Leveraging Functional Assays to Optimize and Validate the IQ DruSafe Safety-77 Panel for Comprehensive Safety Pharmacology

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Introduction

In drug discovery, proactive toxicological risk assessment of candidate compounds is crucial for avoiding clinical-stage failure and post-market withdrawal. However, traditional preclinical safety evaluation strategies have significant limitations: in vivo animal studies exhibit low human relevance (translatability) due to significant species differences and face dual pressures of ethics and cost; while conventional in vitro binding assays are inadequate for predicting functional biological effects. Notably, approximately 75% of clinical adverse drug reactions (ADRs) originate from dose-dependent off-target effects, highlighting an urgent need for more accurate early-risk identification tools. To address these challenges, the IQ DruSafe consortium—a preeminent alliance of global pharmaceutical companies—has championed the enhancement of preclinical predictive power through the expansion and refinement of secondary pharmacology screening strategies. This study is aligned with this initiative and aims to investigate the implementation of an advanced in vitro secondary pharmacology screening system utilizing functional assay formats. By generating richer pharmacological information beyond mere binding affinity, this strategy seeks to enable a more accurate and earlier identification of potential safety liabilities, thereby providing highly translatable safety insights for the optimization of lead compounds.

The ICESTP SAFETYPANEL™ 77 Dose Response platform provides a comprehensive functional screening strategy that integrates single-point primary screening with quantitative dose-response (curve-based) profiling. This strategy is supported by a broad suite of mechanism-relevant functional assay technologies, including FLIPR calcium flux assay, HTRF, ADP-Glo, fluorescence polarization (FP), and other mechanism-relevant functional formats . The entire workflow exemplifies the convergence of functional biology and advanced automation. By accelerating the safety profiling process, minimizing manual error, and delivering mechanistically insightful, quantitative datasets (e.g., IC_{50}/EC_{50}), the platform enables more reliable optimization of candidate compounds early in the drug discovery pipeline.

