Small Molecule Lead Optimization to Increase Selectivity and Minimize Off-target Effects

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Conflict of Interest Statement

Yu (Zoe) Zhong is an employee at Genentech

Objectives/Outline

- Objectives: To share the rationale and approaches on how to increase selectivity and minimize off-target effects of small molecules – an important aspect of safety lead optimization

- Outline:
  - Why we care about off-target effects (secondary pharmacology) of small molecules
  - What are the drivers of secondary pharmacology and How to minimize it
  - How to contextualization the data
    - Case studies
  - Conclusions

Two main sources of toxicity for small molecules

On-Target

- aka ‘exaggerated pharmacology’
- Due to pharmacological engagement of the intended molecular target (primary pharmacology)
- Therapeutic index ~ 1
- **Strategy – Target Safety Assessment:** Does the potential on-target safety liability fit the indication (benefit/risk)?

Off-Target

- Due to **pharmacological engagement of unintended molecular target(s)**; and/or other non-pharmacological toxicity (e.g. membrane damage)
- Physicochemical characteristics-driven
- ADME-related
- **Strategy – Minimizing**

Understanding secondary pharmacology is required by ICH S7A

- Secondary pharmacology –

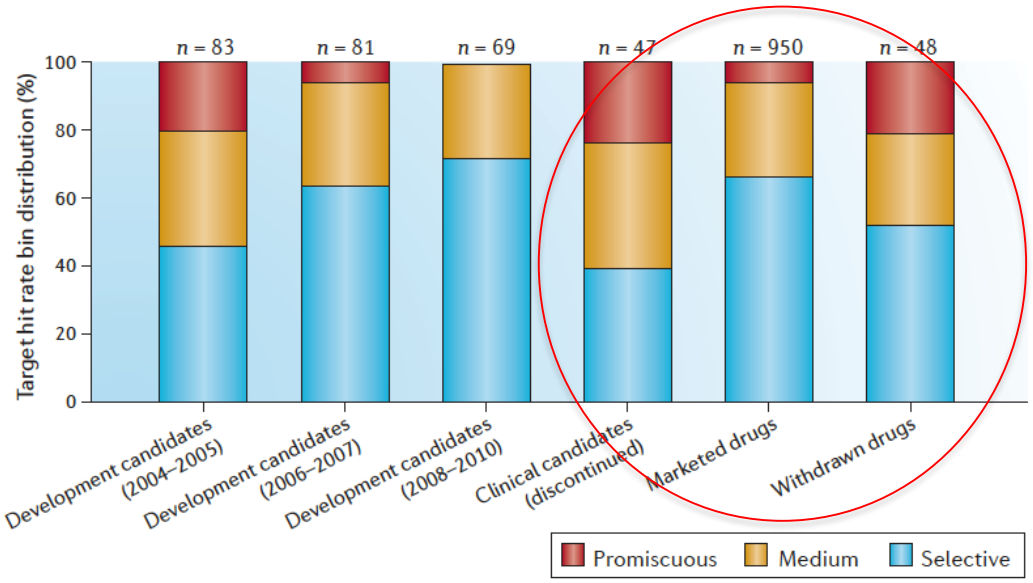
  ‘Studies on the mode of action and/or effects of a substance not related to its desired therapeutic target’

  - Near targets
  - Off-target pharmacological effects

- Data are included in regulatory filing
  - e.g. IND 2.6.2 Pharmacology section

Promiscuity is associated with greater toxicity and attrition

In Vitro Pharmacology Receptor Screens



Promiscuity index:

High: >20% targets with >50% inhibition

Medium: 5-20% targets with >50% inhibition

Low: <5% targets with >50% inhibition

Underlying assumption:

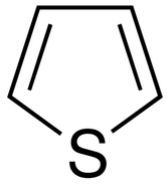
Compounds that bind numerous, unintended targets are associated with greater toxicity

- Marketed Drugs: 5% promiscuous & 66% selective
- Withdrawn or Discontinued Drugs: 20-24% promiscuous; 40-50% selective

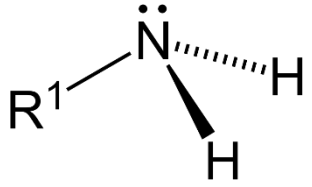
Bowes, et al. 2012. Nature Drug Discovery. 11:909

Physicochemical properties are fixed for each molecule and include the structural features and physical attributes

