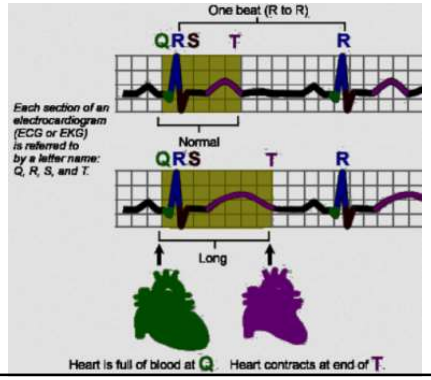
Presentation Summary

1. antitargets (hERG, P-gly, Cyt540)
2. cell membrane
3. bioavailability (pharmacokinetic, drug-likeness, partition and distribution coeficient)
4. Lipinski Ro5 + (PSA and NRB)
5. differences in drug-like selection criteria (Ro3, Ro5)
6. absorption as f(PSA, LogP) SPARC, VCLAB, intestinal and BBB absorption
7. other considerations (toxicity, bad metabolic parameters)
8. from were active compounds (nature, synthesis, virtual)
9. active compounds (screenings: HTS, biophysical, sources: synthetic, fragment, natural productsm, SOSA)
10. New drug development (10y/1-2mld USD, 24new/year)
11. DD flowchart (active => hit => lead => clinical candidate => drug)
12. A2H, H2L, drug candidate, case story
13. what compound should fulfill to become a drug (PKin, PDyn, other: novelty, synt. feasib., scale up synth.)
14. case study
15. pKa universal measure of acidity and basicity
16. pKa different heterocyclic and amines
17. Rational drug design (structure, ligand, fragment)
18. SBDD methodology
19. SBDD (virtual screening + example, building fragments in target active place)
20. Ligand based induced fit, Danijel Kikelj example
21. X-ray structure screening to overcome induced fit
22. LBDD, FBDD
23. Case studies (gefinitib, imatinib, pazopanib)

1

Antitargets

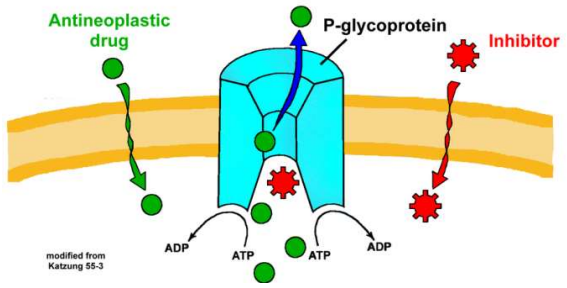
hERG - **potassium ion channel** that **coordinates the heart's beating**. When **this channel is inhibited** by application of drugs **it can result in a potentially fatal disorder called long QT syndrome**; a number of clinically successful drugs in the market have had the tendency to inhibit hERG, and create a concomitant risk of **sudden death, as an unwanted side effect**, **hERG inhibition must be avoided during drug development**



2

Antitargets

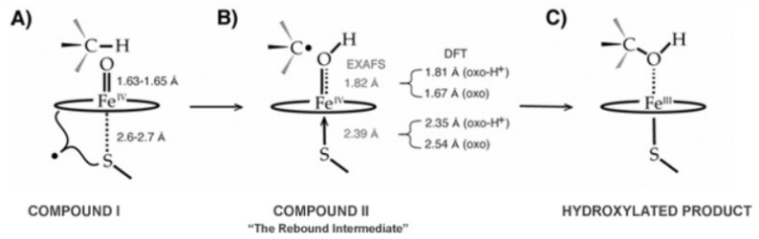
P-glycoprotein **transports substrates across the cell membrane, efflux pump for xenobiotics (e.g. drugs)** with broad substrate specificity. It is responsible for **multidrug-resistantance** and often mediates the development of resistance to anticancer drugs.



3

Antitargets

Cytochrome P450 are the major enzymes involved in **metabolism** (~75%), they catalyze the oxidation of organic substrates, drugs included.



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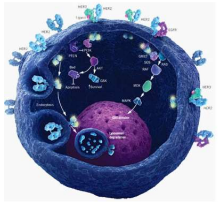
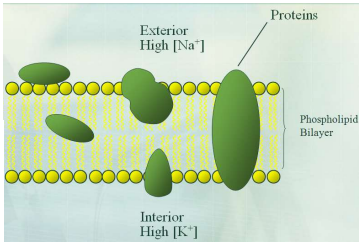
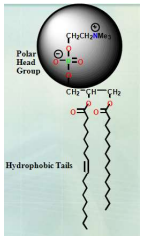
Cell Membrane – protects cell compartment

- The cell membrane provides a **hydrophobic barrier** around the cell, **preventing a passage of water and polar molecules.** Proteins (receptors, ion channels and carrier proteins) are present, floating in the cell membrane.







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Bioavailability
(PK - pharmacokinetic)

- *(in vitro)* **active compound, to perform as a drug, has to reach its target** in the human body (*in vivo*)
- **Drug-likeness** **is** qualitative **concept to estimate bioavailability from** the molecular **structure** **before the substance is synthesized**.

The **drug-like molecule** should have:

- ❑ **an optimal MW** and appropriate **number of HBD**, **HBA** (affecting solubility and absorption)
- ❑ **optimal water and fat solubility, partition coeficient logP** (octanol / water) to penetrate cellular membrane to rich target inside cells. The **distribution coefficient (Log D)** is the correct descriptor for ionisable systems. **logD is pH dependent** (e.g. pH = 7.4 is the physiological value of blood serum)

Lipinski's Rule of Five (Ro5)

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Lipinski Ro5
(an empiric rule, all numbers are multiples of five)

for prediction of bioavailability (**not activity!**) to quickly eliminate compounds that have poor physicochemical properties for an oral bioavailability

- an **orally active drug** has no more than one violation of the **following criteria**:
  - ❑ **MW** ≤ 500
  - ❑ **Lipophilicity** (logP ≤ 5) octanol-water partition coefficient (**better log D ≤ 5** respecting the ionic states present at physiological pH values)
  - ❑ Sum of **hydrogen bond donors** ≤ 5 (NH,OH)
  - ❑ Sum of **hydrogen bond acceptors** ≤ 10 (N,O)

C.A. Lipinski et al. Adv. Drug Del. Rev. **1997**, 23, 3. (Ro5)
G.M. Pearl et al., Mol. Pharmaceutics, **2007,** 4, 556–560. (log D introduced)

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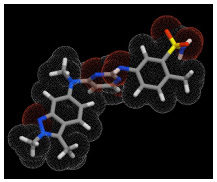
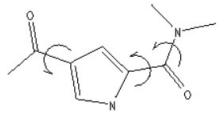
Additional drug-like parameters

- ❑ **MW** ≤ 500
- ❑ **Lipophilicity** (logP ≤ 5) octanol-water partition coefficient
- ❑ Sum of **hydrogen bond donors** ≤ 5 (NH,OH)
- ❑ Sum of **hydrogen bond acceptors** ≤ 10 (N,O)



- ❑ **PSA < 140 Å²** (Molecular Polar Surface Area – sum of surfaces of polar atoms (N,O...with H) that correlates with human intestinal and BBB absorption), or (**PSA < 60 Å²**) for good BBB penetration
- ❑ **Number of rotatable bonds < 10** (high NRB → many conformers)



*Ertl, P. in Molecular Drug Properties, R. Mannhold (ed), Wiley-VCH , **2007**, 111 – 126.*

