Identification of Covalent JNK Inhibitor

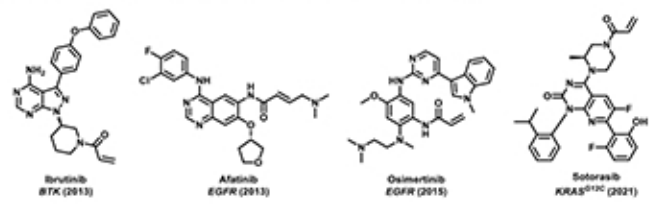
*Hirotoshi Yagishita, Tamotsu Suzuki, Taiichi Ora, Yoshi Nara, Mitsuyo Kondo, Mika Yamaguchi, Satoshi Sogabe, Kazuhiro Tsuchinaga, Masataka Miwa, Kozo Hayashi, Jun Chiba

Axcelead Drug Discovery Partners, Inc.

1. Background

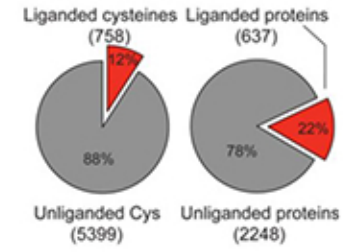
Covalent inhibitor history and recent trends

Covalent kinase inhibitors approved by FDA in recent years



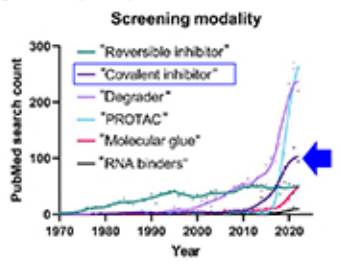
Ligandable cysteine

Nature, **2016**, *534*, 570–574.



HTS trend

Drug Discovery Today, 28, 2023.



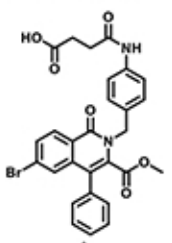
- ◆ Rising of covalent inhibitor: targeted EGFR, BTK, JAK etc. covalent kinase inhibitor
- ◆ Cysteinome: ligandable cysteine, relationship with disease
- ◆ Screening trend: PROTAC > Degrader > **Covalent inh.** > Reversible inh. > RNA binder

2. Concept

c-Jun N-terminal Kinase (JNK)

- ✓ JNKs are key signal transducers in the MAPK signaling pathways, involved in inflammation, cancer, and neurodegeneration.

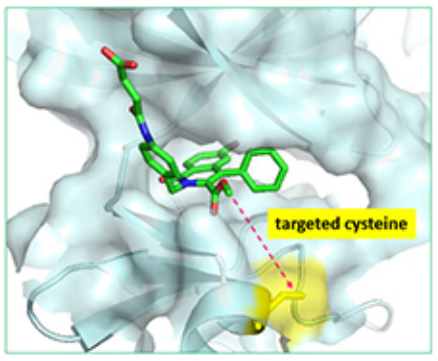
previously reported JNK inhibitor[1)]



A

JNK inhibtion IC_{50} = 9.2 nM

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

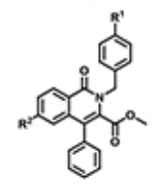


Novel covalent JNK inhibitor

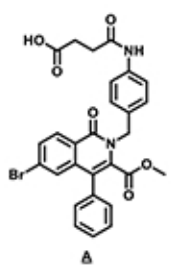
3. Compound design

- **Modification based on SAR information**

Table. SAR information of compound A[1)]

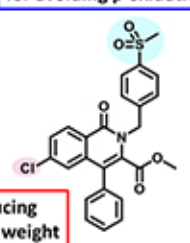


R^1	R^2	JNK1 IC_{50} (nM)
$NHCO(CH_2)_2CO_2H$	Br	9.2
SO_2Me	Br	30
SO_2Me	Cl	28



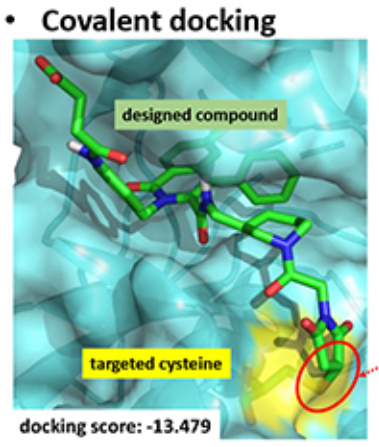
A

for avoiding β oxidation



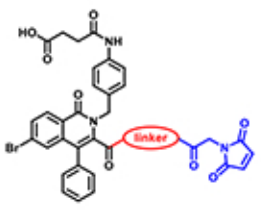
for reducing molecular weight (563.4 ⇒ 482.0)