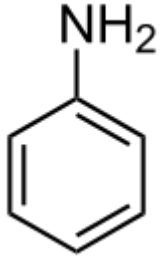
- Examples of physical attributes: molecular weight, lipophilicity (cLogP), acid-ionization constant (pKa), solubility, boiling & melting points, etc.
- Examples of structural features include substructures like thiophenes, anilines, basic amines, & acids, which determine the chemical interactions of a drug



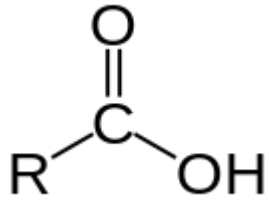
thiophene



Primary amine



aniline



Carboxylic acid

Physicochemical properties are the main drivers of off-target toxicity through multiple mechanisms

Lipophilic drugs	**Promiscuity** (non-selectivity), e.g. cardiovascular & Neuro **In Vivo Toxicity** (tissue accumulation)
Basic amines	**Promiscuity** **In Vivo Toxicity** (accumulation in tissues and acid organelles, e.g. phospholipidosis)
Reactive substructures	**Genetic Toxicity** **Phototoxicity** **In Vivo Toxicity** (interaction with macromolecules and cellular stress)
Acids	**Transporter substrates and/or inhibitors** (hepatic; renal) **Inhibition of enzymes** (hepatic/mitochondrial)

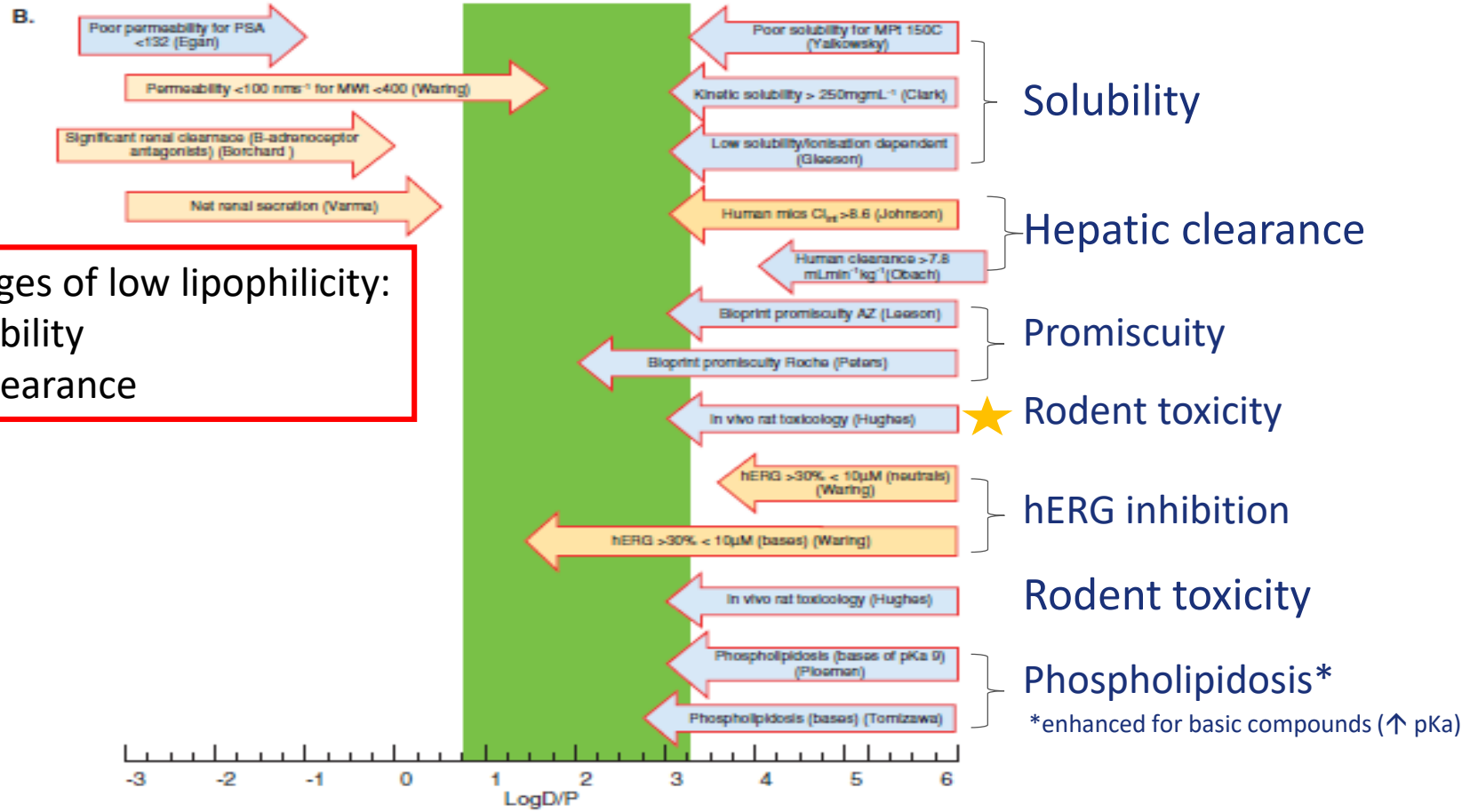
- We do not always understand the mechanism of toxicity; however, known mechanisms are evaluated for all small molecules

