

PHARMACOPHORE MODELING, VIRTUAL COMPUTATIONAL SCREENING AND BIOLOGICAL EVALUATION STUDIES

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Reproducibility, standards and SOP in bioinformatics
Combined CHARME – EMBnet and NETTAB 2016 Workshop,
October 25-26, 2016, Rome, Italy

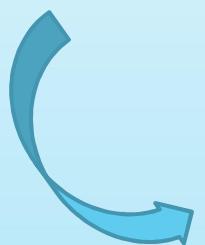
Drug Discovery process



Bioinformatics tools



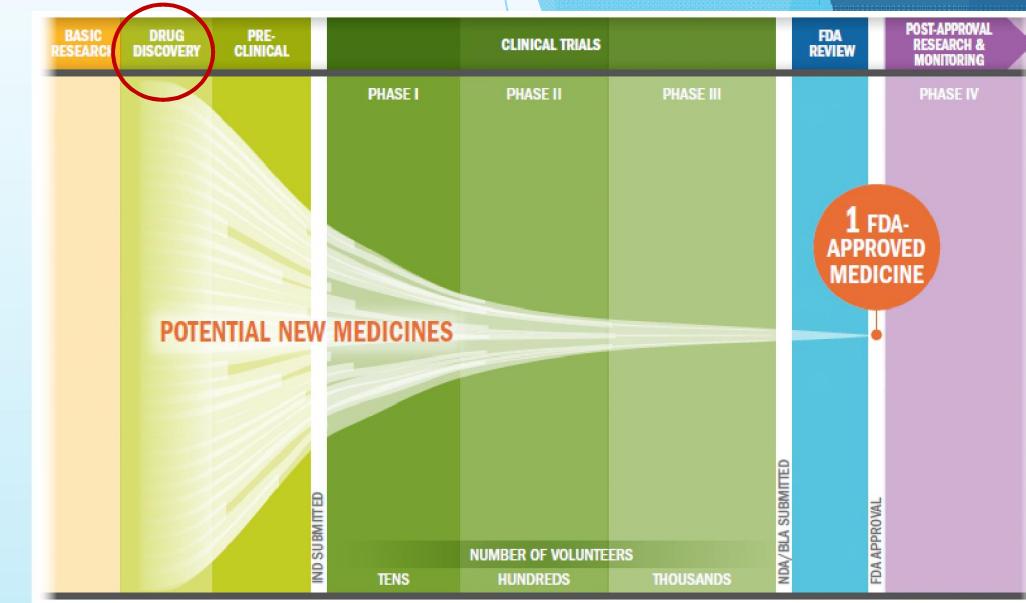
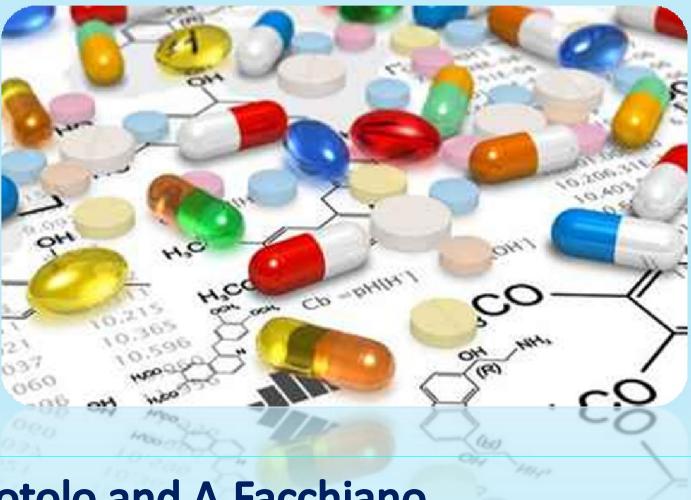
New potential drug-likes



New inhibitors



Specific biological target



Pharmacophore modeling method



a more detailed *screening process*

PHARMIT

- Online interactive environment
- Pharmacophore modeling and virtual screening



DISCOVERY STUDIO

- Platform for simulating small molecules and macromolecules systems



The screenshot shows the PHARMIT homepage. At the top, it says "pharmit interactive exploration of chemical space". Below that is a search bar with fields for "start from PDB:" (set to ligand) and "binding site waters:" (set to ignore), with a "submit" button and a "examples" link. To the right is a "create" section for submitting libraries, with fields for email and password, and links for "log in", "register new account", and "log in as guest". There is also a "code" and "help" link at the top right. A chemical structure with pharmacophore features is displayed in the header area.

