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Identification of CK2 inhibitors with new scaffolds by a hybrid virtual screening approach based on Bayesian model; pharmacophore hypothesis and molecular docking

Lei Di-wu[a], Lin-Li Li[b,*], Wen-Jing Wang[a], Huan-Zhang Xie[b], Jiao Yang[b], Chun-Hui Zhang[a], Qi Huang[a], Lei Zhong[b], Shan Feng[a], Sheng-Yong Yang[a,*]

[a] State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, West China Medical School, Sichuan University, No. 1, Keyuan 4 Road, High Tech Park, Chengdu, Sichuan 610041, China
[b] West China School of pharmacy, Sichuan University, No. 17, Section 3, Renmin South Road, Chengdu, Sichuan 610041, China

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ABSTRACT

Protein kinase casein kinase 2 (CK2), a member of the serine/threonine kinase family, has been established as one of the most attractive targets for molecularly targeted cancer therapy. The discovery of CK2 inhibitors has thus attracted much attention in recent years. In this investigation, a hybrid virtual screening approach based on Bayesian classification model, pharmacophore hypothesis and molecular docking was proposed and employed to identify CK2 inhibitors. We first established a naïve Bayes classification model of CK2 inhibitors/non-inhibitors and pharmacophore hypotheses of CK2 inhibitors. The docking parameters and scoring functions were also optimized in advance. The three virtual screening methods were sequentially used to screen two large chemical libraries, Specs and Enamine, for retrieving new CK2 inhibitors. Finally 30 compounds were selected from the final hits for *in vitro* CK2 kinase inhibitory assays. Five compounds with completely novel scaffolds showed a good inhibitory potency against CK2, which have good potentials for a future hit-to-lead optimization.

1. Introduction

Protein kinase CK2 (an acronym of 'casein kinase 2') is a ubiquitous eukaryotic serine/threonine protein kinase distributed in the nucleus and cytoplasm [1–3]. CK2 exists as a tetramer composed of two catalytic isoforms (CK2α and CK2α′) and two regulatory β subunits (CK2β). It has been established that CK2 phosphorylates multiple substrates and plays important roles in cell proliferation, transformation, apoptosis and senescence [1,2,4]. Dysregulation of CK2 can lead to human diseases [5], particularly in cancers [6]. Indeed CK2 has been found to over-express in all the cancers that have been examined [7]. The presence of elevated CK2 in cancer appears to correlate with the cancer. Recent studies have indicated that CK2 is also a potent suppressor of apoptosis [8,9], further raising its key importance in cancer cell phenotype. All of these show that CK2 is an important target for cancer therapy. In addition, it has been found that some viruses use CK2 to phosphorylate their own proteins [5,10], hence CK2 is also considered as an attractive antivirus target.

Due to the potential therapeutic value of CK2 inhibitors in cancer as well as antivirus, many academic institutes and pharmaceutical companies have been involved in the development of CK2 inhibitors. Up to now, more than one hundred small molecule inhibitors against CK2 have been reported publicly [11–18]. However, except for very few compounds including CX-4945 (see Fig. 1) that has entered Phase I clinical trials for advanced solid tumors and multiple myeloma treatment [19], most of them have just moderate or weak inhibition potency. Additionally, the known CK2 inhibitors, especially those with higher potency, have very limited structural diversity; several known CK2 inhibitors with different chemical scaffolds are shown in Fig. 1. Thus, discovering more potent CK2 inhibitors especially with new scaffolds is still needed and important, which could offer more candidates for drug development targeting CK2.

Currently, virtual screening (VS) has emerged as a complementary method to high-throughput screening of large chemical databases, and has been widely used in the drug discovery. VS methods are designed for searching large compound databases *in silico* and selecting a limited number of candidate molecules for testing to identify novel chemical entities that have the desired biological activity. There are two classical VS approaches: molecular docking-based VS (DB-VS) and pharmacophore-based VS (PB-VS) [20,21]. These methods have been broadly applied and

* Corresponding authors. Tel.: +86 28 85164063; fax: +86 28 85164060.
E-mail addresses: ysylilinli@sina.com.cn (L.-L. Li), yangsy@scu.edu.cn (S.-Y. Yang).

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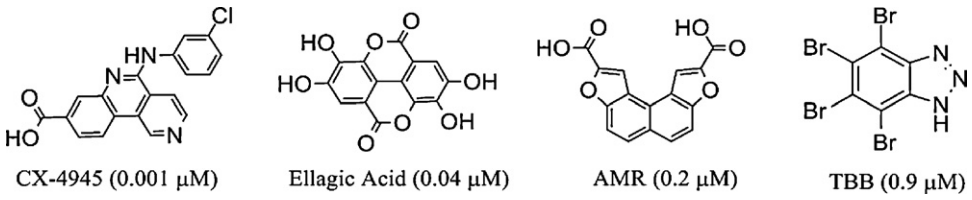

Fig. 1. Chemical structures of typical CK2 kinase inhibitors with novel scaffolds. IC_{50} values against CK2 are given in parentheses.

been becoming a major source of lead compounds in drug discovery. However, these methods are individually far from perfect in many aspects, including a low hit rate and a low enrichment factor, as well as a high false positive rate [22–24]. A combination of DB-VS and PB-VS in a hybrid protocol has been demonstrated to mutually compensate for these limitations and capitalize on their mutual strengths [25,26]. In addition, recent studies have shown that introduction of other newly emerging methods based on statistical learning theory could further increase the performance of the classical VS methods. For example, we recently employed a hybrid VS protocol of PB-VS and DB-VS, as well as support vector machine (SVM)-based VS (SB-VS) to discover novel potent Pim-1 inhibitors [23]. The results showed that the hybrid VS protocol had a much higher performance compared with individual PB-VS and DB-VS or their combination in terms of the screening speed and enrichment factor.