Donna Dambach

High lipophilicity is a risk factor for promiscuity and in vivo toxicity

Challenges of high lipophilicity:
A risk factor for solubility, metabolic stability, promiscuity (including hERG), tissue binding, in vivo toxicity

Challenges of low lipophilicity:
Permeability
Renal clearance



Solubility

Hepatic clearance

Promiscuity

Rodent toxicity

hERG inhibition

Rodent toxicity

Phospholipidosis*

*enhanced for basic compounds (↑ pKa)

Waring, et al. 2010. Expert Opin Drug Discov. 5:235

Increased lipophilicity is associated with toxicity in vivo

- Compounds with low-ClogP/high-TPSA: ~2.5 times more likely to be clean as to be toxic
- High-ClogP/low-TPSA compounds: ~2.5 times more likely to be toxic as to be clean, representing an odds ratio of greater than 6

Table 1
Observed odds for toxicity versus Clog*P*/TPSA

Toxicity	Total-drug		Free-drug	
	TPSA > 75	TPSA < 75	TPSA > 75	TPSA < 75
Clog *P* < 3	0.39 (57)	1.08 (27)	0.38 (44)	0.5 (27)
Clog *P* > 3	0.41 (38)	*2.4 (85)*	0.81 (29)	*2.59 (61)*

*In vivo rat tolerability study (>=4 days); Toxicity assessed at a specific exposure threshold (10 uM Cmax total drug)

TPSA - total polar surface area

Strategy:

1) Work with your chemists to optimize physchem properties; in general decrease lipophilicity, and basicity
2) Utilize receptor panels to understand the promiscuity

Hughes et al, 2008. Bioorganic Med Chem Lett 18: 4872-5.

Secondary pharmacology profiling strategy

- Off target pharmacology can be observed/measured in a variety of systems
  - In vitro recombinant/native cell lines (binding/functional) ← Initial screen
  - In vitro / Ex vivo tissue bath studies
  - In vivo animal studies

  } Tissue/in vivo translation for contextualization of the in vitro finding
- Panels
  - General panels with selected targets important for CNS, CV, GI safety (kinases, G protein-coupled receptors, ion channels, transporters, nuclear receptors and enzymes)
  - Target-specific panels to examine near targets (e.g., kinase panel, protease panel, ion channel panels for respective primary targets in that class)
- Adjust based on primary target, indication, chemical space, company experience

