Supplementary materials

New anticandidal Cu(I) complexes with neocuproine and ketoconazole derived diphenyl(aminomethyl)phosphane: luminescence properties for detection in fungal cells. †

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Abstract

The search for new antifungals is very important because the large genetic variation of pathogenic organisms has resulted in the development of increasingly effective defense mechanisms by microorganisms. Metal complexes as potential drugs are gaining nowadays an interest, because they are characterized by accessible redox states of metal centers and plethora of easily modifiable geometries. In this work we present two new copper(I) iodide or thiocyanide complexes with 2,9-dimethyl-1,10-phenanthroline (dmp) and diphenylphosphane derivative of ketoconazole (**KeP**), where ketoconazole acetyl group is replaced by -CH_2PPh_2 unit, [CuI(dmp)**KeP**] (**1-KeP**) and [CuNCS(dmp)**KeP**] (**2-KeP**) - their synthesis and structural characteristics. The analysis of the intrinsic fluorescence of ketoconazole moiety in the coordinated **KeP** molecule revealed that the copper(I) central atom does not act as a quencher and the observed decrease of fluorescence intensity is a result of a strong inner filter effect caused by the presence of CuXdmp unit. Moreover, the complexes exhibit a remarkable MLCT (metal - ligand charge transfer) based phosphorescence with the emission maximum at 600-615 nm in aqueous media, which probably results from the formation of dimers and higher order oligomers in the most polar solutions. Both complexes proved to be promising antifungal agents towards *Candida albicans*, showing a relatively high efficiency towards the fluconazole resistant strains with - *CDR1* and *CDR2* or *MDR1* efflux pumps overexpression, what suggests that they overcome at least partially these defense mechanisms. Simulations of docking to the cytochrome P450 14α-demethylase (the azoles' primary molecular target) suggested that the compounds studied were rather uncapable to competitively inhibit this enzyme, unlike ketoconazole and the **KeP** ligand. On the other hand, the phosphorescence in aqueous solutions allowed to record the confocal micrographs of the complexes which showed that both of them are situated in spherical structures inside the cells, most likely in the vacuoles.

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16. **Fig.S13** Proliferation rate of NHDF cells measured by MTT assay. NHDF cells were treated with 0.25; 0.5; 1; 2 and 4 x MIC_{50} concentrations of Ke, Flc and 1-KeP for 72 h, incubated with MTT and the number of viable cells measured spectrophotometrically at 540 nm. The bars represent the means ± SD of triplicate values for three independent experiments. ***P<0.001.

17. **Fig.S14** Docking of a) **1-KeP** (orange) and b) **2-KeP** (purple) to the CYP51 of *C. albicans* (grey ribbons). Experimental positions of the heme cofactor (pink) and the inhibitor – posaconazole (cyan) – are taken from the 5FSA PDB deposit. Docking ensembles of 27 conformations are shown for each ligand. The large molecules of the complexes **1-KeP** and **2-KeP** are not able to enter the binding cavity accessible to the posaconazole molecule; in particular, they are not able to interact directly with the heme cofactor. They are, instead, located on the protein surface, possibly obstructing the entrance to the binding cavity.

18. References –extended information on the cited papers in the main article.

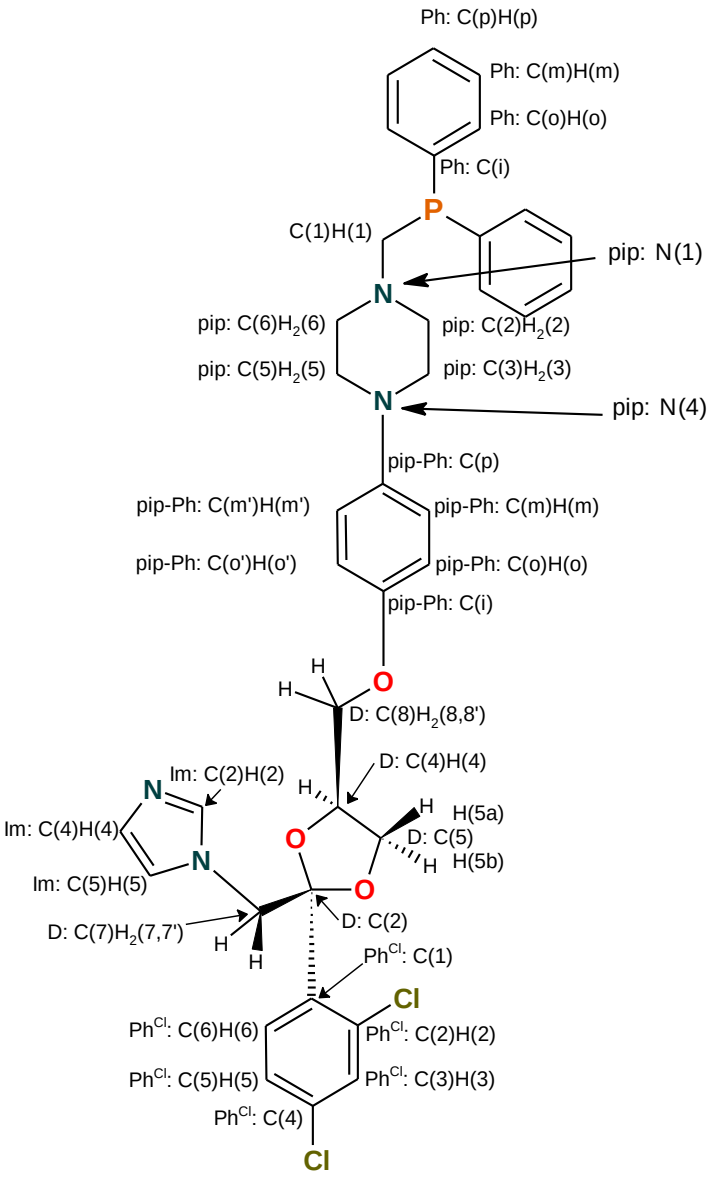

Fig.S1. Molecular scheme of **KeP** with atoms numbering for the NMR data.

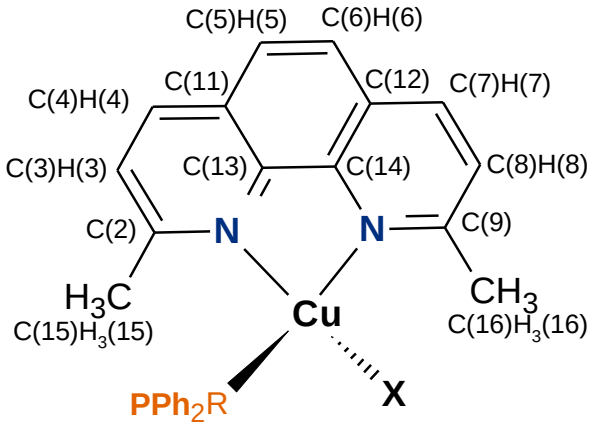

Fig.S2. Molecular scheme of the studied complexes with atoms numbering for the NMR data.

