Applications of in silico modelling in drug metabolism study in pharmaceutical research

Examples on the combined application of experimental data and in silico modelling

Nicolò Milani (postdoc scientist at Hofmann La Roche – PS department – M&S group)

Lecture University of Basel 20-11-2020

Introduction and aim of the talk

University of Perugia

Synthesis in Organic Chemistry

University of Perugia

Synthesis in Organic Chemistry
Drug Metabolism
and Molecular Modelling

Molecular discovery Ltd

Drug Metabolism and
Development of software
for the identification of side
products from chemical processes
(LC-MS/MS data and NMR)

University of Perugia and Roche

Drug Metabolism and
Molecular Modelling –
Hightroughtput systems
(Roche)

Roche and University of Manchester

DMPK modelling with in vitro data processing and data optimization in vitro assay with Liver/Gut-Liver OoC

- The aim of the talk is that to report the basic activities necessary for a full understanding of the human drug metabolism
- I will provide some basics concepts of metabolism and the respective modelling which can be applied for the different kind of studies
- In addition, I will try to contextualize the talk using the experience which I have gained during my educational period

Metabolism Key-Concepts

DRUG CANDIDATE

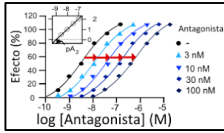
METABOLISM

Decreased drug bioavailability

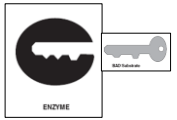
Decreased therapeutic effect

Metabolites mav be toxic for different organs in the body

Interaction with other xenobitics (DDI)

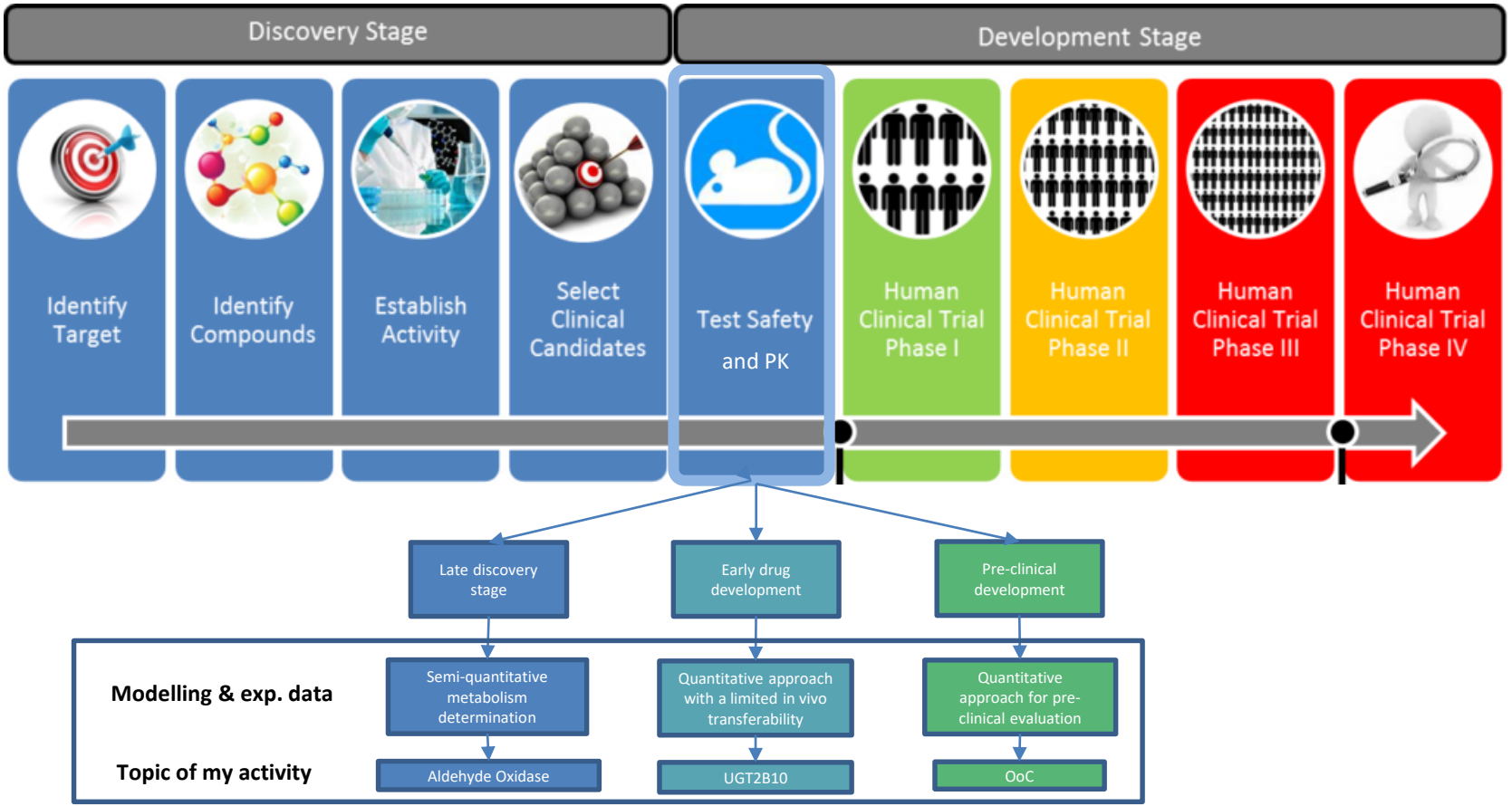


Inactive towards biological target



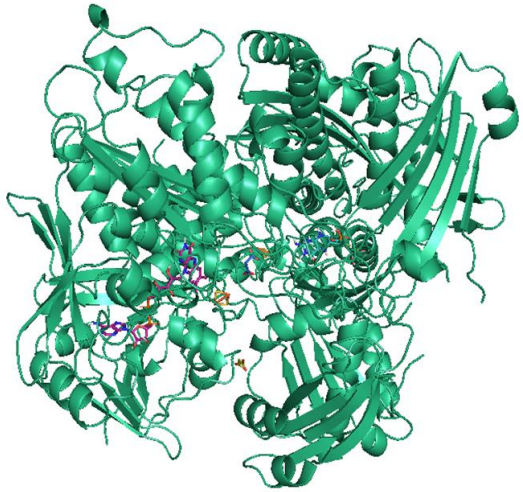
More active than substrate towards biological target => **PRODRUG**

Drug metabolism study in a pharmaceutical company context



Aldehyde Oxidase (AOX)

Crystalized AOX structure



C. Coelho et al. *Nature Chemical Biology* 11, 779–783, **2015**

- Present in several organisms (animals, vegetables, fungus and bacteria…)
- Mainly present in animal cytosol liver
- A Molybdo-flavoprotein
- Homodimeric enzyme with 3 domains
- Uses O_2 as final electron acceptor
- There is a wide intra and interspecific variability

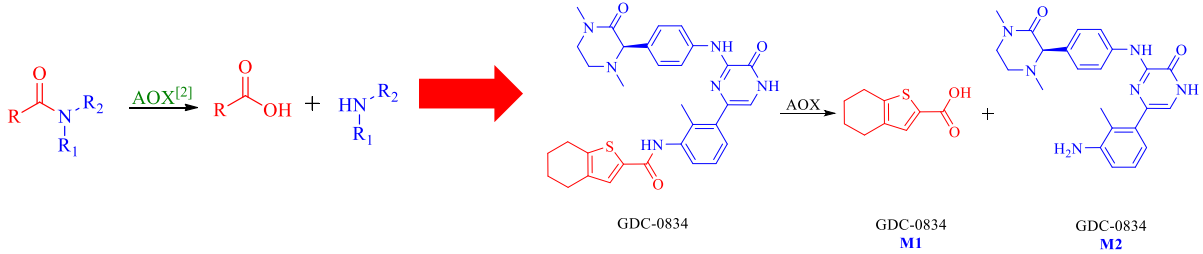
Garattini & Terao *Expert Opin. Drug Discov*. 8, 641-654, **2013**
Pryde et al. *J. Med. Chem.* 53, 8441–8460, **2010**

