FONDAZIONE PER LA RICERCA FARMACOLOGICA GIANNI BENZI ONLUS

UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

XVI FORESIGHT TRAINING COURSE
Repurposing to cover unmet needs: the current scenario in Europe and the proposed changes to the Pharmaceutical Legislation

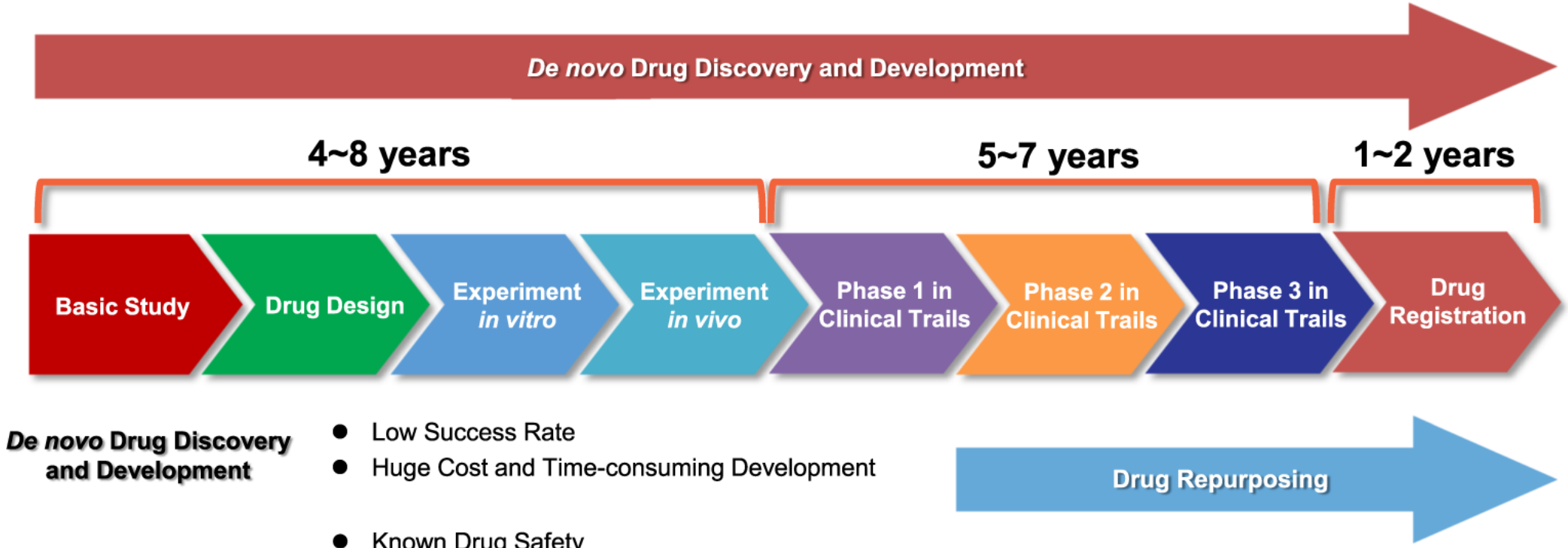
In-silico structure-based platforms for drug repurposing

Cosimo D. Altomare
Department of Pharmacy – Pharmaceutical Sciences
University of Bari Aldo Moro

18 Dec 2023 - Bari, Italy

Drug repurposing

Discovering new indications for existing drugs originally developed to treat different diseases



***De novo* Drug Discovery and Development**

- Low Success Rate
- Huge Cost and Time-consuming Development

Drug Repurposing

- Known Drug Safety
- Reduced Pharmacokinetic Uncertainty

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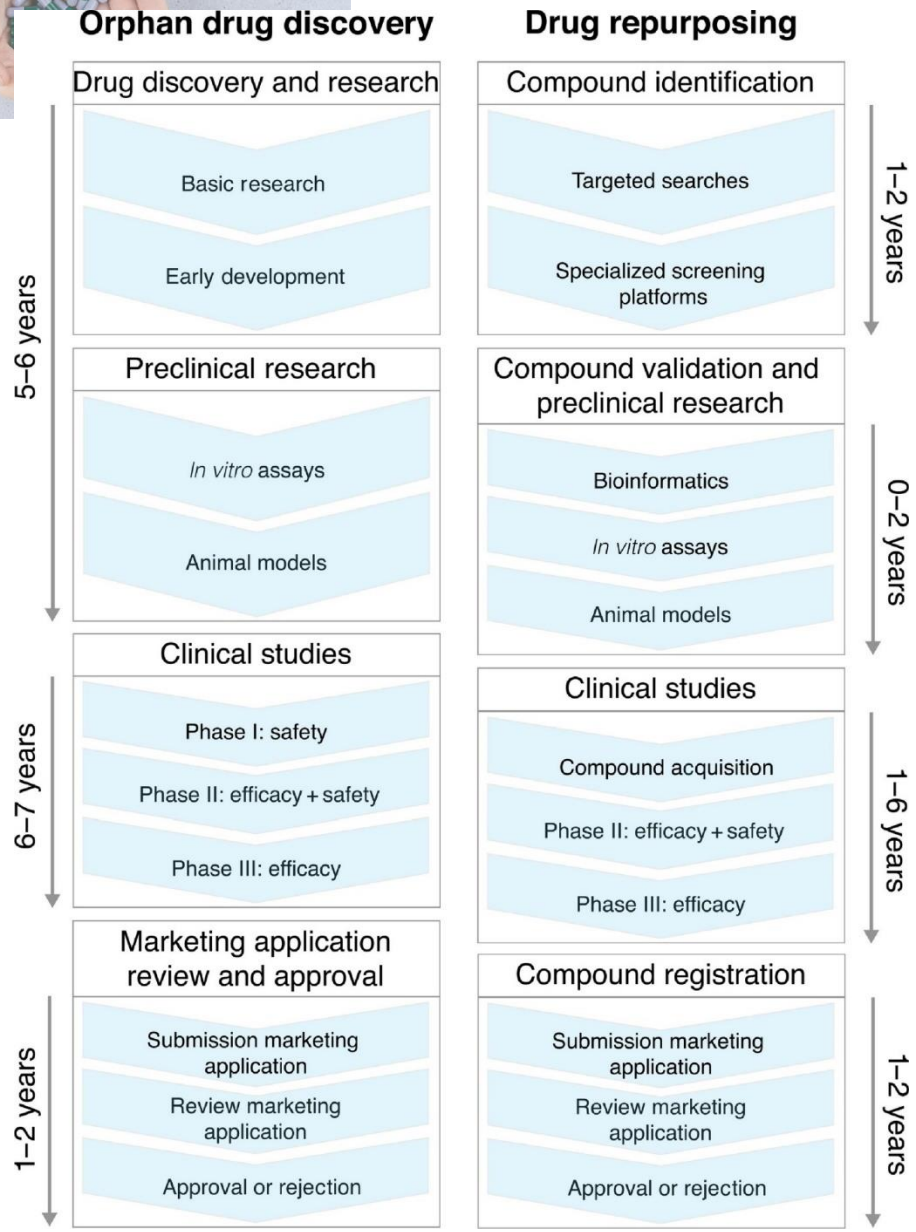
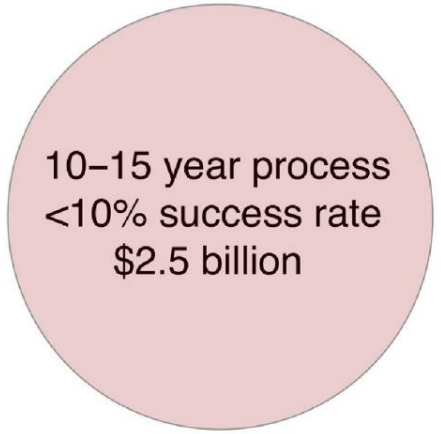
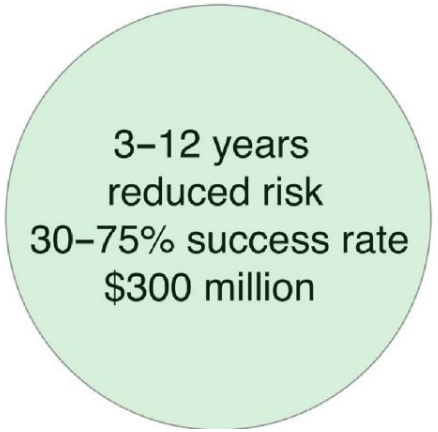
Drug repurposing and orphan drug development







