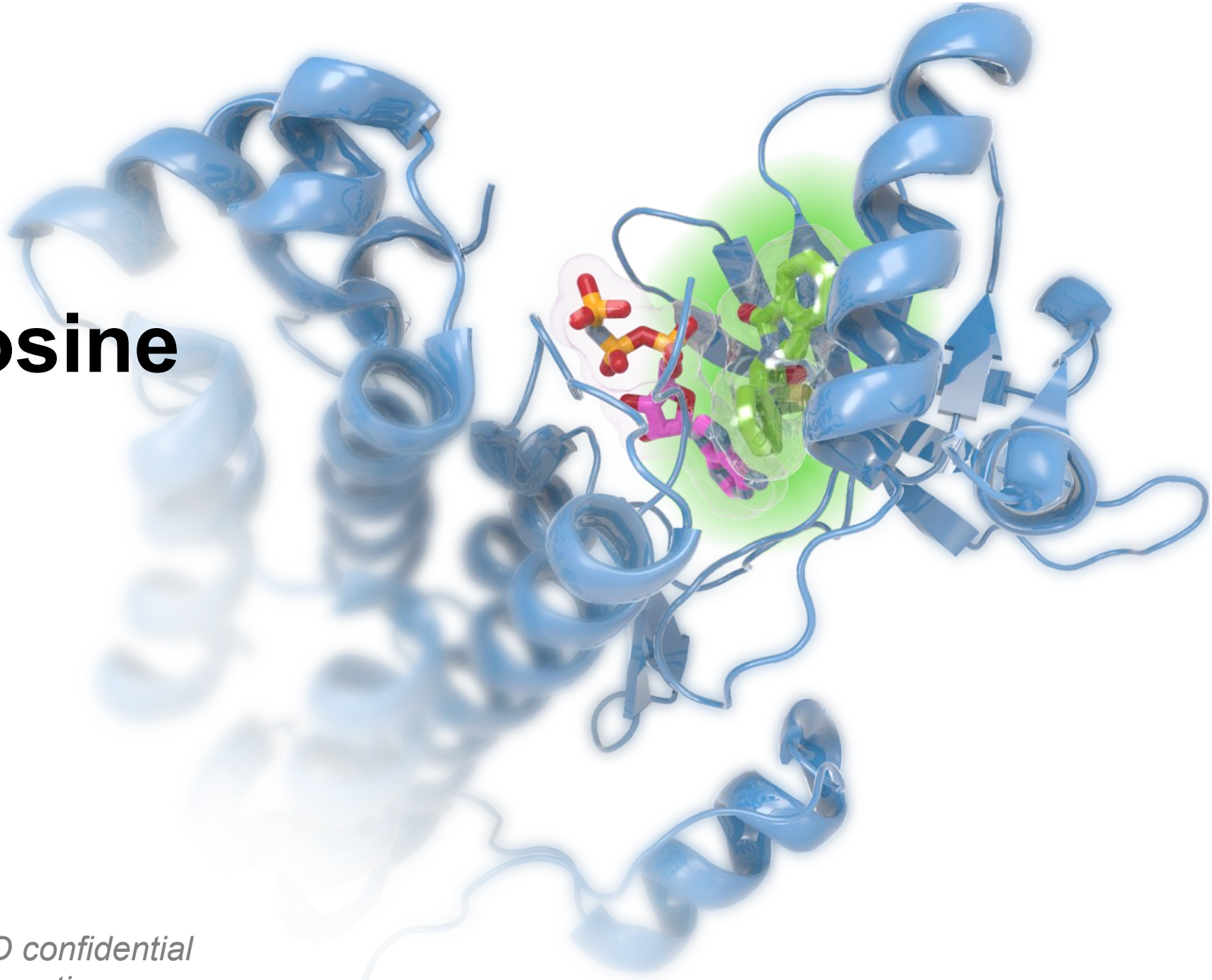
Bruton’s Tyrosine Kinase (BTK)



Jeevan B GC

Computational Chemistry Scientist I

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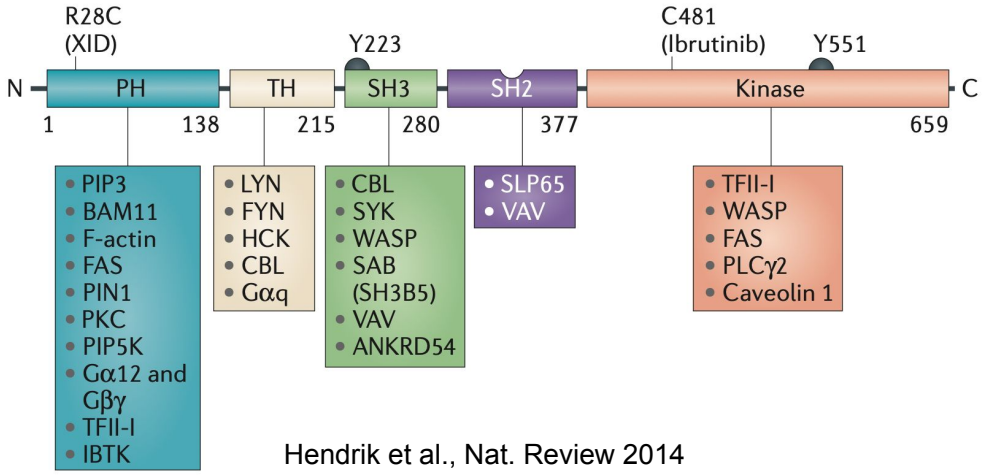
The Center for Protein Degradation

