Integrated HTS platform for innovative drug discovery

Axcelead

Axcelead Drug Discovery Partners Inc.

AXCELEAD
Drug Discovery Partners

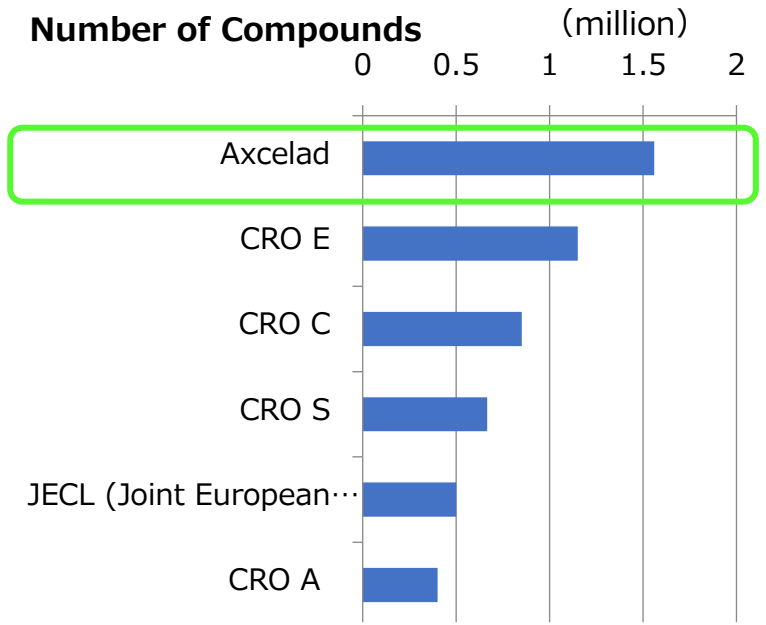
The lead-like and diversity compound library

>1,500,000 Compounds

- **Purchased compounds : Maximally diverse lead-like cpds, >180 different and selected vendors**
- **Inhouse compounds : >600 different programs of small-molecule drug discovery, a rich store of ADMET data**

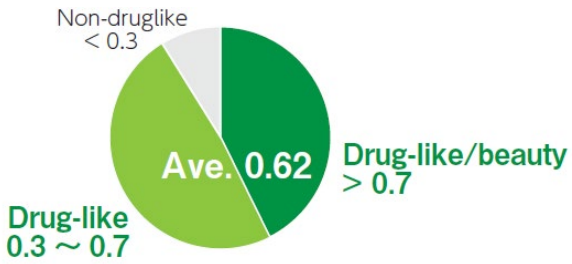
Library design and synthesis : Novelty, chemical properties, and diversity-oriented

Library Size

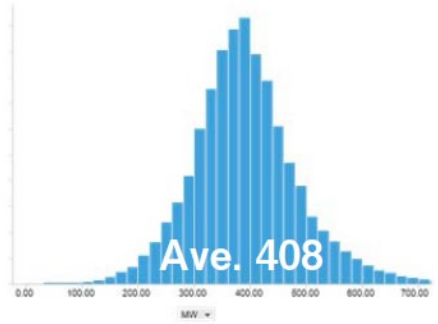


Quality

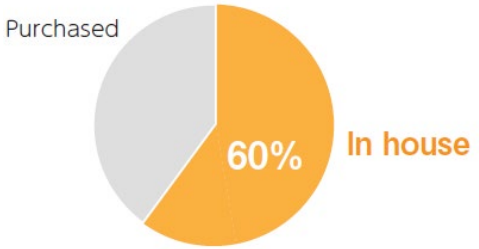
Druglikeness
QED: (Chemical beauty)

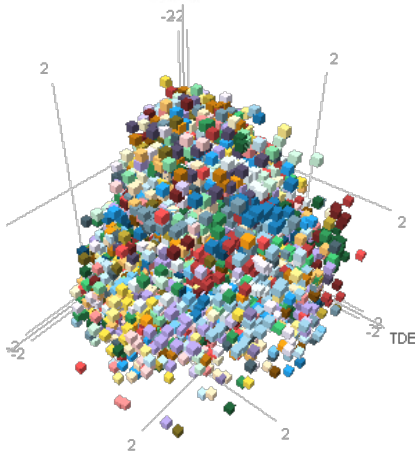


Molecular Weight



In-house compounds





QED: Quantitative Estimate of Drug-likeness
PCA: Principal Component Analysis

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Library Sets for HTS

Axcelead libraries >1,500,000 cpds

Diversity,
Chemical Structure
Solubility data,
Cell toxicity data
Activities data

Diversity Library

Single 200K

Diversity Library
- Pilot screening : 2,560
- Clean-A : 10,240
- Clean-B : 44,180
- CNS : 18,900
- sp^3rich and chirality rich: 7,500
- Phenotypic Sc. : 22,000
- Fragment（Ro3.5): 11,000
- Extended rule of 5 : 6,400
- Diversity set A : 24,000
- Diversity set B : 18,540
- Core Library（FY21) : 33,000

Pooled 720K

Pool:10 different cpds per one sample

Diversity Library
- Pilot screening: 32,000
- # 1 : 292,000 (High Priority)
- # 2 : 396,000

Focused Library 41K

➢ Target class
Kinase : 8,500
GPCR : 9,300
Protease : 401
PPI :4,100
RNA : 6,400
RNA splicing（FY21） :1,280
➢ Macrocyclic: 6,400
➢ Natural product: 3,700
➢ Covalent: 3,800

➢ Phenotypic Sc.
Annotation: 7,000
Covalent fragment（FY21):50

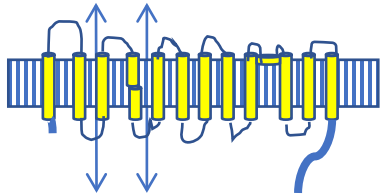
Assay platforms

GPCR

cAMP assay
Ca^{2+} flux assay
Reporter gene assay
Arrestin/Internalization assays
Binding assay, Impedance assay

Ion channel / Transporter

Ion influx assay
Membrane potential
Electrophysiology
Substrate uptake
Binding



Enzyme

Direct assay
- Absorbance, fluorescence, FRET
- ELISA
- Label-free assay (e.g. HT-MS)

Indirect assay
- Coupling assay (e.g. ATP by luciferase)