AOX- promoted reactions

Aldehyde oxidation

Aza-aromatic oxidation

Amide hydrolysis



DRUGBANK

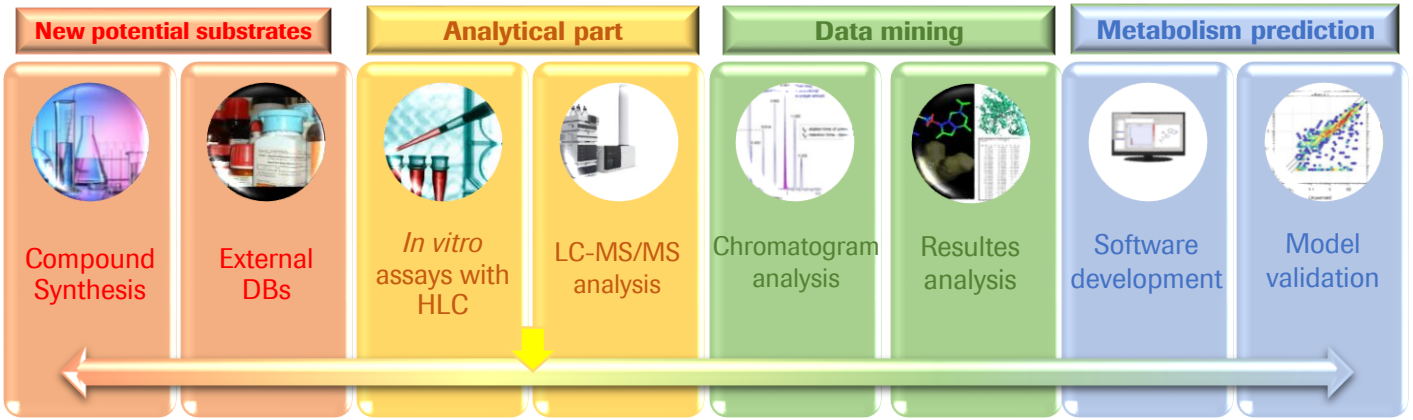
My work was focused on these reactions because:

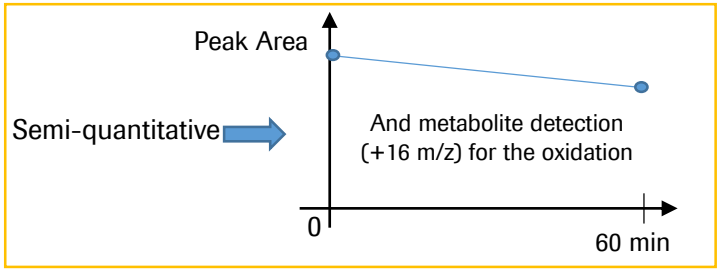
- Several drugs contained aza-aromatic scaffolds
- There are about 35% of molecules with amide functionality in DrugBank

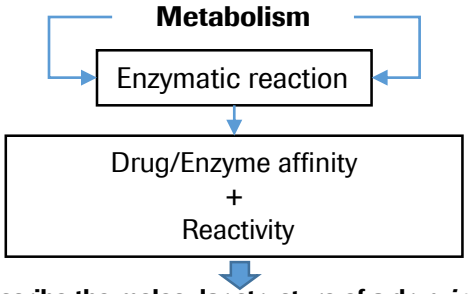
[1] Pryde et al. *J. Med. Chem.* 53, 8441–8460, **2010**
[2] Sodhi et al. *Drug Metab. Dispos.* 43, 908–915, **2015**

Many activities for one purpose

Aim: Purely *in silico* prediction of AOX substrate. Is the compound AOX substrate?







How we can describe the molecular structure of a drug *in silico*?

Molecular description

- Molecular structure
  (SMILES, SDF…)

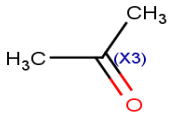
Application: Filter out compounds having a desired functional group

Database with 10 000 molecules

[#6][CX3](=O)[#6]

Query in SMARTS

All ketones



- Global description

Database with 10 000 molecules

ADME predictor software

LogP Ranking

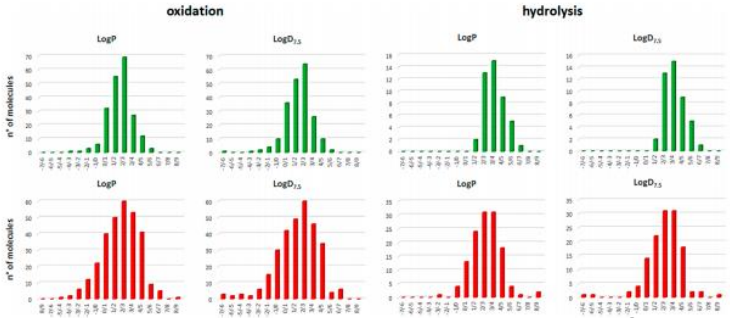
Application: Estimation of logP, logD
From the total hydrophobic/hydrophilic domain

Molecular description

Application of the global molecular description in drug Metabolism

Does the global description of the molecule work to study target-molecule affinity?

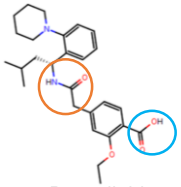
VolSurf



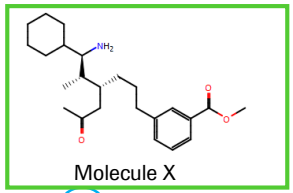
logP cannot discriminate substrate and non-substrate of *h*-AOX

Local interactions with the target make a huge difference

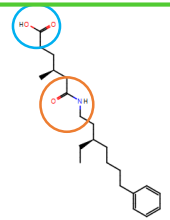
Molecular Similarity (Example)



Repaglinide

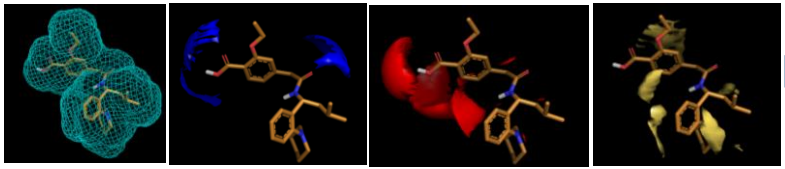


Molecule X



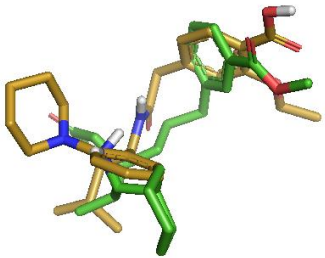
Molecule Y

Using the pure molecular description they seem to be quite different (e.g. no common functional group)



MIFs

Solid MIFs -> Repaglanide
Wireframe MIFs -> Molecule X



They have several common interactions

Repaglanide

In medicinal chemistry these models are mostly base on **Molecular mechanics**

Application of classical mechanics to molecular systems

Molecular description

Application of the global molecular description in drug Metabolism

Does the global description of the molecule work to study target-molecule affinity?