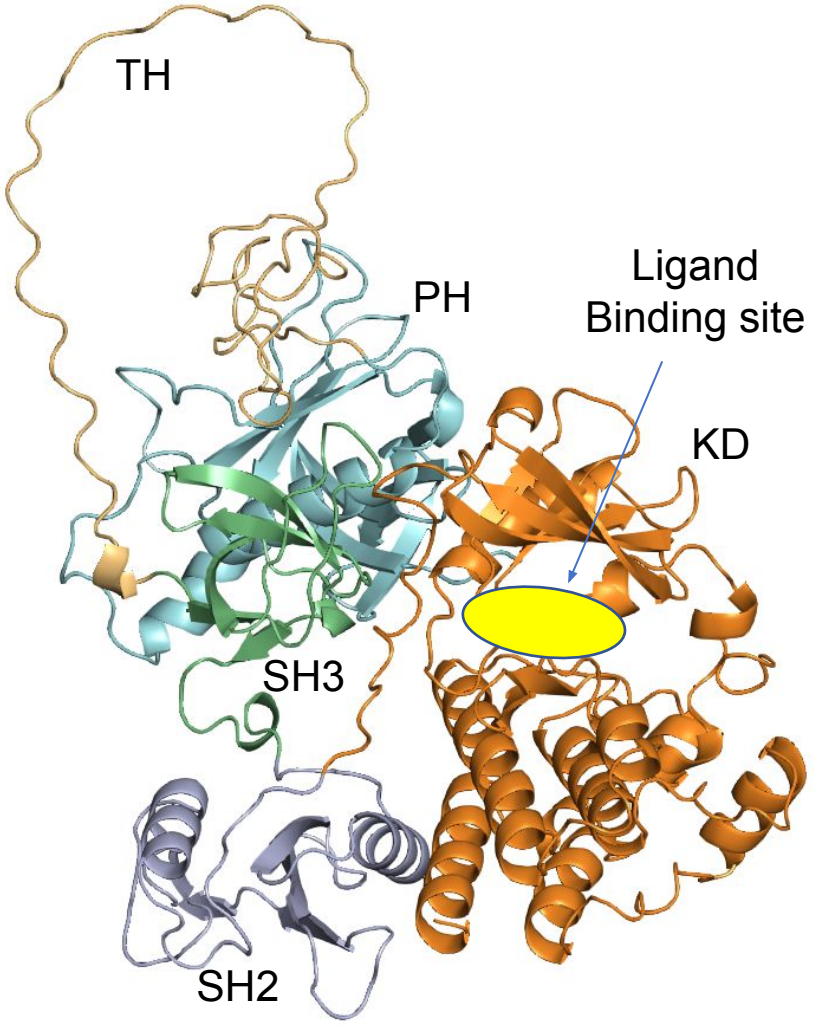
Summary of the Target (BTK)

- BTK – a valuable and effective cancer target implicated in hematological leukemia and non-hodgkin lymphoma, autoimmune diseases such as rheumatoid arthritis, multiple sclerosis etc.
- BTK consist 659 residues (77 kDa) categorized into five domains. KD – ligand binding, PH - plasma membrane localization, SH2-KD domain-domain interaction crucial for BTK activation.
- FDA approved inhibitors such as ibrutinib covalently engages with C481 in the ATP binding site KD.



Hendrik et al., Nat. Review 2014

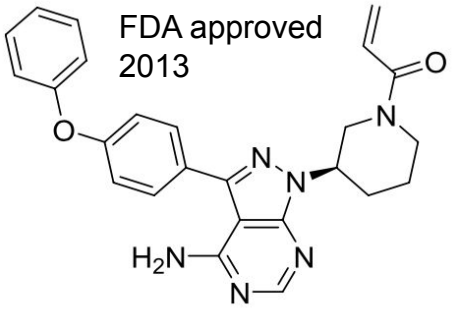
BTK Full-length AlphaFold model



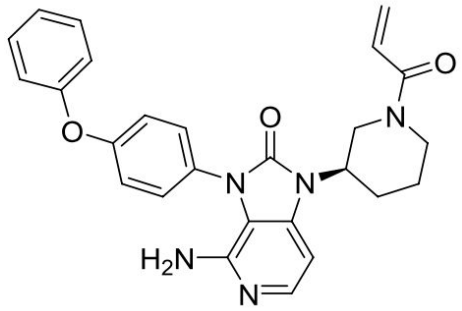
KD – Kinase domain
SH – SRC homology domain
PH – Pleckstrin homology domain
TH – TEC homology domain

