Unexplored features of Ru(II) polypyridyl complexes - towards combined cytotoxic and antimetastatic activity

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Content

1. Computational characterization

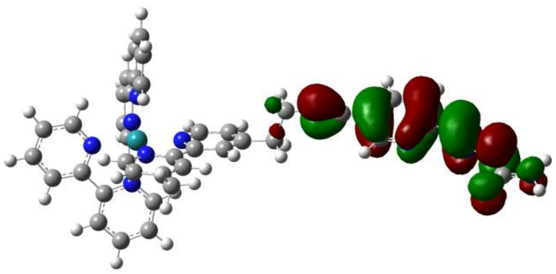
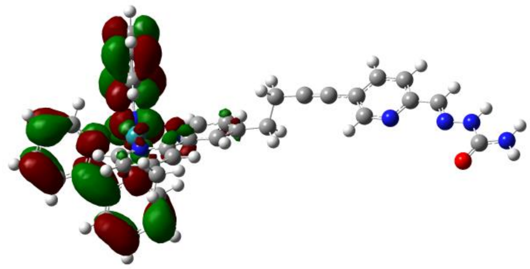
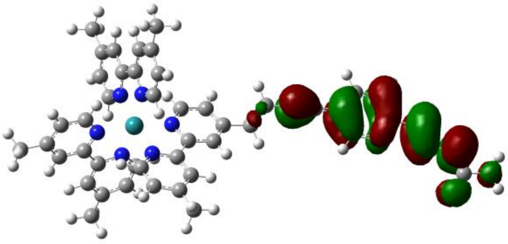
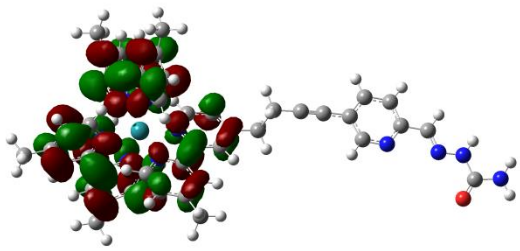
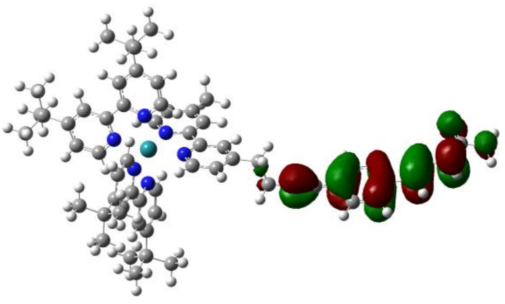
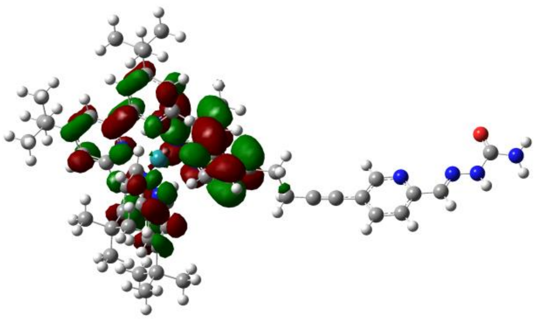
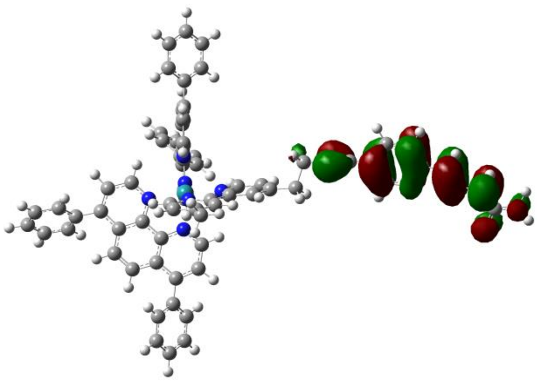
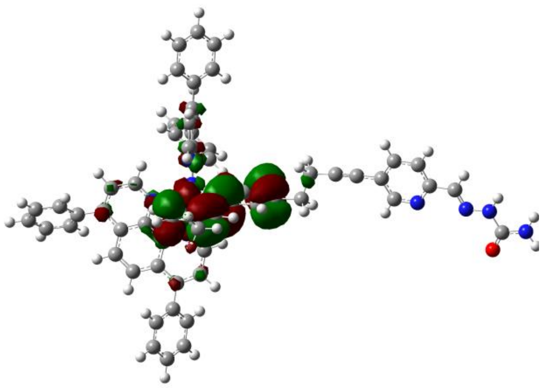
Computational characterization

The ground state geometries and electronic structures of Ru2, Ru3 and Ru5 were obtained within Density Functional Theory (DFT) using hybrid B3LYP functional [2-6] and 6-31G(d,p) basis set [7-10] for C, N, O, S and H atoms. For Ru LANL2DZ basis-set [11,12] was applied. The electronic excitation spectra were computed within Time Dependent – Density Functional

Theory (TD-DFT) with the same functional and basis sets. Eighty lowest laying electronic excitations were determined. The reported calculations were done with Gaussian 09 program [13], and plots of orbitals were done using GaussView [14] software.

DFT geometry structures for **Ru2**, **Ru3** and **Ru5** is given at the end of this file as additional data.

Fig. S1. Plots of HOMO and LUMO of the studied Ru(II) complexes. For Ru1 and Ru 4 data were taken from reference [15]