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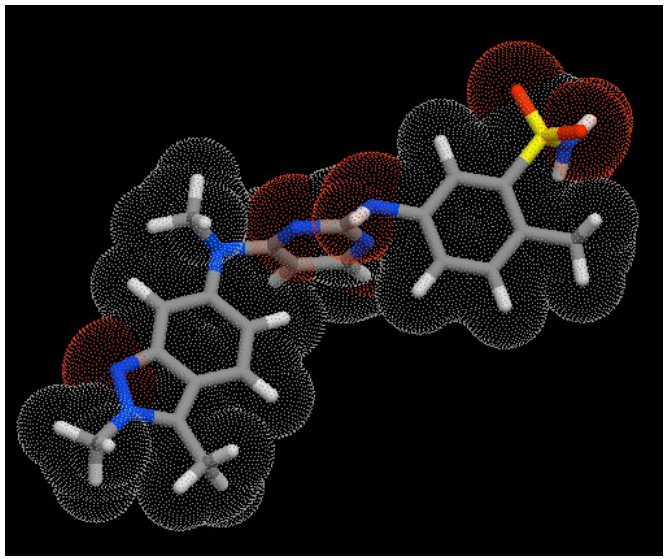

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Differences in drug-like selection criteria

- **Optimization often gives** drugs with **higher molecular weight**, **more rings**, **more rotatable bonds**, and a **higher lipophilicity.**

T. I. Oprea et all. J. Chem. Inf. Comput. Sci. 2001, ***41***, 1308–1315.

	Ro3		Ro5
Fragment[3]		**Lead-Like**[2]	**Drug-Like**[1]
MW < 300		MW < 450	MW < 500
LogP < 3		LogP < 4.5	LogP < 5
Proton Donors < 3		Proton Donors < 5	Proton Donors < 5
Proton Acceptors < 6		Proton Acceptors < 10	Proton Acceptors < 10
Rotatable Bonds < 6		Rotatable Bonds < 10	Rotatable Bonds < 10
tPSA < 60		tPSA < 150	tPSA < 150

Others Properties, Solubility – calculated and measured, LogD, pKa

1/ Lipinski, C.A et al. Adv. Drug Del. Rev. 1997, 23, 3. (Rule of 5)
2/ Verheij, H.J. Molecular Diversity 2006, 10, 377. (Lead-Likeness)
3/ Congreve, M. et al. Drug Discov. Today 2003, 8, 876. (Fragments)

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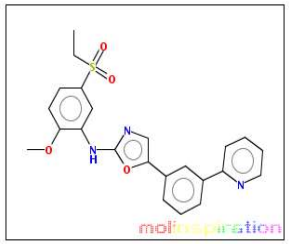
Ro5 determined from 2D tructure

http://www.molinspiration.com

molinspiration

SMILES CCS(=O)(=O)c4ccc(OC)c(Nc3ncc(c2cccc(c1ccccn1)c2)o3)c4



Molinspiration property engine v2009.01

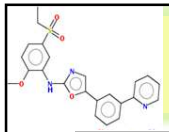
miLogP	4.117
TPSA	94.327
natoms	31
MW	435.505
nON	7
nOHNH	1
nviolations	0
nrotb	7
volume	374.267

Get data as text (for copy / paste).

Get 3D geometry BETA

Ertl, P. et al., *J. Med. Chem.* 2000, 43: 3714-3717. (molecular property prediction toolkit)

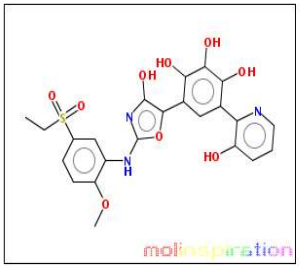
11



Ro5 violations

molinspiration

SMILES CCS(=O)(=O)c4ccc(OC)c(Nc3nc(O)c(c2cc(c1nccc1O)c(O)c(O)c2O)o3)c4



Molinspiration property engine v2009.01

miLogP	2.904
TPSA	195.467
natoms	36
MW	515.5
nON	12
nOHNH	6
nviolations	3
nrotb	7
volume	414.356

Get data as text (for copy / paste).

Get 3D geometry BETA

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Absorption as f(PSA, LogP)

- **pKa** (influences binding Ki and logP)
  https://epoch.uky.edu/ace/public/pKa.jsp (free of charge)
  commercial software SPARC http://www.archemcalc.com/ (5USD/monthly)
- **AlogP** (lipophilicity, water solubility)
  http://www.vcclab.org/ **(Virtual Computational Chemistry Laboratory)**

Intestinal and other **absorption**

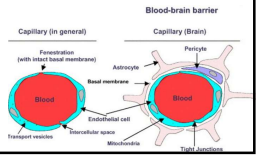
- **% ABS = 109 – 0.345 PSA**
  (good when % ABS > 30 %; **lower PSA, higher absorption**)

Zao YH et al. Pharm Res 2002, 19, 1446-1457.

BBB absorption

- **LogBB = -0.0148 PSA + 0.152 CLogP + 0.139**

CNS drug: logBB > -0.5 (otherwise side effects can be expected)
non CNS drugs: logBB < -1



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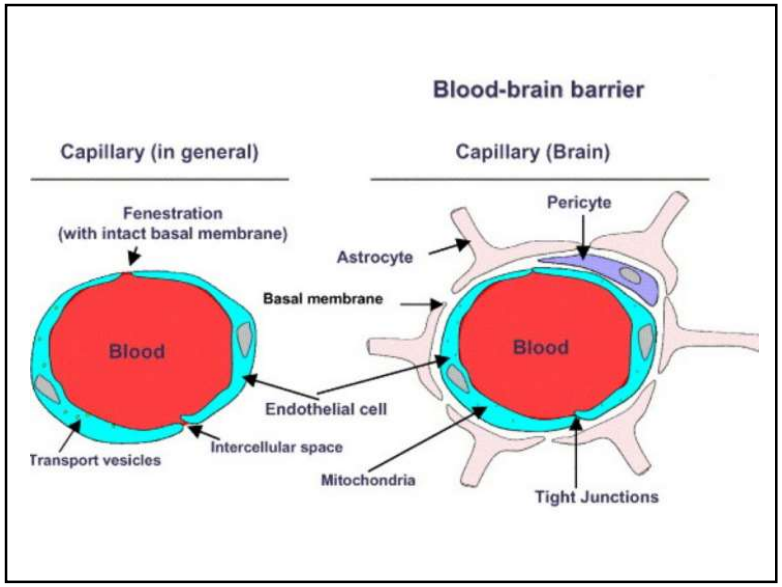

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Other considerations

- **despite good druglikeness** some compounds should be avoided as drug candidates:

  ❑ **substructures with known reactive, toxic, mutagenic or teratogenic properties** affect the usefulness (RCOX, $(RCO)_2O$, Michael acceptors, epoxides, $-NO_2$, -NO, $-N_3$, NH-NH, N=N...)

  ❑ and with **bad metabolic parameters**, e.g. fast metabolism **can quickly destroy the pharmacological activity** of the compound

  (metabolic half life, metabolic clearance should be determined)

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From were to get active compounds?

A) The Natural World
- **Micro-organisms** (bacteria, fungi)
- **Marine chemistry** (corals, bacteria, fish etc)
- **Plant life** (flowers, trees, bushes)
- **Animal life** (frogs, snakes, scorpions)
- **Biochemicals** (neurotransmitters, hormones)

B) The Synthetic World
- **Chemical synthesis** (traditional, combinatorial synthesis, chemical collections, commercial sources)

C) The Virtual World
- **Computer** aided drug design (CADD)

to call them „active compounds“ evaluation through biological screening is essential

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ACTIVE compounds can be obtained as hits in screening focused on selected biological target

- **Screenings** (in vitro: enzymatic, cellular or biophysical assays):
  - ❑ **High-throughput screening (HTS)** rapid screening of large numbers of compounds (up to 100 000/day)
  - ❑ **Biopysical screening** (NMR, PSR, X-ray screening)
- **Sources**
  - ❑ **LMW synthetic compounds** (collections from combinatorial chemistry / parallel synthesis, historical corporate collections…)
  - ❑ **Fragment-based** screening (not direct hit generation)
  - ❑ **Pure natural products, bioextracts** (e. g. plant or microbial) **ethnopharmacology** (Chinese traditional medicine...)
  - ❑ **SOSA approach** based on known Drugs/Clinical development compounds where side effects observed from *in vivo* screenings or human clinical trials
  - ❑ **Drug Repurposing approach** based on known Drugs applied in new therapeutic application

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SOSA = Selective Optimization of Side Activities

Exploitation of **Existing Drugs** as Leads **for the Discovery of New Drugs**

- ❑ **all drugs act on more than one target** (known and unknown), **resulting** in a several **side effects**
- ❑ advantage: drugs and many compounds that underwent clinical development **have an established safety profile.** Many of them **can be** therefore safely **administered to humans**.

