Computational Systems Toxicology and in silico prediction of off-target activities. Case studies and discussion.

SANOFI

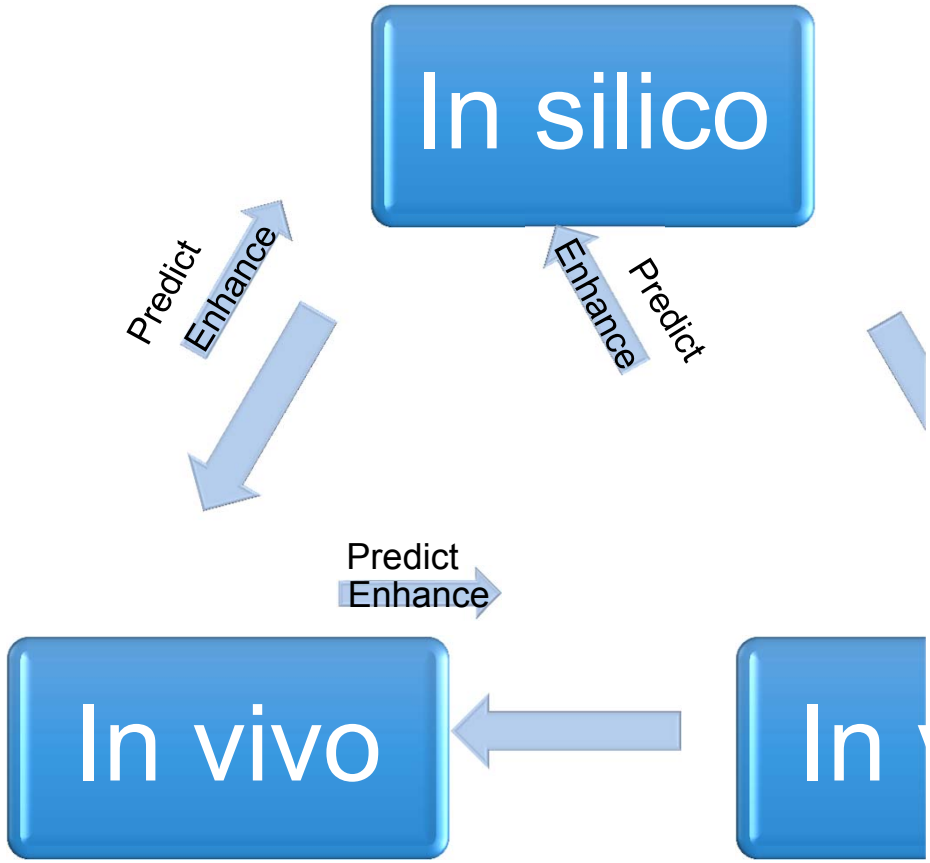
EUFEMED 2017, London, UK

Friedemann Schmidt

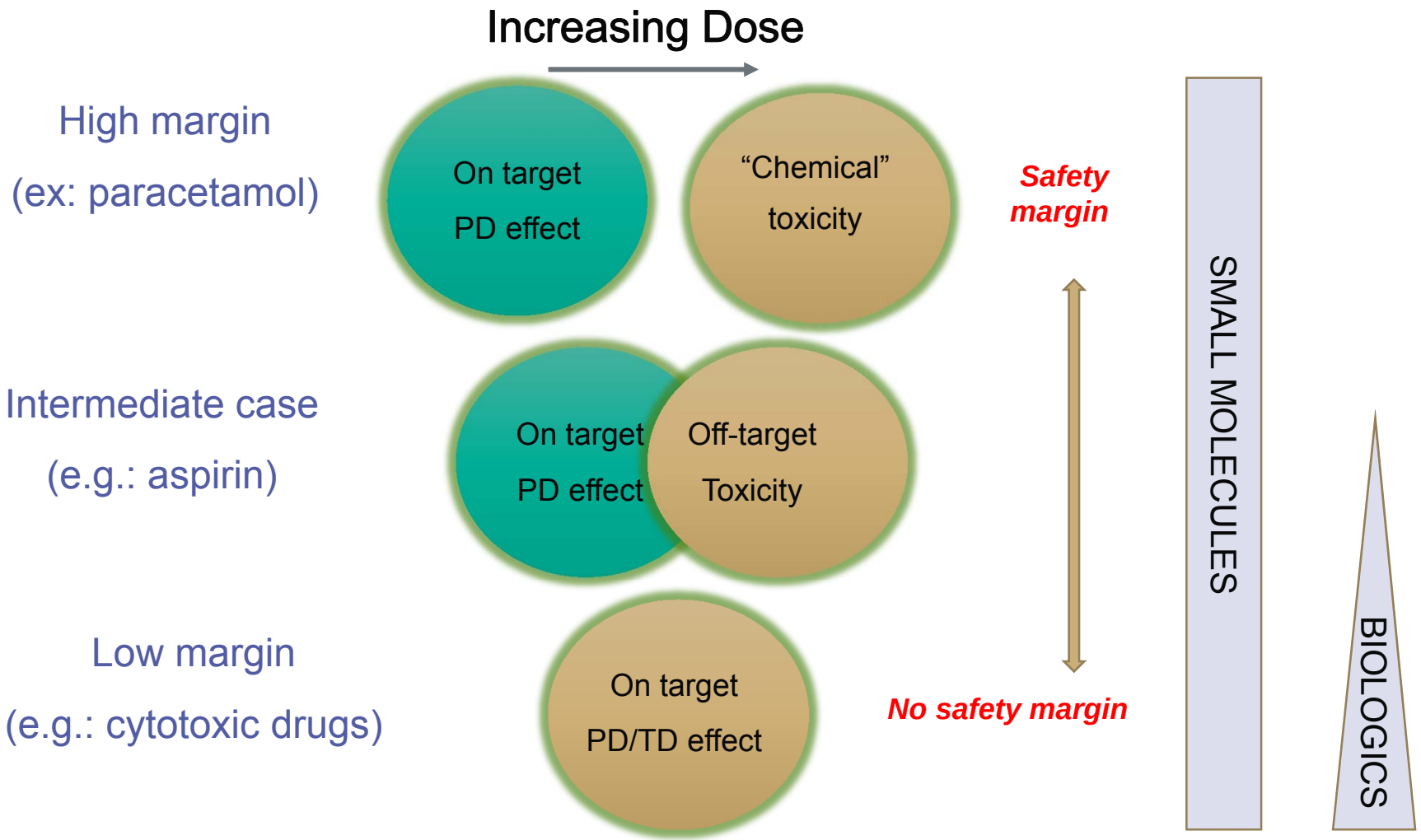
Outline

- Computational Systems Toxicology: Between data mining and mathematical modelling

- Advancing Confidence in Compounds and Prediction Tools. Application Examples.



The main origin of toxicities

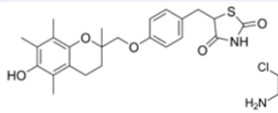


Drug Off-Target Effects & Market Withdrawals

2000-2001

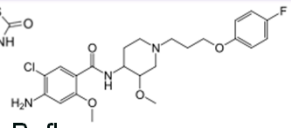
Alpidem
Troglitazone
Alosetron
Cerivastatin
Tegaserod
Sparfloxacin
Rapacuronium
Ardeparin

Troglitazone



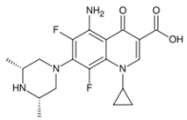
Diabetes
PPARa, PPARg
Hepatotoxicity
FABP4, MRP2, BSEP

Cisapride



Reflux
5-HT4 ag.
Torsade de points
5-HTR2A/2B/2C/3A, DRD3, ADRA1D, KCNH2

Sparfloxacin

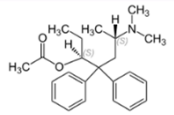


Antibiotic
DNA gyrase, TOP2A
QT syndrome, Phototox
KCNH2, ABCB1

2003-2004

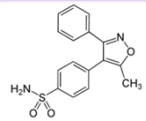
Levomethadyl
Rofecoxib
Benzitramide
Dofetilide
Valdecoxib

Levomethadyl



Dependence
OPRM1/D1/K1
QT-prolongation
CHRNA3, CHRNB4, SLC6A4, KCNH2

Valdecoxib

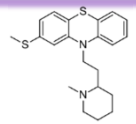


NSAID
PTGS2
Heart attack, stroke
CA2/5B/9/12/14

2005-2006

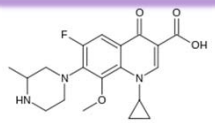
Adderall
Hydromorphone
Thioridazine
Ximelagatran
Gatifloxacin
Alatrofloxacin
Trofloxacin

Thioridazine



Antipsychotic
5-HTR1A/2A/2C, SLC6A2/3/4, DRD2/3
Hepatotoxicity, Cardiotoxicity
KCNH2, CHRM4/5, ADRA1A/2C

Gatifloxacin

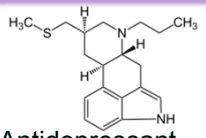


Antibiotic
DNA gyrase, TOP2A
Dysglycemia
Glucagon, AKT1

2007-2008

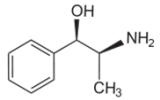
Aprotinin
Clobutinol
Nefazodon
Tegaserod
Lumiracoxib
Pergolide
Propagest
Rimonabant
Veralipride

