Naša cesta nového liečiva

Inhibítory aldózareduktázy v prevencii vzniku diabetických komplikácií

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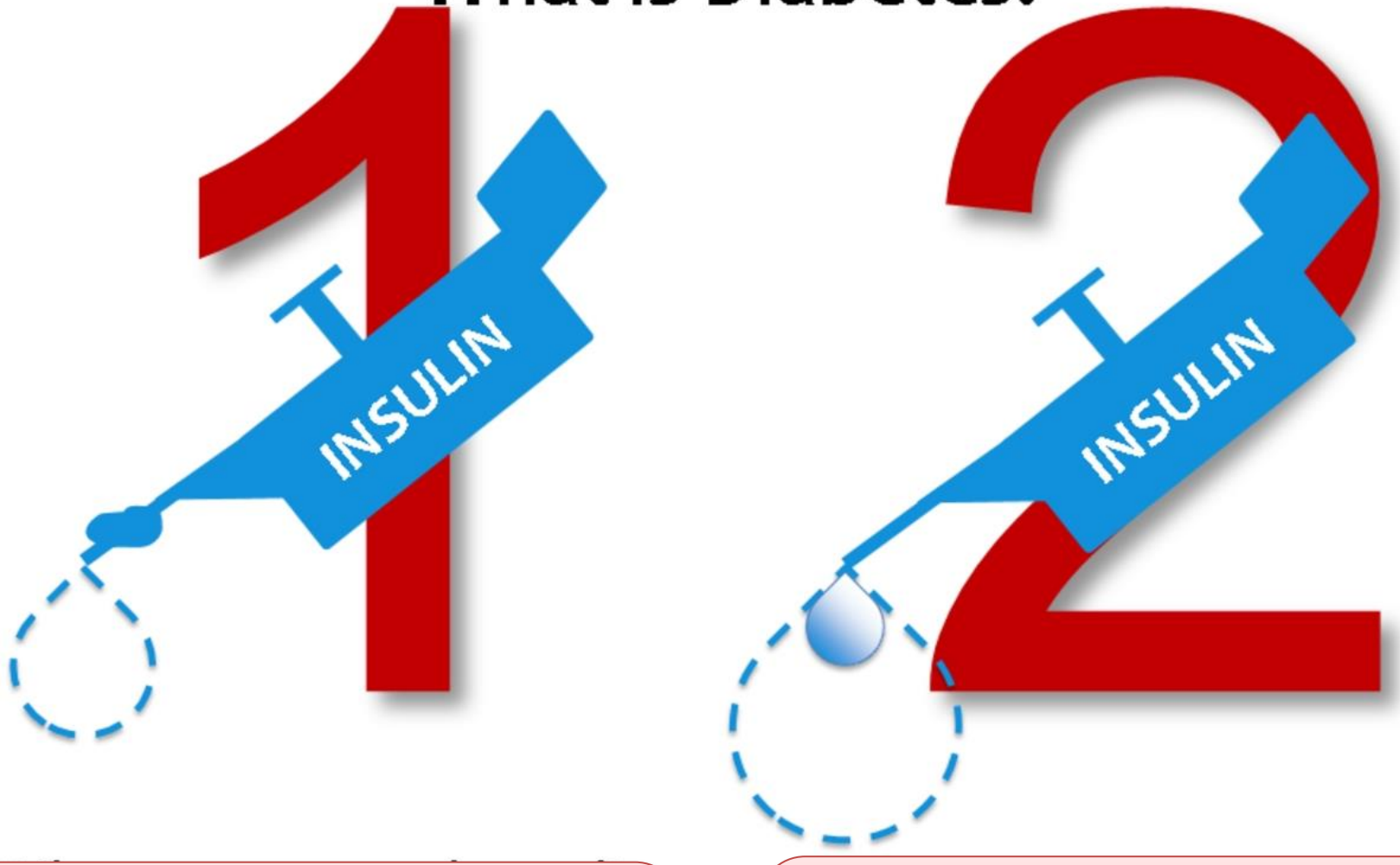
DERIVÁTY KYSELINY INDOLOCTOVEJ AKO INHIBÍTORY ALDÓZAREDUKTÁZY V PREVENCII DIABETICKÝCH KOMPLIKÁCIÍ:

predklinické štúdium na modeloch *in vitro* a za podmienok experimentálneho diabetu *in vivo*

Dizertačná práca

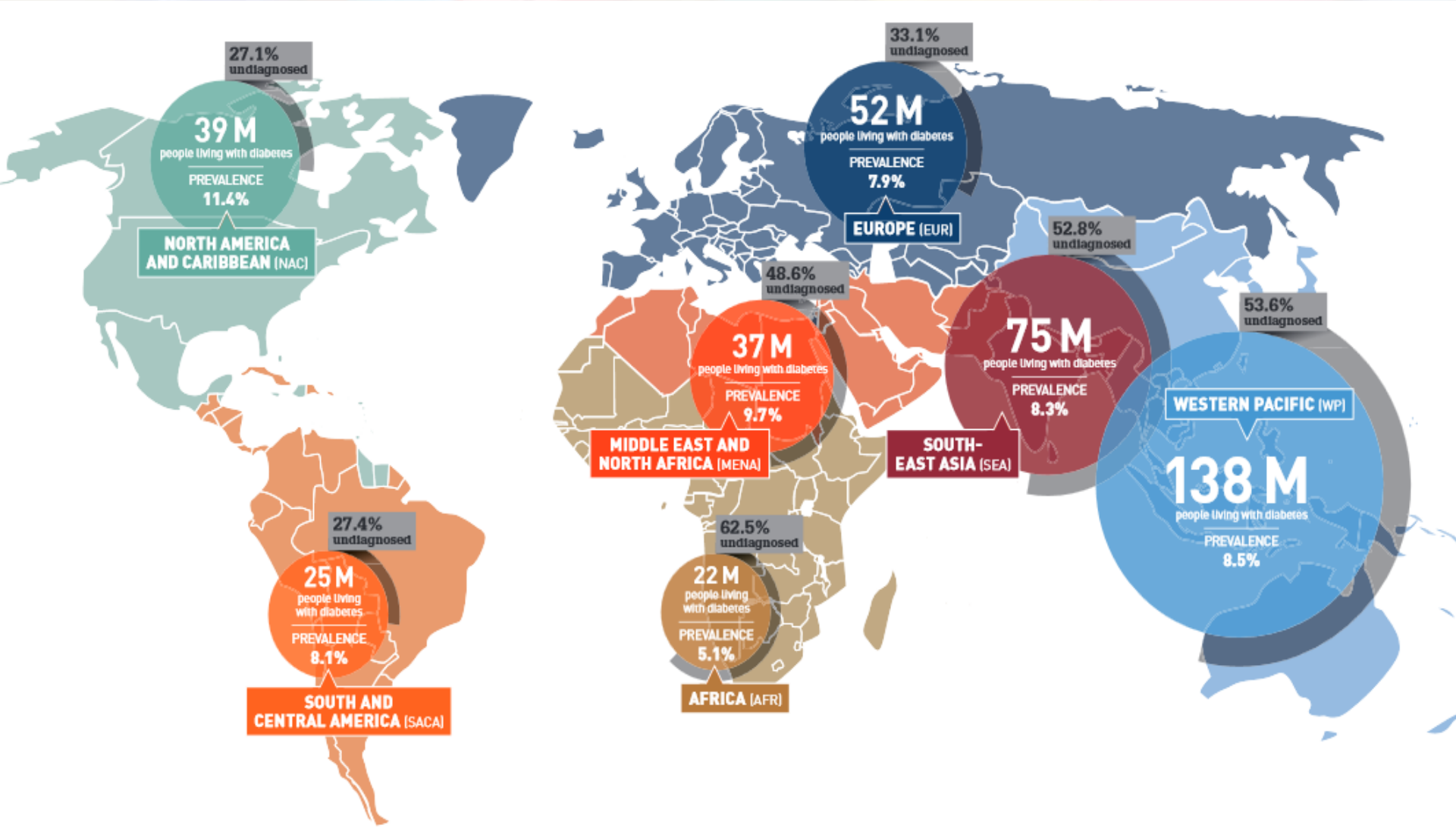
What is Diabetes?



Pankreas neprodukuje inzulín

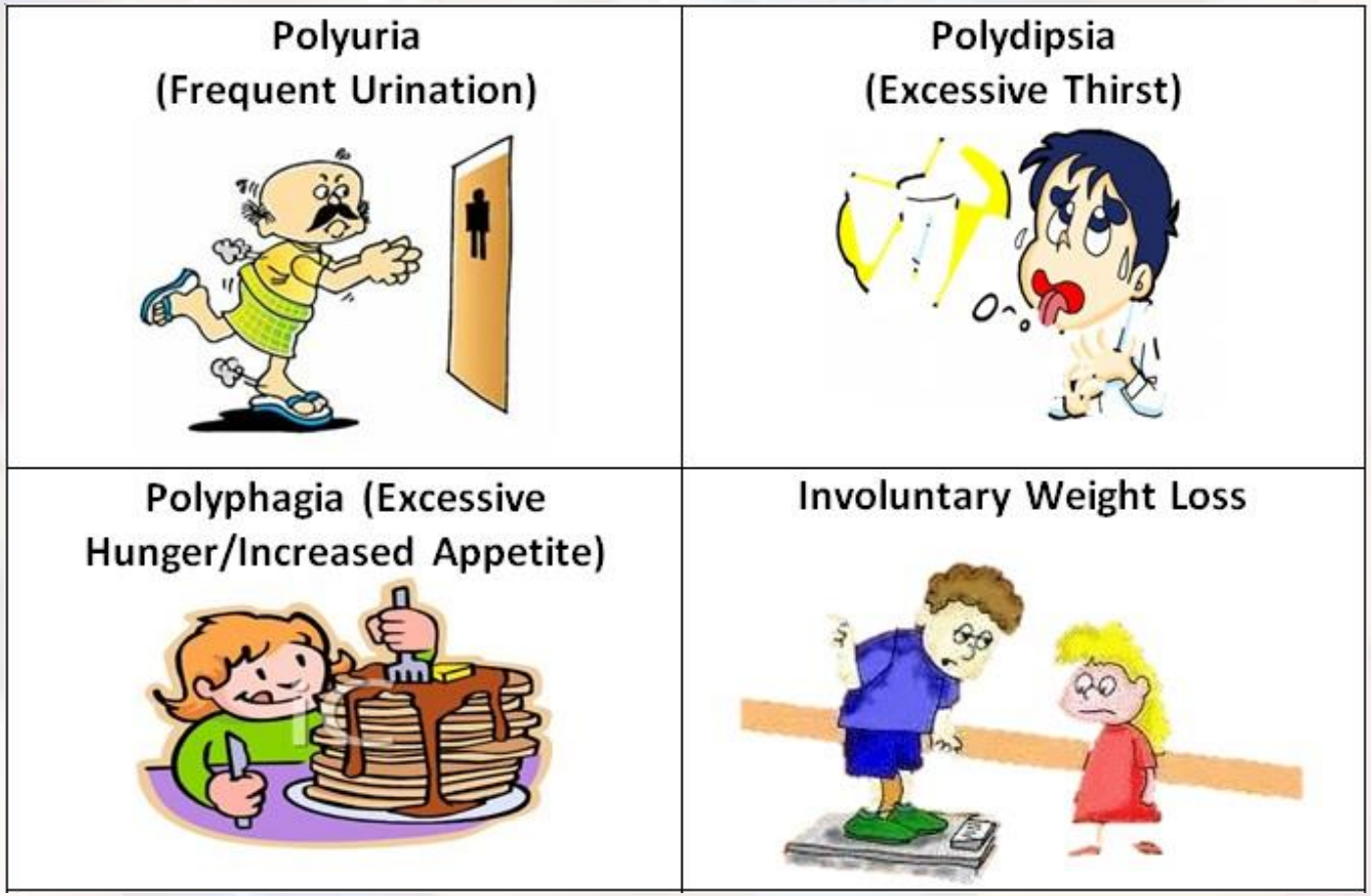
Inzulínová rezistencia

Prevalencia DIABETU vo svete

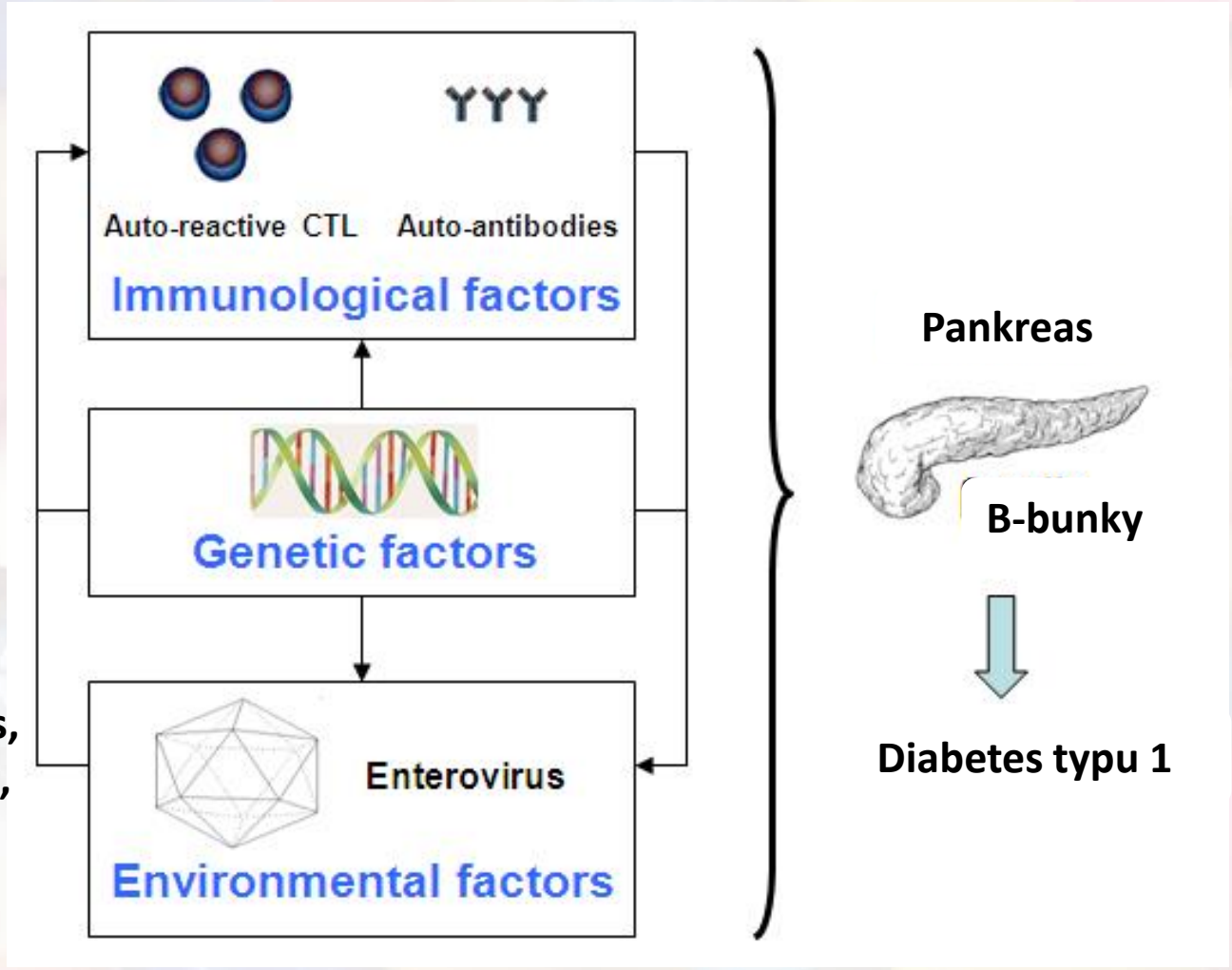


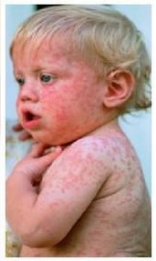
Diabetes melitus typu 1 (T1DM)

- 10 % všetkých diabetikov
- Organizmu nie je schopný spracovávať glukózu v dôsledku nedostatku inzulínu



Diabetes melitus typu 1 (T1DM)





mumps,
rubella,
CMV,
POPs

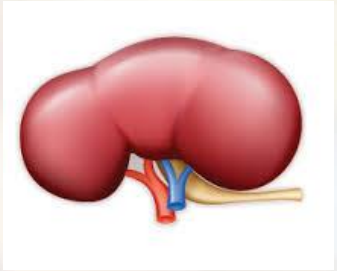
Transport glukózy do bunky

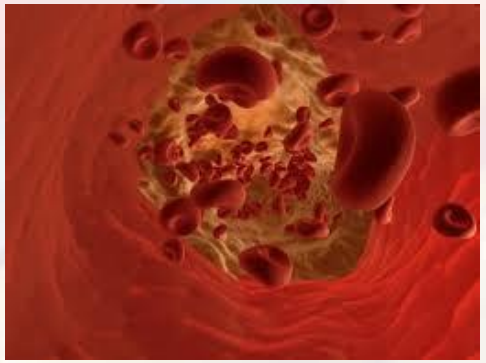
1. Inzulín – závislé tkanivá

- PEČEŇ, SVALY, ADIPOCYTY

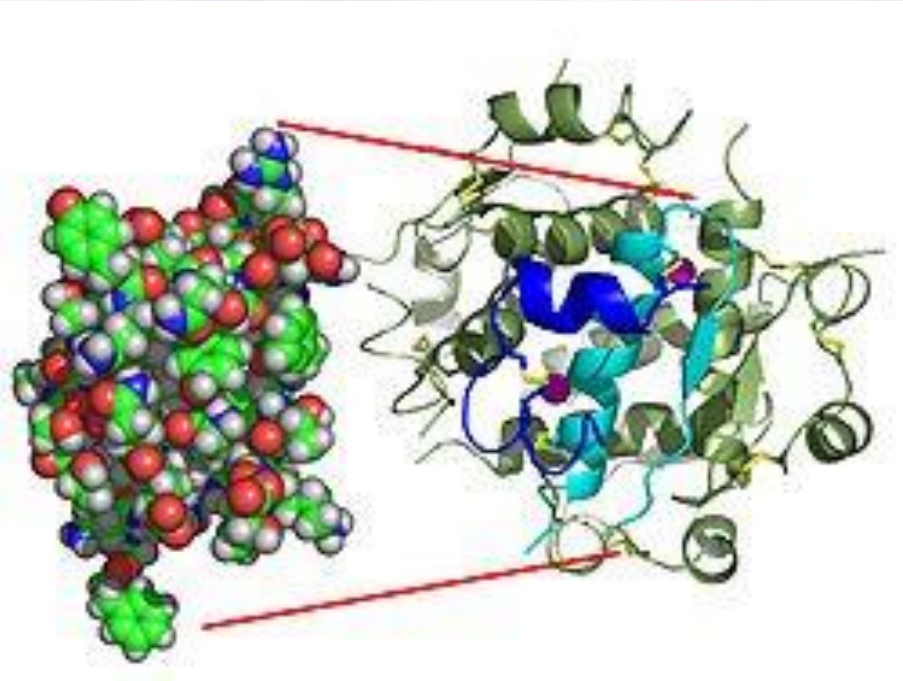
2. Inzulín - nezávislé tkanivá

- OBLIČKY, OČNÁ ŠOŠOVKA, CIEVNY ENDOTEL, PERIFERNE NERVY





Inzulín



- peptidový hormón produkovaný Langerhansovými ostrovčekmi pankreasu
- **Vznik:**

1. Fáza : ribozómy – pre-proinzulín
2. Fáza: endoplazmatické retikulum – proinzulín (A, B, C reťazec)
3. Fáza: Golgiho aparát - inzulín + C-peptid
4. Fáza: sekrečné granule - skladovanie

- Indukcia syntézy glukagónu v pečeni

Diabetes melitus typu 2 (T2DM)

- Metabolické ochorenie
- Organizmus nie je schopný spracovávať glukózu v dôsledku inzulínovej rezistencie
- Najčastejšie sprevádzaná obezitou ???
- Rizikové faktory: životný štýl, málo pohybu, vysoko-kalorická diéta, metabolický syndróm, obezita, genetická predispozícia, znečistené životné prostredie (POPs, konzervanty, chemikálie....)

LOW BLOOD SUGAR
*Hypo*glycemia

Signs and Symptoms


SWEATING


TREMBLING


DIZZINESS


MOOD CHANGES


HUNGER

HEADACHES


BLURRED VISION


EXTREME TIREDNESS AND PALENESS

HIGH BLOOD SUGAR
*Hyper*glycemia

Signs and Symptoms:


DRY MOUTH

EXTREME THIRST


FREQUENT URGE TO URINATE

DROWSINESS

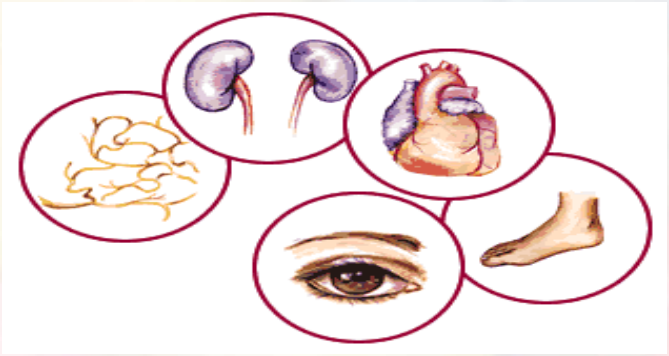

FREQUENT BED WETTING


STOMACH PAIN

Diabetické komplikácie

1) **Akútne / skoré komplikácie**
   - diabetická ketoacidóza, hypoglykémia, diabetická kóma, infekcie dýchacích ciest, periodontálne ochorenia
   - riešenie – optimálna hladina inzulínu – inzulínové pumpy....

