Fragment-based Lead Discovery

Two approaches aiming to prevent phosphorylation of SMARCA4 by CDK9 using WAC™ and in silico screening

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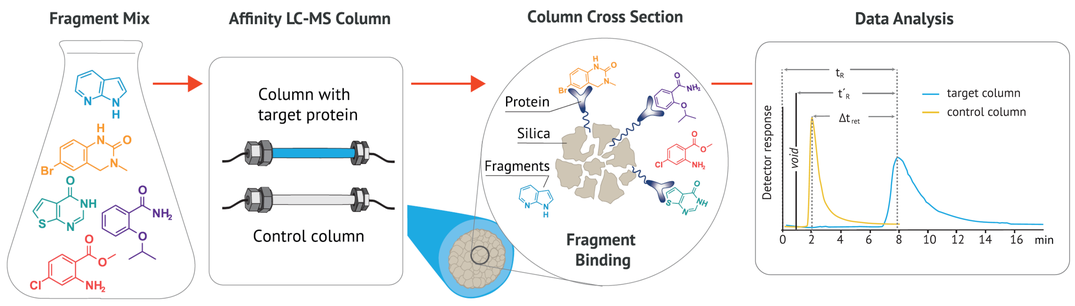
[1]Red Glead Discovery, [2]SARomics Biostructures

Weak Affinity Chromatography

Studying the SMARCA4 bromodomain by WAC™

Introduction to WAC™



Key features

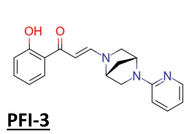
- Affinity chromatography with immobilized target (~mM)
- MS-detection enables screening at low µM, built-in QC
- Affinity range low µM to mM, direct detection
- High throughput (>5000 cpds/week; cocktails of 25-100)

Facts and learnings

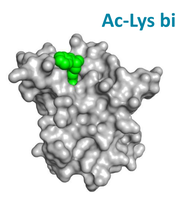
- WAC hit rates from 1% to 20%, avg 6%
- Sensitive to charge effects, buffer and co-factors
- > 50 FBLD projects over 7 years
- One tool in the FBLD toolbox

SMARCA4 FBLD Case Study

PFI-3 used as reference SMARCA4 inhibitor
- Fedorov et al., Sci Adv 2015;1,
- Gerstenberger et al., J Med Chem 2016;59:4800



PFI-3
Xray: 5DKD
TR-FRET: 1.2 µM



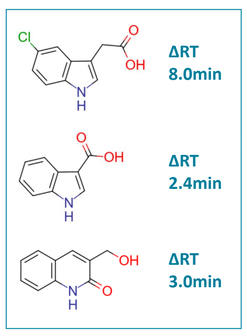
Fragment screen by WAC™
- Ligandability set 250 cpds
- **20 hits (8% hr)**

Hit validation
- TSA (DSF)

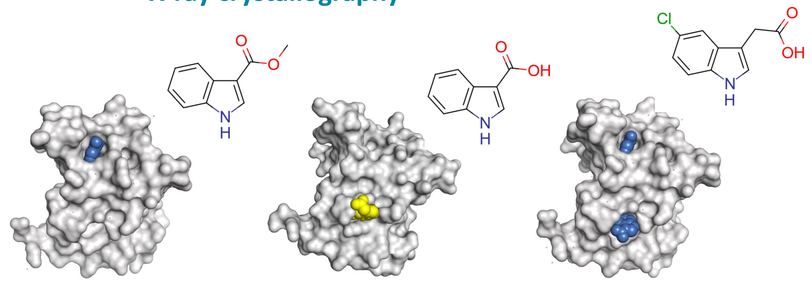
Xray crystallography
- 5 structures
- **New pocket identified**

Hit expansion
- Parallel chemistry, CC, catalogue

Example WAC actives



X-ray crystallography

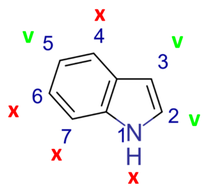


RSF-137
WAC ΔRT: 3.5 min
Xray: 1.85 Å
TR-FRET: 31% @ 250 µM

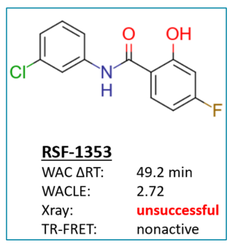
RSF-153
WAC ΔRT: 2.4 min
Xray: 1.5 Å
TR-FRET: 36% @ 250 µM

