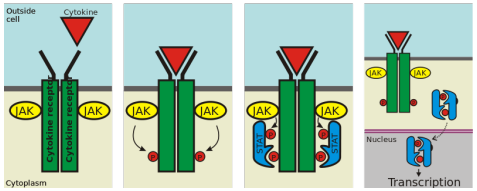
Assessment of Novel JAK2–STAT Pathway Inhibitors with Automated APBS - Brownian Dynamics Simulation Scripts

Anupam Anand Ojha

Department of Chemistry and Biochemistry
University of California San Diego



April 3, 2020

Abstract

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of novel Jak2–Stat pathway inhibitors with extended residence time on target

Huiping Guan [a], Michelle L. Lamb [a], Bo Peng [a], Shan Huang [a], Nancy DeGrace [a], Jon Read [b], Syeed Hussain [b], Jiaquan Wu [c], Caroline Rivard [d], Marat Alimzhanov [c], Geraldine Bebernitz [c], Kirsten Bell [c], Minwei Ye [c], Michael Zinda [c], Stephanos Ioannidis [a,*]

[a] Department of Cancer Chemistry, Oncology Innovative Medicines Unit, AstraZeneca R&D Boston, 35 Gatehouse Drive, Waltham, MA 02451, USA
[b] Discovery Enabling Capabilities and Sciences-Cells, Protein & Structural Sciences AstraZeneca, Alderley Park, Cheshire SK10 4TG, UK
[c] Department of Cancer Bioscience, Oncology Innovative Medicines Unit, AstraZeneca R&D Boston, 35 Gatehouse Drive, Waltham, MA 02451, USA
[d] DMPK Department, Oncology Innovative Medicines Unit, AstraZeneca R&D Boston, 35 Gatehouse Drive, Waltham, MA 02451, USA

- Discovery of novel inhibitors of the JAK2/STAT5 axis, the N-(1H-pyrazol-3-yl) pyrimidine-2-amino derivatives.
- Two lead analogs from this series, compounds 6 and 9, displayed prolonged residence time on JAK2, at an enzymatic level.
- To investigate, a series of python scripts are written which automatically carries out APBS and BrownDye simulations to calculate the second-order rate constants for the ligand-receptor associations and further analyze graphically.

