). Deeb et al. [38] have performed an in silico study to elucidate the observed high ligand-promiscuity of albumin in its binding sites I and II. They have provided evidences that conformational adjustments of the protein structure within these sites in conjunction with ligand conformational adaptation mainly contribute to the interactions of drugs with albumin. Warfarin and ketoprofen were investigated as the representative drugs interacting with albumin’s Sudlow’s site I and Sudlow’s site II, respectively. The obtained binding energies between the albumin protein and Warfarin and ketoprofen molecules were presented through a five nano-second MD simulation. These binding energies have been used as reference energies to confirm our results. Intriguingly, the results obtained by Deeb et al. (binding energy about −7 Kcal/mol) are in line with our results (binding energy about −7 Kcal/mol) which indicates the reliability our results.

Taken together, it could be concluded that albumin as the most abundant protein of the serum could act as the bio-distributor of thyme active ingredients. This would be of great significance to exert the desired therapeutic effects. Given the natural presence of albumin within the serum, the thyme active ingredients do not need a designed bio-carrier to be decimated throughout the body or the target destinations.

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References

1. Wishart DS. Improving early drug discovery through ADME modeling. Drugs R D 2007;8:349–62.
2. Tayman C, Rayyan M, Allegaert K. Neonatal pharmacology: extensive interindividual variability despite limited size. J Pediatr Pharmacol Ther 2011;16:170–84.
3. Peters Jr T. All about albumin: biochemistry, genetics, and medical applications. Academic Press. 0080527043. 1995.
4. Routledge P. The plasma protein binding of basic drugs. Br J Clin Pharmacol 1986;22:499–506.
5. He XM, Carter DC. Atomic structure and chemistry of human serum albumin. Nature 1992;358:209–15.
6. Sudlow G, Birkett D, Wade D. The characterization of two specific drug binding sites on human serum albumin. Mol Pharmacol 1975;11:824–32.
7. Majorek KA, Porebski PJ, Dayal A, Zimmerman MD, Jablonska K, Stewart AJ, et al. Structural and immunologic characterization of bovine, horse, and rabbit serum albumins. Mol Immunol 2012;52:374–82.
8. Ghumar J, Zunszain PA, Petitpas I, Bhattacharya AA, Otagiri M, Curry S. Structural basis of the drug-binding specificity of human serum albumin. J Mol Biol 2005;353:38–52.
9. Sulkowska A. Interaction of drugs with bovine and human serum albumin. J Mol Struct 2002;614:227–32.
10. Sulkowska A, Równicka J, Bojko B, Sulkowski W. Interaction of anticancer drugs with human and bovine serum albumin. J Mol Struct 2003;651–653:133–40.
11. Jaldappagari S, Balakrishnan S, Hegde AH, Teradal NL, Narayan PS. Interactions of polyphenols with plasma proteins: insights from analytical techniques. Curr Drug Metab 2013;14:456–73.
12. Hudaib M, Speroni E, Di Pietra AM, Cavrini V. GC/MS evaluation of thyme (Thymus vulgaris L.) oil composition and variations during the vegetative cycle. J Pharm Biomed Anal 2002;29:691–700.
13. Lee S-J, Umano K, Shibamoto T, Leed K-G. Identification of volatile components in basil (Ocimum basilicum L.) and thyme leaves (Thymus vulgaris L.) and their antioxidant properties. Food Chem 2005;91:131–7.
14. Rota MC, Herrera A, Martínez RM, Sotomayor JA, Jordán MJ. Antimicrobial activity and chemical composition of Thymus vulgaris, Thymus zygis and Thymus hyemalis essential oils. Food Control 2008;19:681–7.
15. Fachini-Queiroz FC, Kummer R, Estevao-Silva CF, Carvalho MD, Cunha JM, Grespan R, et al. Effects of thymol and carvacrol, constituents of Thymus vulgaris L. essential oil, on the inflammatory response. Evid Based Complement Alternat Med 2012;2012:657026.
16. Bónai A, Dalle Zotte A, Kamerer L, Kovacs M. Dietary supplementation of spirulina (arthrosira platensis) and Thyme (Thymus Vulgaris). Part 2: effect on gastrointestinal growth, caecal microbiota and fermentation in rabbits. In: World Rabbit Science Association Proceedings 10th World Rabbit Congress-September, 2012:3–6.
17. Parsaei P, Bahmani M, Naghdi M, Asadi-Samani M, Rafieian-Kopaei M. A review of therapeutic and pharmacological effects of thymol. Der Pharm Lett 2016;8:150–4.
18. Giordani R, Regli P, Kaloustian J, Mikail C, Abou L, Portugal H. Antifungal effect of various essential oils against Candida albicans. Potentiation of antifungal action of amphotericin B by essential oil from Thymus vulgaris. Phytother Res 2004;18:990–5.
19. Taherian AA, Babaei M, Vafaee AA, Jarrahi M, Jadidi M, Sadeghi H. Antinociceptive effects of hydroalcoholic extract of Thymus vulgaris. Pak J Pharm Sci 2009;22:83–9.
20. Khalili S, Rahbar MR, Dezfulian MH, Jahangiri A. In silico analyses of Wilms’ tumor protein to designing a novel multi-epitope DNA vaccine against cancer. J Theor Biol 2015;379:66–78.
21. Jahangiri A, Rasooli I, Gargari SI, Owlia P, Rahbar MR, Amani J, et al. An in silico DNA vaccine against Listeria monocytogenes. Vaccine 2011;29:6948–58.
22. Khalili S, Jahangiri A, Borna H, Ahmadi Zanoos K, Amani J. Computational vaccinology and epitope vaccine design by immuno-informatics. Acta Microbiol Immunol Hung 2014;61:285–307.
23. Mohammadpour H, Khalili S, Hashemi ZS. Kremen is beyond a subsidiary co-receptor of Wnt signaling: an in silico validation. Turkish J Biol 2015;39:501–10.