The screenshot shows the BIOVIA Discovery Studio Version 4.5 interface. It features a dark blue header with the DS BIOVIA / Discovery Studio logo and "Version 4.5". Below the header is a banner for "Solutions for Computational Chemistry and Biology". The main area displays a 3D molecular model of a protein-ligand complex. Text overlays include "Discovery Studio v4.5.0.15071" and "Processing XML Data". At the bottom, there is a copyright notice: "©2015 Dassault Systèmes, all rights reserved" and the Dassault Systèmes logo.

multi-step protocol

1) Search in PDBdb a model structure for target

Study of disease at molecular level
- Genes and proteins involved

Identification a *model crystal structure*
of protein sequence of target in *PDBdb*



Choosing the best model of target



Comparing several structures

RCSB PDB Deposit Search Visualize Analyze Download Learn More MyPDB Login

PDB-101 Worldwide Protein Data Bank EMDDataBank Nucleic Acid Database StructuralBiology Knowledgebase Worldwide Protein Data Bank Foundation

Facebook Twitter YouTube App Store

Structure Summary 3D View Annotations Sequence Sequence Similarity Structure Similarity Experiment Literature

Biological Assembly 1 3D View

3096

Crystal Structure of Human AKT1 with an Allosteric Inhibitor

DOI: 10.2210/pdb3096/pdb

Classification: TRANSFERASE

Deposited: 2010-08-03 Released: 2010-10-13

Deposition author(s): Voegeli, W.C., Wu, W.-I., Lord-Ondash, H.A., Dizon, F.P., Vigers, G.P.A., Brandhuber, B.J.

Organism: Homo sapiens

Expression System: TRICHOPLUSIA NI

Structural Biology Knowledgebase: 3096 (3 models >20 annotations) SBKB.org

Experimental Data Snapshot

Method: X-RAY DIFFRACTION Resolution: 2.7 Å R-Value Free: 0.308 R-Value Work: 0.245

wwPDB Validation

Metric	Percentile Ranks	Value
Rfree	21	0.308
Clashscore	0.8%	21
Ramachandran outliers	7.7%	0.8%
Sidechain outliers	7.9%	7.9%
RSRZ outliers	Worse	Better

Literature

Download Primary Citation

View in 3D: NGL or JSmol or PV (in Browser)

Standalone Viewers

Simple Viewer Protein Workshop Ligand Explorer Kiosk Viewer

Protein Symmetry: Asymmetric (View in 3D)

Protein Stoichiometry: Monomer

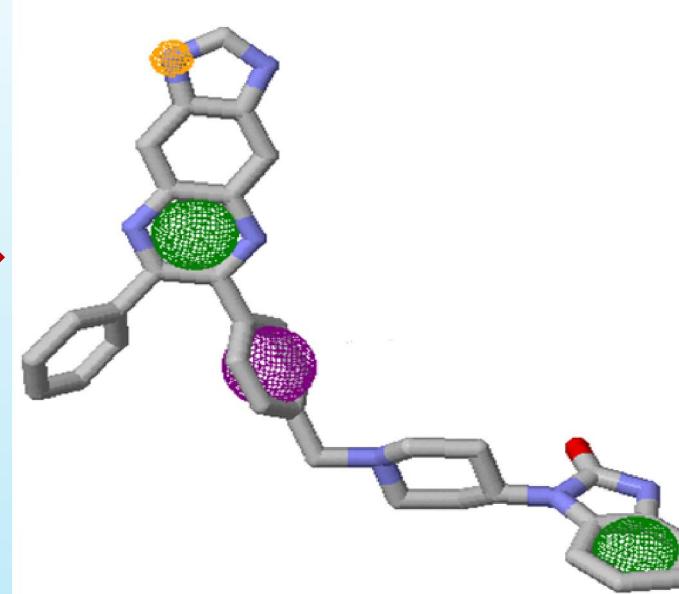
Crystal structure of human AKT1 with an allosteric inhibitor reveals a new mode of kinase inhibition.

2) Pharmacophore modeling/virtual computational screening

Pharmacophore model

Pharmacophore standard features:

- HydrogenBond_Acceptor (orange)
- Hydrophobic group (green)
- Aromatic Ring (magenta)



Pharmacophore models have been created through PHARMIT and Discovery Studio

Investigate all *bioactive* compounds of ZINCdb



Virtual computational screening

Not Authenticated – sign in
Active cart: Temporary Cart (0 items)
60

ZINC 12

About Search Subsets Help Social G+ 0 Quick Search Bar...
Synonyms (2) Vendors (2) Annotations (7) Representations (2) Notes (2) Targets (6) Clustered (3) Reactome (23) Rings (0) Analogs (1)

ZINC28129161

In ZINC since	Heavy atoms	Benign functionality
February 24 th , 2009	42	No

Popular Name: *Akti-1/2*
Find On: PubMed – Wikipedia – Google
CAS Number: 612847-09-3

