Identification of Covalent JNK Inhibitor

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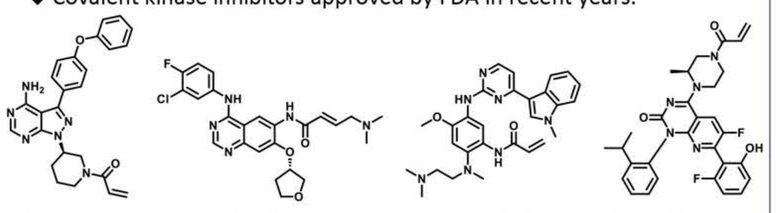
1. Background

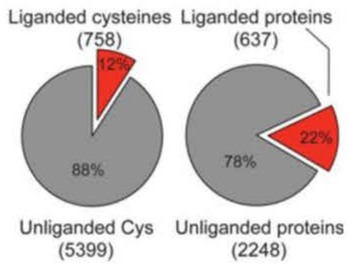
History and recent trends

◆ Covalent kinase inhibitors approved by FDA in recent years.

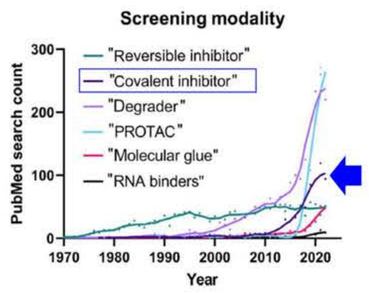


Ibrutinib BTK (2013) | Afatinib EGFR (2013) | Osimertinib EGFR (2015) | Sotorasib $KRAS^{G12C}$ (2021)

◆ Ligandable cysteine[1)]



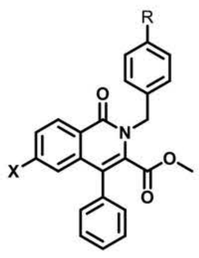
◆ HTS trend[2)]



2. Concept

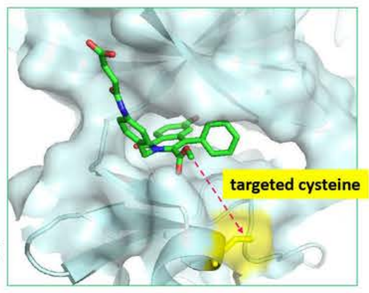
c-Jun N-terminal Kinase (JNK)

- ✓ JNKs are key signal transducers in the MAPK signaling pathways, involved in inflammation, cancer, and neurodegeneration.
- ✓ JNK3, which is predominantly expressed in the brain, is an attractive therapeutic target for neurodegenerative disorders [3)].



JNK1 inhibitor A and B[4)]
A: R=$NHCO(CH_2)_2CO_2H$, X = Br, IC_{50} (JNK1) = 9.2 nM
B: R=SO_2Me, X = Br, IC_{50} (JNK1) = 28 nM

Docking pose of JNK1 inhibitor A and JNK3 protein (PDBID : 2ZDT)



➢ **By exploiting the covalent bond to cysteine, we expect to achieve 1) enhanced JNK3 inhibitory activity and 2) improved selectivity toward JNK3.**

