How Best to Discover Novel Bioactive Small Molecules?

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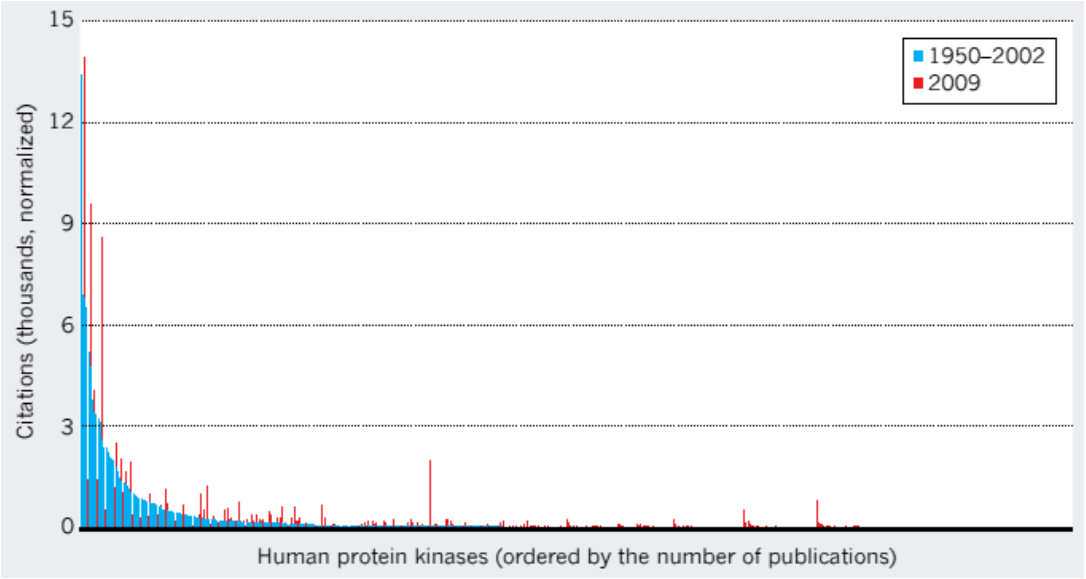
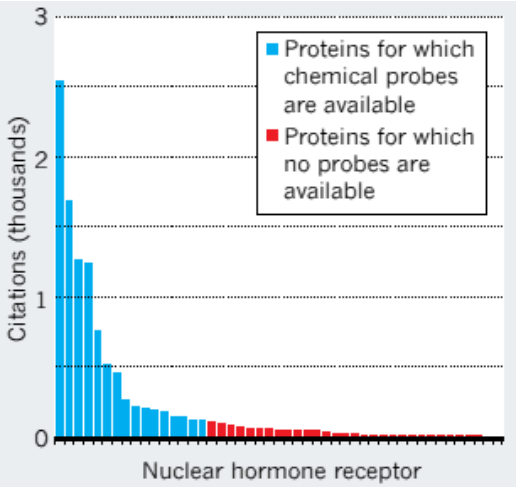
Impact of high-quality chemical probes

1. Biologists' favourite proteins are broadly unchanged in >20 years (data for protein kinases)



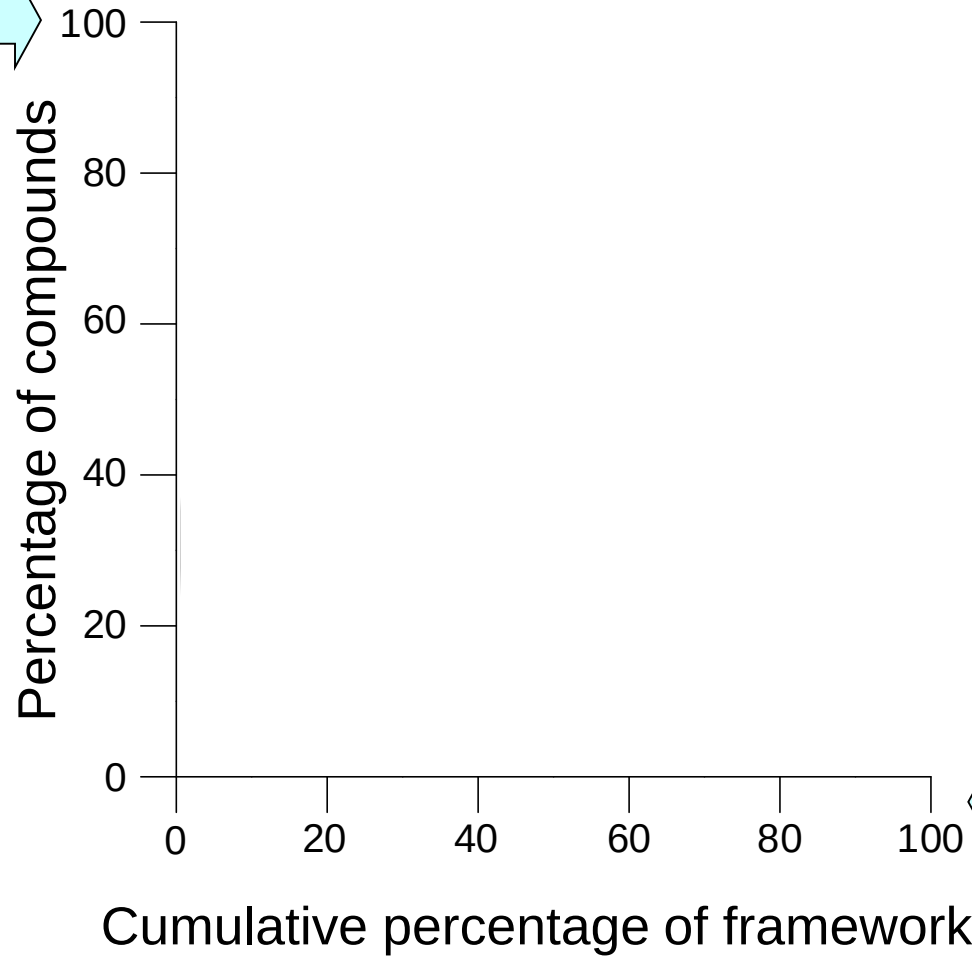


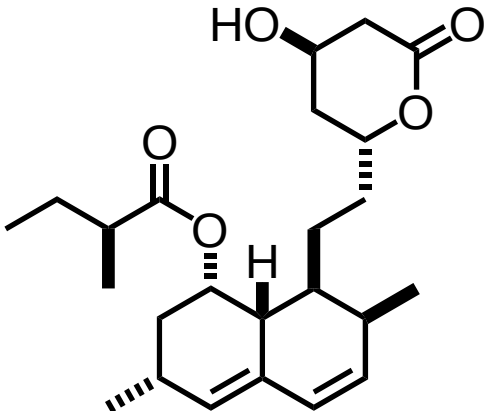
2. **High-quality chemical tools drive biomedical science** (data for nuclear hormone receptors)

See: A. M. Edwards, *Nature* 2011, **470**, 163

The Organic Chemistry Universe is "Top Heavy"

25 million cyclic compounds in CAS registry

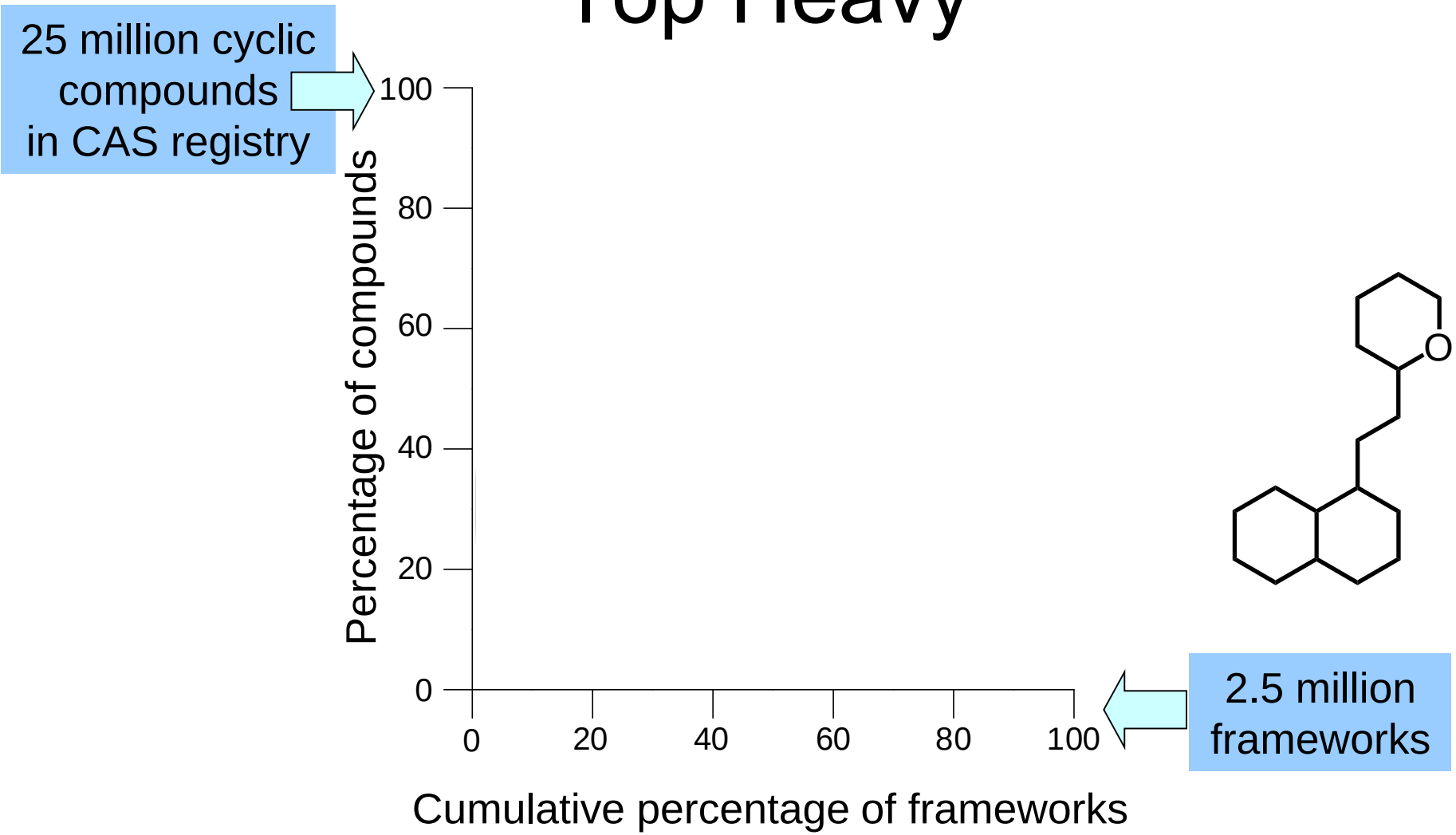




2.5 million frameworks

See A. H. Lipkus *et al*, *J. Org. Chem.* 2008, **73**, 4443

The Organic Chemistry Universe is "Top Heavy"



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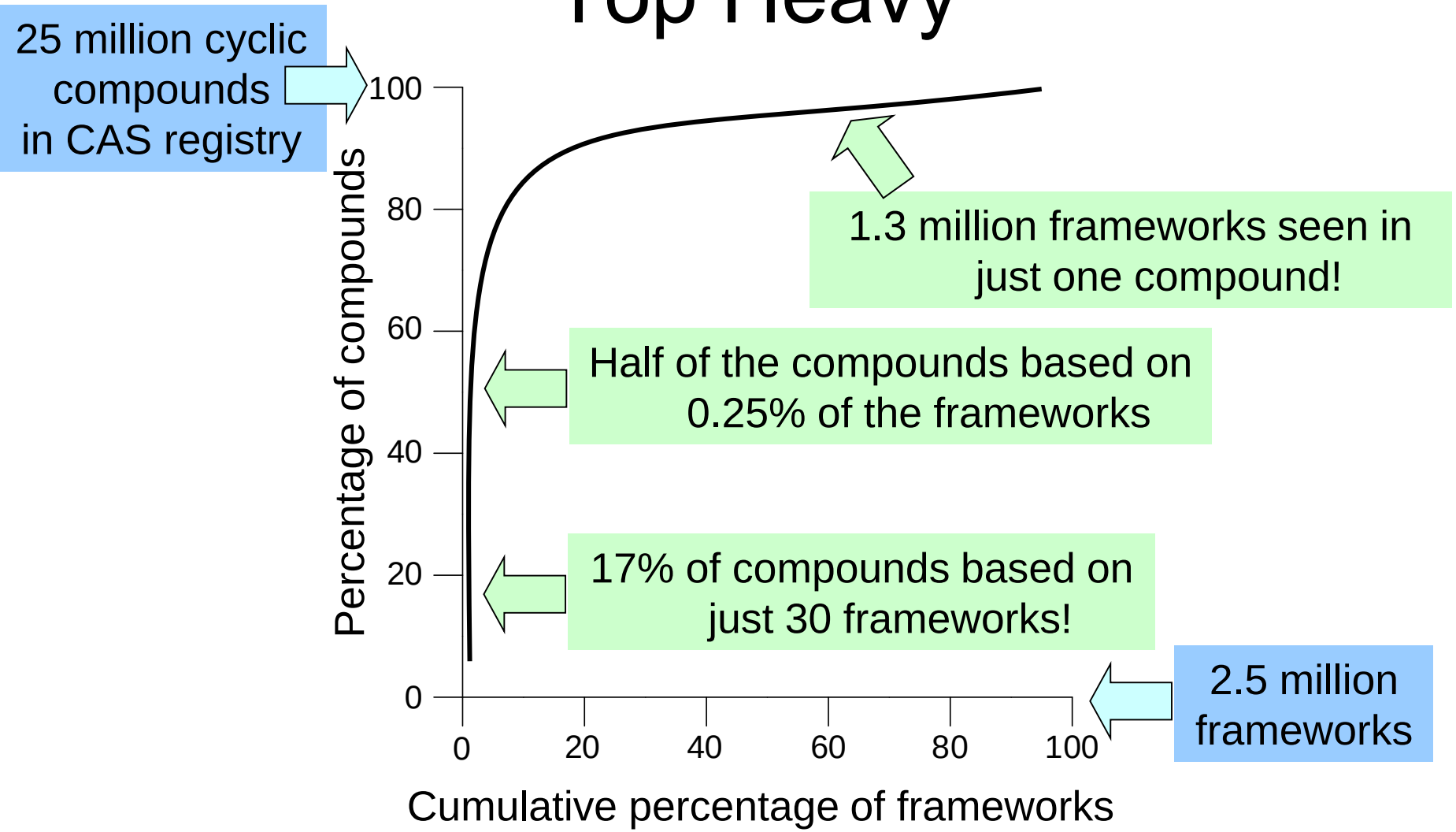
The Organic Chemistry Universe is "Top Heavy"



See A. H. Lipkus *et al*, *J. Org. Chem.* 2008, **73**, 4443

Why such uneven exploration?

Reaction type	Number of reactions	% of all reactions
Amide formation	1165	16.0
N-alkylation	776	10.6
N heterocycle formation	537	7.4
N-arylation	458	6.3
RCO_2H deprotection	395	5.4
N-Boc deprotection	357	4.9
Suzuki cross-coupling	338	4.6
O-substitution	319	4.4
Other NH deprotection	212	2.9
Total	**4557**	**62.4**

Analysis of 7315 reactions from 140 papers published by major pharmaceutical companies: *J. Med. Chem.* 2011, **54**, 3451

Searching chemical space

An uneven search of chemical space...may not reveal the peaks of activity

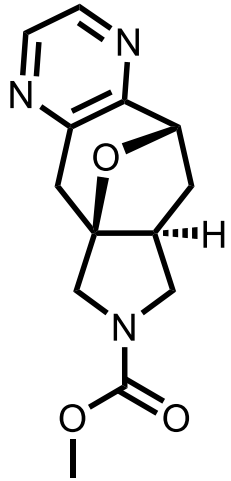
Searching chemical space





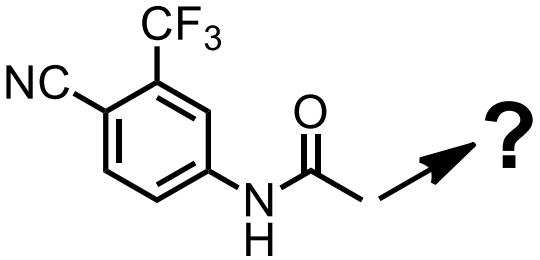

Planned and unplanned synthesis of diverse small molecules

1. Lead-oriented synthesis



Deliberate synthesis of large numbers of lead-like molecular scaffolds

2. Activity-directed synthesis



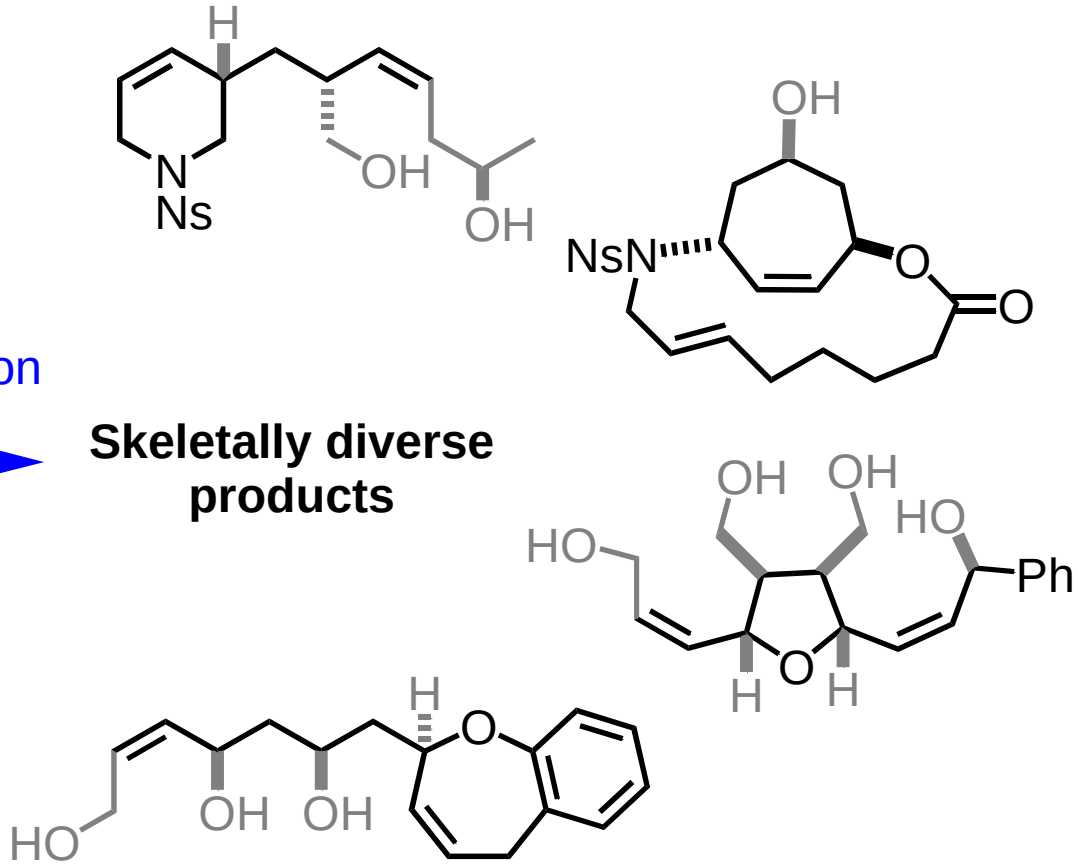
Synthetic targets **not** planned: instead, prioritised on the basis of their **function**

Diverse natural product-like molecules

Simple building blocks

ca. 5 reaction types

Skeletally diverse products



Angew. Chem. Int. Ed. 2009, **48**, 104 (VIP article)

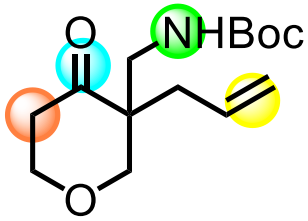
See also highlights in *Nature* (Schreiber), *Nature Chem. Biol.* (Waldmann), *Angew. Chem.* (Spring) and *Nature Chem*.