Pergolide



Antidepressant
DRD2/3/4/5
Valvular degeneration
5-HTR1A/2A/2B/1D, ADRA2A/2B

Propagest

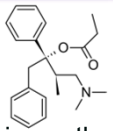


Anorexiant, stimulant
SLC6A2/3
Stroke
ADRA1A/2A/B1 , MAOA, DRD1, MIF

2009-2010

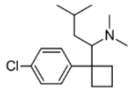
Isoretinoin
Efalizumab
Benfluorex
Propoxyphene
Gemtuzumab
Sibutramine
Sitaxentan
Pemoline
Bufexamac
Rosiglitazon

Propoxyphene



Analgesic, restless legs
OPRM1/D1/K1
Cardiovascular depression
INa, CYP3A4/2C9/2D6/2C8

Sibutramine

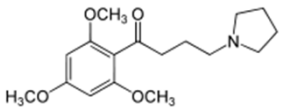


Anorexiant
SLC6A2/3/4
Cardiovascular, renal failure
ADRA2B, KCNC1/H2, CYP3A4

2011-

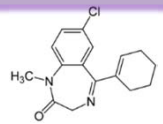
Buflomedil
Drotrecognin
Tetrazepam

Buflomedil



Vasodilation
ADRA1A/2A
Cardiac toxicity
Sigma1, EMP, ERG2

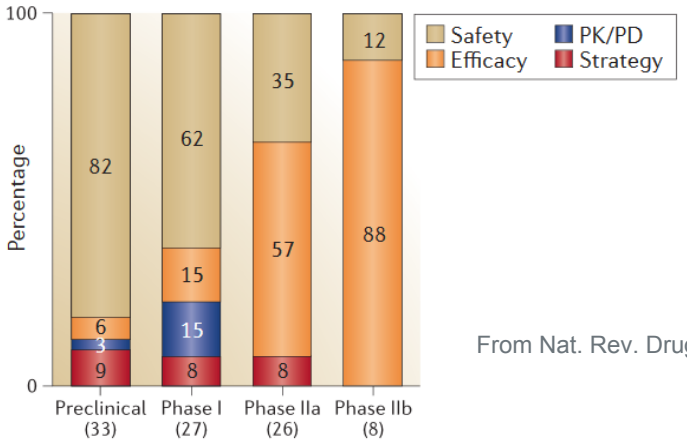
Tetrazepam



Anticonvulant
GABAA,GABAB
Cuteanous toxicity
TSPO, DBI

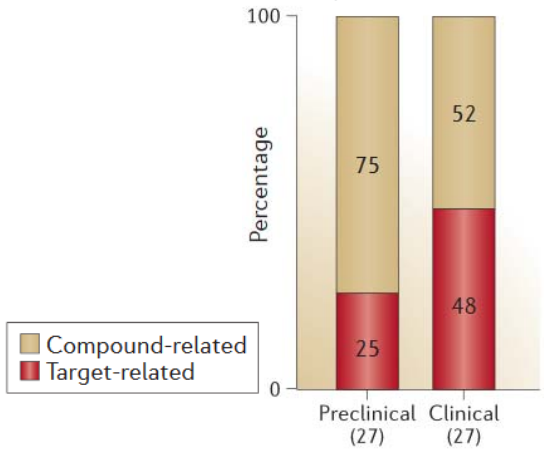
Safety Remains a Cause of Attrition

Project Termination, AZ 2005-2010

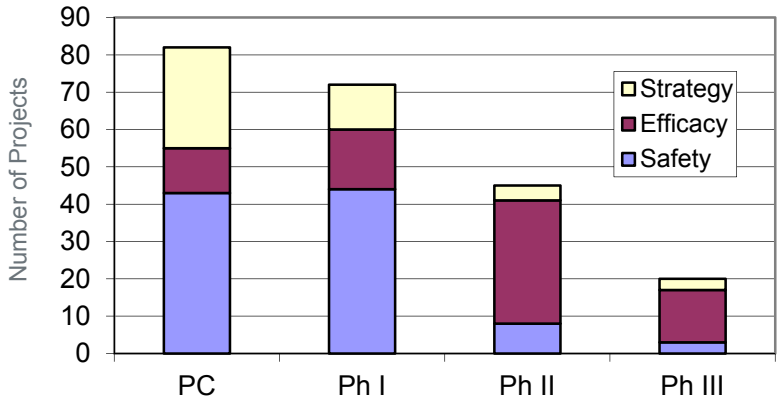


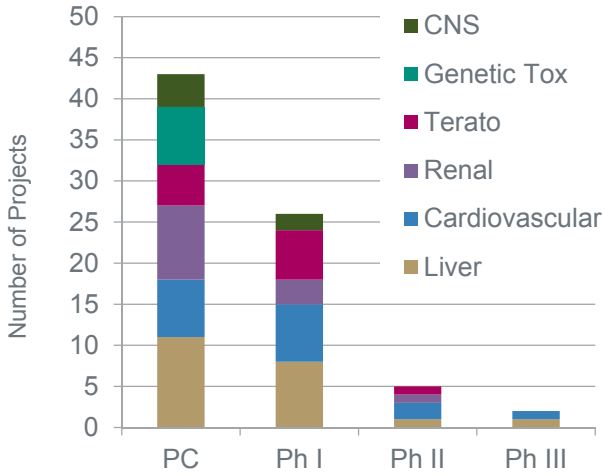
From Nat. Rev. Drug Discov. 10, 749–765 (2014)

Safety failures



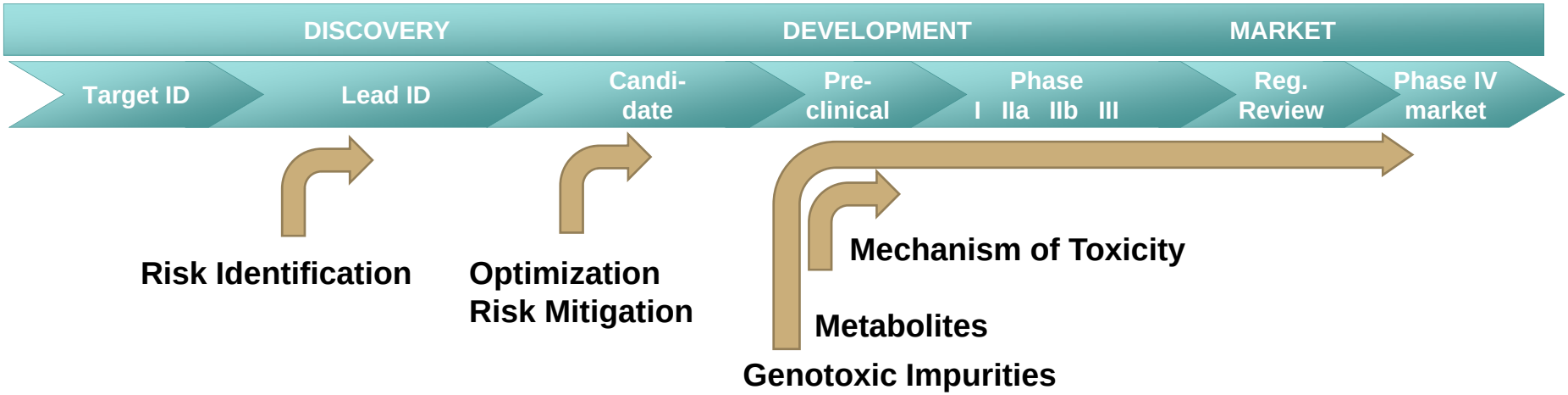
Project Termination, Sanofi 2000-2010



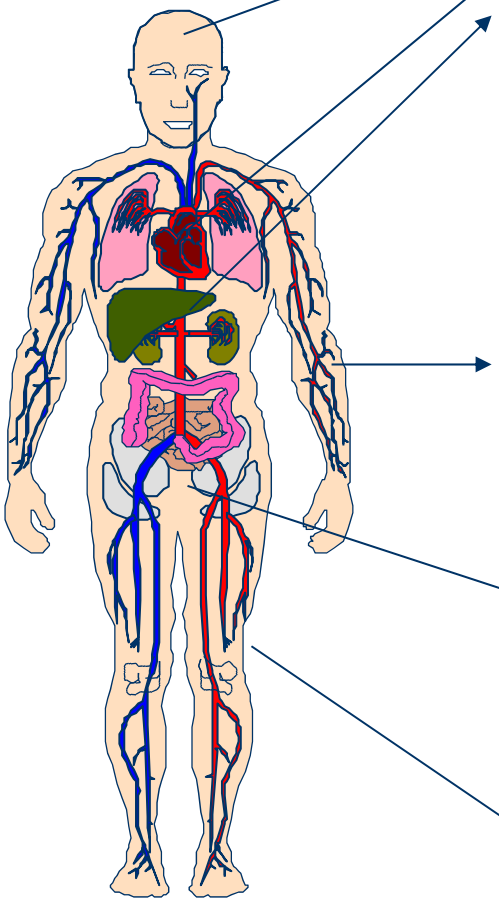


Computational Toxicology

- Drivers to improve current computational/systems toxicology capabilities
  - Low/no physical availability of substances
  - Cost of experiments
  - Speed / turnaround time
  - Reduction of (animal) experiments
  - Generating hypothesis for MoT → design of in vitro and in vivo screening strategy



Systems Toxicology is leveraged by a broad range of computational approaches



Effects on Major Functions (Safety Pharmacology)

