High-Throughput Campaign to Identify Reversible Small Molecule Inhibitors of p97

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Introduction

- p97 (also called VCP in mammals and Cdc48p in yeast) is a prominent member of the magnesium-dependent Walker P loop AAA-ATPases.
- The multifunctional AAA-ATPase p97/VCP is one of the most extensively studied members of this protein family.
- It functions as a homohexamer with two AAA cassettes forming stacked rings, which couple coordinated ATP-hydrolysis cycles to conformational changes of the hexamer.
- p97/VCP has been associated with a wide variety of essential cellular protein pathways : (i) nuclear envelope reconstruction, (ii) cell cycle, (iii) Golgi reassembly, (iv) suppression of apoptosis (v) DNA-damage response and endoplasmic reticulum associated degradation (ERAD).
- The identification of probes that selectively target p97 activity may provide insights into the biological roles of p97.

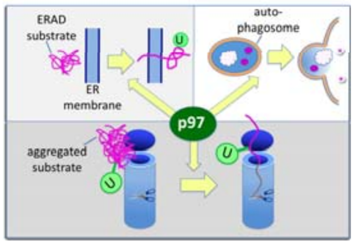
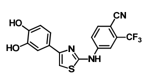


Figure 1. Schematic illustration of the mechanism and function of p97

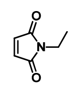
Existing Probes and Prior Art



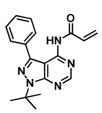
p97/cdc48 Inhibitor Probe
CYM-5654
CID-25110544
ML080

CID/ML	SID	Target Name	Target IC50 [AID]	Anti-target	Anti-target IC50 [AID]	Selectivity *	Secondary Assay: Ub-GFP Assay IC50 [AID]
25110544 /ML080	56432669	p97 ATPase	4.5 μM [1534]	Mutant P97C522A	>50 μM [1551, 1629]	>10	7.5 μM refer to Summary AID (TBD)

Brown S.J., Chou, T.-F., Deshaies, R., Roberts, E., Guerrero, M., Minond, D., Mercer, B. A., Hodder, P., and Rosen, H. R. (**2010**) *Probe Report for P97/cdc48 Inhibitors*.



N-Ethylmaleimide (NEM), a known p97 inhibitor, not p97 selective



ACJI-99C

An irreversible inhibitor of p97, IC_{50} = ~0.6 μM in vitro. However, this compound is exquisitely sensitive to DTT.

Brown, S.J.; Chou, T.F.; *et al.* U.S. Pat. 2009253717 A1(**2009**).

Probe Criteria and Probe Critical Path Assay Flowchart

Probe type: Reversible Inhibitor of p97 ATPase activity

- In vitro ATPase assay: $IC_{50} \leq 0.25$ μM
- Inhibition of Ub-GFP turn over in vivo: $IC_{50} \leq 0.25$ μM
- Apoptosis induction: A suitable probe should be 100-fold less potent in inducing apoptosis compared to inhibiting p97-dependent turnover of Ub-GFP (i.e. EC_{50} for apoptosis divided by EC_{50} for p97 inhibition ≥100)
- Lack of inhibition of ODD-luciferase and ODC-luciferase turn over in vivo: $IC_{50} \geq 20$ μM
- A suitable probe should provoke accumulation of luciferase fusion at a level that is ≤10% of that observed with MG132.

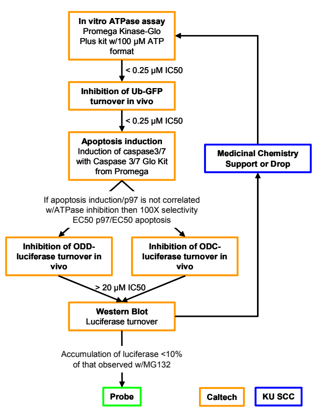
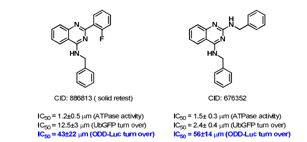


Figure 2. Screening strategy for advancing compounds to probe status

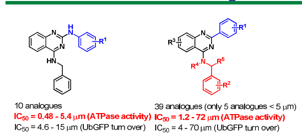
Top "Tier" Compound Hits from HTS and SAR Studies

1 Screening Hits

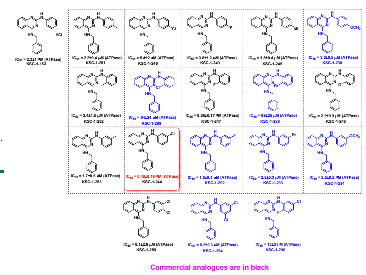


Chou T.-F., et al. Proc. Natl. Acad. Sci. USA **108**:4834-9 (2011).

