Supporting information

Albumin-based transport of non-steroidal anti-inflammatory drugs in mammalian blood plasma

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Table S1. RMSD values between the aligned Cα atoms of ESA-NSAIDs complexes and ligand-free ESA and HSA molecules.

	Etodolac	**Nabumetone**	**6-MNA**	**Ibuprofen**	**Ketoprofen**	**Ligand free ESA (3V08)**	**Ligand free ESA (5HOZ)***	**Ligand free HSA (4K2C)**
Etodolac	-	0.4	0.4	0.8	0.6	1.4	0.7	1.7
Nabumetone	0.4	-	0.3	0.8	0.6	1.4	0.8	1.7
6-MNA	0.4	0.3	-	0.8	0.7	1.3	0.5	1.6
Ibuprofen	0.8	0.8	0.8	-	0.8	1.5	1.0	1.8
Ketoprofen	0.6	0.6	0.7	0.8	-	1.5	1.0	1.9
Ligand-free ESA (3V08)	1.4	1.4	1.3	1.5	1.5	-	1.1	1.7
Ligand-free ESA (5HOZ)*	0.7	0.8	0.5	1.0	1.0	1.1	-	1.5
Ligand-free HSA (4K2C)	1.7	1.7	1.6	1.8	1.9	1.7	1.5	-

*Structure of ligand-free ESA (5HOZ) was obtained from similar conditions as structures presented in this publication.

Table S2. Binding parameters reported for HSA and NSAIDs.

Drug	**n_1**	**K_1 (μM^{-1})**	**n_2**	**K_2 (μM^{-1})**
(*R*)-etodolac[1]	0.53 ± 0.11	0.19 ± 0.04	3.37 ± 0.97	0.0043 ± 0.0024
(*S*)-etodolac[1]	1.15 ± 0.10	0.20 ± 0.02	1.63 ± 0.48	0.0048 ± 0.0035
Ibuprofen[2]	0.8-1.0	1.89-2.73	6.17-7.06	0.0168-0.0225
Ketoprofen[3]	1	2-2.5	4	0.04
6-MNA[4]	1	8.4	-	-

Table S3. List of all known structures of SA complexes with FDA-approved drugs.

Ligand name	Drug's trade name	Drug's classification	DrugBank ID	PDB ID / Organism
Halothane	Fluothane	Anesthetic	DB01159	1E7B,[5] 1E7C[5] / Human
Lidocaine	Xylocaine	Anesthetic	DB00281	3JQZ[6] / Human
Propofol	Diprivan	Anesthetic	DB00818	1E7A[5] / Human
Fusidic acid	Fucidin	Antibiotic	DB02703	2VUF[7] / Human
Bicalutamide	Casodex	Anti-cancer drug	DB01128	4LA0[8] / Human
Cisplatin	Platinol	Anti-cancer drug	DB00515	4S1Y[9] / Human
Etoposide	Etopophos	Anti-cancer drug	DB00773	4LB9[8] / Human
Teniposide	Vumon	Anti-cancer drug	DB00444	4L9Q[8] / Human
Warfarin	Coumadin	Anticoagulant	DB00682	2BXD,[10] 1H9Z,[11] 1HA2[11] / Human
Aripiprazole	Abilify	Antipsychotic	DB01238	6A7P[12] / Human
Amantadine	Gocovri	Antiviral drug	DB00915	3UIV[13] / Human
Diazepam	Valium	Drug for anxiety disorders treatment	DB00829	2BXF[10] / Human
Zidovudine	Retrovir	Antiretroviral drug	DB00495	3B9L,[14] 3B9M[14] / Human
Idarubicin	Idamycin	Drug for leukemia treatment	DB01177	4LB2[8] / Human
Phenylbutyric acid	Ammonaps	Drug for urea cycle disorders treatment	DB06819	5YOQ[15] / Human
Cetirizine	Zyrtec	Histamine antagonist	DB00341	5DQF[16] / Horse
Aspirin	Bayer® Aspirin	Non-steroidal anti-inflammatory drug	DB00945	3B9M,[14] 2I30,[17] 2I2Z[17] / Human
Azapropazone	Rheumox	Non-steroidal anti-inflammatory drug	DB07402	2BXI,[10] 2BX8,[10] 2BXK[10] / Human
Diclofenac	Cambia	Non-steroidal anti-inflammatory drug	DB00586	4Z69[18] / Human 4ZBQ,[19] 4ZBR,[19] 5DBY[19] / Horse
Diflunisal	Dolobid	Non-steroidal anti-inflammatory drug	DB00861	2BXE[10] / Human
Etodolac	Lodine	Non-steroidal anti-inflammatory drug	DB00749	**5V0V** / Horse
Ibuprofen	Advil	Non-steroidal anti-inflammatory drug	DB01050	2BXG[10] / Human, **6U4X,** 6OCI[20] / Horse
Indomethacin	Indocin	Non-steroidal anti-inflammatory drug	DB00328	2BXK,[10] 2BXM,[10] 2BXQ[10] / Human
Ketoprofen	Orudis	Non-steroidal anti-inflammatory drug	DB01009	**6U4R** / Horse, 6QS9[21] / Bovine 6OCK[20] / Leporine
Nabumetone / 6-MNA	Relafen	Non-steroidal anti-inflammatory drug	DB00461	**6CI6, 6U5A** / Horse
Naproxen	Aleve	Non-steroidal anti-inflammatory drug	DB00788	2VDB[22] / Human, 4ZBR,[19] 5DBY[19] / Horse 4OR0[23] / Bovine 4PO0[23] / Leporine
Oxyphenbutazone	Withdrawn	Non-steroidal anti-inflammatory drug	DB03585	2BXB,[10] 2BXO[10] / Human
Phenylbutazone	Butazolidine	Non-steroidal anti-inflammatory drug	DB00812	2BXC,[10] 2BXP,[10] 2BXQ[10] / Human
Suprofen	Withdrawn	Non-steroidal anti-inflammatory drug	DB00870	6OCJ[20] / Horse 6OCL[20] / Leporine
Iodipamide	Sinografin	Radiographic contrast media	DB04711	2BXN[10] / Human
Testosterone	Androderm	Steroid hormone	DB00624	6MDQ[24] / Horse
Levothyroxine	Eltroxin	Thyroid hormone	DB00451	1HK1,[25] 1HK2,[25] 1HK3,[25] 1HK4,[25] 1HK5[25] / Human