Downloading and Testing the Scripts

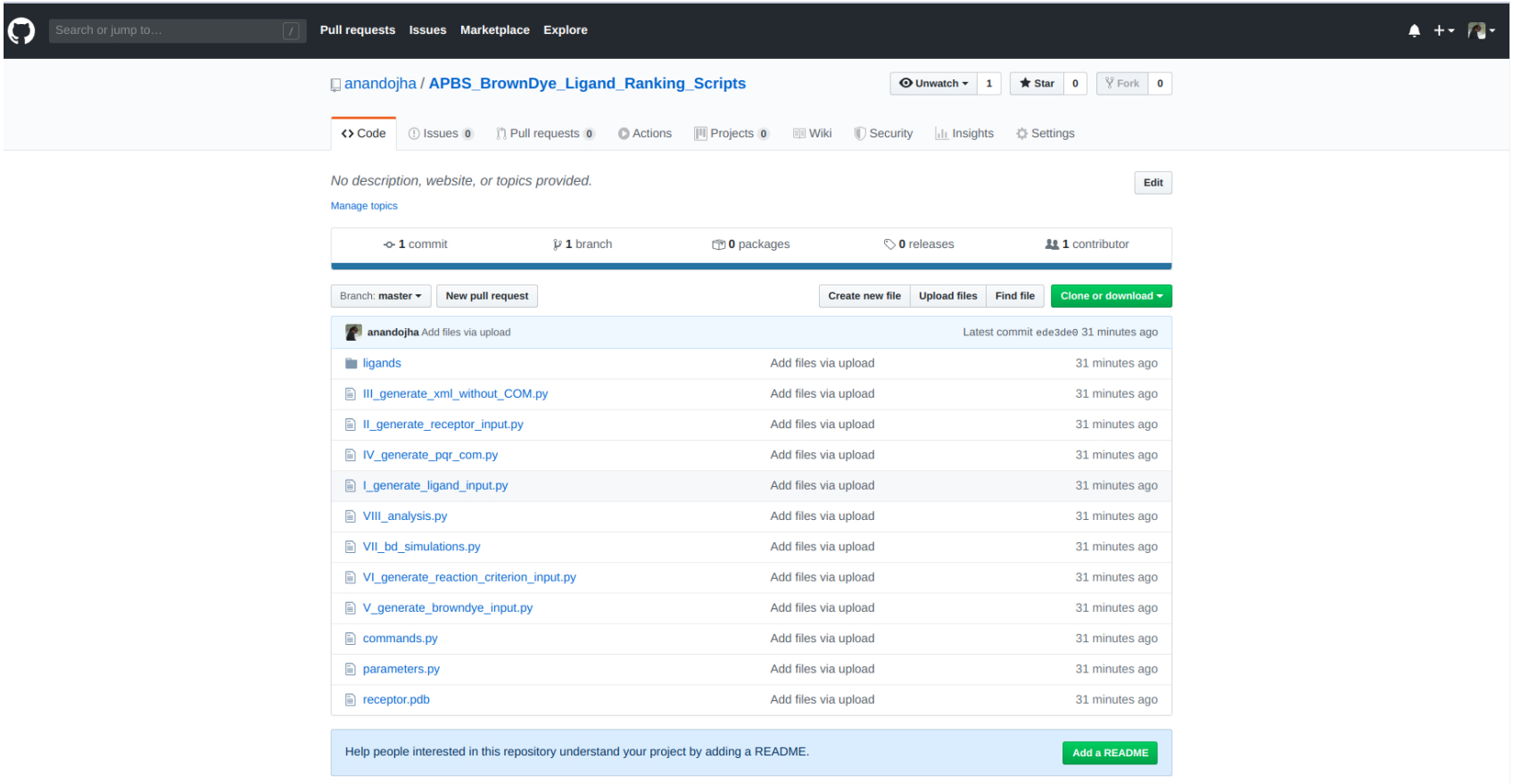


Figure: GitHub Link: https://github.com/anandojha/APBS_BrownDye_Ligand_Ranking_Scripts.git

Installation Requirements

- Python 3 (https://www.anaconda.com/distribution/)
- NumPy (https://numpy.org/)
- pandas (https://pandas.pydata.org/)
- ABPS (https://apbs-pdb2pqr.readthedocs.io/en/latest/apbs/installing.html)
- Browndye (https://browndye.ucsd.edu/)
- VMD (https://www.ks.uiuc.edu/Research/vmd/)

JAK-STAT Signaling Pathway

- JAK (Janus kinase)-STAT (Signal transducers and activators of transcription) pathway is a chain of interactions between proteins in a cell, and is involved in processes such as immunity, cell division, cell death, and tumor formation.
- Signaling pathway communicates information from chemical signals outside of a cell to the cell nucleus, resulting in the activation of genes through transcription.
- There are three key parts of JAK-STAT signaling pathway: Janus kinases (JAKs), signal transducer and activator of transcription proteins (STATs), and receptors (which bind the chemical signals).
- Disrupted JAK-STAT signaling may lead to a variety of diseases, such as skin conditions, cancers, and disorders affecting the immune system.

Mechanism of JAK-STAT Signaling Pathway

Binding of various ligands to cell-surface receptors causes the receptors to dimerize.

↓

This brings the receptor-associated JAKs to close proximity.

↓

JAKs then phosphorylate each other on tyrosine residues which increases the activity of their kinase domains.

↓

Activated JAKs then phosphorylate tyrosine residues on the receptor, creating binding sites for proteins possessing SH2 domains.

↓

STATs binds to the phosphorylated tyrosines on the receptor using their SH2 domains.

↓

STATs are then tyrosine-phosphorylated by JAKs causing the STATs to dissociate from the receptor.

↓

Activated STATs form homo-dimers / hetero-dimers where the SH2 domain of each STAT binds the phosphorylated tyrosine of the opposite STAT.

↓

STAT dimers translocates to the cell nucleus to induce transcription of target genes.

Mechanism of JAK-STAT Signaling Pathway

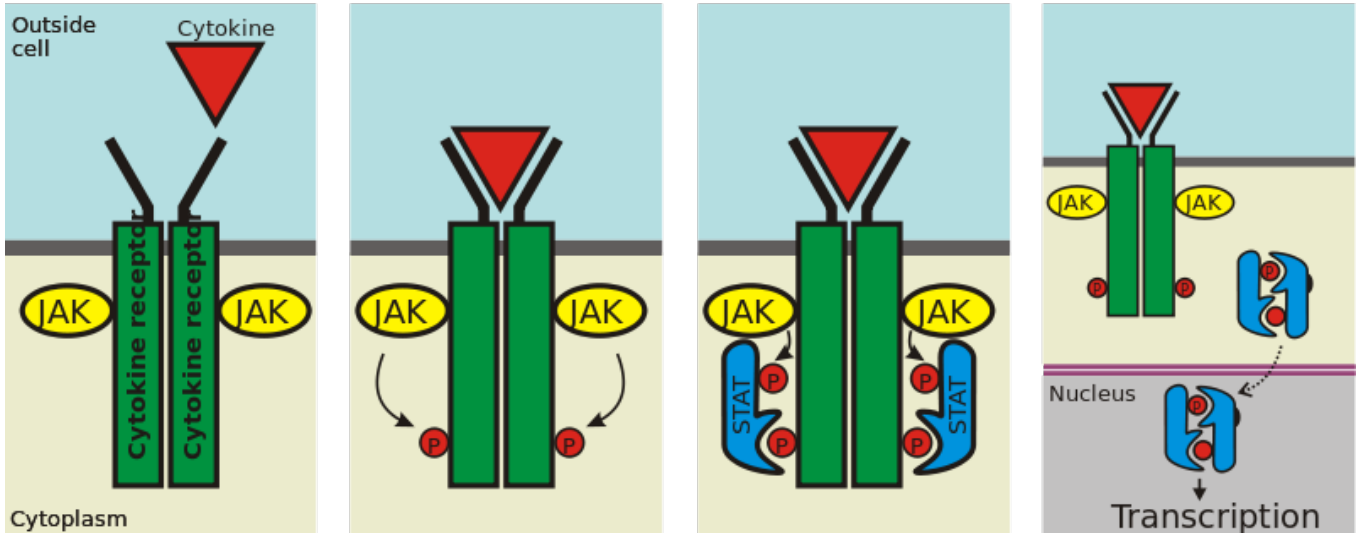


Figure: JAK-STAT signaling is composed of three major proteins: cell-surface receptors, Janus kinases (JAKs), and signal transducer and activator of transcription proteins (STATs). Once a ligand (red triangle) binds to the receptor, JAKs add phosphates (red circles) to the receptor. Two STAT proteins then attach to the phosphates, and then the STATs are phosphorylated by JAKs to form a dimer. The dimer enters the nucleus, binds to DNA, and causes transcription of target genes.