Comparison of alternative approaches to generate molecular diversity

	Diversity-oriented synthesis
Diversity	High
Molecular properties	Low level of control
Reliability of reaction toolkit	High level required
Resource focused on bioactives?	Equal focus on all compounds

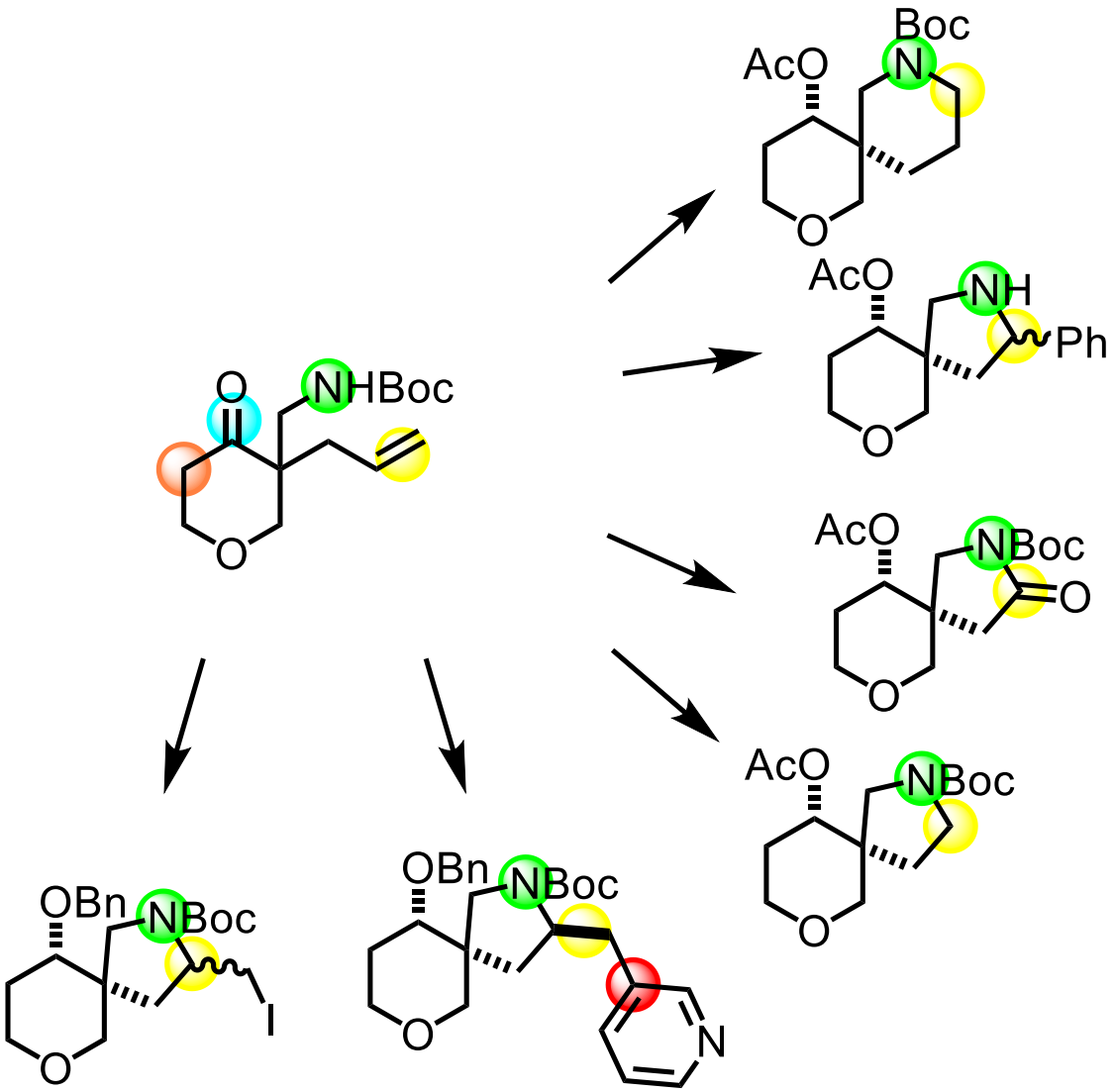
Aligning synthetic methods with molecular property requirements:
Angew. Chem. Int. Ed. 2016, **55**, 13650

Synthesis of CNS lead-like scaffolds



Chem. Comm. 2017, **53**, 12345

Synthesis of CNS lead-like scaffolds



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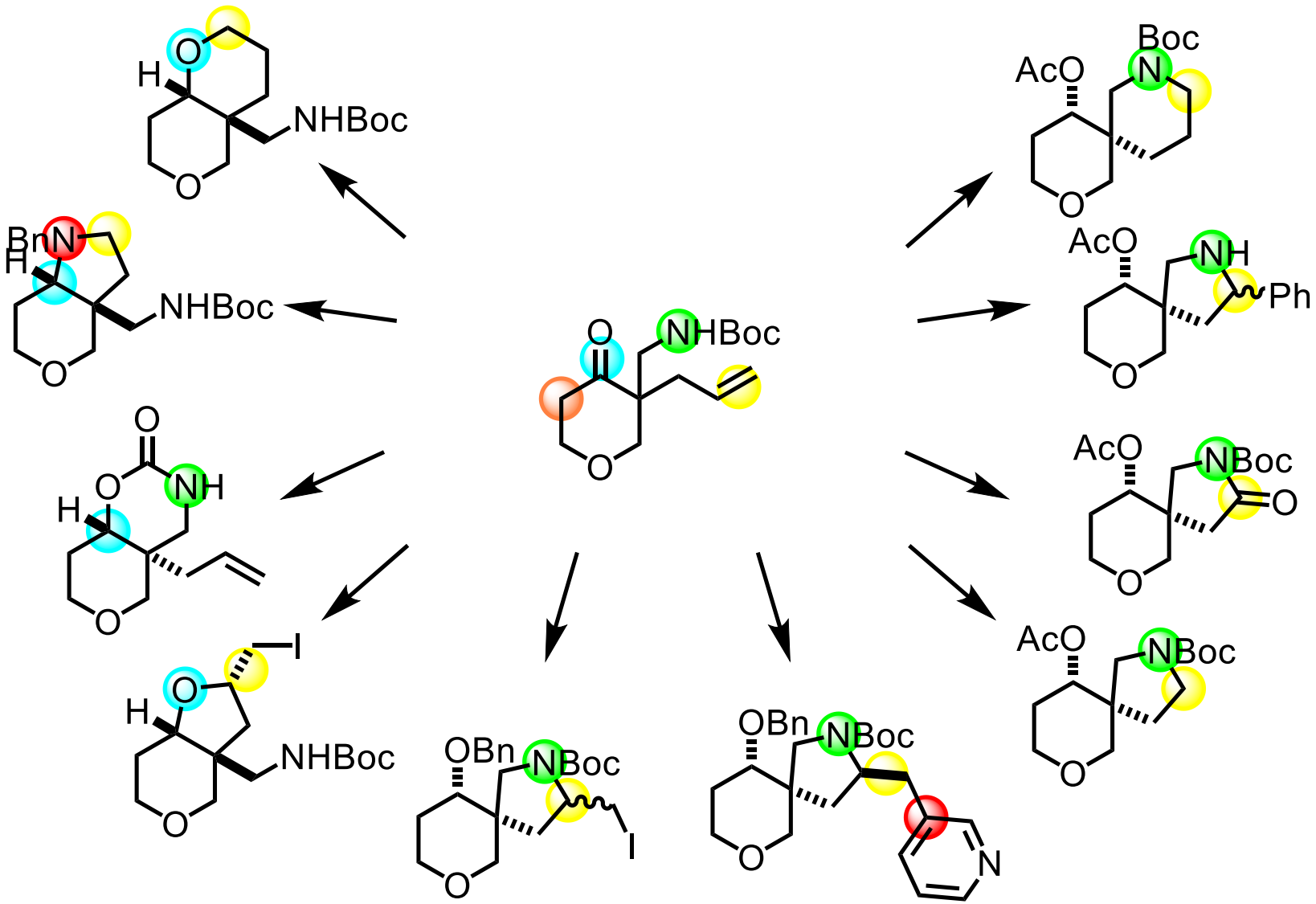
Synthesis of CNS lead-like scaffolds



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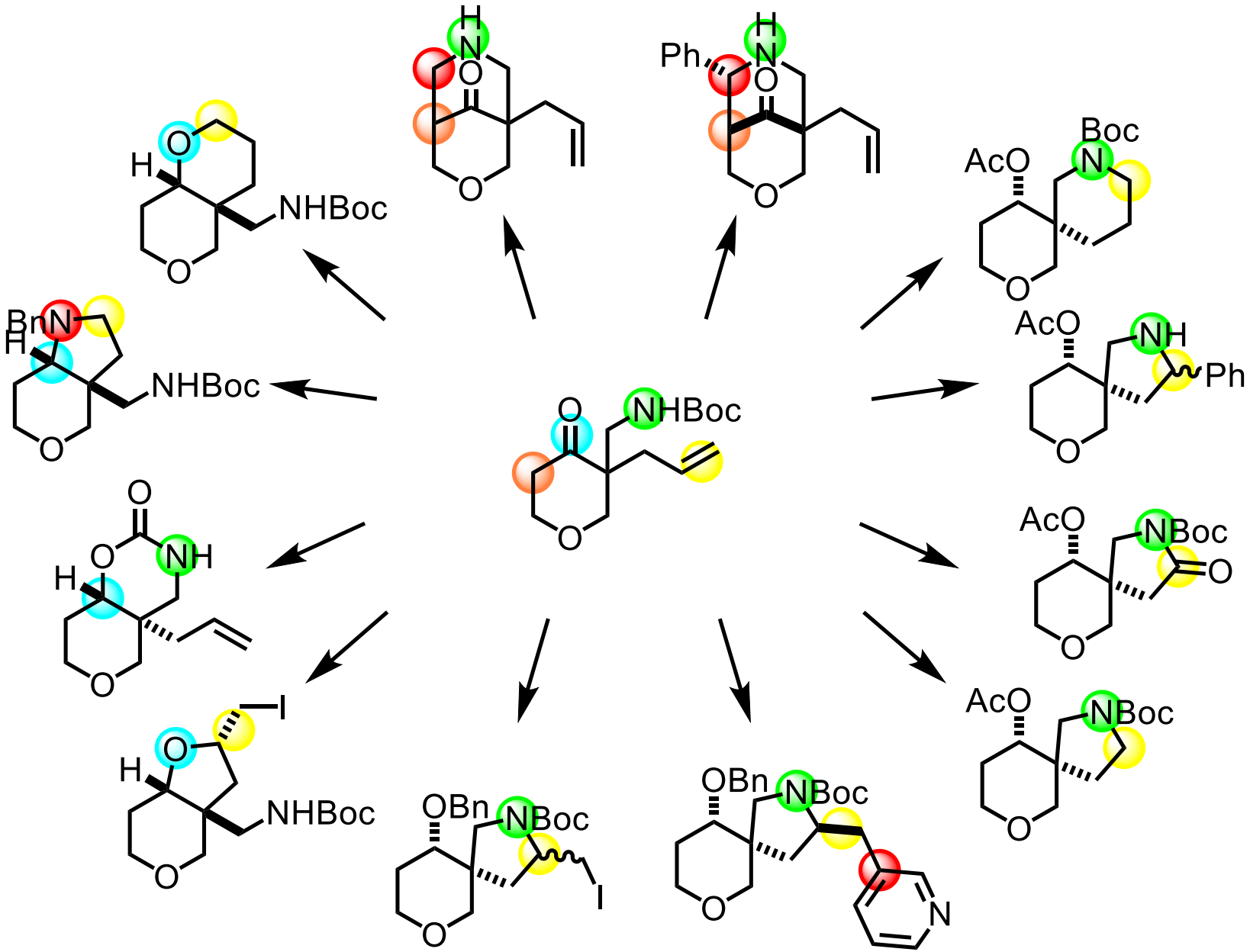

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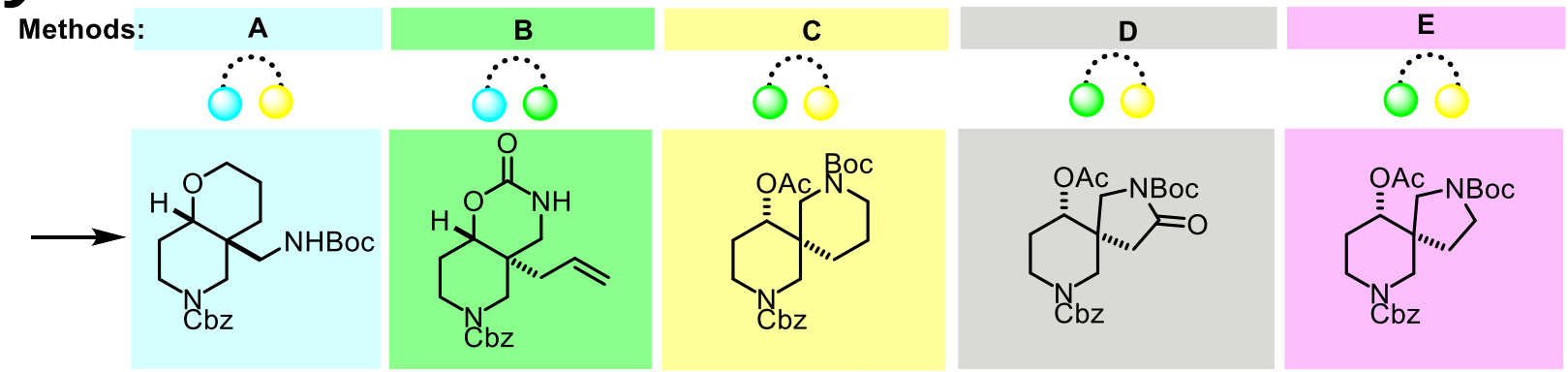