Try to transform one of the side activities into the major effect and strongly reduce their initial pharmacological activity.

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Drug Repurposing (DR)

- is a strategy for **identifying new uses for approved or investigational drugs**

Advantages over developing a new drug

- a/ **the risk of failure is lower**; because the repurposed drug has already been found to be sufficiently safe in preclinical models and humans if early-stage trials have been completed, it is less likely to fail at least from a safety point of view in subsequent efficacy trials.
- b/ **the time frame for drug development can be reduced**, because most of the preclinical testing, safety assessment and, in some cases, formulation development will already have been completed.
- c/ **less investment is needed** The phase III costs may remain more or less the same for a repurposed drug as for a new drug, but there could still be savings in preclinical and phase I and II costs.

- All together DR has the potential to result in a **less risky and more rapid return on investment** (the costs of bringing a repurposed drug to market have been estimated to be US$ 300 million on average, compared with an estimated ~$2–3 billion for a new chemical entity).

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How many new drugs reach the market yearly?

- **DrugDiscovery:**
  10 years / from 1 to 2 000 000 000 USD /1 new drug
- **global production ca 24 innovative drugs**
  (possessing new chemical entity) / year

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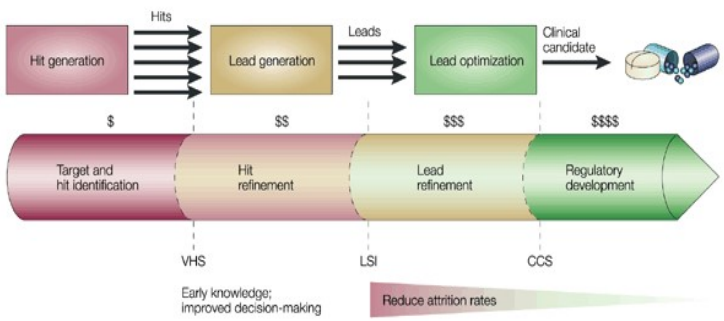
DD - flowchart

Target selection **=> active => hit => lead => clinical candidate => drug**



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Active → Hit → Lead → Drug candidate → Drug

Actives: are indentified **compounds** with a desired target bioactivity (e.g. by HTS or biophysical methods (SPR, ITC – Isothermal Titration Calorimetry, NMR)).

A2H process: Validated **Hits** are **stable active** (< 3 mM in biochemical assay, < 10 mM in vivo assay) **small molecules** with **determined purities, confirmed structures,** and specific **IC_{50} target activities.** **The aim of A2H process is to determine appropriate active compounds possessing diverse chemical structures for further development.**

H2L process: Lead compounds are identified from validated hits. The aim of H2L process is to exclude inappropriate compounds that could fail in subsequent preclinical and clinical trials early in DD, before significant resources are spent.

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OPTIMAL LEADS: have **good target and cell activities, selectivities** (e.g. > 10-fold over related targets), **ADME/Tox properties**: **bioavailability** (lead-likeness, aq **solubility > 100 uM** (e.g. at FW: 500 g/mol → > 1 mg / 20 mL), logP, logD, pKa, plasma albumine binding), **metabolic stability** (not metabolized too quickly) and **low toxicity** (no **undesirable chem functionalities** like nitros, Michael acceptors..., low **antitargets activities**: hERG (30-fold selectivity over hERG), P-glykoprotein, CYP450 (> 1 mM for 4 of 5 major human isoforms), low **cytotoxicity** (30-fold selectivity over chronic (24h) cellular toxicity), low **genotoxicity**...). Leads have **novel patentable structures**. They are **synthetically accessible** (e.g. parallel (convergent) synthesis) and they have **optimization potential**. Focused libraries around the most promising hits (selection of 3-6 structure clusters) are prepared for early and rapid generation of structure activity relationship (SAR) data in order to indentify highly active and selective leads (< 100 nM in vitro assay, < 1 mM in vivo assay) for further DD development.

	HIT	LEAD
In vitro assay	< **3000 uM** (3 mM)	< **0.1 uM** (100 nM)
In vivo assay	< 10 mM	< 1 mM

LEAD-LIKE COMPOUNDS

physicochemical **properties:** MW < 450, logD < 4, H-bond donors (NH,OH) ≤ 4, H-bond acceptors ≤ 8 (N,O)

DRUG CANDIDATE is a **result of further leads development** by *in vivo* assays and clinical trials PhI-III confirming activity and low toxicity on patients to show their clinical benefit and better properties compare to similar marketed drugs.

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Case story from HTS to Leads

- at **Schering** begins with
  - **HTS assay of 700 000** compounds. Afterwards they **repeated HTS** with selected **2 000** compounds. This reduces the compound pool sending **200 compounds forward to IC_{50} assaying**. The result was **100 active compounds with determined IC_{50} values**. Those 100 compounds went for **purity and structure evaluation** bring the number down to **50 validated actives (A)** in about one month (enrichment: 1/14 000).
  - Subsequently **in vitro efficacy, selectivity, and toxicology studies produced 15 compounds as "qualified hits„ (A2H)** by the end of the third month (enrichment: 1/46 667).
  - **Qualified hits were resynthesized to yield more compound for in vitro and in vivo evaluations**. These evaluations concluded with the **identification of 13 lead structures** after 10-months.
  - After 14 months of the above selection process final **13 compounds** (total enrichment: 13 / 700 000 = 1 / 53 846) **were moved** for the **lead optimization process**.

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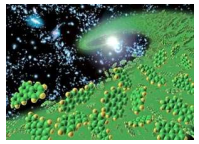
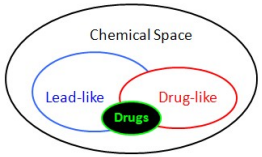
Summary: what should the compound fulfill to become a drug?

- **Biologically active,** chemically **stable compound** possessing appropriate:
  - ❑ **pharmacodynamic properties** (target activity and selectivity)
  - ❑ **pharmacokinetic properties** (bioavailability: ADME/TOX)
  - ❑ **other properties** (novelty, synthetic feasibility, scale up synthesis...)
- **> 30% of all drug failures can be attributed to poor physiochemical properties**: Log P (Log D), pKa, and solubility with **impact on drug absorption and diffusion in vivo**





Chem Space: 10^{60} - 10^{200}
DB of 11 atoms C,N,O, F: 26 400 000 of stable compounds (111 000 000 cmpds if included all stereoisomers) *J.-L. Reimond*

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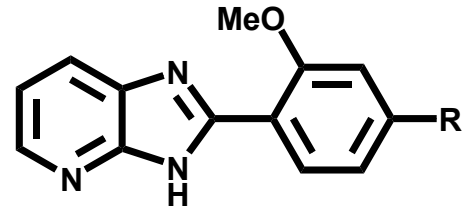
Case study – cardiotonic agent (optimization of physico-chemical properties)



Cardiotonicum

R: MeO- log P (2.59) FW: 255.27 (**CNS side effects bright visions**)
MeSO- lop P (1.17) FW: 287.34 (**Sulmazol**)

LogP round 2.5 allows compound to penetrate to CNS → side effects

2-Aryl-3H-imidazo[4,5-b]pyridine

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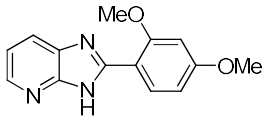
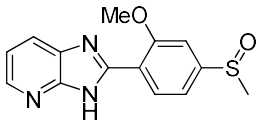


www.MOLINSPIRATION.com

COC1=CC(OC)=CC=C1C2=NC3=CC=CN=C3N2

Molinspiration property engine v2014.11

miLogP	2.69
TPSA	60.04
natoms	19
MW	255.28
nON	5
nOHNH	1
nviolations	0
nrotb	3
volume	227.21



CS(C(C=C1OC)=CC=C1C2=NC3=CC=CN=C3N2)=O

Molinspiration property engine v2014.11

miLogP	1.47
TPSA	67.88
natoms	20
MW	287.34
nON	5
nOHNH	1
nviolations	0
nrotb	3
volume	243.90

What is another problem that occurred by introduction of a sulfoxide group?