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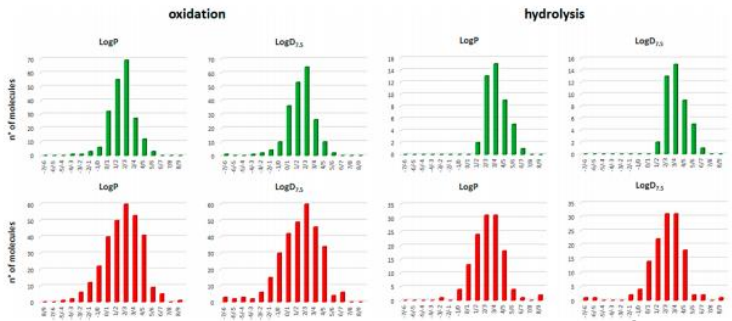

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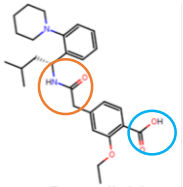
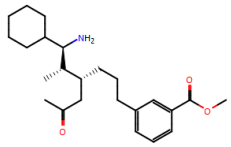


Repaglinide



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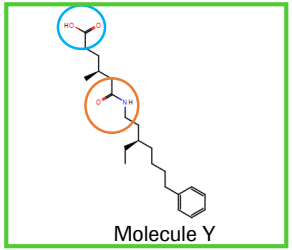

Molecule Y

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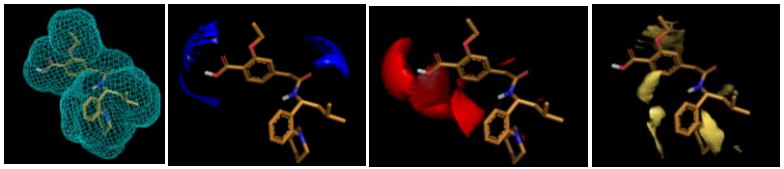

MIFs

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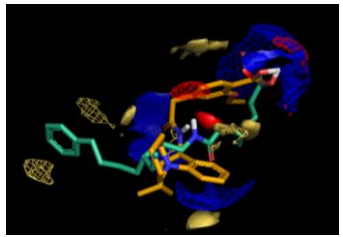
Wireframe MIFs -> Molecule Y



They do not have common interactions

Repaglanide

In medicinal chemistry these models are mostly base on **Molecular mechanics**
Application of classical mechanics to molecular systems

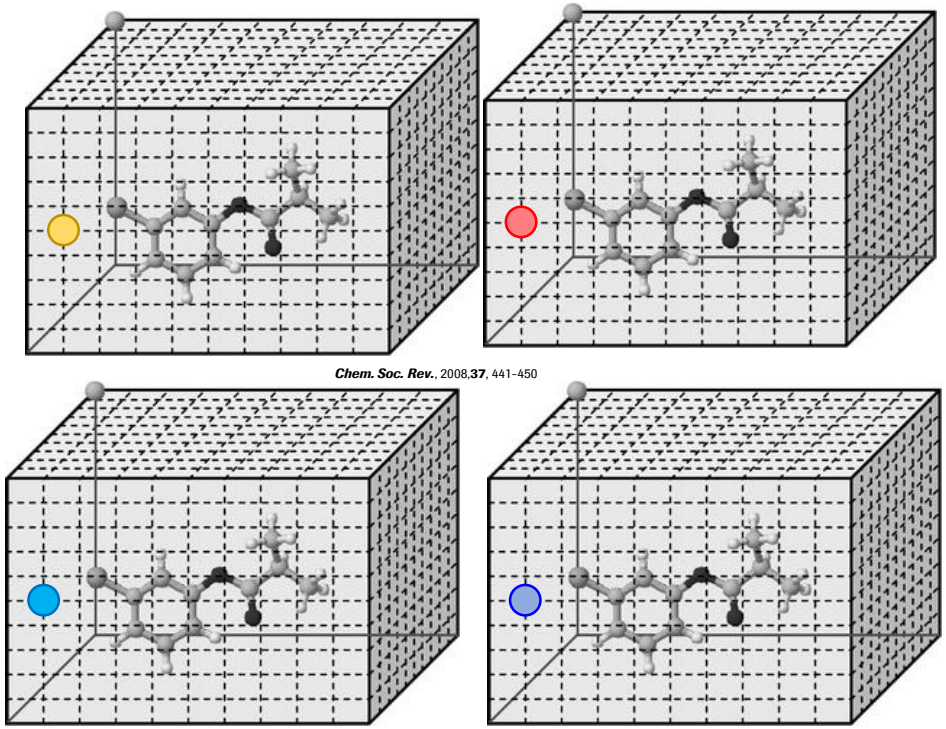
Molecular Interaction Fields: Applications in Drug Discovery and ADME Prediction, Volume 27

Molecular description

Grid Method

- Hydrophobic
- Hydrogen bond donor
- Hydrogen bond acceptor
- Shape

$$E = E_{covalent} + E_{noncovalent}$$

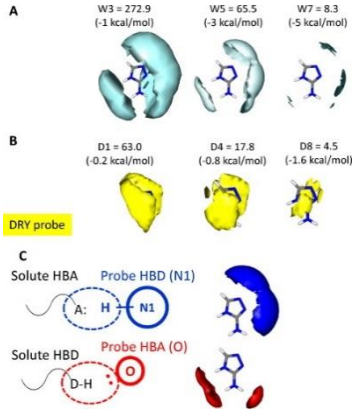


Chem. Soc. Rev., 2008,**37**, 441–450

All points in the grid are used for mapping the molecule

Same approach can be performed for large molecules as protein

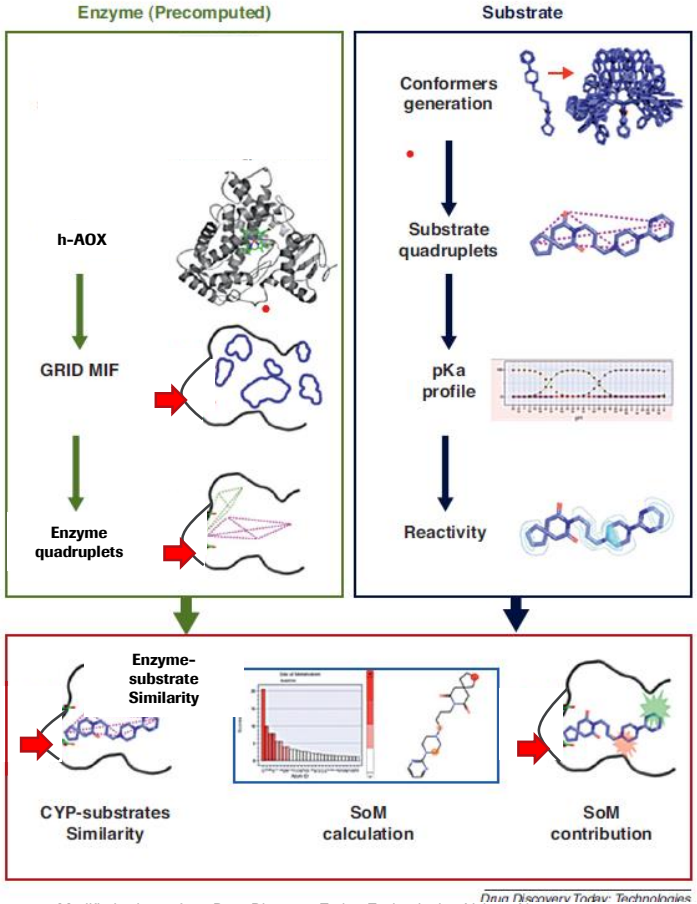
The molecule into the box is flexible (conformations are considered)



Molecular Interaction Fields (MIFs)

Focus on enzymatic reaction

MoCo
(reactive point of the enzyme)



Modified scheme from Drug Discovery Today: Technologies: Vol. 10, No. 1 2013

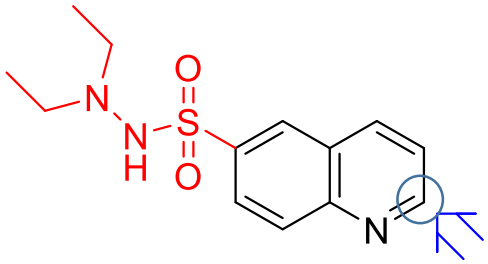
pKa influences both reactivity and ligand-target affinity

R_i is the reactivity of the atom i-th in the appropriate reaction mechanism

It can be approximated using ab initio methods in non-redundant chemical fragments.

