Off-target mapping enhances selectivity of machine learning-predicted CK2 inhibitors

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A key challenge in drug development is identification of druggable targets, the modulation of which attenuates disease progression, while avoiding inhibition of proteins that lead to dose-limiting toxicities. Here, we investigate a drug target Casein kinase 2 (CK2) - a serine/threonine kinase implicated in cancer, for which existing molecules have so far failed clinical trials. Using molecular and pharmacoepidemiology approaches, we show that molecules targeting CDK kinase family members CDK1/2/7/9 - such as the existing CK2 inhibitors - have a higher risk to induce adverse effects or fail in clinical trials. Based on this finding, we establish a machine learning assisted pipeline to redesign more specific and allosteric lead compounds against CK2, with more selective on-target binding and favourable off-target profile. Importantly, we show that such design is possible via machine learning powered, docking assisted discovery pipeline, when standard ML algorithms were combined with an error prediction model. In conclusion, our study reports a simple yet efficient machine learning-powered drug discovery pipeline and novel submicromolar CK2 inhibitors targeted. Importantly, our prediction pipeline was able to achieve a 90% hit-rate, significantly reducing the need for subsequent wet-lab validation.

Small-molecule bioactivity prediction | Kinase inhibitor discovery | Off-target mapping | Cheminformatics

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Introduction

In drug discovery, off-targets refer to proteins whose unintended engagement by a therapeutic agent produces adverse or dose-limiting effects, without contributing to the desired therapeutic efficacy. Unlike primary or secondary efficacy targets, off-targets are liabilities embedded within the proteome that constrain therapeutic selectivity. Well-recognized examples include the hERG potassium channel (KCNH2), the blockade of which leads to cardiac arrhythmias, and cytochrome P450 enzymes, where broad inhibition causes drug–drug interactions (Goldwaser et al. 2022; Zhang et al. 2014). Systematic mapping of such off-targets is becoming a cornerstone of modern pharmacology as available data on genetic dependencies and drug-induced adverse reactions become more available (Isigkeit et al. 2024; Ianevski et al. 2024; Tanoli et al. 2025). Increased off-target awareness enhances early screening cascades and guides medicinal chemistry efforts to reduce off-target binding, informing risk-benefit assessments (Schmidt et al. 2025).

In parallel with awareness of off-targets, drug molecules need to effectively bind proteins driving disease progression or resistance to achieve therapeutic benefit. Whether repurposing or new molecular design, this is increasingly being done in reliance on artificial intelligence (AI) or machine learning (ML) methods, which enable systematic exploration of the small molecular space, in contrast to classical chemical screens (Tibo et al. 2024). While AI/ML holds significant promise for addressing long standing drug discovery challenges, such as target prediction and activity profiling, many applications remain limited by data quality, model generalizability, and biological complexity. Within the kinase inhibitor space, AI/ML methods have begun to show potential for improving predictions of binding affinity and selectivity based on chemical structure and bioactivity data (Schulman et al. 2024; Liu et al. 2023; Cichońska et al. 2021; Theisen et al. 2024). However, selectivity modeling, particularly across closely related kinase families, remains an open challenge. More refined approaches, better datasets, and thoughtful, wider integration into drug discovery workflows are needed to realize the potential of ML in guiding kinase inhibitor design, with improved safety profiles via better off-target awareness.

In this work, we explore the landscape of off-targets across human cell lines and apply ML-driven strategies for the rational design of kinase inhibitors with enhanced selectivity profiles. In particular, we train a support vector machine-based, structurally assisted prediction pipeline, which is able to predict novel small molecules against CK2, a serine/threonine kinase relevant for both solid and hematological cancers (Pizzi et al. 2015; Bulanova et al. 2024). Currently, no clinically approved inhibitors for CK2 exist. We perform deep validation of two of the high-scoring lead molecules in their ability to selectively inhibit CK2 kinase in comparison to other kinases (their off-target profiles). Using both biochemical and cell-based assays of breast cancer origin, we show that our novel molecules effectively inhibit CK2 signalling pathways.

Results

Machine-learning assisted discovery pipeline for CK2 small-molecular inhibitors. Casein kinase 2 (CK2) is a heterotetrameric kinase complex and an attractive drug target as it has been shown to be essential (Pizzi et al. 2015) and over-expressed (Supplementary figure 1; details of the CK2 pan-cancer analysis under Extensive Supplementary Materials) for multiple prevalent malignancies. In addition, its inhibition has been shown to synergize with chemotherapies (Bulanova et al. 2024). To discover selective CK2 inhibitors, we built an ML-assisted pipeline that first performs site-independent affinity prediction to prioritize compounds, followed by site-specific molecular docking and experimental validation (Figure 1A).

In the first part of the ML pipeline, drug/target activity data against CK2 was collected from open databases (Figure 1B). As shown by multiple prior studies attempting to escalate model complexity, the prospective performance of AI/ML based modelling is dominated by factors such as dataset size and harmonicity (Ahlmann-Eltze et al. 2025). Because of this, we placed primary emphasis on optimizing the training dataset and undertook an attempt on systematic data-centric optimization including (i) curated and harmonized CK2 activity records across public sources, (ii) standardized structures and assay units/metadata, removed duplicates and reconciled conflicts, (iii) combined activity labels (IC_{50}, Ki, Kd and their combinations), and (iv) evaluated structural fingerprints alongside chemical-space coverage and similarity-aware splitting to quantify their effects on accuracy and stability. These analyses indicated that IC_{50} labels encoded with a 1024-bit standard fingerprint lead to the most reliable performance (Supplementary figure 2-4; details of the optimization under Extensive Supplementary Materials). Accordingly, we used 1,282 non-redundant IC_{50}-annotated compounds, represented by the standard fingerprint, for model development under a 3:1 train-test split.