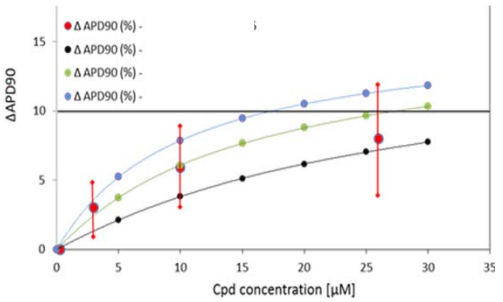
Genetic Toxicity

Carcinogenicity

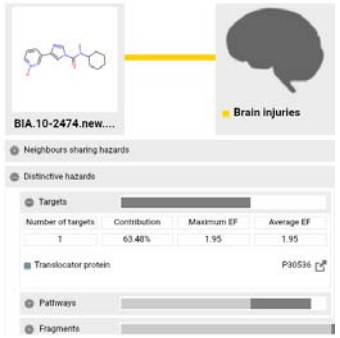
Development & Reproductive Toxicity

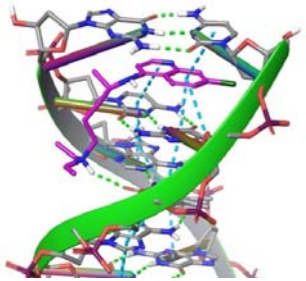
Local Toxicity
Immunotoxicity
Phototoxicity …

Multiparametric simulation

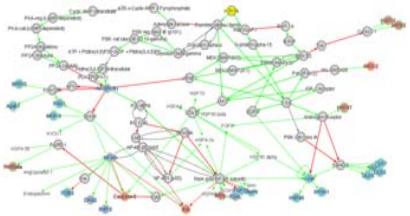


QSAR & Read-across

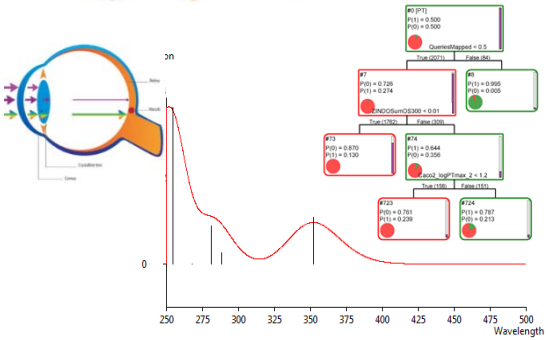




Drug-DNA interaction



Causal target networks



Time-dependent simulation

Basic Technology: Quantitative Structure-Property Relationship: QSPR

Toxicity = f(chemical structure)

$$\text{Toxicity} = c_1 * \text{descriptor}_1 + \ldots + c_k * \text{descriptor}_k + c_0$$

Typical descriptors:

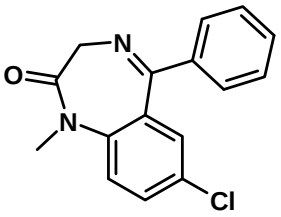
- lipophilicity, H-bonding capacity,
- static or dynamic polar surfaces (PSA)
- free energy of solvation
- properties from *ab initio* calculations
- CoMFA, CoMSIA fields

Goal: Correlate compound properties with interpretable molecular features (2D / 3D)

WIREs Comput Mol Sci 2016, 6:147–172.

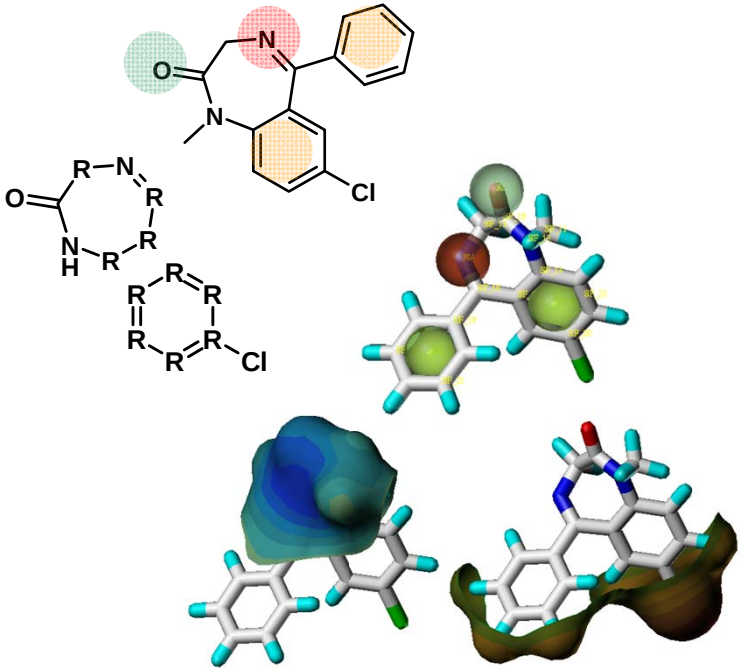
Technology: Molecular Descriptors for Chemical Structures

- Descriptors are provided by chemometrics software, such as DRAGON, MOE, PipelinePilot, MoKa, …
- Global descriptors
  - Atom & bond counts, indices
  - 1D descriptors
    - Molecular weight, physico-chemical properties
    - Diameter, dipole moment, pka, charge
- 2D descriptors
  - Fragments, pharmacophoric indices, topology
- 3D descriptors
  - volume, partial surface area,
  - pharmacophoric features



Diazepam

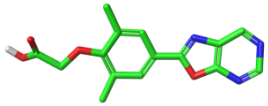
$C_{16}H_{13}ClN_2O$

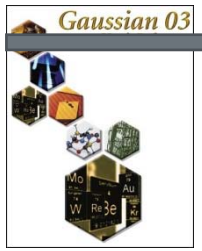


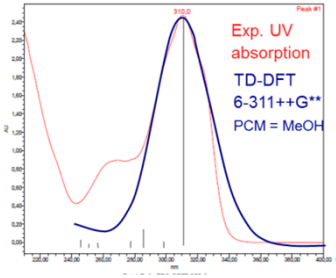
Example 1: Computational Phototoxicity Evaluation

Goal: Identify chromophore, variability and electronic properties to be used in QSPR

- **Simulation of excitation spectra**
  - Quantum-mechanical calculation
    - High level 6-311++G** basis set
    - Vertical excitation, TD-DFT (B3LYP)
    - Implicit solvent effects (MeOH)
    - Results have proven to be rather accurate

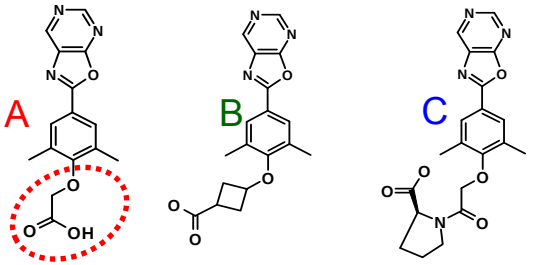


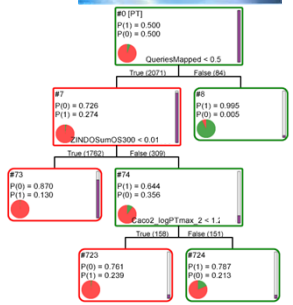




Goal: Correlate structure with interpretable molecular descriptors

- **Liability assessment, calculation of probability of photosensitation**
  - Recursive partitioning classifier
    - Integrated UV absorption permeability, phototoxic fragments
    - Predicted property is the probability of positive in vitro test
    - Good statistical model parameters $ROC_{test} > 0.8$



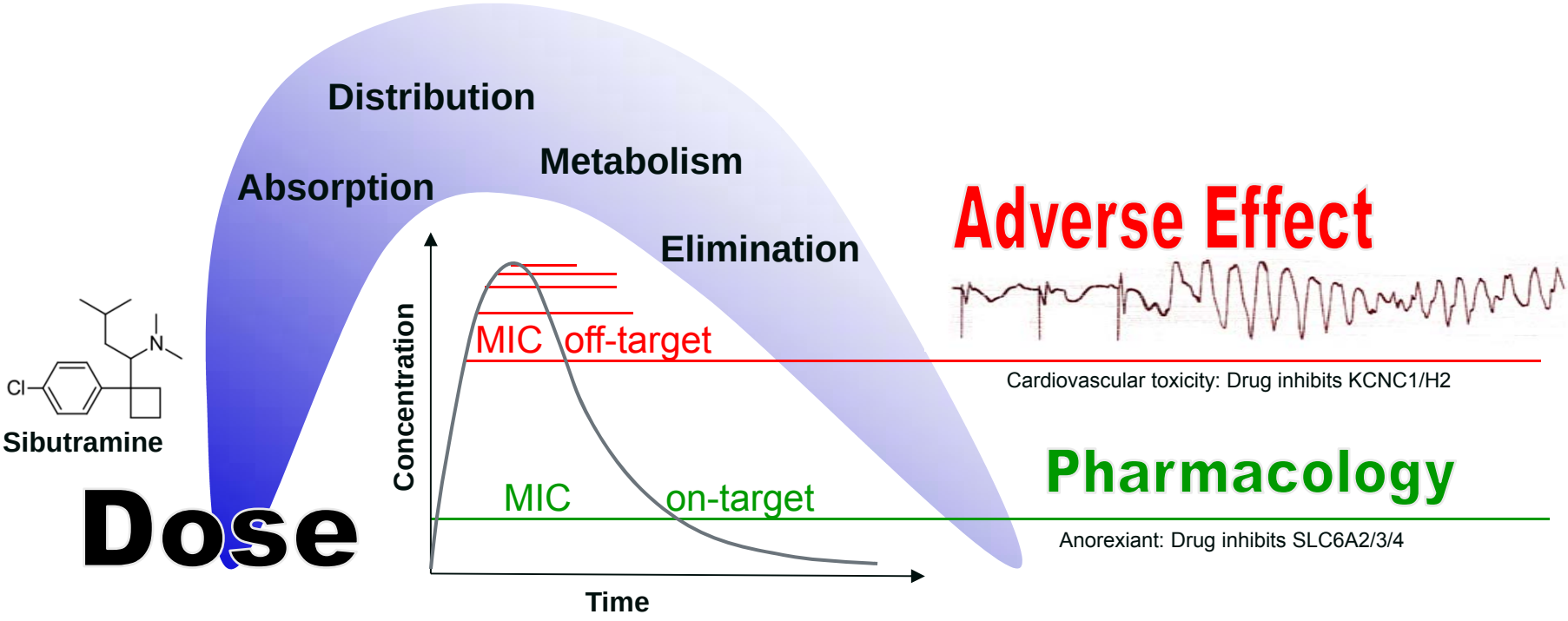


Prediction	phototoxic	safe	safe
Photostability	17 %	99.1 %	100 %

Adverse Polypharmacology:
Toxic effects are often driven by off-target interaction