24. Mohammadpour H, Pourfathollah AA, Zarif MN, Khalili S. Key role of Dkk3 protein in inhibition of cancer cell proliferation: an in silico identification. J Theor Biol 2016;393:98–104.

25. Jahangiri A, Rasooli I, Rahbar MR, Khalili S, Amani J, Ahmadi Zanoos K. Precise detection of L. monocytogenes hitting its highly conserved region possessing several specific antibody binding sites. J Theor Biol 2012;305:15–23.

26. Khalili S, Mohammadpour H, Borough MS, Kokhaei P. ILP-2 modeling and virtual screening of an FDA-approved library: a possible anticancer therapy. Turk J Med Sci 2016;46:1135–43.

27. Venkateswarlu D. Structural insights into the interaction of blood coagulation co-factor VIIa with factor IXa: A computational protein–protein docking and molecular dynamics refinement study. Biochim Biophys Res Commun 2014;452:408–14.

28. Khalili S, Jahangiri A, Hashemi ZS, Khalesi B, Mard-Soltani M, Amani J. Structural Pierce into molecular mechanism underlying Clostridium perfringens Epsilon toxin function. Toxicol 2017;127:90–9.

29. Khalili S, Rasaei M, Bamdad T. 3D structure of DKK1 indicates its involvement in both canonical and non-canonical Wnt pathways. Mol Biol 2017;51:155–66.

30. Jahangiri A, Rasooli I, Owlia P, Fooladi AA, Salimian J. In silico design of an immunogen against Acinetotber bacter baumanni based on a novel model for native structure of outer membrane protein A. Microb Pathog 2017;105:201–10.

31. Liu J, Yue Y, Wang J, Yan X, Liu R, Sun Y, et al. Study of interaction between human serum albumin and three phenanthridine derivatives: fluorescence spectroscopy and computational approach. Spectrochim Acta A 1 Mol Biomol Spectros 2015;145:473–81.

32. Yue Y, Liu J, Liu R, Sun Y, Li X, Fan J. The binding affinity of phthalate plasticizers-protein revealed by spectroscopic techniques and molecular modeling. Food Chem Toxicol 2014;71:244–53.

33. Yue Y, Liu J, Liu R, Dong Q, Fan J. Binding of helicid to human serum albumin: a hybrid spectroscopic approach and conformational study. Spectrochim Acta A 1 Mol Biomol Spectros 2014;124:46–51.