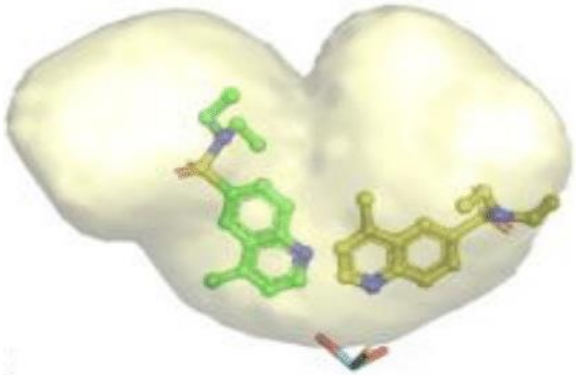
Data mining

Analysis results: SoM exposition



Experimental substrate

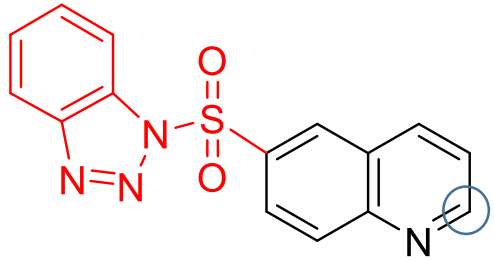
Reactive Site of Metabolism (SoM)



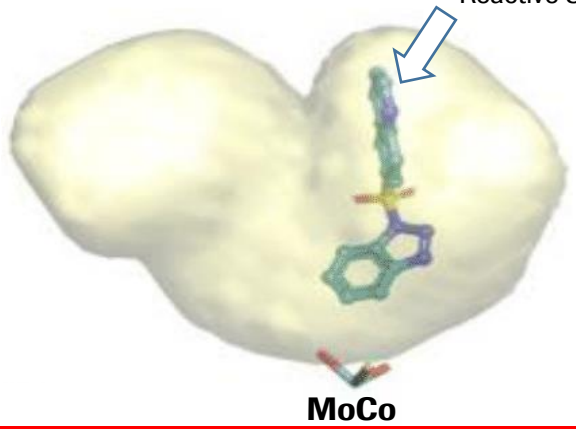
MoCo

Correct exposition

Same principle of docking but the information are collapsed in 4 high informative points (pharmachoporic points)



