molecules

MDPI

Supplementary Material:

In Silico Discovery of a Substituted 6-Methoxy-quinalidine with Leishmanicidal Activity in *Leishmania infantum*

Strahinja Stevanović[1]*, Andrej Perdih[2]*, Milan Senćanski[1], Sanja Glišić[1], Margarida Duarte[3], Anna Tomás[3], Filipa V. Sena[4], Filipe M. Sousa[4], Manuela M. Pereira[4, 5] and Tom Solmajer[2]

[1] *Center of Multidisciplinary Research, Institute of Nuclear Sciences "Vinča", University of Belgrade, 11001 Belgrade, Serbia*

[2] *National Institute of Chemistry, Hajdrihova 19, 1001 Ljubljana, Slovenia*

[3] *i3S-Instituto de Investigação e Inovação em Saúde, Universidade do Porto and IBMC-Institute for Molecular and Cell Biology, 4099-002 Porto, Portugal*

[4] *ICBAS, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, 4099-002 Porto, Portugal*

[5] *Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, 1099-085 Oeiras, Portugall*

[6] *University of Lisbon, Faculty of Sciences, BioISI-Biosystems & Integrative Sciences Institute, Campo Grande, C8,1749-016 Lisboa, Portugal*

* Correspondence: sstevanovic@vin.bg.ac.rs;

* Correspondence: andrej.perdih@ki.si; Tel.: +386-1-4760-376

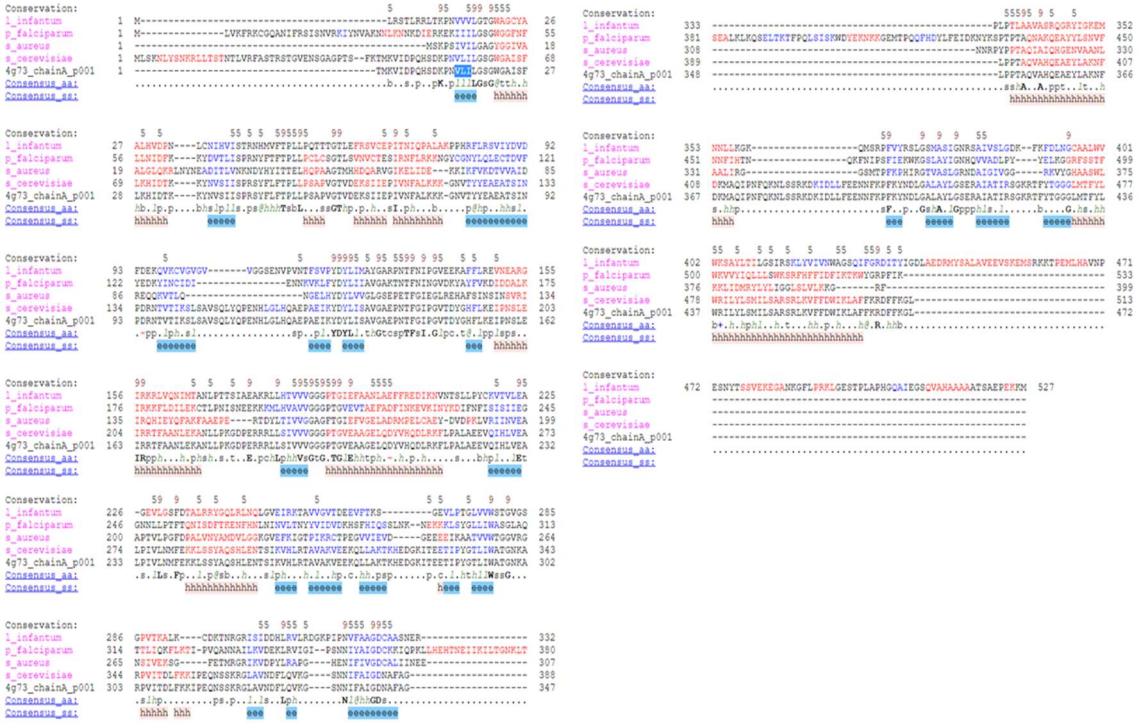


Figure S1. Secondary Structure Alignment generated from LiNDH2, ScNDH2, SaNDH2 and PfNDH2 sequence alignment using PROMALS3D: a tool for multiple sequence and structure alignment. For the reference template—PDB ID 4g73 is used (available crystal structure of ScNDH2). In consensus secondary structure line: With letter "h" chain—alfa-helix is marked, and with "e" chain, beta-sheet.

