Supplementary Material (ESI) for Chemical Communications

Supporting Informations.

Rhenium(IV) Compounds Inducing Apoptosis in Cancer Cells

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Experimental Section

Materials. All chemicals and solvents used in the synthesis were of reagent grade and employed without further purification. Compounds [$ReCl_4$(bpy)] (**1**) and [$ReCl_4$(bpym)] (**2**) as well as ammonium hexachlororhenate(IV), $(NH_4)_2[ReCl_6]$, were prepared according to literature procedures.

Physical Measurements. Elemental analyses (C, H, N) were performed by a CE Instruments EA 1110 CHNS analyzer and the Re/Cl molar ratio of 1:4 was determined for **1-4** by means of a Philips XL-30 scanning electron microscope (SEM) equipped with an X-ray microanalysis system. Infrared spectra were recorded with a Perkin-Elmer 1750 FTIR spectrophotometer as KBr pellets in the 4000-400 cm^{-1} region. High resolution ESI MS spectra of compound **1-4** were acquired on a hybrid Q-Star Pulsar-i (MSD Sciex,

Applied Biosystem, Toronto, Canada) mass spectrometer equipped with an ion spray ionization source. The instrument was calibrated using two standard flavonoids compound to achieve a mass accuracy less than 5 ppm. Analytes, dissolved in a solution of DMSO/H_2O 5:95 at a concentration of 25 mg/L, were introduced by direct infusion (3 μL/min) at the optimum ion spray (IS) voltage of 4800 V. The source nitrogen (GS1) and the curtain gas (CUR) flows were set at pressures of 20 and 25 psi, respectively, whereas the first declustering potential (DP1), the focusing potential (FP), and the second declustering potential (DP2) were kept at 50, 220, and 10 V relative to ground, respectively

Synthesis of [$ReCl_4$(dmbpy)] (3) and [$ReCl_4$(phen)] (4). Compounds **3** and **4** were obtained by following a very similar procedure to that described for [$ReCl_4$(bpy)] (**1**). A mixture of ammonium hexachlororhenate(IV) (1.38 mmol) and dmbpy (**3**) or phen (**4**) (3.20 mmol) in 5mL of DMF was refluxed under argon atmosphere during 2–3 h leading to a dark green solution. After cooling at room temperature, the solution was filtered and heated at 60 °C to eliminate fully the solvent. 50 mL of acetone were added to the residue thus obtained stirring for 20 min. Then, the insoluble solid was removed by filtration and discarded. Finally, 4–5 drops from a 0.5M HCl solution were added and by slow evaporation at room temperature brown-yellowish needles were formed in two weeks. Yield: *ca*. 35–40%. Anal. Calc. for $C_{12}H_8N_2Cl_4Re$ (**3**): C, 28.36; H, 1.59; N, 5.51. Found: C, 28.30; H, 1.60; N, 5.53%. Anal. Calc. for $C_{12}H_{12}N_2Cl_4Re$ (**4**): C, 28.14; H, 2.36; N, 5.47. Found: C, 28.50; H, 2.38; N, 5.12%. IR peaks associated to dmbpy ligand (**3**) appear at (cm^{-1}) 3080m, 1602s, 1550m, 1465s, 1440s, 952m, 770s, 720m and 651m; and those assigned to phen ligand (**4**) at (cm^{-1}) 3084m, 3056m, 1577m, 1516m, 1427s, 854s, 717s and 653w cm^{-1}.

Crystallography.

Crystallographic Data Collection and Structure Determination. X-ray diffraction data werecollected using a Bruker-Nonius X8APEXII CCD area detector diffractometer. Graphite-monochromated Mo-Ka radiation (λ = 0.71073 Å) was used in all cases. The data were processed through the SAINT [1] reduction and SADABS [2] absorption software. The unit cell parameters, which are listed in Table 1 together with a summary of the structure refinement data, were based upon least-squares refinement of 5343 (**1**), 9318 (**3**) and 7277 (**4**) reflections. The structures were solved by direct methods and subsequently completed by Fourier recycling using the SHELXTL software package.[3]