We next assessed the training and test dataset distributions using small molecular descriptors, showing that both of the datasets encompass a large and diverse range of chemical space. Furthermore, the wide chemical space in the training dataset ensured the prediction model has a wide application domain, making it possible to achieve a high accuracy on unknown compounds (Figure 1C). In addition, we also included molecules with extreme values (e.g. Chembl4077554) outside of Lipinski-Egan-Verber's laws, as they could contribute to enhancing the model generalizability and accuracy (Cichońska et al. 2021).

To acquire a predictive machine-learning model for drug/target affinity prediction, we trained and tested multiple supervised machine learning models from different model families, including random forest (RF), artificial neural network (ANN), support vector machine (SVM), extreme gradient boosting (XGBoost) and gradient boosted decision tree (GBDT). We found that the SVM model had the best performance in terms of both Pearson and NRMSE. For the Spearman coefficient, the GBDT was the top performer, but its relative difference with the SVM model was rather small. In addition, we also ensembled the top 3 models (SVM, GBDT, XGBoost). However, no significant improvement was observed (Figure 1D), indicating that the top models make rather similar predictions. In previous studies, SVM has exhibited good performance in the virtual screening (Liang et al. 2020; Han et al. 2008; Jorissen and Gilson 2005), so we selected the SVM model for the subsequent prediction and virtual screening tasks.

In addition, to further assess the applicability domain of the bioactivity prediction model and enhance its prediction reliability, we established an error prediction model (EPM) following the SVM method and identical parameter settings. The EPM predicts the expected error of the bioactivity prediction model for each compound, allowing exclusion of samples with large predicted errors under different confidence levels (α values). Applying the EPM on top of the SVM model led to significant improvements in the correlation metrics (Supplementary Figure 5, details of the model under Extensive Supplementary materials) and reduced RMSE, demonstrating its potential to refine bioactivity predictions within the model's applicability domain.

Next, using our highest performing machine-learning model (SVM model combined with BPM and EPM), we predicted novel CK2 binders out of a large chemical library, sized 12 M small molecules, while filtering out molecules with poor bioavailability by applying a filtering score based on Lipinski's rule of five (see Methods). Out of these molecules, we selected the top 622 molecules (predicted IC_{50} 100 nM or lower) and continued with high-throughput docking predictions for the assessment of molecular affinities, binding modes and site-specificity.

Structure-based prioritisation of novel CK2 hit molecules. Although the SVM model performs well, the models trained merely on CK2 binding data are structurally unaware. Therefore, we next assessed the possible CK2 binding sites for the top 622 molecules predicted to have a high affinity towards the CK2 alpha subunit. Interestingly, CK2 alpha subunit hosts multiple binding pockets on its surface, enabling potential binding of our predicted small molecules (Figure 2A). Between the ATP site, the αD pocket, the CK2α/β interface, and the N-terminal tail, we focused our efforts on the αD pocket which has lower sequence and structural similarity to other kinases and high potential for allosteric modulation of kinase activity (Brear et al. 2020; Iegre et al. 2021). The CK2α/β interface and the N-terminal were not prioritized since they are shallow and partly solvent exposed, often depending on the β subunit, and having multiple previously reported ligands (Kufareva et al. 2019; Brear et al. 2020; Iegre et al. 2021). Guided by this rationale, we ran two complementary docking schemes: a bitopic protocol that searches for poses contacting the ATP region while extending into the αD pocket, and an αD-only protocol restricted to the αD cavity to capture purely allosteric binders (Brear et al. 2020; Iegre et al. 2021).

To enable high-throughput molecular docking, we took advantage of two previously published crystal structures for CK2alpha and CK2alpha' (Figure 2B-C). In both the cases