	HOMO	LUMO
Ru1		
Ru2		
Ru3		
Ru4		

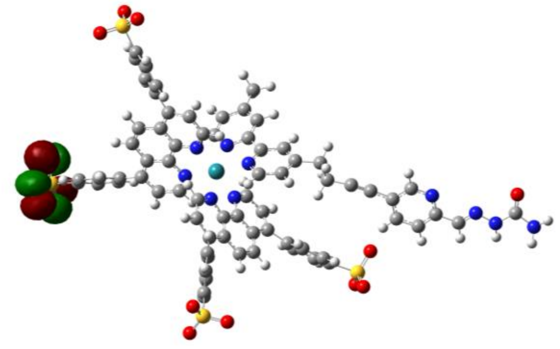
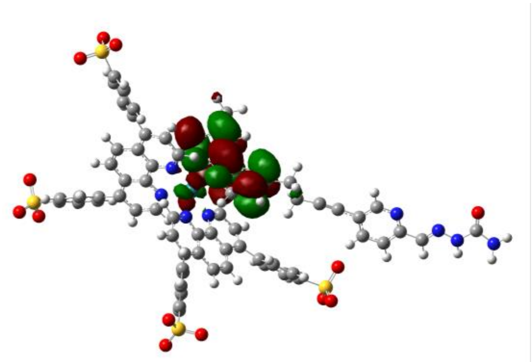
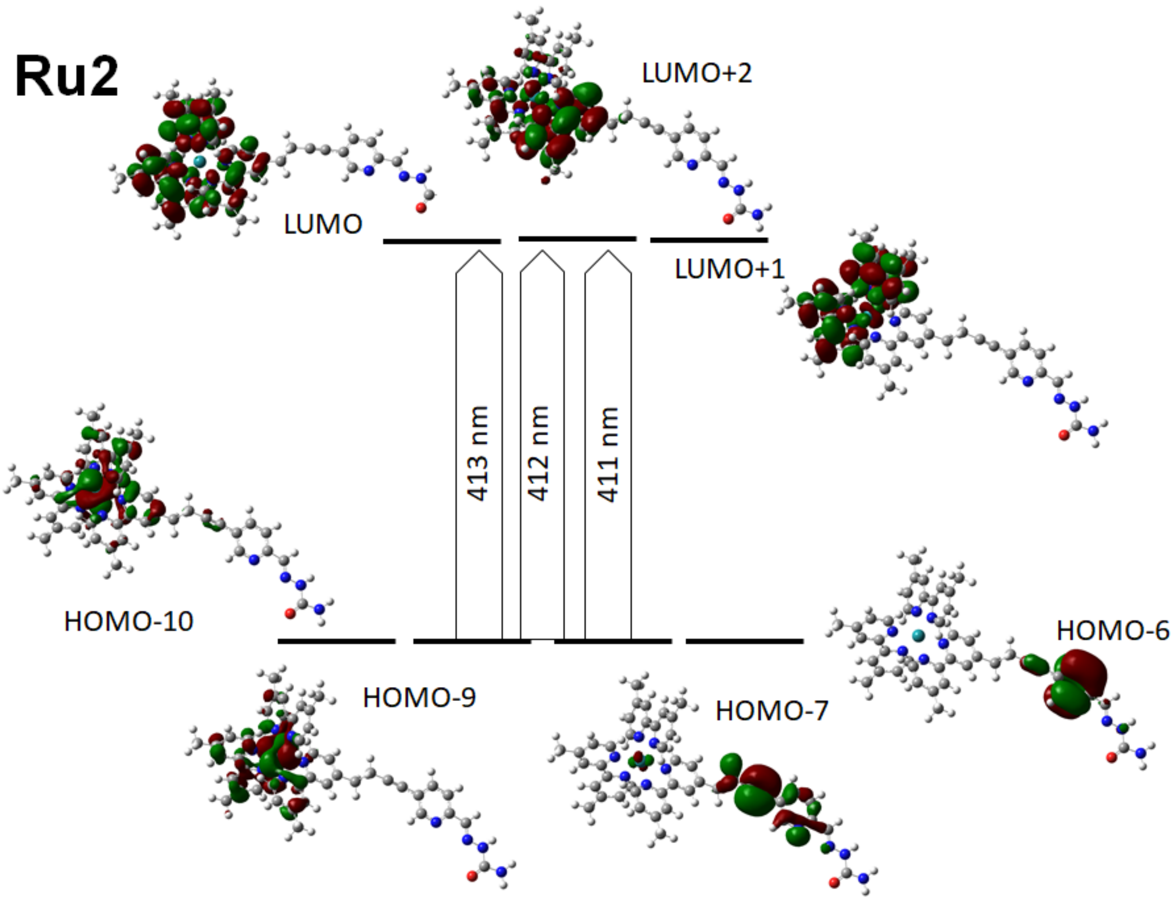
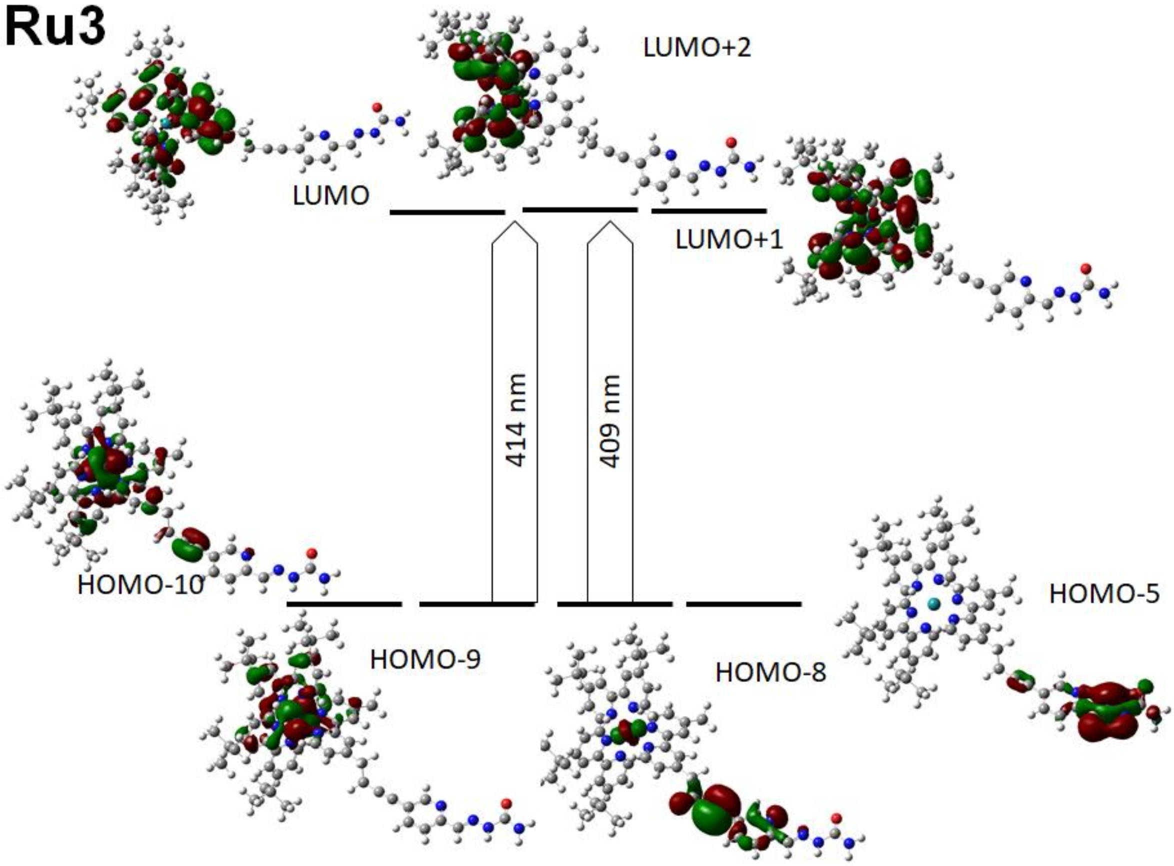
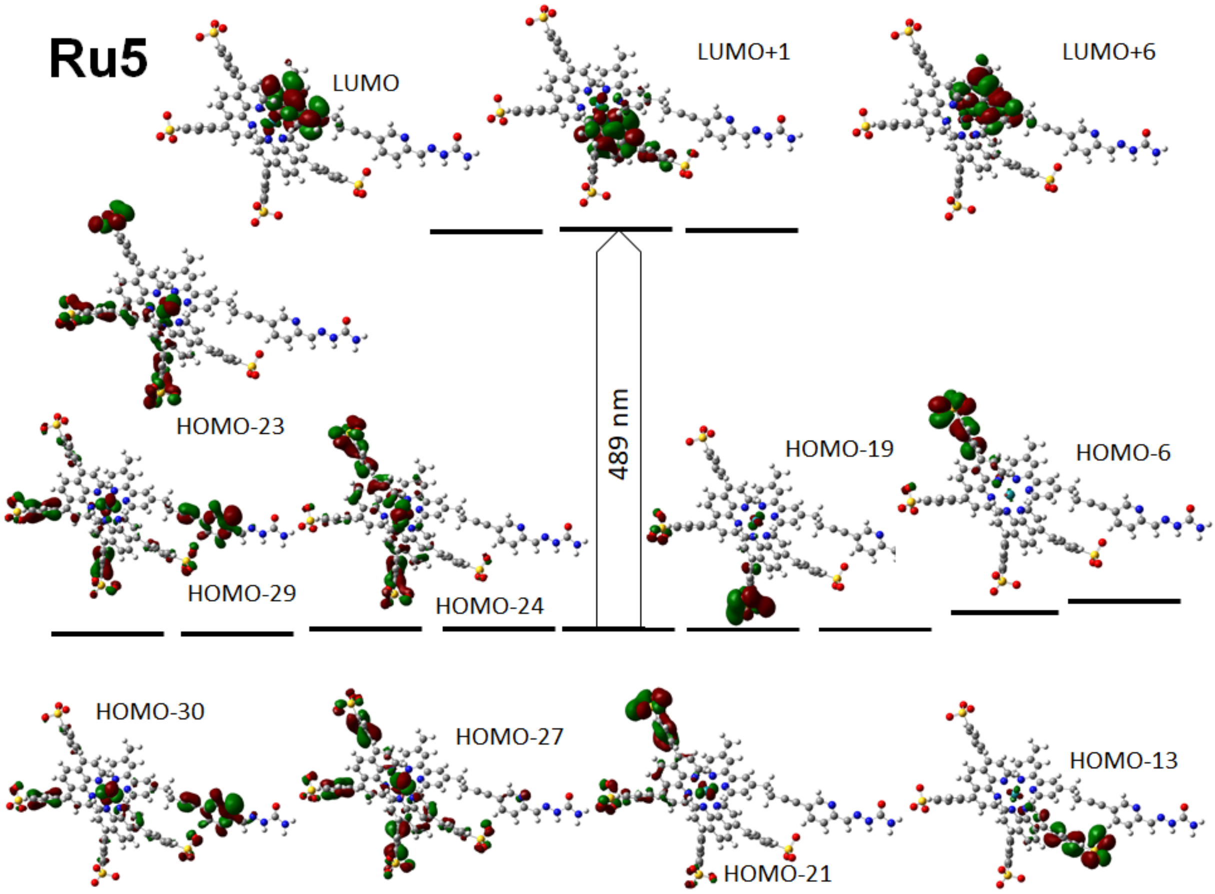
Ru5		

