Electronic Supplementary Information

A Ni(II) Dibridged Complex Manifested by End-on Azide-N and Phenolate-O Atom: Spectral Interpretation, Magnetism and Biological Study

Kuheli Das,[a] Amitabha Datta,[*,b] Soumendranath Nandi,[a] Sandeep B. Mane,[b] Sudipa Mondal,[a] Chiara Massera,[c] Chittaranjan Sinha,[*,a] Chen-Hsiung Hung,[*,b] Tulin Askun,[d] Pinar Celikboyun,[d] Zerrin Cantürk,[e] Eugenio Garribba,[f] Takashiro Akitsu,[g]

Experimental

Apoptosis detection by staining with Annexin V-fluorescein isothiocyanate and propidium iodide (FACS)

Apoptotic effect was evaluated on the A549 (non-small cell lung cancer), MCF¬-7 (breast cancer), Caco-2 (colon cancer cell line) and healthy cell lines, CRL 2522 (human normal fibroblast); through flow cytometry analysis. The Annexin V-fluorescein isothiocyanate (FITC) Apoptosis Detection Kit (BD Biosciences Pharmingen, San Diego, California, USA, Cat. No. 556547) was used to detect apoptosis as described by the manufacturer. Briefly, cancer cell lines and healthy cell line (5×10^5/well) were seeded in six-well plates and treated with different concentrations of HL and complex **1** (IC50/2 and IC50 value) for 24 h. After the treatment, cells were centrifuged at 1200 rpm for 5 min and the pellets were washed twice with 1 ml of cold PBS. The cells were re-suspended in 100 µl of binding buffer and stained with 5 µl of Annexin V-FITC solution and 5 µl propidium iodide (PI) solution for 20 min at room temperature in dark. Then, the samples were diluted with 400 µl of 1 × binding buffer and processed for data acquisition and analysed on Accuri C6 flow cytometry. At least 10000 cells were analysed per sample. The fraction of cell populations in different quadrants was analysed using quadrant statistics. Quadrant settings were based on the control (without compounds). It was possible to detect and quantitatively compare the percentages of gated populations in all the four regions delineated. Four distinct phenotypes were distinguishable: viable [Annexin V (−)/PI (−); lower left quadrant], early apoptotic [Annexin V (+)/PI (−);

lower right quadrant], late apoptotic [(Annexin V (+)/PI (+); upper right quadrant] and necrotic cells [(Annexin V (−)/PI (+); upper left quadrant].

Statistical analysis

The percentage data from MTT experiments were expressed as the mean ± standard error of the mean (SEM) and analysed statistically using one-way analysis of variance (ANOVA). When ANOVA showed significant differences between groups, Turkey's post hoc test was applied to determine the specific pairs of groups showing statistically significant differences. A p-value of less than 0.05 was considered as statistically significant. The apoptotic results (Annexin V-FITC) were evaluated by flow cytometry using BD Accuri™ C6 Software and, the apoptotic cells (early and late apoptotic) were determined as the percentage of cells.

Results and Discussion

Figure S1. ^{1}H NMR spectrum of the ligand, **HL**.

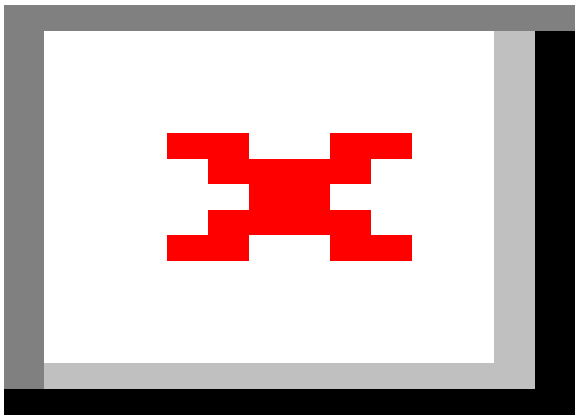

Figure S2. ORTEP view (20% probability level) of complex **1** with the corresponding labelling scheme. Hydrogen atoms have been omitted for clarity.