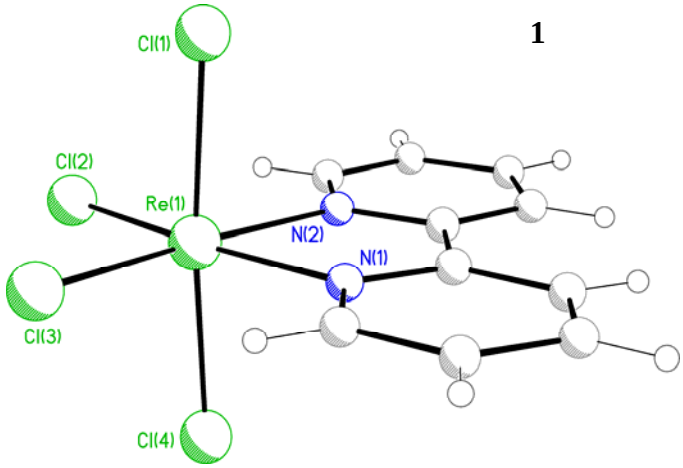
Non-hydrogen atoms were refined anisotropically. Hydrogen atoms were set in calculated positions and refined as riding atoms. Full-matrix least-squares refinements on F2, carried out by minimizing the function $Rw(|Fo| - |Fc|)^2$, reached convergence with values of the discrepancy indices given in Table 1. The graphical manipulations were performed using the XP utility of the SHELXTL system. Testing the two enantiomeric models of the crystal **1** with a final refined Flack parameter of 0.019(4) shows that the handedness was uniquely determined. CCDC reference numbers are 797402, 797403 and 797404 for

complexes **1**, **3** and **4**, respectively.

[1] *SAINT-Ver. 6.45 Copyright (c)*, BrukerAnalyticalX-ray Systems, Inc., Madison, WI, USA, 2003.
[2] G. M. Sheldrick, *SADABS Program for Absorption Correction, Version 2.10*, Analytical X-ray Systems, Madison, WI, USA, 2003.
[3] *SHELXTL, Bruker Analytical X-ray Instruments*,Madison, WI, USA, 1998.

Figure 1. Perspective drawings of the structure of compounds **1**, **3** and **4** showing the atomic numbering scheme.

1



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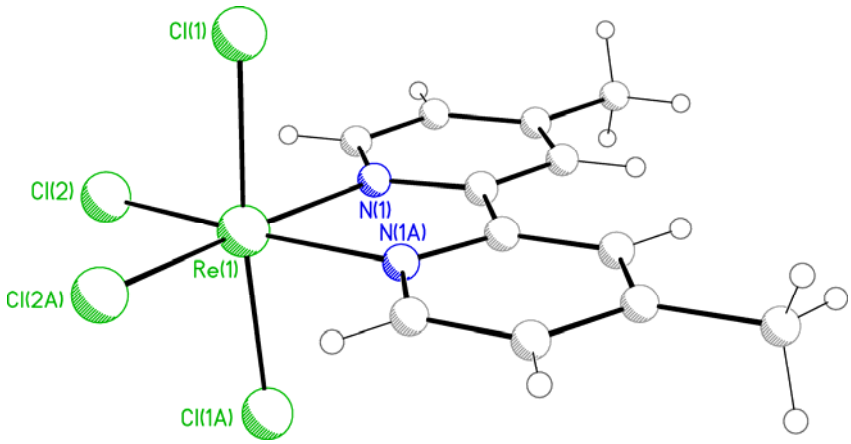

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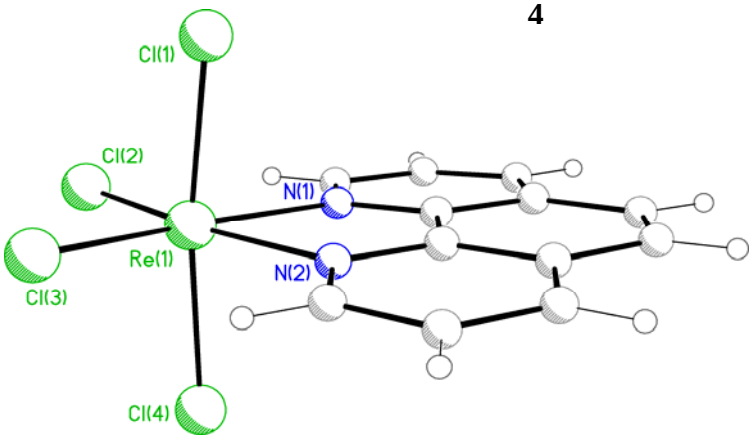


Table 1. Crystal data and structure refinement for compound **1**, **3** and **4**.