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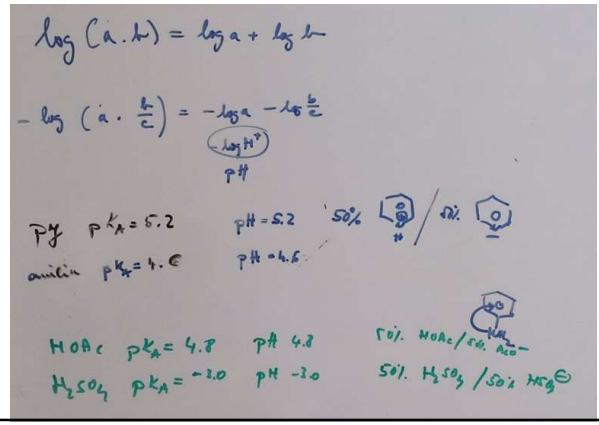
HA ⇌ $H^+ + A^-$ ionised forms $pK_a = -\log_{10}([H^+][A^-]/[HA])$

HB^+ ⇌ $H^+ + B$ $pK_a = -\log_{10}([H^+].[B]/[HB^+])$

pKa = -log {[H⁺].[B] / [HB⁺]} = pH - log [B]/[HB⁺] = pH – log 1 = pH



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pKa a universal measure for both acidity and basicity

Definition of pK_a

$HA \rightleftharpoons H^+ + A^-$ ionised forms

or $HB^+ \rightleftharpoons H^+ + B$

$pK_a = -\log_{10} ([H^+][A^-]/[HA])$

$pKa = -\log_{10} ([H^+].[B]/[HB^+])$

the higher pKa the stronger base (aniline 4.6, py 5.1, methylamine 10.6)
(or the weaker acid: HBr -9.0, HCl -8.0, H_2SO_4 -3.0, H_3O+ -1.7, HF +3.2, HOAc +4.8)

pKa = -log {[H⁺].[B] / [HB⁺]} = pH - log [B]/[HB⁺] = pH – log 1 = pH

Conclusion: if concentration of base and its protonated form is equal than pKa = pH (it is special pH at which the base is protonated on 50 %). Such values can be compared within different bases to estimate their basicity.

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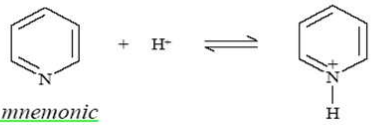
We consider the basicity of pyridine in terms of the pK_a of the conjugate acid - the pyridinium cation



Useful mnemonic

The pK_a designates that pH where the two species are present at equal concentration

i.e. at pH = 5.1 pyridine is 50% protonated

at more basic conditions at pH = 6.1 pyridine is 10% protonated

in more acidic environm. at pH = 4.1 pyridine is 90% protonated

pKa = -log {[H⁺].[B] / [HB⁺]} = pH - log [B]/[HB⁺]

5.1 = 4.1 - log [B]/[HB⁺]
[B] + [HB⁺] = 100%

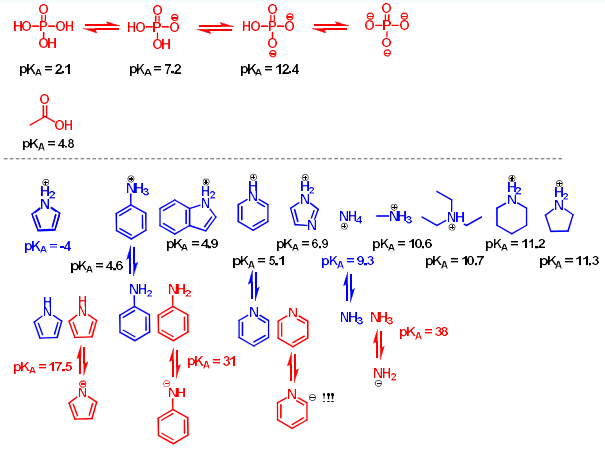
two equations, two variables

9% [B] and 91% [HB⁺]

Aká ionizácia pyridínu bude v krvi pH 7.4? (len 0.5%)

30

The higher pKa the stronger base
(or the weaker acid)



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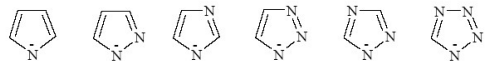
Scheme 14. Basicities of Nitrogen in Heteroaromatic Five-Membered Rings (pK_a values)

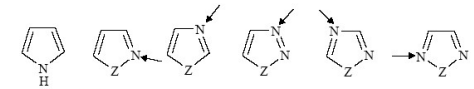
A. Anionic Nitrogen : Azole Anions or Acidity of Neutral Azoles



16.5 14.2 14.4 9.3 10.0 4.9

with increasing number of nitrogen atoms basicity decreases and acidity of conjugate acid (the neutral azole) increases

B. Neutral Nitrogen: Azoles (Acidity of Azole Cations)



(at N)	Z =	NH	2.5	6.9	1.2	2.5	1.2
ca -4		O	-3	0.8	-	-	-
		S	-0.5	2.5	-	-	-4.9

Note: (i) Pyrrole very weakly basic because no pyridine-like nitrogen

(ii) Imidazole much more basic than pyrazole because of base weakening interaction of adjacent N

(iii) S- and especially O- substitution for NH dramatically reduces basicity

(iv) Additional pyridine-like N atoms also strongly reduce basicity

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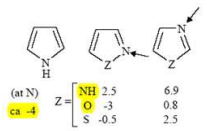
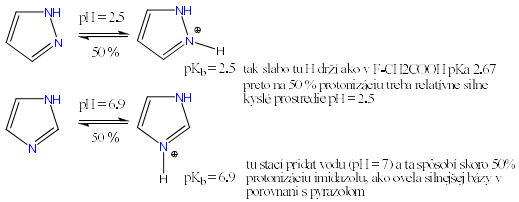
pyrazol ma pKB = 2.5 a imidazol 6.9
teda je tam velky rozdiel bazicity, na pyrazol na jeho 50% protonizaciu treba pH 2.5
ale na imidazol staci už pH = 6.9 teda on je už vo vode skoro na 50% protonizovaný





tak slabo tu H drží ako v F-CH2COOH pKa 2,67
preto na 50 % protonizaciu treba relatívne silne kyslé prostredie pH = 2.5

tu staci pridat vodu (pH = 7) a ta spôsobi skoro 50% protonizaciu imidazolu, ako ovela silnejšej bázy v porovnaní s pyrazolom

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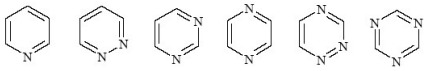
Scheme 15. Basicities of Six-Membered Heteroaromatics and Non-Heteroaromatics

A. Basicities of Azines (pK_a values)

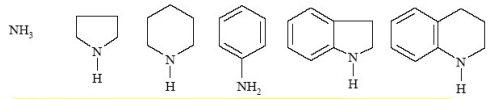


5.1	2.3	1.3	0.6	< 0	< 0
pyridine	pyridazine	pyrimidine	pyrazine	1,2,4-triazine	1,3,5-triazine

Note: (i) Extra N-atoms considerably reduce basicity of pyridine (inductive effect)

(ii) Pyridazine more basic than pyrazine because of the effect of adjacent electron lone pairs (α-effect)