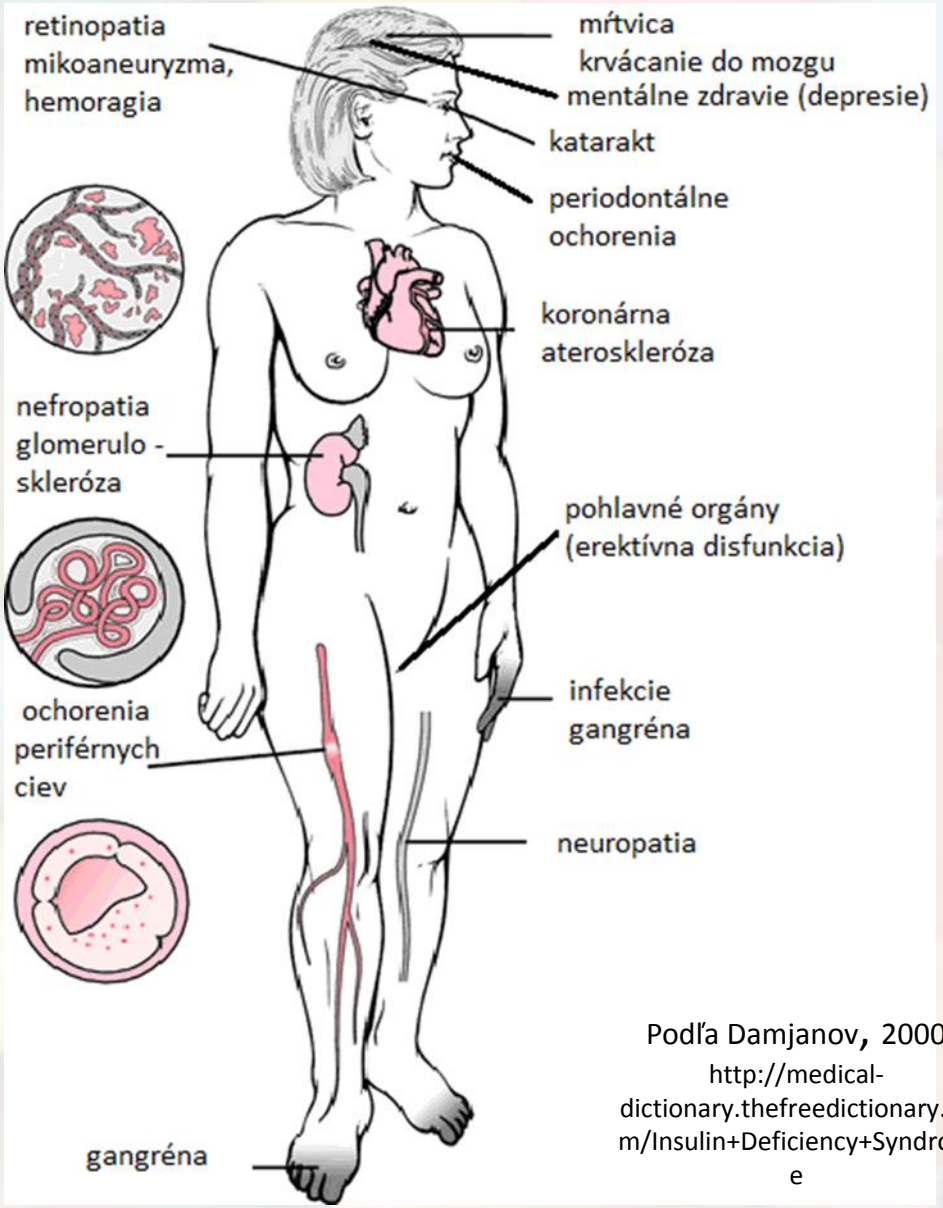
2) **Chronické / neskoré komplikácie**
   - katarakta, retinopatia, nefropatia, neuropatia , angiopatia.....
   - riešenie – ??? Doteraz neexistujú farmaká využívané v klinickej praxi na euro-americkom trhu



DIABETICKÉ KOMPLÍKÁCIE SÚ HLAVNOU PRÍČINOU UMRTIA ĽUDÍ POSTIHNUTÝCH DIABETOM.

Chronické diabetické komplikácie

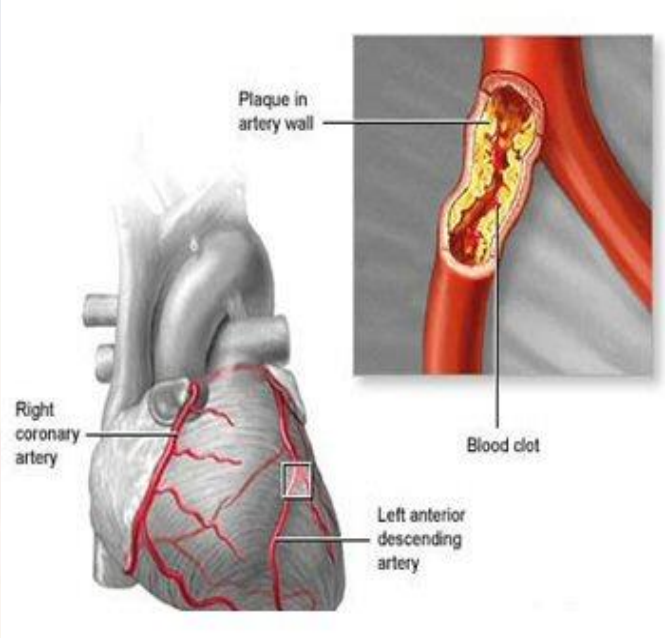
- ANGIOPATIA
- NEFROPATIA
- KATARAKT
- NEUROPATIA
- RETINOPATIA
- OBEZITA
- ZÁPALOVÉ PROCESY



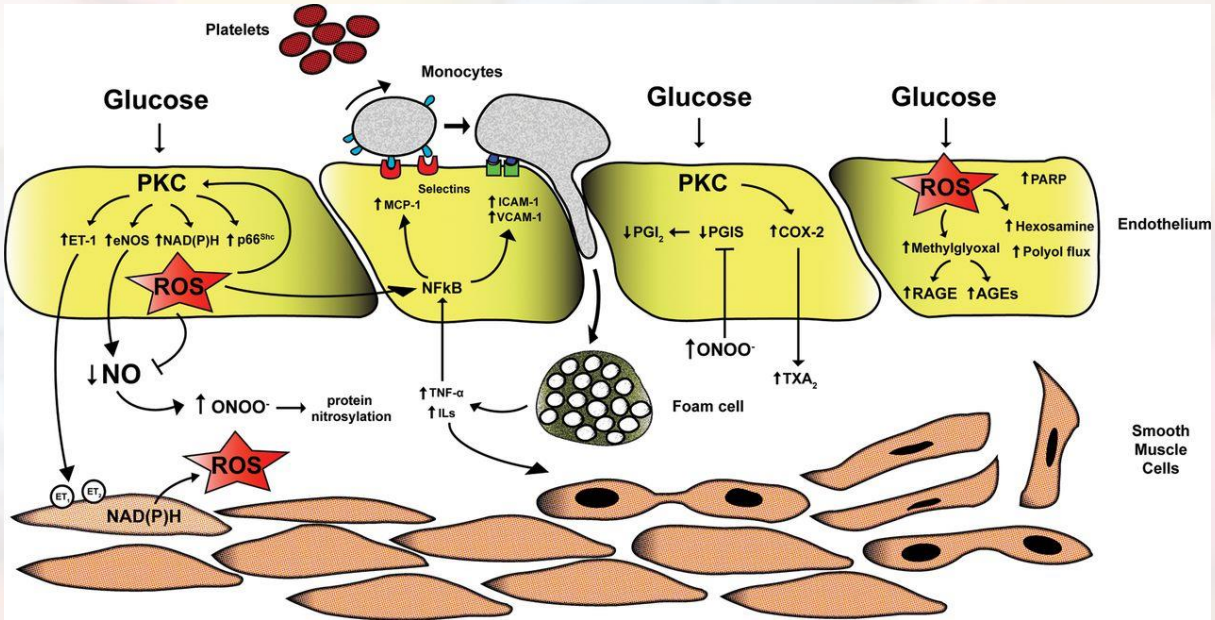
Podľa Damjanov, 2000

http://medical-dictionary.thefreedictionary.com/Insulin+Deficiency+Syndrome

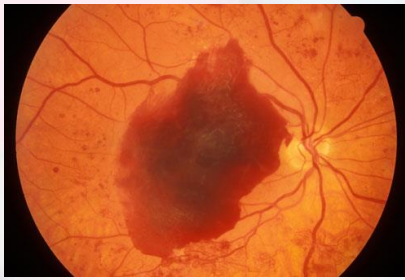
Diabetická angiopatia



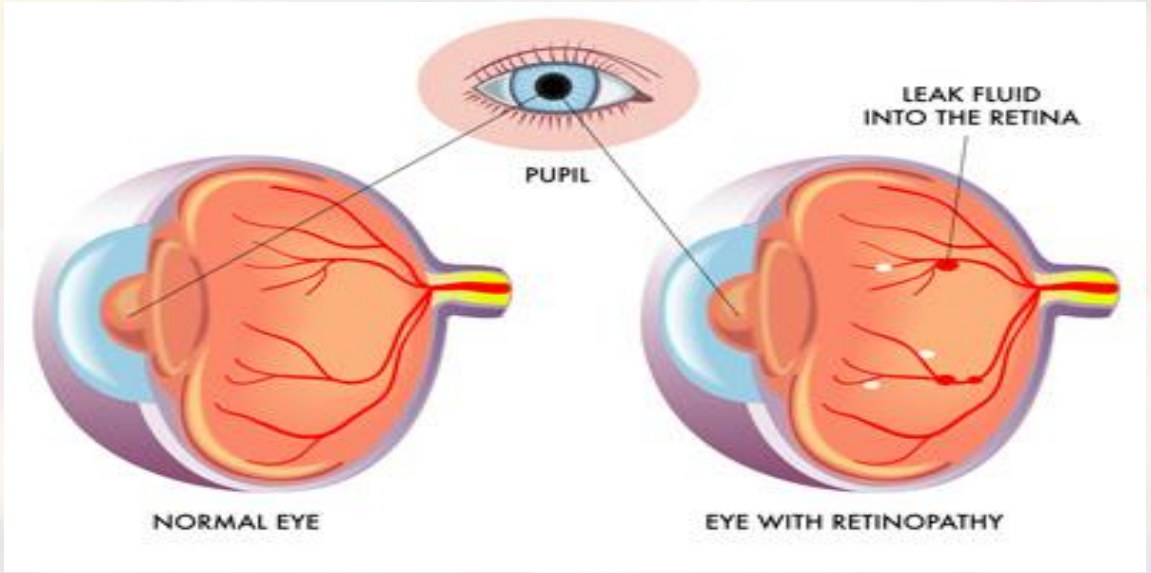
- Tvorba plakov, ktoré spôsobujú zníženú elasticitu ciev
- Znížený transport kyslíka a krvi
- Vedie k ateroskleróze
- Ateroskleróza sa môže prejaviť kdekoľvek, avšak najnebezpečnejšia je ak napáda artérie vedúce k mozgu, srdcu, obličkám a dolným končatinám
- Rizikový faktor - fajčenie



Diabetická Retinopatia

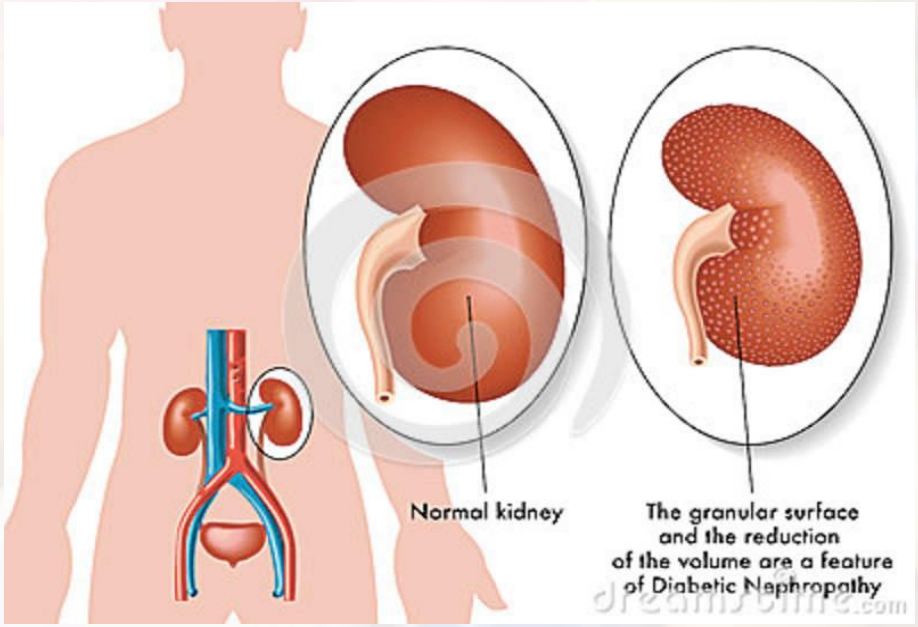


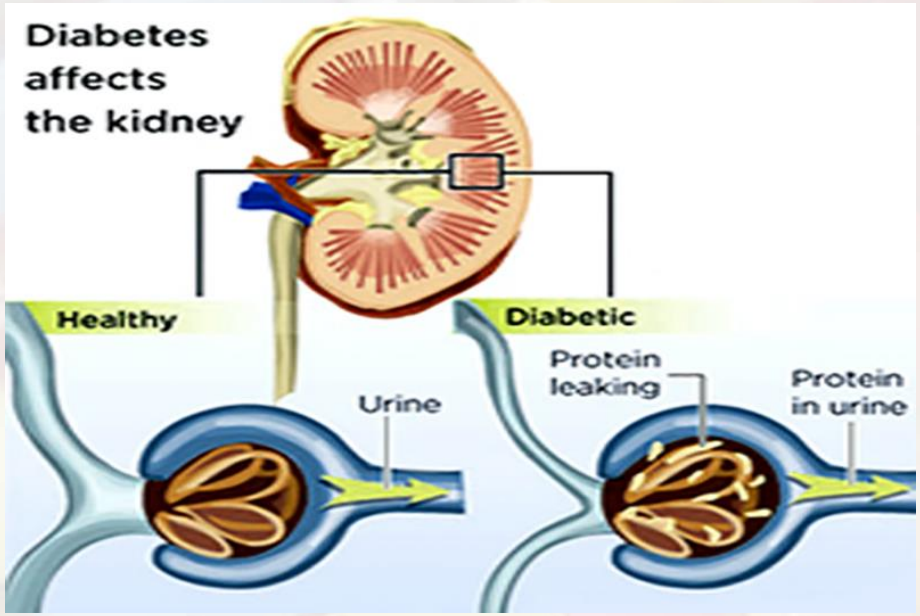
- Hlavná príčina oslepnutia u ľudí vo veku 20-74 rokov
- 80 % ľudí trpiacich diabetom viac ako 10 rokov



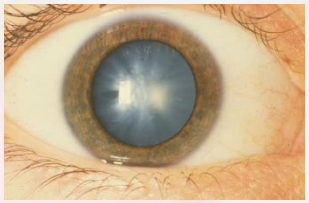
Diabetická Nefropatia

- Je progresívne ochorenie obličiek spôsobené angiopatiou kapilár v globerulách obličiek
- Prejav: nefropatický syndróm a difúznou glomerulosklerózou
- Postihuje pacientov dlho trpiacich na diabetes
- Hlavná príčina nasadenia dialýzy v západných krajinách

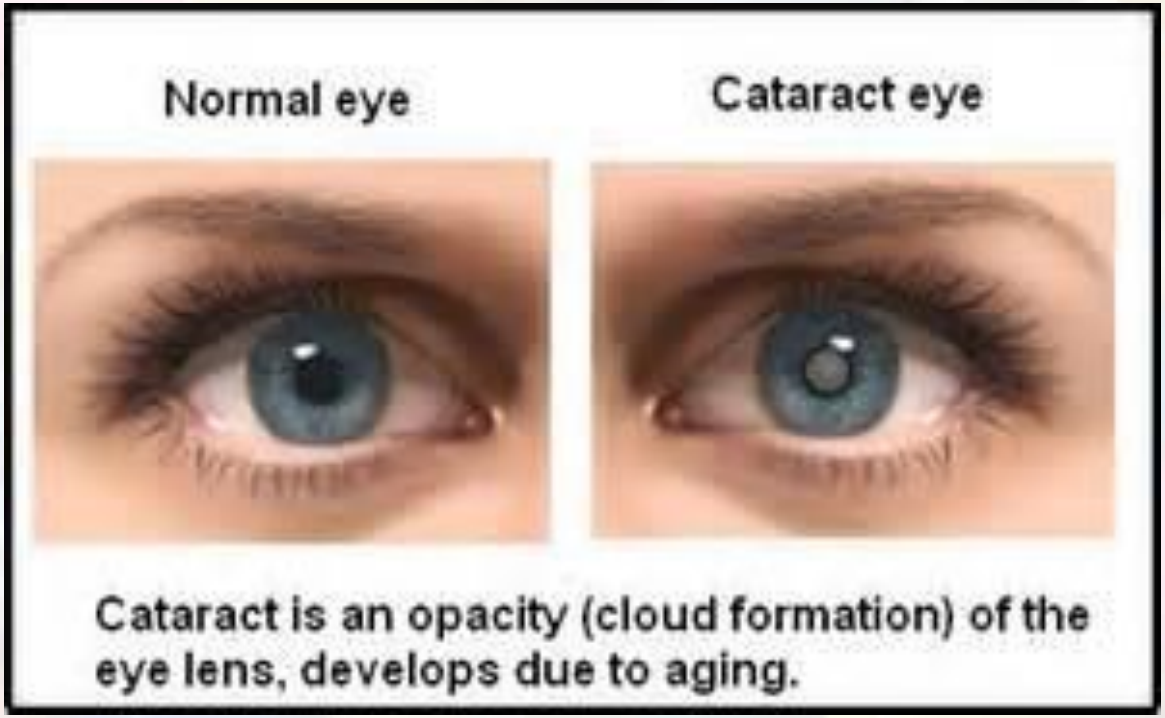




Diabetická Katarakta

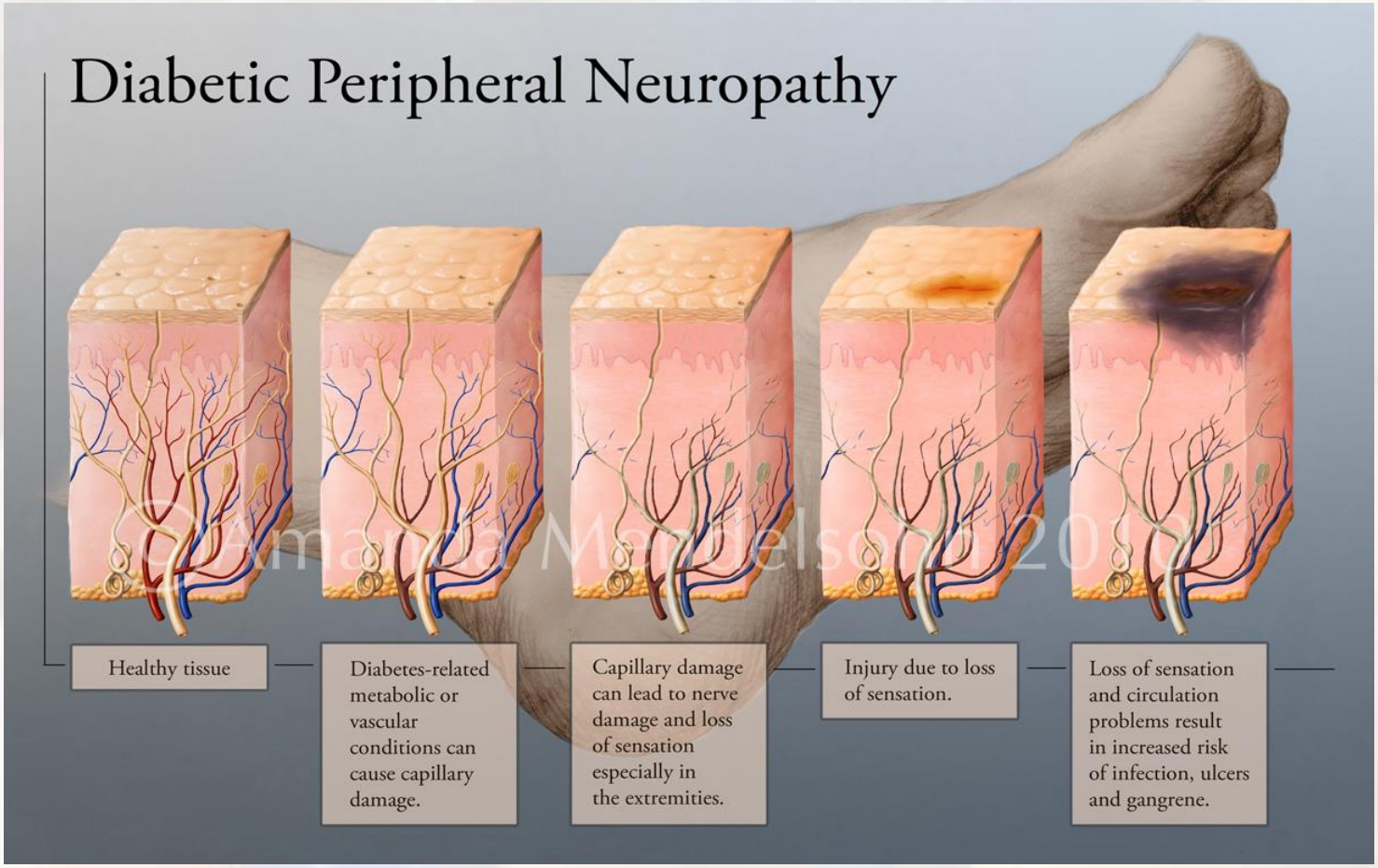


- Vysoká hladina sorbitolu hromadená v očnej šošovka spôsobuje jej poškodenie
- Zvýšená hladina sorbitolu taktiež vedie k zvýšenej tvorbe voľných radikálov poškodzujúcich fibrili šošoviek
- 5.5 milióna ľudí v USA
- 8 miliónov v západnej Európe



