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WuXi AppTec

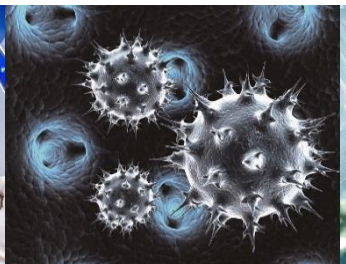
Research Service Division (RSD)
Enabling Unbounded Possibilities

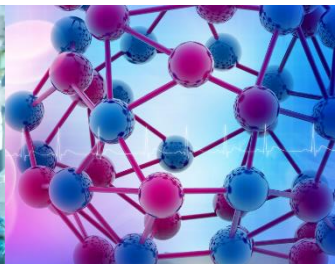
WuXi AppTec
Early *In Vitro* Mini Safety Panel

Comprehensive *In Vitro* Mini Safety Panel Service

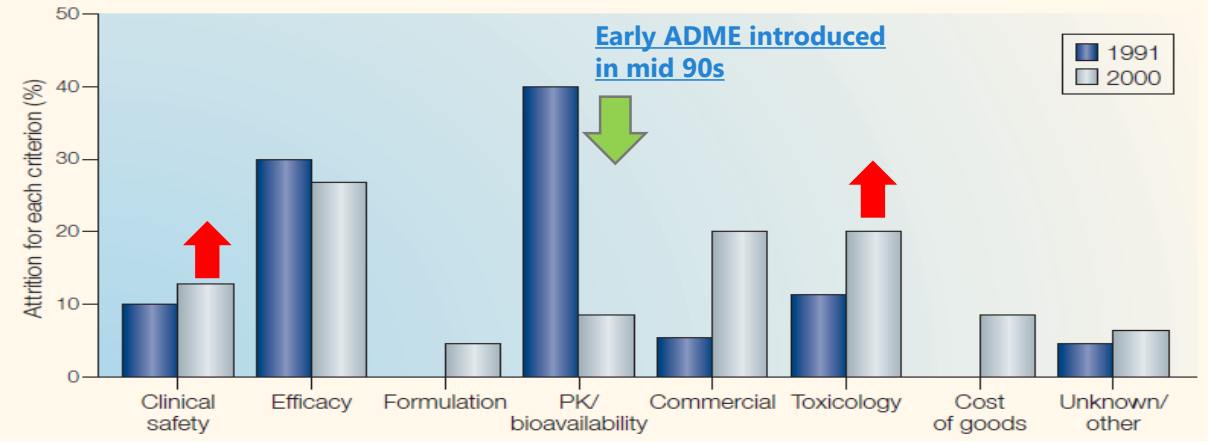
www.wuxiapptec.com







Drug Attritions, Safety and ADR



- Safety (Clinical safety & Toxicology) is one of the major causes of attrition in the clinic (I.Kola and J.Landis, Nature Rev. Drug Discov. 3,711 (2004))
- Adverse Drug Reactions (ADRs) are estimated to be the fourth leading cause of death in the USA (Lazarou et al., JAMA 1998; 279:1200)

Drugs Withdrawn Due to Adverse Drug Reactions (ADRs)

Drugs withdrawn from market	Drug target Mode	Treatment of disease	Time (Yr) (market/withdrawn)	Adverse Drug Reactions	Molecular targets
Fenfluramine (Pondimin)	5HT reuptake inhibitor	Anti-obesity drugs	1973/1997	Heart valve disease, pulmonary hypertension, cardiac fibrosis	5HT2B
Dexfenfluramine (Redux)					
Astemizole (Hismanal)	H1 antagonist	Antihistamine drug	1977/1999	QTc interval prolongation and arrhythmias	hERG
Terfenadine (prodrug)	H1 antagonist	Antihistamine drug	1985/1998		
Grepafloxacin	DNA gyrase inhibitor	Antibacterial drug	1997/1999		
Cisapride	5HT4 agonist	Gastroprokinetic agent	1980/2000s		
Rapacuronium (Raplon)	nAchR blocker	Neuromuscular blocking agent	2000/2001	Fatal bronchospasm	M2
Phencyclidine (PCP)	NMDA receptor antagonist	Anesthesia drug	1950s/1965	Hallucinations, mania, delirium, and disorientation	nAChR, D2
Rofecoxib (Vioxx)	COX2 inhibitor	Nonsteroidal anti-inflammatory drug	1999/2004	Heart attack and stroke	COX2