Experimental Findings

- Several ATP-competitive JAK-STAT pathway inhibitors such as N^4 -(thiazol-2-yl) pyrimidine-2,4-diamine have been discovered before which displayed good activity against JAK2, both in in-vitro and in-vivo settings, and sufficient selectivity against JAK3.
- A modification by design is suggested to N^4 -(thiazol-2-yl) pyrimidine-2,4-diamine by moving the thiazol-2-yl amino hinge template from C4 to C2 of pyrimidine B-ring.

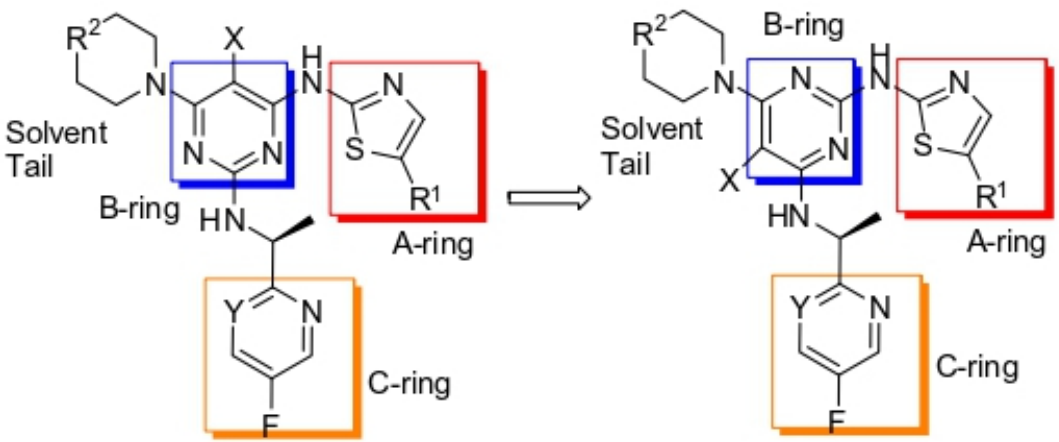


Figure: Thiazol-2-yl amino hinge binder

Modification By Design

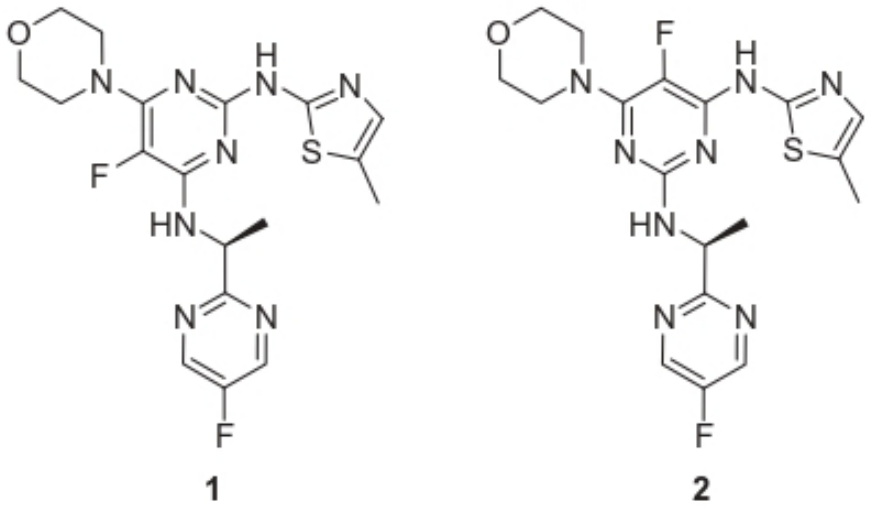


Figure: Compound 1 (Left) and Compound 2 (Right). Modification in compound 2 to derive compound 1 is tolerated since the interaction of thiazoly-3-yl amino group with the kinase hinge residues are retained.

Motivation

- Compound 1 exhibited significant growth inhibition of TEL-JAK2 cells in-vitro but its in-vivo activity was considered sub-optimal with respect to what has been previously observed for compound 2 in a similar setting.
- Encouraged by the favorable cellular activity of compound 1 in the TEL-JAK2 assay, effects of a pyrazol-3-yl amine as a hinge binder motif had been explored.

