
PhenQSAR: A Cell Painting-Based Platform for Gene-Compound Similarity and Drug Repurposing

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Abstract

PhenQSAR is a computational and web-based platform that leverages high-throughput Cell Painting morphological profiling to map gene-compound phenotypic similarity at scale. Using profiles from the JUMP Cell Painting (JUMP-CP) resource (115,000+ compounds; 8,000+ genetic perturbations), PhenQSAR identifies compounds that phenocopy (mimic) or phenopose (oppose) CRISPR knockouts or ORF overexpression, prioritizing candidates for repurposing and lead optimization. In a case study, PhenQSAR ranks moclobemide, a reversible MAO-A inhibitor antidepressant, as a morphological phenocopy of OPRL1 (NOP receptor) CRISPR perturbation, suggesting a potential hypothesis for opioid use disorder (OUD) treatment that aligns with historical IP, case reports, and pharmacology. Consistent with this signal, a 2002 case report described sustained (2-year) opiate abstinence and marked ADHD and functional improvements in a 27-year-old patient treated with moclobemide. Overall, PhenQSAR provides an interpretable, mechanism-suggestive route to drug repurposing. The code and website are available at <https://github.com/phenqsar/> and <https://phenqsarapp.streamlit.app>.

1 Introduction

Traditional drug repurposing methods are fundamentally constrained by pre-existing knowledge of a drug’s mechanism of action [Krishnamurthy et al., 2022] and have relied primarily on literature mining, clinical observation, and molecular target-based analyses [Kulkarni et al., 2023]. Several well-known drugs have been successfully repurposed for new therapeutic uses. For example, Thalidomide, originally a sedative for morning sickness, is now approved to treat leprosy and multiple myeloma. Sildenafil, first developed for hypertension, was repositioned for erectile dysfunction and pulmonary hypertension. Similarly, Minoxidil, an antihypertensive, is now an approved treatment for hair loss (alopecia), and AZT (Zidovudine), once studied for chemotherapy, became a foundational approved therapy for HIV/AIDS [Kulkarni et al., 2023].

Target-based approaches, which screen for compounds interacting with a specific protein, are inherently limited in discovering drugs acting through novel targets, engaging in polypharmacology, or indirect pathway modulation [Park, 2019]. Phenotypic screening offers a target-agnostic alternative

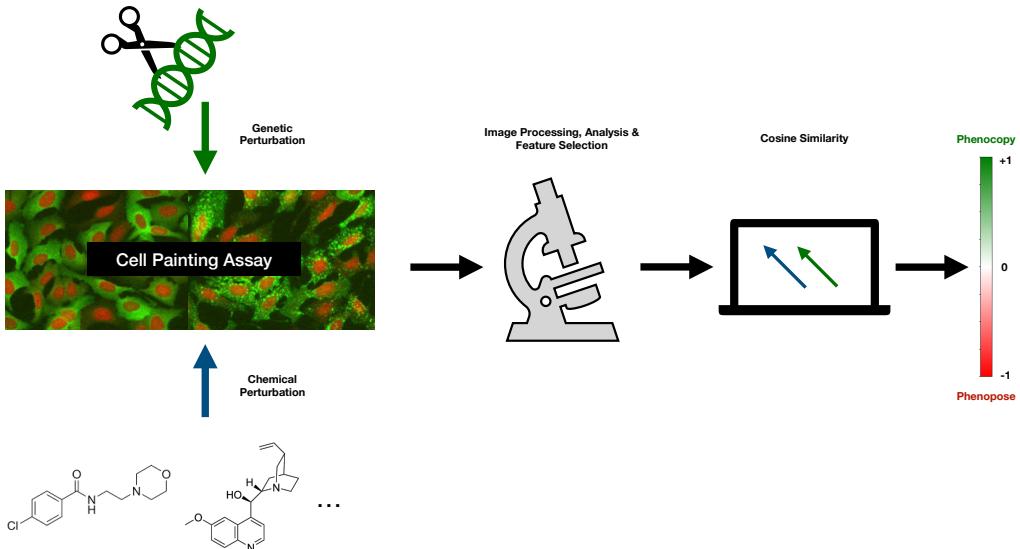


Figure 1: The PhenQSAR workflow. Representations of Morphological profiles from genetic and chemical perturbations in a Cell Painting assay are compared using cosine similarity. This comparison identifies compounds that phenocopy (mimic the genetic effect, score close to +1) or phenopose (oppose the effect, score close to -1).

that circumvents these limitations by prioritizing a desired functional outcome. Cell Painting represents a new approach methodology in unbiased phenotypic profiling, providing a standardized, high-throughput method for capturing cellular morphological changes in response to chemical and genetic perturbations. This fluorescence microscopy-based assay employs six dyes to simultaneously visualize eight cellular compartments: nuclei, endoplasmic reticulum, mitochondria, Golgi apparatus, F-actin cytoskeleton, and plasma membrane [Cimini et al., 2022]. Machine learning algorithms extract thousands of morphological features from these images, creating rich phenotypic signatures that reflect the biological state of cells [Cimini et al., 2022, Pearson et al., 2022, Herman et al., 2023].