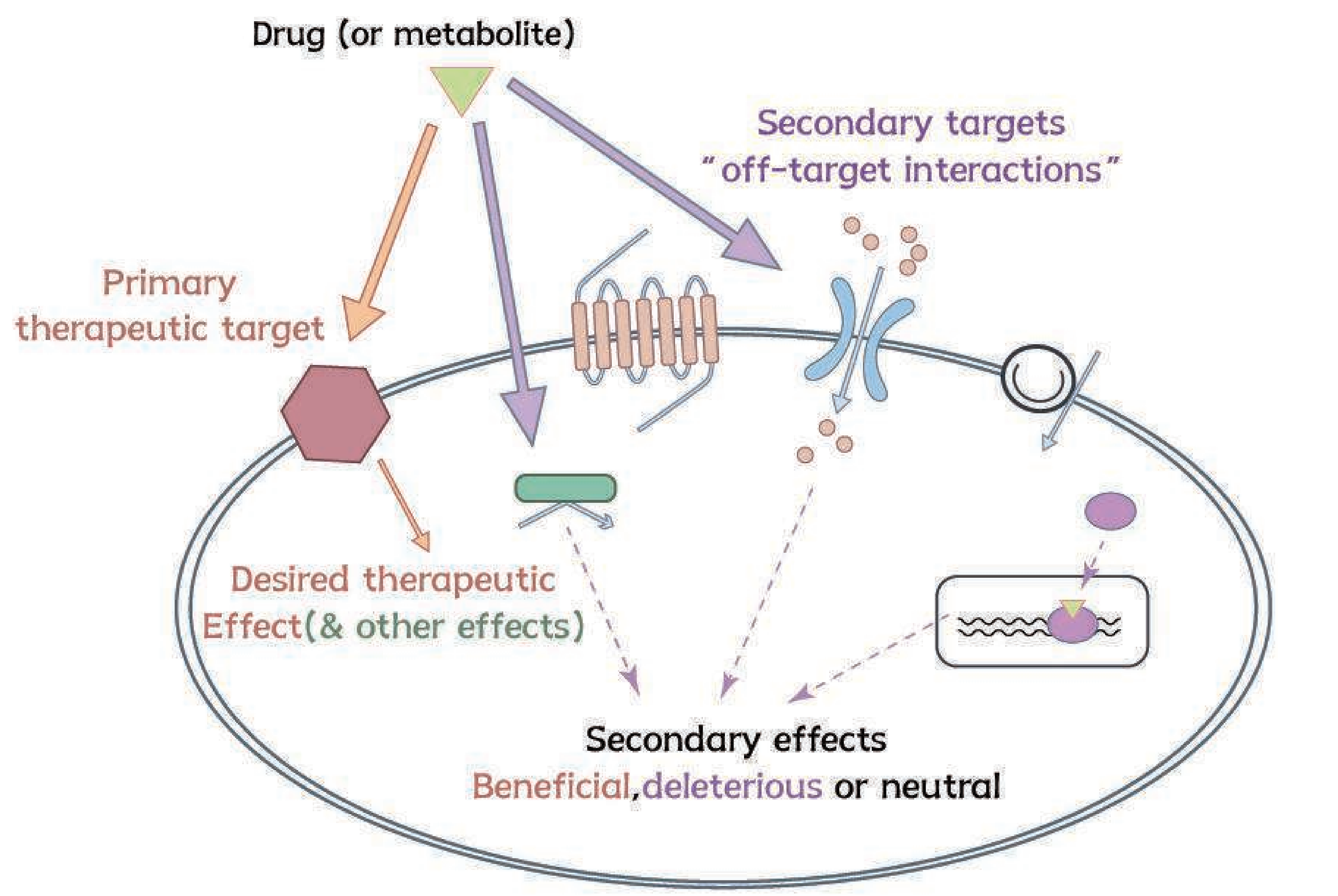


Figure 1: Mechanisms of drug action

Methods

Automated systems supporting the ICESTP SAFETYPANEL™ 77 Dose Response

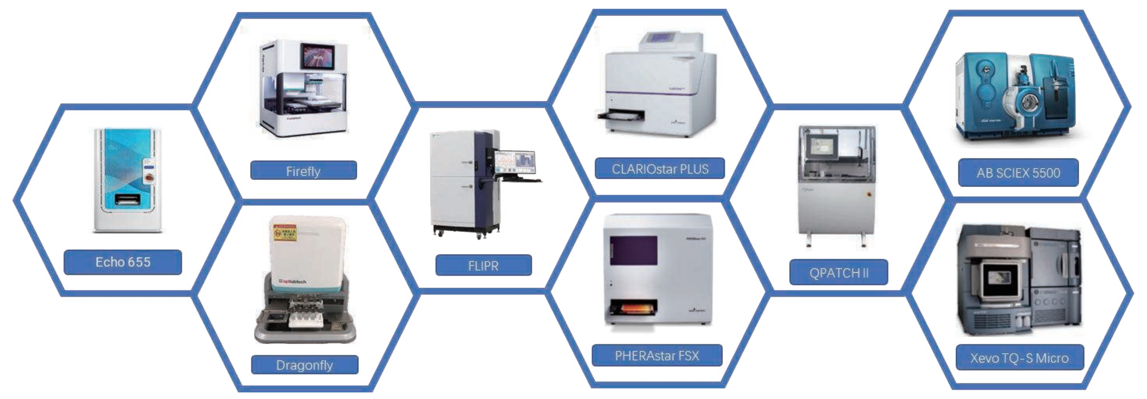


Figure 2. This study adopted a high-throughput screening strategy integrating multiple functional assay technologies (including FLIPR, HTRF, QPatch, ADP-Glo, FP, etc.). The strategy included two modes: single-point primary screening and multi-concentration dose-response curve screening. To achieve high throughput and high precision, the platform integrated automated instruments (such as acoustic liquid handling systems and multi-mode plate readers) to perform curve-based screening tests. We applied this strategy to test the dose-response effects of various antidepressants, pergolide, and osimertinib on a panel covering 77 important safety-related targets.

Overview of the ICESTP SAFETYPANEL™ 77

DAT-Antagonist-Re-uptake
NET-Antagonist-Re-uptake
5-HTT-Antagonist-Re-uptake
GAT1-Antagonist-LCMS

AR-Agonist/Antagonist-Luciferase Reporter
GR-Agonist/Antagonist-Luciferase Reporter
ERα-Agonist/Antagonist-Luciferase Reporter
PR-Agonist/Antagonist-Luciferase Reporter
RARα-Agonist/Antagonist-Luciferase Reporter