Drug repurposing increase success rates, reduce development costs, shorten time to the market, and therefore reduce the overall development risk compared with traditional drug development

Drug Repurposing for Rare Diseases. Trends in Pharmacological Sciences, **2021**, Vol. 42, No. 4, doi.org/10.1016/j.tips.2021.01.003

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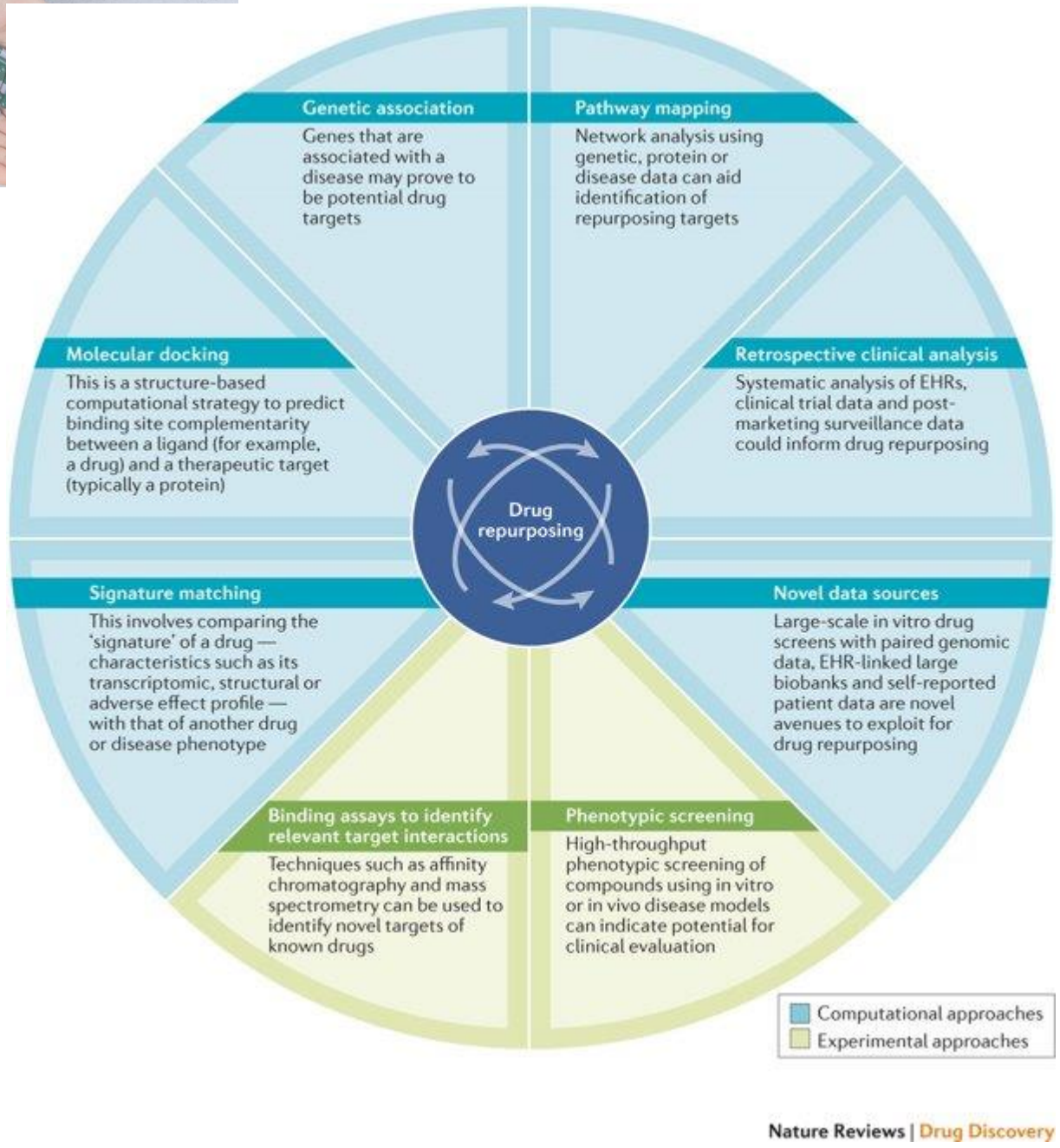
Drug repurposing

Historically, drug repurposing has been largely opportunistic and serendipitous.

Pushpakom et al. Drug repurposing: progress, challenges and recommendations
Nature Reviews Drug Discovery 18, 41–58 **(2019)**

Drug name	Original indication	New indication	Date of approval	Repurposing approach used	Comments on outcome of repurposing
Zidovudine	Cancer	HIV/AIDS	1987	In vitro screening of compound libraries	Zidovudine was the first anti-HIV drug to be approved by the FDA
Minoxidil	Hypertension	Hair loss	1988	Retrospective clinical analysis (identification of hair growth as an adverse effect)	Global sales for minoxidil were US$860 million in 2016 (Questale minoxidil sales report 2017; see Related links)
Sildenafil	Angina	Erectile dysfunction	1998	Retrospective clinical analysis	Marketed as Viagra, sildenafil became the leading product in the erectile dysfunction drug market, with global sales in 2012 of $2.05 billion[5]
Thalidomide	Morning sickness	Erythema nodosum leprosum and multiple myeloma	1998 and 2006	Off-label usage and pharmacological analysis	Thalidomide derivatives have achieved substantial clinical and commercial success in multiple myeloma
Celecoxib	Pain and inflammation	Familial adenomatous polyps	2000	Pharmacological analysis	The total revenue from Celebrex (Pfizer) at the end of 2014 was $2.69 billion (Pfizer 2014 financial report; see Related links)
Atomoxetine	Parkinson disease	ADHD	2002	Pharmacological analysis	Strattera (Eli Lilly) recorded global sales of $855 million in 2016
Duloxetine	Depression	SUI	2004	Pharmacological analysis	Approved by the EMA for SUI. The application was withdrawn in the US. Duloxetine is approved for the treatment of depression and chronic pain in the US
Rituximab	Various cancers	Rheumatoid arthritis	2006	Retrospective clinical analysis (remission of coexisting rheumatoid arthritis in patients with non-Hodgkin lymphoma treated with rituximab[144])	Global sales of rituximab topped $7 billion in 2015 (REF.[145])
Raloxifene	Osteoporosis	Breast cancer	2007	Retrospective clinical analysis	Approved by the FDA for invasive breast cancer. Worldwide sales of $237 million in 2015 (see Related links)
Fingolimod	Transplant rejection	MS	2010	Pharmacological and structural analysis[146]	First oral disease-modifying therapy to be approved for MS. Global sales for fingolimod (Gilenya) reached $3.1 billion in 2017 (see Related links)
Dapoxetine	Analgesia and depression	Premature ejaculation	2012	Pharmacological analysis	Approved in the UK and a number of European countries; still awaiting approval in the US. Peak sales are projected to reach $750 million
Topiramate	Epilepsy	Obesity	2012	Pharmacological analysis	Qsymia (Vivus) contains topiramate in combination with phentermine
Ketoconazole	Fungal infections	Cushing syndrome	2014	Pharmacological analysis	Approved by the EMA for Cushing syndrome in adults and adolescents above the age of 12 years (see Related links)
Aspirin	Analgesia	Colorectal cancer	2015	Retrospective clinical and pharmacological analysis	US Preventive Services Task Force released draft recommendations in September 2015 regarding the use of aspirin to help prevent cardiovascular disease and colorectal cancer[52]



Computational and experimental approaches can also be used to identify repurposing opportunities.

