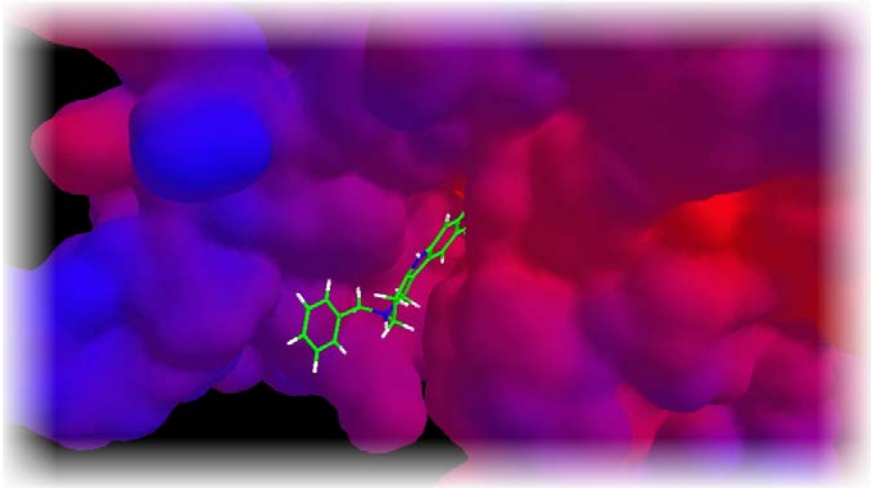
RBC 2018, #REBICON2018
ZREČE, MAY 16TH - 20TH 2018

LIGAND-BASED DRUG DESIGN CONSIDERING SPECIFIC FEATURES OF ALDOSE REDUCTASE

Magdaléna Májeková, Milan Štefek, Marta Šoltésová Prnová, Lucia Kováčiková, Jana Balleková

Department of Biochemical Pharmacology



Institute of Experimental Pharmacology & Toxicology

Centre of Experimental Medicine SAS

SLOVAK ACADEMY OF SCIENCES

841 04 Bratislava, Slovak Republic

Medicinal chemistry of aldose reductase inhibitors

Milan Štefek
(head of Department 1995-2015)
Lucia Kováčiková

Marta Šoltésová Prnová

Ľudmila Križanová - technician

Design, development and testing new multi-target oriented compounds for a treatment and prevention of diabetic complications and other chronic disorders caused by hyperglycemia

Targets: aldo-keto reductases, monoamine oxidases, reactive products and modulators of oxidation stress, modulators of inflammation

SERCA and β–pancreatic cells study

Ľubica Horáková

Jana Lomenová

Petronela Rezbáriková *

Barbora Benešová – undergraduate student

Silvia Michalíková postdoc *

Vladimír Heger - postgraduate student

Denisa Lipsceyová – undergraduate student

Oxidative injury, other postranslation changes and conformational study of Ca2+-ATPase from sarcoplasmic reticulum as a calcium regulatory enzyme.

Targets: SERCA, β–pancreatic cells

G-LUCK - Pharmacological intervention in glucose-toxicity in type 2 diabetes

+

In silico methods: drug design, MM, QCH and MD calculations, QSAR, combinatorial libraries,...

Magdaléna Májeková (head of department)
Kristína Tomeková – undergraduate student

Pavel Majek (ext) - statistical methods

Aldo-keto reductases

cytosolic NAD(P)H dependant oxido reductases (34 - 37 kDa)

Physiology:

- Transformation and detoxification of endogenous and exogenous carbonyl compounds
- Crucial role in the 1st phase of metabolism of eubiotics and xenobiotics

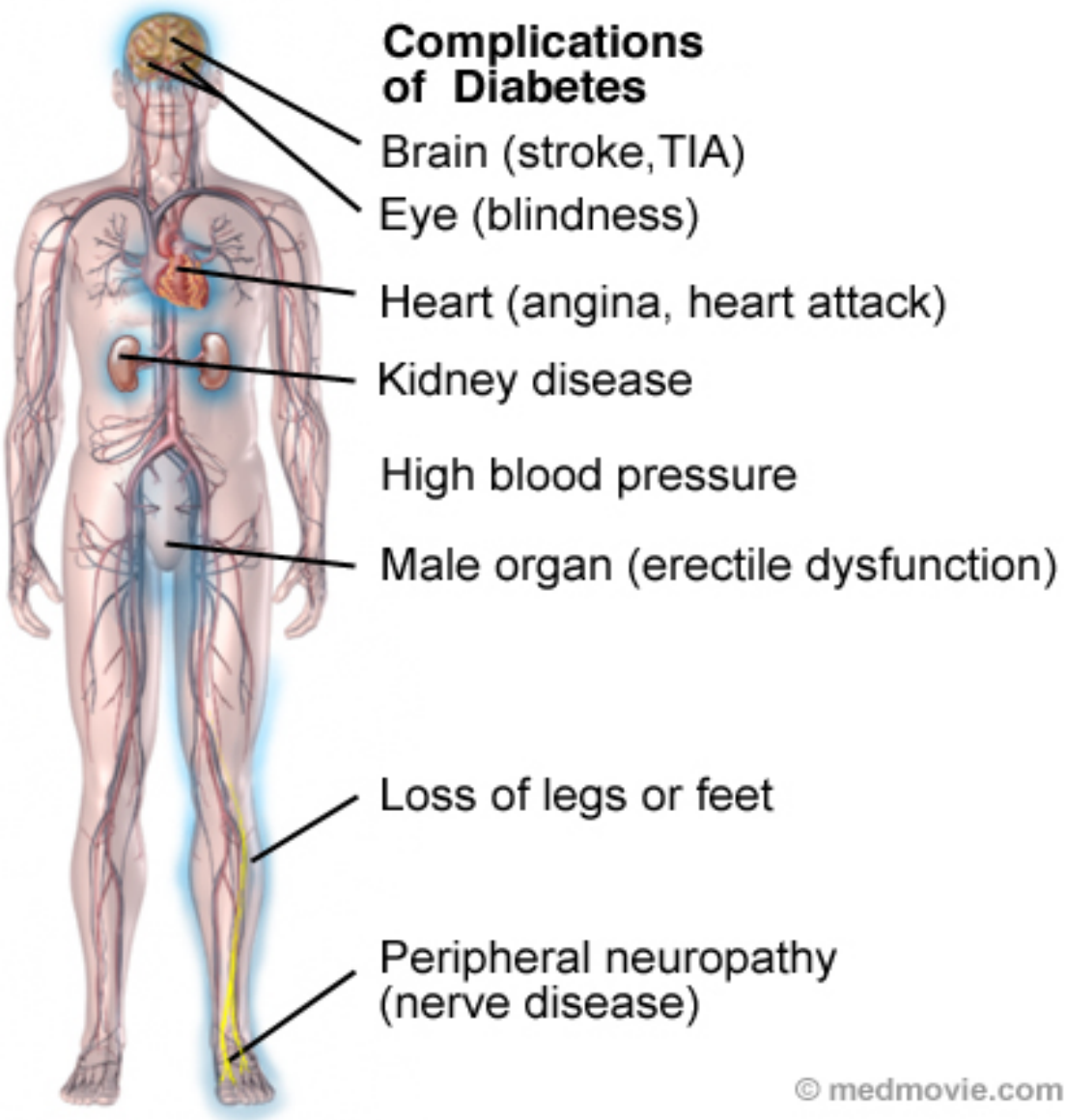
Patophysiology:

- chronic diabetic complications, inflammation, cancer, endocrinologic and metabolic disorder, drug resistance

Perelman School of Medicine
University of Pennsylvania

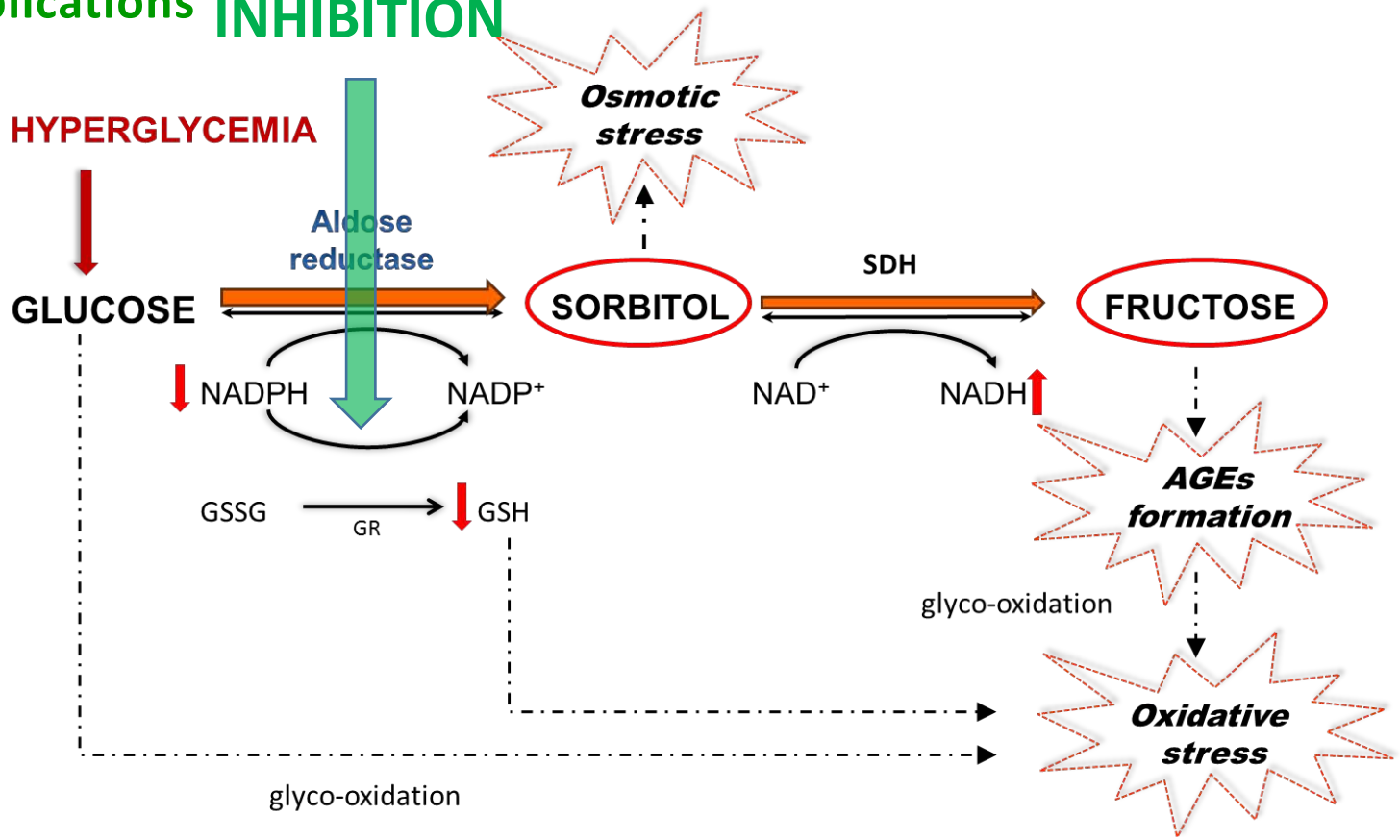
https://www.med.upenn.edu/akr/
AKR- superfamily homepage

