Regular Article

Highlighted Paper selected by Editor-in-Chief

Modeling Analysis of Potential Target of Dolastatin 16 by Computational Virtual Screening

Ting-Ting Liang,^{a,b} Qi Zhao,^c Shan He,^d Fang-Zhou Mu,^e Wei Deng,^{*,a} and Bing-Nan Han^{*,b}^aSchool of Chemical and Environmental Engineering, Shanghai Institute of Technology, Shanghai 201418, China;^bDepartment of Development Technology of Marine Resources, College of Life Sciences, Zhejiang Sci-Tech University, Zhejiang 310018, China; ^cFaculty of Health Sciences, University of Macau, Macau 999078, China;^dLi Dak Sum Yip Yio Chin Kenneth Li Marine Biopharmaceutical Research Center, Ningbo University, Ningbo 315211, China; and ^ePharmaceutical Sciences Division, School of Pharmacy, University of Wisconsin-Madison, Wisconsin WI 53706, U.S.A.

Received November 30, 2017; accepted March 21, 2018

Dolastatin 16 is a cyclic depsipeptide isolated from the marine invertebrates and cyanobacterium *Lyngbya majuscula*, however, its bioactivity has been a historical question. In this study, peptidyl-prolyl *cis-trans* isomerase FKBP1A (FKBP12) was predicted as a potential target of dolastatin 16 via PharmMapper as well as verified using chemical-protein interactome (CPI) and molecular docking. FKBP1A has been previously identified as a target for the natural polyketide FK506 (tacrolimus), an immune suppressor inhibiting the rejection of organ transplantation in clinical use. The comparison study via the reverse pharmacophore screening and molecular docking of dolastatin 16 and FK506 indicated the good consistency of analysis with the computational approach. As the results, the lowest binding energy of dolastatin 16–FKBP1A complex was -7.4 kcal/mol and FK506–FKBP1A complex was -8.7 kcal/mol. The ligand dolastatin 16 formed three hydrogen bonds vs. four of FK506, as well as seven hydrophobic interactions vs. six of FK506 within the active site residues. These functional residues are highly repetitive and consistent with previously reported active site of model of FK506–FKBP1A complex, and the pharmacophore model was shown feasibly matching with the molecular feature of dolastatin 16.

Key words dolastatin 16; PharmMapper; FKBP1A; molecular docking

In modern drug development, target fishing (target identification) is an important step for exploring the mechanism of action of bioactive small molecules.¹⁾ In recent years, using computer tools to identify new indications or new targets for existing drugs has become a hot spot in drug research, such as target identification for Saffron²⁾ and Capsaicin.³⁾ Computational target fishing technologies have increased the accuracy and efficiency of target identification and is expected to have a large impact on drug development.^{4–6)} So far, many computational target identification methods have been developed,^{7–9)} such as the ligand-protein reverse docking approach for finding potential protein targets of a small molecule by computer-automated docking search of a protein cavity database.¹⁰⁾

Our long-term research goal is to isolate bioactive natural products from marine cyanobacteria and to understand their mechanism of action. Just recently we have re-isolated a cyclic peptide, dolastatin 16 from cyanobacterium *Lyngbya majuscula* collected from the South China Sea. In 1997 dolastatin 16 (Fig. 1(a)), was first isolated from sea hare *Dolabella auricularia* and showed impressive cytotoxicity against a variety of cancer cell lines reported.¹¹⁾ In 2015, synthesis of dolastatin 16 was completed.¹²⁾ Surprisingly, synthetic dolastatin 16 loses potency in inhibiting cancer cell growth (growth inhibition, 50% (GI₅₀) > 10 μ g/mL) as opposed to its natural counterpart.¹²⁾ Their analysis suggested that the strong cytotoxicity observed in the 1997 study might be due to a chemically undetected compound other than dolastatin 16. Over the past decade, re-discovery of dolastatin 16 from *L. majuscula* and other microalgae was occasionally reported.¹³⁾ Tan's group isolated dolastatin 16 from *L. majuscula* and showed that it effectively

inhibited the larval settlement and metamorphosis of the barnacle *Amphibalanus amphitrite* with an EC₅₀ value of 0.003 μ g mL⁻¹.¹⁴⁾ The LC₅₀/EC₅₀ ratio of dolastatin 16 is 6000, due to its significant antifouling activity, but low toxicity, therefore, dolastatin 16 was expected to be a promising lead compound alternative for the current use of heavy-metal-based antifouling agents.¹⁵⁾ Since we have successfully obtained the crystal structure of dolastatin 16, and have some primary data showing that it exhibited certain anti-inflammation activity, we conducted a virtual screening to search for its possible targets.