- **Covalent docking**

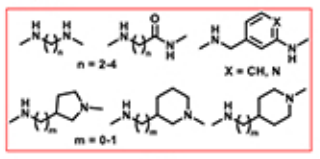


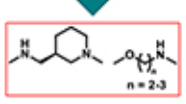
docking score: -13.479

covalent bond formation



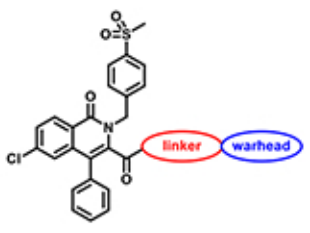
linkers



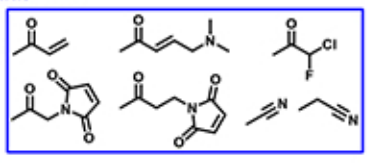


Linkers were prioritized based on **docking score**.

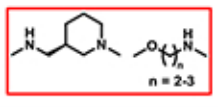
- **Selected compounds to be synthesized**



warheads[2, 3, 4)]

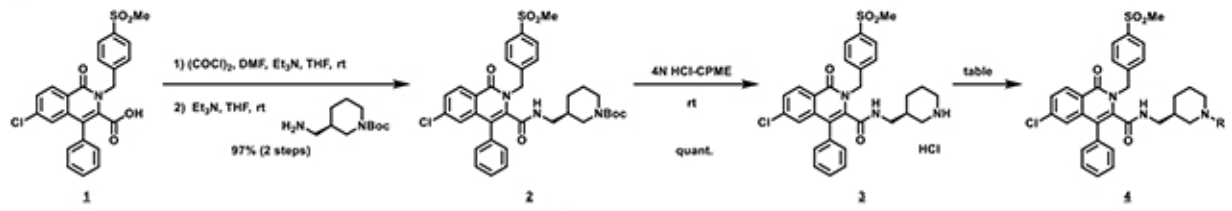


linkers



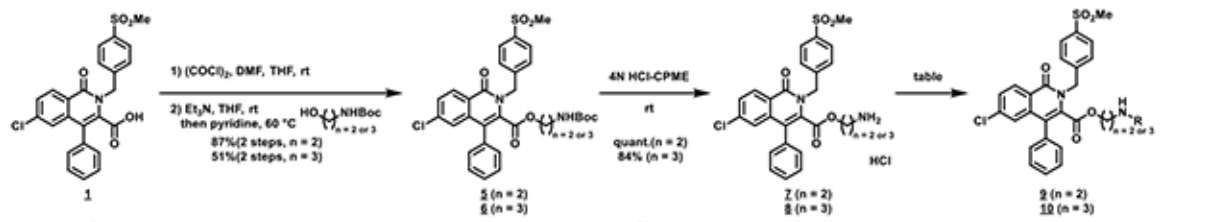
✓ **Compounds to be synthesized prioritized based on docking score and synthetic feasibility.**

4. Synthesis



compound	R	Reaction conditions	Yield
4a		DIPEA, THF	77%
4b		DIPEA, THF	72%
4c		K_2CO_3, MeCN	72%

compound	R	Reaction conditions	Yield
4d		HATU, DIPEA, THF	76%
4e		HATU, DIPEA, THF	82%
4f		HATU, DIPEA, THF	93%



n = 2

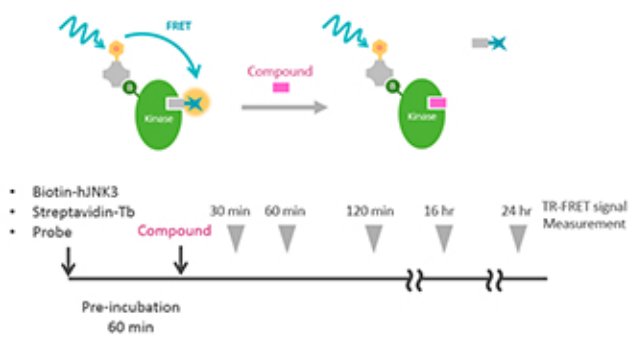
compound	R	Reaction conditions	Yield
9a		DIPEA, THF	84%
9b		DIPEA, THF	81%
9c		HATU, DIPEA, THF	61%
9d		HATU, DIPEA, THF	99%

n = 3

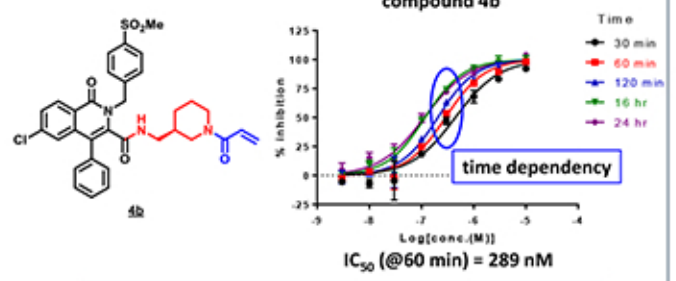
compound	R	Reaction conditions	Yield
10a		DIPEA, THF	87%
10b		DIPEA, THF	82%
10c		HATU, DIPEA, THF	92%
10d		HATU, DIPEA, THF	58%

