COMPREHENSIVE EXPLORATION OF FRAGMENT CHEMISTRY SPACE: AN FBLD APPROACH TO INHIBITORS OF SGK1

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BioBlocks
A CHEMISTRY PARTNER IN DRUG DISCOVERY

FBLD AT BIOBLOCKS

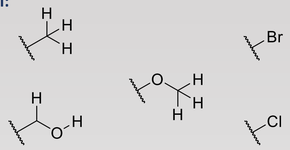
The Comprehensive Fragment Library (CFL) is a set of small, rigid, medicinally interesting fragments. This library originates from a starting set of >3 million potentially synthesizable virtual fragments designed from first principles and is 3D enabled to maximize exploration of target interactions. Extensive clustering analysis allows broad coverage of chemistry space and provides an immediate follow-up strategy from any screening hit.

BUILDING THE VIRTUAL FRAGMENT SET

An exhaustive set of ring structures containing ≤18 heavy atoms with 1 handle atom was built in BIOVIA Pipeline Pilot with several inclusion criteria:

- ≥ 1 ring, including bridged, spiro and fused ring connections
- ≤ 3 unique rings
- ≤ 2 ring assemblies
- ≤ 2 rotatable bonds
- 1 atom has all rotatable bonds
- C,N,S,O atoms only
- ≤ 1 S

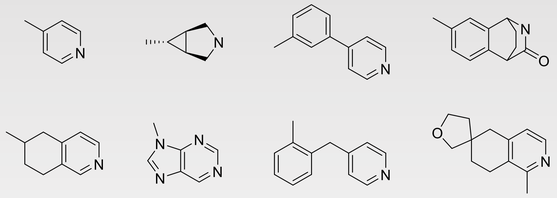
Core structures are decorated with neutral, extendable handles chosen for synthesis potential:



The raw set of > 50 million structures with Me handles was filtered by medicinal chemistry criteria to produce a 7 million candidate fragment set

Example CFL Fragments

Rigid, partially aromatic structures are the highest value 3D enabling subset



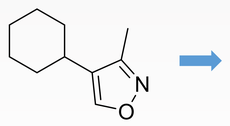
CLUSTERING

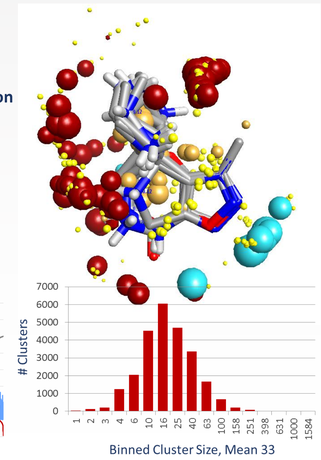
cresset

A high value 580k subset of 2D structures was selected from the raw set
Generated 830k diastereomers for 3D similarity clustering
High quality clusters enable immediate analoging from a screening hit
Entry points to Syntheverse™ virtual library generation

Representative Cluster

One compound suggests multiple related cores and substitution patterns for rapid SAR investigation





Mean intra-cluster similarity: 0.77

Binned Cluster Size, Mean 33

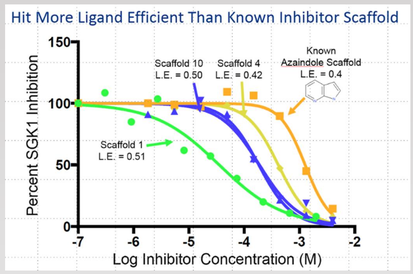
SGK1 IS AN EMERGING ONCOLOGY TARGET

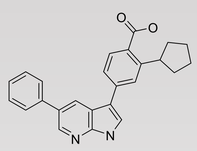
Visionary Pharmaceuticals

Serum and glucocorticoid-regulated kinase 1 (SGK1) has been identified as a pro-survival, anti-apoptotic kinase that is overexpressed in multiple cancer types. SGK1 is a PDK1-dependent AGC kinase downstream of PI3K signaling[1]. This pathway is known to drive invasiveness, motility and the epithelial to mesenchymal transition (EMT). While the full range of SGK1 substrates is not yet defined, it shares some substrates with AKT and can serve as a substitute in relevant signaling pathways. SGK1 has been reported to be upregulated in multiple AKT inhibitor-resistant cell lines, particularly triple-negative breast cancer cells[3].
While inhibitors of SGK1 are expected to have application for a range of oncology indications, compounds reported to date have lacked the drug-like properties required for *in vivo* target validation. We employed a fragment-based lead discovery approach using our proprietary Leap-to-Lead™ platform to identify novel compounds suitable for use as tool compounds and possible future development.

FRAGMENT SCREENING AND ANALOGING

Fragments were screened at 2 mM in a high concentration kinase inhibition assay
Primary hits were followed up with IC_{50} determinations





GSK650394
Standard Compound

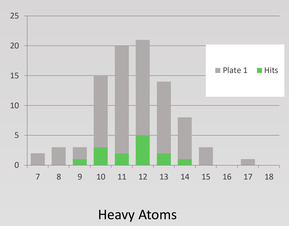
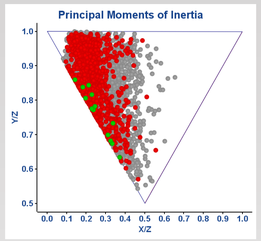


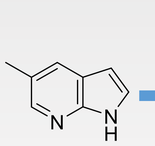
Plate 1 Screened
- 14 hits, 36% contain 3D character
- Mean IC_{50} 400 µM (50-2000)