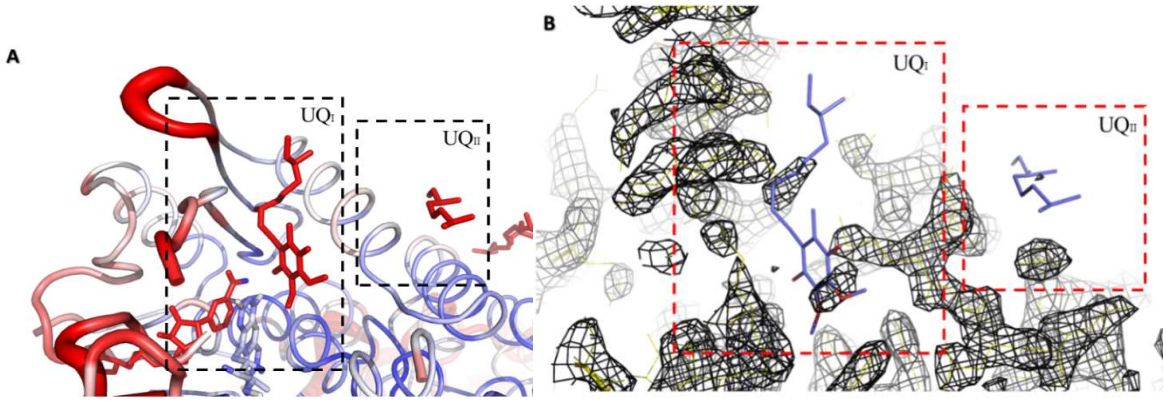


Figure S2. (A) B-factor representation of S. *cerevisiae* NDH-2 (PDB ID: 4G73) focused on binding site—UQI which contains UQ, FAD and NADH as a form of ternary complex. Color code: white(high)-to-red(low) quantification of atom position certainty, traced using pymol software. **(B)** Isomesh displayed using pymol's CCP4—format electron density mapping. Lower electron density population is shown in the region of second—UQ_{II} binding site, thus making it difficult to deduce conformational occupancy of UQ and amino acid residues from X-ray structure.

Table S1. Homology models generated using Phyre 2 server. First two homologues structural models are shown;

Template	Confidence	% i.d.	Template Information
4G6G_B 4G73_B **(same seq.)**	100.0	33	**PDB header:** oxidoreductase **Chain:** B: **PDB Molecule:** rotenone-insensitive nadh-ubiquinone oxidoreductase, **PDBTitle:** crystal structure of NDH-2 with TRT **Resolution:** 2.5 Å
4XDB_C	100	27	**PDB header:** oxidoreductase, membrane protein, flavoprotein **Chain:** C: **PDB Molecule:** nadh dehydrogenase-like protein saouhsc_00878; **PDBTitle:** nadh:quinone oxidoreductase (ndh-ii) from staphylococcus aureus -2 holo protein structure. **Resolution:** 3.32 Å
Note: For both templates alignment coverage is around 80 %			

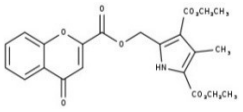
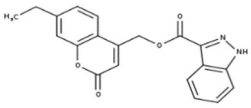
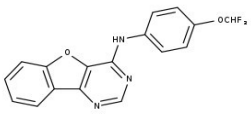
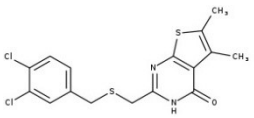
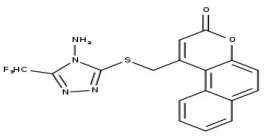
Table S2. Obtained pharmacophore and docking scores for twenty-three selected hit compounds.