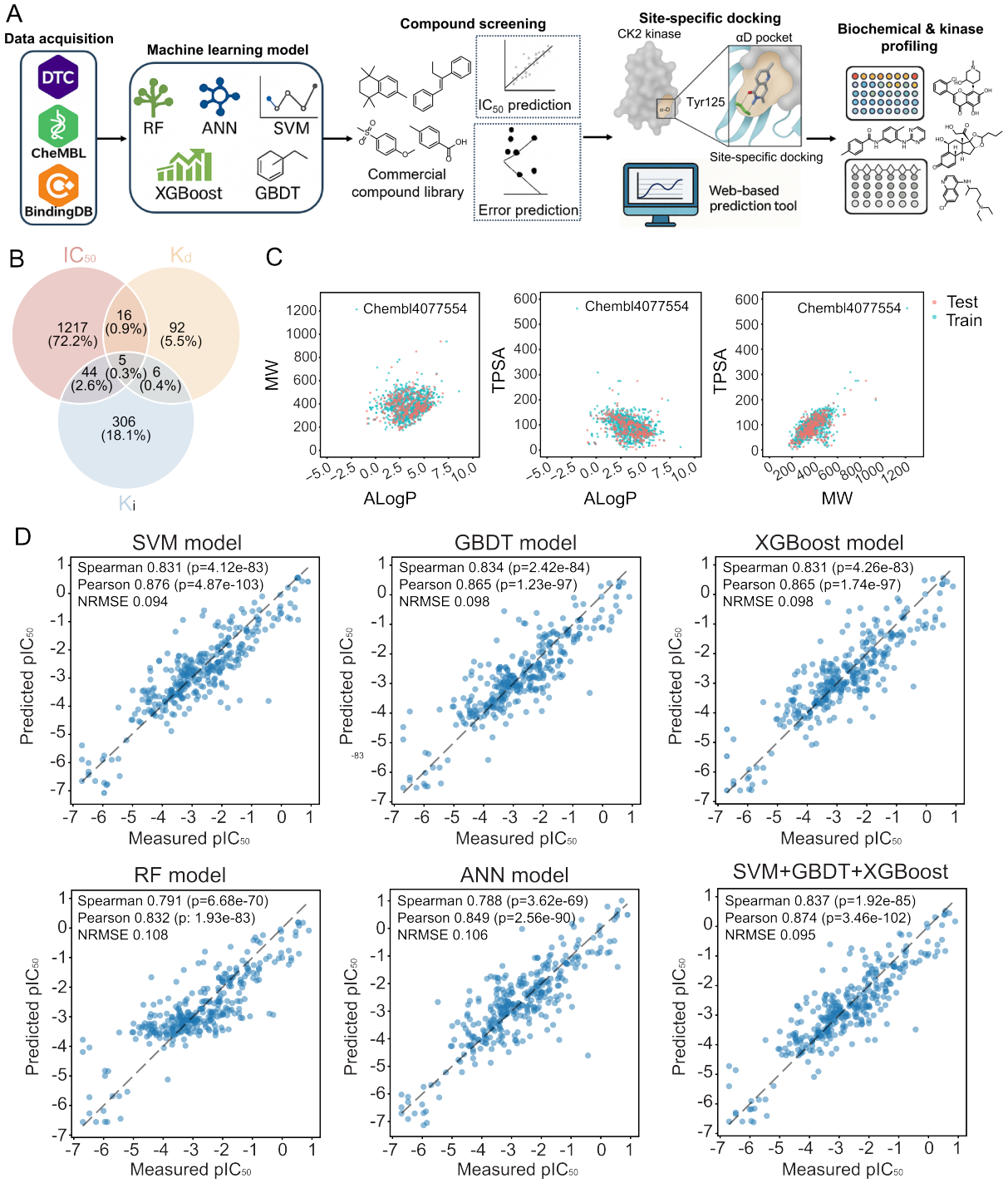


Fig. 1. Machine-learning assisted affinity-based prioritization to find lead CK2 inhibitors. (A) Schematic illustration of the computational prediction framework followed by experimental validation. (B) Venn diagram of compounds with available IC_{50}, Kd, Ki bioactivity data against CK2 kinase. The overlapping portions indicate the compounds with multiple bioactivity types. (C) Scatter plots of molecular properties in the training and test datasets, including MW-ALogP, TPSA-ALogP and TPSA-MW. (D) Scatter plots between the predicted pIC_{50} and measured pIC_{50} values in the test dataset using various ML models and their ensemble. pIC_{50}= -log10(IC_{50}).

(ATP-site + αD pocket or αD-pocket docking), we optimized the docking parameters using control compounds (CAM4066, DPA) in which the affinity and docking pose to these sites are known. We then ran the docking for the top-622 small-molecules towards CK2alpha or CK2alpha' and ranked the compounds based on their binding poses and binding affinity as predicted by autodock vina (Trott and Olson 2010). The top-12 compounds that satisfied one of the two predefined binding modes and showed more favorable docking scores than the control ligands (CAM4066 for CK2α and DPA for CK2α) were selected as the potential allosteric CK2 binders. Predicted binding affinities and IC_{50} values are summarized in Figure 2D, and representative bound conformations are shown in Figure 2E.

Four compounds (Comp1, Comp2, Comp9, and Comp10) bound within the αD pocket, having potential to allosterically inhibit CK2. The remaining eight (Comp3-8, Comp11, and Comp12) adopted bitopic poses that contact the ATP region and extend into the αD pocket. Magnified views of two representatives are provided in Figure 2F-G. Comp2 exemplifies the αD-only mode and forms a hydrogen-bonding network involving Tyr125 and nearby backbone atoms around Pro159, together with aromatic stacking against Met225. Comp3 illustrates the bitopic mode, bridging the ATP hinge region and the αD cavity, with hydrogen-bonding to Asn118, Pi-Pi contacts to Ile174, Val53, His160 and Phe121 and hydrophobic interactions with residues such as Tyr125.

As a conclusion, when using well-harmonized datasets, the SVM machine learning model with parallel error prediction can predict CK2 binders, which can be docked into both ATP or alternative binding pockets, with potential to reveal allosteric inhibitors.

CDK family kinases are essential off-target proteins. Our machine learning assisted discovery pipeline was able to pinpoint multiple small molecules with significant affinity and reasonable docking poses towards CK2. Next, to help selecting the most promising molecules and to maximise the therapeutic window of the lead compounds, we first sought to understand the most central off-targets in drug development. Although previous work has suggested frameworks for safety panel design (Schmidt et al. 2025), we performed a more systematic analysis of possible off-targets leveraging the DepMap resource - a cancer centric database that hosts data from whole-genome loss-of-function screens performed for over 1300 cancer cell lines. We hypothesized that genes crucial for overall cell viability and survival across the majority of cancer cell lines can be considered as potential off-targets, as their cellular function is likely preserved also in non-malignant cells.

We ranked all recorded genes based on their cell line dependence probability, displaying a right-skewed, heavy-tailed distribution, where 1696 of the tested genes (9.5%) were detected as highly dependent overall for cellular growth or survival (Figure 3A). Analysis of the 1696 most dependent genes across all cell lines identified a plethora of protein groups with a statistically significant overrepresentation. These included gene groups encoding essential machinery for ribosome, spliceosome, proteasome and RNA polymerase. Since the most likely off-targets for CK2 targeting molecules are other kinases, due to their structural similarity, we next limited our analysis to cellular kinases only. This analysis highlighted the CDK family of kinases as a key group of kinases necessary for cellular growth or survival. Interestingly, none of the existing safety pharmacology panels list CDK family members as toxicity-inducing targets (Schmidt et al. 2025).

To follow up on these findings, we next performed a more detailed genetic dependency analysis, focusing solely on the CDK family of kinases. Interestingly, cells showed variable responses to knockouts of different CDK family members. Here, cell line growth and survival depended on three kinases CDK1/7/9 with near absolute certainty, kinases CDK2/4/6/12 showing high dependency scores as well (Figure 3B). Since kinases are important targets for multiple small molecules (71 small-molecule kinase inhibitors FDA approved, additional 16 approved by other regulatory agencies) (Attwood et al. 2021), we next explored the safety profiles of clinically approved molecules with activity towards the highlighted CDK family.