Experimental non substrate



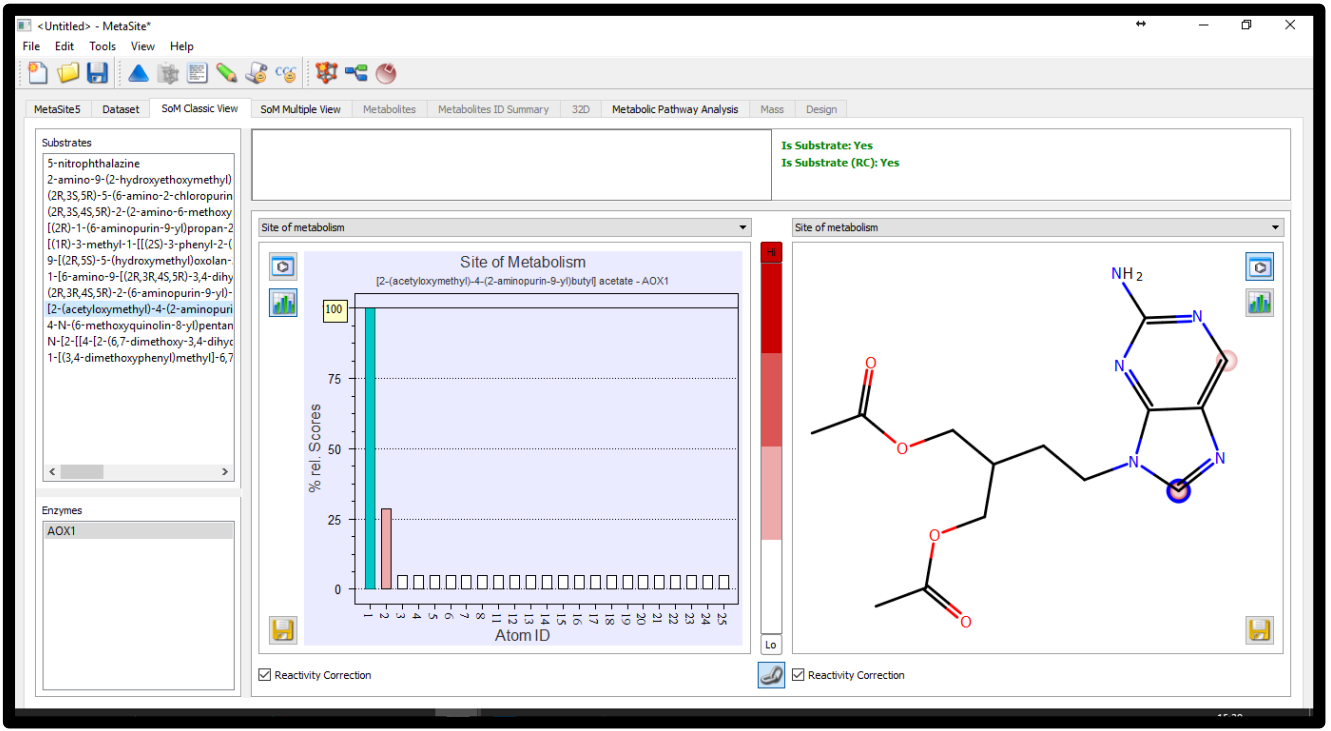
MoCo

Wrong exposition

Proc Natl Acad Sci U S A 2017 Apr 18;114(16):E3178-E3187

Outcome of the project: AOX metabolism prediction model

Software development



Internal Model Validation

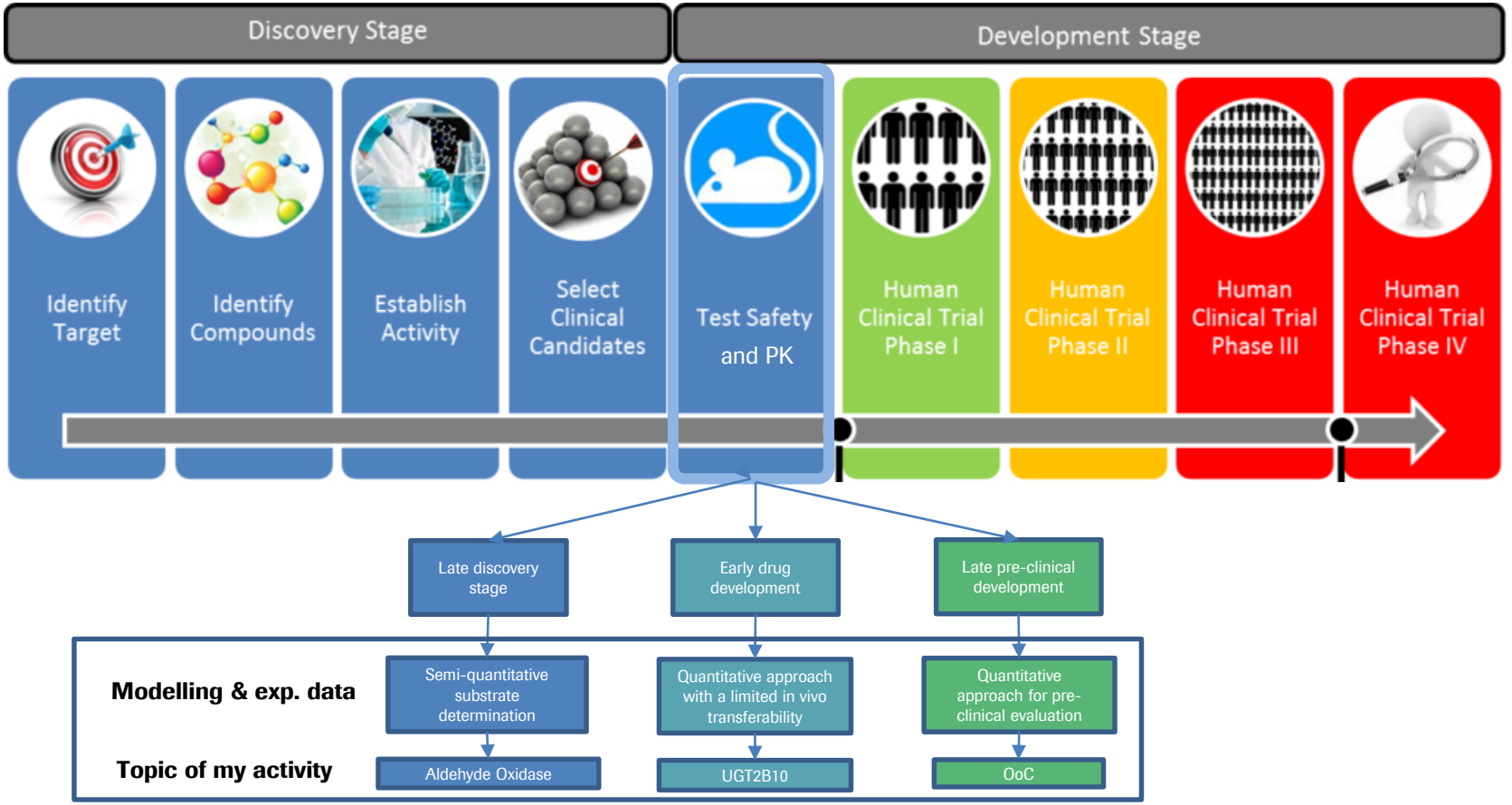
AOX METABOLISM	OXIDATION[a]	HYDROLYSIS[b]
% good prediction	88	98

[a]Database of 535 compounds
[b]Database of 173

This function is considered to be an approximation of the free energy of the process including substrate-enzyme interaction where exposure (E) and reactivity (R) are opportunely weighted (we and wr, respectively).

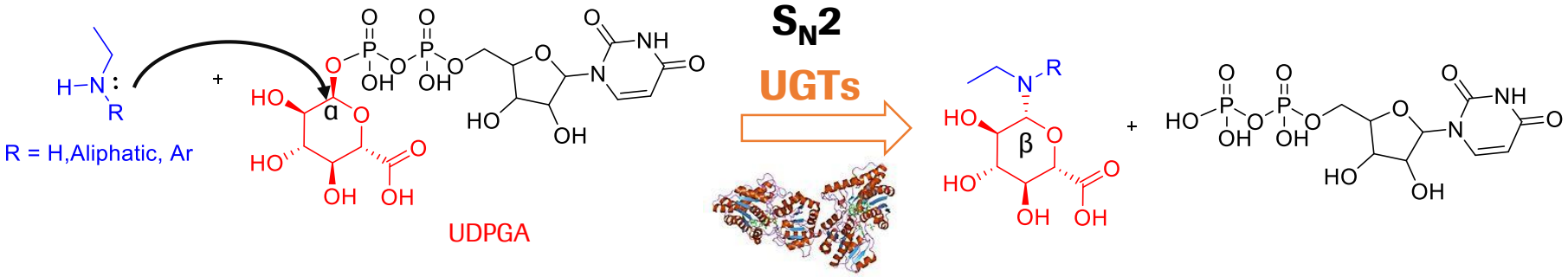
J. Med. Chem., 61, 360−371, **2018**

Drug metabolism study in a pharmaceutical company context

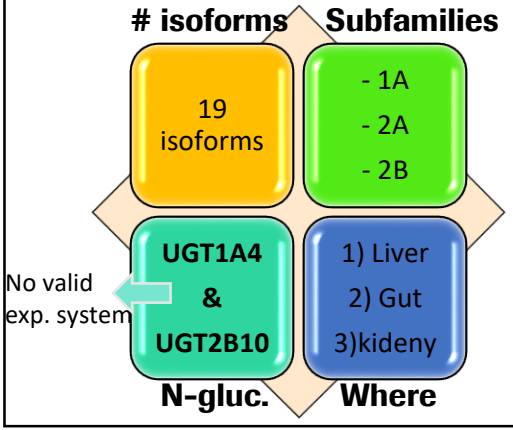


UGTs and glucuronidation reaction

Mechanism reaction

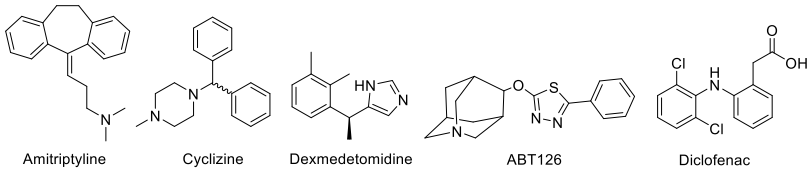


Human UGT isoforms

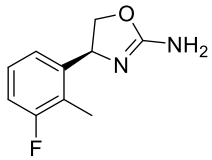


h-UGT2B10

23 known UGT2B10 substrates from the DDI DB of the Univ. of Washington



Roche clinical candidate



3D structure of UGT2B10 is not available
No valid experimental protocol to asses UGT2B10 selective substrate

UGT2B10: bullet points

Aim: find new selective UGT2B10 substrates

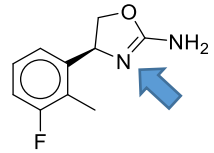
Why is this project useful in the pharmaceutical field?

A UGT2B10 Splicing Polymorphism Common in African Populations May Greatly Increase Drug Exposure

Stephen Fowler, Heidemarie Kletzl, Moshe Finel, Nenad Manevski,[1] Paul Schmid, Dietrich Tuerck, Roger D. Norcross, Marius C. Hoener, Olivia Spleiss, and Victor A. Iglesias

Roche Pharma Research and Early Development, Roche Innovation Center Basel, Basel, Switzerland (S.F., H.K., P.S., D.T., R.D.N., M.C.H., O.S., V.A.I.); and Pharmaceutical Chemistry, Faculty of Pharmacy, University of Helsinki, Helsinki, Finland (M.F., N.M.)

