



16 - 19 AGOSTO 2022
COMPUTATIONAL BIO-ORGANIC CHEMISTRY BOGOTÁ (COBO)

Neglected trypanosomiasis: LBDD, SBDD and experimental models applied to the design of multitarget quinones as candidates of selective T. cruzi growth inhibitors



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MONTEVIDEO - URUGUAY

To Organizers for the kind invitation

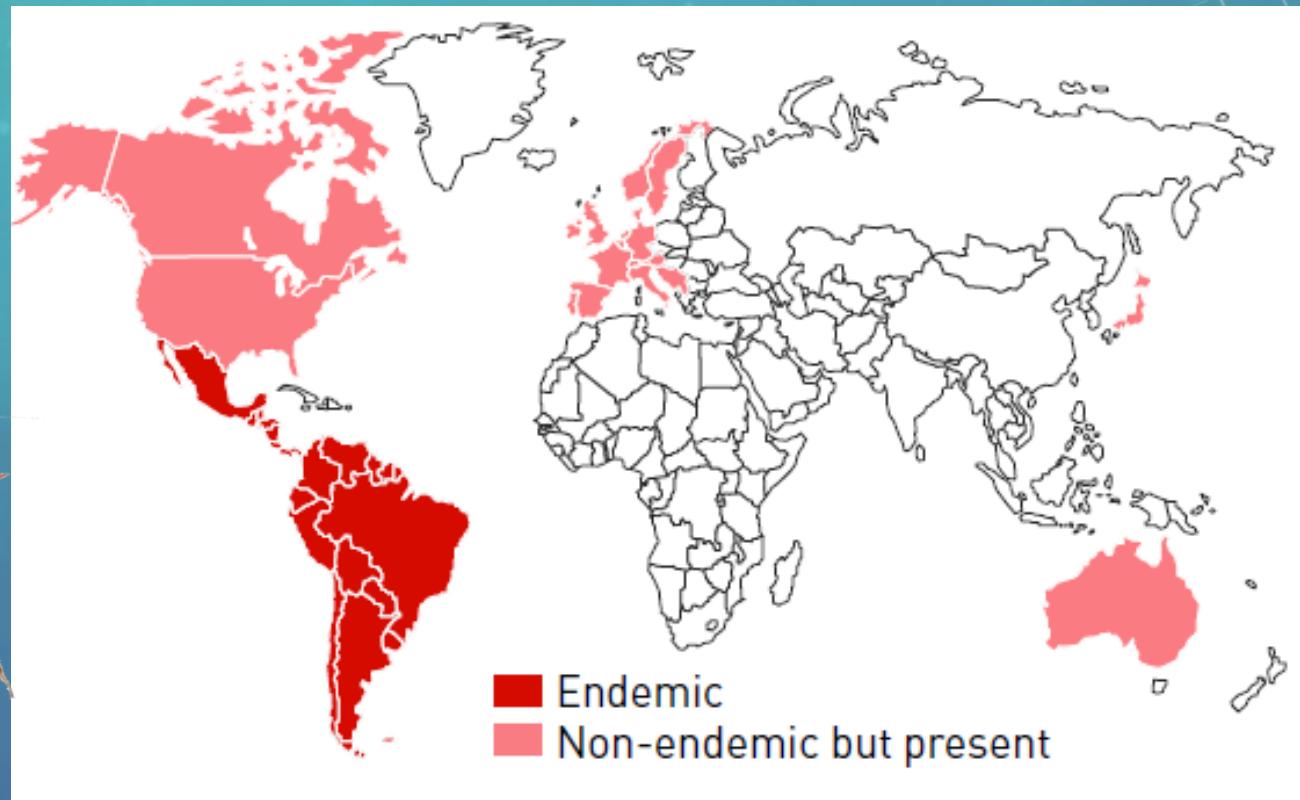
CSIC-I+D (UdelaR) Project. All results here mentioned are been developped in collaboration with: Andrés Camilo Ballesteros, Jorge Cantero (**DETEMA-CeBioinfo Facultad de Química, UdelaR**), Dinorah Gambino, **)Bioinorganic chemistry, FQ UdelaR**), Marcelo Comini, Andrea Medeiros, Cristina Quiroga and Cecilia Ortiz (**Institut Pasteur Montevideo**), Gian Pietro Miscione, Gilles Paul Pieffet and David Ricardo Figueroa (**Universidad de Los Andes, Bogotá**), Cristian Salas y Ricardo Tapia (**Pontificia Universidad Católica de Chile**), Hugo Cerecetto (**Facultad de Ciencias, UdelaR**), DETEMA-CeBioinfo Facultad de Química, UdelaR student and ex students Brenda Vera and Diego Carvalho, Universidad Nacional de Córdoba (Tonino Adessi, Viviana Nicotta and Manuela García)

Consultants: Prof Fabio Polticelli (Universitá Roma III) and Andrea Cavalli (IIT and Universitá di Bologna)

STRATEGY

- To synthetize in the organic chemistry clasical way
- To amplify the chemical space using Deep/Reinforcing Learning
- To develop experimental models
- To do biochemical and in silico studies: LBDD (PH4 AND QSAR) AND SBDD
- To SELECT HOOKS
- To do REVERSE VIRTUAL SCREENING (based on PDB respository)
 - SHAFTS(+IdTarget)
 - PHARMMAPPER
 - Overcome de bias of RVS (PDB information) by CLUSTALW
- To MODEL by HM, Ab Initio, ML, etc +MD to validate
- To FILTER:TriYtyp, BindingDB, essenciality, infectivity, adverse phenotyping
- To RANK the fished targets by DOCKING, MD (basic and advanced as FEP)
- WET LAB VALIDATION AND RECURSIVE CYCLES

Parasitic diseases are a major obstacle to human health and economic development in many parts of the world, and cause high rates of mortality and morbidity. Current therapies against these diseases are unsatisfactory, with treatment failure being common due to widespread resistance and severe side effects. Thus, there is a need for the development of new, efficient and safe drugs.



Distribution of Chagas disease cases reported to WHO, 2012-2013 / Drugs for Neglected Diseases initiative (DNDi), 2015.

Specific Imbalance of the
normal redox balance
(oxidative stress) → One of
the preferred ways to fight
against trypanosomatids

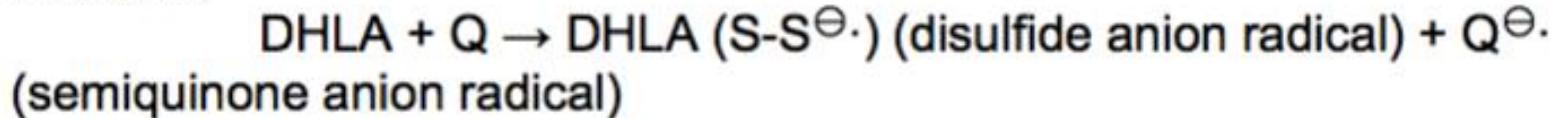
Quinones are considered privileged structures in the development of novel drugs against parasitic diseases such as trypanosomiasis because its capacity to induce oxidative stress

Paulino, M., Iribarne, F., Dubin, M., Aguilera-Morales, S., Tapia, O. & Stoppani, A. O. (2005). *Mini Rev Med Chem* 5: 499-519.

Total reaction:

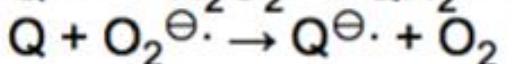
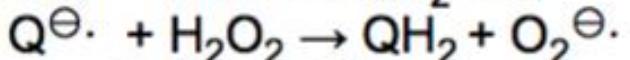
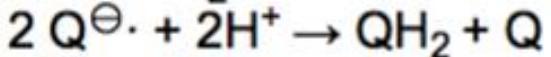
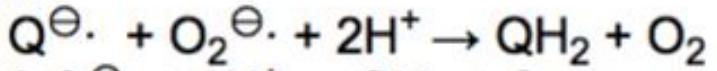


Initiation:

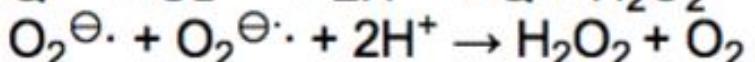
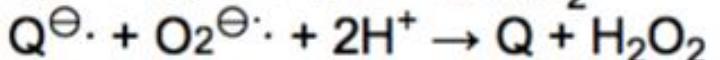
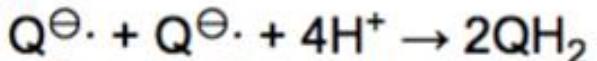


Then the initiation continues until forming the disulfide LA and QH₂.

Propagation:



Termination:



Scheme 1. Initiation, propagation and termination steps involved in the quinones reactivity.

QUINONES AND THE OXIDATIVE STRESS: TWO ELECTRON IN SECUENCIAL STEPS

TARGETTED/NON TARGETTED REDOX REACTIVITY

Objectives:

- to revise the oxidant non targeted capacity of quinones
- to modulate the reactivity of redox metabolism enzymes
 - Trypanothione Reductase
 - Trypanothione Synthetase
 - Other detected by reverse screenings



OBSERVING THE
LIGAND.... LBDD

The Chemotherapy of Chagas' Disease: An Overview

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^b Bioenergetics Research Centre, Universidad de Buenos Aires, Paraguay 2155, Buenos Aires-Argentina

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Journal of Molecular Graphics and Modelling 28 (2009) 371–381

Contents lists available at ScienceDirect

Journal of Molecular Graphics and Modelling

journal homepage: www.elsevier.com/locate/JMGM



Assaying phenothiazine derivatives as trypanothione reductase and glutathione reductase inhibitors by theoretical docking and Molecular Dynamics studies

F. Iribarne ^{a,*}, M. Paulino ^{a,b}, S. Aguilera ^b, O. Tapia ^c

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European Journal of Medicinal Chemistry 43 (2008) 2238–2246

EUROPEAN JOURNAL OF
MEDICINAL
CHEMISTRY

<http://www.elsevier.com/locate/ejmec>

Original article

Studies of trypanocidal (inhibitory) power of naphthoquinones: Evaluation of quantum chemical molecular descriptors for structure–activity relationships

M. Paulino ^{a,*}, E.M. Alvareda ^a, P.A. Denis ^a, E.J. Barreiro ^b, G.M. Sperandio da Silva ^b,
M. Dubin ^c, C. Gastellú ^d, S. Aguilera ^e, O. Tapia ^{f,**}

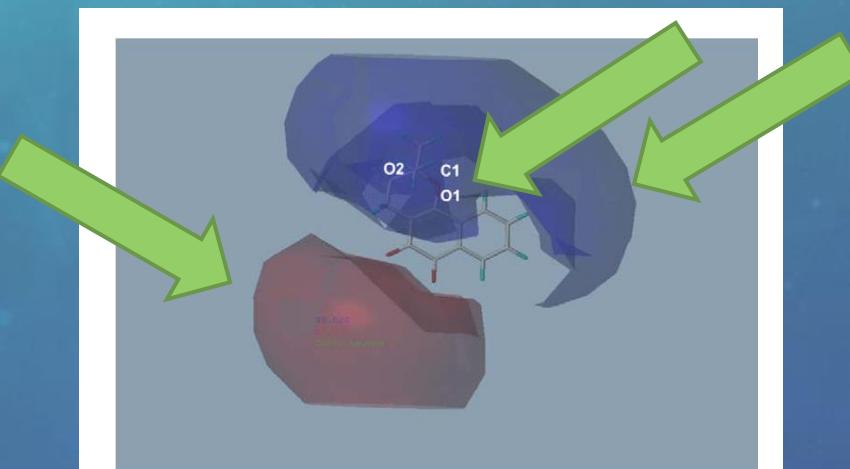
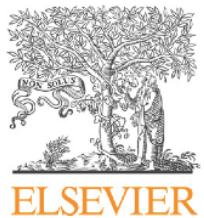


Fig. 5. CoMFA plot showing electrostatic fields for the quinones used. The quinone molecule is represented by sticks and the electronic field contours are represented in blue and red colors. Red field is favorable to negative substituents and blue field is favorable to positive substituents. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl



Synthesis and biological characterization of new aryloxyindole-4,9-diones as potent trypanosomicidal agents

Ricardo A. Tapia ^{a,*}, Cristian O. Salas ^a, Karina Vázquez ^a, Christian Espinosa-Bustos ^a, Jorge Soto-Delgado ^a, Javier Varela ^b, Estefanía Birriel ^b, Hugo Cerecetto ^{b,c}, Mercedes González ^b, Margot Paulino ^d

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^d Centro de Bioinformática Estructural-DETEMA, Facultad de Química, Universidad de la República, C.C. 1157, Montevideo,

R. A. Tapia et al./Bioorg. Med. Chem. Lett. xxx (2014) xxx–xxx

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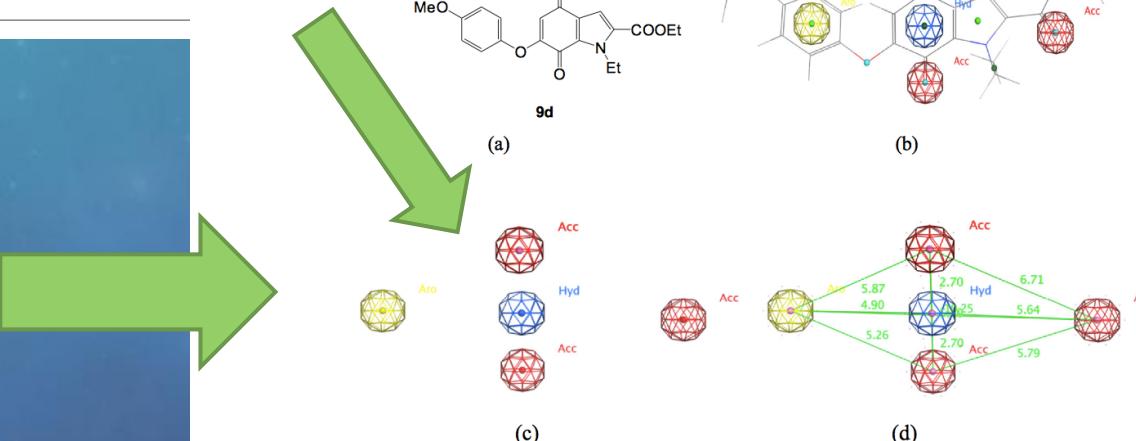
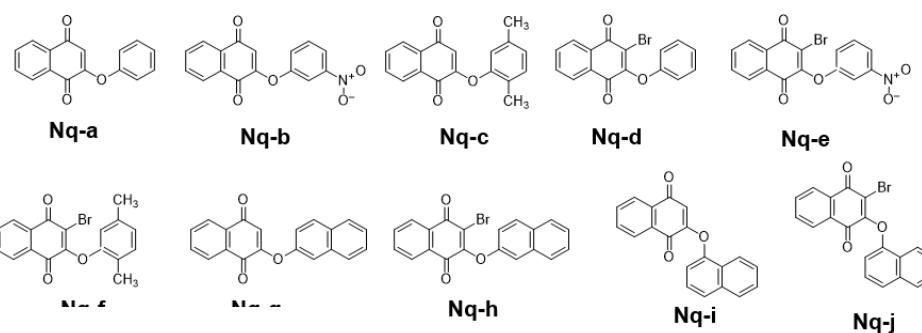
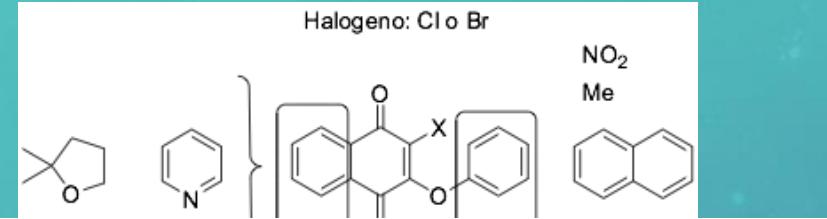


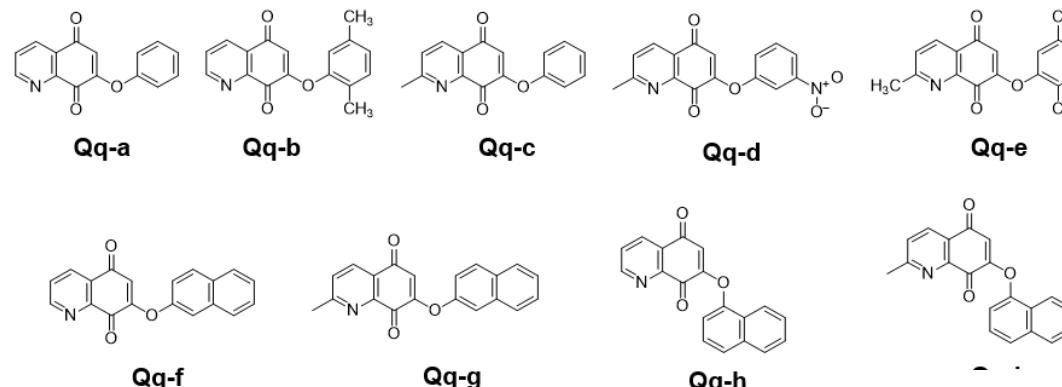
Figure 2. Graphical display of 'Activity Pharmacophore'. (a) 2D picture of the modelled **9d** molecule. (b) 3D drawing of **9d** molecule overlapped with the pharmacophore picture. (c) 3D drawing of isolated pharmacophore. The different features are depicted in yellow (aromatic), red (H-bond acceptor) and blue (hydrophobic). (d) Distances between the centre of pharmacophoric features for **9a** and **9d** molecules are shown in green numbers.

Quinones

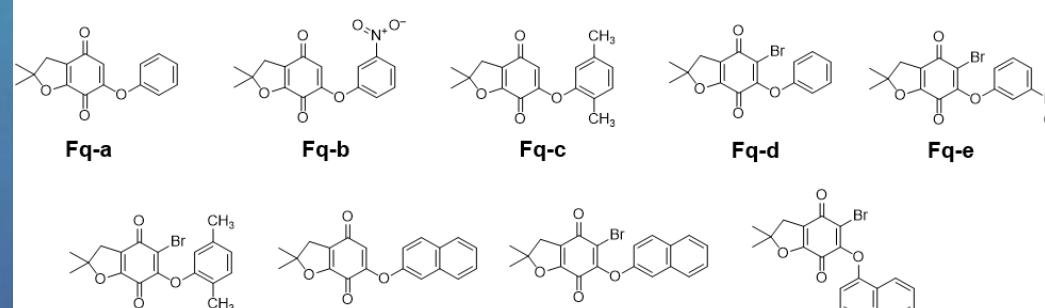
STARTING SAMPLE: 28 synthetic ariloxo-quinones have been synthesized and their tripanosomicidal and putative citotoxic activities were measured [3]



naphtoquinones



quinolinequinones



furanquinones

[3] Vazquez, K., Espinosa-Bustos, C., Soto-Delgado, J., Tapia, R. A., Varela, J., Birriel, E., Segura, R., Pizarro, J., Cerecetto, H., Gonzalez, M., Paulino, M. & Salas, C. O. (2015). *Rsc Advances* 5: 65153-65166.

Test % inhibition of grow in vitro (epi and trypo *T. cruzi*) and in macrophages

	%GI ^{a,b}	IC ₅₀ (μM) ^b	J774 IC ₅₀ (μM)	Selectivity Index ^c	E _{pc1} (V) ^e
3a	87.2 ± 2.1	0.05 ± 0.02	<12.5	<250	-0.610
3b	87.6 ± 1.5	0.02 ± 0.01	12.5	625	-0.526
3c	92.6 ± 1.1	0.17 ± 0.05	<12.5	<73.5	-0.610
3g	79.3 ± 7.8	0.15 ± 0.04	<12.5	<83.3	-0.633
3h	69.6 ± 4.2	0.14 ± 0.05	<12.5	<89.3	-0.456
3i	85.5 ± 1.3	0.17 ± 0.04	<12.5	<73.5	-0.624
3j	99.2 ± 0.7	0.17 ± 0.06	19	111.8	-0.440





Cite this: RSC Adv., 2015, 5, 65153

New aryloxy-quinone derivatives as potential anti-Chagasic agents: synthesis, trypanosomicidal activity, electrochemical properties, pharmacophore elucidation and 3D-QSAR analysis†

Karina Vazquez,^a Christian Espinosa-Bustos,^a Jorge Soto-Delgado,^b Ricardo A. Tapia,^a Javier Varela,^c Estefania Birriel,^c Rodrigo Segura,^d Jaime Pizarno,^d Hugo Cerecetto,^d Mercedes González,^c Margot Paulino,^a and Cristian O. Salas^{**}

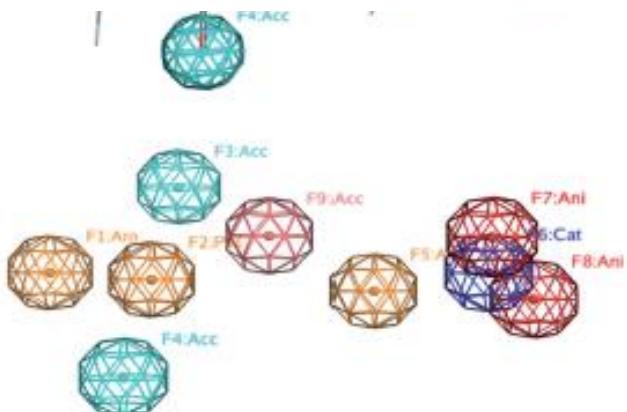


Fig. 2 (a) Pharmacophore generated from 3b overlaid with a rod drawing of molecules; (b) pharmacophoric features depicted in the pharmacophore of 3b. F1 = Aro, aromatic moiety; F2 = Pir, rings with p-orbitalization; F3; F4 = Acc, hydrogen bond acceptor; F5 = Cat, cationic; F7 = Ani, anionic.