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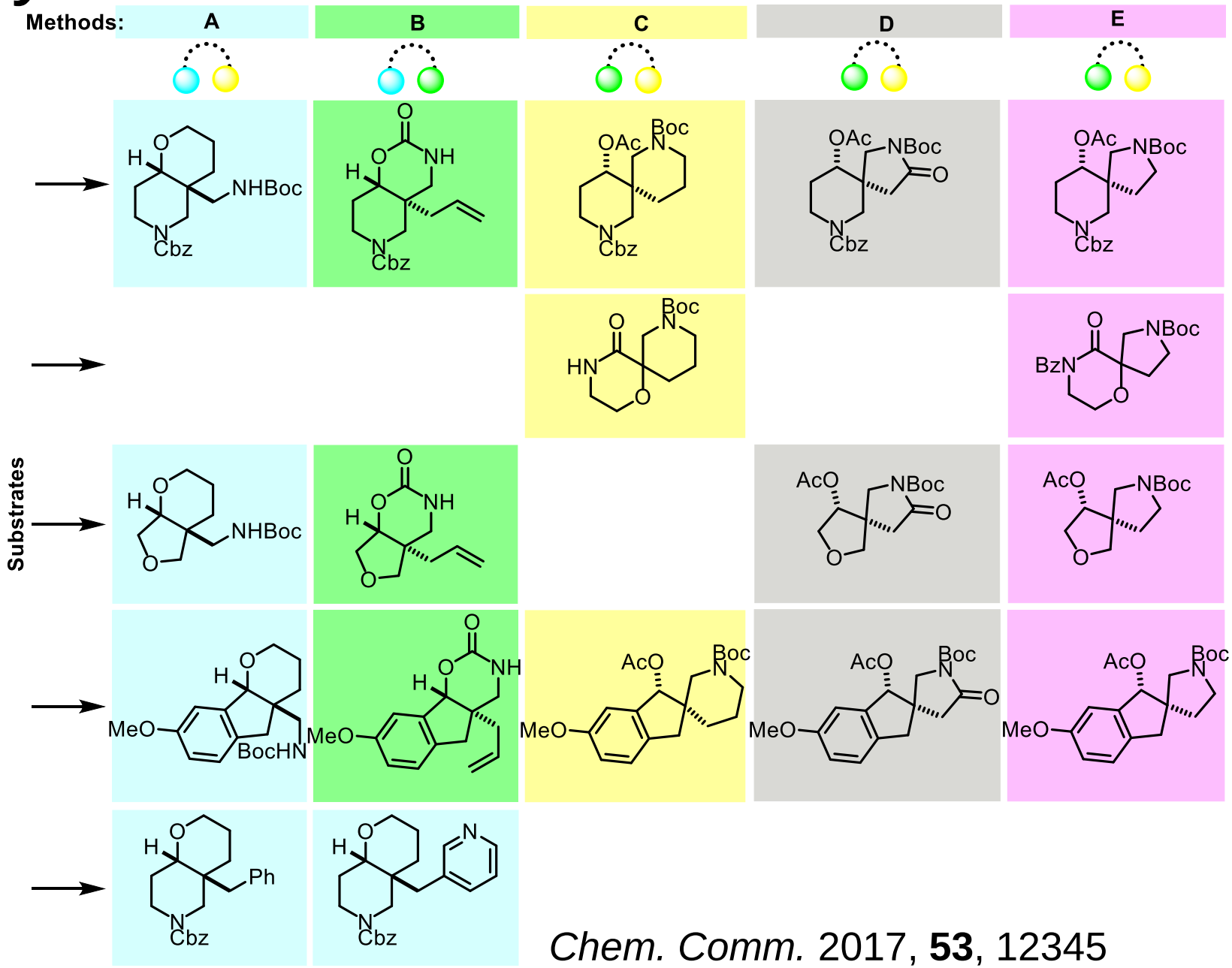
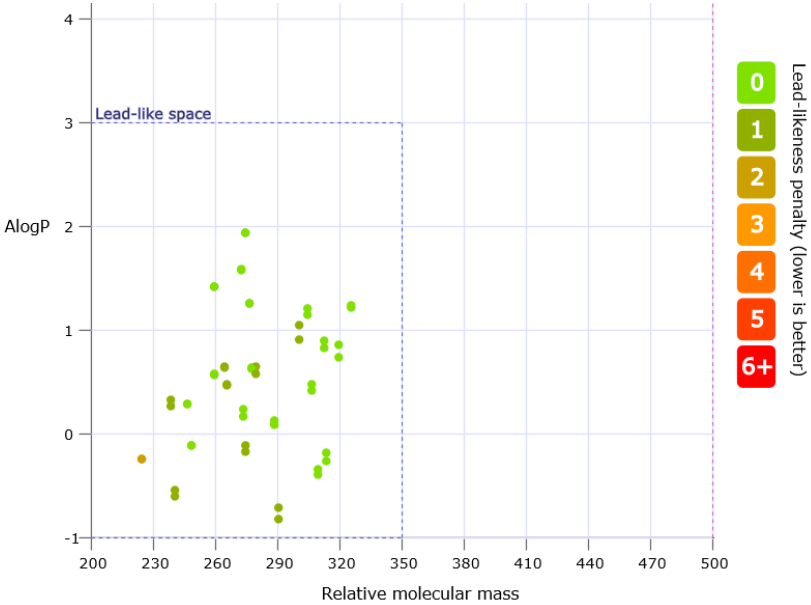
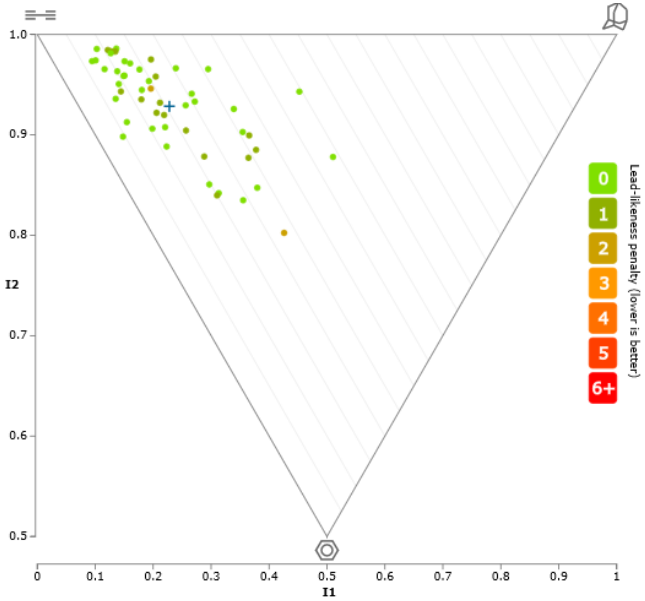
Synthesis of CNS lead-like scaffolds



Chem. Comm. 2017, **53**, 12345

LLAMA, an open-access tool for assessing the lead-likeness of scaffolds





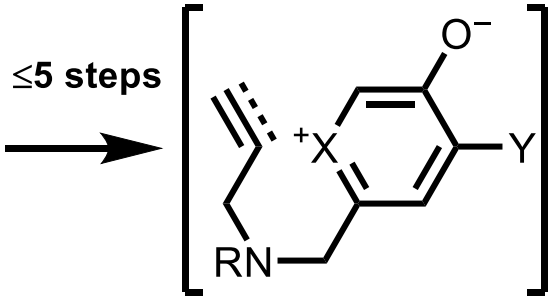
Chem. Commun. 2016, **52**, 7209

A ‘top-down’ synthetic approach

1. Prepare simple precursors

2. Generate complexity

3. Exploit complexity



X= O, N
Y= H, OTBS

[5+2] cycloadditions: Advances in cycloaddition, **1999**, 6, 1
Related: *Nat. Chem.* **2013**, 5, 195; *J. Am. Chem. Soc.* **2015**, *137*, 6327

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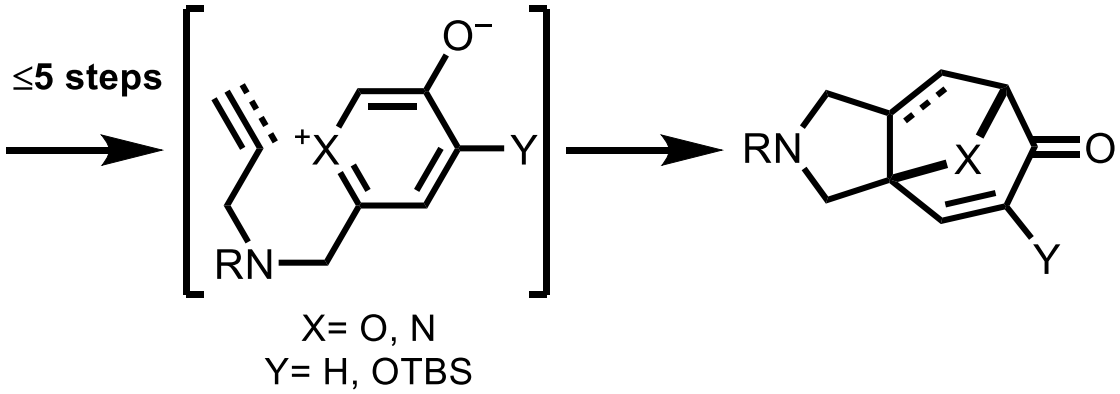

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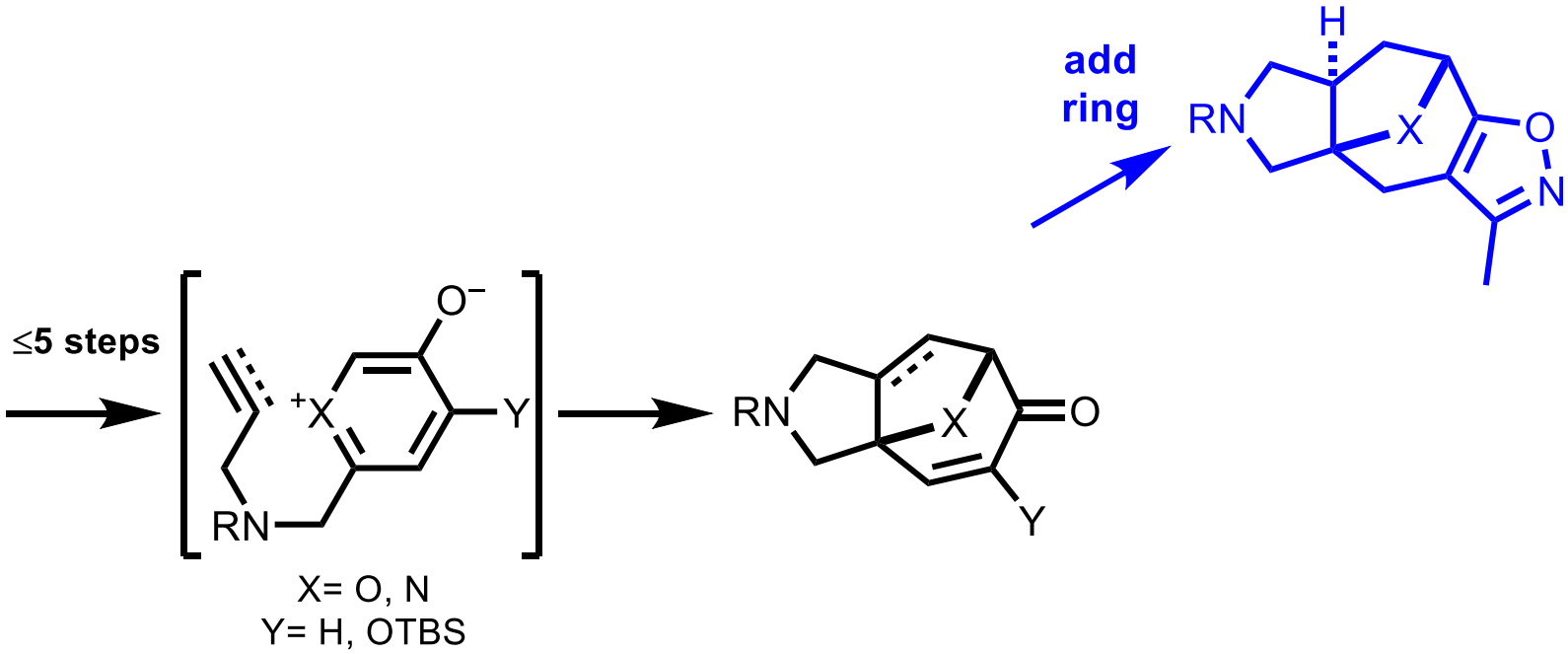

Chem. Eur. J. 2017, **23**, 15227

Translation into ELF: *Drug Discov. Today* 2018, **23**, 1578

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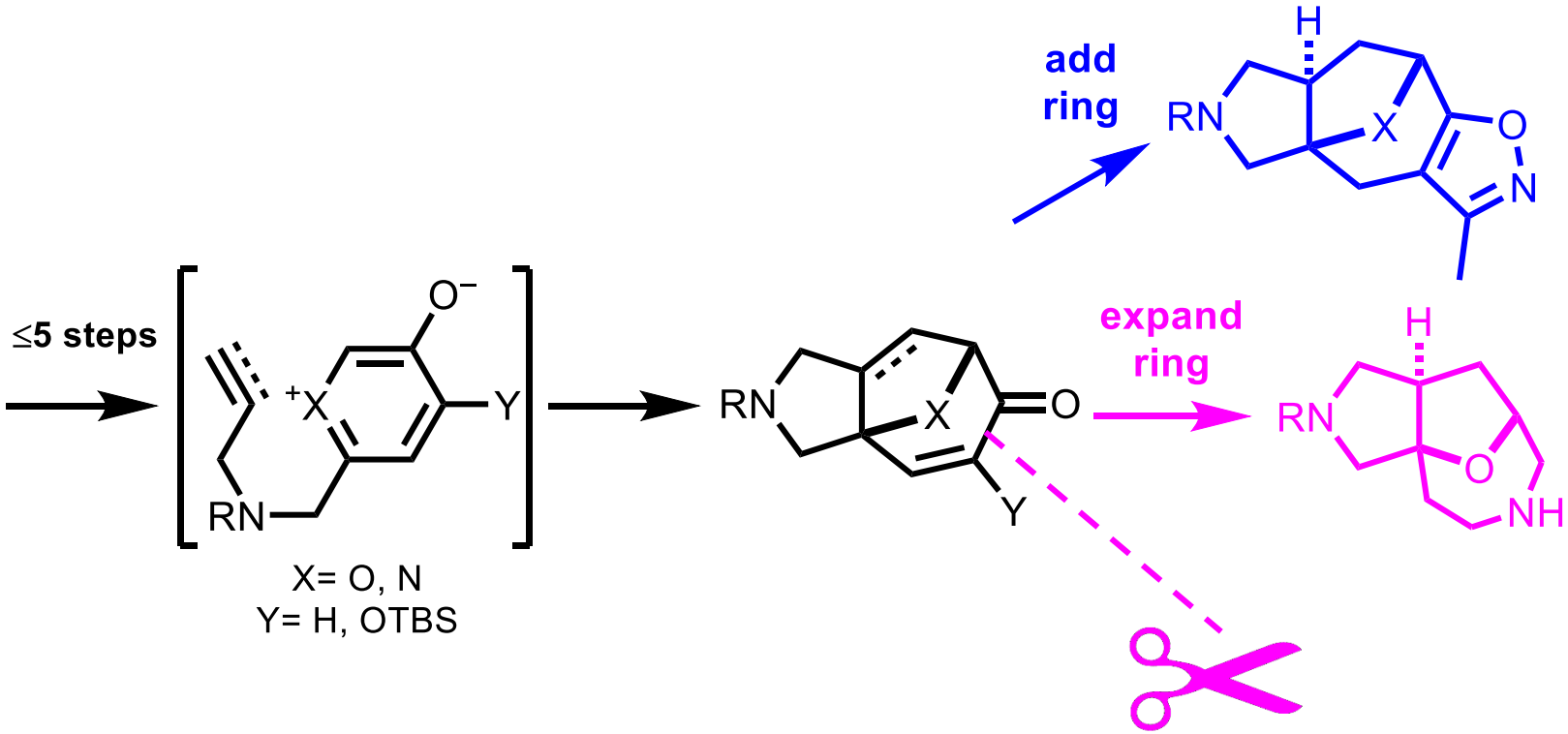

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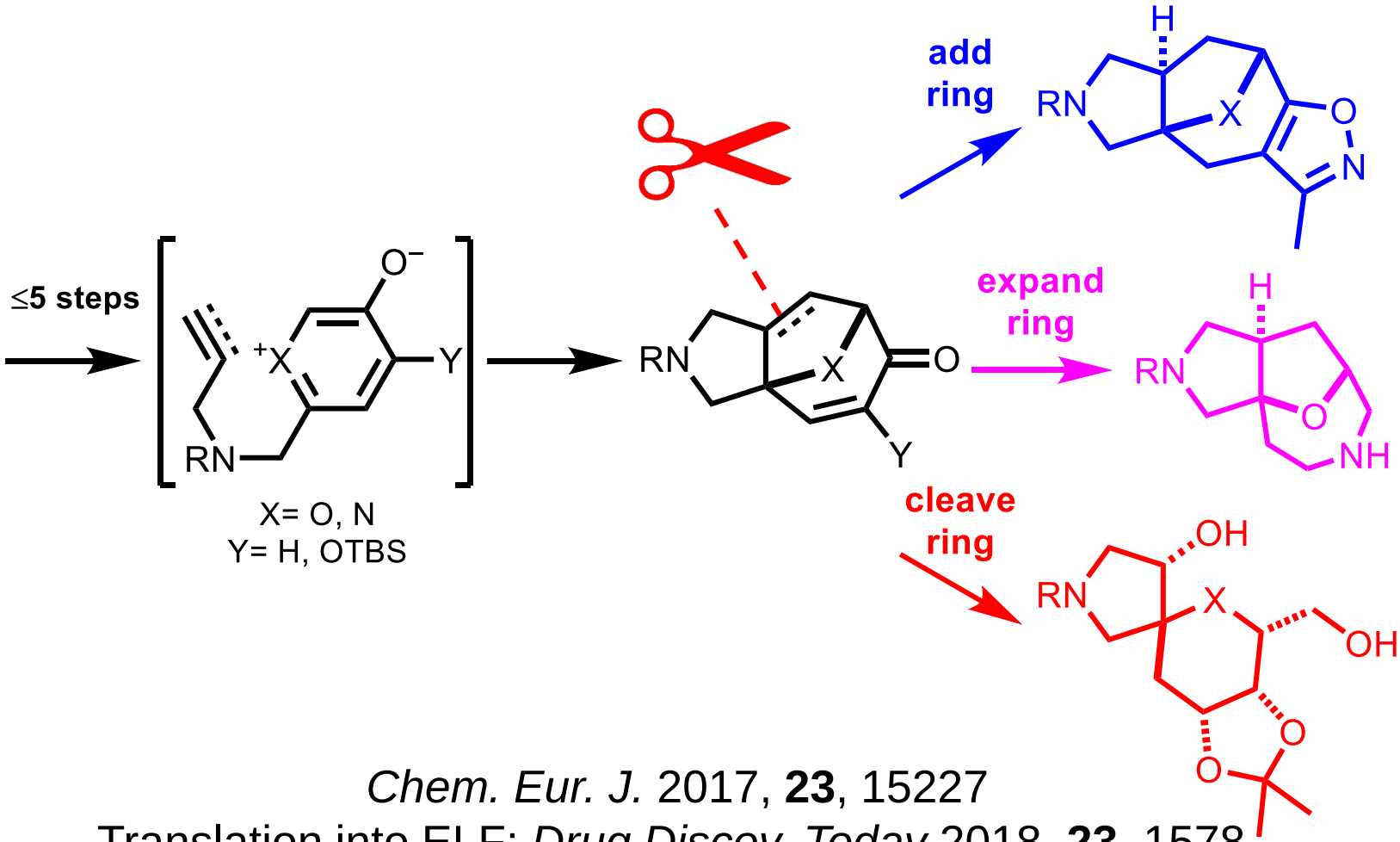