Late (chronic) diabetic complications



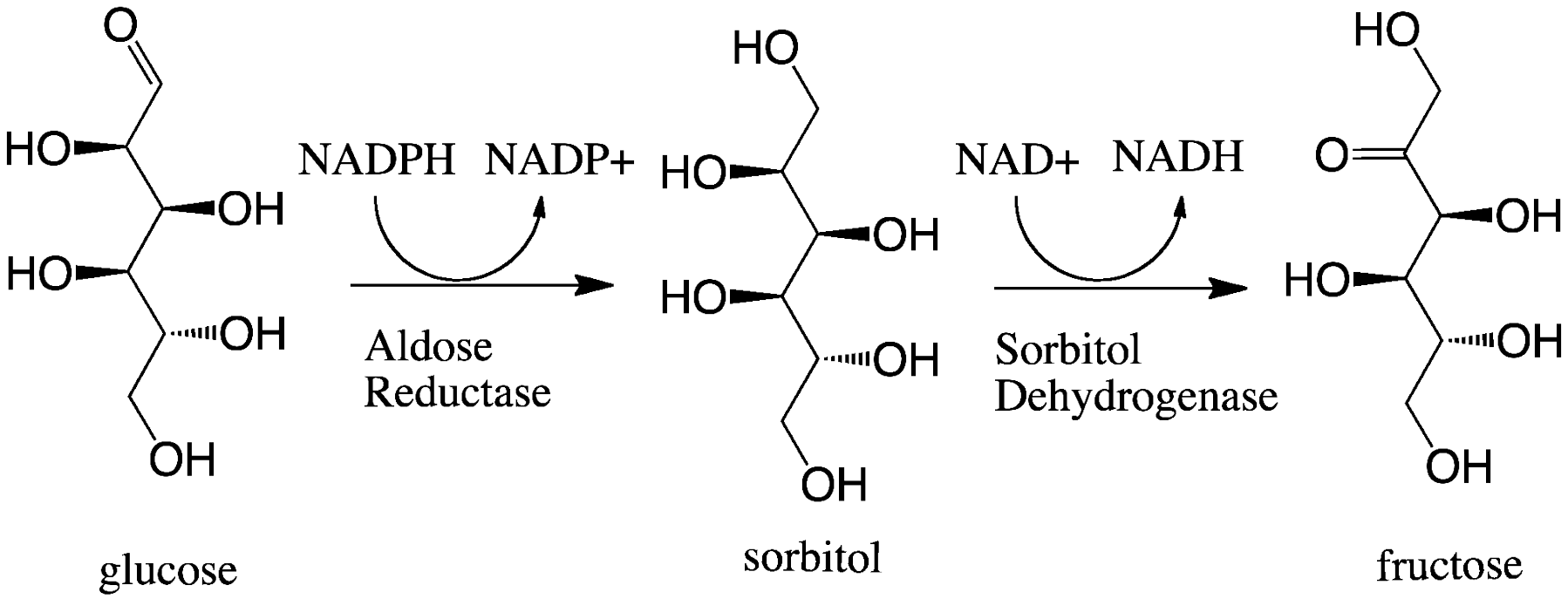
Patients with T2DM – insulin resistance

Hyperglycemia (blood level > 7 mmol/L) long term effect - **chronic diabetic complications** **INHIBITION**



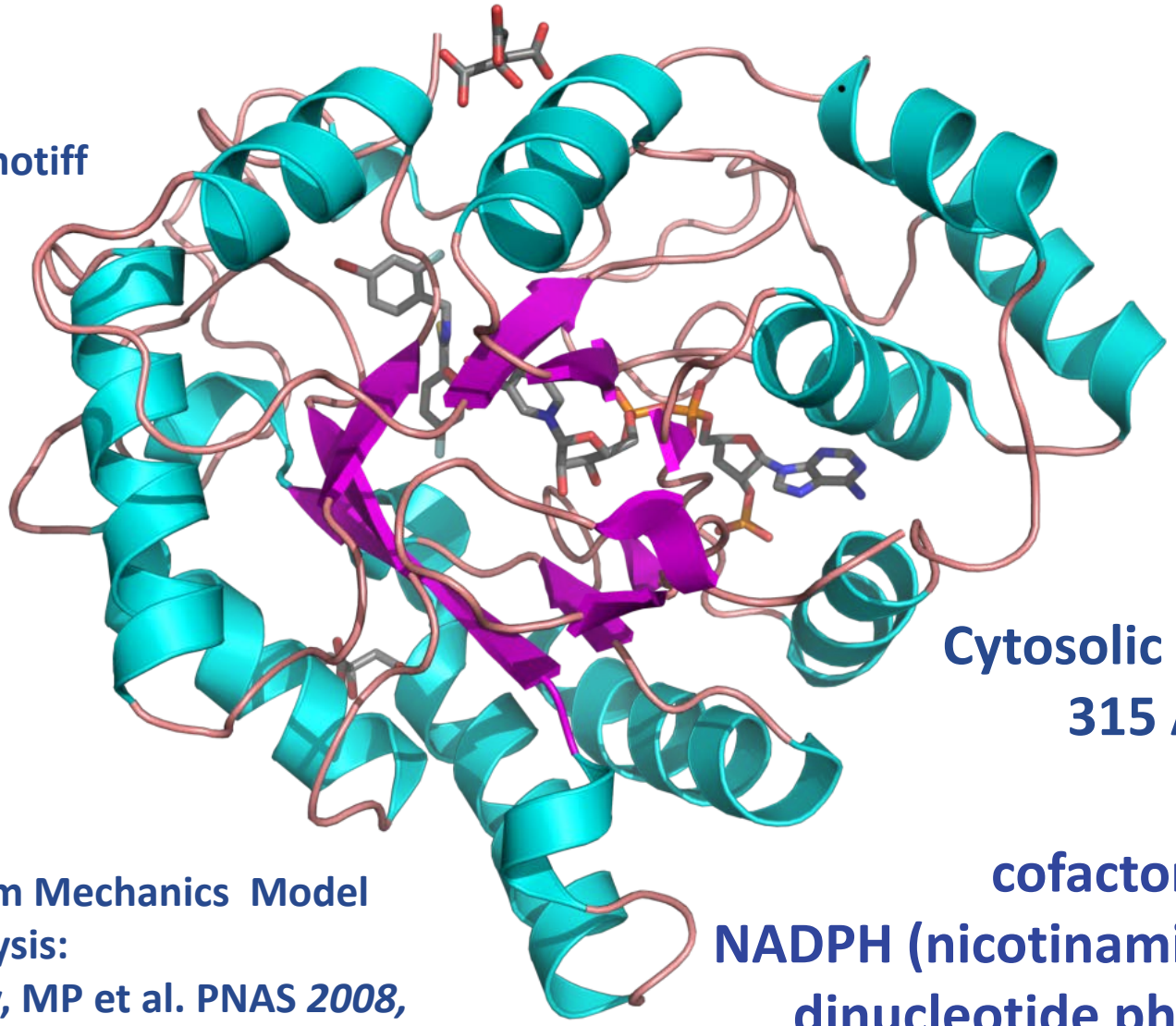
Hyperglycemia –> disorder of the cell calcium regulation (SERCA)

Polyol pathway



ALR2

(αβ)8 motiff



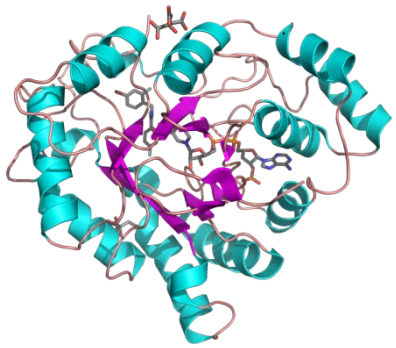
Cytosolic enzyme
315 AA

cofactor:
NADPH (nicotinamide adenine dinucleotide phosphate)

Quantum Mechanics Model of Catalysis:
Blakeley, MP et al. PNAS *2008, 105 (6) 1844-1848*

ALR2

Important !