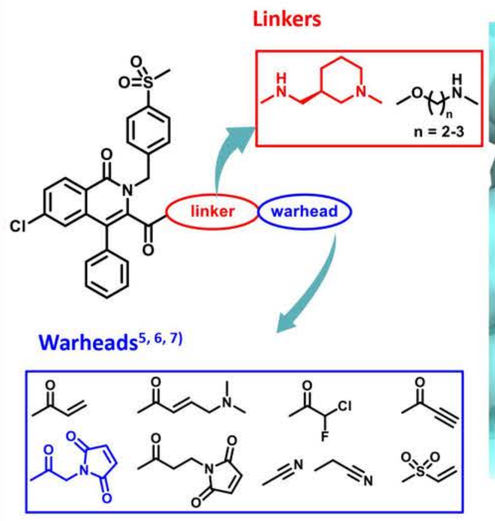
- Inhibition activity ↑
- Selectivity ↑

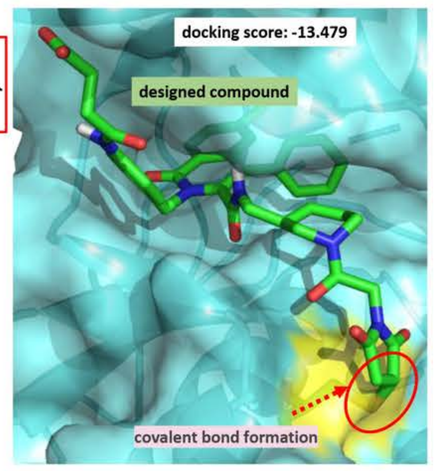
Novel covalent JNK3 inhibitor

3. Compound design

Covalent docking

- ✓ Linkers were prioritized based on docking score.
- ✓ The warheads have been chosen largely from the reported.

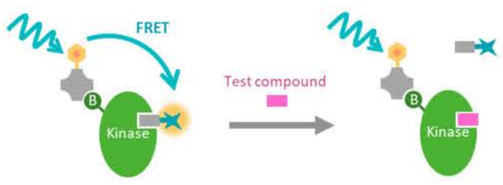


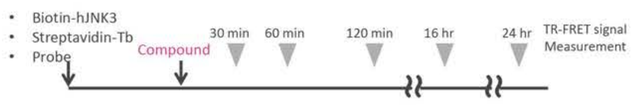


4. Kinase activity, MS, permeability, and solubility

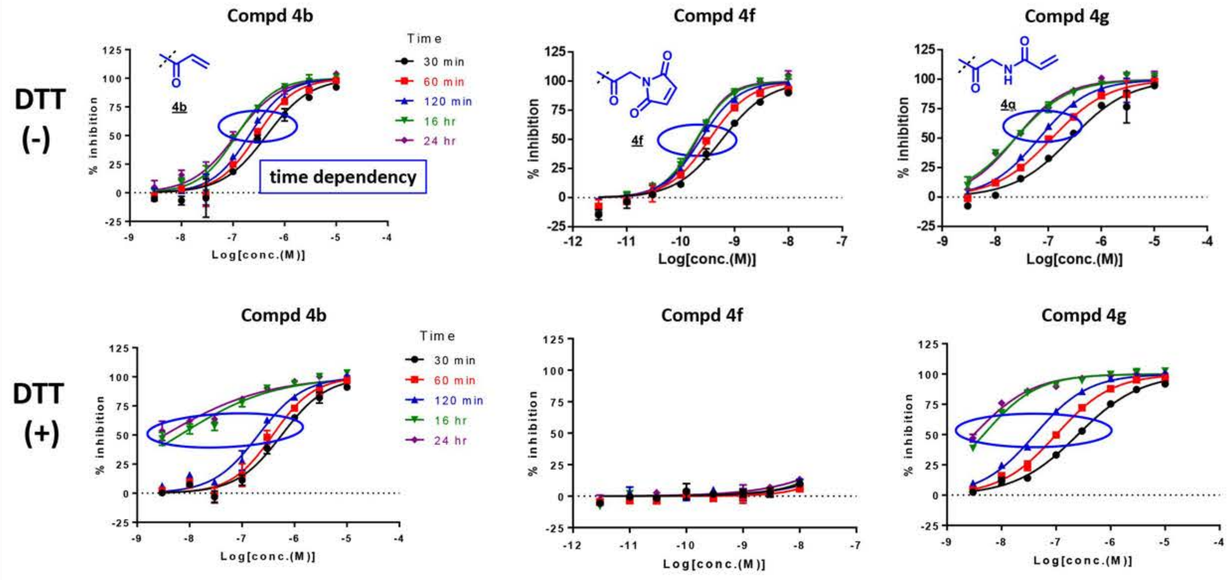
Kinase assay, time dependency and effect of DTT