More specifically, we assessed if CDK off-target activity of approved pharmaceuticals is associated with higher drug toxicity. To understand whether in vitro affinities would be associated with more severe side-effects, we first compiled drug safety information from SafetyVista platform (SafetyVista, Chemotargets SL, 2025. https://safety-vista.com/), establishing an extensive database of adverse reactions associated with clinically used pharmaceuticals. We then assessed whether a CDK off-target activity negatively impacted the safety profiles of already approved pharmaceuticals. For this we employed a post-marketing drug severity score (see Methods), which integrates the percentage of death-related reports and the disproportionality signals of the administered drugs. The severity score ranges from 0 (safer, lower-severity drugs) to 1 (more severe, risk-prone drugs). Our analysis revealed a trend in which off-target activity towards CDK1,2,7 and 9 was associated with more severe drug adverse effects among clinically used pharmaceuticals (Figure 3C). Interestingly, CDK4/6 which is the primary anti-cancer target for multiple malignancies did not show a similar activity-safety correlation.

After establishing the relationship between CDK targeting and increased drug severity score, we wanted to understand whether bioactivity towards any individual CDK family member would link to increased clinical attrition during clinical testing of potential drug candidates. Using the ChEMBL database, we established a dataset of both preclinical and clinical compounds with recorded bioactivity towards the CDK family of kinases (Figure 3D). To identify the CDK family members with the highest negative associations to clinical failure, we performed a univariate regression analysis for each CDK family member, evaluating the association between off-target kinase binding and clinical success (Figure 3E). Interestingly, activity of a molecule towards 6

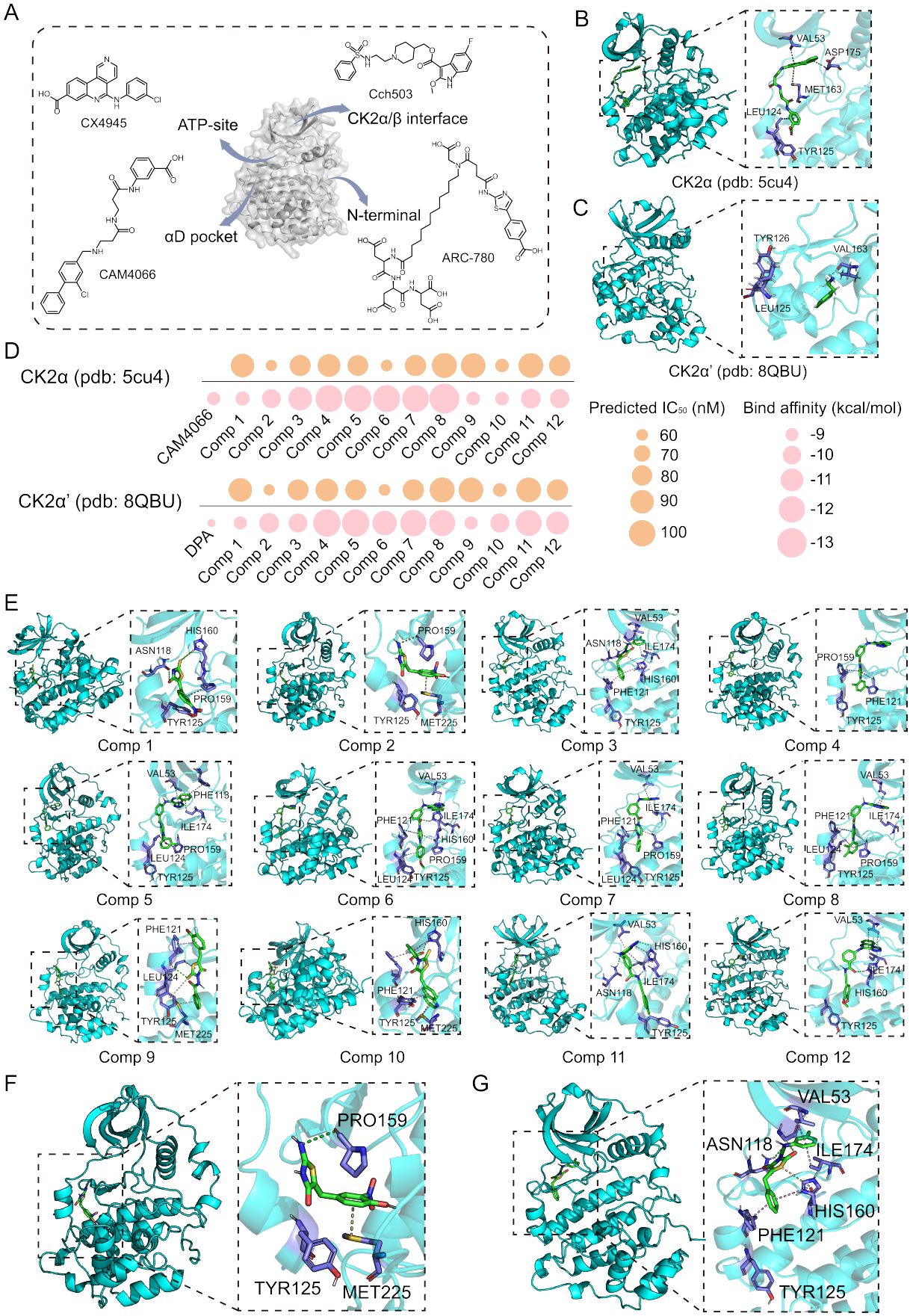


Fig. 2. Molecular-docking assisted structure-based prioritisation for lead CK2 inhibitor. (A) CK2 structure (CK2α unit as an example). Key binding sites and corresponding control compounds are shown (ATP-site/CX4945, αD pocket/CAM4066, CKα/β interface/Cch503, N-termimal/ARC-780). αD pocket as the main docking site to screen the potential inhibitors with two major binding modes with their control compound of (B) CAM4066 binds to both ATP-site and αD pocket; (C) DPA binds to αD pocket. (D) Dot plots illustrate the predicted IC_{50} and binding affinities of the top 12 screened compounds against the αD pocket in both CK2α and CK2α. All selected compounds exhibit stronger binding (i.e., lower affinity values) compared to the control compound (CAM4066: -9.1 kcal/mol; DPA: -5.5 kcal/mol). (E) Conformation of the screened 12 protein-compound structures. (F-G) Magnification of two of the most promising docking poses, compounds 2 and 3.