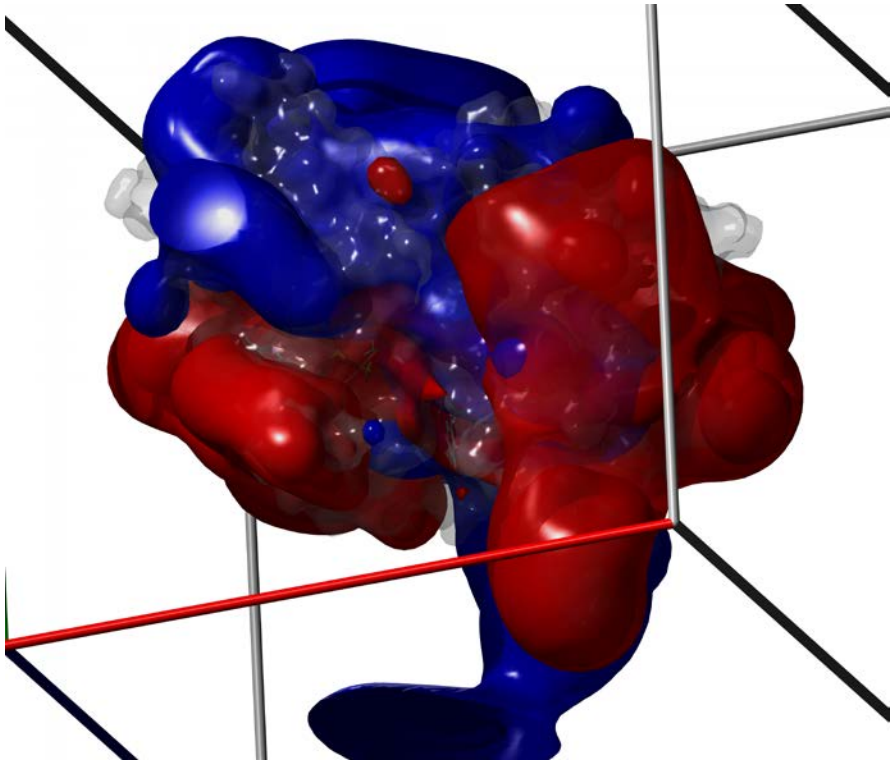
ALR2 – multi-substrate enzyme (aldehydes, HNE, GS-HNE, PGH2, methylglyoxal, doxorubicine …)

ALR2 needs the cofactor NADPH →
2 substrates mechanism
of the catalysis (and inhibition)

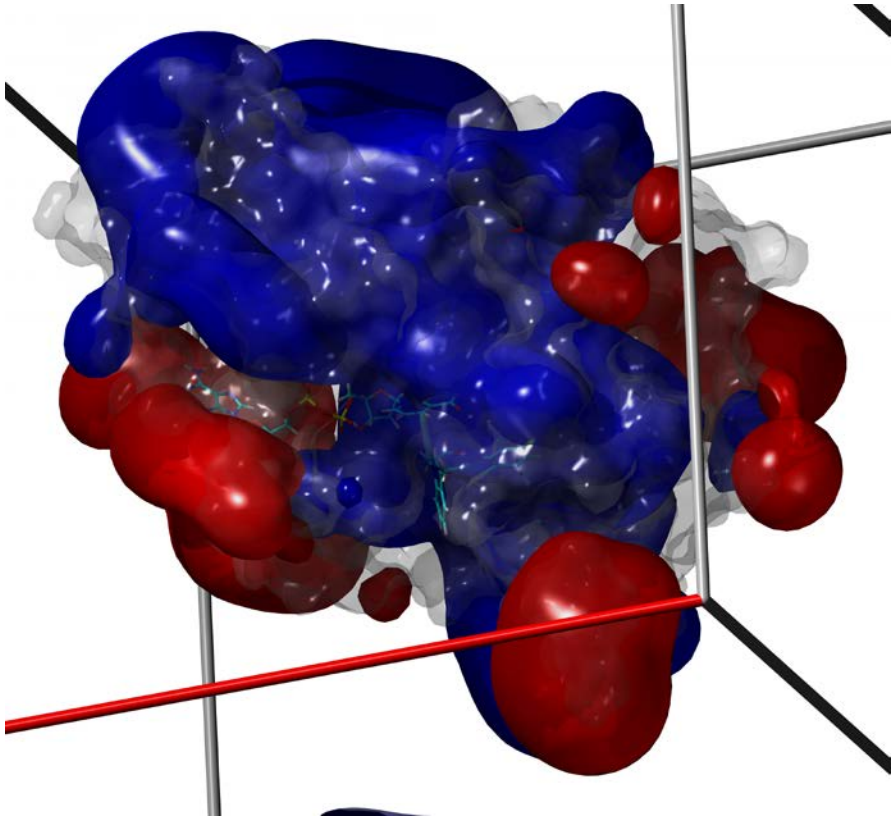
ALR2 – optimal catalytic activity under acidic condition (pH = 6.2) ← protonation state of the catalytic site

