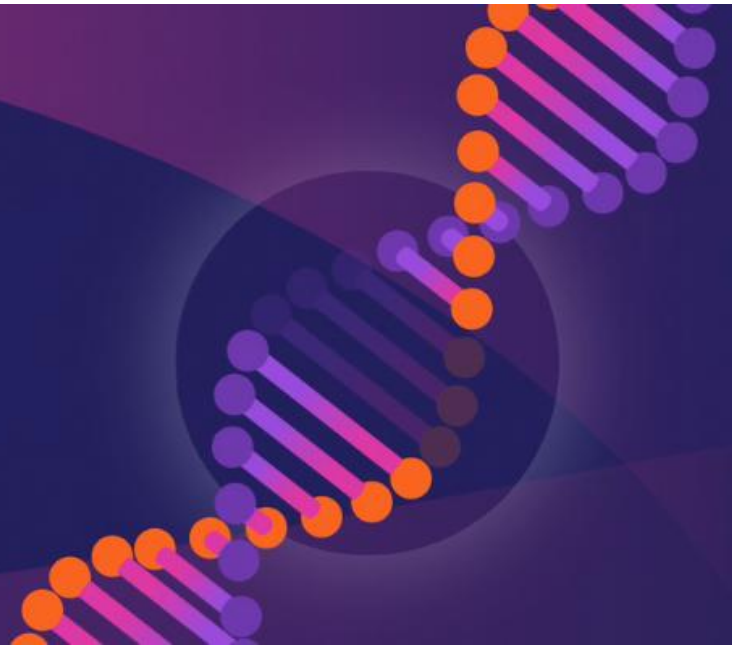
TANGO therapeutics™

Discovery of TNG348

A selective and potent inhibitor of USP1 for treatment of BRCA1, BRCA2-mutant and other HRD+ cancers

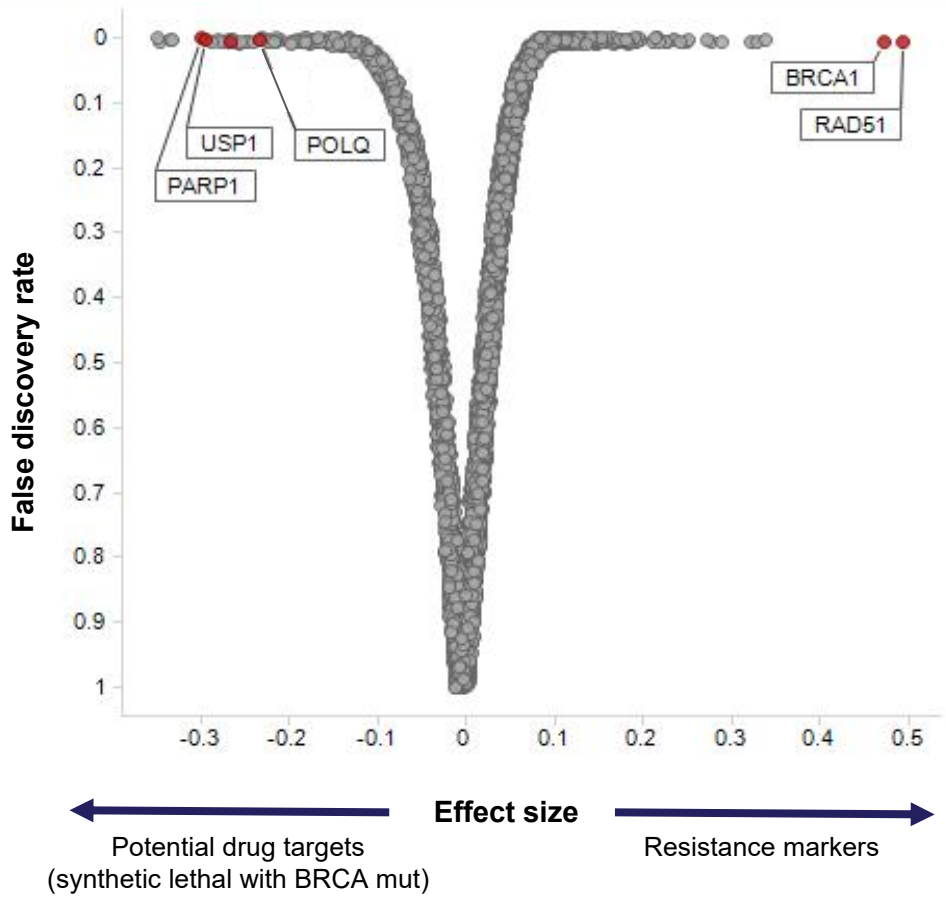


Scott Throner

March 20, 2024
ACS National Meeting, New Orleans

USP1 inhibition is synthetic lethal with BRCA1/2 mutations

USP1 is a strong hit in a druggable genome CRISPR screen



- BRCA1/2 mut and other HRD+ cancers include ~50% ovarian, 25% breast, 10% prostate and 5% pancreatic cancers
- Loss of USP1 results in impaired DNA replication in BRCA1/2 mutant and other HRD deficient cells
- USP1 or related genes are not pan lethal in Achilles CRISPR

Simoneau et al, *Mol. Cancer Ther*. **2023**, 22, 215-226

USP1 and BRCA1 mutant synthetic lethality independently discovered by D'Andrea group at DFCI - Lim et al, *Molecular Cell*, **2018**, 73, 925-941

USP1 inhibition is synthetic lethal with BRCA1/2 mutations

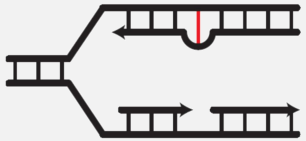
Multiple mechanisms exist to repair damaged DNA

BRCA1/2 mutations (HRD+)

Prevent repair of double strand breaks (homologous recombination repair)

USP1 inhibitors

Prevent efficient repair of single strand breaks (translesion synthesis)



PARP inhibitors

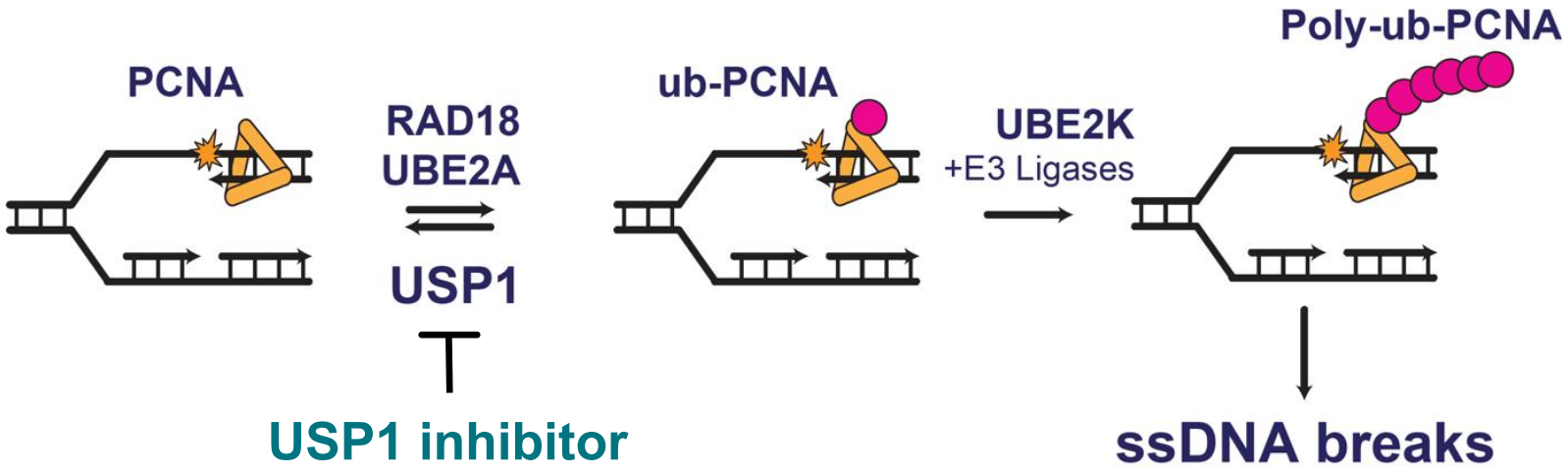
Prevent efficient repair of single strand breaks (base excision repair)

Blocking DNA damage repair causes cell death

- Normal cells have multiple mechanisms to repair damaged DNA and prevent cell death (or cancer)
- BRCA1/2 mutant cells rely on translesion synthesis and base excision repair
- Both USP1 and PARP inhibition severely impair DNA damage repair in BRCA1/2 mutant cells
- Combining USP1 and PARP inhibition largely eliminates DNA damage repair in BRCA1/2 mutant cells

USP1 inhibition blocks an important DNA damage repair pathway

USP1 inhibition blocks translesion synthesis



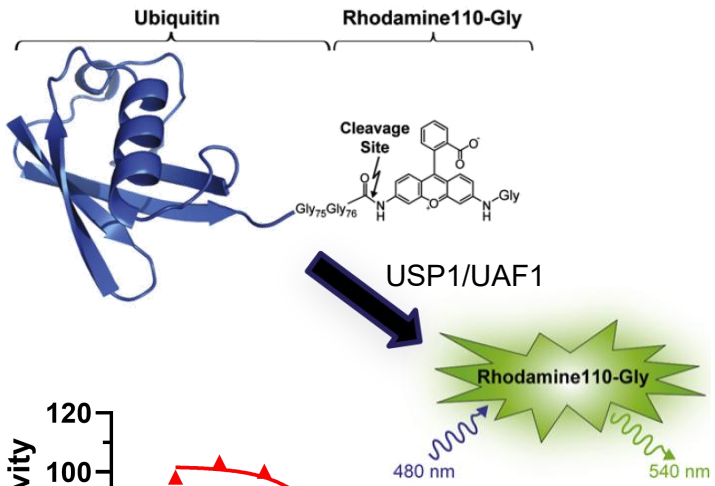
BRCA1/2 mutant cells rely on translesion synthesis because they lack efficient double-strand break repair