Compound	**1**	**3**	**4**
Empirical formula	$C_{10}H_8Cl_4N_2Re$	$C_{12}H_{12}Cl_4N_2Re$	$C_{12}H_8Cl_4N_2Re$
Formula weight	484.18	512.24	508.20
Crystal system	Monoclinic	Orthorombic	Monoclinic
Space group	Pn	Pccn	$P2_1/c$
a/ Å	8.195(1)	6.9640(2)	7.5973(2)
b/ Å	6.737(1)	13.4511(4)	18.9753(6)
c/ Å	12.397(2)	17.4989(5)	10.3352(3)
β/°	104.45(1)		108.249(1)
V/ $Å^3$	662.73(16)	1639.18(8)	1415.00(7)
Z	2	4	4
D_c /g cm^{-3}	2.426	2.076	2.386
Absorption coefficient/mm^{-1}	9.950	8.052	9.327
F(000)	450	964	948
Reflections collected	14857	32431	22670
Independent reflections	4276 [R(int) = 0.0242]	2674 [R(int) = 0.0257]	4255 [R(int) = 0.0290]
Data / restraints / parameters	4276 / 2 / 154	2674 / 0 / 88	4255 / 0 / 172
Goodness-of-fit on F^2	0.767	1.180	1.090
R_1 [I>2sigma(I)]	0.0158	0.0192	0.0721
wR2	0.0278	0.0482	0.1809
Absolute structure parameter	0.019(4)		
Largest diff. peak and hole/ e.$Å^{-3}$	0.510 and -0.519	0.367 and -1.276	4.758 and -5.374

[a] $R1=\sum||F_o|-|F_c||/\sum|F_o|$; [b] $wR2=\{\sum[w(F_o^2-F_c^2)^2]/[(w(F_o^2)^2]\}^{1/2}$; [c] $w = 1/[\sigma^2(F_o^2) + (aP)^2+bP]$; with $P = [F_o^2+2F_c^2]/3$, a = 0 (**1**), 0.0205 (**3**), 0.0623 (**4**); b = 0 (**1**), 1.22 (**3**), 28.10 (**4**).

Table 2. Bond lengths [Å] and angles [°] for **1**.

Re(1)-N(2)	2.118(2)	N(1)-Re(1)-Cl(2)	171.44(6)
Re(1)-N(1)	2.120(2)	Cl(3)-Re(1)-Cl(2)	94.00(3)
Re(1)-Cl(3)	2.3148(7)	N(2)-Re(1)-Cl(4)	87.94(6)
Re(1)-Cl(2)	2.3164(7)	N(1)-Re(1)-Cl(4)	87.92(6)
Re(1)-Cl(4)	2.3278(7)	Cl(3)-Re(1)-Cl(4)	92.32(3)
Re(1)-Cl(1)	2.3312(7)	Cl(2)-Re(1)-Cl(4)	91.11(3)
		N(2)-Re(1)-Cl(1)	87.56(6)
N(2)-Re(1)-N(1)	77.15(8)	N(1)-Re(1)-Cl(1)	88.28(6)
N(2)-Re(1)-Cl(3)	171.67(6)	Cl(3)-Re(1)-Cl(1)	91.72(3)
N(1)-Re(1)-Cl(3)	94.54(6)	Cl(2)-Re(1)-Cl(1)	92.09(3)
N(2)-Re(1)-Cl(2)	94.31(6)	Cl(4)-Re(1)-Cl(1)	174.65(3)

Table 3. Bond lengths [Å] and angles [°] for **3**.