Fig. S2 Plots of orbitals responsible for the most intensive absorption bands in the visible range of Ru2, Ru3 and Ru5 spectra with schematic representation of the most intense absorption bands, as computed with TD-DFT. For Ru1 and Ru4 data are present in reference [15]




Ru3
LUMO+2
LUMO
LUMO+1
414 nm
409 nm
HOMO-10
HOMO-5
HOMO-9
HOMO-8


Ru5
LUMO
LUMO+1
LUMO+6
HOMO-23
489 nm
HOMO-19
HOMO-6
HOMO-29
HOMO-24
HOMO-30
HOMO-27
HOMO-13
HOMO-21

2. Photophysical and physicochemical properties

Spectroscopy measurements – experimental details

UV-Vis absorption spectra of Ru complexes were measured at 25 ºC in water using Perkin Elmer Lambda 35 spectrophotometer (Fig. S3A). Luminescence spectra were registered on Perkin Elmer LS55 spectrofluorimeter in the range 480 - 900 nm upon excitation at the maximum of charge transfer band for each ruthenium complex (~ 460 nm) as the average of three scans (Fig. S3B). For the determination of the quantum yield of luminescence (Φ), aqueous solutions of $[Ru(bpy)_3]^{2+}$ with a small amount of DMSO (<0.008% v/v) were used as standards (Φ = 0.028 [16] and 0.042 [17] for air-equilibrated and deoxygenated conditions, respectively). The spectra for the ruthenium complexes were recorded at a concentration that had an absorbance less than 0.05 units at the excitation wavelength. The quantum yield was calculated according to the following equation:[18]

$$\Phi = \Phi_{ref} \times [A_{ref}/A] \times [I/I_{ref}] \times [n^2/n_{ref}^2]$$

where I is the integrated luminescence intensity, A is the optical density, and n is the refractive index, *ref* refers to the values for the reference. The mean value was calculated from a minimum of three independent experiments.

Luminescence lifetime experiments were performed at room temperature using Fluorolog-3 Horiba Jobin Yvon with single photon counting technique. The complexes were excited at 464 nm with NanoLED diode and photons were counted at the emission maximum for each compound. The DAS6 software (HORIBA Scientific) was used for calculation of the lifetime values.

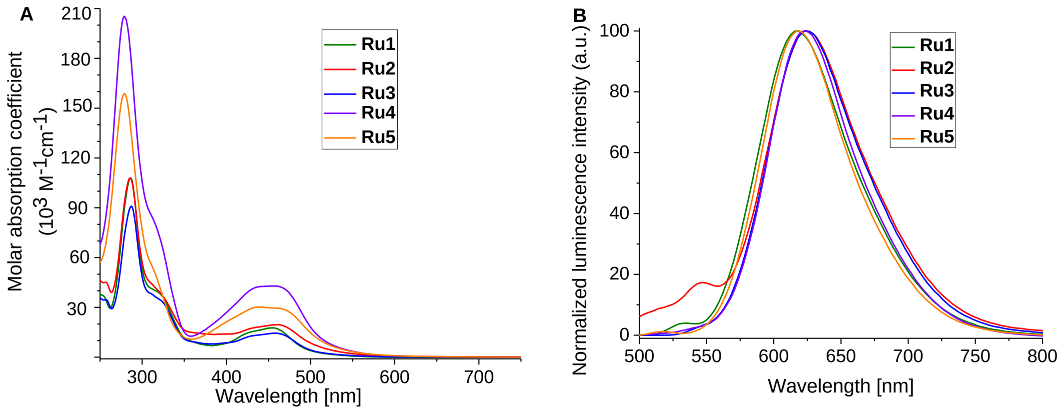


Fig. S3 Absorbance spectra (A) and normalized luminescence spectra (B) of the studied ruthenium(II) complexes recorded at 25 °C in water

Lipophilicity measurements – experimental details

The lipophilicity of studied complexes referred as n-octan-1-ol/water partition coefficient ($\log P_{o/w}$) were measured using shake flask method. The Ru(II) complexes (dissolved in DMSO, final DMSO concentration was 1 %) were diluted in n-octan-1-ol to obtain micromolar concentration solutions. Next the identical volume of water was added and the mixtures were shaking for 20 h. The concentrations of the Ru(II) complexes in water phase were measured spectrophotometrically and $P_{o/w}$ values were calculated as $\frac{C_0 - C_{\text{water after ocanol}}}{C_{\text{water after octanol}}}$ where C_0 is an initial concentration of ruthenium complex. The experiment was conducted in triplicates.

Strong correlation (R_2 =0.97) was observed between measured $\log P_{o/w}$ values for Ru(II) complexes and ones calculated for auxiliary ligands (Fig. S4). So in case of difficulties with experimental determination of $\log P_{o/w}$ for $[Ru(L)_2L1]^{2+}$ compounds, it could be successfully substitute with calculated parameters for distinct ligands.