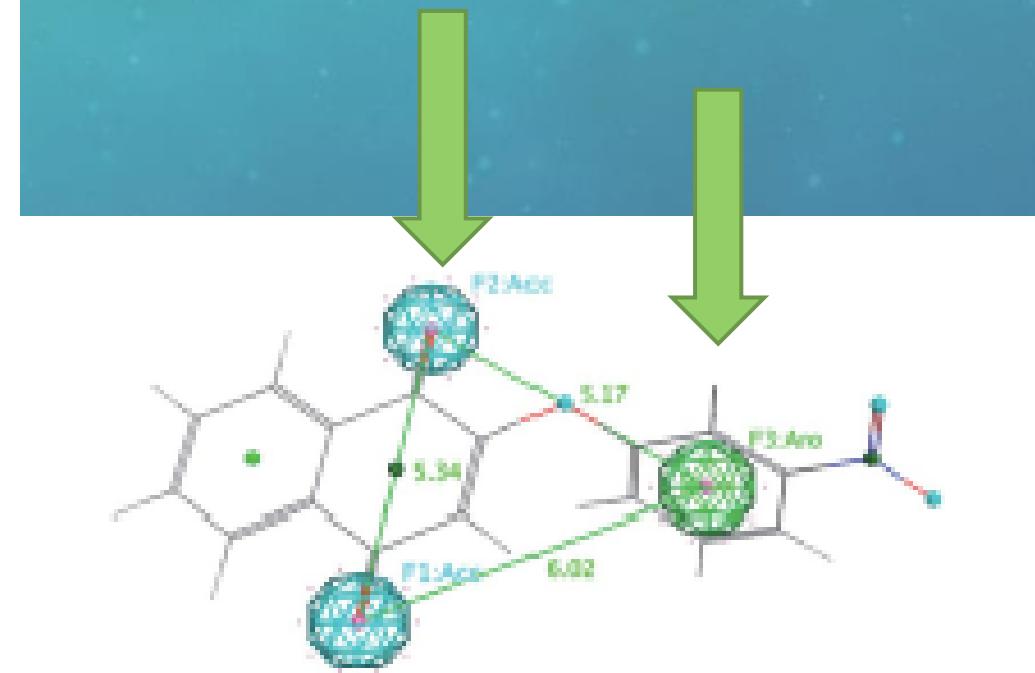
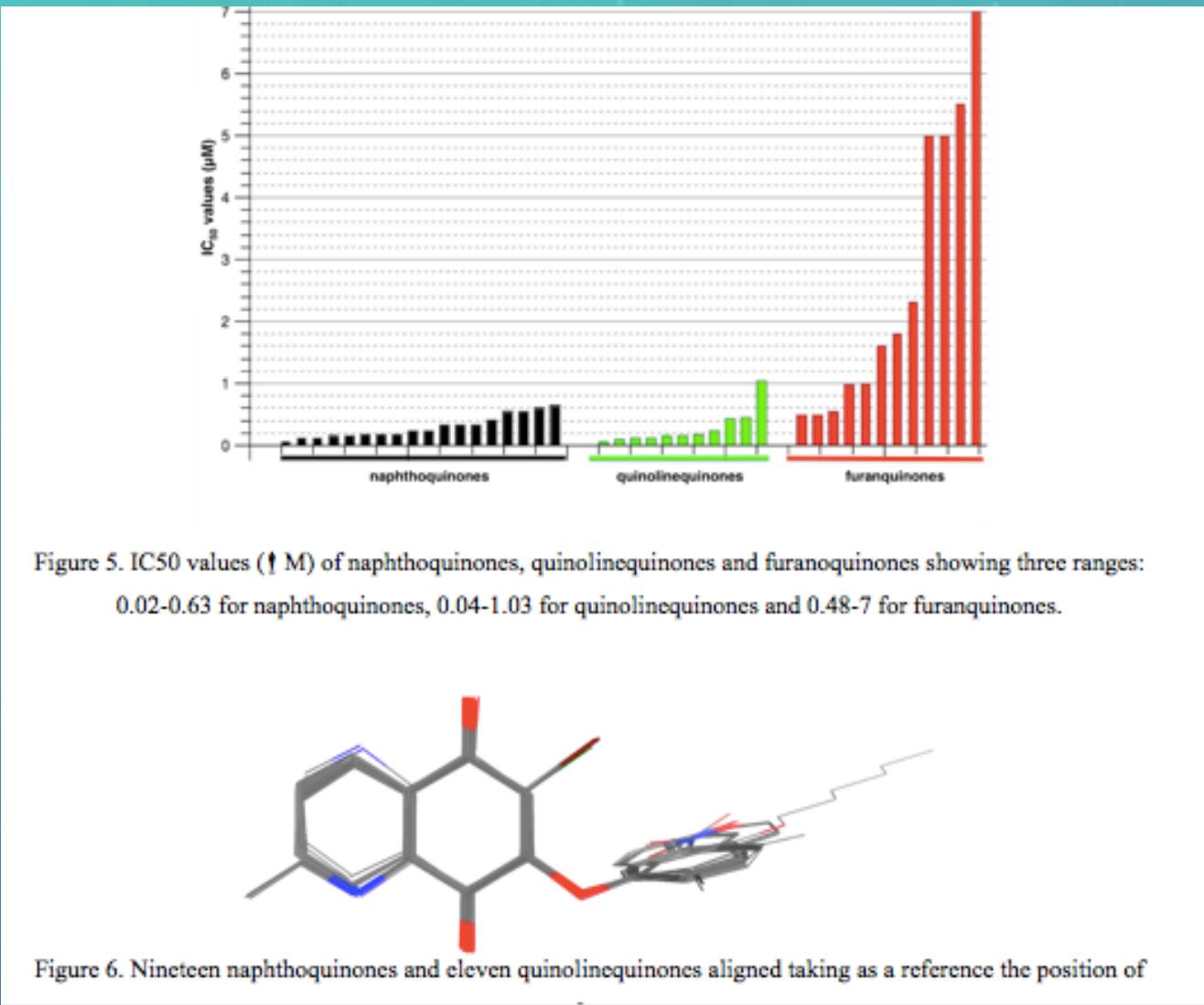


Fig. 3 Selective pharmacophore features and interatomic distances between the essential features Acc (F1 and F2) and Aro (F3).

Exploring the substitution pattern of the aryloxy-quinone scaffold in the development of potential new anti-*T. cruzi* agents: A 3D-QSAR and pharmacophoric analysis. Ch Espinosa-Bustos ^{a*}, K Vázquez ^b, M Gonzalez-Castro ^a, J Mella ^c, J Varela ^d, M Paulino ^e, B Vera ^e, R A. Tapia ^a, H Cerecetto ^{d,f}, M González ^d, and C O. Salas ^{a*}



IMPROVING EXPERIMENTAL AND IN SILICO MODELS TO DILUCIDATE THE MODE OF ACTION

- . AMPLIFIED CHEMICAL SPACE: 28→45→62 QUINONES
- . NEW PARASITOLOGICAL MODELS:
BLOOD CIRCULATING INFECTIVE TRYPOMASTIGOTES
T CRUZI AMASTIGOTES
- . IN SILICO NEW MODELS:
DFT (M05-2X) OF ELECTRONIC DESCRIPTORS
- . SELECTION OF THE BEST CHEMICAL SPACE→ 19

Mode of action of *p*-quinone derivatives with trypanocidal activity studied by experimental and *in silico* models

Andrés Ballesteros-Casallas^{1,2}, Cecilia Ortiz³, Cristina Quiroga³, Diego Benítez³, Pablo Denis⁴, David Figueroa¹, Cristian O. Salas⁵, Jeanluc Bertrand⁵, Ricardo A. Tapia⁵, Patricio Sanchez⁵, Gian Pietro Mischione^{1*}, Marcelo A. Comini^{3*}, Margot Paulino^{2*}

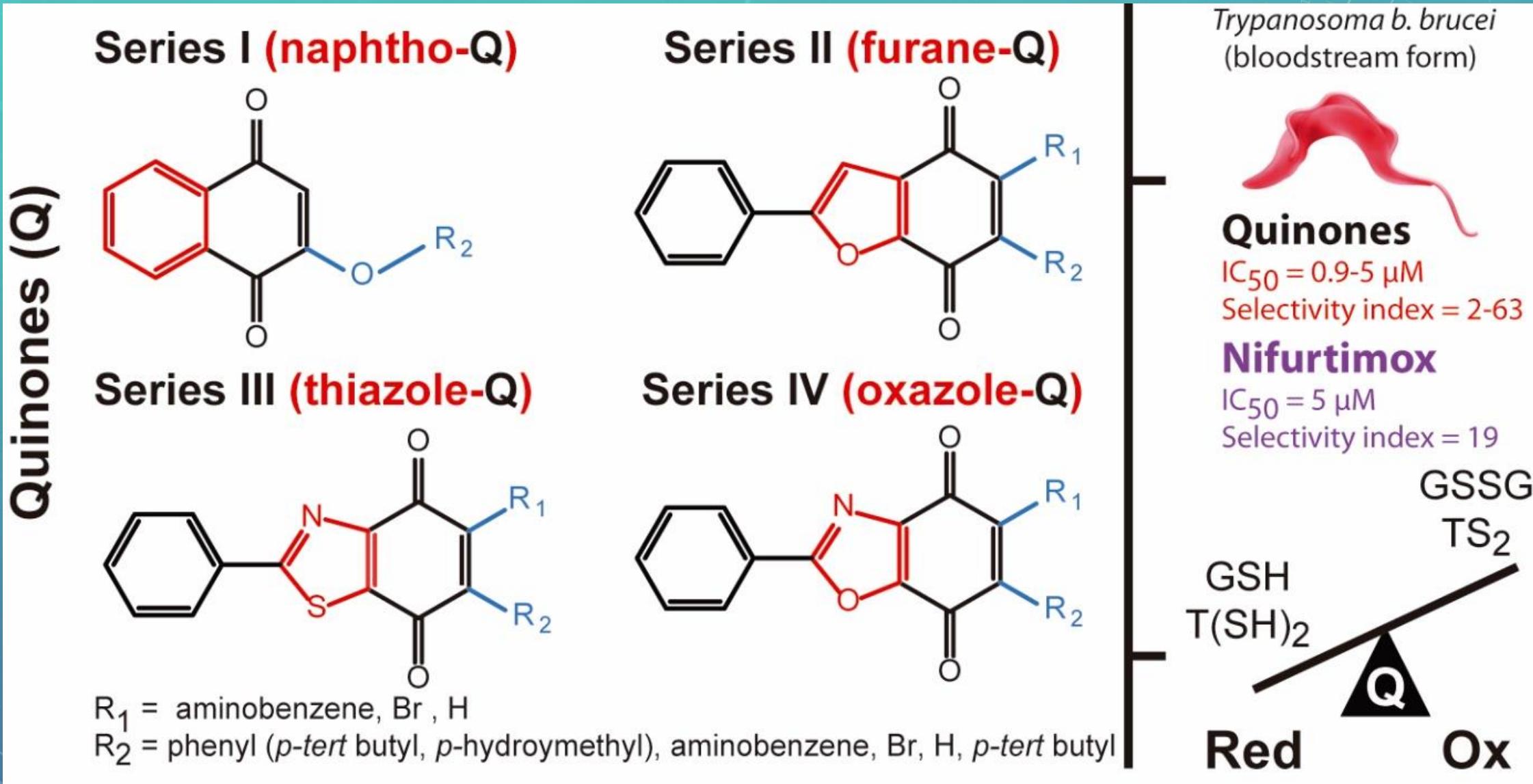
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² Bioinformatics Center, DETEMA Department, Faculty of Chemistry, Universidad de la República, General Flores 2124, Montevideo 11600, Uruguay.

³ Laboratory Redox Biology of Trypanosomes, Institut Pasteur de Montevideo, Mataojo 2020, Montevideo 11400, Uruguay.

⁴ Computational Nanotechnology, DETEMA Department, Faculty of Chemistry, Universidad de la República, General Flores 2124, Montevideo 11600, Uruguay.

⁵ Departamento de Química Orgánica, Facultad de Química y de Farmacia, Pontificia Universidad Católica de Chile, Av. Vicuña Mackenna 4860, Santiago 6094411, Chile.



Trypanosoma b. brucei
 (bloodstream form)



Quinones

$IC_{50} = 0.9\text{--}5 \mu\text{M}$

Selectivity index = 2–63

Nifurtimox

$IC_{50} = 5 \mu\text{M}$

Selectivity index = 19

GSSG

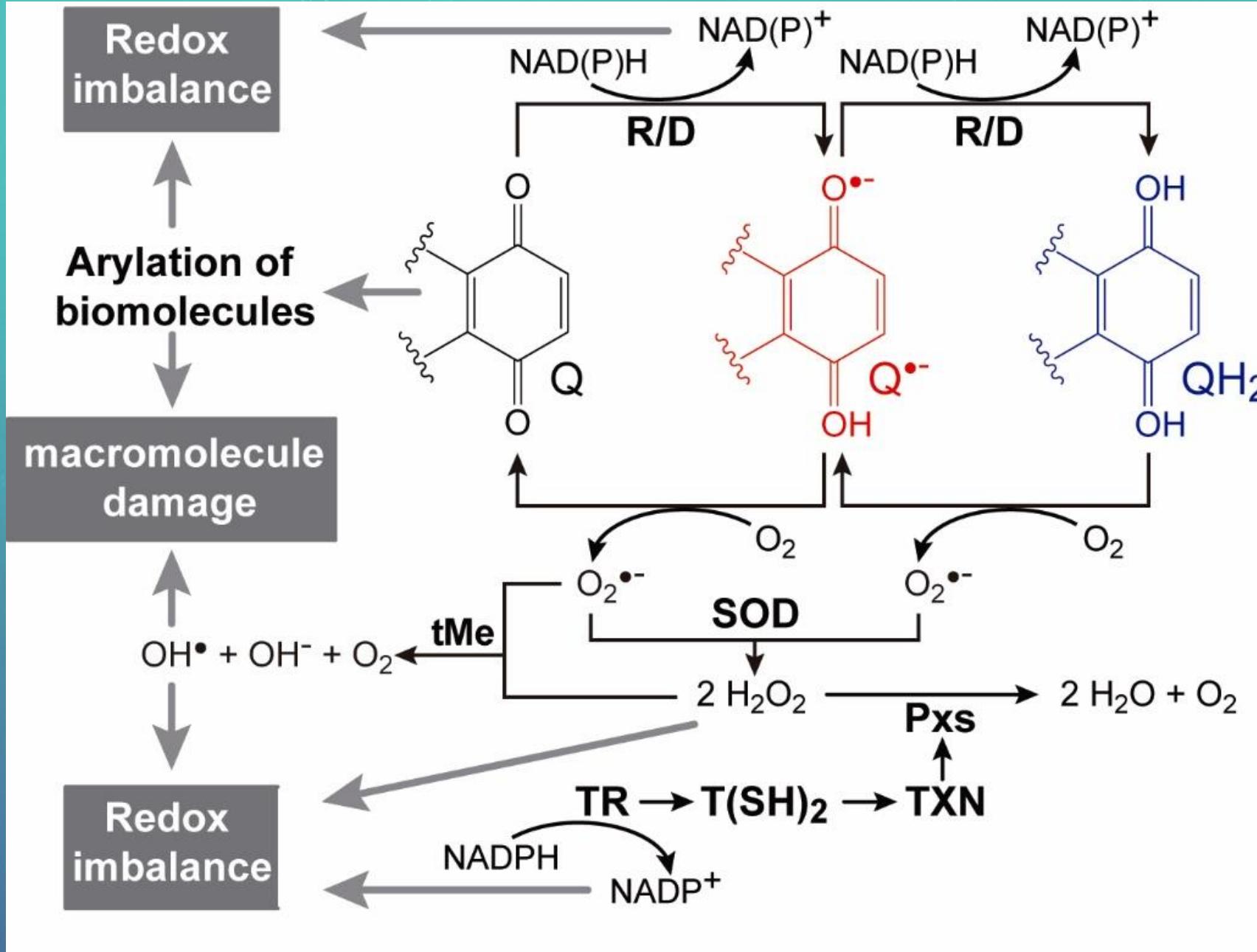
TS₂

GSH
 $T(\text{SH})_2$

Red

Ox





SAR : the description of the chemical space

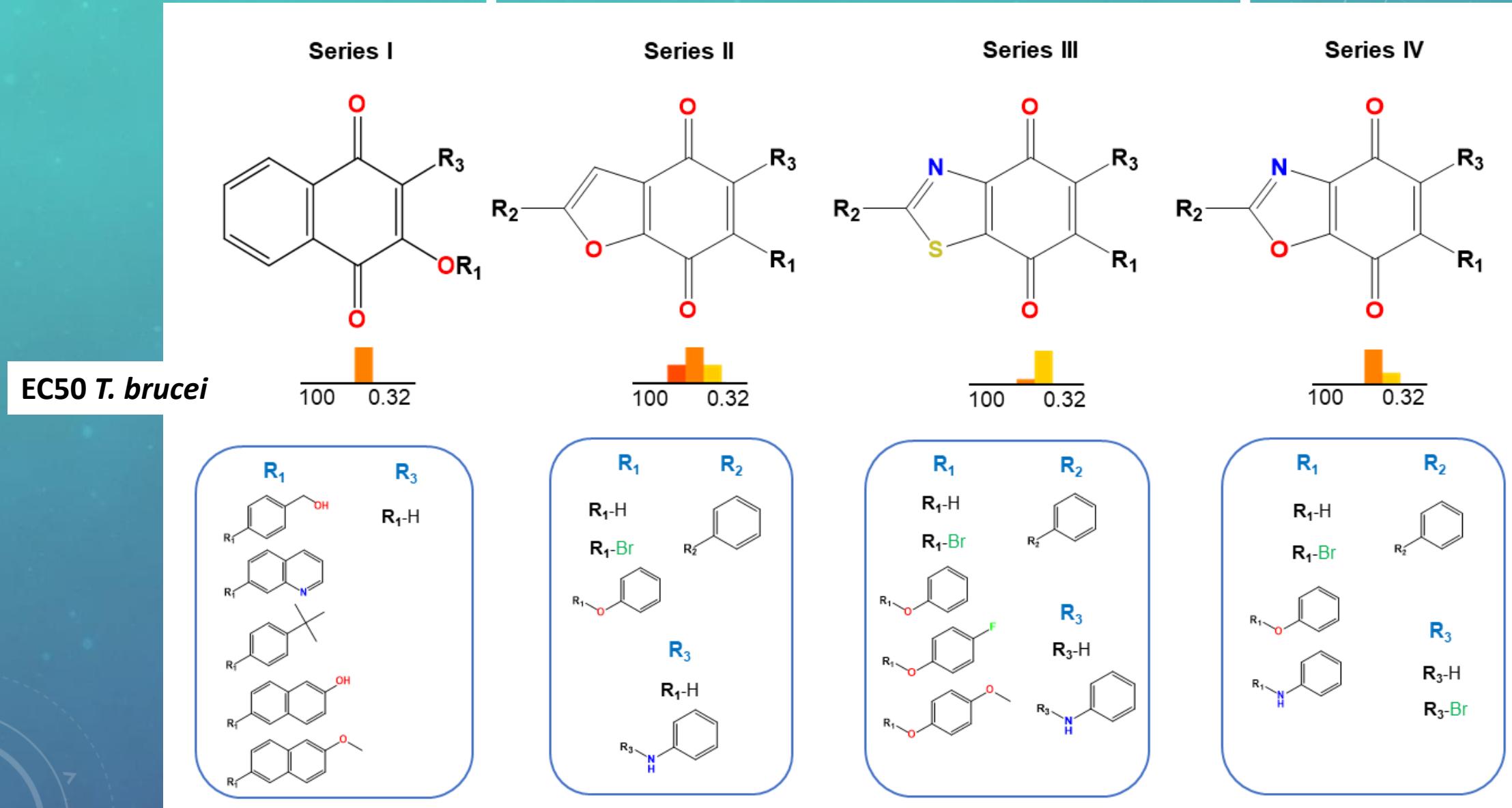


Table 1. Biological activity of *p*-quinones against the infective stages of *T. brucei* (bloodstream form), *L. infantum* and *T. cruzi* (intracellular amastigotes), and murine macrophages (J774 cells).

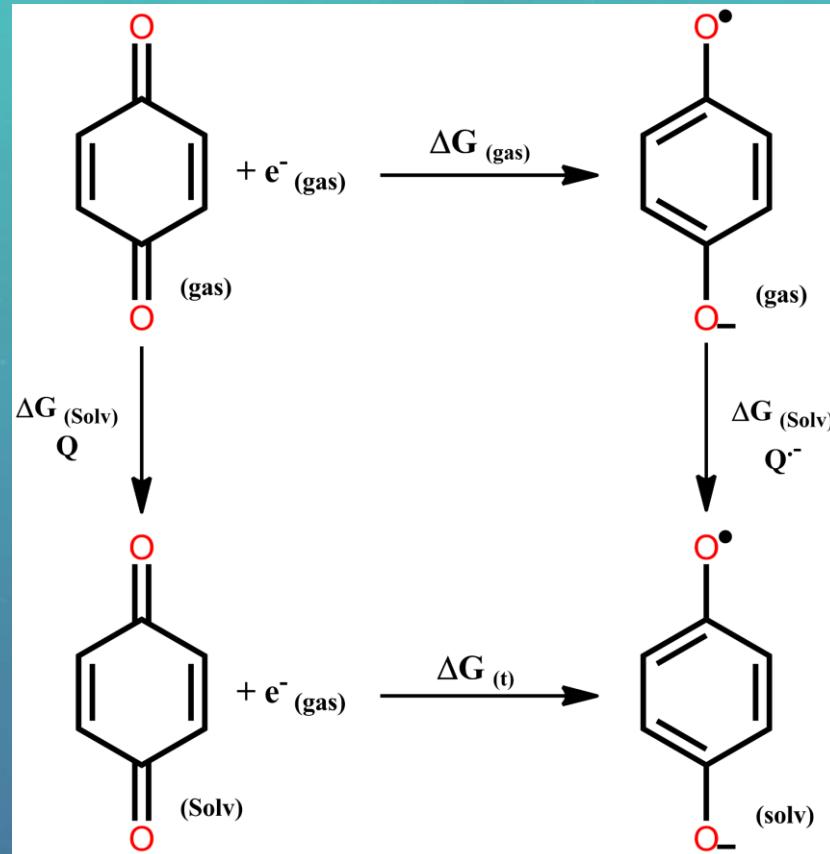
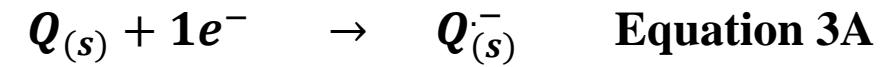
Series	Compound	<i>T. brucei</i>			Selectivity index (SI)		<i>L. infantum</i> * % Viability or <i>EC</i> ₅₀ (μ M)	<i>T. cruzi</i> % Viability or <i>EC</i> ₅₀ (μ M) and SI		
		Murine macrophages			<i>CC</i> ₅₀ (μ M)	<i>CC</i> ₅₀ macrophage / <i>EC</i> ₅₀ <i>T. brucei</i>				
		<i>EC</i> ₅₀ (μ M)	% Viability at 10 μ M	50 μ M						
I	1	5.2	-	57	~50	~10	-	-		
	2	3.8	-	37	< 50	< 13	-	-		
	3	5.5	-	24	< 50	< 9	-	-		
	4	4.3	-	63	~50	~14	-	-		
	5	4.6	-	49	~50	~11	-	-		
II	6	2.7	-	-	118	44	129 (at 10 μ M)	88 (at 10 μ M)		
	7	38	-	-	59	1,6	-	-		
	8	2.9	-	-	37	13	-	-		
	9	0.9	-	-	57	63	102 (at 10 μ M)	0.8 / 71		
	10	1.3	0	-	< 10	< 8	-	-		
	11	1.5	-	-	61	40	91 (at 20 μ M)	78 (at 10 μ M)		

Series	Compound	(μ M)	10 μ M	50 μ M	(μ M)	/ EC ₅₀ <i>T. brucei</i>	EC ₅₀ (μ M)	SI
I	1	5.2	-	57	~50	~10	-	-
	2	3.8	-	37	< 50	< 13	-	-
	3	5.5	-	24	< 50	< 9	-	-
	4	4.3	-	63	~50	~14	-	-
	5	4.6	-	49	~50	~11	-	-
II	6	2.7	-	118	44	129 (at 10 μ M)	88 (at 10 μ M)	
	7	38	-	-	59	1,6	-	-
	8	2.9	-	-	37	13	-	
	9	0.9	-	57	63	102 (at 10 μ M)	0.8 / 71	
III	10	1.3	0	-	< 10	< 8	-	-
	11	1.5	-	61	40	91 (at 20 μ M)	78 (at 10 μ M)	
	12	1.8	40	-	< 10	< 6	-	-
	13	0.45	17	< 10	< 22	-	-	
	14	1.8	-	78	43	90 (at 20 μ M)	1.1 / 71	
	15	5.0	5.0	-	< 10	< 2	-	-