Both approaches are increasingly being used synergistically

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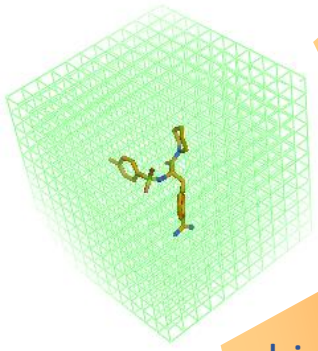
In-silico Drug Discovery/Repurposing

A knowledge-based inventive process



ligands

biological data

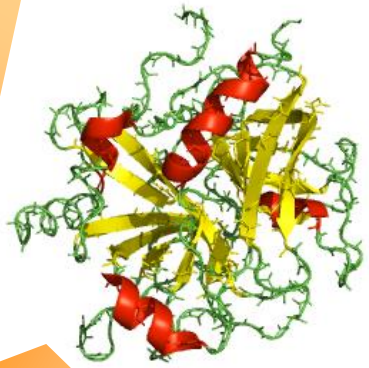
Ligand-Based

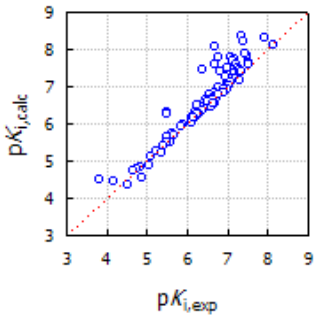
Drug Design

Structure-Based

protein 3D structure

binding mode





Quantitative Structure-Activity Relationships (1-3D-QSAR)

Molecular Docking and Molecular Dynamics

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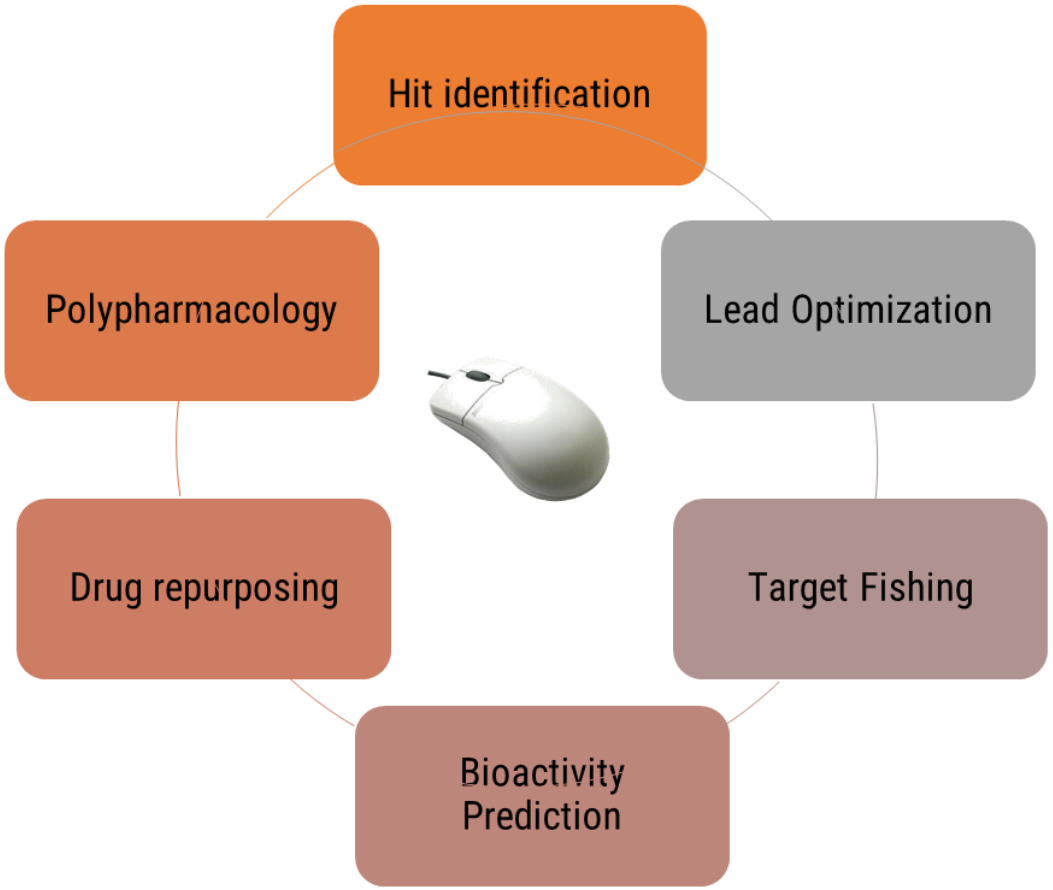
How *in silico* methods can be useful in drug discovery?



- ✓ Hit identification to identify compounds able to interact with confirmed activity against a biological target
- ✓ Lead Optimization improve potency, reduce off-target activities, and physiochemical/metabolic properties
- ✓ Target Fishing: identify potential target proteins that are likely to bind to a given small molecule
- ✓ Bioactivity Prediction: prediction of bioactivity values for a set of potential target proteins.
- ✓ Polypharmacology: interaction of drug molecules with multiple targets.
- ✓ Drug repurposing: discovering new uses for existing drugs originally developed to treat a different disease or condition.

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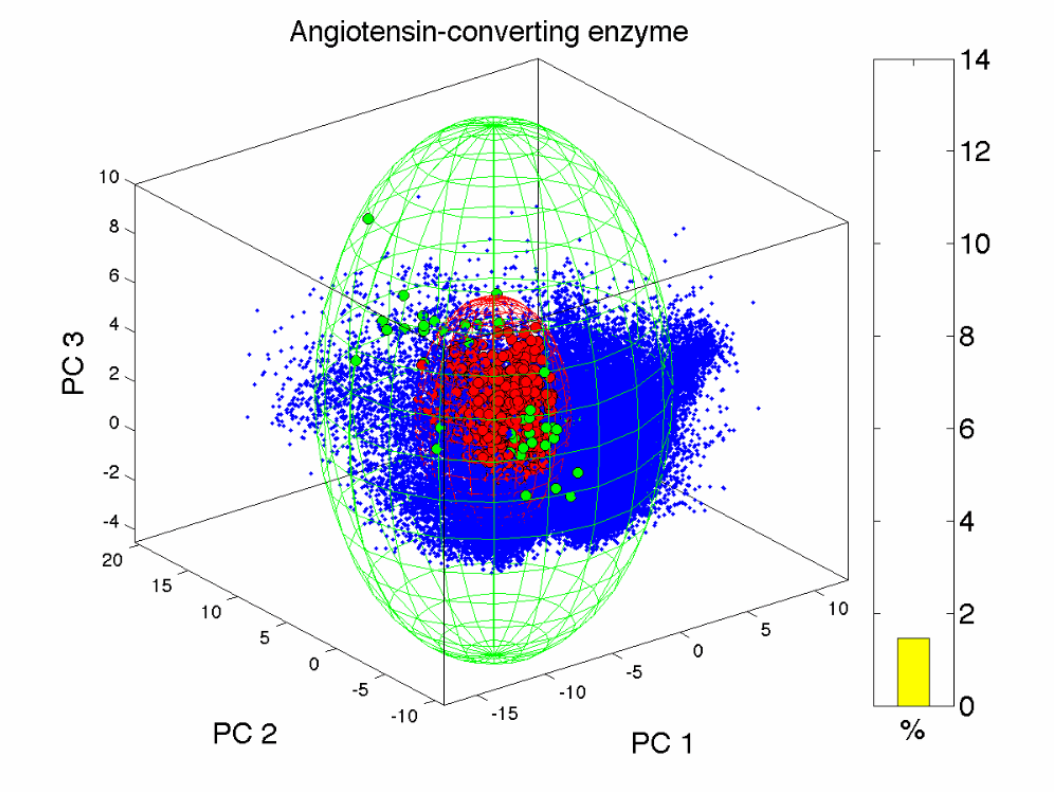
Navigating from chemical to biology and medicine spaces



The continuum of the **chemical space** and the discrete areas of chemical space that are occupied by **target** and **off-target** compounds