Diabetická Neuropatia

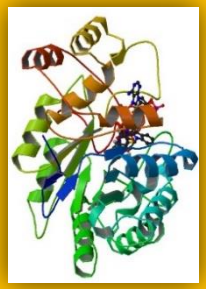
- Ošetrenie zamerané na zmienenie bolesti
- Neliečiteľné a nezvratné ochorenie
- Narastá riziko poranení, pacient stráca cit
- Aj menšie infekcie môžu viesť k nezvratnému poškodeniu tkaniva a tým amputácii



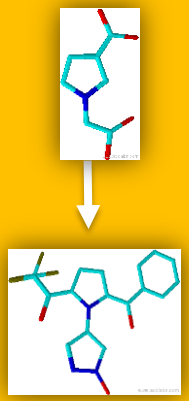
Vývoj liečiv – jednotlivé kroky

1.Identifikácia ochorenia

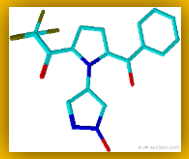
2. Identifikácia cieľa



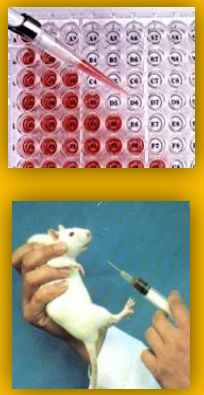
3. HIT to LEAD



4. Lead optimization



5. Predklinické testy



6. Klinické testy

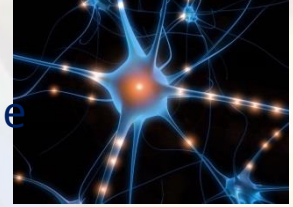
7. Liek

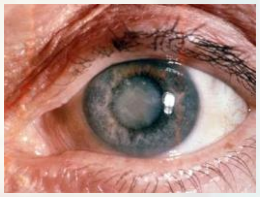
Hyperglykémia

Polyolová dráha

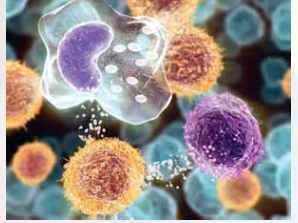
Diabetické komplikácie

Neuro-degeneratívne ochorenia





Katarakta



Zápal

Glykácia proteínov AGE´s

Osmotický stres

hyperglykémia

glukóza → sorbitol → fruktóza

SDH

NADPH NADP+

NAD+ NADH

Stárnutie

↓NAD+/NADH v cytozole

↓ Sirt1

NADH oxidáza

GSSG → GSH

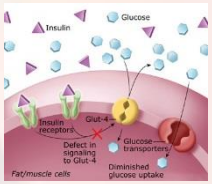
Glutatión reduktáza

Glutatión peroxidáza

ROS

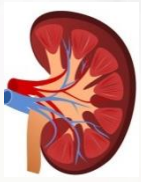
OH°

Inzulínová rezistencia



kataláza

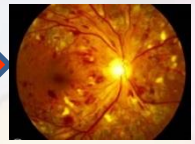
H_2O ← H_2O_2



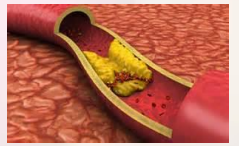
Nefropatia

Neuropatia

Oxidačný stres



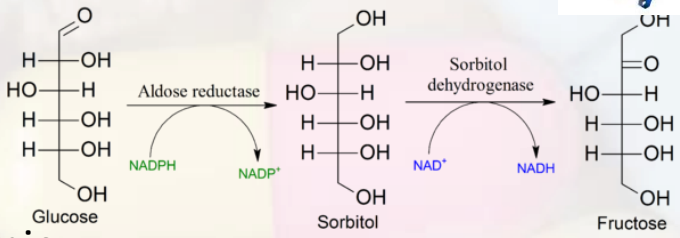
Retinopatia



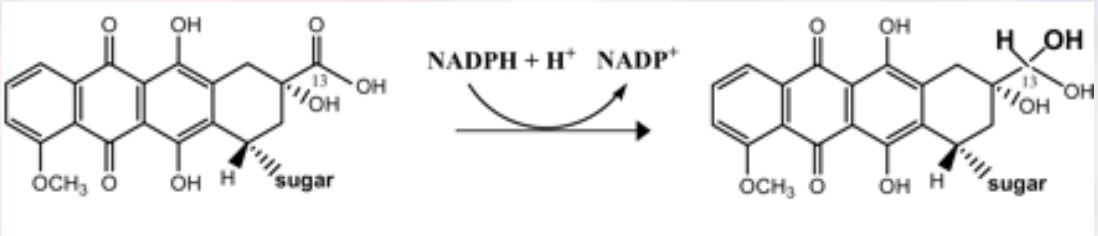
Ateroskleróza

Fyziologické funkcie AKR1B1

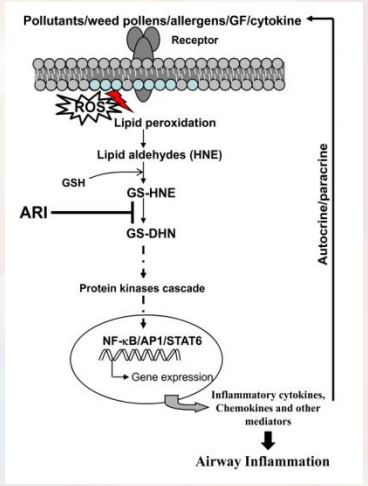
1. **Polyolová dráha**
   A) osmoregulácia
   obličky
   B) produkcia fruktózy – „lacný“ zdroj energie pre spermie



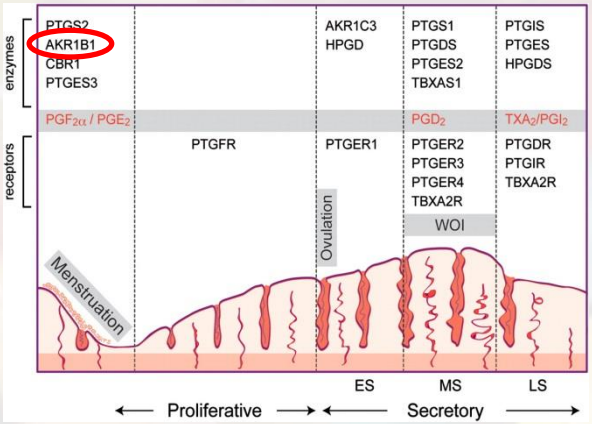
2. **Detoxifikácia**
   A) metabolizovanie enviromentálnych toxínov



   B) redukcia produktov lipidovej peroxidácie



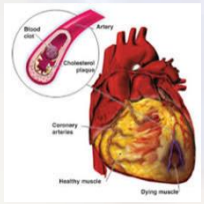
3. **Regulácia menštručného cyklu**
   - prostaglandin F2 syntáza



Civilizačné ochorenia súvisiace s AKR1B1

Kardiovaskulárne ochorenia

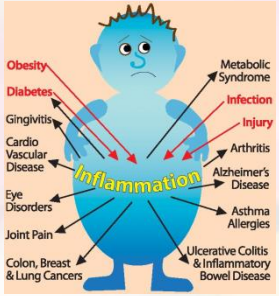
Počas ischémia vzrastá hladina AKR1B1



Rakovina – agresívne typy rakoviny s rýchlim progresom

Poruchy správania, nneurologické a psychiatrické poruchy a ochorenia- Alzheimerova choroba, Downov syndróme, Vaskulárna demencia, Parkinsonova choroba, mániodepresívna psychóza, bipolárna porucha, Huntingtonova choroba...

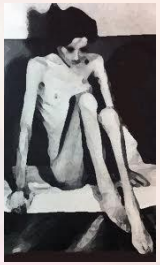
Zápal – astma, sepsa, uveitída, periontitída...



Ochorenia maternice

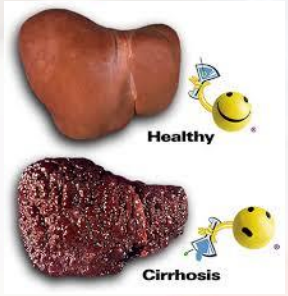
endometrióza, infertilita, cerviakálna rakovina, predčasný pôrod, dysmenorea, ťažké menštruačné krvácanie...

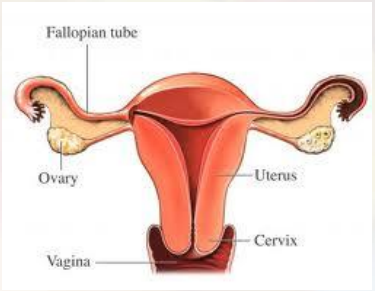
Kachexia



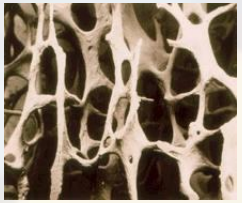
Ochorenia pečene

cirhóza, hepatokancerogenéza





Strata kostnej hmoty



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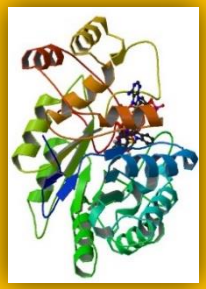
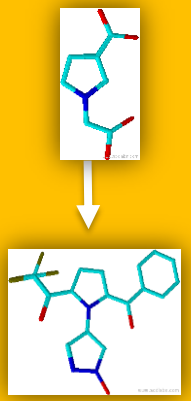
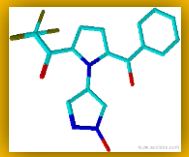


3. HIT to LEAD



4. Lead optimization

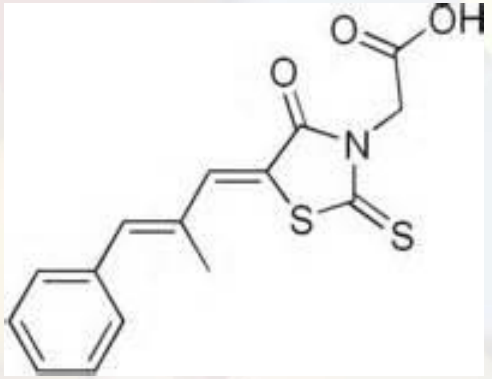


5. Predklinické testy

6. Klinické testy

7. Liek

Epalrestat



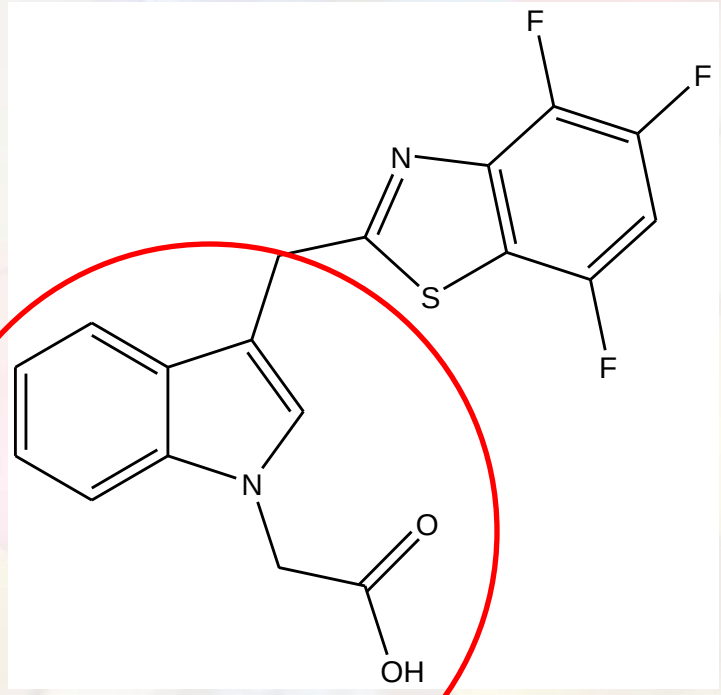
Efektívne znížil prevalenciu **diabetickej neuropatie**

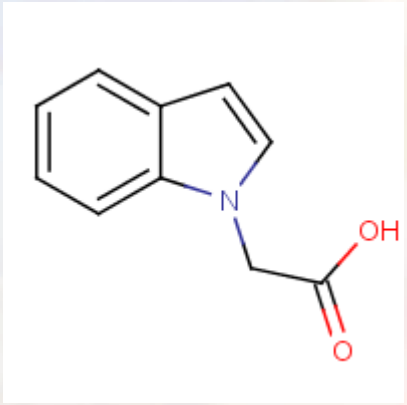
Svetový predaj epalrestatu v roku 2010 bol
154 miliónov USD

Vedľajšie účinky !

Lidorestat

Účinný inhibítor ALR2





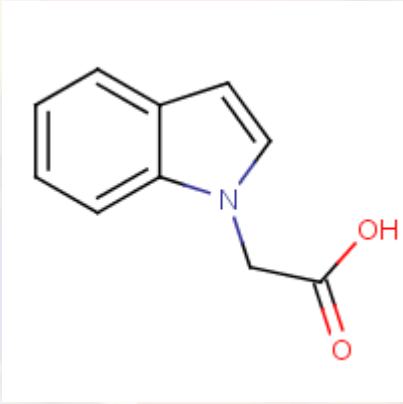
IC(50) ≈ 5 nM

IC(50) ≈ 7 µM

HIT

Hit to Lead

Hits



Typické parametre

MW << 300; IC(50) ~ 1 to 100 uM

Selektivita, biodostupnosť, famakokinetika nie sú dôležité

Lead

?

Typically

MW < 300; IC(50) ~ 1 to 100 nM, log P < 3

Selektivita, biodostupnosť a farmakokinetika dôležité pre pedklinické štúdie *in vivo*

Hit to Lead

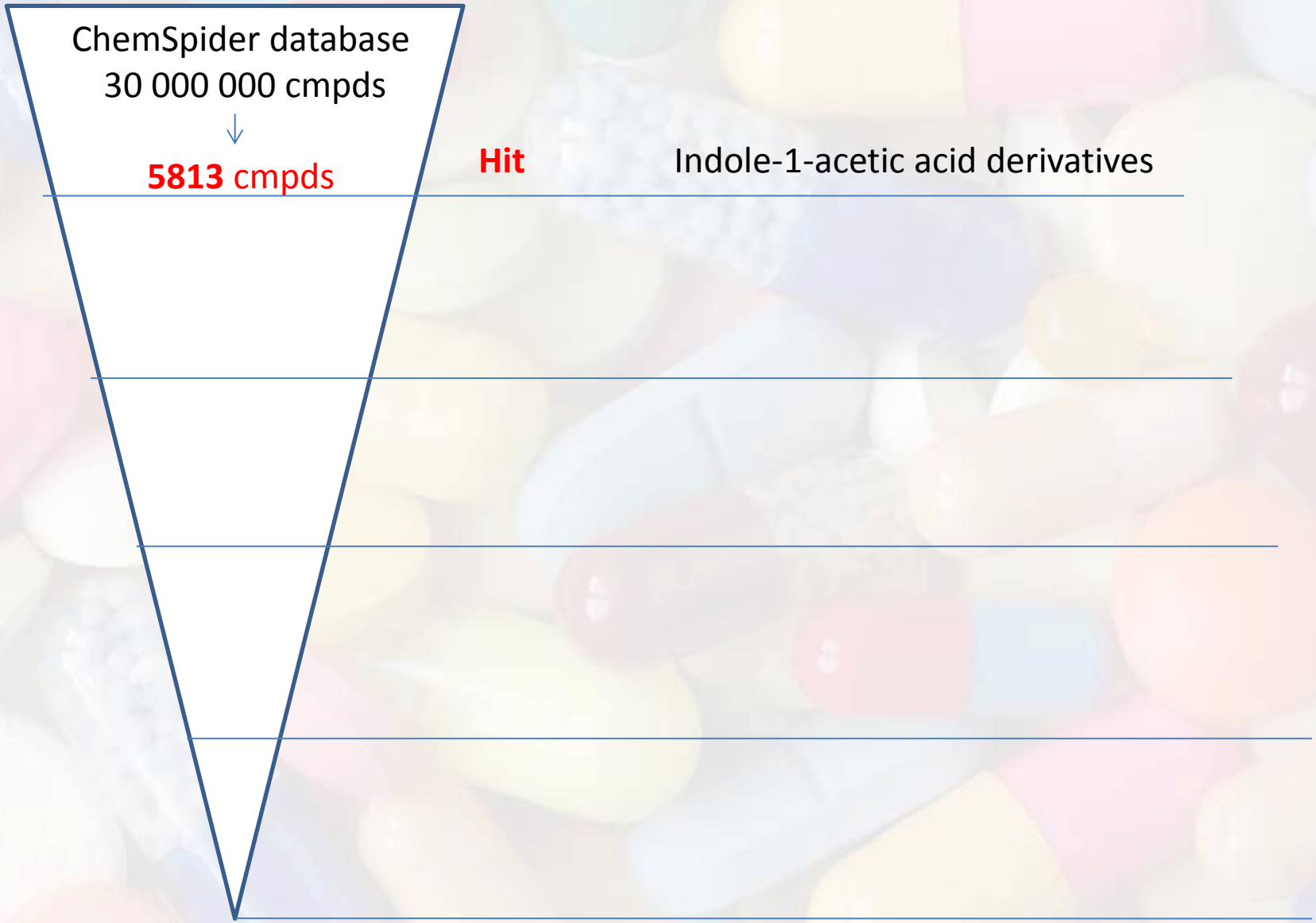
ChemSpider Database
30 000 000 cmpds

5813 derivátov 1-IAA

ChemSpider - databáza chemikálii
- majiteľ: Royal Society of Chemistry
- viac ako 30 miliónov molekúl z viac ako 450 databáz

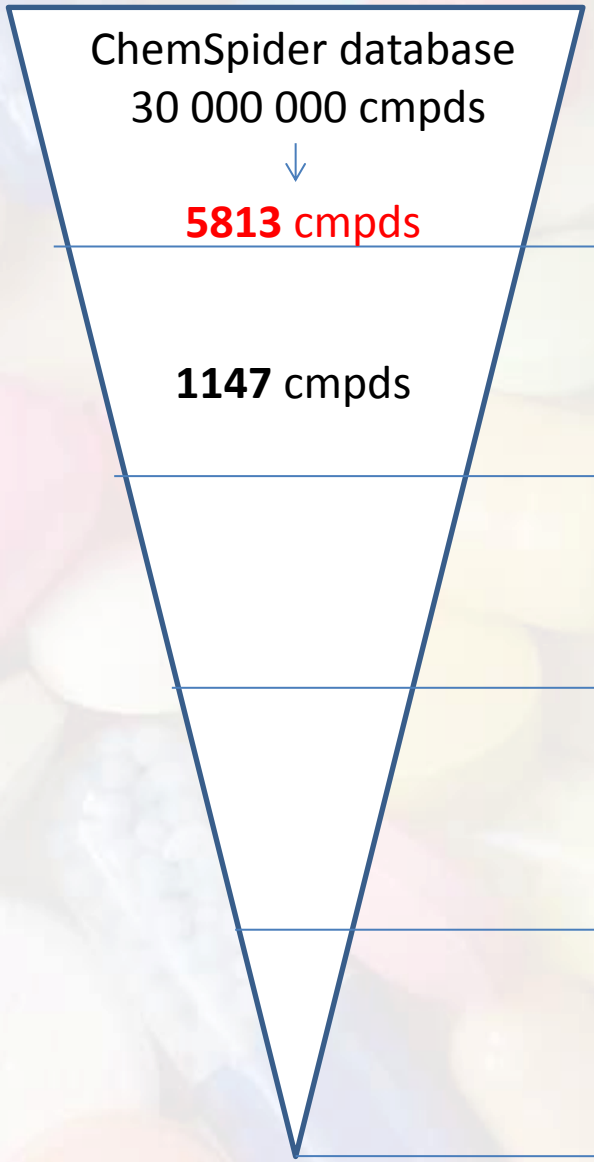
Hit to Lead

Virtuálny screening



Hit to Lead

Virtual screening: Drug likeness



Hit Indole-1-acetic acid derivatives

Drug likeness criteria:

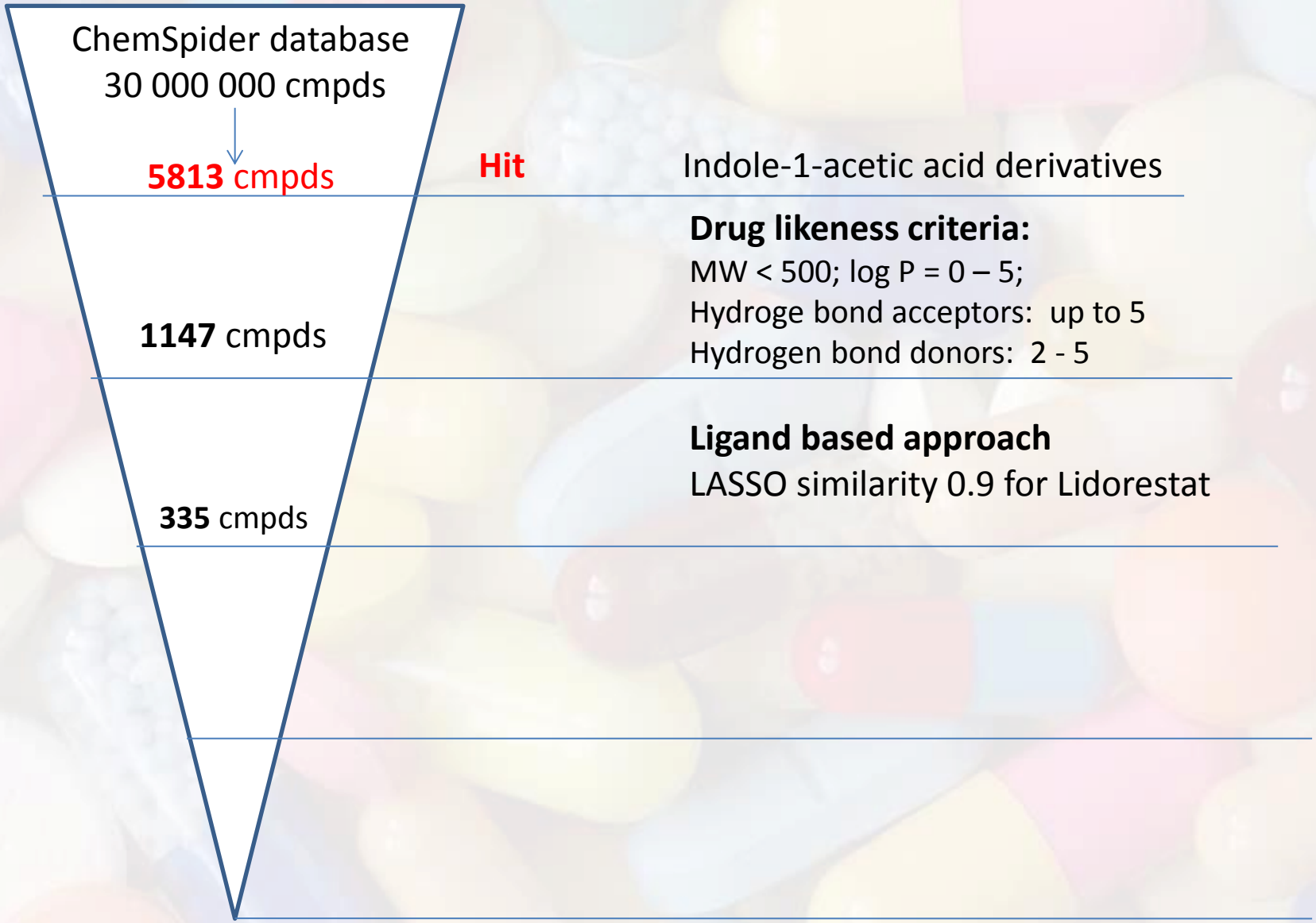
MW < 500; log P = 0 – 5;

Hydroge bond acceptors: up to 5

Hydrogen bond donors: 2 - 5

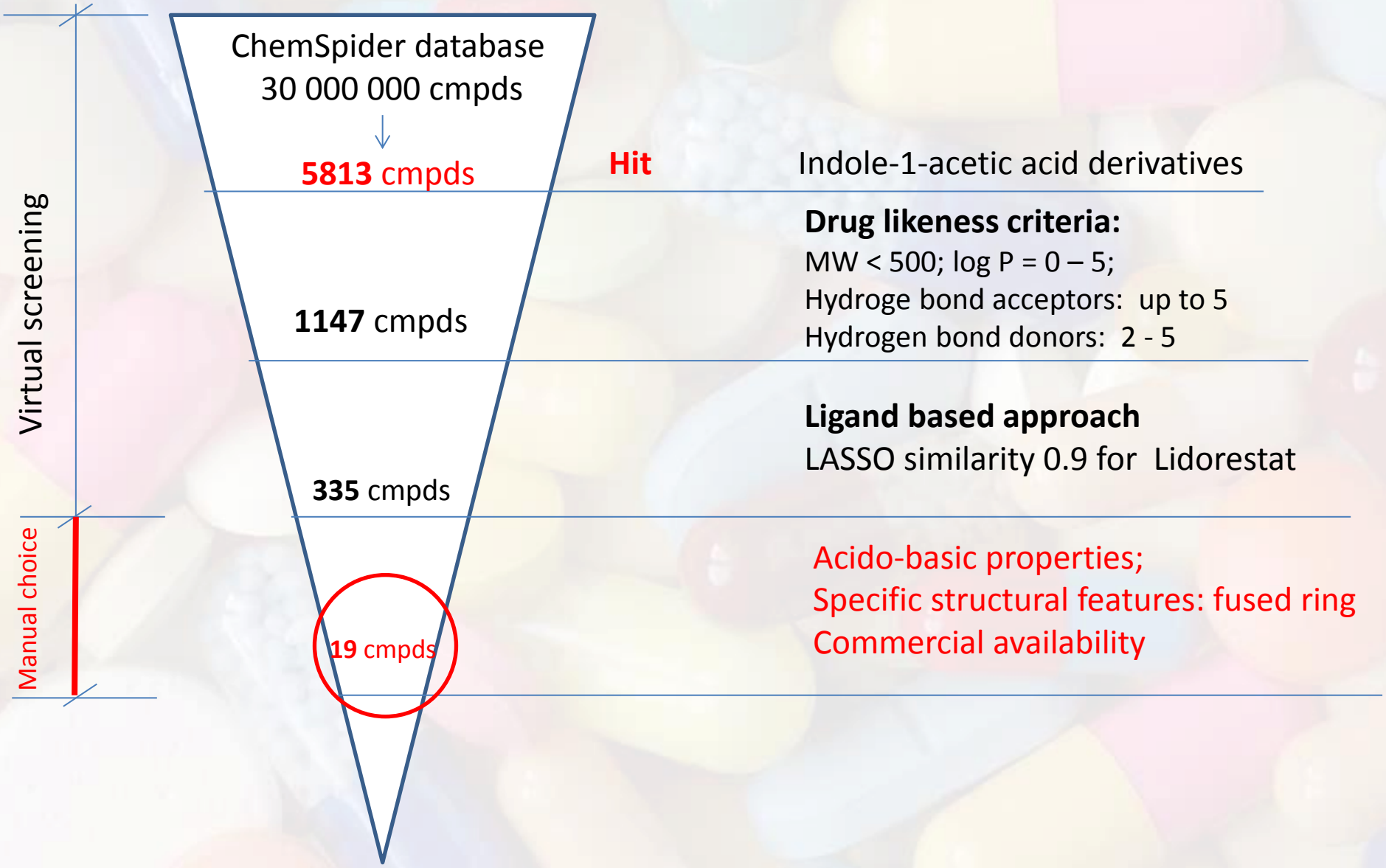
Hit to Lead

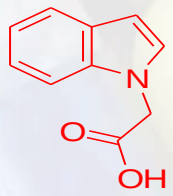
Virtual screening: Ligand-based strategy



Hit to Lead

Manual choice





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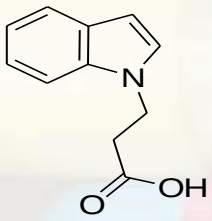

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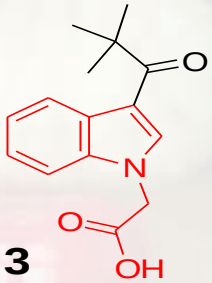

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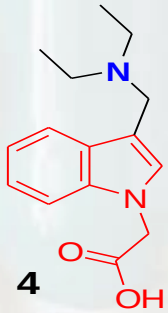

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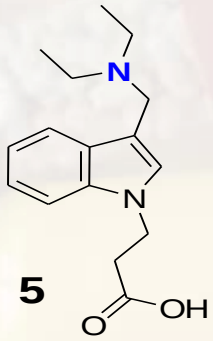

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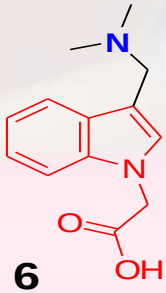

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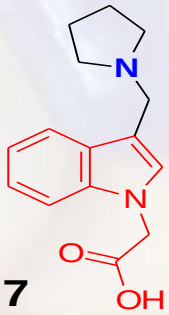

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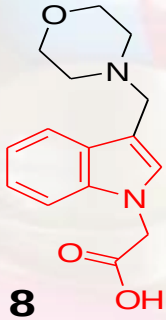

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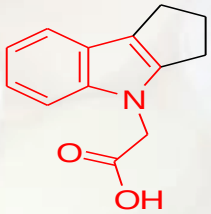

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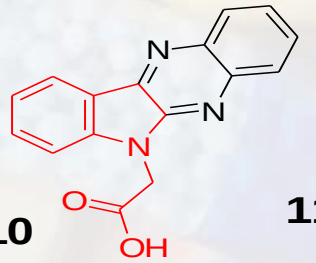

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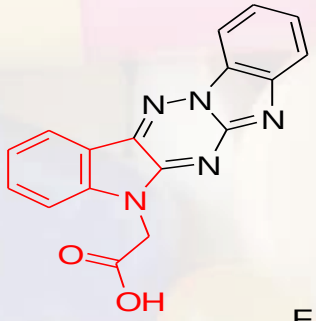

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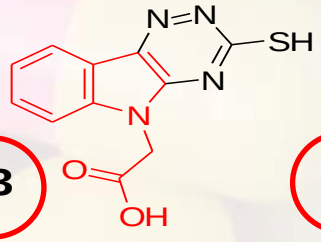

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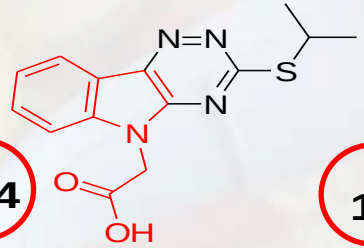

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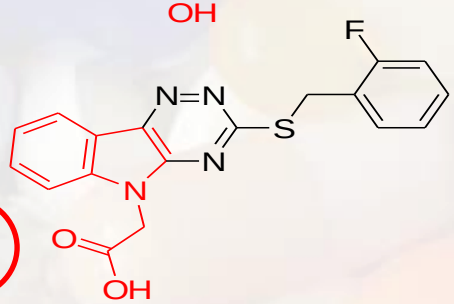

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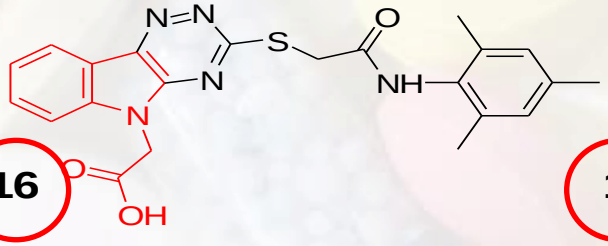

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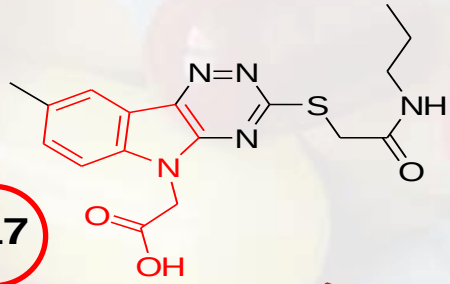

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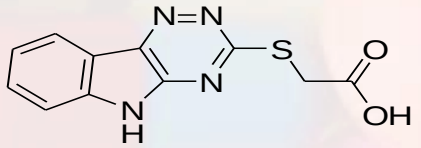

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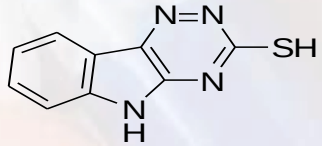

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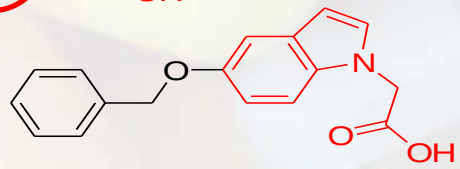
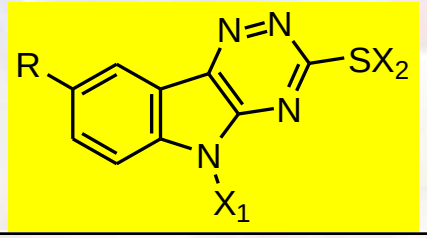
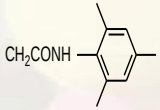


20

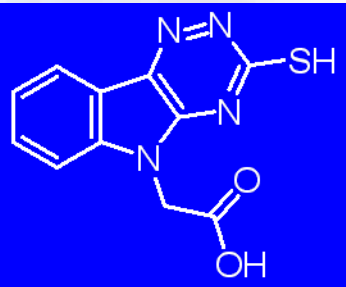
Hit to lead

Identifikácia „lead“ štruktúry



Compound	R	X_1	X_2
13	H	CH_2COOH	H
14	H	CH_2COOH	$CH(CH_3)_2$
15	H	CH_2COOH	
16	H	CH_2COOH	
17	CH_3	CH_2COOH	$CH_2CONHCH_2CH_2CH_3$

CMTI



LEAD

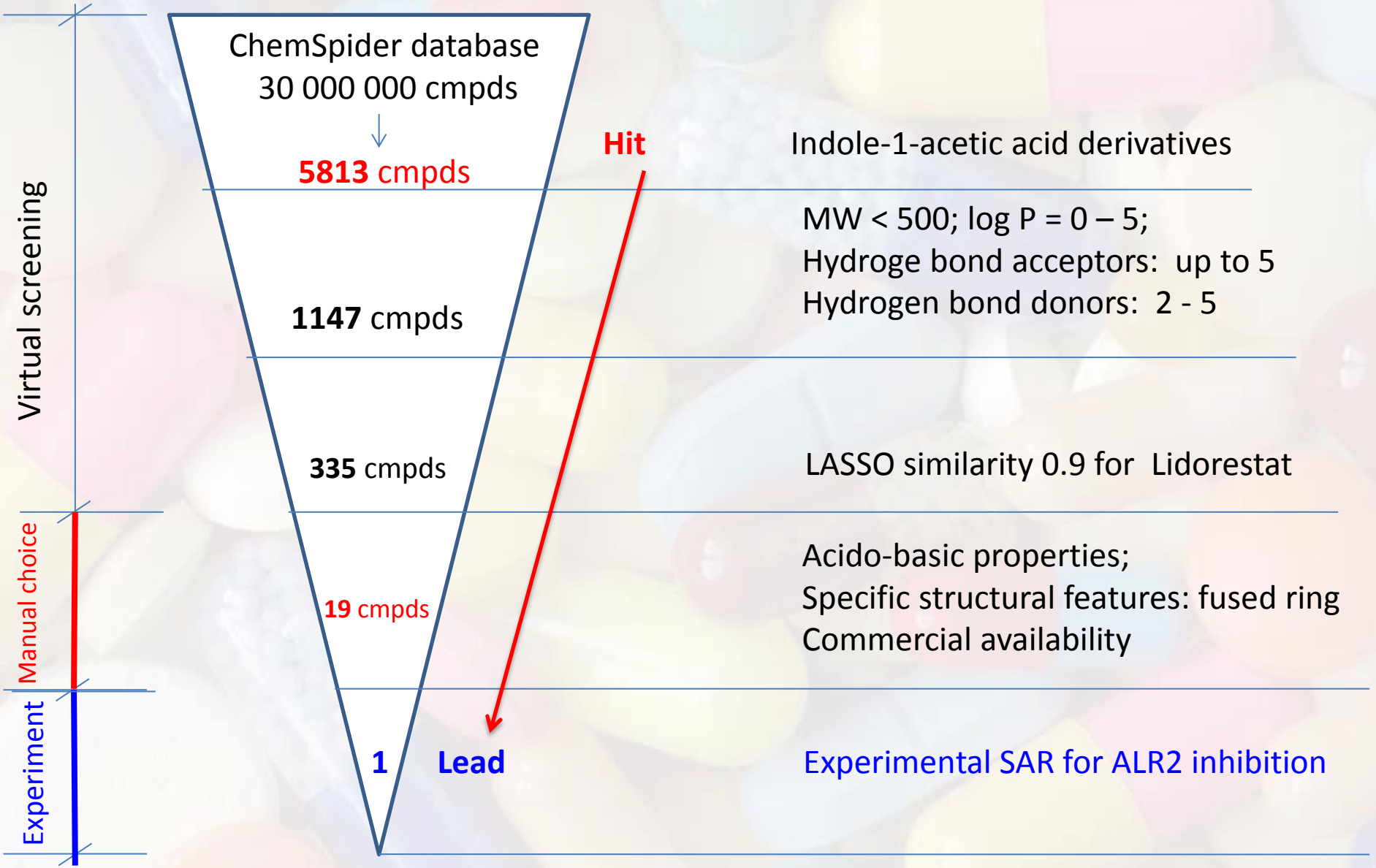
Rat ALR2 : 97 nM

Human AKR1B1 : 57 nM

Rat ALR1 : 40 550 nM

Human AKR1B10 : 21 400 nM

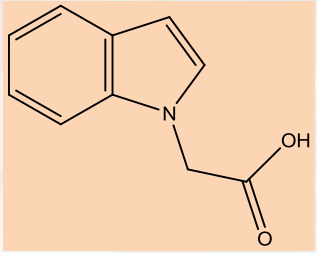
Hit to Lead


Virtual screening
Manual choice
Experiment
ChemSpider database
30 000 000 cmpds
5813 cmpds
1147 cmpds
335 cmpds
19 cmpds
1
Lead
Hit
Indole-1-acetic acid derivatives
MW < 500; log P = 0 – 5;
Hydroge bond acceptors: up to 5
Hydrogen bond donors: 2 - 5
LASSO similarity 0.9 for Lidorestat
Acido-basic properties;
Specific structural features: fused ring
Commercial availability
Experimental SAR for ALR2 inhibition

Hits and Lead

ALR2 inhibition

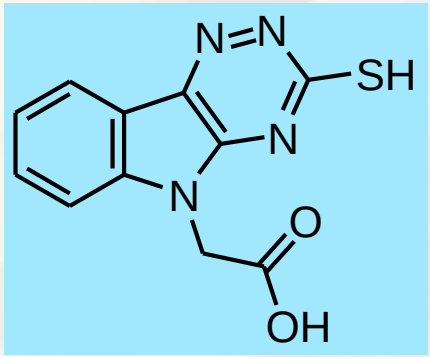
Hit



1-IAA

MW = 175; IC(50) = 7 uM

Lead



CMTI

MW = 260; IC(50) ~ 97 nM, log P = 1,7

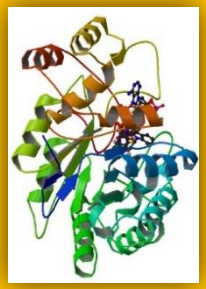
Selectivity factor relative to ALR1 > 400

