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Original article

3D-QSAR and molecular docking studies on benzothiazole derivatives as *Candida albicans* *N*-myristoyltransferase inhibitors

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Abstract

N-Myristoyltransferase has been a promising new target for the design of novel antifungal agents with new mode of action. Molecular docking and three-dimensional quantitative structure–activity relationship (3D-QSAR) methods, CoMFA and CoMSIA, were applied to a set of novel benzothiazole *Candida albicans* *N*-myristoyltransferase (CaNmt) inhibitors. The binding mode of the compounds at the active site of CaNmt was explored using flexible docking method and various hydrophobic and hydrogen-bonding interactions were observed between the benzothiazole inhibitors and the target enzyme. The best CoMFA and CoMSIA models had a cross-validated coefficient q^2 of 0.733 and 0.738, respectively, which showed high correlative and predictive abilities on both the test set and training set. The 3D contour maps of CoMFA and CoMSIA provided smooth and interpretable explanation of the structure–activity relationship for the compounds. The analysis of the 3D contour plots permitted interesting conclusions about the effects of different substituent groups at different position of the benzothiazole ring, which will guide the design of novel CaNmt inhibitors with higher activity.

Keywords: 3D-QSAR; Molecular docking; Benzothiazole; *N*-Myristoyltransferase inhibitors; Antifungal

1. Introduction

During the past two decades, the life threatening infections caused by pathogenic fungi are becoming increasingly common, especially in those individuals with immunocompromised hosts, such as patients undergoing anticancer chemotherapy or organ transplants and patients with AIDS [1]. Clinically, candidosis, aspergillosis and cryptococcosis are three major fungal infections in immunocompromised individuals [2,3]. The common antifungal agents currently used in clinic are azoles (such as fluconazole, ketoconazole and itraconazole), polyenes (such as amphotericin B and nystatin), echinocandins (such as caspofungin and micafungin) and allylamines (such as naftifine and terbinafine). However, their use can suffer from limited efficiency, narrow antifungal spectrum, drug related toxicity, severer drug resistance, non-optimal pharmacokinetics and serious drug–drug interactions. Therefore, there is an emergent need to develop novel fungicidal drugs with a new mode of action.

Myristoyl-CoA:protein *N*-myristoyltransferase (Nmt) is a cytosolic monomeric enzyme that catalyzes the transfer of the myristoyl group from myristoyl-CoA to the N-terminal glycine of a number of eukaryotic cellular and viral proteins [4,5]. Myristoylation relates to diverse biological processes including signal transduction cascades and apoptosis. Genetic experiments have shown that Nmt is an essential enzyme for the viability of some important pathogenic fungi including *Candida albicans* and *Cryptococcus neoformans* [6,7]. Thus, Nmt has been a promising target enzyme for the development of novel fungicidal drugs having a broad antifungal spectrum

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[8]. Although Nmt also exists in human, the differences in the protein substrate specificities of fungal and human Nmts have been utilized to develop species-selective inhibitors that are fungicidal and safe [8].

Up to now, peptidomimetic inhibitors [9–12], myristic acid analogues [13,14], *p*-toluenesulfonamide inhibitors [15], benzofuran inhibitors [16–19] and benzothiazole inhibitors [20] have been reported to be Nmt inhibitors. Among them, the benzofuran and benzothiazole inhibitors showed high selectivity over human Nmt and exhibited good antifungal activity. Benzofuran inhibitors have been well studied *in vivo* and *in vitro* [16-19]. The structure–activity relationship (SAR) of benzofuran inhibitors has been studied by three quantitative structure–activity relationship (3D-QSAR) methods [21,22]. The binding mode of the benzofuran inhibitors was further clarified by the determination of the crystal structure of *Candida albicans* Nmt (CaNmt) in complex with benzofuran inhibitors [23]. Benzothiazole derivatives are relatively new class of Nmt inhibitors and no molecular modeling studies have been reported until now.