Re(1)-N(1)	2.114(2)	Cl(2A)-Re(1)-Cl(2)	93.24(4)
Re(1)-Cl(2)	2.3174(6)	N(1)-Re(1)-Cl(1a)	88.99(6)
Re(1)-Cl(1)	2.3290(7)	N(1)-Re(1)-Cl(1)	86.42(6)
		Cl(2A)1-Re(1)-Cl(1)	91.60(3)
N(1A)-Re(1)-N(1)	76.91(10)	Cl(2)-Re(1)-Cl(1)	92.43(3)
N(1)-Re(1)-Cl(2a)	171.64(5)	Cl(1)-Re(1)-Cl(1a)	174.14(4)
N(1)-Re(1)-Cl(2)	94.95(5)		

Symmetry transformations used to generate equivalent atoms: a) -x+5/2,-y+3/2,z

Table 4. Bond lengths [Å] and angles [°] for **4**.

Re(1)-N(1)	2.143(8)	N(2)-Re(1)-Cl(3)	93.8(2)
Re(1)-N(2)	2.164(8)	Cl(2)-Re(1)-Cl(3)	95.7(2)
Re(1)-Cl(2)	2.166(4)	N(1)-Re(1)-Cl(1)	87.6(3)
Re(1)-Cl(3)	2.289(3)	N(2)-Re(1)-Cl(1)	86.3(2)
Re(1)-Cl(1)	2.331(3)	Cl(2)-Re(1)-Cl(1)	92.6(2)
Re(1)-Cl(4)	2.344(3)	Cl(3)-Re(1)-Cl(1)	90.6(1)
		N(1)-Re(1)-Cl(4)	88.2(2)
N(1)-Re(1)-N(2)	76.8(3)	N(2)-Re(1)-Cl(4)	86.0(2)
N(1)-Re(1)-Cl(2)	93.7(3)	Cl(2)-Re(1)-Cl(4)	94.6(2)
N(2)-Re(1)-Cl(2)	170.5(2)	Cl(3)-Re(1)-Cl(4)	92.5(1)
N(1)-Re(1)-Cl(3)	170.5(3)	Cl(1)-Re(1)-Cl(4)	171.9(1)

HRESIMS

compound **1** *m/z* 447.9296 $[M\text{-}Cl]^+$ (calculated *m/z* 447.9305), *m/z* 465.9416 $[M\text{-}Cl+H_2O]^+$ (calculated *m/z* 465.9410).

compound **2** *m/z* 449.9223 $[M\text{-}Cl]^+$ (calculated *m/z* 449.9210), *m/z* 467.9321 $[M\text{-}Cl+H_2O]^+$ (calculated *m/z* 467.9315).

compound **3** *m/z* 475.9625 $[M\text{-}Cl]^+$ (calculated *m/z* 475.9618), *m/z* 493.9740 $[M\text{-}Cl+H_2O]^+$ (calculated *m/z* 493.9723).

compound **4** *m/z* 472.9389 $[M\text{-}Cl]^+$ (calculated *m/z* 472.9383), *m/z* 490.9475 $[M\text{-}Cl+H_2O]^+$ (calculated *m/z* 490.9483).

Biological characterization.

