ICESTP SAFETYPANEL™: From Off-Target Identification to Clinical Risk Management

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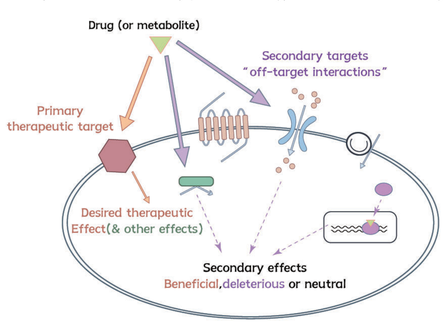
Abstract Number:

Abstract

Background and Purpose:
Early assessment of candidate drug safety is critical in pharmaceutical development to mitigate post-market withdrawals and reduce potential risks. Approximately 75% of Adverse Drug Reactions (ADRs) are dose-dependent (Type A), often mediated by interactions with unintended off-target proteins. Traditional binding assays have limitations in predicting functional biological effects. To address this challenge, this study aims to investigate how an in vitro secondary pharmacology screening strategy, utilizing a functional assay format, can enable more accurate identification of potential safety liabilities early in drug discovery by providing richer pharmacological data.
Methods:
This study employed a high-throughput screening strategy integrating various functional assay technologies (including FLIPR calcium flux, HTRF, ADP-Glo, FP, etc.). The strategy comprised two phases: single-point primary screening and multi-concentration dose-response curve screening. To achieve high throughput and precision, the platform incorporated automated instrumentation (e.g., acoustic liquid handlers, multimode plate readers) for curve-based screening tests. We applied this strategy to test the dose-response effects of Pergolide and several antidepressant compounds against a panel of 77 predefined safety-relevant targets.
Results:
The ICESTP SAFETYPANEL™ dose-response platforms provide robust support for addressing translational safety barrier. Its functional assays precisely quantify interaction potency with both therapeutic and non-target receptors, identify non-linear dose-toxicity relationships to enable personalized testing protocols, and effectively circumvent traditional false-positive bias via physiological ATP kinase milieu simulation (1 mM) and solubility correction. The high-throughput capabilities driven by the integrated automation (Echo/Firefly/BMG) significantly accelerate the translation of preclinical safety data into clinical decision-making.
Conclusions:
The ICESTP SAFETYPANEL™ framework reshapes the translational medicine defense through secondary pharmacology. Systematic evaluation of off-target across key target classes enhances the clinical predictability of Type A ADRs. Its mechanistic safety profiles underpin individualized dosing regimens, proactively mitigating clinical attrition. This establishes a reliable pathway from in vitro safety pharmacology to clinical risk management.



Methods

Automated systems and workflow supporting the ICESTP SAFETYPANEL™ 77 Dose Response

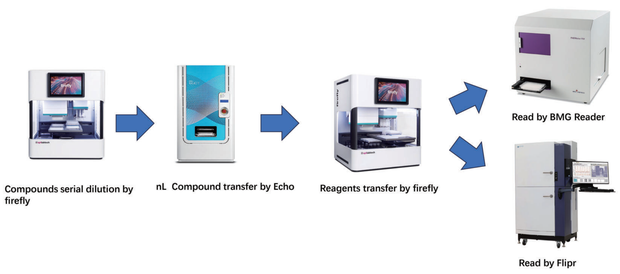


Figure 1. Utilizing technological platforms including FLIPR, HTRF, ADP-Glo, FP, and FI, the SPT Labtech firefly® automated liquid handling system and the ECHO655 acoustic liquid handler enable high-throughput sample processing, significantly improving the efficiency and detection accuracy of ICESTP™ safety assessment.

Overview of the ICESTP SAFETYPANEL™ 77

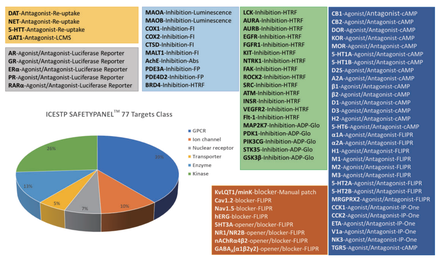


Figure 2. The ICESTP SAFETYPANEL ™ 77 comprises 116 assays, integrating both single-point and dose-response screening methodologies within a functional assay framework.

