

**NOVEL SMALL-MOLECULE INHIBITORS OF C-TERMINAL Src  
KINASE (Csk)**

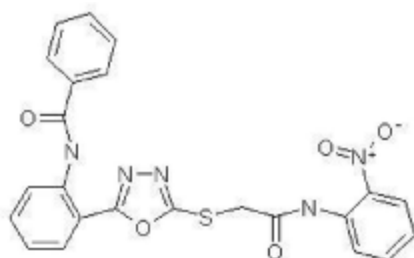
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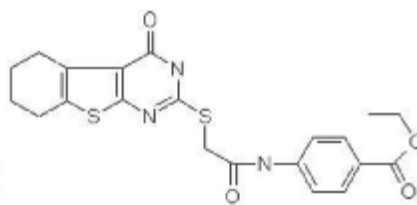
Src-family kinases, a closely related group of nonreceptor tyrosine kinases play key role in eukaryotic signal transduction. The Src kinases are recognized as important targets for therapeutic interventions to treat cancer and immunological diseases.

The specific aim of this work was to select new leads for development of ATP-site directed low molecular weight inhibitors of Src kinase family. A prototypic Src-like PTK, c-terminal Src kinase (Csk) was used as the model enzyme.

About 2000 derivatives of s-triazolo[4,3-a]quinoline-1-thione, thieno[3,2-e][1,2,4]thiazolo[4,3-c]pyrimidine-3-thione, 2-thioxo-2,3-dihydro-1H-thieno[2,3-d]pyrimidin-4-one and [1,3,4]oxadiazole-2-thione were selected from ASINEX compound libraries, based on the initial *in silico* screening procedure. Compounds were filtered according to Lipinski's "Rule of Five"[1]. All modeling studies were performed using the program Sybyl 6.8. Three-dimensional structure of Csk was retrieved from the RCSB Protein data bank. The fast docking of ligands was generated using the program Flex X (Tripos Inc.). As the result, 384 compounds were chosen for the *in vitro* screening using microplate ELISA-based inhibition assay.



**A**  
IC<sub>50</sub> = 1,9 μM



**B**  
IC<sub>50</sub> = 4.1 μM

Of selected compounds, 223 (58%) inhibited catalytic activity of Csk with IC<sub>50</sub> <100 μM; 46 (12%) inhibited the enzyme with IC<sub>50</sub> 0,050-5 μM. Most potent Csk inhibitors contained the [1,3,4]oxadiazole-2-thione nucleus (A), whereas the greatest number of inhibitors was discovered within the derivatives of 2-

thioxo-2,3-dihydro-1H-thieno[2,3-d]pyrimidin-4-one (B). Analysis of 172 most potent Csk inhibitors of groups A and B with Ser/Thr kinase p38, using the identical assay format, did not show any significant decrease in catalytic activity of these enzymes, thus indicating that selected compounds possess specific inhibitory activity towards Tyr kinases. Several potent inhibitors were chosen for further lead optimization.

These findings allow us to assume that two novel templates may offer good perspectives for further research in the protein tyrosine kinase inhibition field.

#### REFERENCE

1. Lipinski, C.A., Lombardo, F., Dominy, B.W. and Feeney, P.J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. **Adv. Drug Deliv. Rev.** 23 (1997) 3-25.