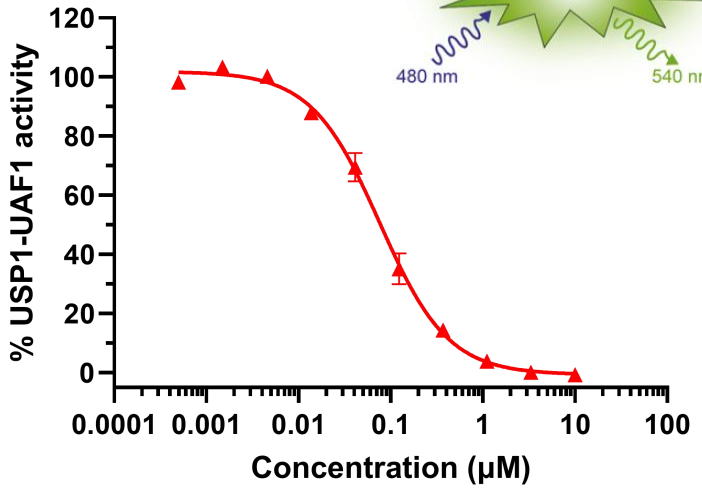
- USP1 is a de-ubiquitinating enzyme (DUB) in complex with UAF1
- USP1•UAF1 is required to complete single stranded DNA break repair via **translesion synthesis**
- **Mono-ubiquitinated PCNA** is required to read through damaged DNA
- USP1 inhibition causes accumulation of poly-Ub PCNA
- **poly-UB PCNA** blocks translesion synthesis mediated DNA repair

Assays to measure biochemical and cellular selectivity

Biochemical

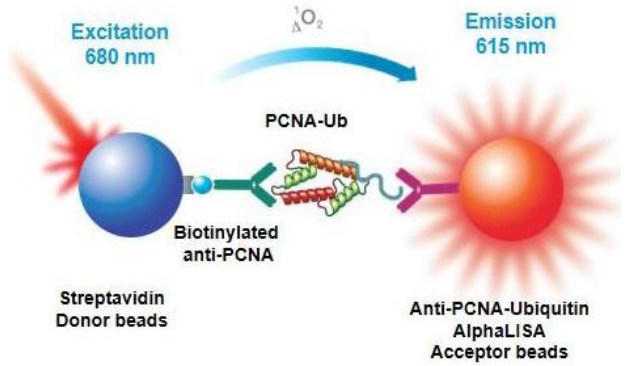
USP1-UAF1 Ubiquitin Rho110-glycine cleavage fluorescence assay

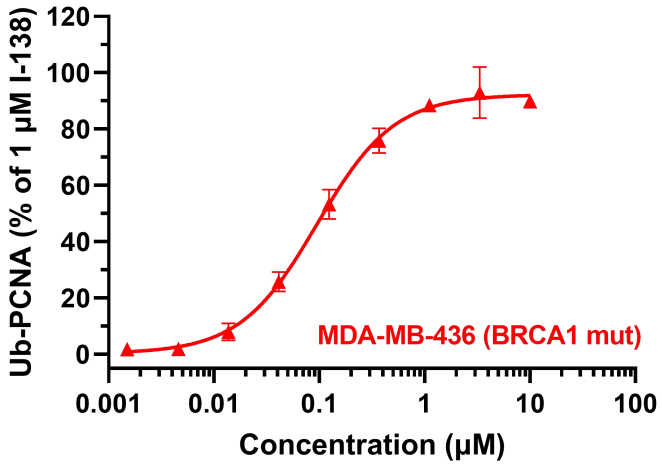




Cellular PD

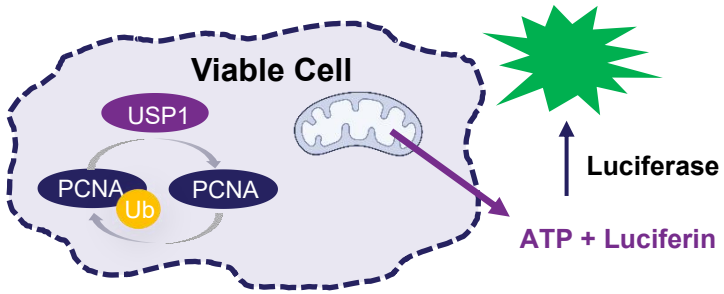
AlphaLISA detection of ub-PCNA in BRCA1 mut cell line

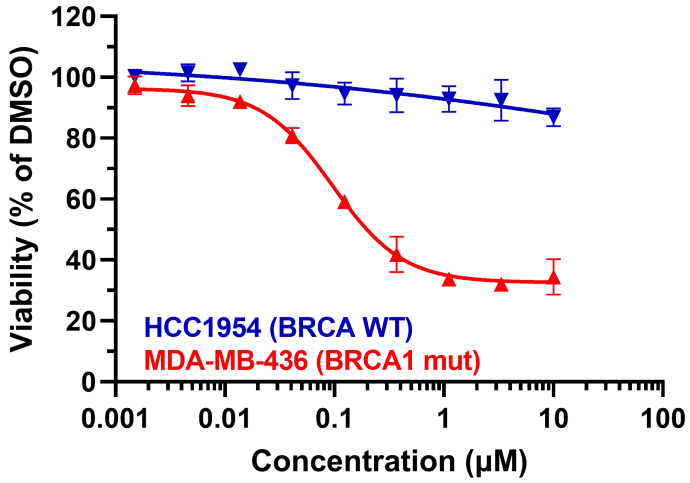




Cellular viability

10-day viability assay assessed by CellTiter-Glo in BRCA1 mut and WT cell lines

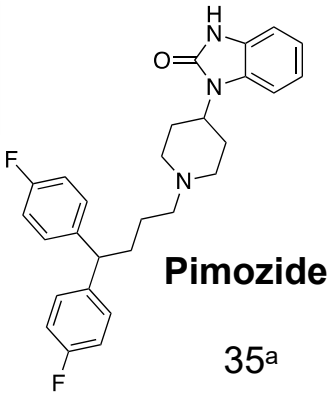
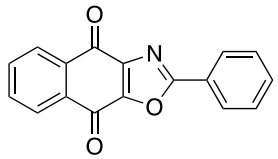
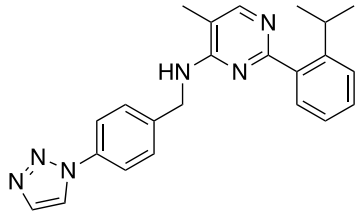
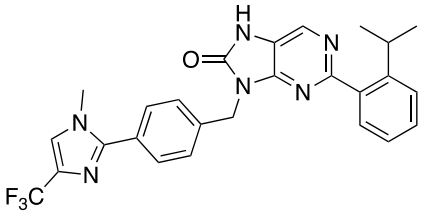




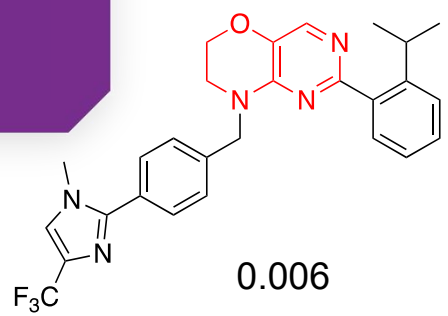
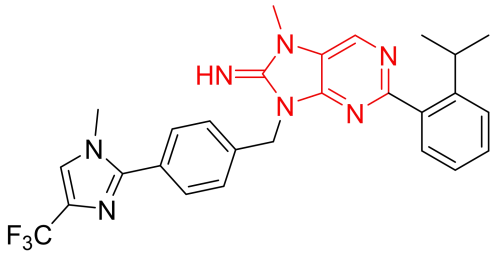
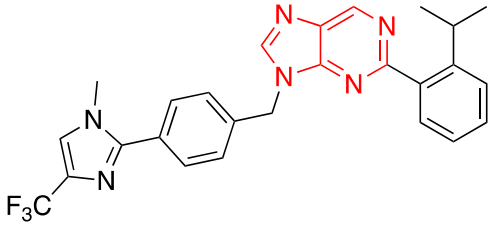
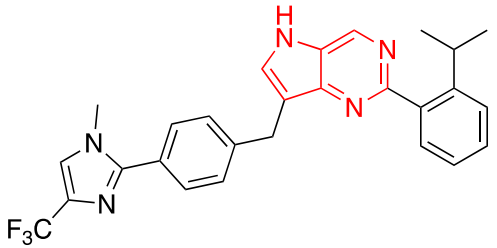
Example of Ub-Rho110 cleavage assay by Chen et al. *Chem. Biol.* **2011**, 18(11), 1390-1400

Known USP1 inhibitors and examples of scaffolds investigated at program initiation

Published USP1 inhibitors (2019)

	Pimozide	SJB2-043	ML323	I-138
				
USP1 IC_{50} (μM)	35[a]	0.54[b]	0.076[c]	< 0.05[d]