Table S4. List of residues comprising NSAIDs' binding sites that may be affected by glycation.

Drug site	Residues in HSA known to be glycated	Analogous residues in ESA	Drugs whose binding may be affected by glycation
DS1	Lys195, Lys199, Arg257	Lys194, Lys198, Arg256	(*S*)-etodolac
DS2	Arg410, Lys414, Arg485	Arg409, Lys413, Arg484	(*S*)-ibuprofen, nabumetone, 6-MNA
DS3	Arg114, Arg145, Arg186	Lys114, Arg144, Lys185	(*R*)-etodolac
DS4	Lys20	Lys20	(*S*)-ibuprofen, (*S*)-ketoprofen
DS6	Lys541, Lys545	Lys540, Lys544	(*S*)-ketoprofen, nabumetone, 6-MNA
DS7	Arg209, Lys212, Lys351	Arg208, Lys211, Lys350	(*S*)- and (*R*)-etodolac, 6-MNA
DS10	Lys73	Lys73	(*S*)-ketoprofen

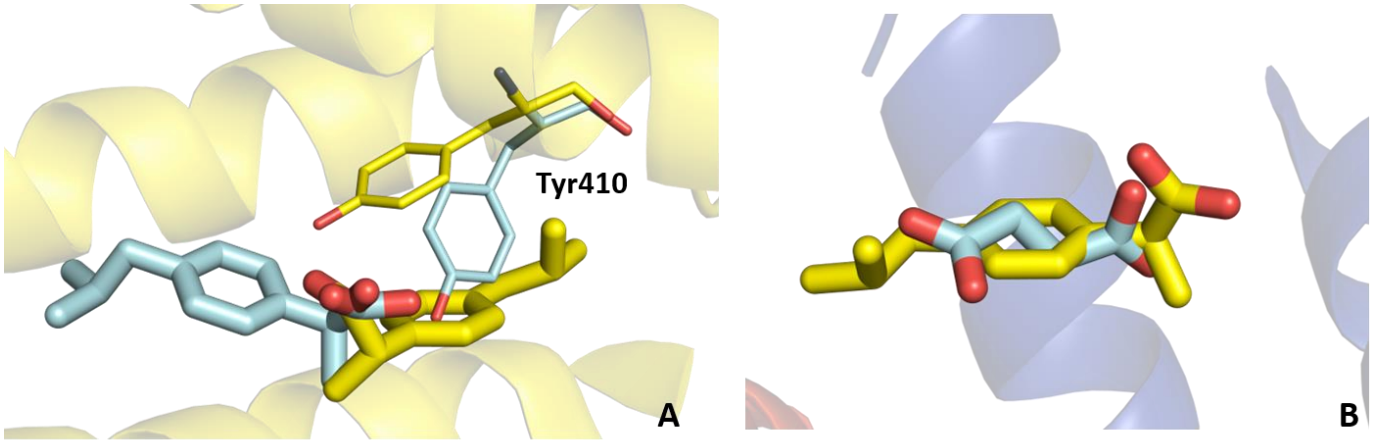

Figure S1. Superposition of the crystal structures of ESA complexes with ibuprofen obtained from different crystallization conditions. For ligands in yellow – ESA was crystallized with the crystallization conditions composed of 0.2 M lithium sulfate, 1.8-2.4 M ammonium sulfate, and 0.1 M Tris buffer pH 7.4 (PDB ID: 6U4X); for ligands in cyan - ESA was crystallized with the crystallization conditions composed of 75% Tacsimate at pH 6.0 (PDB ID: 6OCI). A) Drug site 2. Two different binding modes of ibuprofen and conformations of Tyr410. B) Drug site 4. Succinic acid (shown in cyan) binds at drug site 4 (PDB ID: 6OCI) and may prevent binding of ibuprofen at this site, which was observed in the structure reported in this study (PDB ID: 6U4X). At drug site 7, electron density for ibuprofen was observed only in PDB ID: 6OCI ESA structure; in our ESA-ibuprofen structure (PDB ID: 6U4X), drug site 7 is unoccupied.

