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Exploring the structure requirement for PKCθ inhibitory activity of pyridinecarbonitrile derivatives: an *in silico* analysis

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ABSTRACT

Presently, an *in silico* modeling was carried out on a large series of 263 PKCθ inhibitors using 3D-QSAR, molecular docking and molecular dynamics (MD) simulations for the first time. Based on different alignment rules, several computational models were established with their statistical results compared. The resultant models derived from the database alignment exhibit satisfying internal and external predictive capabilities with q^2 of 0.503, 0.616 and r^2_{pred} of 0.568, 0.602 for CoMFA and CoMSIA, respectively. The consistency of conclusion among 3D contour maps of CoMFA and CoMSIA, molecular docking and molecular dynamics proves the reliability of the developed models. The analysis of the 3D contour plots permits interesting conclusions about the effects of different substituent groups at different positions of the common scaffold. In addition, Leu461 and Asn509 have been identified as the key amino acid residues to form H-bond interaction with the ligand compound. The developed models will provide a clue to the design of novel PKCθ inhibitors.

1. Introduction

Protein kinase Cθ (PKCθ), a Ca^{2+}-independent novel PKC subfamily member, is a key T-cell receptor (TCR) signaling molecule [1]. PKCθ is expressed primarily in the lymphocytes, skeletal muscle and platelets, which is independently cloned and characterized by three different groups [2–4]. It has been reported that the key translocation of PKCθ results in an activation of several transcription factors required for the T cell activation. PKCθ plays a central role in the TCR-mediated activation of transcription factors which, in turn, upregulate the interleukin-2 (IL-2) gene expression [5]. The previous experiment illustrates PKCθ knockout mice have shown diminished responses in various T-cell mediated disease models including the type II collagen-induced arthritis model [6], the experimental autoimmune encephalomyelitis model of multiple sclerosis [7] as well as the ovalbumin challenge model of asthma [8], etc. It is interesting that, in spite of their defective T cell activation pathway, PKCθ knockout mice have been shown to have normal Th1 cell response in the lung and normal viral clearance [9]. For these reasons, the inhibition of PKCθ has recently become an attractive target for treatment of autoimmune and inflammatory diseases. Up to date several kind of small molecular PKCθ inhibitors have been reviewed [10] including the Boehringer Ingleheim-, Millennium-, Altana- and Wyeth-based PKCθ inhibitors [11–22] and so forth. However, among them, a large number of PKCθ inhibitors are from the patent literatures. Therefore, for many of these compounds, no absolute value for the inhibition of PKCθ or selectivity against other PKC isoforms is disclosed except those PKCθ inhibitors reported by Wyeth Research available to the public. The SAR assessment of these Wyeth-based PKCθ inhibitors, based on the common pyridinecarbonitrile structure, illustrates both their potential against PKCθ and the excellent selectivity over a variety of PKC isoforms.

However, the production of such a large quantity of SAR information using a trial and error method is time-consuming and costly. The application of quantitative structure–activity relationship (QSAR) methodologies to the development of new leads, thus, has the potential to decrease substantially the time and effort required to discover new medicines or improve current ones in terms of their efficacy [23]. Therefore, *in silico* approaches have been successfully applied to various biochemical fields [24–26].

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However, up to now, to our best knowledge, there is still no report of *in silico* modeling on the interactions between PKCθ and its inhibitors except the recent 2D-QSAR study using the random forest (RF) algorithm based on a series of 208 structurally diverse PKCθ inhibitors [27]. By using the RF built-in measure of the relative importance of the descriptors, an important predictor – the number of group donor atoms for H-bonds (with N and O) – has been identified to play a crucial role in PKCθ inhibitory activity. Although the results of the RF method indicate the importance of hydrogen bonding but do not precisely locate the site of hydrogen bonding. 3D-QSAR, if successful, might provide that additional information. Consequently, using an enlarging data set consisting of 263 molecules, we performed 3D-QSAR analyses, as a supplement for our previous report [27] to gain further insights into the structural and chemical features required for the PKCθ inhibitory activity.

CoMFA [28] and CoMSIA [29] are powerful and versatile tools to build and design an activity model (QSAR) for a given set of molecules in rational drug design and related applications, which have already been successfully applied to different classes of compounds. As potential-based approaches, CoMFA and CoMSIA methods correlate the biological data with the field properties generated around the aligned molecules using certain probes at a space grid, and can offer information of interaction between ligand and and putative receptors. The success of a CoMFA and CoMSIA study is usually determined by the quality of the alignment rule, or the choice of superimposition of the molecules in the study. Many successful 3D-QSAR models have been generated employing a wide variety of alignment procedures. Thus, in this work, both the ligand-based (i.e., database alignment and field fit alignment) and receptor-based (docking alignment) superimposition schemes were employed to build 3D-QSAR models, for purpose of comparing and seeking for the optimal one between the two alternatives for the 3D-QSAR model development.

