Reducing Time and Cost in Drug Candidate Prioritization

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Table of Contents

Introduction.

Drug discovery is a complex process involving the screening of vast libraries of chemical compounds to identify those suitable for preclinical and clinical development. The process is iterative and resource-intensive, with significant investments of time and capital occurring in the latter stages, particularly during clinical trials. The overarching aim is to identify a single therapeutic agent that is both safe and effective for market introduction. However, the current attrition rates in drug discovery programs remain high, with only approximately 10% of drug candidates successfully reaching the market. We will discuss how INDIGO's extensive portfolio of cell-based assays can assist in prioritizing drug candidates prior to pre-clinical animal studies and human trials.

Current Drug Discovery Strategy.

The drug discovery pipeline begins with the screening of tens of thousands of compounds, followed by several stages of optimization aimed at identifying lead candidates with promising pharmacological profiles (see Figure 1). Once a disease and its associated drug target are identified, high-throughput screening (HTS) methods allow for rapidly identifying compounds capable of interacting with the target. The objective is to prioritize those compounds that exhibit favorable pharmacodynamic and pharmacokinetic properties before advancing to more costly and time-consuming stages, such as preclinical animal studies and clinical trials. Despite technological advances in HTS and drug target identification, the overall success rate remains low, with failure often attributed to a lack of efficacy or unacceptable toxicity profiles emerging during clinical trials.

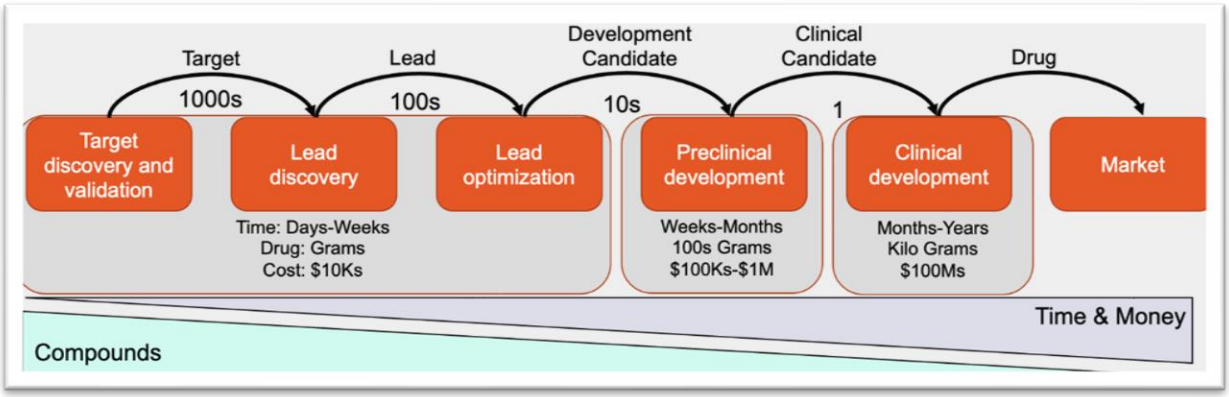


Figure 1. Basic drug discovery process.

Pharmacophore Concepts.

Pharmacophore is "an ensemble of steric and electronic features necessary to ensure the optimal supramolecular interactions with a specific biologic target and to trigger (or block) its biologic response". This refers to the spatial arrangement of atoms or functional groups within a molecule that are necessary for its interaction with a specific biological target, typically a macromolecule such as a protein, receptor, or enzyme. The interaction between a drug and its target is often described using a "lock and key" analogy, where the drug acts as a key fitting into the receptor's binding pocket. This binding is mediated by non-covalent interactions, including hydrogen bonds, hydrophobic interactions, and electrostatic forces (Figure 2). While this analogy provides a basic understanding of drug-receptor interactions, it oversimplifies the complexity of binding affinity and does not

adequately account for the functional consequences of binding, such as agonism, antagonism, or partial agonism. A more nuanced understanding is that the drug induces a conformational change in the receptor, depending on the internal structure of both the ligand and the binding pocket.

Structure-Activity Relationships and Lead Optimization.

In drug discovery, identifying a "hit" compound that interacts with a druggable target initiates the lead optimization phase. Related compounds (pharmacophores) are synthesized and tested during this phase to optimize the desired pharmacological properties, such as binding affinity and specificity. This process is informed by structure-activity relationships (SAR), which correlate chemical structure modifications with changes in biological activity. Computational tools and high-throughput synthesis techniques enable the rapid generation of compound libraries based on a lead scaffold. HTS is then employed to assess the interactions between these compounds and the target receptor, while considering both the structure of the ligand and the receptor's binding pocket.