Profiling in the Cell Painting can detect subtle morphological changes that correlate with biological activity, often revealing relationships between compounds and biological processes [Seal et al., 2025]. Image-based morphological profiling, specifically the Cell Painting assay, leverages the visual complexity of the cell to capture a holistic, high-dimensional fingerprint of its physiological state, detecting phenotypic signatures that are less expensive and higher-throughput than other high-dimensional profiling methods [Seal et al., 2025, Chandrasekaran et al., 2021]. This approach, supported by open-source analysis software, enables the elucidation of unexpected mechanisms of action [Verma et al., 2024].

In this work we aimed to determine which compounds induce morphological phenotypes similar (or opposite) to the phenotypes induced by genetic perturbations. Our hypothesis is that these compounds may modulate the same biological pathways or targets [Rohban et al., 2021]. This pheno-copying (or pheno-opposing) concept suggests that mechanistic similarity manifests through comparable representations of morphological profiles, functional redundancy produces comparable phenotypic changes across different perturbations affecting the same biological process, and therapeutic potential emerges when compounds mimic disease-relevant genetic perturbations.

A recently released resource, the JUMP-CP dataset, comprising profiles for over 115,000 compounds and 8,000 genetic perturbations, represents the largest publicly available resource for morphological profiling [Chandrasekaran et al., 2023]. This study uses the JUMP-CP dataset to develop PhenQSAR as a computational platform for identifying gene-compound morphological similarities, demonstrate the platform's utility using a OPRL1/moclobemide case study, and establish methodological frameworks for prioritizing repurposing candidates.

2 Methods

We analyzed JUMP-CP morphological profiles spanning 115,000+ compounds and 8,000+ genetic perturbations (CRISPR knockouts and ORF overexpression) across primarily U2OS cells [Chandrasekaran et al., 2023]. Imaging used the standard Cell Painting protocol, and data were aggregated at the well and perturbation levels. Images were processed via CellProfiler pipelines (illumination correction; nucleus/cell segmentation; feature measurement) [Cimini et al., 2022, Stirling et al., 2021]. We built consensus signatures by median aggregation per compound. Compound chemical structures were curated and standardized using RDKit (Landrum 2013). SMILES strings were canonicalized, and compounds with molecular weight >1000 Da or containing reactive functional groups were excluded to focus on drug-like molecules. An overview of the PhenQSAR experimental workflow is provided in Figure 1.

Morphological similarity between genetic perturbations and compounds was quantified using cosine similarity, chosen for its robustness to feature scaling and interpretability:

$$Sim(g, c) = \frac{v_g \cdot v_c}{\|v_g\| \|v_c\|}$$

where v_g and v_c represent L2-normalized morphological feature vectors for genetic perturbation g and compound c , respectively. The cosine similarity values range from -1 (perfectly inverse or antagonistic profiles) to 1 (perfectly similar), with 0 indicating orthogonal (uncorrelated) profiles.

PhenQSAR was implemented as a web-based platform providing an intuitive interface to explore gene-compound similarities. Key platform features include searchable gene selection from available genetic perturbations, molecular structure visualization using RDKit and chemical structure databases, and downloadable results tables with statistical annotations. PhenQSAR successfully processed the complete JUMP Cell Painting dataset, enabling similarity analysis between 8,000+ genetic perturbations and 115,000+ compounds.

3 Case Study: OPRL1 and Moclobemide

To validate PhenQSAR's predictive capability, a detailed analysis was conducted of the nociceptin/orphanin FQ peptide receptor (OPRL1) and its top-ranking compound matches. The analysis revealed over 105 compounds with cosine similarity >0.30 (and moclobemide was found with a cosine similarity of **0.314**).

Moclobemide is characterized as a reversible MAO-A inhibitor (RIMA) with established use as an antidepressant approved in 50+ countries [noa]. Moclobemide's primary action is as a selective, reversible inhibitor of monoamine oxidase A (MAO-A), an enzyme responsible for the breakdown of neurotransmitters such as serotonin, norepinephrine, and dopamine [Alia-Klein et al., 2008]. Moclobemide is also established to act on the serotonin system [PubChem, c] (5-HT1A receptor), which is known for addressing mood dysregulation and neural plasticity deficits commonly associated with OUD. Moclobemide is active (5uM) against MAO(A) and 5-HT1A, per PubChem [PubChem, c]. Also, it shows activity against D3 dopamine receptors[PubChem, b], adrenergic receptors (AR) [PubChem, e], estrogen receptors (ER) [PubChem, d], and CYP2D6 [PubChem, a].