Nuclear receptor

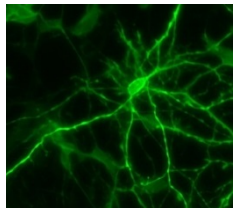
Binding assay
Cofactor recruit assay
Reporter gene assay
Nuclear translocation assay

PPI (protein-protein interaction)

TR-FRET/Alpha screen assay
ELISA
NanoBit/BRET
Two-hybrid assay
Biophysical assay (e.g. Surface Plasmon Resonance)

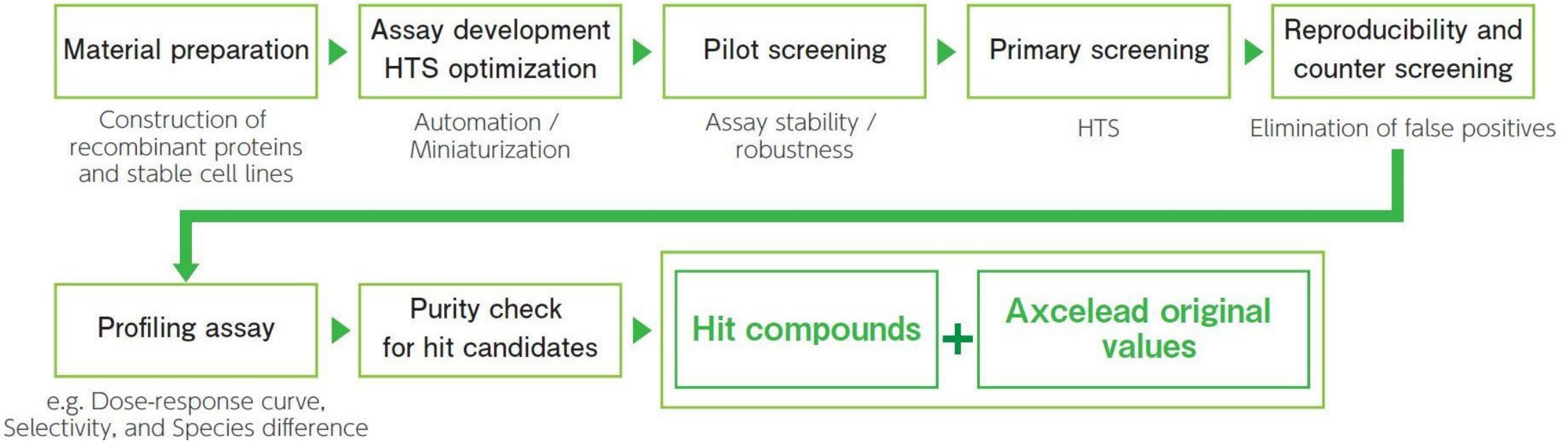
Phenotypic screening

High-content assay
Reporter gene assay
Cell growth
qPCR
CRISPR Cas KO screen etc.



General hit finding process

■ Axcelead comprehensive screening services

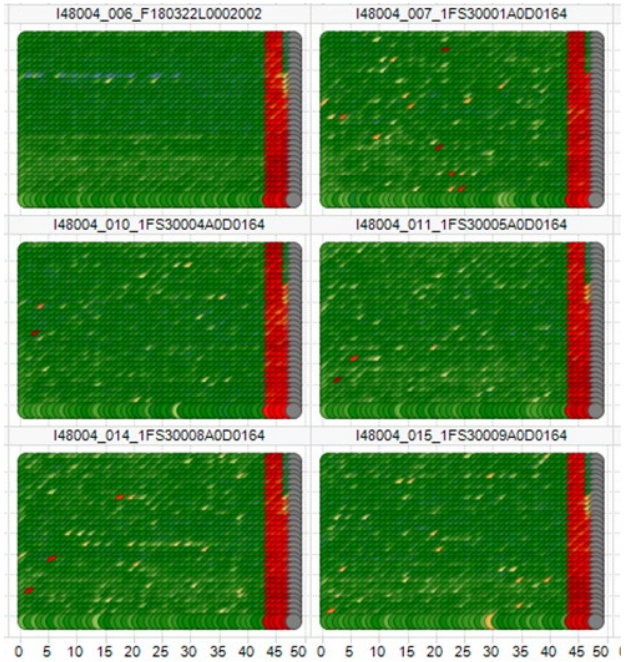


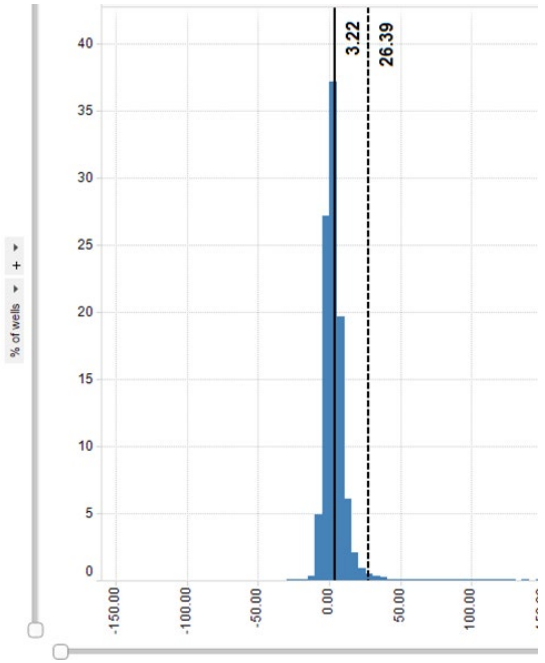
We can also conduct HTS campaigns using each client's assay system.

High throughput Screening (HTS)

Target assessment → Assay development → **Screening** → Hit to Lead → Lead optimization → IND

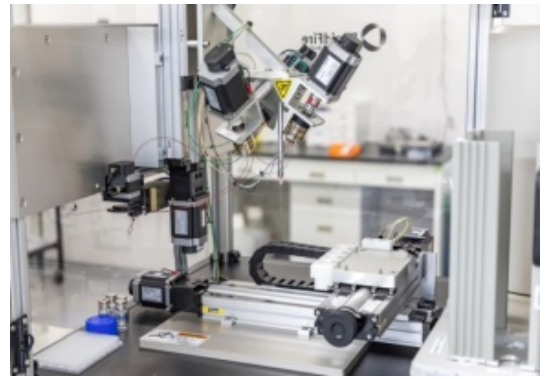
- HTS adaptation
  - Fully automation system
  - Miniaturization (384/1536 well format)
- Pilot screening
  - Feasibility
  - Robustness
- Implementation of HTS
- Data analysis
  - Primary screen
  - Counter assay
  - EC_{50}/IC_{50}
  - Purity of compounds
- Profiling
  - Species difference
  - Selectivity
  - Mode of action etc.