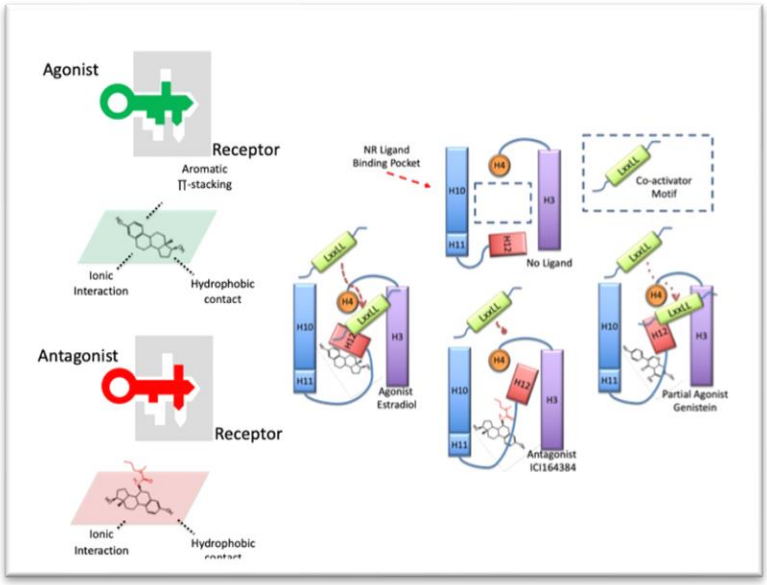


Figure 2. "Lock-and-key" model for drug-receptor interactions. Shown is example of estrogen receptor (ER) full agonist, partial agonist and antagonist. The key feature is conformational shape of the receptor and its ability to attract the co-activator complex (LXXLL motif) and regulate transcription.

The interaction between a ligand and its target receptor is not isolated; a compound may interact with multiple receptors, especially those that are phylogenetically related (see Figure 3). This phenomenon, known as polypharmacology, presents a challenge in drug discovery, as off-target effects may lead to adverse events. For example, a compound designed to interact with the retinoic acid receptor-related orphan receptor gamma (RORγ) may also interact with other nuclear receptors that share structural similarities, necessitating the evaluation of both on-target and off-target interactions.

Affinity, Efficacy, and Potency in Drug-Receptor Interactions. Not all binding events between a drug and a receptor result in the same biological outcome. Parameters such as binding affinity (the strength of the interaction), efficacy (the maximal biological effect), and potency (the