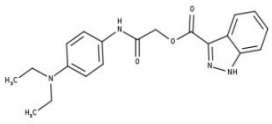
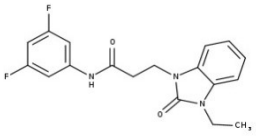
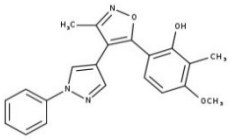
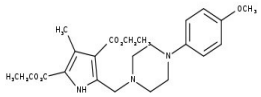
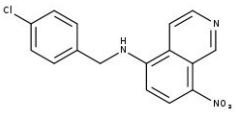
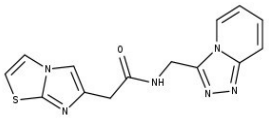
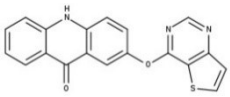
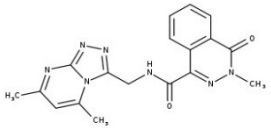
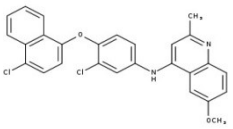
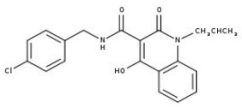
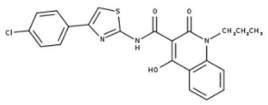
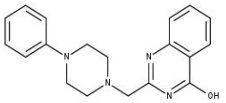
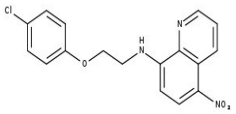
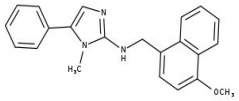
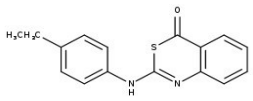
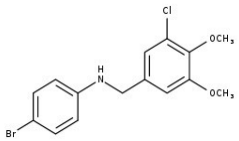
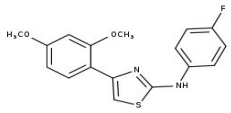
No.	Scaffold	Pose/conformation	PharmFit	GOLD.fitness
1	PYRROLE	1	48.2	78.9353
		2	48.2	78.8489
		3	48.2	73.5001
2	CHROMEN	1	47.4	71.5822
		2	47.4	71.1047
		3	47.4	67.7467
3	benzofuro[3,2-d]pyrimidin	1	46.62	71.1031
		2	46.62	67.5164
		3	46.62	67.4288
4	THYENO[3,2-d]PYRIMIDIN	1	49.46	67.0032
		2	49.46	66.7081
		3	49.46	64.9063
5	CHROMEN	1	47.02	70.0423
		2	47.02	67.3608
		3	47.02	66.6311

6	quinazoline	1	48.01	71.0211
		2	48.01	70.8991
		3	48.01	69.7889
7	INDAZOLE	1	47.28	74.1601
		2	47.28	72.2617
		3	47.28	60
8	BENZYMIDAZOLE	1	46.25	73.6621
		2	46.25	71.6988
		3	46.25	65.6762
9	O,M-METOXY	1	49.57	66.1606
		2	49.57	66.0541
		3	49.57	66.189
10	PYRROLE	1	47.41	83.8593
		2	47.41	82.4897
		3	47.41	80.6411
11	nitroisoquinoline	1	46.43	69.5886
		2	46.43	68.1004
		3	46.43	66.7446
12	TRIAZOLO[4,3-a]PYRIDIN	1	46.83	72.574
		2	46.83	70.5994
		3	46.83	60
13	THYENO[3,2-d]PYRIMIDIN	1	46.33	73.0864
		2	46.33	72.0532
		3	46.33	69.4898
14	dihydrophthalazine	1	50.63	72.2082
		2	50.63	71.9494
		3	50.63	71.0051
15	quinoline	1	47.45	79.2522
		2	47.45	77.9944
		3	47.45	77.1914
16	quinazoline	1	47.72	77.3913
		2	47.72	75.1822
		3	47.72	71.6327
17	quinoline	1	49.78	74.6217
		2	49.78	74.0581

		3	49.78	73.5339
18	QUINAZOLINE	1	49.8	70.3329
		2	49.8	69.9571
		3	49.8	68.429
19	nitroquinoline	1	49.45	74.248
		2	49.45	73.1649
		3	49.45	69.6986
20	naphthalene	1	46.1	73.5739
		2	46.1	72.3605
		3	46.1	60
21	benzothiazine	1	46.04	64.2407
		2	46.04	63.8897
		3	46.04	62.9287
22	aniline	1	46.53	63.8863
		2	46.53	61.1532
		3	46.53	60
23	pyrrole	1	47.52	71.4297
		2	47.52	70.6303
		3	47.52	60
REF	ubiquinone	1	50	80-84
		2	50	80-84
		3	50	80-84

Table S3. Selected commercially available compounds derived from ligand-based virtual screening campaign against *Li*NDH2.