Probe displacement TR-FRET assay





Buffer : 50 mM HEPES (pH 7.5), 10 mM MgCl2, 1 mM EGTA, 0.01% Brig-35, w/wo 0.1 mM DTT
Reagent : Biotin-hJNK3, 1 nM; Streptavidin-Tb, 1/800 stock; Probe 10 nM



Warhead	Compd	JNK3 IC_{50} (nM)* DTT (-) [8)] 1h	DTT (-) 24h	DTT (+) 1h	DTT (+) 24h	Kinase Panel [9)] DTT (-)** JNK1 IC_{50} (nM) 1h	JNK1 9h	JNK2 IC_{50} (nM) 1h	JNK2 9h	JNK3 IC_{50} (nM) 1h	JNK3 9h	hMS (μL/min/mg)	PAMPA (nm/sec at pH 7.4)	Solubility (μg/ml at pH 6.8)
	4a	660	600	630	430	720	1400	590	550	260	140	NT	NT	NT
	4b	290	110	390	< 3.0	1100	470	740	160	560	130	487	349	28
	4c	550	1300	580	1200	NT	NT	NT	NT	NT	NT	NT	NT	NT
	4d	350	280	740	88	NT	NT	NT	NT	NT	NT	NT	NT	NT
	4e	410	310	420	250	NT	NT	NT	NT	NT	NT	NT	NT	NT
	4f	0.34	0.20	> 10	> 10	17	9.2	78	52	20	8.8	NT	TN	NT
	4g	120	24	100	3.1	370	49	310	44	160	16	121	223	44

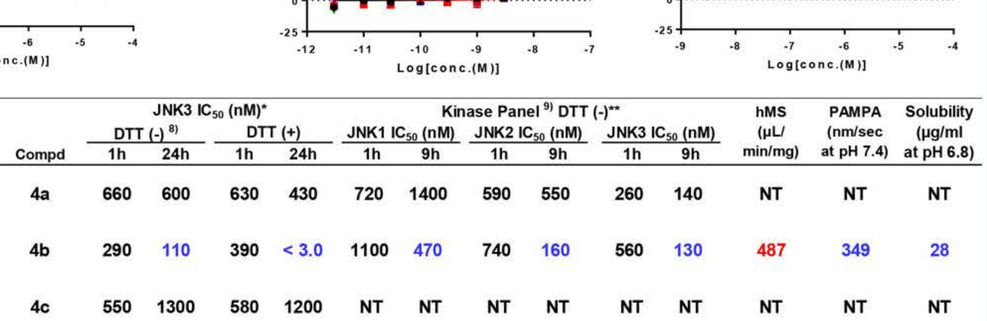
*, Full length protein; **, Truncated protein

- ✓ **4b, 4d, and 4g** retained inhibition of JNK3 and showed a time dependent manner.
- ✓ Although **4f** showed a potent activity in absence of DTT, **4f** lost activity in presence of DTT.
- ✓ **4g** showed moderate human metabolic stability, but **4b** showed low metabolic stability.
- ✓ **4b** and **4g** had a good membrane permeability and solubility.

5. PD result

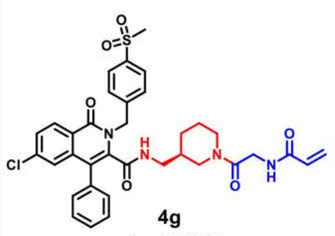
PD assay Procedure

293T plating
↓ 24 hr
+Compound Treatment
↓ 24 h
+ Anisomycin Stimulation
↓ 15 min
Sample collection
↓
P-c-Jun (Ser63) / c-Jun



- ✓ **4b** inhibited phosphorylation of c-Jun in a dose dependent manner.
- ✓ The trend of c-Jun degradation was observed at a high dose of **4b** and **4g**.