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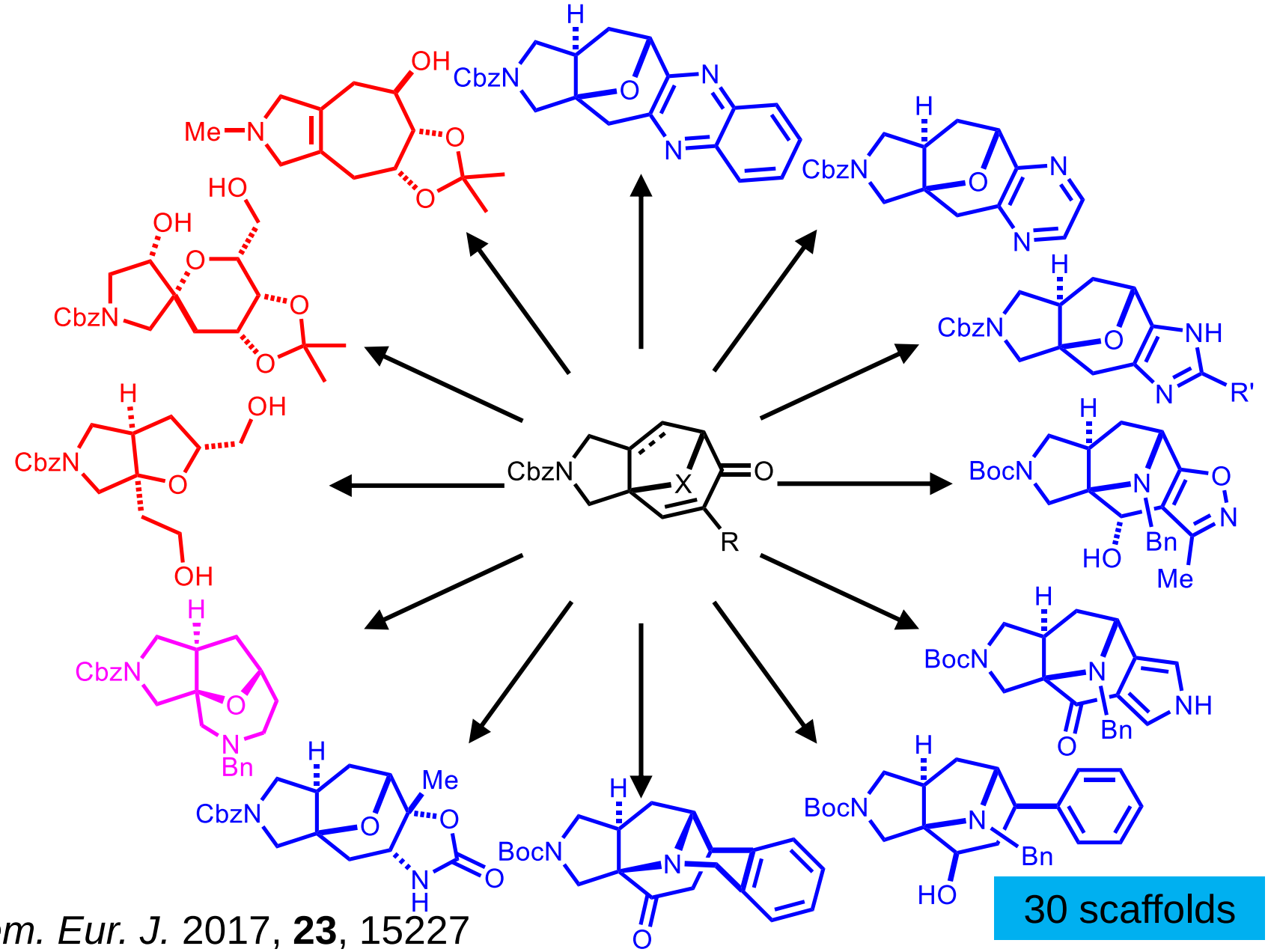

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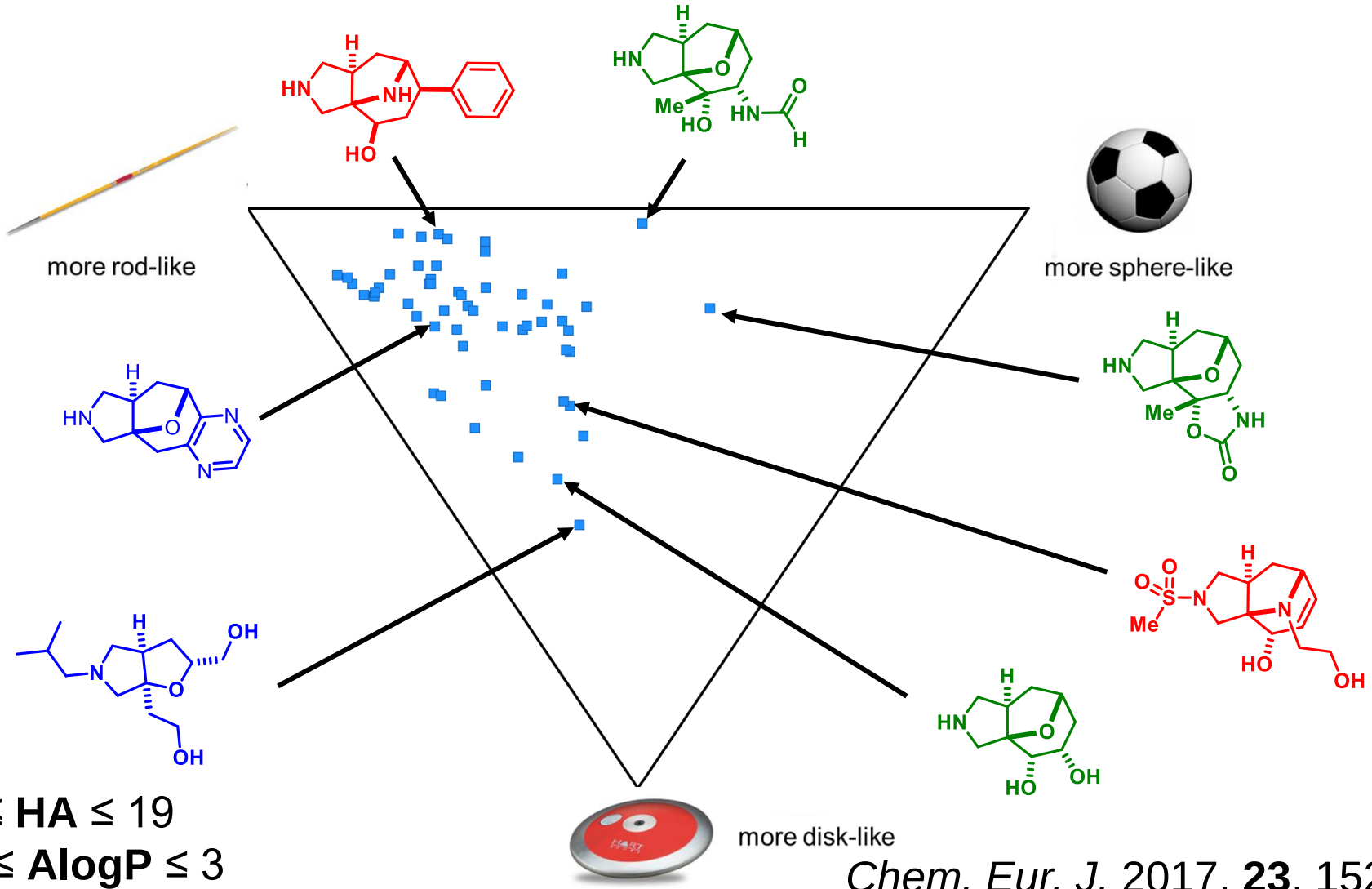
Shape diversity of our fragments

Decoration yielded a shape-diverse fragment set (54 fragments):

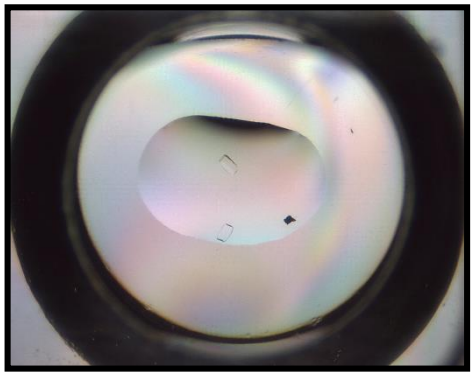


9 ≤ **HA** ≤ 19

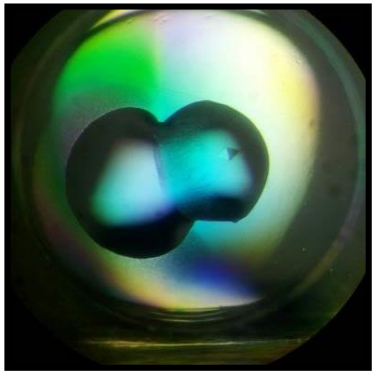
−1.5 ≤ **AlogP** ≤ 3

Chem. Eur. J. 2017, **23**, 15227

Assessment of biological relevance



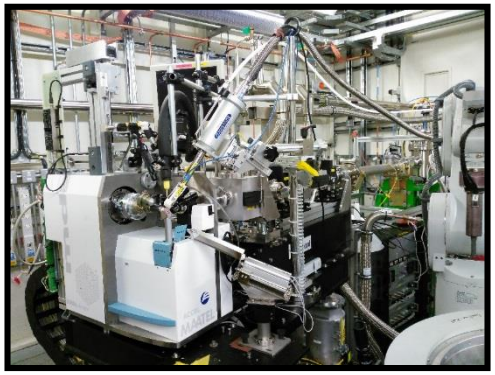
Grow crystals



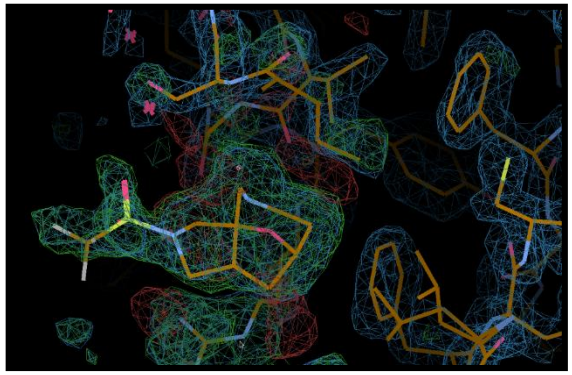
Soak fragments

Pick crystals

X-ray diffraction



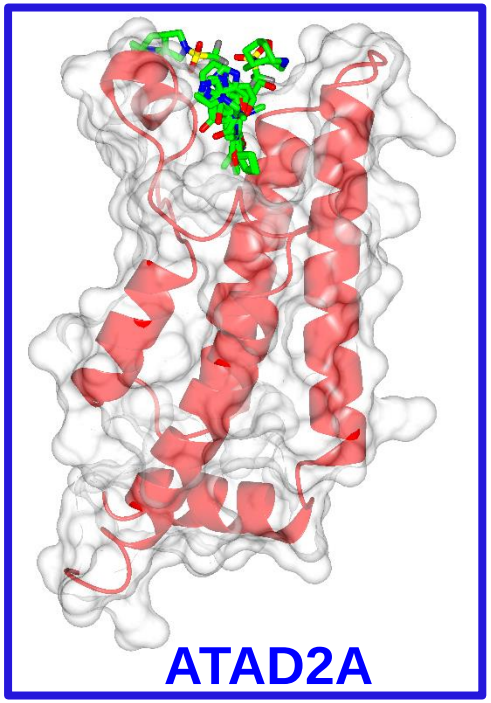
Fit ligands

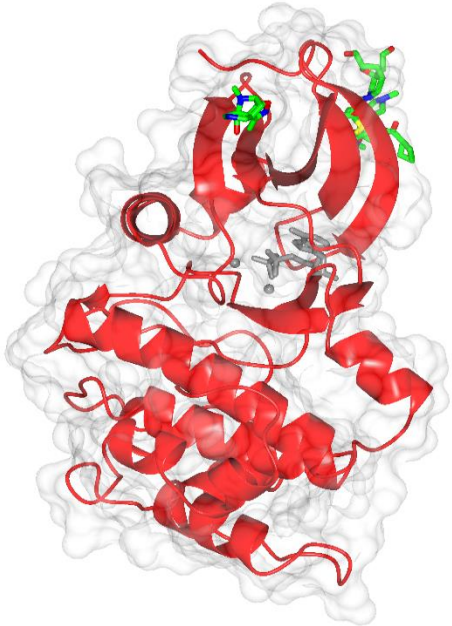


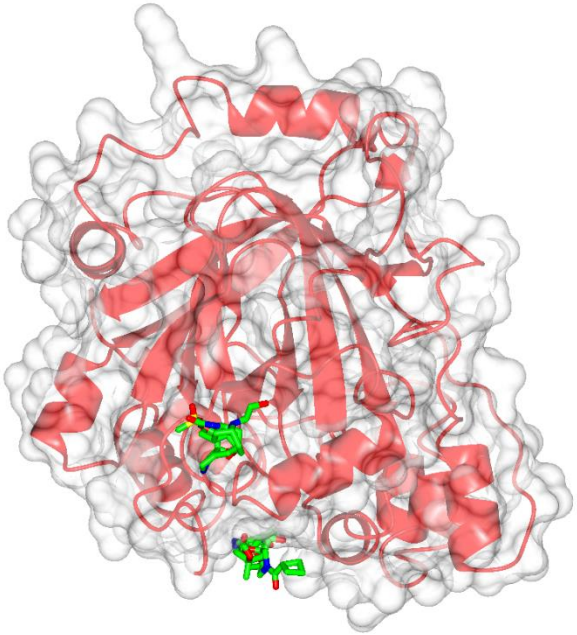
- Collaboration with Structural Genomics Consortium and Diamond

Assessment of biological relevance

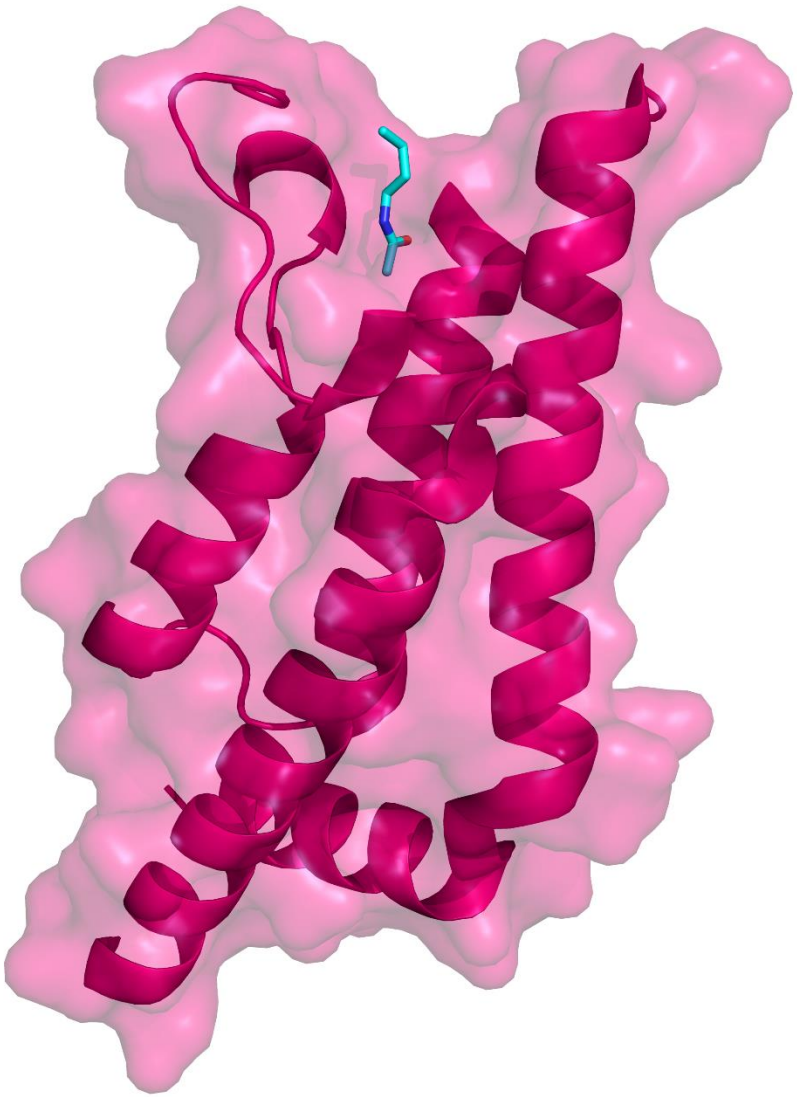
- The library was screened against 4 distinct proteins (3 classes):
  - ATAD2A (bromodomain): 8 hits
  - AURA (protein kinase): 4 hits
  - BRD1A (bromodomain): 10 hits
  - JMJD2DA (histone demethylase): 3 hits



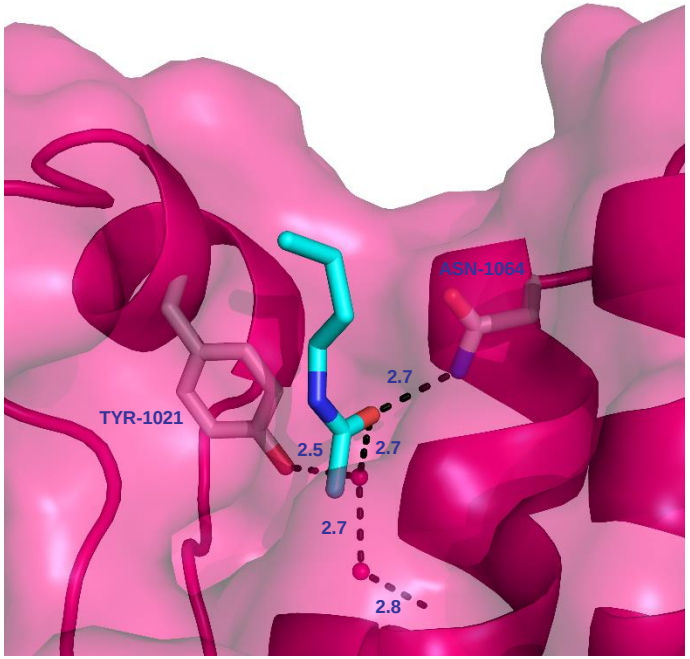




Assessment of biological relevance



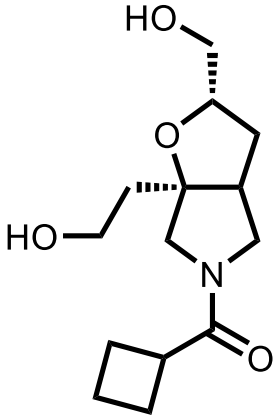
- ATAD2 (ATPases Associated with Diverse cellular activities)
- Acetyl-lysine-binding bromodomain
- Over-expressed in several cancers
- Binding site is polar and shallow