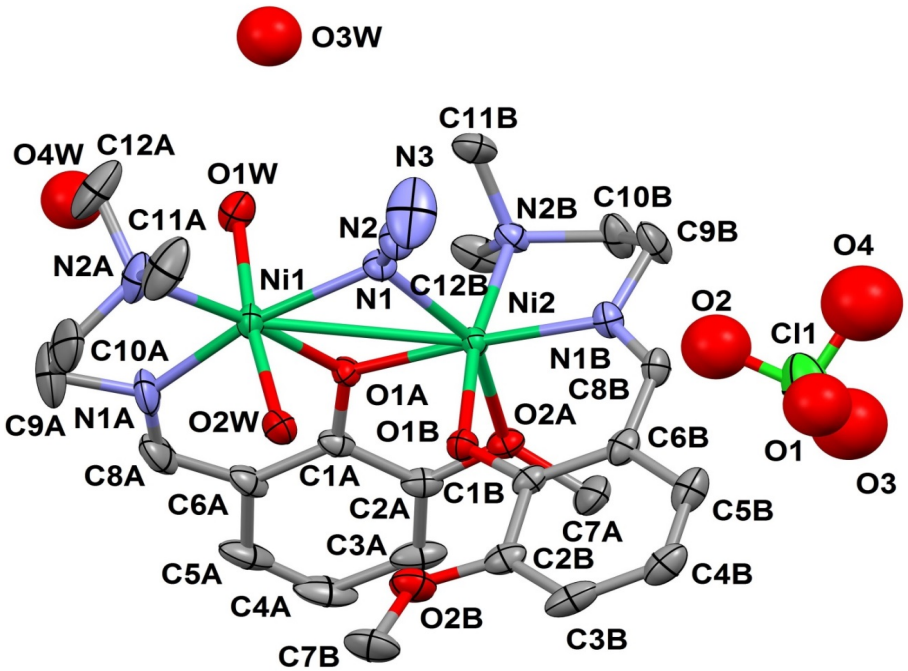

Figure S3. UV-Vis spectra of the ligand HL and of complex **1**.

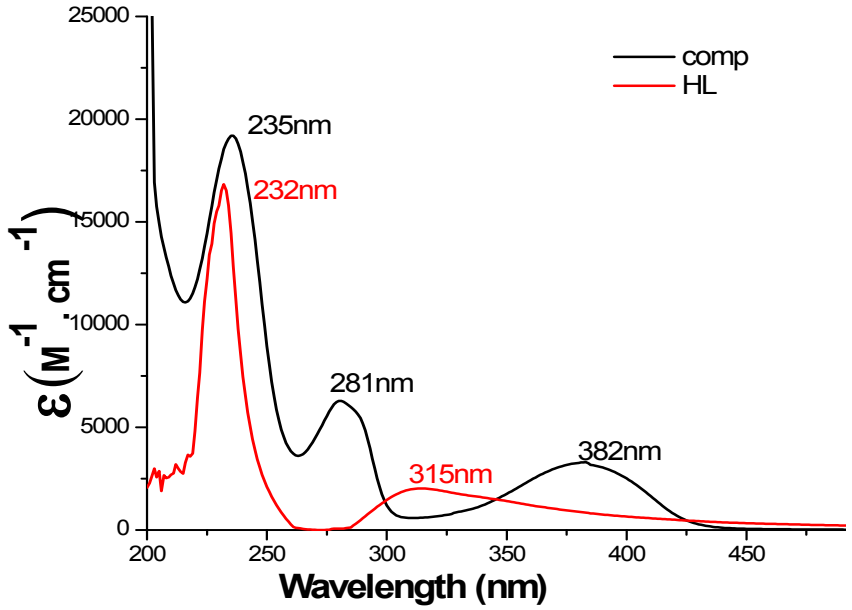

Figure S4. Best docked pose of pyrollidium carboxamides inside the binding pocket of enoyl acyl carrier protein reductase (PDB id 4U0K) of *M. tuberculosis* $H37R_v$ (2D view).