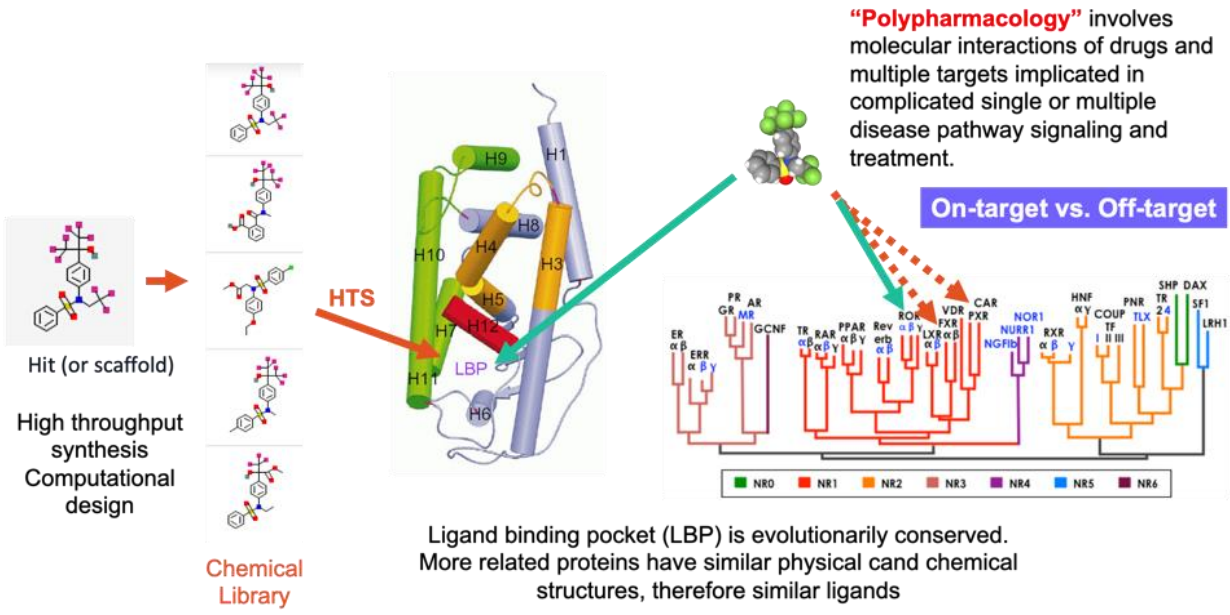


Figure 3. Concept of polypharmacology.

concentration of the drug required to elicit a specific effect) must be considered when evaluating potential drug candidates. Quantifying these parameters allows for the prioritization of candidates based on their predicted clinical relevance. However, the interaction between a drug and its target is only one aspect of drug efficacy; off-target interactions and systemic effects must also be evaluated to fully assess a candidate's therapeutic potential.

Affinity. Affinity refers to the strength of the interaction between a drug (ligand) and its target receptor. It is quantitatively expressed in terms of the dissociation constant (K_d), which represents the concentration of the ligand at which half of the available receptor sites are occupied. A lower K_d value indicates higher affinity, suggesting the drug binds more tightly to the receptor. Measurement of affinity can be influenced by several factors, including:

- **Chemical Structure**: Modifications in the chemical structure of a ligand can significantly impact its binding affinity. For instance, introducing functional groups may enhance hydrogen bonding or hydrophobic interactions, thereby increasing affinity.
- **Receptor Conformation**: Receptors exist in multiple conformational states, which can influence ligand binding. Allosteric sites, where ligands bind to sites other than the active site, can also modify the receptor's affinity for primary ligands by stabilizing specific conformations.

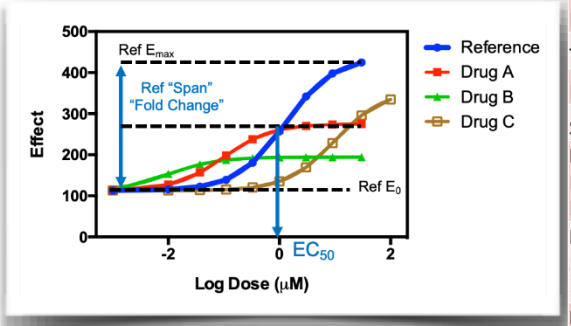
Efficacy. Efficacy describes the ability of a bound ligand to induce a biological response upon receptor activation. This parameter is often categorized as:

- **Full Agonists**: These compounds bind to a receptor and activate it to produce the maximum possible biological response. Full agonists stabilize the receptor in a conformation that promotes a robust signaling cascade.

- **Partial Agonists**: These ligands bind to the receptor but activate it to a lesser extent than a full agonist. Partial agonists can modulate receptor activity, acting as agonists in the presence of antagonists but exhibiting a reduced effect compared to full agonists.
- **Antagonists**: These molecules bind to the receptor without activating it, effectively blocking the receptor from being activated by other agonists. Antagonists can be competitive (competing with agonists for the same binding site) or non-competitive (binding to a different site and preventing receptor activation).
- **Inverse agonists:** Certain receptors have a certain amount of activity in the absence of a ligand, (also referred to as basal or constitutive activity). Ligands that reduce this basal level of activity in nuclear receptors are known as inverse agonists.

Potency. Potency refers to the amount of drug required to produce a specific effect. It is often assessed in terms of the effective concentration (EC_{50}) or effective dose (ED_{50}) at which 50% of the maximum response is achieved. A drug with high potency will elicit a therapeutic effect at a lower concentration than a drug with low potency. Potency is influenced by:

- **Affinity**: Higher affinity generally increases potency, as less drug is needed to achieve receptor occupancy and subsequent activation.
- **Efficacy**: Drugs with higher efficacy tend to demonstrate greater potency because they can produce a more substantial effect at lower concentrations.
- **Pharmacokinetics**: A drug's absorption, distribution, metabolism, and excretion (ADME) properties affect its effective concentration in the system. For instance, a drug that is rapidly metabolized may require higher doses to maintain efficacy.
- **Biological Variability**: Individual differences in physiology, receptor expression levels, and genetic factors can lead to variations in potency among different patients, necessitating personalized dosing strategies in clinical practice.