In our study, FKBP1A (FK506 binding protein) was ranked as the top target for dolastatin 16 according to PharmMapper, a reverse docking server for computational virtual screening. This enzyme is thought to play a role in accelerating protein folding.¹⁶⁾ FKBP modulates the release of calcium from skel-

Fig. 1. The Chemical Structure of (a) Dolastatin 16 and (b) FK506

* To whom correspondence should be addressed. e-mail: wdeng@sit.edu.cn; hanbingnan@zstu.edu.cn

eton, and is necessary for T-cell receptor mediated activation of interleukin-2 transcription.¹⁶⁾ This target was evaluated and analyzed *via* chemical–protein interactome (CPI) and molecular docking methods. Our results indicated that dolastatin 16 has good virtual binding capability to FKBP1A comparable to its natural substrate FK506.

Experimental

Targets Predicted by PharmMapper PharmMapper is a freely accessed web server that uses a pharmacophore mapping approach to identify potential target candidates for the given probe small molecules.¹⁷⁾ PharmMapper contains 7302 pharmacophore models and its pharmacophore database contains annotation from TargetBank, DrugBank, BindingDB and potential drug target database. It automatically searches for the best mapping poses in all pharmacophore models of PharmTargetDB when a query molecule is uploaded, and lists the top N best-fit hits with appropriate target annotations, as well as the aligned poses of the respective molecules.¹⁷⁾

Molecular file of dolastatin 16 was submitted to the PharmMapper server. The parameters were set as follows; the generate conformers were set to yes, the maximum generated conformations were set to 300, targets setting was set to “All Targets (7302),” and the number of reserved matched targets was set to 300.^{9,18)} Other parameters were set to default.

Targets Checked by the CPI The chemical–protein interactome (CPI) refers to the interaction information of a panel of chemicals across a panel of target proteins in terms of binding strength and binding conformation for each chemical–protein pocket pair.¹⁹⁾ Both drug–drug interactions (DDI)–CPI and drug repositioning potential and adverse drug reactions (DRAR)–CPI are free servers for predicting these interactions *via* the CPI.^{20,21)}

The molecular file of FK506 was extracted from the complex of the target protein (PDB ID: 1FKF), which was downloaded from the RCSB Protein Data Bank, and together with the molecular file of dolastatin 16 were then pretreated following the web instructions as described previously.²¹⁾ Then they were submitted to the DDI–CPI and DRAR–CPI servers, parameters used default values.

Molecular Docking Molecular docking is a computational procedure, which attempts to predict binding of a small molecule (ligand) and a macromolecule (receptor) efficiently.²²⁾ Autodock vina is a new open-source program, useful for molecular docking and virtual screening which, when compared to autodock 4, performs docking at higher speed and accuracy,

and has a new scoring function.²³⁾ At the same time, it also greatly increases the efficiency of the optimization and the accuracy of the binding mode predictions. PyRx0.8 is a valuable tool for molecular docking, which contains autodock vina wizard with easy-to-handle user interface, as well as chemical spreadsheet-like functionality and powerful visualization engine. Autodock vina in PyRx0.8 is being widely used as a new program for molecular docking and virtual screening.

The crystal structures of the potential target proteins were obtained from the RCSB Protein Data Bank (1FKF, 1BL4, <http://www.rcsb.org/pdb>), and prepared using the protein preparing tool in PyMOL. Which was used to extract ligand from binding site, deleting all water molecules and hydrogen atoms were then added, in addition, showed the ligand coordinates and radius information.²⁴⁾ During the docking procedure, the grid box covered the binding site residues and the ligand was allowed to move freely. The box was set to 40×40×40, and the center coordinate was shown in Tables 4 and 5. Other parameters were kept to default values.

Visualization Visualization of the complex structure was performed using PyMOL molecular graphics system, and the protein–ligand interactions were shown by discovery studio version 2016.