Bioavailability : readily taken up by RBCs and isolated eye lenses

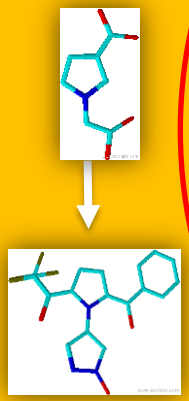
Vývoj liečiv – jednotlivé kroky

1.Identifikácia ochorenia

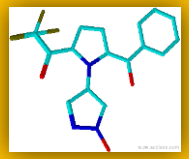
2. Identifikácia cieľa



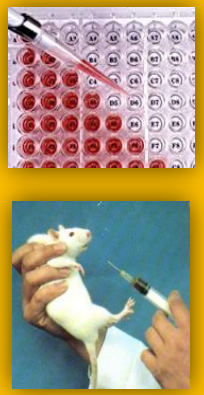
3. HIT to LEAD



4. Lead optimization



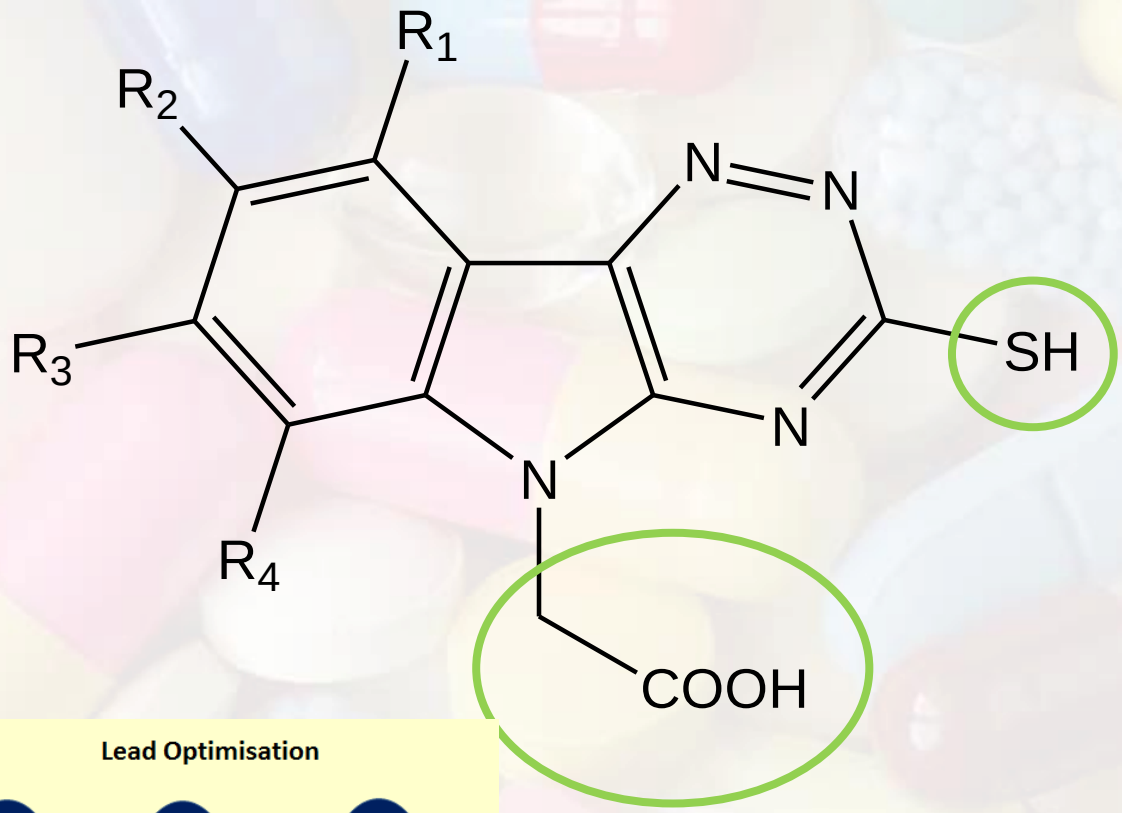
5. Predklinické testy



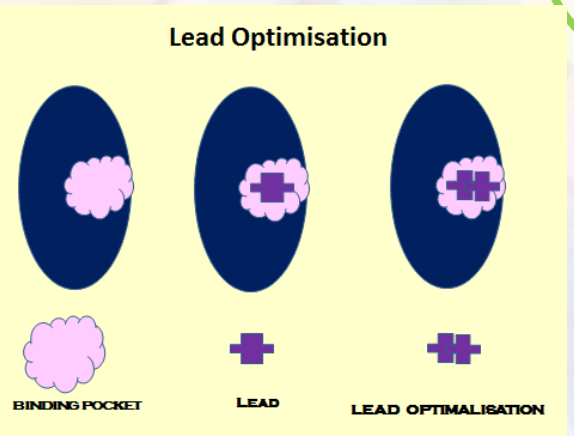
6. Klinické testy

7. Liek

Lead optimalisation



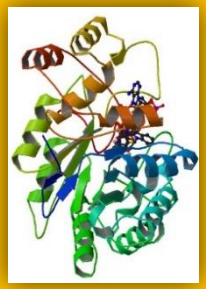
- ↓ toxicita
- ↑ biodostupnosť
- ↑ Inhibičná aktivita
- ↑ selektivita



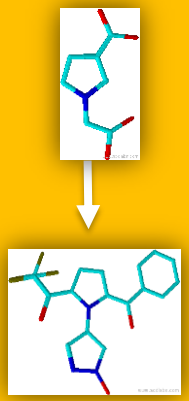
Vývoj liečiv – jednotlivé kroky

1.Identifikácia ochorenia

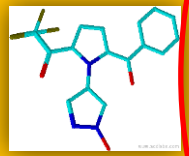
2. Identifikácia cieľa



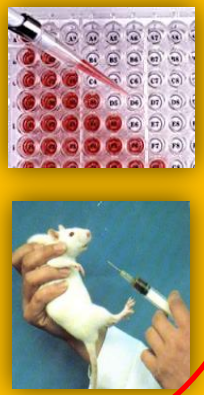
3. HIT to LEAD



4. Lead optimization



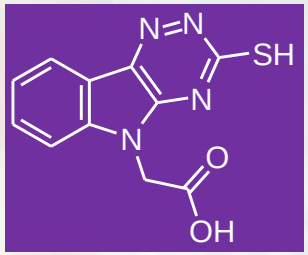
5. Predklinické testy



6. Klinické testy

7. Liek

Inhibícia aldózareduktázy



Stefek M. et al. EU Patent Application: PCT SK 2014/000021

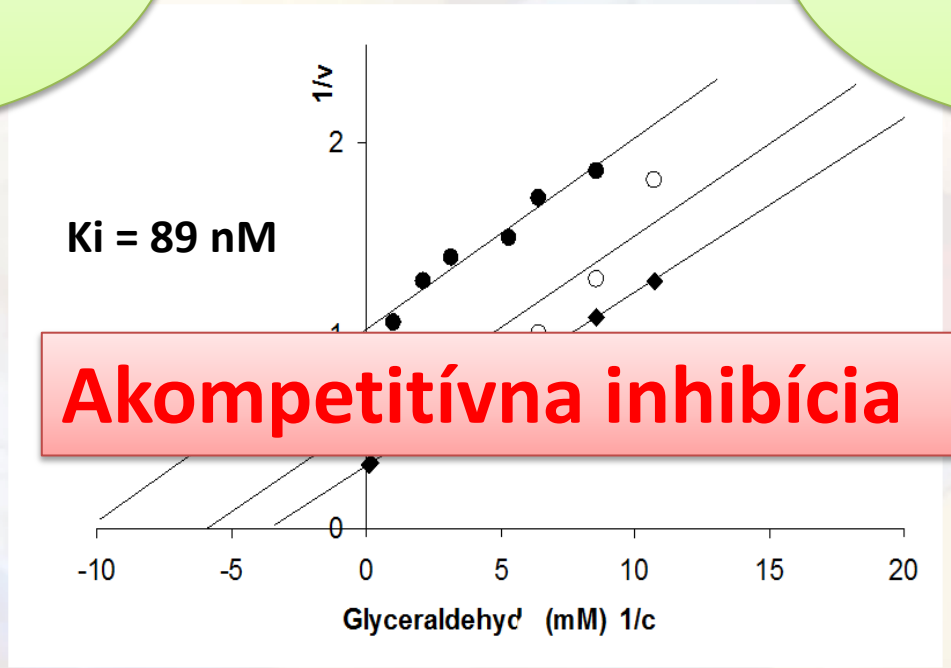
Humánny AKR1B1

IC(50)≈ 57 nM

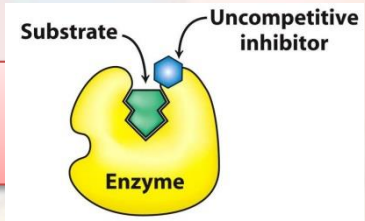
Potkaní ALR2

IC(50)≈ 97 nM

Ki = 89 nM

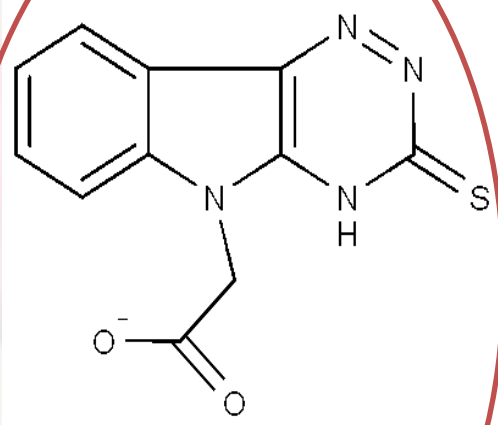
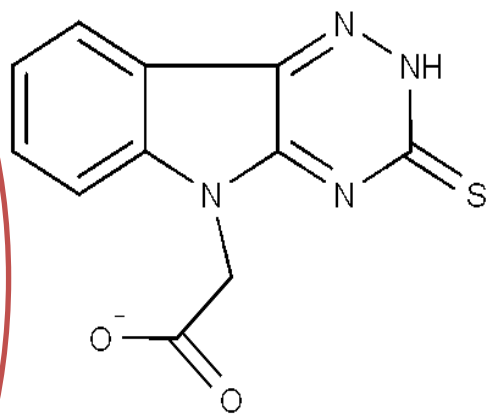
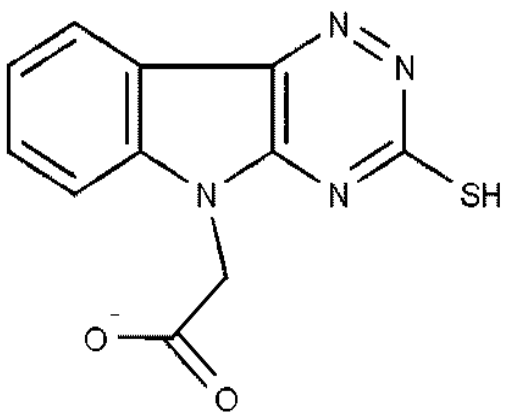


Akompetitívna inhibícia



Stefek et al., J.Med.Chem., 2015

Tautomérna analýza

1	2	3
		
13-1	13-2	13-3
98%	~ 1%	< 1%

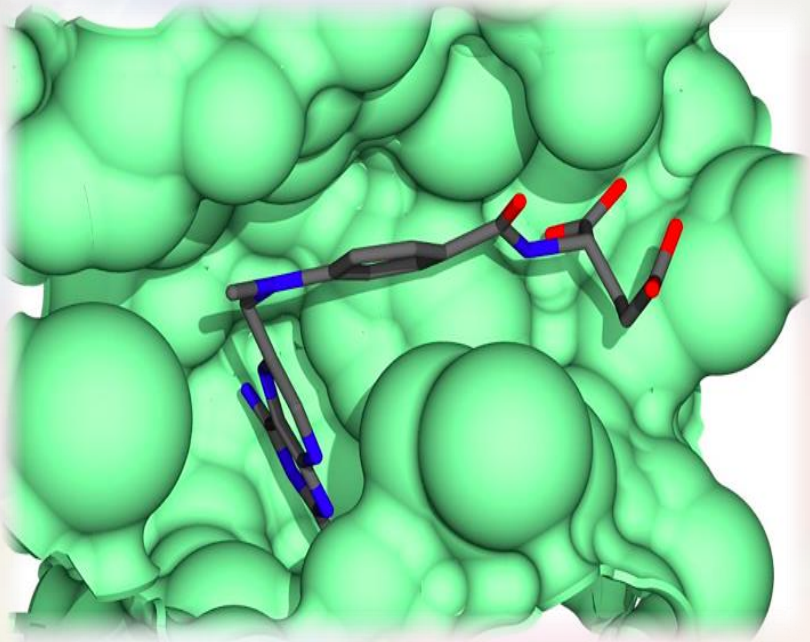
Stefek et al., J.Med.Chem., 2015

Selektivita

Hľadanie energeticky najvýhodnejšieho objatia,

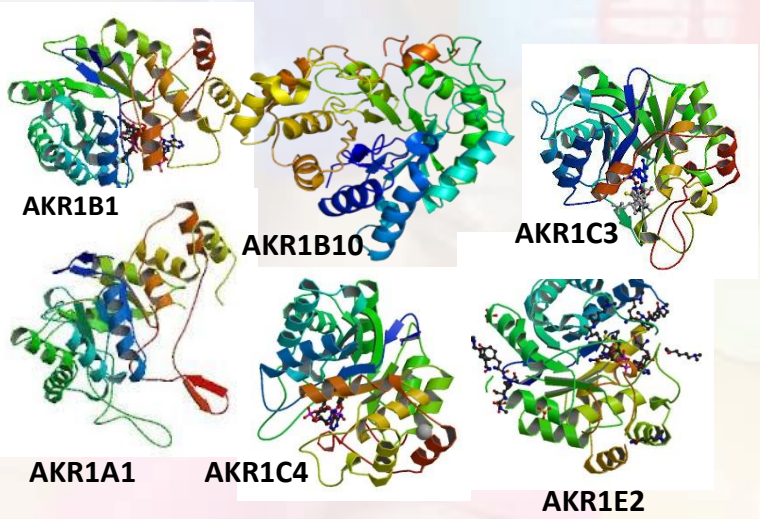
ktoré lieči

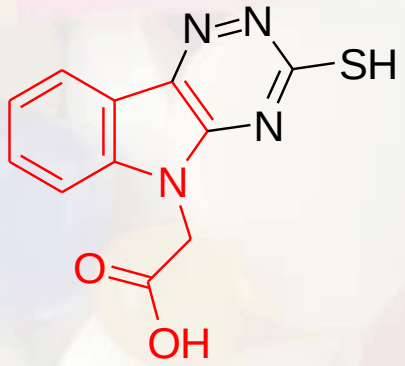
E_i (IC50)= ?