Compound	%BO	$\Delta G(Q\cdot)$	$\Delta G(QH_2)$	SUMO ($Q\cdot$)	HOMO Q	LUMO Q	HOMO QH_2	LUMO QH_2
1	59.07	-404	-3345	-556.4	-706.0	-237.1	-673.1	-42.7
2	7.92*	-415	-3321	-558.1	-762.6	-249.3	-677.6	-83.3
3	81.70	-413	-3308	-554.6	-761.8	-245.9	-670.8	-35.2
4	40.54	-402	-3370	-555.6	-826.8	-237.1	-671.8	-36.8
5	87.33	-403	-3365	-556.2	-716.7	-236.9	-673.1	-44.9
6	95.33	-431	-3295	-584.0	-745.1	-261.5	-702.7	32.4
7	99.25	-445	-3290	-591.3	-749.5	-280.0	-679.6	-68.8
8	85.40	-425	-3317	-574.1	-744.3	-258.5	-657.3	-58.4
9	71.31	-423	-3283	-575.8	-709.1	-253.2	-676.9	-70.8
10	99.42	-439	-3340	-591.2	-794.2	-272.8	-715.6	-104.3
11	96.99	-453	-3290	-599.7	-797.1	-291.3	-714.9	-104.6
12	95.51	-434	-3338	-583.4	-783.5	-271.3	-705.6	-101.9
13	85.67	-431	-3283	-580.9	-724.5	-266.8	-690.6	-119.5
14	89.22	-424	-3345	-595.4	-763.3	-269.9	-697.1	-103.5
15	92.06	-436	-3307	-596.9	-789.0	-271.2	-699.6	-104.4
16	100	-445	-3357	-601.7	-795.9	-273.2	-709.3	-81.7
17	20.95	-459	-3304	-610.0	-798.7	-292.2	-732.9	-91.4
18	94.92	-439	-3344	-596.2	-789.1	-265.6	-719.4	-87.5
19	95.87	-436	-3298	-587.7	-724.6	-270.5	-698.4	-93.1

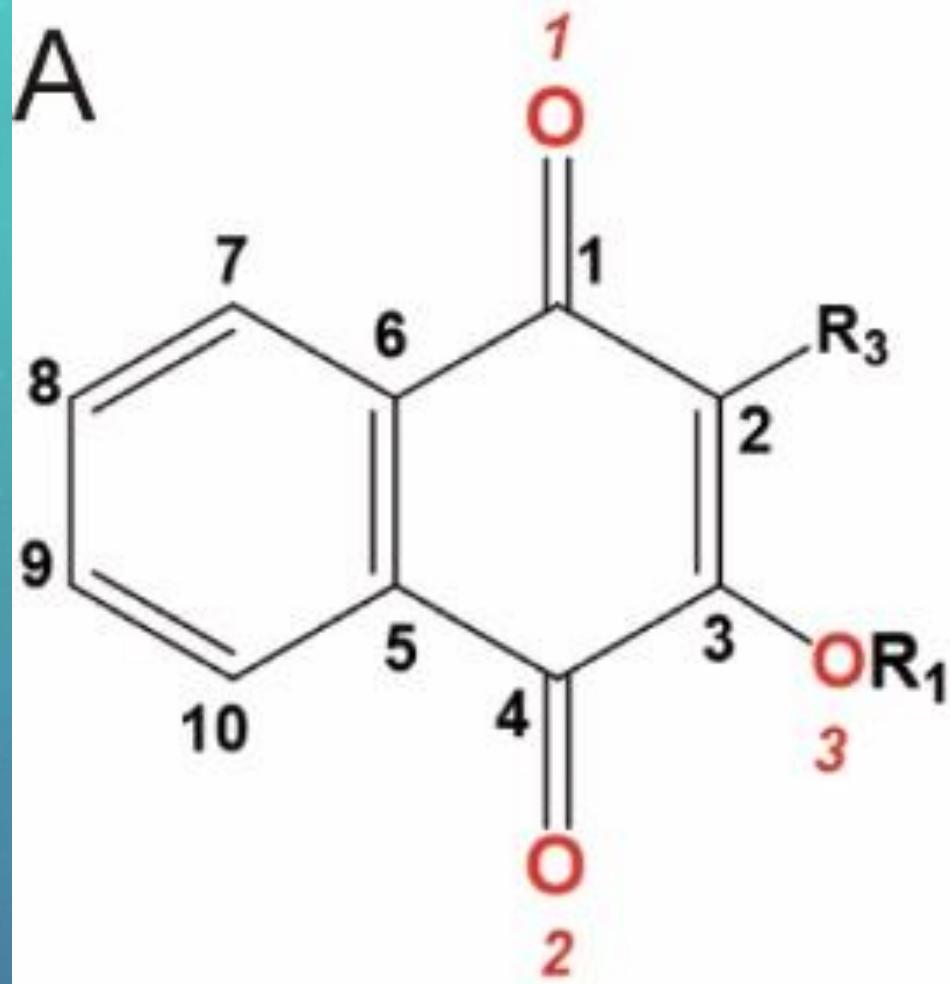
Table 2. Percentage of biosensor oxidation (%BO) and DFT parameters for *p*-quinones.

TWO ELECTRON IN SECUENCIAL STEPS



$$\Delta G_t^\circ = \Delta G_{(g)}^\circ + \Delta G_{(Solv)}^\circ(Q^{\bullet-}) - \Delta G_{(s)}^\circ(Q) \quad \text{Equation 4}$$

A



B

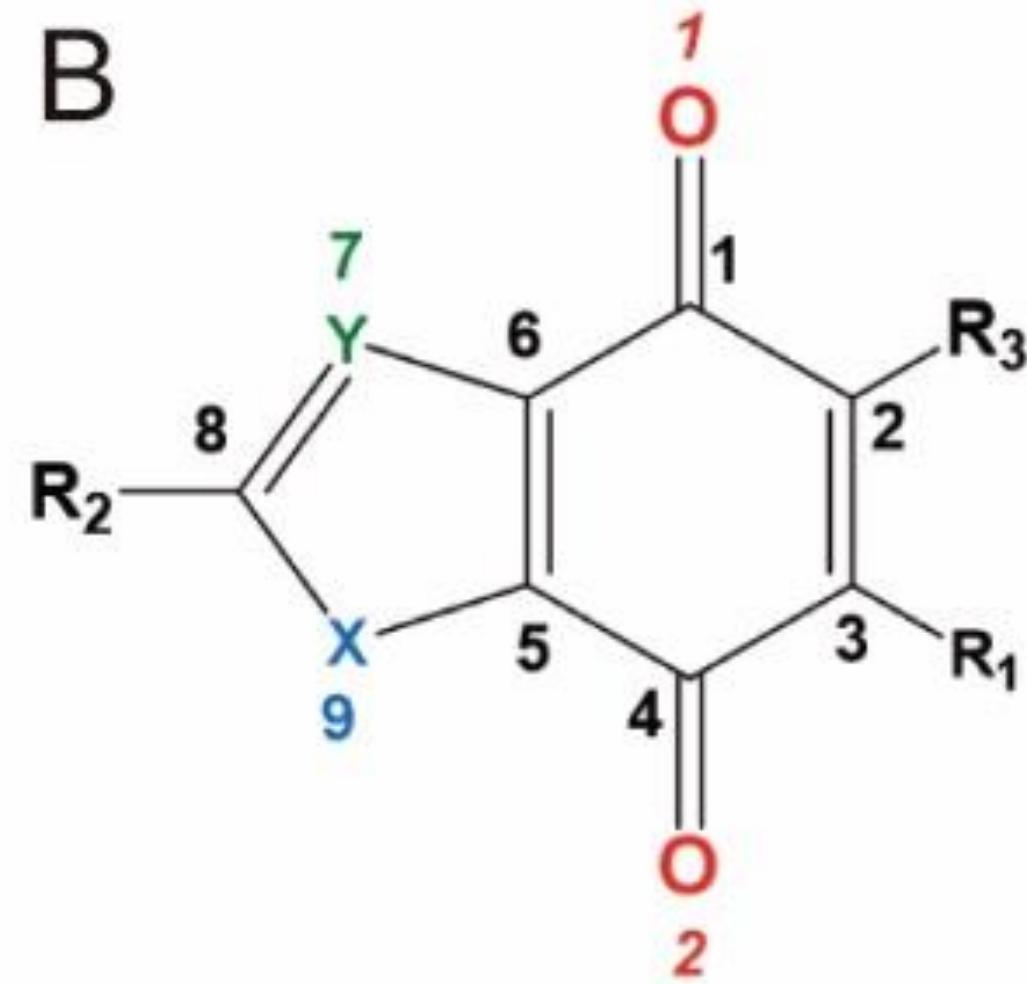


Table 3. Correlation indices of the electronic descriptors with pEC50 and with the percentage oxidation of the biosensor (%BO). Asterisk * is annotated to statistically significant correlations.

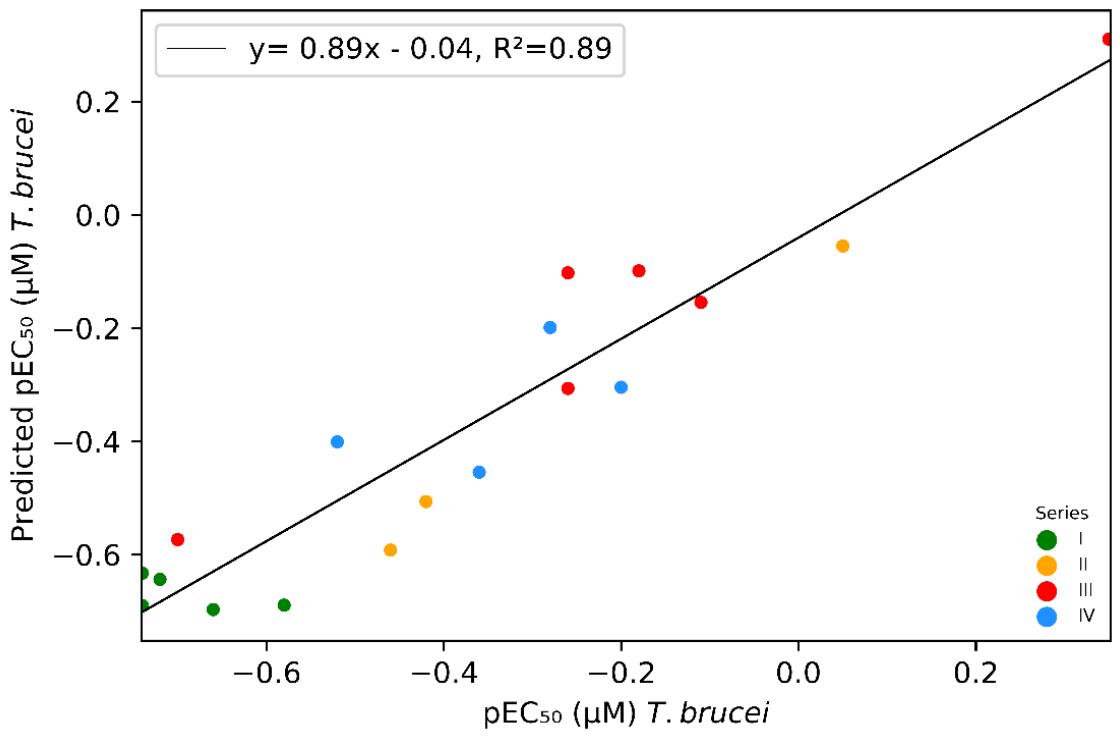
	pEC50	%OB
Index	r/rho	r/rho
$\Delta G (Q^-)$	-0.52*	-0.89*
$\Delta G (QH_2)$	0.53*	0.13
SUMO (Q^-)	-0.48*	-0.78*
HOMO Q	0.16	-0.43
LUMO Q	-0.55*	-0.87*
HOMO QH_2	-0.37	-0.76*
LUMO QH_2	-0.63*	-0.43
η	-0.46	0.16

	pEC50	%OB
Index	r/rho	r/rho
O1-Q	0.46	0.20
O2-Q	-0.08	0.14
C1-Q	-0.60*	-0.85*
C2-Q	-0.60*	0.43
C3-Q	0.87*	0.52*
C4-Q	-0.66*	-0.48
C5-Q	0.53*	0.49*
C6-Q	-0.23	0.17
Y7-Q	0.07	-0.35
C8-Q	0.74*	0.29
X9-Q	0.71*	0.36

	pEC50	%OB
Index	r/rho	r/rho
O1-Q ⁻	0.26	-0.13
O2-Q ⁻	0.43	-0.09
C1-Q ⁻	-0.50*	-0.70*
C2-Q ⁻	-0.42	0.08
C3-Q ⁻	0.79*	0.20
C4-Q ⁻	-0.47*	-0.26
C5-Q ⁻	0.48*	0.46
C6-Q ⁻	-0.21	0.16
Y7-Q ⁻	-0.28	-0.17
C8-Q ⁻	0.48*	-0.08
X9-Q ⁻	0.44	0.51*

Normalized estimated QSAR 2D linear model, obtained dividing the coefficients by the standard deviation of the variables:

$$\text{pEC50 } T. brucei = -5.9439 - 0.1492 * \Delta G(Q\cdot) - 0.6555 * C1Q\cdot - 0.8227 * C2Q$$

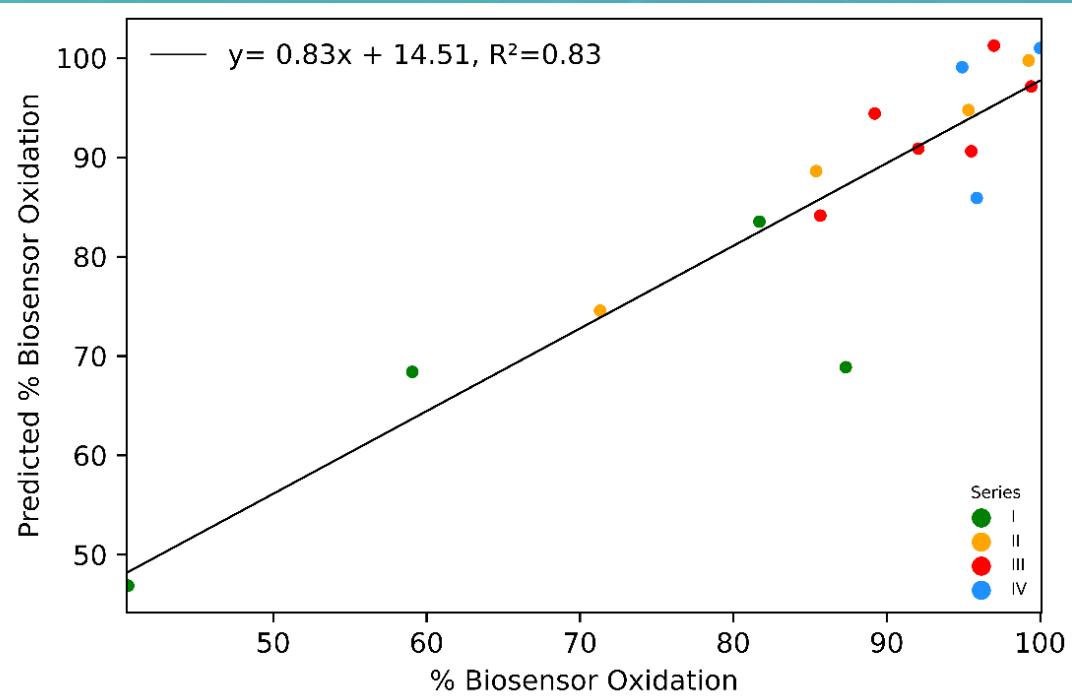


Statistical values: $R^2 = 0.89$ RMSE = 0.09.
After cross validation Leave One Out (LOO):
 $R^2_{cv} = 0.82$, $RMSE_{cv} = 0.12$

→good quality models: $R^2 > 0.6$ and low RMSE (there is not a limit value)

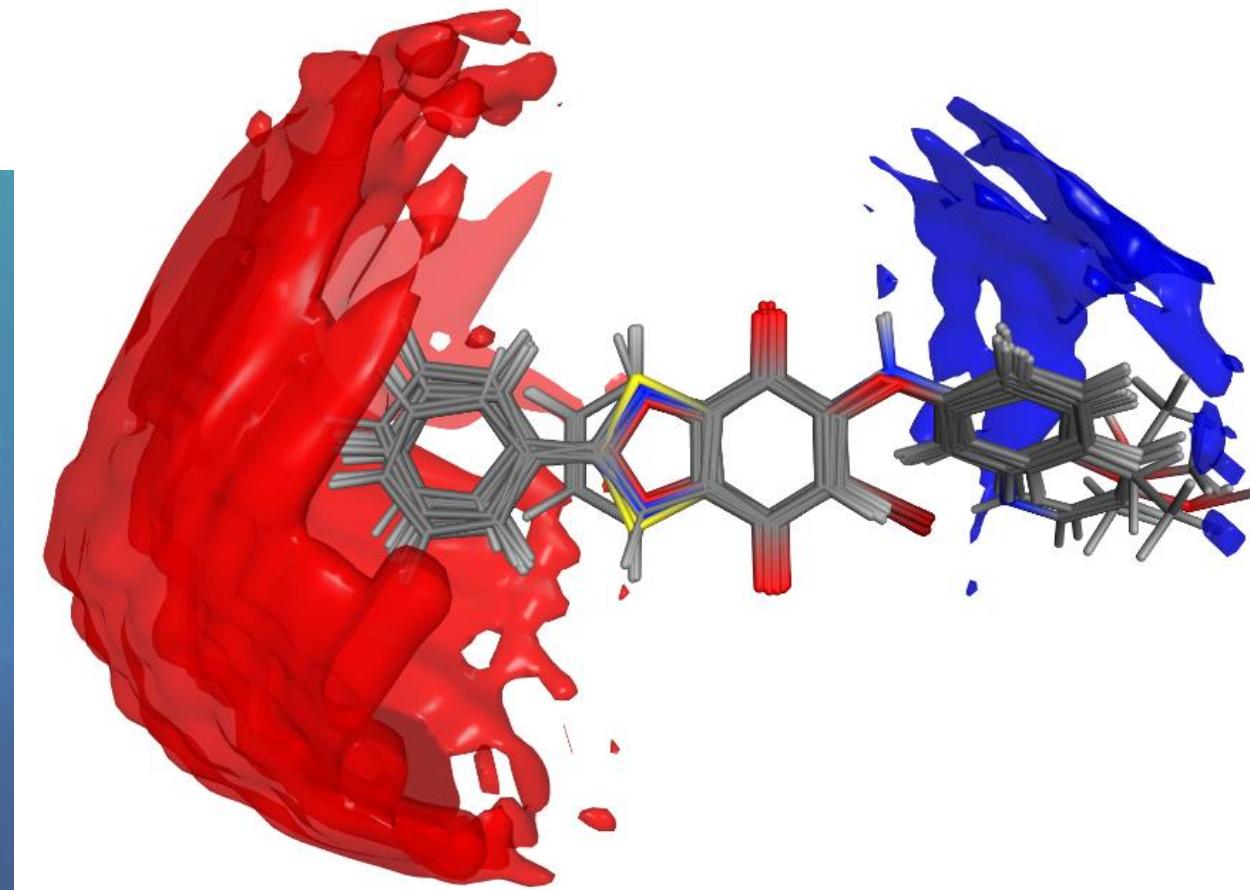
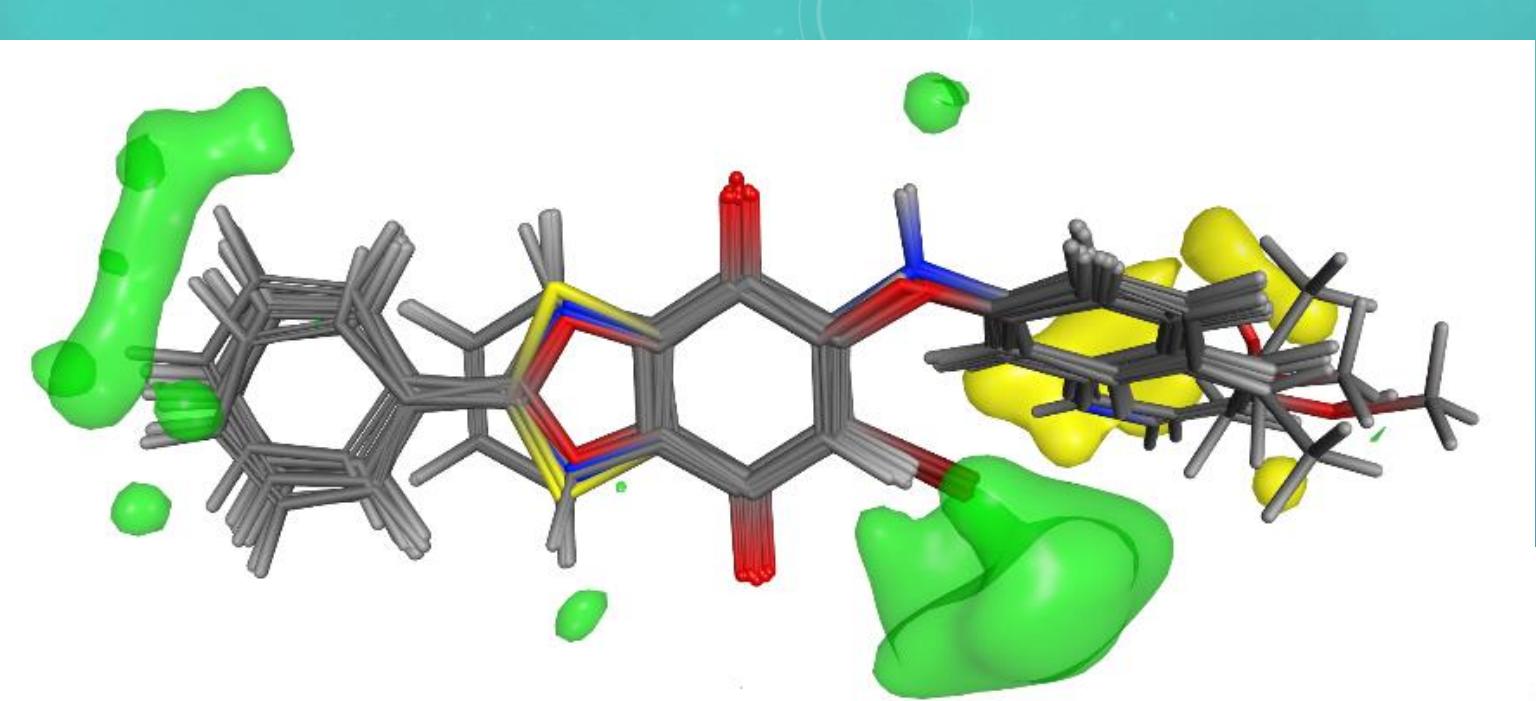
Normalized estimated QSAR 2D linear model, obtained dividing the coefficients by the standard deviation of the variables:

$$\%BO = -9.3040 + 0.6822 * C2Q\cdot + 0.8430 * C3Q - 0.3675 * O1Q\cdot$$



The statistical values obtained:
 $R^2 = 0.83$ RMSE = 6.36.
After cross validation leave one out procedure were: $R^2_{cv} = 0.70$, $RMSE_{cv} = 8.52$.