Tab.S1. Chemical shifts and coupling constants from the $^{13}C\{^{1}H\}$ NMR spectra of **KeP**, **KeOP**, **1-KeP** and **2-KeP** in $CDCl_3$.

	$^{13}C\{^{1}H\}$	**KeP ***		**KeOP ***		**1-KeP**		**2-KeP**		dmp
$-CH_2PPh_2$	C(1) d	61.40	3.6	58.43	88.1	59.08	27.2	59.16	26.3	
	C(i) d	138.38	12.7	132.42	97.2	not obs.		134.87	25.4	
	C(o) d	132.83	18.2	131.28	9.1	133.06	12.7	132.64	13.6	
	C(m) d	128.36	6.4	128.50	11.8	128.23	8.2	128.37	8.2	
	C(p) s/d	128.52	-	131.86	2.7	129.30		129.36		
pip	C(2;6) d	54.55	9.1	55.54	8.2	55.23	5.4	55.07	6.4	
	C(3,5) s	50.46		50.43		49.94		50.07		
pip-Ph	C(i) s	152.19		152.28		152.01		152.15		
	C(o) s	117.99		117.98		117.62		117.77		
	C(m) s	115.15		115.18		115.06		115.13		
	C(p) s	146.18		146.05		145.89		145.94		
D	C(2) s	107.97		108.00		107.84		107.97		
	C(4) s	74.79		74.81		74.74		74.80		
	C(5) s	67.71		67.75		67.68		67.74		
	C(7) s	51.27		51.31		51.30		51.32		
	C(8) s	67.57		67.60		67.53		67.59		
Im	C(2) s	131.30		131.35		131.28		131.33		
	C(4) s	127.17		127.21		127.17		127.21		
	C(5) s	129.48		129.51		129.48		129.51		
PhCl	C(1) s	135.80		135.85		135.80		135.84		
	C(2) s	134.60		134.61		134.47		134.59		
	C(3) s	138.74		138.78		139.01		139**	s**	
	C(4) s	132.94		132.98		132.90		132.96		
	C(5) s	128.55		128.56		128.44		128.5*	s*	
	C(6) s	121.07		121.12		121.16		121.2*	s*	
dmp	$C^{2,9}$ s					159.28		158.96		159.07
	$C^{3,8}$ s					124.81		124.88		123.29
	$C^{4,7}$ s					136.35		136.47		136.07
	$C^{5,6}$ s					125.28		125.36		125.22
	$C^{11,12}$ s					142.98		142.93		145.03
	$C^{13,14}$ s					126.96		126.91		126.58
	CH_3 s					26.76		26.36		26.64

*** - data from** de Almeida R.F.M., Santos F.C., Marycz K., Alicka M., Krasowska A., Suchodolski J., Panek J.J., Jezierska A., Starosta R., New diphenylphosphane derivatives of ketoconazole are promising antifungal agents. Sci. Rep. 2019, 9, 16214 https://doi.org/10.1038/s41598-019-52525-7

Tab.S2. Chemical shifts and coupling constants from the $^{31}P\{^{1}H\}$ and ^{1}H NMR spectra of **KeP**, **KeOP**, **1-KeP** and **2-KeP** in $CDCl_3$.

		KeP *		**KeOP ***		**1-KeP**		**2-KeP**	
	$^{31}P\{^{1}H\}$	-27.39		27.18		-19.3*		-18.6*	
	^{1}H NMR:								
$-CH_2PPh_2$	H(1) d	3.28	2.9	3.31	6.9	3.51*		3.30*	
	Ph	7.32-7.38 7.47-7.50	6H 4H	7.46-7.55 7.83-7.87	6H 4H	7.22-7.32 7.60-7.63	6H 4H	7.22-7.32 7.41-7.44	6H 4H
pip	H(2;6) m	2.83		2.81		2.54		2.50	
	H(3,5) m	3.12		3.03		2.66		2.70	
pip-Ph	H(o) AA'	6.89		6.84		6.69		6.71	(one
	H(m) BB'	6.75		6.73		6.67		6.71	singlet)
D	H(4) dddd	4.34	6.6; 6.5; 5.1; 5.0	4.34	6.6; 6.5; 5.1; 5.0	4.32	6.6; 6.5; 5.1; 5.0	4.34	6.5; 6.5; 5.1; 5.0
	H(5a) dd	3.74	8.4; 4.9	3.73	8.4, 4.9	3.71	8.3; 4.9	3.73	8.4; 4.9
	H(5b) dd	3.88	8.3; 6.6	3.87	8.4; 6.5	3.86	8.5; 6.6	3.88	8.3; 6.6
	H(7) A	4.51	14.68	4.51	14.88	4.49	14.7	4.51	14.7
	H(7') B	4.41	14.68	4.42	14.88	4†.39	14.7	4.49	14.7
	H(8) dd	3.74	9.7; 5.1	3.74	9.6, 5.1	3.71	9.9; 5.1	3.73	9.6; 5.2
	H(8') dd	3.33	9.6; 6.7	3.32	9.6, 6.8	3.30	9.6; 6.8	3.33	9.7; 6.9
Im	H(2) d	under Ph		under Ph		7.46	1.9	7.47	1.9
	H(4) dd	7.25	8.4; 2.1	7.25	8.4; 2.2	7.25	8.4; 1.9	under Ph	
	H(5) d	7.58	8.4	7.58	8.4	7.57	8.4	7.58	8.4
PhCl	H(3) s*	7.00		6.99		6.97		6.99	
	H(5) s*	6.97		6.96		6.95		6.97	
	H(6) s*	7.51		under Ph		7.56		7.52	
dmp#	$H^{5,6}$ s					7.75		7.76	
	$H^{4,7}$ d					8.18	8.2	8.20	8.0
	$H^{3,8}$ d					7.47	8.2	7.51	~8
	CH_3 s					2.82		2.79	

- Free dmp ligand: 1H NMR $H^{5,6}$ s: 7.62 ppm; $H^{4,7}$d: 8.04 ppm d (J=8.2 Hz); $H^{3,8}$ d: 7.42 ppm (J=8.2 Hz); CH_3 s: 2.90 ppm;

* - **data from** de Almeida R.F.M., Santos F.C., Marycz K., Alicka M., Krasowska A., Suchodolski J., Panek J.J., Jezierska A., Starosta R., New diphenylphosphane derivatives of ketoconazole are promising antifungal agents. Sci. Rep. 2019, 9, 16214 https://doi.org/10.1038/s41598-019-52525-7

