Identifying novel CDK9 inhibitors by traditional virtual screening and AI-based approaches

Example of Hit Finding at Red Glead Discovery

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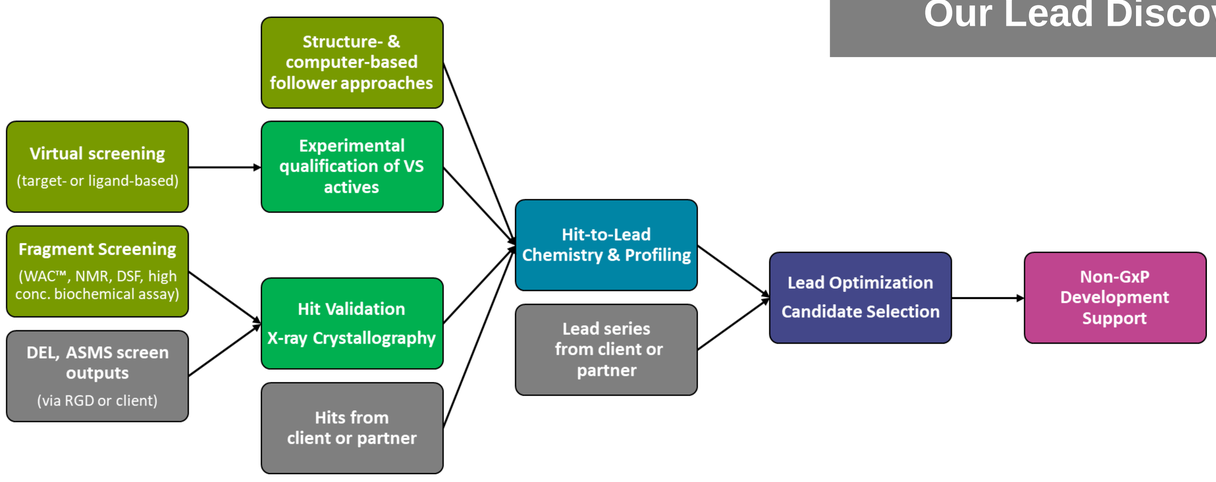
Our Lead Discovery platform



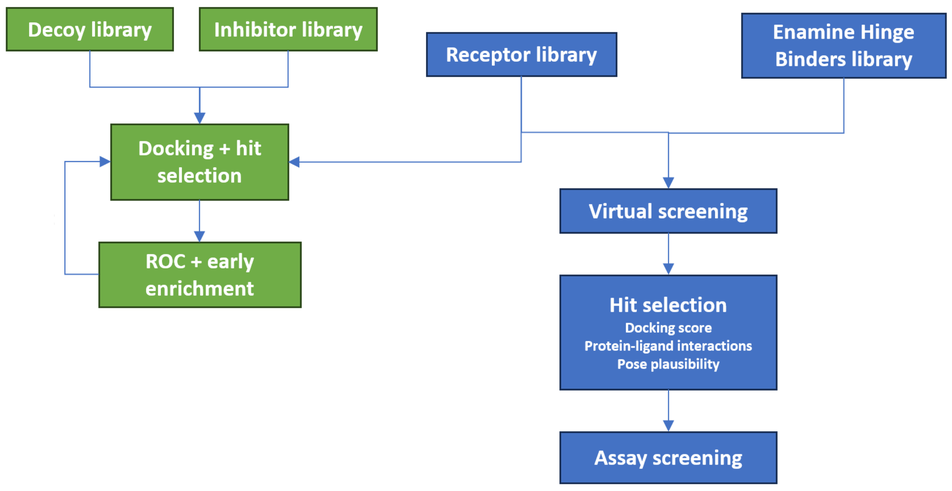
Key principles

- Designing and setting up efficient and robust screening cascades is key
- Ligandibility assessment by FBLD (1D $^{1}H/^{19}F$ NMR or WAC)
- Virtual screen followed by focused biochemical/cell-based screen
- Fragment-based or SBDD approach (X-ray data strongly preferred)
- If no structure - generate SAR by synthesis and biochemical/cell-based assay
- Developing Hit Series, seeing SAR early is major predictor of success

Traditional virtual screening: methodology and results

Screening cascade

- 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 (29% HR/validation rate), selected for 8-dose response test
- 9 out of 10 compounds showed clear dose response curves and IC50 values were determined

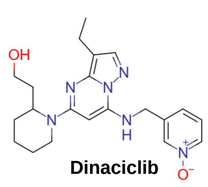


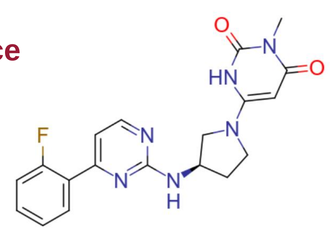
Validation of VS hits

CDK9 inhibition data

ID	IC50 (µM)	LE	cLog D	MW
Dinaciclib (ref)	0.002	0.42	2.1	396.50
RG-3290	**0.48**	**0.32**	**1.4**	**382.40**
RG-3277	**0.74**	**0.37**	**1.9**	**332.36**
RG-3271	**1**	**0.37**	**2.6**	**312.42**
RG-3284	**2.5**	**0.44**	**2.5**	**236.27**
RG-3283	2.9	0.34	3.3	321.41
RG-3285	5.8	0.39	2.7	250.31
RG-3298	**8.5**	**0.42**	**2.7**	**242.30**
RG-3293	10.7	0.36	3	302.34
RG-3294	22.5	0.24	1	281.32