MAOA-Inhibition-Luminescence
MAOB-Inhibition-Luminescence
COX1-Inhibition-FI
COX2-Inhibition-FI
CTSD-Inhibition-FI
MALT1-Inhibition-FI
AchE-Inhibition-Abs
PDE3A-Inhibition-FP
PDE4D2-Inhibition-FP
BRD4-Inhibition-HTRF

LCK-Inhibition-HTRF
AURA-Inhibition-HTRF
AURB-Inhibition-HTRF
EGFR-Inhibition-HTRF
FGFR1-Inhibition-HTRF
KIT-Inhibition-HTRF
NTRK1-Inhibition-HTRF
FAK-Inhibition-HTRF
ROCK2-Inhibition-HTRF
SRC-Inhibition-HTRF
ATM-Inhibition-HTRF
INSR-Inhibition-HTRF
VEGFR2-Inhibition-HTRF
Flt-1-Inhibition-HTRF
MAP2K7-Inhibition-ADP-Glo
PDK1-Inhibition-ADP-Glo
PIK3CG-Inhibition-ADP-Glo
STK35-Inhibition-ADP-Glo
GSK3β-Inhibition-ADP-Glo

KvLQT1/minK-blocker-Manual patch
Cav1.2-blocker-FLIPR
Nav1.5-blocker-Automated patch clamp
hERG-blocker-Automated patch clamp
5HT3A-opener/blocker-FLIPR
NR1/NR2B-opener/blocker-FLIPR
nAChRα4β2-opener/blocker-FLIPR
$GABA_A$(α1β2γ2)-opener/blocker-FLIPR

CB1-Agonist/Antagonist-cAMP
CB2-Agonist/Antagonist-cAMP
DOR-Agonist/Antagonist-cAMP
KOR-Agonist/Antagonist-cAMP
MOR-Agonist/Antagonist-cAMP
5-HT1A-Agonist/Antagonist-cAMP
5-HT1B-Agonist/Antagonist-cAMP
D2S-Agonist/Antagonist-cAMP
A2A-Agonist/Antagonist-cAMP
β1-Agonist/Antagonist-cAMP
β2-Agonist/Antagonist-cAMP
D1-Agonist/Antagonist-cAMP
D3-Agonist/Antagonist-cAMP
H2-Agonist/Antagonist-cAMP
5-HT6-Agonist/Antagonist-cAMP
α1A-Agonist/Antagonist-FLIPR
α2A-Agonist/Antagonist-IP-One
H1-Agonist/Antagonist-IP-One
M1-Agonist/Antagonist-FLIPR
M2-Agonist/Antagonist-FLIPR
M3-Agonist/Antagonist-FLIPR
5-HT2A-Agonist/Antagonist-FLIPR
5-HT2B-Agonist/Antagonist-FLIPR
MRGPRX2-Agonist/Antagonist-IP-One
CCK1-Agonist/Antagonist-IP-One
CCK2-Agonist/Antagonist-IP-One
ETA-Agonist/Antagonist-IP-One
V1a-Agonist/Antagonist-IP-One
NK3-Agonist/Antagonist-IP-One
TGR5-Agonist/Antagonist-cAMP