Facilities and capability for general HTS

- Ultra High Throughput Screening capabilities (full automation,1536-wells format)
- State-of-the-art equipment compatible with diverse assays
  - Acoustic liquid handling system for ensuring high accuracy at low volume
  - Biochemical screening (Fluorescence, Absorbance, Luminescence, qPCR)
  - High-content screening
  - HT-Mass spectrometry screening
  - Electrophysiological screen with HT-autopatch systems
- Data analysis platform to support all screening processes
- Ability to perform HTS under BSL2 conditions
- Ability to perform Radioactive HTS



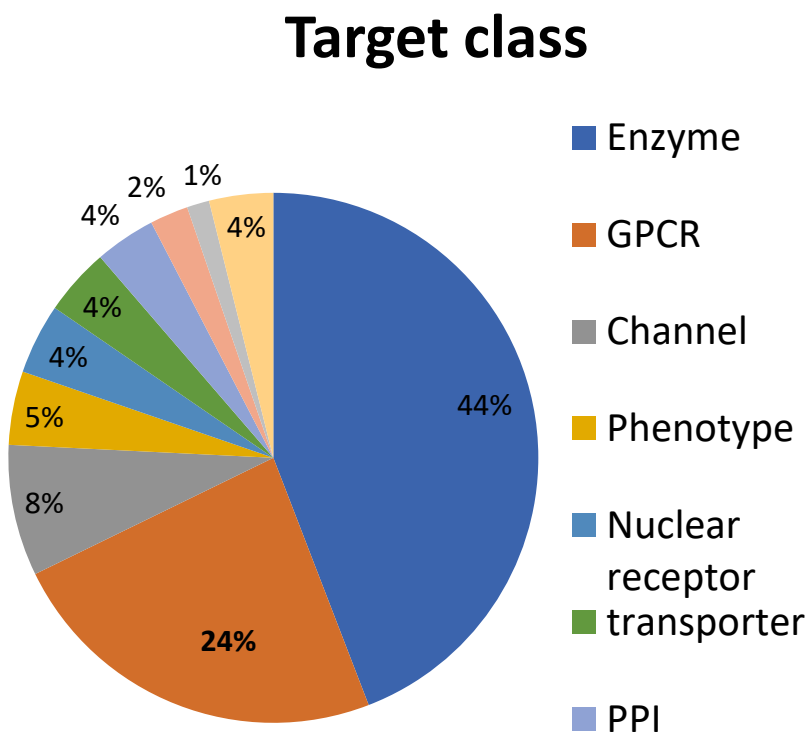
➢ RapidFire-MS/MS systems (Agilent)

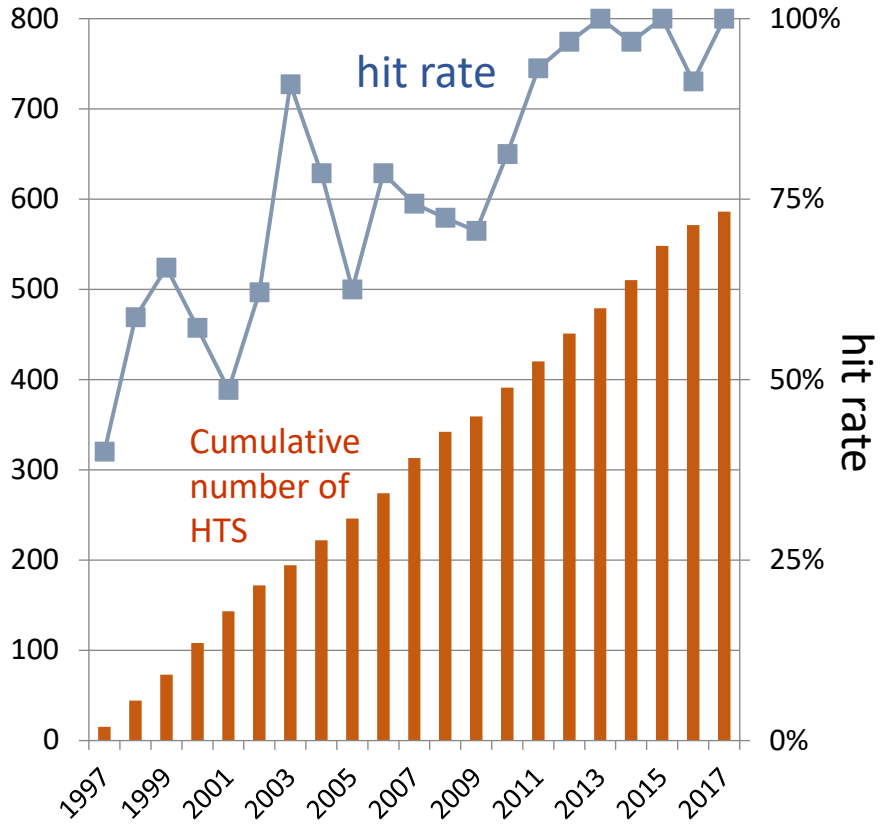
➢ Fully automated screening system (FUJIFILM Wako)

➢ Acoustic droplet dispensing system (Labcyte)

Syncropatch 384 (Nanion)

HTS track record





- **Our team has achieved more than 600 HTS campaigns for multiple target classes with high hit rates**

General Cascade of Hit Finding

Primary screen (n1, 1dose)

↓

Deconvolution assay (for active samples from pooled lib.) (3,200 cpds)

Counter assay (To exclude false positives)

↓

Clustering

Dose response tests(IC_{50}/EC_{50}) (n2, 6 dose, 320 cpds)

Purity check of compounds

↓

Hit compounds

Hit report

↓

Hit expansion

- Evaluation of related compounds
- Parallel synthesis with HT-chemistry
- SAR analysis

Profiling

- Selectivity/Species difference
- Mode of inhibition/activation
- in vitro ADME-tox assay
- Cellular assays