Preparation Scheme of Compound 3

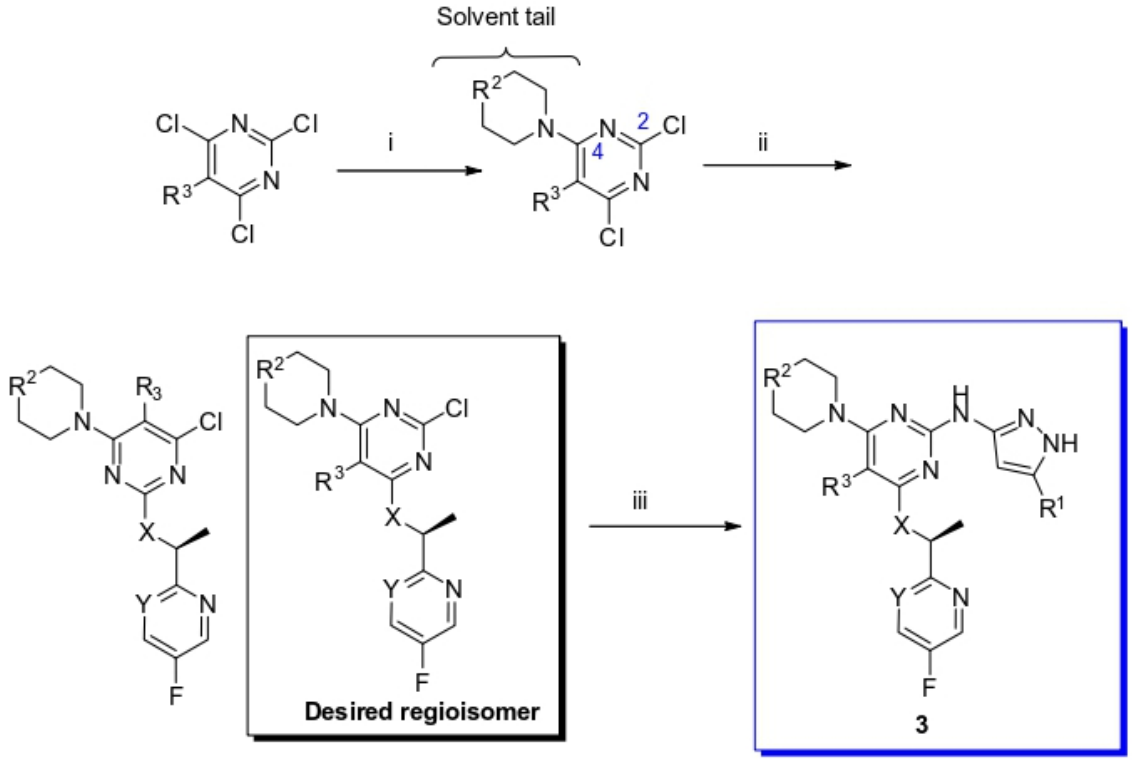


Figure: (i) Amine (such as morpholine), EtOH, 20°C (ii) for X = N: amine such as (S)-1-(5-fluoropyridin-2-yl)ethanamine, hydrochloride DIPEA, n-BuOH, 130°C , o/n. For X = O: alcohol such as 1-(5-fluoropyridin-2-yl)ethanol t-BuOH, t-BuOK, 50°C , o/n (iii) Boc -protected pyrazole, $Pd_2(dba)_3$, BINAP, Cs_2CO_3 , 180°C

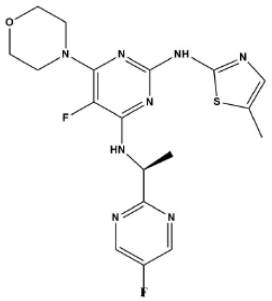
Preparation of Analogus Compounds 4 -12

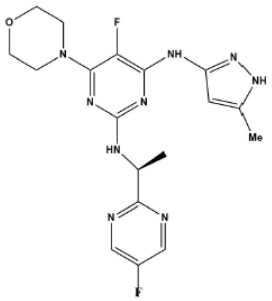
- Several 4-morpholino- N-(1H-pyrazol-3-yl)pyrimidin-2-amine derivatives were prepared and screened in both enzymatic and primary cellular assays keeping compound 3 as a reference.

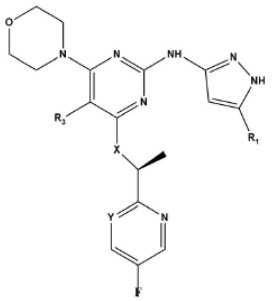
Compd	R^1/R^3	X/Y	Jak2 IC_{50}[a] (μM)	Jak3 IC_{50}[a] (μM)	Tel-Jak2 GI_{50}[b] (μM)	Tel-Jak3 GI_{50}[b] (μM)
4	OMe/F	NH/N	0.037	11.23	0.16	9.4
5	Me/F	NH/N	0.003	0.229	0.015	0.39
6	Me/F	O/N	<0.003	0.020	0.006	0.69
7	Me/F	NH/CH	0.006	0.262	0.019	0.85
8	Me/F	O/CH	0.004	0.087	0.016	0.24
9	Me/F	NH/CF	0.003	0.008	0.009	0.30
10	Me/F	O/CF	0.004	0.045	0.010	0.43
11	Me/H	NH/N	0.005	0.095	0.058	n.d
12	Me/Cl	NH/CH	0.006	0.262	n.d	n.d

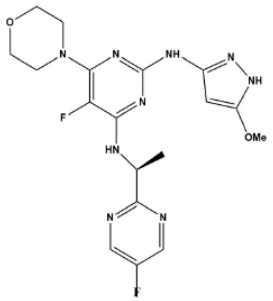
Figure: Biochemical and cellular evaluation of 6-morpholino- N^2 -(1H-pyrazol- 3-yl)pyrimidin-2-amine derivatives (compounds 4–12)