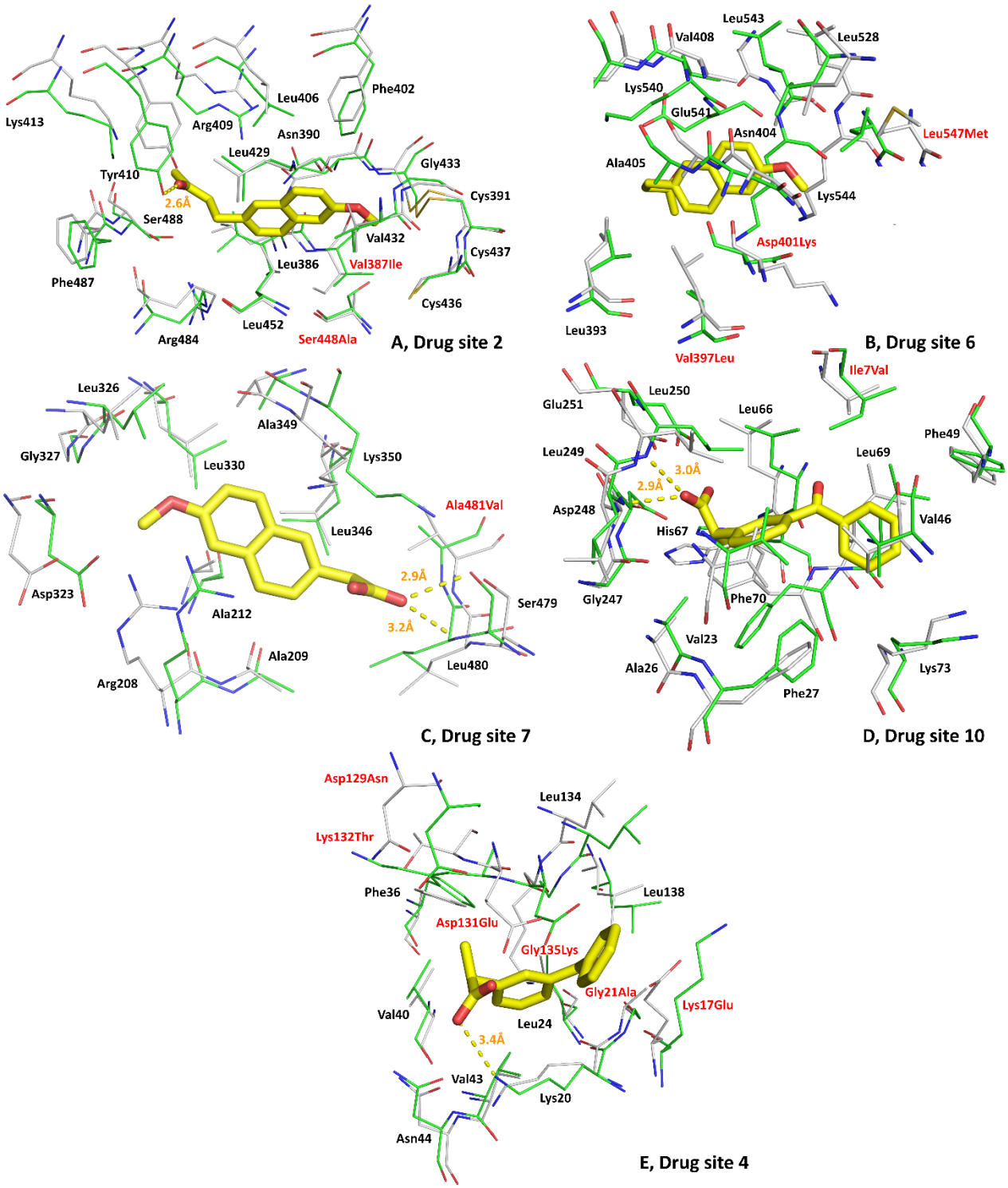

Figure S2. Superposition of NSAIDs binding sites in ESA and analogous sites in HSA (PDB ID: 4K2C). A-B) nabumetone (PDB ID: 6CI6); C) 6-MNA (PDB ID: 6U5A); D-E) ketoprofen (PDB ID: 6U4R). Carbon atoms in ESA and HSA are shown in green and gray, respectively. Oxygen atoms are shown in red, nitrogen in blue; NSAID molecules are shown with carbon atoms in yellow. Residue numbers correspond to positions in ESA; naming scheme is as follows: residue from ESA, residue number, residue from HSA (if different). Models can be compared interactively at https://molstack.bioreproducibility.org/p/dupc/ .

