Molecular Docking of 3-Methylindole-containing Drugs Binding into CYP3A4

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Abstract Drugs SPD-304(6,7-dimethyl-3-{[methyl-(2-{methyl-[1-(3-trifluoromethyl-phenyl)-1*H*-indol-3-ylmethyl]-amino}-ethyl)-amino]-methyl}-chromen-4-one) and zafirlukast contain a common structural element of 3-substituted indole moiety which closely relates to a dehydrogenated reaction catalyzed by cytochrome P450s(CYPs). It was reported that the dehydrogenation can produce a reactive electrophilic intermediate which cause toxicities and inactivate CYPs. Drug L-745,870(3-{[4-(4-chlorophenyl)piperazin-1-yl]-methyl}-1*H*-pyrrolo-2,3-*β*-pyridine) might have similar effect since it contains the same structural element. We used molecular docking approach combined with molecular dynamics(MD) simulation to model three-dimensional(3D) complex structures of SPD-304, zafirlukast and L-745,870 into CYP3A4, respectively. The results show that these three drugs can stably bind into the active site and the 3-methylene carbons of the drugs keep a reasonable reactive distance from the heme iron. The complex structure of SPD-304-CYP3A4 is in agreement with experimental data. For zafirlukast, the calculation results indicate that 3-methylene carbon might be the dehydrogenation reaction site. Docking model of L-745,870-CYP3A4 shows a potential possibility of L-745,870 dehydrogenated by CYP3A4 at 3-methylene carbon which is in agreement with experiment *in vivo*. In addition, residues in the phenylalanine cluster as well as S119 and R212 play a critical role in the ligands binding based on our calculations. The docking models could provide some clues to understand the metabolic mechanism of the drugs by CYP3A4.

Keywords CYP3A4; Molecular docking; Molecular dynamics(MD) simulation

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1 Introduction

Cytochrome P450s(CYPs) constitute a superfamily of heme-containing biotransformation enzymes involved in the oxidative metabolism of a wide variety of endogenous and exogenous compounds[1,2]. CYP3A4 as a major CYP isoform in human liver and intestine is responsible for the oxidative metabolism of half marketed drugs[3]. In this study, we have focused our attention on three drugs, SPD-304 (6,7-dimethyl-3-{[methyl-(2-{methyl-[1-(3-trifluoromethyl-phenyl)-1*H*-indol-3-ylmethyl]-amino}-ethyl)-amino]-methyl}-chromen-4-one), zafirlukast and L-745,870(3-{[4-(4-chlorophenyl) piperazin-1-yl]-methyl}-1*H*-pyrrolo-2,3-*β*-pyridine) which are designed for different targets but share a common structural element of 3-substituted indole moiety. SPD-304 is screened as an anti-TNF(tumor necrosis factor-*α*) drug[4]; Zafirlukast is a leukotriene receptor antagonist used for asthma treatment[5]; and L-745,870 is an antagonist for the dopamine D4 receptor and a potential drug compound for the treatment of amyotrophic lateral sclerosis(ALS)[6,7]. Sun *et al*.[8] reported that SPD-304 can be bioactivated by CYP3A4 through a dehydrogenation pathway which involves the abstraction of an initial hydrogen atom from 3-methylene group, producing a reactive electrophilic intermediate. The intermediate could cause toxicities and inactivate CYP3A4. Similar intermediates are also proved to exist in zafirlukast and L-745,870 metabolism process[9,10]. However, for zafirlukast dehydrogenated by CYP3A4, it is uncertain that 3-methylene carbon is the initial reaction site[9]; for L-745,870, it is experiment *in vivo* that was available, thus whether CYP3A4 takes part in the dehydrogenation needs to be further studied.

Computational approaches have been permeating all the aspects of drug discovery today[11], which provide a series of approaches, for instance, homology modeling, molecular dynamics(MD) simulations and molecular docking, to illustrate the function of proteins and the modes of substrates binding[12]. These methods have been successfully used in CYP systems and provided useful information for further studies[1]. We conducted molecular docking combined with MD simulations to identify the binding modes of zafirlukast and SPD-304 dehydrogenation by CYP3A4 and to seek the possibility of L-745,870 dehydrogenated by CYP3A4 from the computation.