 **1** (T5251861)	 **2** (T5338693)	 **3** (T5357635)
 **4** (T5418152)	 **5** (T5510138)	**6** (T5550562)

 **7** (T5826550)	 **8** (T5925359)	 **9** (T5268007)
 **10** (T5368230)	 **11** (T5320777)	 **12** (T5803358)
 **13** (T5811637)	 **14** (T5973959)	 **15** (STK061205)
 **16** (STK047376)	 **17** (STK002301)	 **18** (STK149674)
 **19** (STK085541)	 **20** (STK118160)	 **21** (STK042244)
 **22** (STK140911)	 **23**(T5330742)	

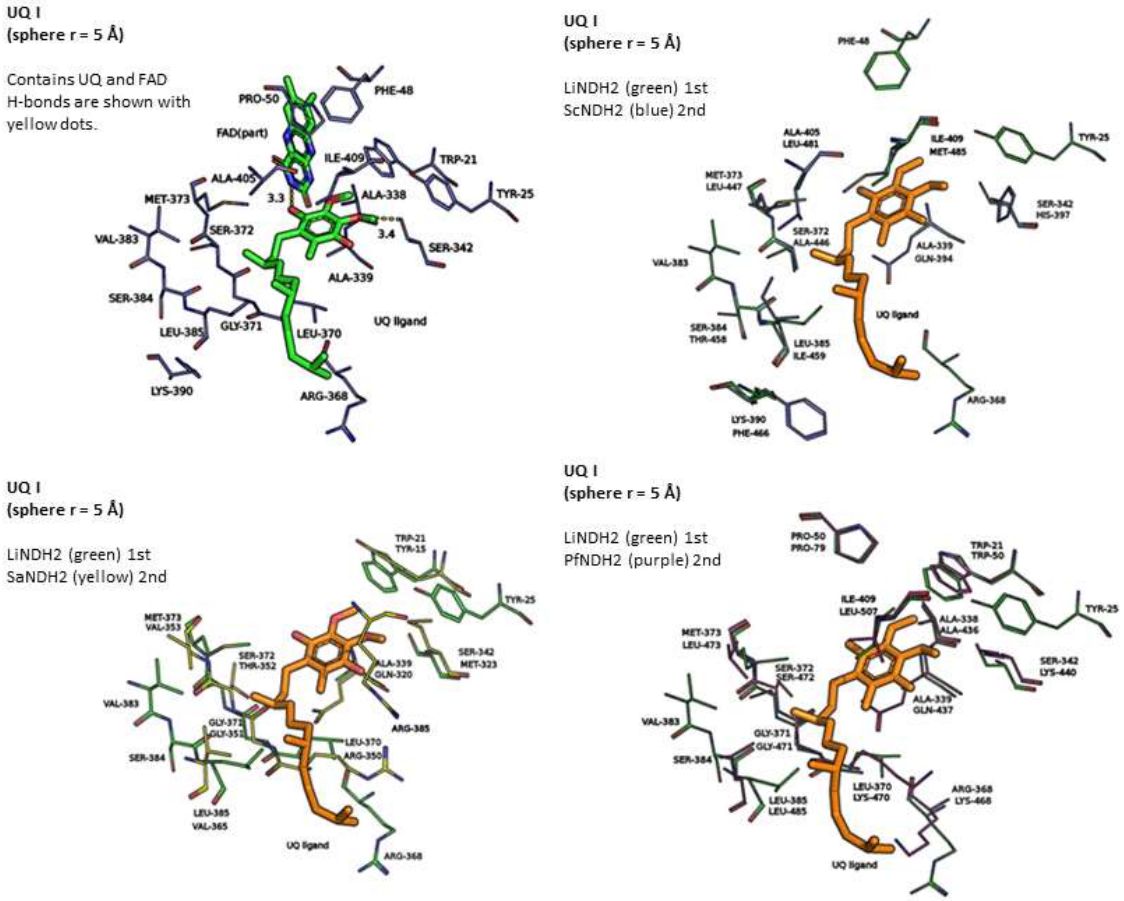