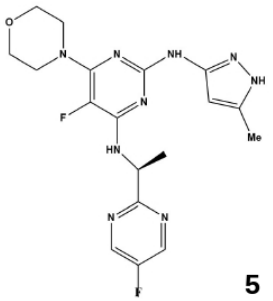
Analogus Compounds

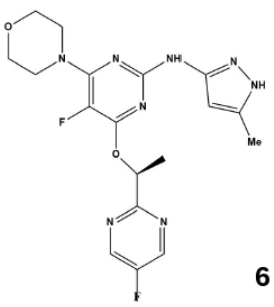

1

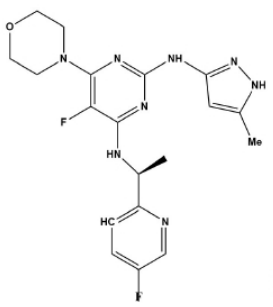

2

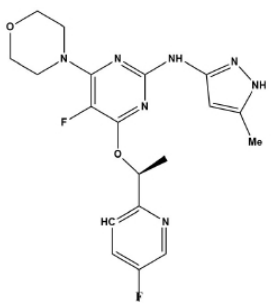

3

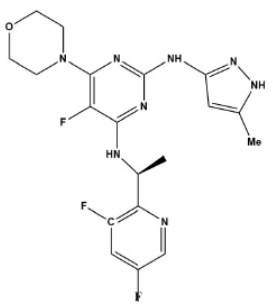

4

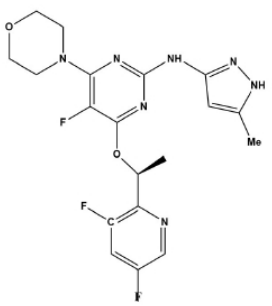

5

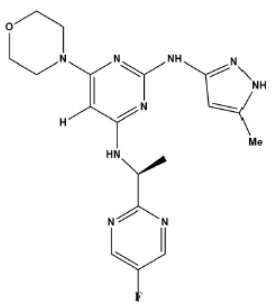

6

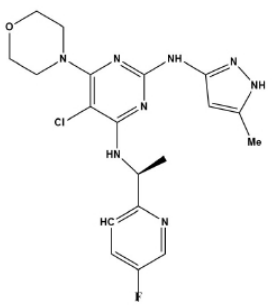

7

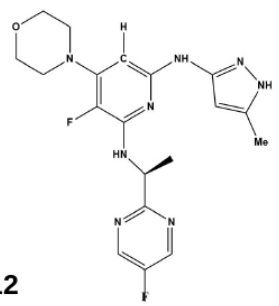

8

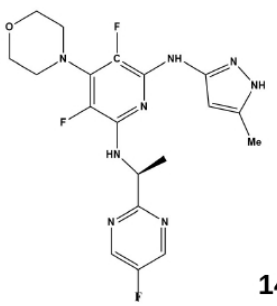

9


10

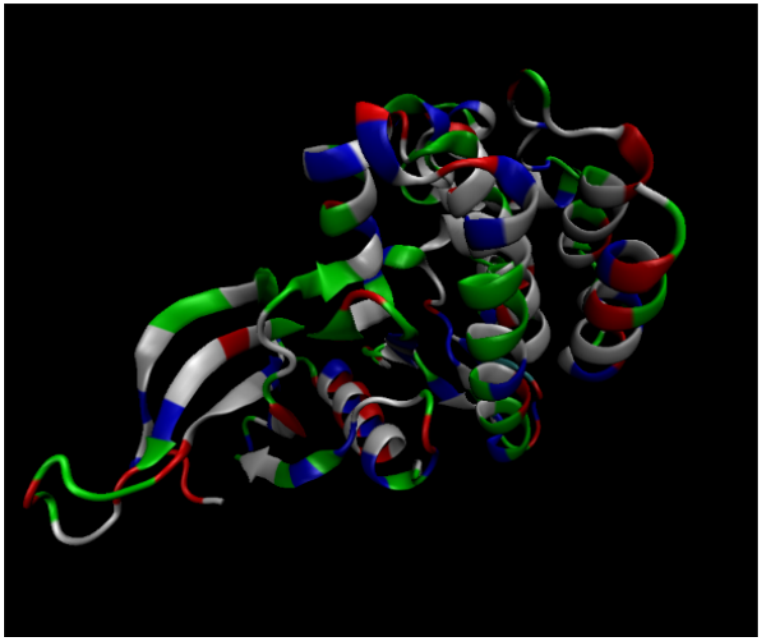

11


12


13


14

JAKs



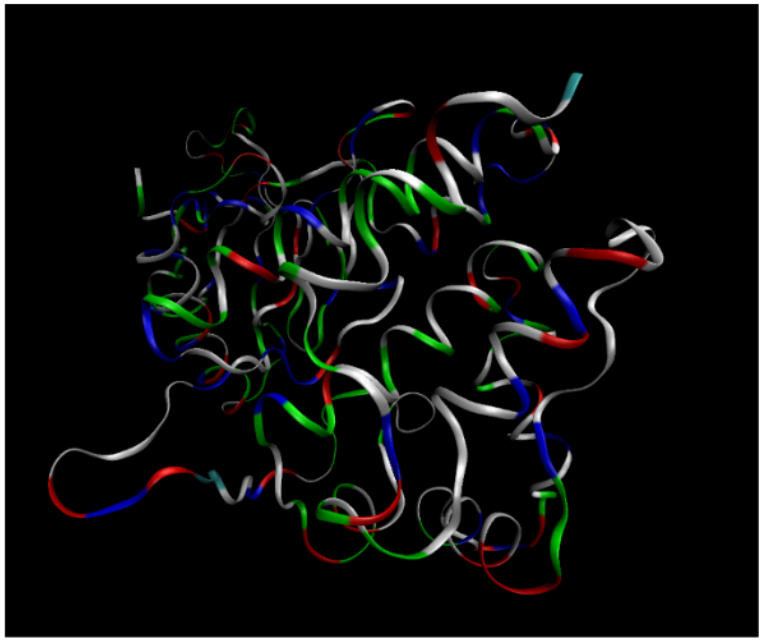


Figure: Restrained Optimised Structures of JAK2 (Left) and JAK3 (Right)