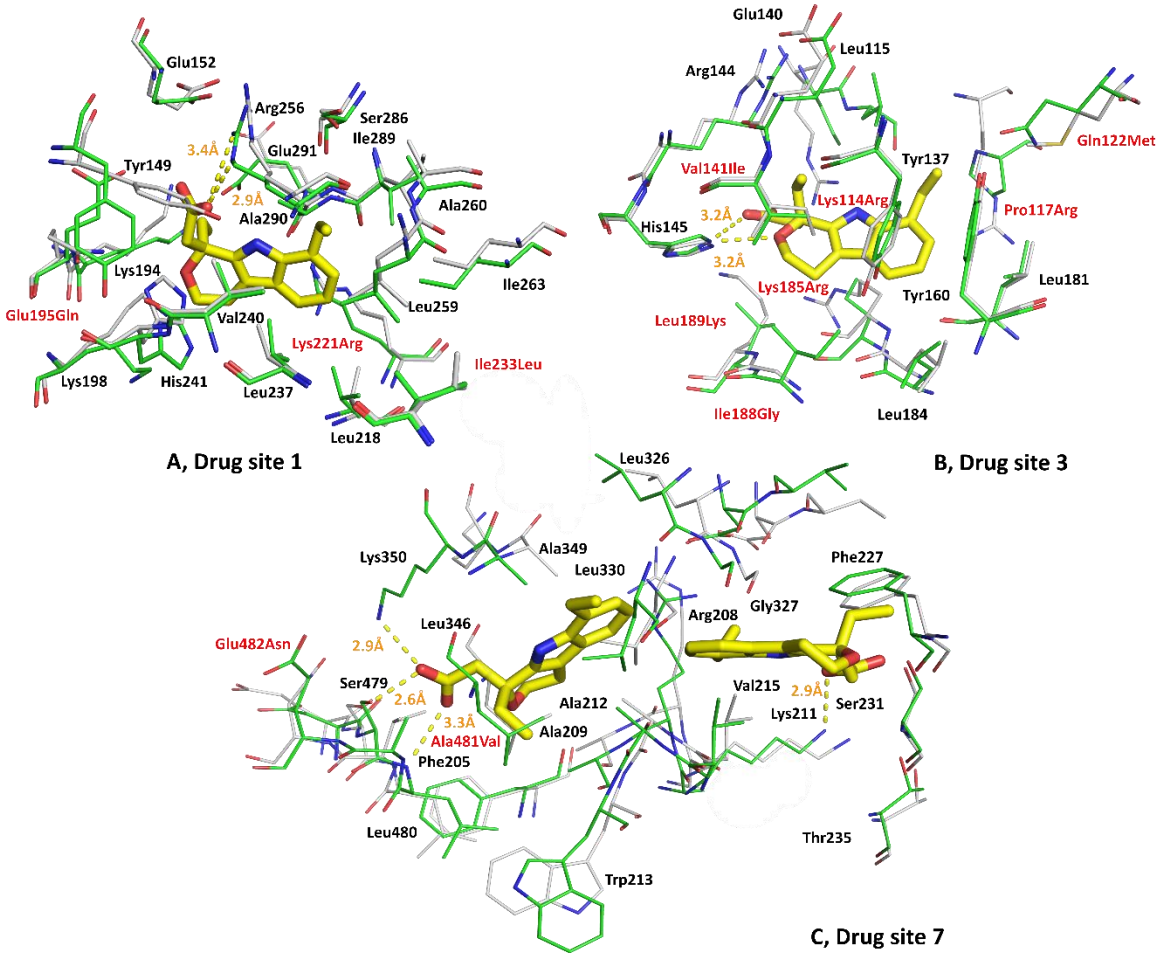

Figure S3. Comparison of residues involved in etodolac binding in ESA (PDB ID: 5V0V) and residues comprising the analogous sites in HSA (PDB ID: 4K2C). Carbon atoms in ESA and HSA are shown in green and gray, respectively. Oxygen atoms are shown in red, nitrogen in blue; etodolac molecules are shown with carbon atoms in yellow. Residue numbers correspond to positions in ESA; naming scheme is as follows: residue from ESA, residue number, residue from HSA (if different). Models can be compared interactively at https://molstack.bioreproducibility.org/p/dupc/.