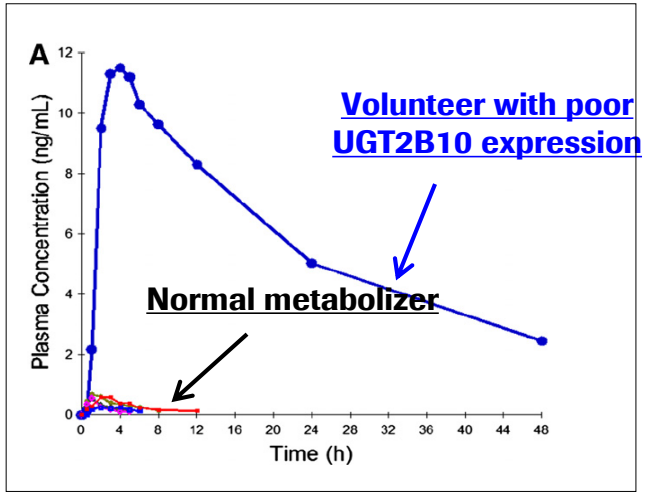
Received September 26, 2014; accepted December 10, 2014



RO5263397

Roche

Clinical candidate against Schizophrenia disease



Journal of Pharmacology and Experimental Therapeutics 2015, 352 (2) 358-367

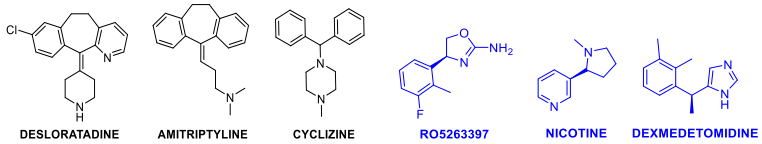
Application of in vitro and in silico modelling

There are two broad categories of computational techniques for virtual screening: Structural Based and Ligand Based

Ligand-based. It is based on that similar chemical-physical features implicate a similar target affinity. IUPAC defines a pharmacophore to be "an ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target and to trigger (or block) its biological response Glossary of terms used in medicinal chemistry (IUPAC Recommendations 1998)

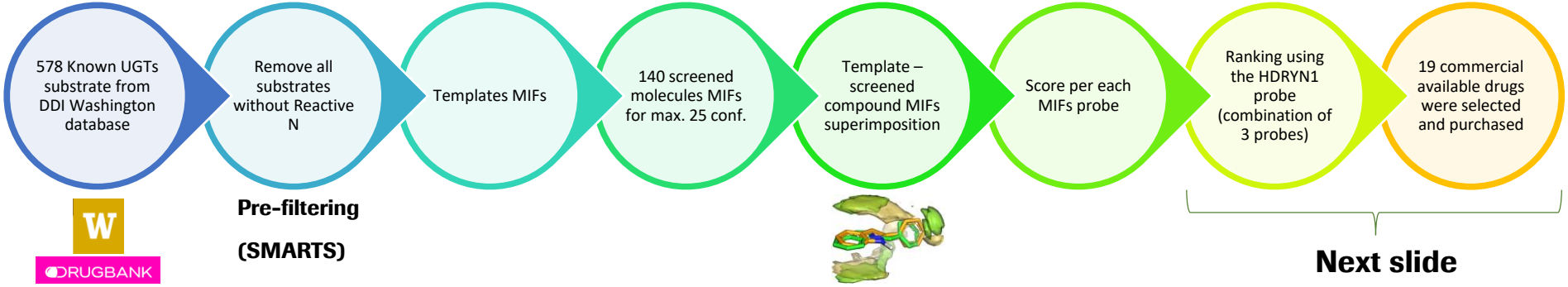
Ligand based – Method used for UGT2B10 project

Known UGT2B10 substrates used as templates



Repeated for all 140 screened compounds and for all 6 templates

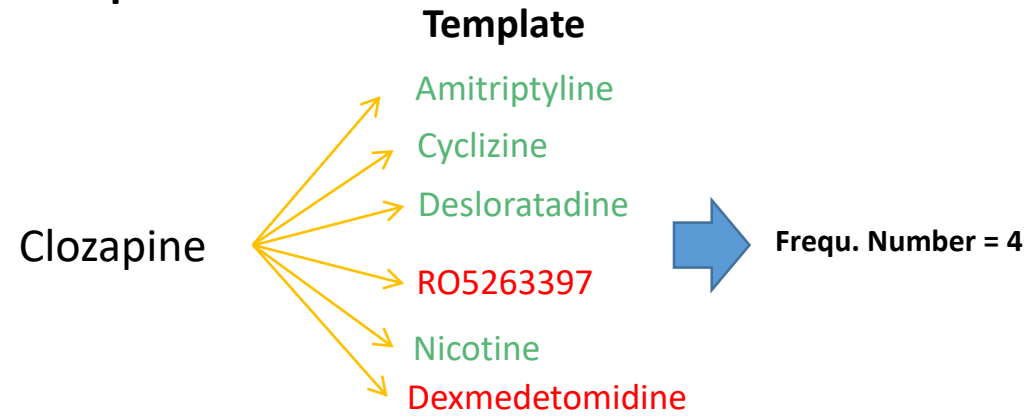
Steps



Final results

A frequency number of 1 is added for each screened compound in the 20 top rank of each LB with the 6 templates

Example



In the top 20

NO in the top 20

Results

Best compounds with at least 2 as frequency number

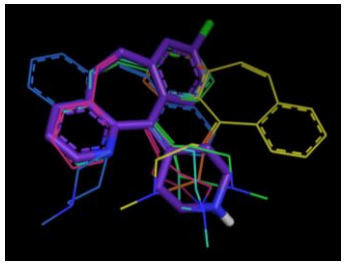
Drug	Frequency Number	Templates	Known metabolism (UGT isoform)	Literature ref.
tioconazole	5	amitriptyline, cyclizine, desloratadine, dexmedetomidine, nicotine	UGT1A3, UGT1A4, UGT2B7	(Bourcier et al., 2010)
bifonazole	4	amitriptyline, cyclizine, desloratadine, nicotine	UGT1A3, UGT1A4, UGT1A9, UGT2B7	(Bourcier et al., 2010)
loxapine	4	amitriptyline, cyclizine, desloratadine, RO5263397	UGT1A4	(Bourcier et al., 2010)
clozapine	4	amitriptyline, cyclizine, desloratadine, dexmedetomidine	UGT1A1, UGT1A4	(Green, 1998 ;Erickson-Ridout, 2012; Mori, 2005)
econazole	4		UGT1A6, UGT1A3, UGT1A4, UGT2B7	(Bourcier et al., 2010)
lidocaine	4		UGT	(Zanelli et al., 2012; Jinno et al., 2014; Green et al., 1998)
chlorpromazine	4		UGT1A4	(Green, et al., 1995)
cyproheptadine	3	cyclizine, desloratadine, RO5263397	UGT1A3, UGT1A4	(Green, et al., 1998)
etodolac	3	amitriptyline, cyclizine, dexmedetomidine	UGT, UGT1A10, UGT1A9, UGT2B7	(Nakamori et al., 2012; Kutsuno et al., 2014; Oda, et al., 2015; Furukawa et al., 2014)
miconazole	3	amitriptyline, cyclizine, desloratadine	UGT1A1, UGT1A3, UGT1A4, UGT2B4, UGT2B7	(Bourcier et al., 2010)
oxymetazoline	3	amitriptyline, nicotine, RO5263397	UGT1A9	(Mahajan et al., 2011)
propranolol	3	amitriptyline, dexmedetomidine, RO5263397	UGT, UGT1A9, UGT2B7	(Yu, et al., 2010; Jinno, et al., 2014)
sertraline	3	cyclizine, desloratadine, dexmedetomidine	UGT2B7	(Obach et al., 2005)
tamoxifen	3	amitriptyline, cyclizine, desloratadine	UGT1A4	(Greer et al., 2014)
asenapine	2	cyclizine, RO5263397	UGT1A4	(Xu et al., 2016)
alprenolol	2	desloratadine, dexmedetomidine	UGT	(Zanelli et al., 2012)
norclozapine	2	amitriptyline, dexmedetomidine	UGT1A1, UGT1A4	(Algeelani et al., 2018)
carbamazepine	2	amitriptyline, RO5263397	UGT2B7	(Staines et al., 2004)
imidafenacin	2	amitriptyline, cyclizine	UGT1A4	(Kanayama et al., 2007)
toremifene	2		UGT1A4	(Greer et al., 2014)
lamotrigine	2	desloratadine, RO5263397	UGT, UGT1A3, UGT1A4, UGT2B7	(Mimura, et al., 2011)
ornidazole	2	dexmedetomidine, nicotine	UGT1A9, UGT2B7	(Du, et al., 2013)
varenicline	2		UGT2B7	(Obach, et al., 2006)
sulconazole	2	amitriptyline, desloratadine	UGT1A1, UGT1A3, UGT1A4, UGT2B7	(Bourcier et al., 2010)
tapentadol	2	nicotine, RO5263397	UGT	FDA (2008) New Drug Application, in: *022304*
edaravone	2		UGT, UGT1A6, UGT1A1, UGT1A9, UGT2B7	(Ma et al., 2012)