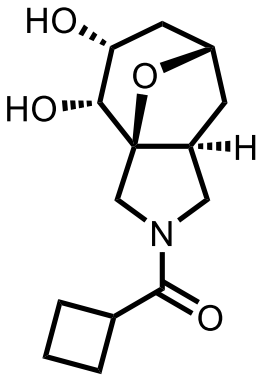


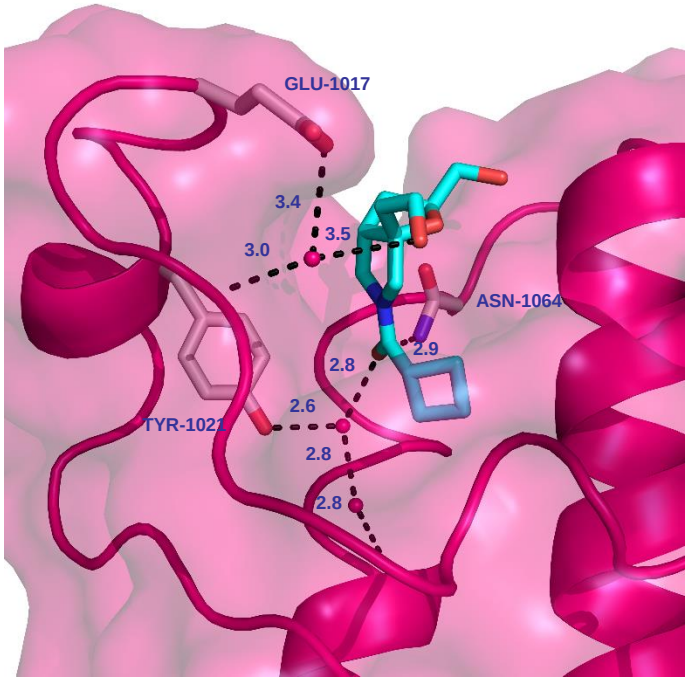
Med. Chem. Commun. **2014**, *5*, 1843

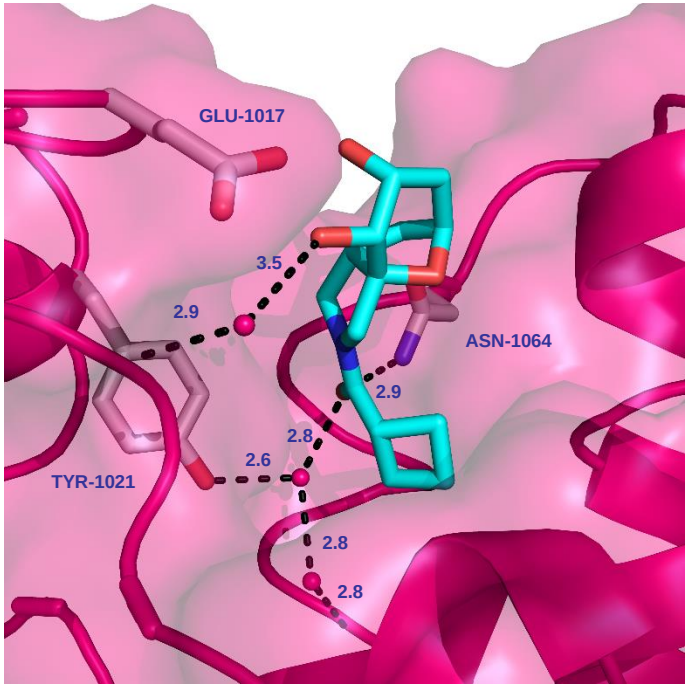
Assessment of biological relevance

2 hits with similar binding modes:





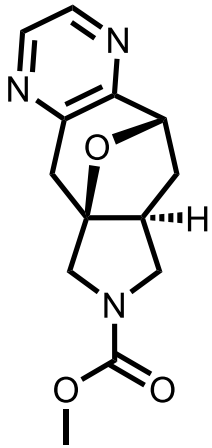


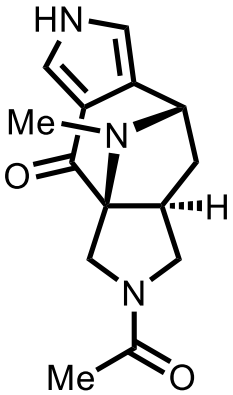


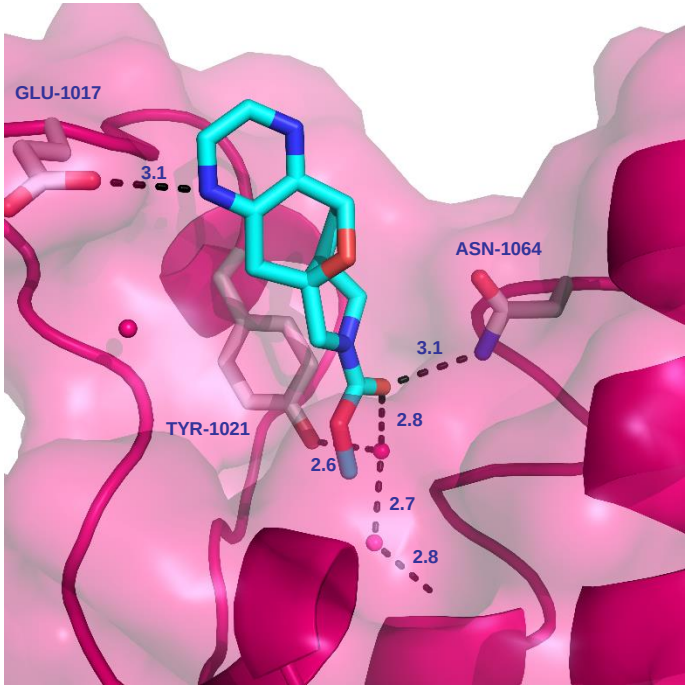
Chem. Eur. J. 2017, **23**, 15227

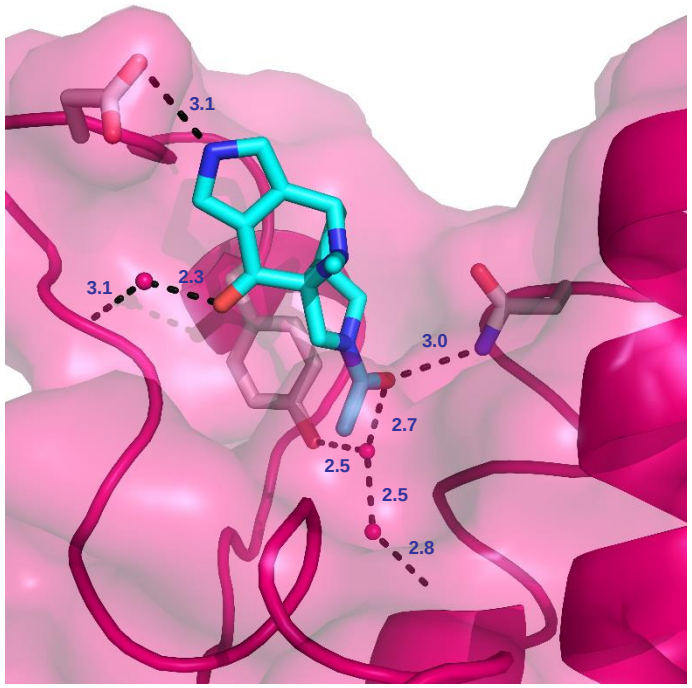
Assessment of biological relevance

2 more hits with similar binding modes:









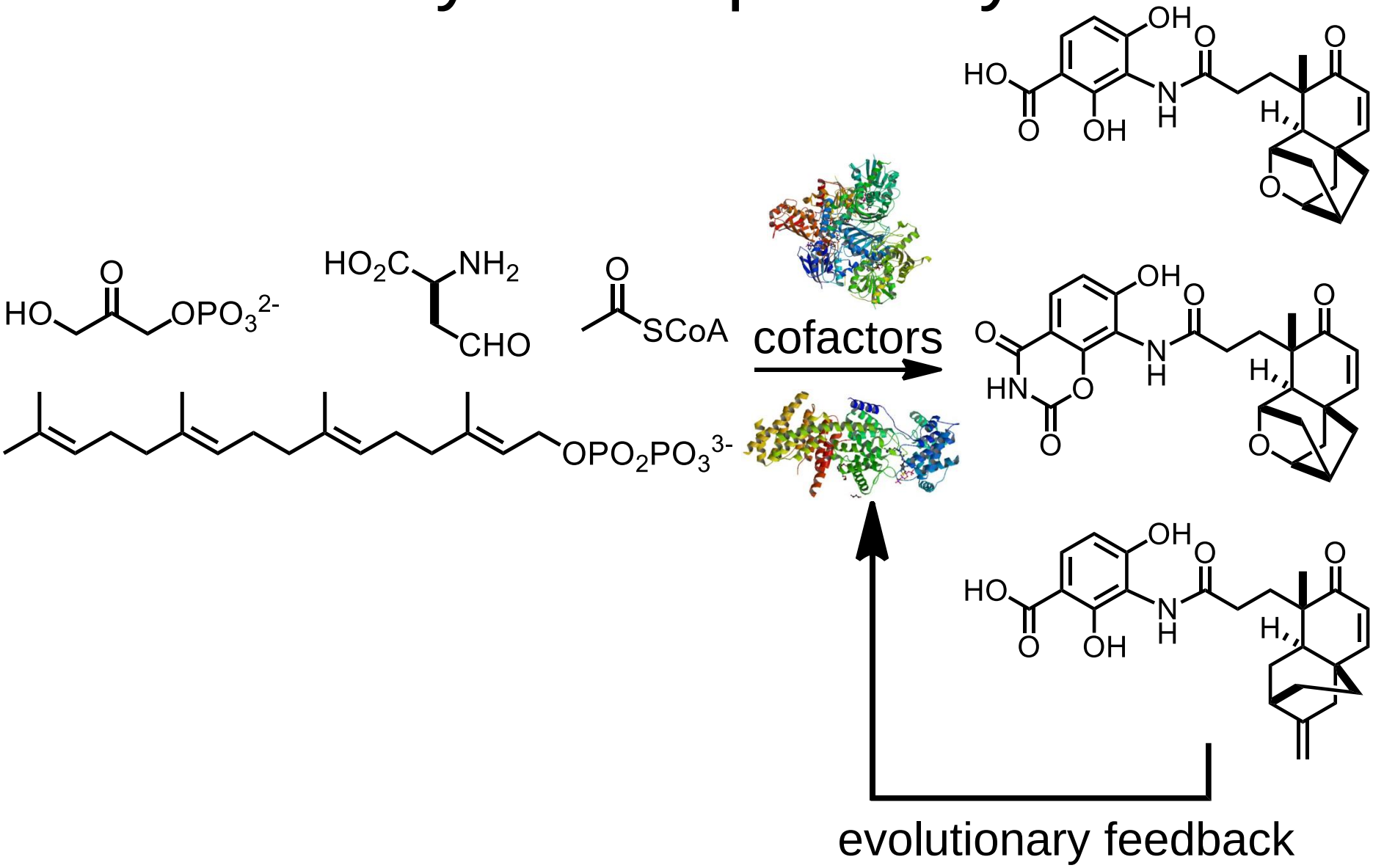
Chem. Eur. J. 2017, **23**, 15227

Comparison of alternative approaches to generate molecular diversity

	Diversity-oriented synthesis	Lead-oriented synthesis	Activity-directed synthesis
Diversity	High	High within defined chemical space	
Molecular properties	Low level of control	High level of control	
Reliability of reaction toolkit	High level required	High level required	
Resource focused on bioactives?	Equal focus on all compounds	Equal focus on all compounds	

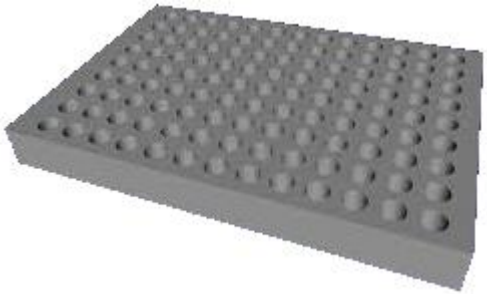
Focusing the search of chemical space

Can we learn from the evolution of biosynthetic pathways?

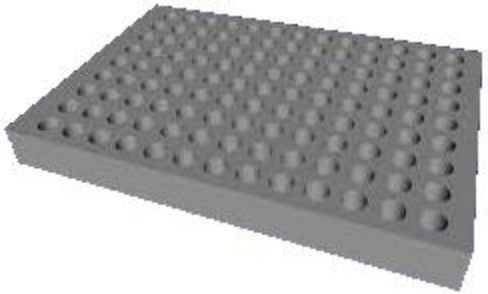


Experimental set-up

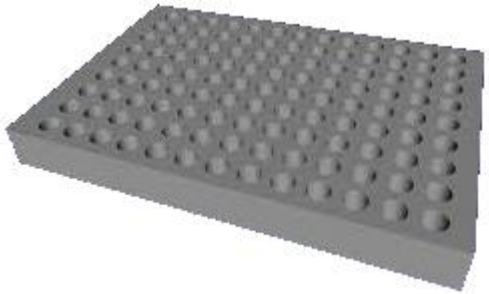
Reaction array
[S] = 100 mM



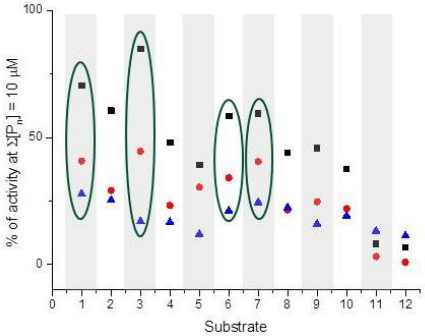
1. Scavenge
2. Evaporate
3. Dissolve in
0.1% DMSO in buffer



Stock array of crude products



Assay array
Decrease $\Sigma[P_n]$
in each round

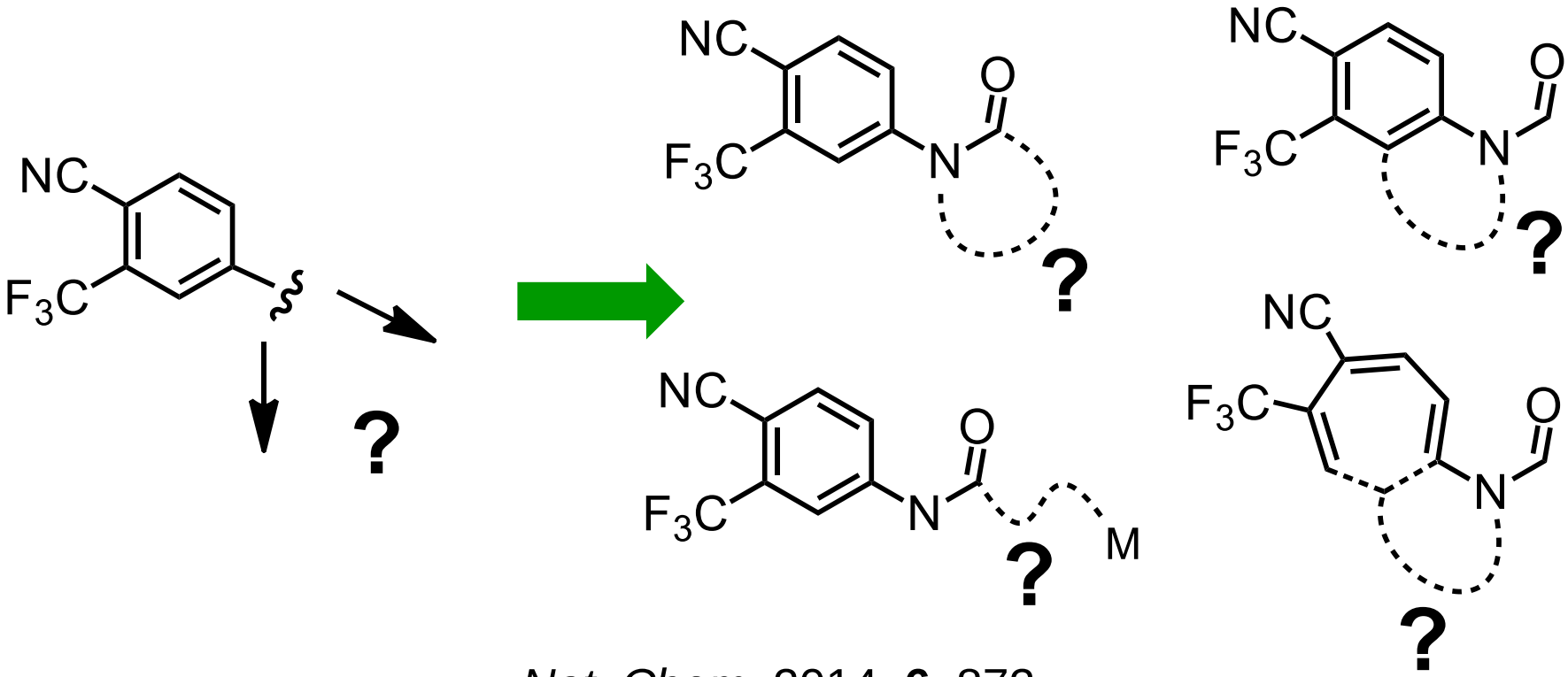


1. Analyse results
2. Design
next array

1. Scale-up
2. Purify
3. Assay

Nature Chemistry 2014, **6**, 872

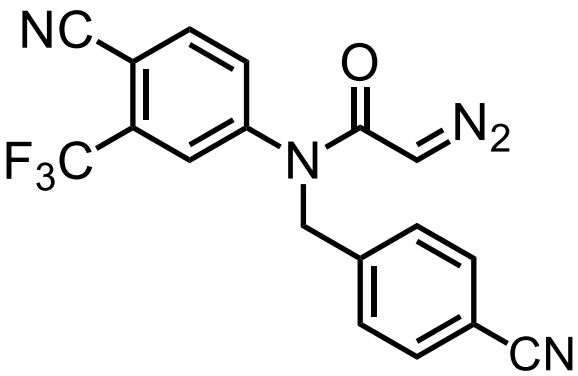
Objective: To develop a known fragment into novel chemotypes that agonise the androgen receptor