Results and Discussion

Protein Target Prediction by PharmMapper We performed a virtual screening to identify the potential targets of dolastatin 16 that match the pharmacophore models within the protein database of PharmMapper. Our result shows 300 hits, with the top ten listed in Table 1. Peptidyl–prolyl *cis*–*trans* isomerase FKBP1A (PDB ID: 1BL4) ranked number one virtual target for dolastatin 16, showing five hydrophobic sites and four acceptors (Fig. 2) within the pharmacophore groups. FKBP1A is the target of FK506,²⁵⁾ which is a novel immunosuppressant agent. The alignment of FK506 and pharmacophore models using PharmMapper was depicted in Fig. 3, showing six hydrophobic sites, one donor, as well as two acceptors. Furthermore, alignment information of the two small molecules and the pharmacophore model candidates shown in Figs. 2 and 3, further support the PharmMapper results. Molecular modeling study of FK506–FKBP1A has been extensively conducted, which provides applicable and comparable pharmacophore information for modeling analysis of potential target of dolastatin 16 by computational virtual screening.^{26–28)} Therefore, a detailed comparison study of dolastatin 16–FKBP1A *vs.* FK506–FKBP1A interactions was

Table 1. Top Ten Potential Disease-Related Targets of Dolastatin 16 Predicted by PharmMapper

Rank	PDB ID	Name	Fit score
1	1BL4	Peptidyl–prolyl <i>cis</i> – <i>trans</i> isomerase FKBP1A	4.308
2	1NC6	Cationic trypsin	4.292
3	1HWK	3-Hydroxy-3-methylglutaryl-coenzyme A reductase	4.184
4	1LEE	Plasmeprin-2	4.098
5	1HEF	Gag-Pol polyprotein	4.097
6	1L7H	Neuraminidase	3.998
7	1ILH	Nuclear receptor subfamily 1 group I member 2	3.963
8	1BWA	Gag-Pol polyprotein	3.896
9	1K6R	β -Lactamase PSE-2	3.893
10	1SKX	Nuclear receptor subfamily 1 group I member 2	3.875

Fig. 2. Alignment of Dolastatin 16 and Pharmacophore Model of FKBP1A (PDB ID: 1BL4)

(a) Dolastatin 16 features. (b) Pharmacophore model of FKBP1A. (c) Molecular and pharmacophore model.

Fig. 3. Alignment of FK506 and Pharmacophore Model of FKBP1A (PDB ID: 1FKF)

(a) FK506 features. (b) Pharmacophore model of FKBP1A. (c) Molecular and pharmacophore model.

Table 2. Results of FK506–FKBP Interactome by DDI–CPI and DRAR–CPI

DDI–CPI			DRAR–CPI			
PDB ID	Name	Docking score/(kcal/mol)	PDB ID	Name	Docking score/(kcal/mol)	Z'-Score
1FKB	FKBP1A	–8.4	1J4I	FKBP1A	–45.1127	–1.44483
1PBK	FKBP3	–7.4	1C9H	FKBP1B	–45.0239	–1.645

Table 3. Results of Dolastatin 16–FKBP Interactome by DDI–CPI and DRAR–CPI

DDI–CPI			DRAR–CPI			
PDB ID	Name	Docking score/(kcal/mol)	PDB ID	Name	Docking score/(kcal/mol)	Z'-Score
1PBK	FKBP3	–8.9	1C9H	FKBP1B	–46.8008	–2.60766
1FKB	FKBP1A	–7.2	1CYN	PP1aseB	–48.7681	–2.0365

conducted by software PyMOL and Discovery studio version 2016. The pharmacophores of dolastatin 16 and FK506 share highly similar hydrophobic sites and two identical acceptors.

Targets Verified by Chemical–Protein Interactome The information displayed by CPI can result in better understanding of the complex structures. The DDI–CPI server will dock it across 611 human proteins when a drug is uploaded to the server, then a CPI profile can be generated, which can be used as a feature vector of the pre-constructed prediction model.²⁰⁾ As Table 2 shown, FKBP1A and FKBP3 were docked with FK506. Docking score of FK506–FKBP1A was –8.4kcal/mol. It can be observed from the DDI–CPI results

of the FK506 of that it is reasonable and feasible to use this platform to verify the prediction results of PharmMapper. As displayed in Table 3, there have two isoforms, containing FKBP3, and FKBP1A, docked with dolastatin 16. Docking score of dolastatin 16–FKBP1A was –7.2 kcal/mol. Moreover, visualization of dolastatin 16–FKBP1A and FK506–FKBP1A were presented in Fig. 4.