MEP dependance on pH

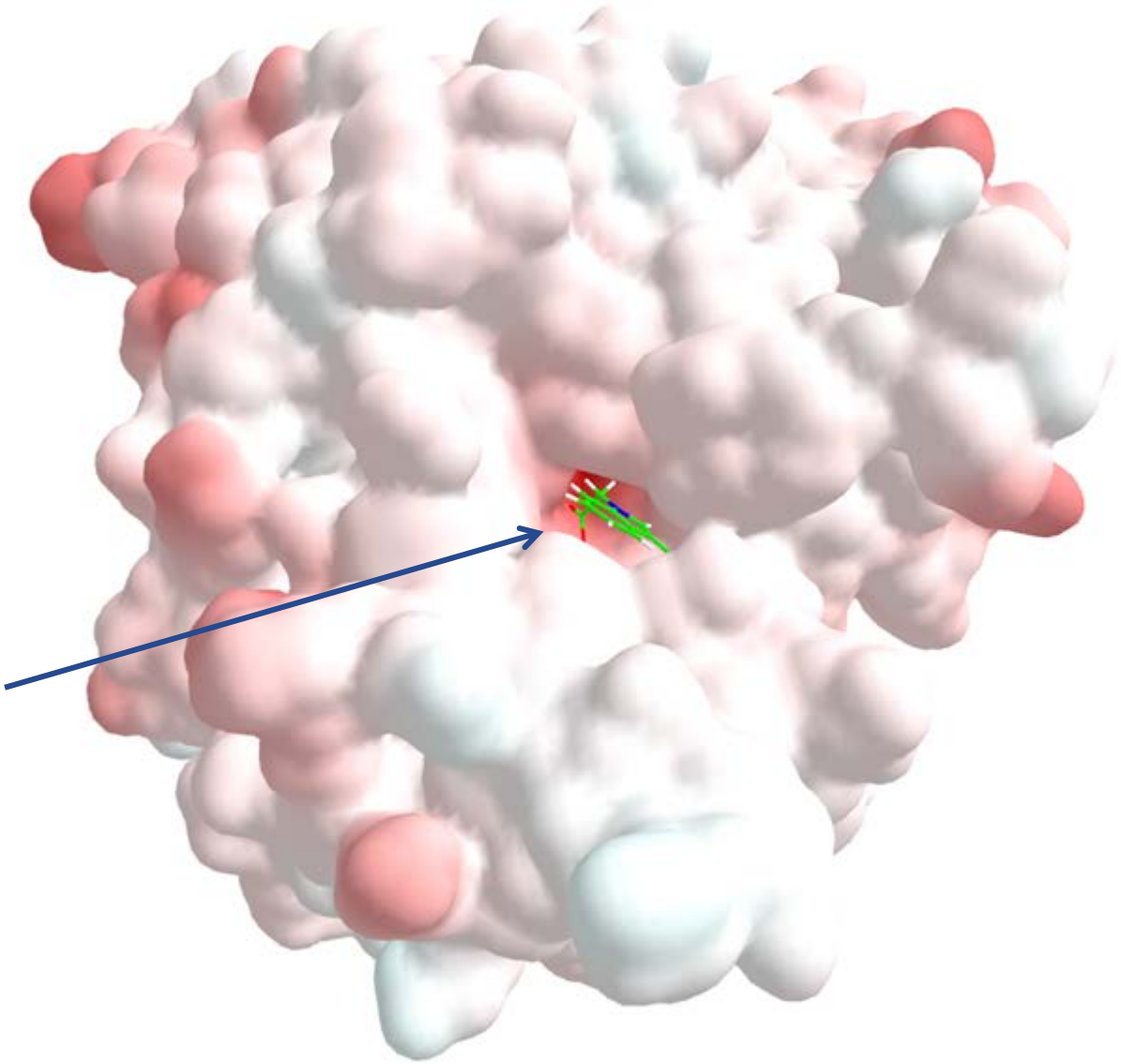
pH=7.4



pH=6.2



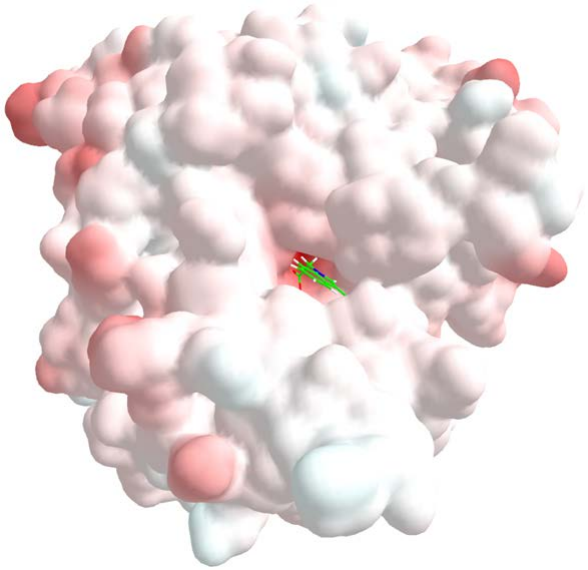
MEP of aldose reductase



cavity of the active site

+ value red

MEP of aldose reductase

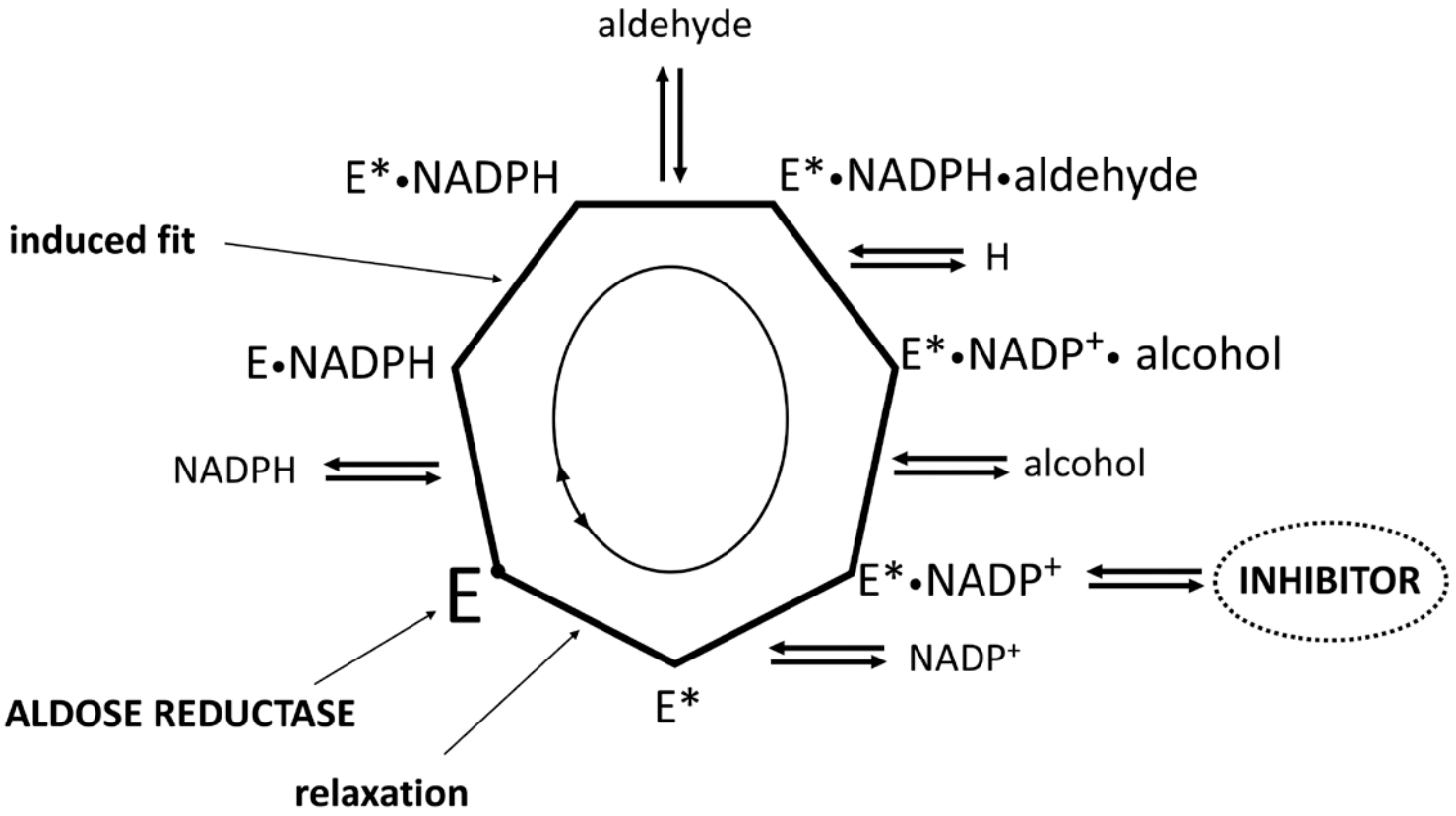


long-range (+) electrostatic driving force ->
for organic acids, e.g.

lidorestat, zopolrestat,
epalrestat (clinical use), …
dissociation – negative charge
activity ⇧ bioavailability ⇩

carboxymethyl substituent
?
lipophilic scaffold
with good permeation

2 substrates mechanism



Inhibitors

Two steps when inhibition could be achieved by external compound:

i) Before NADPH binding and subsequent fixation of the "safety-belt", i.e. pushing the loop of residues Gly213–Leu226 to ;

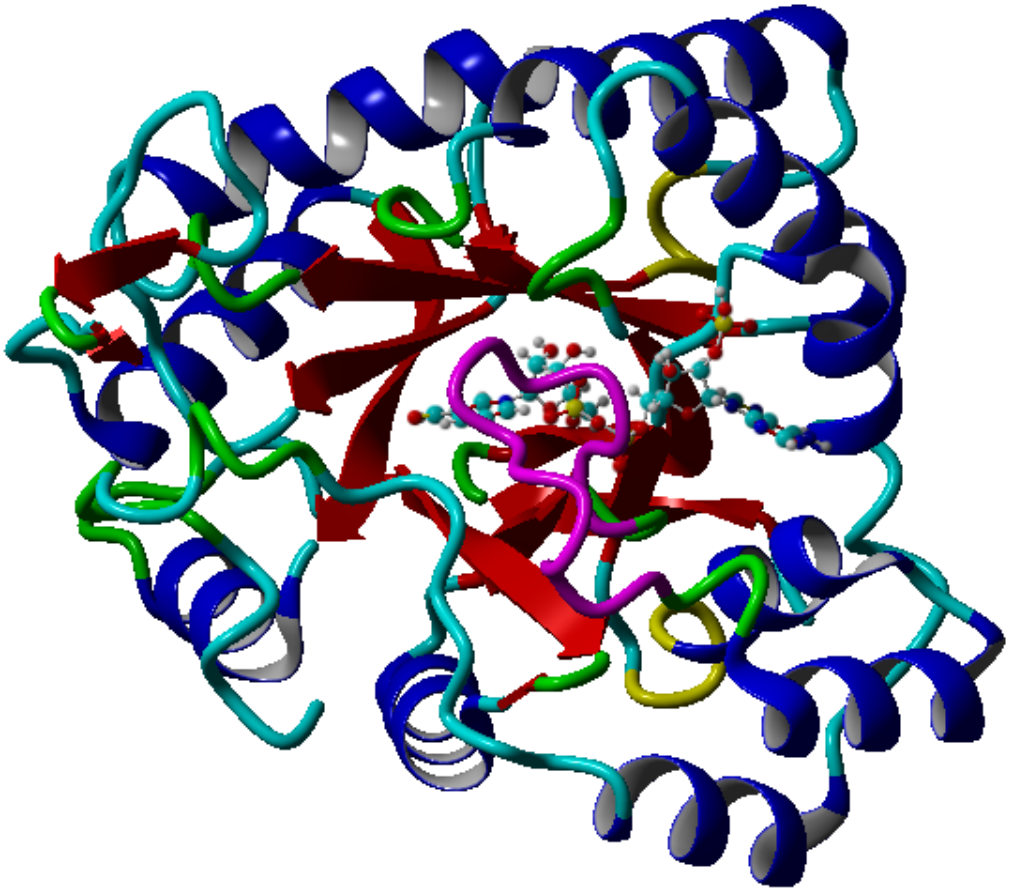
ii) After leaving the second product, i.e. by attacking the complex E*•NADP+

i) Substrate-like inhibitors (glutathione analog)

ii) Products like inhibitors

- **organic acids (carboxymethyl substituent)**
- **hydantoins (tautomeric balance, large dipoles)**
- **polyphenols (? PAINS)**

Safety belt



HISTORY

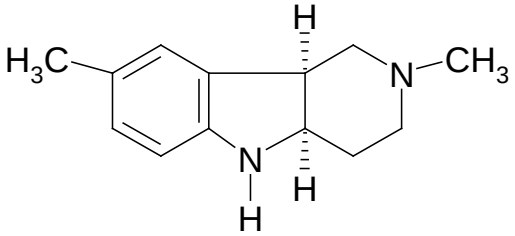
IEPT SAS - 20 years ago

Hexahydropyridoindoles -

efficient radical scavengers

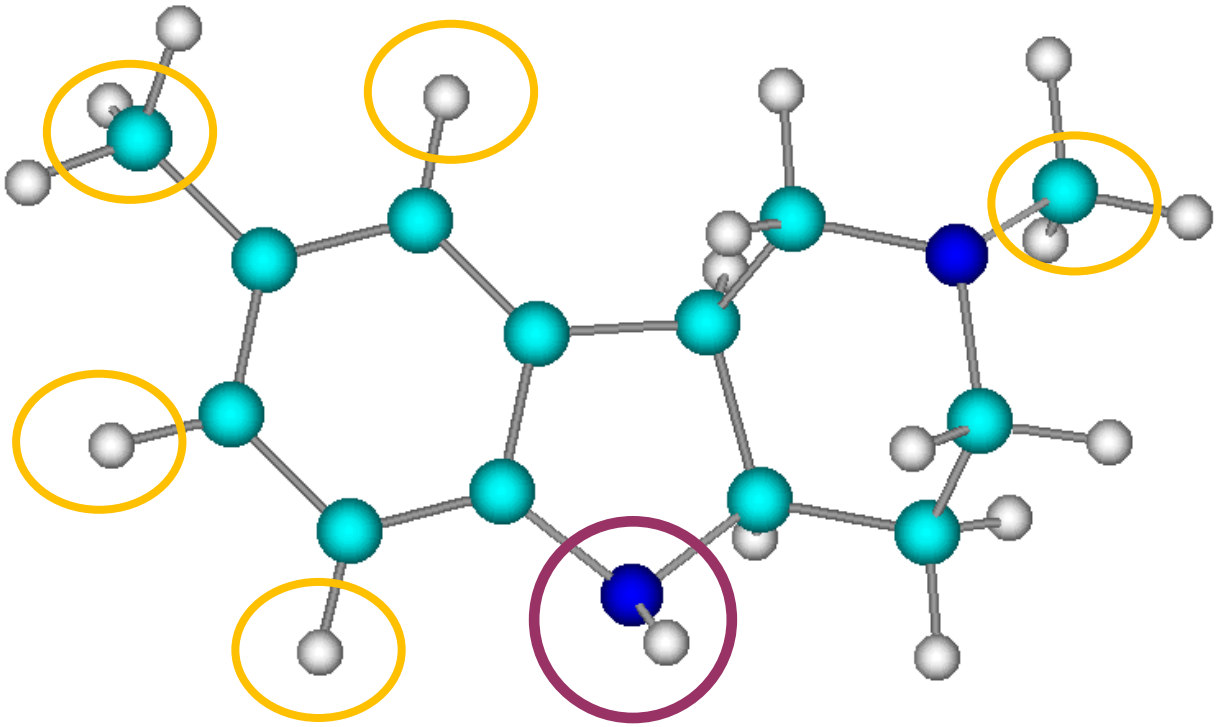
Hexahydropyridoindoles – efficient radical scavengers