B. Basicities of Some Other Neutral Nitrogen (Non-heteroaromatic) Compounds



9.3	11.3	11.2	4.6	4.9	5.0
ammonia	pyrrolidine	piperidine	aniline	indoline	tetrahydroquinoline

Note: (i) Most aliphatic amines have pK_a around 10 - 11; (ii) Most anilines have pK_a around 4 - 5

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pKa predictions

https://epoch.uky.edu/ace/public/pKa.jsp **(zdarma!!!!)**

pKa (influences binding Ki and logP) c
http://www.archemcalc.com/

SPARC CAPABILITY

All molecular species presented as a **function of pH**
concentrations of tautomeric species
Carbon (sp3) as an acid can be calculated
LogD (water/user solvent partitioning)
Non aqueous pKa can be calculated for any organic solvent that the user specifies
Hydrolysis rate constants for carboxylic acid esters in water and selected solvents
Hydrolysis rate constants for **phosphorous acid esters in water**
Batchmode pKa calculations using **SMILES or SDF files**

Vapor Pressure as a function of temperature
Boiling point as a function of ambient pressure
Diffusion coefficient (air and water) as a function of temperature and pressure
Molecular volume as a function of temperature
Density as a function of temperature
Polarizability
Electron affinity

Solubility (includes crystal energy contributions) as a function of temperature
Liquid/liquid partitioning including octanol/water Kow. The user can specifiy any two user defined solvents if desired. These can be calculated as a function of temperature
Reduction potential (E 1/2)

We also provide a pay-per-calc option. A credit card is used to sign up for a one week trial subscription to the SPARC basic plan. During the one week trial period no charges will be made to your card. At the end of the trial week, you will be charged a monthly $6 subscription. This subscription will allow free calculations up to the limit described below under "Free per month".

Affiliations with more than one user can sign up and share a billing ID.

Free per month

Commercial	5 free per month
Government	20 free per month
Academic	100 free per month

Cost above free

Calcs/mo	Cost/calc
1-10	$6.00
11-100	$5.00
101-500	$4.00
501+	$3.00

ZAVER: 5 USD/monthly/100 vypoctov

AlogP (lipophilicity, water solubility)
http://www.vcclab.org/ **(Virtual Computational Chemistry Laboratory)**
http://www.vcclab.org/web/alogps/ non-java environment

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Rational drug design

frequently relies on **computer modeling techniques**
(*computer-aided drug design **CADD***)

AIM:

- **to discover**, or enhance **molecules with ability to bind to a selected target** and **to estimate the power of** binding **before compounds are synthesized**
- to **estimate drug-like properties** and use them for elimination of undesirable structures

it still takes several iterations of design, synthesis, and testing **before an optimal molecule is discovered**

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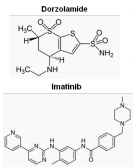
Rational methods in DD

- **Structure-based drug design SBDD**
  - ❑ requires 3D information about target (X-ray crystallography or NMR spectroscopy, PDB database) http://www.rcsb.org/pdb/



  - ❑ first example **Dorzolamid** Carbonic anhydrase inhibitor: Greer J, et al. *JMCH* 1994, 37, 1035–54. (Merck 1995)
  - ❑ **Imatinib** (Gleevec , Novartis 2001) the first tyrosine kinase inhibitor designed for the *bcr-abl* fusion protein (Philadelphia chromosome-positive receptor in chronic myelogenous leukemia (CML)

- **Ligand-based drug design LBDD**
- **Fragment-based drug design FBDD**

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Structure Based Drug Design SBDD (direct DD)

- based on **knowledge of 3D structure of the biological target** obtained through **X-ray crystallography or NMR spectroscopy** (http://www.rcsb.org/pdb/)
- SBDD can be divided into two methodologies:
  - ❑ **"finding" ligands for a given receptor** (database searching / **virtual screening**). A large number of potential ligand structures are screened to find those fitting the binding pocket of the receptor. **It saves synthetic effort** to obtain new active compounds.
  - ❑ **"building" ligands in a target active place**. In this case, **ligand molecules are built up** within the constraints of the binding pocket **by assembling small pieces (atoms, fragments) in a stepwise manner.** The key advantage: **novel structures, not contained in any database, can be suggested**.

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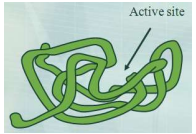
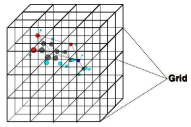
„Finding ligand" methodology

- relies on **known target structure** (PDB complex)
- active side identification
  - ❑ protein, ligand atoms and virtual grid spots need to be classified by their atomic properties as
    - **hydrophobic atom**: all carbons
    - **H-bond donor**: OH,NH
    - **H-bond acceptor**: O,N
    - **polar atom**: O,N,S,P,X,M,C-HETATOM



The space inside the ligand binding region **would be studied with virtual probe atoms** of the four types above **to determine what kind of chemical fragments can be put into their corresponding spots in the ligand binding region of the receptor**.



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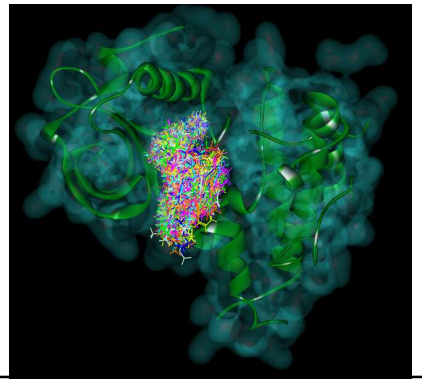
Virtual SBDD screening („finding ligands")

- 500 potentially actives / 1 500 000 mol. in library



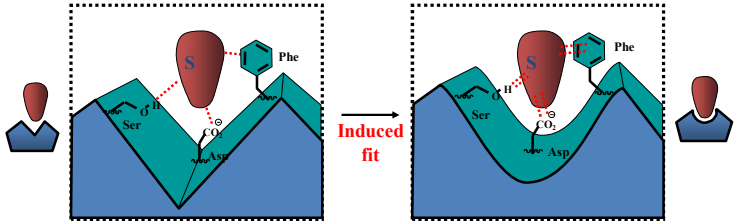
1 : 3000

40

Ligand-based induced fit

(different conformers of the same protein)

- **Binding of ligand alters the shape of the protein active site to maximise intermolecular interactions. This will result 3D changes in protein binding site => induced fit**



Intermolecular bonds not optimum length for maximum bonding

Intermolecular bond lengths optimized (conformational changes in AA residues and in backbone observed in the protein)

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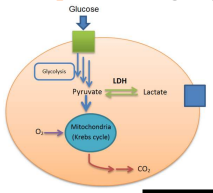
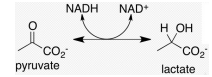
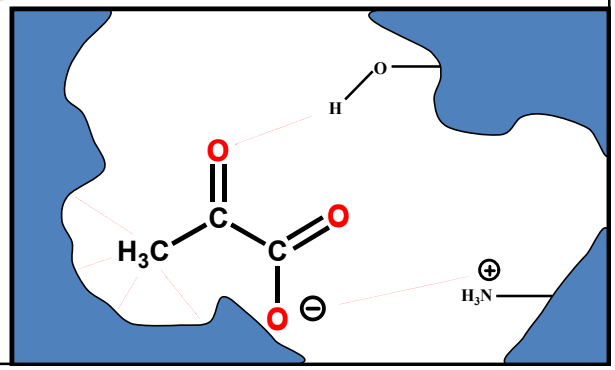
Example: Binding of pyruvic acid in Lactate Dehydrogenase LDH





LDH converts pyruvate, the final product of glycolysis, to lactate when oxygen is absent or in short supply



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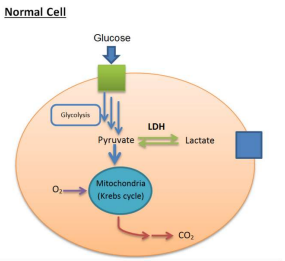
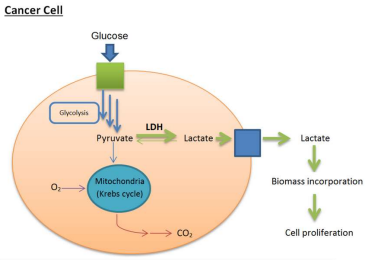


Oxidative phosphorylation- high ATP: glucose ratio, low LDH activity in both directions results in high energy production.



