

# ChemBioChem

Supporting Information

## The Antimicrobial Properties of Pd<sup>II</sup>– and Ru<sup>II</sup>–pyta Complexes

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## Preliminary antimicrobial screening against the ESKAPE pathogens.

Table S1: The results from the preliminary screening of the pyta complexes at a single concentration against a selection of bacteria and fungi.

Compound name	Percentage growth inhibition*						
	Bacteria (Gram+)	Bacteria (Gram-)				Fungi	
	<i>Staphylococcus aureus</i> (Sa)	<i>Escherichia coli</i> (Ec)	<i>Klebsiella pneumoniae</i> (Kp)	<i>Pseudomonas aeruginosa</i> (Pa)	<i>Acinetobacter baumannii</i> (Ab)	<i>Candida albicans</i> (Ca)	<i>Cryptococcus neoformans</i> (Cn)
	ATCC 4330	ATCC 25922	ATCC 700603	ATCC 27853	ATCC 19606	ATCC 90028	ATCC 208821
<b>Pd1</b>	97.99	18.2	18.38	-6.42	63.33	105.36	114.35
<b>Pd2</b>	3.61	29.08	20.37	15.76	58.21	95.63	105.2
<b>Pd3</b>	-26.8	25.43	22.15	15.24	41.22	108.47	120.58
<b>Pd4</b>	66.52	40.95	17.85	11.01	32.96	101.07	100
<b>Pd5</b>	53.27	43.8	19.15	13.14	53.7	104.4	109.56
<b>Pd6</b>	91.29	0.13	1.36	8.12	30.87	4.01	7.66
<b>Pd7</b>	-6.64	18.88	13.04	18.83	35.6	95.75	135.76
<b>Pd8</b>	-44.67	14.06	17.03	15.66	26.14	99.74	106.65
<b>Pd9</b>	-0.14	11.53	11.6	10.09	34.41	94.84	103.95
<b>Pd10</b>	11.66	31.24	29.94	73.72	42.63	96.63	127.08
<b>Pd11</b>	-7.55	31.76	16.11	15.72	49.84	97.45	105.65
<b>Ru1</b>	-9.41	-1.21	6.15	7.15	5.76	62.11	-36.56
<b>Ru2</b>	45.94	17.72	10.36	1.96	42.93	99.71	-40.12
<b>Ru3</b>	-20.72	-1.06	4.1	8.91	6.35	82.95	-20.48
<b>Ru4</b>	98.27	34.53	18.32	14.42	44.63	5.99	106.99
<b>Ru5</b>	-19.55	6.55	6.67	11.3	24.68	4	-17.88
<b>Ru6</b>	97.04	12.16	13.38	14.77	35.28	6.66	102.49
<b>Ru7</b>	-16.84	11.67	12.42	18.83	35.24	5.33	-26.6

\* Initial screening was performed at compound concentrations of 32.00 µg/mL. The percentage growth rate was calculated relative to the negative control (media only), therefore negative values indicate bacterial growth in the presence of the compounds.

## MIC determination, cytotoxicity, and Haemolysis determination

Table S2: The MIC values of the complexes evaluated against the ESKAPE pathogens as well as the human embryonic kidney cells (HEK-293) and human red blood cells (RBC) to assess the cytotoxicity and haemolytic properties of the compounds.

	Minimum inhibition concentration (µg/mL)								
	Bacteria (Gram+)	Bacteria (Gram-)				Fungi		HEK-293	RBC
	Sa	Ec	Kp	Pa	Ab	Ca	Cn	Hk	Hm
<b>Pd1</b>	32	>32	>32	>32	>32	2	0.5	32	>32
<b>Pd2</b>	>32	>32	>32	>32	>32	≤0.25	≤0.25	>32	>32
<b>Pd3</b>	>32	>32	>32	>32	>32	16	0.5	>32	>32
<b>Pd4</b>	>32	>32	>32	>32	>32	2	≤0.25	>32	>32
<b>Pd5</b>	>32	>32	>32	>32	>32	≤0.25	1	>32	>32
<b>Pd6</b>	>32	>32	>32	>32	>32	>32	>32	>32	>32
<b>Pd7</b>	>32	>32	>32	>32	>32	≤0.25	≤0.25	>32	>32
<b>Pd8</b>	>32	>32	>32	>32	>32	≤0.25	≤0.25	>32	>32

