Engineering Serendipity: Rapid Single-Iteration Hit Discovery using Plate Analogs in µSpaceM1

In just four weeks we: (1) designed a focused library of over 1000 compounds with Plate Analogs, (2) completed synthesis in a week, (3) confirmed hits using ASMS, (4) resynthesized the top hits, and further confirmed them in a secondary assay.

1. Challenge

Turning ideas into experimentally validated hits takes far too long.

2. Molecule.one's Approach

µSpaceM1: Weekly Synthesis of Highly Novel Compounds

We built µSpaceM1: the first generative, deep-learning chemical space trained on more than 300,000 experimentally observed reaction outcomes.
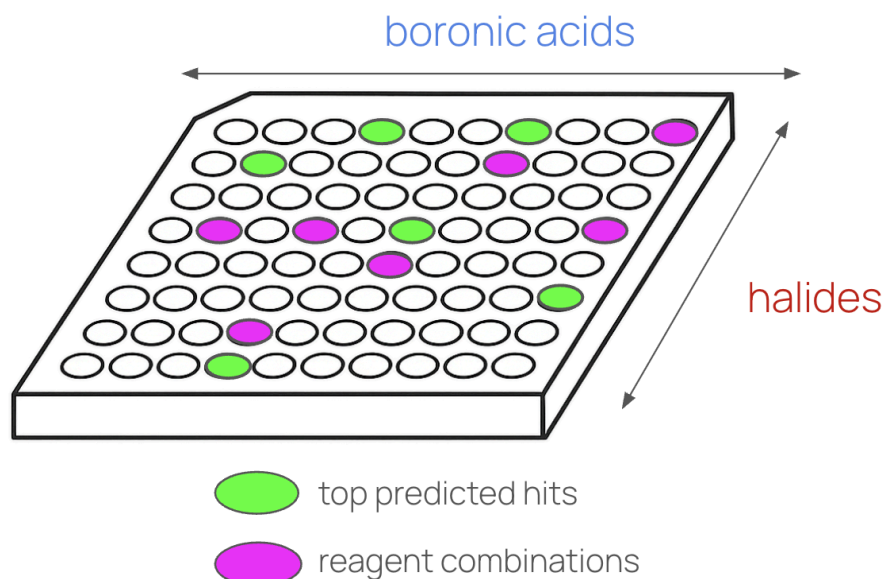
The main benefits are **speed** and **novelty**:

- We can operate at microliter scale, enabling synthesis of a 1000 membered library in direct-to-biology mode **in a week**.
- Our compounds are highly novel thanks to discovering nontrivial chemistry such as Suzuki couplings in DMSO. This significantly broadens the range of makeable chemistry beyond standard constraints.

Plate Analogs: Engineering Serendipity

Computational methods have limited predicted power for high novelty targets and ligands. To augment them, we add to the screening library a large number of structurally related compounds.

Consider the plate design shown below, where green circles mark computationally predicted top hits. Each column and row represent substrates used in their synthesis. We leverage the fact that those building blocks are ready for use and enrich the library with their novel combinations. We call those Plate Analogs.

We discovered and shown in two case studies (this included) that Plate Analogs turn out often to be the most active compounds.
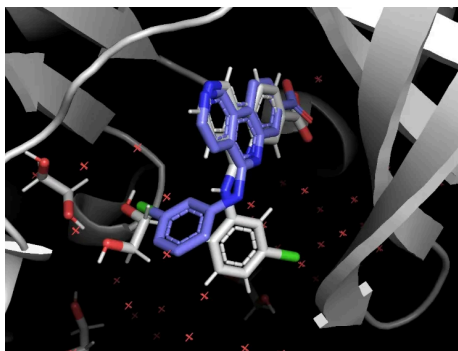

3. CK2

CK2 is a protein kinase implicated in cell survival and stress-response pathways, making it a relevant target in oncology and inflammation. The enzyme possesses a highly conserved ATP-binding pocket, which constitutes the main catalytic site.