34. Yue Y, Dong Q, Zhang Y, Li X, Yan X, Sun Y, et al. Synthesis of imidazole derivatives and the spectral characterization of the binding properties towards human serum albumin. Spectrochim Acta A 1 Mol Biomol Spectros 2016;153:688–703.

35. Yue Y, Sun Y, Dong Q, Liu R, Yan X, Zhang Y, et al. Interaction of human serum albumin with novel imidazole derivatives studied by spectroscopy and molecular docking. Luminescence 2016;31:671–81.

36. Yue Y, Sun Y, Yan X, Liu J, Zhao S, Zhang J. Evaluation of the binding of perfluorinated compound to pepsin: Spectroscopic analysis and molecular docking. Chemosphere 2016;161:475–81.

37. Prentis R, Lis Y, Walker S. Pharmaceutical innovation by the seven UK-owned pharmaceutical companies (1964–1985). Br J Clin Pharmacol 1988;25:387–96.

38. Deeb O, Rosales-Hernández MC, Gómez-Castro C, Garduño-Juárez R, Correa-Basurto J. Exploration of human serum albumin binding sites by docking and molecular dynamics flexible ligand–protein interactions. Biopolymers 2010;93:161–70.

39. Paal K, Shkarupin A, Beckford L. Paclitaxel binding to human serum albumin—Automated docking studies. Bioorg Med Chem 2007;15:1323–9.

40. Bertucci C, Domenici E. Reversible and covalent binding of drugs to human serum albumin: methodological approaches and physiological relevance. Curr Med Chem 2002;9:1463–81.

41. Sleep D, Cameron J, Evans LR. Albumin as a versatile platform for drug half-life extension. Biochem Biophys Acta 2013;1830:5526–34.

42. Cohen S, Margalit R. Binding of porphyrin to human serum albumin. Structure—activity relationships. Biochem J 1990;270:325–30.

43. Yang F, Zhang Y, Liang H. Interactive association of drugs binding to human serum albumin. Int J Mol Sci 2014;15:3585–95.

44. Li Y, Wang Q, He J, Yan J, Li H. Fluorescence spectroscopy and docking study in two flavonoids, isolated tectoridin and its aglycone tectorigenin, interacting with human serum albumin: a comparison study. Luminescence 2016;31:38–46.

45. Dockal M, Carter DC, Rüker F. The three recombinant domains of human serum albumin structural characterization and ligand binding properties. J Biol Chem 1999;274:29303–10.

46. Curry S, Mandelkow H, Brick P, Franks N. Crystal structure of human serum albumin complexed with fatty acid reveals an asymmetric distribution of binding sites. Nat Struct Mol Biol 1998;5:827–35.

47. Bhattacharya AA, Grüne T, Curry S. Crystallographic analysis reveals common modes of binding of medium and long-chain fatty acids to human serum albumin. J Mol Biol 2000;303:721–32.

48. Curry S. Lessons from the crystallographic analysis of small molecule binding to human serum albumin. Drug Metab Pharmacokinet 2009;24:342–57.

49. Kanakis CD, Tarantilis PA, Tajmir-Riahi HA, Polissiou MG. Crocetin, dimethylcrocetin, and safranal bind human serum albumin: stability and antioxidative properties. J Agric Food Chem 2007;55:970–7.

50. Xie M-X, Long M, Liu Y, Qin C, Wang Y-D. Characterization of the interaction between human serum albumin and morin. Biochim Biophys Acta 2006;1760:1184–91.

51. Zsila B, Bíkádi Z, Simony M. Induced chirality upon crocetin binding to human serum albumin: origin and nature. Tetrahedron Asymmetr 2001;12:3125–37.

52. Zsila B, Bíkádi Z, Simony M. Further insight into the molecular basis of carotenoid–albumin interactions: circular dichroism and electronic absorption study on different crocetin–albumin complexes. Tetrahedron Asymmetr 2002;13:273–83.

53. Kratz F. Albumin as a drug carrier: design of prodrugs, drug conjugates and nanoparticles. J Control Release 2008;132:171–83.

54. Fasano M, Curry S, Terreno E, Galliano M, Fanali G, Narciso P, et al. The extraordinary ligand binding properties of human serum albumin. IUBMB life 2005;57:787–96.