Other Names:
*1,3-Dihydro-1*H*-1*H*-[6-phenyl-1*H*-imidazo[4,5-*g*]quinoxalin-2-yl]phenylmethyl-4-piperidinyl-2*H*-benzimidazol-2-one*

SMILES: C1CCO(C=C1)c2cnc3cc(C=O)c(N)c4c3cc(C=C4)C(=N)C5CCCC(C=C5)n7cccc8[nH]c78
Download: MOL2 SDF SMILES Flexibase

Vendors
ChemBio Pharma KB-74550
Toronto Research Chemicals A450300

Annotations
BindingDB.org 95058
ChEMBL12 CHEMBL258844
ChEMBL12 10uM CHEMBL258844
ChEMBL19 CHEMBL258844
Collaborative Drug Discovery 1208023
Protein Databank 1Q0
PubChem 16218054, 44476122, 19196499, 68904072

Draw Identity 99% 90% 80% 70%

The parameters applied for realizing virtual screening



Choosing only the good compounds

Ligand-target molecular interactions through AutoDock (Blind docking)

- lowest binding energy
- estimated inhibition constant



Choosing only the best candidates

Analysis by means of Discovery Studio:

- min/max features for pharmacophores
 - Selectivity score
 - Good predicted activity

Virtual computational screening parameters

ZINC natural purchasable database

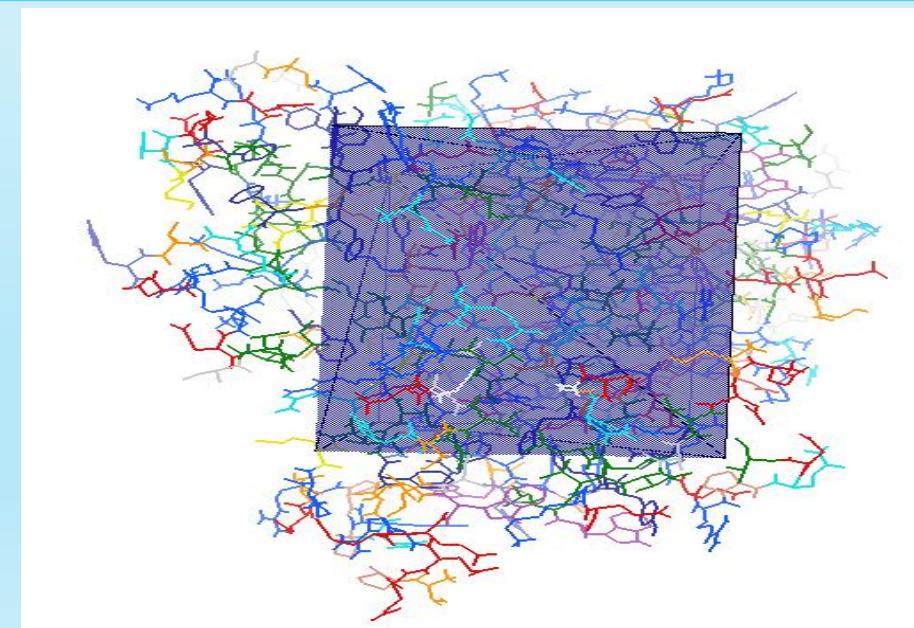
Bioactive compounds

RMSD range values

MW range values

RBnds (rotational angle) not exceeding 15

Physical-chemical features for interaction between protein-ligand



3) Molecular validation

Underline of *aminoacids pocket*
