Integrated Screening in Hit Identification: Combining DEL Hit Results with FBDD and Computational Approaches

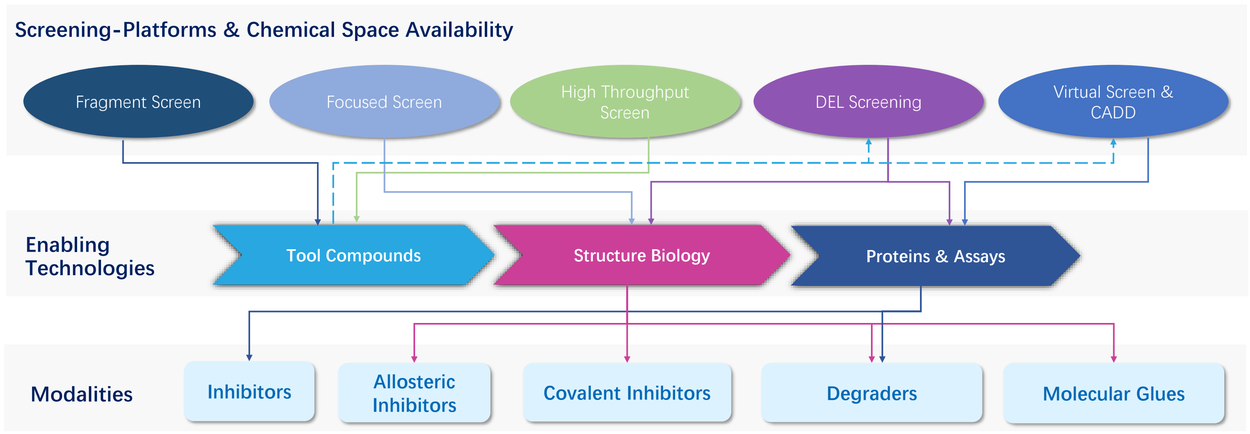
WuXi Biology

Zuyuan Shen, Zaihong Zhang, Jianyu Li, Yunjing Jia, Qi Zhang, Junkun Lei, Shuai Han, Wen Luo, Haozhen Han, Huan Liu, Jingsi Zhao, Weiren Cui, Robert Byrne, Sandra Postel, Andreas Schoop, Alex Satz, Wenji Su, Letian Kuai

Abstract

Despite the availability of various modern screening technologies and the growing experience with new modalities, poorly druggable targets still remain a challenge in the identification of hits, leads, and clinical candidates for novel targets.
Often, a deeper understanding of the target and its interaction partners is required and enabling technologies like structure biology with crystal structures or cryo-EM structures, tool compounds, and specifically designed assays are key to advance projects to a stage in which medicinal chemistry can gain traction. Generating hit matter and tool compounds with one screening technology and screening with a second technology can then become quite powerful.
Here, we present a case of p38α screening in a combination of three screening technologies and structure biology using fragment screening, DEL screening, and a computational model to identify and advance hits into nanomolar small molecules.

Integration of Hit-ID Strategies & Identification of a Modality

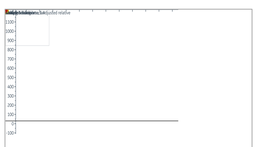


Fragment Screening & Structure Biology

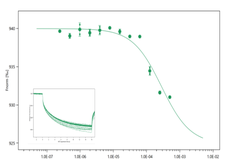
Fragment Screening Workflow:

Target for p38α (MAPK14) plays a pivotal role in initiating different disease states such as inflammatory disorders, neurodegenerative diseases, cardiovascular cases, and cancer. As a result, the identification of potent small molecule p38α inhibitors has been actively pursued by many academic and industry groups.

Primary Screen p38α → **Hit Confirmation** → **Hit Expansion** → **Crystallography** → **Hit Evolution**



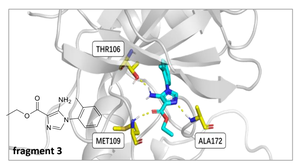
3000+ fragments screened for p38α by SPR



33 fragments confirmed by Kd determination

Compound Design & Synthesis

Similarity search (HTS / Mcule library)



One Co-crystal structure obtained

Compound Design & Synthesis

Fragment Hits Submitted to Crystallography:

Fragment 1

SPR K_D = 26.6 μM
LE = 0.35
HAC = 18
MW = 244
IC_{50} = 30.5 μM
ΔT_m = 3.5 ℃

Fragment 2

SPR K_D = 66.6 μM
LE = 0.30
HAC = 19
MW = 279
IC_{50} = 25.6 μM

Fragment 3

SPR K_D = 71.6 μM
LE = 0.31
HAC = 18
MW = 245
ΔT_m = 2.2 ℃

Fragment 4

SPR K_D = 117 μM
LE = 0.32
HAC = 17
MW = 250
IC_{50} = 21.1 μM
ΔT_m = 4.1 ℃

Fragment 5

SPR K_D = 150 μM
LE = 0.33
HAC = 16
MW = 217
ΔT_m < 0.5 ℃

Fragment 6

SPR K_D = 313 μM
LE = 0.32
HAC = 15
MW = 205.31
ΔT_m < 0.5 ℃

Fragment 7

SPR K_D = 208 μM
LE = 0.51
HAC = 10
MW = 151
IC_{50} > 100 μM
ΔT_m = 1.5 ℃

Fragment 8

SPR K_D = 243 μM
LE = 0.35
HAC = 14
MW = 191
ΔT_m < 0.5 ℃

Fragment 9

SPR K_D = 365 μM
LE = 0.43
HAC = 11
MW = 250
ΔT_m = ~1.3 ℃

Fragment 10

SPR K_D = 372 μM
LE = 0.42
HAC = 11
MW = 217
ΔT_m <0.5 ℃

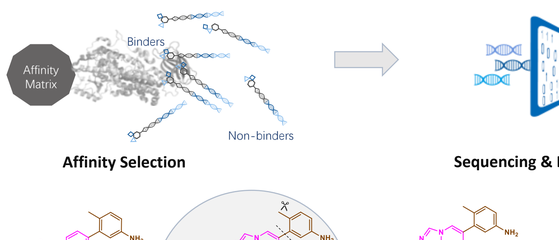
Fragment 11

IC_{50} was determined using ADP-Glo assay

ΔT_m was determined by nanoDSF

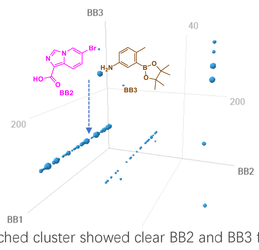
DEL Screening

Primary hit Identified from DEL Screening



Affinity Selection

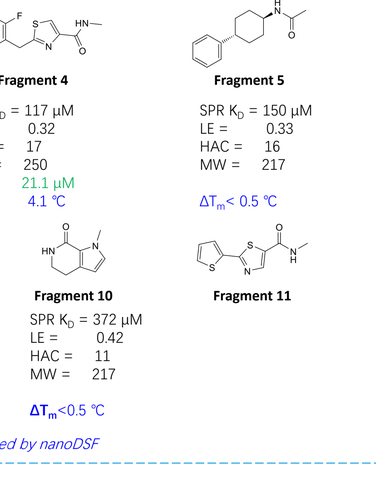
Sequencing & Recoding



An enriched cluster showed clear BB2 and BB3 features

Off-DNA Re-synthesis

3 representative potential binders selected for off-DNA re-synthesis



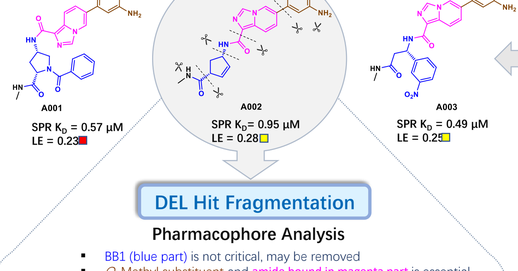
- The amide group shows best LE

Kd Determination

DEL Hit Fragmentation

Pharmacophore Analysis

- BB1 (blue part) is not critical, may be removed
- *O*-Methyl substituent and amide bound in magenta part is essential

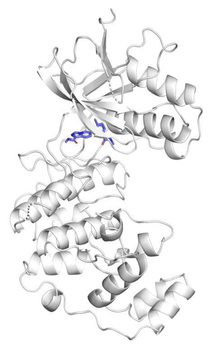


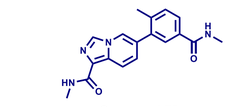
1st Gen. Evolution

- Molecules design by MedChem
- Molecules evaluation by CADD
- 6 compounds were synthesized
- K_D identification by SPR & Enzymatic inhibition activity measurement by ADPglo assay

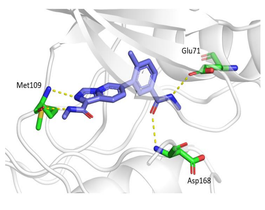
2nd Gen. Evolution

Co-crystal structure confirmation

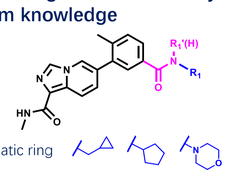




Compound F5



Molecule design based on co-crystal structure and MedChem knowledge

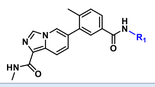


- Add aliphatic ring
- Add substituted benzene ring
- Add aromatic ring
- Add urea linked group
- Cyclization in amide
- Change the amide group to other functional groups