Select examples of pursued scaffolds

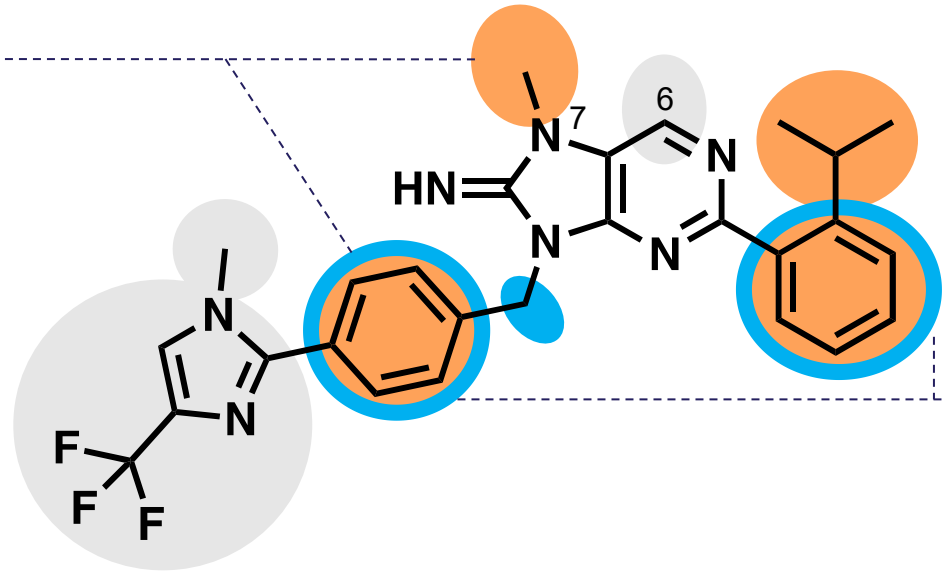
				
USP1 IC_{50} (μM)	0.006	0.010	0.059	0.004
$LogD_{7.4}$	> 4	3.7	4.3	> 4.5
Mic hCL_{int} (μL/min/mg)	> 300	51	146	88

(a) Chen et al. *Chem. Biol.* **2011**, 18(11), 1390-1400; (b) Mistry et al. *Mol. Cancer Ther.* **2013**, 12(12), 2651-2662; (c) Liang et al. *Nat. Chem Biol.* **2014**, 10(4), 298-304 and Dexheimer et al. *J Med Chem.* **2014**, 57(19), 8099-8110; (d) Buckmelter et al. WO2017087837

Initial medchem focus on lipophilicity, metabolism, potency

Potency

- Heteroaromatic alternatives
- Phenyl isosteres
- Heteroalkyl N-substituents



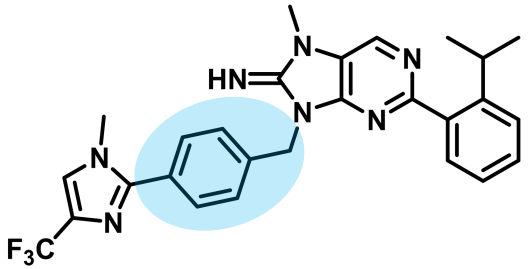
Properties/ADME

- Oxidative blocking groups
- Conformational restrictions
- Underexplored space

Early pharmacophore anchors

- Broad exploration
- Good balance of attributes

Side chain ring modification has high impact on potency

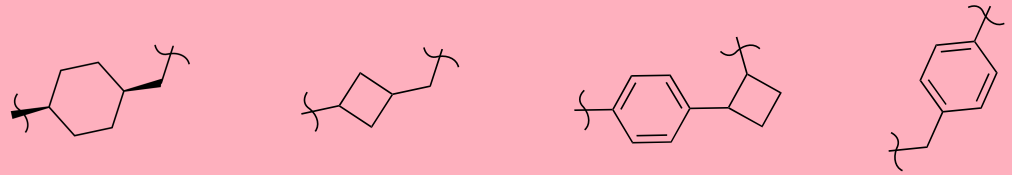


USP1 IC_{50} (μM)	0.009
Cell viability IC_{50} (μM)	0.038
Mic hCL_{int} (μL/min/mg)	52

< 100 nM Biochem

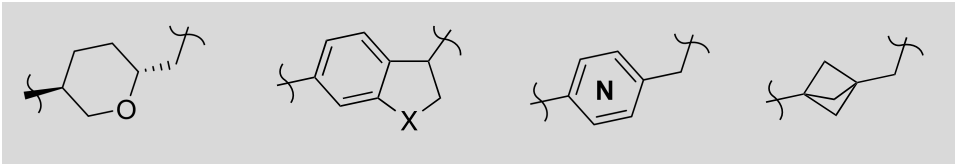
100 - 200 nM Biochem

> 1 μM Biochem

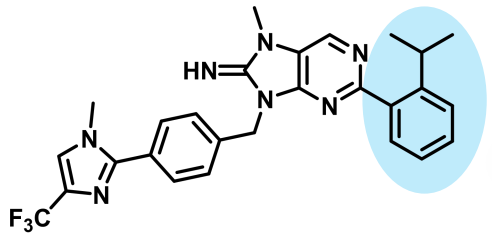


- Phenyl preferred, small *ortho* substituents tolerated
- Small benzylic substitution can decrease CLint
- Constrained linkers give significant decrease in potency

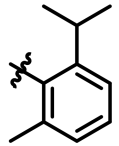
Other linkers in separate contexts unproductive, e.g.



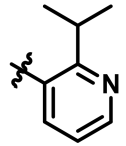
2-position aryl moiety significantly impacts potency and properties



Methyl and nitrogen scan

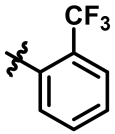


0.026 / 0.16
78 / 3.9

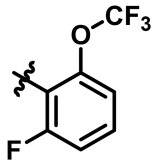


0.074 / 0.38
30 / 2.8

isopropyl replacement



0.030 / 0.076
< 9.6 / 3.5



0.017 / 0.052
26 / 3.3

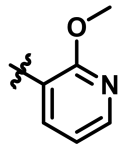
USP1 IC_{50} (μM) | Cell viability IC_{50} (μM)

0.009 / 0.038
52 / 3.7

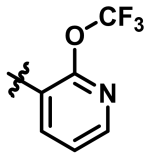
Hu Mic CL_{int} (μL/min/mg) | $LogD_{7.4}$

Potency | Properties

Potency 😊 | Properties ☺

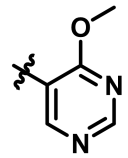


0.109 / 1.1
< 9.6 / 2.3

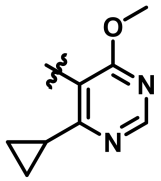


0.029 / 0.077
14 / 3.4

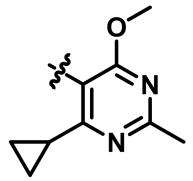
Reducing LogD



1.71 / –
25 / 2.0



0.135 / 0.15
< 9.6 / 2.2

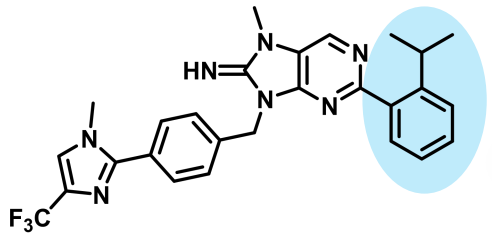


0.064 / 0.068
15 / 2.9

Potency 😊 | Properties 😊

Potency 😊 | Properties 😊

2-position aryl moiety significantly impacts potency and properties



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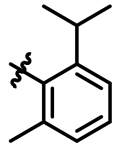
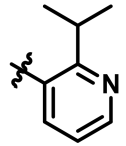
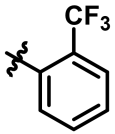


0.026 / 0.16
78 / 3.9
–

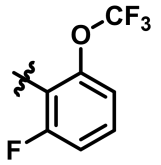


0.074 / 0.38
30 / 2.8
0.99

isopropyl replacement



0.030 / 0.076
< 9.6 / 3.5
1.2

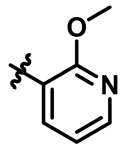


0.017 / 0.052
26 / 3.3
0.08[a]

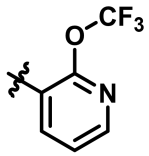
USP1 IC_{50} (μM) | Cell viability IC_{50} (μM)

0.009 / 0.038
52 / 3.7
0.94

Hu Mic CL_{int} (μL/min/mg) | $LogD_{7.4}$ | hERG IC_{50} (μM)

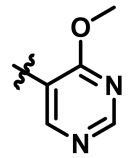


0.109 / 1.1
< 9.6 / 2.3
1.7

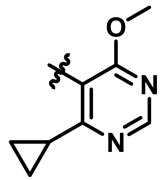


0.029 / 0.077
14 / 3.4
0.38[a]

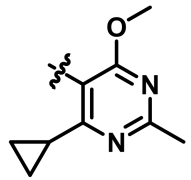
Reducing LogD



1.71 / –
25 / 2.0
–



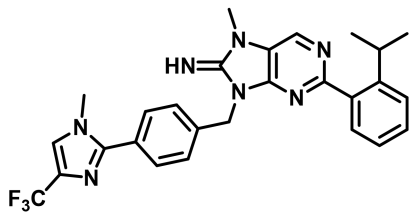
0.135 / 0.15
< 9.6 / 2.2
1.8



0.064 / 0.068
15 / 2.9
2.6

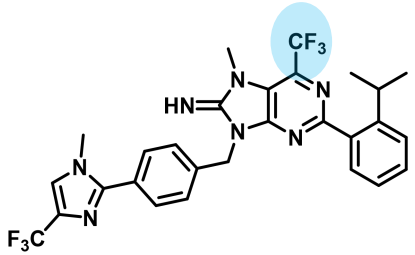
(a) hERG [^{3}H]-dofetilide binding IC_{50} (μM)

Basicity, polarity, and steric modifications in approach to attenuate hERG channel inhibition