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2 Materials and Methods

2.1 Docking Study

The initial structures of SPD-304, zafirlukast and L-745,870 were obtained from the DrugBank database[13], then optimized by means of conjugated gradient(CG) energy minimization until root mean square(RMS) gradient energy was lower than 0.4 kJ·mol^{-1}·nm^{-1}. Their structures are shown in Fig.1.

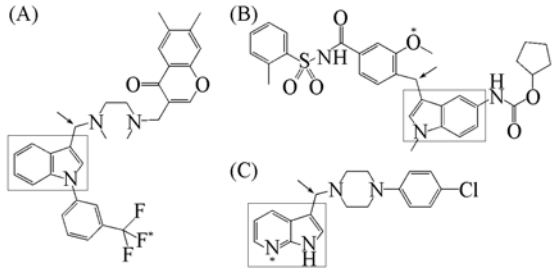

Fig.1 Structures of SPD-304(A), zafirlukast(B) and L-745,870(C)

Arrows point to the possible reaction sites being studied for each ligand. 3-Methylindole portion for each ligand is highlighted in a box and asterisks sign the possible H-bond acceptors.

The Affinity[14] module of InsightII was employed to dock the three ligands into CYP3A4, respectively. Affinity combination of Monte Carlo(MC) and Simulated Annealing(SA) method was used to search for the optimal orientation of ligand binding. This is a semi-flexible docking in that only the binding site atoms and ligand atoms are allowed to move during the docking process. The water molecules in crystal structures were reserved to model the solvent effect. The final ligand-protein complex structures were chosen by virtue of the criteria of interacting energy and geometrical matching quality.

2.2 Molecular Dynamics Simulations

To further consider flexibility of protein, MD simulation was conducted for each ligand-protein complex obtained from docking step. Complexes were gradually heated from 50 K to 300 K over 60 ps followed by the equilibration dynamics simulation at 300 K for 100 ps. Then 2 ns MD production runs were performed in the NPT ensemble at 101.3 kPa and 300 K with time step 1 fs and a nonbond interaction cutoff distance 1 nm. Coordinates were saved at 1 ps time intervals during the production run. The average structures from the last 25 ps were generated and energy minimized *via* CG until RMS gradient energy was lower than 4 kJ·mol^{-1}·nm^{-1}. All the energy minimization and MD simulations mentioned above were accomplished *via* Discover Studio 2.1 with CHARMm force field[15].

3 Results and Discussion

Since Williams *et al*.[16] and Yano *et al*.[17] independently resolved the first crystal structure of CYP3A4 in 2004, seven CYP3A4 crystal structures(two apo and five holo structures) have been available in the PDB database[16—19]. CYP3A4 has broad substrate specificity, and is able to have conformational changes within the active site and metabolize some large substrates[20]. Two points within our studies are therefore determined by these features. First, the apo structure of CYP3A4 should be used to avoid those conformational changes induced by other ligands. At the same time, in consideration of the resolution of the crystal structures, the structure with resolution 0.205 nm from Yano *et al*.(PDB ID: 1TQN) was chosen to do docking studies. Second, after initial docking step with affinity, we conducted 2 ns MD simulations for each ligand-protein complex to include the full flexibility of protein and ligand. The potential energies and root mean square deviation(RMSD) kept equilibrate after 1 ns MD simulations for the three protein-ligand complexes, indicating the stable binding of the drugs in the CYP3A4(Fig.2). The average complex structures from the last 25 ps MD simulations were generated and analyzed in the following section.

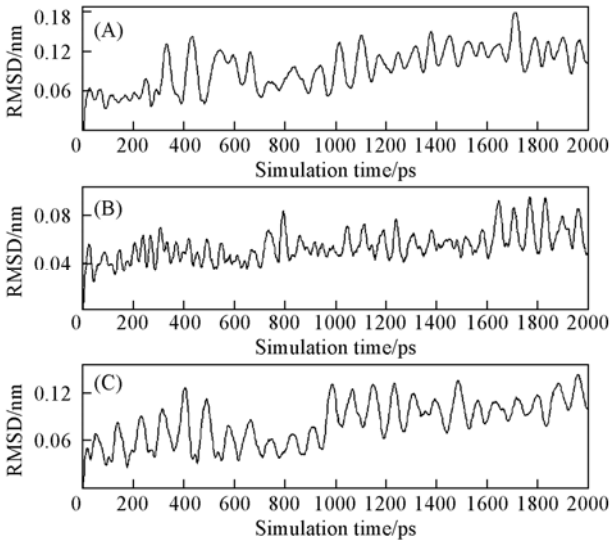

Fig.2 Variations of Cα RMSD during 2 ns MD simulations on SPD-304-CYP3A4(A), zafirlukast-CYP3A4(B) and L-745,870-CYP3A4(C)