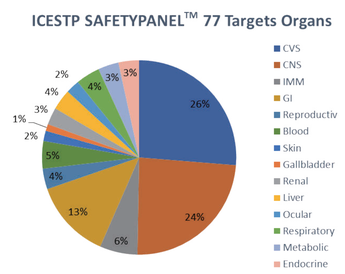


Figure 3. Relative distribution of different target organs.

Results

Pharmacological Profiling of Antidepressants Using ICESTP SAFETYPANEL™ 77

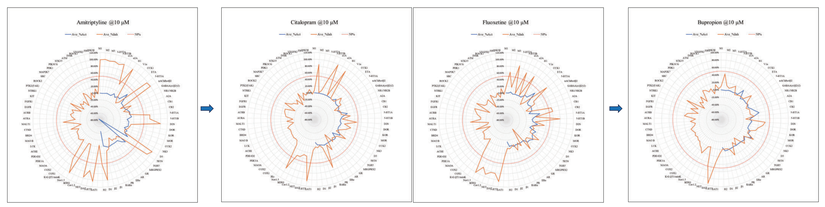


Figure 4: Rader maps The resulting radar maps provide a clear and intuitive visualization of each drug's binding activity across a diverse range of molecular targets. The data robustly corroborate established pharmacological knowledge and explain their distinct clinical side effect profiles: Amitriptyline exhibited the broadest and strongest off-target activity, demonstrating significant antagonism at muscarinic M1 receptors and histaminergic 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. Both Citalopram and Fluoxetine showed a much cleaner profile, with high selectivity for the serotonin transporter (SERT), consistent with their SSRI classification. Their minimal off-target interactions, particularly at cholinergic and histaminergic receptors, explain their superior tolerability over TCAs and their establishment as first-line therapeutics. Bupropion displayed a unique and distinct profile characterized by minimal activity at serotonergic targets but notable interactions with norepinephrine and dopamine transporters. 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.

Table 1: Pharmacological Profiles of Four Representative Antidepressants

	Amitriptyline (TCA)	Citalopram(SSRI)	Fluoxetine (SSRI)	Bupropion (NDRI)
Generation	**First-Generation**	**Second-Generation**	**Second-Generation**	**Novel agents**
Primary Target	Non-selective inhibition Of SERT & NET	Selective inhibition of SERT	Selective inhibition of SERT	Selective inhibition of NET & DAT
Key Mechanisms	Antagonism: mAChR, H1,1 adrenergic receptors	Minimal affinity for other receptors	Mild antagonism at 5-HT2C receptor; Long half-life	No direct action on 5-HT, mAChR, or H1 receptors
Clinical Applications	Major Depressive Disorder (MDD) Chronic neuropathic pain, Migraine prophylaxis	MDD, Generalized Anxiety Disorder (GAD), Panic Disorder	MDD,Obsessive-Compulsive Disorder(OCD),Bulimia nervosa	MDD, Smoking cessation, SSRI-induced sexual dysfunction
Common Side Effects	Frequent: Dry mouth, Constipation, Sedation, Weight gain, Tachycardia	Nausea, Headache, Sweating, Sexua dysfunction	Anxiety,Insomnia, Nausea, Sexua dysfunction	Insomnia, Dry mouth, Minima sexualside effects
Serious Risks	High toxicity in overdose (cardiac arrhythmia), QT prolongation, Reduced seizure threshold	QT prolongation (dose-dependent) Serotonin syndrome risk	Activation, Drug interactions, Serotonin syndrome	Dose-dependent risk of seizures Contraindicated in eating disorders
Advantages	Potent analgesic, Effective for severe depression, Low cost	Favorable tolerability, Fewer drug interactions	Once-weeklydosing option, Activative for fatigued patients	Lacks sexual Side Effects & weight gain,Activative, aids smoking cessation
Disadvantages	Poor tolerability, Multiple drug interactions, Requires monitoring	Slow onset, Sexual Side Effects	Long half-life (risks interactions), Activation can be harmful	Not for anxious patients, Risk of insomnia and agitation

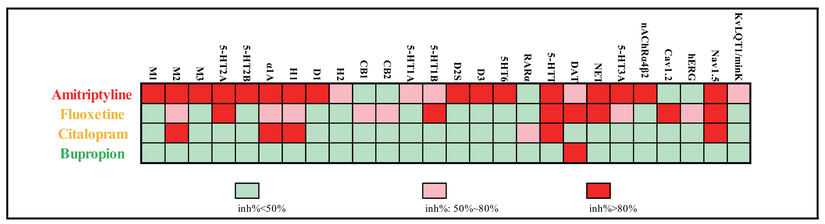


Figure 5: Heatmap Differential off-target receptor inhibition profiles underlie the distinct side effect patterns of these antidepressants.