BTK Inhibitors and Challenges

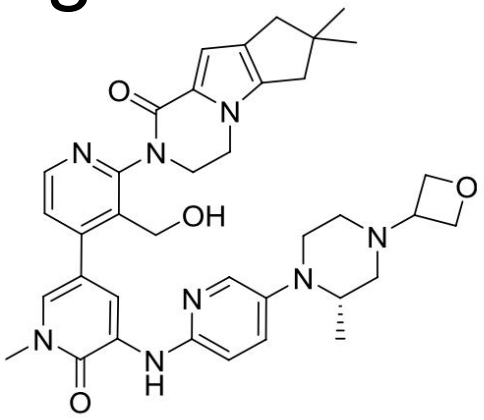
FDA approved 2013



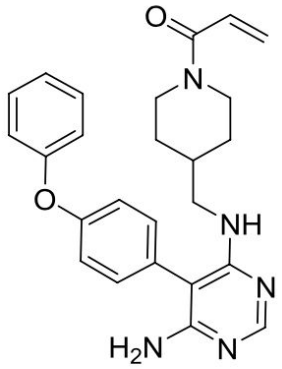
1
Ibrutinib



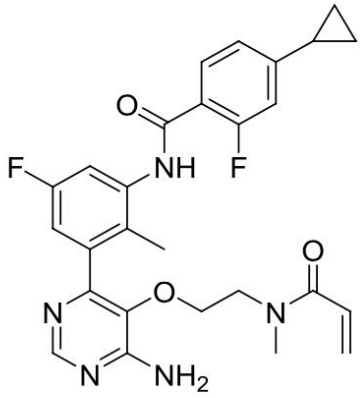
2
Tolebrutinib
Sanofi



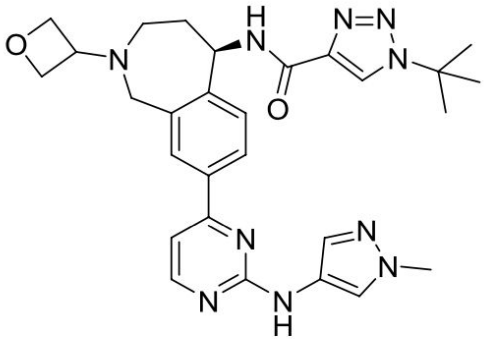
3
Fenebrutinib
Roche



4
Evobrutinib
EMD - serono



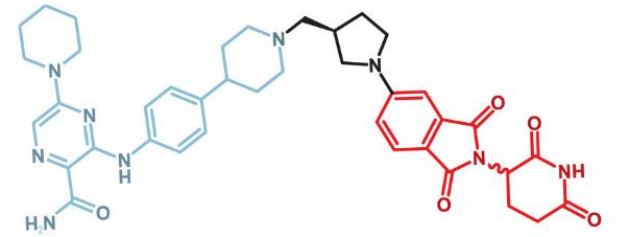
5
Remibrutinib
Novartis



6
BIIB091
Biogen

Dual degrader of BTK and IKZF1/IKZF3

NX-2127



Skye Montoya et al. Science 2024

Challenges:

1. Toxicity: In addition to BTK's C481, covalent drugs might covalently engage with other surface cysteine on other kinases causing off-target toxicity.
2. Drug resistance: Resistance may develop, primarily driven by mutations in BTK (C481S, T474, I443 etc.) leading to impaired drug binding and ineffective kinase activity inhibition.
3. Intolerance and infections.
4. Low membrane permeability of protacs, large MW (720) of NX-2127, Caco2 permeability < 0.02×10^{-6} cm/s.

Alternative approach:

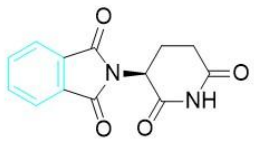
- Molecular glues (MGs), low MW small molecules that influence the function of targets by modulating the protein-protein interactions (PPI). Examples are pan-RAS MGs from Revolution medicines, Immunomodulatory drugs (IMiDs), Rapamycin etc.
- Novel approach to drug undruggable proteins such as transcription factors.

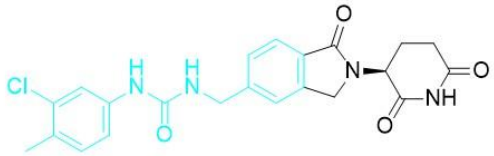
Examples of Molecular Glues (MGs), degrader and non-degrader

- Examples of MGs; degraders and non-degraders. Solvent exposed region colored in cyan, recruits protein-of-interest (neo-substrates) to form a ternary complex.
- Can we utilize these chemical motifs and design BTK MGs to disrupt BTK activation?

- **Objective 1**: MG stabilized BTK KD and protein-of-interest ternary complex, leading to disruption of plasma membrane localization.
- **Objective2**: MG stabilized BTK KD and SH2 domain in a non-functional state, disrupting kinase activation. Literature suggest SH2 domain plays a crucial role in the kinase activation.