→good quality models: $R^2 > 0.6$ and low RMSE (there is not a limit value)



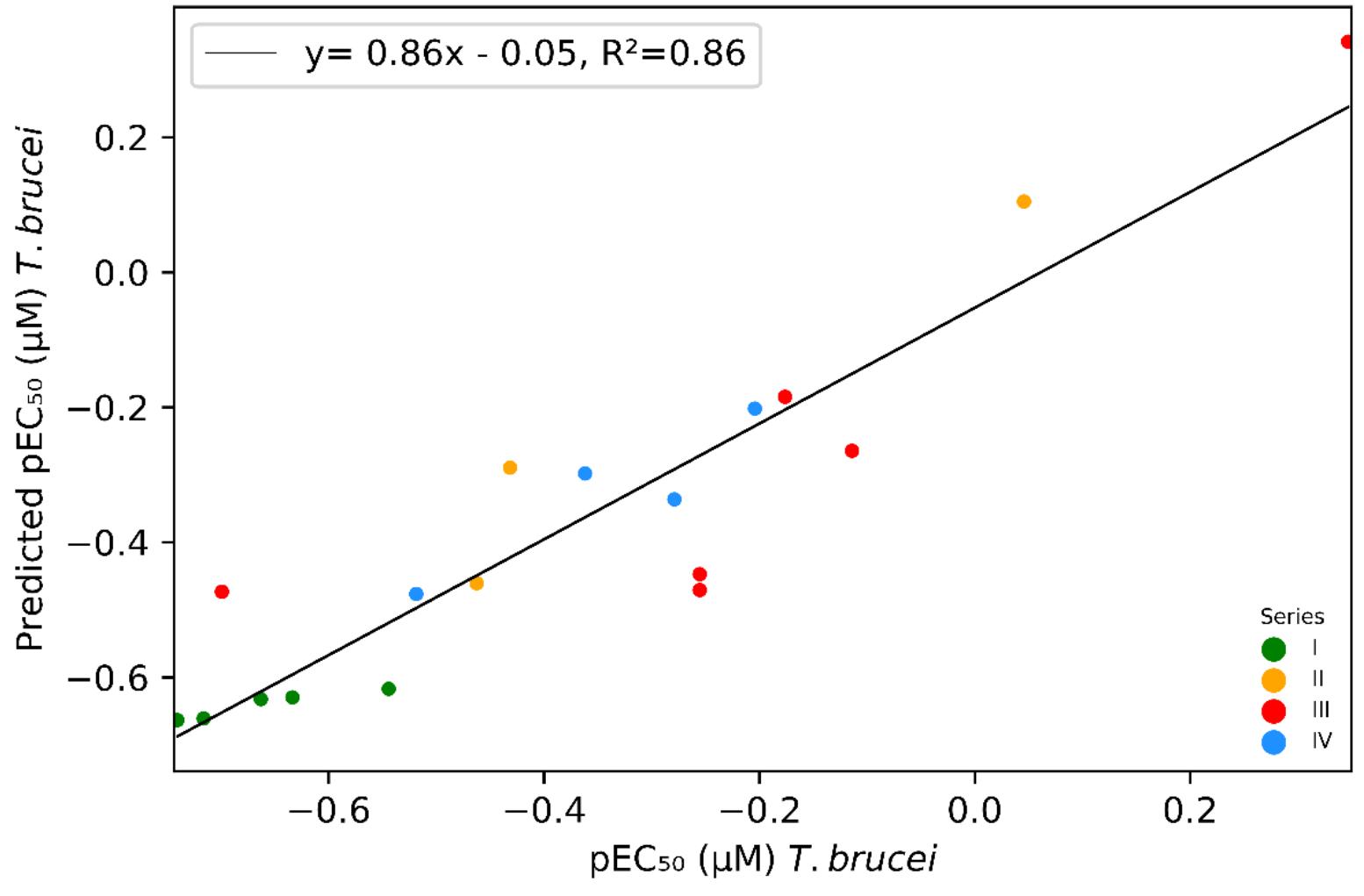
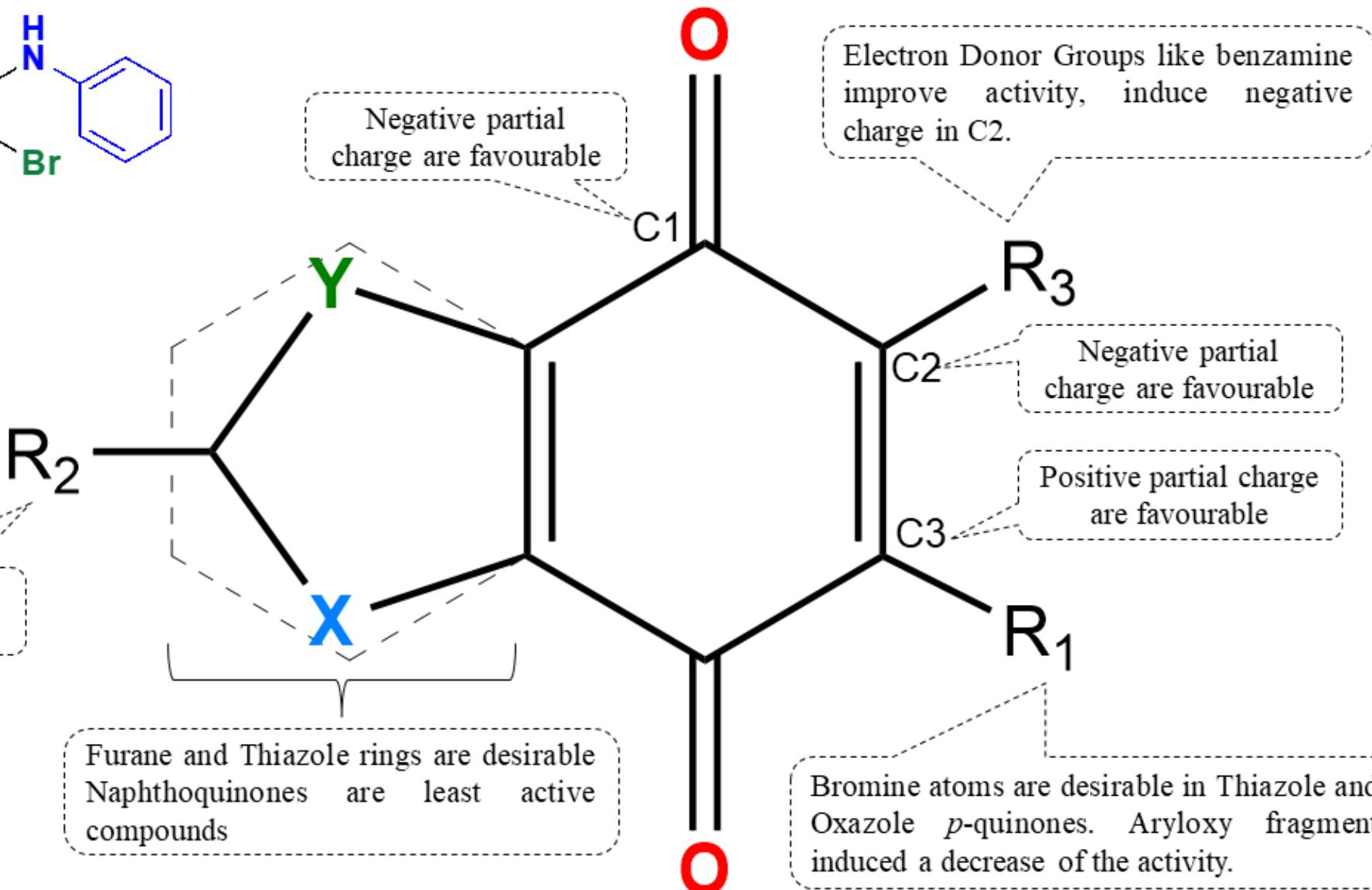
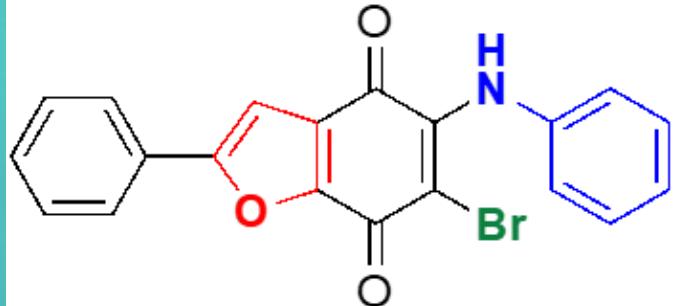
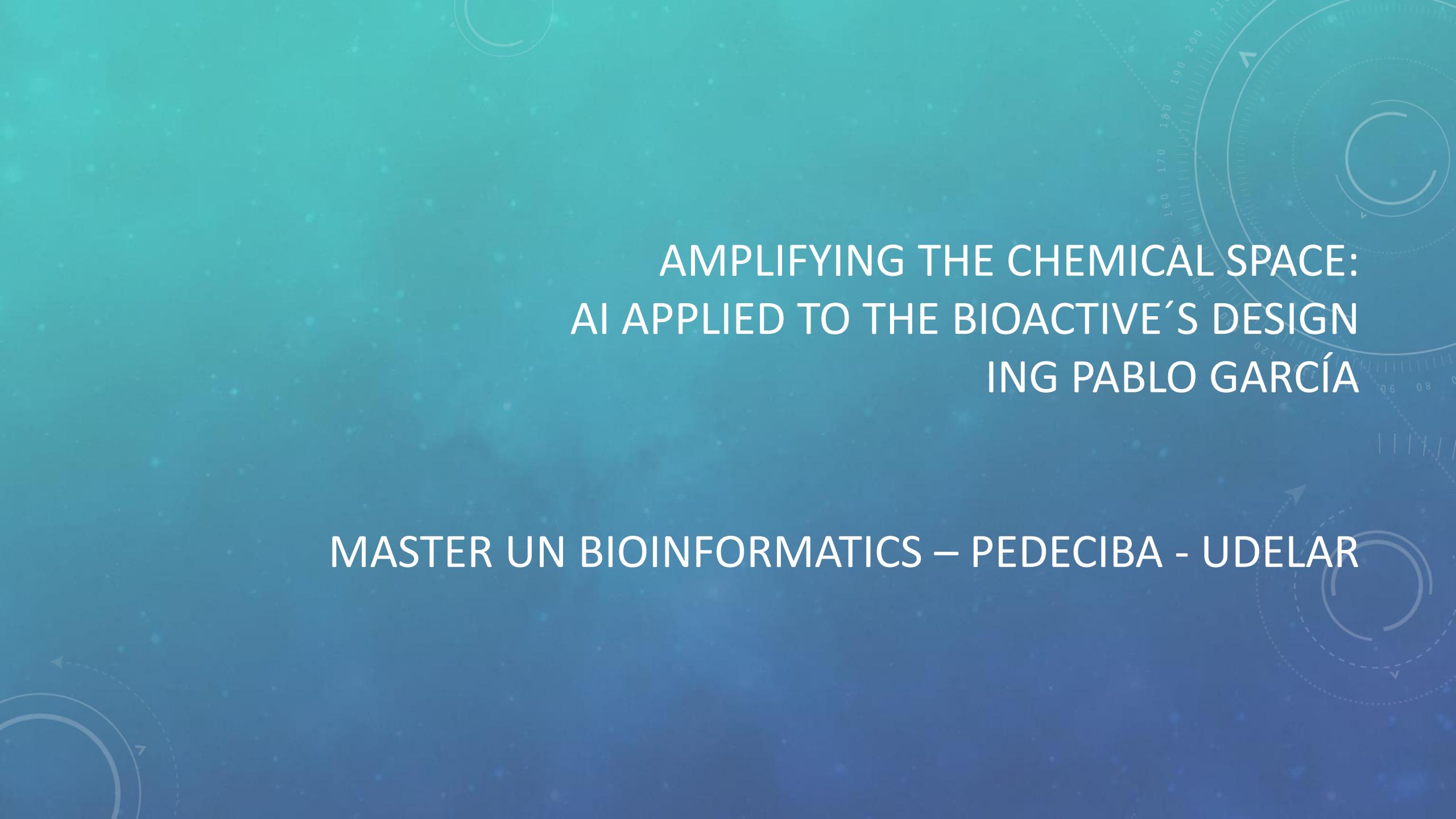


Table 4. Statistic parameters of the QSAR COMFA model.

PC	RMSE	q^2
0	0.299	-0.121
1	0.195	0.524
2	0.155	0.701
3	0.151	0.713
4	0.160	0.679
5	0.162	0.671





AMPLIFYING THE CHEMICAL SPACE: AI APPLIED TO THE BIOACTIVE'S DESIGN ING PABLO GARCÍA

MASTER UN BIOINFORMATICS – PEDECIBA - UDELAR

CHALLENGES AND TOOLS



Tensorflow and Tensorflow probabilistic platforms
Networks feeded by graphs convolution (to matrix)
Pockets identified by fpocket
Scores based on gnina, autodock vina, Networks trained with qm9, tox21 and delaney

To acquire data on interactions of molecules with bioactivity in proteins

To represent the information in such a way that a neural network can use it to learn

generate new ligands (originals)

To transfer knowledge to a network designed to experiment in the generation of new ligands for a new target

To optimize new ligands until they can be validated by traditional bioinformatics methods

FILTERING AND TRAINING...

PDB Bind structure

PDB 30000 → Protein-ligand =
23700 pairs

Filter → $-\log K_i < 5 \mu M$

→ 18800 pairs with which to learn



OBSERVING THE ASSOCIATION OF THE QUINONES WITH TARGETS SBDD

SBDD (in TR/GR)

The 28 synthetic ariloxy-quinones have been studied by biochemical and *in silico* methods in their interaction with an essential enzyme of the parasite, trypanothione reductase (TR) and its mammalian counterpart, glutathione reductase (GR) [4].



Journal of Biomolecular Structure and Dynamics

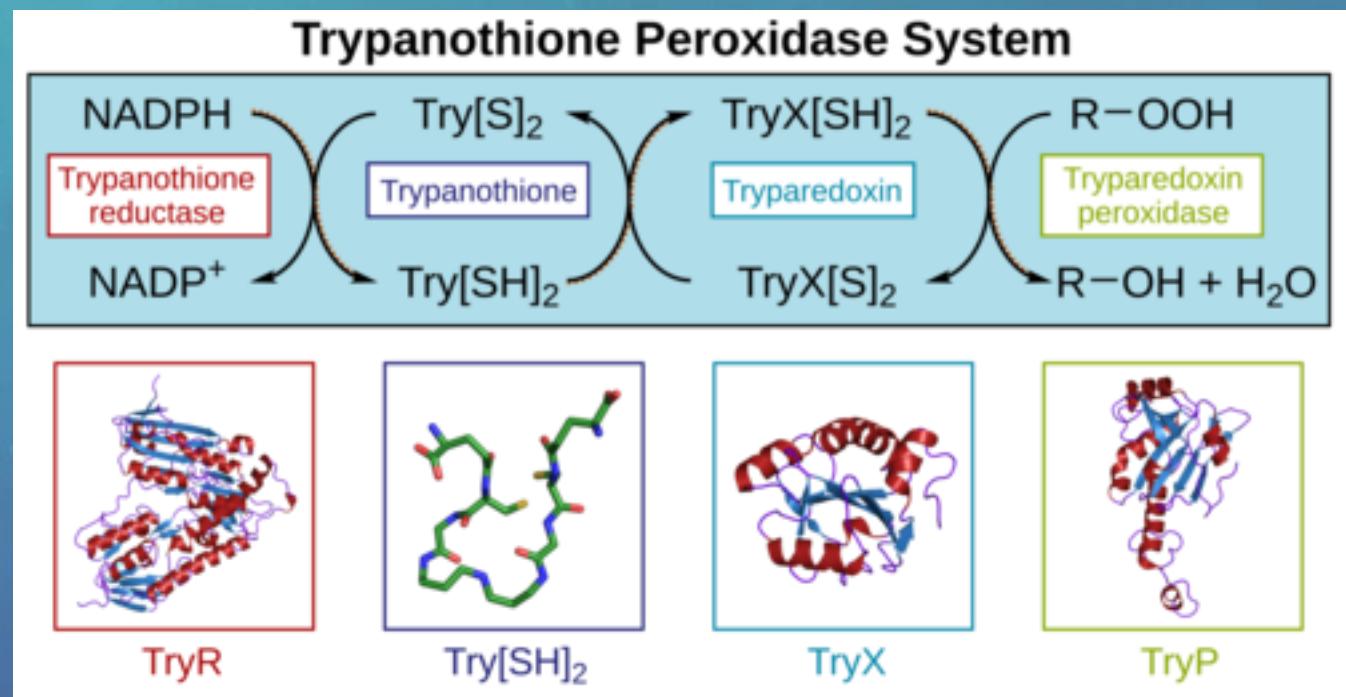
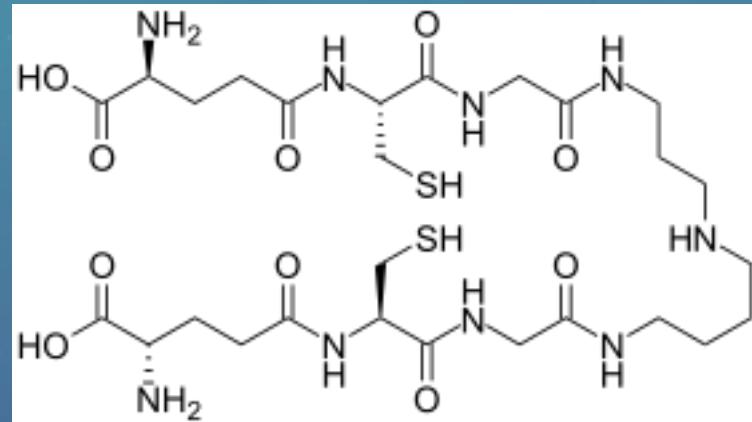
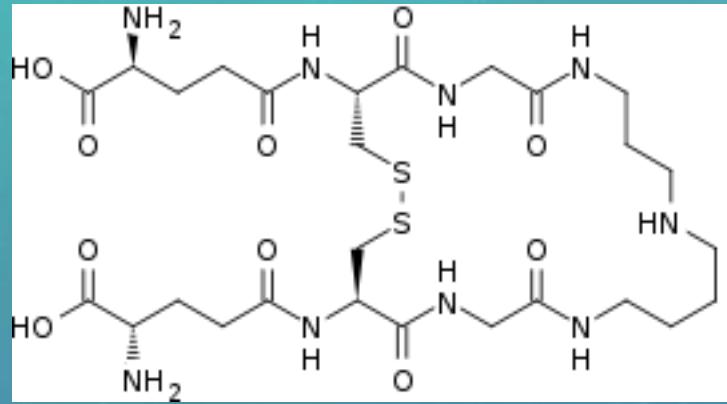
Taylor & Francis
Taylor & Francis Group

ISSN: 0739-1102 (Print) 1538-0254 (Online) Journal homepage: <http://www.tandfonline.com/loi/tbsd20>

Structural Analysis and Molecular Docking of Trypanocidal Aryloxy-quinones in Trypanothione and Glutathione Reductases: A Comparison with Biochemical Data

Brenda Vera, Karina Vázquez, Carolina Mascayano, Ricardo A. Tapia, Victoria Espinosa, Jorge Soto-Delgado, Cristian O. Salas & Margot Paulino

Trypanothione Reductase: crucial (essencial) key target of redox oxidative defenses in parasites → NADPH consumption



BIOCHEMISTRY (in TR/GR)

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Table 1: First column, coded name of quinones. Second and Third columns: IC₅₀ for the *T. cruzi* growth inhibition and cytotoxicity in J-774 IC₅₀ in μ M. Fourth and Fifth columns: percentages of trypanothione and glutathione reductase inhibition and in parentheses are annotated the related concentrations in μ M. * Data are from Vazquez et al 2015.

Molecule	IC ₅₀ <i>T. cruzi</i> Epimastigote*	J-774 IC ₅₀ *	% TR inhibition	% GR inhibition
Naphthoquinones				
Nq-a	0.05 ± 0.04	< 12.5	62 % (5)	50 % (7.32)
Nq-b	0.02 ± 0.01	12.5	57 % (100)	50 % (1.4)
Nq-c	0.17 ± 0.05	< 12.5	46 % (2.5)	50 % (3.9)
Nq-d	0.11 ± 0.04	18	53 % (5)	50 % (>100)
Nq-e	0.11 ± 0.04	46	41 % (10)	50 % (>100)
Nq-f	insoluble	insoluble	insoluble	50 % (>100)
Nq-g	0.15 ± 0.04	< 12.5	43 % (2.5)	50 % (>100)
Nq-h	0.14 ± 0.05	< 12.5	64 % (5)	50 % (>100)
Nq-i	0.17 ± 0.04	< 12.5	precipitated	50 % (>100)
Nq-j	0.17 ± 0.06	19	precipitated	50 % (>100)
Furanquinones				
Fq-a	1.00 ± 0.02	21	24 % (12.5)	50 % (>100)
Fq-b	2.30 ± 0.03	61	4 % (1.5)	50 % (>100)
Fq-c	0.54 ± 0.13	44	25 % (10)	50 % (>100)
Quinolinquinones				
Qq-b	2.30 ± 0.03	18	52 % (50)	50 % (>100)
Qq-c	0.14 ± 0.05	12.5	65 % (10)	50 % (>100)
Qq-d	0.44 ± 0.09	12.5	46 % (10)	50 % (1.69)

THE SITES AND THE KINETICS

COMPETITIVE—> CATALITIC SITE

A COMPETITIVE—> INTERFASE ... NADP-FAD?

NON COMPETITIVE—> Z SITE....Q SITE?

Figure 9. Furanequinones docked in the catalytic site 2 of a) glutathione reductase and b) trypanothione reductase. In red. poses of higher energetic range and in yellow. lower energetic scored ranges.