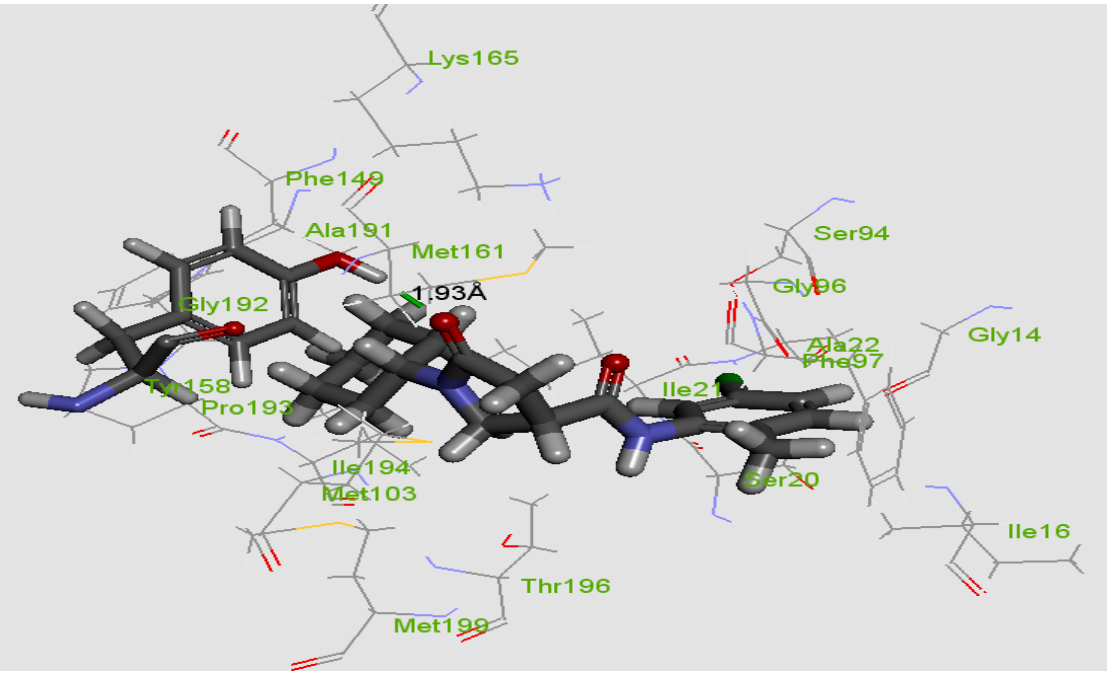

Table S1. MO composition of complex **1**.

MO	Energy (eV)	Ni	L	Azide	OH_2
LUMO+10	-2.04	22	6	71	1
LUMO+9	-2.14	36	4	60	0
LUMO+8	-2.24	60	13	27	0
LUMO+7	-2.36	4	94	2	0
LUMO+6	-2.54	3	96	1	0
LUMO+5	-4.34	22	78	0	0
LUMO+4	-4.4	7	92	0	1
LUMO+3	-4.51	38	61	0	1
LUMO+2	-4.92	38	11	11	40
LUMO+1	-6.43	42	38	17	3
LUMO	-7.02	8	25	2	65
HOMO	-7.77	7	73	2	18
HOMO-1	-8.19	15	11	14	60
HOMO-2	-8.34	15	17	6	62
HOMO-3	-8.39	16	71	5	8
HOMO-4	-8.49	18	14	9	59
HOMO-5	-8.56	11	17	11	61
HOMO-6	-8.93	22	64	5	9
HOMO-7	-9.02	8	36	10	46
HOMO-8	-9.34	19	48	30	3
HOMO-9	-9.45	18	37	15	30
HOMO-10	-9.75	40	17	34	9

Table S2. IC50 values of cytotoxic effects of HL and complex **1** on A-549, MCF-7, CaCo-2 and CRL-2522 with MTT method.

Substances	**A-549 cellline IC50(µg/mL)**	**MCF-7 cellline IC50(µg/mL)**	**Caco-2 cellline IC50(µg/mL)**	**CRL-2522 cellline IC50(µg/mL)**
HL	55.38	79.71	24.82	306.75
Complex **1**	42.301	287.4	38.24	146.5

Table S3. Percentages of early apoptotic, late apoptotic, necrotic values on A-549 (non-small cell lung cancer); MCF-7 (breast cancer); Caco-2 (colon cancer) and healthy cell lines CRL-2522 (human normal fibroblast).

Cell lines and concentrations	**Viable % LL (Lower left)**	**Early Apoptotic % LR (Lower Right)**	**Late Apoptotic % UR (Upper Right)**	**Necrotic % UL (Upper Left)**
A549 Control	100	0	0	0
A549 HL: IC50/2	47.6	13.3	37.3	1.8
A549 HL: IC50	48.9	16.6	33.0	1.5
A549 Complex 1: IC50/2	37.5	18.2	43.6	0.8
A549 Complex 1: IC50	48.7	16.9	33.9	0.6
Caco2 Control	93.5	0.6	3.0	3.0
Caco2 HL: IC50/2	92.3	0.6	3.3	3.7
Caco2 HL: IC50	90.6	0.7	2.9	5.8
Caco2 Complex 1: IC50/2	91.2	1.1	2.6	5.1
Caco2 Complex 1: IC50	32.8	0.4	1.6	5.2
MCF7 Control	91.2	0.1	0.2	8.5
MCF7 HL: IC50/2	70.7	4.1	2.6	22.6

MCF7 HL: IC50	63.0	2.9	9.3	24.8
MCF7 Complex 1: IC50/2	65.4	2.2	0.3	32.1
MCF7 Complex 1: IC50	69.6	0.4	2.5	27.5
CRL2522 Control	98.0	0.1	0.1	1.9
CRL2522 HL: IC50/2	98.7	0.1	0.1	1.1
CRL2522 HL: IC50	98.2	0.1	0.3	1.4
CRL2522 Complex 1: IC50/2	98.6	0.2	0.0	1.3
CRL2522 Complex 1: IC50	98.2	0.2	0.0	1.6