↓

Advanced hit

Hit finding

Hit follow-up services

HTS report

Compound No	Cluster ID	Primary assay IC50 (M)	Counter assay IC50 (M)	Primary assay (Graph)	Counter assay (Graph)	Chemical properties				Purity (%)	Notes
						MW	HBD	HBA	HA		
AXL1	1	8.0E-08	>1.0E-05			・・・	・・・	・・・	・・・	95.5	kinase A inhibitor
AXL2	1	2.9E-07	>1.0E-05			・・・	・・・	・・・	・・・	90.5	kinase A inhibitor
AXL3	1	4.9E-06	>1.0E-05			・・・	・・・	・・・	・・・	95.3	kinase A inhibitor
AXL4	2	3.2E-08	>1.0E-05			・・・	・・・	・・・	・・・	85.4	promiscuous
AXL5	3	1.2E-06	>1.0E-05			・・・	・・・	・・・	・・・	97.2	
AXL6	3	2.5E-06	>1.0E-05			・・・	・・・	・・・	・・・	98.1	
AXL7	3	4.2E-06	>1.0E-05			・・・	・・・	・・・	・・・	95.4	

- **Legacy assay data including annotation (target classes) and cell toxicity etc.**
- **Chemical properties (QED,HBA/HBD, AlogP, tPSA, Fsp3 etc.)**
- **Clustering, Comments by medicinal chemists**

We offer an HTS report with above information for clients

Capability for Lead Generation/Optimization

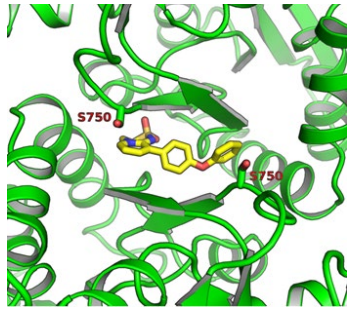
Target assessment → Assay development → Screening → Hit to Lead → Lead optimization → IND

- Biochemical assay (potency/selectivity/species for SAR study)
- Mode of action/kinetics analysis and profiling assay
- Cell-based assay (Cellular target engagement, Cellular function etc.)
- Biophysical analysis for target-compound interaction assay
  - AS-MS, TSA, NMR, ITC, SPR, X-ray crystallography

Kinetics assay with SPR

Thermodynamics assay with ITC

Electrophysiology assay with auto patch clamp

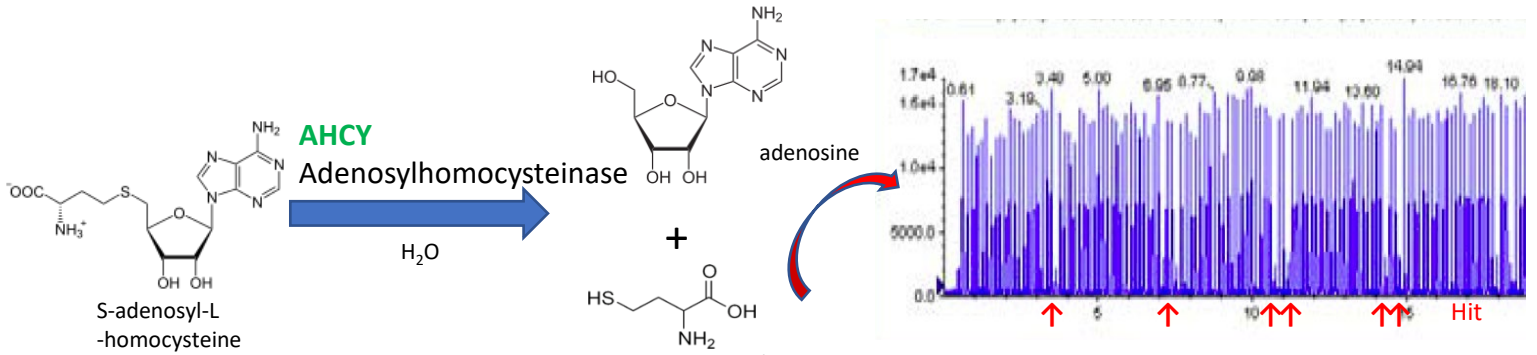


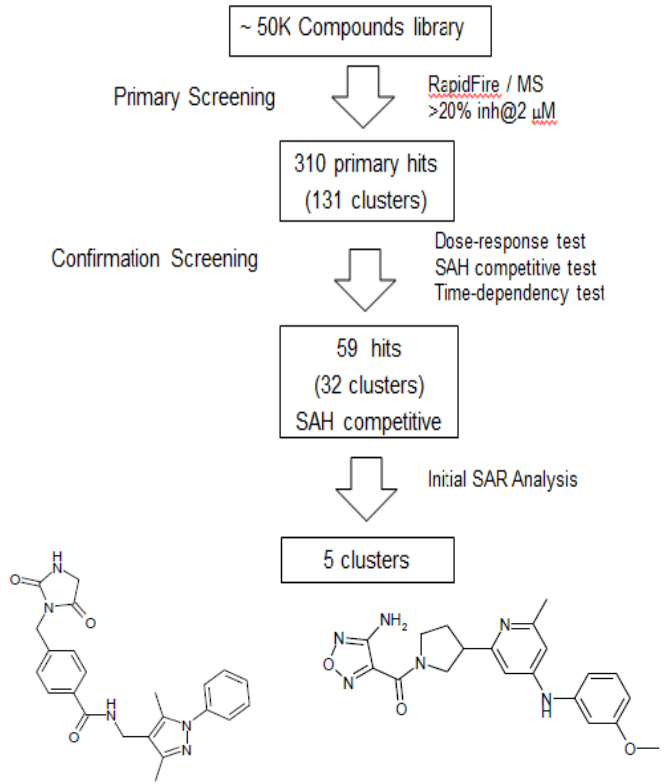
Neuropsychopharm **44** 961–70 (2019)

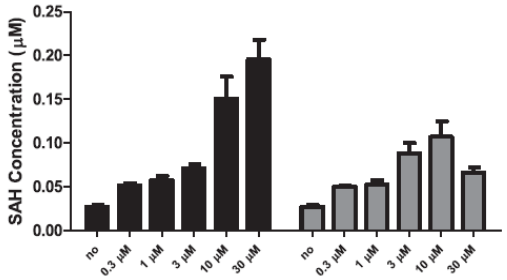
X-ray crystallography

We provide useful data for drug discovery at the LG / LO stage by using various in vitro assay techniques.