involved in molecular interactions



To find only **good lead compounds**



Molecular *focused docking*
protein-ligand

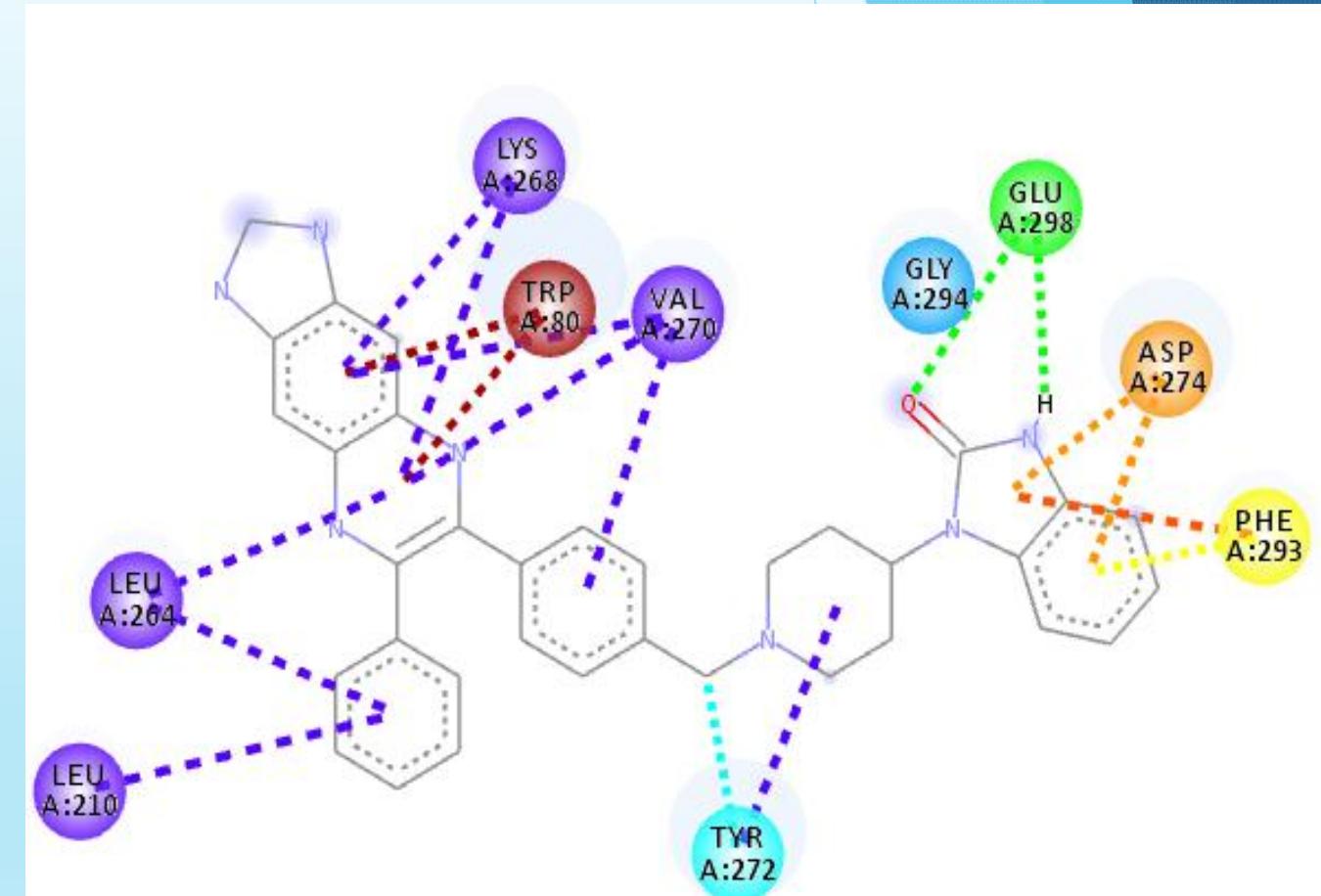


Physical-chemical properties

Pharmacokinetics and
Pharmacodynamics models



characterize *the new lead compounds*



4) Physical-chemical properties and pharmacokinetics/pharmacodynamics models

physical-chemical properties
of the best candidates



To trace their origin and their
features



ALogP
Solubility
pKa
Chemical stability



Chemicalize
FooDB/HMDB
PubChem Compound
SciFinder



**PharmacoKinetics and
PharmacoDynamics models**

Biosynthetic pathways to predict plausible enzyme-catalyzed reaction



Select only good lead compounds



Pharmacokinetics and Pharmacodynamics models



Bioavailability
ADMET/Toxicity
Run simulation (MD)



PathPred: Pathway Prediction server

PathSearch **PathComp** **PathPred** **KEGG2**

About PathPred

PathPred is a web-based server to predict plausible enzyme-catalyzed reaction pathways from a query compound using the information of [RDM patterns](#) and chemical structure alignments of substrate-product pairs. This server provides plausible reactions and transformed compounds, and displays all predicted reaction pathways in tree-shaped graph.