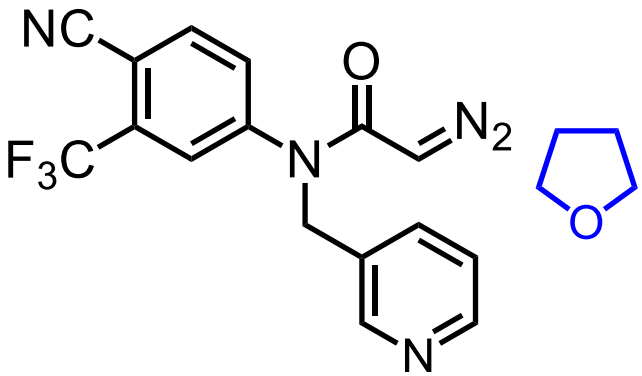


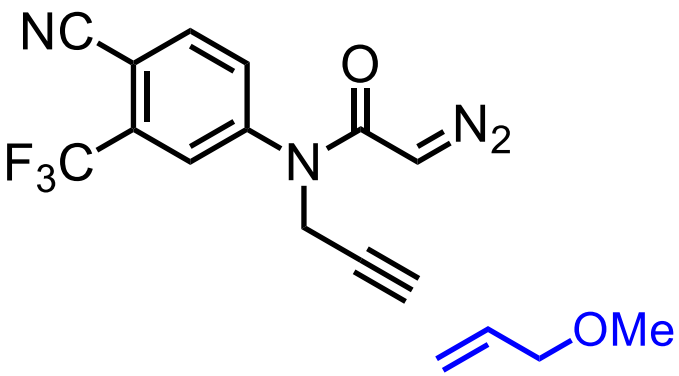
Nat. Chem. 2014, **6**, 872

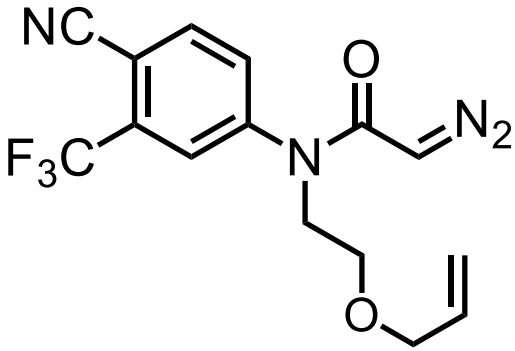
See also News & Views (Lowe), interview, editorial and themed web issue in *Nature Chemistry*

Rationale for choice of chemistry





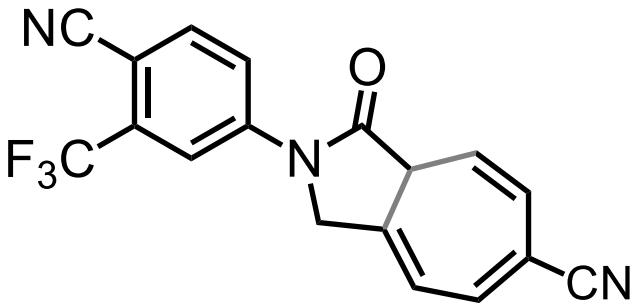


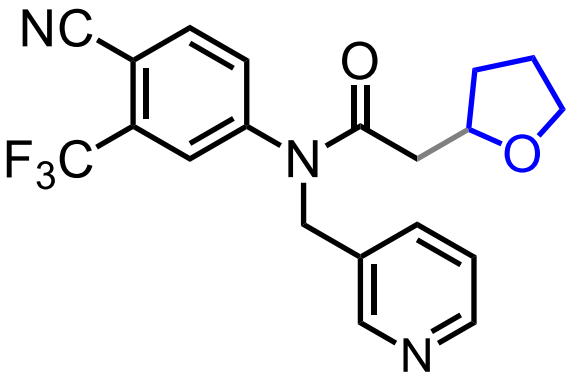


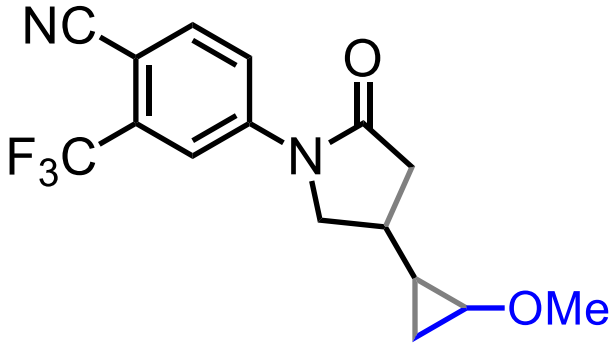
Ideally require:

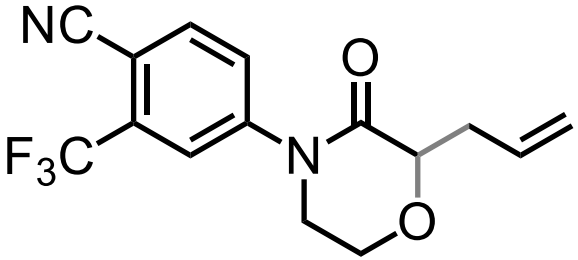
- Diverse reactivity
- Potential for asymmetric synthesis
- Multiple possible pathways for each substrate
- Fate tunable through choice of catalyst and ligand
- Either inter- or intramolecular

Rationale for choice of chemistry









Ideally require:

- Diverse reactivity
- Potential for asymmetric synthesis
- Multiple possible pathways for each substrate
- Fate tunable through choice of catalyst and ligand
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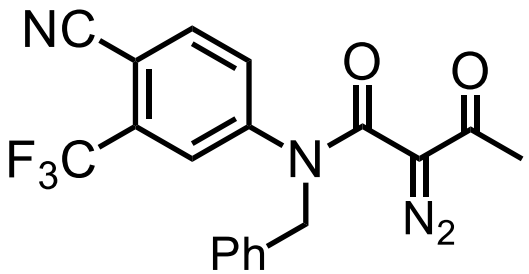
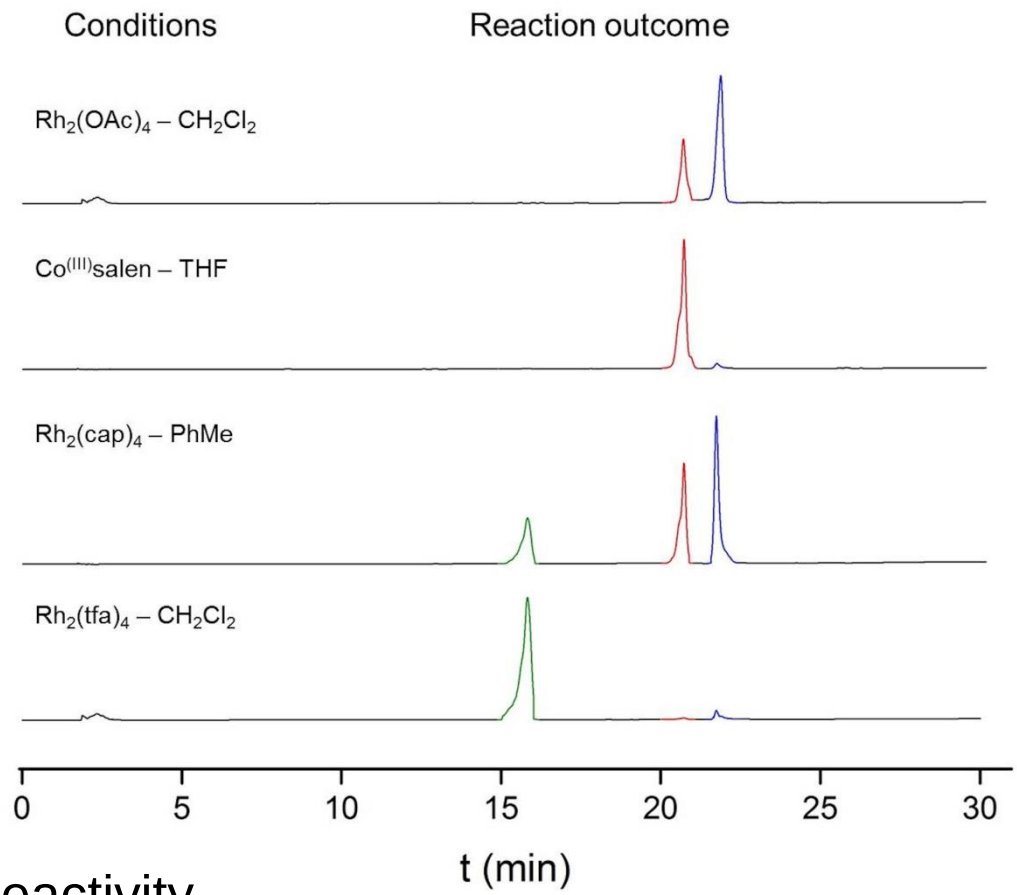
Rationale for choice of chemistry

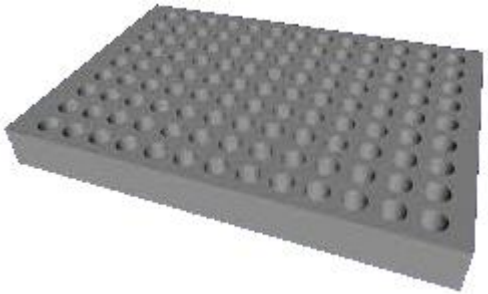




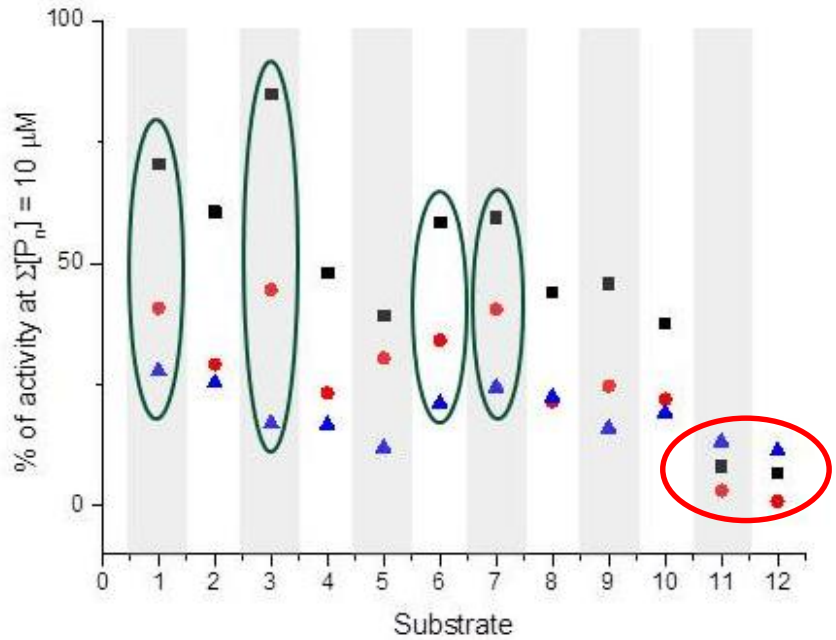
Ideally require:

- Diverse reactivity
- Potential for asymmetric synthesis
- Multiple possible pathways for each substrate
- Fate tunable through choice of catalyst and ligand
- Either inter- or intramolecular
- Compatible with other catalyst systems

Evolution of bioactivity: First array

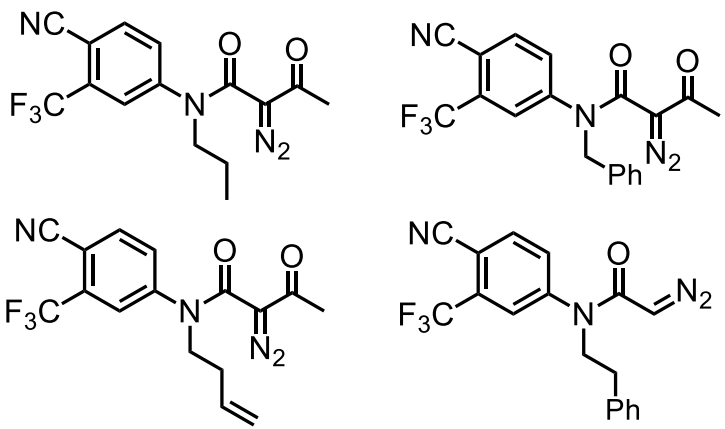


- 36 reactions
- 12 substrates x 3 catalysts
- CH_2Cl_2 as solvent
- Rh catalysts only



Activities are expressed relative to 5 μM testosterone

4 promising substrates used in next array



plus two control substrates

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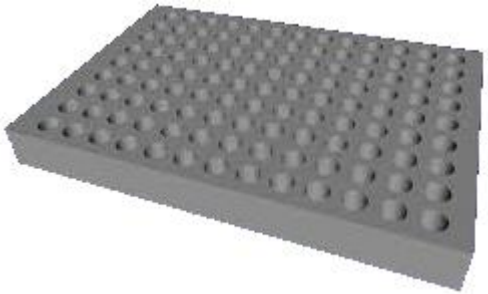
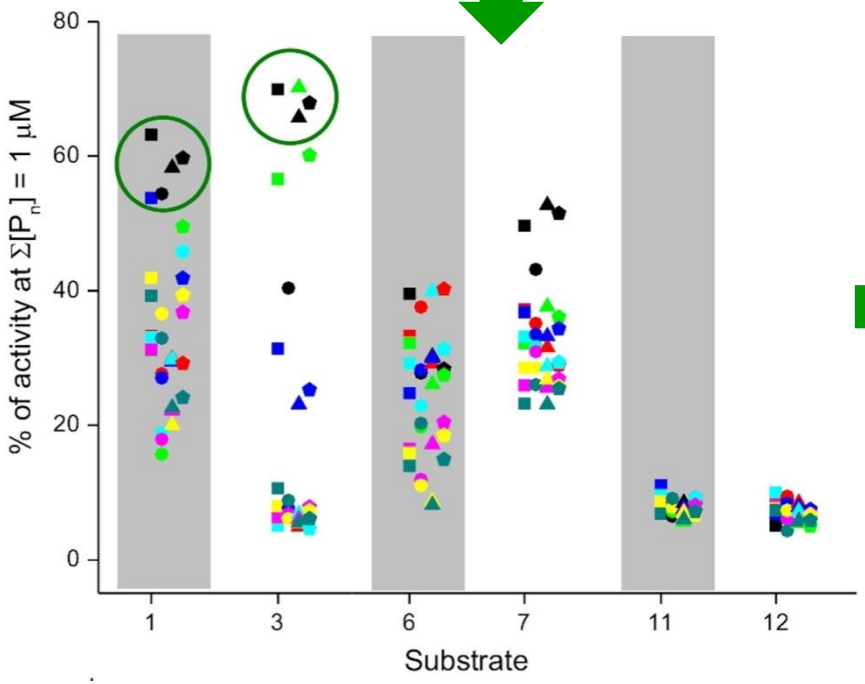
Evolution of bioactivity: Second array



- 192 reactions
- 6 substrates x 8 catalysts x 4 solvents
- Solvents: CH_2Cl_2, THF, EtOAc, PhMe
- Catalysts: 5 x Rh, 1xCu, 1xPd, 1xCo



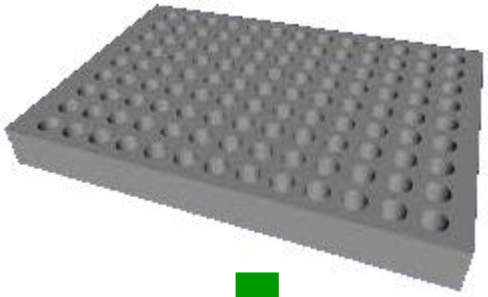
Next array designed based on:

- 2 substrates and 4 new substrates
- 3 x Rh catalysts
- 3 x solvents

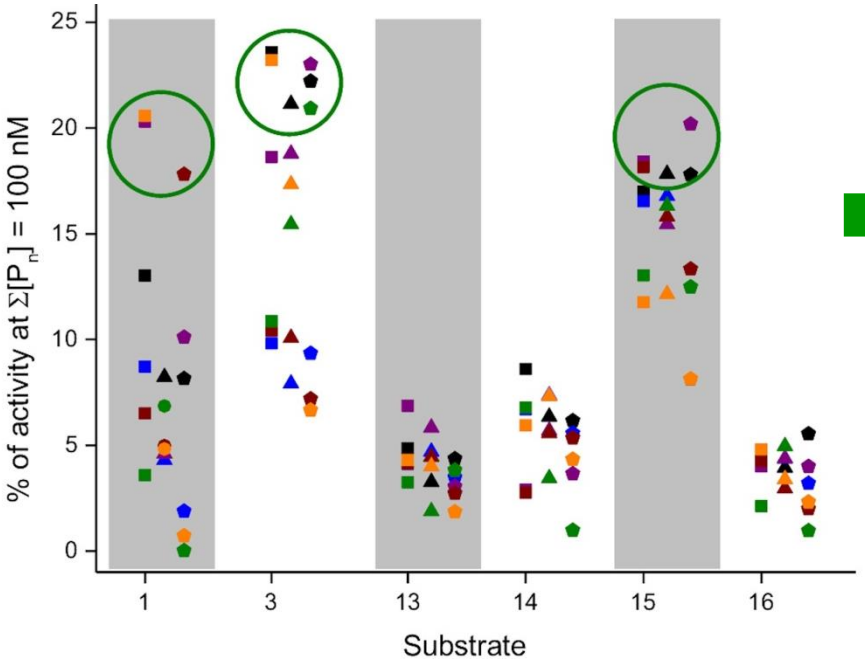
Activities are expressed relative to 5 μM testosterone

Nature Chemistry 2014, **6**, 872

Evolution of bioactivity: Third array



- 108 reactions
- 6 substrates x 6 catalysts x 3 solvents
- Solvents: CH_2Cl_2, EtOAc, PhMe
- Catalysts: Specific class of Rh complex