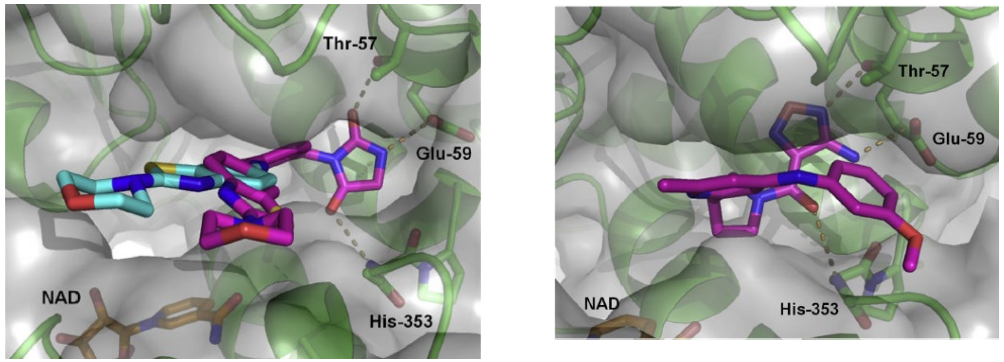
Practical example- hit finding using HT-MS-







SAH levels in cell culture supernatant



Crystal structures of AHCY in complex with hit compounds

Biochem Biophys Res Commun. 2017;491(1):1-7

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Practical example - GPCR biased ligands-

GPR39 positive allosteric modulators

Primary screening

Library: >600,000 cpds at 3μM

↓ hGPR39 cAMP assay (Gs) with EC_{20} Zn^{2+}

Counter assay

↓

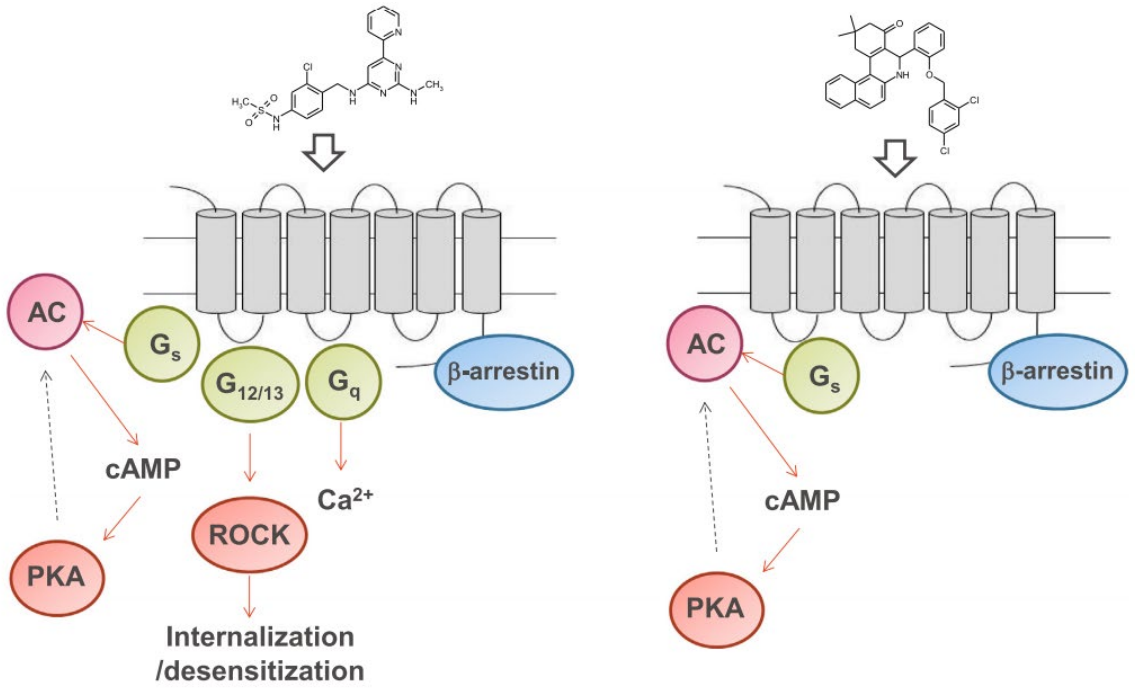
Profiling assay

hGPR39 cAMP assay (Gs)
Calcium/IP1 assay (Gq)
SRE-Luc assay (G12)
Arrestin assay

↓

Gs Biased ligands

Gs biased



Biochemical Pharmacology 140 (2017) 105–114

Practical example-Fragment-Based Approach -

BCL6 inhibitors (PPI inhibitors)

Primary screening

Fragment library (1494 compounds)

SPR
BIACORE 4000
Single point assay at 1 mM
$mtBCL6^{BTB}$, $wtBCL6^{BTB}$, wtBCL6, Neturavidin

200 mM stock in DMSO
<350 Da (ave. 180 Da)

➢ **64 compounds (hit rate: 4.3%)**

Hit confirmation

Dose response (@ 0.25, 0.5, 1, 2 mM)