out of the 20 CDK kinases had a statistically significant negative association with clinical trial success, suggesting CDK off-target activity towards these molecules contributes to clinical attrition of small molecules during clinical trials. CDK1/2 were among the most statistically significant molecular targets associated with clinical attrition (Figure 3D). Out of the CDK family, the only kinase showing slight positive association was CDK6, a known drug target for multiple cancers with many clinically approved molecules binding CDK4/6 as their primary target. CK2 kinase inhibitor development is one example where molecules targeting the primary target (CK2) have significant off-target effects via the CDK family of kinases and the previous inhibitors have failed in clinical trials (Figure 3F). To gain mechanistic insight how existing CK2 inhibitors cause toxicities, we integrated data from chemical and toxicity databases, linking small molecular inhibitors with toxicities via their reported CDK family bioactivities (Supplementary figure XX). This analysis flagged many CDKs, CDK1/2/7 in particular, as relevant off-targets to be considered in preclinical drug discovery, especially since currently used safety pharmacology panels, Safety-77 (Brennan et al. 2024) and Safety-44 (Bowes et al. 2012) do not include any CDK kinases in their recommended off-target list (Schmidt et al. 2025).

Based on these results, we argue that previous drug development processes have not adequately steered away from targeting the CDK family of kinases, increasing clinical failure rates. To enable CK2 inhibitor development with enhanced safety profiles and less CDK binding, we next focused on a more refined molecule selection, taking into account both efficacy and toxicity, and aiming for more balanced therapeutic profiles of the molecules targeting CK2.

Mechanistic assessment of the novel CK2 inhibitors. Next, we sought to experimentally test the performance of the top predicted molecules (12 hits, 2 positive control molecules and 2 negative molecules). To assess IC_{50} values for each of the molecules, we set up an in vitro biochemical assay to measure the efficacy of the predicted molecules. This plate-based assay records the amount of ATP used by the kinase, while different compounds can be titered in (see Methods). Importantly, 11 of the 12 molecules (discovery success rate >90%) showed a clear inhibition of the target kinase, whereas negative controls did not cause inhibition (Fig. 4A). Of note, although the positive control molecule CX4945 was more efficient in inhibiting CK2 kinase in this reductionist assay, it has so far failed clinical trials, potentially due to the off target effects and CDK binding. As shown in Supplementary Table S3, CX-4945 displays measurable binding affinity toward multiple CDK family members, including CDK1 (IC_{50} = 56 nM, Kd = 3035 nM), CDK2 (IC_{50} = 1800 nM), CDK5 (Kd = 514 nM), and CDK16 (Kd = 1208 nM). Such broad cross-reactivity across cell-cycle kinases suggests that its cellular effects may not arise solely from CK2 inhibition but also from off-target CDK blockade, which could contribute to the toxicity and limited clinical tolerability observed for CX-4945. Since molecules 2 and 3 showed promising inhibition of CK2 (IC_{50} = 156.2 nM and 198.6 nM), we next assessed their off-target binding to other kinases.