- PathPred help

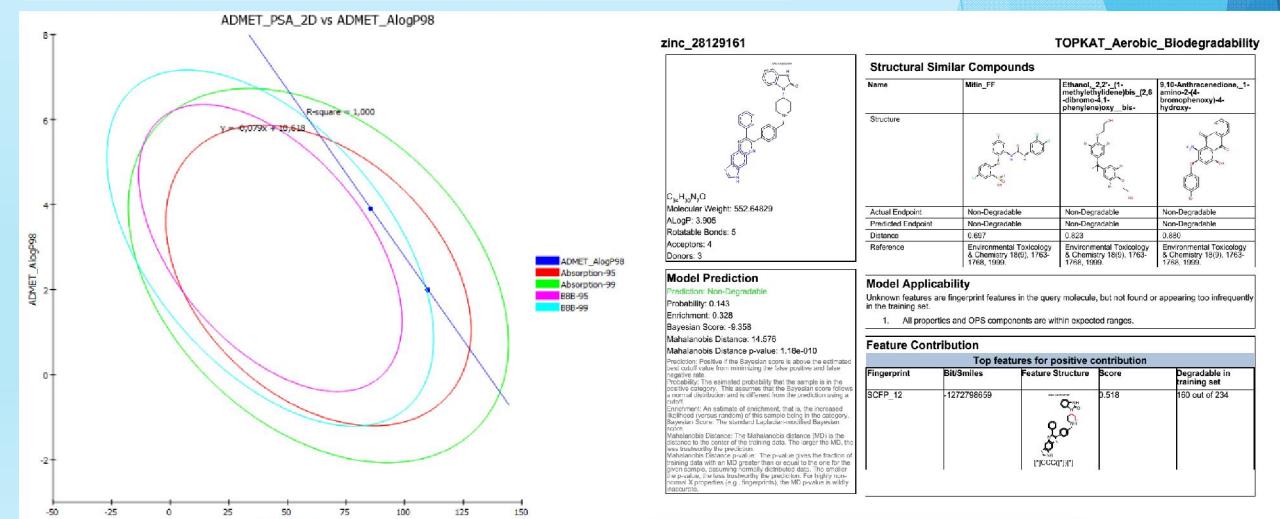
Reference pathway:

- Xenobiotics Biodegradation (Bacteria)
- Biosynthesis of Secondary Metabolites (Plants)