Activities are expressed relative to 5 μM testosterone

Select most productive combinations for scale-up, purification and evaluation

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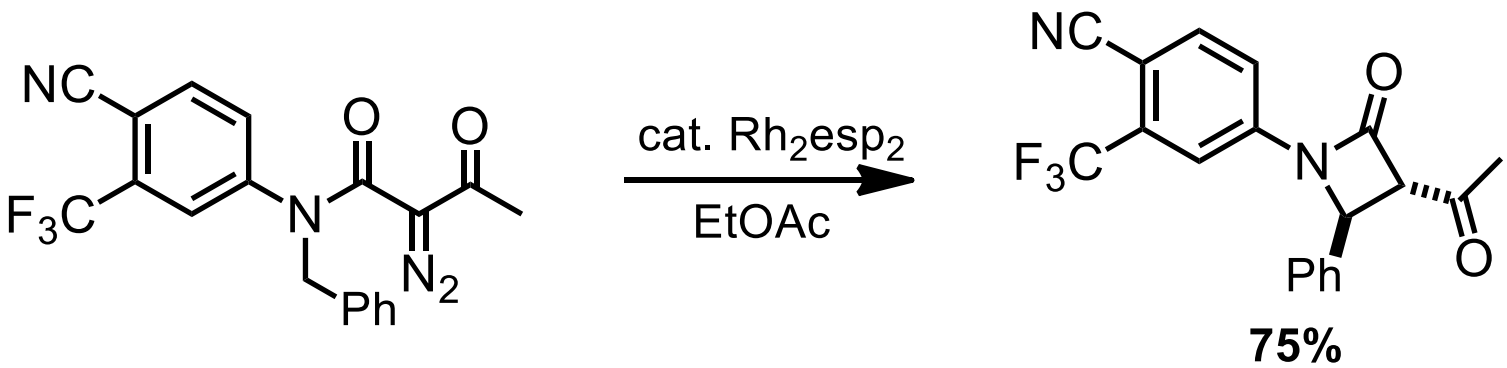
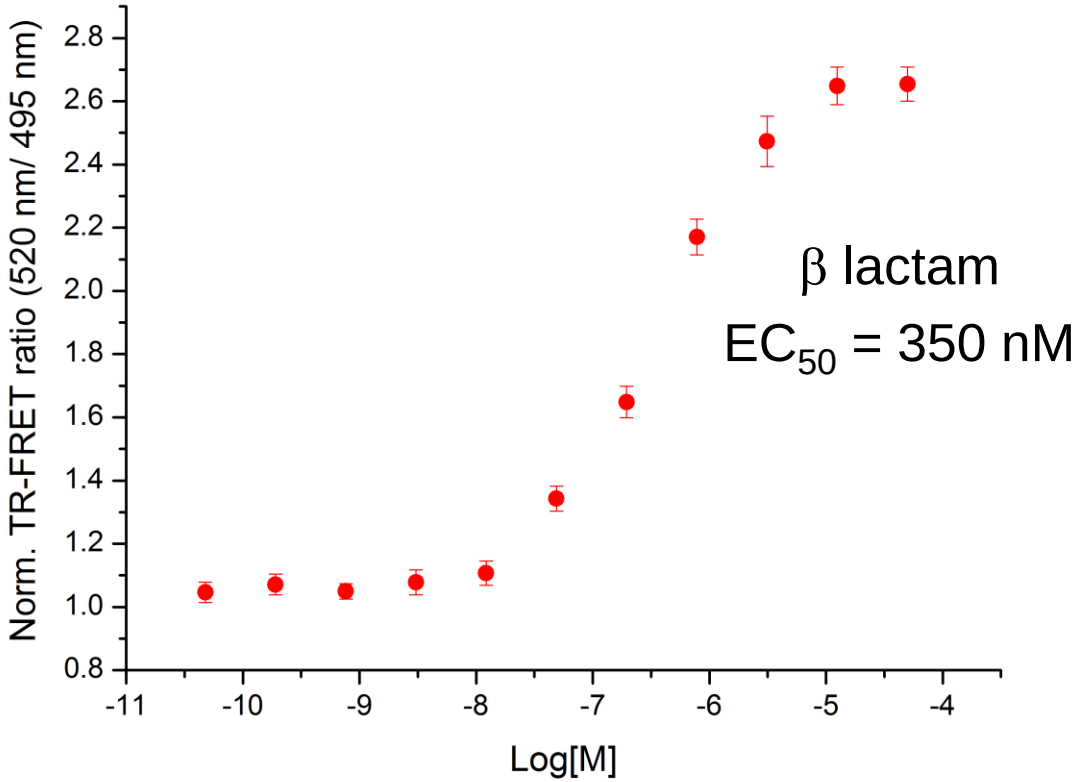
Scale-up, purification and assay





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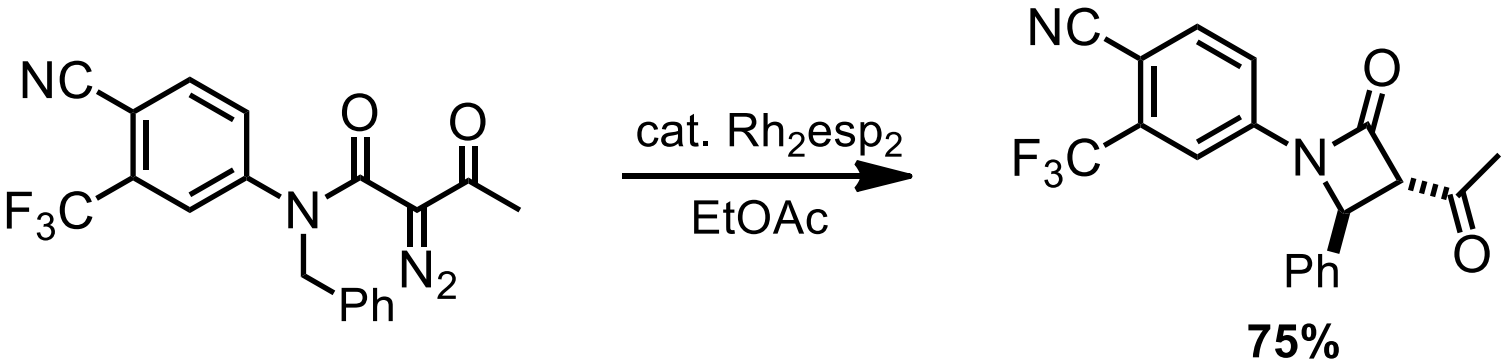
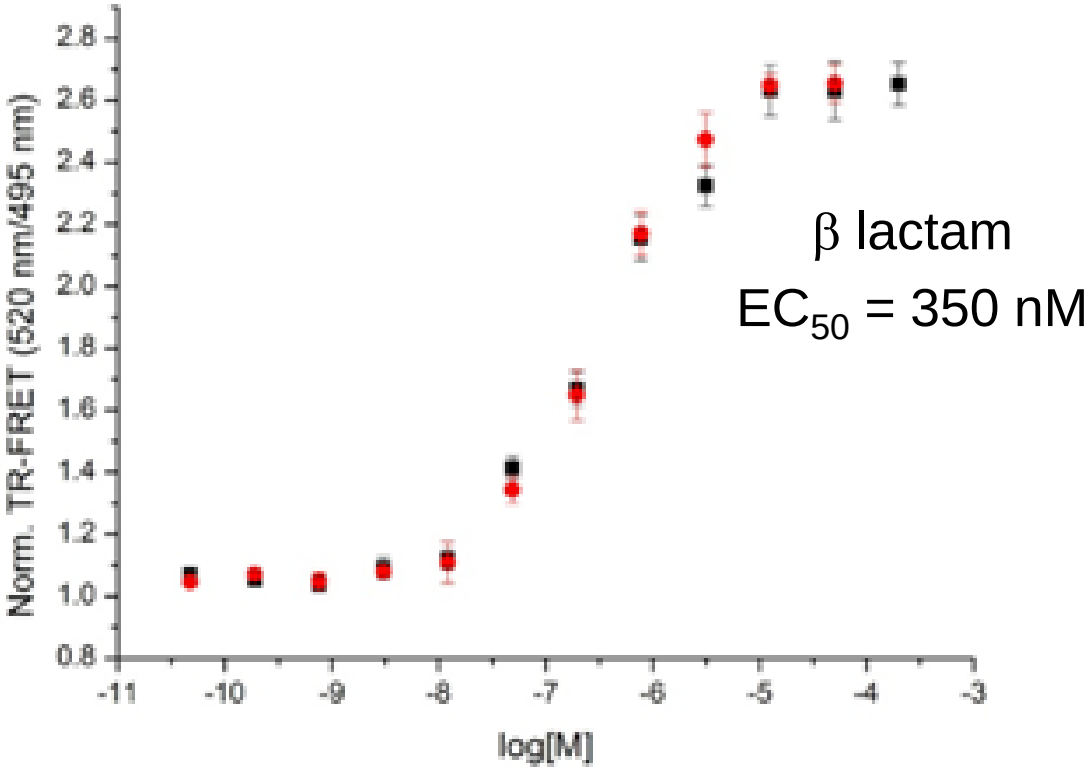
Comparison with independent sample





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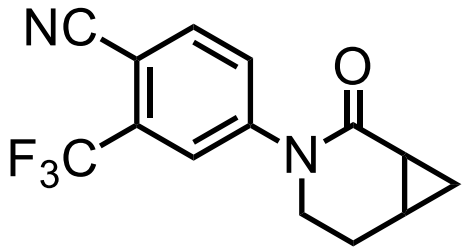
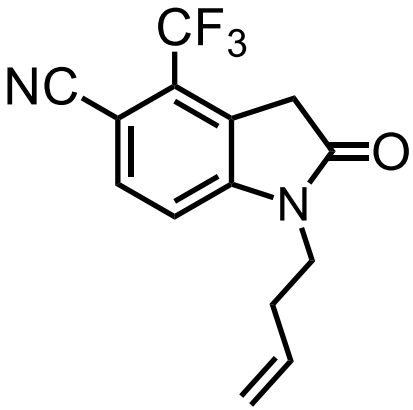
Overview of some chemotypes explored

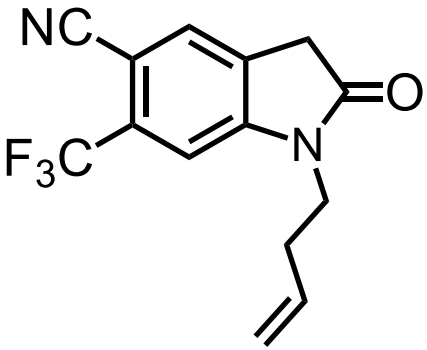
From a reaction from round 1 (with borderline activity at 10 μM)



EC_{50} = 700 nM
(26%; partial agonist)



EC_{50} = 11 μM
(13%)



(EC_{50} > 500 μM)
(12%)

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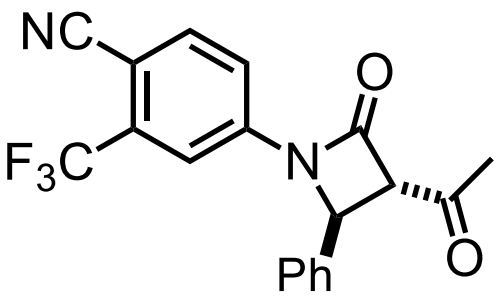
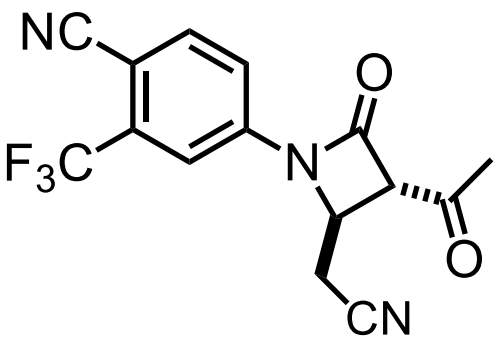
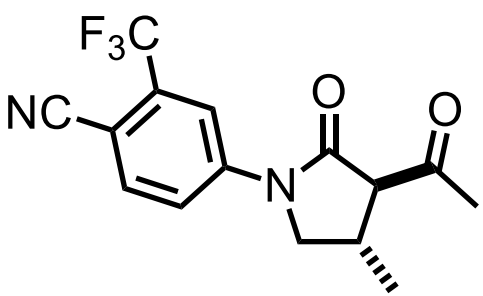
Overview of novel chemotypes discovered



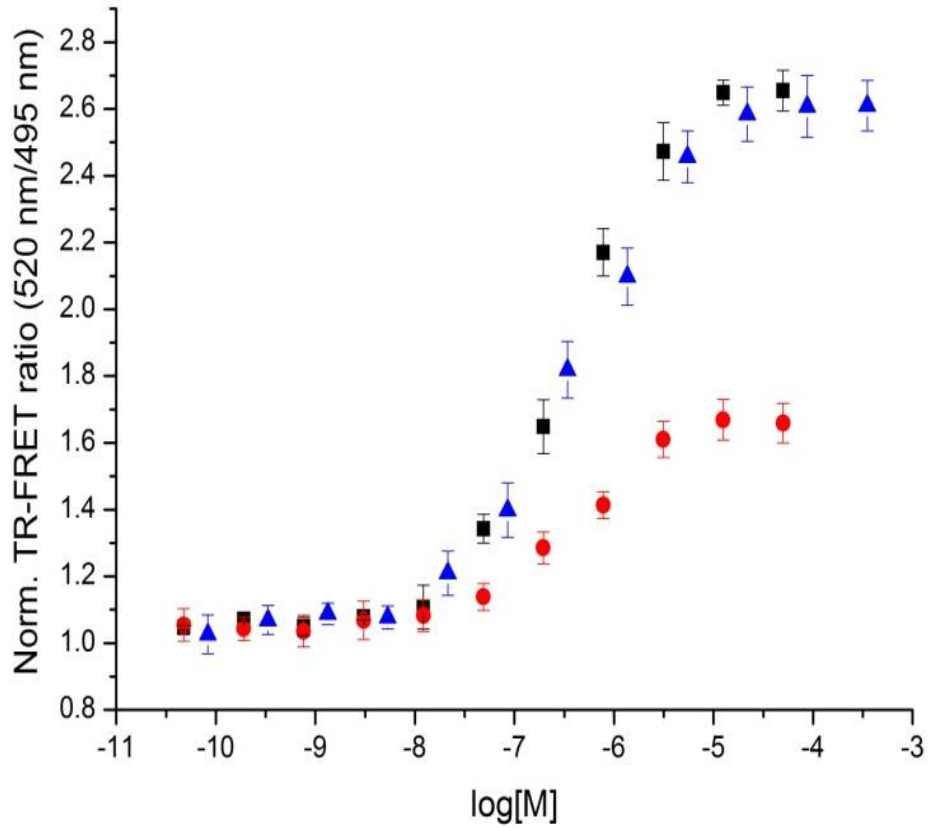
EC_{50} = 350 nM
(75%)
(full agonist)



EC_{50} = 440 nM
(78%)
(full agonist)



EC_{50} = 470 nM
(90%)
(partial agonist)



In total, •16 substrates
- 336 reactions
- 3 products purified

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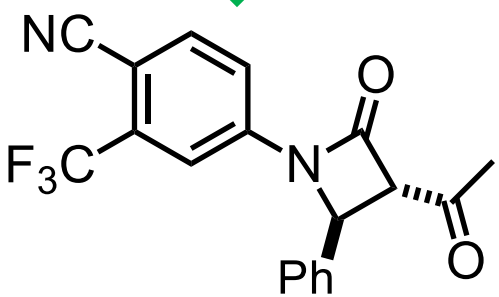
How did these activity-directed syntheses develop?

Substrate 3

Round 1



EC_{50} = 350 nM

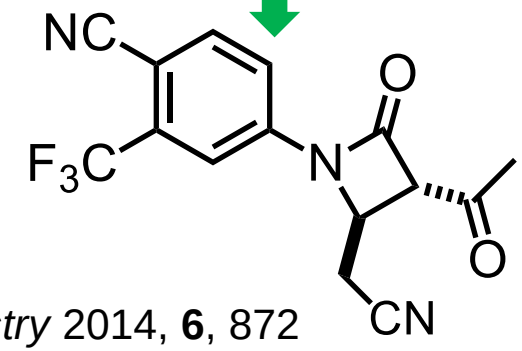
increase in yield

Round 2

(75% yield)

New substrate 15

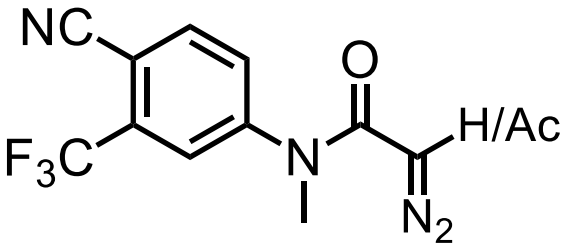
Round 3

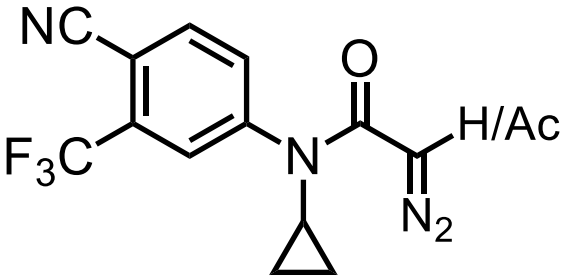


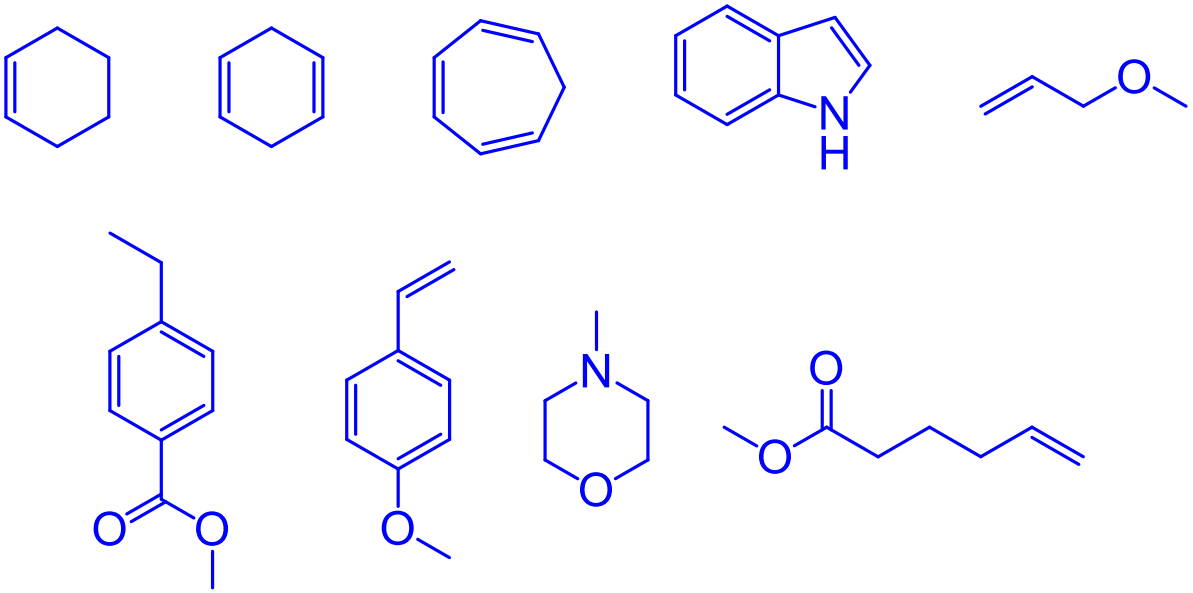
EC_{50} = 440 nM
(78% yield)

Nature Chemistry 2014, **6**, 872

Moving to intermolecular reactions







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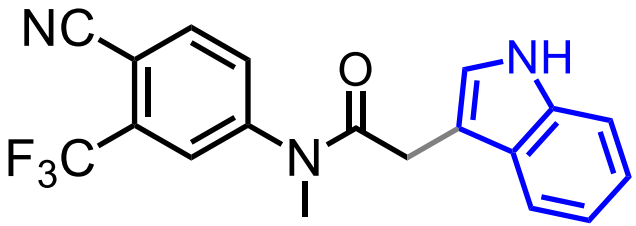
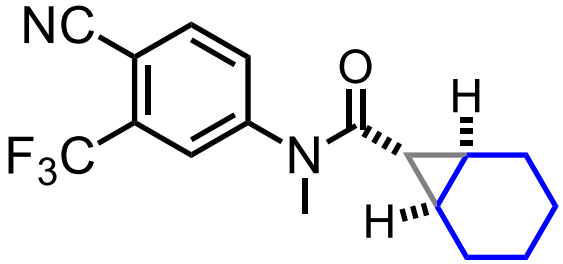
How did the ADSs develop?