Aerobic glycolysis (Warburg effect) - low ATP: glucose ratio, high LDH activity in one direction. Results in biomass incorporation and cell proliferation.

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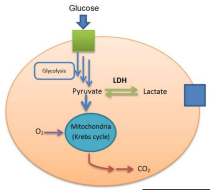
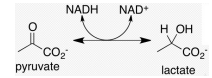
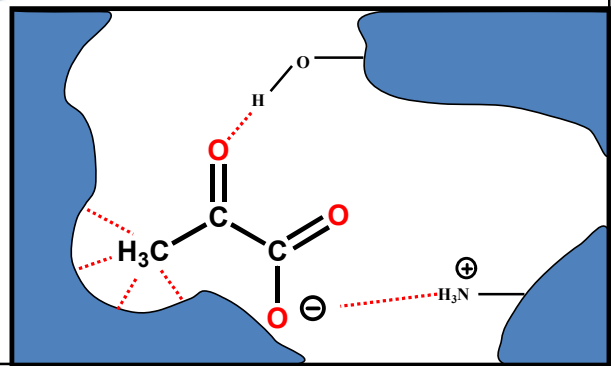
Example: Binding of pyruvic acid in Lactate Dehydrogenase LDH





LDH converts pyruvate, the final product of glycolysis, to lactate when oxygen is absent or in short supply



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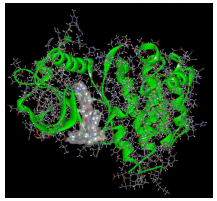
VEGFR2 TK – induced fit

(different 20 conformers of the same protein differently accommodating the same set of compounds, **induced fit is a complication for CADD**)

PDB code	The best binder kcal/mol
1Y6B	-35.75
3C7Q	-36.03
2RL5	-35.20
3B8Q	-26.80
2QU5	-3.46
2P2I	-30.65
1YWN	-33.60
3B8R	-36.60
3DTW	-30.75
3CP9	-28.27
3CPB	-18.01
3EFL	-35.40
3EWH	-27.10
3CJF	-46.96

PDB code	The best binder kcal/mol
3CPC	-27.23
2OH4	-31.38
1Y6A	-38.11
2P2H	-36.12
2QU6	-37.69
3CJG	-45.16



nM inhibitors should rich the relative level of interaction energy ca -50 kcal/mol. 3CJF and 3CJG are only receptor conformers that almost give this level for the best of a set of 16 docked compounds.

45

X-ray Structure Screening is overcoming induced fit problems

Procedure:

- **crystallize** target protein with your ligand
  (e.g. receptor + inhibitor)
- **acquire 3D structure** of complex by X-ray crystallography
- **identify a binding site** (region where ligand is bound)
- identify **binding interactions** between ligand and target
- identify **vacant regions** for extra binding interactions
- **'Fit' analogues** into binding site to test binding capability

Carry out **drug design based on the more accurate interactions** between your lead compound and the target binding site.

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Ligand Based Drug Design LBDD (indirect DD)

- based on **knowledge of molecules that bind to the biological target** (their structure and IC_{50} bioactivity)

  ❑ These molecules (ligands) may be used **to derive a pharmacophoric model which defines** the minimum necessary **structural characteristics** a molecule must possess in order to bind to the target.

  Virtual screening (based on pharmacophore models; high-throughput docking) including drug **property filtering** (Zinc 35 000 000 / Lipinski)
  http://zinc.docking.org/

  ❑ Alternatively, a **quantitative structure-activity relationship (QSAR)** in which a **correlation between calculated properties** of molecules **and** their **experimentally determined biological activity** may be derived. These may be used **to predict the activity of new analogues**.

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Fragment Based Drug Design FBDD

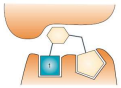
- Screening of **small** (MW < 300), **low potency fragments** (**epitops**, "seed templates"), which are **subsequently developed into higher potency structures**

  ❑ we need a **database** of **fragments** to choose ligands

  ❑ although the diversity of organic structures is infinite, **the number of basic fragments is rather limited**

  ❑ seed is put into the binding pocket, and **add other fragments one by one**

  ❑ **new molecules can be regarded as combinations of two or more individual binding epitopes**



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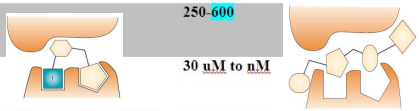
	FBDD	HTS
Output	Efficiency of binding	Potency in activity
Screened compounds	few 100 to several 1000	several 100 000
MW	150-300	250-600
Activity threshold	mM to 30 uM	30 uM to nM
Screening based on	Biophysical assays (NMR, X-RAY, SPR), direct measurements of ligand-protein interactions (direct measurements of inactive forms of kinases)	In vitro assay not ideal compounds available some functional groups not involved in interactions
Hit to Lead	synthesis of only few designed compounds	more extensive synthesis
Requirements	Expertise in protein-ligand binding interactions and fragment design	Requires intensive infrastructure, data processing



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Ligand efficiency / Ro3

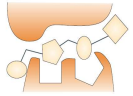
- **Ligand Efficiency LE**

$$LE = -\Delta G/HAC \approx -RT\ln(IC_{50})/HAC$$

(HAC = Number of heavy atoms)

fragments typically exhibit higher ligand efficiency than higher MW compounds identified through HTS (not ideal interactions)

*I. D. Kuntz et al. Proc. Natl. Acad. Sci. USA **1999**, 96, 9997.*



- The "rule of three, Ro3" for fragment design:

TABLE 1	Rule of Three Criteria[1]
MW	≤ 300
cLogP	≤ 3.0
H-Bond Acceptors	≤ 3
H-Bond Donors	≤ 3
Rotatable bonds (Flexibility Index)	≤ 3
Polar Surface Area	≤ 60Å²

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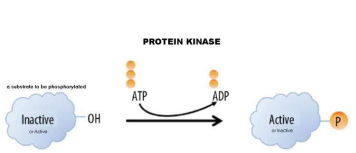
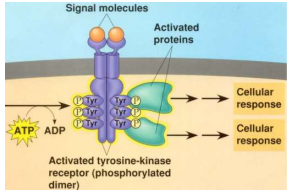
Protein Kinase Inhibitors

Protein kinases (PKs) (tyrosine, serin-threonine and histidine kinases) **phosphorylate** specific amino acids in **protein substrates**.





There are **over 500 different types of hu-protein kinases**. Many PKs are **enzymes (TK)** within cytoplasm, others traverse the cell membrane **and** play **dual role as receptor and enzyme (TKR)**. **Growth factors through TKRs signalling control transcription of genes leading to cell division. In many cancers excess of growth factor or PK receptor has been observed.** Therefore **PK inhibitors are useful anticancer agents.** All PK use ATP as the phosphorylation agent.

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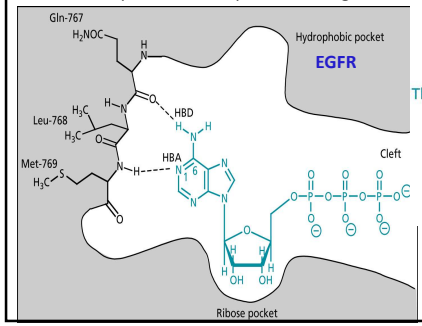
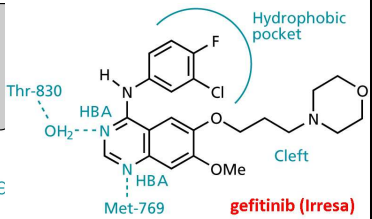
EGFRI gefinitib (Irresa) (AstraZeneca, FDA 2003)

EGFR (ErbB, HER1) **tyrosine kinase receptor**: **abnormal or over-expressed in** the breast, lung, brain, prostate, gastrointestinal tract, ovaries **cancer**. EGFR is a receptor for EGF growth factors (Nobel Prize 1986). **Upon activation** by EGF, **EGFR forms active homodimer possessing intracellular TK activity** that initiate several signal transduction cascades **leading to DNA synthesis and cell proliferation**. **Gefitinib inhibits EGFR by binding to the ATP-binding site**. Thus this receptor and its dependent **malignant cells are inhibited**.