The DRAR–CPI server use the DOCK program to “hybridize” the molecule with all targets after a molecule is submitted to the server.²¹⁾ Then to rank the interactomes of drugs across the targetable proteins Z'-scores are used. In a DRAR–CPI job, potential targets of uploaded drug with Z'-score < –1 were

Fig. 4. (a) Visualization of a Dolastatin 16–FKBP1A Complex Captured from the DDI–CPI Server; (b) Visualization of a FK506–FKBP1A Complex Captured from the DDI–CPI Server

Note: protein chain: rocket; drug: stick.

Table 4. Dolastatin 16, the Center Coordinates of the Binding Site and the Lowest Binding Energy by Molecular Docking

PDB ID	Name	Center ($x \times y \times z$)/Å	Binding affinity/(kcal/mol)
1BL4	FKBP1A	12.33×14.68×21.97	−7.4

Table 5. FK506, the Center Coordinates of the Binding Site and the Lowest Binding Energy by Molecular Docking

PDB ID	Name	Center ($x \times y \times z$)/Å	Binding affinity/(kcal/mol)
1FKF	FKBP1A	23.24×29.17×23.61	−8.7

Fig. 5. Molecular Interactions between Dolastatin 16 and FKBP1A

(a) 3D structure of the FKBP1A (1BL4)-dolastatin 16 complex by PyMOL. Hydr ogen bond: dash line. (b) 3D interaction scheme by Discovery studio version 2016. Hydrophobic interaction: arrow.

treated as advantageous targets while those with Z' -score>1 were treated as advantageous targets.²¹⁾ The results of FK506–peptidyl–prolyl *cis*–*trans* isomerase (FKBP1A, and FKBP1B) as well as dolastatin 16–peptidyl–prolyl *cis*–*trans* isomerase (FKBP1B, and B) were presented in Tables 2 and 3.

Molecular Docking To further validate the selected protein target, exploration of the binding mode and mechanism of small molecule and screened target protein, the interaction of dolastatin 16 and FK506 with FKBP1A were investigated by molecular docking. The similarities were found by comparing the interaction between dolastatin 16–FKBP1A and FK506–FKBP1A. Autodock vina in PyRx0.8 was used to further the investigation, the lowest binding energy of dolastatin 16–FKBP1A complex was −7.4kcal/mol (Table 4) and FK506–FKBP1A complex was −8.7kcal/mol (Table 5). The docking results were consistent with the DDI–CPI validations. As shown in Fig. 5(a), the ligand dolastatin 16 formed three hydrogen bonds with the active site residues (Tyr26, Ile56, and Tyr82), as well as seven hydrophobic interactions (Fig. 5(b)), including Val36, Phe46, Ile56, Ile90, Ile91, Leu97, and Phe99. Meanwhile, the ligand FK506 formed four hydrogen bonds (Fig. 6(a)) with the active site residues (Asp37, Glu54, Ile56, and Tyr82), and six hydrophobic interactions (Fig. 6(b)), including Phe36, Phe46, Ile56, Ile90, Ile91, and Phe99. These functional residues are highly repetitive, and they are significant residues that make up the active site. Results of molecular docking indicated that the hydrogen bonds and hydrophobic interactions of dolastatin 16 and FK506 with the active sites

Fig. 6. Molecular Interactions between FK506 and FKBP1A

(a) 3D structure of the FKBP1A (1FKF)-FK506 complex by PyMOL. Hydrogen bond: dash line. (b) 3D interaction scheme by Discovery studio version 2016. Hydrophobic interaction: arrow.

of the protein were consistent with the previously produced pharmacophore models. The fit score and binding energy of target proteins with small molecules are both high, indicating that small molecules have a strong inhibitory effect on these protein targets and thus play a key role in the treatment of related diseases.