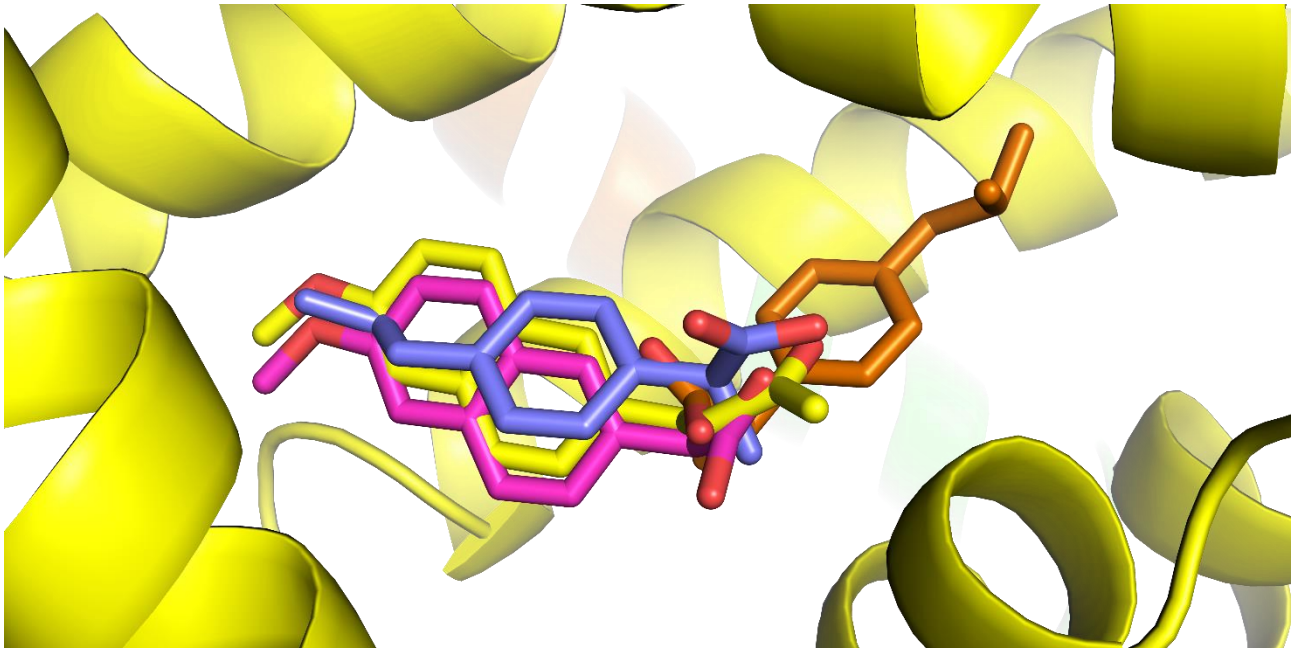

Figure S4. Drug site 2; superposition of ESA complexes with ibuprofen (PDB ID: 6U4X), nabumetone (PDB ID: 6CI6), and 6-MNA (PDB ID: 6U5A) and HSA complex with ibuprofen (PDB ID:2BXG). Orange – ibuprofen in ESA; yellow – nabumetone; magenta – 6-MNA; blue – ibuprofen in HSA.

(1) Mignot, I.; Presle, N.; Lapicque, F.; Monot, C.; Dropsy, R.; Netter, P. Albumin Binding Sites for Etodolac Enantiomers. *Chirality* **1996**, *8* (3), 271–280.

(2) Whitlam, J. B.; Crooks, M. J.; Brown, K. F.; Pedersen, P. V. Binding of Nonsteroidal Anti-Inflammatory Agents to Proteins--I. Ibuprofen-Serum Albumin Interaction. *Biochem. Pharmacol.* **1979**, *28* (5), 675–678.

(3) Rahman, M. H.; Yamasaki, K.; Shin, Y. H.; Lin, C. C.; Otagiri, M. Characterization of High Affinity Binding Sites of Non-Steroidal Anti-Inflammatory Drugs with Respect to Site-Specific Probes on Human Serum Albumin. *Biol. Pharm. Bull.* **1993**, *16* (11), 1169–1174.

(4) Ogata, K.; Takamura, N.; Tokunaga, J.; Ikeda, T.; Setoguchi, N.; Tanda, K.; Yamasaki, T.; Nishio, T.; Kawai, K. A Novel Injection Strategy of Flurbiprofen Axetil by Inhibiting Protein Binding with 6-Methoxy-2-Naphthylacetic Acid. *Eur. J. Drug Metab. Pharmacokinet.* **2016**, *41* (2), 179–186.

(5) Bhattacharya, A. A.; Curry, S.; Franks, N. P. Binding of the General Anesthetics Propofol and Halothane to Human Serum Albumin: High Resolution Crystal Structures. *J. Biol. Chem.* **2000**, *275* (49), 38731–38738.