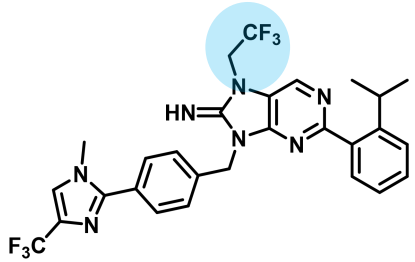


USP1 IC_{50} (μM)	0.009
pKa	7.1
hERG IC_{50} (μM)	**0.94**

Reduce core basicity

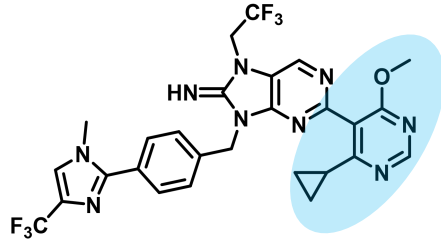


0.023	
6.1[a]	
1.6[b]	



0.043	
5.3[a]	
4.3	

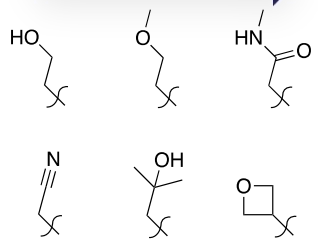
Reduce LogD

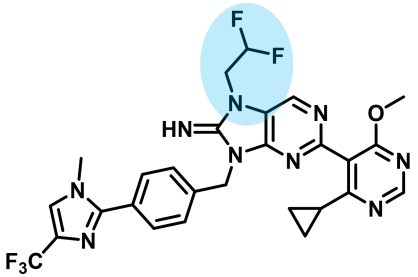


0.099	
4.8	
9.5	

Explore *N*-7 substituents

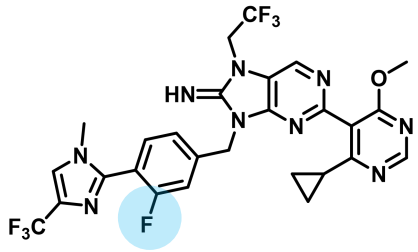
e.g.



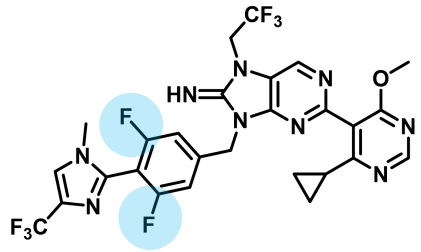


0.045	
5.4	
10.6	

Fluorinate phenyl linker



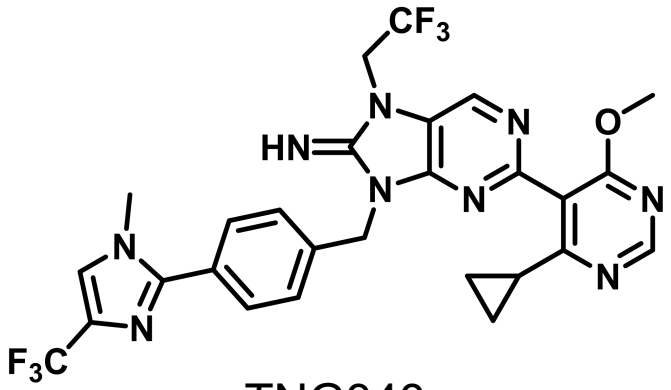
0.117	
4.7[a]	
10.8	



0.078	
4.5[a]	
24	

(a) modeled pKa, (b) hERG [^{3}H]-dofetilide binding IC_{50} (μM)

TNG348 preclinical profile

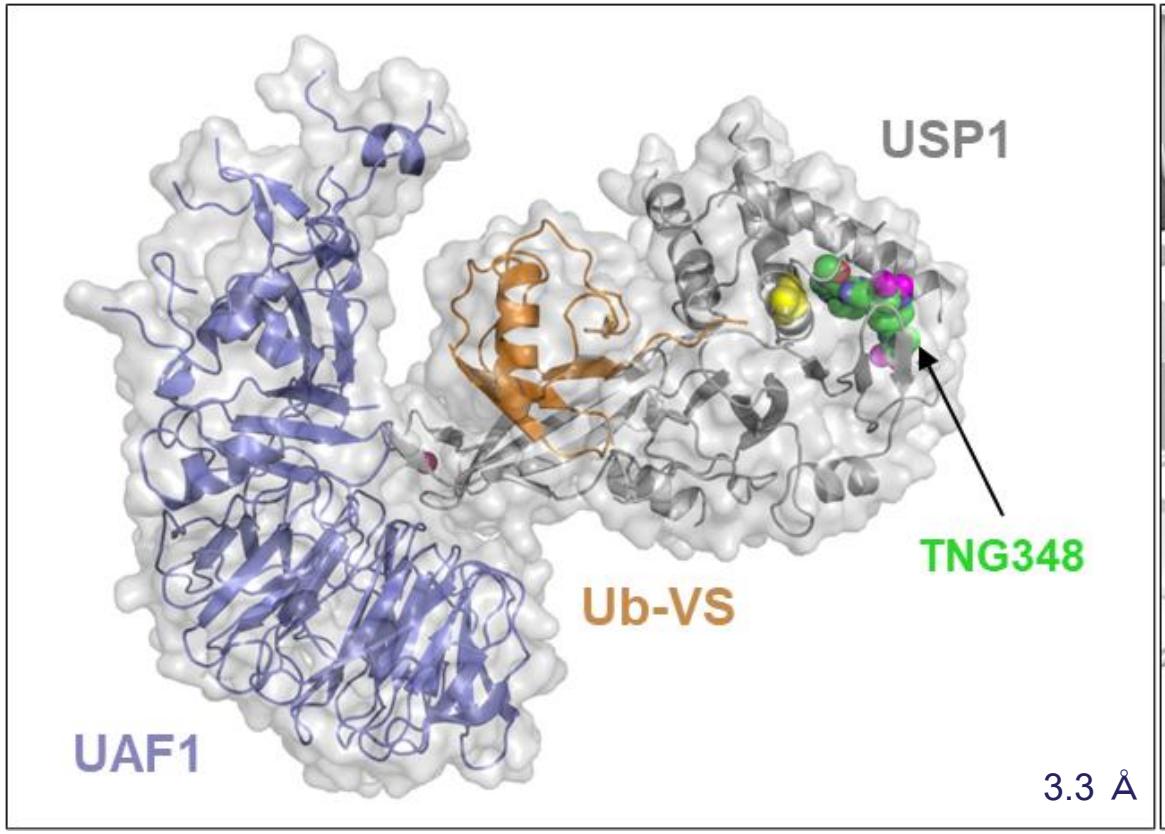


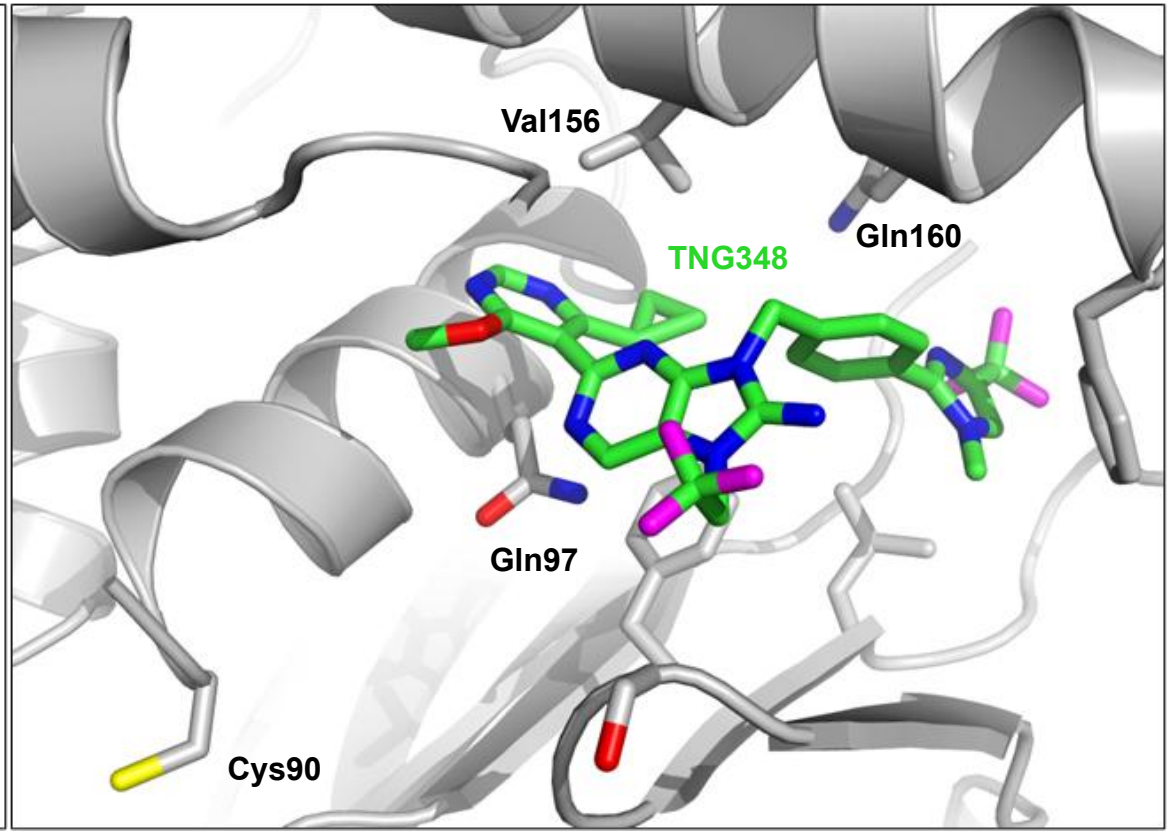
TNG348

Select preclinical properties	
MW, $LogD_{7.4}$, TPSA	604, 3.2, 112
Cellular PD IC_{50}, viability IC_{50} (nM)	95, 68
MDCKII P_{app} (cm/s x 10^{-6}), Mdr1 ER	31, 29
$Cl_{int, hep}$ h, r, d, c (µL/min/10^6)	< 0.9, < 0.9, 1.2, 1.0
CL r, d, c (mL/min/kg)	13, 11, 13.5
$T_{1/2}$ r, d, c (hrs)	3.5, 5.8, 1.4
%F r, d, c	74, ≈100, 67
PPB (% unbound) h, r, d, c	21, 30, 21, 20
GLP hERG IC_{50} (µM)	19.6
Deubiquitinase panel*	Highly selective
Eurofins safety and kinase panels	No concerns