Optimization supported by screening of virtual molecules

Screening by docking → Inspection of docking pose → Checking conformation of the ligand → Evaluating Docking Score and virtual LE calculation → Select molecules for synthesis

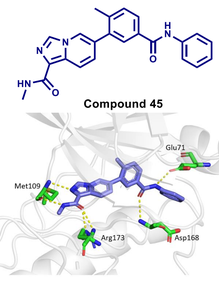
Enzymatic assay of 2nd gen. modified molecules



Compound	R_1	p38α IC_{50} (nM)[a]	LE[b]
48		81.9	0.36
16		185.0	0.33
83		12059	0.21
82		181.7	0.27
45		65.7	0.34
47		31.7	0.31
13		13.8	0.39

[a] p38α inhibition activity of compounds is tested ADPglo assay
[b] LE value is calculated by Ki

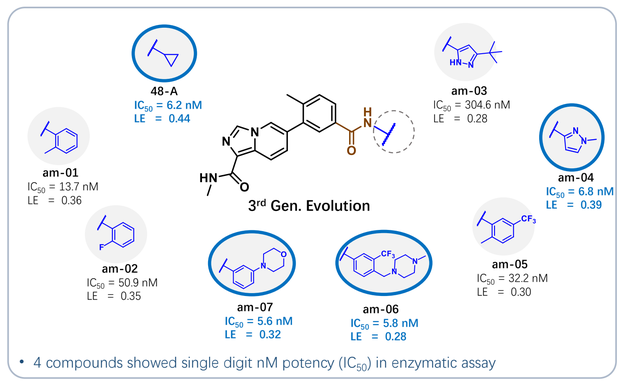
- Co-crystal structure of p38α/Compound 45 complex



SAR Analysis:

- Hydrophobic group is more potent than polar group for substitution
- Cyclopropyl (3-membered) substituted group shows very high ligand efficiency
- Aryl and aromatic rings are very potent

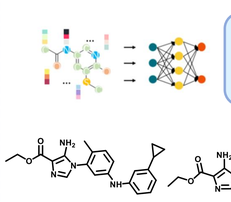
3rd Gen. Evolution



- 4 compounds showed single digit nM potency (IC_{50}) in enzymatic assay

DEL Hit Fragment Evolution - Summary

Primary DEL Hit → Fragment Hit → 1st Gen. Evolution → 2nd Gen. Evolution → Advanced Hit



Fragment Hit: K_D = 950 nM, MW = 389, LE = 0.28

- Molecule truncates
- K_D identification & LE calculation
- Pharmacophore analysis

1st Gen. Evolution: IC_{50} = 1060 nM, MW = 265, LE = 0.41

- MedChem + CADD
- K_D identification & Enzymatic inhibition activity measurement

2nd Gen. Evolution: IC_{50} = 115 nM, MW = 322, LE = 0.39

- Design and molecule docking of 120 analogues
- Docking pose analysis & selecting 20 of them for synthesis
- Enzymatic inhibition activity measurement

Advanced Hit: IC_{50} = 66 nM, MW = 384, LE = 0.34

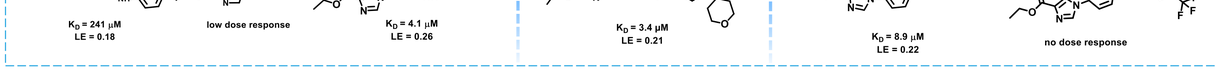
- MedChem + CADD
- Enzymatic inhibition activity measurement

IC_{50} = 6 nM, MW = 469, LE = 0.32

Computational Model

Molecular Generation Method:

Computational Evaluation Tools:



Summary

- As an integrated screening approach, we have been combining DEL-hits with fragment-based optimization methods and DEL data to improve fragment hits with computational screening approaches.
- Our DEL-screen delivered a micromolar DEL-hit that was first fragmented, and the fragment then subjected to fragment evolution to obtain nanomolar potent hits.
- Enumerating a large virtual chemical space of 35K compounds around fragment 3 derived from the fragment screen and selecting compounds for synthesis based on a DEL-model, Reaxys data or by docking resulted in micromolar potent hits.

药明康德 WuXi AppTec

www.wuxibiology.com
Business Contact: Mahnaz_Arjomand@wuxiapptec.com (US)
Technical Contact: DB_Early_Discovery_Business_Transformation@wuxiapptec.com
Business Contact: dave_madge@wuxiapptec.com (EU and Israel)
Business Contact: sycho@wuxiapptec.com (Korea)
Business Contact: fumio_itoh@wuxiapptec.com (Japan)