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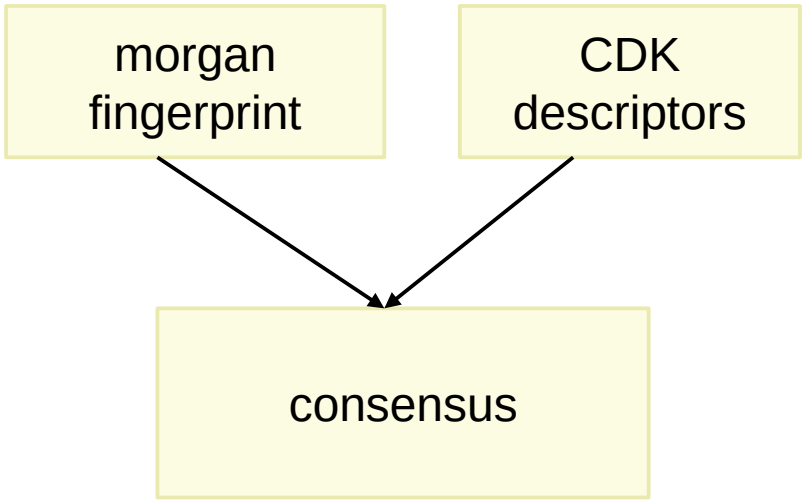
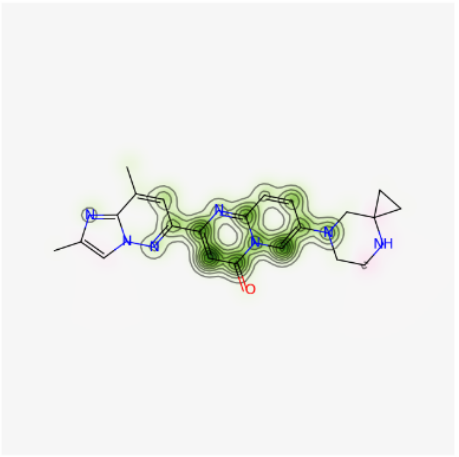
Platforms for biological activity/ADMETox prediction

- Pred-herg : http://predherg.labmol.com.br/predict
- Venompred : http://www.mmvsl.it/wp/venompred/
- SwissADME : http://www.swissadme.ch/index.php

Pred-hERG: platform for cardiotoxicity prediction



Pred-hERG Results

Prediction / Potency	Confidence	Applicability domain (AD)	Probabilty Map
Potential cardiotoxic (+)	60%	No (Value= 0.24 and limit = 0.26)	
Weak or Moderate	60%		

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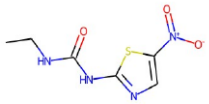
VenomPred : platform for toxicity prediction

fingerprint based:
1. Morgan
2. RDKit
3. Pharm2D
4. PubChem
5. LINGO

ML models:
1. Random Forest
2. Support Vector Machine
3. K Nearest Neighbour
4. MultiLayer Perceptron

VenomPred

VenomPred is a Machine Learning based platform for qualitative prediction of ***mutagenicity***, ***carcinogenicity***, ***hepatotoxicity*** and ***estrogenicity*** of chemicals.



Name	Entry_1
SMILES	CCNC(=O)NC1=NC=C(S1)[N+](=O)[O-]

Endpoint	Probability
Mutagenicity	89 %
Carcinogenicity	78 %

Endpoint	Probability
Hepatotoxicity	37 %
Estrogenicity	7 %

The main article describing the web service and its underlying methodologies is: VenomPred: A Machine Learning Based Platform for Molecular Toxicity Predictions. *Int. J. Mol. Sci.* **2022**, *23*, 2105

Università di Pisa
Molecular Modeling & Virtual Screening Laboratory
Dipartimento di Farmacia

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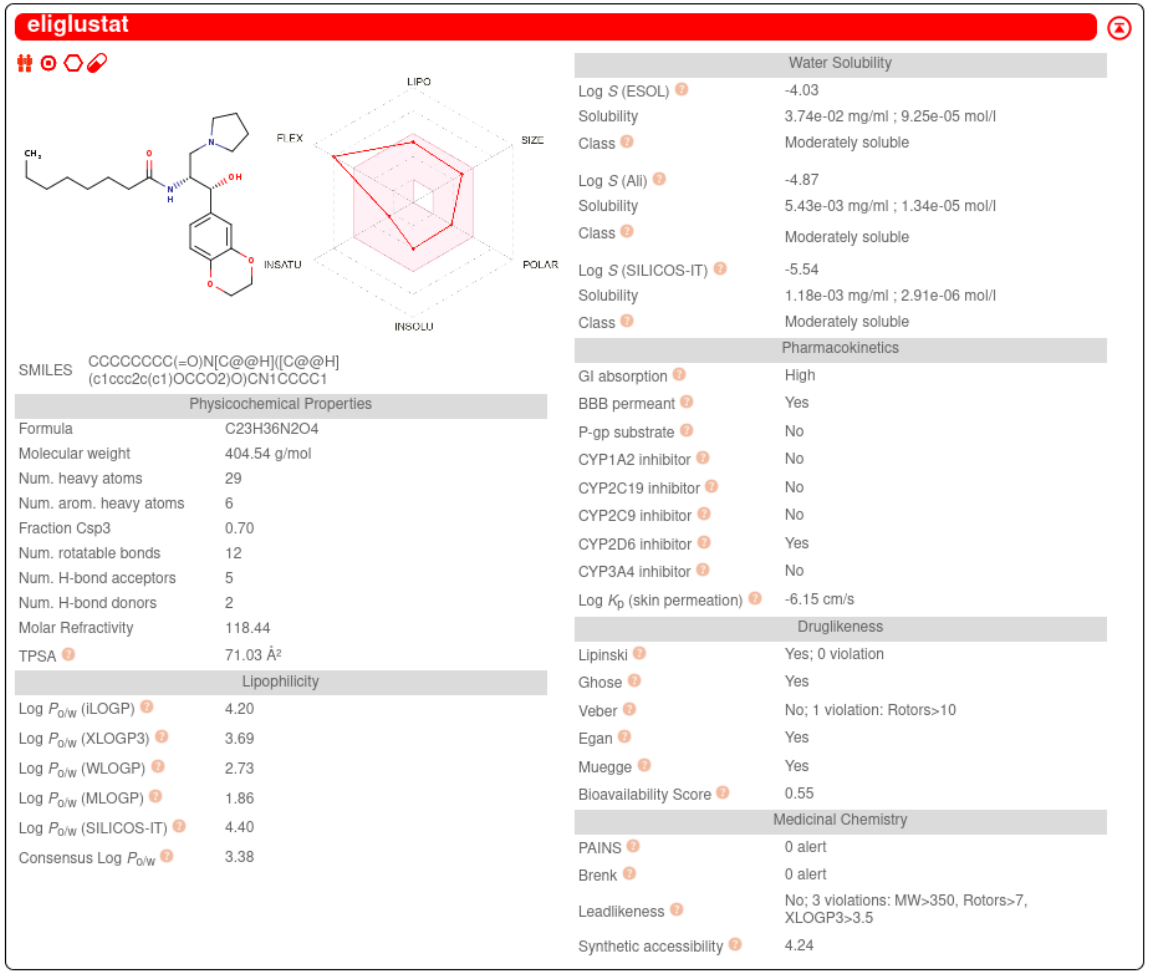
SwissADME

A website for computing descriptors and predicting ADME parameters, druglikeness of small molecules to support drug discovery



ALERTS MedChem

- **PAINS**: Pan-assay interference compounds. i.e., chemical compounds that often give false positive results in high-throughput screens. PAINS tend to react nonspecifically with numerous biological targets rather than specifically affecting one desired target.
- **Brenk**: hint to toxicity, but also poor stability or dying properties

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Prometheus Lab

PLATO

POLYPHARMACOLOGY PLATFORM PREDICTION

SCAN ME

TIRESIA

TOXICOLOGY INTELLIGENCE AND REGULATORY EVALUATIONS FOR SCIENTIFIC AND INDUSTRY APPLICATIONS

SCAN ME

CIRCE

CANNABINOID ITERATIVE REVALUATION FOR CLASSIFICATION AND EXPLAINABILITY

SCAN ME

TISBE

TIRESIA IMPROVED BY STRUCTURE-BASED EXPLAINABILITY

<< JUST SUBMITTED>>

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Plato

Polypharmacology pLATform for predictiOn

A ligand-based polypharmacology web drug discovery predictive platform designed for two-fold main objectives

a) target fishing

Target	score	reliable
Integrin alpha-IIb/beta-3:Homo sapiens	13.00	yes
Nicotinate phosphoribosyltransferase:Homo sapiens	13.00	yes
Cyclooxygenase-1:Bos taurus	13.00	yes
Gamma-glutamyltranspeptidase 1:Homo sapiens	13.00	yes
Cyclooxygenase-2:Homo sapiens	13.00	yes
Cyclooxygenase-1:Ovis aries	13.00	yes
Cyclooxygenase-2:Ovis aries	13.00	yes
Cyclooxygenase-1:Homo sapiens	13.00	yes
Complement factor D:Homo sapiens	8.66	yes
Complement factor D:Rattus norvegicus	8.66	yes
Carbonic anhydrase IX:Homo sapiens	7.54	yes

b) quantitative activity profiling

Target	IC50	EC50	Ki	Kd	σ_P
Cyclooxygenase-1:Ovis aries	6.57μM		1.78μM	33.1μM	1.5: IC50
Cyclooxygenase-2:Homo sapiens	758nM	330nM	6.17μM	172nM	1.5: IC50
Nicotinate phosphoribosyltransferase:Homo sapiens			0.39nM		1.5: Ki
Cyclooxygenase-2:Ovis aries	1.90μM		12.5μM		1.5: IC50
Cyclooxygenase-1:Bos taurus	1.13μM				1.5: IC50
Cyclooxygenase-1:Homo sapiens	5.50μM		49.2μM	266nM	1.5: IC50
Integrin alpha-IIb/beta-3:Homo sapiens	231nM	33.1nM	192nM	4.96nM	1.6: IC50