(6) Hein, K. L.; Kragh-Hansen, U.; Morth, J. P.; Jeppesen, M. D.; Otzen, D.; Møller, J. V; Nissen, P. Crystallographic Analysis Reveals a Unique Lidocaine Binding Site on Human Serum Albumin. *J. Struct. Biol.* **2010**, *171* (3), 353–360.

(7) Zunszain, P. A.; Ghuman, J.; McDonagh, A. F.; Curry, S. Crystallographic Analysis of Human Serum Albumin Complexed with 4Z,15E-Bilirubin-IXalpha. *J. Mol. Biol.* **2008**, *381* (2), 394–406.

(8) Wang, Z.; Ho, J. X.; Ruble, J. R.; Rose, J.; Rüker, F.; Ellenburg, M.; Murphy, R.; Click, J.; Soistman, E.; Wilkerson, L.; Carter, D. C. Structural Studies of Several Clinically Important Oncology Drugs in Complex with Human Serum Albumin. *Biochim. Biophys. Acta* **2013**, *1830* (12), 5356–5374.

(9) Ferraro, G.; Massai, L.; Messori, L.; Merlino, A. Cisplatin Binding to Human Serum Albumin: A Structural Study. *Chem. Commun. (Camb).* **2015**, *51* (46), 9436–9439.

(10) Ghuman, J.; Zunszain, P. A.; Petitpas, I.; Bhattacharya, A. A.; Otagiri, M.; Curry, S. Structural Basis of the Drug-Binding Specificity of Human Serum Albumin. *J. Mol. Biol.* **2005**, *353* (1), 38–52.

(11) Petitpas, I.; Bhattacharya, A. A.; Twine, S.; East, M.; Curry, S. Crystal Structure Analysis of Warfarin Binding to Human Serum Albumin: Anatomy of Drug Site I. *J. Biol. Chem.* **2001**, *276* (25), 22804–22809.