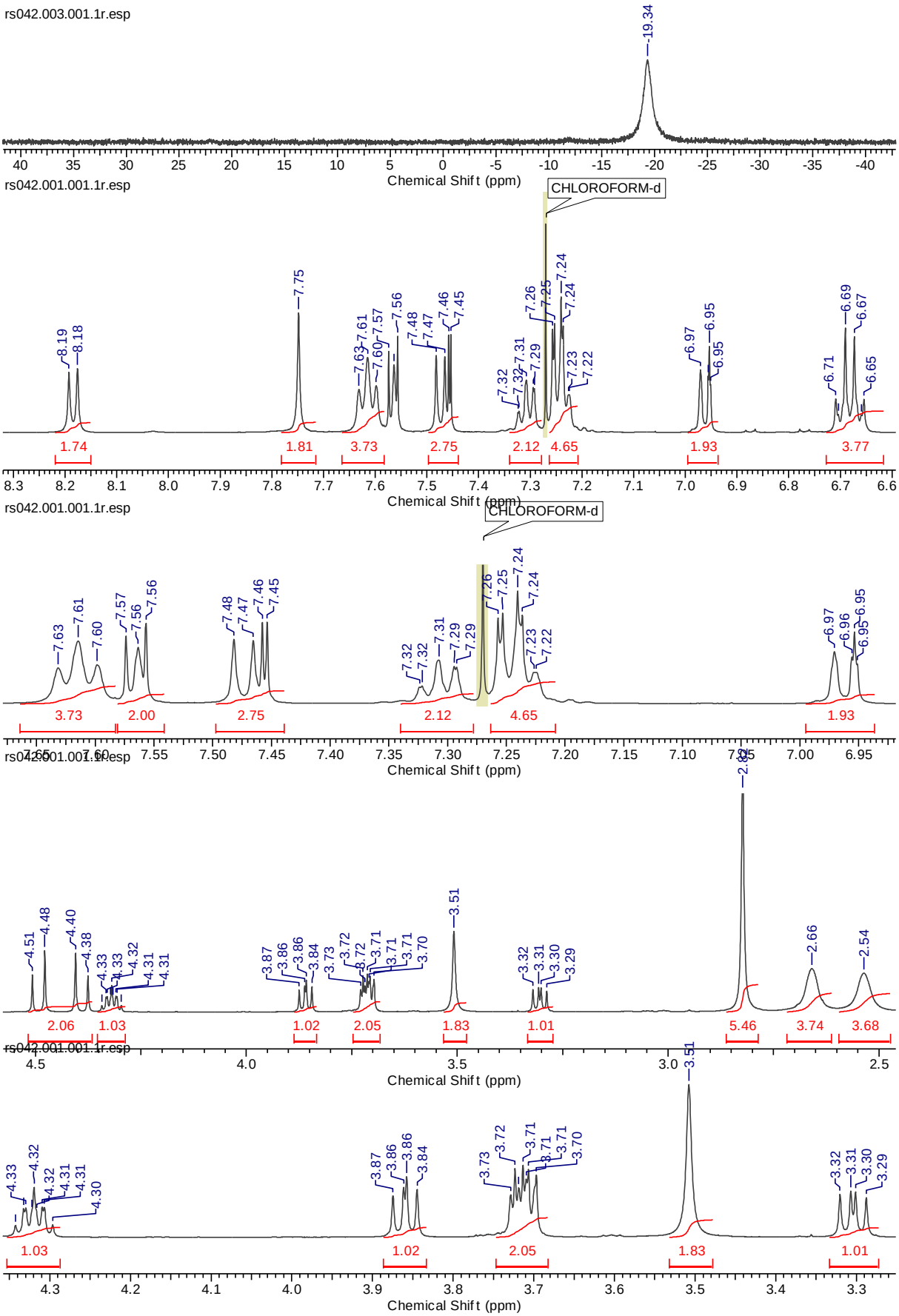

Fig. S3. $^{31}P\{^{1}H\}$ and ^{1}H NMR spectra of **1-KeP** in $CDCl_3$. (T = 300K)