Mestres 2009*: a drug hits 6.3 targets on average

- The majority of adverse drug reactions are time and dose-dependent**
- Adverse reactions often depend on the pharmacology profile of the candidate molecule

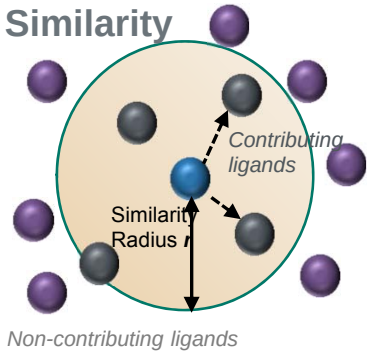


*Mestres et al., DDT 14 (9), 479-485.
**Bowes et al., NRDD 11, 909-922, 2012.

Predicting Adverse Polypharmacology

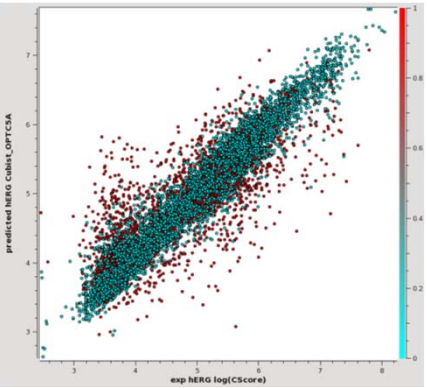
- **Experimental information**
  - Large target activity databases are available with millions of data points
  - Screening collections with well annotated biological data are included in phenotypic screening sets (Deorphaning set)

- **CTlink (Chemotargets)**
  - Similarity and QSAR-based
  - Multiple descriptors: PHRAG, FPD, SHED.
  - Five statistical approaches for activity prediction.



Today 5818 target models from public data, 1811 Sanofi-models

- **Off-target QSAR models (predictProfile)**
  - Models are generated for off-target panels automatically
  - Applicability domain defined by similarity to training set.
  - Predictive models for 400 targets SAR sets.
  - Reflecting Sanofi off-target profiling



D. Vidal, J. Mestres, *Mol. Inf.* **2010**, *29*, 543–551.

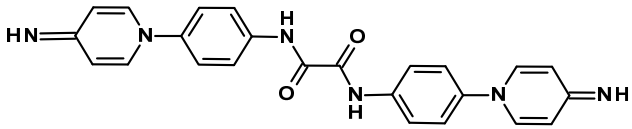
SANOFI

http://www.chemotargets.com/

Example 2: Which molecular initiating events are causal for severe cardiotoxic effects of an antifungal lead compound?

Problem: Cardiotoxicity observed in exploratory rodent study at mid and high dose

@10 mg/kg severe dyspnea, tachycardia and trembling, mortality at high dose



Antifungal Lead

CTlink prediction

- 5HT1A (h)
- 5HT2A (h)
- **AchE (h)**
- ADORA1 (h)
- ADORA2A (h)
- ADRA1A (h)
- ADRA2A (h
- ADRB1 (h)
- ADRB2 (h)
- AKT1 (h)
- BTK (h)
- CAMK2D (h)
- CB1 (h)
- CB2 (h)
- **CHRM1 (h)**
- **CHRM2 (h)**
- **CHRM3 (h)**
- Cl-Chan (h)
- cRAF (h)
- D1 (h)
- D2s (h)

- Weak hits on 35 other targets

Antitarget Panel prediction

- ABL1_Hu
- AKT1_Hu
- ALK_Hu
- AMPKa2b1g1_Hu
- Aurora1_Hu
- BTK_Hu
- BrSK2_Hu
- CAMK1_Hu
- CAMK2D_Hu
- CDK1_cyclinB1_Hu
- Cav3.1_Hu
- ERG_Hu
- KCNK2_Hu
- Kir4.1_5.1_Hu
- Kv1.3_Hu
- Kv4.3_Hu
- NAV_Hu
- Nav1.7_Hu
- SCN2A_Hu
- SCN5A_Hu

10 µM — IC50/EC50/KI — 0.1 µM

Predicted interactions:

AchE IC_{50} = 300 nM, CHRM1 IC_{50} = 6 µM, CHRM2,3 IC_{50} = 3 µM, hERG (inactive), 60 kinases (inactive)

Observed: interactions:

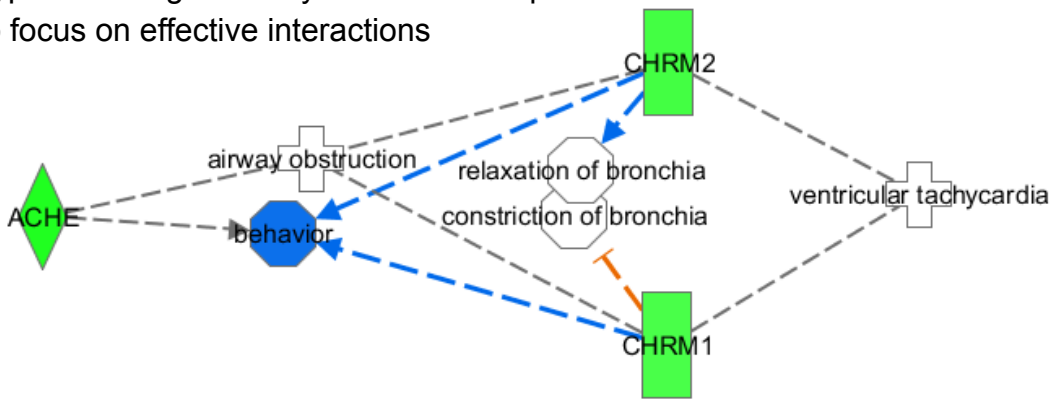
AchE IC_{50} = 90 nM, CHRM1 IC_{50} = 7 µM, CHRM2,3 (nt), hERG (inactive), 60 kinases (inactive)

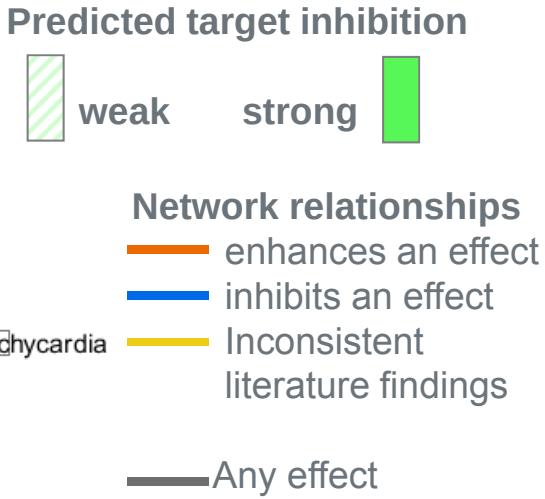
Proceedings of the 54th SOT, (2015), #1306.

Example 2: Two distinct interactions putatively responsible

IPA® (Ingenuity), MetaCore®, WikiPathways, Reactome are systematically exploited for off-target engagement in adverse drug reactions

- Typical off-target activity threshold <10 µM to focus on effective interactions