3.1 Docking of SPD-304 into CYP3A4

The binding 3D conformation of SPD-304-CYP3A4 is displayed in Fig.3(A). Interaction energies were calculated between each amino acid on active sites and the ligand, aiming at evaluating the docking result in general and identifying the significant residues at binding-site in the model. A residue is proposed as a key residue in the ligand binding complex if it binds the ligand with interaction energy lower than –5 kJ/mol. The interaction energies of SPD-304 with each residue at the active site of CYP3A4 were calculated(Table 1).

SPD-304 is positioned firmly into the active site of CYP3A4 with a favorable total interaction energy of –308.44 kJ/mol. The 3-substituted indole plane of SPD-304 simultaneously parallels with the heme porphyrin ring and the chromene moiety of SPD-304, and is between them. This spatial arrangement leads to remarkable π-π interaction, stabilizing both ligand pose and its interaction with the heme of the enzyme. F304 and F213, two members of the "phenylalanine cluster"[16], have large hydrophobic interaction energies with

SPD-304 from two sides of the active site boundary. A hydrogen bond formed between the hydrogen atom of carboxyl group of E374 and one fluorine atom in trifluoromethyl-phenyl group of SPD-304further enhances the stability of the ligand binding. As a consequence, SPD-304 binds stably into CYP3A4 with the 3-methylene carbon in indole moiety being 0.49 nm far from the heme iron[2,21,22], which facilitates the 3-methylene hydrogen absorption and is in agreement with experimental data[8].

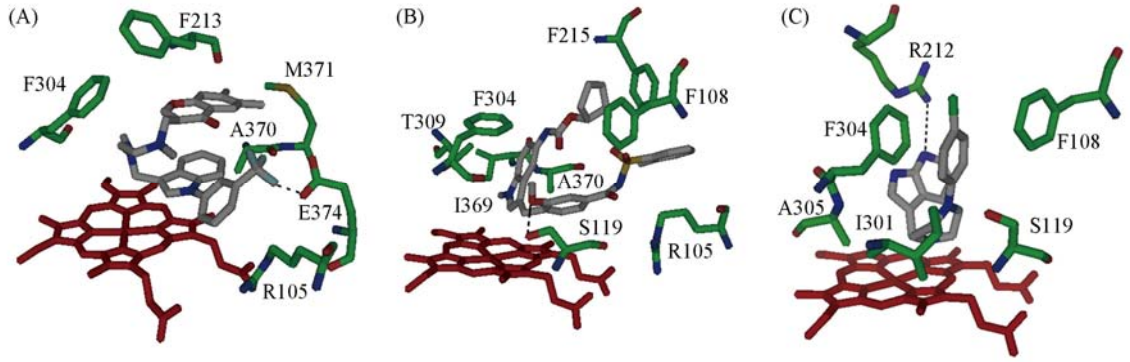

Fig. 3 Binding modes of SPD-304-CYP3A4(A), zafirlukast-CYP3A4(B) and L-745,870-CYP3A4(C)

The heme group is represented by a red stick and the hydrogen bonds are shown in black dashed lines.

Table 1 Total energies(E_{total}), van der Waals energies(E_{vdW}) and electrostatic energies(E_{ele}) between SPD-304 and each of residues in CYP3A4

Residue	E_{vdW}/(kJ·mol^{-1})	E_{ele}/(kJ·mol^{-1})	E_{total}/(kJ·mol^{-1})
Total	–235.10	–73.34	–308.44
HEM508	–31.96	–7.20	–39.16
PHE304	–23.30	–3.97	–27.27
PHE213	–16.36	–4.10	–20.46
ALA370	–15.56	–4.23	–19.79
ARG105	–11.42	–3.60	–15.02
MET371	–9.33	–2.97	–12.30
ILE369	–6.65	–5.56	–12.21
ILE301	–8.62	–2.72	–11.34
ALA305	–10.50	0.04	–10.46
PHE108	–7.40	–2.47	–9.87
LEU482	–10.70	1.17	–9.53
ILE120	–6.78	–2.43	–9.21
PHE215	–5.65	–3.47	–9.12
SER119	–3.93	–4.85	–8.78
THR309	–9.37	1.09	–8.28
PHE57	–7.78	–0.42	–8.20
GLU374	–7.82	0.08	–7.74
ASP214	–3.43	–3.14	–6.57
GLY481	–2.47	–3.85	–6.32
ARG106	–1.21	–3.81	–5.02

