

EVALUATION OF 3-CARBOXY-4-(1H)-QUINOLONES AS INHIBITORS OF HUMAN PROTEIN CASEIN KINASE 2

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ABSTRACT

Inhibition of protein kinase CK2 by small organic molecule inhibitors, besides of its application in scientific research, could be used in anticancer, antiviral and anti-inflammatory therapeutics. Here we report and discuss the inhibitory activity of the class of 3-carboxy-4-(1H)-quinolones towards the human CK2. The inhibitors were found by virtual screening and further *in vitro* evaluation of Otava collection containing around 70,000 compounds. Two compounds, 5,6,8-trichloro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (L1) and 4-oxo-1,4-dihydrobenzo[h]quinoline-3-carboxylic acid (L2), were the most potent compounds of the series (IC₅₀ values 0.3 and 1.0 μM respectively). According to the obtained data and with the aid of theoretical calculations, we developed a structural model describing the key features of (1H)-quinolones binding in CK2 active site. In our model, the critical points of interaction are the hydrogen bonding between the ligand 3-carboxy group and residues Lys68, Asp175 and Glu81 of the CK2 ATP-binding cleft and also hydrophobic contacts with the set of hydrophobic residues, among of them the Val66, Ile174 and Phe113 are the most important. The proposed binding mode appears to be in good agreement with X-ray data for the complex of CK2 - [[5-oxo-5,6-dihydro-indolo[1,2-a]quinazolin-7-yl]acetic acid] (IQA), a known CK2 inhibitor [1]. *In vitro* tests with CDK5 and GSK3 kinases indicated the absence of activity of the obtained CK2 inhibitors, indicating at least some degree of selectivity towards CK2.

RESULTS

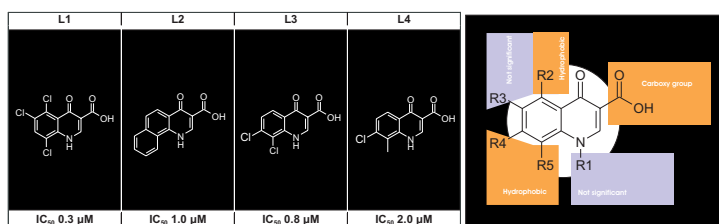


Fig.1 Structure and IC₅₀ of active ompounds

Fig.2 Structure - activity relationships of 3-Carboxy-4-(1H)-Quinolones

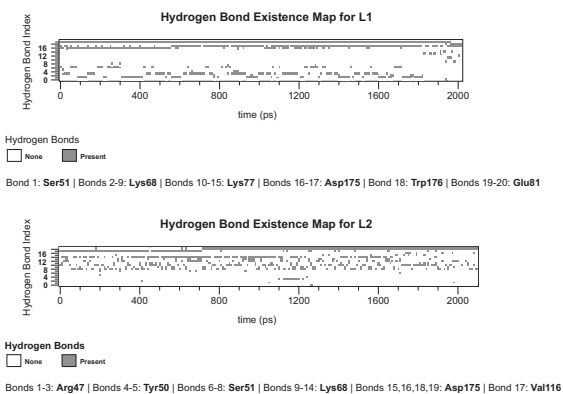


Fig.3 Hydrogen bond existence maps of L1-CK2 and L2-CK2 complexes upon 2 ns molecular dynamics simulation

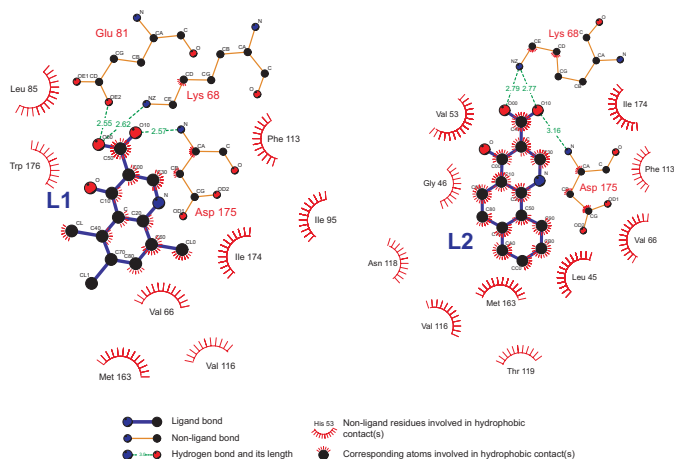


Fig.4 Schematic plots of L1 and L2 binding modes. The plots were produced with LigPlot software.