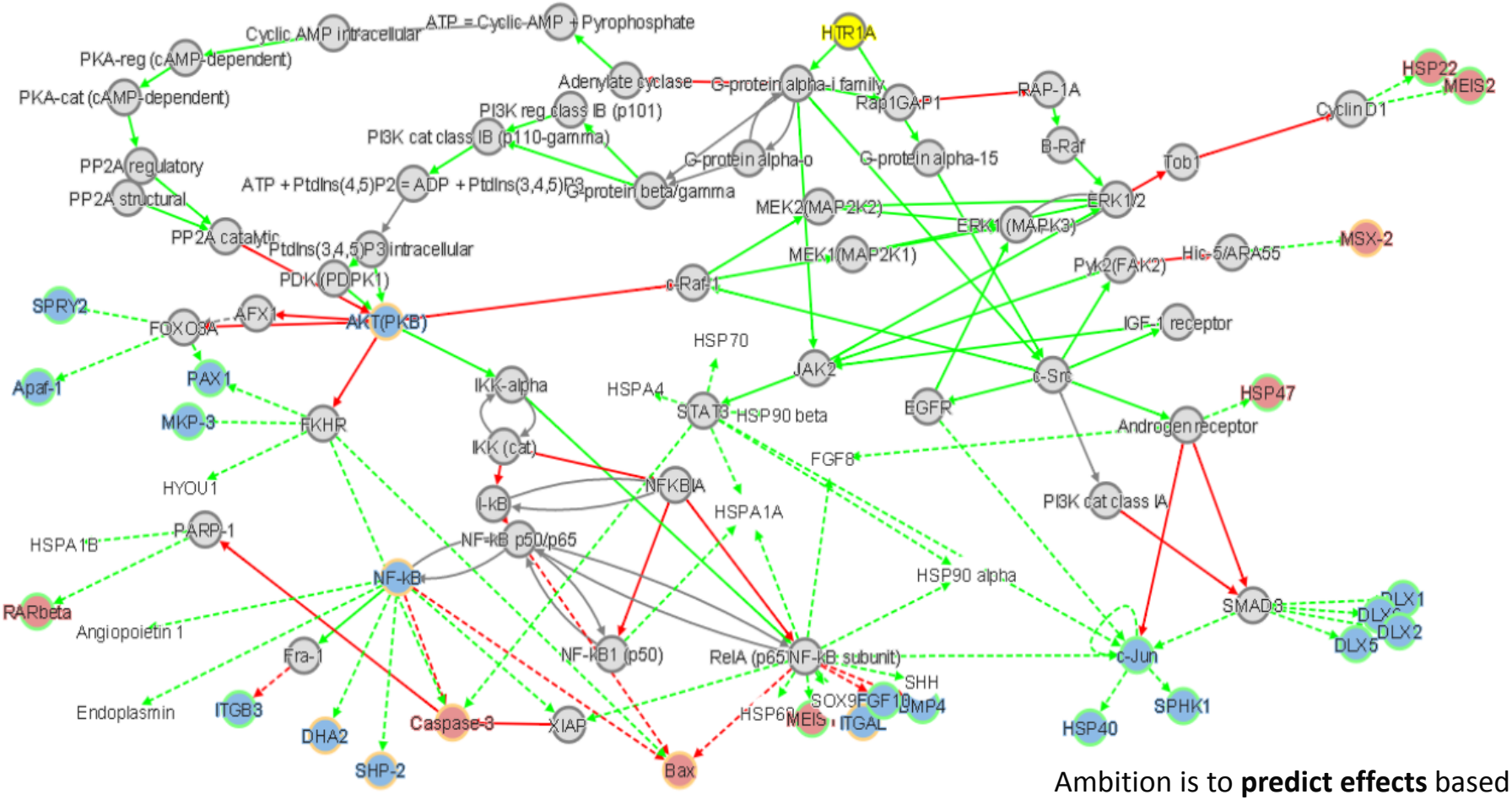
- AchE: Airway obstruction, behavioural effects
- CHRM1: ventricular tachycardia, constriction of bronchia

Network representation: Compound interactions with muscarinic CHRM1 and acetylcholinesterase AchE are considered causal for the observed effects.

Proceedings of the 54th SOT, (2015), #1306.

Network enhancements using biomedical knowledge to model developmental toxicity

- Target: HTR1A; Biomarkers: skeletal
  - Yellow: target; Node color: biomarker change (up(red)/down(blue)
  - Node border color: correct prediction (green) or incorrect (orange)



Ambition is to **predict effects** based on target activity profiles

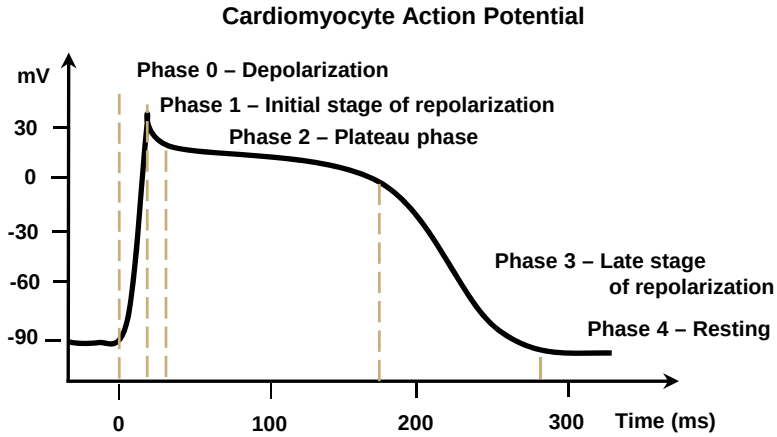
Example 3: Off-target assessment for cardiosafety

Problem: A small molecule pre-candidate compound with MoA related to nuclear receptors, has several critical off-target interactions to cardiac ion channels. Is this a no-go?

$IC50^{hERG}$ ~ 2 µM,

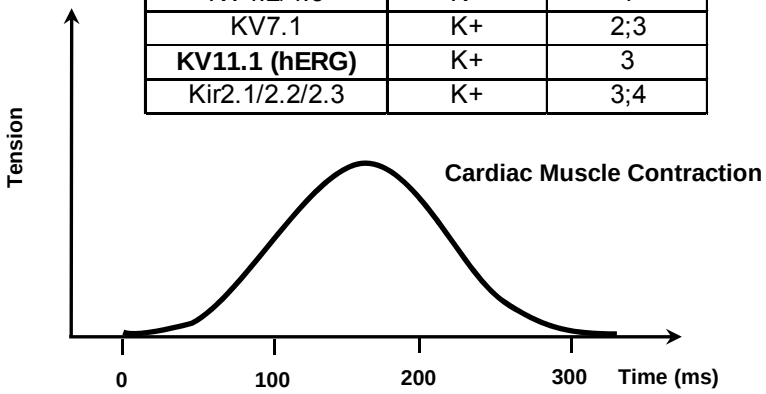
$IC50^{Cav1.2}$ ~3 µM

$IC50^{Nav1.5}$ >30 µM



- **The standard model implies the engagement of key ion-channels, which are highly expressed in cardiomyocytes**

Channel	Ion	Phase
NaV1.5	Na+	0
CaV1.2	Ca2+	0;1;2
KV4.2/4.3	K+	1
KV7.1	K+	2;3
KV11.1 (hERG)	K+	3
Kir2.1/2.2/2.3	K+	3;4



Multiscale Modelling suggests non-prohibitive APD shortening.

Simulation

Model Variables and Parameters

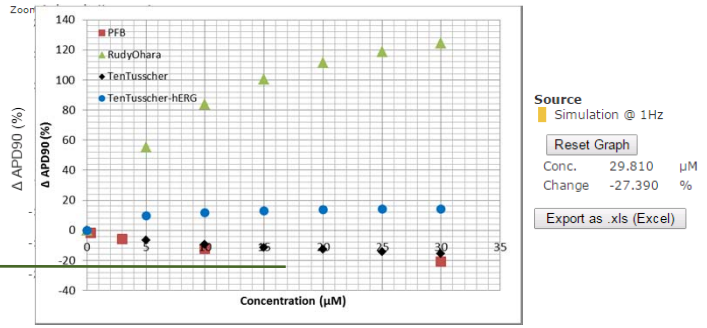
Effective change of action potential duration by compound concentration using different models
Shortening!

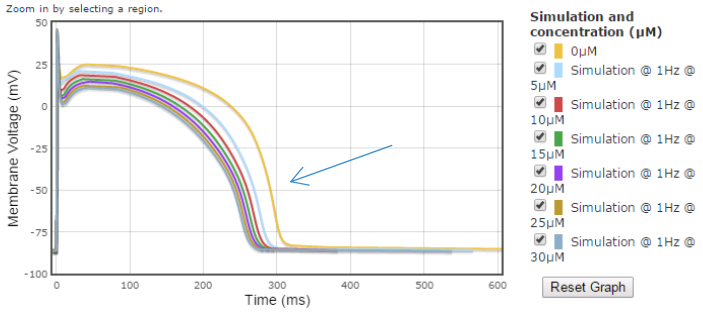
Cell Model		
Model	TenTusscher	TenTusscher et al. (2006) human ventricular cell model (epicardial)
Pacing Details		
Pacing frequency	1	(Hz)
Maximum pacing time	5	(minutes)
Ion Channel Current Inhibitory Concentrations		

Note 1 : No value for Inhibitory Concentration implies "no effect"
Note 2 : Unless otherwise assigned Hill Coefficients default to 1, and Saturation Levels default to 0.

Ion current	pIC50 (-log(M))	Channel protein	Gene
IKr	5.59	$K_v11.1$	*hERG or KCNH2*
INa		$Na_v1.5$	*SCN5A*
ICaL	5.47	$Ca_v1.2$	*CACNA1C*
IKs		$K_v7.1$	*KCNQ1/minK*
IK1		$K_{ir}2.1$	*KCNJ2*
Ito		$K_v4.3$	*KCND3*

Compound Concentrations		
Min.	0	(μM)
Max.	30	(μM)
Intermediate point count	5	
Intermediate point log scale	false	

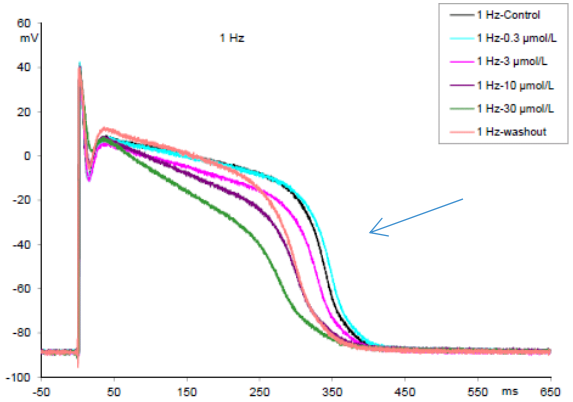




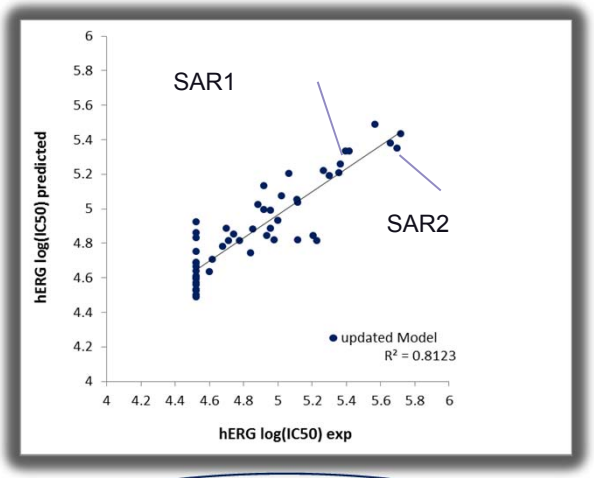
Simulated plot of membrane voltage over time