In Vitro WuXi Mini Safety Panel

Target selection

- **Cross-pharma knowledge**
  - AZ, GSK, Novartis, Pfizer
  (Bowes J. et al. Nat Rev Drug Discov. 2012)
- **44 Targets**
  - 24 GPCRs
  - 8 ion channels
  - 7 enzymes
  - 3 monoamine transporters
  - 2 nuclear hormone receptors

Application

- Off target interaction profiling
- Early safety risk assessment
- Reducing safety-related drug attrition
- Vital screening in pre-clinical R&D and for IND filing
- Widely accepted and utilized by pharma

WuXi mini safety panel

- **Sources of Targets for assays**
  - Human origin of targets overexpressed in stable cell lines
  - Recombinant human enzymes
  - Rat cerebral cortex (2 Channels for binding)
  - Cancer cell lines (AR and GR for binding
- **Assay format**
  - Function assays: FLIPR (Ca); reporter gene assay
  - Biochemical assay: Enzymatic assay
  - Radioligand binding assay (Plate filtration assay)

Why WuXi?

- Both binding and functional assays
  - More information
  - Fast follow-up
- Faster: turnaround time; no international shipping for SAR programs run in China
- Cost effective
- Flexible in target selection; expendable to other assay formats, eg. cAMP, IP1
- Enabling IND filing: documentation and on-site inspection in China

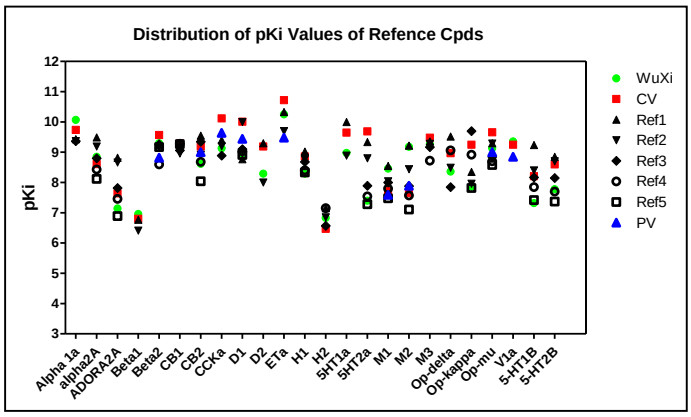
WuXi Mini Safety Panel Targets, Affecting Organ Class or System and Assay Methods

GPCRs	Receptor (Human)	Main organ class or system	Radioligand Binding	Function Assay (Ca^{2+} Assay)
Adenosine receptor	Ad2A	CVS, CNS	√	√
Adrenergic receptors	Alpha1A	CVS, GI, CNS	√	√
	alpha2A	CVS, CNS	√	√
	Beta1	CVS, GI	√	√
	Beta2	Pulmonary, CVS	√	√
Cannabinoid receptor	CB1	CNS	√	√
	CB2	Immune	√	√
Cholecystokinin receptor	CCKa	GI	√	√
Dopamine receptors	D1	CVS, CNS	√	√
	D2	CVS, CNS, endocrine	√	√
Endothelin receptor	ETa	CVS, development	√	√
Histamine receptor	H1	CVS, immune	√	√
	H2	GI, CVS	√	√
5-Hydroxytryptamine receptor	5HT1A	CNS, endocrine	√	√
	5HT1B	CVS, CNS	√	√
	5HT2A	CVS, CNS	√	√
	5HT2B	CVS, pulmonary, development	√	√
Muscarinic receptor	M1	CNS, GI, CVS	√	√
	M2	CVS	√	√
	M3	GI, pulmonary	√	√
Opioid receptor	Op-delta	CNS, CVS	√	√
	Op-kappa	GI, CNS, CVS	√	√
	Op-mu	CNS, GI, CVS	√	√
Vasopressin receptor	V1a	Renal, CVS	√	√
Channels	**Ion Channel (human or rat)**	**Main organ class or system**	**Radioligand Binding**	**Function Assay (Manual patch clamp)**
Ca^{2+} Channel	L-Ca^{2+} channel	CVS	√	√
K^+ Channel	hERG	CVS	√	√
	Kv or hKCNQ1+MinK	CVS	√	√
Na^+ channel	hNav1.5	CVS	na*	√
Serotonin	5-HT3a	GI, endocrine	√	√
Nicotinic	nAChR, α7	CNS, CVS, GI, pulmonary	√	√
GABA	$GABA_AR$, α1	CNS	√	√
Glutamate	NMDAR	CNS	√	na*
Transporter and NR	**Transporter NHR (human)**	**Main organ class or system**	**Radioligand Binding**	**Function Assay**
Transporter	DAT	CNS	√	Dye Uptake
	5HTT	CNS, CVS	√	Dye Uptake
	NET	CNS, CVS	√	Dye Uptake
Steroid Nuclear Receptors	AR	Endocrine	√	Reporter gene assay
	GR	Endocrine, immune	√	Reporter gene assay
Enzymes	**Enzyme (human)**	**Main organ class or system**	**Function (Assay Type)**	
Protein-Tyrosine Kinase - CTK	LCK	Immune	HTRF	
Phosphodiesterase	PDE3A	CVS	HTRF	
	PDE4D	CNS, immune	HTRF	
Cyclooxygenase	COX1	GI, pulmonary, renal	Fluorescence	
	COX2	Immune, CVS	Fluorescence	
Monoamine & NRT Synthesis & Metabolism	ACHE	CVS, GI, pulmonary	Optical Density	
	MAO-A	CVS, CNS	Luminescent	