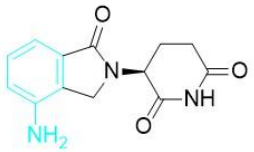
Thalidomide
(IKZF1, IKZF3)

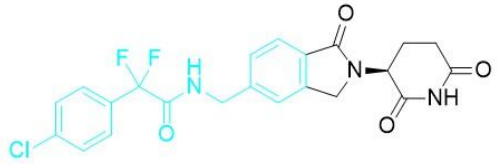




CC-885
(GSPT1)

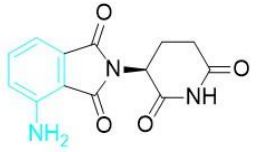
Lenalidomide
(IKZF1, IKZF3, CK1α)

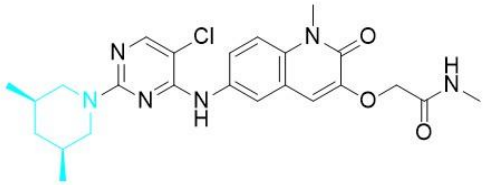




CC-90009
(GSPT1)

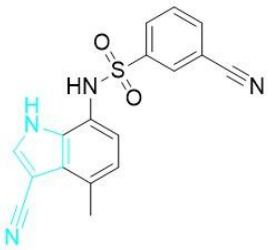
Pomalidomide
(IKZF1, IKZF3)

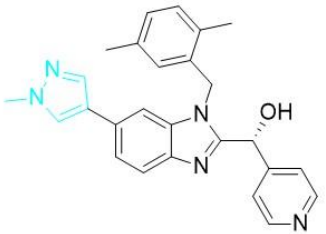


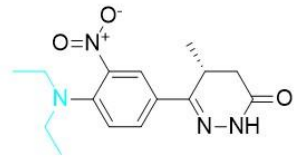


BI-3802
(BCL6)

Indisulam
(DCAF15-RBM39)

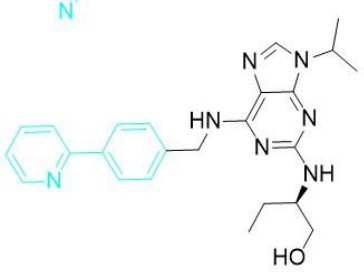




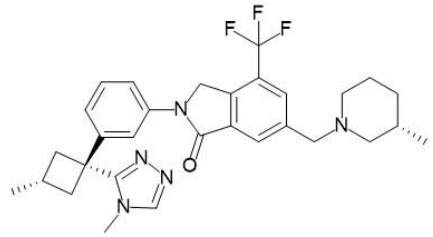


DNMDP
(PDE3A-SLFN12)

CR8
(DDB1-Cyclin K)

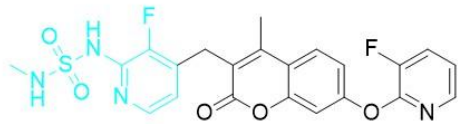


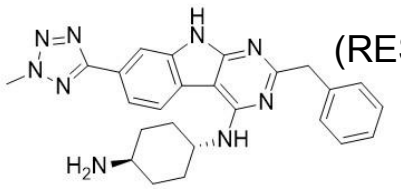
JH-RE06



NX-1607
(CBLB)

NST-628
(panRAF-MEK1)

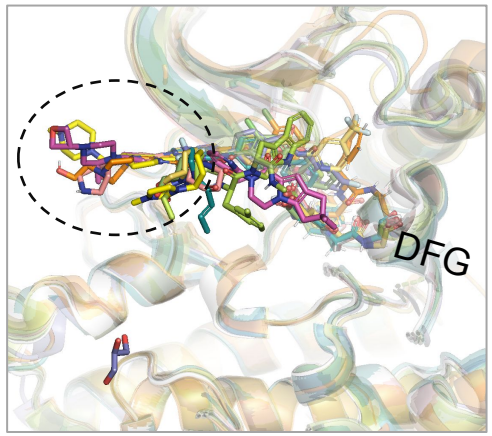


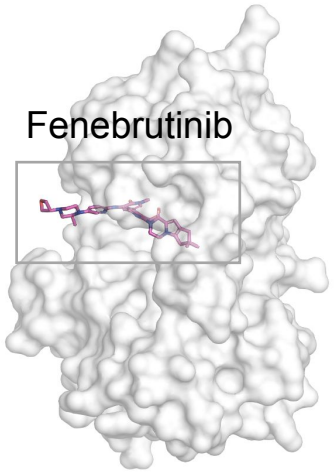


UM-171
(REST corepressor 1)

BTK Molecular Glue

- Structural alignment of BTK-ligand complex X-ray structures.
- Due to solvent exposed moiety oxetenyl piperazine, fenebrutinib was selected as a starting point for further designs.
- Fenebrutinib, a highly selective BTK inhibitor has the potential to be the best immuno-oncology drug.