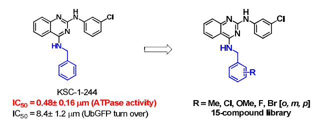
2 SAR for Commercial Analogues



3 SAR for 2,4-diaminoquinazoline Chemotype

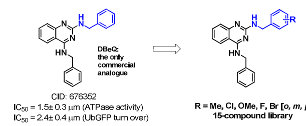


4 Library "A" SAR



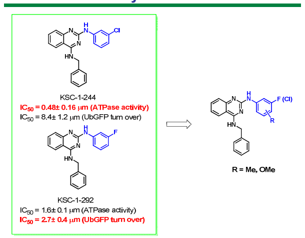
ATPase activity: IC_{50} = 1.0~5.0 μM,
UbGFP turn over: IC_{50} = 5.7~18 μM

5 Library "B" SAR



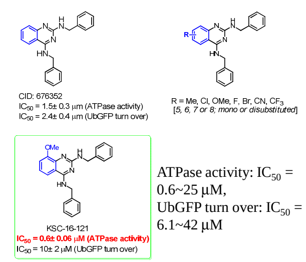
ATPase activity: IC_{50} = 2.0~5.3 μM,
UbGFP turn over: IC_{50} = 1.3~3.9 μM

6 Library "C" SAR



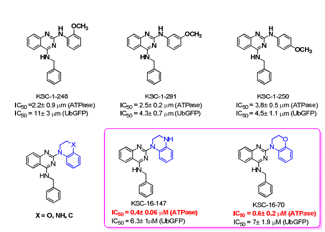
ATPase activity: IC_{50} = 0.7~6.7 μM,
UbGFP turn over: IC_{50} =3.7~16 μM
Most of the analogues are <1 μM (ATPase)

7 Library "D" SAR

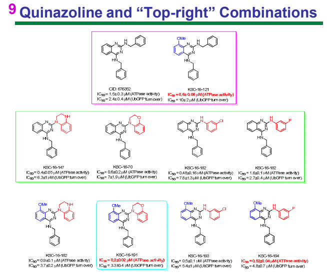


ATPase activity: IC_{50} = 0.6~25 μM,
UbGFP turn over: IC_{50} = 6.1~42 μM

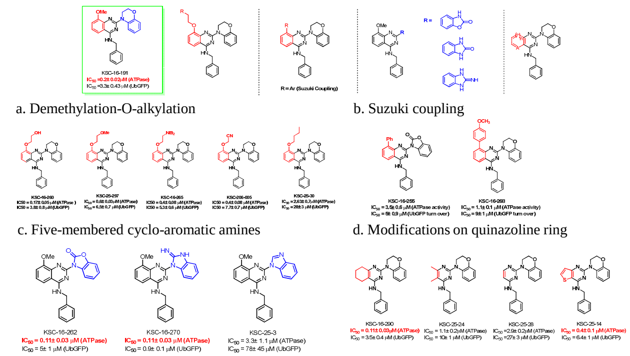
8 Constrained Analogues SAR



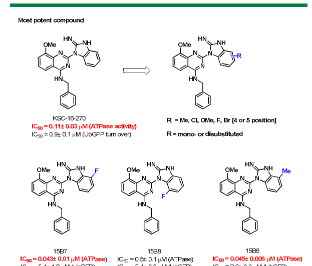
9 Quinazoline and "Top-right" Combinations



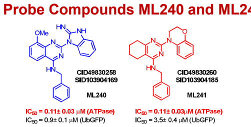
10 More Analogues Aimed at Improving ATPase and Ub-GFP Turn Over Activities



11 Modifications on 2-aminobenzimidazole



The Probe Compounds ML240 and ML241

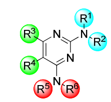


ML240: IC_{50} = 0.11± 0.03 μM (ATPase); IC_{50} = 0.9± 0.1 μM (UbGFP)

ML241: IC_{50} = 0.11± 0.03μM (ATPase); IC_{50} = 3.5± 0.4 μM (UbGFP)

CID/ML#	Target Name	IC_{50}/EC_{50} (nM) [SID, AID]	Anti-target Name(s)‡	IC_{50}/EC_{50} (μM) [SID, AID]	Fold Selective	Secondary Assay(s) Name: IC_{50}/EC_{50} (nM) [SID, AID]
49830258 **ML240**	p97ATPase Ub^{G76V}-GFP	110 nM [SID103904169, AID504650] 900nM [SID103904169, AID504649]	170 Kinase Panel ODD-Luc	<50% I at 20 μM 28,000nM [SID103904169, AID504654]	N/A 31-fold	Caspase 3/7(SW403): 29-fold at 10,000 nM [SID103904169, AID504653]
49830260 **ML241**	p97ATPase Ub^{G76V}-GFP	110 nM [SID103904185, AID504650] 3,500nM [SID103904185, AID504649]	170 Kinase Panel ODD-Luc	>50% I at 20 μM (JAK1, PIP5K3, DNAPK) 46,000nM [SID103904185, AID504654]	N/A 13-fold	Caspase 3/7(SW403): No Activation up to 20,000 nM [SID103904185, AID504653]

SAR Summary – ATPase Activity



- Changes to the region defined by R[1]/R[2] (turquoise) produced the most dramatic improvement in potency
- Changes to the region defined by R[3]/R[4] (green) seemed to be well-tolerated, across the range of substituents introduced at R[1]/R[2]
- Changes to R[5]/R[6] (red) were not well-tolerated, with R[5] = H and R[6] = benzyl being preferred at these positions
- Future efforts to improve potency against p97 ATPase might involve exploring the region defined by R[1]/R[2], while a strategy for improving compound physiochemical properties, for example, improving compound solubility by introducing basic amine functional groups, may well involve exploring the region defined by R[3]/R[4]

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