5. Assay results

- **kinase probe assay**



compound 4b



IC_{50} (@60 min) = 289 nM

✓ **4b** retained inhibition of JNK3.
✓ **4b** inhibited JNK3 in a time dependent manner.

linker	warhead	compound	time dependency	IC_{50} (nM) @ 60 min.
		4a	–	655
		4b	○	289
		4c	–	553
		4d	–	347
		4e	–	408
		4f	○	0.34
		4g	○	116

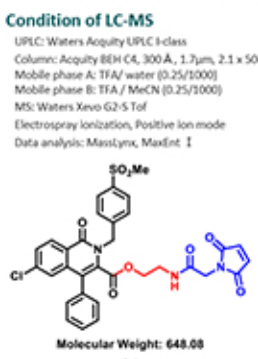
linker	warhead	compound	time dependency	IC_{50} (nM) @ 60 min.
		9a	–	196
		9b	–	251
		9c	–	177
		9d	○	0.23
		10a	–	102
		10b	–	102
		10c	○	72
		10d	○	0.14

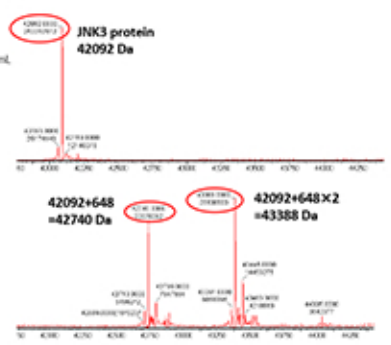
✓ **6 compounds** inhibited in a time dependent manner.
✓ Inhibition activities of **4f**, **9d**, **10d** were improved.

- **Protein-MS analysis**

Condition of LC-MS

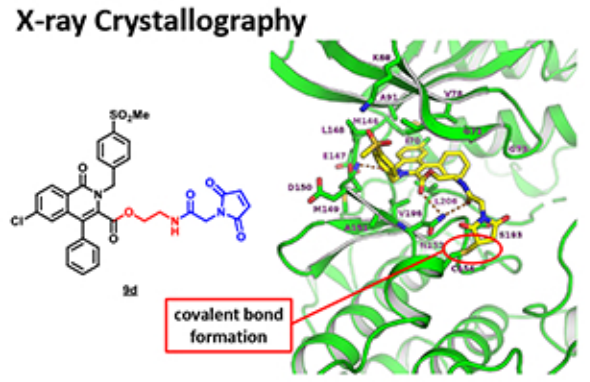
UPLC: Waters Acquity UPLC I-class
Column: Acquity BEH C4, 300 Å, 1.7μm, 2.1 x 50mmL
Mobile phase A: TFA/ water (0.25/1000)
Mobile phase B: TFA / MeCN (0.25/1000)
MS: Waters Xevo G2-S Tof
Electrospray Ionization, Positive Ion mode
Data analysis: MassLynx, MaxEnt 1





✓ Compound **9d**'s adduct with the JNK3 protein were detected by protein-MS.

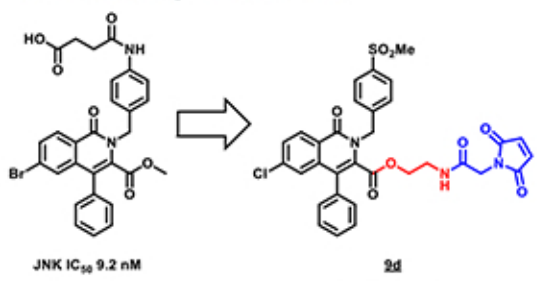
- **X-ray Crystallography**



JNK3/comp. 9d (1.7 Å)

✓ Covalent binding to the expected cysteine residue was confirmed.

6. Summary & Tasks



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 X-ray crystallography.

Tasks to do in future

- selectivity assay (JNK3/1 and 2)
- site identification
- in vivo assay

reference

1) Y. Asano, S. Kitamura, et al. *Bioorg. Med. Chem.* **2008**, *16*, 4715-4732.
2) M. E. Flanagan, J. A. Abramite, et al. *J. Med. Chem.* **2014**, *57*, 10072-10079.
3) N. Shindo, H. Fuchida, et al. *Nat. Chem. Biol.* **2019**, *15*, 250-258.
4) W. H. Parsons, J. D. Bois, *J. Am. Chem. Soc.* **2013**, *135*, 10582-10585.