Example: General secondary pharmacology panel

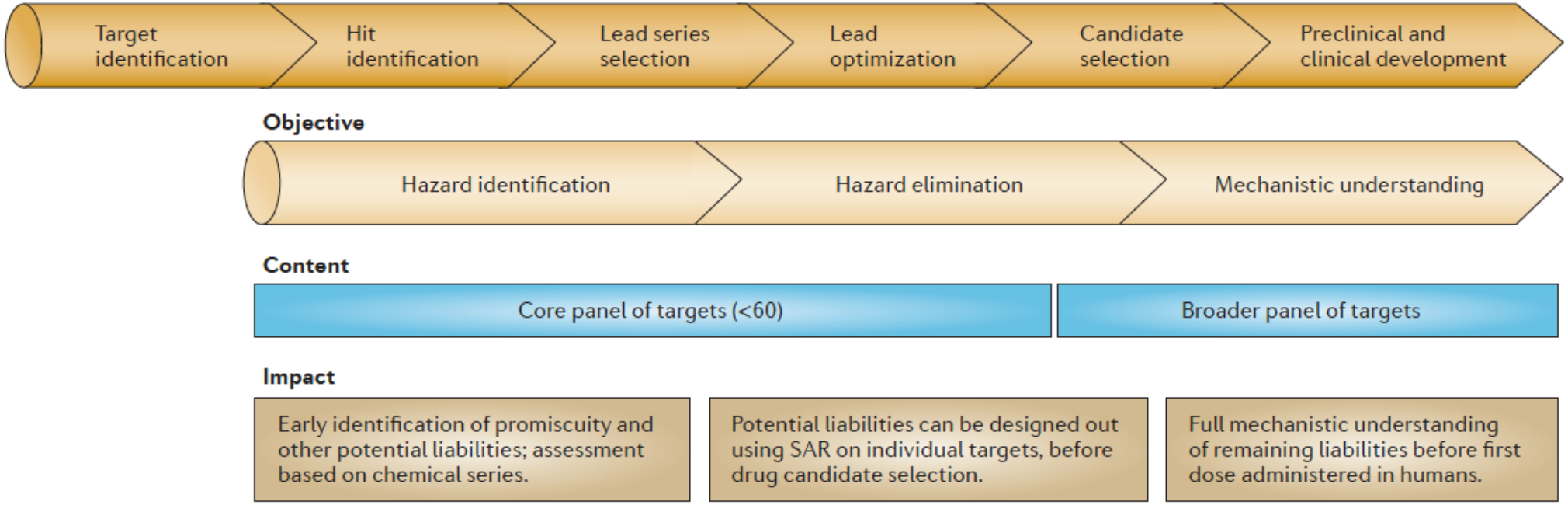
Table 1 | **Recommended targets to provide an early assessment of the potential hazard of a compound or chemical series**

Targets (gene)	Hit rate*		Main organ class or system	Effects		Refs§
	Binding	Functional or enzymatic		Agonism or activation	Antagonism or inhibition	
G protein-coupled receptors						
Adenosine receptor A_{2A} (*ADORA2A*)	High	Low (agonist)	CVS, CNS	Coronary vasodilation; ↓ in BP and reflex; ↑ in HR; ↓ in platelet aggregation and leukocyte activation; ↓ in locomotor activity; sleep induction	Potential for stimulation of platelet aggregation; ↑ in BP; nervousness (tremors, agitation); arousal; insomnia	57
α_{1A}-adrenergic receptor (*ADRA1A*)	High	Low (agonist); high (antagonist)	CVS, GI, CNS	Smooth muscle contraction; ↑ in BP; cardiac positive ionotropy; potential for arrhythmia; mydriasis; ↓ in insulin release	↓ in smooth muscle tone; orthostatic hypotension and ↑ in HR; dizziness; impact on various aspects of sexual function	58
α_{2A}-adrenergic receptor (*ADRA2A*)	High	Low (agonist); medium (antagonist)	CVS, CNS	↓ in noradrenaline release and sympathetic neurotransmission; ↓ in BP; ↓ in HR; mydriasis; sedation	↑ in GI motility; ↑ in insulin secretion	59
β_1-adrenergic receptor (*ADRB1*)	Medium	NA	CVS, GI	↑ in HR; ↑ in cardiac contractility; electrolyte disturbances; ↑ in renin release; relaxation of colon and oesophagus; lipolysis	↓ in BP; ↓ in HR; ↓ in CO	60
β_2-adrenergic receptor (*ADRB2*)‡	High	Medium (agonist); medium (antagonist)	Pulmonary, CVS	↑ in HR; bronchodilation; peripheral vasodilation and skeletal muscle tremor; ↑ in glycogenolysis and glucagon release	↓ in BP	61
Cannabinoid receptor CB_1 (*CNR1*)	Medium/ high	Medium (antagonist)	CNS	Euphoria and dysphoria; anxiety; memory impairment and poor concentration; analgesia; hypothermia	↑ in weight loss; emesis; depression	62
Cannabinoid receptor CB_2 (*CNR2*)	Medium	Medium (agonist)	Immune	Insufficient information	↑ in inflammation; ↓ in bone mass	63

Bowel et al. (2012) *Nat Rev Drug Discov. 11: 909.*

Alignment of secondary pharmacology profiling to the drug discovery and development process

- Early lead identification: Hazard identification of initial lead series; influence SAR, and compound optimization
- Candidate selection: Risk assessment – functional follow up, safety margin estimation
  - Sufficient safety margin to $hC_{max,\ free}$ at efficacious exposure?
- Investigation: Contextualization of in vivo non-clinical / clinical findings
  - Can in vivo findings be explained by coverage of the off-target?



