

Structural Insights for Design of Potent Spleen Tyrosine Kinase Inhibitors from Crystallographic Analysis of Three Inhibitor Complexes

Digest 19220318

Summary

Spleen tyrosine kinase (Syk) – lymphocyte signalling molecule
Inhibitors potentially useful for autoimmune and allergic diseases
Novel protein–ligand interactions and protein conformational changes revealed in co-crystallization experiments could guide design of second generation inhibitors

Introduction

Spleen tyrosine kinase (Syk) is a cytosolic non-receptor tyrosine kinase in B lymphocytes, mast cells and macrophages. Phosphorylates downstream targets which triggers cytokine and lipid mediator release.
Future inhibitor design guided by co-crystal structures of Syk complexed with its inhibitors YM193306, the 7-azaindole derivative, and R406.

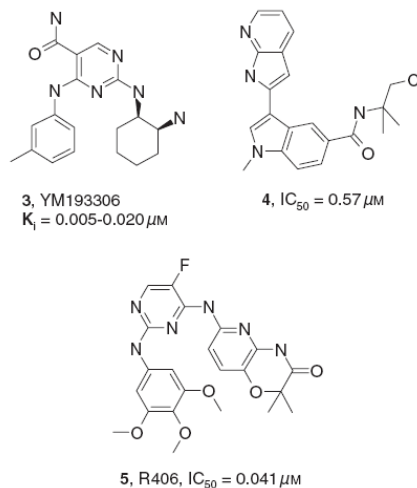
Key new finding in this study

First structure of a 2-substituted 7-azaindole (compound 4 in paper) bound to a protein kinase. Observed interactions with side chains will guide substitutions to improve potency of this weakly active compound.

Methods

TK domain (amino acids 356–635) expressed in Sf9 cells.
Diffraction images from synchrotron data processed with DENZO and SCALEPACK.
Structure model built with MOLOC.

Results



- Syk–YM193306 complex cis-cyclohexyldiamino moiety forming a hydrogen bond via its secondary amine and a salt bridge via its primary amine to the carboxy side chain of D512.
- Contrasts to published Syk crystal structures where the side chain of D512 is not pointing away from but towards the ligand allowing highly favorable protein–ligand interaction motif .
- Primary amino group of YM193306 3 is donating hydrogen bonds to the side chain of N499 and the backbone carbonyl of R498 in the catalytic loop.
- The salt bridge and hydrogen bonding interactions between the cis-cyclohexyldiamino moiety Syk protein contribute to the binding affinity of compound 3 This motif can be used to increase the affinity of Syk (and other) kinase inhibitors.
- Crystal structure of Syk kinase domain complexed inhibitor 4 (first structure of a 2-substituted 7-azaindole bound to a protein kinase).

- Acceptor and donor nitrogens of the 7-azaindole core form H bonds with the backbone NH and carbonyl of the hinge residue A451, respectively.
- Ligand undergoes an aromatic CH–O interaction to the backbone carbonyl of E449 via the azaindole 6-position.
- 1-methylindole-5-amide moiety of 4 is sandwiched between hinge residues G454 / P455 and the Gly-rich loop residues L377 / G378.
- R406 (compound 5) is positioned higher up in the ATP binding pocket compared with YM193306 and 4, inducing a ~3.5 Å shift of the Gly-rich loop away from the ligand.
- Syk–R406 structure presented indicates that direct polar interactions with D512 side chain are not necessary for achieving high potency.

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Key Background References

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