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PLATO predictions

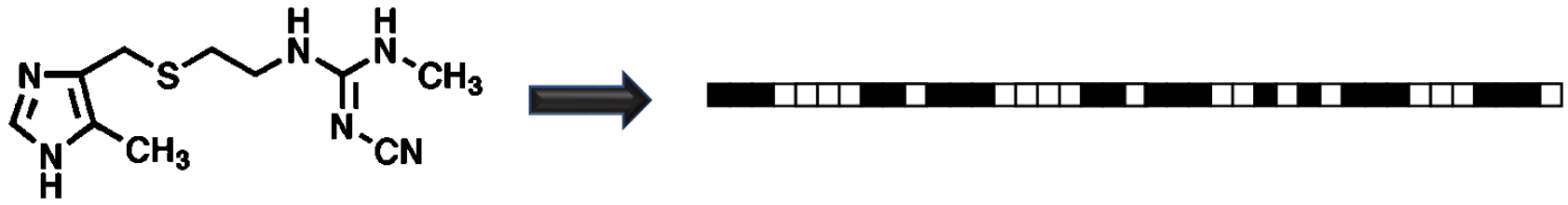
ChEMBL v 32

released on June 2023

Based the similarity principle, through a reverse ligand-based screening, a large collection of experimentally active chemicals on **> 6000 protein targets** implementing two just optimized **multifingerprint similarity-based algorithms**, which have been recently published.

FINGERPRINTS

bit string or feature set representations of molecular structure and properties



cimetidine

molecular fingerprint

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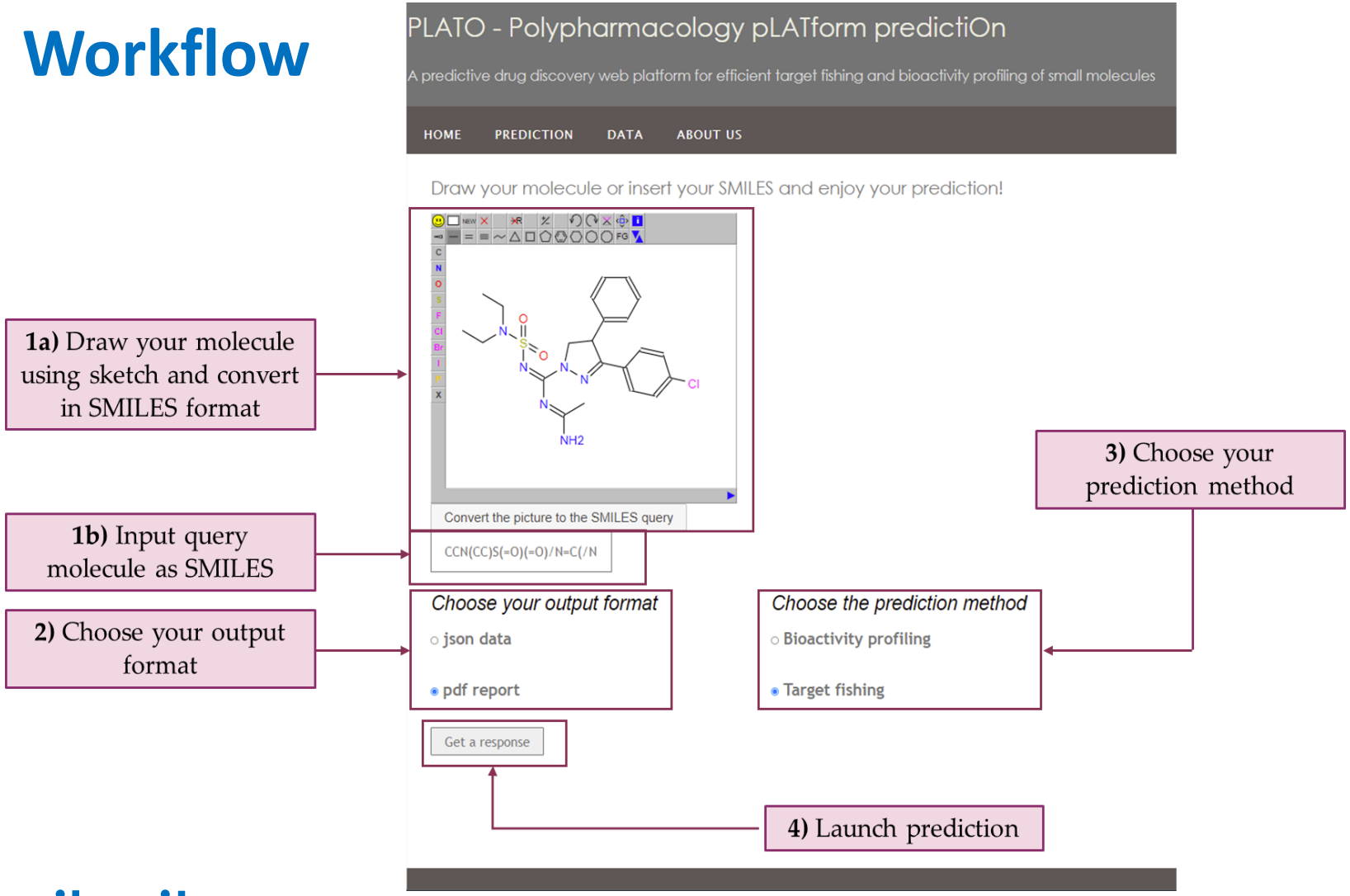
Workflow



http://plato.uniba.it

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SCAN ME

DevTox is the study of adverse effects on the development of the organism:

- Abnormal Growth
- Any Alteration which interferes with Homeostasis
- Structural or Functional Alteration

TIRESIA

TIRESIA: An eXplainable Artificial Intelligence Platform for Predicting Developmental Toxicity

Maria Vittoria Togo, Fabrizio Mastrolorito, Fulvio Ciriaco, Daniela Trisciuzzi, Anna Rita Tondo, Nicola Gambacorta, Loredana Bellantuono, Alfonso Monaco, Francesco Leonetti, Roberto Bellotti, Cosimo Damiano Altomare, Nicola Amoroso*, and Orazio Nicolotti*

Cite this: *J. Chem. Inf. Model.* 2023, 63, 1, 56–66
Publication Date: December 15, 2022
https://doi.org/10.1021/acs.jcim.2c01126

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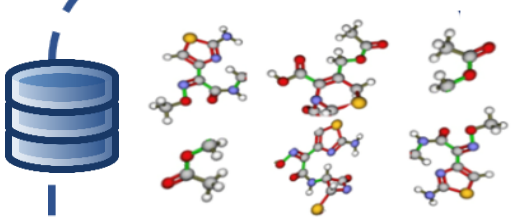
XAI framework for DevTox

(i) Feature Representation



SMILES	Feature 1	Feature 2	Feature 3	Feature N	Label
CC(C)(C1=CC=C(C=C1)O)C2=CC=C	39	39	1	25	Tox
CC1C(C(CC(O1)OC2CC(CC3=C(C4=C	39	39	1	24	Tox
CC(=O)NC1=CC=C(C=C1)O	39	39	1	26	No-Tox
O=C(Nc1ccc(O)cc1)C3	39	39	1	24	Tox
O=C(Nc1nnc(s1)S(=O)(=O)N)C4	38	38	1	23	No-Tox
O=C(NC1CCCCC1)NS(=O)(=O)c2ccc	37	37	1	22	No-Tox
O=C(O)C=C(C=CC=C(C=CC1=C(C)	41	41	1	28	Tox
O=C(N)NC1NC(=O)NC1(=O)7	37	37	1	24	Tox
O=C(NC(=N)N)c1nc(c(nc1(N))N)Cl8	37	37	1	21	Tox