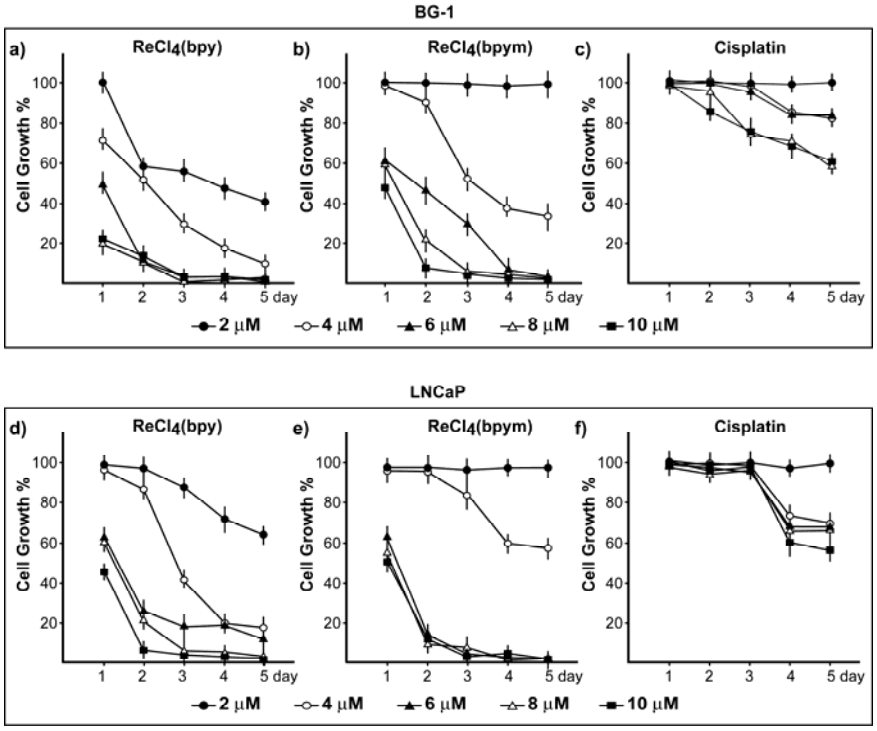
Cell culture. MCF-7 breast and LNCaP prostate cancer cells were cultured in DMEM and RPMI 1640, respectively, with phenol red supplemented with 10% fetal bovine serum (FBS). BG-1 ovarian cancer cells were maintained in DMEM without phenol red supplemented with 10% FBS. Cells were switched to medium without serum the day before experiments for immunoblots.

Growth assay. The effects of each compound on cultured cells were measured using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay, which is based on the conversion of MTT to MTT-formazan by mitochondrial enzyme. Complexes **1-4** and cisplatin were dissolved in DMSO and quickly mixed with the growth medium (final percentage of DMSO < 0.1%). Cells were seeded in regular growth medium and grown until 70-80% confluence. Cells were washed once they had attached and then treated with different concentrations of agents (2-10 μM) for the indicated time (for one day up to 5 days). Relative cell viability was monitored/determined by MTT assay according to the manufacturer's protocol (SigmaAldrich, Milan, Italy).

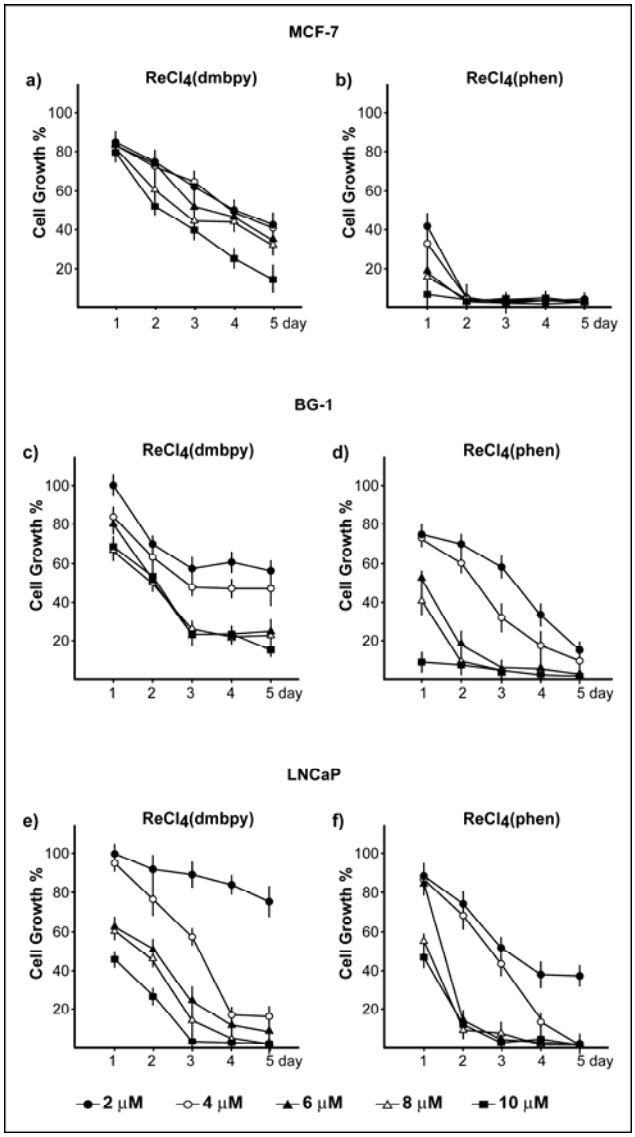
Immunoblotting. Cells were grown in 10-cm dishes, exposed to treatments and then lysed in 50 mM HEPES (pH 7.5), 150 mM NaCl, 1.5 mM MgCl2, 1 mM EGTA, 10% glycerol, 1% Triton X-100, 0.1% SDS, and a mixture of protease inhibitors (Boeringher). Equal amounts of whole protein extract were resolved on a 10% PAGE , transferred to a nitrocellulose membrane (Amersham Biosciences, Arlington Heights, IL), probed overnight at 4°C with the antibodies against p-c-JunS73 and c-Jun, (from Santa Cruz Biotechnology) and then revealed using the enhanced chemiluminescence system (Amersham Biosciences).