6. Integrated Mass Spectrometry Results

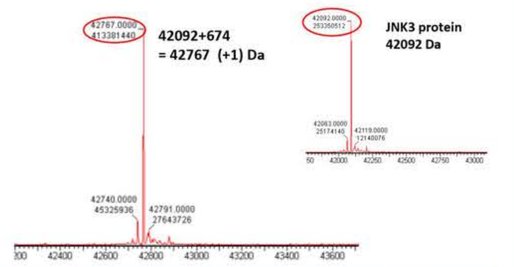


4g
m/z = 674.20

Protein-MS analysis

Condition of LC-MS

UPLC : Acquity UPLC I-class
Column: Acquity BEH C4, 300 Å, 1.7μm, 2.1 x 50mmL
Mobile phase A: TFA/ water (0.25/1000)
Mobile phase B: TFA / MeCN (0.25/1000)
MS: Xevo G2-S Tof Electrospray ionization, Positive ion mode
Data analysis: MassLynx, MaxEnt Ⅰ



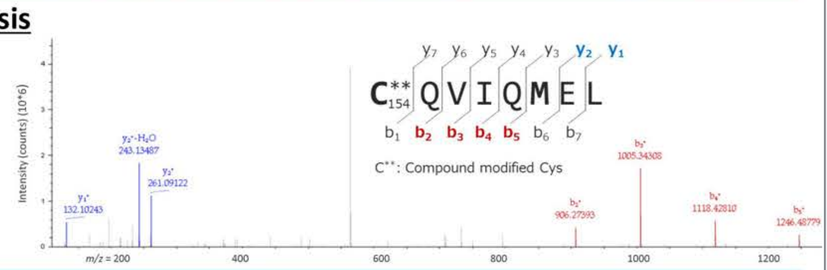
✓ Compound **4g**'s adduct with the JNK3 protein was detected by protein-MS.

Binding site identification analysis

Condition of LC-MS

UPLC: Easy-nLC 1000
Column: Reprosil-Pur C18 AQ 3 μm, 0.075 x 150 mm
Mobile phase A: MeCN/H_2O/Formic acid (2:98:0.1, v/v/v)
Mobile phase B: MeCN/H_2O/Formic acid (90:10:0.1,v/v/v)
MS: Orbitrap Fusion Tribrid mass spectrometer
Data analysis: Proteome Discoverer 1.4

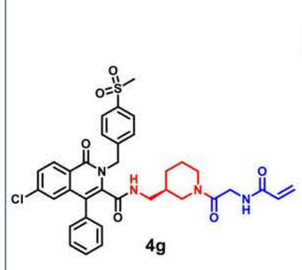
*, Enzymic digestion was conducted by chymotrypsin



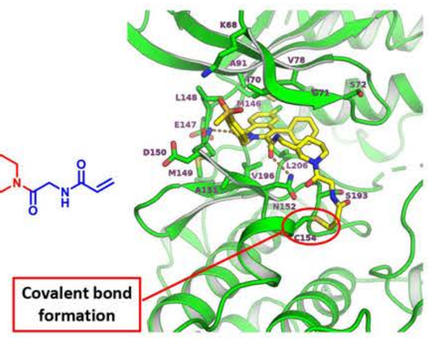
✓ By proteomics approach, the covalent binding site of compound **4g** was confirmed as Cys154.