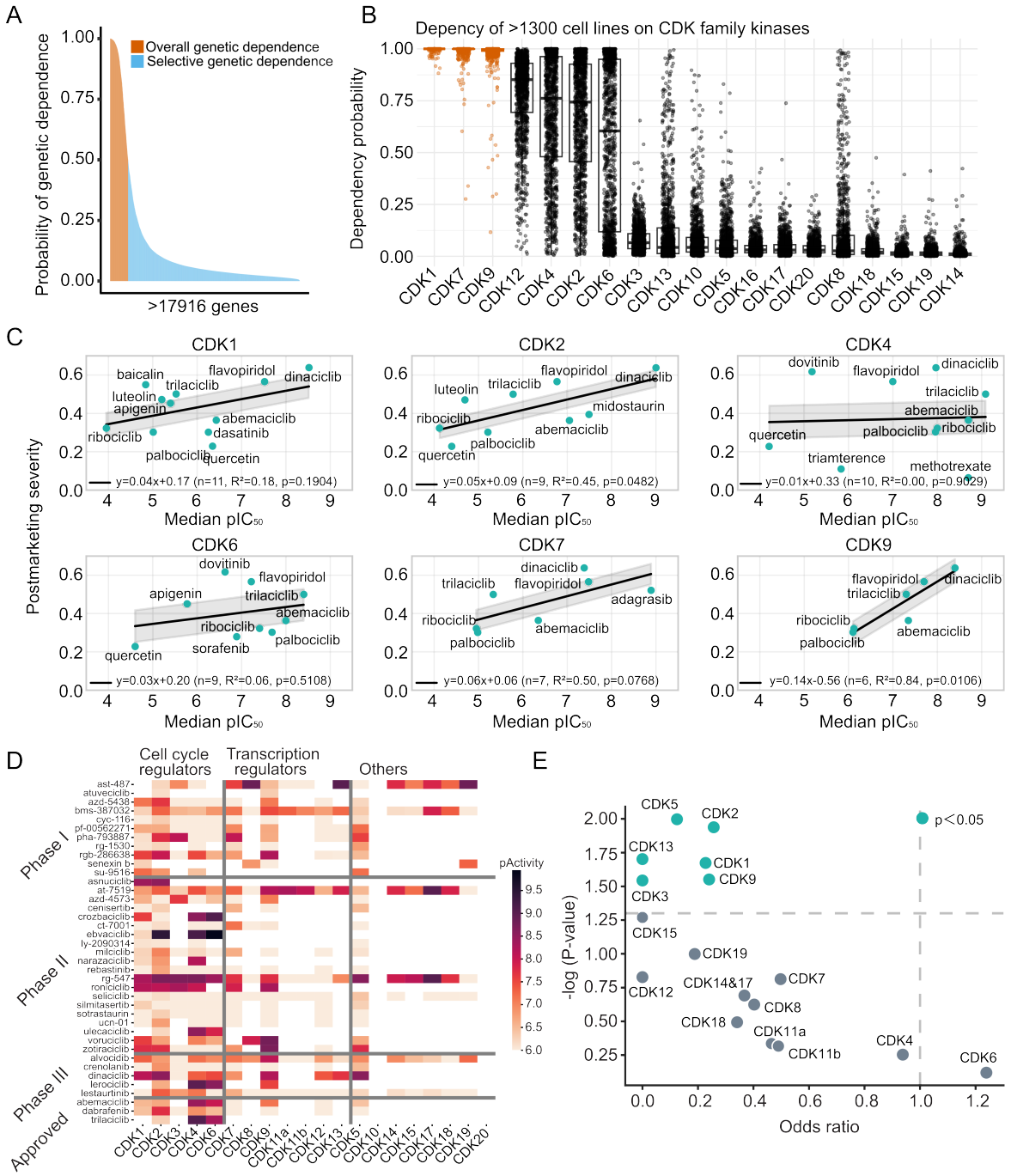
To anticipate potential safety liabilities of our top CK2 inhibitors, we tested the bioactivity of compounds2 and 3 against a panel of 216 kinases. Importantly, both of the top molecules were confirmed as strong inhibitors for CK2 with only minor activity towards CDK family members (Fig. 4B). Previous studies have shown that CK2 inhibition alone is not efficient in killing breast cancer cells, however, its inhibition can synergize with potent PARP inhibitors (Bulanova et al. 2024). To assess if our new, more specific CK2 inhibitors synergize with PARP inhibition in breast cancer, we first treated a panel of 8 different breast cancer cell lines with compounds 2 and 3. The effect of single-agent treatments was limited and high concentrations of the compounds were needed to reduce breast cancer cell viabilities (Fig. 4C-D). Next, we performed similar assays, treating breast cancer cells with combinatorial therapies of different PARP inhibitors plus CK2 compounds 2 and 3. Especially compound 2 was able to synergize with rucaparib and olaparib in PARP sensitive models, but not in cell models that are intrinsically resistant to PARP inhibition (SK-BR-3) (Fig. 4E).

In conclusion, we suggest compounds 2 and 3 as potent and selective CK2 inhibitors and seek next to assess the scaffold novelty of these compounds.

Structural similarity and scaffold novelty of the new CK2 inhibitors. To assess scaffold novelty, we first made a ChemBL database search revealing Comp2 as pre-existing molecule with known affinity towards DYRK1A. However, Comp3 molecule did not exist in these database searches.The PCA projection based on ECFP4 fingerprints revealed that the two new compounds (Comp2 and Comp3) occupied a distinct region of the chemical space compared with the ten known CK2 inhibitors (Fig. 5A). The known inhibitors clustered according to their chemical series, such as benzimidazoles (TBB, TDB, TBI, DMAT, and TMCB) and indole-based inhibitors (CX-4945, CX-5011, CX-5279), whereas Comp2 and Comp3 formed a separate cluster, indicating chemical distinctness.

Quantitative similarity analysis confirmed the low structural overlap of Comp 2 and 3 with known CK2 inhibitors. The maximum ECFP4 Tanimoto similarities were 0.111 for Comp2 and 0.157 for Comp3, and the corresponding ECFP6 values were 0.103 and 0.122, respectively (Fig. 5C). Both compounds showed the highest similarity to SGC-CK2-1 but remained well below the typical threshold (0.6-0.7) for scaffold-level similarity (Zahoránszky-Kőhalmi et al. 2016), suggesting novel chemical frameworks.

MCS analysis further demonstrated limited substructure overlap between the new hits and SGC-CK2-1, with shared substructures consisting of only 7-8 atoms (corresponding to 34-39% molecular coverage in the hits; Fig. 5B-C). Murcko scaffold comparison confirmed that neither Comp2 nor Comp3 shared any scaffold with the reference CK2 inhibitors, indicating that both represent novel chemotypes distinct from previously known CK2 inhibitor classes.



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	CX4945	DMAT	TBB
CK2	1 nM (IC_{50})	140 nM (IC_{50})	900 nM (IC_{50})
CDK1	56 nM (IC_{50})	ND	ND
CDK2	60% (Inhibition, at 0.5 μM)	82% (Inhibition, at 10 μM)	52% (Inhibition, at 10 μM)
REF	Cancer Res (2010) 70 (24): 10288–10298. Sci. Adv. (2019) 5 : eaau9060	Cell Tissue Res. (2010) 340 (2): 371-9. Sci. Adv. (2019) 5 : eaau9060	FEBS Letters (2001) 496 (1): 44-48. Sci. Adv. (2019) 5 : eaau9060

Fig. 3. Off-target bioactivity of small molecules against the CDK family contributes to clinical trial attrition. (A) Distribution of genetic dependencies across >1300 cell lines (DepMap release 25Q2). Gene dependency probabilities for each gene were averaged over all cell models plotted from high to low. Here, we defined the anti-target cut-off to be 0.5 which led to 1696 genes to be defined as highly dependent, or anti-targets. (B) Dependency probabilities of >1300 cell lines of different CDK family members (DepMap release 25Q2). Cell lines are overly dependent on CDK1/7/9 (C) Correlation between CDK binding and drugs post-marketing severity. (D) The bioactivity profiles of selected investigational and approved kinase inhibitors against the CDK family. See Supplementary Figure 7 for the complete heatmap. (E) Left-tailed Fisher's exact test was used to assess whether approved drugs show significantly less activity against CDK family members compared to investigational compounds. (F) Existing CK2 inhibitors CX-4945, DMAT and TBB exhibit bioactivity towards CDK1/2. ND: Not determined.