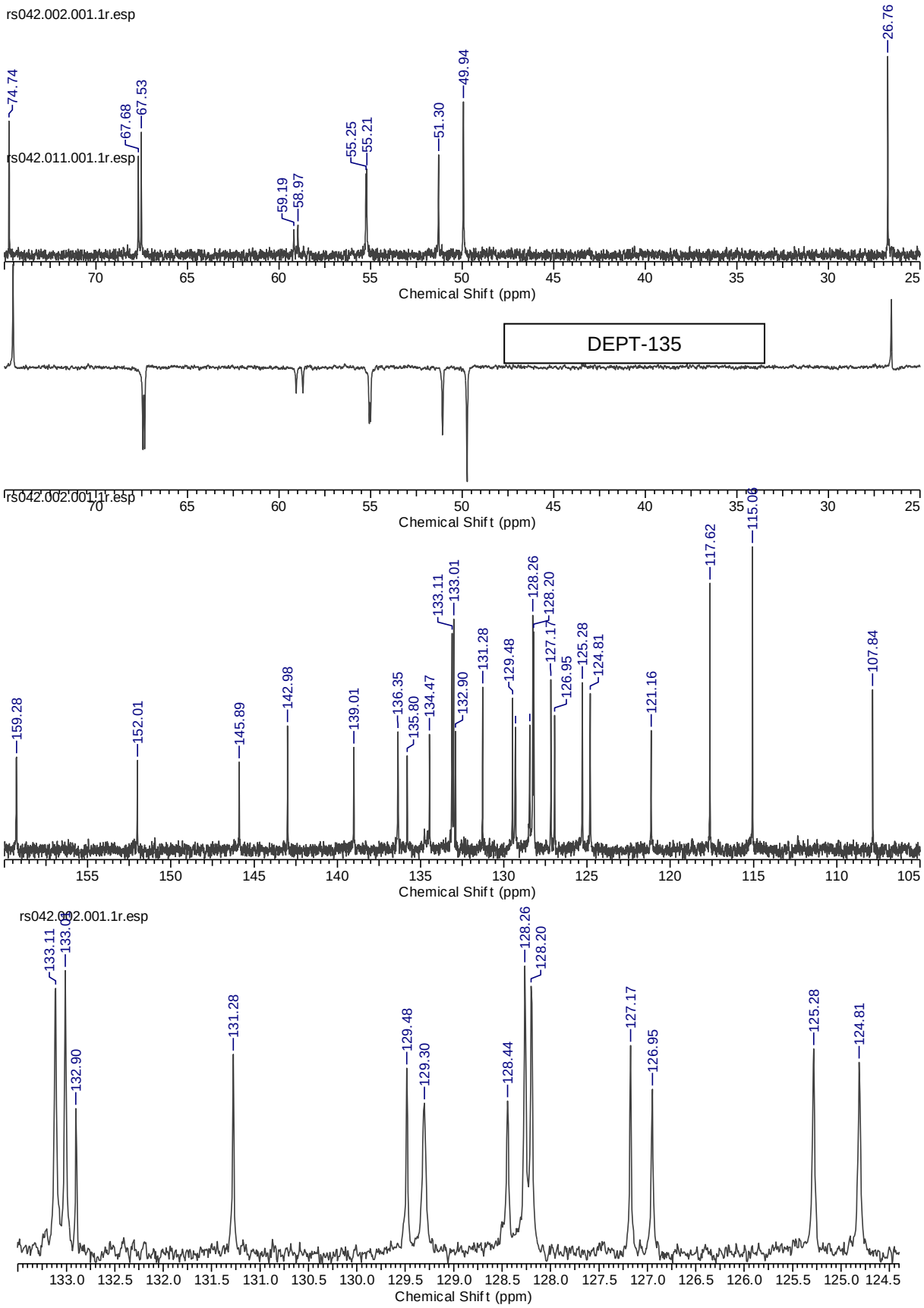

Fig. S4. $^{13}C\{^{1}H\}$ NMR spectra of **1-KeP** in $CDCl_3$. (T = 300K)

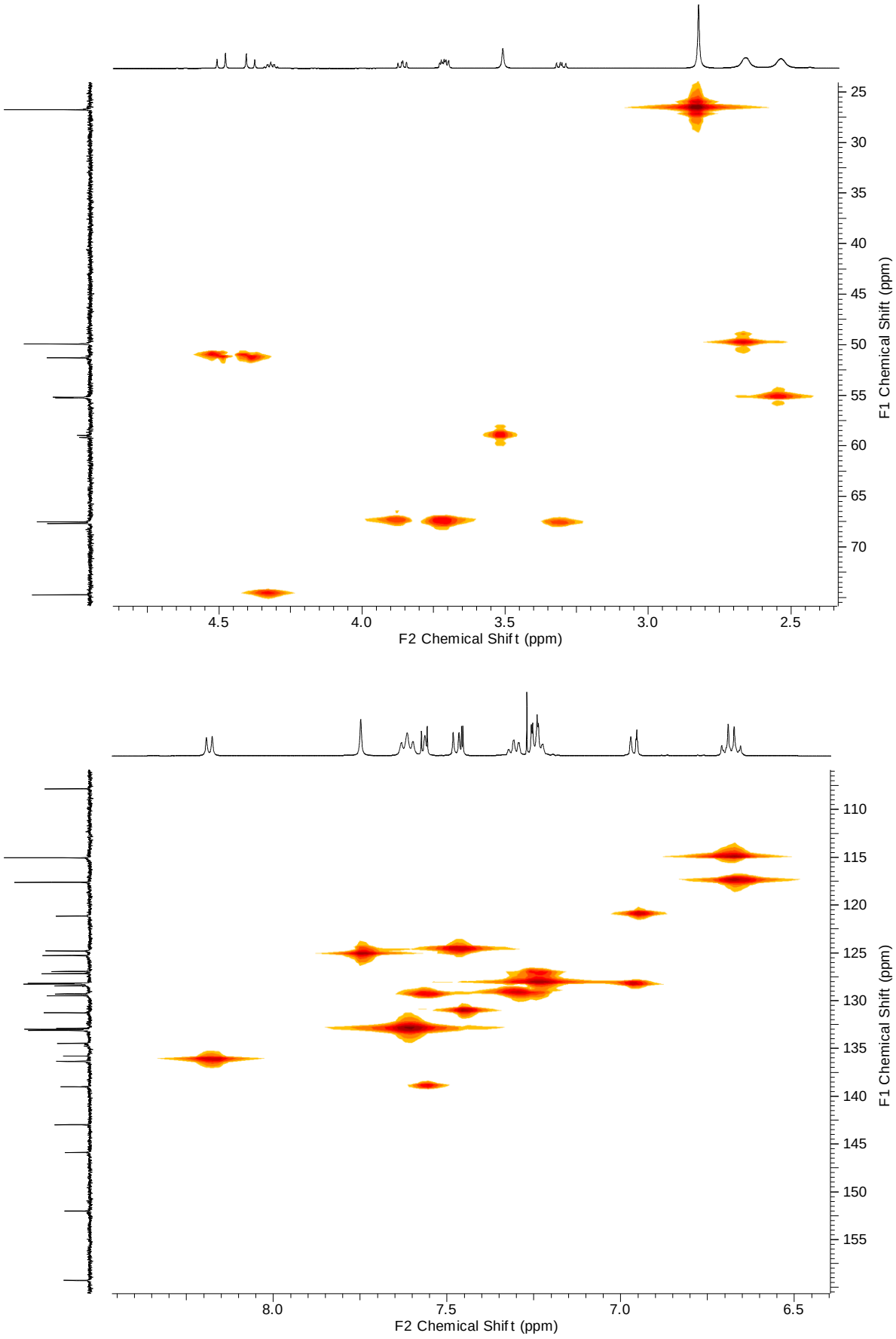

Fig.S5. 2D ^{1}H-^{13}C HMQC NMR spectra of **1-KeP** in $CDCl_3$. (T = 300K)