Ligand-Receptor(JAK2) Binding Site

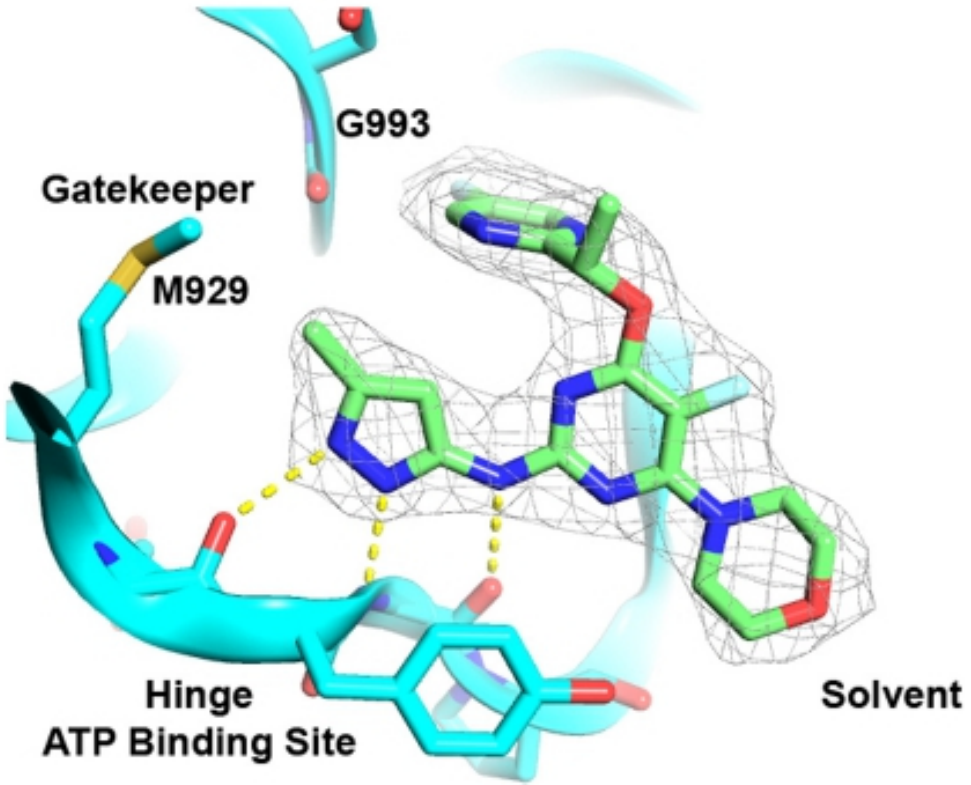


Figure: X-ray crystal structure of compound 6 in complex with human JAK2 kinase domain (PDB code: 3zmm).

Basics of Brownian Dynamics and APBS - BrownDye Simulation Softwares

- Assumption I : There is large separation in timescales between the rapid solute particles and slow solvent particles.
- Assumption II : Inertia of particles are neglected i.e. $F_i^{tot} = 0$.
- Equation : $F^{tot}_i = F^d_i + F^B_i + F_i^{nh} = 0$ where F^d_i represents the drag forces (Stokes drag), F^B_i represents the Brownian force (random collisions of solvent with the particle) and F^{nh}_i represents the non-hydrodynamic forces (external body forces, spring forces and excluded volume interactions).

Basics of Brownian Dynamics and APBS - BrownDye Simulation Softwares

- $F_i{}^d$ = - C_d ($\frac{dr_i}{dt} - u^\infty$ (r_i)) where C_d is the drag coefficient and u^∞ (r_i) is the unperturbed velocity of the solvent evaluated at the position of the particle.
  $\implies \frac{dr_i}{dt} = u^\infty$ (r_i) - $\frac{1}{C_w} F_i{}^d$
  $\implies \frac{dr_i}{dt} = u^\infty$ (r_i) - $\frac{1}{C_w}(F^B{}_i + F_i{}^{nh}(r_j))$ since $F^d{}_i + F^B{}_i + F_i{}^{nh} = 0$ and $F_i{}^{nh}(r_j)$ depends on the set of all particle positions rj.
- The above equation is a stochastic differential equation since Brownian force is taken from a random distribution.

Basics of Brownian Dynamics and APBS - BrownDye Simulation Softwares

- Due to its stochastic nature, one must perform many independent trajectories that are averaged together, producing time evolution to an ensemble averaged property.
- BrownDye : Composed of 2 Simulation and 25 Auxiliary Programs. It computes the second order rate constant of the encounter of two rigid bodies composed of spheres. The software communicate with each other by writing out and reading in XML format . Only the grids used by APBS uses openDX format. The units of Length, Time and Energy are Angstroms, picoseconds and kT respectively.