Rabbit purkinje fiber

- Concentration-dependent decrease in action potential duration from 3 μmol/L
- Putatively consistent with an effect on Na+ and/or Ca++channels.
- **Not suggestive of a pro-arrhythmic profile**



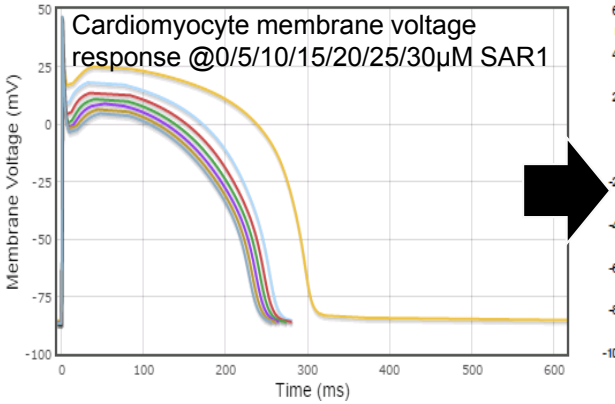
General Workflow



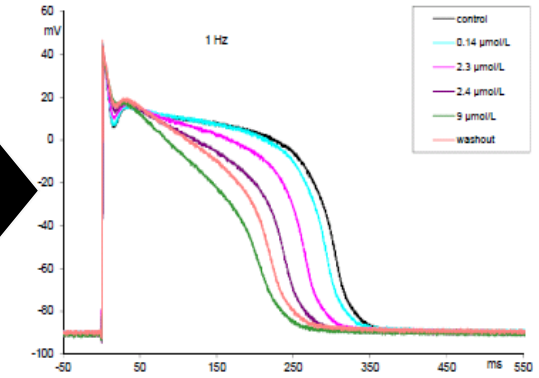
QSAR in silico prediction
hERG, metabolic stability, permeability

Multiparametric selection

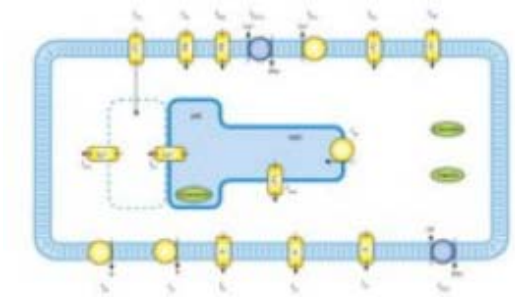
hERG IC50 = 3.0 µM (man)
Nav1.5 IC50 = N/A
Cav1.2 IC50 = 1.7 (man)



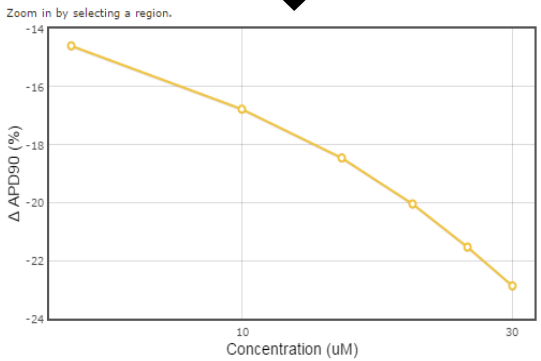
Multiparametric simulation



Experimental validation



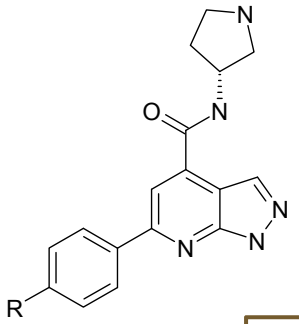
Parametrization of differential equation model for cardiac action potential model



Result: Non-prohibitive decrease in APD90

Example 4: Comparative Genotoxicity assessment for Kinase Inhibitors

- Focal-adhesion Kinase FAK, Tie2 receptor tyrosine kinase and Kinase insert domain Receptor KDR are tyrosine kinases with implication in cellular proliferation and a decisive role in angiogenesis
- Protein kinases often share a significant similarity of the binding pocket, thus Polypharmacology is often observed, and selectivity is difficult to achieve



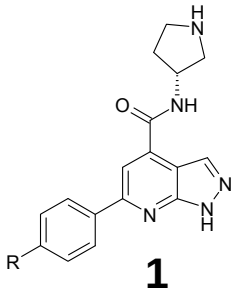
IC50 = 0.35 µM
Passive Permeability = low
logD = 1.6

Problem: Unselective on various protein kinases

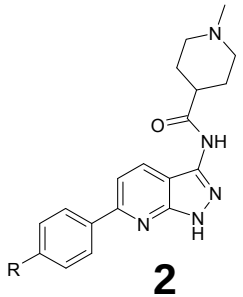
- Risk for genetic toxicity (aneugenicity) implicated by cooperative inhibition of Aurora and CDK kinases
- Hits include: AMPK, Aurora1/2, CDK1/5, RSK2, SGK1, p70S6K

Example 4: Comparative Genotoxicity assessment for Kinase Inhibitors

- Assumption: Side effects are driven by specific compound features
- Model-assisted assessment of Kinase selectivity:

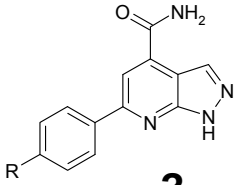


1



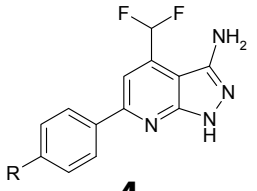
2

Variation of basic amine



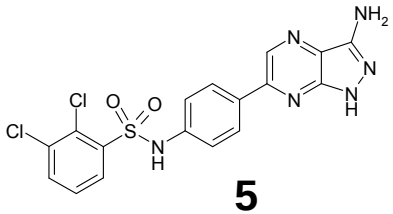
3

Truncation of basic amine



4

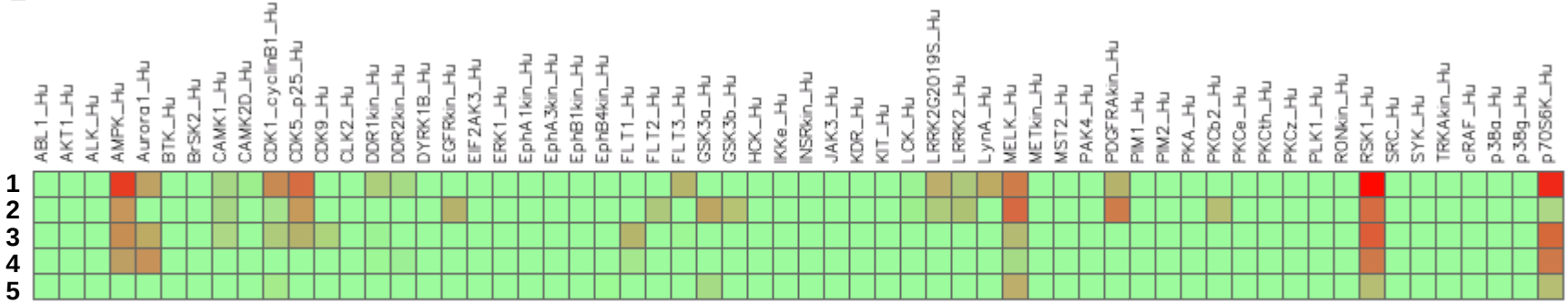
Lipophilic substitution of amide substituent



5

Rescaffolding

Cpd 5 can be differentiated as highly selective inhibitor of SGK1 kinase*



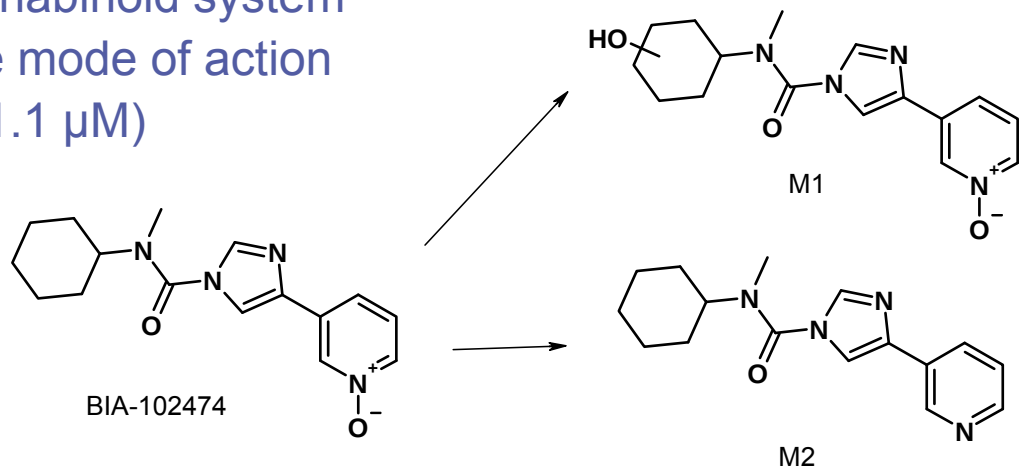
30 µM — IC50/EC50/KI — 0.1 µM

SANOFI

*ACS Med Chem Lett. 2015;6(1):73-8

Example 5: AE prediction of a FAAH Inhibitor

- **Fatty-acid-amide-hydrolase (FAAH serine hydrolase) inhibitor**
  - FAAH is mediator in the endocannabinoid system
  - Potentially (partially?) irreversible mode of action
  - Low potency in rodents: (IC_{50}^{rat}=1.1 µM)
  - Significant metabolism
    - Unknown level of metabolite