Stobadine



neuroprotective, antiarhythmic, local anesthetic, alpha-adrenolytic, antihistaminic, myorelaxant, antiulcerogenic,

inhibits development of chronic disorders in diabetes …



~ 80 compounds

AO activity in IC_{50} for the inhibition of lipoperoxidation

1.5 order > Trolox

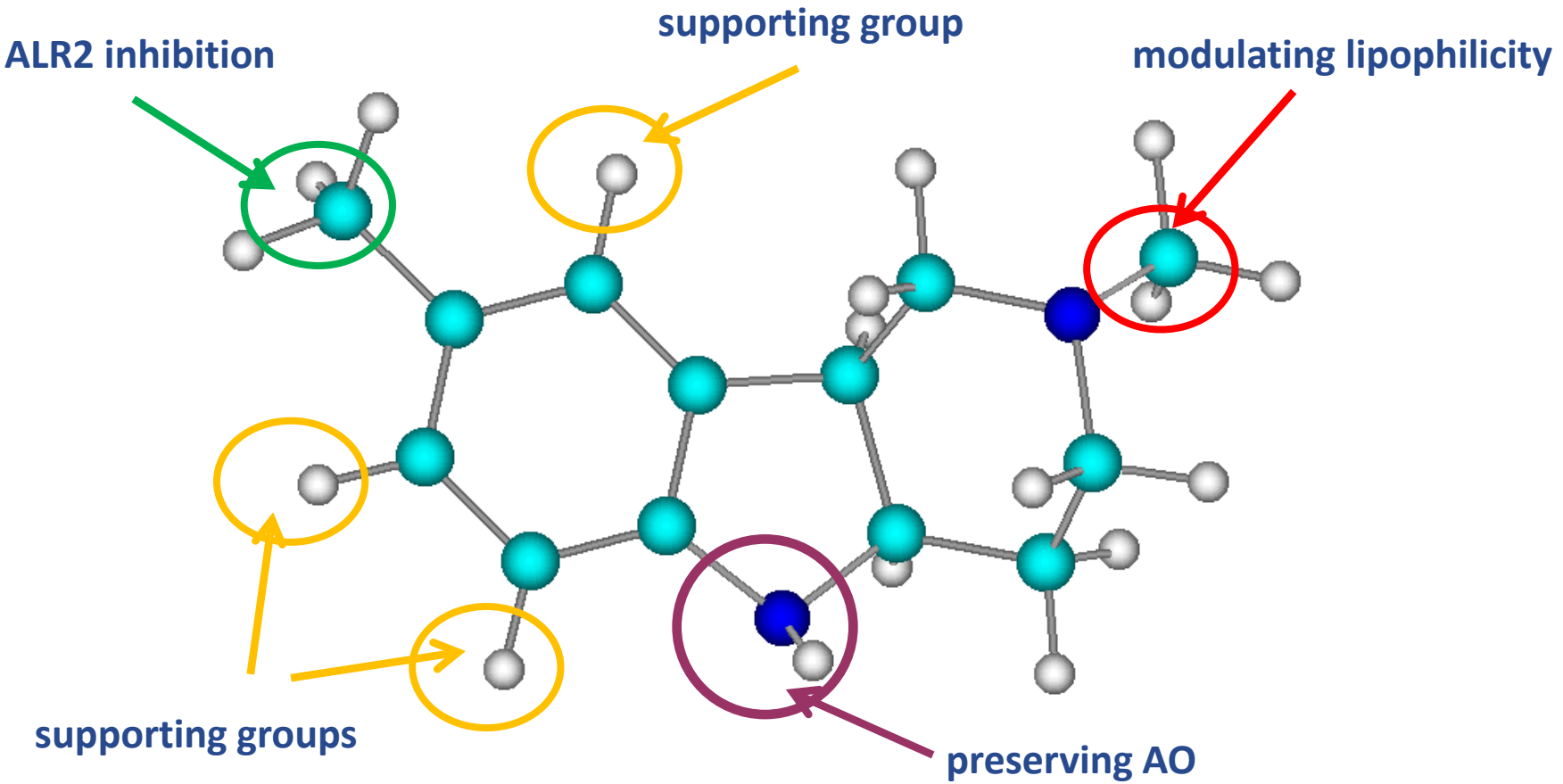
+

specific effect inhibition of ALR2 ?

Hexahydropyridoindoles – efficient radical scavengers

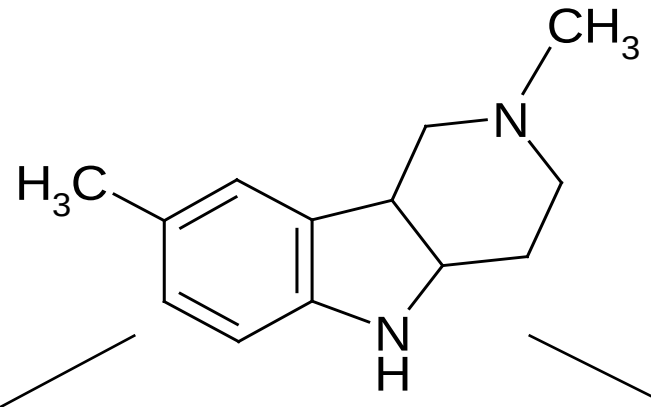
?