Figure S3. Binding site comparison of different NDH-2s. Upper left—binding site UQ_I in the model *Li*NDH2 showing UQ molecule and important residues. Upper right—comparison of UQ_I between *Li*NDH2 and S*c*NDH2. Lower left—comparison of UQ_I between *Li*NDH2 and S*a*NDH2. Lower right—comparison of UQ_I between *Li*NDH2 and *Pf*NDH2. For clarity reasons, only the differences in amino acid between structures are shown in comparisons.

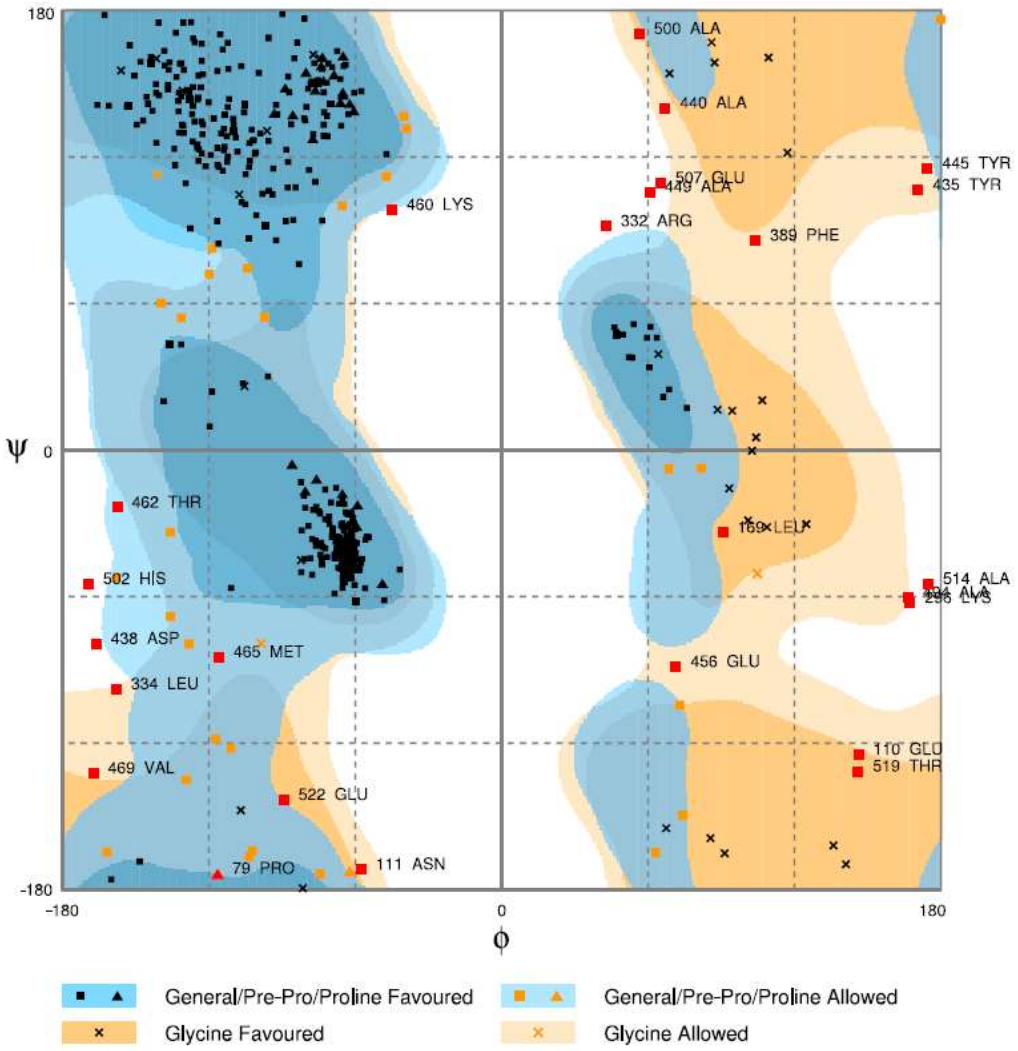


Figure S4. Ramachandran plot for *Li*NDH-2, calculated using RAMPAGE.