RSF-024
WAC ΔRT: 8 min
Xray: 1.3 Å
TR-FRET: nonactive

SAR studies and hit expansion by parallel chemistry



- ~30 indole analogs tested by WAC
- Substitution not tolerated in positions 1, 4, 6- and 7
- Substitution tolerated in positions 2, 3 and 5



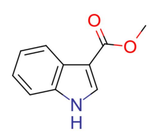
RSF-1353
WAC ΔRT: 49.2 min
WACLE: 2.72
Xray: unsuccessful
TR-FRET: nonactive

- ~150 cpds made by efficient parallel chemistry (72 h) → WAC
- Three series explored:
  - Substitution on indole
  - Scaffold hopping → quinoline
  - Ring opening to aniline
- Longest ΔRT from aniline series – RSF-1353

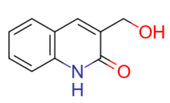
Site-selectivity screening by WAC

ID	Binding site	WAC ΔRT (min)	%change after PFI-3 saturation
RSF-137	1	3.5	-57%
RSF-136	1	2.9	-63%
RSF-153	1 & 2	2.4	-16%
RSF-1648	2	9.4	-9%
RSF-1654	2	8.9	-7%
RSF-1353	2	49.2	-12%

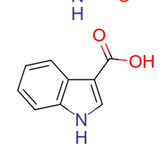
Ac-Lys binding site ("Site 1") binders



RSF-137
TR-FRET: 31% @ 250 µM

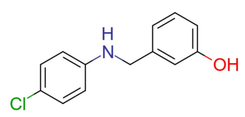


RSF-136
TR-FRET: 65% @ 250 µM

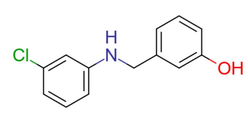


RSF-153
TR-FRET: 36% @ 250 µM

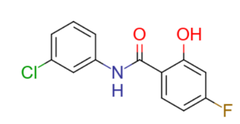
New site ("Site 2") binders?



RSF-1648
TR-FRET: nonactive

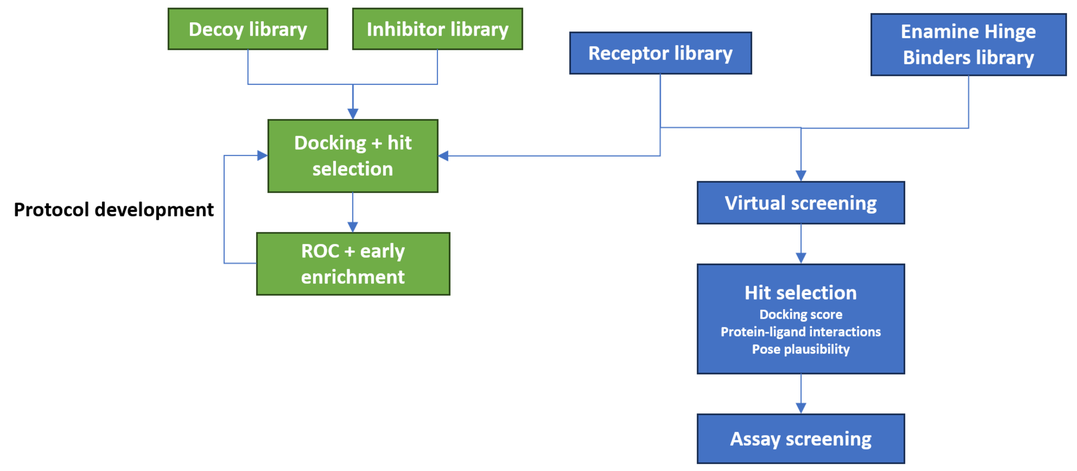


RSF-1654
TR-FRET: nonactive



RSF-1353
TR-FRET: nonactive

Identifying novel CDK9 inhibitors in silico



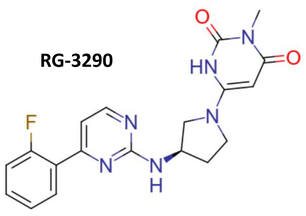
- 24.000 compound library (Enamine hinge binders) screened
- 60 VS actives total → 34 selected for purchasing
- CDK9/Cyclin T1 ADP-Glo assay set-up and validated
- All 34 tested at 2 doses (10 and 1 µM)
- 10 VS hits confirmed (30% validation rate)
- 9 out of 10 compounds with IC50 < 30 µM