This docking conformation is very similar to the result from Sun *et al*.[8] with AutoDock 3.5 calculations. Even if using different sampling algorithms(MC combined with SA in Affinity *vs*. Lamarckian genetic algorithm in AutoDock), the 3-substituted indole portion actually locates at the same place on the active site of CYP3A4. Only difference between the conformations is the orientation of chromene moiety of SPD-304. It is parallel with the 3-substituted indole portion in our docking model but has slight deviations from indole portion in Sun's. This might be related to AutoDock 3.5 semi-flexibility docking strategy, that is, ligand is flexible and protein keeps rigid[23]. In general, the two conformations can be regarded as the same and this agreement convinces the configuration of SPD-304 binding into CYP3A4.

3.2 Docking of Zafirlukast into CYP3A4

For zafirlukast-CYP3A4 complex, the interaction energies of zafirlukast with each residue on the active site were calculated(Table 2). Residues with an interaction energy of lower than –8 kJ/mol for them with ligand are regarded as essential ones for ligand binding. The complex has a favorable total interaction energy of –389.8 kJ/mol, in which van der Waals and electrostatic energies are –261.84 kJ/mol and –127.99 kJ/mol, respectively.

Table 2 Total energies(E_{total}), van der Waals energies (E_{vdW}) and electrostatic energies(E_{ele}) between zafirlukast and each of residues in CYP3A4

Residue	E_{vdW}/(kJ·mol^{-1})	E_{ele}/(kJ·mol^{-1})	E_{total}/(kJ·mol^{-1})
Total	–261.84	–127.99	–389.83
HEME	–32.97	–3.81	–36.78
PHE215	–22.13	–3.81	–25.94
ARG105	–20.12	–5.06	–25.18
PHE108	–19.25	–3.26	–22.51
PHE304	–17.28	–3.18	–20.46
SER119	–11.38	–7.11	–18.49
ALA370	–10.92	–4.81	–15.73
ILE369	–8.95	–5.65	–14.60
THR309	–11.84	–1.84	–13.68
ARG372	–7.36	–5.98	–13.34
ALA305	–11.30	–0.71	–12.01
GLU374	–6.94	–4.52	–11.46
ILE301	–8.91	–0.75	–9.66
MET371	–3.39	–5.44	–8.83
ARG106	–10.92	2.38	–8.54
ASP214	–6.86	–1.51	–8.37

As seen from Fig.3(B), zafirlukast is stabilized by hydrophobic interactions and hydrogen bond in the center of the active site. Dominant interaction between zafirlukast and CYP3A4 is a hydrophobic cluster comprised of F215, F108 in CYP3A4, the cyclopentyl ring in the one end of zafirlukast and the methylbenzene moiety in the other. Additionally, R105 also contributes to the hydrophobic cluster with its alkyl side chain.

3-Substituted indole plane is surrounded by residues F304, T309 located on Helix I and I369, A370 on the loop connecting Helix K and β2(nomenclature from ref.[24]). Moreover, S119 forms a hydrogen bond with oxygen atom in the anisole moiety of zafirlukast to further stabilize the conformation.

Different from the docking pose of SPD-304, 3-substituted indole plane of zafirlukast is approximately perpendicular to the heme group on the active site of CYP3A4. The perpendicular conformation gives rise to 3-methylene carbon being 0.436 nm far from heme iron which is a reasonable distance as a reaction site. This is in agreement with experimental data, that is, a high reactive electrophilic intermediate could be formed initially from an initial hydrogen atom abstraction from 3-methylene group[9].