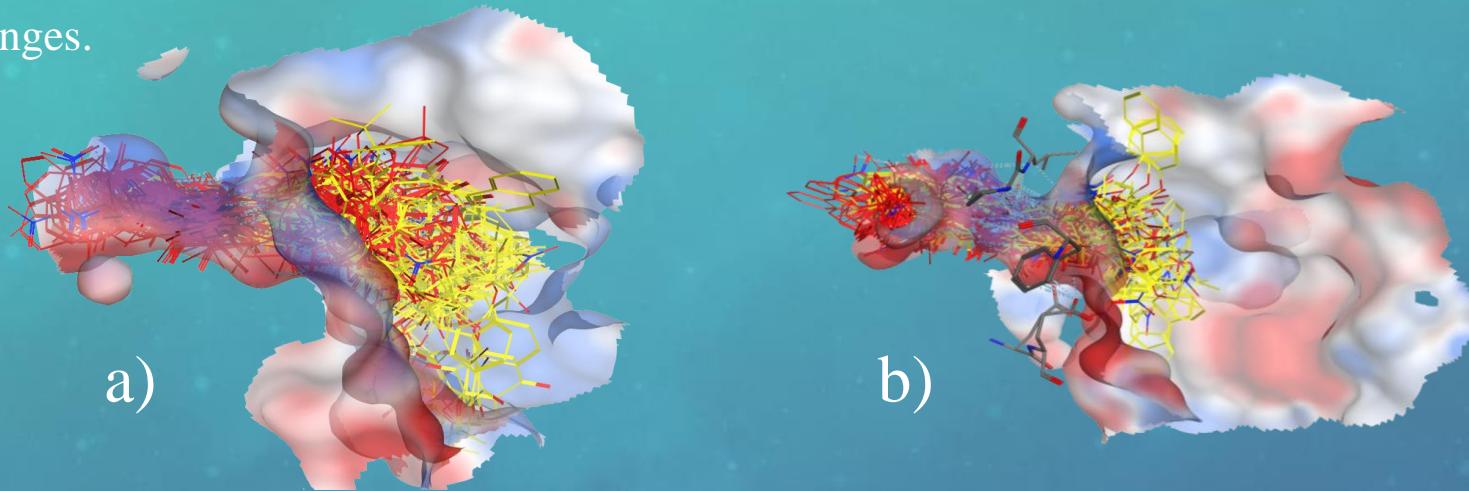
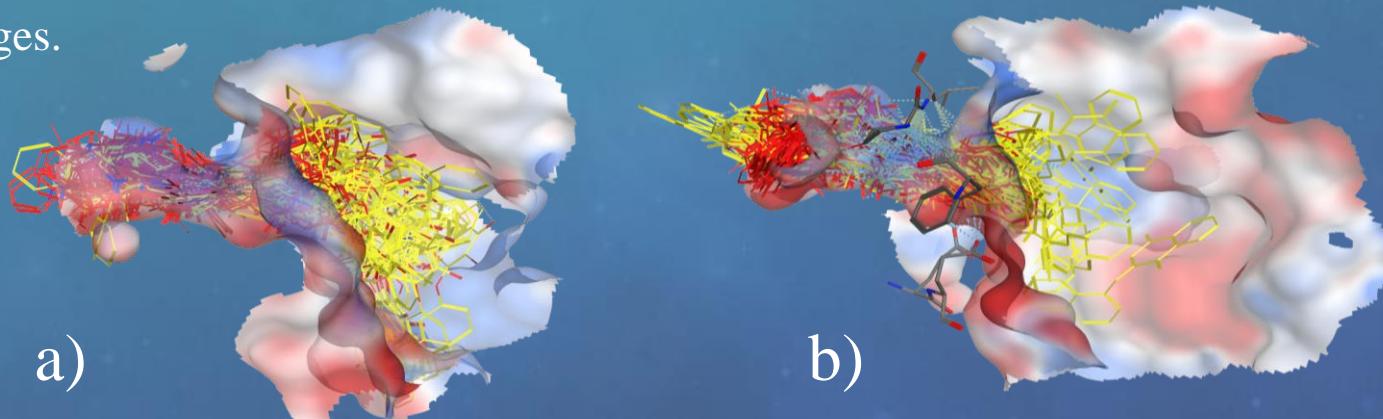
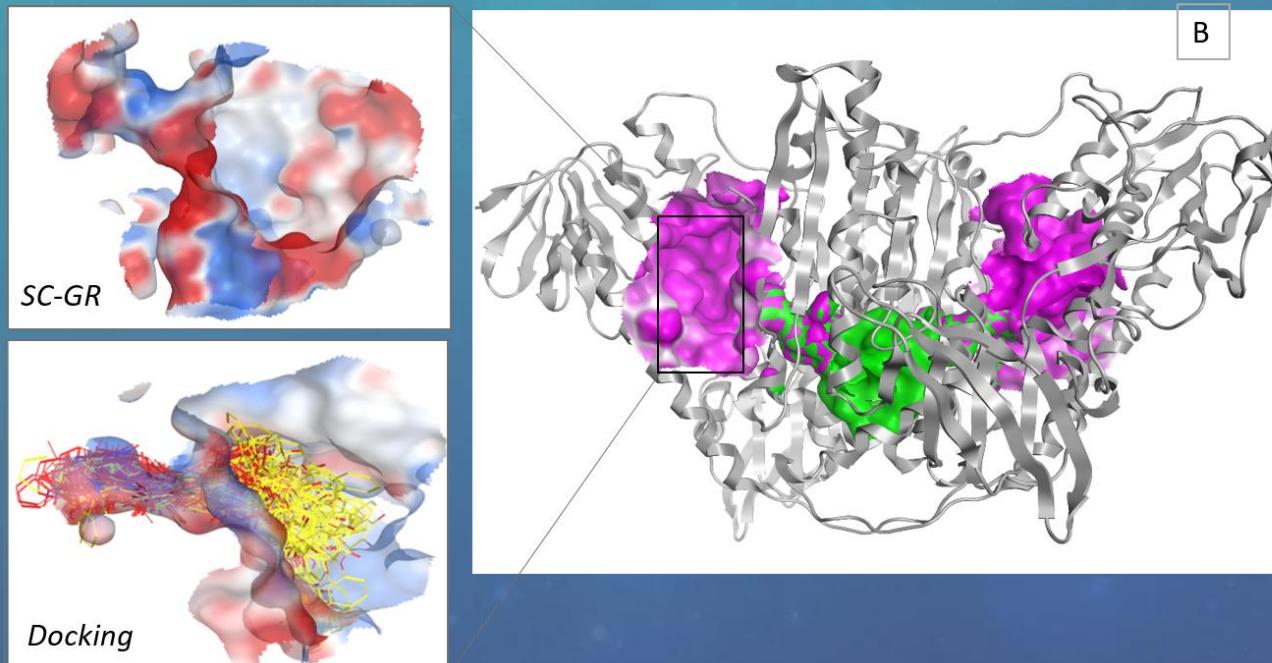
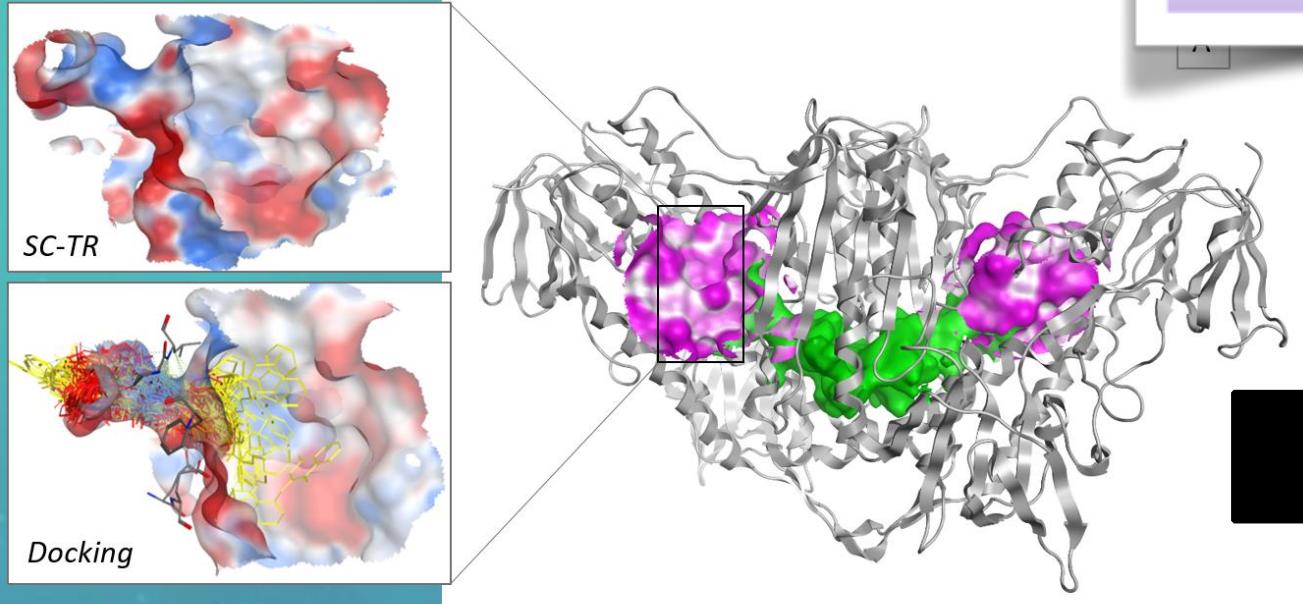


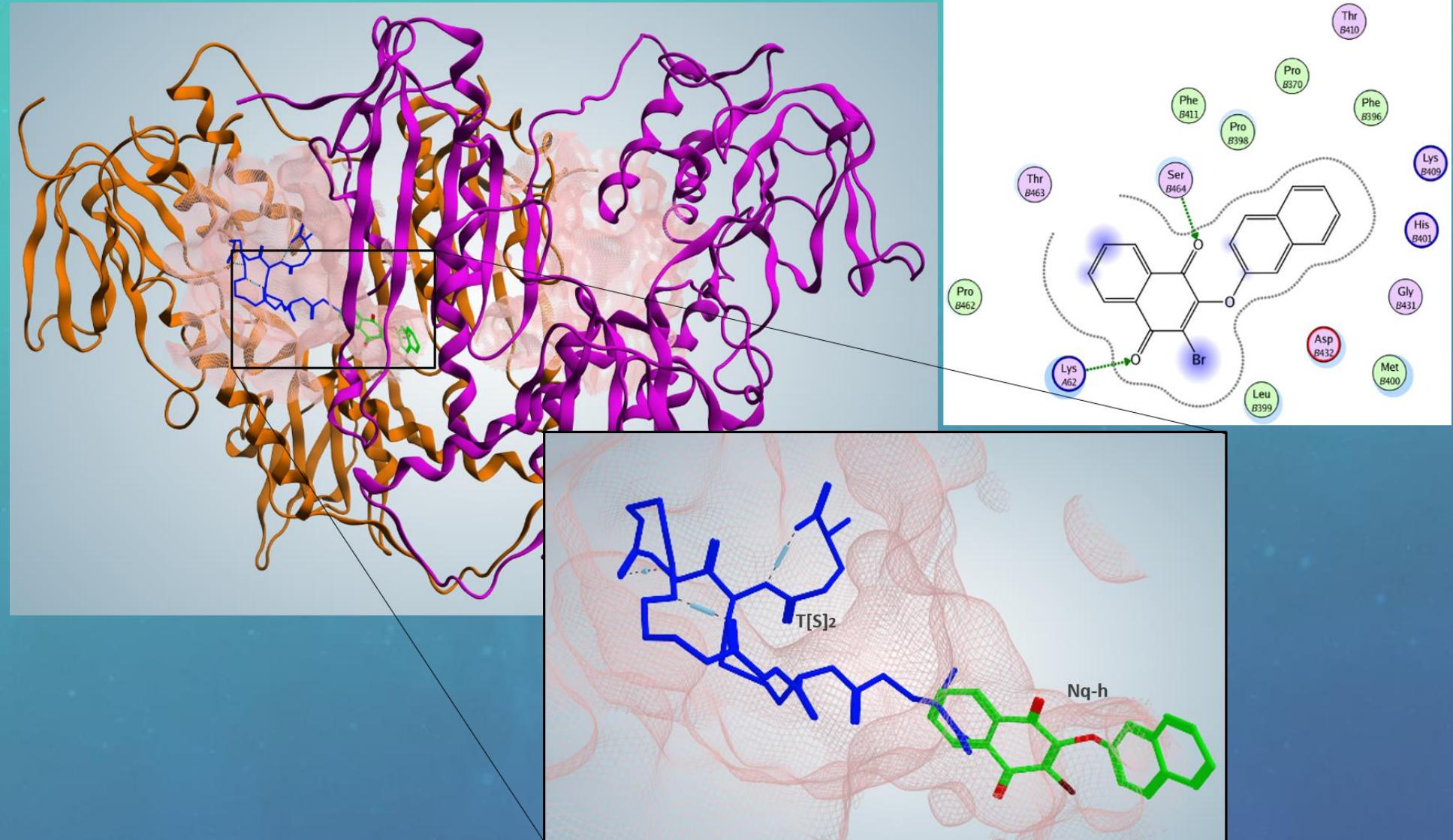
Figure 10. Naphthoquinones docked in the catalytic site 2 of a) glutathione reductase and b) trypanothione reductase. In red. poses of higher energetic range and in yellow. lower energetic scored ranges.



THE “Q” SITE IN TR

SBDD (in TR/GR)



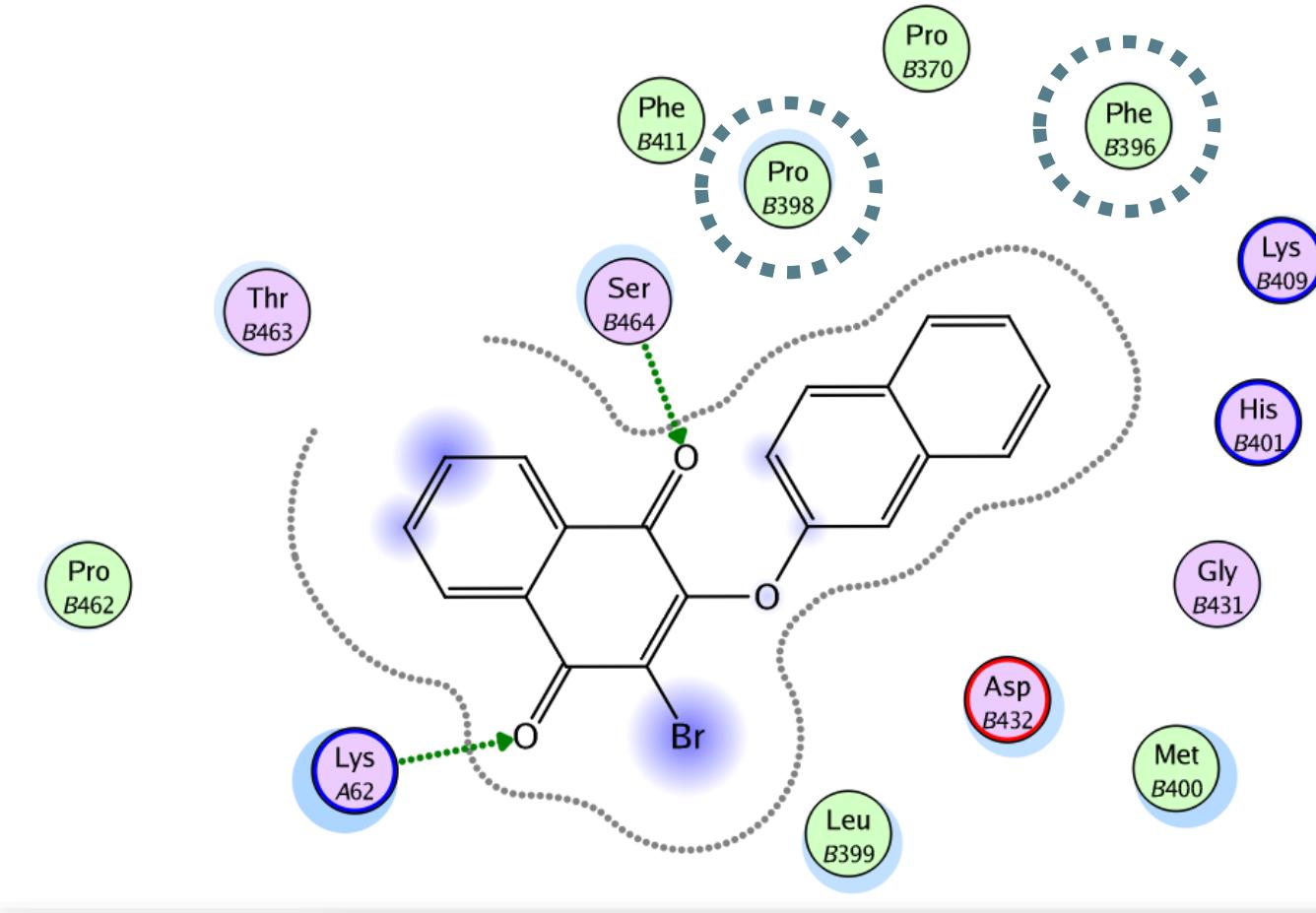


Putative complex TR-TS2-Nq-h. A dotted light pink surface is showing the catalytic site and interface surroundings. A black rectangle is amplified in the middle: the TS₂ is shown in blue rods. The Nq-h quinone is show in green rods. **Bottom:** Ligand Interactions analysis showing in a 2D picture the contacts made by Nq-h in TR. Green arrows indicate H-bond side chain interactions. Blue light shadows are places where there is ligand exposition

LIGAND INTERACTION

2D LIGAND INTERACTION

TR-3H



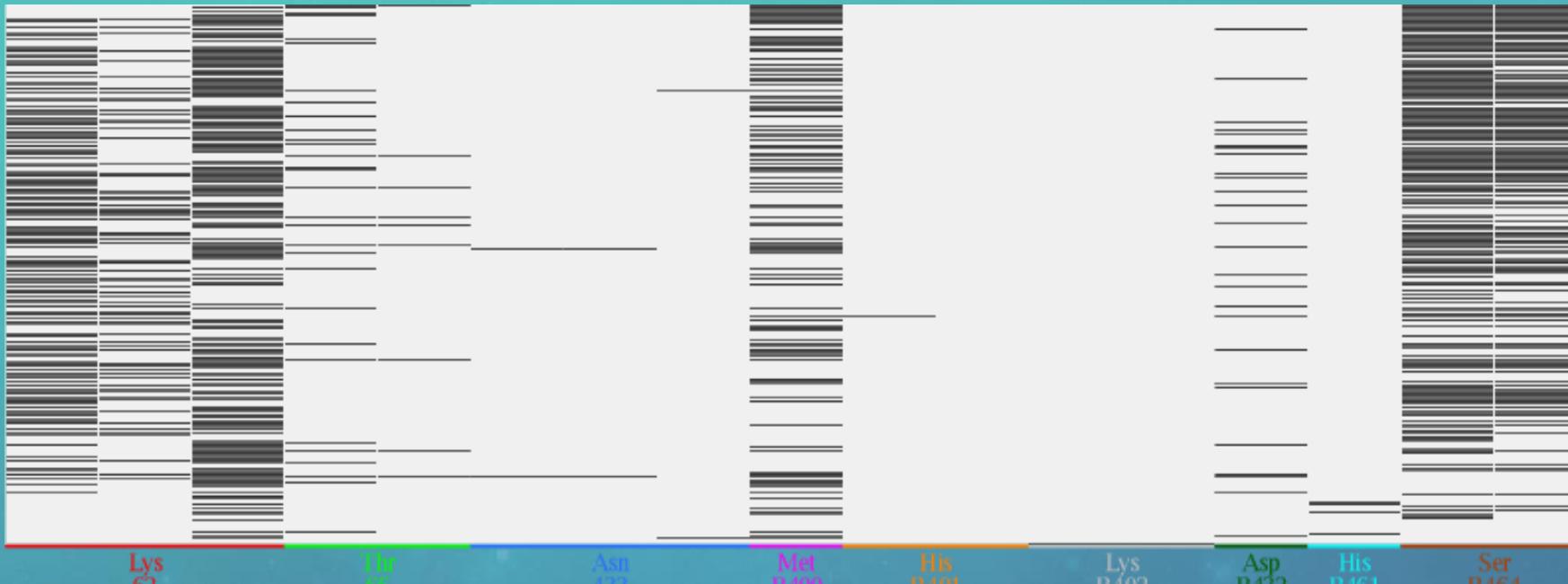
THE Z SITE IN T

Phe 396. Pro

Q SITE IN TR?

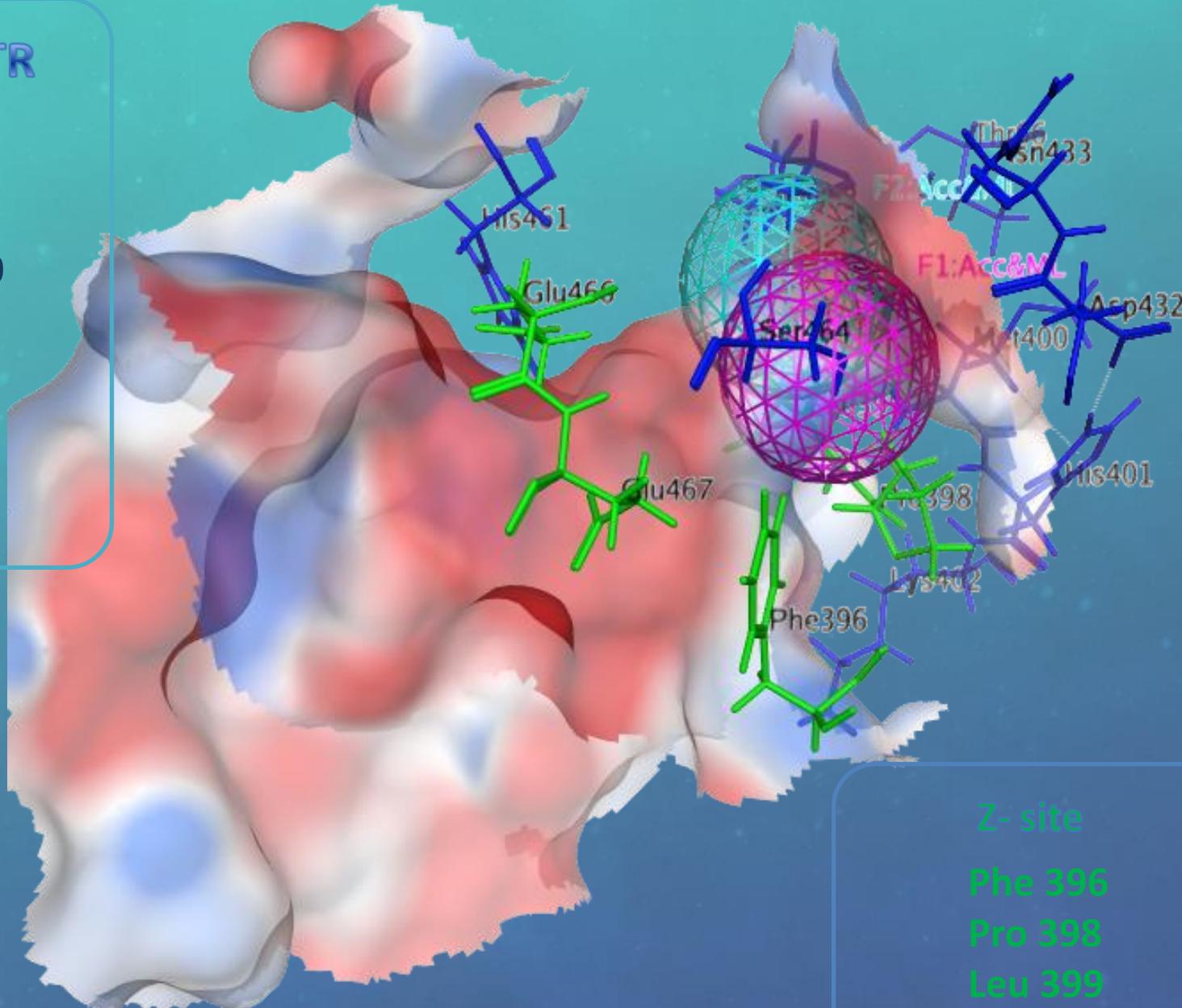
Lys 62 Ser 464' Met 400'

TR FINGERPRINTS



PLIF in TR

Lys 62
Thr 66
Asn 433
Met B400
His B401
Lys B402
Asp B432
His B461
Ser B464



Z- site

Phe 396
Pro 398
Leu 399
Glu 466
Glu 467



ARE QUINONES ASSOCIATED TO AN UNIQUE TARGET?

ONE DRUG →

ONE TARGET? →

ONE EFFECT? →

IN A UNIQUE SITE?