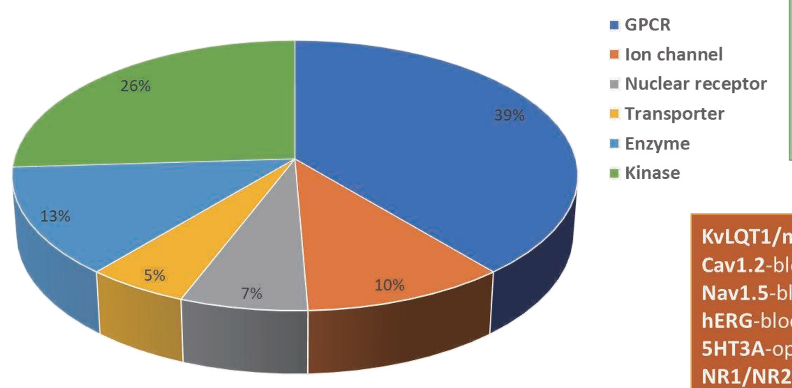
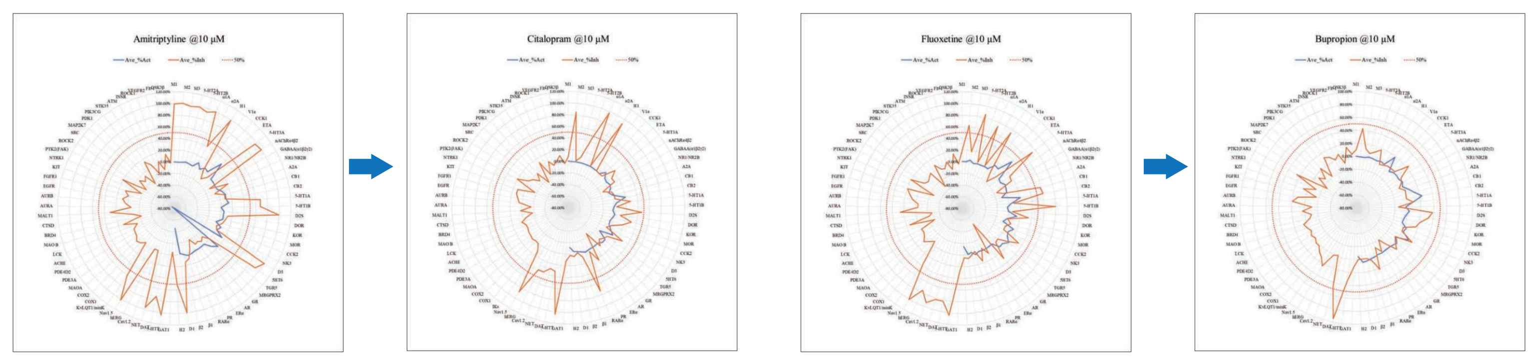


Figure 3. The ICESTP SAFETYPANEL ™ 77 comprises 116 assays, integrating both single-point and dose-response screening methodologies within a functional assay framework.

Results

A



B

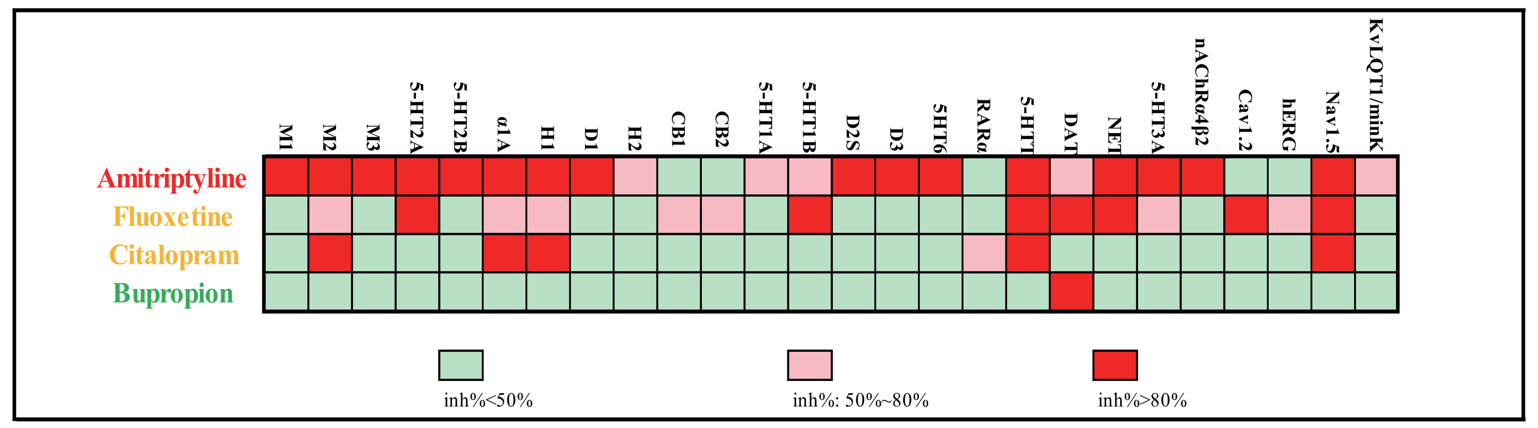


Figure 4: Pharmacological Profiling of Antidepressants Using ICESTP SAFETYPANEL™ 77 platform

The resulting radar maps provide a clear and intuitive visualization of each drug's binding activity across a diverse range of molecular targets. As shown in Figure 4A, pharmacological profiling of antidepressants (amitriptyline, citalopram, fluoxetine, bupropion) at 10 μM via ICESTP SAFETYPANEL™ 77, showing %Activity (blue) & %Inhibition (orange) across 77 targets. Single concentration (10 μM) clustered heatmap (partial) for 4 antidepressants tested in ICESTP SAFETYPANEL™ 77 (Figure 4B),. Differential off-target receptor inhibition profiles underlie the distinct side effect patterns of these antidepressants.