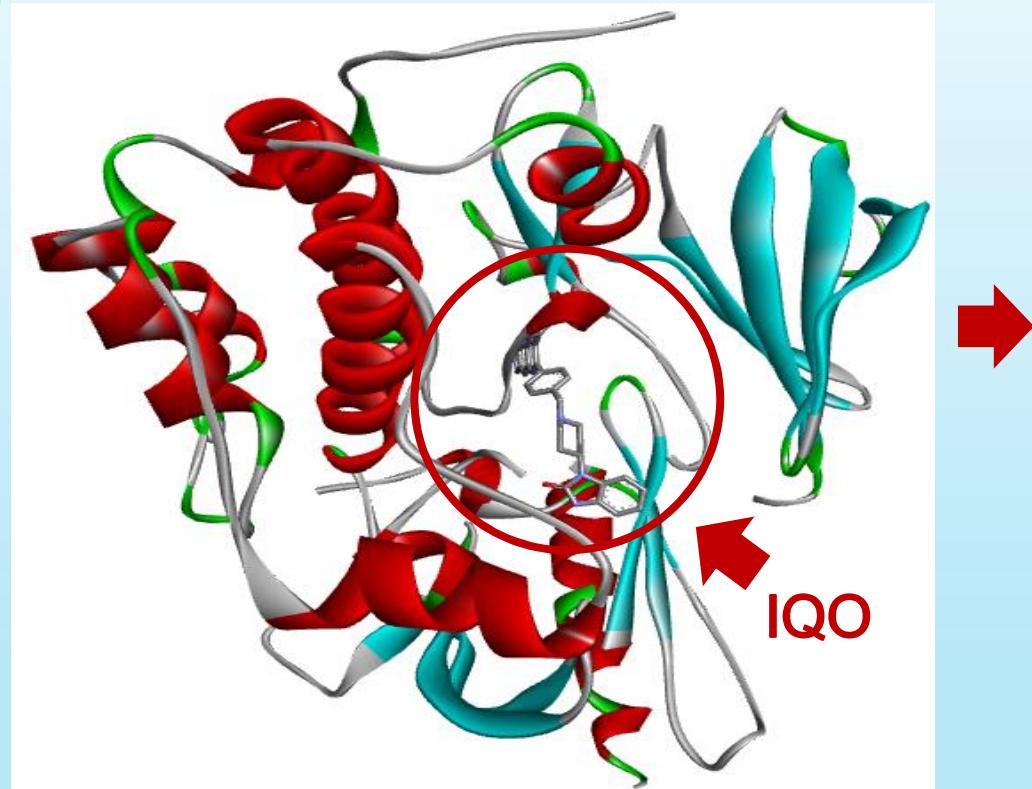
Next

Pathway Prediction server Ver. 1.13

Feedback **KEGG** **GenomeNet** Kyoto University Bioinformatics Center



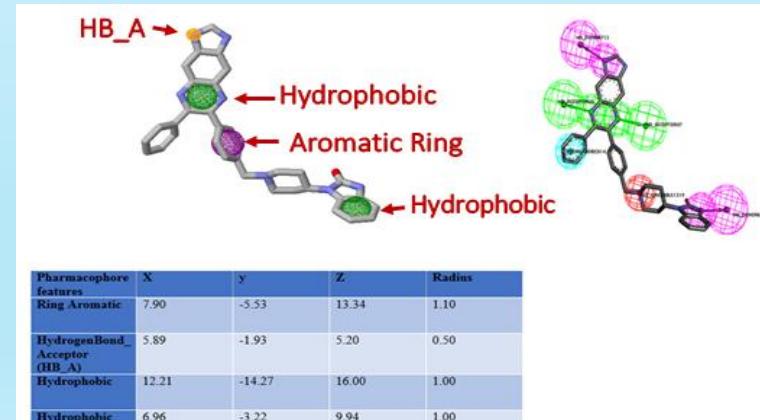
Search of model structure and pharmacophore modeling for AKT1 (modulator of PI3K)



3096 as reference crystal structure

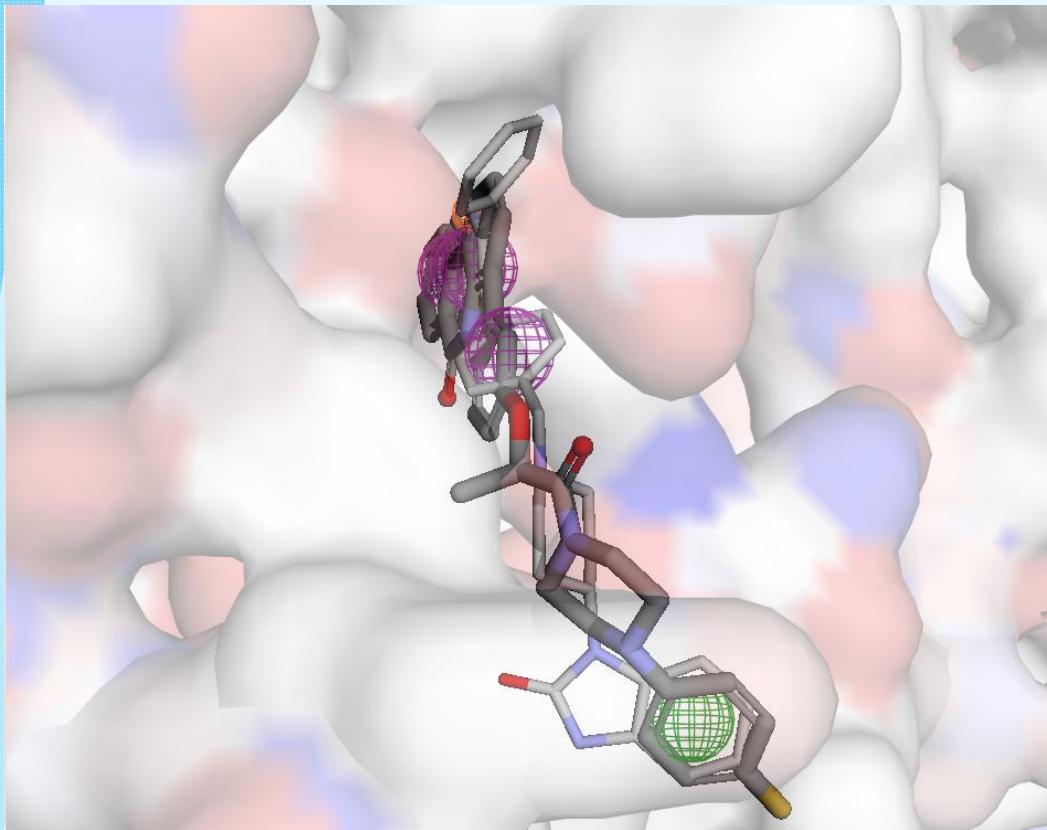
Accession	Max score	Total score	Query cover	E Value	Ident
3096	922	922	92%	0.0	100%
4EJN	922	922	92%	0.0	99%
3OCB	699	699	70%	0.0	99%
4GV1	698	698	70%	0.0	99%
3CQU	698	698	70%	0.0	99%
1MRV	613	613	70%	0.0	86%