“Chemogenomics is this emerging research field aimed at systematically studying the biological effect of a wide array of small molecular-weight ligands on a wide array of macromolecular targets”

OPEN  ACCESS Freely available online



Multi-Target Drugs: The Trend of Drug Research and Development

Jin-Jian Lu, Wei Pan, Yuan-Jia Hu*, Yi-Tao Wang*

State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Taipa, Macau SAR, China

Shifting from the single to the multitarget paradigm in drug discovery

Reviews • POST-SCI

José L. Medina-Franco^{1,2}, Marc A. Giulianotti²,
Gregory S. Welmaker² and Richard A. Houghten²

¹ Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, México, D.F. 04510, Mexico

² Torrey Pines Institute for Molecular Studies, 11350 SW Village Parkway, Port St. Lucie, FL 34987, USA



MULTITARGETING






**KEEP
CALM
AND
FIND
ANSWERS**

	%GI ^{a,b}	IC ₅₀ (μ M) ^b	J774 IC ₅₀ (μ M)	Selectivity Index ^c	E _{pcl} (V) ^e
3a	87.2 ± 2.1	0.05 ± 0.02	<12.5	<250	-0.610
3b	87.6 ± 1.5	0.02 ± 0.01	12.5	625	-0.526
3c	92.6 ± 1.1	0.17 ± 0.05	<12.5	<73.5	-0.610
3g	79.3 ± 7.8	0.15 ± 0.04	<12.5	<83.3	-0.633
3h	69.6 ± 4.2	0.14 ± 0.05	<12.5	<89.3	-0.456
3i	85.5 ± 1.3	0.17 ± 0.04	<12.5	<73.5	-0.624
3j	99.2 ± 0.7	0.17 ± 0.06	19	111.8	-0.440



Table 1: First column, coded name of quinones. Second and Third columns: IC ₅₀ for the <i>T. cruzi</i> growth inhibition and cytotoxicity in J-774 IC ₅₀ in μ M. Fourth and Fifth columns: percentages of trypanothione and glutathione reductase inhibition and in parentheses are annotated the related concentrations in μ M. * Data are from Vazquez et al 2015.					
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Nq-e	0.17 ± 0.05	<12.5	46 % (2.5)	50 % (3.9)	
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Nq-e	0.11 ± 0.04	46	41 % (10)	50 % (>100)	
Nq-f	insoluble	insoluble	insoluble	50 % (>100)	
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Furanquinones					
Fq-a	1.00 ± 0.02	21	24 % (12.5)	50 % (>100)	
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Qq-d	0.44 ± 0.09	12.5	46 % (10)	50 % (1.69)	

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It has been proved that these targets are not necessarily the only ones who interact with these molecules, suggesting a multitargetted action

multitargeting repositioning

STRATEGY

To synthetize and make trypanocidal and citotoxic assays in an starting set of quinones

To do biochemical and in silico studies in TR/GR

To SELECT HOOKS—> the two aryloxy-quinones with the best selective trypanocidal and TR/GR interesting activities.

To do TARGET FISHING

- SHAFTS(+IdTarget)
- PHARMMAPPER
- CLUSTALW

To model by HOMOLOGY/IN SILICO

To compare with TriTrypDB

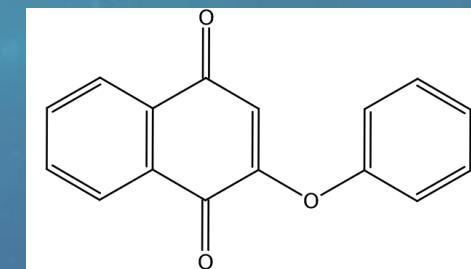
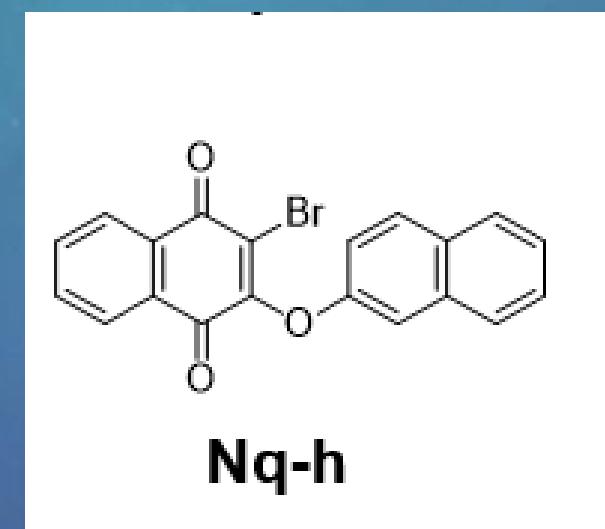
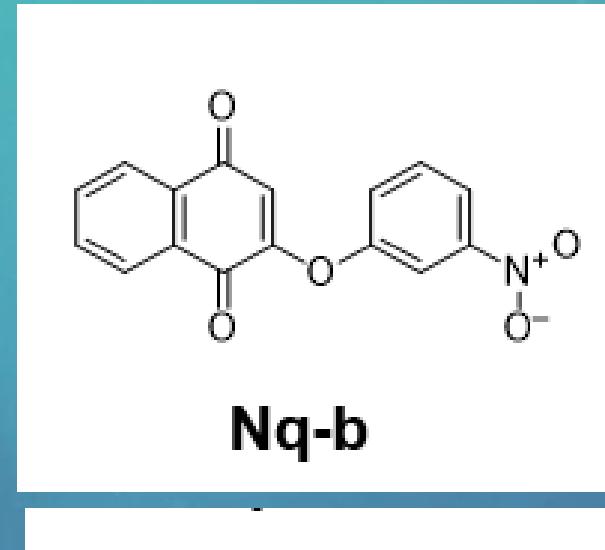
MODELLING (I-TASSER) + MD VALIDATION

To RANK the fished targets by DOCKING

Therefore, in a first step, a reverse virtual screening strategy was used to find putative new targets, using a selected *p*-naphthoquinone (alpha naphtoxi napthoquinone 3h) as a “hook” and an automated on-line protocol developped to "fish" in the Protein Data Bank.

THE HOOKS

Three query
compounds: the best
trypanocidal and TR
inhibitors compounds

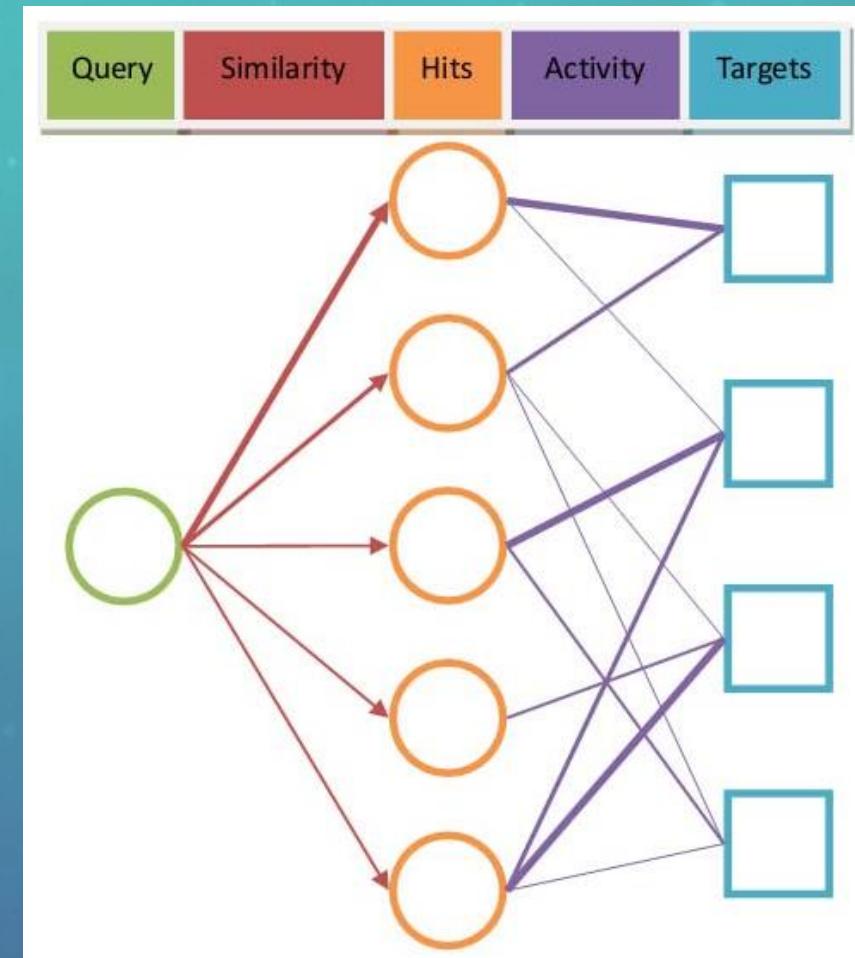
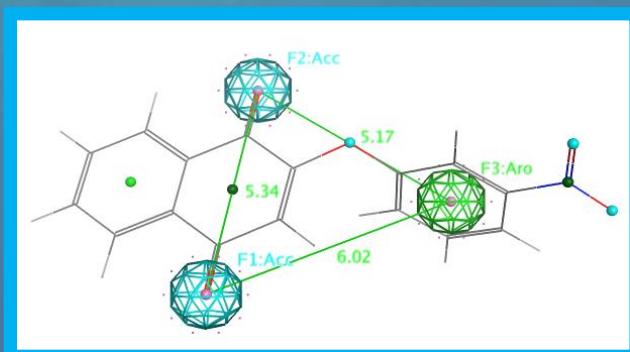


TARGET FISHING METHODOLOGIES:

SHAFTS (SHApe-FeaTure Similarity) ligand similarity and pharmacophore
Id Target: Random walk to ranking

LIBRA (Ligand Binding site Recognition Application) L. Viet Hung, S. Caprari, M. Bizai, D. Toti, F. Polticelli. Bioinformatics. (2015). doi:10.1093/bioinformatics/btv489.

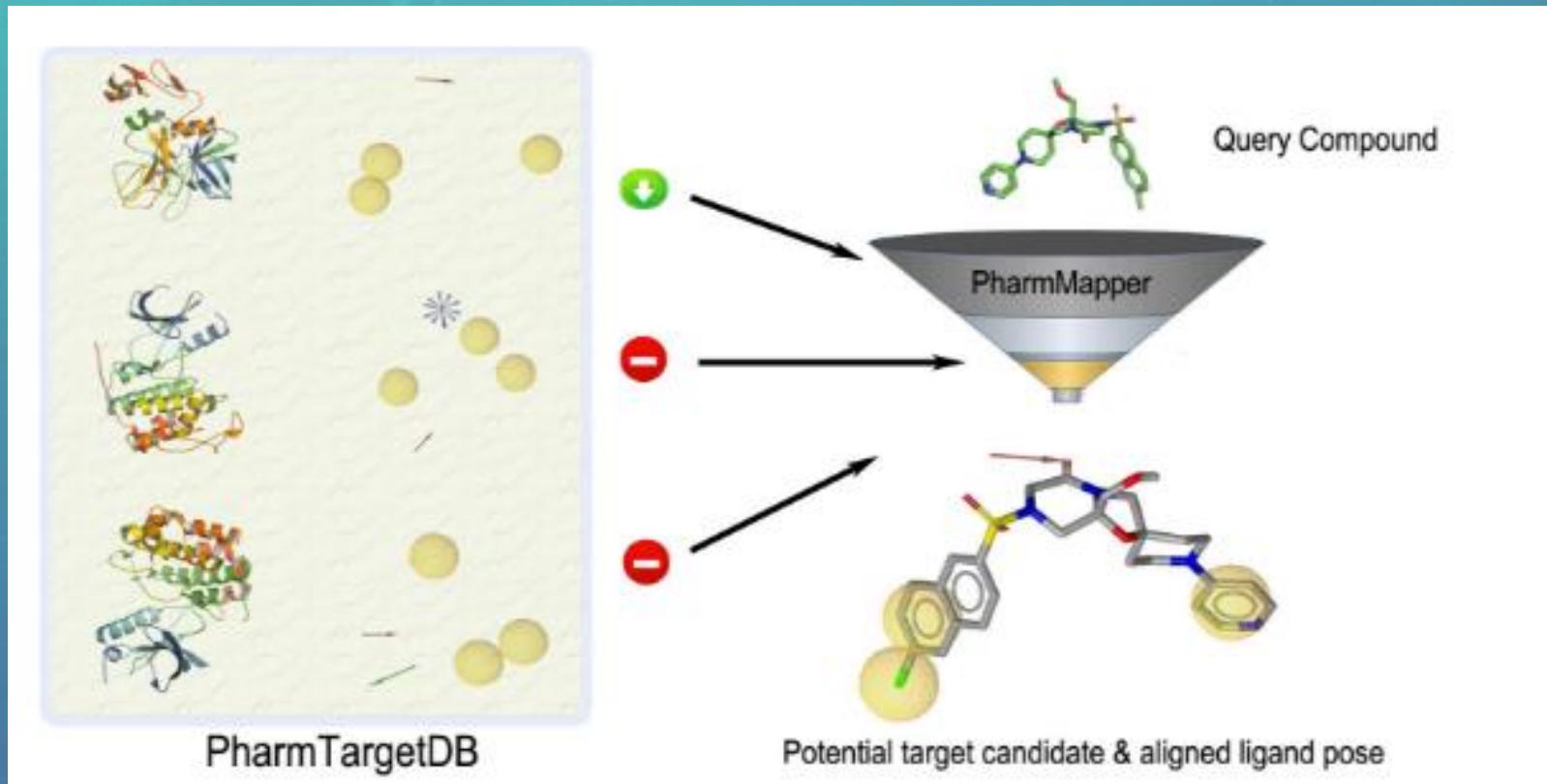
Query



- [5] W. Lu, X. Liu, X. Cao, M. Xue, K. Liu, Z. Zhao, et al., SHAFTS: a hybrid approach for 3D molecular similarity calculation. 2. Prospective case study in the discovery of diverse p90 ribosomal S6 protein kinase 2 inhibitors to suppress cell migration., J. Med. Chem. 54 (2011) 3564–74. doi:10.1021/jm200139j.
- [6] J. Wang, P. Chu, C.-M. Chen, J. Lin, idTarget: a web server for identifying protein targets of small chemical molecules with robust scoring functions and a divide-and-conquer docking approach., Nucleic Acids Res. 40 (2012) W393–9. doi:10.1093/nar/gks496.

TARGET FISHING METHODOLOGY: PHARMMAPPER: ph4 sites similarity

In parallel a second kind of reverse docking was made using the PharmMapper platform to make a similar fishing buy using the hydrogen bond acceptor and donor capacity of ligands, the aromaticity and the negative charge. In this case the “hook” was the best tripanocidal compound, a nitro phenoxy napthoquinone (3b).



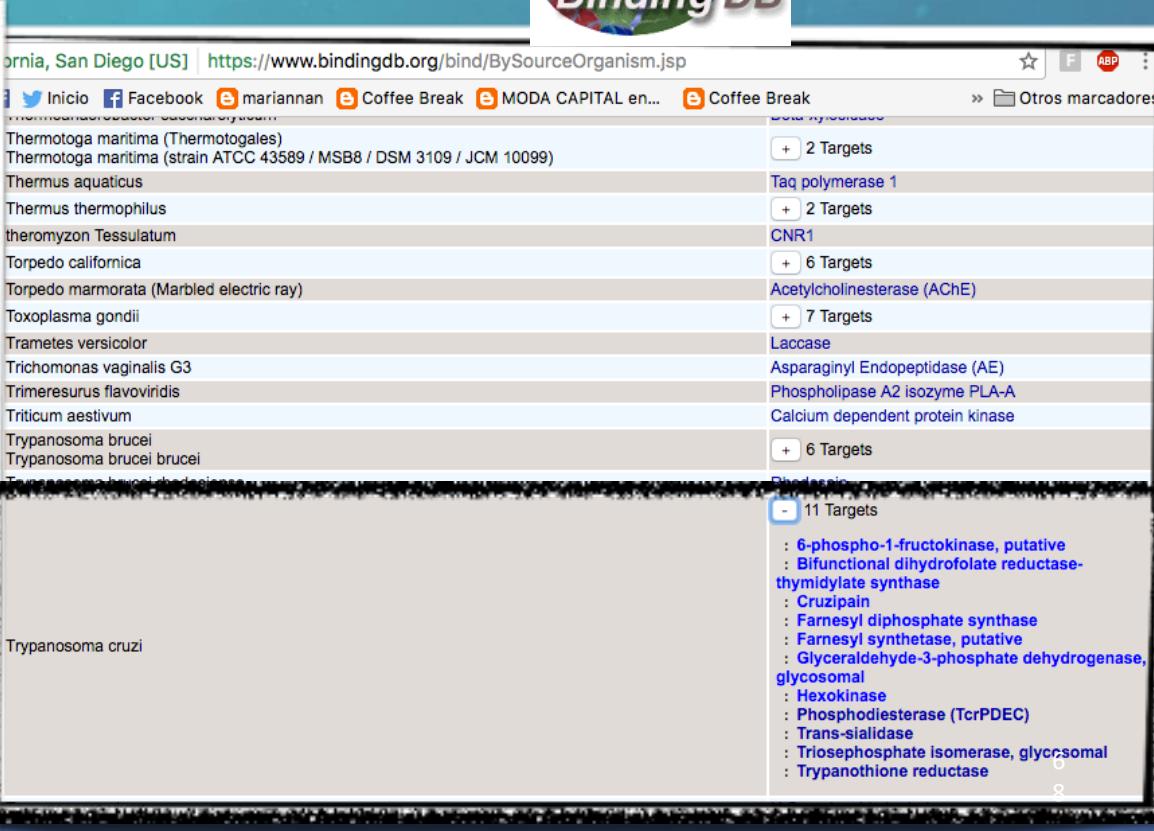
VALIDATION IN REPOSITORIES

Tritryp (kinetoplastids) , inside the EuPathDB (eukariotes). Search the genes associated to proteins reported in the PDB, text mine and analize.

Comparison with the BindingDataBases, a repository of scientific journals with revised information. —> Apps (BindingDB Data by Source Organism) to classify targets by organism—> ELEVEN TARGETS



The screenshot shows the TriTrypDB interface with a sidebar menu and a main content area displaying a hierarchical tree of trypanosomatid species under the 'Tritryp' category. The tree includes sub-categories like 'Cercozoa', 'Endomastigomorpha', 'Leishmeria', 'Leptomonida', and 'Trypanosomatidae'. A search bar at the top is set to 'Tritryp'.



The screenshot shows the BindingDB interface with a sidebar menu and a main content area. The main content displays a list of targets for 'Trypanosoma cruzi' with their respective target counts: 'Taq polymerase 1' (2 Targets), 'CNR1' (6 Targets), 'Acetylcholinesterase (AChE)' (7 Targets), 'Laccase' (6 Targets), and 'Phospholipase A2 isozyme PLA-A' (11 Targets). The 'Phospholipase A2 isozyme PLA-A' section is expanded, showing a detailed list of targets including '6-phospho-1-fructokinase, putative', 'Bifunctional dihydrofolate reductase-thymidylate synthase', 'Cruzipain', 'Farnesyl diphosphate synthase', 'Farnesyl synthetase, putative', 'Glyceraldehyde-3-phosphate dehydrogenase, glycosomal', 'Hexokinase', 'Phosphodiesterase (TcrPDEC)', 'Trans-sialidase', 'Triosephosphate isomerase, glycosomal', and 'Trypanothione reductase'.

T. cruzi ELEVEN EMERGING TARGETS

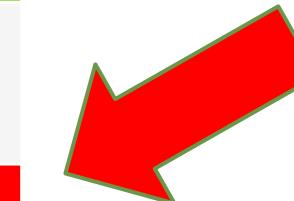


Aslett et al. TriTrypDB: a functional genomic resource for the Trypanosomatidae Nucleic Acids Research 2010 38(Database issue):D457-D462; doi:10.1093/nar/gkp851

- **6-phospho-1-fructokinase**
- **Bifunctional dihydrofolate reductase-thymidylate synthase**
- **Cruzipain**
- **Farnesyl diphosphate synthase**
- **Farnesyl synthetase, putative**
- **Glyceraldehyde-3-phosphate dehydrogenase**
- **Hexokinase**
- **Phosphodiesterase (TcrPDEC)**
- **Trans-sialidase**
- **Triosephosphate isomerase, glycosomal**
- **Trypanothione reductase**

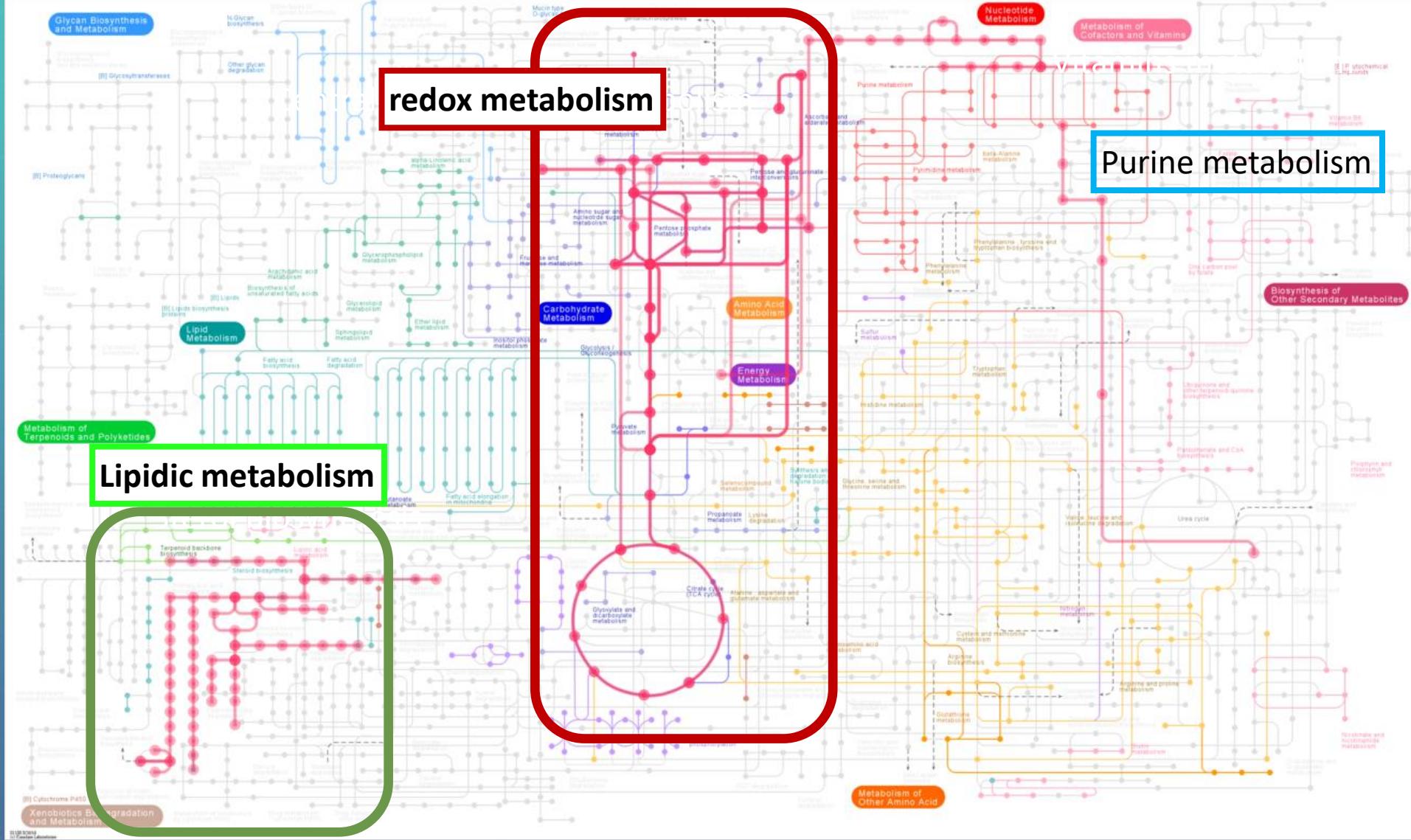
TRITRYP	Metabolism	SHAFTSNqh	PHARMNqh	SHAFTSNqb	PHARMNqb	Highest S	Nqb S	Nqh S	Nqa S
Farnesyl diphosphate synthase /synthetase, Transferase- FPS	Terpenoid backbone biosynthesis	X(H. sapiens)	X (R. norvegicus)		X (R. norvegicus)	-14.02	-10.98	-11.04	-11.41
Trypanothione reductase TR	Trypanothione metabolism	X (T. cruzi) CLUSTALW (GR)	X (T. cruzi)	X (T. cruzi)	X (T. cruzi)	-13.13	-11.74	-13.43	-10.08
Hexokinase HK	Glycolysis / Gluconeogenesis	X (H. sapiens)				-12.87	-10.09	-11.15	-8.06
Glutathione reductase	Glutathione metabolism	X (H. sapiens)	X (H. sapiens)	X (H. sapiens)	X (H. sapiens)	-13.19	-11.1	-11.6	-10.5
Phosphodiesterase (TcrPDEC)	Nucleotide metabolism		X (H. sapiens)	X (H. sapiens)	X (H. sapiens)	-13.07	-9.82	-11.10	-9.11
Cruzipaín	Nutrition of T. cruzi by digestion of host proteins				X (T. cruzi)	-11.93	-9.36	-9.54	-7.81
Triosephosphate isomerase TIM, glycosomal TPS	Glycolysis / Gluconeogenesis	X (T. cruzi)	X (T. cruzi)		X (Saccharom ices)	-12.49	-9.31	-7.28	-7.13