➢ **64 compounds**

STD-NMR
Bruker 600 MHz w. cryoprobe

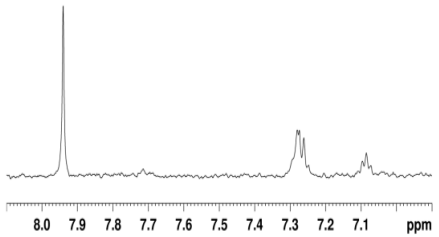
➢ **7 compounds (0.47%)**

Competition experiment by SPR

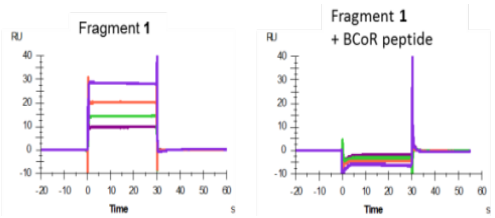
X-tal

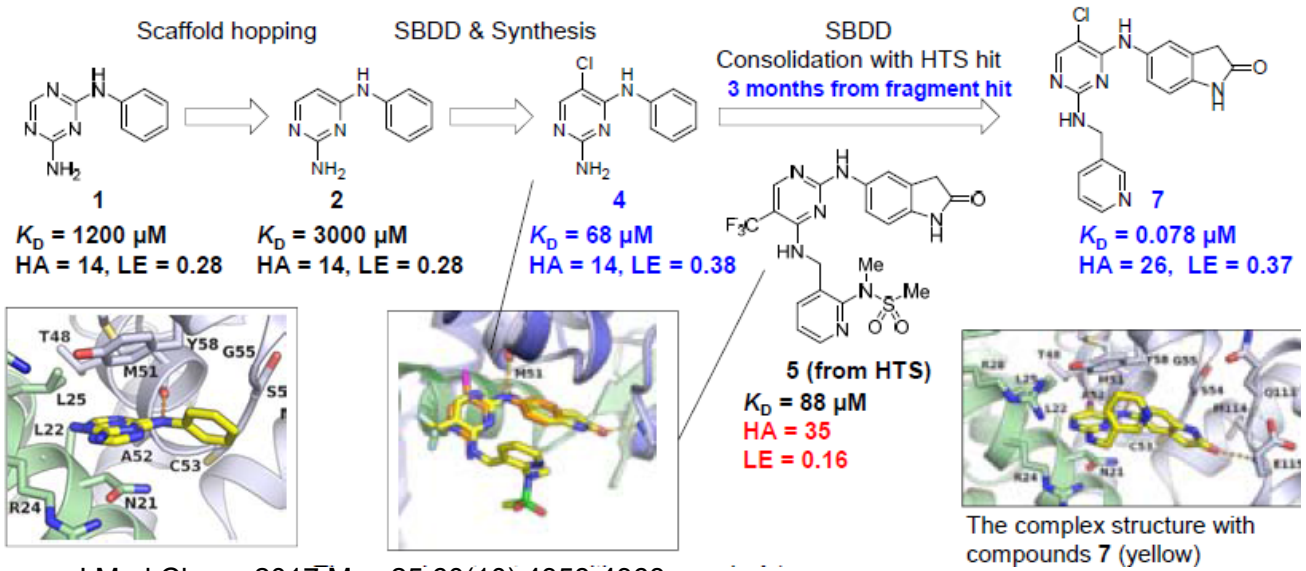
➢ **1 compound (0.067%)**

STD-NMR



Competition experiment





The complex structure with compounds **7** (yellow)

J Med Chem. 2017 May 25;60(10):4358-4368.

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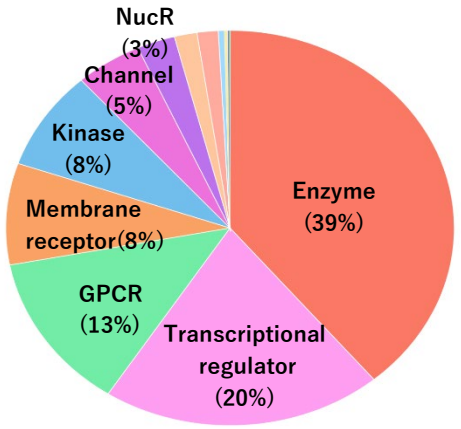
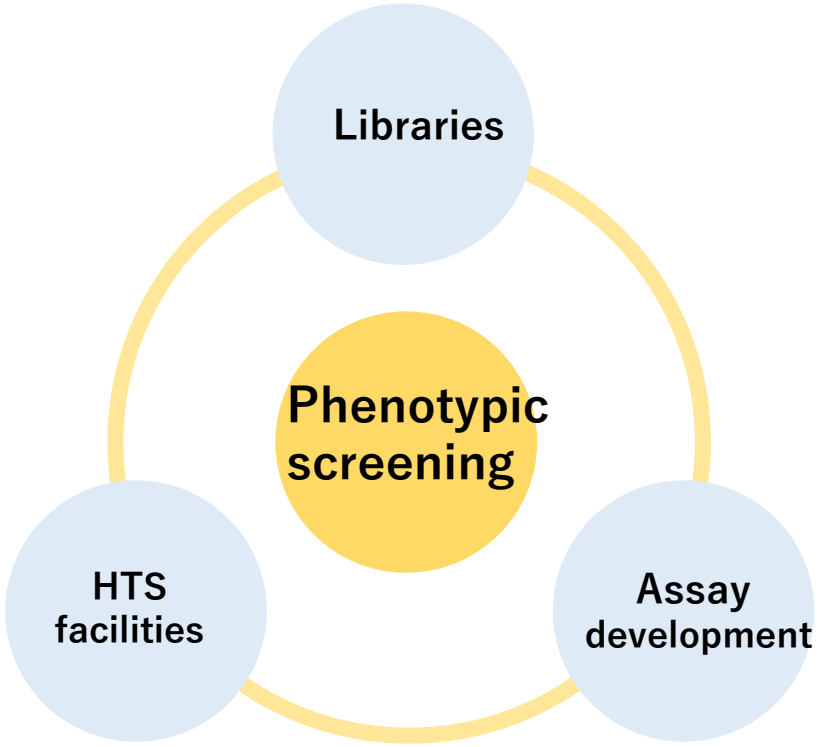
Phenotypic screening

◆ **Objectives**
- **Hit-Lead finding**
- **Drug repositioning**
- **Target discovery**

Target profiles of annotated library
(7,000 cpds)



◆ **High quality and attractive libraries**
- Diversity
- Biologically anotatation
- FDA approved
- Focused



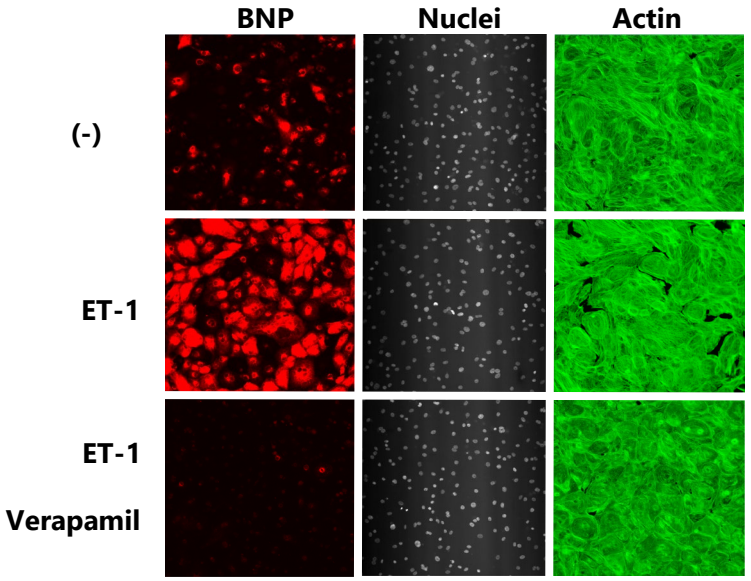
◆ **Cutting edge facilities**
- Full auto system
- Wide rage of devices
  Envision
  Incell6000
  qRT-PCR system　etc.
- BSL2 laboratory