Example hit structures and Dinaciclib reference


Dinaciclib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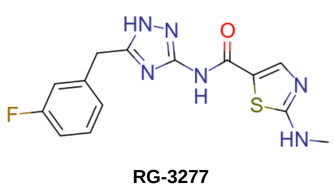
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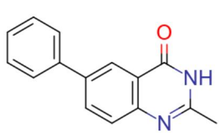
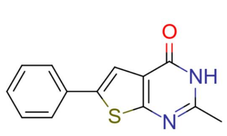
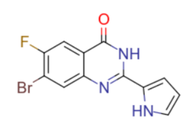
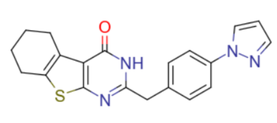
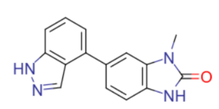
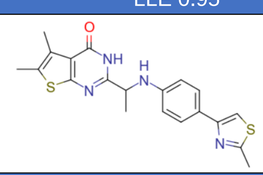

RG-3284


RG-3298

Benchmarking with AI-based approaches

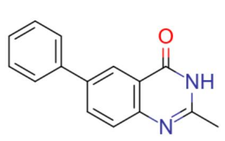
- Same library as used in traditional VS (Enamine hinge binders)
- Hits purchased and tested in the biochemical assay

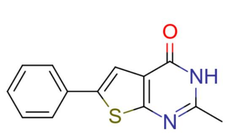
	DESUPERVISED[1]	AnyoLabs[2]
Methodology	• Equivariant deep probabilistic graph neural network • Quantifies uncertainty for each prediction	• Hybrid AI model incorporating various machine learning methods • 1D models for rapid screening
Hit rate (>30% inhibition at 10 uM)	**15.6 %** (29.4% classic VS)	**8.9 %** (29.4%classic VS)
IC50 < 10 uM	**6.3 %** (26.5% classic VS)	**4.4 %** (26.5% classic VS)
IC50 < 1 uM	**0 %** (8.8% classic VS)	**0%** (8.8% classic VS)
Example hit structures		
	IC_{50} 2 µM LogD 2.6 LE 0.44 LLE 3.10	IC_{50} 7 µM LogD 3.6 LE 0.28 LLE 0.95
		
	IC_{50} 10 µM LogD 2.3 LE 0.35 LLE 2.7	IC_{50} 9 µM LogD 4.8 LE 0.26 LLE 0.46

[1]Desupervised: Johan Gudmundsson, Michael Green [2]Anyo Labs: S. Jalil Mahdizadeh, Leif A. Eriksson

Hit expansion

Hit expansion by SBC and focused library synthesis


RG-3284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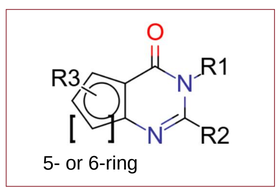
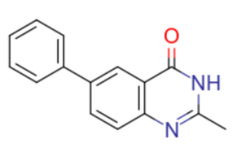
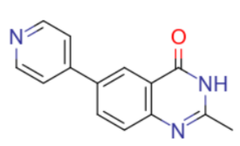




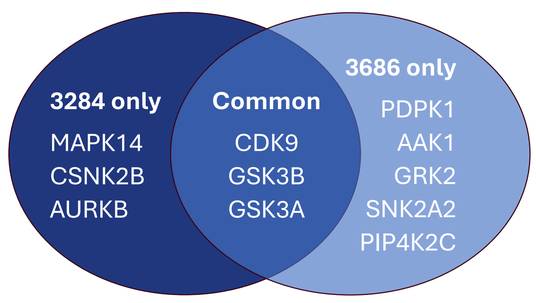


- Fragment-sized hits prioritized for hit expansion
- SAR-by-catalogue, **15 cpds purchased**
- A focused library of analogues: **25 cpds synthesized at RGD**
- Best compounds profiled and one example of a "matched pair" is shown

	RG-1003284	RG-1003686
CDK9 IC_{50} (pIC_{50})	**2 490 nM (5.6)**	**757 nM (6.1)**
LE	**0.44**	**0.48**
LLE	**2.4**	**4.6**
MW	236.2	237.2
LogD pH 7.4 (*cLogD*)	3.2 *(2.5)*	1.5 *(1.4)*
cLogP (CDD)	*2.5*	*1.4*
PSA (topological, CDD)	46 Å^2	59 Å^2
Solubility pH 7.4	6.3 µM	>95 µM
hMics / CL	96 µL/min/mg	<10 µL/min/mg

Selectivity Profiling by CETSA® Explore

Unbiased target engagement assay with a proteome coverage of more than 5,000 proteins.



- Significant observed engagement of kinase targets at 100µM is shown
- 3686 also demonstrated target engagement for CDK9 and GSK3 at 30µM and 10µM

Pelago Bioscience: Tomas Friman, Christina Velasco, Oskar Alsing

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