It has been reported that the conformation of *cis*, *trans* of the cyclic peptides may cause a change in activity of the peptide.²⁹⁾ Therefore, we investigated the effect of molecular interaction by altering the *cis*, *trans* conformation of dolastatin 16 (Fig. 7). The original conformation of the three prolines of dolastatin 16 were *trans*, *trans*, *cis*, which were computationally transformed as follows: (1) the conformation of Pro¹ was

Fig. 7. Chemical Structures of Analogues of Dolastatin 16

Table 6. Potential Disease-Related Targets of Dolastatin 16a, Dolastatin 16b, Dolastatin 16c, Dolastatin 16d, Predicted by PharmMapper

Molecule	Rank	Fit score
Dolastatin 16a	174	3.189
Dolastatin 16b	NONE	NONE
Dolastatin 16c	11	3.866
Dolastatin 16d	86	3.569

converted to *cis*, named dolastatin 16a; (2) the conformation of Pro² was converted to *cis*, named dolastatin 16b; (3) the conformation of Pro³ was converted to *trans* was named dolastatin 16c; (4) the conformation of 3 prolines were converted to *cis*, named dolastatin 16d. These transformations were directly modified on the crystal structure. Then submitted the mol2 file of these four modified compounds to PharmMapper server. Target peptidyl-prolyl *cis*-*trans* isomerase FKBP1A (PDB ID: 1BL4) ranking and scoring were shown in Table 6. The conformation transformation of Pro¹ or Pro² changed the target prediction results greatly, however, the result of three *trans* prolines showed less change. This indicates that *cis*, *trans* isomerism of prolines have a prodigious influence on target prediction.²⁹ From our prediction results it is shown that the original conformation is still the best fit for the prediction.

Near the FKBP binding pocket, there are thirteen important residues: Tyr26; Phe36; Asp37; Phe46; Phe48; Val55; Ile56; Trp59; Tyr82; Ile90; Ile91; Leu97; and Phe99.^{30,31} In the previous report, the hydrophobic surface of the active site cavity of FKBP contains the residues Tyr26; Phe36; Trp59; Tyr82; and Phe99, along with five hydrogen bonds Asp37; Gln53; Glu54; Ile56; and Tyr82 formed between FKBP and FK506.³⁰ It can be seen that the hydrogen bonds and hydrophobic interactions of dolastatin 16 with the active sites of the protein was similar to the previously reported. In the preliminary study, we have found that dolastatin 16 exhibited potent anti-inflammatory effect, which is likely relevant to the predicted target. Further study to explore its pharmacological activities is underway.

Conclusion

In this study, potential targets of dolastatin 16 were predicted *via* PharmMapper as well as verified using CPI and molecular docking. Our study identified FKBP1A as a potential target of dolastatin 16. The lowest binding energy of dolastatin 16-FKBP1A complex (−7.4kcal/mol) and FK506-FKBP1A complex (−8.7kcal/mol) were computed through autodock vina. In the above modeling system, the ligand dolastatin 16 formed three hydrogen bonds with active site residues, as well as seven hydrophobic interactions. Meanwhile, the ligand FK506 formed four hydrogen bonds with the active site residues and six hydrophobic interactions. These functional residues are highly repetitive. This shows the potential for using dolastatin 16 in the interactions of FKBP1A. Validation of reverse pharmacophore screening and molecular docking afforded highly consistent data for our virtual screening approach. Our result may provide practical insight for future experimental characterization of dolastatin 16's biological activity.

Acknowledgment The work was supported by the Natural Science Foundation of China (Grant no. 81373321).

Conflict of Interest The authors declare no conflict of interest.

References

- 1) Lomenick B., Olsen R. W., Huang J., *ACS Chem. Biol.*, **6**, 34–46 (2011).
- 2) Bhattacharjee B., Vijayarathay S., Karunakar P., Chatterjee J., *Asian Pac. J. Cancer Prev.*, **13**, 5605–5611 (2012).
- 3) Ye X. Y., Ling Q. Z., Chen S. J., *Evid-Based. Compl. Alt. Med.*, **2015**, 1–9 (2015).
- 4) Rask-Andersen M., Almén M. S., Schiöth H. B., *Nat. Rev. Drug Discov.*, **10**, 579–590 (2011).
- 5) Cereto-Massague A., Ojeda M. J., Valls C., Mulero M., Pujadas G., Garcia-Vallve S., *Methods*, **71**, 98–103 (2015).
- 6) Peón A., Dang C. C., Ballester P. J., *Front. Chem.*, **4**, 624–632 (2016).
- 7) Hammell M., *Semin. Cell Dev. Biol.*, **21**, 738–744 (2010).
- 8) Chitrula K. N., Yeguvapalli S., *PLOS ONE*, **9**, e109185 (2014).