	Reference	Drug A	Drug B	Drug C
Bottom	113	112	103	113
Top	438	275	194	357
EC_{50}	1.2	0.1	0.01	10.0
Span	325	163	91	244
Relative Potency EC_{50}Ref/ EC_{50}Drug	1	12.81	153.3	0.13
Relative Efficacy Signal to Background	1	0.5	0.28	0.75
Relative Efficiency (Span/EC_{50})	1	6	43	0.09

Measures of affinity: K_d
Measures of activity:
Potency (EC_{50}, IC_{50}, AC50)
Efficacy (Peak/Span, Signal to Background)

Figure 4. Concepts of efficacy and potency. Although important for prioritizing hits and leads, more information is required to know the safety of each candidate.

Interrelation of Affinity, Efficacy, and Potency. While affinity, efficacy, and potency are distinct concepts, they are interrelated and collectively influence the therapeutic profile of a drug (Figure 4). For instance, a compound may have a high affinity for a receptor but low efficacy, resulting in high binding with limited therapeutic effect. Conversely, a drug with lower affinity but high efficacy might still demonstrate significant therapeutic benefits, albeit at higher doses. Understanding these parameters is essential for drug development. Researchers utilize *in vitro* assays to characterize these interactions, often employing computational models to predict how structural modifications can enhance affinity and efficacy while optimizing potency. Ultimately, achieving the right balance among these factors is crucial for the successful development of new therapeutic agents.

Challenges in Predicting Drug Toxicity.

A major obstacle in drug discovery is the unpredictability of adverse effects, particularly in preclinical models. While a cornerstone of preclinical testing, animal studies often fail to accurately predict human responses due to inherent species differences, leading to costly failures in later-stage clinical trials. This discrepancy underscores the need for improved screening methods to assess toxicity earlier in the drug discovery process, where the costs and time investments are lower. Screening methods that assess known toxicity pathways, such as nuclear receptor-mediated pathways, provide a valuable tool for identifying compounds with unfavorable safety profiles before advancing to more expensive preclinical and clinical stages (Figure 5). Such tools can be used for multiple applications such as prospective screening, signal generation screening, and retrospective or repurposing screening.

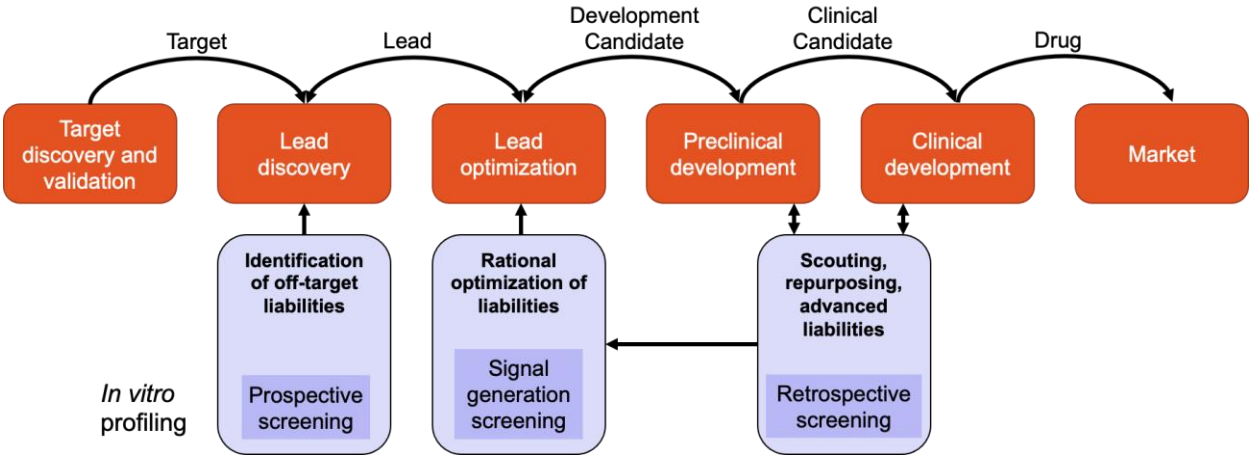


Figure 5. Use of cell-based assays in safety pharmacology including prospective, signal generation, and retrospective screening approaches.