◆ **Comprehensive services**
- Cell construction
- Assay platforms
  Reporter, RT-PCR, HCA etc.
  iPS/Primary cells
  CRISPR CAS KO screen
- HTS adaptation

Practical example of HTS using iPS cells

Heart hypertrophy assay with iPS differentiated cardiomyocytes

IN Cell Analyzer 6000(GE Healthcare)



① 1st screening

ICC — Library:biologically annotated library_3683 compounds, 3uM, N=1
Image analyzing of BNP by INCell 6000

② Reproducibility

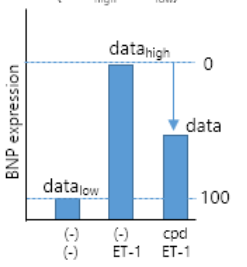
ICC
ELISA — 3uM, N=2

③ Dose-dependent activity

ICC
ELISA
TaqMan — 0.03,0.1,0.3,1,3,10uM, N=2

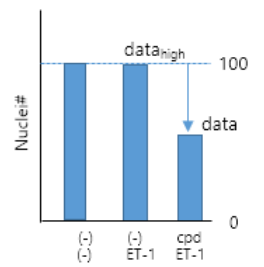
BNP:

$$\text{\% inhibition: } \left(1 - \frac{(data_{high}\text{-}data)}{(data_{high}\text{-}data_{low})}\right) * 100$$



Nuclei:

$$\text{\% expression: } data/data_{high} * 100$$



➢ **High content screen using iPS cells resulted in identification of hit compounds including FDA-approved drugs and the related pathways.**

AXCELEAD Drug Discovery Partners

Integrated HTS platform

- Pharmaceutical origin, huge, high-quality and diverse library

2. State-of-the-art infrastructure

- Fully automated screening systems
- Comprehensive platforms covering diverse target classes and phenotypic screens
- A proven track record of more than 600 HTS campaigns for drug discovery

3. High quality and comprehensive services

- Comprehensive services in hit identification including strategy planning, assay development, HTS and profiling
- Hit expansion services including SAR analysis, design and synthesis of related compounds by medicinal chemists
- High-throughput-ADMET profiling services with extensive experience and sophisticated protocols

➢ ***We efficiently offer high-quality hit compounds through our integrated HTS platform***

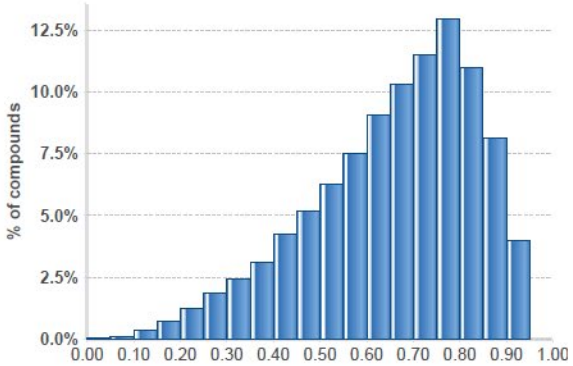
We are Your Best Partner

Chemical properties of Diversity Library（pooled）

Diversity library (ca.320K)