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Interpretation and contextualization of binding hits

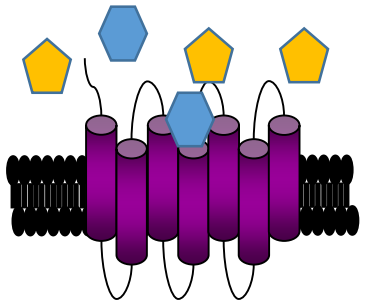
- **Promiscuity**:
  - Measured as: ratio (%) of targets with ≥50% binding inhibition over total N of assays at 10 μM
  - A measure of propensity of a molecule to bind other targets
  - Promiscuity in a small panel is a surrogate for promiscuity across proteins in the body

Promiscuity index:
High: >20% targets with >50% inhibition
Medium: 5-20% targets with >50% inhibition
Low: <5% targets with >50% inhibition

- **Selectivity:**
  - Measured as: targets with ≥75% binding inhibition in radioligand binding assays
  - Evaluate each target “hit” for potential functional translation and in vivo implication

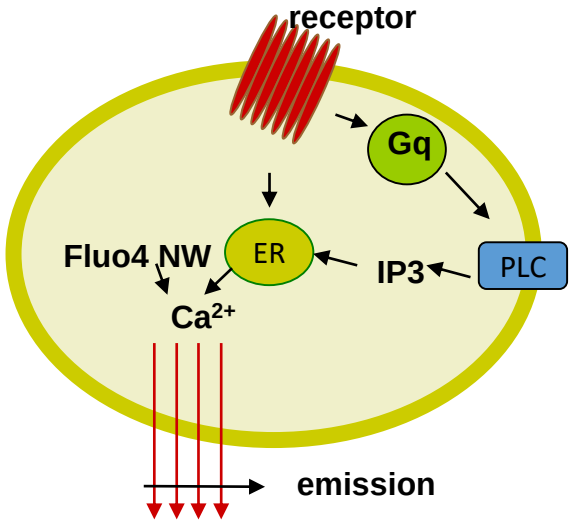
Follow-up for binding hits: Determining functional translation and in vivo relevance

Binding Assay



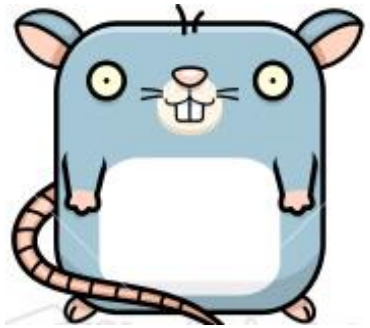
- Direct measure of affinity
- Single, defined site of binding only on the target
- No differentiation of modes of action
- Multiple binding sites on one target

Functional Assay



- Functional Translation - end result of binding at any site on the target
- **Agonist or Antagonist**

In vivo



Assumptions:

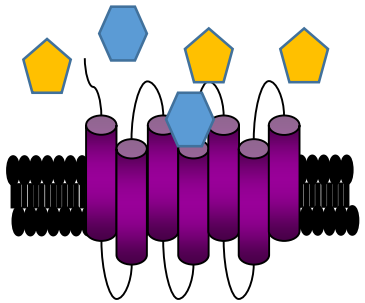
- Free Drug Hypothesis: only free (unbound) drug is available to interact with the target
- Free Cmax frequently used as a relevant (and more conservative) measure of drug exposure

Dolo Diaz

Follow-up for binding hits: Determining functional translation and in vivo relevance

Decision making:
- How many off-targets are affected?
- Which off-targets are affected?
- What is the safety margin?

Binding Assay



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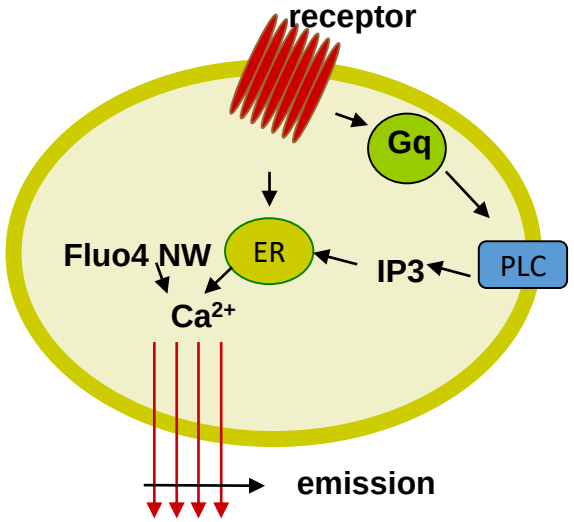