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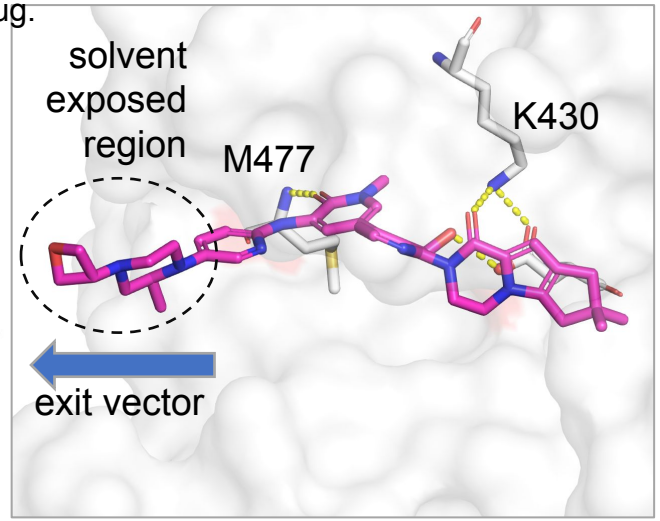
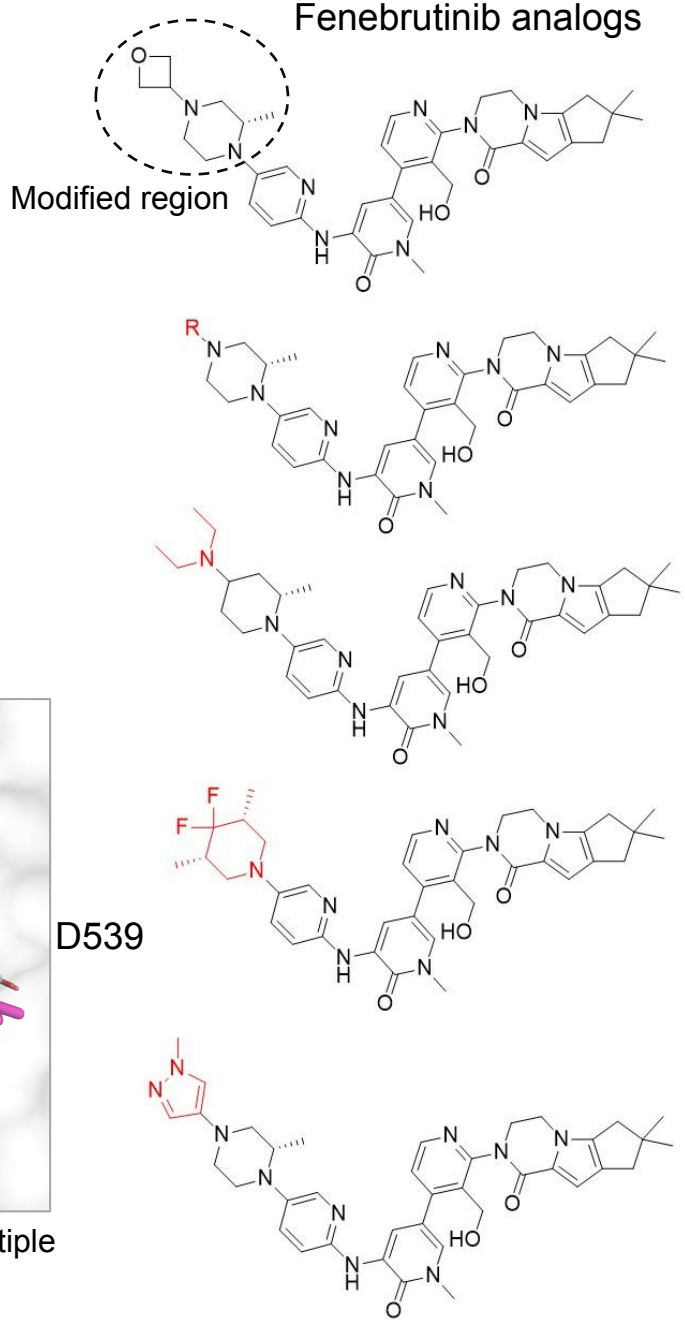
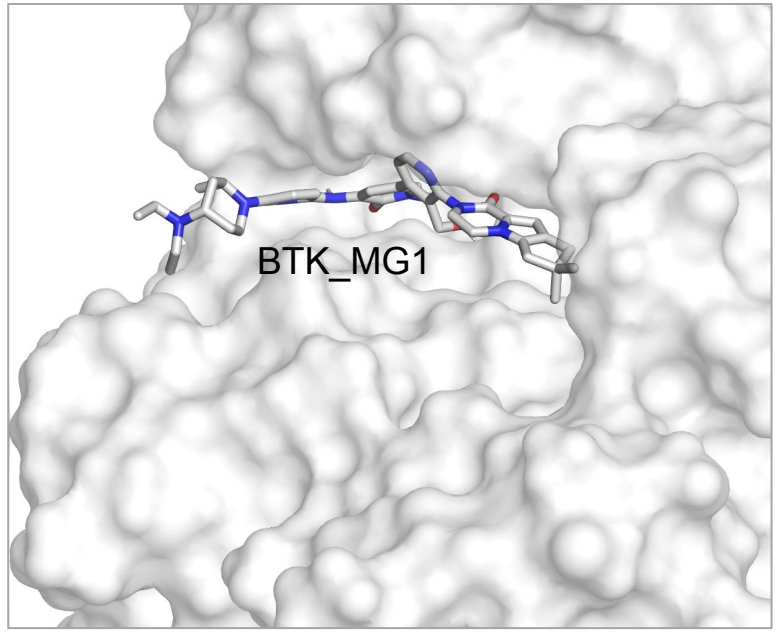
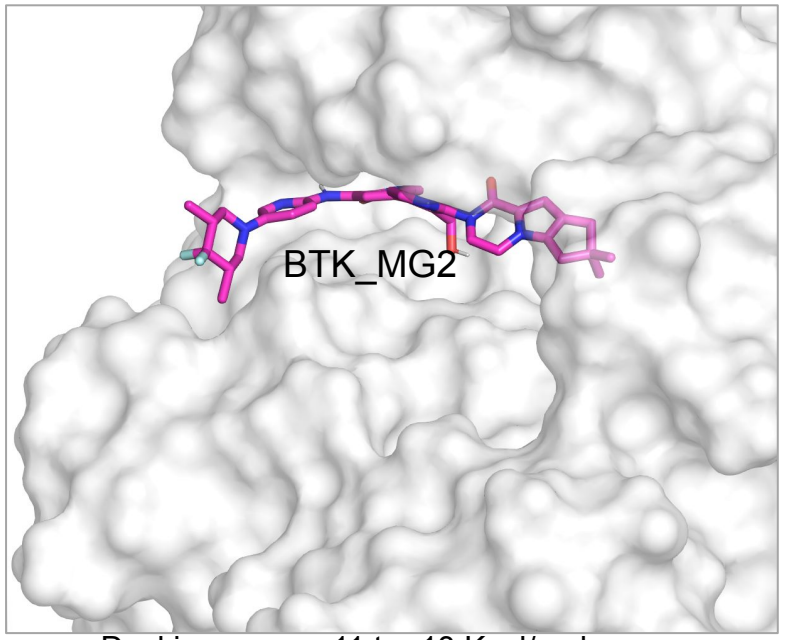