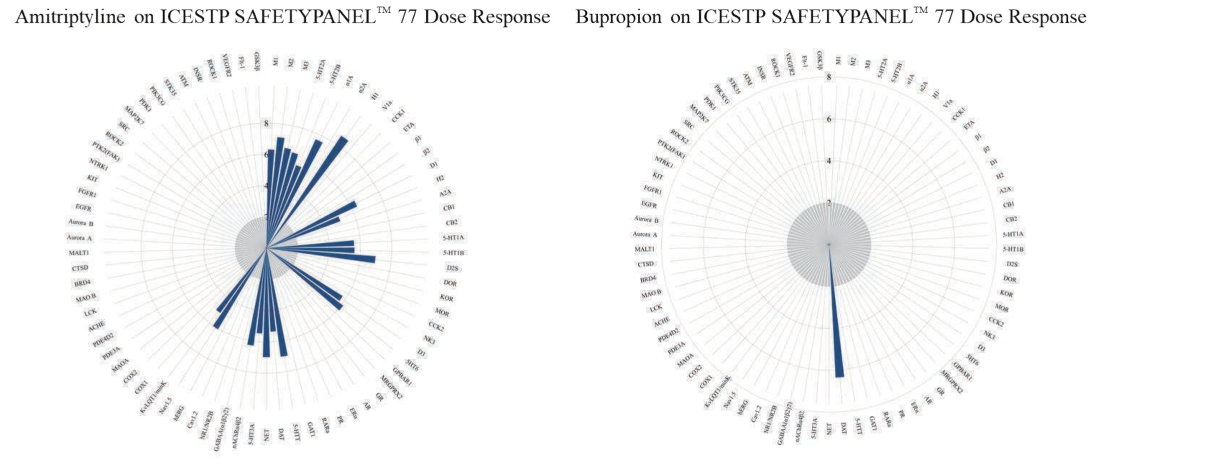


Figure 5: Rose plots show the distinct inhibitory profiles of amitriptyline and bupropion on the ICESTP SAFETYPANEL™ 77 Dose Response, with potency as pIC_{50}

IC50 values are expressed as the negative logarithm (base 10) of the IC_{50} value in molar (i.e., $pIC_{50} = 9-\log10(IC_{50})$). A higher bar represents a more potent response. Amitriptyline exhibited the broad off-target activity, demonstrating significant antagonism at M1 and H1 receptors. This pronounced inhibition aligns perfectly with its well-documented, dose-limiting anticholinergic (e.g., dry mouth, constipation, blurred vision) and sedative side effects. Additionally, its activity on adrenergic and other channels underscores its cardiovascular risks. Bupropion displayed a unique and distinct profile characterized by minimal activity at serotonergic targets but notable interactions with dopamine transporter. Its clean lack of antagonism at muscarinic and histaminergic receptors provides a mechanistic basis for its clinically advantageous absence of sexual dysfunction, weight gain, and sedation, which are common limitations of SSRIs.

Functional Assays and Physiological ATP Enhance Prediction of Compound Safety Profiles