  - No apparent reactivity
  - Compound specificity
    - Selectivity of compound and metabolites
    - Serine hydrolases: monoacylglycerol lipase (MAGL), a carboxyl esterase and acetylcholinesterase (10 fold selectivity for FAAH rat, 50 fold for FAAH human).
    - 100 fold against dopamine-beta-hydroxylase, glutamic acid decarboxylase, the monoamine oxidases A and B and choline acetyl transferase.
  - Other inhibitors have higher selectivity margins (e.g. PF 04457845 Pfizer)
    - Disproportionate PK may lead to tissue exposure exceeding the margin

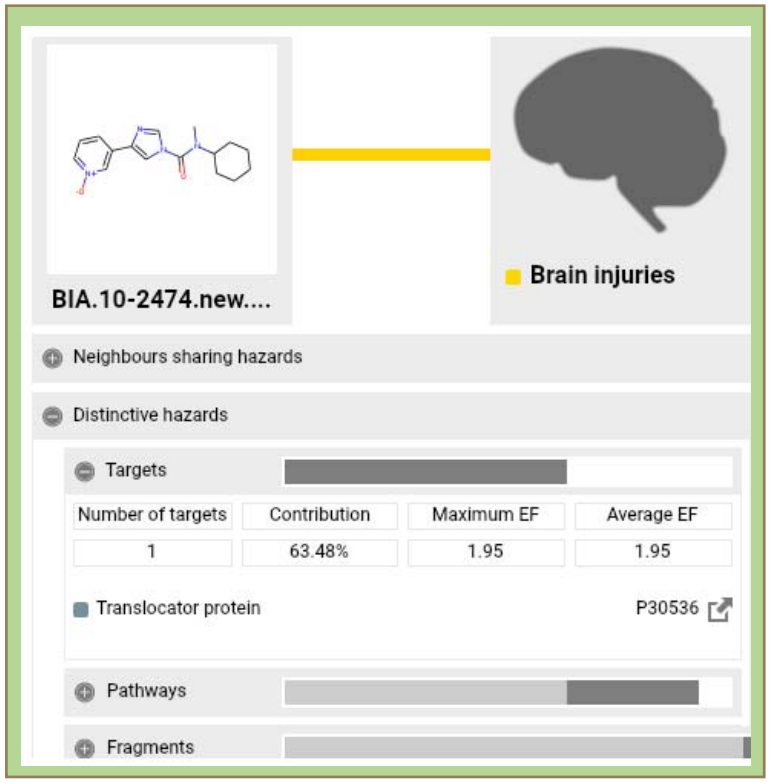
Supporting Risk Assessment by Off-target Prediction

- **CTlink off-target assessment may be used to suggest potentially unexplored interactions of the substance and/or metabolites with endocannabinoid system**
  - Refine in vitro safety screening strategy to include predicted targets (starting with predictions having a high confidence factor)

	Identifier	I...		p... 1 ▾		M...	Con... 2 ▾	Target name	UniProt
★	M1	P		7.1			0.47	Fatty-acid amide hydrolase 1	O00519
★	BIA.10-2474....	P		7.1			0.28	Fatty-acid amide hydrolase 1	O00519
★	M1	P		6.8			0.68	Metabotropic glutamate receptor 1	Q13255
★	M1	P		6.4			0.28	Cannabinoid receptor 2	P34972
★	M2	P		6.2			0.23	Translocator protein	P30536
★	BIA.10-2474....	P		6.1			0.23	Translocator protein	P30536
★	M1	P		6.1			0.23	Translocator protein	P30536
★	BIA.10-2474....	P		A			0.24	Metabotropic glutamate receptor 1	Q13255
☆	BIA.10-2474....	P		A			0.15	HCV RNA-directed RNA polymerase	P26663.11
☆	M1	P		A			0.15	Protein DBF4 homolog A	Q9UBU7
☆	BIA.10-2474....	P		A			0.15	Photosystem II protein D1	P83755
☆	M1	P		A			0.15	HIV-1 Reverse transcriptase/ribonuclease H	P03367.8
☆	M1	P		5.2			0.14	Mitochondrial import inner membrane transloc...	P32897

Supporting Risk Assessment by Off-target Prediction

- CNS predicted as potential target organ
- Support for hypothesis generation, e.g. interactions with the endocannabinoid system could contribute to adverse effects at high tissue exposure.

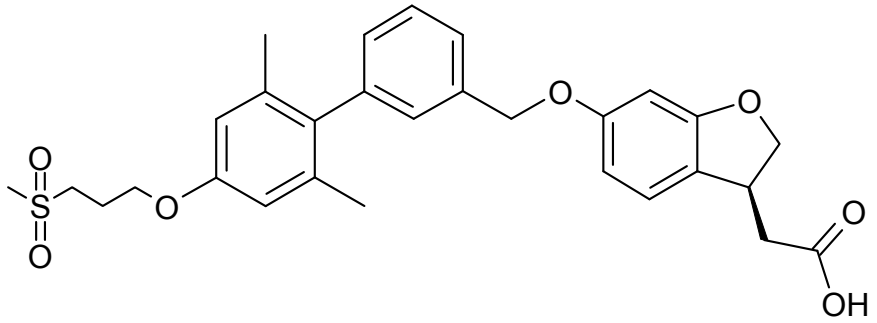


SUMMARY

S-links	69
Known	0
Predicted	69
Max affinity	7.0
Min affinity	6.0
Max confidence	0.55
Min confidence	0.30
By safety category	
CardioTox	3
EndocrinoTox	1
EyeTox	2
NeuroTox	12

Identifier	Main term	Category	I	M...	Confidence...	pAct
M2	Cns disorder	NeuroTox	P		0.55	6.0
BIA.10-2474....	Headache	NeuroTox	P		0.53	6.0
BIA.10-2474....	Nervous system disorder	NeuroTox	P		0.53	6.0
M2	Nervous system disorder	NeuroTox	P		0.52	6.0
M2	Headache	NeuroTox	P		0.51	6.0
BIA.10-2474....	Spinal diseases	NeuroTox	P		0.48	6.0
BIA.10-2474....	Brain injuries	NeuroTox	P		0.34	6.0
M2	Neuropathy	NeuroTox	P		0.33	6.0
BIA.10-2474....	Encephalopathies	NeuroTox	P		0.32	6.0
M1	Cns disorder	NeuroTox	P		0.30	7.0
BIA.10-2474....	Cns disorder	NeuroTox	P		0.30	7.0

Example 6: FFAR1 Agonist Fasiglifam



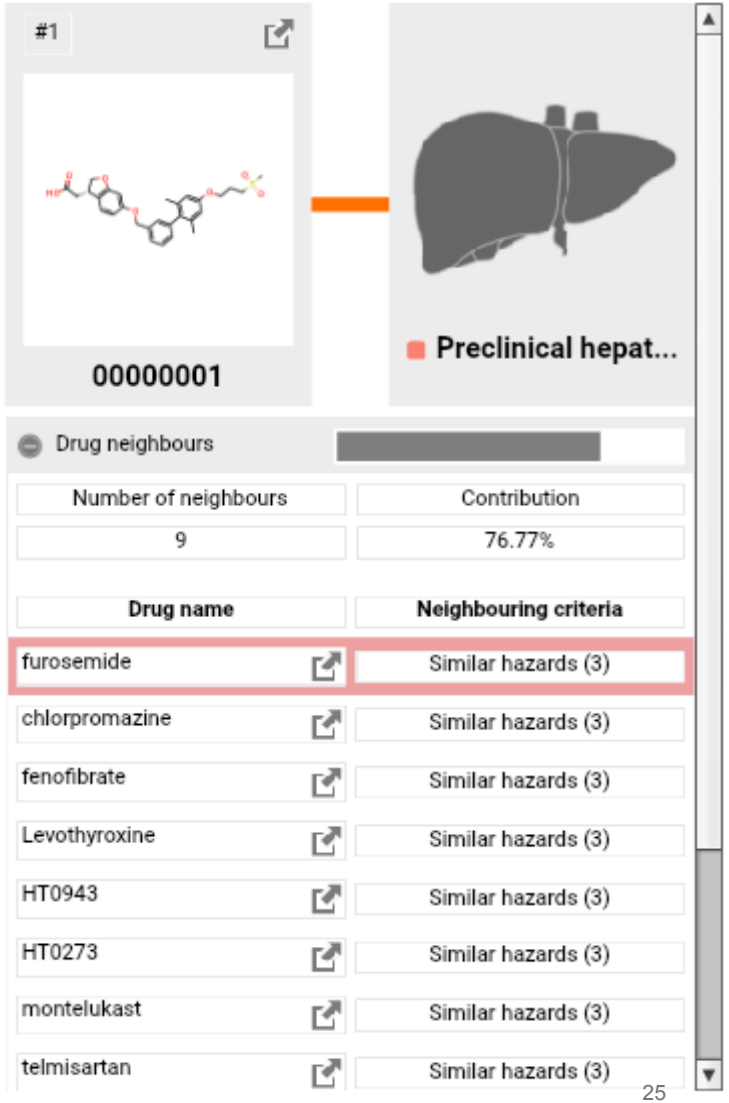
- **Potent orally available, selective partial agonist of human FFAR1**
  - Advanced for type 2 diabetes before stopped in Phase III clinical development
  - Once daily oral administration to type 2 diabetic patients for 24 weeks was well tolerated and led to reduced HbA1c levels and reduction of fasting plasma glucose.
  - A slightly higher incidence of 3-fold elevated alanine transaminase (ALT) in the fasiglifam compared to placebo groups was observed. Although a majority of patients with elevation of aminotransferases had confounding factors, in some cases, drug-induced liver injury couldn't be excluded completely leading to a termination.