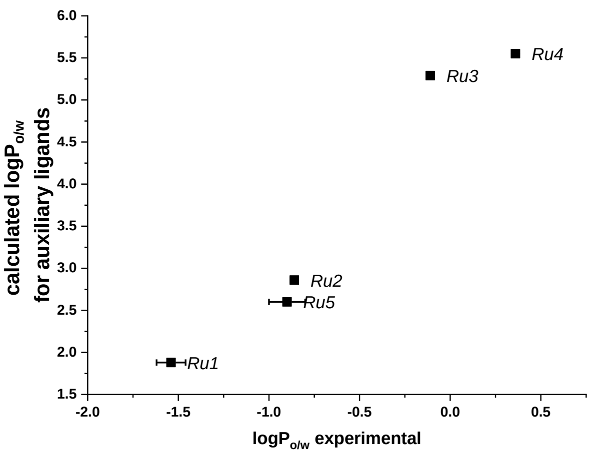


Fig. S4. The correlation between experimental $\log P_{o/w}$ values and calculated for auxiliary ligands using ChemDraw Professional 17.1.

3. Cytotoxicity

Table S1. IC_{50} (µM) of the studied Ru(II) complexes and cisplatin against PANC-1 cells

Compound	IC_{50} (µM)
Ru1	>240
Ru2	102 ± 28
Ru3	7.6 ± 0.2

Ru4	15 ± 3
Ru5	121 ± 11
cisplatin	268 ± 54

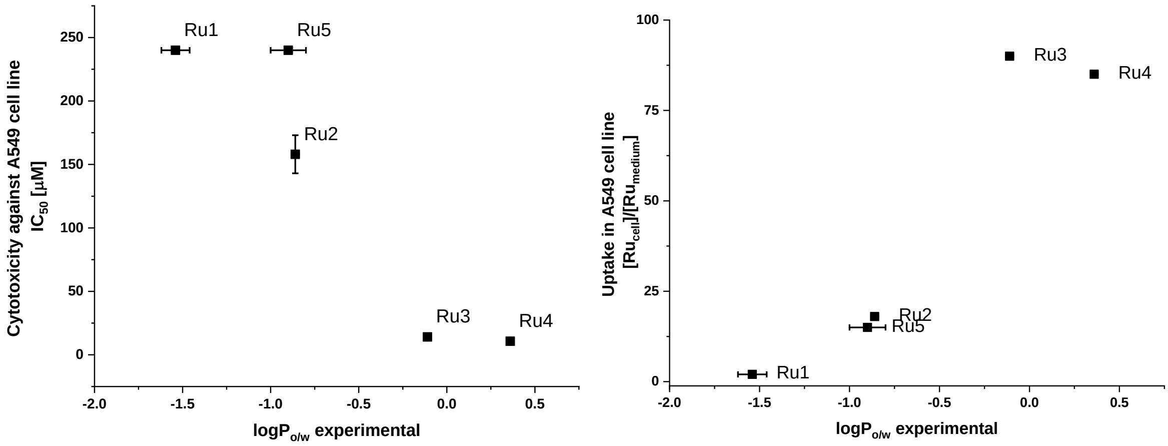


Fig. S5. Correlation between cytotoxicity of Ru complexes against A549 cell lines (A) or their uptake (B) with the compounds lipophilicity. Pearson's *r* are 0.971 and 0.936, respectively; $p < 0.05$.

4. Adhesion properties

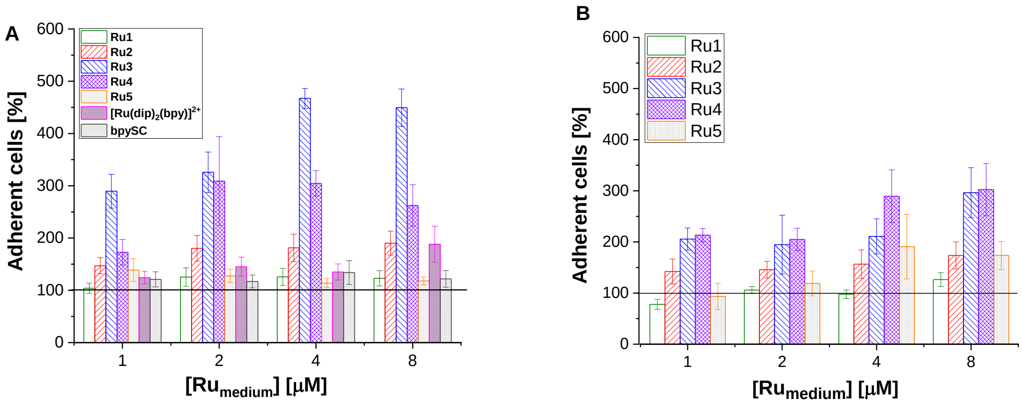


Fig S6. PANC-1 cell adherence to A) plastic or B) collagen coated surface evaluated as the percentage of remained adherent cells upon controlled trypsin treatment. Cells were treated for 24 h with Ru1 (green), Ru2 (red), Ru3 (blue), Ru4 (purple), Ru5 (orange), $[Ru(dip)_2(bpy)]^{2+}$ (pink) and bpySC (grey). Untreated cells were used as control (100%).

5. Change in activity of matrix proteinases

Table S2 IC_{50} values for inhibition of MMP2 and MMP9 by Ru3 and Ru4 as well as the reference metalloproteinase inhibitor GM6001. Experimental conditions: [FS-6] = 2.5 μM, [enzyme] = 0.5 nM, 0.1M Tris, pH 7.4 at 37 ºC (MMP9 was activated using 1 mM APMA, 4ºC, overnight and buffered was supplemented with 0.1M NaCl, 10mM $CaCl_2$, 0.1mM $ZnCl_2$ and 0.05% Brij35. Bars represent mean and SD from triplicate experiments.

	Ru3	Ru4	GM6001
Inhibitory activity against MMP2			
IC50/μM	1.7 ± 0.6	0.4 ± 0.1	34 ± 5
Inhibitory activity against MMP9			
IC50/μM	17 ± 1	1.8 ± 0.5	0.8 ± 0.1

6. Oxidative stress evaluation

To examine the generation of singlet oxygen in the cells singlet oxygen sensor green (SOSG) was used. 2,7-dichlorodihydrofluorescein diacetate (H_2DCF-DA) was used to determine the general ROS production. It is cell-permeable dye, which diffuses into cells and is deacetylated by cellular esterases to form 2,7-dichlorodihydrofluorescein (H_2DCF). In the presence of ROS H_2DCF is rapidly oxidized to fluorescent 2,7-dichlorofluorescein (DCF). It exhibits high sensitivity towards H_2O_2 but also other ROS such as hydroxyl radical, hydroperoxides or peroxynitrite can oxidized this dye, however with the reduced sensitivity as compare to H_2O_2. Hydroethidium (HE) was used to qualify the production of superoxide anion radical and aminophenyl fluorescein (APF) was used to evaluate the production of hydroxyl radical, hypochloride or peroxynitrite. Weak response of APF sensor allowed to presume that response of cells to H_2DCF arise mainly from H_2O_2 production by cell upon treatment with Ru(II) complexes.