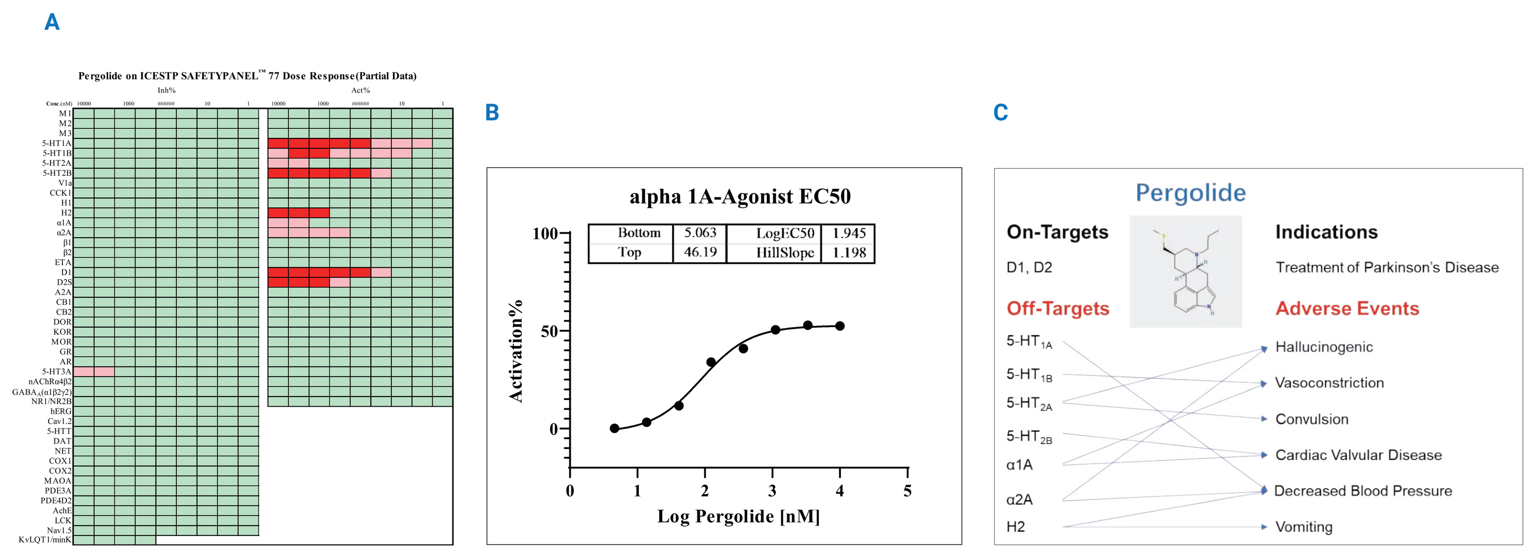


Figure 6: Pergolide with functional assay format ICESTP SAFETYPANEL™ 77 Dose Response.

Using Pergolide as a case study, we systematically evaluated its off-target profile using the ICESTP SAFETYPANEL™ 77 Dose Response assay. As shown in Figure 6A, the heatmap visualization revealed distinct inhibition and activation patterns across multiple targets—particularly showing strong agonist activity at 5-HT1A, V1a, and α1A receptors. Subsequent dose-response characterization (Figure 6B) confirmed pergolide acts as a partial agonist at α1A ($LogEC_{50}$ = 1.945, Top = 46.19). Finally, we integrated these off-target effects into a mechanistic network (Figure 6C), linking its promiscuous receptor activity (e.g., 5-HT2B, α1A) to clinical adverse events such as valvulopathy and vasoconstriction. This approach provides actionable insights for predictive drug safety assessment.

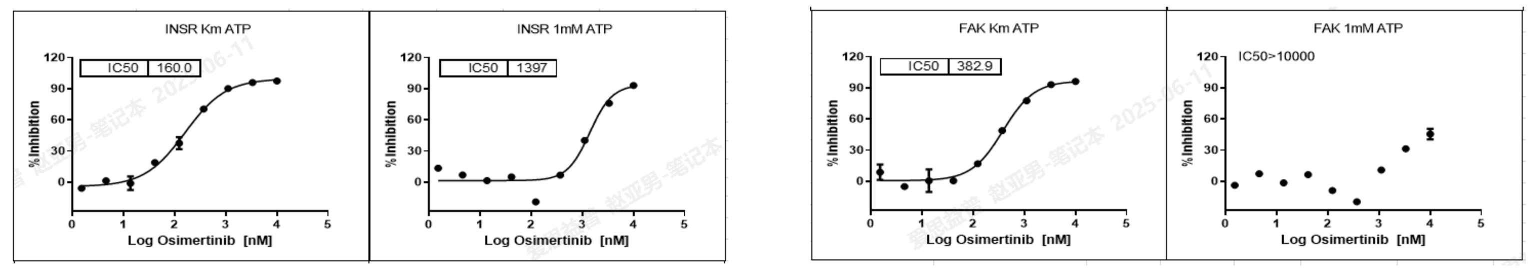


Figure 7: Physiological ATP (1 mM) Enables Clinically Relevant Prediction of Osimertinib's Kinase Off-Target Profiles

A key advantage of the ICESTP SAFETYPANEL ™ 77 Dose Response kinase assay is the uniform use of 1 mM ATP—a physiologically relevant concentration that mirrors the intracellular milieu—thereby significantly enhancing the clinical translatability of off-target predictions. While osimertinib potently inhibits INSR and FAK under low, non-physiological ATP (Km), its efficacy is drastically reduced under 1 mM ATP (INSR IC_{50} increases to 1397 nM; FAK inhibition is nearly abolished). This stark contrast demonstrates that only low IC_{50} values obtained under physiological ATP conditions are clinically meaningful, preventing the overestimation of off-target risks that can occur in non-physiological assays.