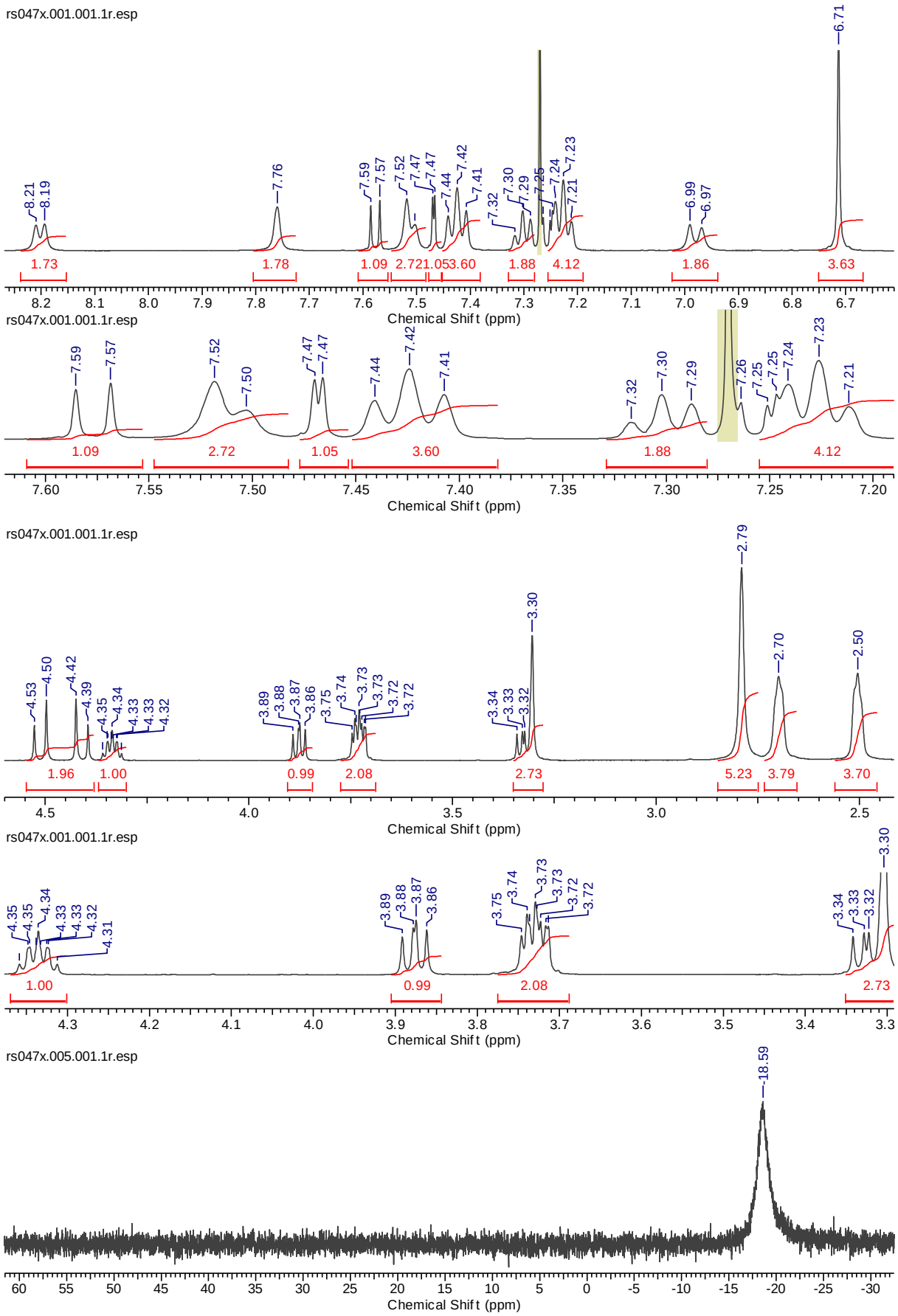

Fig. S6. ^{1}H and $^{31}P\{^{1}H\}$ NMR spectra of **2-KeP** in $CDCl_3$. (T = 300K)

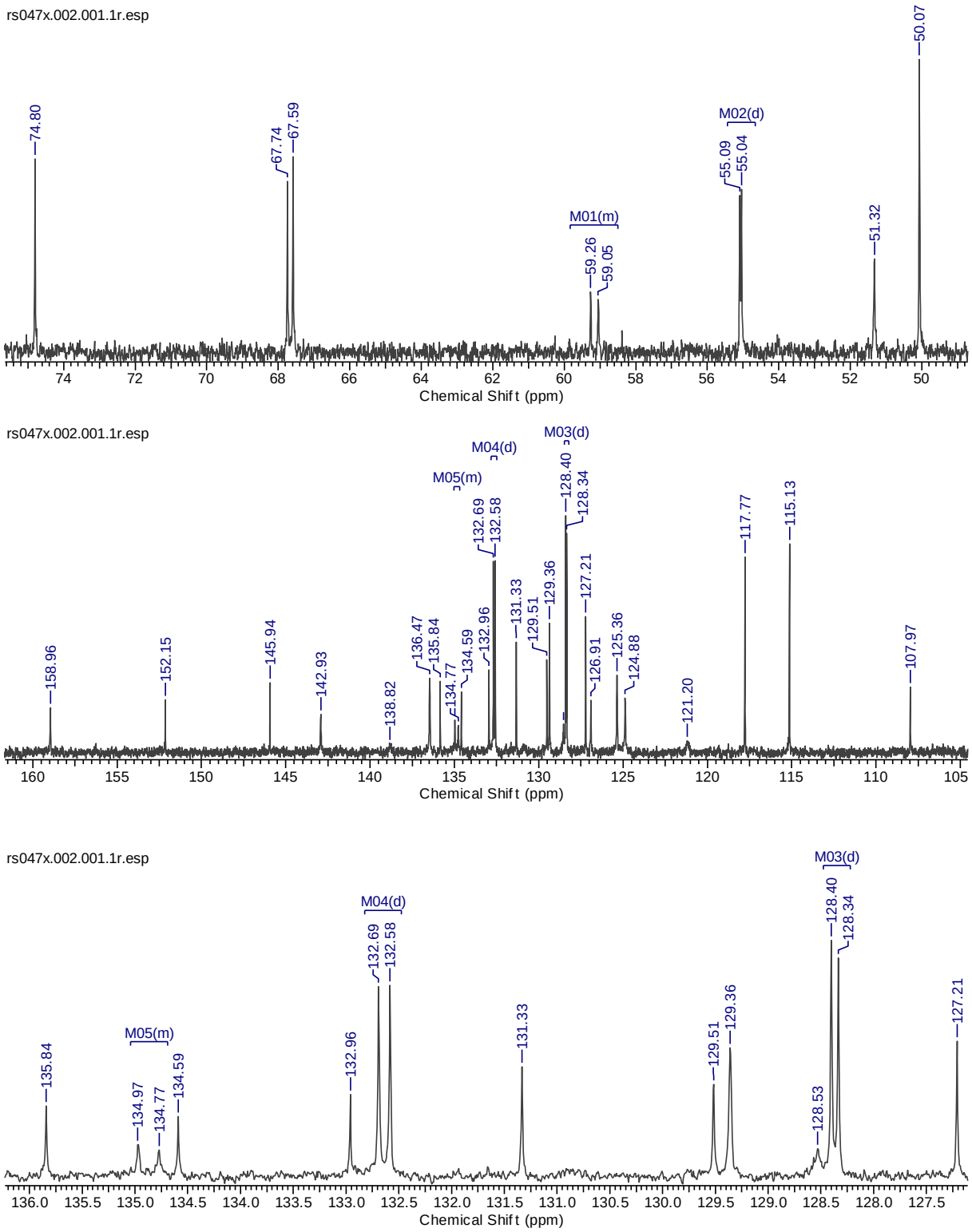

Fig. S7. $^{13}C\{^{1}H\}$ NMR spectra of **2-KeP** in $CDCl_3$. (T = 300K)