<b>Pd9</b>	>32	>32	>32	>32	>32	<=0.25	<=0.25	>32	>32
<b>Pd10</b>	<=0.25	>32	>32	>32	>32	<=0.25	<=0.25	>32	>32
<b>Pd11</b>	>32	>32	>32	>32	>32	<=0.25	<=0.25	>32	>32
<b>Ru2</b>	>32	>32	>32	>32	>32	>32	>32	>32	>32
<b>Ru3</b>	>32	>32	>32	>32	>32	>32	>32	>32	>32
<b>Ru4</b>	32	>32	>32	>32	>32	>32	8	32	>32
<b>Ru6</b>	>32	>32	>32	>32	>32	>32	>32	>32	>32
<b>Ru7</b>	>32	>32	>32	>32	>32	>32	>32	>32	>32

## MIC ( $\mu\text{M}$ ) against the extended fungal panel

Table S3: The MIC ( $\mu\text{M}$ ) concentrations of the compounds evaluated against the extended fungal panel.

Compound name	MIC ( $\mu\text{M}$ )					
	<i>Candida albicans</i>	<i>Cryptococcus neoformans</i>	<i>Candida tropicalis</i>	<i>Candida glabrata</i>	<i>Cryptococcus deuterogattii</i>	<i>Candida auris</i>
	ATCC 90028	ATCC 208821	ATCC 750	ATCC 90030	ATCC 32609	CBS10913
<b>Pd1</b>	0.602 - 1.20	0.301	0.301	0.144 - 0.301	0.602	0.0722 - 0.301
<b>Pd2</b>	<0.659	<0.659	2.64	42.2	0.0422	0.659
<b>Pd3</b>	40.5	1.27	n.d.	n.d.	n.d.	n.d.
<b>Pd4</b>	1.12 - 4.47	0.280 - 0.559	1.12 - 2.24	2.24 - 4.47	0.559 - 2.24	0.13 - 2.24
<b>Pd5</b>	9.32 - 18.6	1.16 - 2.33	18.6 - 37.3	18.6 - 37.3	4.66 - 9.32	4.66 - 9.32
<b>Pd6</b>	>69.4	>69.4	n.d.	n.d.	n.d.	n.d.
<b>Pd7</b>	0.478 - 1.91	<0.478	1.91	0.956 - 1.91	0.956 - 1.91	0.956 - 1.91
<b>Pd8</b>	0.837 - 1.67	0.100 - 0.435	0.837	1.67 - 3.35	0.418 - 0.837	0.418 - 1.67
<b>Pd9</b>	0.817 - 1.63	0.204	0.817 - 1.63	0.817 - 3.27	0.409 - 0.817	0.817 - 3.27
<b>Pd10</b>	0.371 - 1.48	0.371	0.742 - 1.48	0.742 - 2.97	0.742 - 2.97	0.742 - 2.97
<b>Pd11</b>	3.78 - 7.55	1.89	3.78 - 7.55	15.1	3.78	1.89 - 7.55
fluconazole	0.816 - 1.63	26.1	13.1	>104	>104	>104

## In vivo toxicity evaluation

Table S4: The results from the in vivo toxicity studies in *G. mellonella* larvae.

Compound name	Concentration in 2% DMSO	Toxicity result (1)	Concentration in 10%	
			DMSO	Toxicity test result (2)
<b>Pd4</b>	40 $\mu\text{M}$	Non-toxic	100 $\mu\text{M}$	Non-toxic
<b>Pd5</b>	10 $\mu\text{M}$	Non-toxic	100 $\mu\text{M}$	Non-toxic
<b>Pd6</b>	160 $\mu\text{M}$	Non-toxic	1000 $\mu\text{M}$	Non-toxic
<b>Pd7</b>	20 $\mu\text{M}$	Non-toxic	100 $\mu\text{M}$	Non-toxic
<b>Pd8</b>	20 $\mu\text{M}$	Non-toxic	100 $\mu\text{M}$	Non-toxic
<b>Pd9</b>	160 $\mu\text{M}$	Non-toxic	1000 $\mu\text{M}$	Non-toxic
<b>Pd10<sup>a</sup></b>	NA	NA	NA	NA

<b>Pd11</b>	40 $\mu$ M	Non-toxic	100 $\mu$ M	Non-toxic
DMSO	2%	Non-toxic	10%	Non-toxic

<sup>a</sup> The complex did not fully dissolve at the concentrations tested and was therefore not injected into the larvae.