$hCl_{int, hep}$: intrinsic clearance in human hepatocytes; GLP hERG: manual patch clamp; *Ubiquigent DUB*profiler*™

Cryo-EM structure of TNG348 bound to USP1•UAF1•Ub-VS complex



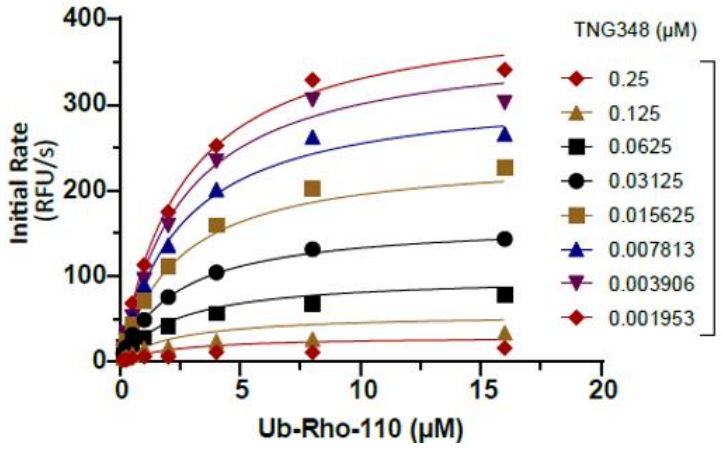


- Induced fit in cryptic allosteric pocket normally occupied by N-terminus loop
- TNG348 binding induces movement of catalytic triad residues, impeding USP1 peptidase activity

For cryo-EM structure of ML323 in USP1-UAF1 and discussion on mode of USP1 inhibition see Rennie et al., *Sci. Adv.* **2022**, 8, eabq6363

Non-competitive inhibition negated by V156K point mutation

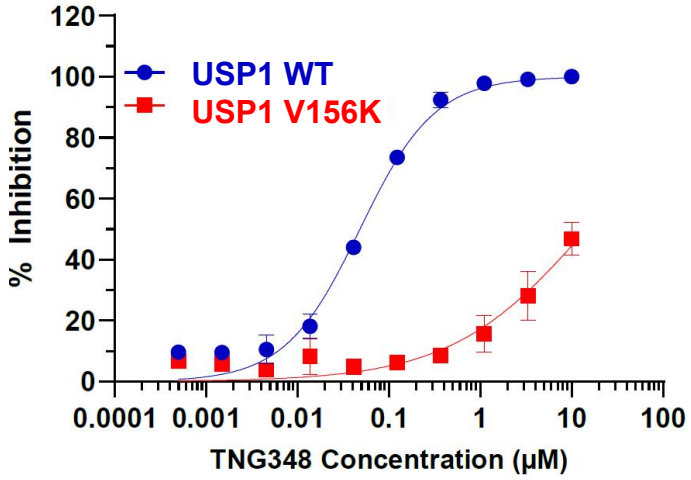
Non-competitive inhibition



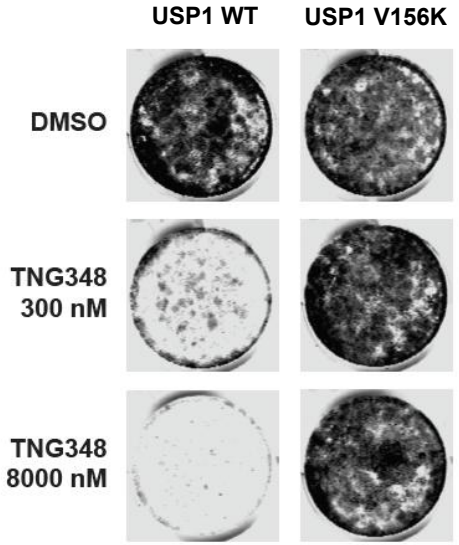
- Lowers apparent Vmax
- No effect on Km of ubiquitin

> 100-fold reduced activity with allosteric pocket mutant

TNG348 Biochemical Potency
Ub-Rho110 assay



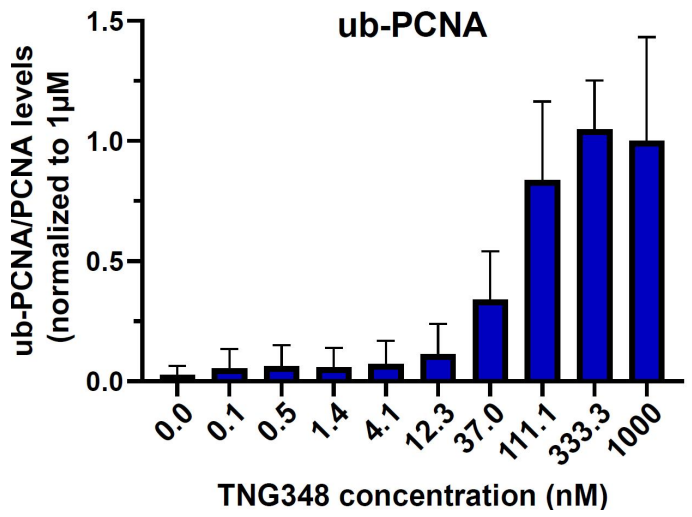
TNG348 Cell viability
MDA-MB-436



- Cellular validation of structural and biochemical findings

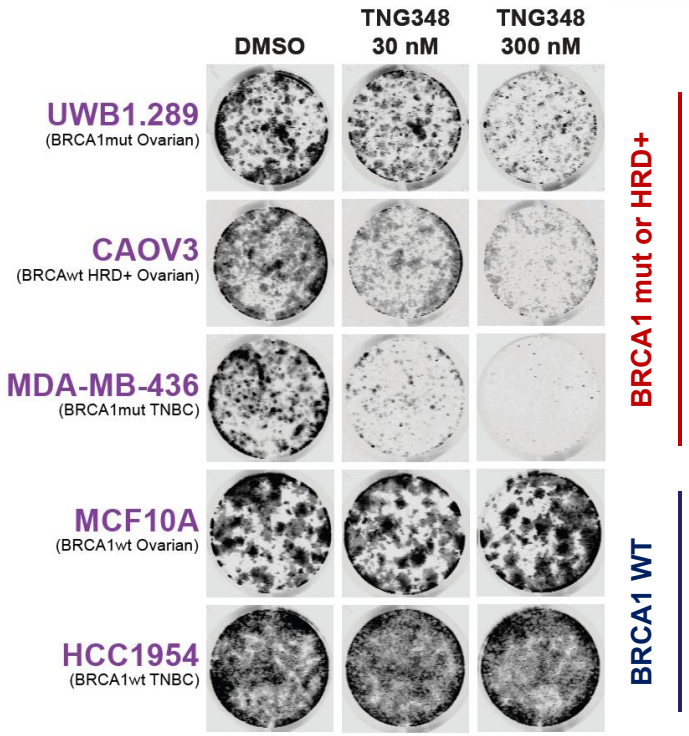
TNG348 on-target activity as single agent and combined with PARPi

Cellular PD



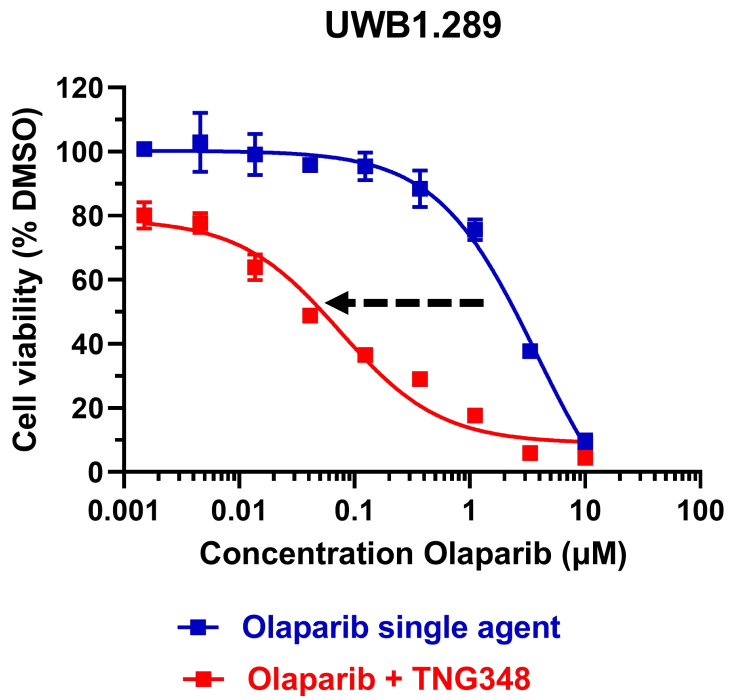
Ubiquitin-PCNA increase in MDA-MB-436 breast cancer cell line