Examination of Cell Painting profiles revealed that both OPRL1 CRISPR perturbation and moclobemide treatment induced mitochondrial redistribution away from the cell periphery, altered cytoskeletal organization, modified endoplasmic reticulum morphology, and changes in nuclear texture features (Figure 2). These phenotypes have been described as typical in psychosis and unusual in depression and modulated by sufentanil [Cataldo et al., 2010]. We found that, as a class, serotonin receptor antagonists impact this mitochondrial redistribution. Together, this suggests a connection between the cell-based phenotypes in Cell Painting and human clinical impact. This morphological similarity aligns with prior clinical evidence suggesting moclobemide's potential in opioid use disorder treatment, where OPRL1 represents a therapeutic target.

In 1999, a method-of-use patent application (WO2000006139A2) proposed the use of moclobemide for substance abuse and withdrawal treatment [Klein and Lederman, 2000]. To our knowledge, no formal clinical trials occurred for substance abuse/withdrawal, potentially for entirely business reasons [Klein and Lederman, 2002]. In 2002, a publication described the 'successful treatment' of a 27-year-old patient with comorbid opiate addiction and residual-type ADHD; moclobemide was prescribed

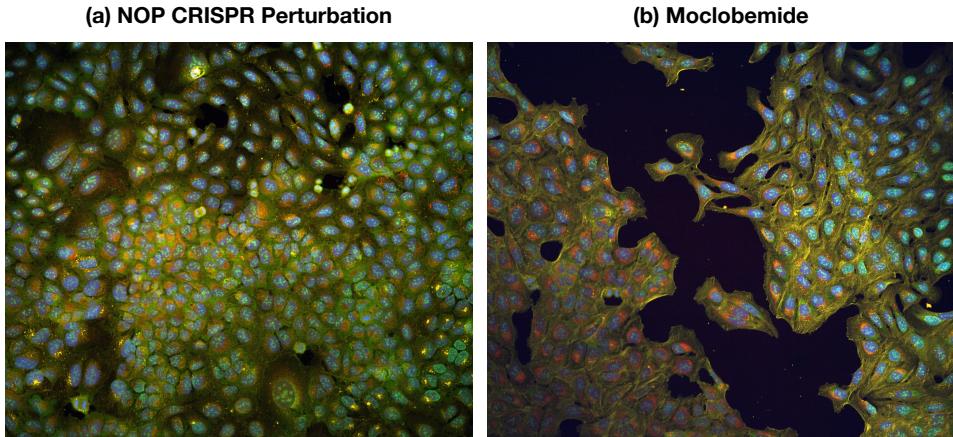


Figure 2: Representative morphological profiles from the Cell Painting assay for cells treated (a) with NOP CRISPR perturbation and (b) with moclobemide. The images showcase the morphological phenotypes in cellular structures, including nuclei, cytoplasm, and organelles induced by the compound and NOP perturbation.

after heroin cessation and symptom management with clonidine [Vaiva et al., 2002]. The patient's significant improvements in ADHD symptoms, work performance, and social interactions were thought to have prevented a previous pattern of substance use relapse, which had been characterized by impulsivity and inattention in the past. The patient remained opiate-use-free over two years of follow-up.

Moclobemide has a well-documented safety profile and global availability, making it a compelling candidate for repurposing. Overall, this case-study demonstrates the PhenQSAR platform's ability to predict therapeutically relevant relationships that are subsequently partially supported by independent clinical evidence.

4 Limitations and Considerations

Several limitations warrant consideration in the interpretation and application of PhenQSAR results. Morphological profiles are cell line-dependent, potentially limiting generalizability across tissue types, indicating cell type specificity. Similarity patterns vary with compound concentration, requiring careful consideration of dosing relationships and concentration dependencies. Morphological similarities may reflect downstream consequences rather than direct target interactions, suggesting potential for indirect effects. Random similarities may occur, necessitating orthogonal validation approaches to address false positives.

5 Conclusions

This study establishes PhenQSAR as a platform for drug repurposing through gene-compound morphological similarity analysis. The platform identifies biologically relevant compound-gene relationships across a large-scale Cell Painting dataset, demonstrating platform validation. The moclobemide/OPRL1 case study demonstrates the platform's ability to predict relevant relationships, partially supported by previous publications.

Identification of drug-like molecules for new indications addresses unmet clinical needs in areas that create therapeutic opportunities. The study establishes standardized approaches for morphological similarity analysis that can be applied to other datasets and disease areas, representing methodological

advancement. The convergence of high-throughput imaging, machine learning, and large-scale biological datasets creates many opportunities for understanding drug action and identifying new therapeutic applications. As phenotypic profiling datasets continue to expand, platforms like PhenQSAR will play increasingly important roles in modern drug discovery and development.

Data and Code Availability

JUMP-CP data are publicly available per the cited publications. PhenQSAR code, processed matrices to reproduce figures/tables will be released under an open license at the project repository <https://github.com/phenqsar/>

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