Experimental details for oxidative stress evaluation

Cells were seeded into 96-well plate with the density of 3×10^4 cells per cm^2 in complete medium and cultured for 24 h. Then medium was removed and various concentration of the studied complexes were added for 24 h incubation. After the treatment cells were washed with PBS and ROS probes were added to the culture (H_2DCF-DA (20μM), SOSG (5 μM), APF (5 μM) and HE (10 μM)) for 30 min incubation. Then, the probes were removed, cells were washed with PBS, and fluorescence of the cells was quantified by a Tecan Infinite 200 plate reader. (λ_{ex} = 535 nm and λ_{em}= 485 nm for H_2DCF-DA, SOSG, APF and λ_{ex} = 520 nm and λ_{em}= 605 nm for HE). Experiments were performed in triplicates and each experiment was repeated three times to get mean values and standard deviation of mean.

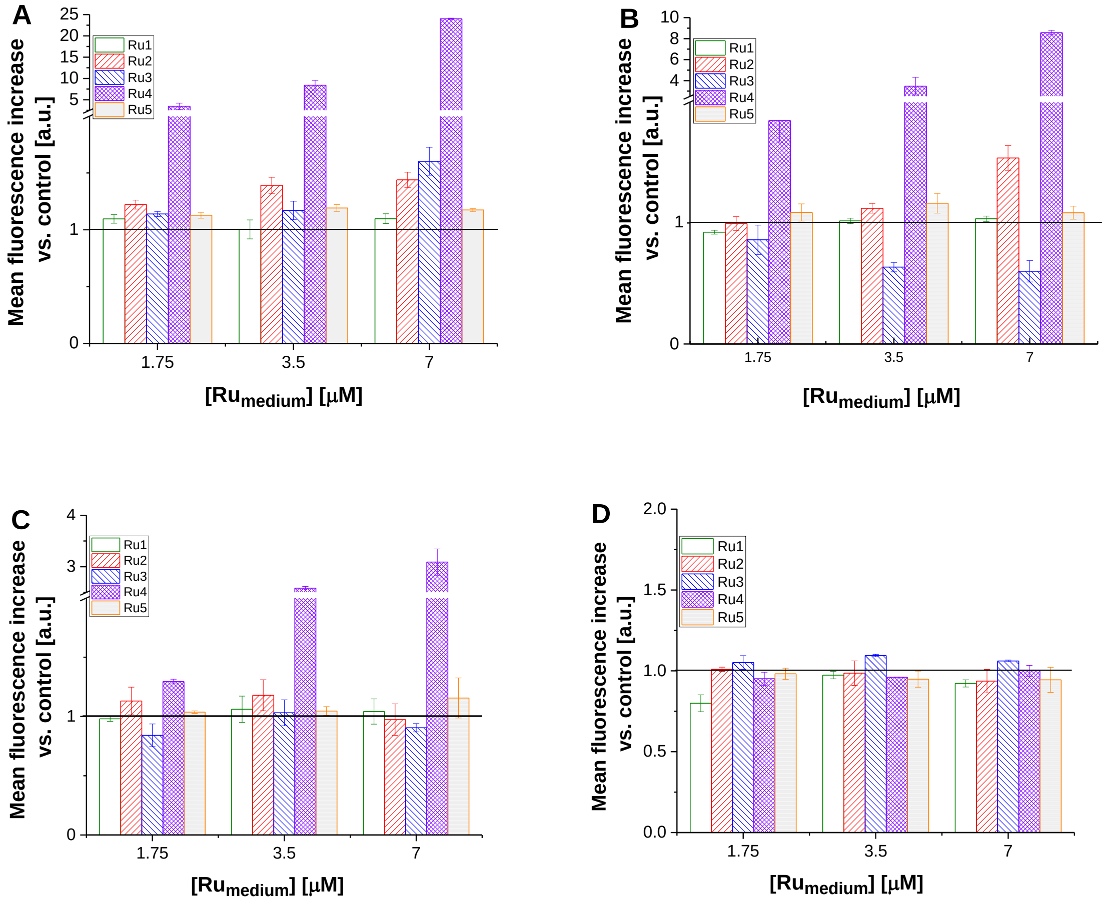


Fig S7. The level of oxidative stress measured by the selected fluorescent probes A) $^1O_2$ measured by SOSG probe, B) H_2O_2 measured by H_2DCF-DA probe, C) $O_2^{\bullet-}$ measured by HE and D)·OH, ONOO and OCl^- measured by APF probe probe induced in A549 cells after 24 h treatment with Ru1 (green), Ru2 (red), Ru3 (blue), Ru4 (purple) and Ru5 (orange).

7. Changes in mitochondria membrane potential

Mitochondria membrane potential was evaluated using JC-1 probe (AAT Bioquest). Red fluorescence corresponds to the fluorescence of J-aggregates, while green fluorescence is related to the fluorescence of J-monomers. The decrease in a ratio of red to green fluorescence values indicates depolarization of mitochondria membrane potential.

Experimental details

Cells were seeded into 96-well plate with the density of 3×10^4 cells per cm^2 in a complete medium and cultured for 24 h. Then, medium was removed and various concentration of the studied Ru(II) complexes were added and incubated for 24 h. Next, cells were washed and JC-1 solution (10 µM) was added to each well and incubated for 30 min. Then, the probe was removed and cells were analyzed by a Tecan Infinite 200 microplate reader (λ_{ex} = 490 nm and λ_{em} = 525 and 590 nm). Valinomycin (20 µM) and gramicidin (10 µM) were used as a positive control of a decrease in membrane potential. Results were calculated as a red/green fluorescence intensity relation. The correction was made on fluorescence of the Ru(II) compounds by subtracting the fluorescent intensity values of Ru treated cells without JC-1 probe. Experiments were performed in triplicates and each experiment was repeated three times to get mean values and standard deviation of mean.

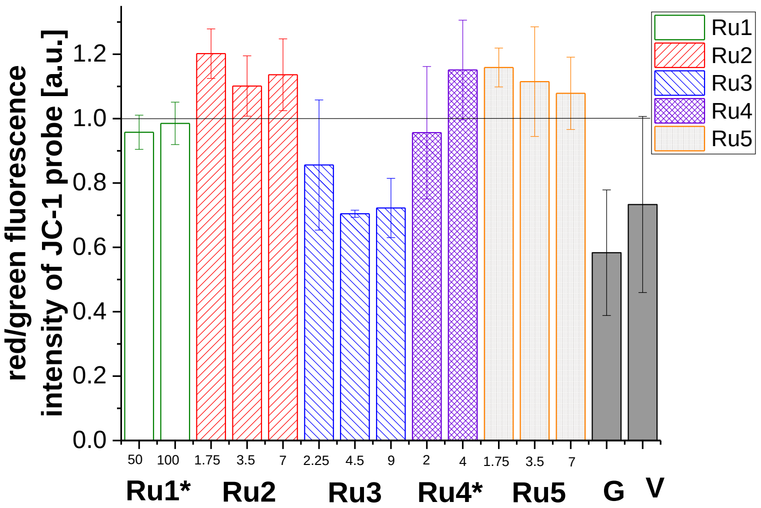


Fig. S8. Changes in mitochondria membrane potential in A549 cells after 24 h incubation with Ru(II) complexes measured using JC-1 probe. All concentrations are given in µM. Gramicidin (G, 10 µM) and valinomycin (V, 20 µM) were used as positive controls. *The results for Ru1 and Ru4 were taken from reference ([15]).