+ specific activity - ALR2 inhibition

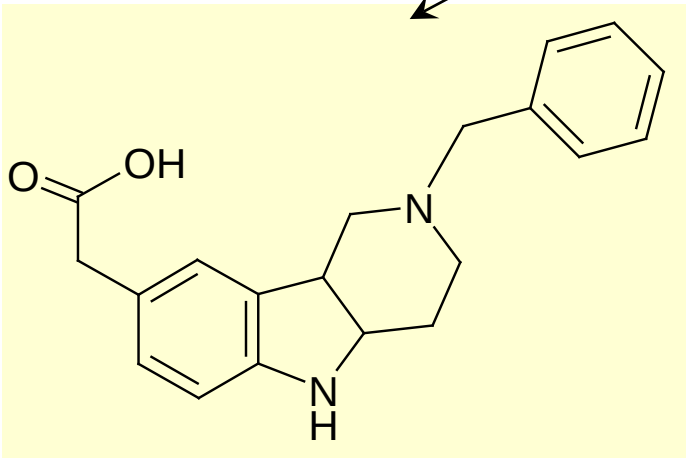


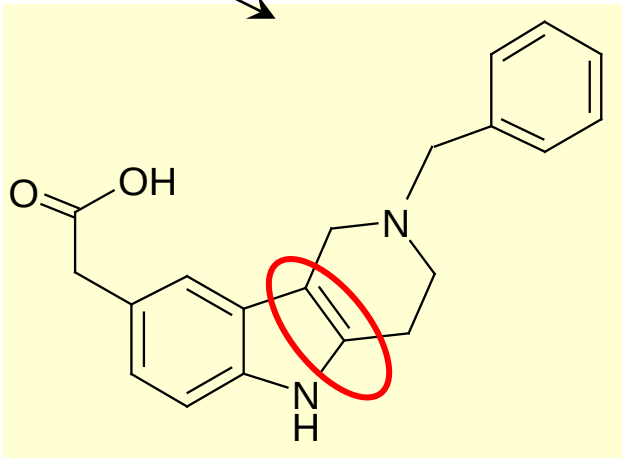
Carboxymethylated pyridoindoles

Inhibition of rat lens aldose reductase



stobadine



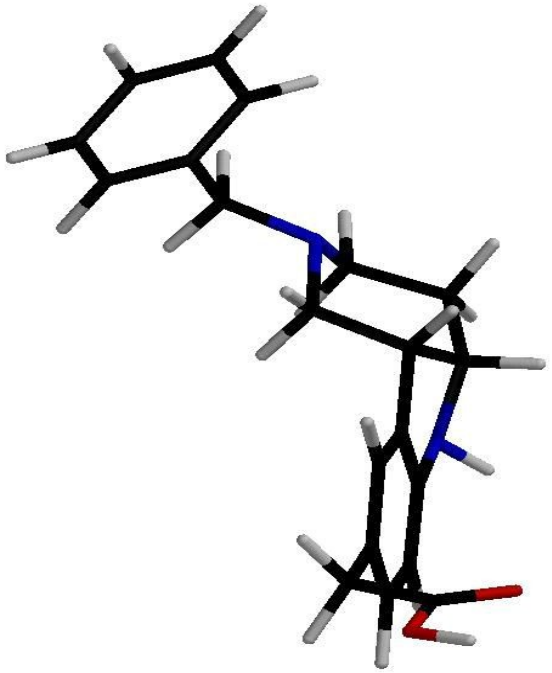


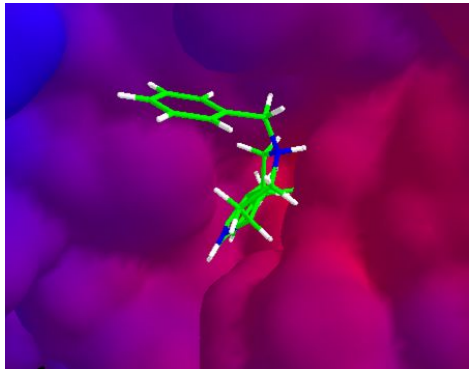
IC_{50} = 266.0 ± 4.1 μM

IC_{50} = 18.2 ± 1.2 μM

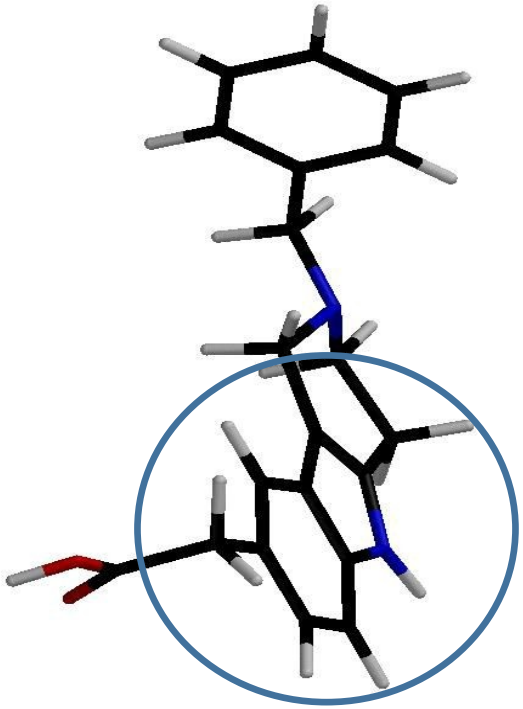
3-D structure

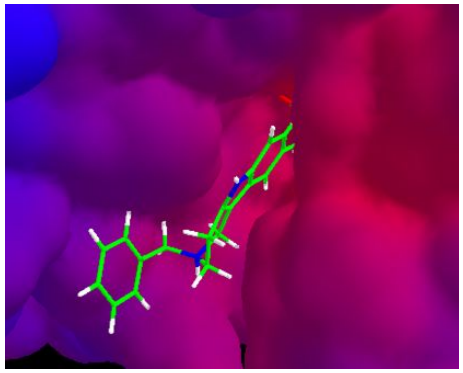
hexahydropyridoindolej

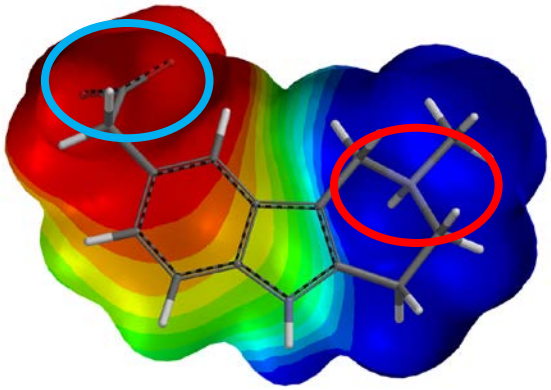




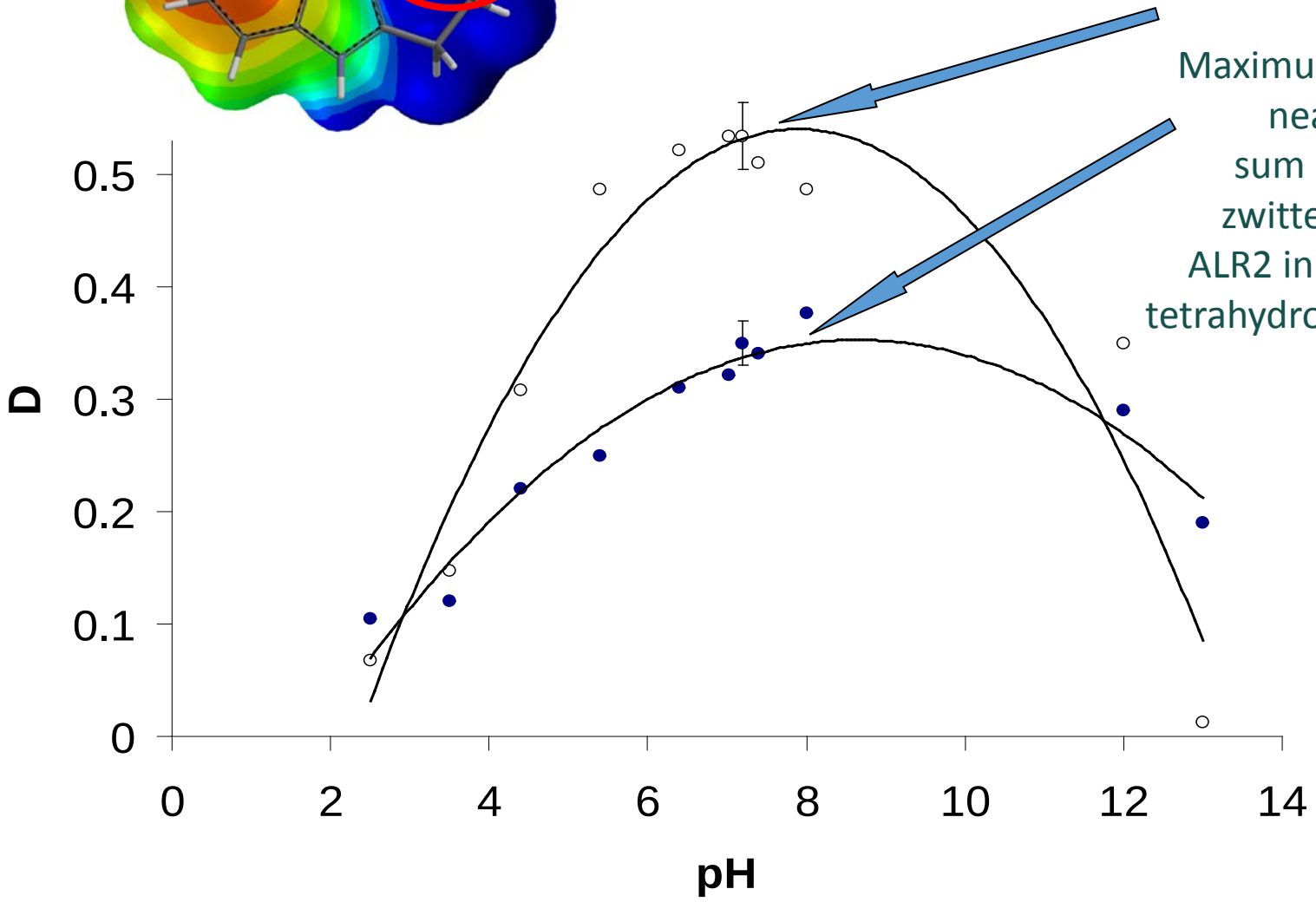
tetrahydropyridoindole







basic (protonated) nitrogen
+ acidic (dissociated) >COO^-
=> zwitterion



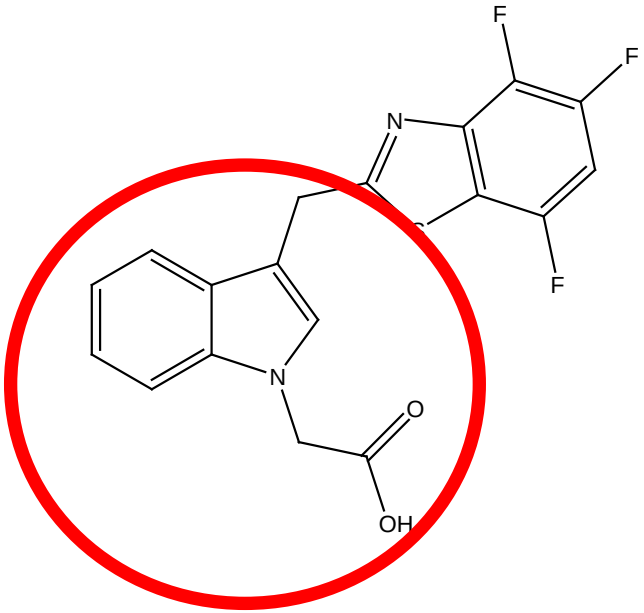
Maximum distribution near neutral pH; sum of neutral and zwitterionic form of ALR2 inhibition active tetrahydropyridoindoles

tetrahydropyridoindoles

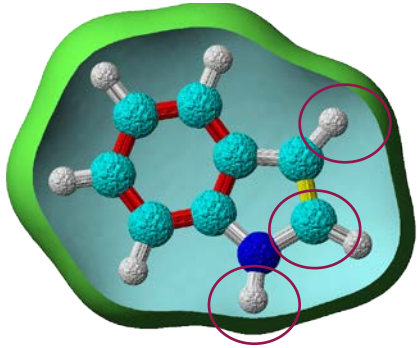
substituted indoles

lidorestat

IC_{50} = 5 nM



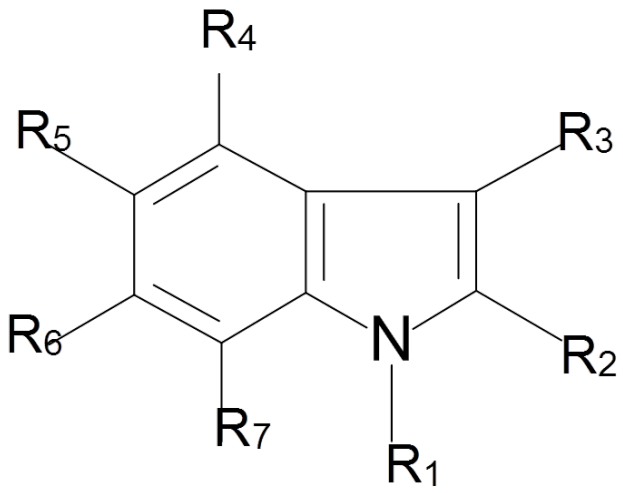
Indole acetic acids



Compound	AKR1B1		AKR1A1	
	IC_{50}(μM)	$I_{\%}$ (100μM)	IC_{50}(μM)	$I_{\%}$ (100μM)
Indole 1 acetic acid	7.98	99.26±5.90	80.17	55.49
Indole 2 acetic acid	~~0~~		~~0~~	
Indole 3 acetic acid	~~0~~		-	

Structure Coding

R1-R2-R3-R4-R5-R6-R7



Substituents

1	-H
2	-CH2COOH
3	-CH3
4	- C2H5
5	- OCH3
6	- NH2
7	- N(CH3)2
8	- O-Bz
9	- O-CH2-Bz
10	-SH

Database of indole derivatives for in silico ADMET and activity study

1-2-R3-R4-R5-R6-R7; **1-5-R3-R4-R5-R6-R7**; with the exception of compounds with R3=C2H5, R7=NH2 or N(CH3)2

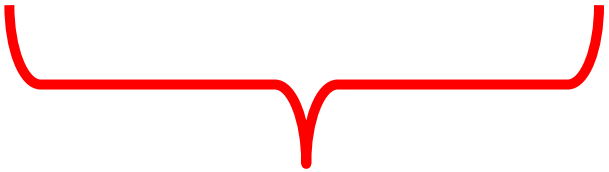
2-3-R3-R4-R5-R6-R7; **2-5-R3-R4-R5-R6-R7** with the exception of compounds with R3=C2H5, R7=NH2 or N(CH3)2, R5= O-C6H6

Compounds with R2=SH - cardiotoxicity expected, in spite of the good predicted absorption.

