In silico Screening for Signalosome Inhibitors

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Introduction

The COP9 signalosome (CSN), a multi enzyme complex, is involved in cell cycle regulation. CSN possesses kinase activity that phosphorylates proteins such as p53 and c-Jun [1] and is therefore an interesting therapeutic target for anti-tumor drugs. There are known inhibitors (e.g. Curcumin) of the kinase activity associated with the signalosome but they exhibit low binding constants and specificity. Using them we have carried out a 3D similarity screening for our in-house database of about 10^6 available organic compounds. The superposition procedure is very fast enabling the explicit consideration of conformers reflecting the structural flexibility. Upon selection we used a number of property filters to achieve drug-like lead compound [2]. First experimental tests show significant improvement of inhibition.

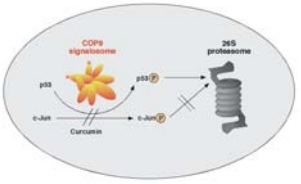

Figure 1: COP9 signalosome (CSN) mediated degradation of p53 and c-Jun. [3]

Methods

The search for new inhibitors is based on a 3D similarity screening. By using known inhibitors as lead compounds the screening is performed with our in-house database. A selective search can be ensured by multifaceted settings of our search program. There are tolerance-settings in terms of molecule-size and deviation of atom-number. Further on the hits are assessed by rms (root mean square: represents the similarity of two structures when they are superimposed at the best or the average deviation of the gaging surface to the target surface). An ensuing 2D screening determines the properties of the potential inhibitors for drug-design. Important attributes are the molecular weight, the number of H-donors and –acceptors and the solubility in different solutions. These attributes are combined in the Lipinski-Rule-of-5 [4]. Compounds who follow these rules are afterwards validated in experimental tests.

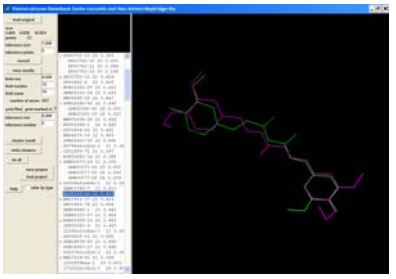

Figure 2: An example for an *in silico* screening is shown. On the left side a variety of tolerance-settings specify the search. The middle part shows the clustered hits of the search and on the right side one can see the superposition of the target molecule (green) and the potential inhibitor (red).

Results

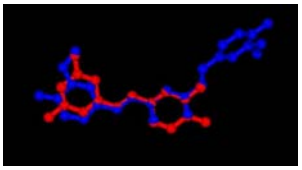

Figure 2: Superposition of Curcumin, shown in blue and Piceatannol shown in red. Curcumin is a pigment from the medical plant *curcuma domestica* and piceatannol is a hydroxylated analog of the chemopreventive agent resveratrol. The superposition was calculated by an overlay program and drawn by DS ViewerPro.

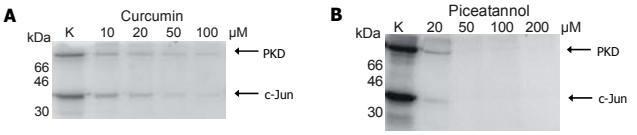

Figure 3: PKD phosphorylates c-Jun (~41kDa). Recombinant c-Jun was used for *in vitro* kinase assays. (**A** + **B**) Recombinant c-Jun was incubated with recombinant PKD in presence of [γ- ^{32}P]ATP and Curcumin (A) resp. Piceatannol (**B**) in different concentrations (K (0 µM), 10, 20, 50, 100 µM). After 1 h incubation at 37°C the reaction mix was separated by SDS-PAGE and the dried gel was autoradiographed. The autoradiography shows a decreased phosphorylation of c-Jun by increasing inhibitot concentrations.

Inhibitor	CSN	CK2	PKD
Curcumin	2.6	11.8	4.1
Piceatannol	1	2.46	0.46

Table 1: K_i values (µM) for the inhibition of CSN-associated kinases, recombinant CK2 and PKD by curcumin [5] and piceatannol.

Conclusions

- With the aid of *in silico* screening we found various potential inhibitors
- *in vitro* experiments show an improved inhibition compared to known inhibitors
- *in vivo* experiments are carried out just now
- *in silico* Screening is a powerful method to evaluate more effective inhibitors

References

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