Functional Assays and Physiological ATP Enhance Prediction of Compound Safety Profiles

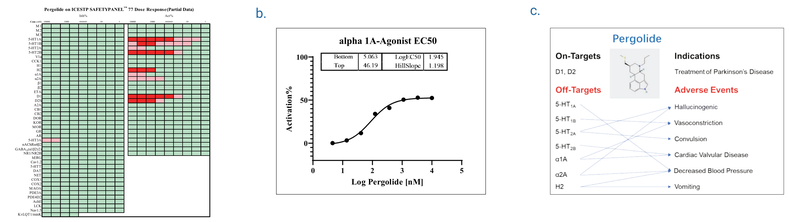


Figure 6: Pergolide with functional assay format ICESTP SAFETYPANEL™ 77 Dose Response.
Using Pergolide as a case study, we systematically evaluated its off-target profile using the ICESTP SAFETYPANEL ™ 77 Dose Response assay. As shown in **Figure 6a**, the heatmap visualization revealed distinct inhibition 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.

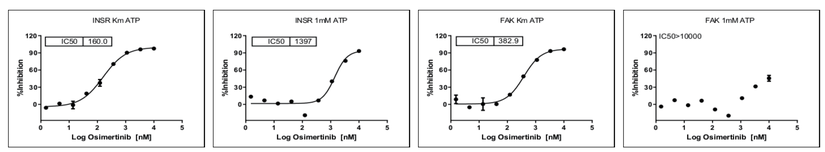


Figure 7: Physiological ATP (1 mM) Enables Clinically Relevant Prediction of Osimertinib's Kinase Off-Target Profiles
A key advantage of the ICESTP SAFETYPANEL ™ 77 Dose Response kinase assay is the uniform use of 1 mM ATP—a physiologically relevant concentration that mirrors the intracellular milieu—thereby 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.

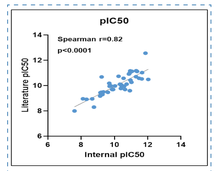


Figure 8: Nonparametric correlation analysis between literature and experimental data.
Excellent correlation (Spearman r = 0.82, p < 0.0001) validates the robustness and reproducibility of our experimental data and screening platform.

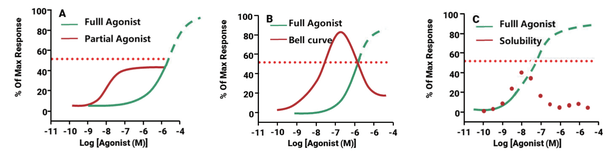


Figure 9: Core Advantages of Functional Dose-Response Curve Analysis
This figure illustrates three pivotal advantages of employing functional dose-response curve analysis in ICESTP SAFETYPANEL™ 77 Dose Response : Accurately distinguishing full from partial agonism (Fig. A) by analyzing curve shape and maximal efficacy, providing critical insights into the mode of target engagement; 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. Curve analysis proficiently identifies these artifacts caused by physico-chemical properties rather than true pharmacological activity, ensuring accurate data interpretation.

Conclusion and discussion

This study utilized the ICESTP SAFETYPANEL ™ 77 to systematically evaluate the off-target profiles of various pharmaceuticals, including pergolide, osimertinib, and four antidepressants. The platform efficiently and accurately delineated complex poly-pharmacological mechanisms underlying the clinical safety profiles of these compounds.
This research successfully validates the significant utility of the ICESTP SAFETYPANEL ™ 77 for drug safety assessment. The high concordance between the in vitro results and the established clinical profiles of the tested drugs demonstrates the platform's strong clinical translatability and predictive accuracy.
Methodological Advantage: The uniform use of a physiological ATP concentration (1 mM) for kinase targets prevents the overestimation of off-target risks under non-physiological conditions, providing more reliable and translatable data for the safety profiling of kinase inhibitors.
Mechanistic Elucidation: The results not only confirmed known off-target but may also reveal novel potential mechanisms, offering molecular-level insights into understanding drug ADRs.
Application Prospect: This technology platform can be applied to guide lead optimization, support candidate drug selection based on safety, and provide compelling in vitro evidence for elucidating clinical ADR mechanisms, thereby significantly reducing late-stage drug development attrition.

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