3.3 Docking of L-745,870 into CYP3A4

For L-745,870-CYP3A4 complex, the docking pose of L-745,870 is shown in Fig.3(C) and the critical residues for binding are listed in Table 3. The total interaction energy is –210.83 kJ/mol in which vdW and electrostatic energies are –162.34 kJ/mol and –48.49 kJ/mol, respectively. The L-745,870 is fixed into the active site of CYP3A4 with 3-methylindole carbon atom being 0.402 nm far from the heme iron. The interaction between L-745,870 and the enzyme arises from two moieties: on the one hand, the chlorophenyl group is sandwiched by F304 and F108, thereby generating a strong π-π interaction to anchor L-745,870; on the other hand, the 3-substituted-7-azaindole moiety over the heme group is stabilized through a hydrogen bond formed between the nitrogen at 7-position and the side chain of R212. Additionally, I301, S119 and A305 also contribute large interaction energies to L-745,870 binding.

We superimposed the docking complex to the holo crystal structures of ketoconazole-CYP3A4(PDB ID: 2V0M)[18] and ritonavir-CYP3A4(PDB ID: 3NXU)[19] and found that the chlorophenyl group of L-745,870 actually located where the dichlorophenyl group of ketoconazole in 2V0M and the phenyl of ritonavir in 3NXU did. The interaction between F304 and the chlorophenyl group of L-745,870 also exists for the counterparts in the holo crystal structures. Moreover, the pose of L-745,870 is similar to that of SPD-304 in terms of occupation of 3-substituted indole moiety on the active site of CYP3A4.

Table 3 Total energies(E_{total}), van der Waals energies (E_{vdW}) and electrostatic energies(E_{ele}) between L-745,870 and each of residues in CYP3A4

Residue	E_{vdW}/(kJ·mol^{-1})	E_{ele}/(kJ·mol^{-1})	E_{total}/(kJ·mol^{-1})
Total	–162.34	–48.49	–210.83
PHE304	–26.32	–1.88	–28.20
ARG212	–7.03	–19.87	–26.90
HEME	–36.74	9.87	–26.87
ILE301	–13.81	–4.73	–18.54
SER119	–10.12	0.17	–9.95
PHE108	–9.25	0.42	–8.83
ALA305	–9.25	0.84	–8.41
ALA370	–4.69	–2.59	–7.28
LEU483	–4.64	–1.05	–5.69
PHE213	–4.77	–0.67	–5.44
THR309	–5.56	0.33	–5.23

L-745,870 is structurally similar(containing an analogue of 3-substituted indole) to SPD-304 and zafirlukast which have been proved to be bioactivated by CYP3A4[8,9]. Experiment *in vivo* also suggests that L-745,870 could undergo biotransformation to a reactive electrophilic intermediate, which can be comparable with 3-methylindole metabolism[10,25]. Our studies show a reasonable binding mode and binding stability of L-745,870 into CYP3A4, which indicates the possibility of L-745,870 dehydrogenated at 3-methylene carbon by CYP3A4.

4 Conclusions

The 3D complex structures of SPD-304, zafirlukast and L-745,870 binding into CYP3A4 obtained by means of computational approaches show that 3-methylene carbons are potential dehydrogenation sites by CYP3A4 for the three drugs. It is in agreement with experimental data conducted on SPD-304[8]. A stack of three aromatic planes(indole and chromene ring by means of SPD-304 and porphyrin from heme) is proposed to stabilize the conformation of SPD-304 in the CYP3A4 and facilitate 3-methylene dehydrogenation based on our calculations. For zafirlukast, the 3-methylene carbon might be responsible for the dehydrogenation process from calculations. L-745,870-CYP3A4 complex model indicates that L-745,870 could also be dehydrogenated by CYP3A4 at 3-methylene carbon which is similar to SPD-304. Thus metabolism profile of L-745,870 by CYP3A4 may deserve further investigation. In addition, F108, F213, F215 and F304, known as members of phenylalanine cluster in CYP3A4, as well as S119 and R212 contribute main interaction energies to the binding of the three ligands.