Some structure of AKT1 in PDBdb



Pharmacophore model

Virtual computational screening and molecular validation



Virtual screening of all compounds

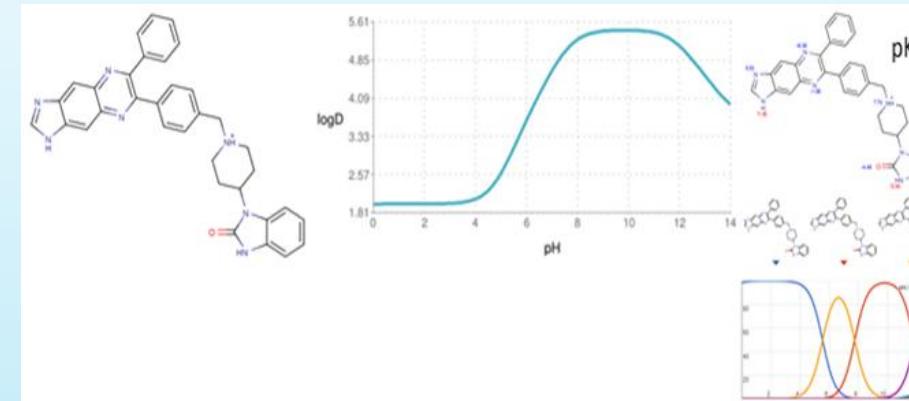


COMPOUNDS	BINDING ENERGY Kcal/mol	Ki
ZINC 4259855	-11.00	737,43 nM
ZINC 3869685 (quercetin)	-6.55	15,85 μM
ZINC 1237912	-10.00	1,12 μM
ZINC 1447881	-9.36	1,36 μM
ZINC 02154548	-9.16	1,04 μM
ZINC 2429155	-10.00	2,21 μM
ZINC 02666313	-8.91	1,6 μM
ZINC 03851635	-9.00	99,14 nM
ZINC 13691379	-9.30	1,13 μM
ZINC 14611917	-8.73	2,16 μM
ZINC 22161363	-10.11	1,41 μM
ZINC 54307082	-8.72	2,2 μM

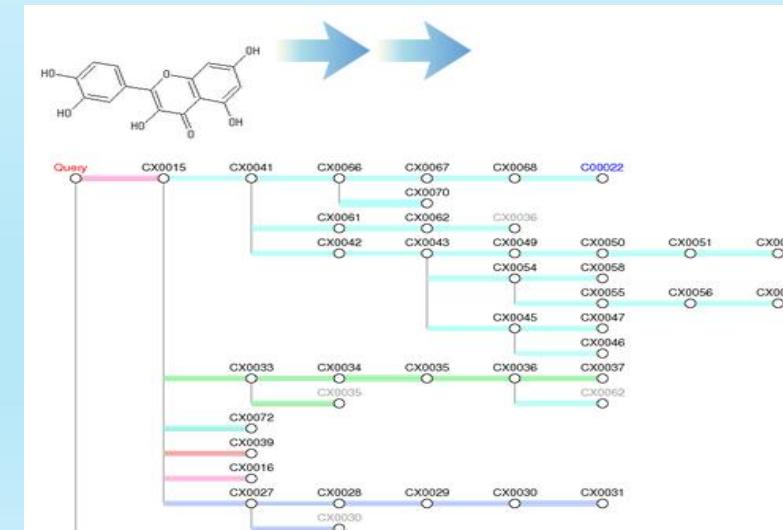
Direct molecular docking

Physical-chemical properties and pharmacokinetics-pharmacodynamics models

Study of physical-chemical
Properties of selected compounds



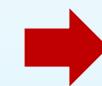
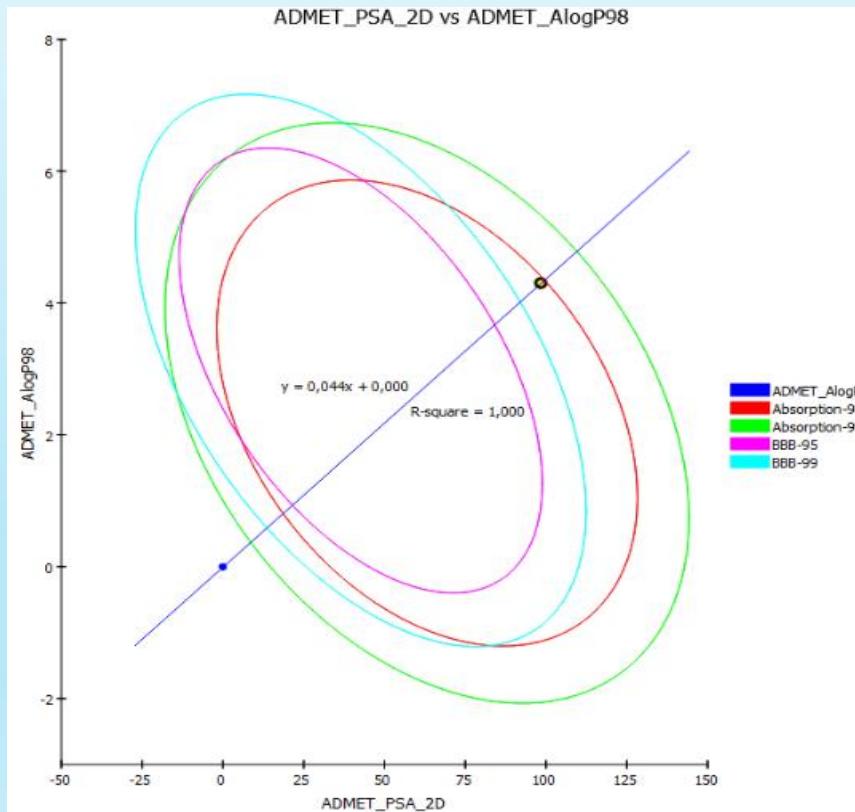
Prediction of *metabolic pathways*



