Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Medicinal Chemistry 41 (2006) 373–378
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://france.elsevier.com/direct/ejmech

Original article

3D QSAR for GSK-3β inhibition by indirubin analogues

Na Zhang [a], Yongjun Jiang [b,*], Jianwei Zou [b], Bing Zhang [a], Haixiao Jin [a], Yanhua Wang [a], Qingsen Yu [a,b]

[a] *Department of Chemistry, Zhejiang University, 310027 Hangzhou, Zhejiang Province, China*
[b] *Key Laboratory for Molecular Design and Nutrition Engineering of Ningbo City, Ningbo Institute of Technology, Zhejiang University, 315100 Ningbo, Zhejiang Province, China*

Received 23 May 2005; received in revised form 20 October 2005; accepted 26 October 2005
Available online 25 January 2006

Abstract

Glycogen synthase kinase 3 (GSK-3) plays an important role in a diverse number of regulatory pathways by phosphorylation of several different cellular targets and its inhibitors have been evaluated as promising drug candidates. Indirubin analogues show favorable inhibitory activity targeting GSK-3β, which is closely related to the property and position of substituents. Two methods were used to build 3D-QSAR models for indirubin derivatives. The conventional 3D-QSAR (ligand-based) studies were performed based on the lower energy conformations employing atom fit alignment rule. The receptor-based 3D-QSAR models were also derived using bioactive conformations obtained by docking the compounds to the active site of GSK-3. Conclusions of models based on two methods are similar and reliable. The results indicate that both ligand-based and receptor-based are feasible tools to build 3D-QSAR models. Contour maps of the receptor-based CoMSIA model ($q^2 = 0.766$, $r^2 = 0.908$, N (number of components) = 5) including the steric, electronic and hydrophobic fields were taken as representative to explain factors affecting activities of inhibitors.

Keywords: GSK-3β; Indirubins; Molecular docking; 3D QSAR; CoMFA; CoMSIA

1. Introduction

Glycogen synthase kinase 3 (GSK-3) is a cytosolic serine/threonine protein kinase found in two closely related isforms, GSK-3α and GSK-3β, which are expressed ubiquitously in mammalian tissues [1]. Both isoforms have nearly identical biochemical functions and substrate affinities, sharing a sequence identity of some 95% in the catalytic domain [2]. Recent reviews give a more detailed description of the roles GSK-3 plays in the different cellular pathways including cell differentiation, cellular growth and proliferation, metabolic processes, apoptosis control, and mechanisms involved in neuronal function [3,4]. Consequently, GSK-3 could be regarded as a potential therapeutic target and its small molecule inhibitors may have a therapeutic potential in numerous human diseases such as cancer, type 2 diabetes and neurological diseases such as bipolar disorders or Alzheimer's disease [5,6].

Structure–activity relationship studies define some structural requirements for potency of inhibitors. And the 3D-QSAR studies of pallones [7], maleimides [8], and aloisines [9] have been reported. Indirubin is an active ingredient of Danggui Longhui Wan, a traditional Chinese medicine recipe used to treat chronic diseases such as leukemias [10]. A variety of indirubins were identified as powerful inhibitors of GSK-3 while other structurally related indigoids were inactive [11]. It is noteworthy that indirubin's analogues such as indiribin-3′-oxime, 5-chloro-indirubin etc. show better pharmacological effect and less toxicity than those of indirubin. The biological activities are closely related to substituents' property and position of indirubins. However, there is no relevant study concerning QSAR studies of indirubin derivates presently, relationships between structures and activities of indirubins could guide us to understand their pharmacological mechanism and design more favorable inhibitors.

* Corresponding author. Tel.: +86 574 8822 9517; fax: +86 574-8822 9516.
E-mail address: yjjiang@nit.net.cn (Y. Jiang).

0223-5234/$ - see front matter © 2006 Elsevier SAS. All rights reserved.
doi:10.1016/j.ejmech.2005.10.018

In 3D-QSAR studies, molecular conformations and alignment rules are usually one of key steps for the meaningful results. Molecular docking is an attractive way to get putative bioactive conformations for building 3D-QSAR models, and several applications of docking alignment have been reported in [12–14]. In this paper, lower energy conformation with atom fit alignment and docking conformation are applied to seek the structure–activity relationship of indirubins. Further, we will focus on the models based on docking conformation to explore the favorable or unfavorable factors affecting the inhibitory activity.