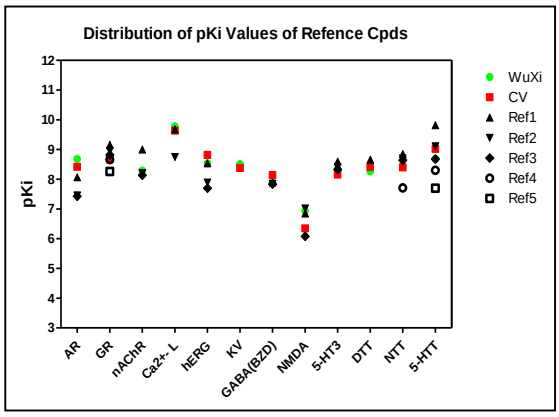
*na: not available

Cross Lab Data Comparison: Radioligand Binding Assay

GPCRs



NR, Channels and Transporters

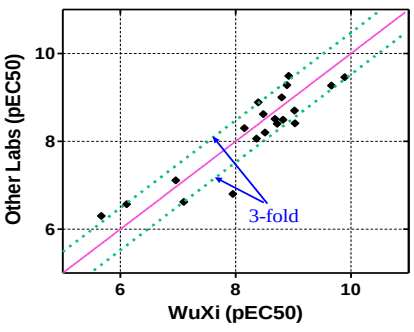


CV, PV: vendor 's values; Ref: values in publications

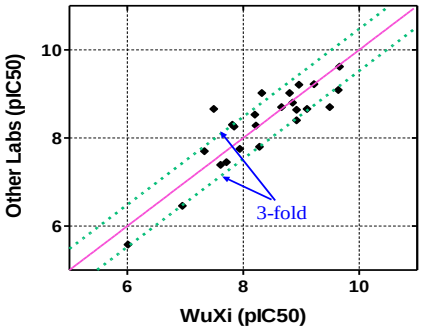
- WuXi and public data of binding affinities (pKi) of reference compounds against GPCRs, NRs, channels and transporters are plotted for their distribution to show data correlation.
- WuXi data are well in the range of the published values, indicating good data correlations among labs.

Cross Lab Data Comparison: Safety GPCR Function Assay

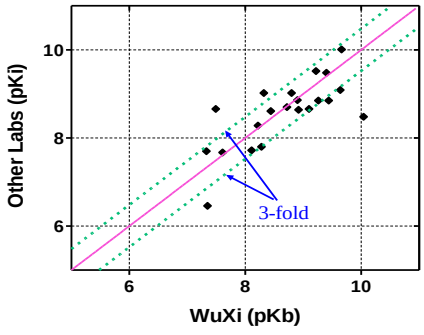
Agonists



Antagonists



Binding Affinity



- Values of pEC50, pIC50 and pKi/pKb are analyzed for correlations between WuXi and other reputable labs' data for same reference compounds
- Strong correlations are observed not only for pEC50 or pIC50 values but between Kb calculated from IC50 in function assays and Ki values obtained from radioligand binding assays as well.

Testing Format, Timeline, Data Delivery

- **Testing format**
  - 96- or 384-well format
  - Single concentration in duplicate (10 uM in general)
  - 8-10 dose response curves (as follow-up) to confirm positive hits (potency > 50% activation or inhibition comparing to that of control)
- **Assay timeline**
  - 15 days
- **Data delivery**
  - Excel or word report
  - Assisting CFDA on-site inspection

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