+

Pharmacophore (lidorestat-type) aligning (similarity ≥ 70%):

2-3-5-1-1-8-1, 2-3-5-1-1-5-1, 2-3-5-1-1-9-1,
2-1-4-1-8-1-1, 2-1-3-1-8-1-1, 2-1-4-1-1-8-1



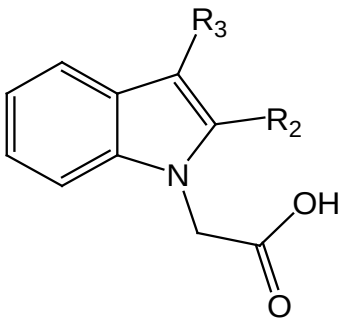
methylation at R2 and R3

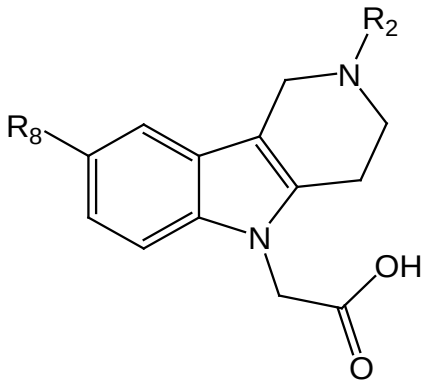
tricyclic structure of cemtirestat

+

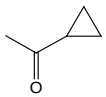
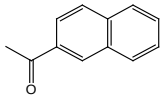
positive effect of methylation in R2 and R3