Reductase thymidylate synthase, DHFR	Metabolism				(Microbacterium)	-	-	-
Glyceraldehyde-3-phosphate dehydrogenase, glycosomal GPDH	Glycolysis / Gluconeogenes is		X (T. cruzi)		X (Bacillus)	-9.12	-7.12	-7.45
Trans-sialidase TS	activate Ca2+ signal							
		5/11	6/11	4/11	9/11			
Dihydroorotate Reductase (SHAFTS/PHARMMAPPER) - DHODH	<u>de novo pyrimidi ne biosynthesi</u> s.	X (T. cruzi)	X (P. falciparum)(H. sapiens)	X (T cruzi)	X (Rattus norvegicus))	-13.16	-11.69	-10.07
Tcruzi MODEL KINASE (Calcium- Dependent Protein Kinase) (SHAFTS)	Parasite division	X (Tgondii)				-10.17	-8.21	-8.90
Dihydrolipoamide dehydrogenase (CLUSTALW)	Glycolysis / Gluconeogenes is	X (T. cruzi)	X (T. cruzi)	X (T. cruzi)	X (T. cruzi)	-13.90	-10.80	-12..23
NQ2/NQ1 (SHAFTS/PHARMMAPPER)	Metabolism of xenobiotics by cytochrome P450	X (H. Sapiens)		X (H. Sapiens)		-12.10	-9.36	-9.02
		10/15	8/15	7/15	11/15			



TO LOCALIZE TARGETS IN KEGG
DATABASE: TWO FILTERS
(OXIDOREDUCTASES AND
ASSOCIATED PATHWAYS)

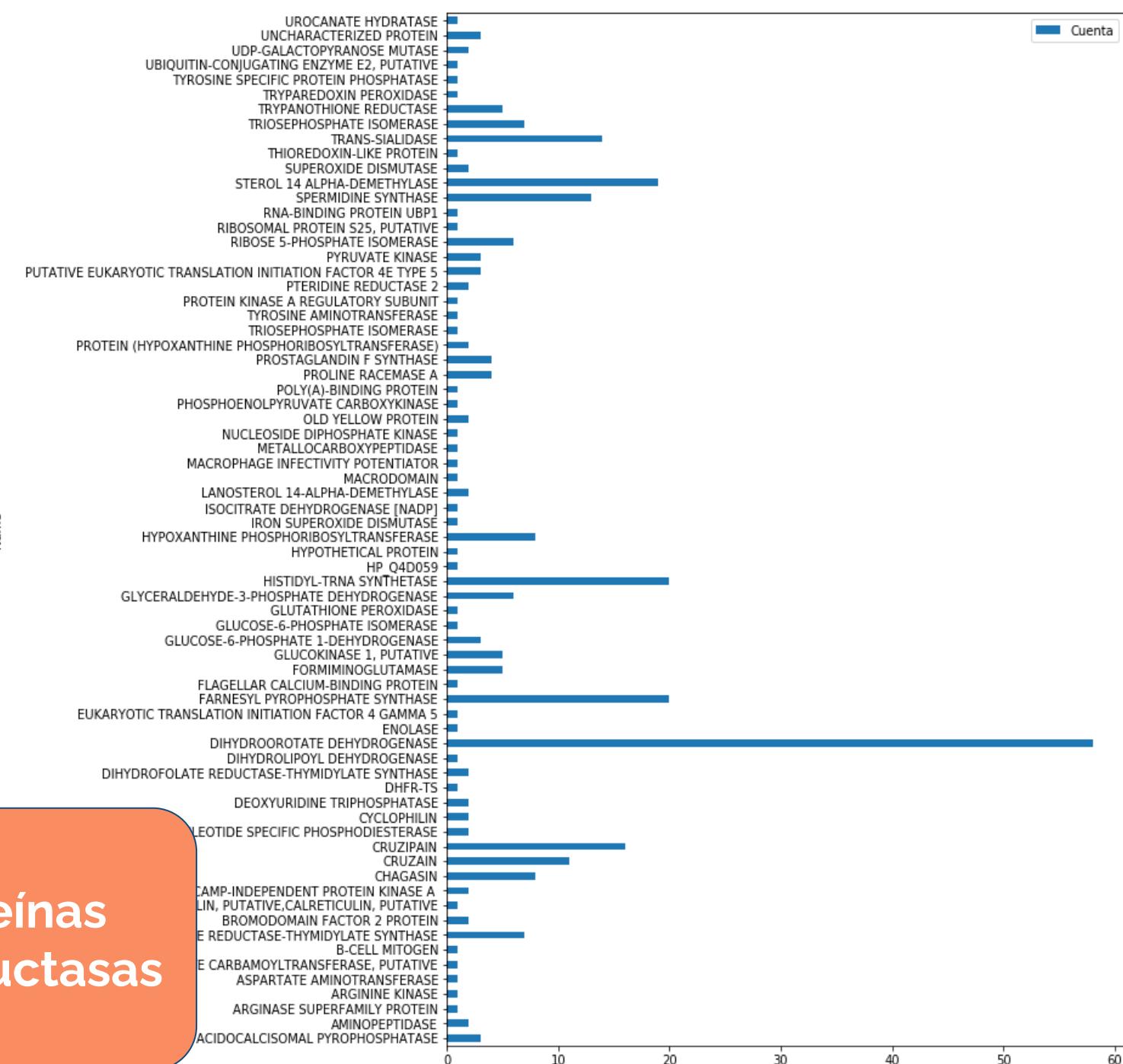
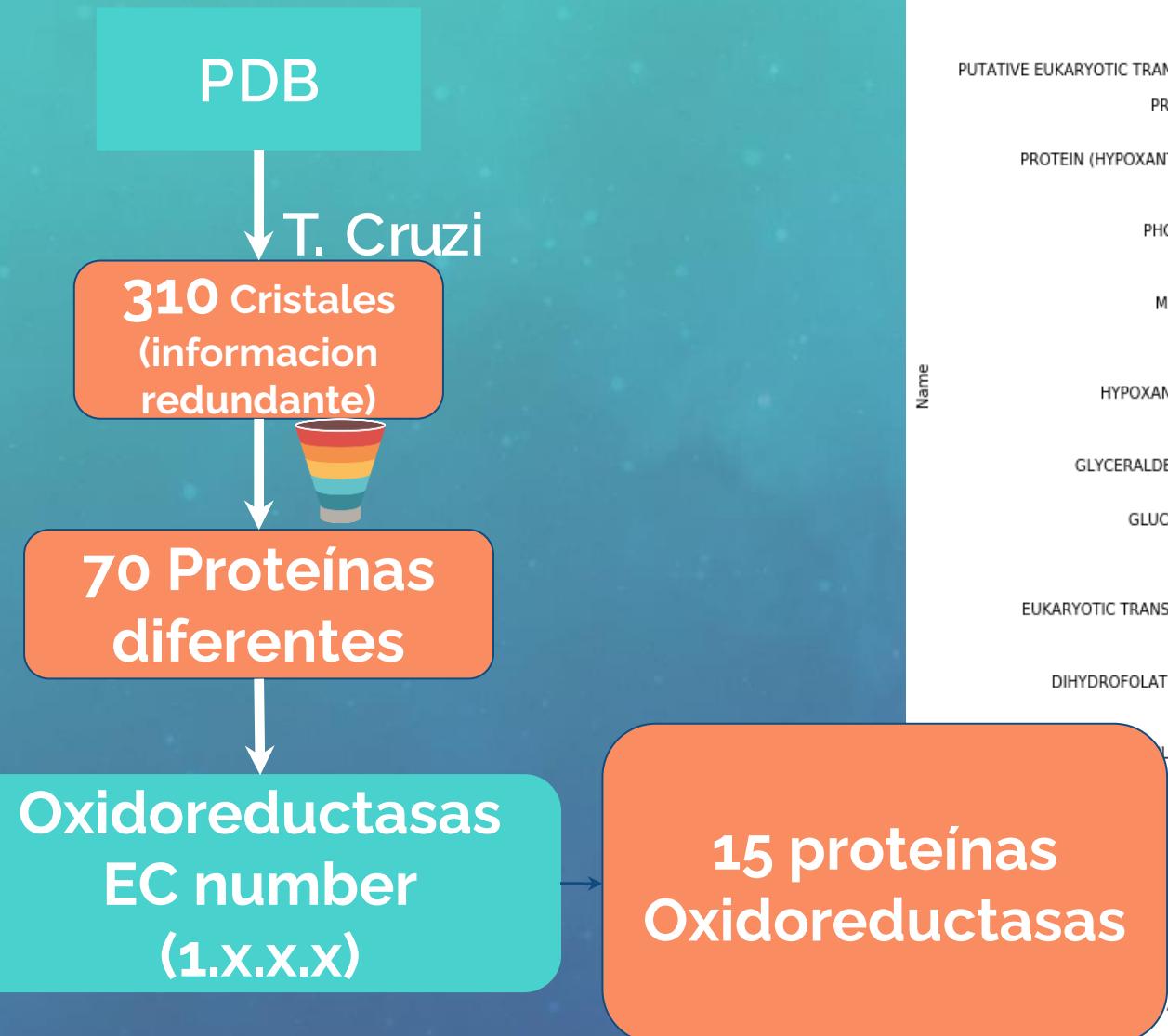
T. cruzi/H sapiens TARGETS Metabolism location



METABOLISM MINING

TO LOOK INSIDE METABOLISMS
ASSOCIATED TO THE OXIDATIVE, LIPIDIC
AND PURINE/PIRIMIDIN METABOLISMS

PDB AND “OXIDOREDUCTASES” FILTER



AMPLIFYING: MINING IN OXIDATIVE, LIPIDIC AND PURINE/PIRIMIDINE METABOLISMS

Kegg

T.
Cruzi

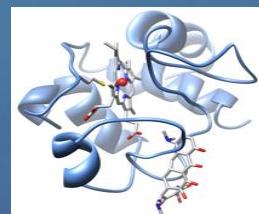
NAD(P)
NADH

Biosíntesis de esteroides
Biosíntesis de terpenos
Metabolismo de nucleótidos

Pentosas Fosfato
Glucolisis/Glucon
eogenesis
Ciclo citrato (TCA)
Metabolismo
Glutation
Fosforilación
Oxidativa

TriTrypDB

Leishm
ania
Trypan
osoma
45/52

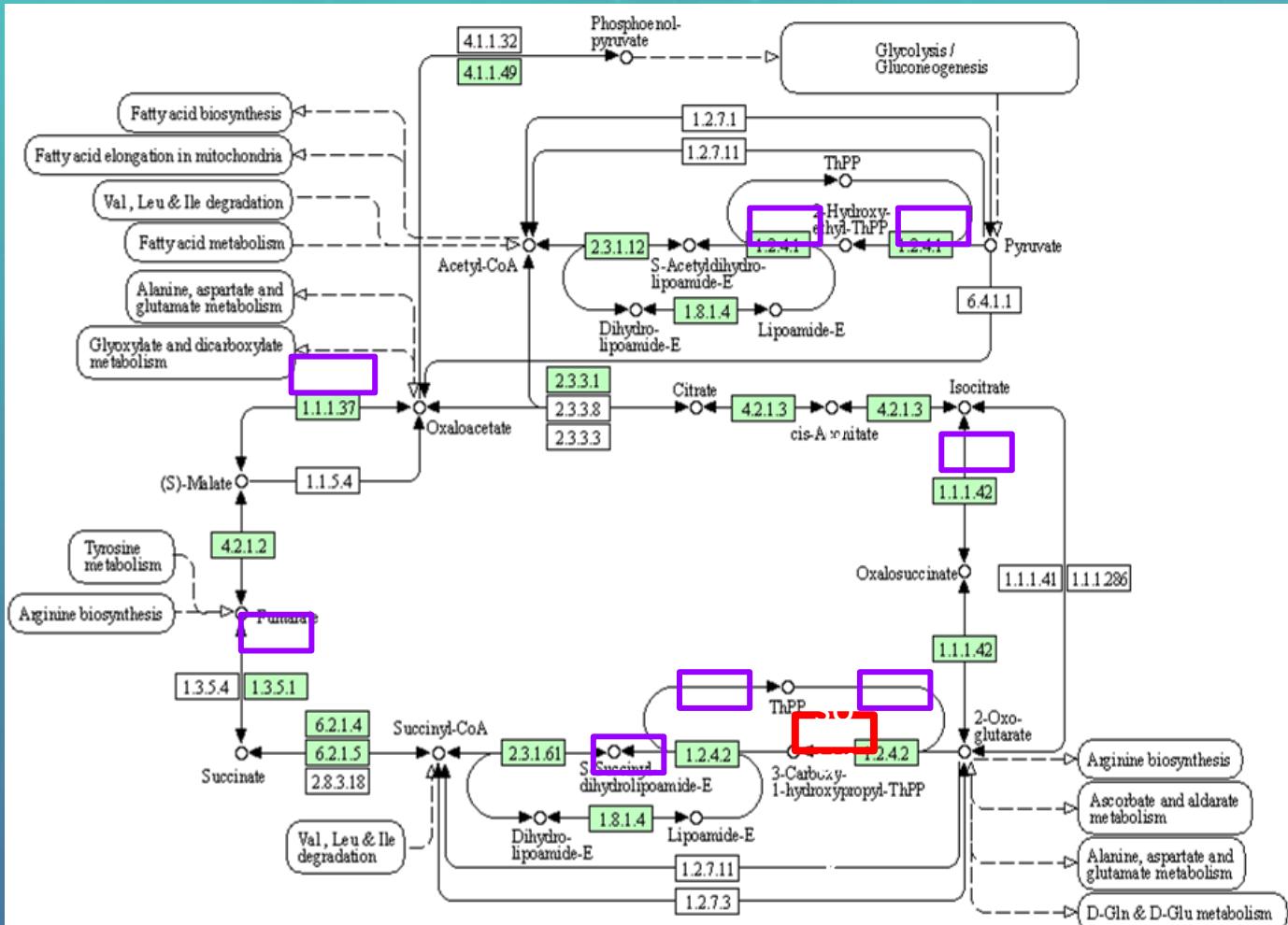


32
Oxidoreductases

Oxidoreducta
sas
EC number
(1.x.x.x)

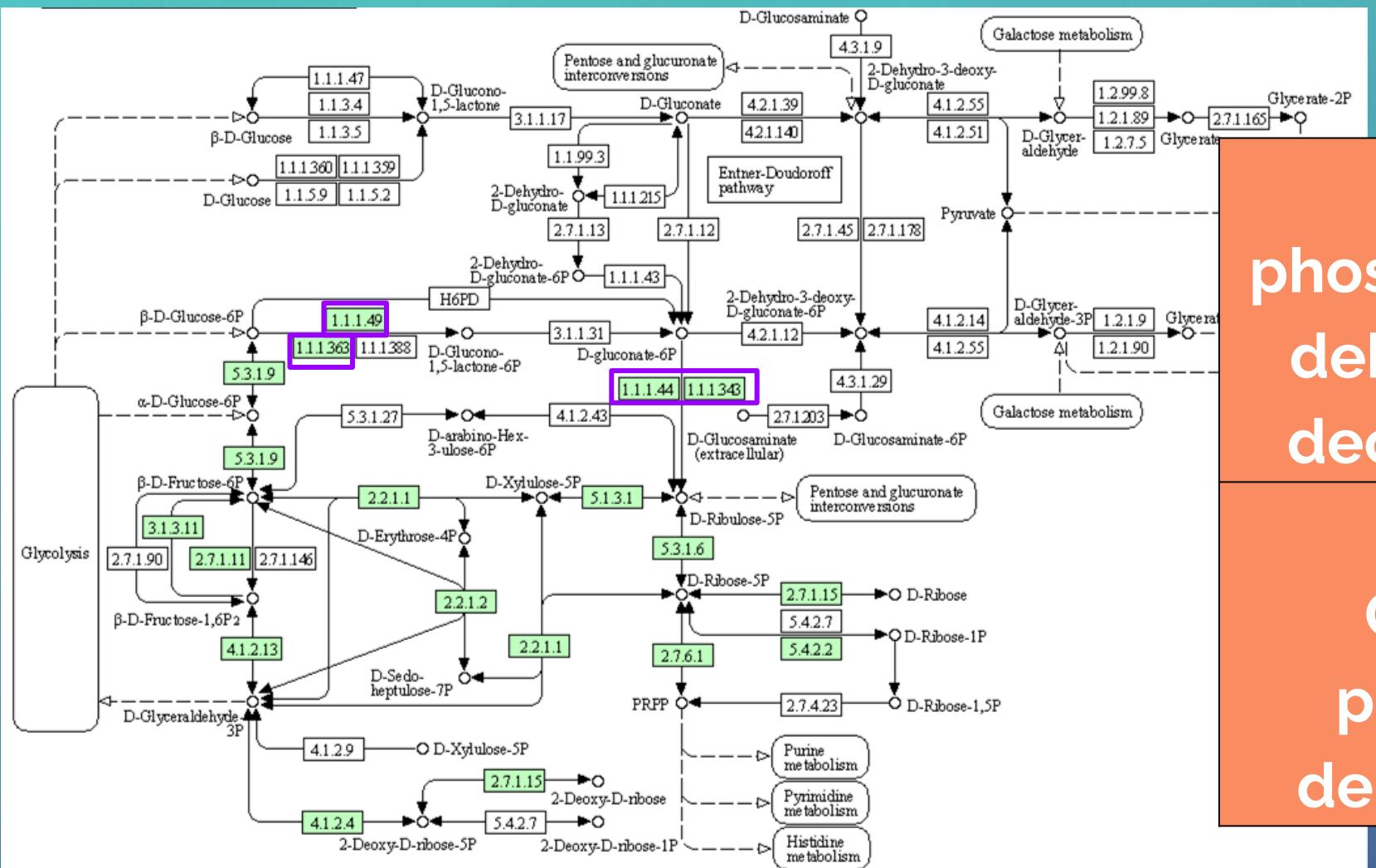


CICLO DEL CITRATO (TCA): PATHWAY SOURCE KEGG



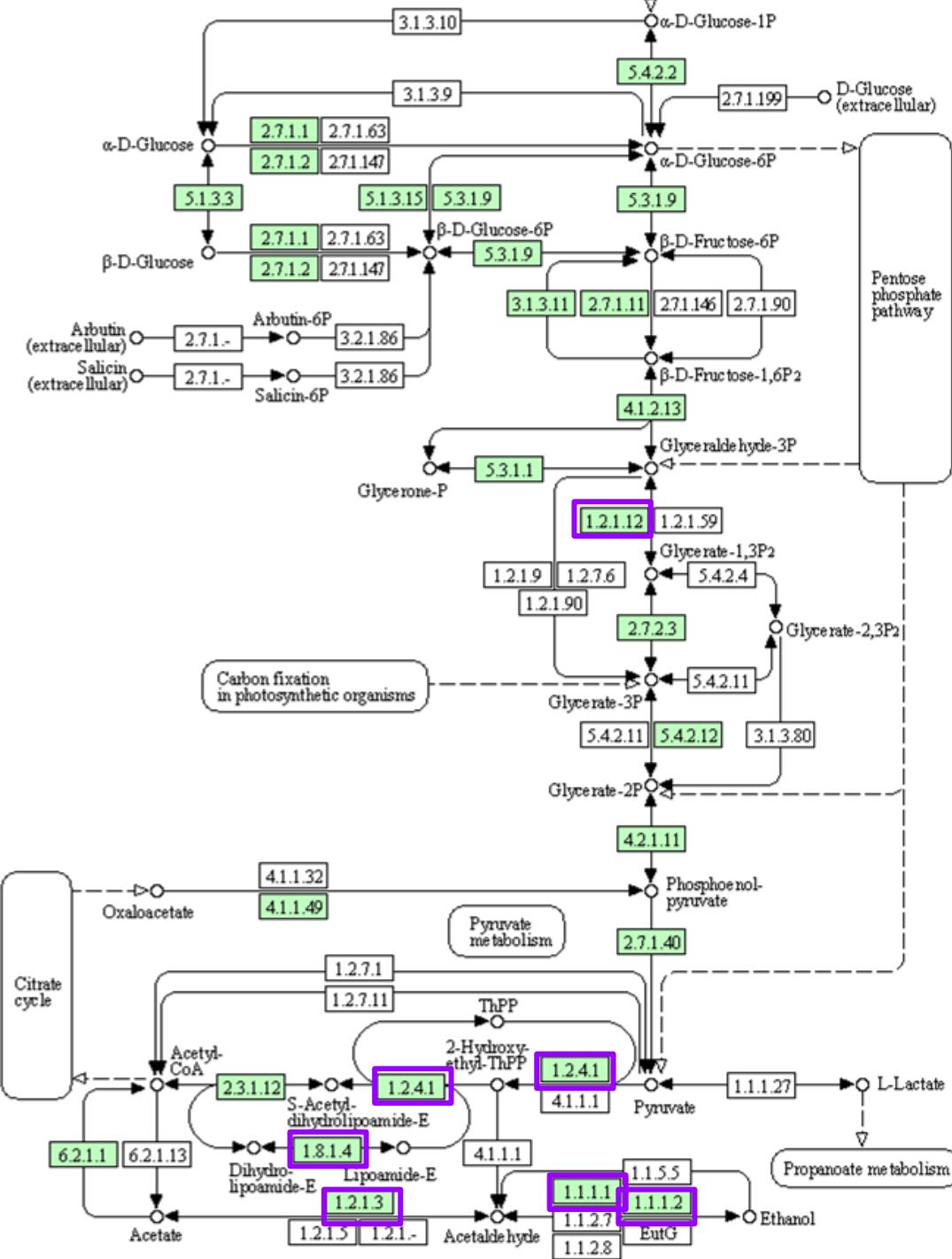
Malate dehydrogenase	1.1.1.37
Pyruvate dehydrogenase E1 alpha subunit	1.2.4.1
2-Oxoglutarate dehydrogenase subunit, putative	1.2.4.2
Dihydrolipoamide dehydrogenase	1.8.1.4
Succinate dehydrogenase	1.3.5.1
Isocitrate dehydrogenase	1.1.1.42

PENTOSAS FOSFATO (P5P)