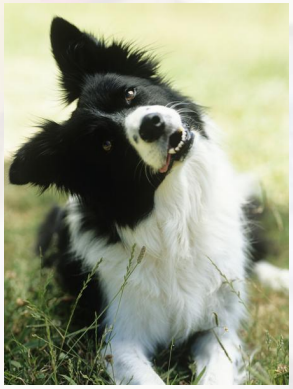
Selektivita interakcie

Aldoe-keto reductase family



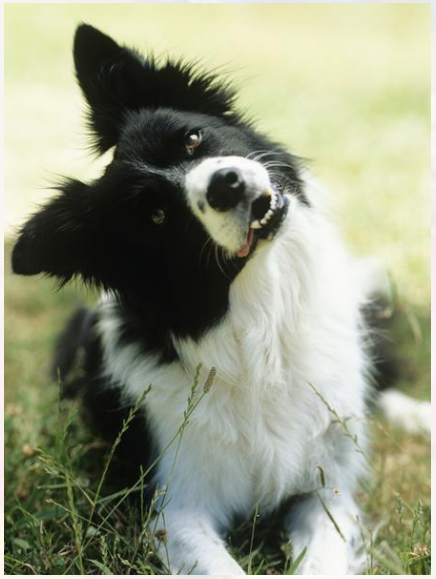


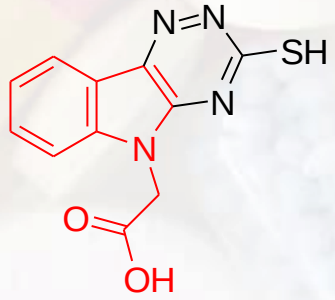
CMTI



Border collie family

Selectivity vs. Side efect

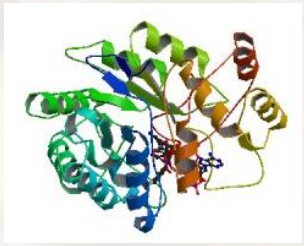




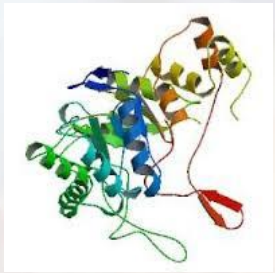
$IC_{50} \approx 0.057\ \mu M$

$IC_{50} \approx 40.55\ \mu M$

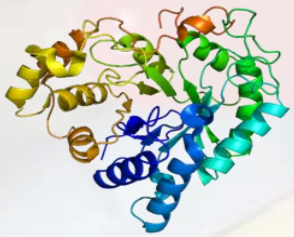
$IC_{50} \approx 27.37\ \mu M$



AKR1B1



AKR1A1



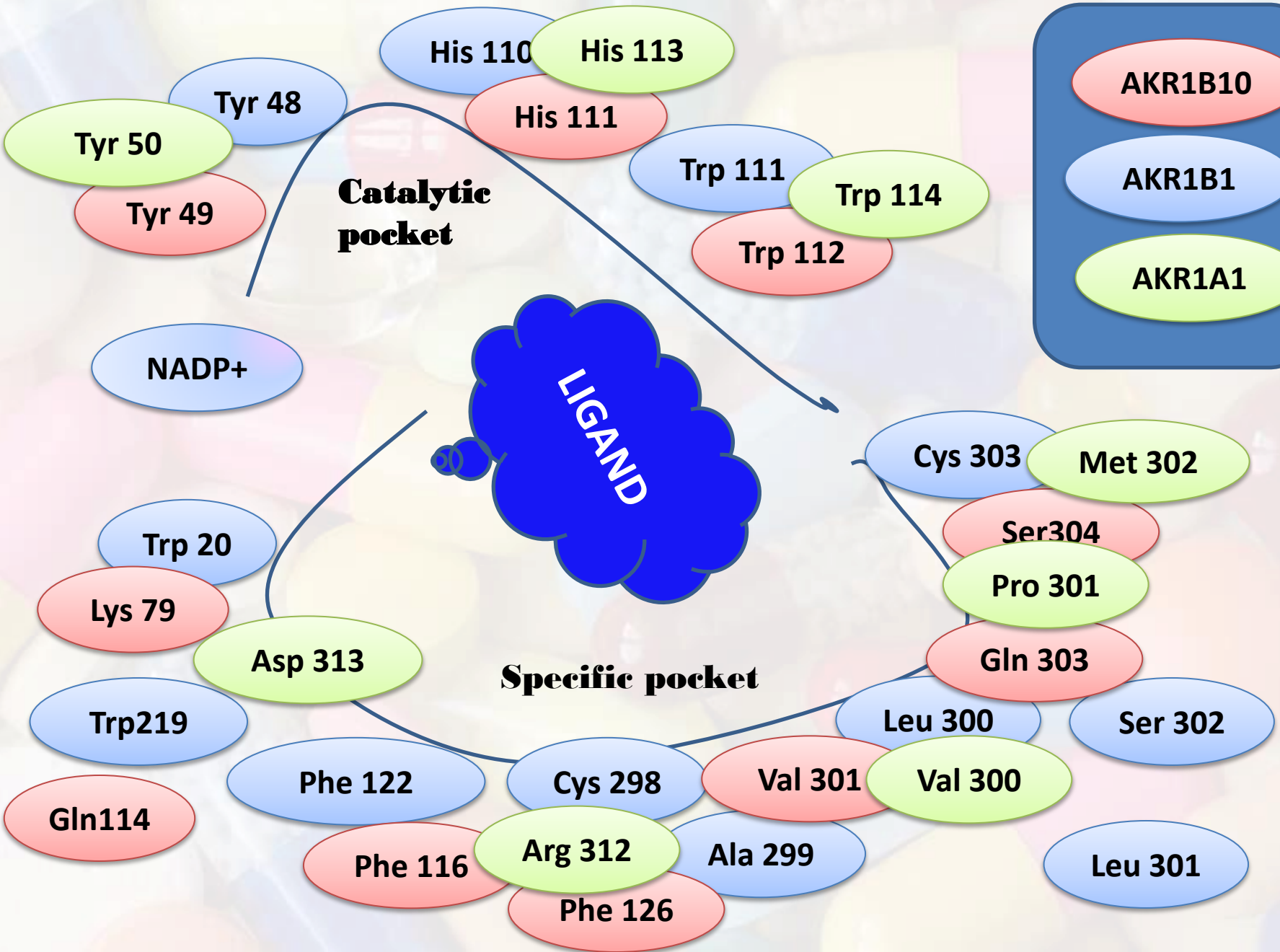
AKR1B10

Eshly

Engie

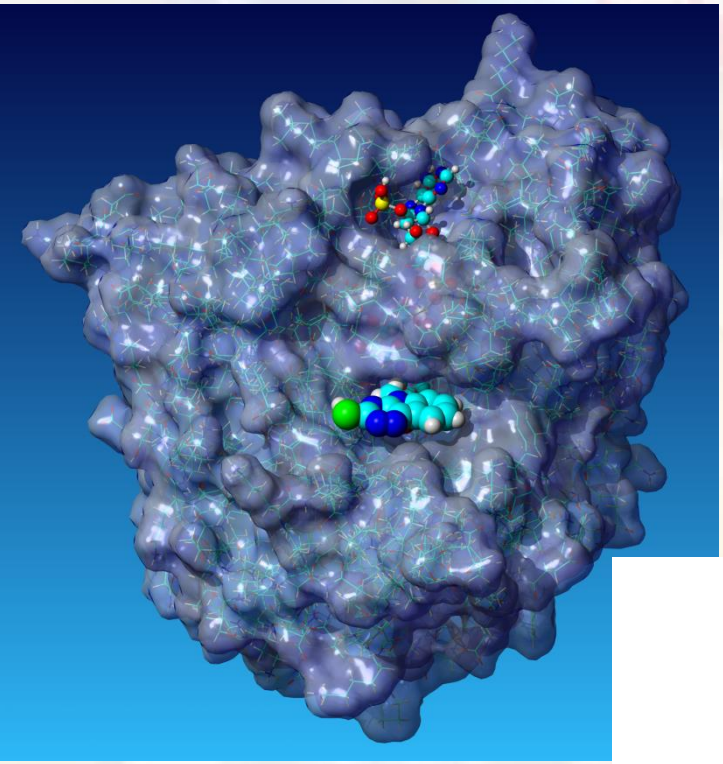
Eliee

Väzbové vrecko AKR1´s

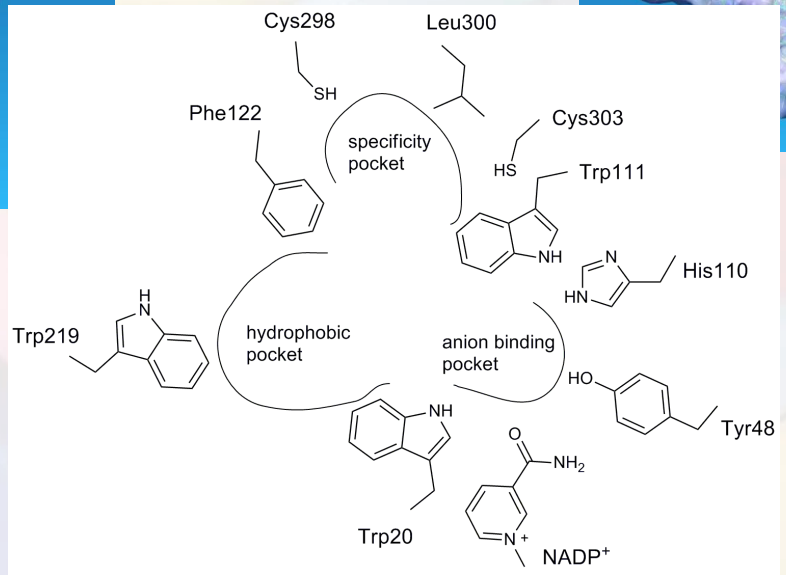


AKR1B1 surface

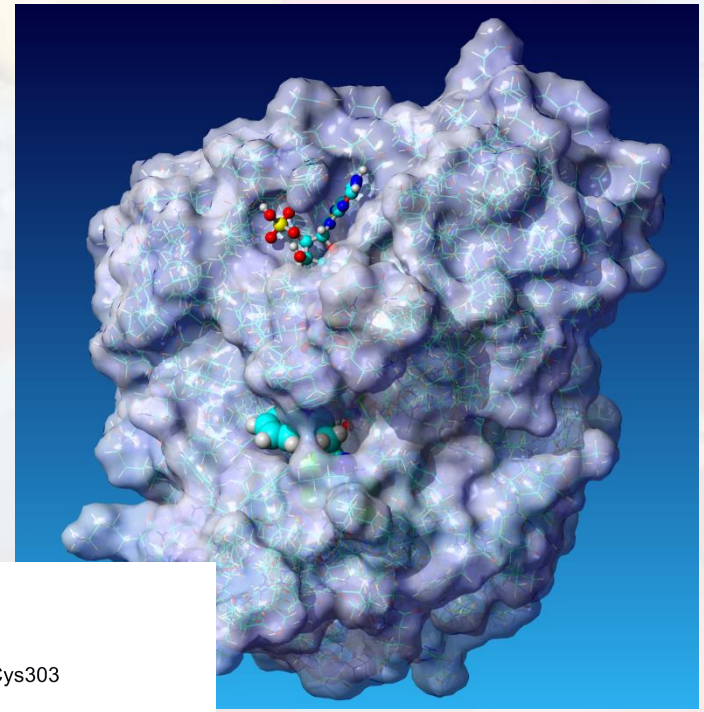
CMTI



$IC_{50} \approx 57$ nM



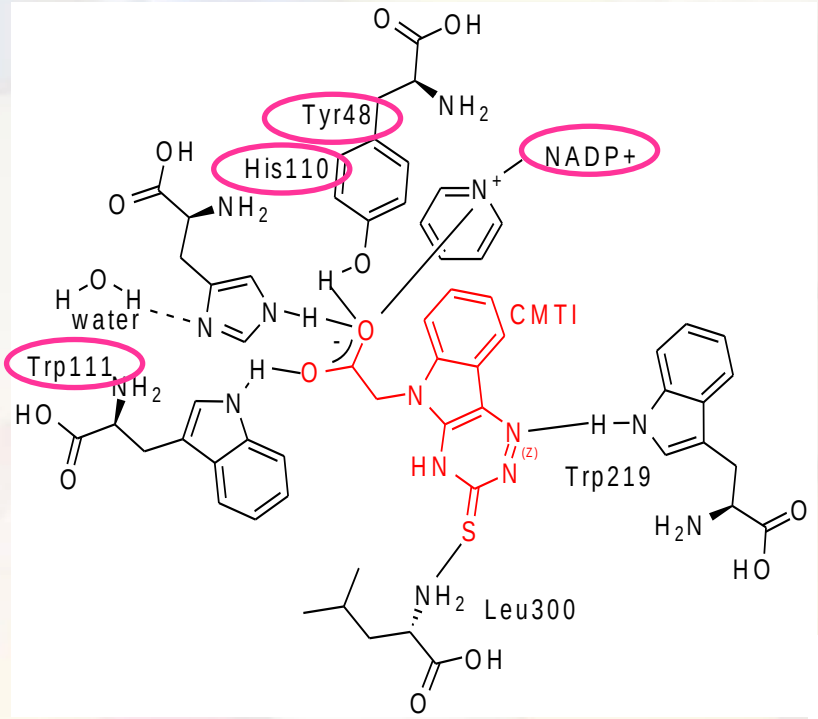
Lidorestat



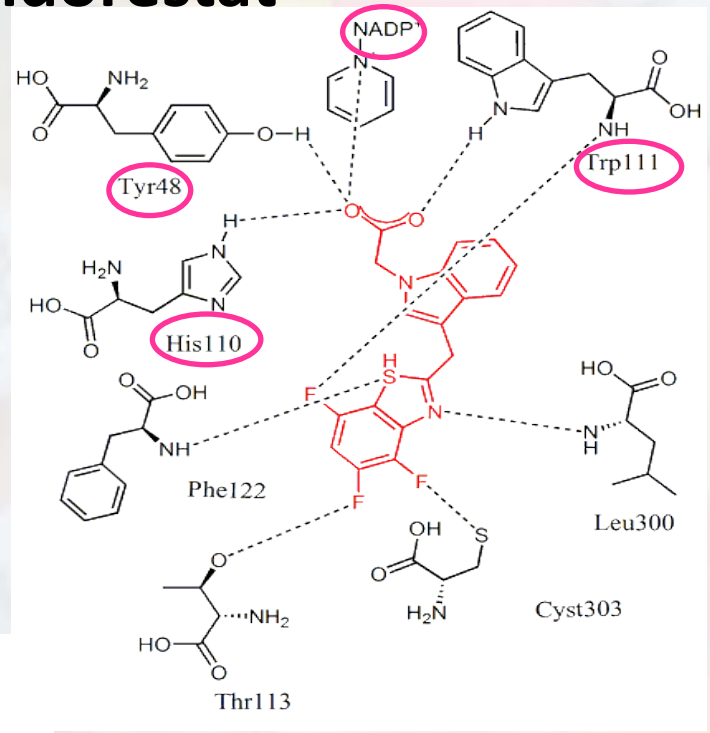
$IC_{50} \approx 5$ nM

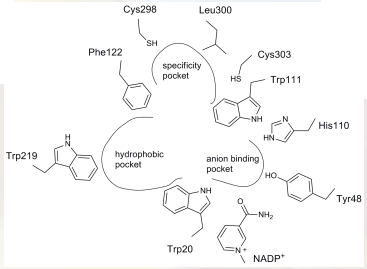
Väzbové vrecko AKR1B1

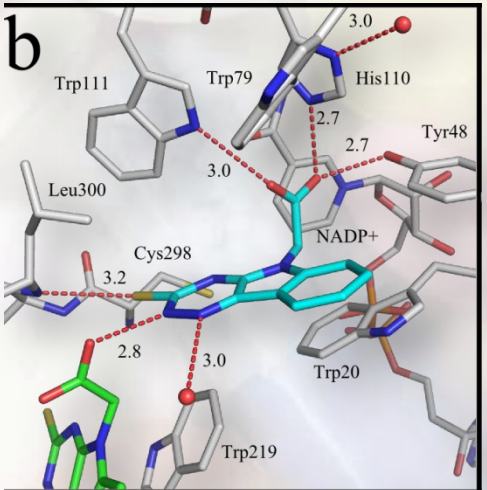
CMTI

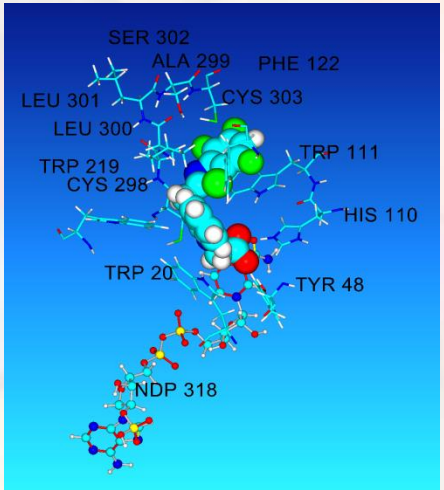


Lidorestat



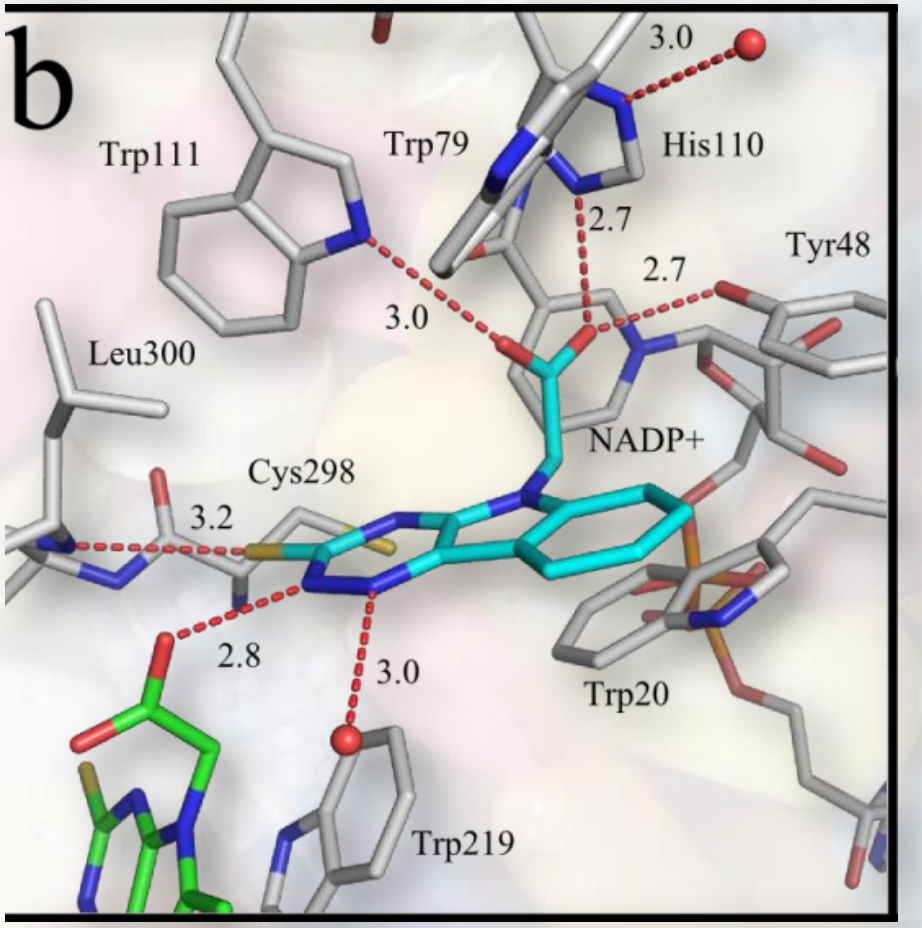






Kryštálová štruktúra

PDB:4QX4



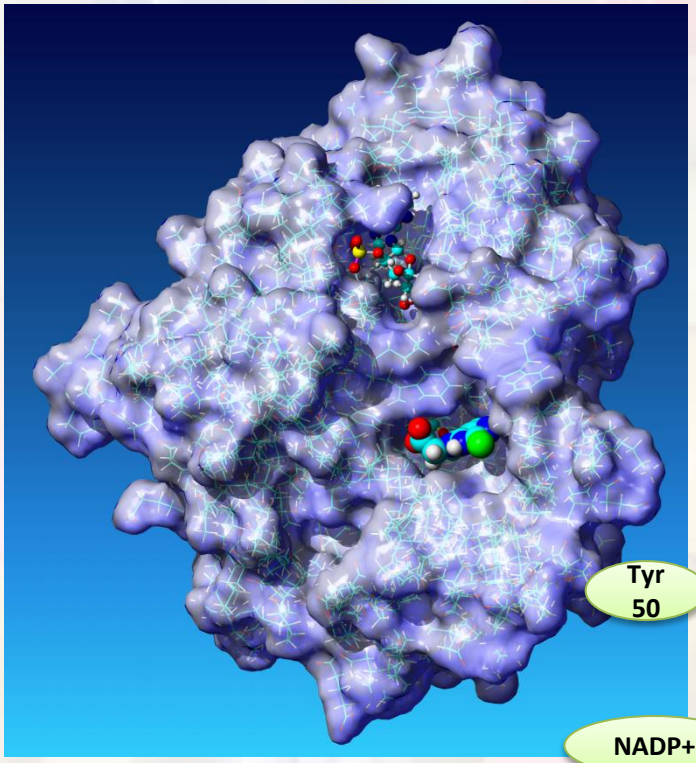
Chris Rechlin

Heine Andreas

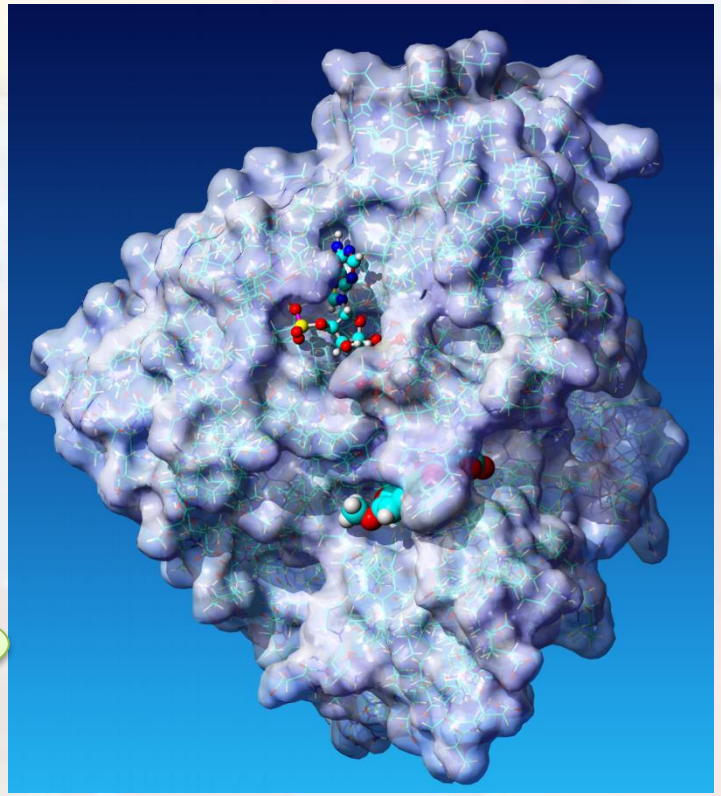
Klebe Gerhard

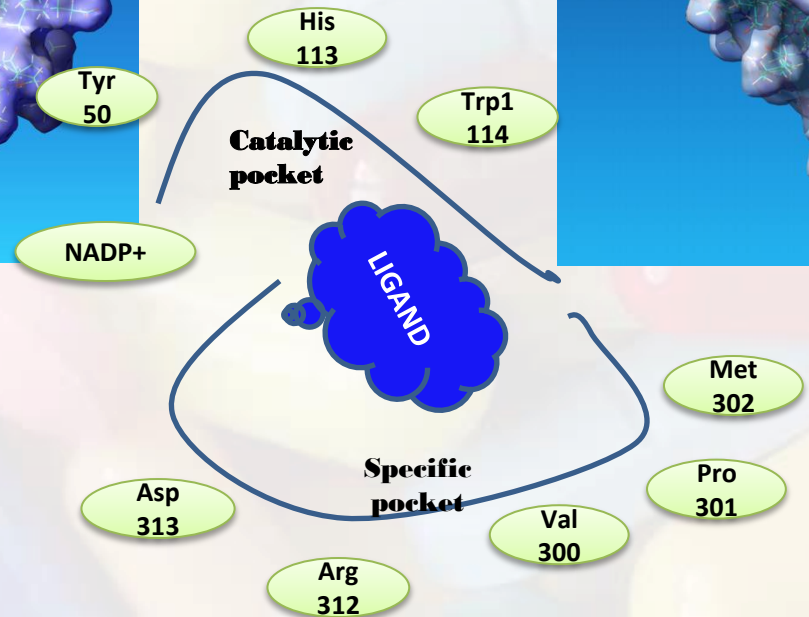
AKR1A1 surface

CMTI



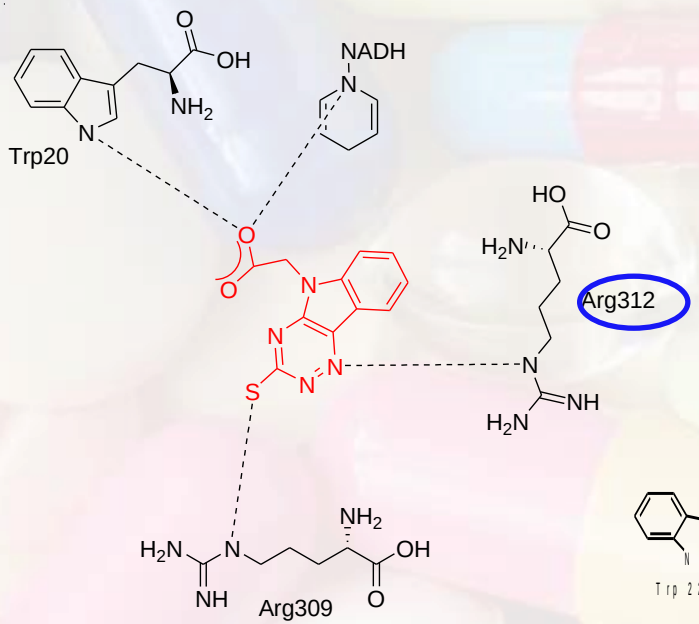
[(5Z)-5-{[3-(CARBOXYMETHOXY)-4-METHOXYPHENYL]METHYLIDENE}- 2,4-DIOXO-1,3-THIAZOLIDIN-3-YL]ACETIC ACID