References

[1] de Graaf C., Vermeulen N. P., Feenstra K. A., *J. Med. Chem.*, **2005**, *48*, 2725

[2] Denisov I. G., Makris, T. M., Sligar S. G., Schlichting I., *Chem. Rev.*, **2005**, *105*, 2253

[3] Dresser G. K., Spence, J. D., Bailey D. G., *Clin. Pharmacokinet*, **2000**, *38*, 41

[4] He M. M., Smith A. S., Oslob J. D., Flanagan W. M., Braisted A. C., Whitty A., Cancilla M. T., Wang J., Lugovskoy A. A., Yoburn J. C., Fung A. D., Farrington G., Eldredge J. K., Day E. S., Cruz L. A., Cachero T. G., Miller S. K., Friedman J. E., Choong I. C., Cunningham B. C., *Science*, **2005**, *310*, 1022

[5] Danese S., De Vitis I., Gasbarrini A., *Ann. Intern. Med.*, **2001**, *135*, 930

[6] Patel S., Freedman S., Chapman K. L., Emms F., Fletcher A. E., Knowles M., Marwood R., McAllister G., Myers J., Curtis N., Kulagowski J. J., Leeson P. D., Ridgill M., Graham M., Matheson S., Rathbone D., Watt A. P., Bristow L. J., Rupniak N. M., Baskin E., Lynch J. J., Ragan C. I., *J. Pharmacol. Exp. Ther*., **1997**, *283*, 636

[7] Tanaka K., Okada Y., Kanno T., Otomo A., Yanagisawa Y., Shouguchi-Miyata J., Suga E., Kohiki E., Onoe K., Osuga H., Aoki M., Hadano S., Itoyama Y., Ikeda J. E., *Exp. Neurol*., **2008**, *211*, 378

[8] Sun H., Yost G. S., *Chem. Res. Toxicol*., **2008**, *21*, 374

[9] Kassahun K., Skordos K., McIntosh I., Slaughter D., Doss G. A.,

Baillie T. A., Yost G. S., *Chem. Res. Toxicol*., **2005**, *18*, 1427

[10] Zhang K. E., Kari P. H., Davis M. R., Doss G., Baillie T. A., Vyas K. P., *Drug. Metab. Dispos*., **2000**, *28*, 633

[11] Jorgensen W. L., *Science*, **2004**, *303*, 1813

[12] Zhang J. L., Guo Y. H., Gao C. H., Wang E. S., Ren Z. Y., Fang X. X., Wang J. D., *Chem. Res. Chinese Universities*, **2010**, *26*(2), 256

[13] Wishart D. S., Knox C., Guo A. C., Shrivastava S., Hassanali M., Stothard P., Chang Z., Woolsey J., *Nucleic Acids Research*, **2006**, *34*, D668

[14] *Affinity In Accelrys Inc.*, Accelrys Inc., San Diego, **1999**

[15] Brooks B. R., Bruccoleri R. E., Olafson B. D., States D. J., Swaminathan S., Karplus M., *Journal of Computational Chemistry*, **1983**, *4*, 187

[16] Williams P. A., Cosme J., Vinkovic D. M., Ward A., Angove H. C., Day P. J., Vonrhein C., Tickle I. J., Jhoti H., *Science*, **2004**, *305*, 683

[17] Yano J. K., Wester M. R., Schoch G. A., Griffin K. J., Stout C. D., Johnson E. F., *J. Biol. Chem*., **2004**, *279*, 38091

[18] Ekroos M., Sjogren T., *Proc. Natl. Acad. Sci. USA*, **2006**, *103*, 13682

[19] Sevrioukova I. F., Poulos T. L., *Proc. Natl. Acad. Sci. USA*, **2010**, *107*, 18422

[20] Guengerich F. P., Hosea N. A., Parikh A., Bell-Parikh L. C., Johnson W. W., Gillam E. M., Shimada T., *Drug Metab. Dispos.*, **1998**, *26*, 1175

[21] Payne V. A., Chang Y. T., Loew G. H., *Proteins-Structure Function and Genetics*, **1999**, *37*, 204

[22] Li W. H., Tang Y., Liu H., Cheng J. G., Zhu W. L., Jiang H. L., *Proteins-Structure Function and Bioinformatics*, **2008**, *71*, 938

[23] Morris G. M., Goodsell D. S., Halliday R. S., Huey R., Hart W. E., Belew R. K., Olson A. J., *Journal of Computational Chemistry*, **1998**, *19*, 1639

[24] Poulos T. L., Finzel B. C., Howard A. J., *J. Mol. Biol.*, **1987**, *195*, 687

[25] Kartha J. S., Yost G. S., *Drug Metab. Dispos.*, **2008**, *36*, 155