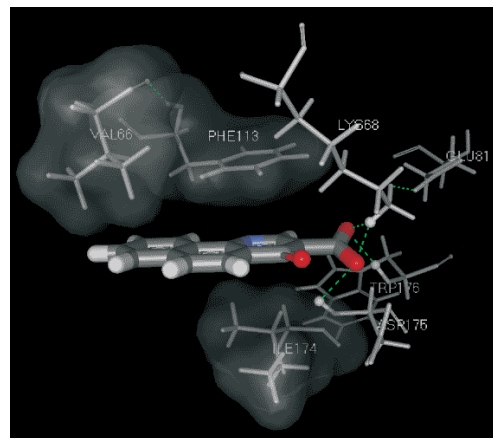


Fig.5 3D model of interaction of L2 and CK2. Molecular dynamics snapshot.

CONCLUSIONS

- 3-carboxy-4-(1H)-quinolones were identified as a new class of CK2 inhibitors.
- Docking and molecular dynamics studies allowed to propose a binding model in which the activity of the disclosed inhibitors is explained by forming of hydrogen bonds between the ligand 3-carboxy group and residues Lys68, Asp175 and Glu81 of the CK2 ATP-binding cleft as well as by hydrophobic contacts with a set of hydrophobic residues, among which Val66, Ile174 and Phe113 are the most important.
- Obtained model correlates well with X-ray data for the complex between CK2 and the IQA inhibitor
- In vitro* tests with CDK5 and GSK3 kinases indicated the absence of activity of the obtained CK2 inhibitors, indicating at least some degree of selectivity towards Ck2.

EXPERIMENTAL SECTION

Virtual screening:

Receptor molecule processing (PDB ID: 1jwh): GROMACS molecular dynamic package (structure optimization, GROMOS96 force field), Connolly MS (surface calculation), and Grid program (calculation of scoring grids) from DOCK 4.0 package.

Ligand molecules processing: GAMESS QM package (assignment of partial charges and complete structure optimization, semi empirical methods), GROMACS MD package (fast structure optimization, GROMOS96 forcefield) and our own program TOPBUILDER (generation of .mol2 formatted 3D structures from ISIS .sdf file).

Flexible docking and scoring: DOCK program of DOCK 4.0 package.

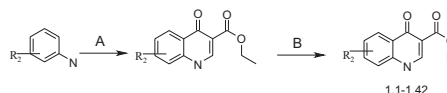
Molecular dynamics

Two nanosecond molecular dynamics simulation of each complex CK2-active ligand was performed with the GROMACS 3.1.2 package (GROMACS forcefield). Initial positions of each ligand into complex were generated by the DOCK. The conditions of the MD simulation consider the water solvent and temperature 300K. Only polar hydrogens of protein and ligand molecules were taken into account.

Chemical synthesis

A series of 4-(1H)-quinolone derivatives were prepared as described at Scheme 1:

Scheme 1. Synthesis of compounds 1.1-1.42.



A: diethylethoxymethylmalonate, 220-230 °C
B: 1. NaOH, H₂O, refluxing; 2. HCl

Biological testing

The selected compounds were tested using *in vitro* kinase assay. CDK1/cyclin B and GSK-3α/β were prepared and assayed as described previously [2]. Selectivity tests were performed at Laboratory of Molecular & Cellular Neuroscience, The Rockefeller University under the direction of Dr. Laurent Meijer and Dr. Olivier Lozach.

REFERENCES

- Sarno, S.; De Moliner, E.; Ruzzene, M.; Pagano, M. A.; Battistutta, R.; Bain, J.; Fabbro, D.; Schoepfer, J.; Elliott, M.; Furet, P.; Meggio, F.; Zanotti, G.; Pinna, L. A. Biochemical and Three-Dimensional-Structural Study of the Specific Inhibition of Protein Kinase CK2 by [5-Oxo-5,6-Dihydroindolo-(1,2-A)quinazolin-7-yl]acetic Acid (IQA). *Biochem. J.* **2003**, *374*, 639-646.
- Meijer, L.; Skaltsounis, A. L.; Magiatis, P.; Polychronopoulos, P.; Knockaert, M.; Leost, M.; Ryan, X. P.; Vanica, C. D.; Bivanlou, A.; Dajani, R.; Taricone, A.; Musacchio, A.; Roe, S. M.; Pearl, L.; Greengard, P. GSK-3 selective inhibitors derived from Tyrain purple indubins. *Chem. & Biol.* **2003**, *10*, 1255-1266.