CDK9 inhibition data (ADP-Glo™ assay)

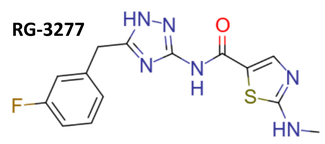
ID	IC50 (µM)	LE	cLog D	MW
Dinaciclib (ref)	0.002	0.42	2.1	397
RG-3290	**0.48**	**0.32**	**1.4**	**382**
RG-3277	**0.74**	**0.37**	**1.9**	**332**
RG-3271	1	0.37	2.6	312
RG-3284	**2.5**	**0.44**	**2.5**	**236**
RG-3283	2.9	0.34	3.3	321
RG-3285	5.8	0.39	2.7	250
RG-3298	**8.5**	**0.42**	**2.7**	**242**
RG-3293	10.7	0.36	3.0	302
RG-3294	22.5	0.24	1.0	281

Example of actives

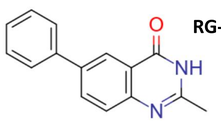
RG-3290



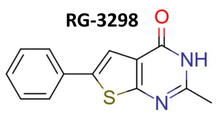
RG-3277



RG-3284



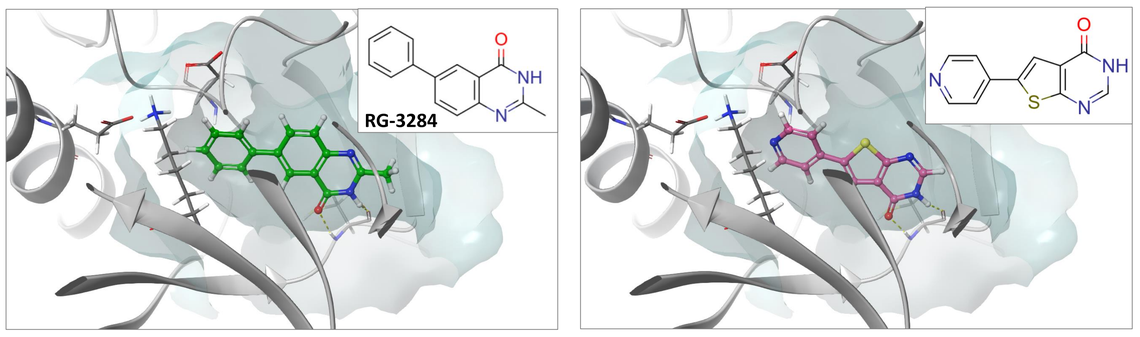
RG-3298

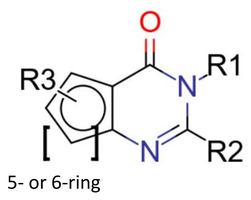


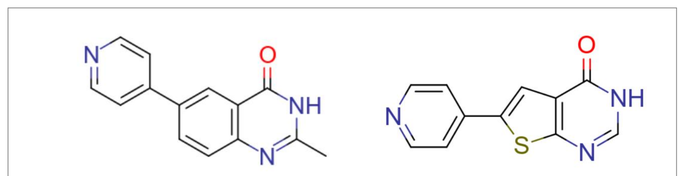
Initial hit expansion

- Two fragment-sized hits, RG-3284 and RG-3298, prioritized for hit expansion
- SAR-by-catalogue, **15 cpds purchased**
- A focused library of analogues: **25 cpds synthesized in-house**
- Most ligand-efficient compound in the "6-core series" identified as **RG-3686**
- Using learnings from the "6-core series", **RG-4577** is identified

Structure-based design







	RG-3686	RG-4577
MW	237	229
logP	**1.4**	**1.3**
HAC	18	16
CDK9 IC_{50}	**0.7 µM**	**1.1 µM**
LE	**0.48**	**0.53**

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