Table S4. Docked UQ in homology structure of *Li*NDH2 against UQ experimental posing from template structure 4G73. RMSD value refers to conformational differences in active site features, amino acid environment.

Docked UQ vs reference—UQ experimental	RMSD (Å)
|UQ| dock 1—reference	1.9159
|UQ| dock 2—reference	1.9501
|UQ| dock 3—reference	1.2439
|UQ| dock 4—reference	1.7549

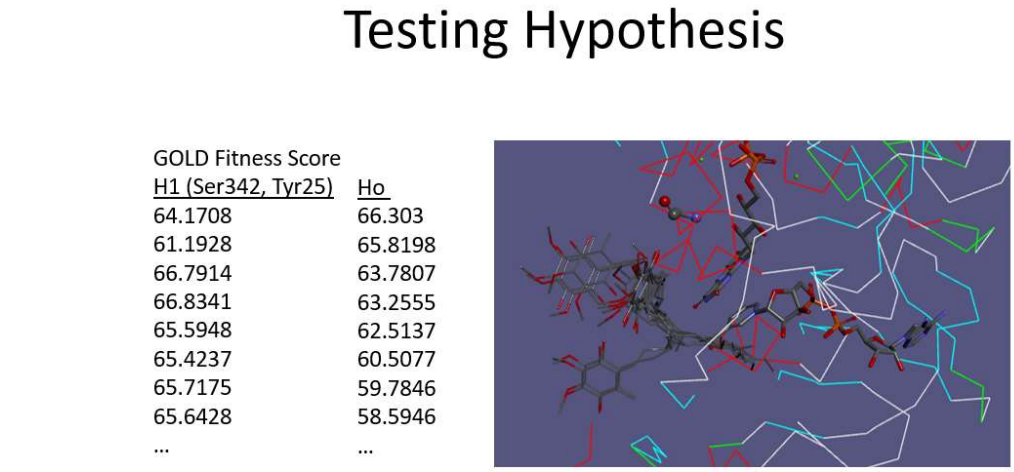


Figure S5. Results of testing of the docking modes of UQ without Ser324 and Tyr25

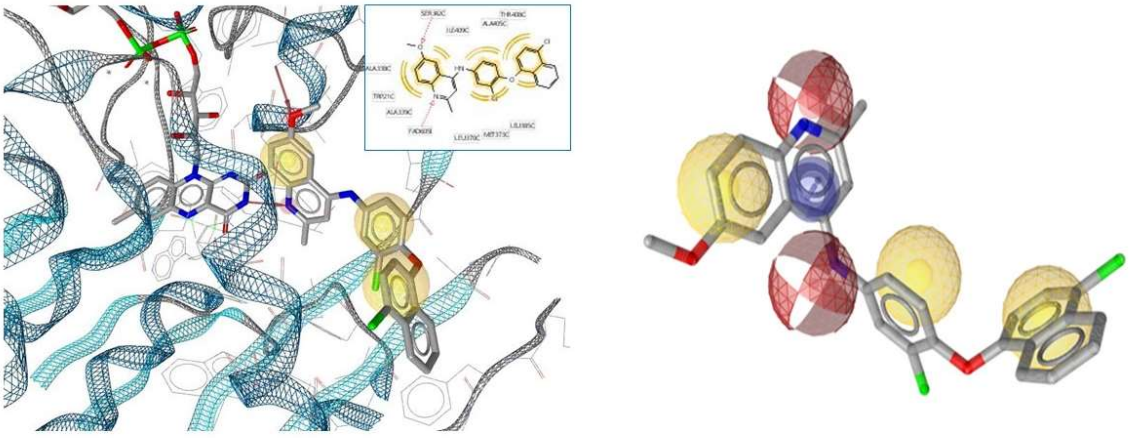


Figure S6. Proof of structure-based and ligand-based consensus in case of compound **15**. Pharmacophore model generated from active compounds predicts functional group composition as a blueprint necessary for interaction with *Li*NDH2 structure.