Pharmacokinetics and Pharmacodynamics models



ADMET properties



TOXICITY level



**Select only lead compounds
for the next assays**

	ZINC3869685 (quer 1G/1F)	ZINC1237912 (1G /1F)	ZINC1447881(1G /1F)	ZINC2161363 (1G /1F)
AEROBIC BIODEGRADABILITY	NON DEGRADABLE	DEGRADABLE	NON DEGRADABLE	DEGRADABLE
AMES MUTAGENICITY	MUTAGEN	NON MUTAGEN	NON MUTAGEN	NON MUTAGEN
DTP	NON TOXIC	NON TOXIC	NON TOXIC	NON TOXIC
FDA MOUSE FEMALE	NON CARCINOGEN	NON CARCINOGEN	NON CARCINOGEN	NON CARCINOGEN
NTP MOUSE FEMALE	NON CARCINOGEN	NON CARCINOGEN	NON CARCINOGEN	NON CARCINOGEN
FDA MOUSE MALE	NON CARCINOGEN	NON CARCINOGEN	NON CARCINOGEN	NON CARCINOGEN
NTP MOUSE MALE	CARCINOGEN	CARCINOGEN	CARCINOGEN	CARCINOGEN
OCULAR IRRITANCY	NON IRRITANT	NON IRRITANT	NON IRRITANT	NON IRRITANT
RAT FEMALE FDA	NON CARCINOGEN	NON CARCINOGEN	NON CARCINOGEN	NON CARCINOGEN
RAT FEMALE NTP	NON CARCINOGEN	NON CARCINOGEN	NON CARCINOGEN	NON CARCINOGEN
RAT MALE FDA	NON CARCINOGEN	NON CARCINOGEN	NON CARCINOGEN	NON CARCINOGEN
RAT MALE NTP	CARCINOGEN	NON CARCINOGEN	NON CARCINOGEN	CARCINOGEN
SKIN IRRITANCY	MILD	MODERATE_SEVERE	MILD	MODERATE_SEVERE
SKIN SENSITIZER	SENSITIZER	SENSITIZER	SENSITIZER	SENSITIZER
WRC	NON CARCINOGEN	NON CARCINOGEN	NON CARCINOGEN	NON CARCINOGEN
CARCIN POTENCY TD50 MOUSE	44,985 mg/kg	28,686 mg/kg	35,307 mg/kg	47,569 mg/kg
CARCIN POTENCY TD50 RAT	48,799 mg/kg	0,726 mg/kg	5,265 mg/kg	0,575 mg/kg
CHRONIC LOAEL	0,007 g/kg	0,012 g/kg	0,010 g/kg	0,014 g/kg
DAPHNIA EC50	5,772 g/l	0,187 g/l	0,162 g/l	0,219 g/l
FATHEAD MINNOW LD50	0,074 g/l	0,0 g/l	0,000 g/l	0,0 g/l
RAT INHALATIO LC50	806,217 mg/m3/h	3933,276 mg/m3/h	3581,196 mg/m3/h	4152,921 mg/m3/h
RAT MTDF	1,594 g/kg	0,047 g/kg	0,045 g/kg	0,046 g/kg
RAT MTDG	0,0 g/kg	0,001 g/kg	0,0 g/kg	0,003 g/kg
RAT ORAL LD50	1,191 g/kg	1,841 g/kg	1,943 g/kg	1,710 g/kg

Conclusions and Future Works

This procedure is robust and efficient for obtaining “*lead compounds*”:

- Selective for the allosteric site
- Complete for the number of performed analyses

Good strategy for the validation of *SOPs in bioinformatics* in case of *drug discovery*

The next planned steps

- Detailed analysis of metabolic pathways
- Molecular dynamics simulations for behavior
- The experimental analysis “*in vitro/in vivo*”

Acknowledgements and Credits

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Bioinformatics and Computational
Biology Laboratory

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THANK YOU FOR YOUR
ATTENTION