In the present study, the calculated octanol/water coefficient ($\log P$) parameter was included as an additional descriptor to determine if its adding improves the statistical quality of the standard CoMFA and CoMSIA models and, if so, to what degree. Additionally, to maximize the predictive ability of the present 3D-QSAR model, we also explored several variations in establishing the CoMFA and CoMSIA models with respect to the alignment scheme, the partial charge formalism, region focusing, etc. We also employed molecular dynamics calculations to validate the reliability of the docking model. The developed models will be of help for predicting the activity of new PKCθ inhibitors.

2. Experimental

2.1. Datasets

A large dataset of 263 PKCθ inhibitors and experimental IC_{50} values were collected from the literatures [11–22]. These large series of compounds were synthesized by Wyeth Research continuous efforts. The PKCθ inhibitory activity of all these molecules was measured by the same assay on human PKCθ by employing a modified IMAP protocol from molecular devices [11]. Here, the converted molar pIC_{50} ($-\log IC_{50}$) values, spanning 4 log units, were used as the dependent variables in the QSAR regression analysis to improve the normal distribution of the experimental data points. The whole data set was divided into a training set of 197 compounds to develop the models and a test set of 66 compounds to evaluate the models in an approximate ratio of 3:1. To diversify the training and test sets, all compounds were grouped so that molecules possessing diverse functionalities and biological activity of wide range were included in both sets. The mean biological activity of the chosen training and test set molecules was 7.63 and 7.62, respectively. All information about the structures and activity of the 263 compounds are provided in Table S1 (Supplementary Information), with several representative ones together with their activity shown in Table 1.

2.2. 3D-QSAR modeling and structure alignment

The three-dimensional structures of all compounds in the dataset were obtained using the SYBYL 6.9 program (SYBYL 6.9 Tripos Inc.). Partial atomic charges calculated using the Gasteiger–Hückel method were assigned to each atom and the energy minimization of each molecule was performed using Powell method and Tripos standard force field with a distance-dependent dielectric function. The minimization was terminated when the energy gradient convergence criterion of 0.05 kcal/mol was reached or when the 1000-step minimization cycle limit was exceeded.

Molecular alignment of the compounds is a crucial step in the development of CoMFA and CoMSIA models, since the prediction accuracy of the model depends greatly on the structural alignment of the molecules. In this work, for comparison and development of the optimal models, three different alignment methods including two ligand-based, i.e., the database and field fit alignments, and one receptor-based (the docking-based alignment) superposition approaches were performed.

2.2.1. Database alignment

The superimposition of the molecules was based on an attempt to minimize the root-mean-squares differences in the fitting of the selected common framework with a template molecule. Presently, compound 174 who has the highest activity was used as the template, and all other molecules were superimposed to the common scaffold on this template using the database alignment option available in SYBYL. All molecules were aligned based on the assumption that the common substructure contributes equally to the biological activity of the molecules and can be assumed together as a constant [30]. Conformations which exhibited minimum of root-mean-squares after superimposition procedure were selected and stored in the database for the next step. Fig. 1A shows the common part for the database alignment and the resulting alignment model is shown in Fig. 1B.

2.2.2. Field fit

The field fit procedure was used as the second alignment criterion to increase field similarity within a series of molecules. In the field fit operation, the root-mean-squares difference in the sum of steric and electrostatic interaction energies averaged across all (possibly weighted) lattice points between molecules in the training set molecule and template molecule was minimized to find the best fit. The same template molecule (compound 174) was also used in field fit alignment. All molecular conformations obtained from superimposition were used to calculate the steric and electrostatic field around the molecules to find the best field fit.

2.2.3. Molecular docking

To explore the interaction and illustrate the accurate binding model for the active site of PKCθ with ligands, molecular docking was performed by using the Surflex-dock module (V 2.51) of another advanced version of SYBYL package (X 1.1). This module utilizes a so-called "whole" molecule alignment algorithm based on the morphological similarity between the ligand and the target [31]. This docking approach aligns the ligand to a "protomol" (called also idealized ligand) in the active site of the target. For our studies, the X-ray crystal structure of PKCθ with high resolution (2.0 Å) was retrieved from RCSB Protein Data Bank (PDB entry code: 1XJD). Prior to docking, all original ligands and water molecules in 1XJD were extracted from the crystal structure. Hydrogen atoms were added in standard geometry using the Biopolymer module