Clonogenic assays



Differentiated activity in BRCA1 mut or HRD+ cells

TNG348 potentiates PARPi



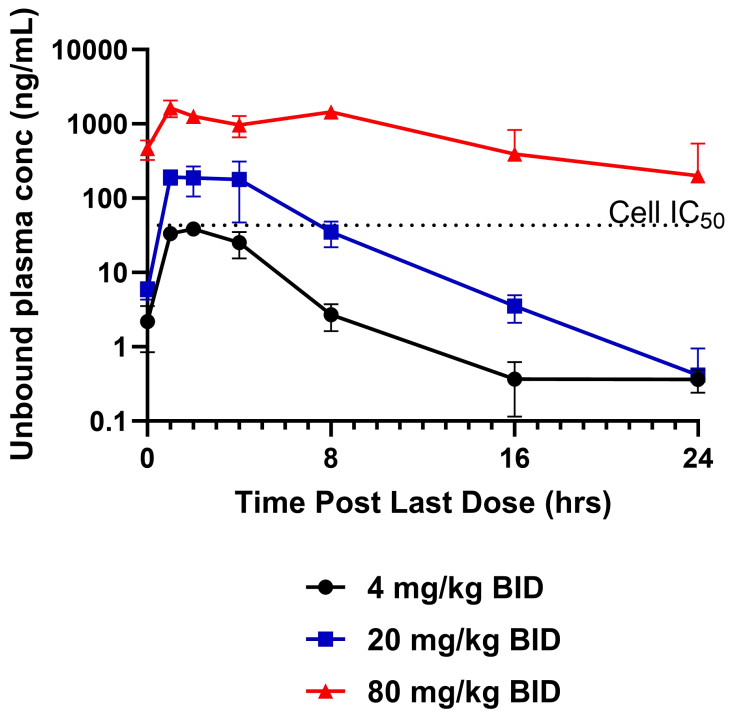
TNG348 potentiates PARPi and significantly lowers its IC50

In vivo dose dependent PK/PD relationship observed with TNG348

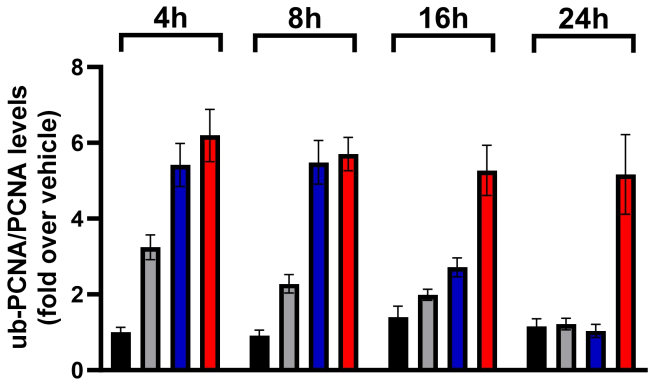
MDA-MB-436 CDX in vivo

(*BRCA1* mut triple negative breast cancer)

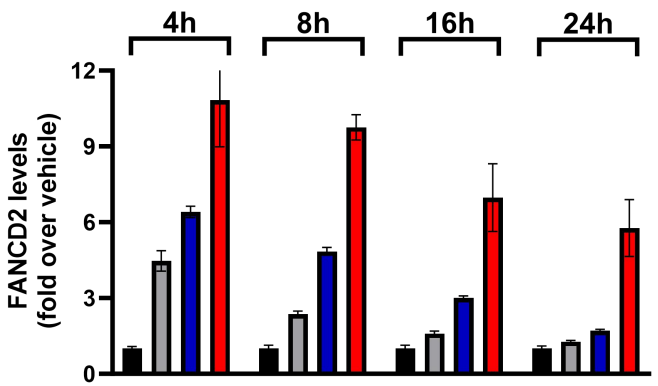
Mouse PO PK



Ub-PCNA



Ub-FANCD2



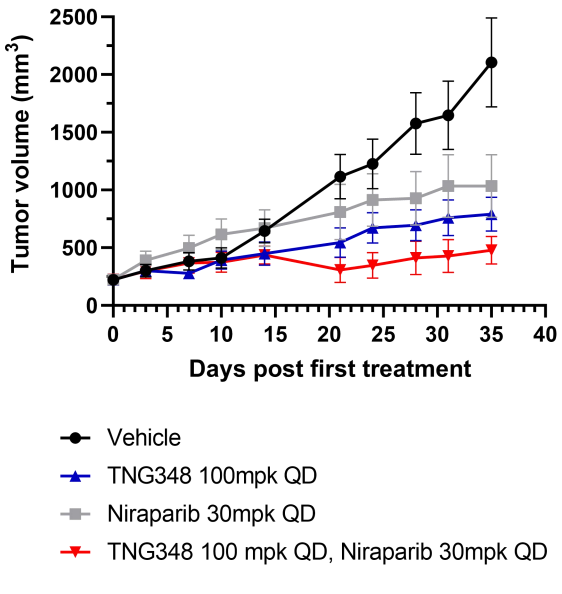
Vehicle
TNG348, 4 mg/kg, BID
TNG348, 20 mg/kg, BID
TNG348, 80 mg/kg, BID

**TNG348 saturates modulation of multiple PD markers*

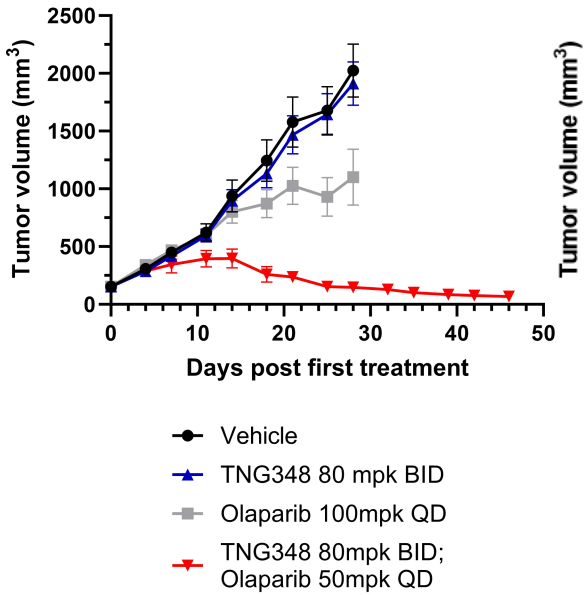
TNG348 is active alone and in combination with PARP inhibitors

In vivo efficacy in PDX models

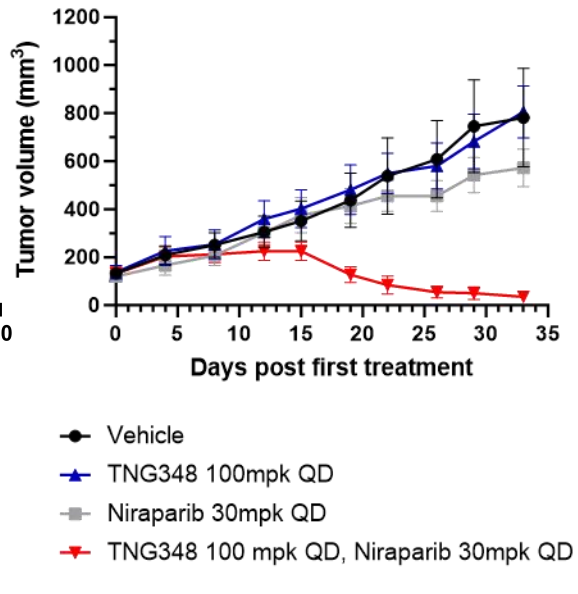
BRCA2 mut
Ovarian cancer



BRCA1 mut
Pancreatic cancer



BRCA wt HRD+
Triple negative breast cancer



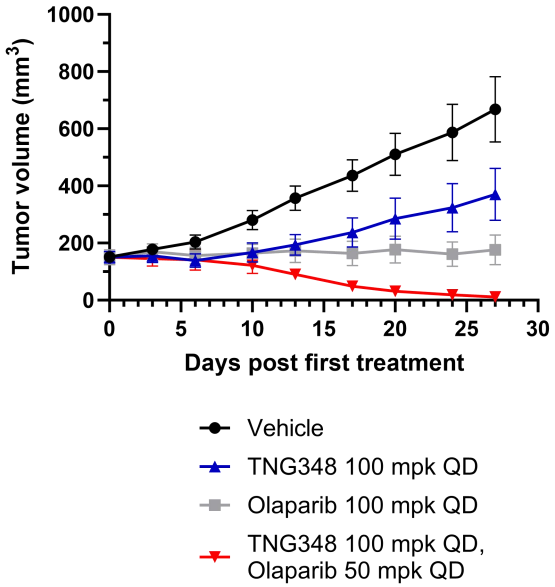
TNG348

- Single-agent activity equivalent to olaparib in multiple models
- Synergy with PARP inhibition in both PARPi sensitive and resistant models
- Strong anti-tumor activity in HRD+ BRCA WT xenograft models broadens the potential addressable patient population

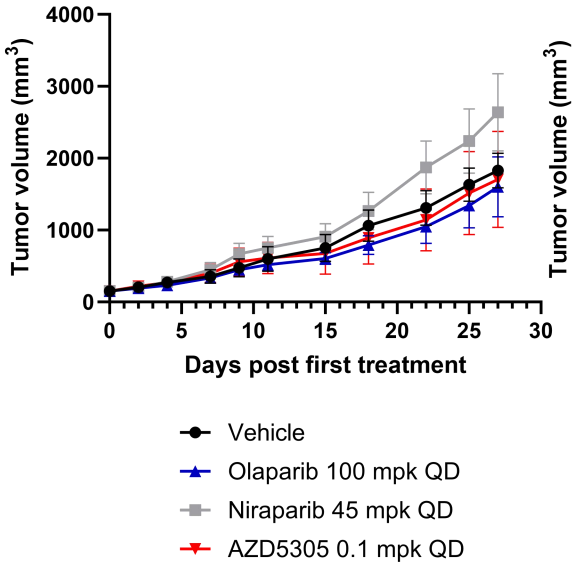
USP1 inhibitors can overcome acquired PARP inhibitor resistance