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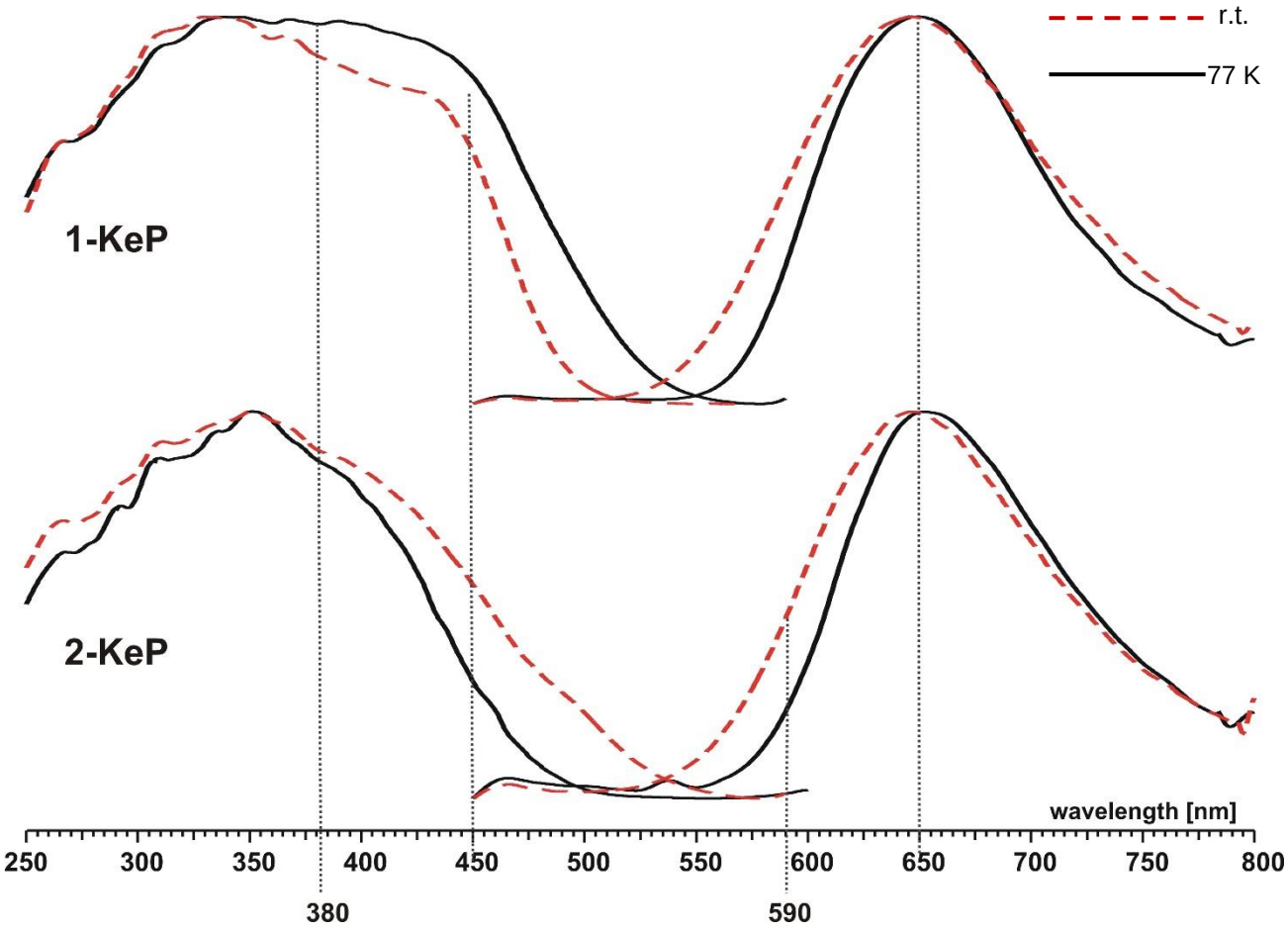

Fig.S8. Normalized solid state luminescence (upon the excitation at 380 nm; right) and excitation (for the emission at the band maximum; left) spectra of the studied complexes studied at room temperature (r.t. - dashed red lines) and at 77K (solid lines).

Tab.S3 Spectroscopic data for the compounds in organic solvents

	Ke	**KeP**	**KeOP**	**1-KeP**	**2-KeP**
MeOH					
ABS: λ_{max}	1: 297 * 2: 243 *	1: *sh* * 2: 246 *	1: 299* 2: 243 *		
$EM^{(295)}$ λ_{max}	368 *	373 *	369 *	362	361
$EX^{(370)}$ λ_{max}	293 *	297 *	295 *	289 ^{sh},302#	289 ^{sh},302#
LT: (280→370) τ /ns (a) [χ^2]	1: 0.28 (0.087) * 2: 1.63 (0.913) * [1.525] * **<τ>: 1.51 ***	0.11 (0.464) * 1.37 (0.536) * [1.434] * **<τ>: 0.78 ***	0.29 (0.506) * 0.58 (0.494) * [1.446] * **<τ>: 0.43 ***	1.43 (0.964) 2.66 (0.036) [1.225] **<τ>: 1.47**	1.34 (0.866) 2.08 (0.536) [1.321] **<τ>: 1.44**
EtOH					
ABS: λ_{max}	1: 295 * 2: 245 *	1: *sh* * 2: 247 *	1: 295 * 2: 245 *		
$EM^{(295)}$ λ_{max}	362 *	365 *	363 *	368	369
$EX^{(370)}$ λ_{max}	291 *	296 *	295 *	291 ^{sh},303#	291 ^{sh},302#
LT: (280→370) τ /ns (a) [χ^2]	1: 1.05 (0.209) 2: 1.68 (0.791) [1.283] **<τ>: 1.55**	1: 0.62 (0.233) 2: 1.37 (0.767) [1.509] **<τ>: 1.19**	1: 0.46 (0.981) 2: 1.95 (0.019) [1.362] **<τ>: 0.49**	1: 0.50 (0.513) 2: 1.57 (0.487) [2.131] **<τ>: 1.02**	1: 0.49 (0.521) 2: 1.52 (0479.) [2.185] **<τ>: 0.98**
CH_3CN					
ABS: λ_{max}	1: 299 * 2: 247 *	1: *sh* * 2: 250 *	1: 305 * 2: 249 *		
$EM^{(295)}$ λ_{max}	370 *	372 *	372 *	371	373
$EX^{(370)}$ λ_{max}	301 *	306 *	303 *	291 ^{sh},306#	291 ^{sh},306#
LT: (280→370) τ /ns (a) [χ^2]	1: 1.22 (0.355) 2: 1.71 (0.645) [1.051] **<τ>: 1.53**	1: 1.13 (0.779) 2: 1.62 (0.221) [1.189] **<τ>: 1.24**	1: 0.85 (0.979) 2: 2.10 (0.021) [1.277] **<τ>: 0.88**	1: 0.67 (0.463) 2: 1.47 (0.537) [1.343] **<τ>: 1.10**	1: 0.59 (0.413) 2: 1.45 (0.587) [1.203] **<τ>: 1.09**
DMSO					
ABS: λ_{max}	303	*sh*	306		
$EM^{(295)}$ λ_{max}	374	374	377	378	377
$EX^{(370)}$ λ_{max}	300	307	305	291 ^{sh},311#	291 ^{sh},310#
LT: (280→370) τ /ns (a) [χ^2]	1: 1.33 (0.986) 2: 3.27 (0.014) [1.151] **<τ>: 1.36**	1: 1.03 (0.979) 2: 2.44 (0.021) [1.352] **<τ>: 1.06**	1: 0.54 (0.995) 2: 4.48 (0.005) [1.544] **<τ>: 0.56**	1: 0.99 (0.987) 2: 3.87 (0.013) [1.456] **<τ>: 1.03**	1: 1.02 (0.987) 2: 3.86 (0.015) [1.572] **<τ>: 1.06**