7. X-ray Crystallography

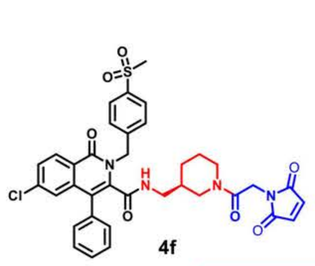
Crystal structural analysis



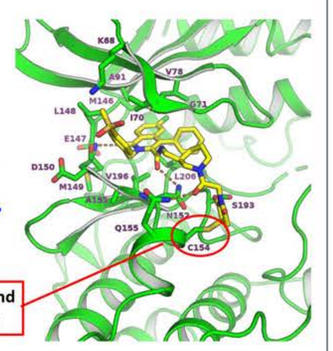
4g



JNK3/comp. 4g (1.8 Å)



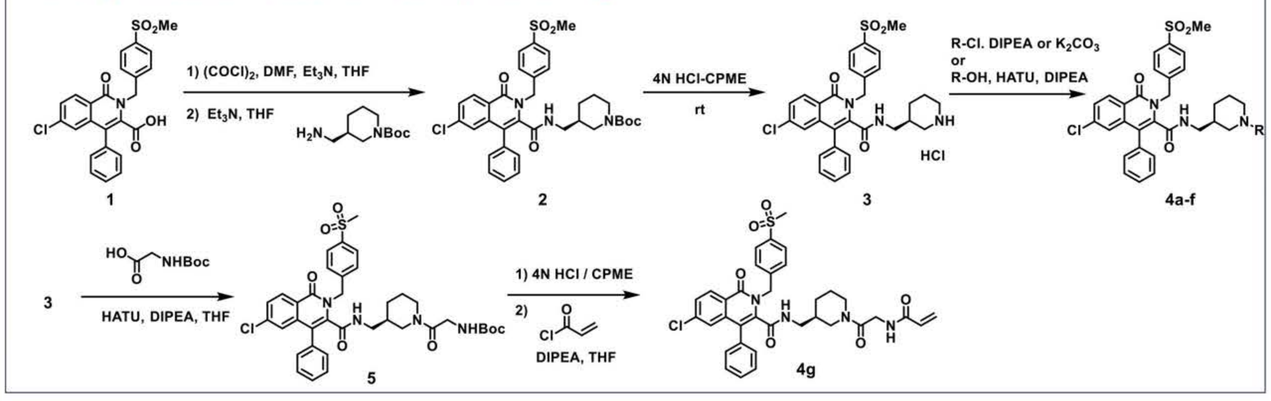
4f



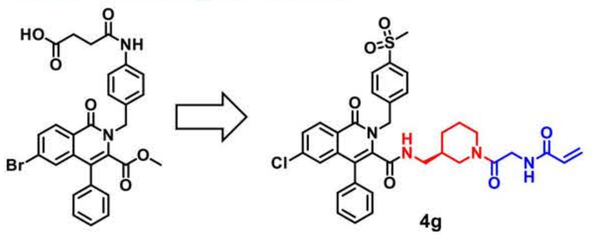
JNK3/comp. 4f (1.5 Å)

✓ The covalent binding to the expected Cys154 was also confirmed by X-ray crystallography.

8. Synthesis route of compound 4g



9. Summary & Tasks



JNK1 IC_{50} [1h. (-) DTT] = 9.2 nM

4g

JNK3 IC_{50} [1/24h. (-) DTT, full length] = 116/**24** nM
JNK3 IC_{50} [1/24h. (+) DTT, full length] = 101/**3.1** nM
JNK1 IC_{50} [1/9h. (+) DTT, truncated] = 366/49 nM
JNK2 IC_{50} [1/9h. (+) DTT, truncated] = 309/44 nM
JNK3 IC_{50} [1/9h. (+) DTT, truncated] = 159/**16** nM

Summary

- ✓ Synthesized compounds **retained inhibition of JNK3**.
- ✓ Several compounds inhibited JNK3 **in a time dependent manner**.
- ✓ **Adducts of synthesized compound with JNK3 protein** were detected by Protein-MS.
- ✓ **Covalent binding to the expected cysteine** was confirmed by proteomics approach and X-ray crystallography .

Tasks to do in future

- Derivatization for metabolic stability improvement
- In vivo assay.

Reference

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