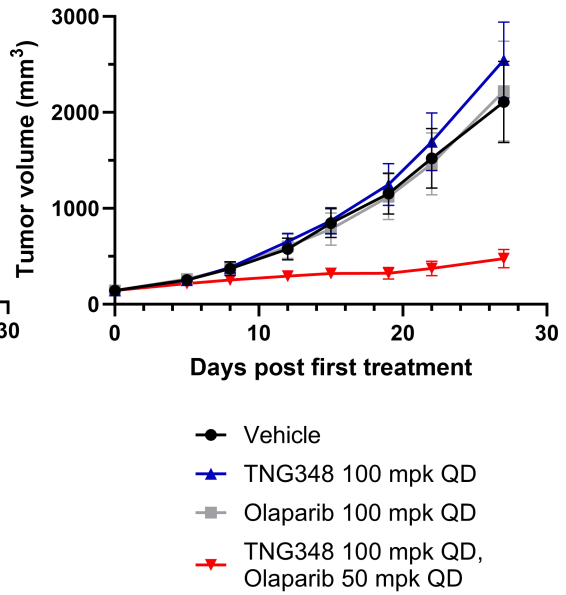
TNG348 in a xenograft with acquired PARPi resistance

Parental
BRCA1 mut breast



PARPi-resistant
BRCA1 mut breast



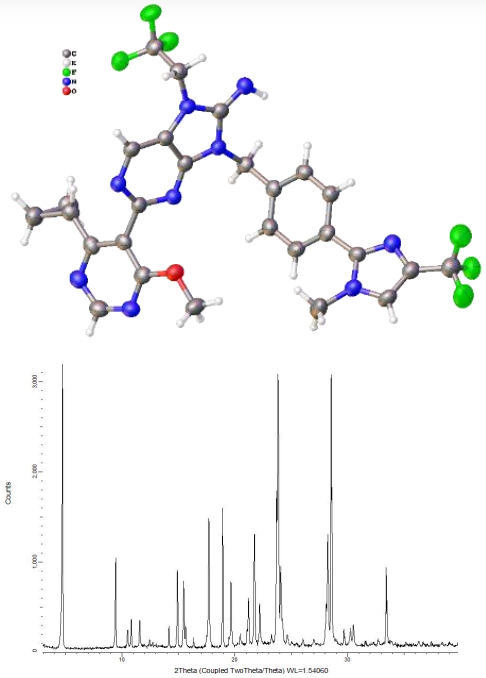


Summary

- Acquired resistance to multiple PARP inhibitors induced by consecutive passage in mice with constant olaparib exposure
- TNG348 + olaparib overcomes acquired PARPi resistance

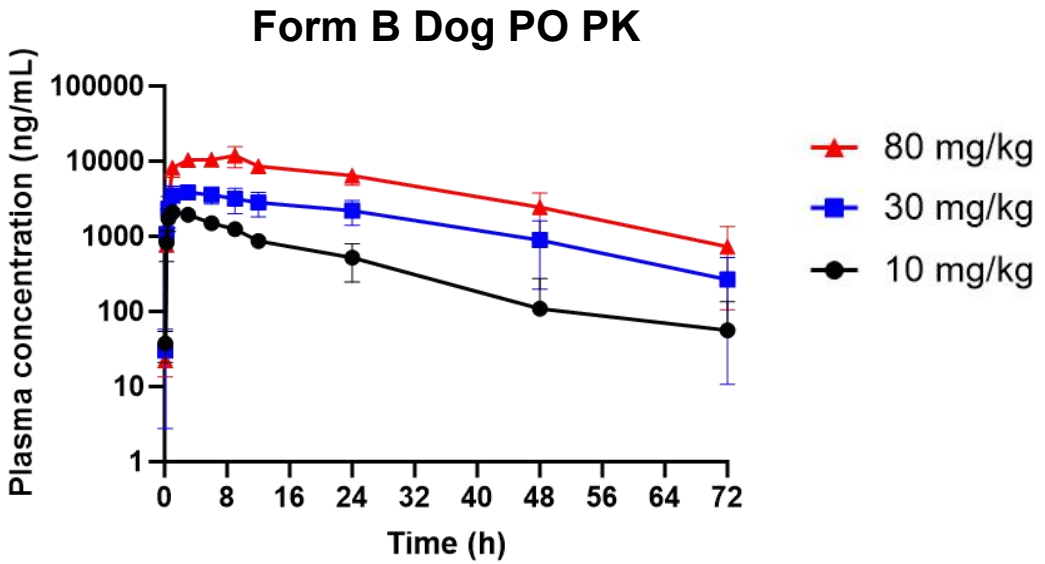
Stable form identified during late preclinical evaluation

Form B



	SGF	FaSSIF
Solubility (mg/mL)	2.7	0.47

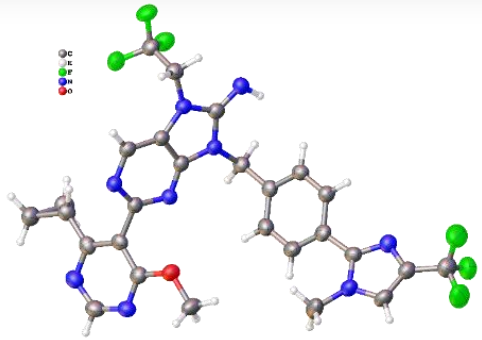
Dog PO PK: dose proportional AUC

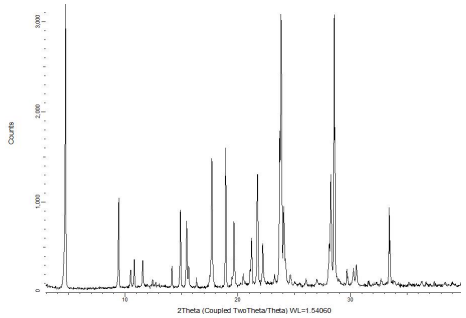


SGF: simulated gastric fluid; FaSSIF: fasted state simulated intestinal fluid

Stable form identified during late preclinical evaluation

Form B

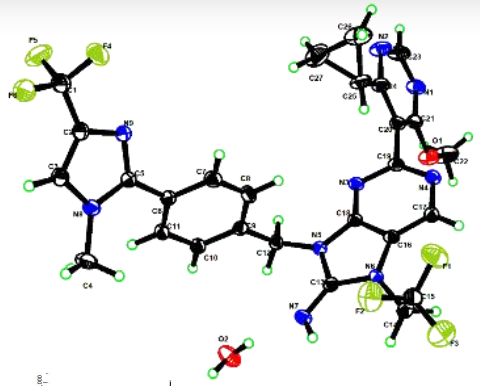


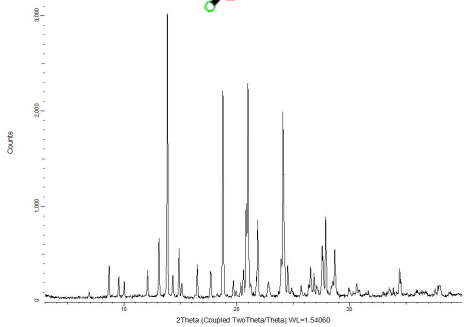


	SGF	FaSSIF
Solubility (mg/mL)	2.7	0.47

Dog PO PK: dose proportional AUC

Form H - monohydrate





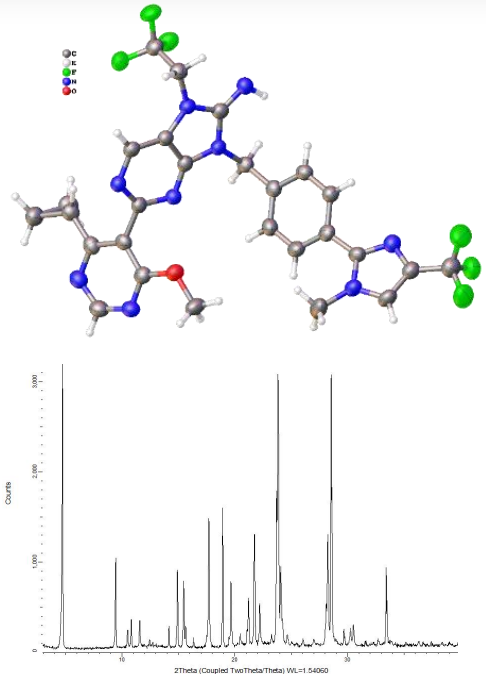
- Crystalline
- Thermodynamical stable polymorph

- Omnipresent

SGF: simulated gastric fluid; FaSSIF: fasted state simulated intestinal fluid

Stable form identified during late preclinical evaluation

Form B



	SGF	FaSSIF
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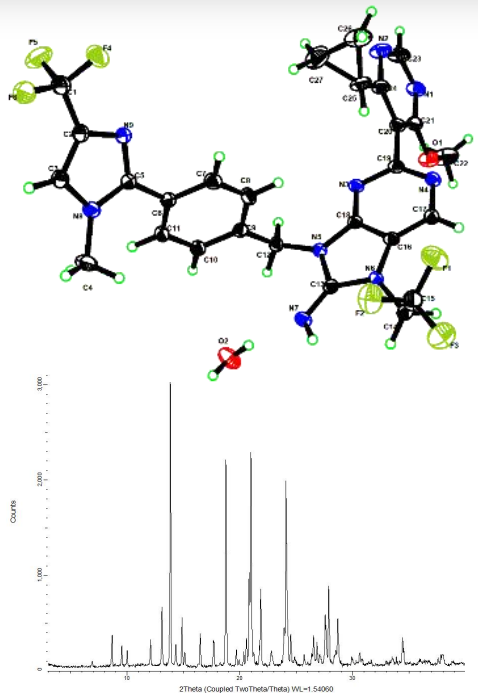
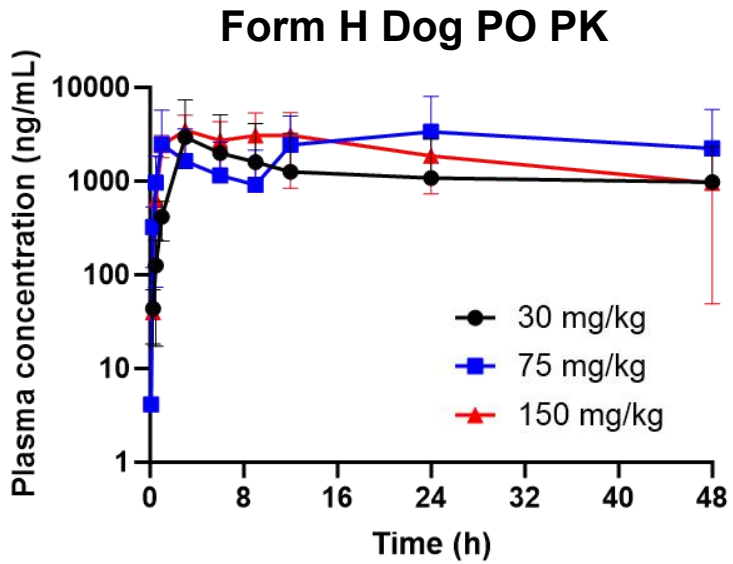
Dog PO PK: dose proportional AUC