Endpoint:

Developmental toxicity

Label:

Tox, No-Tox

(ii) Learning Framework

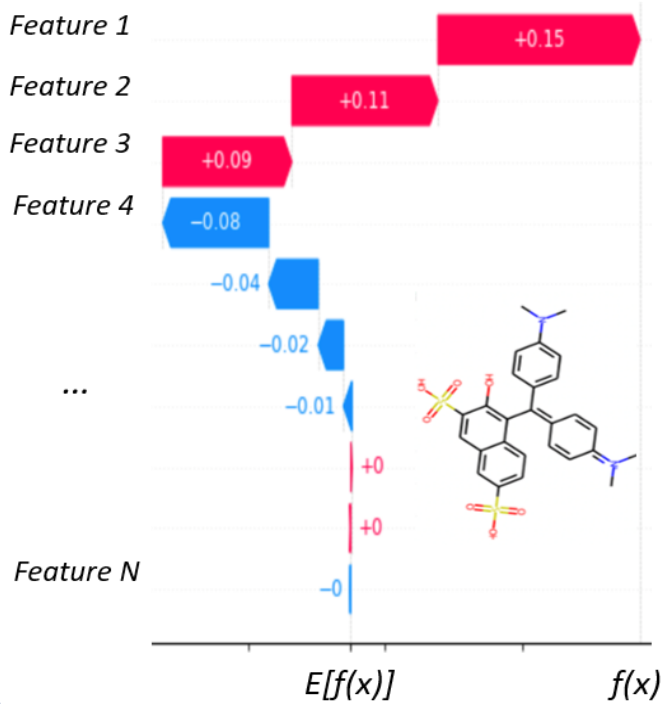
10-fold cross-validation

Embedded Feature Importance

Model selection

Independent test set

(iii) Explainability



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Case study: ELIGUSTAT

A ceramide glucosyltransferase inhibitor as first line oral therapy for adults with Gaucher disease type 1 (a rare genetic disorder)

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PLATO

main target: pharmacological effect

palpitations: advertized collateral effect

recognized collateral effects for eliglustat are also: analgesia, sedation, low blood pressure, itching, nausea and constipation due to reduced intestinal mobility

Target	IC50	EC50	Ki	Kd	σ_P
Ceramide glucosyltransferase:Homo sapiens	48.6nM				1.1: IC50
Protein kinase C delta:Homo sapiens	138nM	121nM	357nM	421nM	2: Ki
Sigma-1 receptor:Cavia porcellus	191nM		53.8nM	2.90nM	2: Ki
Renin:Homo sapiens	24.0nM		53.7nM	23.6nM	2.1: IC50
NADH-ubiquinone oxidoreductase chain 1:Bos taurus	1.90μM				2.1: IC50
Mu opioid receptor:Homo sapiens	222nM	94.9nM	39.2nM	0.79nM	2.1: Ki
Delta opioid receptor:Homo sapiens	60.2nM	39.3nM	104nM	24.9nM	2.1: Ki
Kappa opioid receptor:Homo sapiens	482nM	34.2nM	34.9nM	1.67nM	2.1: Ki
Peroxisome proliferator-activated receptor gamma:Homo sapiens	229nM	331nM	132nM	172nM	2.2: Ki
Protein kinase C epsilon:Homo sapiens	395nM	11.3μM	42.5nM	106nM	2.2: Ki
Protein kinase C gamma:Homo sapiens	486nM		743nM	0.47nM	2.2: Ki
Protein kinase C beta:Homo sapiens	160nM	0.56nM	50.1nM	372nM	2.2: Ki
Protein kinase C theta:Homo sapiens	23.9nM	6.20μM	140nM	252nM	2.2: Ki
Protein kinase C eta:Homo sapiens	61.1nM		32.8nM	67.9nM	2.2: Ki
Protein kinase C alpha:Homo sapiens	206nM		110nM	497nM	2.2: Ki
Peroxisome proliferator-activated receptor alpha:Homo sapiens	627nM	387nM	320nM	331nM	2.2: EC50
Dopamine D2 receptor:Homo sapiens	137nM	38.2nM	142nM	61.3nM	2.3: Ki
Serotonin 1a (5-HT1a) receptor:Rattus norvegicus	37.0nM	229nM	39.0nM	0.59nM	2.3: Ki
Dopamine D3 receptor:Homo sapiens	105nM	11.0nM	26.6nM	9.75nM	2.3: Ki
Serotonin 7 (5-HT7) receptor:Rattus norvegicus	1.20μM		68.2nM		2.3: Ki
Cannabinoid CB2 receptor:Homo sapiens	277nM	48.4nM	79.0nM	13.2nM	2.3: Ki