APBS - BrownDye WorkFlow

- Obtain PQR files for the two molecules (Ligand and Receptor in our case).
- Convert PQR files to equivalent XML files.
- Obtain electrostatic fields for both molecules in openDX format using APBS software.
- Generate files defining the reaction criteria.
- Prepare an input file for the front end program bd_top.
- Run single trajectory simulations using nam_simulation (Northup-Alison-McCommon Algorithm).
- Calculate the second reaction rate constant.

APBS - BrownDye WorkFlow : Challenges and Automation

- **Generating PQR files for Ligands** : Scripts automate the preparation of input files using Amber utility "antechamber" that runs AM1 (Austin Model 1) - BCC (Bond Charge Corrections) semi-empirical quantum mechanical calculations to determine partial charges and writes PQR file for each of the ligand.
- **Grid Dimensions for APBS Electrostatic Calculations** : Scripts automate the preparation of APBS input files with grid dimensions adjusted according to the size of each ofd the ligand.
- **Defining Reaction Criterion for BrownDye Simulations**: VMD based TCL scripts calculate the center of mass of the ligand and the receptor and adds them as a **Ghost Atom** in PQR files of ligand and the receptor. Reactions are assumed to be complete when the COM (Ligand)-COM(Receptor) achieves a defined distance.

APBS - BrownDye WorkFlow : Challenges and Automation

- **Variable Parameters** : A parameter file is incorporated that defines variables such as COM (Ligand)-COM(Receptor) distance, number of ligands, number of trajectories , etc. Only the parameter file has to be changed for variables.
- **Manual Input** : The current working directory should have the PDB files for ligands and the receptor (Only Requirement). Running series of scripts through another script achieves the goal of ligand-receptor second order rate constant calculation and visualization.

Workflow for APBS-BrownDye Simulation Script

Script I : Writes a script for Amber utility "antechamber" to run an AM1-BCC semi-empirical quantum mechanical calculation to dertermine partial charges of atoms within the ligand, prepares PQR files from the previous calculations, prepares APBS input file for ligands with adjusted grid parameters, and runs APBS calculations for all ligands.

Script II : Generates PQR input file the receptor followed by generation of APBS input file and carrying out APBS calculations for the receptor.

Script III : Generates XML input files for BrownDye simulations without Center of Mass consideration for the receptor and the ligand.

Script IV : Generates TCL scripts for VMD to read and extract COM for the ligand and the receptor. It then edits each of the PQR file, adds an extra ghost atom in these files and generates XML files again for BrownDye simulations.

Workflow for APBS-BrownDye Simulation Script

Script V : Generates Browndye input files for all ligand-receptor combinations.

Script VI : Generates input files for reaction criteria which extracts the center of mass information from PQR files and TCL scripts.

Script VII : Runs Brownian Dynamics simulations for all ligand-receptor combinations and calculates the second order rate constant.

Script VIII : Graphical Analysis of corresponding rate constants for different systems.