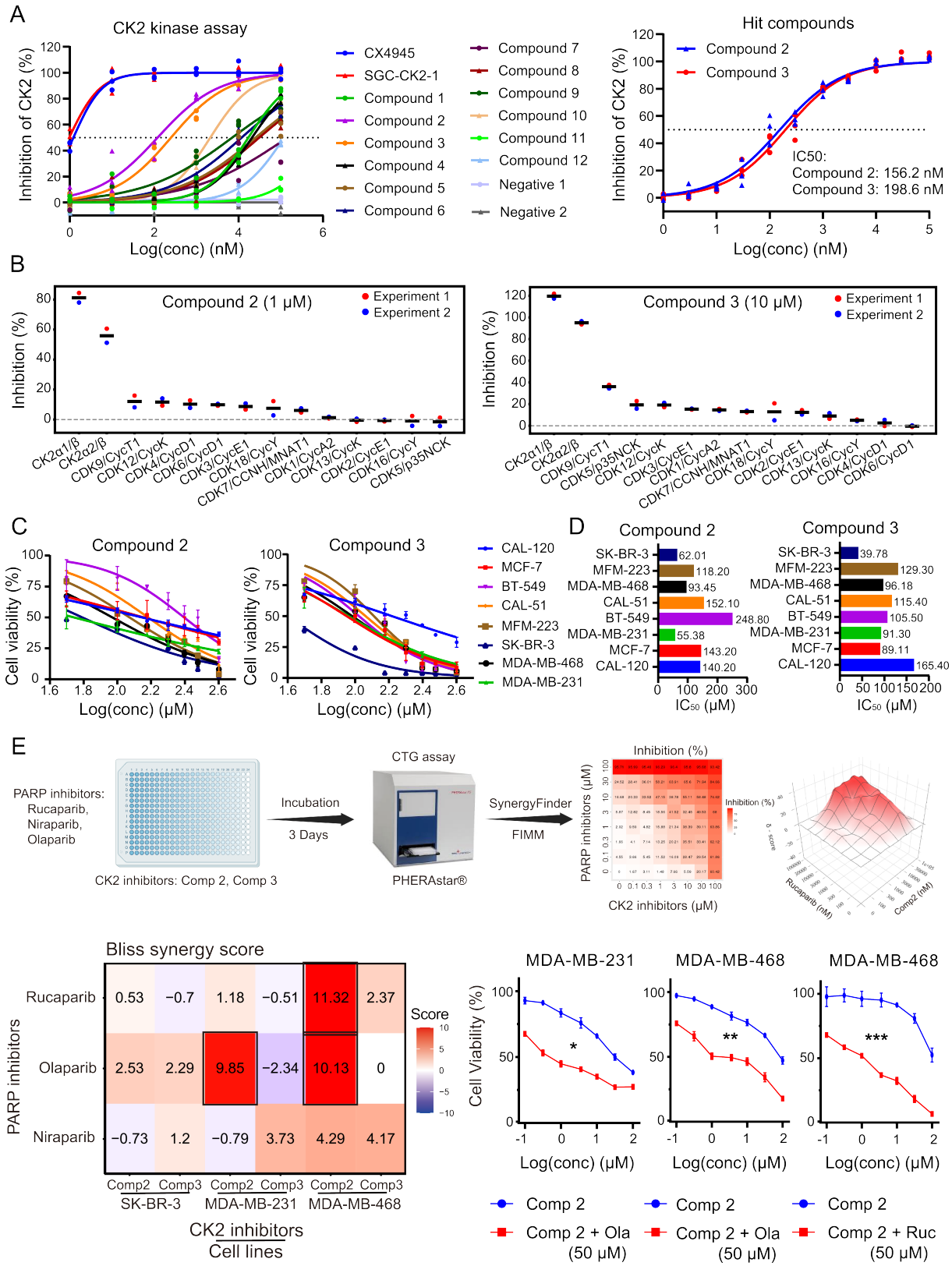
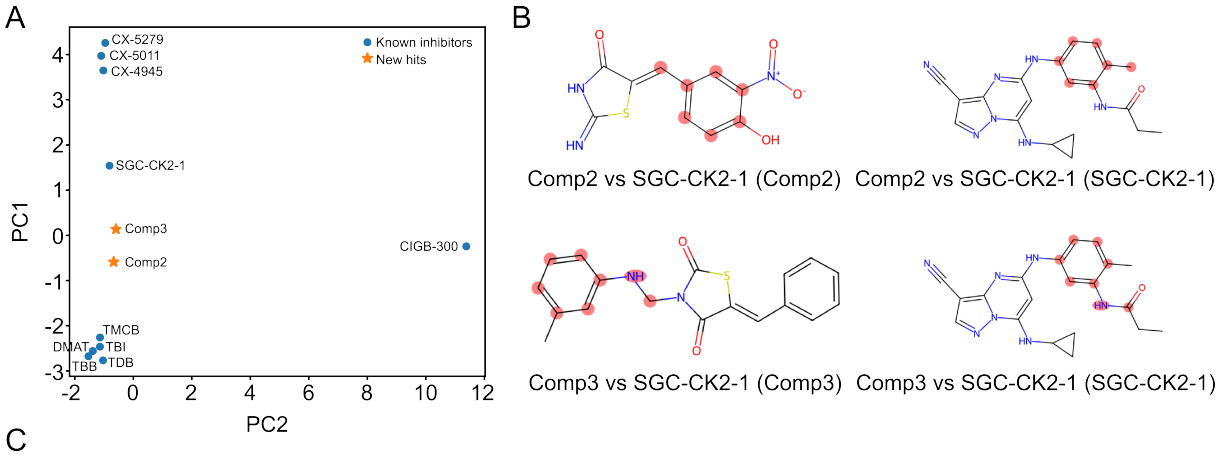