return to tetrahydropyridoindoles



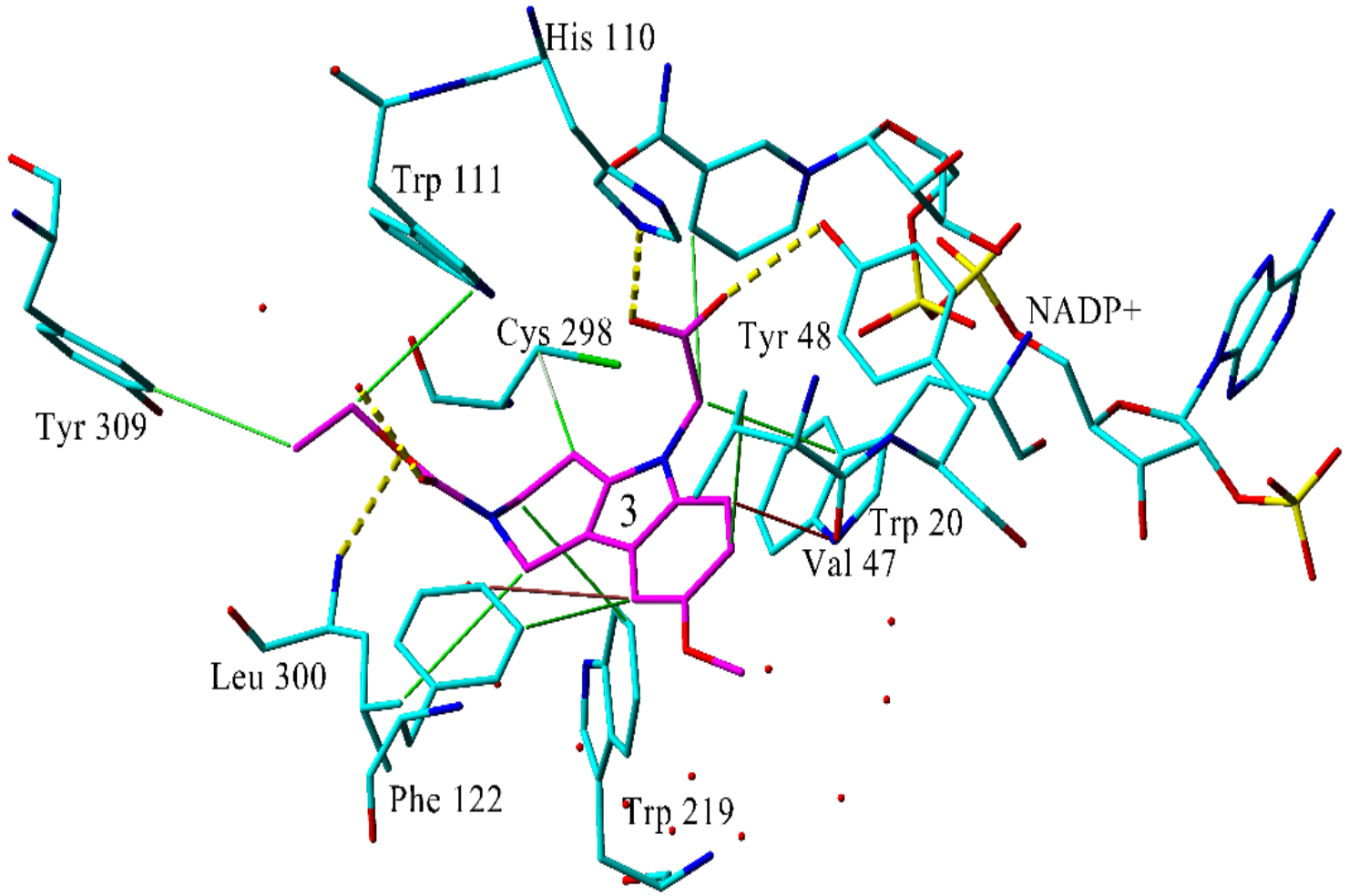


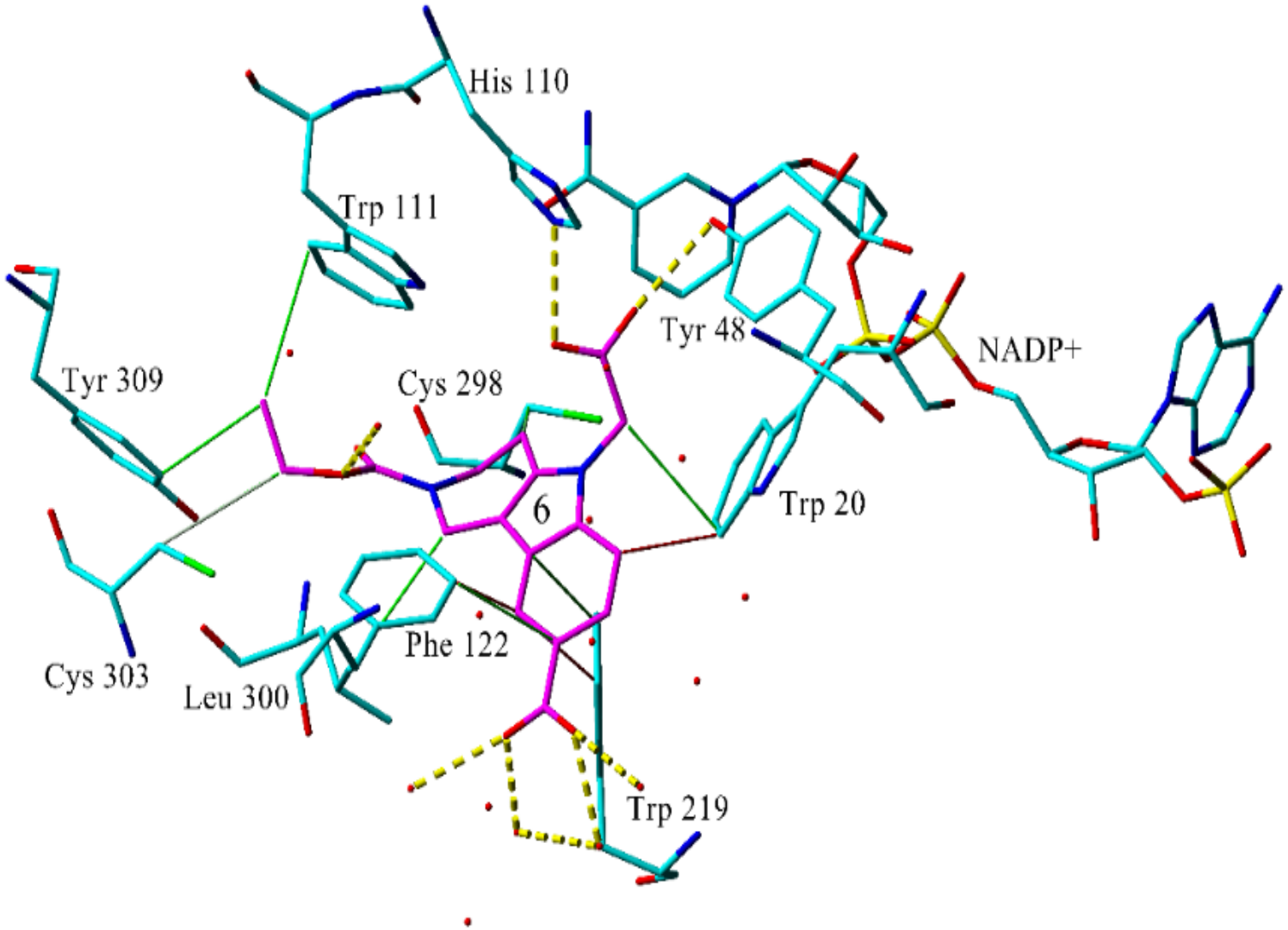
	R_2	R_3
1	CH_3	CHO
2	CH_3	$COCH_3$

	R_2	R_8
3	$COOC_2H_5$	OCH_3
4	$COCH_3$	H
5		H
6	$COOC_2H_5$	COOH
7		F
8	$CH(CH_3)_2$	H

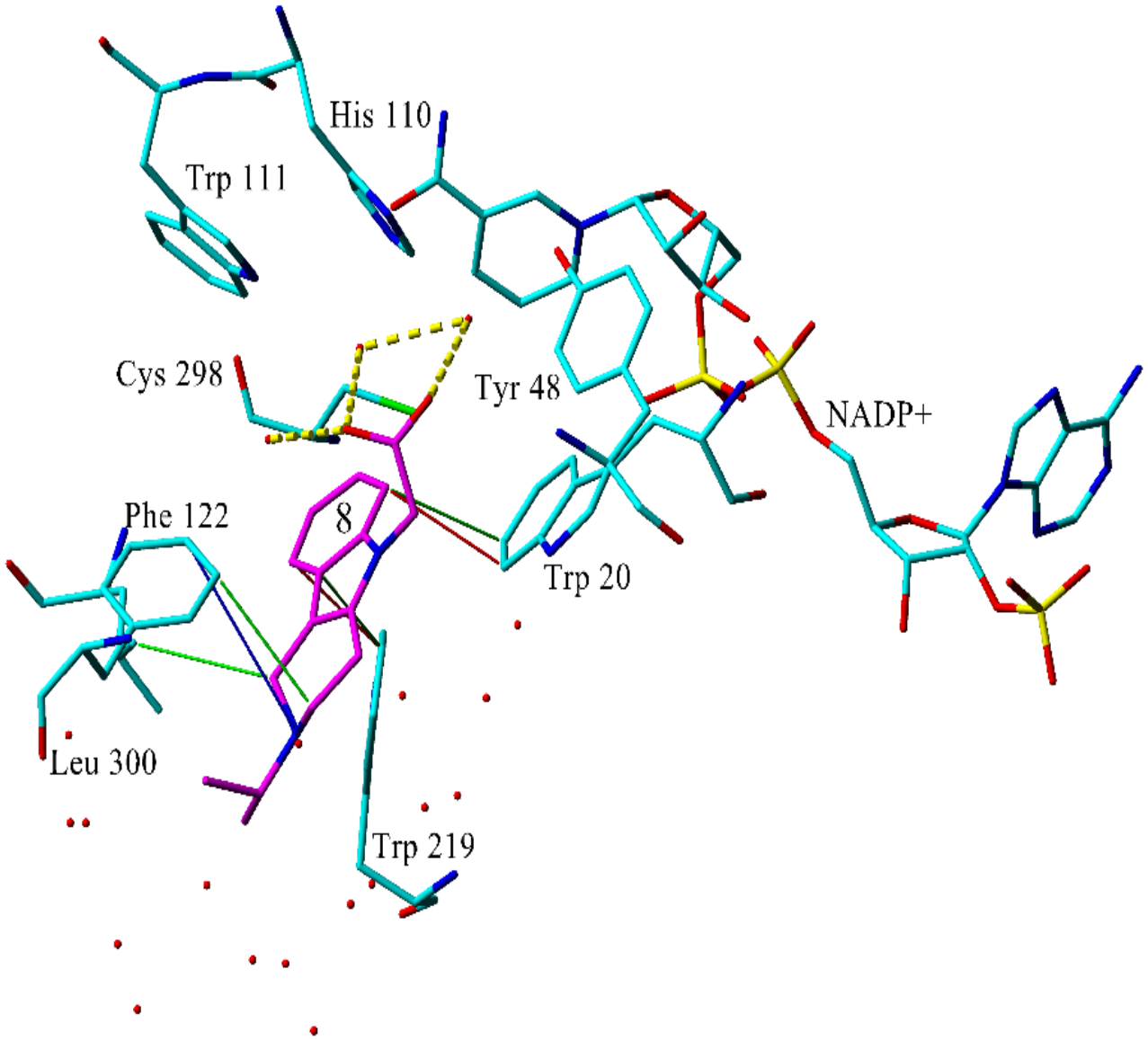
Rat ALR2

Compound	IC_{50} (nM)
1	340.2 ± 37.1
2	169.1 ± 13.6
3	12.6 ± 2.20
4	20.5 ± 1.67
5	57.5 ± 3.96
6	12.7 ± 1.6
7	141.2 ± 53.0
8	34 250.0 ± 4 717.2
Epalrestat	250


His 110
Trp 111
Cys 298
Tyr 48
NADP+
Tyr 309
3
Trp 20
Val 47
Leu 300
Phe 122
Trp 219


His 110
Trp 111
Tyr 48
NADP+
Tyr 309
Cys 298
6
Trp 20
Phe 122
Cys 303
Leu 300
Trp 219

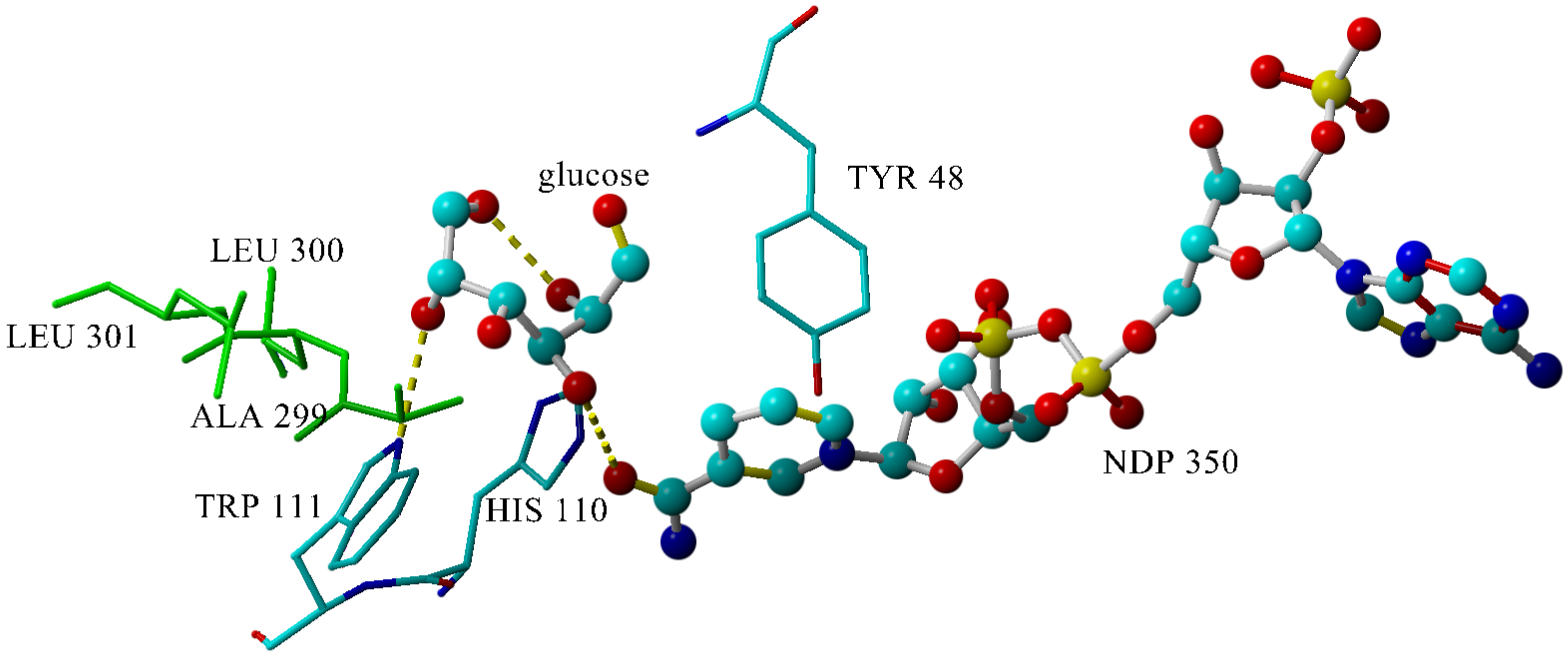
Return to the beginning



- **DPI-1 is a novel, highly potent and selective ALR2 inhibitor**
- Effective in *ex vivo* model of DC – **significant inhibition of sorbitol accumulation in rat lens**
- Effective in *in vivo* model of DC – DPI-1 (50 mg/kg/day) **significantly decreased sorbitol level in erythrocytes and sciatic nerves → uptake of DPI-1 into the central compartment after *i.g.* administration**
- Inhibitory effect on **reduction of inflammation mediator GS-HNE**
- Binding site – the most important interactions **(Trp20, Val47, Tyr48, Trp111, Phe122, Trp219 and NADP+)**
- **Excellent 'lead-likeness',** suitable for further structure optimization, emphasized by structural features fitting 'the rule of three', and the corresponding 'lead-like' values of molecular obesity indices

Patent

2nd substrate-protein-cofactor



Software

Spartan'08 – conformational search (systematic, MC)
QCH calculations
multiprocessing (DFT B3LYP 6-31G*)

YASARA – protein modeling, docking,
AMBER, YASARA, YAMBER3, NOVA FF
molecular dynamics, homology modeling
multiprocessing

DRAGON - calculation of molecular descriptors > 3000
estimation of pharmacodynamic properties

STATISTICA – statistical analysis

GAUSSIAN 09 – exact QCH calculations, HPC klaster STU

MOE – molecular modeling, combinatorial libraries, MD,
pharmacophore constructing

Acknowledgements:

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Anna Tsantili Kapoulidou – Athens University

#ReBiCon2018 Organization Team

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