Round 1

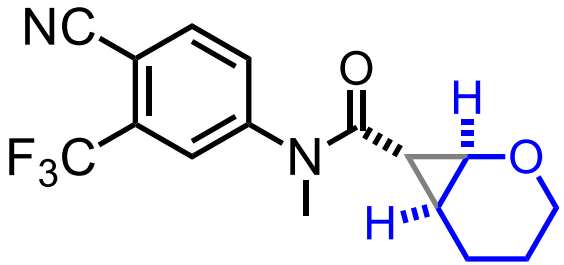


EC_{50} = 8.8 μM; 80%

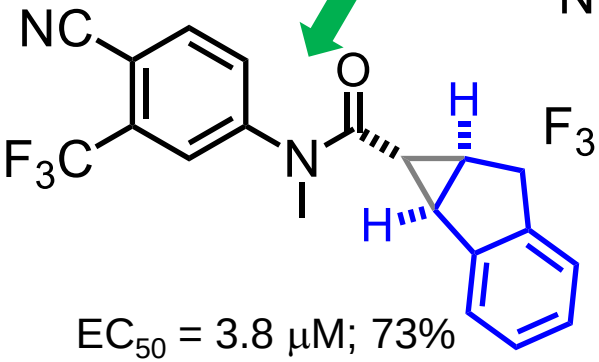


EC_{50} = 7.3 μM; 71%

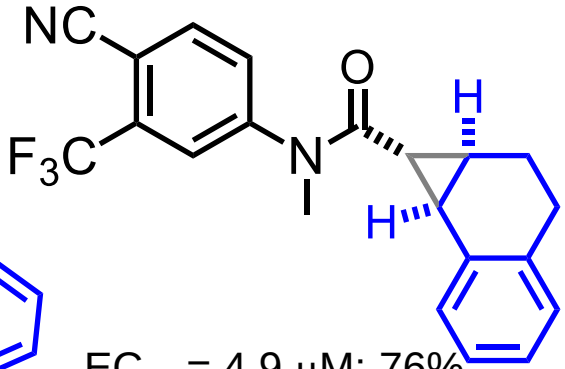
Round 2



EC_{50} = 4.7 μM; 82%

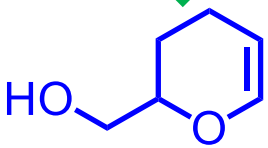


EC_{50} = 3.8 μM; 73%

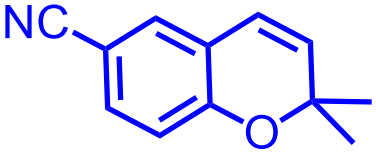


EC_{50} = 4.9 μM; 76%

Round 3



[with Rh_2(*R*-DOSP)$_4$]



[with $Rh_2(OAc)_4$]

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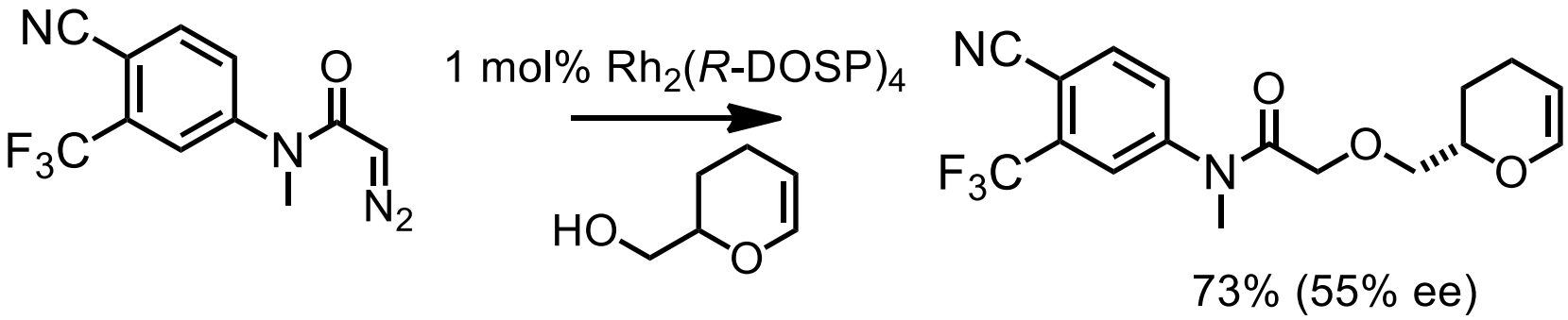
Discovery of an enantioselective reaction



73% (55% ee)

S product: 860 nM

R product: 4.5 μM

Rh-catalysed asymmetric O-H insertion not previously known!

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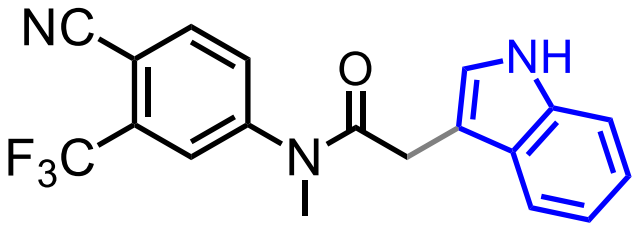
How did the ADSs develop?

Round 1



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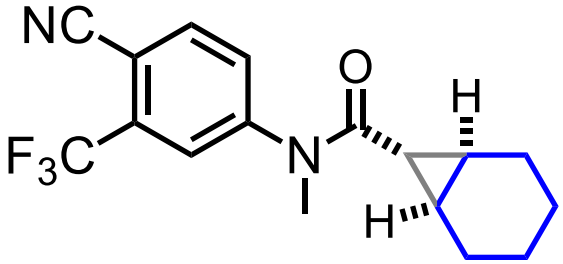

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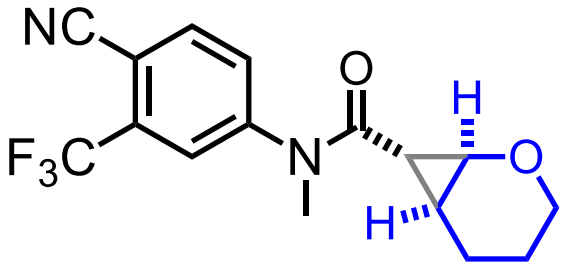
Round 2



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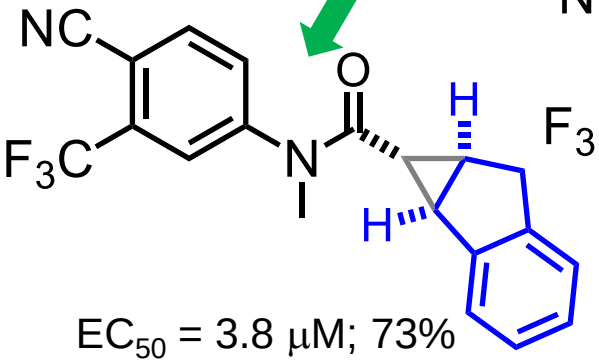

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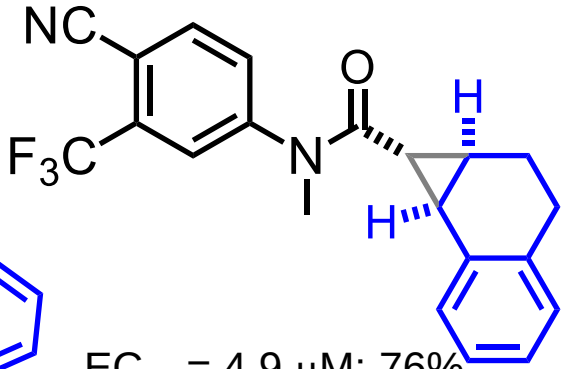

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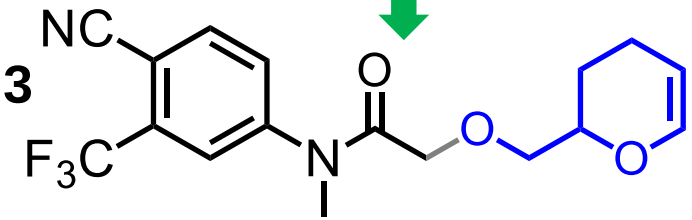
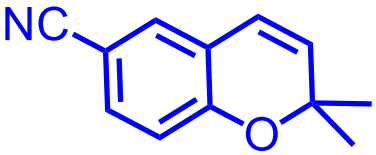
Round 3



EC_{50} = 860 nM (for >98% ee sample)

73% (55% ee)

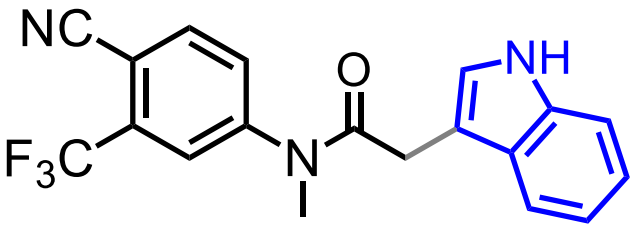


[with $Rh_2(OAc)_4$]

Angew Chem Int Ed 2015, **54**, 13538

How did the ADSs develop?

Round 1



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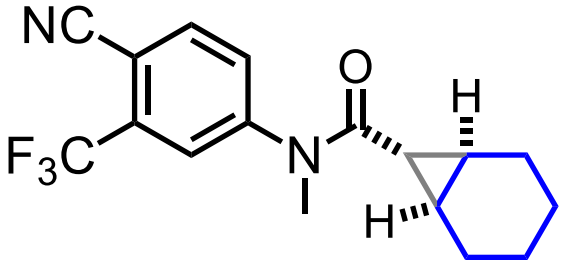
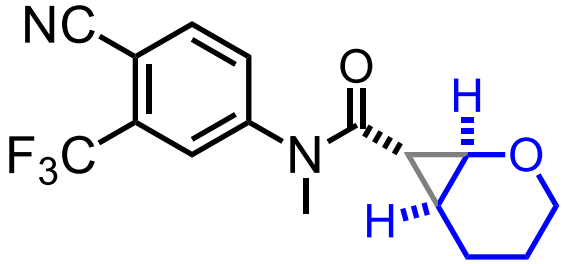


EC_{50} = 7.3 μM; 71%

Round 2



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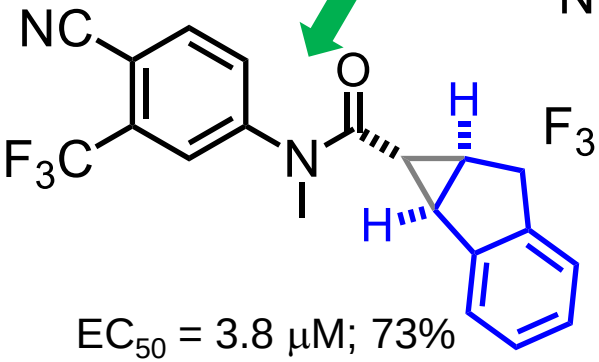

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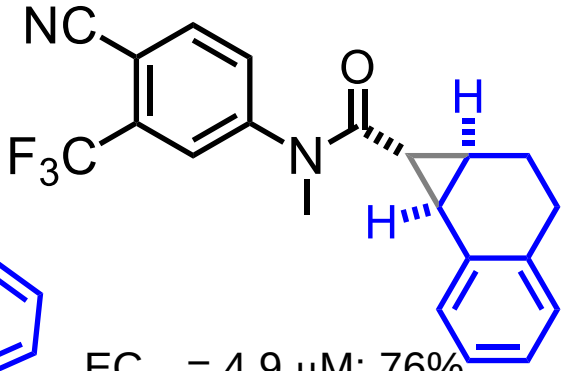
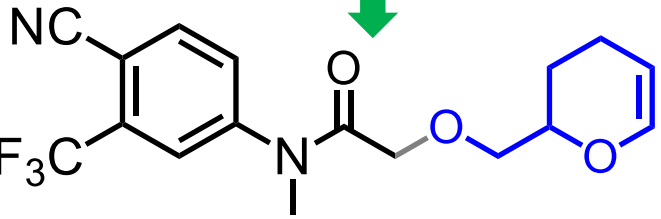


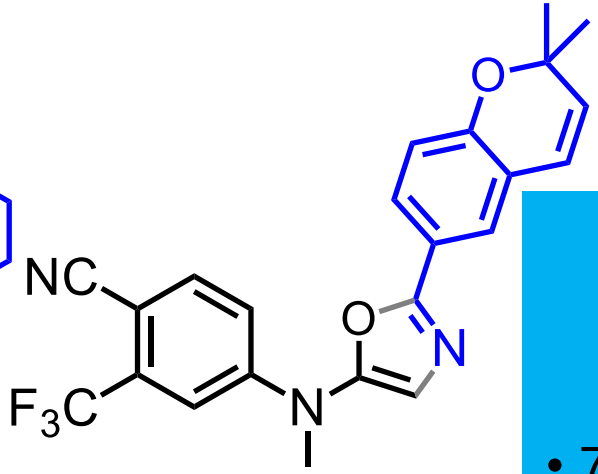
EC_{50} = 4.9 μM; 76%

Round 3



EC_{50} = 860 nM (for >98% ee sample)

73% (55% ee)



EC_{50} = 730 nM; 75%

In total,

- 4 substrates
- 326 reactions
- 7 products purified

Features of Activity-Directed Synthesis

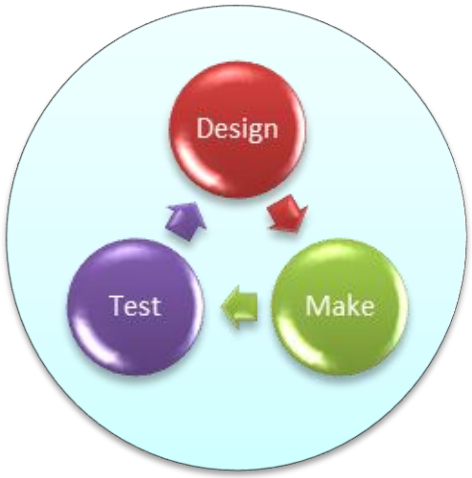
Feature	Comment
Chemical Diversity	• Very high – promiscuous reactions preferred! • Multiple chemotypes explored in parallel
Molecular Properties	Controllable if required
Focus of resources	Highly focused on actives
Nature of workflow	All stages integrated and parallel
Autonomy	Potential for fully autonomous discovery
Generality	• Only requirement is robust high-throughput assay • Can be exploited in different ways e.g. to elaborate fragments or to hop between scaffolds

Towards integrated autonomous bioactive molecular discovery?

Streamlining bioactive molecular discovery through integration and automation

Shiao Chow, Samuel Liver and Adam Nelson

Abstract | The discovery of bioactive small molecules is generally driven via iterative design–make–purify–test cycles. Automation is routinely harnessed at individual stages of these cycles to increase the productivity of drug discovery. Here, we describe recent progress to automate and integrate two or more adjacent stages within discovery workflows. Examples of such technologies include microfluidics, liquid-handling robotics and affinity-selection mass spectrometry. The value of integrated technologies is illustrated in the context of specific case studies in which modulators of targets, such as protein kinases, nuclear hormone receptors and protein–protein interactions, were discovered. We note that to maximize impact on the productivity of discovery, each of the integrated stages would need to have both high and matched throughput. We also consider the longer-term goal of realizing the fully autonomous discovery of bioactive small molecules through the integration and automation of all stages of discovery.



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Comparison of alternative approaches to generate molecular diversity

	Diversity-oriented synthesis	**Lead-oriented synthesis**	**Activity-directed synthesis**
Diversity	High	High within defined chemical space	**Very high**
Molecular properties	Low level of control	High level of control	**Controllable if required**
Reliability of reaction toolkit	High level required	High level required	**Ideally promiscuous reactions**
Resource focused on bioactives?	Equal focus on all compounds	Equal focus on all compounds	**Highly focused on bioactives**


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