6-	1.1.1.3
phosphogluconate	43
dehydrogenase,	1.1.1.4
decarboxylating	4
Glucose-6-	1.1.1.4
phosphate 1-	9
dehydrogenase	1.1.1.3
	63

GLUCOLISIS/GLUCONEOGÉNESIS (GG)



NADP-dependent alcohol dehydrogenase

1.1.1.1

Glyceraldehyde 3-phosphate dehydrogenase

1.2.1.12

Aldehyde dehydrogenase

1.2.1.3

Pyruvate dehydrogenase E1 component alpha subunit

1.2.4.1

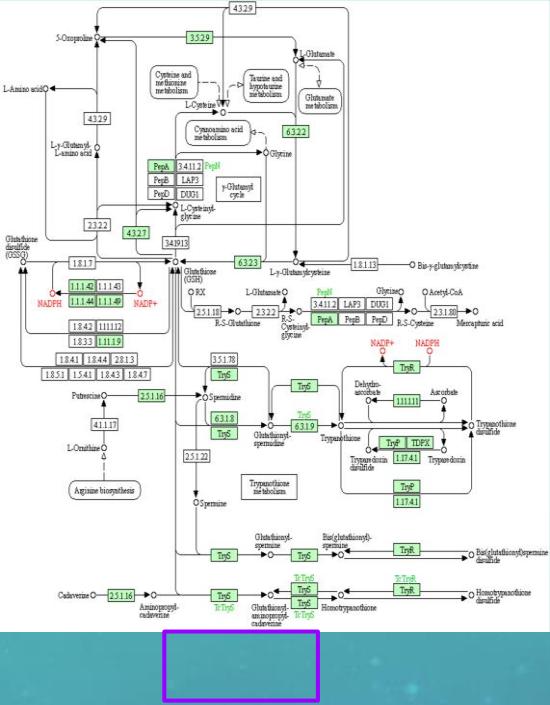
Dihydrolipoamide dehydrogenase

1.8.1.4

NADP-dependent alcohol hydrogenase

1.1.1.2

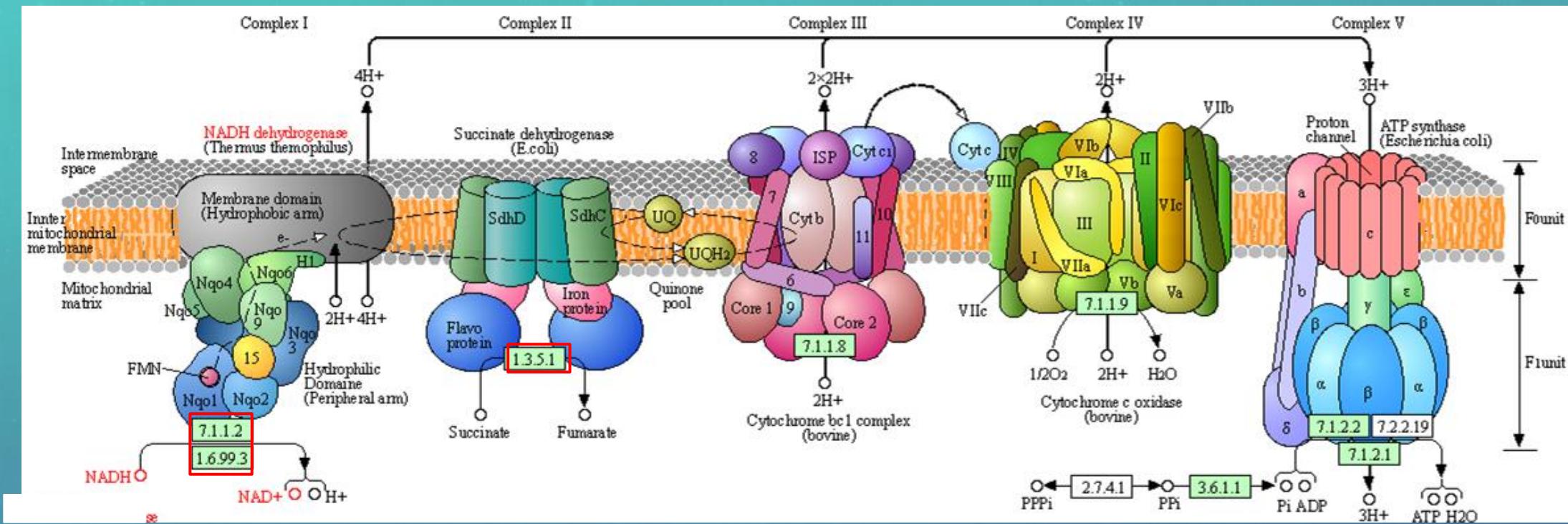
METABOLISMO GLUTATIÓN (GT)



Isocitrate dehydrogenase	1.1.1.42
6-phosphogluconate dehydrogenase	1.1.1.44
Glucose-6-phosphate dehydrogenase	1.1.1.49
Ascorbate peroxidase	1.11.1.11
Tryparedoxin peroxidase	1.11.1.15
Glutathione peroxidase	1.11.1.9
Ribonucleoside-diphosphate reductase	1.17.4.1
Trypanothione reductase	1.8.1.12

TryS: trypanothione synthetase
 TryR: trypanothione reductase
TryP: tryparedoxin peroxidase
 TDPX: glutathione peroxidase

FOSFORILACIÓN OXIDATIVA (OP)

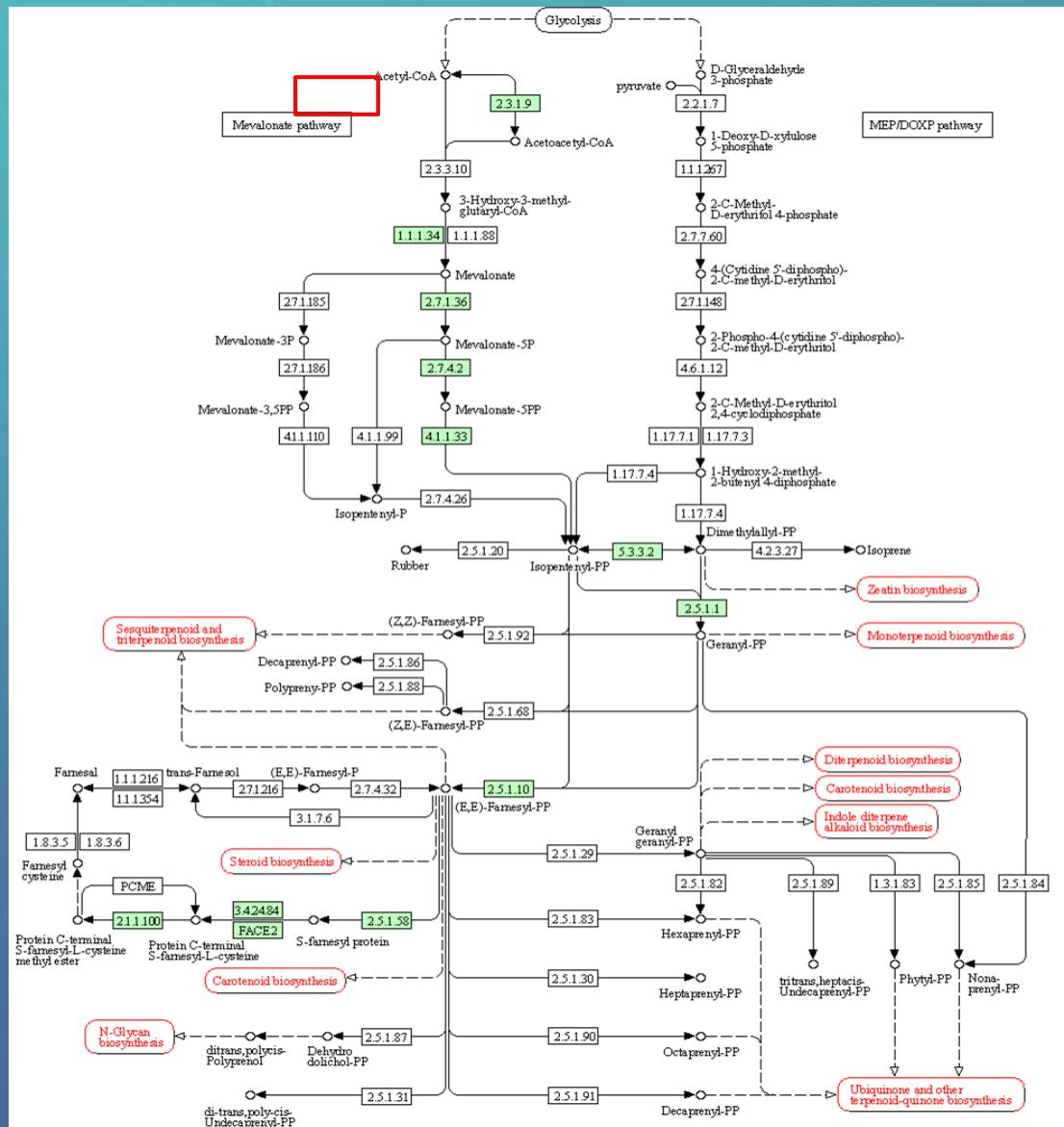


NADH dehydrogenase	1.6.99.3
Succinate dehydrogenase flavoprotein	1.3.5.1
NADH-ubiquinone oxidoreductase	7.1.1.2

BIOSÍNTESIS DE TERPENOIDES (ERGOSTEROL)

3-hydroxy-3-methylglutaryl-CoA reductase

1.1.1.34



BIOSÍNTESIS DE ESTEROIDES (ERGOSTEROL)

Steroid dehydrogenase

1.1.1.170

Lanosterol 14-alpha demethylase

1.14.14.154

Squalene monooxygenase

1.14.14.17

Lathosterol oxidase-like protein

1.14.19.20

Sterol 22-desaturase

1.14.19.41

C-14 sterol reductase

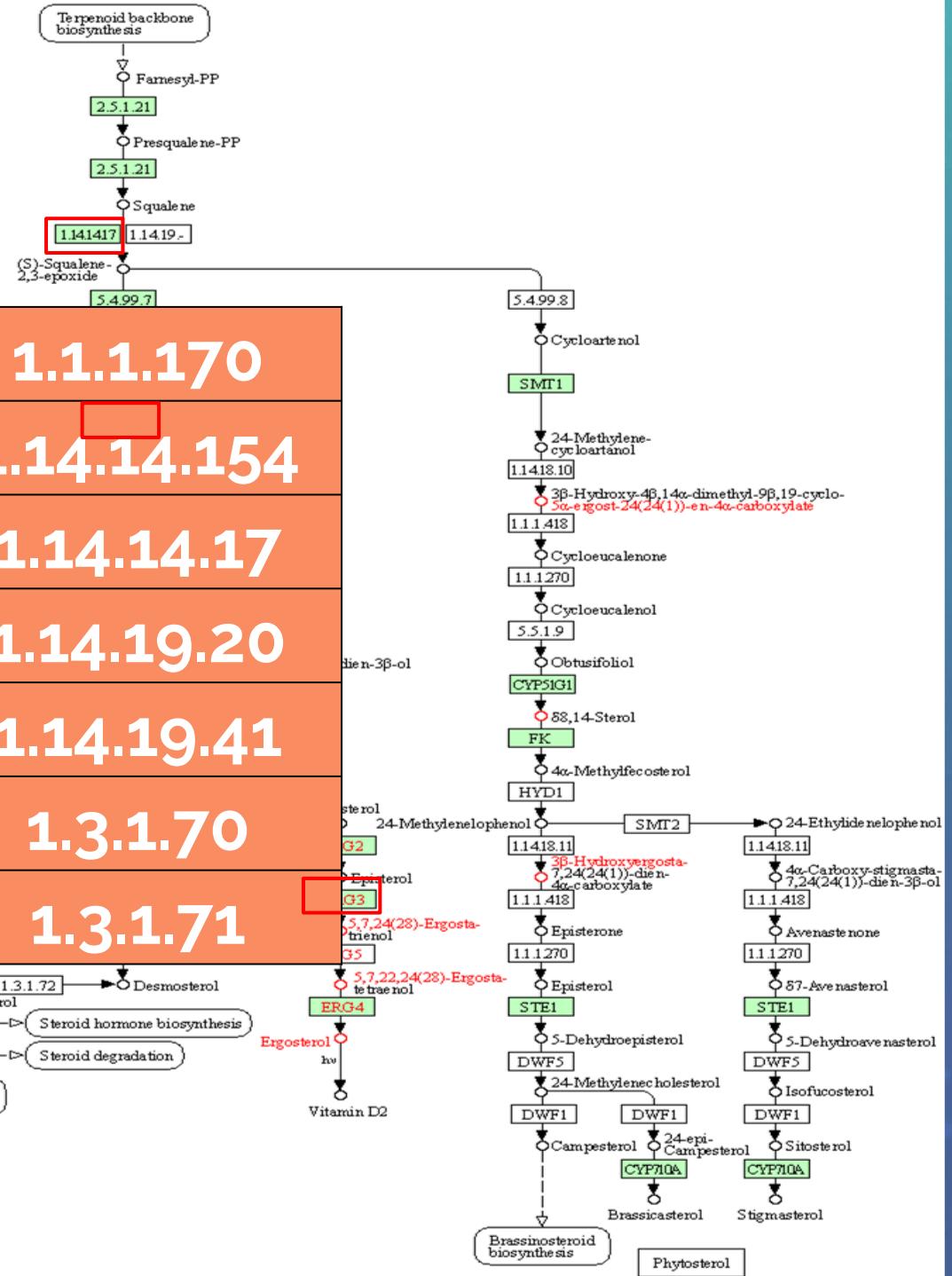
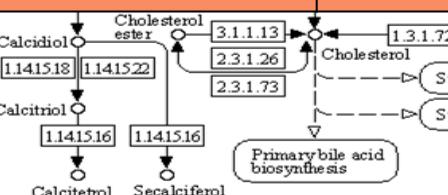
1.3.1.70

Sterol C-24 reductase

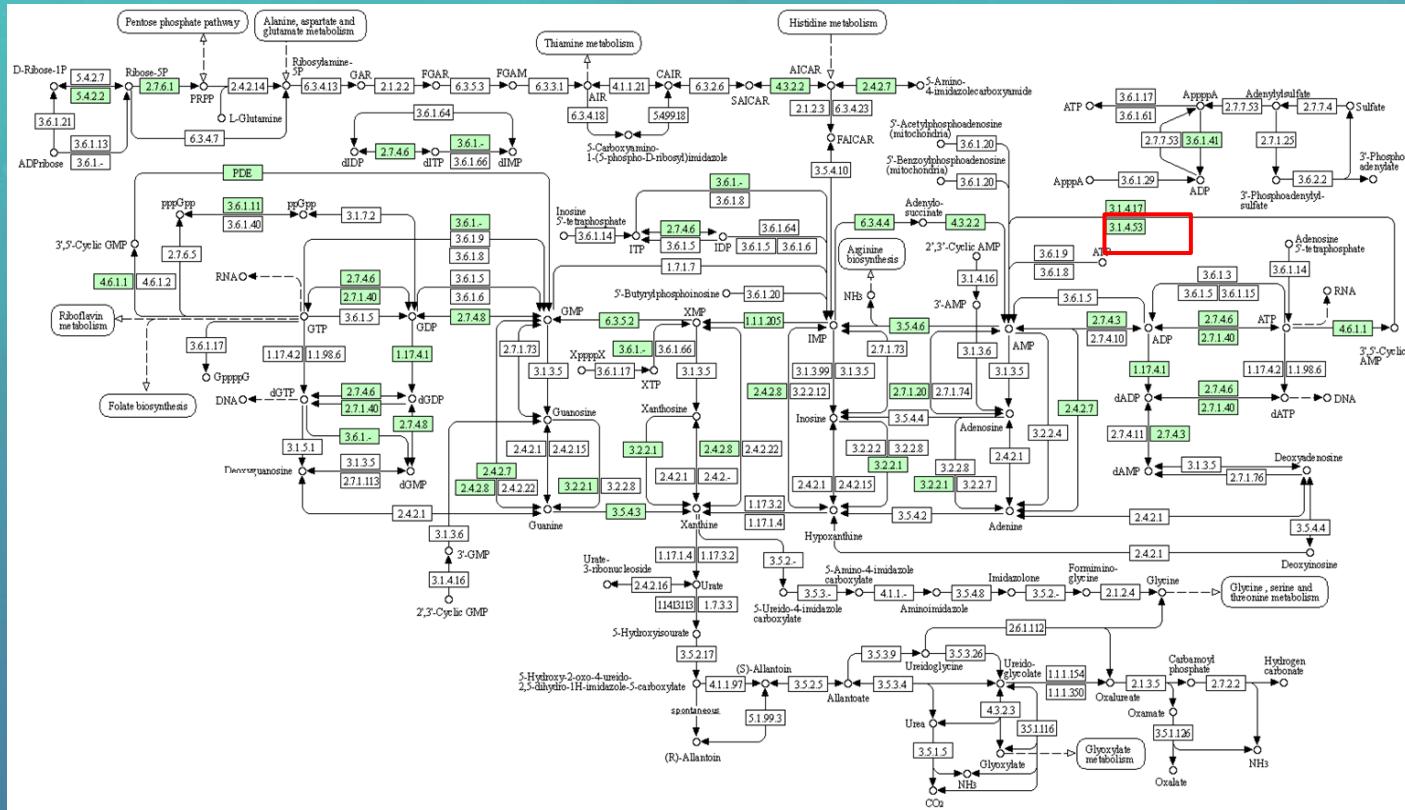
1.3.1.71

ERG3: Lathosterol oxidase

ERG4: Sterol C-24
reductase



METABOLISMO DE PURINAS



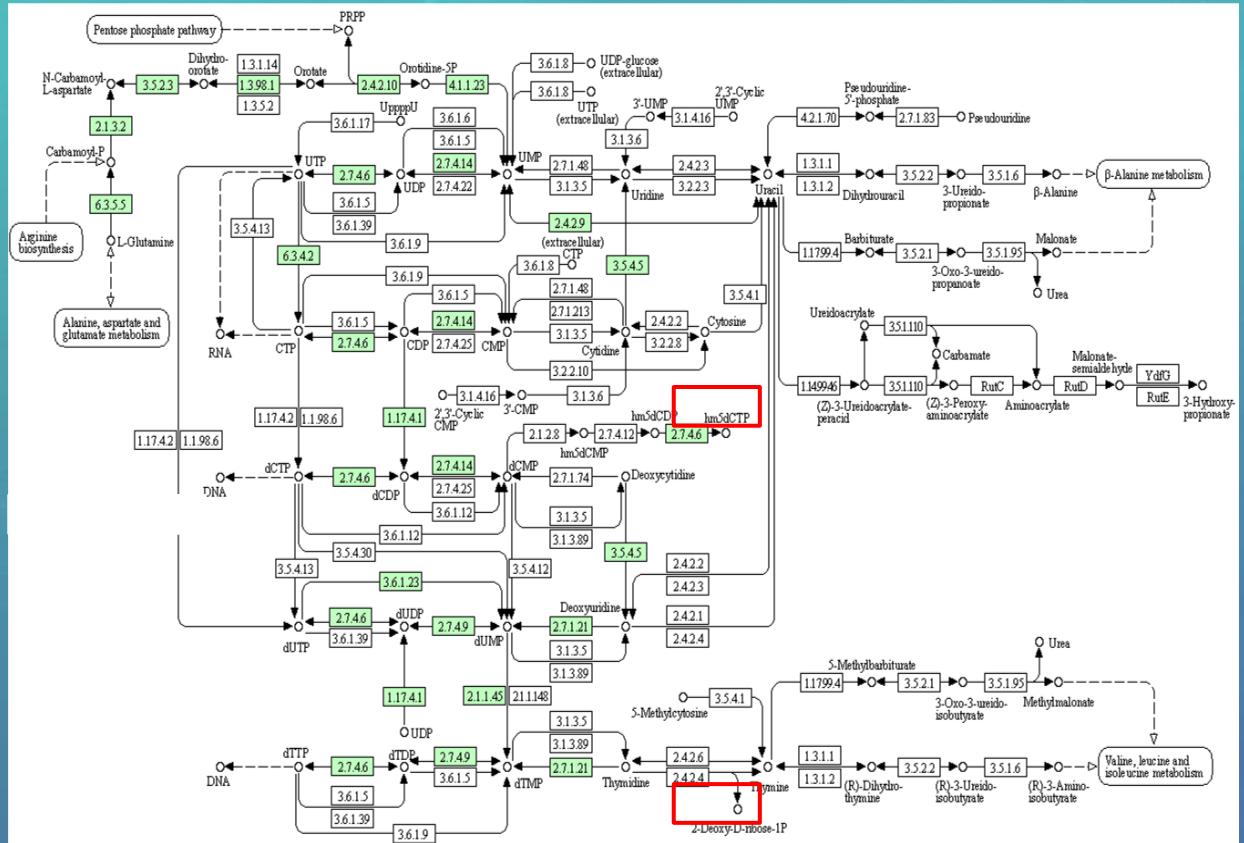
Inosine-5'-monophosphate dehydrogenase

1.1.1.20
5

Ribonucleoside-diphosphate reductase small chain

1.17.4.1

METABOLISMO DE PIRIMIDINAS



Ribonucleoside-diphosphate reductase **1.17.4.1**
Dihydroorotate dehydrogenase (fumarate) **1.3.5.2**

Essentiality, Infectivity, Adverse phenotyping

FILTERS

ESSENTIALITY

LipDH, G6PDH, TR and glutathione peroxidase

PMID: 21631607, Lipoamide dehydrogenase

PMID: 33445584, Glucose 6-P dehydrogenase

PMID: 24722489, Glutathione peroxidase

PMID: 10672177 , Trypanothione reductase

Peroxiredoxine. Wilkinson et al. 2004 JBC

INFECTIVITY of T. brucei:

Trypanothione synthetase (Comini et al. 2004). Interaction studies with epselen and epsulfur: Cristina Quiroga, Marcelo Comini, Andrea Medeiros (IPMON Montevideo Uruguay), Andrés Camilo Ballesteros, Margot Paulino (FQ, Udelar),

ADVERSE PHENOTYPING

MINING IN OXIDATIVE, LIPIDIC AND PURINE/PIRIMIDINE METABOLISMS

Malate dehydrogenase
Pyruvate dehydrogenase E1 alpha subunit
2-Oxoglutarate dehydrogenase
Dihydrolipoamide dehydrogenase
NADP-dependent alcohol dehydrogenase
G 3PDH
Aldehyde dehydrogenase
Isocitrate dehydrogenase
6-phosphogluconate dehydrogenase
NADP-dependent alcohol hydrogenase

Glucose-6-phosphate dehydrogenase
Ascorbate peroxidase
Tryparedoxin peroxidase
Glutathione peroxidase
Ribonucleoside-diphosphate reductase
Trypanothione reductase
NADH dehydrogenase
Succinate dehydrogenase flavoprotein
NADH-ubiquinone oxidoreductase
Aldehyde dehydrogenase

NAD(P)-dependent steroid dehydrogenase
Lanosterol 14-alpha demethylase
Squalene monooxygenase
Lathosterol oxidase-like protein
Sterol 22-desaturase
C-14 sterol reductase, putative
Sterol C-24 reductase, putative
3-hydroxy-3-methylglutaryl-CoA reductase
Inosine-5'-monophosphate dehydrogenase
Ribonucleoside-diphosphate reductase
Dihydroorotate dehydrogenase



Pablo Garcia

<http://detema.fq.edu.uy/Bioinformtica.html>

<http://detema.fq.edu.uy/Bioinformtica.html>



Uruguay

Thank you