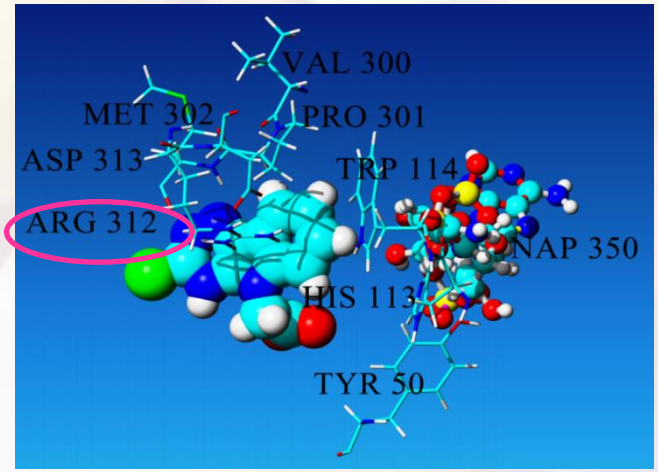


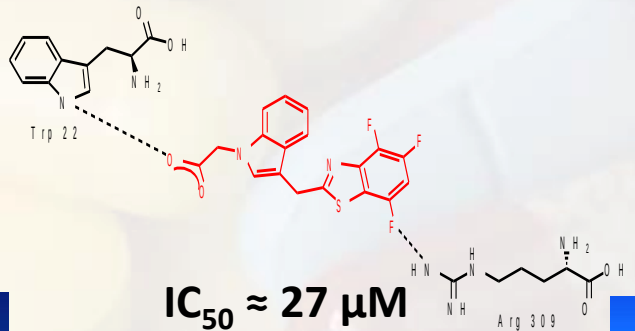
AKR1A1 BINDING POCKET

CMTI



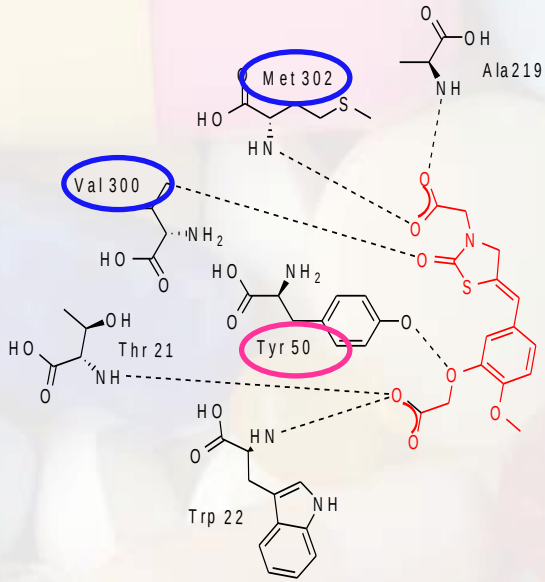
IC_{50} ≈ 40.55 µM



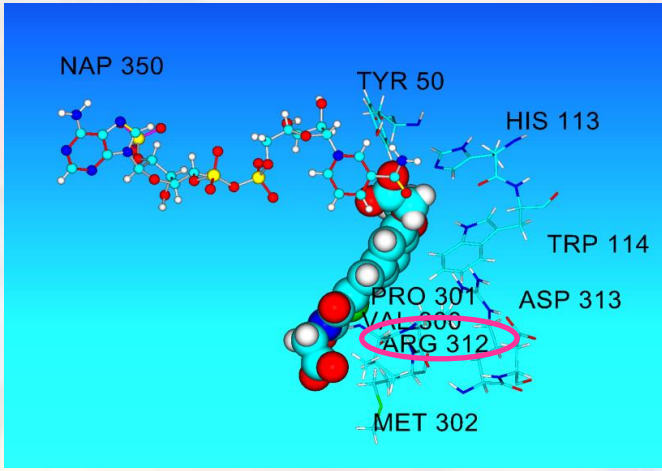


IC_{50} ≈ 27 µM

[(5Z)-5-{[3-(CARBOXYMETHOXY)-4-METHOXYPHENYL]METHYLIDENE}- 2,4-DIOXO-1,3-THIAZOLIDIN-3-YL]ACETIC ACID

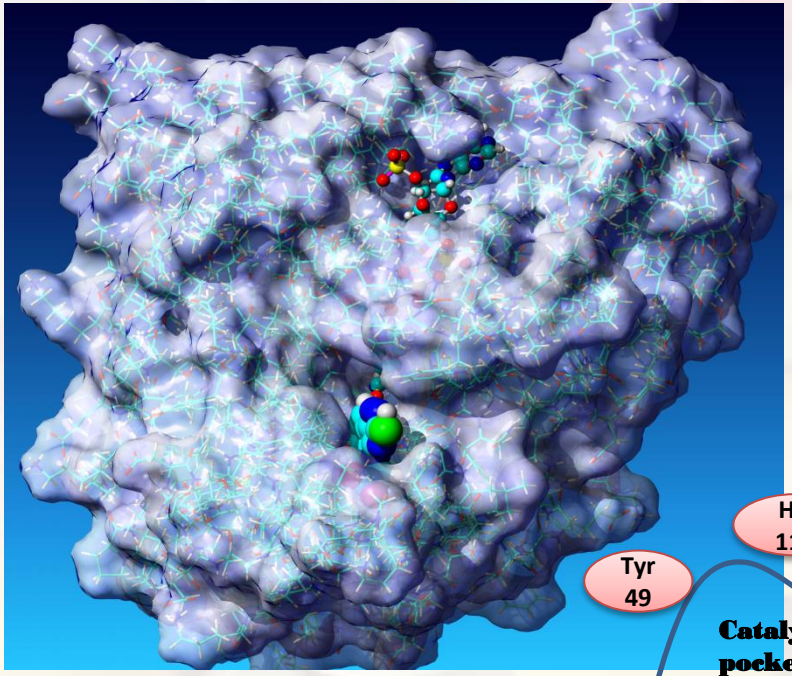


IC_{50} ≈ 5.4 µM

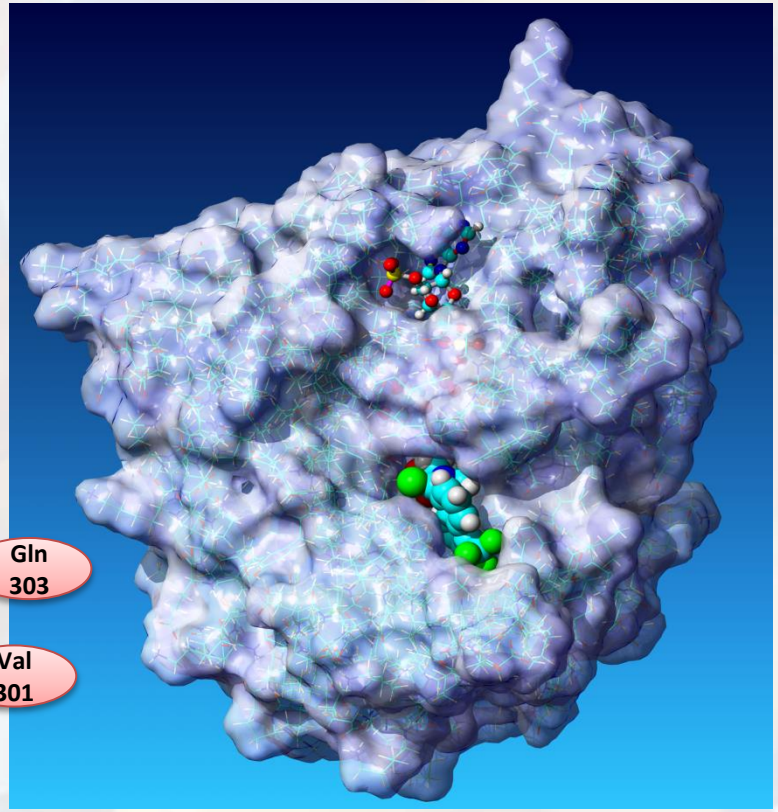


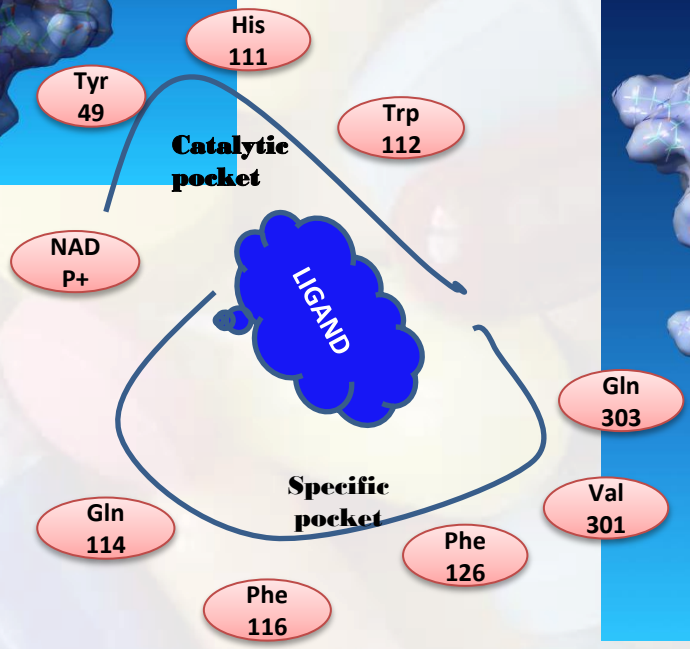
AKR1B10 surface

CMTI



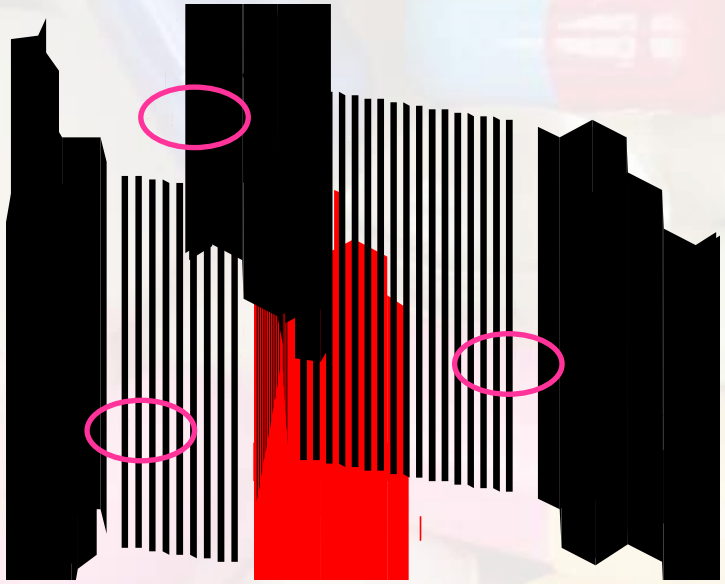
TOLRESTAT



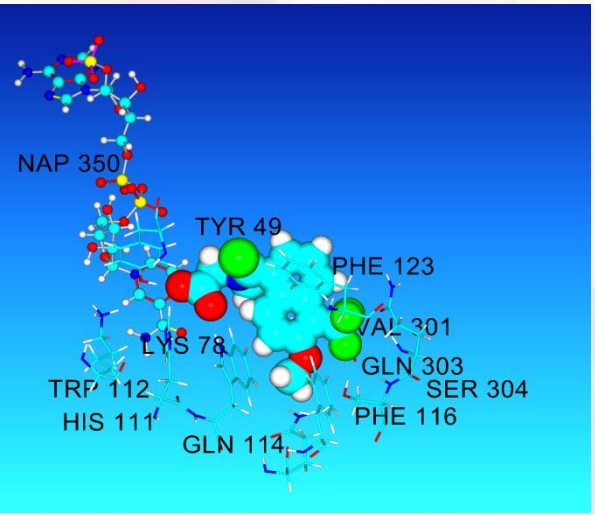


Binding pocket of AKR1B10

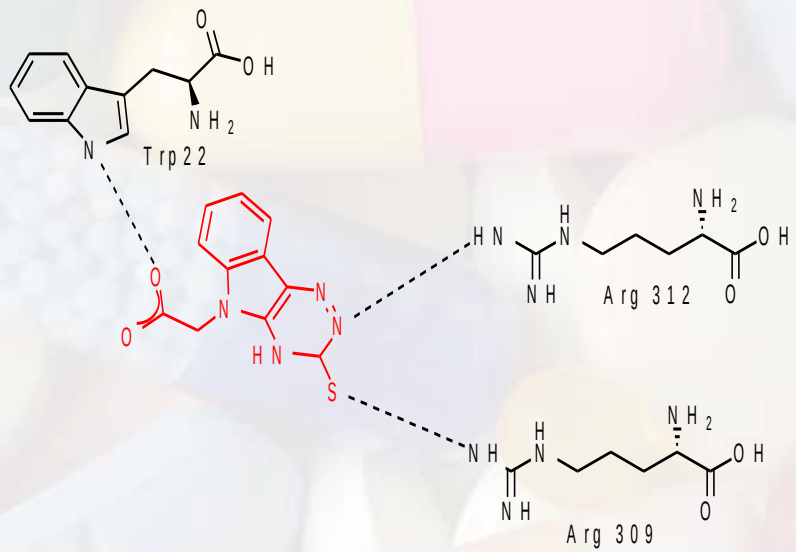
TOLRESTAT



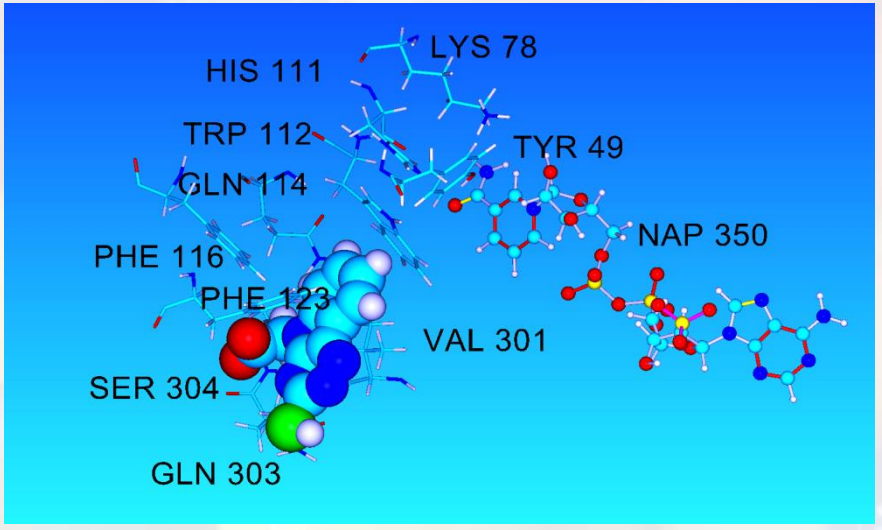
IC_{50} ≈ 0.054 µM



CMTI

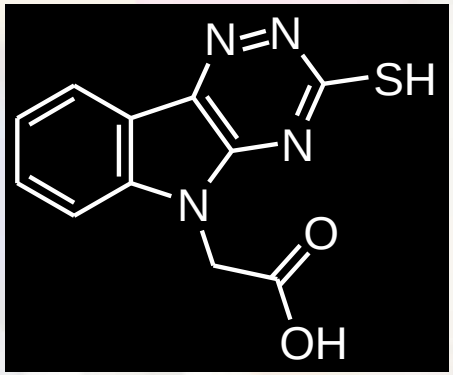


IC_{50} ≈ 21.37 µM



Látka 13 (CMTI)

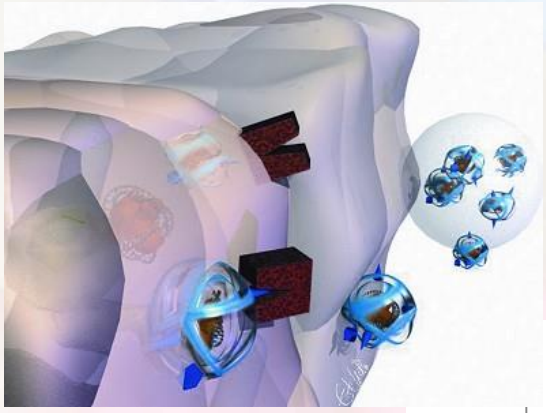
Fyzikálno-chemické vlastnosti

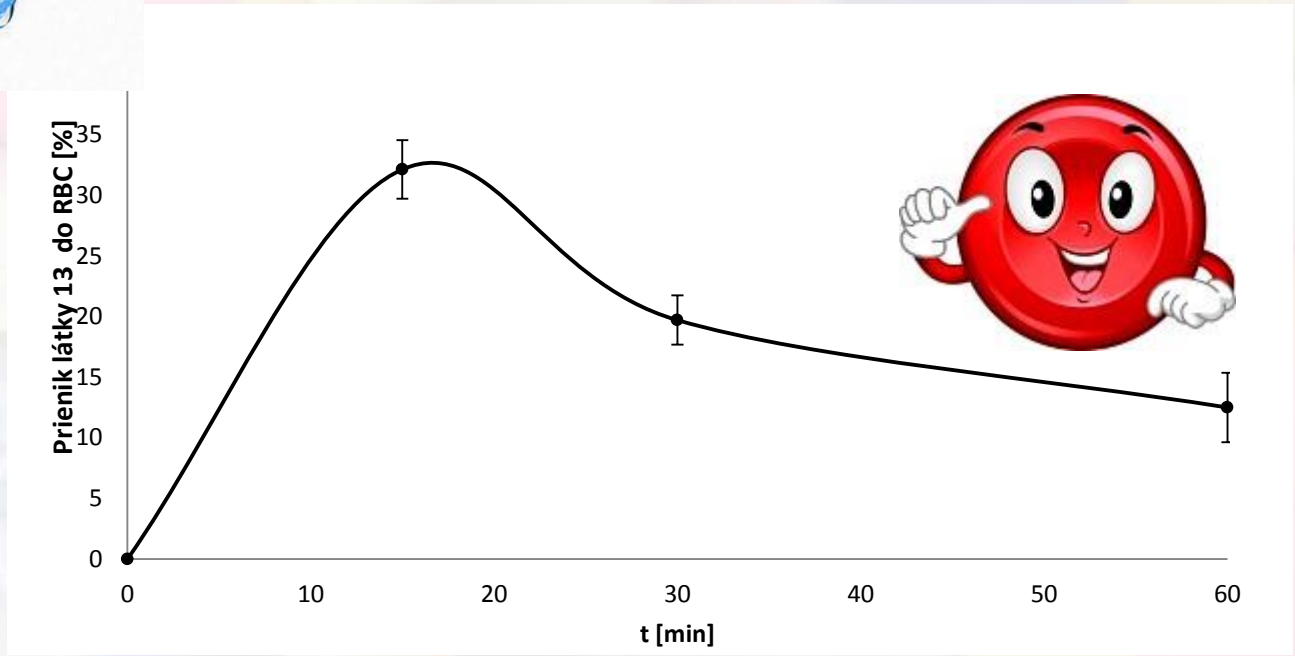


- MW: 260.28
- Log P: 1.68
- Log $D_{7.4}$: -2.03
- (Log $D_{7.4}$)exp : -1.90 ± 0.03
- Rozpustnosť vo vode: >10 mmol/l

Stefek et al., J.Med.Chem., 2015

Prienik CMTI do izolovaných erytrocytov potkana



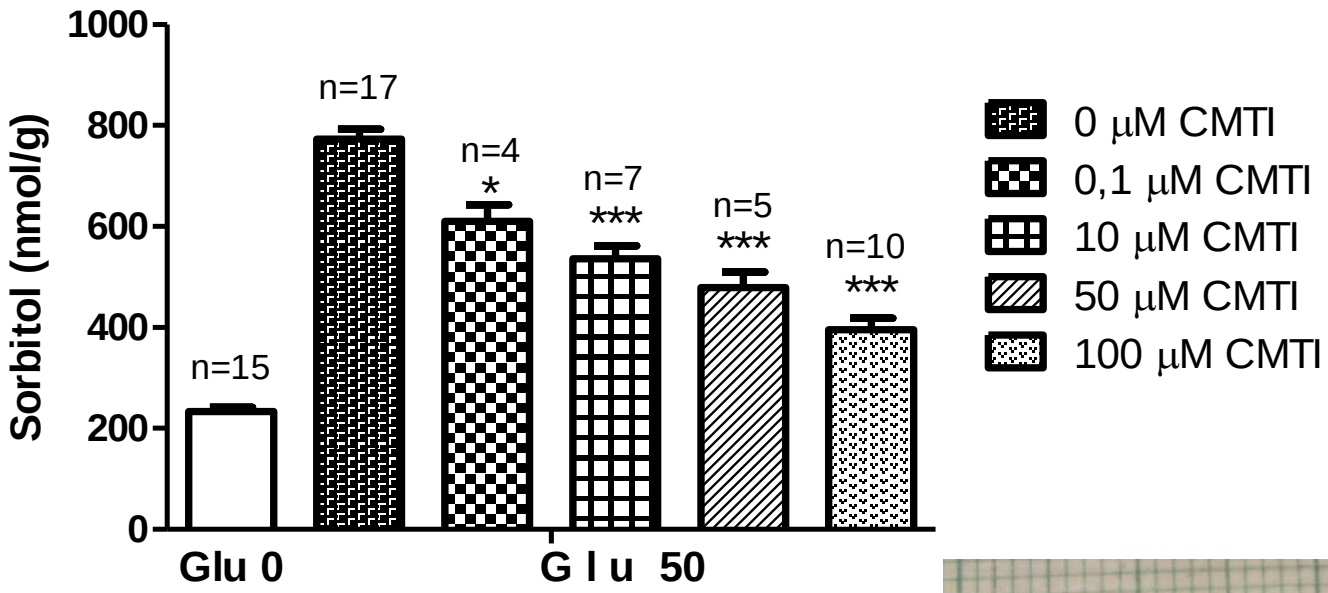


1 mM CMTI, hematokrit 20%

Soltesova Prnova et al., Redox.Report., 2015

Inhibícia polyolovej dráhy ex vivo

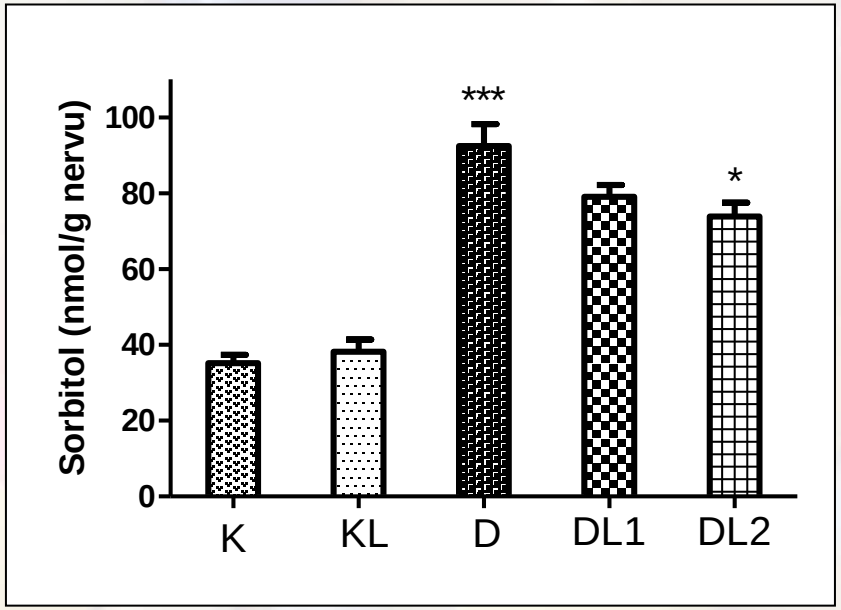
Akumulácia sorbitolu v očných šošovkách potkanov inkubovaných v prítomnosti CMTI