Example: Results from LB-VS

578 Known UGTs substrate from DDI Washington database → Remove all substrates without Reactive N → Template MIFs → 140 screened molecules MIFs for max. 25 conf. → Template – screened compound MIFs superimposition → Score per each MIFs probe → Ranking using the HDRYN1 probe (combination of 3 probes) → 19 commercial available drugs were selected and purchased

Good similarity

Candidate: Cyproheptadine



Desloratadine

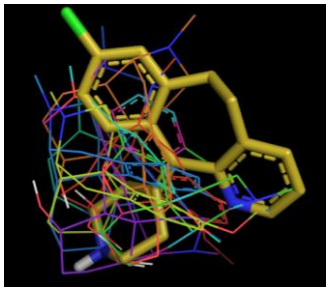
Top 20 in RO5263397, Desloratadine, and Cyclizine

For clarity the MIFs of the ligands are switched off

Best 10 poses

Low similarity

Candidate: dihydrocodeine

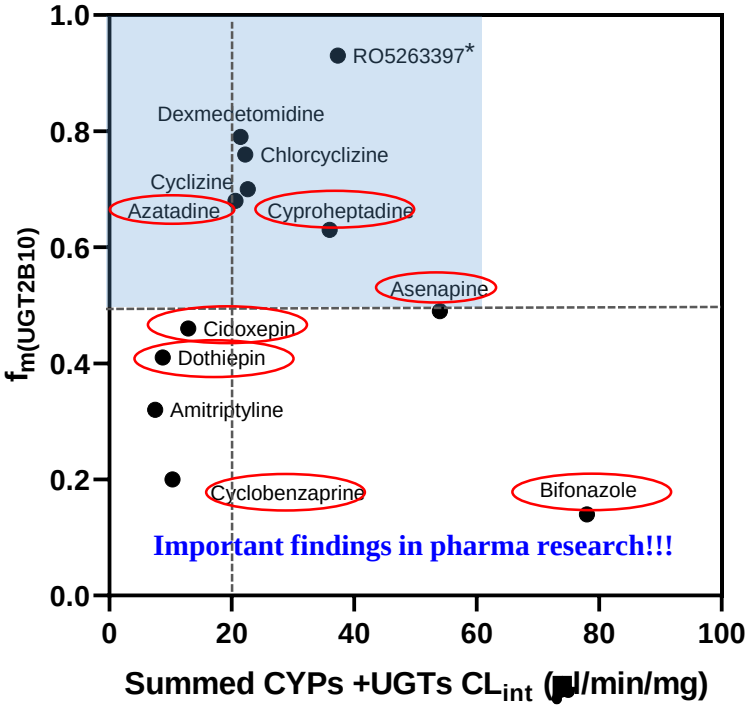


Desloratadine

Never in the top 20

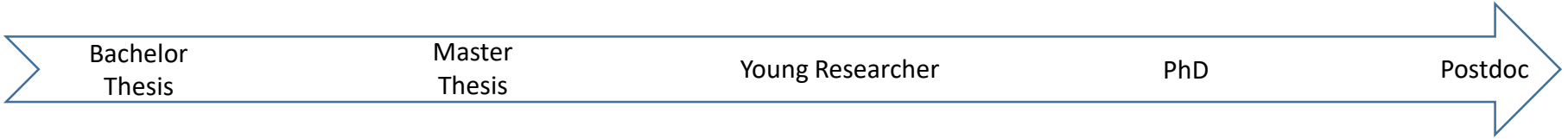
Outcome of the project: new drugs potentially subjected to UGT2B10 polymorphism

f_m(UGT2B10) and potential polymorphism exposure



Drug Metab Dispos 48:176–186, 2020

Quantitative Modelling in DMPK



University of Perugia

Drug metabolism and Molecular Modelling

University of Perugia

Synthesis in Organic Chemistry Drug Metabolism and Molecular Modelling

Molecular discovery Ltd

Drug Metabolism and Development of software for the identification of side products from chemical processes (LC-MS/MS data and NMR)

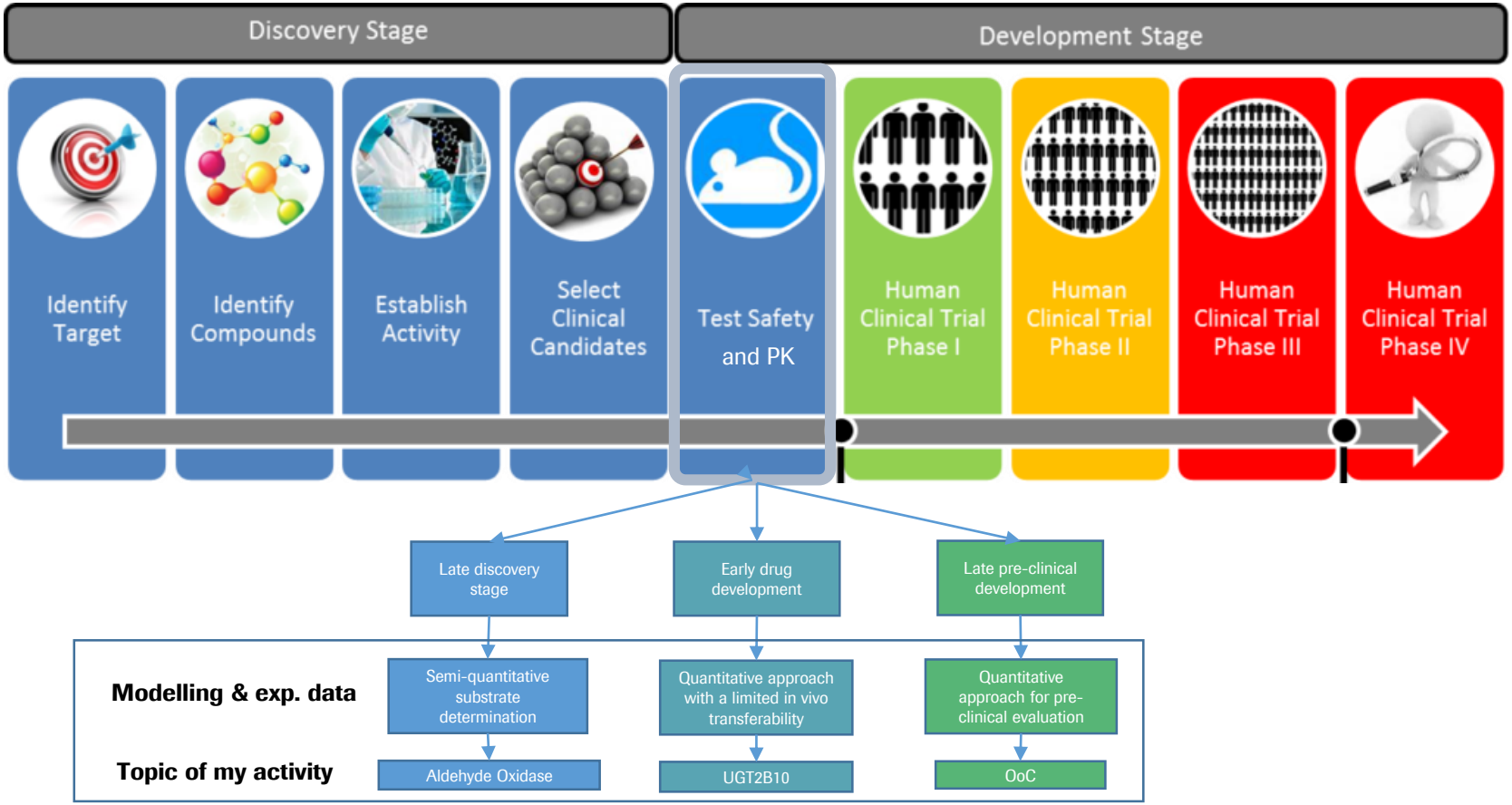
University of Perugia and Roche

Drug Metabolism and Molecular Modelling – Hightroughtput systems (Roche)