## Stability of Pd4 in DMSO

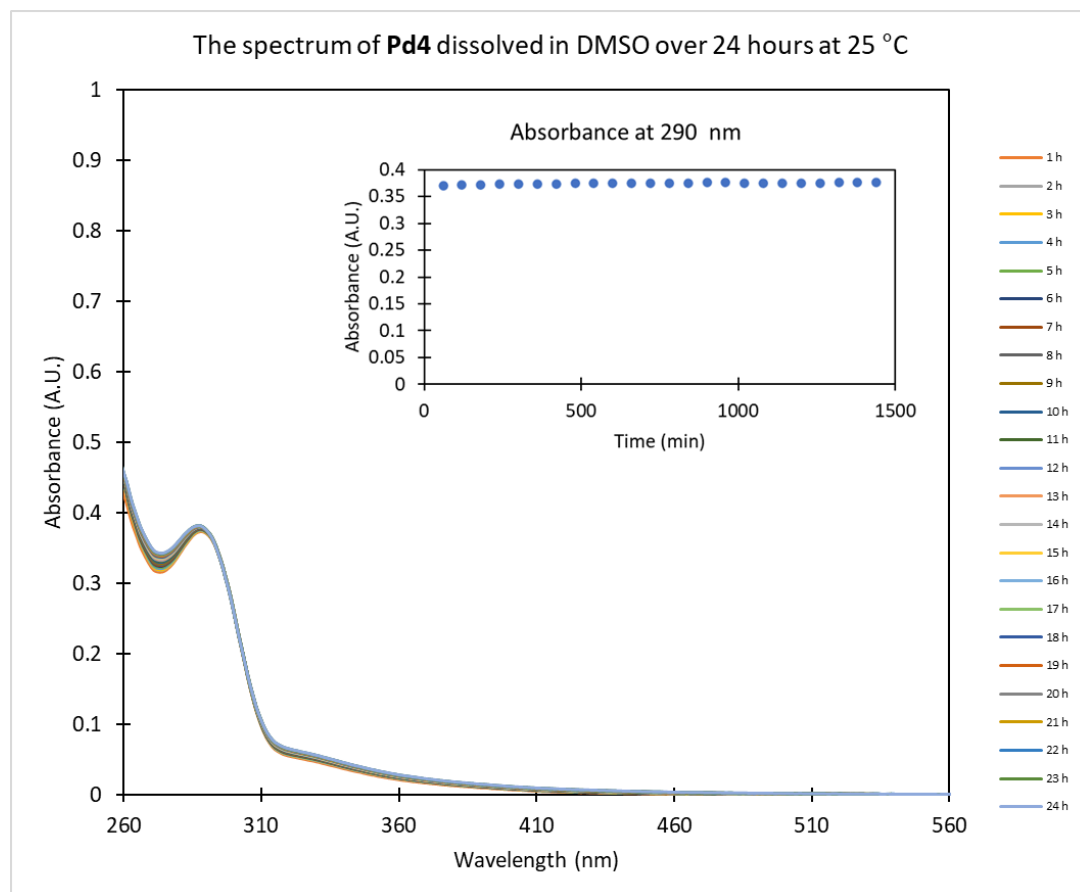


Figure S1: The stability of Pd4 in DMSO measured over time using the UV-vis spectroscopy.

Table S4: The MIC ( $\mu$ M) concentrations of the ligands and starting palladium compound evaluated against the extended fungal panel.