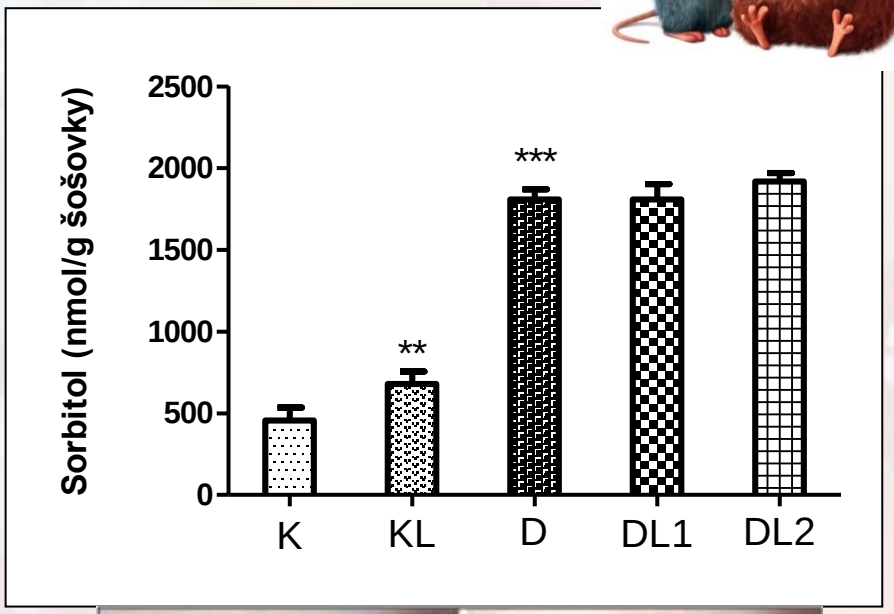


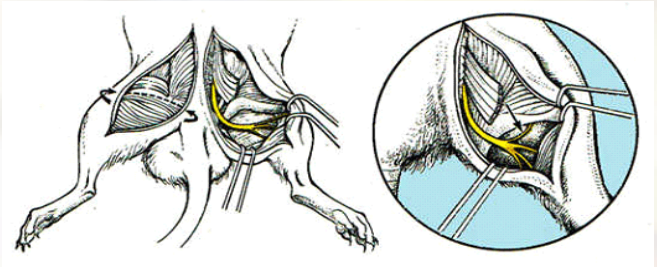
Stefek et al., J.Med.Chem., 2015

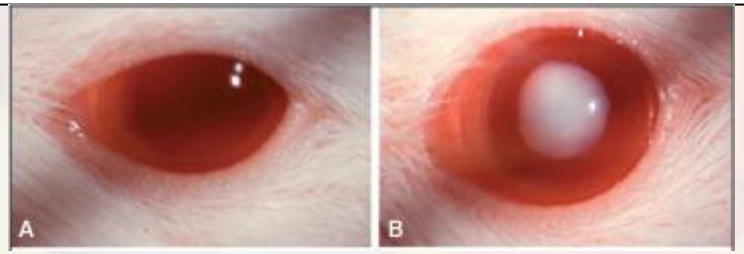
Inhibícia polyolovej dráhy in vivo

10 dňový experiment







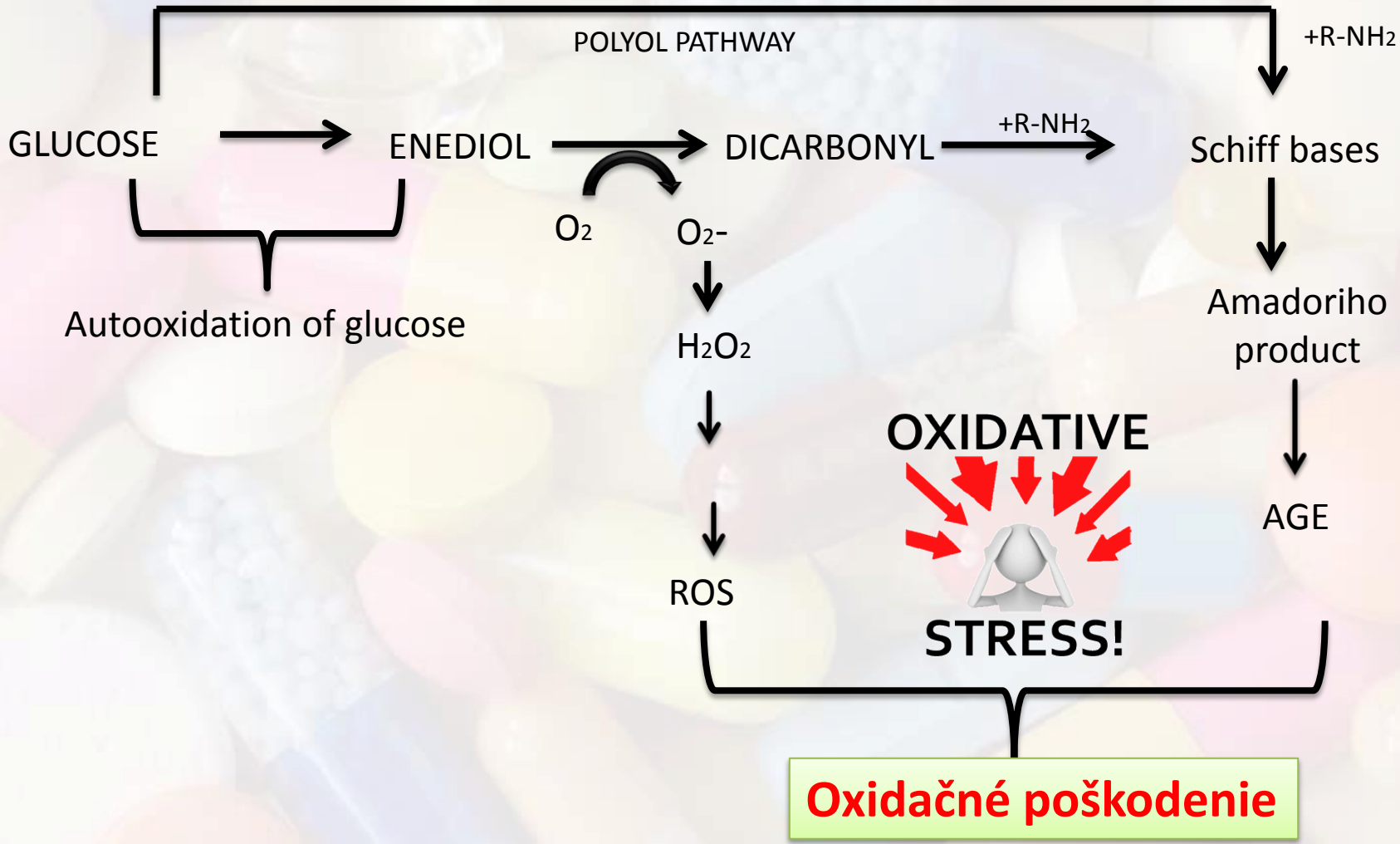


K – kontrola, zdravé zvieratá; KL – zdravé zvieratá + CMTI 50 mg/kg/deň ; D – Diabetické zvieratá ; DL1 – CMTI 25 mg/kg/deň ; DL2 – CMTI 50 mg/kg/deň

Soltesova Prnova et al., Physiol.Res., 2015

Mechanizmus glukózovej toxicity

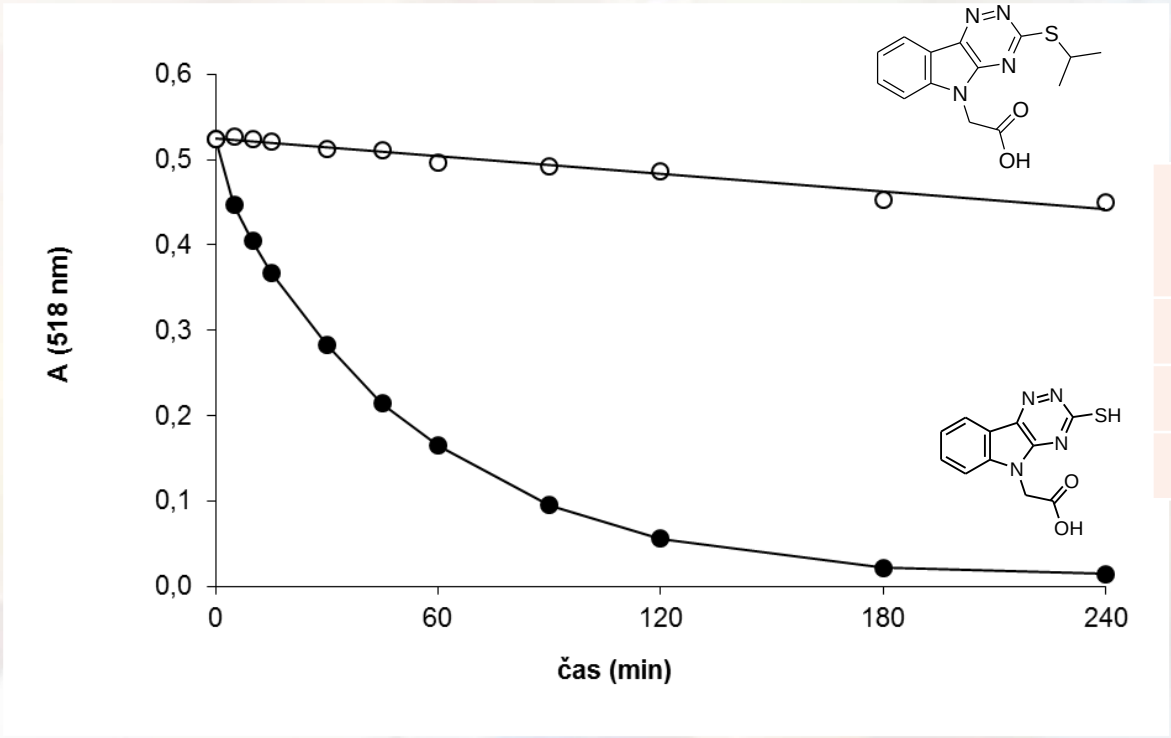
Oxidačný stred

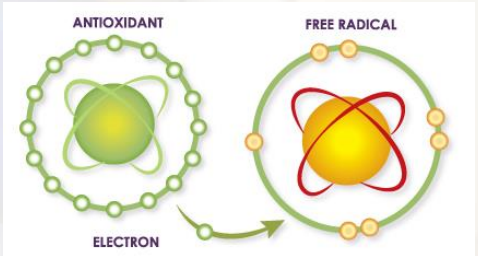


AO aktivita CMTI

DPPH test

DPPH• + AH → DPPH-H + A•





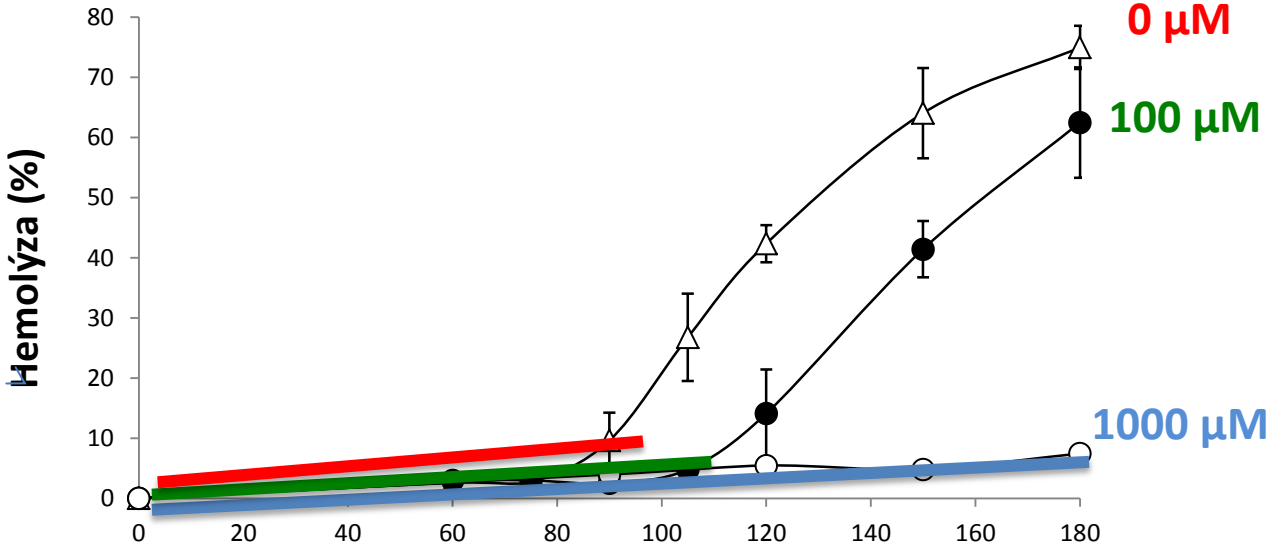
Testovaná látka	C(µM)	-ΔA/30 min ± SD
13	200	0,219 ± 0,005
14	200	0,009 ± 0,001
Melatonín	200	0,022 ± 0,006

Soltesova Prnova et al., Redox.Report., 2015

t-BuOOH– indukovaná peroxidácia RBC

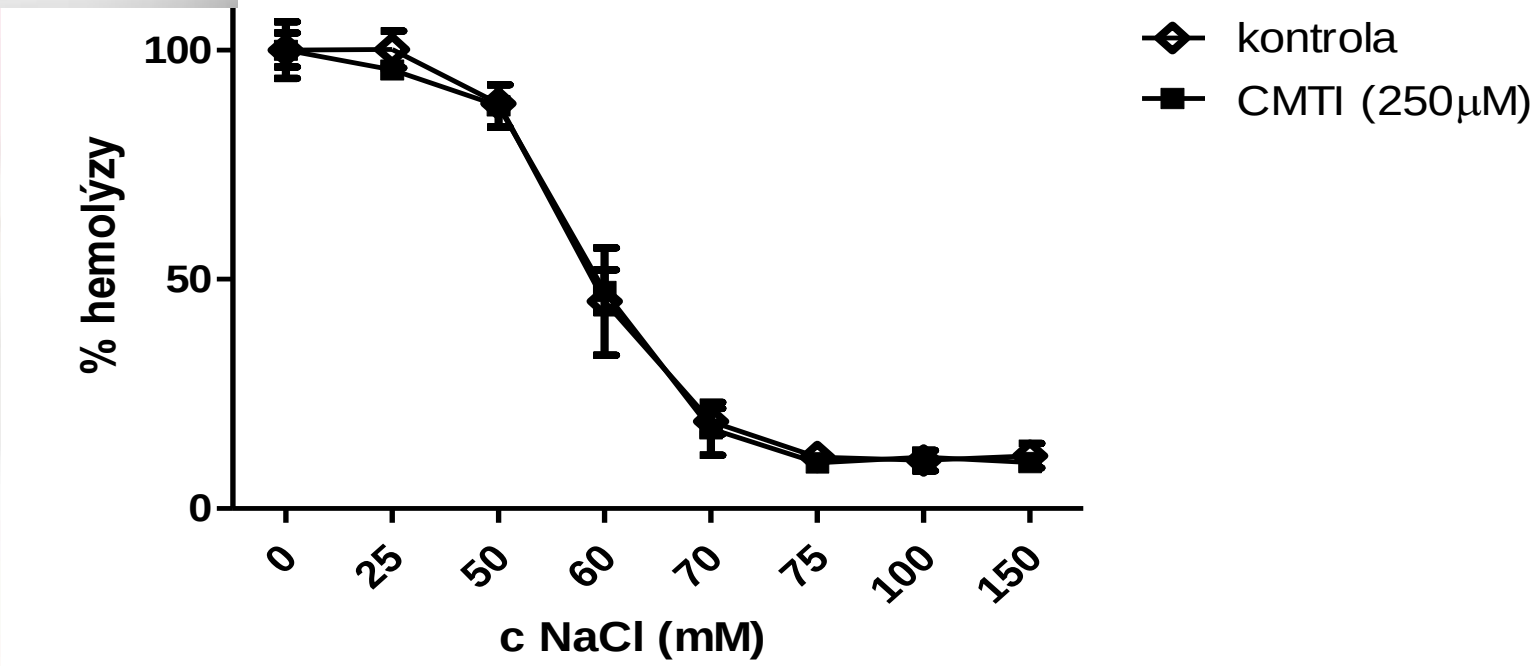
Vplyv CMTI

t-BuOOH



Soltesova Prnova et al., Redox.Report., 2015

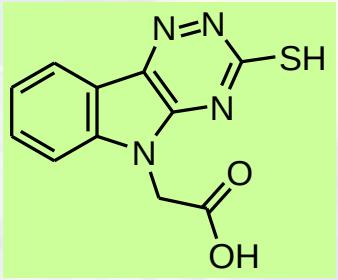
Vplyv CMTI na osmotickú fragilitu potkaních erytrocytov

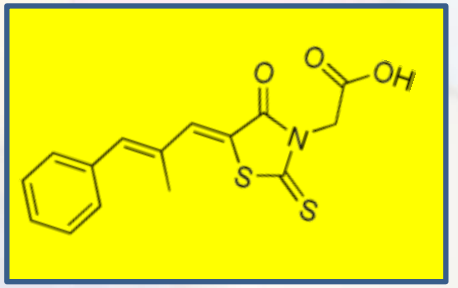


Hematokrit 0,4 %, 37°C, 60 min

Soltesova Prnova et al., Redox.Report., 2015

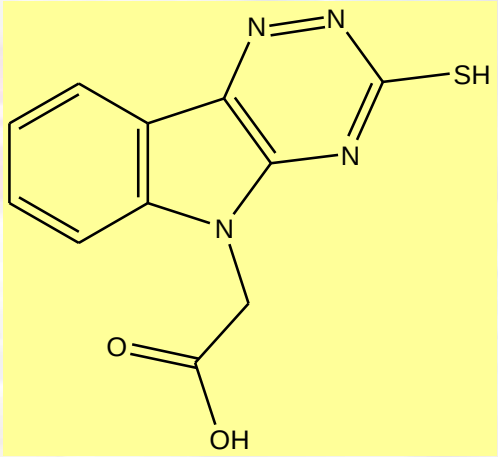
CMTI vs. Epalrestat





	CMTI	Epalrestat
MW	260	319
Rozpustnosť vo vode	> 10 mmol/l	< 3 mmol/l
ARI (IC_{50})	100 nM	250 nM
Sorbitol v očných šošovkách ex vivo, I(%)	38 %	25 %
Antioxidačná aktivita	Ano	Nie

Záver



- **Vysokoúčinný a selektív**
- **Účinný na úr**
- **P**

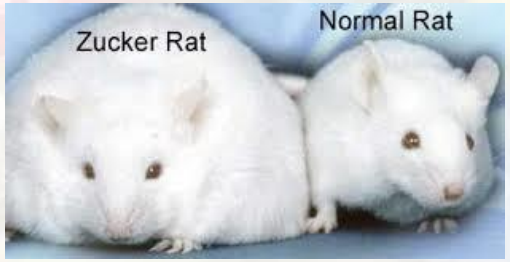
„MULTI-TARGET“

ptimálna „nosná“ štruktúra

Perspektívy

Dlhodobý test na zvieracom modely diabetu

- Streptozotocín
- Zucker



Farmakokinetika po PO podaní

Toxicita

- **Akútna**
- **chronická**

Ing. Milan Štefek, PhD.

RNDr. Lenka Májeková, PhD.

Mgr. Ivana Miláčková

RNDr. Lucia Kovačiková, PhD.

Lidka Križanová

Mgr. Jana Balleková

Chris Rechlin

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