2. Methods

2.1. Molecules preparation

From available series of 42 compounds, a training set and a test set including, respectively, **34** and **8** compounds have been selected randomly. The geometries of molecules except indirubin-3′-oxime (No. 16, directly extracted from PDB ID: 1Q41) were constructed using BioMedCache version 6.0 [15] with AM1 method to obtain lower energy conformations. Gasteiger–Hückel charges were assigned on inhibitors by the software package Sybyl6.8 [16]. The structures and experimental activities ($pIC_{50} = -\log_{10}{}^{IC}{}_{50}$) are listed in Table 1 [17] (the test set with asterisk).

2.2. Molecular modeling

Computational docking was performed using Sybyl 6.8 and Autodock3.05 [18]. To explore the best orientations of substituents, inhibitors were docked into the active site of GSK-3β. The crystal structure of indirubin-3′-oxime complexed with GSK-3β taken from the protein data bank (PDB ID:1Q41) was used to optimize docking parameters and test the docking quality achieved by Audock3.05. Firstly, lost residues of GSK-3β were added by loop search method of Sybyl 6.8. GSK-3β was checked for polar hydrogens and assigned for partial atomic charges, the PDBQs file was created, and the atomic solvation parameters were also assigned for the macromolecule. The torsion angles of inhibitors were defined in order to explore conformations during the process of docking. Secondly, the 3D-grid maps with grid spacing 0.375 Å and $60 \times 60 \times 60$ points were created by the AutoGrid algorithm to evaluate the binding energies between the inhibitors and the GSK-3. During docking processes, the number of generations, energy evaluations, and docking runs were set to 370,000, 1500,000 and 10, respectively. Finally, the best docking conformation of each inhibitor was selected according to the criteria of interacting energy combined with geometrical matching quality for 3D-QSAR studies. Then, other cases were docked sequentially into the binding pocket of GSK-3β using the parameters previously optimized.

2.3. CoMFA and CoMSIA 3D QSAR models

CoMFA and CoMSIA studies require the bioactive conformations and suitable alignment rule. Receptor-based method means the best conformations of all inhibitors except No. 16 (bioactive conformation extracted from 1Q41) clustering in the binding pocket of receptor obtained from molecular docking were directly used to build models. For ligand-based method, the lower energy conformations of inhibitors were superimposed to alignment template compound **16**, indirubin-3-oxime in the crystal complex of 1Q41, reference atoms are four rings almost in one plane and the oxygen atom shown in Table 1.

The CoMFA steric and electostatic fields were calculated at grid points using the Lennard–Jones and coulomb potential functions of the tripos field with a default energy cutoff, 30 kcal mol^{-1}. An sp^3 carbon atom with a charge of + 1.0 and a VDW radius of 1.52 Å was probed on 2 Å spaced lattice, which extended 4 Å units beyond the dimensions of aligned molecules in all directions. For CoMSIA, steric, electrostatic and hydrophobic three field descriptors among the five field descriptors (steric, electrostatic, hydrophobic, hydrogen bond donor and acceptor) were evaluated using the probe atom with + 1 charge, radius of 1 Å and + 1 hydrophobicity on the same lattice as the CoMFA used, since the additions of hydrogen bond donor and acceptor fields in CoMSIA model do not significantly improve the model.

PLS regression analysis was used to explore a linear relationship between field descriptors (as independent variables) and biological activities (pIC_{50} as dependent variables). The leave-one-out (LOO) and leave-some-out (LSO, 10 groups) cross-validation with 2 kcal mol^{-1} column filtering were performed to determine optimum number of components and cross-validated coefficient q^2, which indicates the consistency and predictiveness of models. Then, non-cross-validation was performed to derive the final PLS regression models.

3. Results and discussion

3.1. Molecular docking

One angstrom RMS deviation between the docked and crystal structure of indirubin-3′-oxime (Fig. 1), as well as the hydrogen bonds predicted by molecular docking just like those in the crystal structure validates the reliability of these docking parameters. The crystal structure of indirubin-3′-oxime complexed with GSK-3β shows some characteristic of binding pocket [19] divided into three parts. The pocket sandwiches the inhibitor between Ile62, Val70, and Ala83 on the top and Leu188 on the bottom. The hinge segment is formed using the chain of Leu132-Asp133-Tyr134-Val135-Pro136. There are three hydrogen bonds involved Asp133 and Val135 with indirubin-3′-oxime. The N1 atom of the inhibitor, as the donor, forms a hydrogen bond with the carbonyl oxygen of Asp133. The backbone nitrogen and carbonyl oxygen of Val 135 are involved to form the other two hydrogen bonds with O2 and