pharmAI

Off-Target Identification

High Accuracy Prediction of MAPK14 Off-Targets – a case study

Dr. Joachim Haupt (CEO), Dr. Florian Kaiser (CTO)
PharmAI GmbH, Dresden, Germany
in cooperation with
2bind GmbH, Regensburg, Germany

Get to Know Your Compounds

pharmAI

Introduction

During the discovery and development of new drugs, candidates with undesired and potentially harmful side-effects can arise at all stages, which poses significant scientific and economic risks. Most of such phenotypic side-effects can be attributed to binding of the drug candidate to unintended proteins, so-called off-targets. The early identification of potential off-targets is therefore of utmost importance to mitigate any downstream risks.

To demonstrate the validity and usability of our *DiscoveryEngine* as an off-target identifier, PharmAI and 2bind jointly applied a combination of knowledge-based in silico off-target screening and state-of-the-art biophysics to rapidly identify off-targets for MAPK 14 inhibitor in a showcase study.

Out of 13 predicted off-targets, six were confirmed to interact with the inhibitor in vitro, which translates to an **exceptional hit rate of 46%.**

PharmAI's off-target identification is a unique service with exceptionally high hit rate.

Off-Target Services

A typical off-target screening project can be completed within 7 weeks, excluding protein sourcing and compound procurement. The synergistic benefits of latest AI technology with MicroScale Thermophoresis (MST) from 2bind, allows to launch *in silico* and *in vitro* pipelines instantaneously and in parallel upon knowledge of the target.

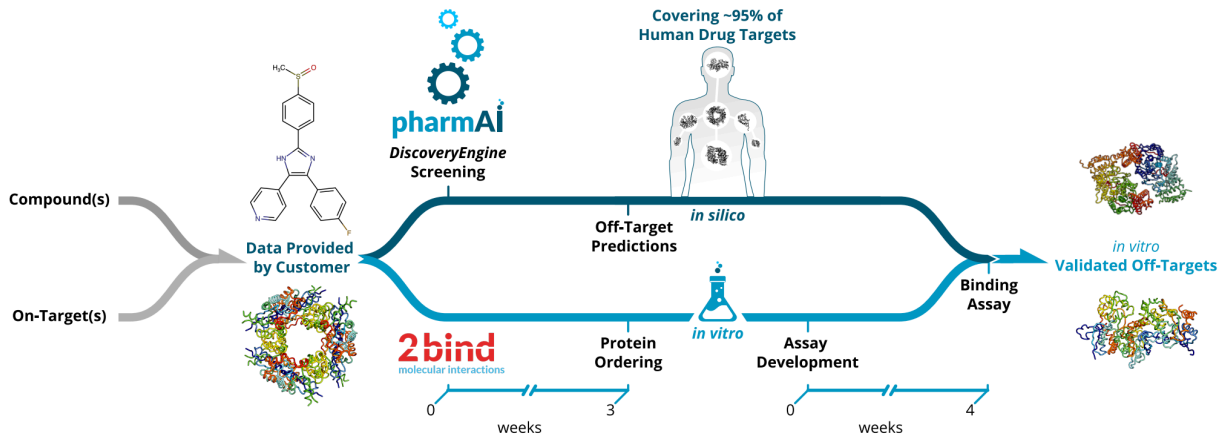

Figure 1: Workflow of the PharmAI-2bind Off-Target Identification Service. Upon the definition of the compound(s) and/or on-targets(s) of interest, *in silico* and *in vitro* pipelines are launched in parallel. The PharmAI *DiscoveryEngine* rapidly predicts potential off-targets, covering 95% of the known human drug targets. Predicted proteins are then purchased and assay development starts as soon as the *in silico* data is ready.

Version 2020.1

Showcase: MAPK14 Off-Target Screening

Starting from the MAPK14 inhibitor SB203580, we applied the PharmAI *DiscoveryEngine* and identified 167 proteins as potential off-targets for SB203580. Out of these proteins, 56 were already known to bind SB203580 *in vitro*. From the remaining 111 unknown targets, the top 13 which were commercially available were purchased in small quantities for biophysical testing.
SB203580 showed binding to six of these proteins in the 2bind Off-Target MST assay, translating to an exceptional hit rate of 46% (see Table 1).