TUNEL assays. Cells were seeded in 2-well Lab-TekII chamber slides at a density of $1x10^5$/well and incubated for 24 h in the corresponding maintenance media. Cells were then switched to medium without serum and treated with vehicle (DMSO), compound **1**, compound **2** and cisplatin. For the detection of DNA fragmentation at the cellular level, cells were stained using DeadEndTM Fluorometric TUNEL System (Promega Milan, Italy) following the manufacturer's instructions. Nuclei of cells were stained with propidium iodide (1:1000) (Sigma-Aldrich Milan, Italy). Leica AF6000 Advanced Fluorescence Imaging System supported by image processing software Leica Application Suite Advanced Fluorescence (Leica Microsystems CMS, GbH Mannheim, Germany) were used for experiment evaluation.

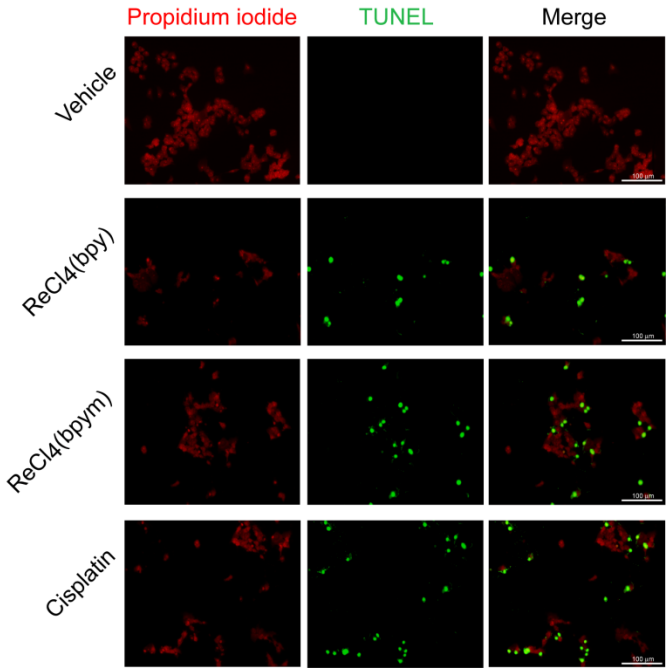
Evaluation of cell growth by compounds 1, 2 and cisplatin in BG-1 ovarian (a-c) and LNCaP prostate (d-f) cancer cells, as determined using the MTT assay.



Evaluation of cell growth by compounds 3 and 4 in MCF-7 (a-b), BG-1 (c-d) and LNCaP (e-f) cancer cells, as determined using the MTT assay.



Representative images of TUNEL staining for apoptosis evaluation in MCF-7 cells treated for 24 h with 10 µM of 1, 2 or cisplatin. In left panels, propidium iodide staining of nuclei; in middle panels, TUNEL staining and in right panels merged images. Magnification is indicated by the bars (100 µm). Each experiment shown is representative of 20 random fields observed.