Fenebrutinib in Clinical Phase III for Multiple Sclerosis and autoimmune disease

Fenebrutinib analogs

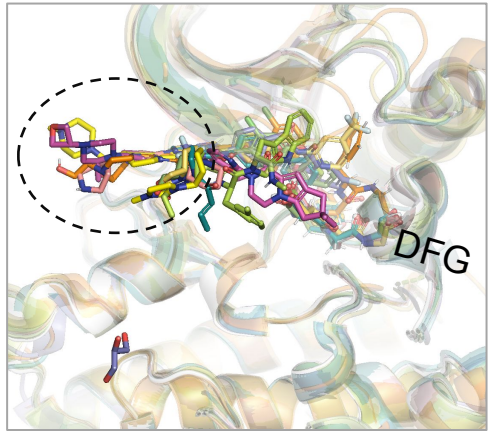


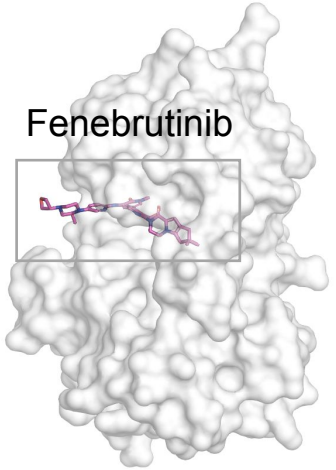




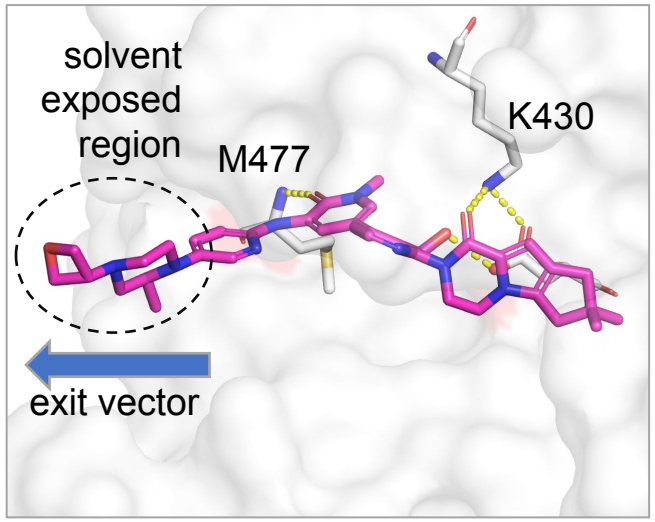
Docking score: -11 to -13 Kcal/mol
Docking method: Autodock Vina
Docking pose visualization: Pymol