Theoretical /Simulation Findings

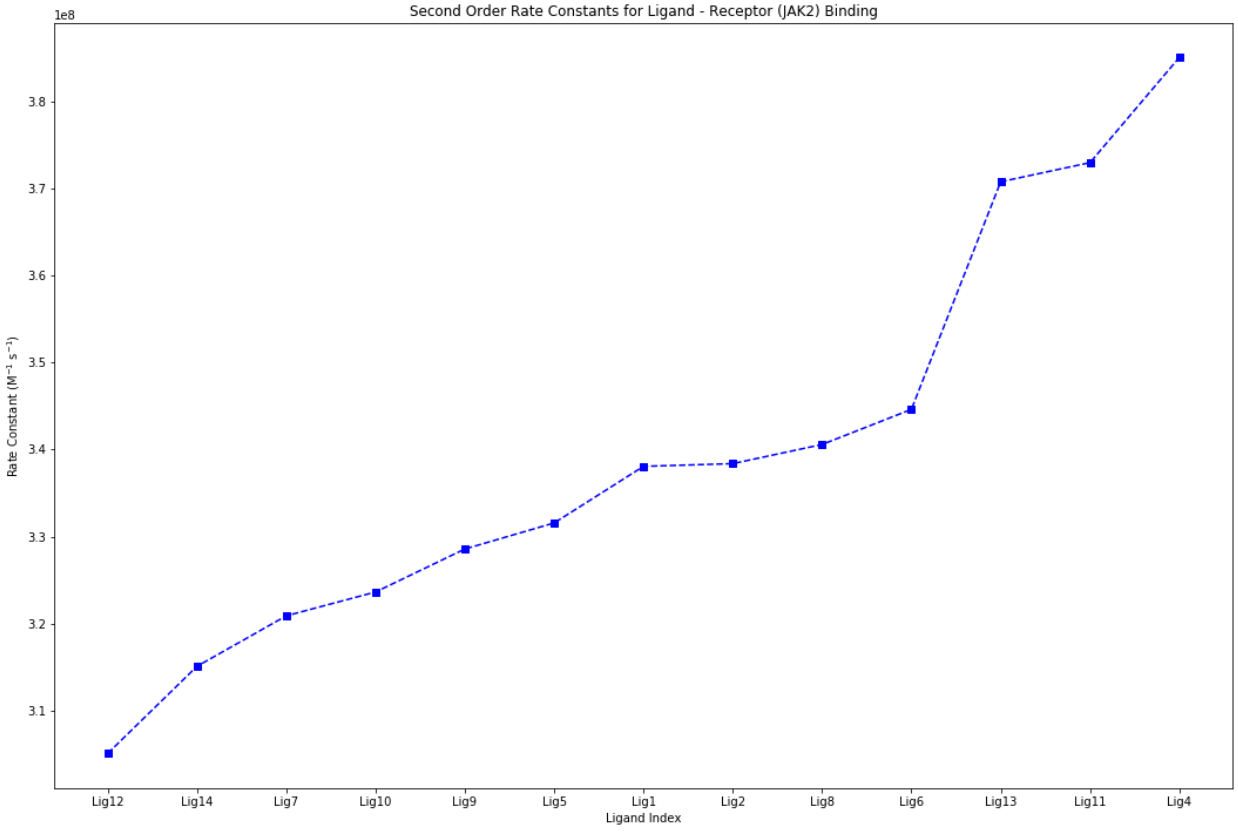


Figure: Second Order Rate Constants for Ligand - Receptor (JAK2) Binding

Theoretical /Simulation Findings

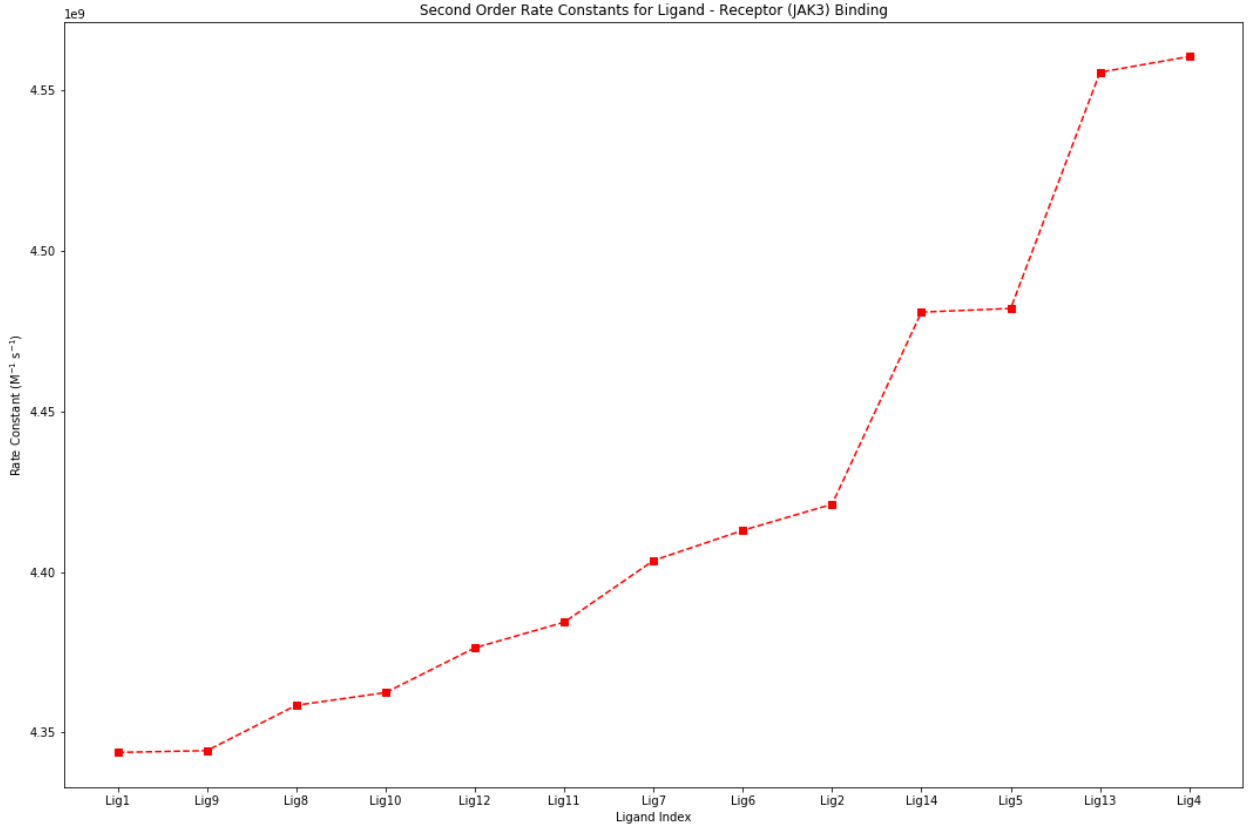


Figure: Second Order Rate Constants for Ligand - Receptor (JAK3) Binding

Conclusion

- A novel series of N^2-(1H-pyrazol-3-yl)pyrimidines has been discovered that display activity against JAK2.
- Theoretical investigations showed a clear selectivity of these ligands against JAK2.
- Ligands were ranked accordingly based on second-order rate constants.
- Ligand 4 showed the highest rate constant for JAK2 and JAK3 but with a distinct selectivity.
- Automated scripts could incorporate any series of ligands with the receptor and can rank them accordingly.

Future Improvements

- Incorporation of more collective variables (Planar and Dihedral Angles to determine reaction criteria).
- Determination of active binding site of the receptor and considering the center of mass of the binding site to perform simulations.
- Incorporation of Classification and Clustering algorithms to determine active binding site using BrownDye simulations.