4. Results

Library Design: Using Consensus Docking and Plate Analogs

For CK2, candidates were selected from µSpaceM1 using consensus docking (AutoDock Vina + Boltz2) combined with deliberate diversity sampling. Docking-based virtual screening yielded a set of virtual hits. For their synthesis, a defined set of building blocks (BBs) was required, from which additional BB-recombined analogs (Plate Analogs) were subsequently designed.

D2B Execution

The resulting grid was advanced directly into D2B plate synthesis in microliter scale, followed by minimal work-up. Out of 1,152 targeted reactions, 887 products were successfully formed, corresponding to a ~77% synthesis success rate, in close agreement with ML-predicted feasibility.

ASMS Screening

When exploring large compound collections, early screening methods must balance throughput, cost, and sensitivity. Affinity-selection mass spectrometry (ASMS) is well suited for this purpose, enabling direct detection of protein–ligand interactions without labeling or compound purification. Importantly, ASMS aligns well with D2B workflows, as it supports screening of pooled compounds directly from crude reaction mixtures. Accordingly, the D2B product set was first evaluated using ASMS. 10 CK2 binders were identified by ASMS, corresponding to a hit rate of ~1.1%, exceeding typical ASMS benchmarks. Notably, 90% of the ASMS hits were BB-recombined analogs generated by reusing building blocks originally selected for the virtual hit set, with 8 out of 10 being novel structures.
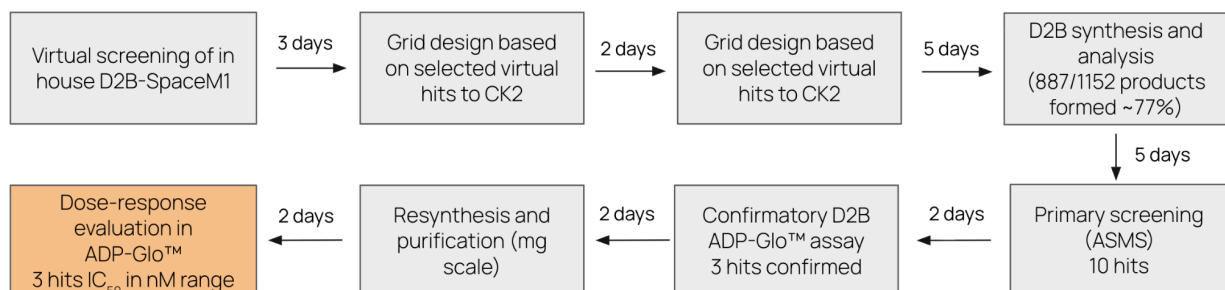
Confirmatory assay

The orthogonal biochemical ADP-Glo™ luminescent kinase assay was used to confirm the activity of selected hits. Ten compounds were initially tested at a single concentration using D2B crude reaction mixtures with Silmitasertib as positive

control. 3 out of 10 ASMS hits exhibited inhibitory activity as D2B post reaction crudes activity and were therefore resynthesized and purified at milligram scale. Dose–response evaluation was subsequently done, revealing one compound with an IC_{50} of approximately 200 nM and two compounds with IC_{50} values below 80 nM. Exact potency values will be determined upon retesting at lower concentrations; nevertheless, the most potent compounds reduced enzyme activity to below 5% at an 80 nM concentration and one structure remains highly novel.

5. Summary

We observed that close analogs often achieve higher potency than the originally designed molecules. This effect reflects what can be described as engineered serendipity: while computational methods are inherently approximate, systematic analog exploration—enabled by low-cost, plate-scale synthesis—provides tolerance to design error and increases the probability of discovering high-quality hits. As a result, this approach is particularly well suited for challenging or poorly characterized targets.

Collectively, the data show that our chemistry—diverse, feasibility-anchored SpaceM1 and rapid HTE execution—streamlines DMTA cycles and cuts resource use in early discovery allowing for hit identification **within 4 weeks, while requiring minimal FTE involvement.**