Virtual screening and structure based drug design of new Fes/Fps kinase domain inhibitors.

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Motivation

The fps/fes proto-oncogene (fps, Fujinami poultry sarcoma; fes, Feline sarcoma) encodes a structurally unique member of the non-receptor protein-tyrosine kinase (PTK) family. Its expression was initially detected in myeloid haematopoietic cells and vascular endothelial cells, but recent works has shown Fes expression also in neurons. Activated form of this kinase, can mediate cellular transformation; moreover, it has been demonstrated that Fes PTK is involved in the regulation of cell-cell and cell-matrix interactions mediated by adherens junctions and focal adhesion. Fes PTK consists of a FCH (Fps/Fes/Fer/CIP4 Homology) N-terminal domain, which distinguish Fes from all the other PTK, followed by three coiled-coil domains, a SH2 domain (Src-homology-2) and a C-terminal kinase domain. We aim to solve the crystal structure of the kinase domain of Fes PTK and to design molecular inhibitors by rational drug design and in-silico docking and molecular dynamics simulations.

Tyrosine kinase inhibitors are, in fact, among the most novel anticancer drugs in current development. Here we report the results of a virtual screening study performed to identify novel inhibitors of Fes kinase domain. Moreover, the expression, purification and crystallization conditions as well as the preliminary X-ray data collection parameters will be discussed.

Methods

The catalytic domain of Fes have been engineered in order to carry a histidine-tag (6His-tag) at the N-terminal and a FLAG-tag at its C-terminal, to facilitate protein purification. The recombinant plasmid was harboured by E. coli M15pREP4 bacteria leading to a yield of about 5.5 mg of protein per litre of culture. Protein concentrated to 5 mg/ml crystallized using vapour diffusion techniques, by equilibrating 600l solution containing 1.6M MgSO4 and 0.1M MES pH 6.5 with a 2 l drop made by 1 l of the reservoir solution and 1 l of protein mixed with AMP-PNP (Adenylylimido-diphosphate) in a 10:1 AMP-PNP:protein ratio. Under these conditions small crystals were observed after two weeks at 300K.

In parallel, we performed docking simulations using the program AUTODOCK 3.5. A series of 20 new chemical entities endowed with micromolar cytotoxic activity in cell based assay, has been docked inside Fes/Fps ATP binding site. The predicted bioactive conformations have been evaluated by their Ki values and the most promising complexes were further refined through a simulated annealing protocol (starting temperature 5000 K) followed by powell minimization (120 steps).

Results

The virtual screening allowed us to identify potential Fes inhibitors endowed with micromolar predicted Ki values. The selected derivatives are currently under evaluation in enzyme-based assay. Crystal diffraction data have been collected up to a resolution of 3.8 ï¿½ the search for structural solution and for a better diffracting crystal are in progress.

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