Fig. 4. Structural similarity and scaffold novelty of the newly identified CK2 inhibitors. (A) Principal component analysis (PCA) of molecular fingerprints (ECFP4) for known CK2 inhibitors (blue circles) and newly identified hits Comp2 and Comp3 (orange stars). (B) Maximum common substructure (MCS) comparisons between Comp2 or Comp3 and the most similar known inhibitor (SGC-CK2-1). Shared atoms identified by RDKit FindMCS are highlighted in red. (C) Summary of computed molecular similarity metrics for Comp2 and Comp3. Maximum and mean Tanimoto coefficients were calculated based on both ECFP4 and ECFP6 fingerprints. The nearest known inhibitor, number of atoms in the MCS, molecular coverage in each compound, and scaffold overlap status are listed.



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Hit	Max ecfp4	Mean ecfp4	Max ecfp6	Mean ecfp6	Nearest ref	Mcs atoms	Hit coverage	Ref coverage	Shared with refs
Comp2	0.111	0.069	0.103	0.063	SGC-CK2-1	7	38.9%	25.0%	FALSE
Comp3	0.157	0.084	0.122	0.070	SGC-CK2-1	8	34.8%	28.6%	FALSE

Fig. 5. Structural similarity and scaffold novelty of the newly identified CK2 inhibitors. (A) Principal component analysis (PCA) of molecular fingerprints (ECFP4) for known CK2 inhibitors (blue circles) and newly identified hits Comp2 and Comp3 (orange stars). (B) Maximum common substructure (MCS) comparisons between Comp2 or Comp3 and the most similar known inhibitor (SGC-CK2-1). Shared atoms identified by RDKit FindMCS are highlighted in red. (C) Summary of computed molecular similarity metrics for Comp2 and Comp3. Maximum and mean Tanimoto coefficients were calculated based on both ECFP4 and ECFP6 fingerprints. The nearest known inhibitor, number of atoms in the MCS, molecular coverage in each compound, and scaffold overlap status are listed.

Together, these findings indicate that Comp3 is a fully novel CK2 inhibitor with distinct scaffold over existing CK2 kinases, and could be refined further in design-make-test cycles to enhance bioactivity.

Conclusions

Machine intelligence is quickly becoming a core engine in drug discovery, enabling efficient lead identification and speeding-up the whole discovery pipeline (Schneider et al. 2020). Despite this, many open problems such as balanced safety/efficacy prediction remain. Here, we explored the off-target landscape of human cells using the largest available resources and showed that molecules with higher affinity towards off-targets such as specific CDK family kinases cause more drastic adverse toxicities or fail clinical trials. By using this knowledge, we designed an ML-powered drug discovery pipeline, which circumvents the key toxicity-inducing targets, while retaining high affinity towards the intended target, CK2. In doing so, we focus both on safety and efficacy aspects of ML-powered drug discovery.

Focusing on molecular safety, we showed that approved pharmaceuticals with high bioactivity towards CDK1/2/7/9 are associated with more severe adverse events. Congruently, drug candidates with activity towards CDK1/2/7/9 have an increased likelihood of being eliminated during the clinical trials. These analyses do not only strengthen our current knowledge of the roles and cellular dependencies of different CDK family members, but they also provide means to perform more broad-scale and nuanced off-target landscape mapping, covering a wide range of targets. We argue that currently existing drug discovery pipelines fail to appreciate the full spectrum of the anti-target landscape, and broader mapping efforts will form a cornerstone for efficient AI/ML driven drug discovery in the future, enabling more balanced safety/efficacy predictions.

To advance drug efficacy prediction, we coupled a data-centric ML strategy (curated IC_{50} labels from multiple databases, and applicability-domain filtering via an error prediction model) with a structure-specific prioritization to enrich CK2 binders. In this hybrid pipeline we attempted to minimize the ML model error with an adjacent error prediction model. To our knowledge, such error modelling has not been previously applied in drug design and it was able to increase ML model performance (Supplementary Figure 6), to deliver a high biochemical hit rate as validated using orthogonal assays. Importantly, the simple ML models, such as SVM, together with the error modelling seemed superior even to more advanced models such as ANN.

CK2 protein has multiple suitable binding interfaces for a drug. Here, based on the error modelling and SVM, we were able to predict molecules which either bridged the ATP hinge and the αD pocket (bitopic binding mode) or occupied the αD pocket alone (allosteric inhibition). This is especially reassuring as targeting allosteric regulation sites, which have less sequence/structural conservation across kinases, can help to circumvent major off-target effects compared to active site targeting. Consistent with previous findings (Bulanova et al. 2024), the newly discovered CK2 inhibitors show synergistic effects with PARP inhibitors.

Supplementary Note 1: Methods

Methods can be found in GitHub Supplementary Methods

A. Data and Code Availability. The harmonised datasets, the codes to produce the analyses and trained models will be available in github after peer review here

Supplementary material

Supplementary material can be found from github here.
The Extensive Supplementary Materials including ML details will be added for the peer review process.

Declaration of Interests

Jordi Mestres is the founder and research director of Chemotargets. The authors declare no other competing interests.

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