- 9) Manivannan J., Silambarasan T., Kadarkarairaj R., Raja B., *Rsc. Advances*, **5**, 77042–77055 (2015).
- 10) Chen Y. Z., Zhi D. G., *Proteins*, **43**, 217–226 (2001).
- 11) Pettit G. R., Xu J. P., Hogan F., Williams M. D., Doubek D. L., Schmidt J. M., Cerny R. L., Boyd M. R., *J. Nat. Prod.*, **60**, 752–754 (1997).
- 12) Pettit G. R., Smith T. H., Arce P. M., Flahive E. J., Anderson C. R., Chapuis J. C., Xu J. P., Groy T. L., Belcher P. E., Macdonald C. B., *J. Nat. Prod.*, **78**, 476–485 (2015).
- 13) Nogle L. M., Gerwick W. H., *J. Nat. Prod.*, **65**, 21–24 (2002).
- 14) Tan L. T., Goh B. P. L., Tripathi A., Lim M. G., Dickinson G. H., Lee S. S. C., Teo S. L. M., *Biofouling*, **26**, 685–695 (2010).
- 15) Casalme L. O., Yamauchi A., Sato A., Petitbois J. G., Nogata Y., Yoshimura E., Okino T., Umezawa T., Matsuda F., *Org. Biomol. Chem.*, **15**, 1140–1150 (2017).
- 16) Islam M. S., *UCSD-Nat. Mol. Page.*, (2007).
- 17) Liu X., Ouyang S., Yu B., Liu Y., Huang K., Gong J., Zheng S., Li Z., Li H., Jiang H., *Nucleic Acids Res.*, **38** (suppl. 2), W609–W614 (2010).
- 18) Chen S. J., Ren J. L., *Asian Pac. J. Cancer Prev.*, **15**, 111–116 (2014).
- 19) Yang L., Wang K. J., Wang L. S., Jegga A. G., Qin S. Y., He G., Chen J., Xiao Y., He L., *Interdiscip. Sci.*, **3**, 22–30 (2011).
- 20) Luo H., Zhang P., Huang H., Huang J. L., Kao E., Shi L. M., He L., Yang L., *Nucleic Acids Res.*, **42** (W1), W46–W52 (2014).
- 21) Luo H., Chen J., Shi L., Mikailov M., Zhu H., Wang K., He L., Yang L., *Nucleic Acids Res.*, **39** (suppl. 2), W492–W498 (2011).
- 22) Chen S. J., *Asian Pac. J. Cancer Prev.*, **15**, 4301–4305 (2014).
- 23) Trott O., Olson A. J., *J. Comput. Chem.*, **31**, 455–461 (2010).
- 24) Seeliger D., de Groot B. L., *J. Comput. Aided Mol. Des.*, **24**, 417–422 (2010).
- 25) Jayaraman T., Brillantes A. M., Timmerman A. P., Fleischer S., Erdjument-Bromage H., Tempst P., Marks A. R., *J. Biol. Chem.*, **267**, 9474–9477 (1992).
- 26) Becker J. W., Rotonda J., McKeever B. M., Chan H. K., Marcy A. I., Wiederrecht G., Hermes J. D., Springer J. P., *J. Biol. Chem.*, **268**, 11335–11339 (1993).
- 27) Aldape R. A., Futer O., DeCenzo M. T., Jarrett B. P., Murcko M. A., Livingston D. J., *J. Biol. Chem.*, **267**, 16029–16032 (1992).
- 28) Kissinger C. R., Parge H. E., Knighton D. R., Lewis C. T., Pelletier L. A., Tempczyk A., Kalish V. J., Tucker K. D., Showalter R. E., Moomaw E. W., Gastinel L. N., Habuka N., Chen X. H., Maldonado F., Barker J. E., Bacquet R., Villafranca J. E., *Nature* (London), **378**, 641–644 (1995).
- 29) Pelay-Gimeno M., Meli A., Tulla-Puche J., Albericio F., *J. Med. Chem.*, **56**, 9780–9788 (2013).
- 30) Van Duyne G. D., Standaert R. F., Karplus P. A., Schreiber S. L., Clardy J., *Science*, **252**, 839–842 (1991).
- 31) Fanghänel J., Fischer G., *Front. Biosci.*, **9**, 3453–3478 (2004).