	Validated Off-Target	Gene	UniProt AC	K_D	Sequence-Identity to MAPK14	Closest Binder (% Similarity)
	Serine/threonine-protein kinase/endoribonuclease IRE1 *endoplasmatic reticulum stress sensor* [24]	ERN1	O75460	2.05±0.52 μM	8.30%	 CHEMBL3660376 (36%)
	Hematopoietic prostaglandin D synthase *production of prostanoids in the immune system* [25]	HPGDS	O60760	30.87±4.54 μM	9.61%	 CHEMBL442461 (41%)
	Glutamate receptor 2 *excitatory synaptic transmission* [26]	GRIA2	P42262	>500 μM	6.76%	 CHEMBL3799697 (31%)
	Nicotinamide/nicotinic acid mononucleotide adenylyltransferase 1 *part of NAD biosynthesis* [27]	NMNAT1	Q9HAN9	>500 μM	8.59%	n/a
	Fatty acid-binding protein 4 *lipid transport protein* [28]	FABP4	P15090	>500 μM	6.65%	 CHEMBL248145 (42%)
	Menin *scaffold protein and regulator of gene transcription* [29]	MEN1	O00255	>500 μM	8.71%	 CHEMBL3696205 (37%)

Table 2: Validated off-targets for the kinase inhibitor SB203580. For each protein, the determined K_D to SB203580 and the sequence identity to MAPK14 is given. Additionally, the chemically most similar compound to SB203580 with reported activity [14] for each protein is shown.

The results clearly show that our methodology allows to fine-tune target selectivity right from the very beginning of the drug discovery pipeline, reducing the downstream risks and costs. Knowing the target classes can also shed light on whether the compound will be a specific binder. Furthermore, the comprehensive knowledge of potential binding partners besides the primary target can guide and aid molecular design in order to increase selectivity during the medicinal chemistry optimization.

Our showcase clearly demonstrates the synergy between highly accurate knowledge-based *in silico* off-target prediction with the PharmAI *DiscoveryEngine* and fast and ultra-low sample consumption biophysical testing with the 2bind Off-Target MST assay. Our combined efforts efficiently identify and validate off-targets at large scale.

Detailed Case Study Publication

Kaiser, F., Plach, M.G., Leberecht, C., Schubert, T. and Haupt, V.J. (2020). *In Silico Driven Prediction of MAPK14 Off-Targets Reveals Unrelated Proteins with High Accuracy.* bioRxiV, doi:10.1101/2020.07.24.219071 https://www.biorxiv.org/content/10.1101/2020.07.24.219071v1

References

[1] Plach, M.G., Kaiser, F., Schubert, T. and Haupt, V.J. (2020). *Focus Your Screening Library: Rapid Identification of Novel PDE2 Inhibitors with in silico Driven Library Prioritization and MicroScale Thermophoresis*. bioRxiV, doi:10.1101/2020.04.22.021360

[2] Salentin, S, Schreiber, S, Haupt, VJ, Adasme, MF, Schroeder, M (2015). *PLIP: fully automated protein-ligand interaction profiler*. Nucleic Acids Res., 43, W1:W443-7.

[3] Haupt, VJ, Daminelli, S, Schroeder, M (2013). *Drug Promiscuity in PDB: Protein Binding Site Similarity Is Key*. PLoS ONE, 8, 6:e65894.

[4] Kaiser, F., Plach, M.G., Leberecht, C., Schubert, T. and Haupt, V.J. (2020). In Silico Driven Prediction of MAPK14 Off-Targets Reveals Unrelated Proteins with High Accuracy, bioRxiV, doi: https://doi.org/10.1101/2020.07.24.219071

The Chemical Universe at Your Hands.

pharmAI

Interested?

Get in touch! We are eager to know how we can help you.

hello@pharm.ai

PharmAI GmbH
Tatzberg 47 | Dresden
www.pharm.ai

pharmAI

Geschäftsführer: Dr. Joachim Haupt
Amtsgericht Dresden, HRB 39032
Umsatzsteuer-Identifikationsnummer: DE325663575