8. Changes in cytosolic calcium concentration

Experimental details

Fluo-8 AM probe (AAT Bioquest) was used to determine changes in cytosolic calcium concentration after incubation with the studied complexes. Cells were seeded into 96-well plate with the density of 3×10^4 cells per cm^2 in complete medium and cultured for 24 h. Then, medium was removed and various concentrations of the studied complexes were added and incubated with cells for 24 h. Next, cells were washed, and Fluo-8 AM solution (4 µM) was added to each well for 1 h incubation. After that, the probe was washed away and fluorescence of the probe was measured by a Tecan Infinite 200 microplate reader (λ_{ex} = 490 nm and λ_{em}= 525 nm). Carbachol (100 μM) causes increase in cytosolic Ca level so was used as a positive control. Experiments were performed in triplicates and each experiment was repeated three times to get mean values and standard deviation of mean

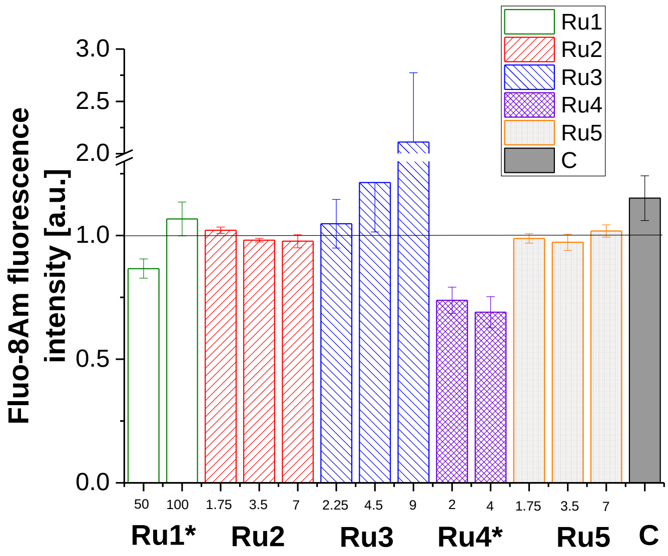


Fig. S9. Changes in cytosolic calcium concentration in A549 cells after 24 h incubation with Ru(II) complexes measured using Fluo-8AM probe. C denotes positive control (carbachol). *The results for Ru1 and Ru4 were taken from reference ([15]).

9. Cellular re-adhesion properties – preliminary studies

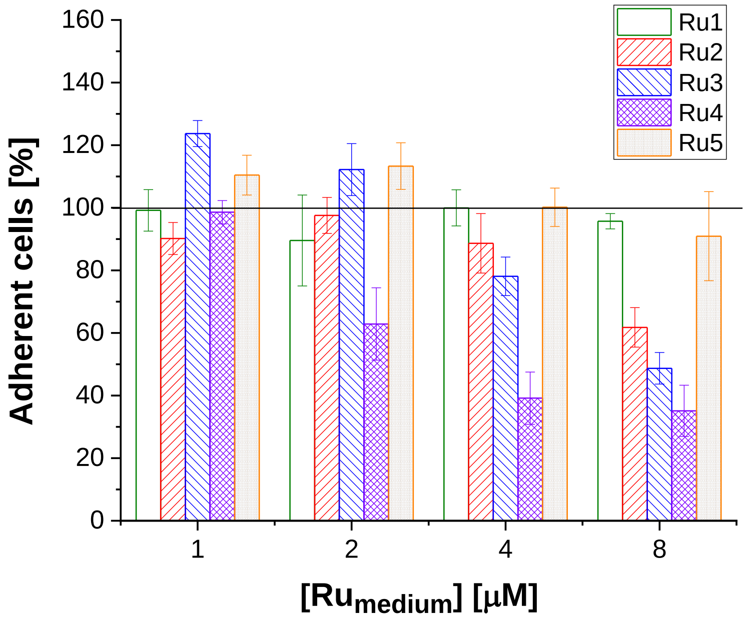


Fig S10. Influence of Ru1 (green), Ru2 (red), Ru3 (blue), Ru4 (purple), Ru5 (orange) and $[Ru(dip)_2(bpy)]^{2+}$ (pink) on PANC-1 cells' ability to re-adhere to plastic surface, measured after 24 h incubation of cells with Ru(II) complexes.

10. References

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11. Additional data: DFT geometry structures for Ru2, Ru3 and Ru5

Ru2:

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Ru	-3.233182	-0.085300	0.057857
O	13.983074	-2.667147	0.241456
N	-3.618659	-1.677538	-1.281753
N	-5.257876	-0.548296	0.463248
N	-3.088425	1.606504	1.320719
N	-1.164325	0.103859	-0.347329
N	-3.894442	1.483987	-1.198009
N	-2.375427	-1.491320	1.387357
N	9.825616	-0.042473	0.108277
N	12.591681	-0.317763	-0.127300
N	13.923422	-0.399748	-0.226337
N	15.943548	-1.513607	-0.098959
C	-2.731868	-2.215235	-2.142931
C	-3.042102	-3.271930	-2.985815
C	-4.328369	-3.831320	-2.960114
C	-4.875176	-2.200783	-1.237482
C	-5.240883	-3.268024	-2.063214
C	-5.795398	-1.559592	-0.273630
C	-6.044956	0.076904	1.361494
C	-7.372628	-0.264884	1.570562
C	-7.952278	-1.302241	0.824903
C	-7.128767	-1.944300	-0.104577
C	-3.455450	2.795066	0.766169
C	-2.649042	1.604370	2.595026
C	-2.560975	2.753559	3.366464
C	-2.937180	3.990648	2.822212
C	-3.386641	3.983904	1.498496
C	-4.350757	3.836751	-1.364675
C	-3.918715	2.724482	-0.636507

C	-4.301623	1.352843	-2.476055
C	-4.774110	3.710607	-2.691131
C	-4.738824	2.421827	-3.244073
C	0.761987	1.033363	-1.445838
C	1.631316	0.214559	-0.711551
C	1.044016	-0.656925	0.213078
C	-0.341472	-0.702190	0.382023
C	-0.607599	0.949909	-1.236035
C	-1.019627	-1.606112	1.335657
C	-0.342299	-2.523071	2.145172
C	-1.035355	-3.349666	3.034107
C	-2.430718	-3.207487	3.069856
C	-3.051911	-2.283992	2.242763
C	3.126007	0.295476	-0.869807
C	-0.326105	-4.347553	3.907801
C	3.756590	1.390846	0.045637
C	5.209835	1.407745	-0.056878
C	6.418375	1.369696	-0.160867
C	7.836744	1.288204	-0.259618
C	9.999374	2.160458	-0.853230
C	10.574721	0.957316	-0.401925
C	8.514576	0.125610	0.173342
C	8.624090	2.327854	-0.784540
C	12.025785	0.779111	-0.490162
C	14.569499	-1.632986	0.013442
C	-2.866482	5.257251	3.630350
H	-3.681740	4.919958	1.039587
H	-4.361211	4.816005	-0.901145
C	-5.256083	4.893223	-3.485698
C	-4.698937	-4.986475	-3.849243
H	-6.245066	-3.671859	-2.013441
H	-7.539023	-2.750973	-0.700177
C	-9.392198	-1.693775	1.013796
H	12.601592	1.626080	-0.887301
H	-1.744432	-1.769040	-2.146964
H	-2.281969	-3.656300	-3.658378
H	-5.577620	0.874086	1.927597
H	-7.951011	0.276783	2.312117
H	-2.362366	0.639590	2.996553
H	-2.198202	2.682316	4.386864
H	-4.265732	0.349585	-2.883888
H	-5.049771	2.247820	-4.269210
H	1.145926	1.729950	-2.184275
H	1.683234	-1.307142	0.798916
H	-1.296574	1.570707	-1.796573
H	0.736698	-2.602430	2.087627
H	-3.034377	-3.814052	3.737375
H	-4.128157	-2.159267	2.254578
H	3.581715	-0.667736	-0.622726
H	3.383320	0.517880	-1.909479
H	0.758383	-4.284431	3.798997
H	-0.575315	-4.188851	4.962066
H	-0.634434	-5.368168	3.655804
H	3.451823	1.205407	1.084917
H	3.340797	2.370254	-0.225541
H	10.629408	2.948418	-1.254726
H	7.940197	-0.699974	0.590985
H	8.156930	3.244870	-1.129278
H	14.448629	0.359980	-0.658042
H	16.436539	-2.347940	0.185798