sh – shoulder; # inner-filter effect; **<τ>** = $\tau_1a_1 + \tau_2a_2$ [ns]; * - data from: Starosta R., de Almeida R.F.M. "Luminescence properties of the antifungal agent ketoconazole and its diphenylphosphane derivatives" J. Lumin. 2020, 220, 116956; DOI: 10.1016/j.jlumin.2019.116956

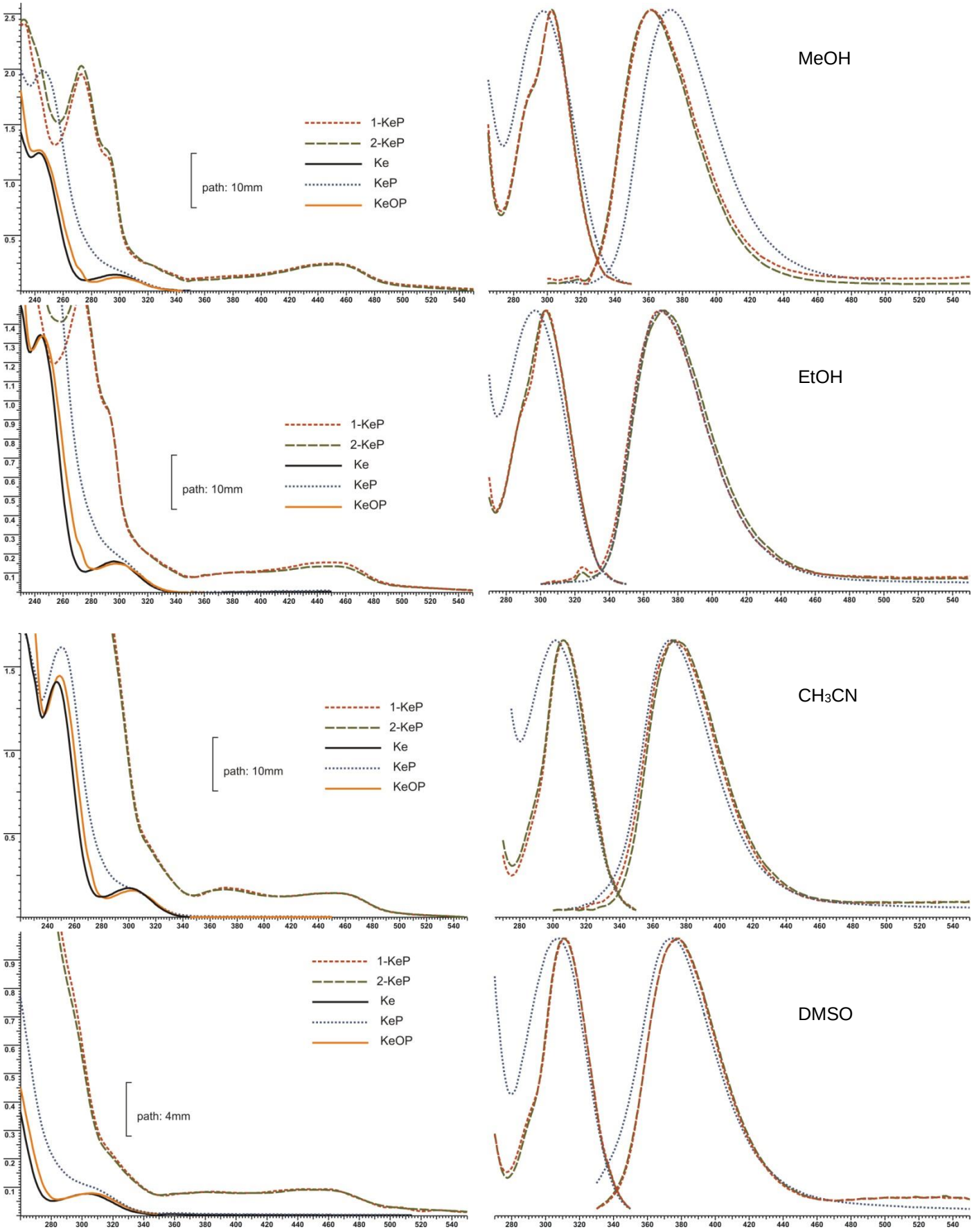

Fig.S9. Absorption, normalized excitation (for the emission at 370 nm) and emission spectra (for the excitation at 295 nm) for the complexes and organic compounds (c = 100μM) in four different solvents.