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Eligustat on TIRESIA

Summary

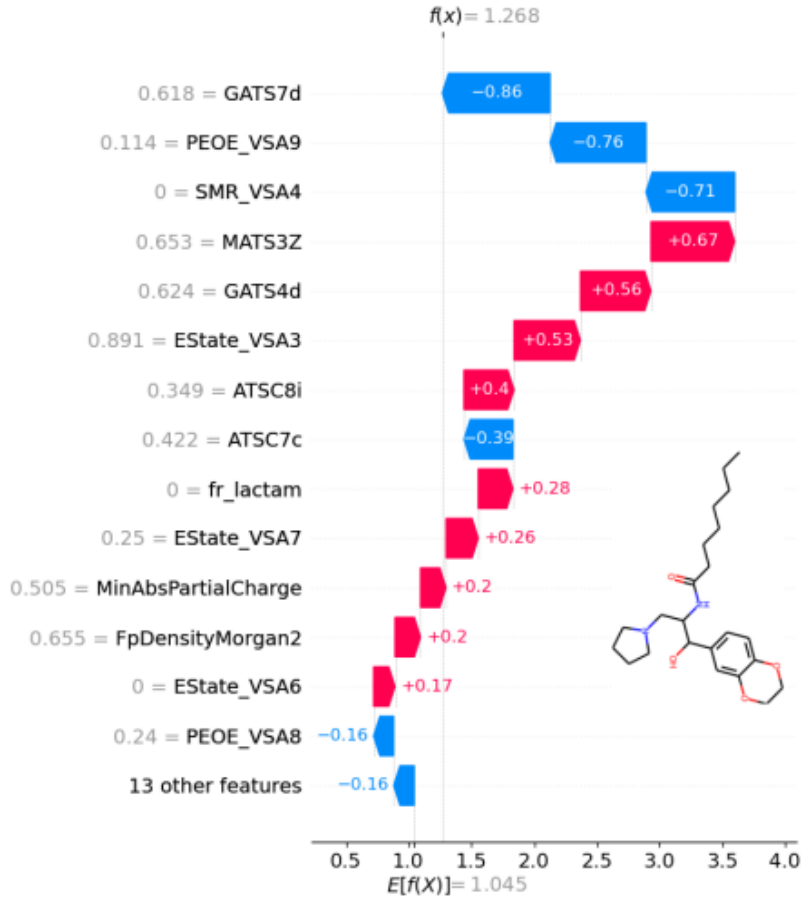
Predicted Data
Toxicant

Source Dataset
Unknown

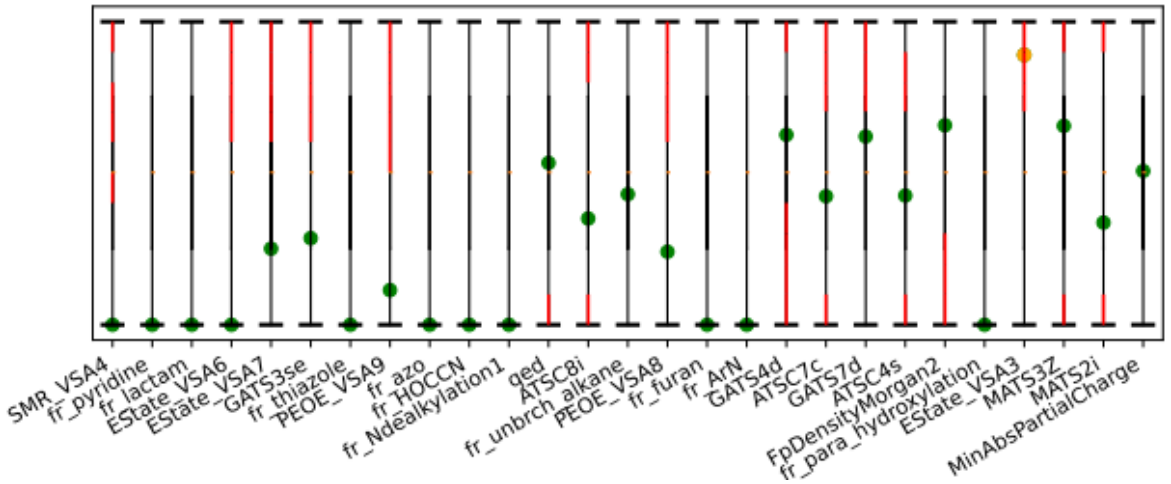
Applicability Domain
Suspicious

Max Similarity Value
0.82

Explainability



Applicability Domain

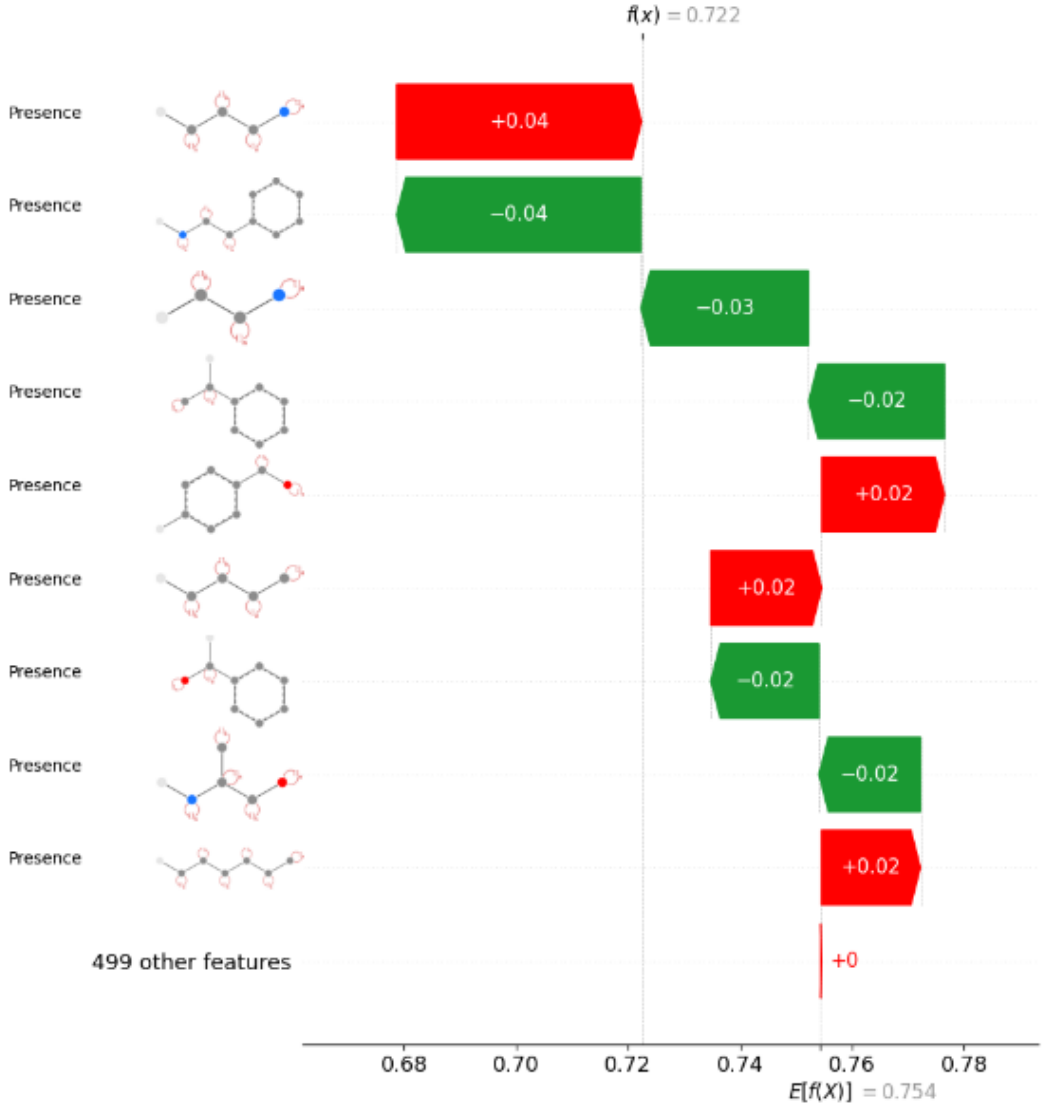


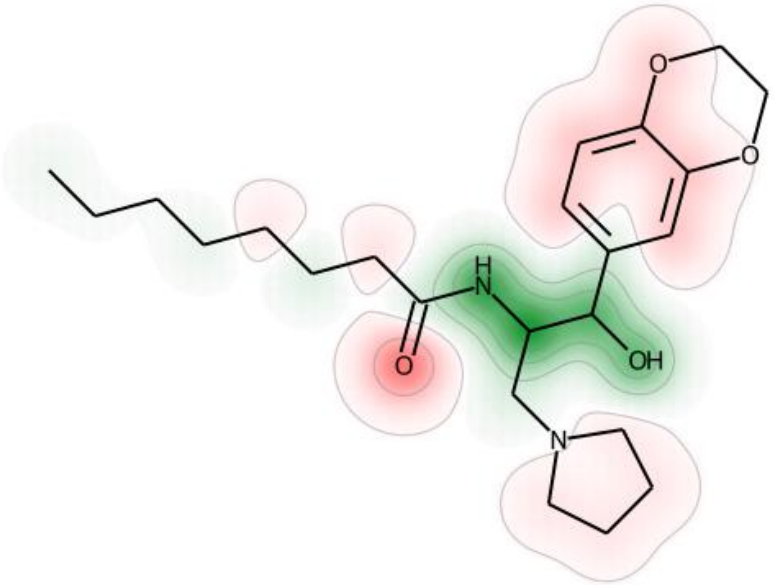
Eligustat on TISBE

Prediction
Toxicant (score=0.72)