Roche and University of Manchester

DMPK modelling for in vitro data processing and data optimization in vitro assay with Liver/Gut-Liver OoC

Drug metabolism study in a pharmaceutical company context



Application of modelling for complex OoC

Aim: Application of high physiological system as OoC (Organ-on-a Chip) for DMPK investigation

- Seeded hepatocytes
- Microflow to mimic the physiological blood flow
- High cell longevity

Application

Single tissue (liver OoC)

- Long incubation (compound with low metabolic turnover)
- DDI study
- fm estimation

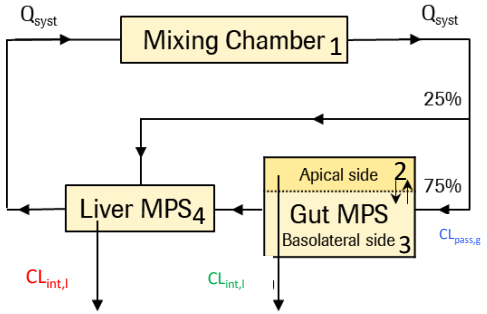
Multi-tissue (Gut-Liver OoC)

- First pass metabolism (GI)
- Intestinal absorption
- Hepatic metabolism
- Intestinal and liver cross-talking (high physiological environment)

Better IVIVE

Complexity needs a mathematical deconvolution

Tsamandours-Chen model



d/dt(A1) = Q * (C4 - C1)
d/dt(A2) = $CL_{pass,g}$ * (fum * C3 - C2) - $CL_{int,g}$ *N3* C2
d/dt(A3) = $CL_{pass,g}$ * (C2 - fum * C3) + fQ * Q * (C1 - C3)
d/dt(A4) = Q * (fQ * C3 + fQ2 * C1 - C4)- $CL_{int,l}$ * fum * N4 *C4

Assumption

$CL_{pass,g} = P_g SA_g >> CLint,_g$
$CL_{pass,h} = P_h SA_h >> CLint,_h$

Metabolism is the rate limiting step

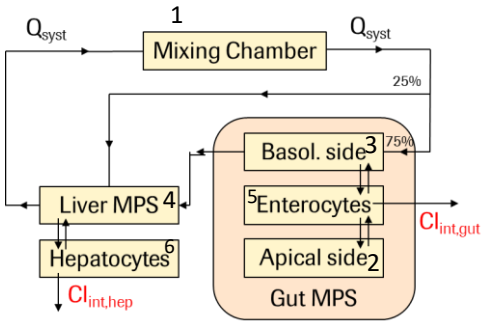
Gut and Liver OoC

Compartmental model is described by ODEs

Simple PBPK

Otherwise

General model



d/dt(A1) = - Q * (C1 - C4 / KP4)
d/dt(A2) = - $CL_{pass,g}$ * NPERM * (C2 * fum2 - C5)
d/dt(A3) = $CL_{pass,g}$ * NPERM * (C5- C3 * fum) - Q * fQ * C3 / KP3 + Q * fQ * C1
d/dt(A4) = Q * fQ * C3 / KP3 + Q * fQ2 * C1 - Q * C4 / KP4 - $CL_{pass,l}$ * (C4 * fum - C6)
d/dt(A5) = $CL_{pass,g}$ * NPERM * (C2 * fum2 - C5) - $CL_{pass,g}$ * NPERM *(C5 - C3 * fum) - $CL_{int,g}$ * N5 *C5
d/dt(A6) = $CL_{pass,l}$ * (C4 * fum - C6) - $CL_{int,l}$ * N6 * C6

These kind of experiments are very expensive and time consuming (no high-throughput system)

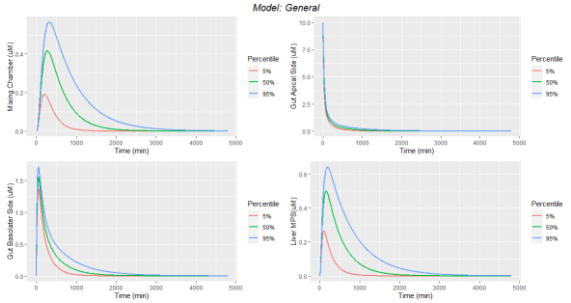
The AAPS Journal **volume 19**, pages1499–1512(2017)

Example: the importance of the model description

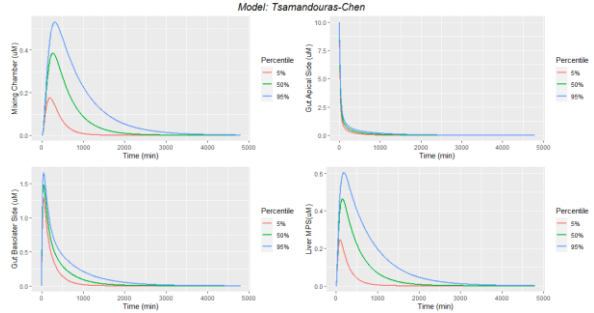
$P_{app,gut}$ = 30-fold

Tsamandouras-Chen conditions are met

General model



Tsamandouras-Chen model



Same profiles in all compartments regardless of the model

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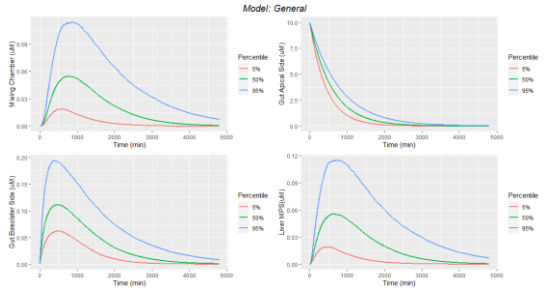
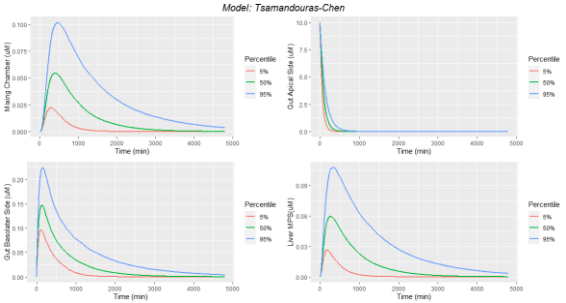
Tsamandouras-Chen conditions are NOT met

General model



Tsamandouras-Chen model



Only the general model represents the real profile

Acknowledgment

- All my previous colleagues in the group of Prof Gabriele Cruciani (Chemoinformatic and Chemometrics, Department of Chemistry, Biology, and Biochemistry at University of Perugia)
- All Molecular Discovery employees for the nice collaboration
- All my current mentors in Roche
  - Dr Benjamin Ribba and Neil John Parrott (M&S)
  - Dr Michael Gertz (DPL group)
  - Dr Stephen Fowler (i-safe)
- Prof Aleksandra Galetin (University of Manchester, Department of Pharmacy)

Thanks for your kind attention