H	16.388052	-0.647504	0.172361
H	-1.885449	5.365450	4.103196
H	-3.610546	5.241596	4.434833
H	-3.052267	6.141198	3.016925
H	-5.056568	5.835763	-2.971811
H	-6.336481	4.824706	-3.657145
H	-4.776822	4.927347	-4.468813
H	-9.611204	-1.882050	2.069582
H	-9.647586	-2.589941	0.444780
H	-10.055897	-0.885019	0.687854
H	-4.145752	-5.886903	-3.559236
H	-4.442886	-4.775228	-4.892208
H	-5.765480	-5.214052	-3.796203

Ru3:
138

Ru	-2.193141	0.170582	-0.282730
O	14.930627	2.831425	-0.780135
N	-2.560726	2.051983	0.612834
N	-4.195316	0.562063	-0.833582
N	-2.068127	-1.783183	-1.085251
N	-0.137590	0.043567	0.203819
N	-2.914343	-1.027889	1.300418
N	-1.275723	1.184091	-1.898657
N	10.829828	0.208363	-0.080830
N	13.585470	0.597455	0.109379
N	14.913722	0.733119	0.199767
N	16.910414	1.838235	-0.160540
C	-1.678927	2.773024	1.333332
C	-1.977037	4.015358	1.870151
C	-3.245459	4.589469	1.674933
C	-3.798245	2.578099	0.406806
C	-4.149423	3.829334	0.924708
C	-4.719180	1.735815	-0.388079
C	-4.988154	-0.249807	-1.560722
C	-6.301904	0.059116	-1.874840
C	-6.875090	1.264117	-1.431772
C	-6.040020	2.095227	-0.677159
C	-2.463015	-2.792149	-0.262718
C	-1.615789	-2.107484	-2.312717
C	-1.538594	-3.413395	-2.769650
C	-1.935625	-4.480634	-1.944953
C	-2.401989	-4.128085	-0.673838
C	-3.427187	-3.258418	2.036347
C	-2.953090	-2.367585	1.067248
C	-3.350739	-0.580451	2.494647
C	-3.881623	-2.809690	3.281249
C	-3.829130	-1.420031	3.487764
C	1.743299	-0.611197	1.550958
C	2.644757	-0.000480	0.668441
C	2.097149	0.623822	-0.458269
C	0.718053	0.636869	-0.676250
C	0.380917	-0.567284	1.286749
C	0.081097	1.282313	-1.844447
C	0.796186	1.946562	-2.845354
C	0.140494	2.531991	-3.932272
C	-1.256991	2.413692	-3.963958
C	-1.916366	1.745181	-2.943245

C	4.133543	-0.043013	0.888773
C	0.891480	3.264462	-5.010336
C	4.792806	-1.298658	0.238361
C	6.244339	-1.270414	0.364518
C	7.450134	-1.185795	0.472501
C	8.864964	-1.050864	0.564638
C	11.037032	-1.709374	1.363588
C	11.592755	-0.628748	0.652719
C	9.523867	-0.002424	-0.117537
C	9.666790	-1.922203	1.322098
C	13.038259	-0.399140	0.711162
C	15.535863	1.893198	-0.312195
C	-1.850643	-5.929936	-2.439258
H	-2.721216	-4.902845	0.008809
H	-3.443163	-4.315847	1.813817
C	-4.411954	-3.746912	4.373134
C	-3.584635	5.965501	2.261143
H	-5.140980	4.215542	0.736200
H	-6.421315	3.035483	-0.304980
C	-8.329744	1.613059	-1.769122
H	13.626433	-1.116552	1.299438
H	-0.703673	2.323175	1.477992
H	-1.211144	4.530470	2.438516
H	-4.535786	-1.175005	-1.897410
H	-6.872809	-0.648475	-2.465080
H	-1.311097	-1.278060	-2.940102
H	-1.165182	-3.588992	-3.771996
H	-3.305689	0.491833	2.644352
H	-4.160450	-0.980116	4.421344
H	2.096001	-1.111743	2.447035
H	2.762142	1.108862	-1.163522
H	-0.334110	-1.025185	1.960203
H	1.875909	2.013353	-2.785328
H	-1.832556	2.840118	-4.779289
H	-2.995023	1.641949	-2.947456
H	4.598620	0.851028	0.463223
H	4.357213	-0.044353	1.959509
H	1.969464	3.109928	-4.930460
H	0.568571	2.940004	-6.004234
H	0.700044	4.341756	-4.945843
H	4.506854	-1.343069	-0.821685
H	4.382570	-2.204085	0.704483
H	11.677992	-2.367775	1.942162
H	8.937645	0.690754	-0.719016
H	9.214716	-2.746576	1.864347
H	15.448493	0.107782	0.801737
H	17.388815	2.596184	-0.626255
H	17.378292	0.944412	-0.220014
C	-0.378467	-6.250872	-2.799193
C	-2.737268	-6.081673	-3.700305
C	-2.328419	-6.936756	-1.376483
C	-4.376811	-5.224587	3.940932
C	-5.876842	-3.359108	4.695018
C	-3.541041	-3.580600	5.643160
C	-8.494163	1.647832	-3.309008
C	-8.748443	2.980430	-1.197708
C	-9.254858	0.522712	-1.173166
C	-2.594654	7.010607	1.688990
C	-3.438551	5.901838	3.801924
C	-5.019734	6.406655	1.919374

H	-2.669501	7.074730	0.598765
H	-1.556613	6.780052	1.946134
H	-2.823173	7.998005	2.100529
H	-2.421636	5.641204	4.109983
H	-4.125102	5.168258	4.236660
H	-3.672991	6.878947	4.234297
H	-5.176894	6.496096	0.839055
H	-5.210446	7.390680	2.355434
H	-5.770298	5.721619	2.328278
H	-6.518833	-3.464678	3.814680
H	-5.961326	-2.330268	5.057297
H	-6.267877	-4.016052	5.477474
H	-2.496882	-3.845051	5.447686
H	-3.912563	-4.239944	6.433037
H	-3.566264	-2.557736	6.030447
H	-5.002474	-5.411801	3.061585
H	-4.764241	-5.848394	4.750661
H	-3.358647	-5.567818	3.727911
H	-0.304585	-7.280838	-3.160276
H	0.275529	-6.151725	-1.926989
H	0.005872	-5.598181	-3.588538
H	-2.246372	-7.951302	-1.774881
H	-3.377755	-6.781055	-1.103686
H	-1.718925	-6.896120	-0.467305
H	-2.678143	-7.109426	-4.070389
H	-2.417644	-5.421497	-4.511996
H	-3.786858	-5.864369	-3.477692
H	-9.032979	-0.470953	-1.573425
H	-10.296700	0.750693	-1.416905
H	-9.165691	0.478296	-0.083001
H	-8.256807	0.685550	-3.772096
H	-7.852165	2.410690	-3.761037
H	-9.530811	1.887585	-3.562854
H	-8.146103	3.799388	-1.605487
H	-8.684457	3.006982	-0.104605
H	-9.789169	3.181767	-1.464420