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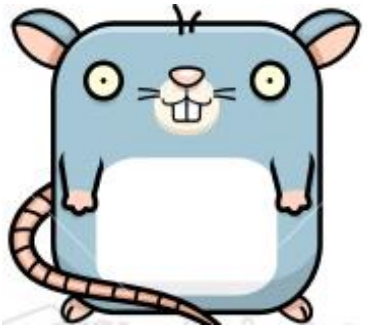

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Dolo Diaz

Case study: Syncope observed in dogs with a small molecule - consistent with alpha adrenergic receptor blockade

In Life Observations (PK study GNE#1 at 1 mg/kg intravenously in dogs):

- Hypoactive at 2 min post dose and could not stand up (4 min post dose)
- Heart rates doubled - 115-121 bpm (baseline) to 200-208 bpm (~12 min post dose)
- Heart rate and normal activity recovered by ~ 45 - 58 min post dose

* Binding does not discriminate agonists vs antagonists; ** nonspecific subtypes that do not indicate subtypes

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- Tachycardia is a normal physiologic response to hypotension in attempt to maintain blood pressure (BP)
  - Alpha adrenergic receptors maintain normal BP
- GNE#1 potently antagonizes alpha 1a receptors (IC_{50} = 86 nM)
  - Unbound plasma GNE#1 at C_0 (1.2 uM) is ~14x higher than IC50
  - Recovery could be based on cleared drug or compensation to raise BP
- GNE#2 was tolerated in dogs without similar clinical effects
  - GNE#2 is ~10x less potent at the alpha 1a receptor and
  - Unbound plasma GNE#2 at C_0 ~40 nM is 21x below the alpha 1a IC_{50}.
- Weaker inhibition observed at alpha 1b, but not other alpha receptors

Receptor Binding Data* (% displacement of ligand at 10 uM)		
	GNE#1	GNE#2
alpha1**	82	75
alpha2**	62	39
Functional Assay - IC50 (nM)		
Receptor	GNE#1	GNE#2
alpha1a	86	840
alpha1b	4700	920
alpha2a	32000	37000
alpha2b	29000	15000
alpha2c	-	71000

* Binding does not discriminate agonists vs antagonists; ** nonspecific subtypes that do not indicate subtypes

Eric Harstad

Selectivity on near targets important for safety margin considerations

- Target-specific
- Between primary pharmacological target and its homologues/isoforms
- Need to be considered early in project so assays can be in place for proactive lead optimization
- In vivo therapeutic index maybe smaller than in vitro selectivity, e.g.
  - IC_{75} or IC_{90} for efficacy vs. IC_{50} or IC_{20} for toxicity
  - C_{trough} needed for efficacy vs. C_{max}-driven toxicity
- Assess off-target risks in the context of expected in vivo exposures (free) to understand potential therapeutic index

Conclusions

- Off-target pharmacology (secondary pharmacology) and promiscuity can be a significant safety attrition source
- Strategies to minimize promiscuity and enhance selectivity can be achieved by
  - Optimize the physicochemical properties of a molecule, avoid known structural alerts
  - Measure secondary pharmacology on near targets and unrelated targets
    - *In vitro* ligand binding and cell-based function assays for hazard identification, and risk assessment
- Pay special attention to near targets where selectivity might be challenging
- Contextualize in vitro selectivity data with in vivo findings, taking into account unbound exposure at efficacious dose range

Reference

- Bowel et al. 2012. *Nat Rev Drug Discov.* 11: 909
- Hughes et al. 2008. *Bioorganic Med Chem Lett*. 18: 4872
- Waring et al. 2010. *Expert Opin Drug Discov*. 5: 235

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- Satoko Kiyota

Abbreviations

- ICH - The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
- IND - investigational new drug application
- LLE - ligand-lipophilicity efficiency
- CNS - central nervous system
- CV - cardiovascular
- GI - gastrointestinal
- SAR - structure activity relationship
- Cmax - maximum (or peak) serum concentration of a drug
- Ctrough - lowest concentration of a drug
- hERG - the human Ether-à-go-go-Related Gene, codes Kv11.1, the alpha subunit of a potassium ion channel
- cLogP - Calculated logP value of a compound, which is the logarithm of its partition coefficient between n-octanol and water log(coctanol/cwater), a well established measure of the compound's hydrophilicity
- TPSA - topological polar surface area of a molecule
- BP - blood pressure
- IC_{50} - the concentration of an inhibitor that corresponding to 50% of maximum inhibition effect