Among the current 3D-QSAR methods, comparative molecular field analysis (CoMFA) [24] and comparative molecular similarity indices analysis (CoMSIA) [25] are widely used in drug design, because they allow rapid prediction of the biological activities of newly designed molecules. To gain further insight into the SAR of the benzothiazole Nmt inhibitors, binding mode of this series of compounds was clarified by molecular docking, and CoMFA and CoMSIA were further applied to build robust 3D-QSAR models on them. The results deduced from the present investigation provided the useful information for the structural requirements of the benzothiazole Nmt inhibitors and could guide the rational design of novel CaNmt inhibitors.

2. Materials and methods

2.1. General

The crystallographic coordinates of CaNmt in complex with benzofuran inhibitor [23] (0.32 Å resolution, $R_{cryst} = 0.284$) were obtained from the Brookheaven Protein Databank as entries 1IYL. In the crystal structure, there are four asymmetric units (molecules A to D) with the inhibitor bound to the A and C asymmetric unit. In the present study, asymmetric unit C was used in our following docking study. All calculations were performed with commercially available SYBYL6.9 [26] and InsightII 2000 [27] software packages. All calculations were performed on an Origin 300 Server.

2.1.1. Data set

A total of 44 benzothiazole compounds from the literature [20] were used as a data set in the following 3D-QSAR analysis (Fig. 1). The selection of training set and test set is based on structural diversity and frequency of distribution of biological data by doing principal component analysis. As a result, eight compounds were selected as a test set, which represented a range of inhibitory activity similar to that of a training set and was used to evaluate the predictive power of the resulting models. In order to reinforce the reliability of their 3D-QSAR models, four benzofuran Nmt inhibitors (compounds **45**–**48**) [19] were selected as real external compounds (Fig. 2) and were added into the test set. The biological activity of each compound was expressed as inhibitory concentrations (IC_{50}) against CaNmt and $-\log(IC_{50})$ was used for the 3D-QSAR analysis.

2.1.2. Docking analysis

In order to find a suitable docking method for the CaNmt system, DOCK 4.0.1 [28], FlexX [29] and InsightII/Affinity [30] were investigated to reproduce the position for the benzofuran inhibitor in the crystal structure 1IYL. For DOCK 4.01, a Connolly surface of each active site was generated using QCPE429 program. A flexible docking was performed starting with a selection and matching of an anchor atom within a maximum of 500 orientations, followed by growth of the ligand with 25 configurations per cycle. The final step included relaxation of 100 simplex minimizations to a convergence of 0.1 kcal/mol. For FlexX, all default parameters, as implemented in the 6.9 release of SYBYL [26], were used. Cscore calculations were performed for ranking, and all 30 poses were inspected. For Affinity, Monte Carlo docking protocol was used by default parameters. For the docking results from each method, the corresponding RMSD between the crystal and docked conformation was computed. The comparison of the docking results revealed that Affinity was the most powerful method. As a result, Affinity was selected to study the binding mode of benzothiazole inhibitor with CaNmt.

Compound **39**, the most active compound in the data set, was used to investigate the binding mode of the benzofuran inhibitors. First, compound **39** was manually docked into the active site of CaNmt on the basis of the binding mode of benzofuran inhibitors to construct the starting enzyme–inhibitor complex [23]. Then, the flexible ligand docking procedure in the Affinity module within InsightII was used to define the lowest energy position for the substrate using a Monte Carlo docking protocol. All the atoms within a defined radius (8 Å) of the inhibitor were allowed to move. The solvation grid supplied with the Affinity program was used. If the resulting substrate/enzyme system was within a predefined energy tolerance of the previous structure, the system was subjected to minimization. The resulting structure was accepted on the basis of energy check, which used the Metropolis criterion, and also a check of RMS distance of the new structure versus the structure found so far. The final conformation was obtained through a simulation annealing procedure from 500 K to 300 K, and then 5000 rounds of energy minimization were performed to reach a convergence, where the resulting interaction energy values were used to define a rank order.

2.1.3. Molecular modeling and alignment

In the 3D-QSAR studies, pharmacophoric conformation and alignment rule are two major critical steps to get meaningful results. Because the crystal structure of CaNmt in complex with benzothiazole inhibitors has not been reported, molecular