Form H - monohydrate



SGF	FaSSIF
0.93	0.0004

variable, low exposure

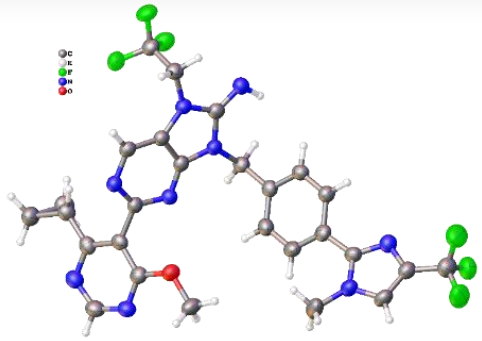


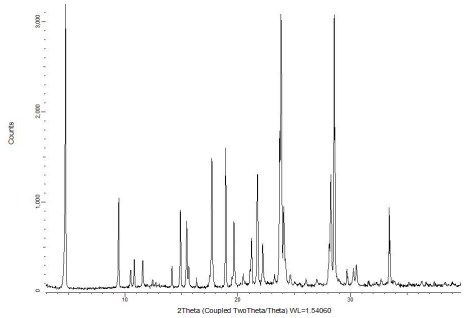
SGF: simulated gastric fluid; FaSSIF: fasted state simulated intestinal fluid

Bauer et al. “Ritonavir: An Extraordinary Example of Conformational Polymorphism” *Pharmaceutical Research*, **2001**, 18(6), 859-866

Challenges of monohydrate overcome with enabling formulation

Form B

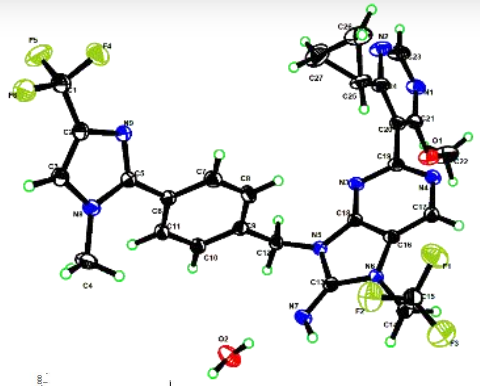


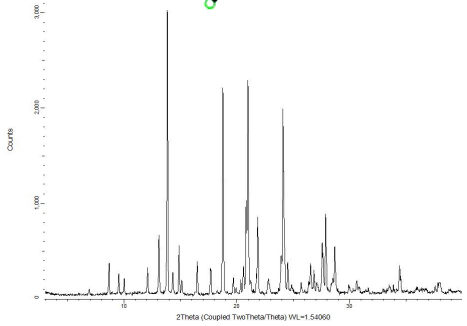


	SGF	FaSSIF
Solubility (mg/mL)	2.7	0.47

Dog PO PK: dose proportional AUC

Form H - monohydrate

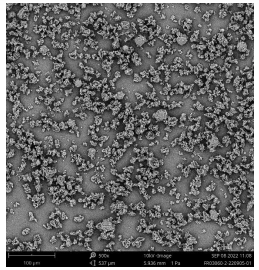


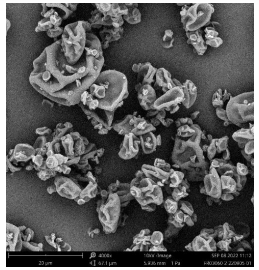


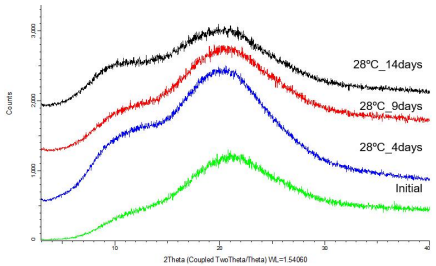
	SGF	FaSSIF
	0.93	0.0004

variable, low exposure

Spray dried dispersion







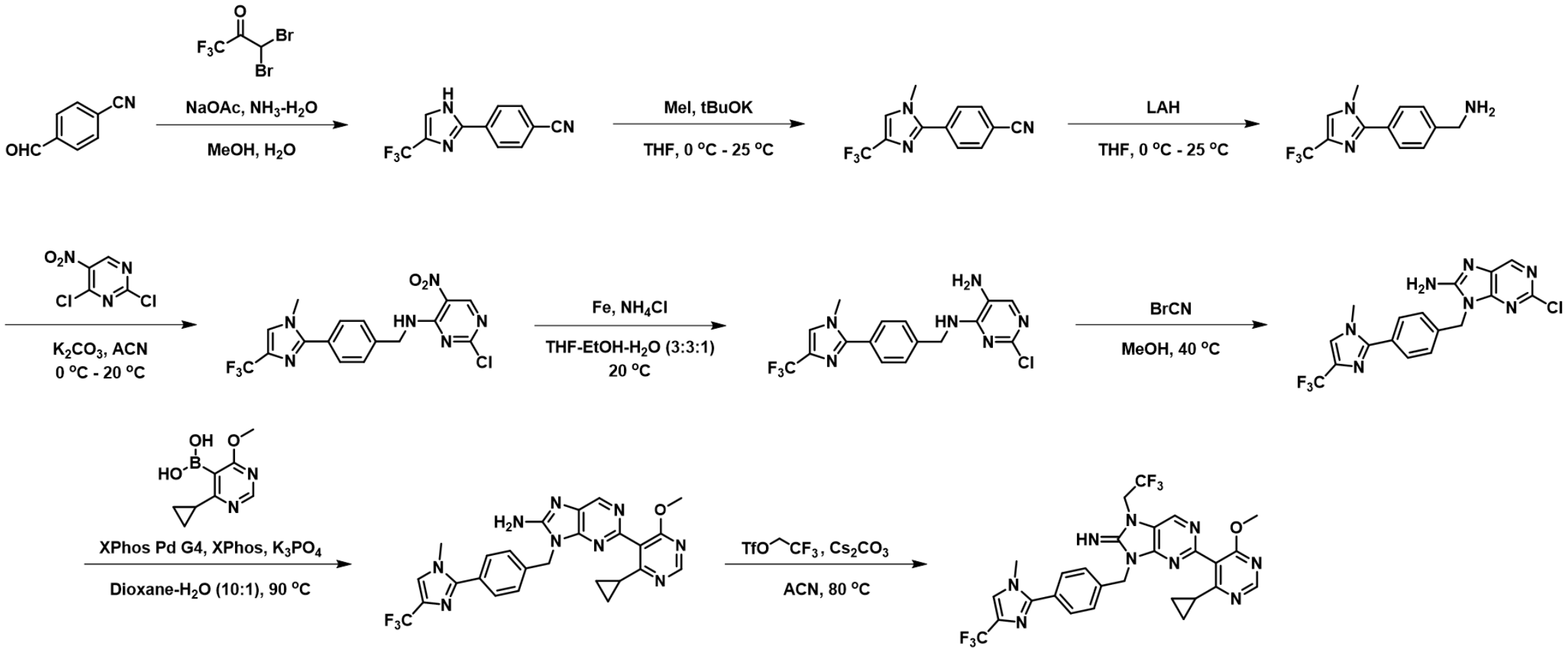
	SGF	FaSSIF
	3.3	0.60

dose proportional AUC

SGF: simulated gastric fluid; FaSSIF: fasted state simulated intestinal fluid

Bauer et al. “Ritonavir: An Extraordinary Example of Conformational Polymorphism” *Pharmaceutical Research*, **2001**, 18(6), 859-866

Med chem synthesis of TNG348



Many thanks to Enamine (Anton Tkachenko, Team Lead) and WuXi STA (Qiao Shuo, CSU PM)

TNG348: A novel, selective inhibitor of USP1

- Potent and selective, reversible allosteric inhibitor of USP1
- Single agent efficacy in BRAC1/2 mutant and HRD+ breast and ovarian cancer models
- Synergistic with PARP inhibition in both PARPi sensitive and resistant models
- Currently in a Phase 1/2 clinical trial (NCT06065059)

Further biological mechanism of action characterization at AACR, abstract #4527

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William Mallender

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Dimitris Papoutsakis
Truc Pham
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Caroline Proulx-Lafrance
Magnus Ronn
Doug Whittington
Minjie Zhang
Wenhai Zhang

Anton Tkachenko
Tetiana Galushka
Enamine Chemistry team

Qiao Shuo, Deju Shang
WuXi Chemistry teams

Fang Liu, Kinsley Zhao
STA DFR team