EGFR positive patients have shown an impressive **60% response rate** which exceeds the response rate for conventional chemotherapy.

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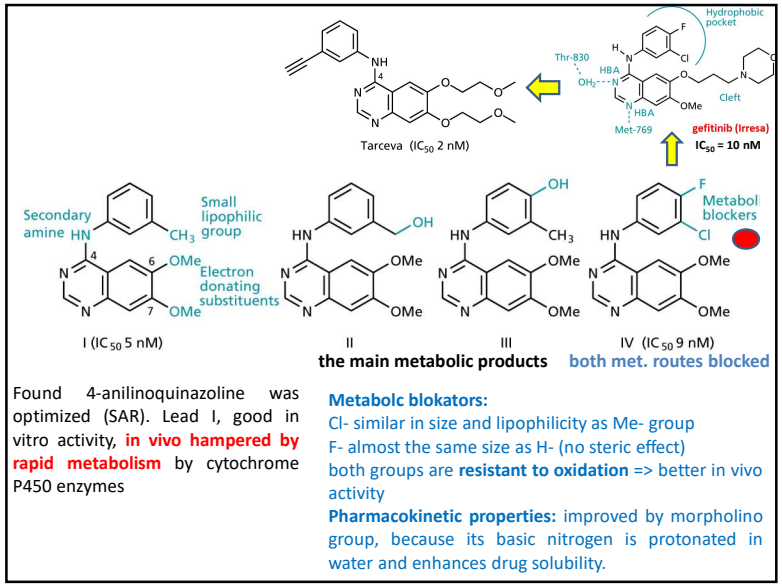

Found 4-anilinoquinazoline was optimized (SAR). Lead I, good in vitro activity, **in vivo hampered by rapid metabolism** by cytochrome P450 enzymes

Metabolc blokators:
Cl- similar in size and lipophilicity as Me- group
F- almost the same size as H- (no steric effect)
both groups are **resistant to oxidation** => better in vivo activity
Pharmacokinetic properties: improved by morpholino group, because its basic nitrogen is protonated in water and enhances drug solubility.

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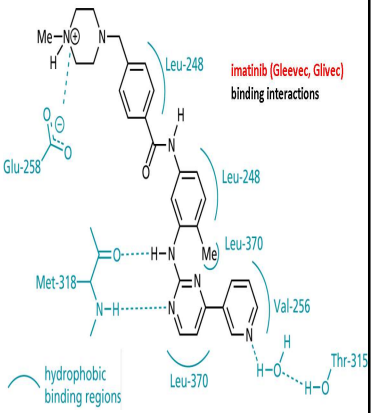
TKI imatinib (Gleevec) (Novartis, FDA 2001)

The first PKI that reach the marked.

Imatinib treats **CML** (Chronic Myeloid Leukemia) a cancer of white blood cells characterized by the **increased and unregulated growth of myeloid cells** in the bone marrow and the accumulation of these cells in the blood. These **cancer cells contain an abnormal heterodimeric protein kinase (Bcr-Abl)** that is **not found in normal cells**. It is **associated with Philadelphia chromosome**. TK active site is on Abl portion of Bcr-Abl receptor. **Imatinib is selective inhibitor successful in 90% of patients.** This is a **first drug targeting unique mol. structure observed only in cancer cells**. Treatment of CML by imatinib dramatically improved patient five year survival from 31% to 59%.



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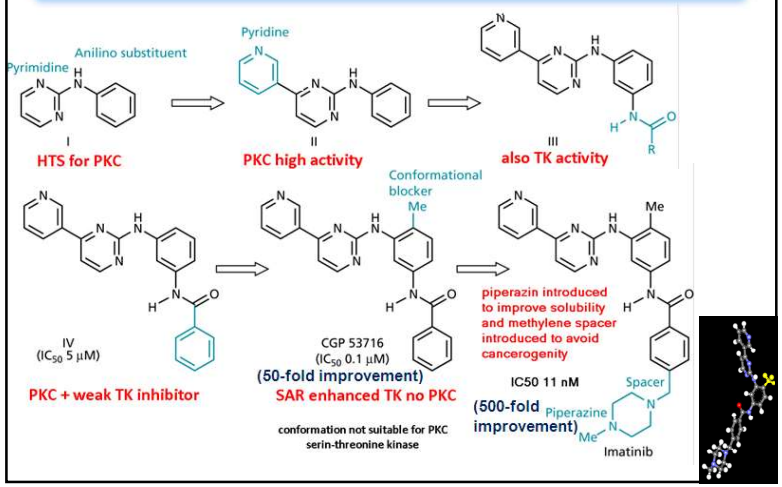
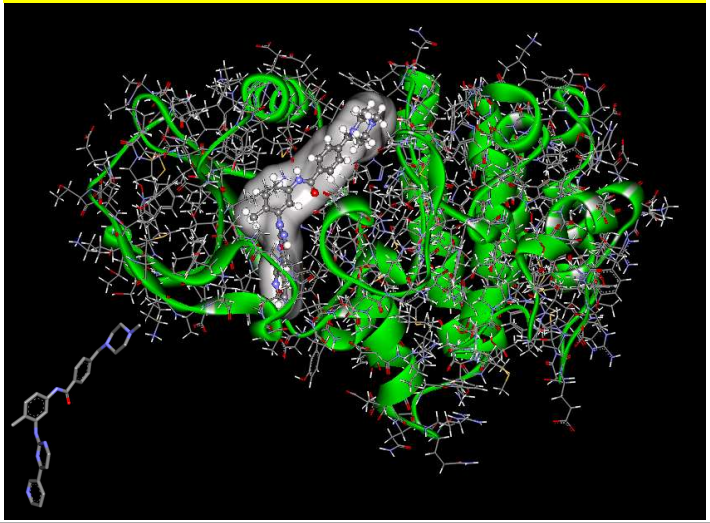
H2L optimization in imatinib DD



55

Abl kinase in complex with imatinib (PDB: 2HYY)



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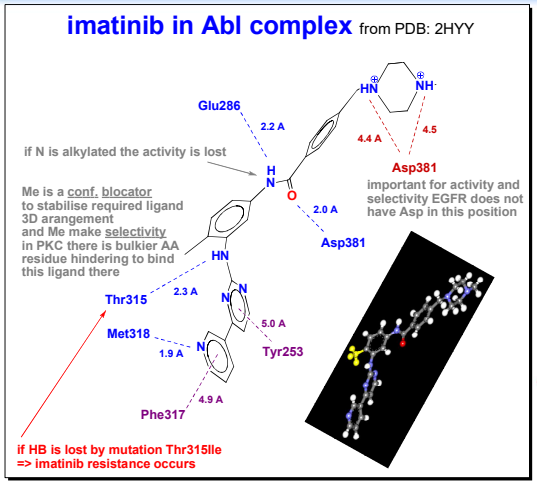
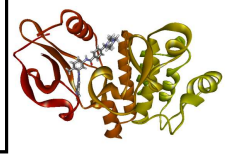
Binding Interactions Map





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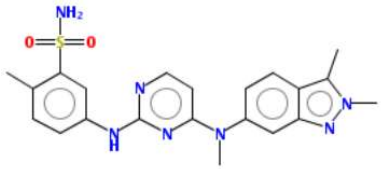
pazopanib (VOTRIENT) (GSK, FDA 2009)