BTK Molecular Glue



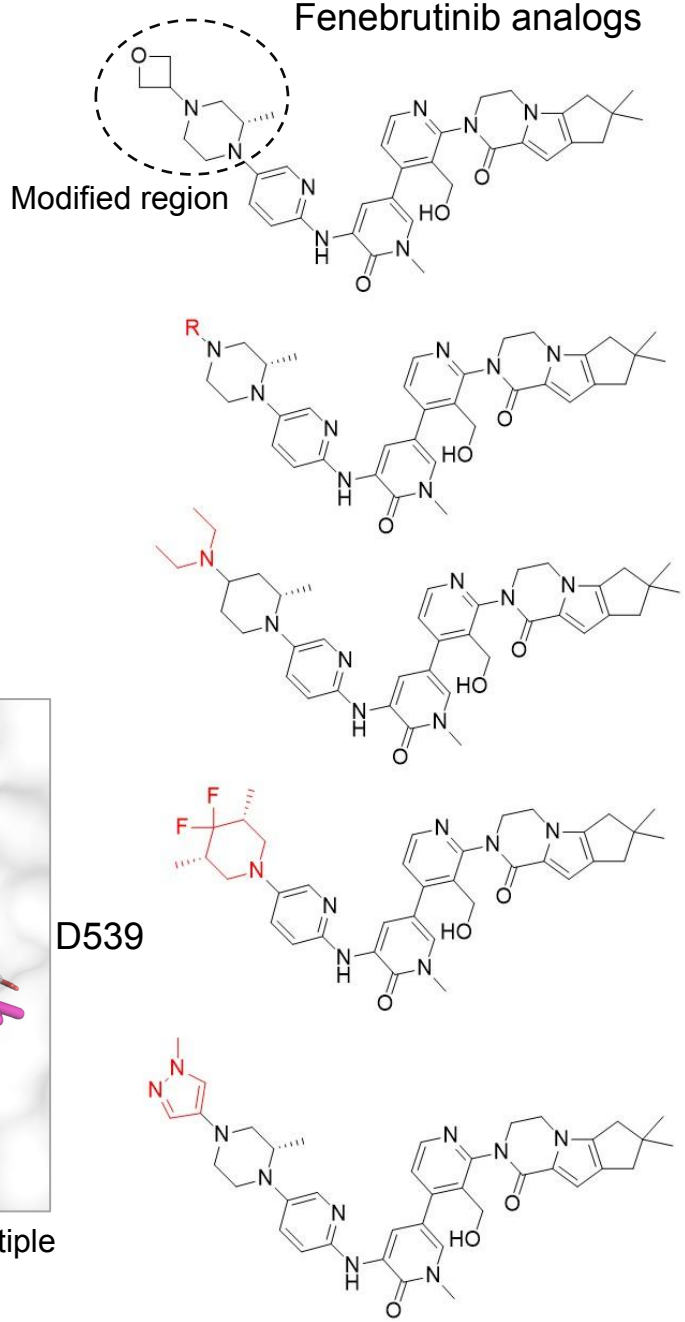


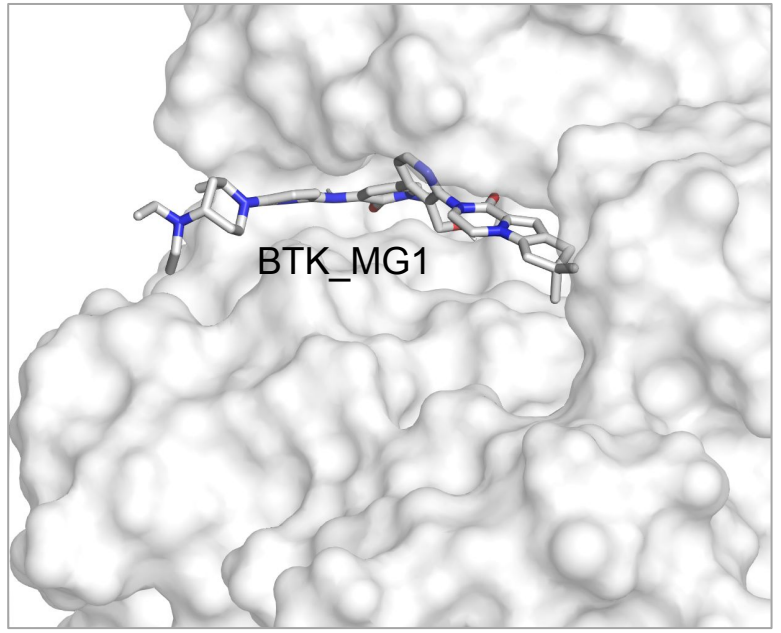
PDB ID 5VFI

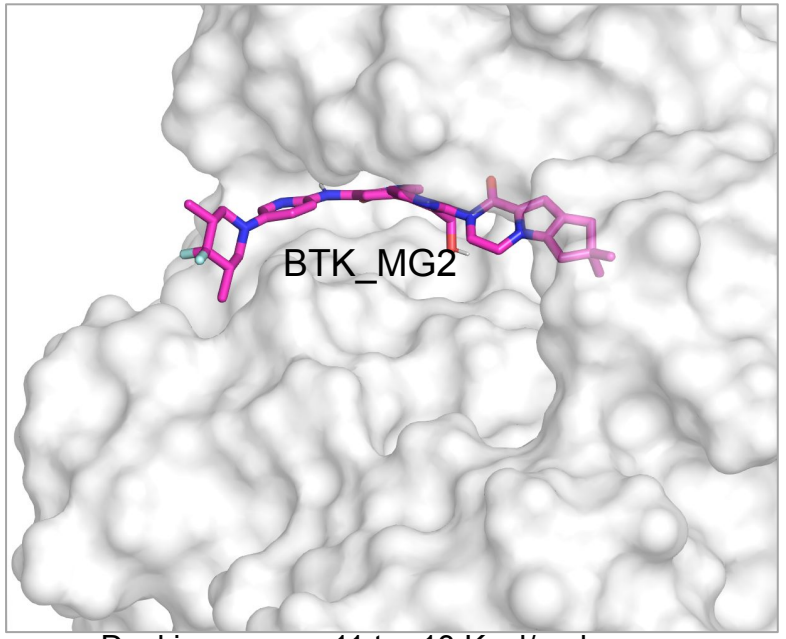


Fenebrutinib in Clinical Phase III for Multiple Sclerosis and autoimmune disease

Fenebrutinib analogs

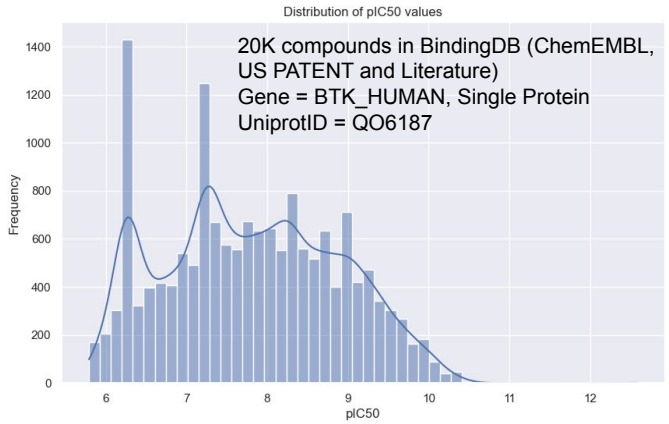


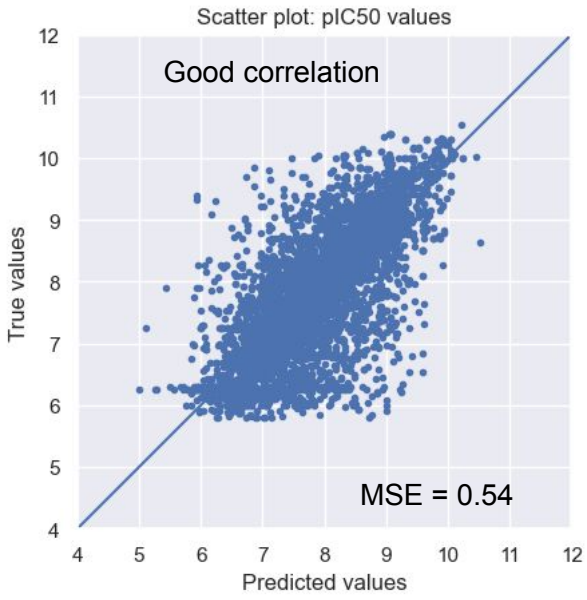


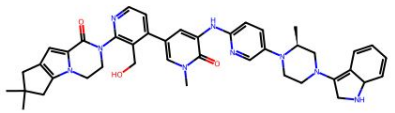


Docking score: -11 to -13 Kcal/mol
Docking method: Autodock Vina
Docking pose visualization: Pymol

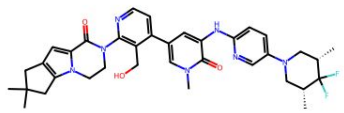
DNN Predicted Binding Affinity of Fenebrutinib Analogs



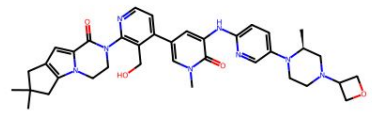




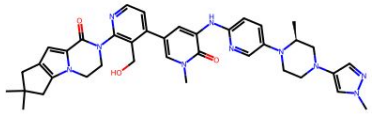
IC50 value: 14.76 nM



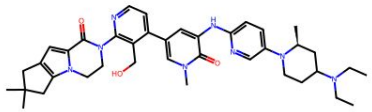
IC50 value: 19.12 nM



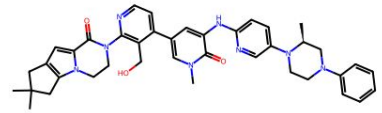
IC50 value: 4.20 nM



IC50 value: 17.64 nM



IC50 value: 2.75 nM



IC50 value: 23.93 nM

- Deep neural network trained using BindingDB 20K compounds and the binding affinity (IC50) of fenebrutinib analogs were predicted.

Dana-Farber Cancer Institute | The Center for Protein Degradation

CPD confidential

Experimental IC50 of Fenebrutinib: min: 1.2, max: 31 (nM) from 9 assay(s), J. Med. Chem. 2018, 61, 2227−2245