Prioritization of Drug Candidates Through Prospective Screens. Cell-based reporter assays provide a robust platform for screening drug candidates based on their interaction with nuclear receptors and other transcription factors. These assays utilize engineered cells that express a reporter gene, such as luciferase, under the control of a nuclear receptor response element. Upon ligand binding to the nuclear receptor, the receptor activates the transcription of the

reporter gene, producing a quantifiable signal that reflects the receptor's activity. This technology allows for the high-throughput screening of large compound libraries to assess both on-target efficacy and off-target liabilities.

A prospective or triage screen is an approach used to eliminate leads that may cause toxicity later in the drug discovery process. Several cell-based assays examine molecular initiating events (MIEs) in important adverse outcome pathways (AOPs), as shown in Table 1.

Table 1. Important prospective screens involving NRs.

AOP	MIEs
Drug-drug interactions (DDI)	PXR, CAR, AhR
Endocrine disruption (EDC)	ERs, AR, PGR, MR, GR, THR
Steatosis	LXR, PPARG
Cholestasis	FXR, PXR, CAR, BSEP

For example, nuclear receptors such as PXR can be screened using these assays to evaluate a compound's potential to induce drug-drug interactions. PXR regulates the expression of enzymes involved in drug metabolism, such as cytochrome P450 3A4 (CYP3A4), which metabolizes approximately 50% of prescription drugs. Activation of PXR by a drug candidate suggests the potential for adverse drug-drug interactions,

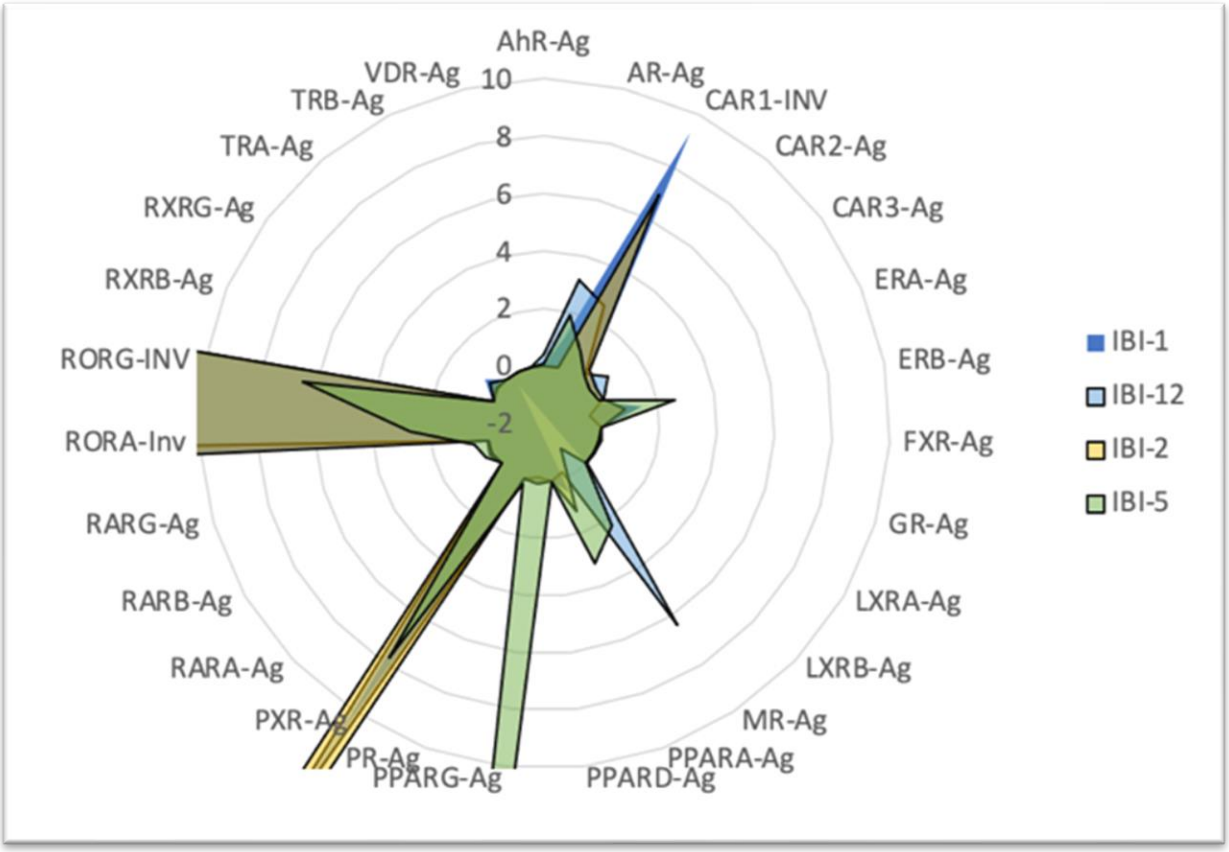


Figure 6. Transcription factor profiling. What off-target liabilities do your lead compounds exhibit

informing the decision to deprioritize or modify the compound. This information helps researchers deprioritize or modify problematic compounds early.