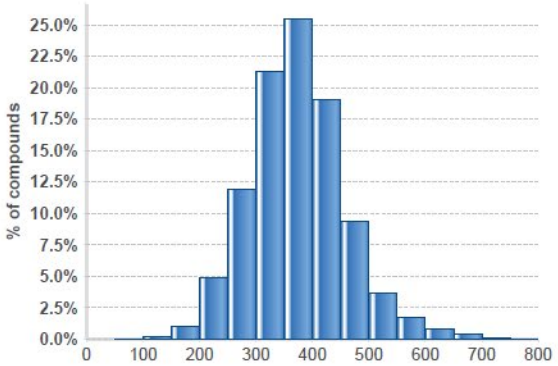
Published at 09/24/20

QED



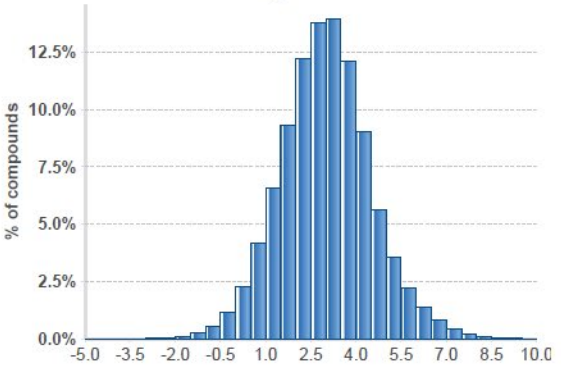
Average: 0.656

MW



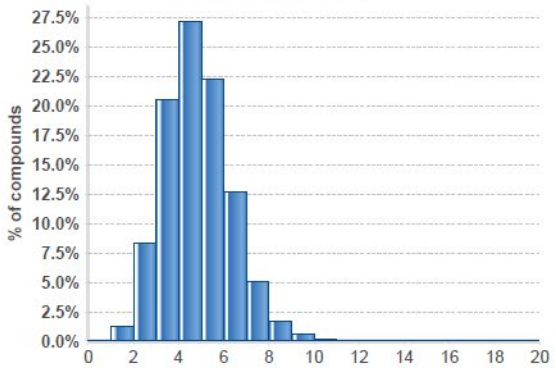
Average: 374.5

AlogP



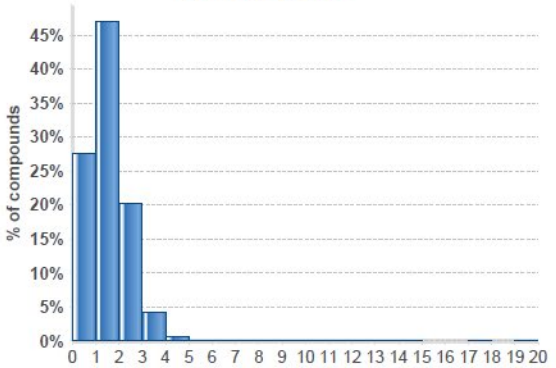
Average: 3.00

Num_H_Acceptors



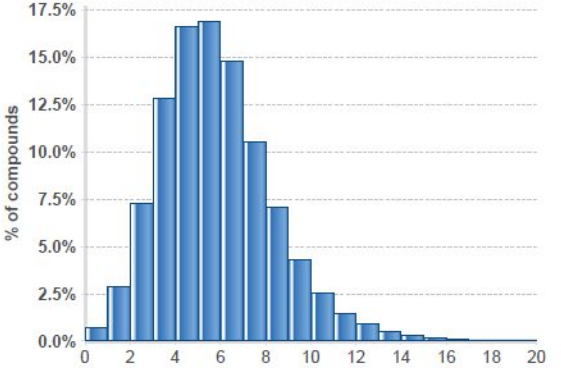
Average: 4.34

Num_H_Donors



Average: 1.05

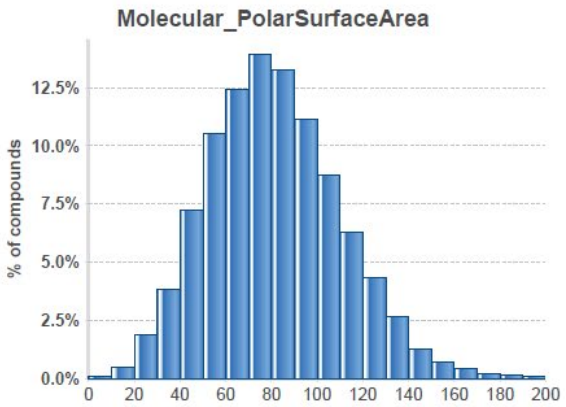
Num_RotatableBonds



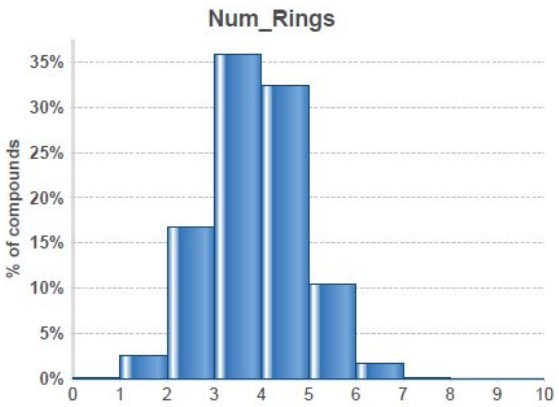
Average: 5.35

Chemical properties of Diversity Library（pooled）

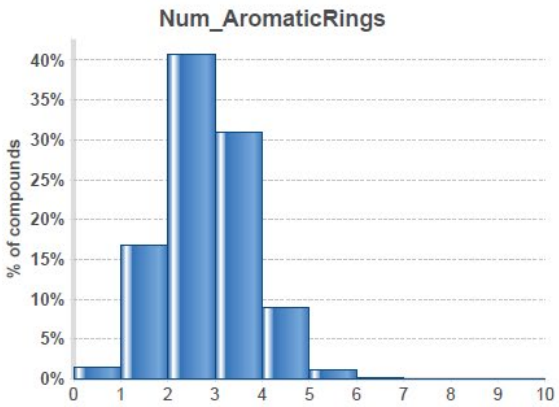
Diversity library (ca.320K)



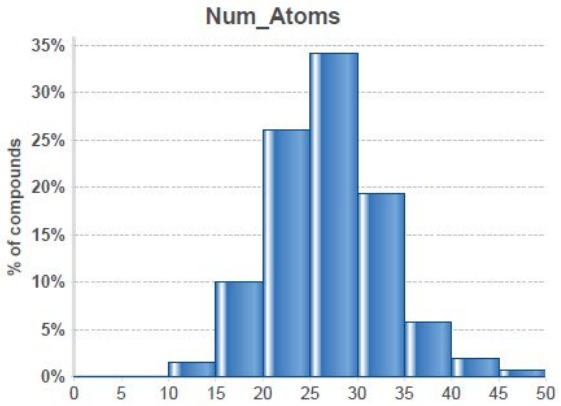
Average: 81.8



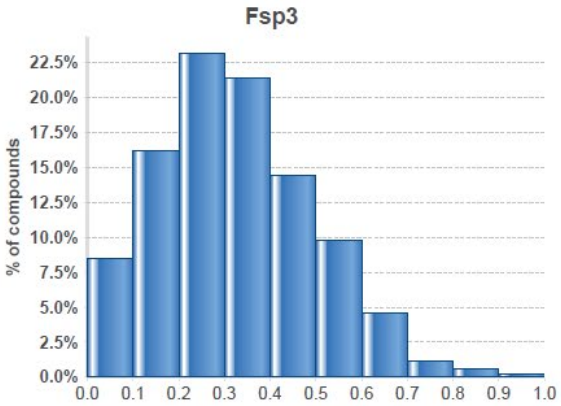
Average: 3.37



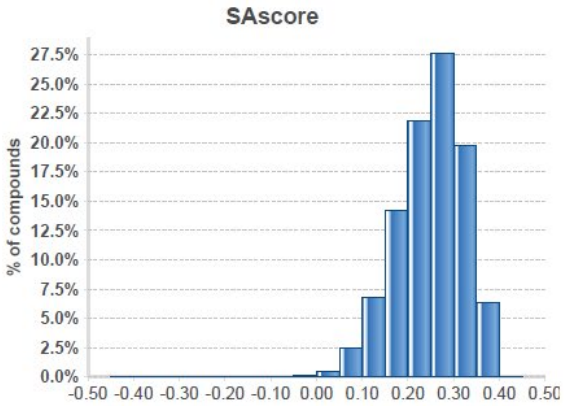
Average: 2.33



Average: 26.5



Average: 0.319



Average: 0.249