(12) Sakurama, K.; Kawai, A.; Tuan Giam Chuang, V.; Kanamori, Y.; Osa, M.; Taguchi, K.; Seo, H.; Maruyama, T.; Imoto, S.; Yamasaki, K.; Otagiri, M. Analysis of the Binding of Aripiprazole to Human Serum Albumin: The Importance of a Chloro-Group in the Chemical Structure. *ACS omega* **2018**, *3* (10), 13790–13797.

(13) Yang, F.; Lee, P.; Ma, Z.; Ma, L.; Yang, G.; Wu, X.; Liang, H. Regulation of Amantadine Hydrochloride Binding with IIA Subdomain of Human Serum Albumin by Fatty Acid Chains. *J. Pharm. Sci.* **2013**, *102* (1), 84–92.

(14) Zhu, L.; Yang, F.; Chen, L.; Meehan, E. J.; Huang, M. A New Drug Binding Subsite on Human Serum Albumin and Drug-Drug Interaction Studied by X-Ray Crystallography. *J. Struct. Biol.* **2008**, *162* (1), 40–49.

(15) Kawai, A.; Yamasaki, K.; Enokida, T.; Miyamoto, S.; Otagiri, M. Crystal Structure Analysis of Human Serum Albumin Complexed with Sodium 4-Phenylbutyrate. *Biochem. Biophys. reports* **2018**, *13*, 78–82.

(16) Handing, K. B.; Shabalin, I. G.; Szlachta, K.; Majorek, K. A.; Minor, W. Crystal Structure of Equine

Serum Albumin in Complex with Cetirizine Reveals a Novel Drug Binding Site. *Mol. Immunol.* **2016**, *71*, 143–151.

(17) Yang, F.; Bian, C.; Zhu, L.; Zhao, G.; Huang, Z.; Huang, M. Effect of Human Serum Albumin on Drug Metabolism: Structural Evidence of Esterase Activity of Human Serum Albumin. *J. Struct. Biol.* **2007**, *157* (2), 348–355.

(18) Zhang, Y.; Lee, P.; Liang, S.; Zhou, Z.; Wu, X.; Yang, F.; Liang, H. Structural Basis of Non-Steroidal Anti-Inflammatory Drug Diclofenac Binding to Human Serum Albumin. *Chem. Biol. Drug Des.* **2015**, *86* (5), 1178–1184.

(19) Sekula, B.; Bujacz, A. Structural Insights into the Competitive Binding of Diclofenac and Naproxen by Equine Serum Albumin. *J. Med. Chem.* **2016**, *59* (1), 82–89.

(20) Zielinski, K.; Sekula, B.; Bujacz, A.; Szymczak, I. Structural Investigations of Stereoselective Profen Binding by Equine and Leporine Serum Albumins. *Chirality* **2020**, *32* (3), 334–344.

(21) Castagna, R.; Donini, S.; Colnago, P.; Serafini, A.; Parisini, E.; Bertarelli, C. Biohybrid Electrospun Membrane for the Filtration of Ketoprofen Drug from Water. *ACS omega* **2019**, *4* (8), 13270–13278.

(22) Lejon, S.; Cramer, J. F.; Nordberg, P. Structural Basis for the Binding of Naproxen to Human Serum Albumin in the Presence of Fatty Acids and the GA Module. *Acta Crystallogr. Sect. F. Struct. Biol. Cryst. Commun.* **2008**, *64* (Pt 2), 64–69.

(23) Bujacz, A.; Zielinski, K.; Sekula, B. Structural Studies of Bovine, Equine, and Leporine Serum Albumin Complexes with Naproxen. *Proteins* **2014**, *82* (9), 2199–2208.

(24) Czub, M. P.; Venkataramany, B. S.; Majorek, K. A.; Handing, K. B.; Porebski, P. J.; Beeram, S. R.; Suh, K.; Woolfork, A. G.; Hage, D. S.; Shabalin, I. G.; Minor, W. Testosterone Meets Albumin - the Molecular Mechanism of Sex Hormone Transport by Serum Albumins. *Chem. Sci.* **2019**, *10* (6), 1607–1618.

(25) Petitpas, I.; Petersen, C. E.; Ha, C.; Bhattacharya, A. A.; Zunszain, P. A.; Ghuman, J.; Bhagavan, N. V; Curry, S. Structural Basis of Albumin-Thyroxine Interactions and Familial Dysalbuminemic Hyperthyroxinemia. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100* (11), 6440–6445.