In this investigation, we shall adopt a new hybrid VS protocol, which involves the classical PB-VS and DB-VS, as well as naïve Bayesian (NB) classification model-based VS (BB-VS), to identify novel CK2 inhibitors. A NB classification model or naïve Bayes classifier is a probabilistic classifier based on applying Bayes' theorem with strong independence assumptions. It is one of the most versatile machine learning algorithms, and has been widely applied to text classification [27,28], assignment of rRNA sequences [29], and recently to drug discovery [30–32]. We shall first establish and validate a NB classification model of CK2 inhibitors and non-inhibitors, followed by development of a pharmacophore hypothesis based on the known CK2 inhibitors. Then BB-VS, PB-VS and DB-VS will be sequentially applied to screen Specs and Enamine chemical libraries. We shall select some compounds from the final hits obtained in the screening to carry out further *in vitro* CK2 kinase assays.

2. Materials and methods

2.1. Bayesian classification modeling

The Bayesian classification model was developed using Discovery Studio (DS) version 2.5.5 (Accelrys Inc., San Diego, CA). A total of 209 ATP-competitive compounds were collected. The training set consists of 102 compounds, including 73 active and 29 inactive compounds (see Table S1 in Supporting information). The descriptors used include AlogP, molecular weight, number of aromatic rings, number of hydrogen bond acceptors, number of hydrogen bond donors, number of rings, number of rotatable bonds, molecular fractional polar surface area and functional class fingerprints (FCFP_6 descriptors) [33]; these descriptors were chosen since they represent the most important chemical features. Validation of the Bayesian model was carried out by leave-one-out cross validation method and external testing set, which contains 77 active and 30 inactive compounds (see Table S2 in Supporting information). Performances of the Bayesian model were evaluated by calculating true positives (TP), true negatives (TN), false negatives (FN), false positives (FP), sensitivity (SE), and specificity (SP).

2.2. Pharmacophore modeling

Pharmacophore modeling was carried out by using "common feature pharmacophore generation" protocol (HipHop algorithm) [34] in DS 2.5.5. The seven active CK2 inhibitors together with two CK2 non-inhibitors (see Fig. 2) were selected to form the training set. The most active compound, CX-4945, was taken as 'reference compound' specifying a 'principal' value of 2 and a 'MaxOmitFeat' value of 0. The 'principal' and 'MaxOmitFeat' values of the remaining 6 compounds were set to 1 and 0, respectively. The 'principal' and 'MaxOmitFeat' values of compounds 6 and 7 were set to 0 and 1, respectively. The 'excluded volume' value was set to 3. Three features, including hydrogen-bond acceptor, hydrogen-bond donor and hydrophobic, were selected as the initial input features. The "minimum interfeature distance" value was set to 2.5 Å. The other parameters were kept at their default values.

2.3. Docking study

All of the molecular docking studies were carried out by GOLD 4.0 [35]. GOLD adopts the genetic algorithm to dock flexible ligands into binding site of protein. The crystal structure of CK2 combined with emodin (PDB ID: 3BQC) [36] was chosen as the structure of reference protein. All water molecules in the crystal structure except one, which is a conserved water molecule, were removed. The Charmm force field [37] was assigned. The binding site was defined as a sphere containing the residues that stay within 7.5 Å from the ligand, which is large enough to cover the ATP binding region at the active site. Hydrogen atoms were added by DS 2.5.5.

2.4. Chemistry

Compounds that were selected to conduct *in vitro* kinase inhibitory assays were purchased from the market, which are shown in Table S4 (see Supporting information). According to the information provided by the suppliers, the purity of these compounds is >98%, which were confirmed by HPLC (high performance liquid chromatography) analysis.

2.5. CK2 kinase inhibitory assays

CK2 kinase inhibitory assays were performed using labeled γ-33P-ATP method, and the incorporation of labeled phosphate onto the peptide RRRDDDSDDD substrate was monitored. The kinase buffer used contains: 20 mM HEPES, pH 7.6, 0.15 M NaCl, 0.1 mM EDTA, 5 mM DTT, 0.1% Triton X-100, 165 μM RRRDDDSDDD, 10 mM MgAcetate and [γ-33P-ATP] (specific activity approx. 500 cpm/pmol, Km Assay concentration (15 μM) was used). The reaction was initiated by the addition of the Mg ATP mix. After incubation for 40 min at room temperature, the reaction was stopped by the addition of 5 μL of a 3% phosphoric acid solution. 10 μL of the reaction was then spotted onto a P30 filtermat and washed three times for 5 min in 75 mM phosphoric acid and once in methanol prior to drying and scintillation counting.

The inhibition profiles of the test compounds were expressed as the percentage of the residual kinase activity for an inhibitor