- multi-targeted tyrosine kinase receptor inhibitor (**PDGFR-α,β, VEGFR-1,2,3, KIT**, for **renal cell carcinoma** (RCC) and **imatinib-resistant gastrointestinal stromal tumor** (GIST)



5-(4-((2,3-dimethyl-2H-**indazol**-6-yl)(methyl)amino)**pyrimidin**-2-ylamino)-2-methylbenzenesulfonamide

JMCH 51 **2008** 4632

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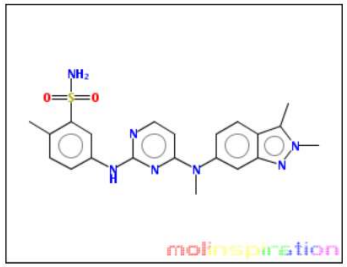
pazopanib / drug-like properties



Molinspiration property engine v2009.01

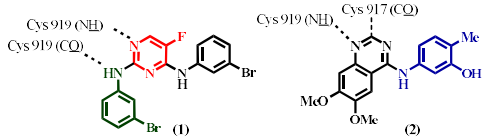
miLogP	3.272
TPSA	119.04
natoms	31
MW	437.529
nON	9
nOHNH	3
nviolations	0
nrotb	5
volume	377.901

Get data as text (for copy / paste).

Get 3D geometry BETA

59

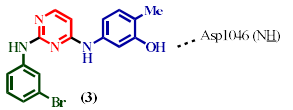
1st HTS GSK / SBDD optimization



IC_{50} = 400 nM (VEGFR2)

IC_{50} = 0.6 nM (VEGFR2)
hit from 2002, even highly active
quinazoline was not patentable

Asp1046 (NH)



IC_{50} = 6.3 nM (VEGFR2) 63-fold improvement to (1)
IC_{50} = 540 nM (HUVEC / VEGF)
poor PK: high clearance 83ml/min/kg
due rapid HO- glucuronidation or sulfation
low bioavailability 11% (mouse),
HO-Ar shoud be omitted

pyrimidine 1 and chinazoline 2 (both bind similarly in ATP binding pocket) → **pyrimidine 3**

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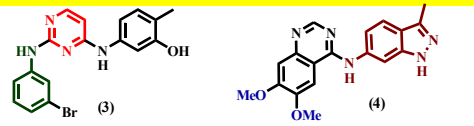
2nd screening – house kinase library, hit (4)

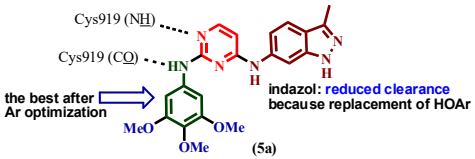


(3)

IC_{50} = 6.3 nM (VEGFR2)
IC_{50} = 540 nM (HUVEC/VEGF)

(4)

IC_{50} = 1.7 nM (VEGFR2)
excellent, but chinazoline not IP



(5a)

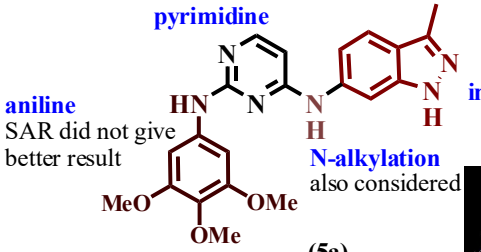
IC_{50} = 6.3 nM (VEGFR2) **good afinity**
IC_{50} = 180 nM (HUVEC/VEGF) **improved cellular act.**
PK improved significantly: clearance 16 ml/min/kg
oral bioavailability 85% (rat)

pyrimidine 3 and chinazoline 4 → pyrimidine 5a

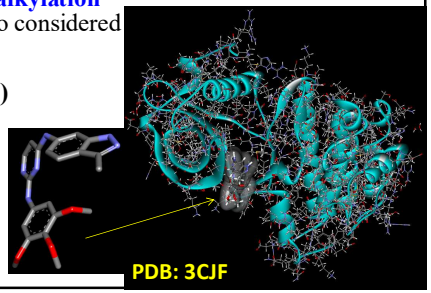
61

Further SAR optimization of 2nd screening result (5a)

(for better affinity, activity, PK properties for oral therapy)



(5a)



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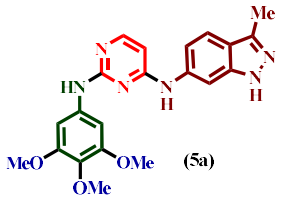
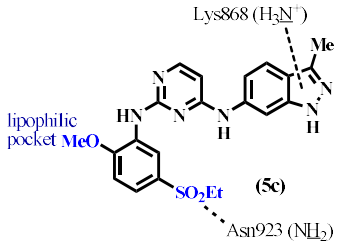
SAR → optimization on an aniline fragment



(5a)

IC_{50} = 6.3 nM (VEGFR2)
IC_{50} = 180 nM (HUVEC/VEGF)
PK: clearance 16 ml/min/kg
oral bioavailability 85% (rat)



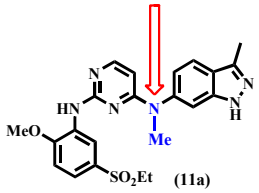
(5c)

IC_{50} = 130 nM (VEGFR2)
IC_{50} = 13 000 nM (HUVEC/VEGF)
moderate clearance 40 ml/min/kg (rat)
moderate bioavailability (28 %)

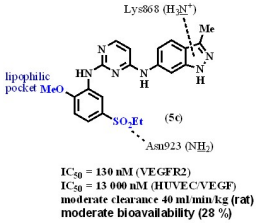
63

SAR → via N-alkylation (conf. stabilization)

NH could formed HBD (worster absorption) and potential place of metabolic changes
NMe stabilizes S conformer, better PK properties



(11a)



(5c)

IC_{50} = 130 nM (VEGFR2)
IC_{50} = 13 000 nM (HUVEC/VEGF)
moderate clearance 40 ml/min/kg (rat)
moderate bioavailability (28 %)

affinity comparable with (5c, NH)
but better PK properties: clearance 10ml/min/kg (from 40), bioavailability 65 % (from 28 %)

IC_{50} < 10 uM (CYP P450)
N from indazole coordinates hem iron in P450

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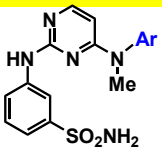
SAR → optimization on a heterocycle to avoid CYP binding



Cpd	Ar	VEGFR2[a] IC_{50} nM	HUVEC[b] IC_{50} µM	Inhibition of P450 Isozymes IC_{50} µM[c]			
				2C9	2C19	2D6	3A4[d]
12a		0.6	0.094	0.7	7.2	4.8	7.6 / 16
12b		36	1.4	1	9.3	6.3	13 / 1.7
12c		5.6	0.023	1.4	20	16	16 / 31
12d		2.6	11	0.5	1.4	66	74 / 79
12e		7.6	0.11	2.9	9.2	18	4.7 / 3.9
12f		63	2.8	15	26	25	26 / 1.7
12g		17	1.4	9	33	33	27 / 45

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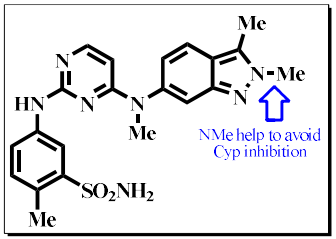
pazopanib (Votrient, GSK)



IC_{50} = 10, 30, 47 (VEGFR1-3) good affinity
IC_{50} = 21 nM (HUVEC / VEGF) high activity
IC_{50} = 720 nM (HUVEC / bFGFR) good selectivity
IC_{50} (CYP P450) > 10 000 nM low antitarget inhib.
very good PK properties: low clearance 1.7 ml/min/kg
good oral bioavailability (72 %) at dose 10 mg / kg
in vivo and **clinical Ph I - III trials succesfull**
FDA approved 19 Oct 2009 as
globally 3rd antiangiogenic drug to treat cancer

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