Signal Generation Screening. Safety pharmacology encompasses the evaluation of a compound's off-target effects and the biological relevance of these effects. Signal generation screens, including transcriptomics and transcription factor profiling (Figure 6), provide detailed insights into the molecular pathways activated by a drug. These screens can be applied early in drug discovery to prioritize compounds based on their selectivity and safety profile.

Polypharmacology refers to the phenomenon where a single drug interacts with multiple biological targets, significantly influencing its therapeutic efficacy and safety profile. In drug discovery, understanding polypharmacology is crucial for optimizing therapeutic outcomes while minimizing adverse effects. Nuclear receptor assays are a powerful tool for assessing polypharmacology by evaluating compounds' agonistic and antagonistic activities across related receptor families. By systematically screening compounds against a panel of nuclear receptors, researchers can identify not only the desired receptor interactions that confer therapeutic effects but also unintended activations or inhibitions that may lead to toxicity. For instance, a compound designed to activate a specific nuclear receptor selectively may also exhibit activity against other receptors within the same family, potentially resulting in off-target effects.

By thoroughly examining the interactions within a gene family, drug developers can prioritize candidates based on their comprehensive pharmacological profiles, facilitating the identification of compounds with favorable therapeutic indices and reduced risk of adverse events. This approach enhances safety pharmacology efforts by proactively addressing potential toxicities associated with polypharmacological interactions, ultimately leading to more effective and safer therapeutic agents.

Retrospective Screening and Drug Repurposing. Retrospective screening involves analyzing compounds that have progressed through preclinical or clinical stages but exhibit unexpected toxicities or other adverse effects. These screens aim to identify the molecular initiating events responsible for these outcomes, providing insight into the mechanisms underlying adverse effects. Species-specific differences in nuclear receptor activity can complicate the extrapolation of preclinical findings to humans, highlighting the need for human-specific assays.

In some cases, retrospective screening can reveal off-target activities that may be therapeutically beneficial, opening the door to drug repurposing. For example, a compound initially developed for its anti-inflammatory properties through interaction with RORγ may exhibit off-target anti-androgenic activity, suggesting its potential use in the treatment of prostate cancer. By repurposing existing compounds, researchers can bypass much of the early-stage development process, reducing costs and accelerating the timeline to clinical application.

Conclusion. Integrating nuclear receptor profiling and cell-based reporter assays into the drug discovery process addresses key efficacy, safety, and cost challenges. By screening for both on-target and off-target effects early in development, researchers can prioritize candidates with favorable safety profiles and reduce the risk of costly late-stage failures. Advances in high-throughput screening, coupled with a deeper understanding of nuclear receptor biology and polypharmacology, provide a powerful framework for optimizing drug discovery and developing safer, more effective therapies.

Citations

Vanden Heuvel, J.P., (2023). *Save Time and Money When Prioritizing Drug Candidates: using cell-based Receptor Assays for Nuclear Receptor Profiling* [Webinar]. Scientist.com. https://info.indigobiosciences.com/save-time-and-money-when-prioritizing-drug-candidates-using-cell-based-reporter-assays-for-nuclear-receptor-profiling

Yadav J, Maldonato BJ, Roesner JM, Vergara AG, Paragas EM, et al. 2024. *Enzyme-mediated drug-drug interactions: a review of in vivo and in vitro methodologies, regulatory guidance, and translation to the clinic*. Drug Metab Rev:1-33

Ma Q. 2008. *Xenobiotic-activated receptors: from transcription to drug metabolism to disease*. Chem Res Toxicol 21:1651-71

Pinne M, Raucy JL. 2014. *Advantages of cell-based high-volume screening assays to assess nuclear receptor activation during drug discovery*. Expert Opin Drug Discov 9:669-86

Manen-Freixa L, Antolin AA. 2024. *Polypharmacology prediction: the long road toward comprehensively anticipating small-molecule selectivity to de-risk drug discovery*. Expert Opin Drug Discov 19:1043-69

Peters JU. 2013. *Polypharmacology - foe or friend?* J Med Chem 56:8955-71