Already in Dataset
False

Explainability





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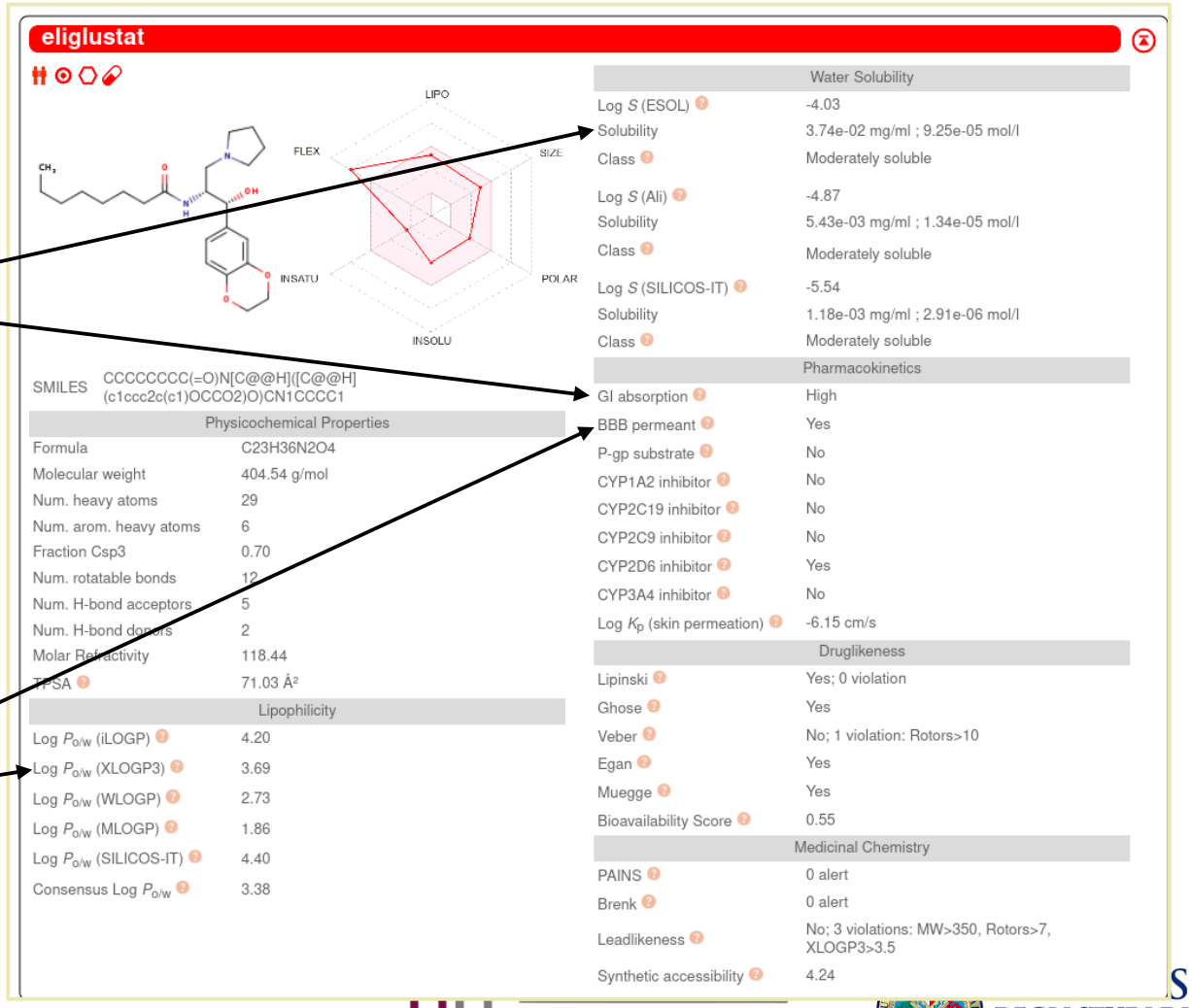
SwissADME

not recommended during breast-feading

nausea, strong headhaches



eliglustat

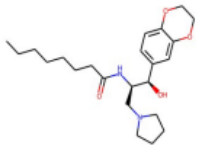
SMILES CCCCCCCC(=O)N[C@@H]([C@@H](c1ccc2c(c1)OCCO2)O)CN1CCCC1

Physicochemical Properties	
Formula	C23H36N2O4
Molecular weight	404.54 g/mol
Num. heavy atoms	29
Num. arom. heavy atoms	6
Fraction Csp3	0.70
Num. rotatable bonds	12
Num. H-bond acceptors	5
Num. H-bond donors	2
Molar Refractivity	118.44
TPSA	71.03 Å²
Lipophilicity	
Log $P_{o/w}$ (iLOGP)	4.20
Log $P_{o/w}$ (XLOGP3)	3.69
Log $P_{o/w}$ (WLOGP)	2.73
Log $P_{o/w}$ (MLOGP)	1.86
Log $P_{o/w}$ (SILICOS-IT)	4.40
Consensus Log $P_{o/w}$	3.38

Water Solubility	
Log *S* (ESOL)	-4.03
Solubility	3.74e-02 mg/ml ; 9.25e-05 mol/l
Class	Moderately soluble
Log *S* (Ali)	-4.87
Solubility	5.43e-03 mg/ml ; 1.34e-05 mol/l
Class	Moderately soluble
Log *S* (SILICOS-IT)	-5.54
Solubility	1.18e-03 mg/ml ; 2.91e-06 mol/l
Class	Moderately soluble
Pharmacokinetics	
GI absorption	High
BBB permeant	Yes
P-gp substrate	No
CYP1A2 inhibitor	No
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	Yes
CYP3A4 inhibitor	No
Log K_p (skin permeation)	-6.15 cm/s
Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	No; 1 violation: Rotors>10
Egan	Yes
Muegge	Yes
Bioavailability Score	0.55
Medicinal Chemistry	
PAINS	0 alert
Brenk	0 alert
Leadlikeness	No; 3 violations: MW>350, Rotors>7, XLOGP3>3.5
Synthetic accessibility	4.24

VenomPred

VenomPred is a Machine Learning based platform for qualitative prediction of ***mutagenicity***, ***carcinogenicity***, ***hepatotoxicity*** and ***estrogenicity*** of chemicals.



Name	Entry_1
SMILES	CCCCCCCC(=O)N[C@H](CN1CCCC1)[C@H](O)c1ccc2c(c1)OCCO2

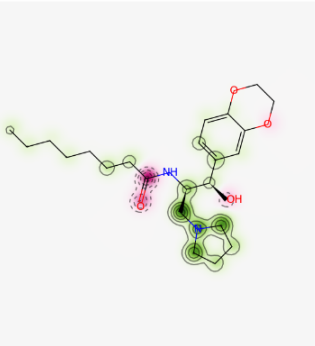
Endpoint	Probability
Mutagenicity	14 %
Carcinogenicity	33 %

Endpoint	Probability
Hepatotoxicity	55 %
Estrogenicity	6 %

The main article describing the web service and its underlying methodologies is: VenomPred: A Machine Learning Based Platform for Molecular Toxicity Predictions. *Int. J. Mol. Sci.* **2022**, *23*, 2105

Università di Pisa
Molecular Modeling & Virtual Screening Laboratory
Dipartimento di Farmacia

Pred-hERG Results

Prediction / Potency	Confidence	Applicability domain (AD)	Probabilty Map
Non-cardiotoxic (-)	50%	Yes (Value= 0.27 and limit = 0.26)	
Not applicable	Not applicable		

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Take-home message

- Better integrative platforms for data analysis
- Greater access to data from industry-sponsored phase 2b-4 clinical trials
- Newer safety liabilities of repurposed drugs should be studied
- Funding opportunities for drug repurposing in general, but especially for rare diseases

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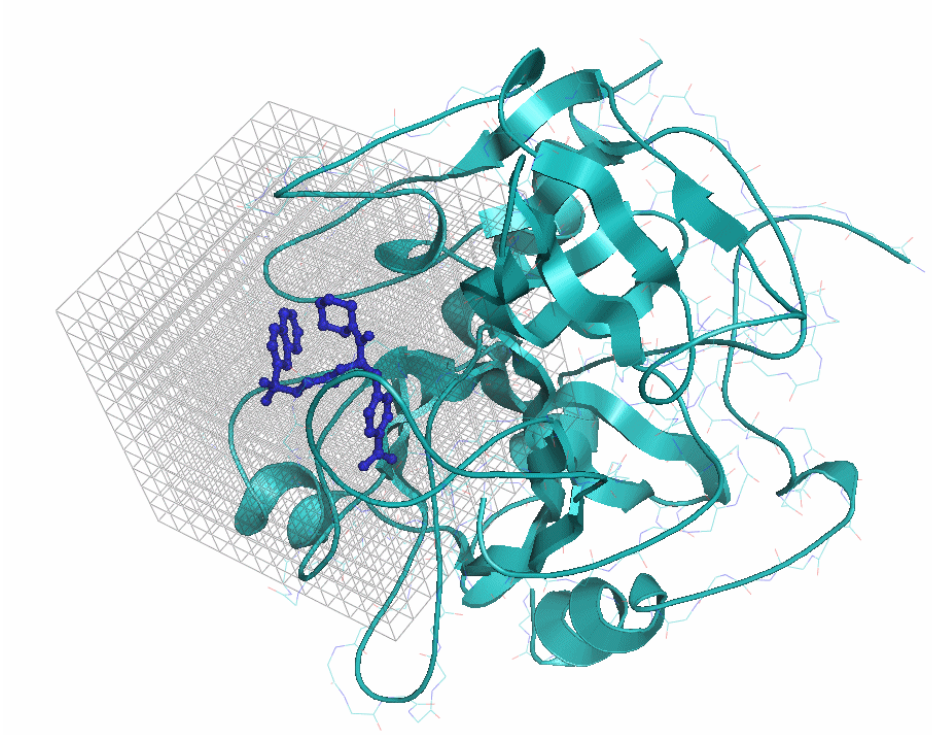
Caterina De Ruvo

Thanks for the attention



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