Compound name	MIC ( $\mu$ M)					
	<i>Candida albicans</i>	<i>Cryptococcus neoformans</i>	<i>Candida tropicalis</i>	<i>Candida glabrata</i>	<i>Cryptococcus deuterogattii</i>	<i>Candida auris</i>
	ATCC 90028	ATCC 208821	ATCC 750	ATCC 90030	ATCC 32609	CBS10913
L1	>32.0	>32.0	>32.0	>32.0	>32.0	>32.0
L2	>32.0	>32.0	>32.0	>32.0	>32.0	2.00
L4	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
L5	>32.0	>32.0	>32.0	>32.0	>32.0	>32.0
L6	>32.0	>32.0	>32.0	>32.0	>32.0	>32.0
L7	>32.0	>32.0	>32.0	>32.0	>32.0	>32.0

<b>L8</b>	>32.0	>32.0	>32.0	>32.0	>32.0	>32.0
fluconazole	0.816 - 1.63	26.1	13.1	>104	>104	>104

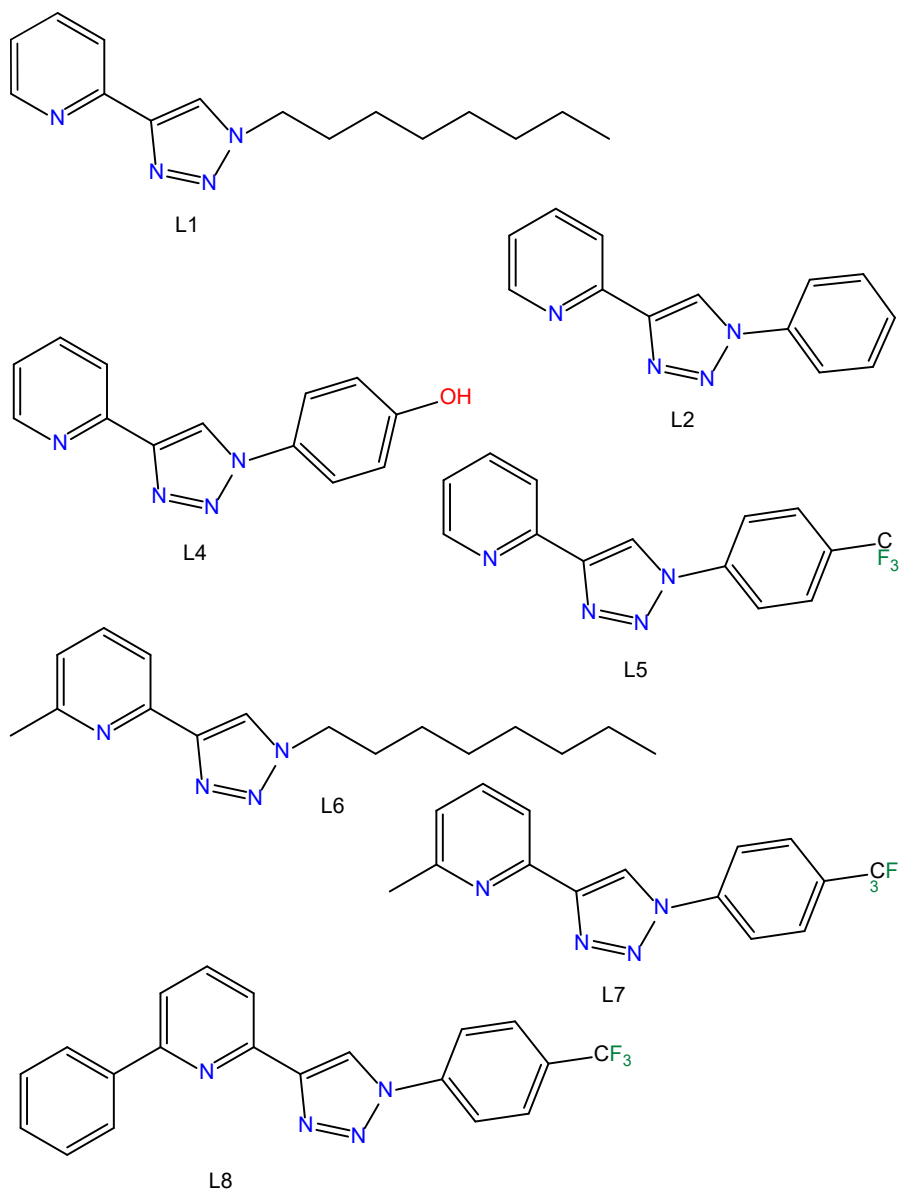


Figure S1: Structures of the tested ligands. (Ligand L3 was not available anymore)