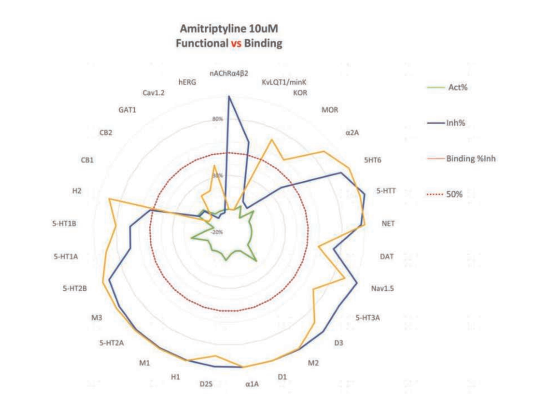


Figure 8: Radar Map of Amitriptyline Multi-Target Binding vs. Functional Comparative.

The manual patch clamp and FLIPR method were employed to verify that amitriptyline exerts a significant antagonistic effect on the nACHRα4β2 target; however, the binding assay demonstrated no such effect.

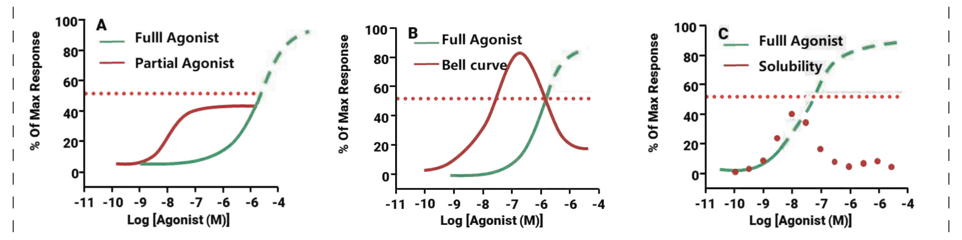


Figure 9: Core Advantages of Functional Dose-Response Curve Analysis

This figure illustrates three pivotal advantages of employing automated functional dose-response curve analysis in ICESTP SAFETYPANEL™ 77 Dose Response : Accurately distinguishing full from partial agonism (Fig. A) by analyzing curve shape and maximal efficacy; Identifying non-linear responses (Fig. B), such as bell-shaped curves or aberrant plateaus, which may signal potential off-target effects or toxicity, thereby preventing false positives/negatives; Avoiding solubility-driven misreads (Fig. C), where precipitation of compounds at higher concentrations causes an anomalous decrease in response. Integrated into high-throughput platforms, this analysis enhances data integrity and accelerates the identification of truly selective compounds, advancing drug safety through innovation.

Conclusion and discussion

This study employed the high-throughput ICESTP SAFETYPANEL™ 77 to systematically profile the off-target activities of multiple drugs. The platform's automated functional analysis accurately delineated polypharmacological mechanisms underlying clinical safety profiles. Utilizing physiological conditions (e.g., 1 mM ATP for kinases) prevented risk overestimation and increased data reliability. Providing human-relevant safety insights with high predictive value. These results validate the platform's utility for mechanistically informed, early safety screening.

Looking ahead, high-throughput functional safety screening platforms represented by the ICESTP SAFETYPANEL™ 77 are poised to be deeply embedded into the entire drug discovery workflow, from target validation to clinical candidate selection. Their application can span multiple critical decision points: identifying and eliminating high-risk chemical scaffolds during the hit-to-lead stage; iteratively assessing the improvement in safety windows of different derivatives during lead optimization; and providing important mechanistic supplementary data for non-clinical safety evaluation during candidate drug nomination. By proactively and scalably applying such highly predictive in vitro safety profiling to R&D pipelines, drug discovery teams can identify potential safety risks at earlier stages and optimize resource allocation. This will significantly reduce late-stage development attrition due to safety issues and accelerate the development of new drugs with greater clinical advantages and improved safety profiles.

Reference

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