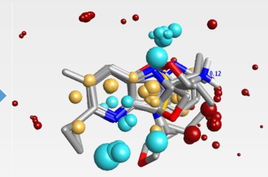
3D Cluster Coverage[4]
- 14 hit clusters, 83% contain 3D
- 2500 CFL structures
- Covers valuable 3D space

Representative Hit

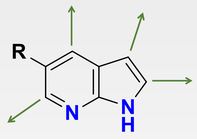
Serves as a positive control for kinases
Putative hinge binder motif enables fragment SAR interpretation



CFL000025

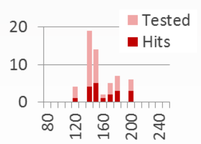


Overlay of 20 closest in cluster

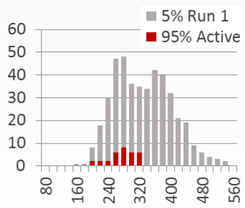


Independent Analogs	CFL000025	14 Fragment Hits
Handle Analogs	**10**	196
Handle Vectors	4	67
Same 2D Scaffold	37	2181
Same HBond Pattern	274	27370
Same 3D Cluster	47	1374
Total CFL Compounds	873	37984
% Unique to Compound	100%	80%

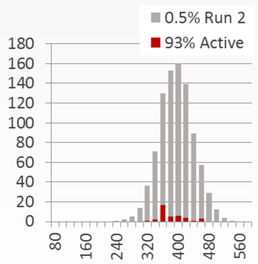
Leap-to-Lead™ analoging enabled rapid exploration of alternate cores and handle vectors
Explored Syntheverse™ chemistry space for readily synthesizable information-rich analogs
Syntheverse™ virtual expansion combined with BindingSIGHTS™ modeling provided steady improvement towards a Lead series



MW
A single scaffold
- Mean MW 148
- Mean IC50 160 µM (20-750)
- Expansion along multiple SAR vectors



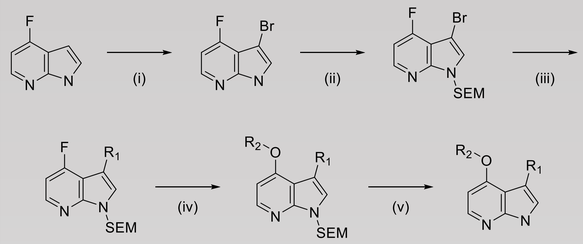
MW
Syntheverse™ Run 1 Results
- 9k products from 2 schemes
- 32 synthesized, 31 active
- Mean MW 263
- Mean IC50 10 µM (2-60)



MW
SVS Run 2 in progress
- 170k products from 1 scheme
- 42 synthesized, 39 active
- Mean MW 372
- Mean IC50 4.5 µM (0.5-120)

FRAGMENT HIT-TO-LEAD CHEMISTRY

Initial fragment hit series progressed to medicinal chemistry
Expansion along key SAR vectors required new chemistry development
3,4-Disubstituted 7-azaindoles surprisingly underrepresented in the literature



Reagents and conditions:
(i) NBS, DMF, RT, 16 h, 89%
(ii) SEM-Cl, NaH, DMF, 0 °C, 2 h, 85%
(iii) Arylboronic acid, $PdCl_2$(dppf), 2M K_2CO_3, 1,4-dioxane, 100 °C, 2-3 h, 56-84%
(iv) R_2OH, NaH, DMSO, RT, 30 min, 58-95%
(v) 1. 6M HCl, 1,4-dioxane, 50 °C, 16-20 h
2. 5N NaOH, RT, 30 min, 70-90%

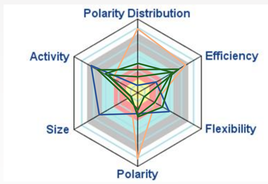
N-Alkylation activates 4-position for S_NAr reactivity under mild conditions
Optimized route enables rapid diversification along key SAR vectors and among potential selectivity groups
Enhanced novelty led to provisional filing

LEADING COMPOUNDS

Compound	IC_{50} (µM)	CLogP	MW (# HA)	LE	Cell EC_{50} (µM)	Caco-2 $P_{app,A-B}$ (ratio)
GSK650394	0.10	5.5	382 (29)	0.34	5	1.8 (3.8)
A	9.8	2.0	301 (21)	0.34	10	66 (0.6)
B	6.6	1.7	315 (22)	0.34	20	
C	0.62	0.7	345 (24)	0.37	>20	
D	0.43	1.5	428 (30)	0.30	10	

Comprehensive Fragment Library screening identified multiple chemotypes
First scaffold expanded through property-driven Leap-to-Lead™ analoging
Maintaining good ligand efficiency as heavy atom count increases
Cell potency achieved in thyroid cell line
- Collaboration with Albert Einstein College of Medicine
- Preliminary evidence of selectivity for SGK1

Substantially improved properties compared to literature lead



- Literature Lead
- BioBlocks Fragment Analog
- BioBlocks Fragment Leads

REFERENCES

[1]Pearce, L.R.; Komander, D.; Alessi, D.R. *Nat. Rev. Mol. Cell Biol.*, **2010**, *11*, 9.
[2]Di Cristofano, A. *Current Topics in Developmental Biology*, **2017**, *123*, 49.
[3]Alessi, D. R. et al. *Biochem. J.*, **2013**, *452*, 499
[4]Metz, J.T. Principal Moments of Inertia Protocol, Pipeline Pilot Community.

CONTACT

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BioBlocks is developing Leap-to-Lead™ for use in Lead Discovery Collaborations. While the CFL is not available for independent purchase, we welcome new collaborators. For additional information or to discuss using the CFL and the Leap-to-Lead™ platform in a drug discovery effort, please contact wwade@bioblocks.com or visit our website: www.bioblocks.com