Slink Systems Toxicology Analysis

Identifier	Main term	Alert type	Co...
00000001	Preclinical hepatotoxicity findings	Preclinical	0.53
00000001	Mitotoxicity	Preclinical	0.62

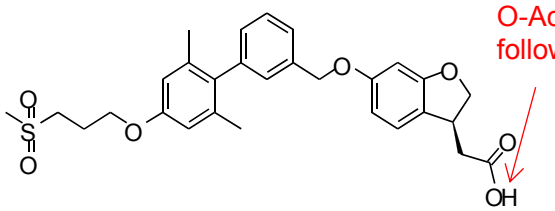
pACT(P)	Effect	Methods	Confidence score	Target name
7.4	AGO[K]		0.85	Free fatty acid receptor 1
6.4	---		0.77	Peroxisome proliferator-activated receptor alpha
5.9	---		0.77	Peroxisome proliferator-activated receptor delta
A	---		0.38	Bile acid receptor
A	---		0.37	Prostacyclin receptor
A	---		0.37	Lysophosphatidic acid receptor 3
A	---		0.37	Cysteinyl leukotriene receptor 1
A	---		0.37	Leukotriene B4 receptor 2
A	---		0.34	Glycogen [starch] synthase, muscle

- **Liver predicted as target organ**
- **Metabolic pathways predicted:**
  - Oxidative decarboxylation
  - Glucuronidation
  - Hydroxylation, Dehydrogenation
  - Sulfatation
- **Putative interactions**
  - FFAR1, PPARa/d,, bile acid receptor, BSEP



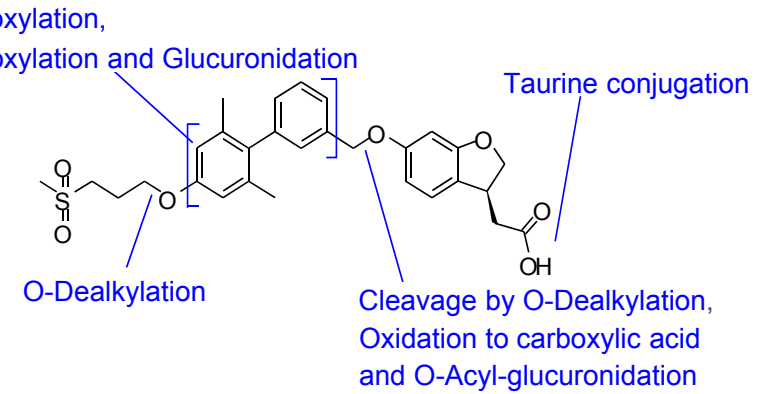
Metabolic Pathways

Abundant pathways in hepatocytes of human, dog and rat



Formation of TAK875-GlcA:	**Human**	**HEP: 13%**
	Dog	**HEP: 2-4%**
	Rat	**HEP: <LOD**

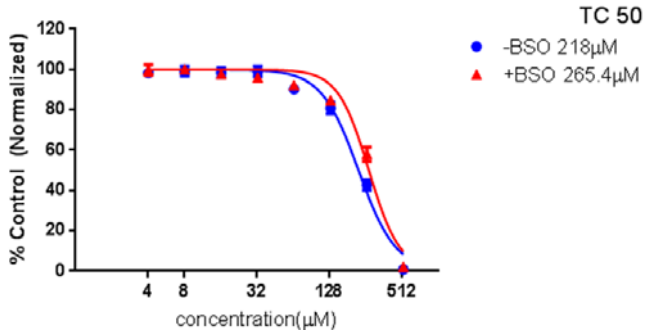
Subordinate metabolic pathways in all species



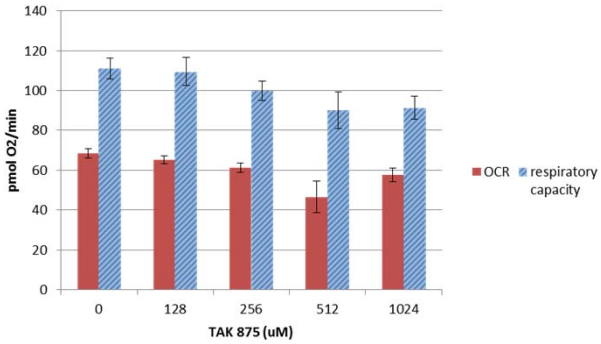
- **In vitro metabolism of human dog and rat**
  - **low (< 20%) in hepatocytes of all species,**
  - **low (< 20%) in liver microsomes with NADPH in all species,**
  - **moderate (38-62%) in human and dog liver microsomes with UDPGA**
  - **O-Acyl-glucuronide in hepatocytes is disproportionate in human**
  - **The rate of disappearance ($t_{½}$) of the 1-O-acyl-glucuronide of was 2.6 h, clearly below the reference compound furosemide-O-acyl-glucuronide with 3.8 h, indicating a potential risk of reactivity.**

In vitro Assessment

- **Cytotoxicity in primary human hepatocytes /GSH depleted hepatocytes**
  - No biologically significant difference in the cytotoxicity
  - No alteration of the total GSH decrease in total GSH content at non-toxic concentration



- **Mitochondrial Toxicity**
  - Effects on human mitochondria and cellular metabolism do not appear to be the primary mechanism of cytotoxicity following 4hr exposure of human hepatocytes.



- **Transporter engagement**

	Fasiglifam	Fasiglifam-Glca
Passive permeability	high	Very low
Uptake	Passive	active
OATP1B1	inhibitor	Inhibitor/substrate
OATP1B3	inhibitor	inhibitor
MRP2	-	Inhibitor/substrate
BSEP	inhibitor	-

Reactive Metabolites

- **No significant covalent binding in liver microsomes of all species.**
- **In human hepatocytes a slight increase of covalent binding compared to the animal species.**
- **The slight potential of covalent binding confirmed in liver microsomes of human and dog after addition of UDPGA in 6h**
- **O-acyl-glucuronide metabolites solely suspected for covalent binding potential, as their abundancies were far below 5% in animal hepatocytes and the other human metabolites were observed at least in one *in-vitro* animal species with similar or higher abundances, as seen in human hepatocytes**

Mean drug related material recovered in pellet[1], given in [pmol eq./mg protein]												
Cofactors/ Species	*-NADPH*	*+NADPH*	*+NADPH +GSH*	*-NADPH +UDPGA*	*+NADPH +UDPGA*	*-NADPH*	*+NADPH*	*-NADPH +UDPGA*	*+NADPH +UDPGA*	HEPs	HEPs	HEPs
Incubation	***1h***	***1h***	***1h***	***2h***	***2h***	***6h***	***6h***	***6h***	***6h***	***2h***	***4h***	***6h***
Number of replicates	*1*	*1*	*1*	*7*	*7*	*3*	*3*	*7*	*7*	*3*	*3*	*5*
Rat M	0	0	0	nd[2]	nd	nd	nd	nd	nd	nd	2	nd
Dog M	12	13	2	14	12	8	9	23	21	5	8	10
Human M/F	5	1	0	12	15	11	15	27	26	46	99	84

1: after repeated wash steps with methanol/sulphuric acid solution and solubilizing with Solvable™, corrected with 0h results
2: nd: not determined
HEPs: hepatocytes; M: Male; F: Female; M/F: Pool of male and female subject

Summary

- Computational systems toxicology
  - Tailored databases, prescriptive and predictive methods to improve compound safety
- Computational profiling complements and supports in vitro/in vivo screening strategy
  - QSPR/QSAR is an extensible technology for various predictive models
  - Cost-effective tools to select compounds and plan experiments early
  - Better planning and more focused design of mechanistic studies (in vitro/in vivo) to explore MoT
  - Risk mitigation
- Regulatory acceptance is increasing
  - e.g. ICH M7: no further investigations if in silico models + expert knowledge conclude negative result

→**Toxicological in-silico analyses aim for a significant reduction in potential risks resulting from unforeseen drug adverse events in all phases of drug discovery and development**

Acknowledgement

- Andreas Czich
- Richard Brennan
- Philippe Detilleux
- Douglas Keller
- Katharina Mertsch
- Jacques Richard
- Veronique Thybaud
- Alexander Amberg
- Lennart Anger
- Manuela Stolte
- Denis Muliner
- Jan Wenzel
- Th. Kissner
- Peter Block
- Axel Dietrich
- Clemens Giegerich
- Nis Halland
- Uwe Heinelt
- Gerhard Hessler
- S. Güssregen
- Hans Matter
- David Machnik
- Patrick Mougenot
- Roy Vaz
- Andrea Cavalli
- Valentina Piretti
- Jordi Mestres
- Ricard Garcia
- Nikita Remez
- David Vidal

SANOFI

SANOFI