Additional Computational Details

All calculations were performed with GAUSSIAN 03 [1] program at Density Functional Theory level, using the hybrid B3LYP functional, composed by Becke's [2] three-parameter hybrid exchange functional (B3) and the correlation functional of Lee, Yang and Parr (LYP) [3]. Geometry optimizations without symmetry constraints were carried out with a 6-31G(d) basis sets for all atoms except the Rhenium atom, which was described by the quasi-relativistic Stuttgart-Dresden pseudopotentials [4]. In order to confirm proper convergence to equilibrium and transition state geometries, vibrational frequency analysis were done based on analytical second derivatives of Hamiltonian at this level of theory. Solvent effects were taken into account performing single point calculations (SP) by the CPCM method employing a dielectric constant of $\varepsilon = 80$ [5]. Klamt radii were used for constructing the solute cavity [6]. On the optimized structures, SP energy calculations were also carried out with the larger basis set 6-31++G(2df,2pd) in gas phase and in water.

[1] Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A.; Vreven, J., T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Knox, X. L., J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, H.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. Gaussian 03, Revision A.1; Gaussian, Inc.: Pittsburgh PA, 2003.

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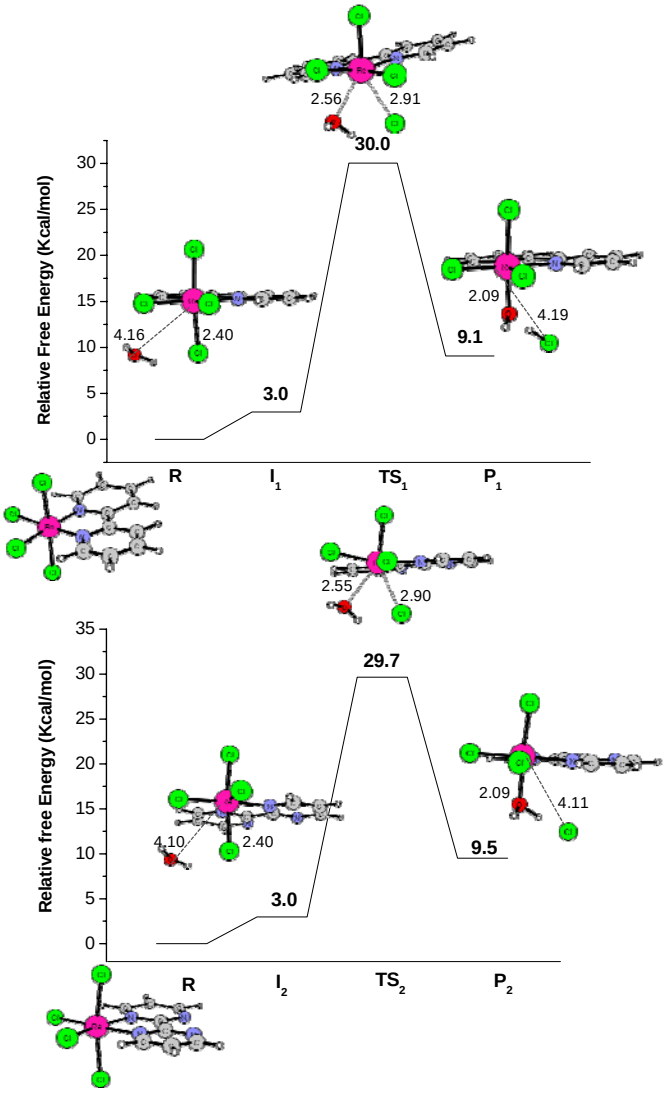
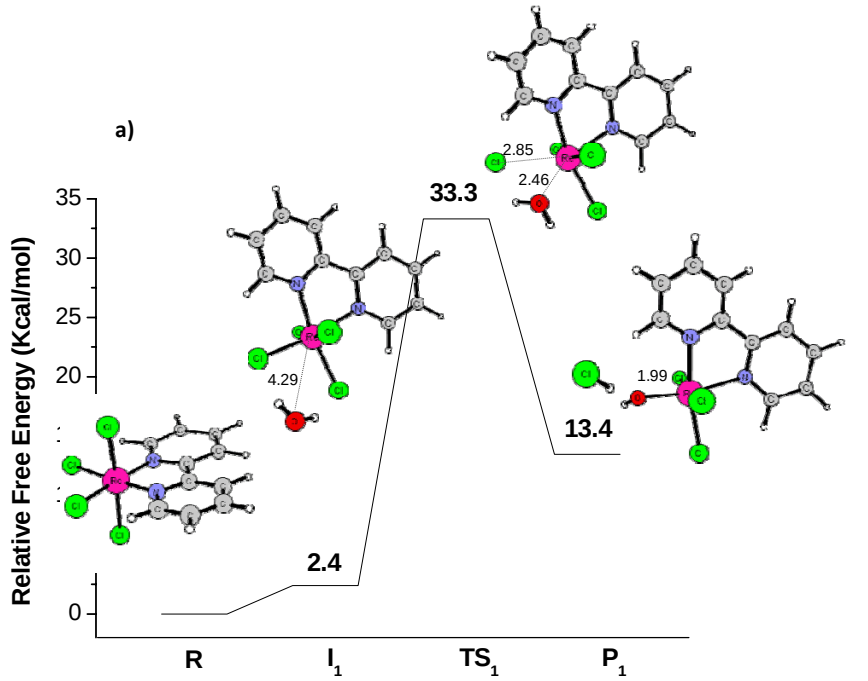
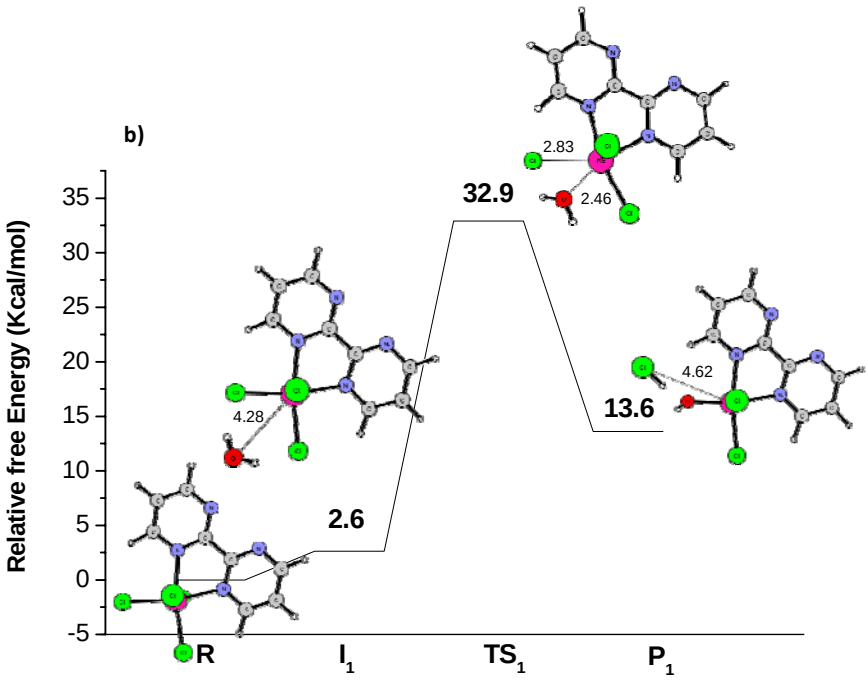
[6] Klamt, A.; Jonas, V.; Burger, T.; Lohrenz, J. C.W.; J. Phys. Chem. A, 1998, 102, 5074.

Computed relative free energies profiles (at 298.15 K) and optimized structures of the stationary points for the hydrolysis process of 1 and 2, in water phase.



Computed relative free energies profiles (at 298.15 K) and optimized structures of the stationary points for the equatorial Cl^- substitution in water phase. a) compound 1, b) compound 2.





Computed relative free energies profiles (at 298.15 K) and optimized structures of the stationary points for the release of ligand in water phase. a) compound 1, b) compound 2.