Ru5:
146

Ru	-1.436786	0.524683	-0.138242
O	16.019468	5.018834	1.304449
N	17.613018	3.640207	0.375618
N	15.379682	3.109400	0.159078
N	14.049158	3.268620	0.368702
N	11.231829	3.334940	0.707772
N	-1.329766	2.386269	-1.130183
N	-1.229077	-1.313231	0.878452
N	0.207047	1.472415	0.831283
N	0.116075	-0.422999	-1.232230
N	-3.102896	-0.196790	-1.194045
N	-3.038320	1.311475	0.981848
C	16.300503	4.012548	0.686256
C	13.278162	2.470335	-0.277894
C	9.692280	1.546155	-0.833703
C	9.908604	3.306607	0.802794
C	11.816354	2.474339	-0.152259
C	11.077281	1.572757	-0.934102
C	9.076610	2.436851	0.063587
C	7.658565	2.444786	0.212693

C	6.449074	2.383421	0.279858
C	5.003633	2.199689	0.266636
C	-0.906936	6.339172	-2.834905
C	4.239726	2.854555	1.454978
C	-2.221602	2.864590	-2.020155
C	-2.113892	4.122421	-2.594540
C	-1.043544	4.959251	-2.248003
C	-0.123038	4.456400	-1.326651
C	-0.277939	3.177068	-0.784573
C	0.984865	0.922012	1.780915
C	0.652998	2.596138	0.204519
C	1.927760	3.106936	0.455196
C	2.782915	2.476723	1.366099
C	2.260478	1.388121	2.074869
C	-6.729867	0.542357	-0.813432
C	-6.687536	1.375748	0.269723
C	-7.894714	-1.805588	-2.614447
C	-8.989940	-2.222988	-3.363895
C	-8.959985	-2.159047	-4.761259
C	-7.804156	-1.702837	-5.404629
C	-6.700481	-1.303981	-4.662785
C	-6.729321	-1.329776	-3.251737
C	-7.316551	4.118232	1.740725
C	-8.267959	4.984623	2.268628
C	-8.401377	5.133151	3.652693
C	-7.567171	4.401899	4.506949
C	-6.602242	3.550494	3.985838
C	-6.458660	3.388063	2.590879
C	-0.417450	-5.025947	4.332941
C	-0.171850	-6.190931	5.048335
C	0.181216	-7.368698	4.380527
C	0.267362	-7.369063	2.983598
C	0.044541	-6.201045	2.263546
C	-0.293099	-5.001903	2.927396
C	4.522100	-1.787927	-3.627670
C	5.882449	-1.895390	-3.894305
C	6.799914	-2.007637	-2.844412
C	6.336307	-2.076625	-1.527428
C	4.974873	-1.984262	-1.256670
C	4.042196	-1.813380	-2.302578
C	-1.675126	-2.972487	2.552836
C	-0.570359	-3.755630	2.199197
C	-1.967118	-1.780587	1.891480
C	-0.140437	-2.050033	0.491701
C	0.251976	-3.250655	1.133481
C	1.485632	-3.852861	0.713759
C	2.255224	-3.311168	-0.277840
C	1.860881	-2.120967	-0.972641
C	2.633881	-1.494128	-2.007423
C	2.011039	-0.452887	-2.701844
C	0.775684	0.049164	-2.294004
C	0.628375	-1.525840	-0.604431
C	-5.545903	-0.061042	-1.354446
C	-5.528863	-0.921728	-2.505868
C	-4.270263	-1.356212	-2.937337
C	-3.102404	-0.985826	-2.273184
C	-4.311989	0.256156	-0.733487
C	-5.454995	1.706287	0.923716
C	-4.271395	1.110662	0.421503
C	-5.349136	2.585276	2.055714

C	-4.082354	2.690488	2.642084
C	-2.970911	2.060188	2.087378
H	17.824553	2.650565	0.381513
H	18.280800	4.194220	0.893740
H	15.695732	2.442614	-0.543475
H	9.122826	0.848232	-1.445898
H	9.458729	4.007893	1.504614
H	11.569598	0.889209	-1.619414
H	4.785001	1.123321	0.256302
H	4.589642	2.593379	-0.672198
H	-1.767877	6.961275	-2.566913
H	-0.868107	6.296167	-3.928618
H	-0.002978	6.840064	-2.480920
H	4.669135	2.507818	2.399178
H	4.368051	3.941179	1.413743
H	-3.042973	2.203544	-2.267883
H	-2.869825	4.447922	-3.302170
H	0.710061	5.075312	-1.013040
H	0.576418	0.052554	2.282353
H	2.285485	3.963463	-0.105733
H	2.857924	0.870593	2.818575
H	-7.679254	0.333217	-1.289451
H	-7.606634	1.798141	0.656223
H	-7.917032	-1.902176	-1.532453
H	-9.875714	-2.628887	-2.886273
S	-10.425139	-2.617217	-5.745328
H	-7.791211	-1.685228	-6.489518
H	-5.813721	-0.932997	-5.171228
H	-7.189278	4.055833	0.663560
H	-8.897315	5.589771	1.624404
S	-9.654851	6.262665	4.344634
H	-7.675242	4.540198	5.577729
H	-5.962959	2.983548	4.658316
H	-0.671359	-4.110495	4.862088
H	-0.258913	-6.219608	6.129554
S	0.578725	-8.874334	5.328865
H	0.486592	-8.305482	2.481111
H	0.075077	-6.225391	1.177649
H	3.816913	-1.699538	-4.450483
H	6.261074	-1.892993	-4.911126
S	8.589951	-1.957758	-3.178739
H	7.058590	-2.195531	-0.726302
H	4.633916	-1.990465	-0.225361
H	-2.340499	-3.309922	3.339098
H	-2.825412	-1.181212	2.175330
H	1.817880	-4.751151	1.218341
H	3.182143	-3.793399	-0.563257
H	2.533849	0.041591	-3.512517
H	0.329183	0.900132	-2.797618
H	-4.198636	-2.024298	-3.787681
H	-2.132306	-1.338170	-2.606617
H	-3.943034	3.334170	3.502802
H	-1.982619	2.191474	2.515314
H	13.688415	1.722355	-0.972304
O	-0.222733	-8.722590	6.569022
O	8.714045	-2.455123	-4.568061
O	9.179028	-2.813460	-2.122796
O	8.892821	-0.501710	-3.024480
O	-10.817555	5.368461	4.569634
O	-9.839144	-3.134357	-7.007943

O	-9.005991	6.765400	5.582244
O	-9.836656	7.270624	3.270089
O	0.155444	-9.972586	4.425593
O	2.046772	-8.749930	5.516369
O	-11.109818	-3.626395	-4.900653
O	-11.138140	-1.321196	-5.876761