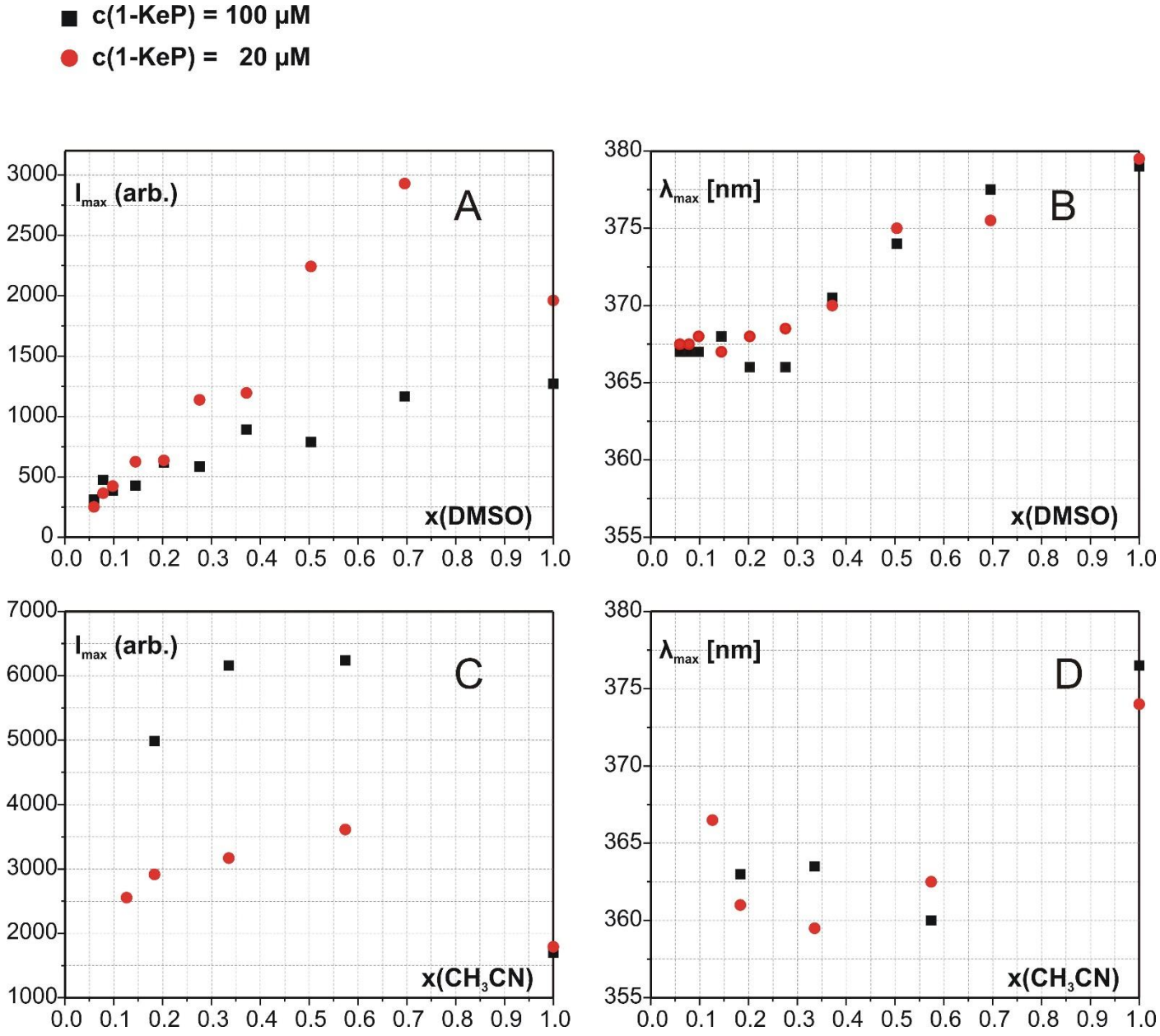

Fig.S10. Luminescence data for **1-KeP** in H_2O+DMSO (A, B) and H_2O+CH_3CN (C, D) in two concentrations: 100 µM (▪black squares) and 20 µM (•red circles). A, C: fluorescence intensity at the **Ke** fluorescence band maximum (excitation: 295 nm) B, D: position of the fluorescence band maximum (excitation: 295 nm) as function of the mole fraction of the organic solvent (DMSO or CH_3CN).

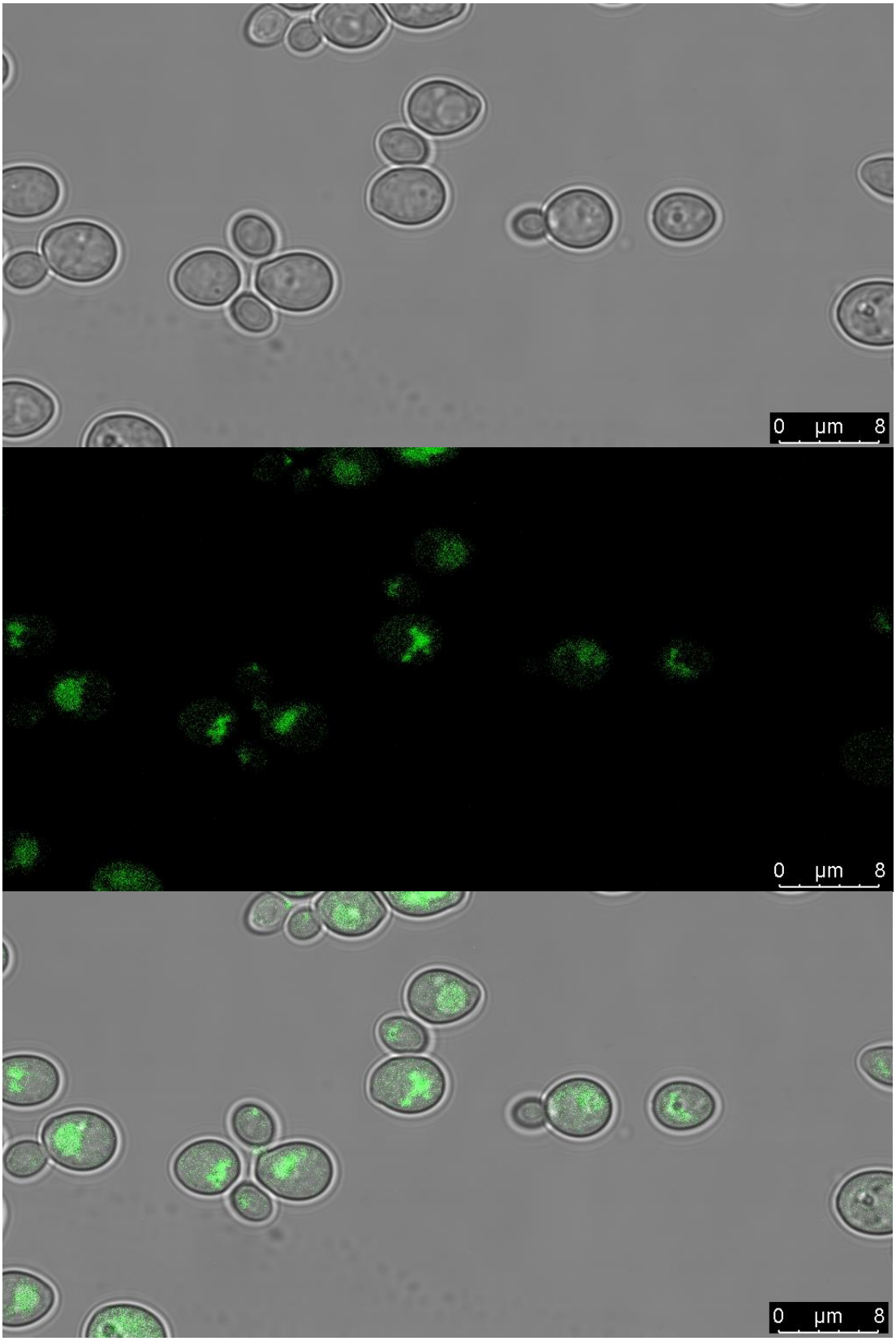

Fig.S11-A. Staining of the DSY1050 strain with **1-KeP** imaged by confocal microscopy (excitation at 405 nm, emission 600 - 700 nm signal collected). Top: bright field; Middle: luminescence; Bottom: merged.

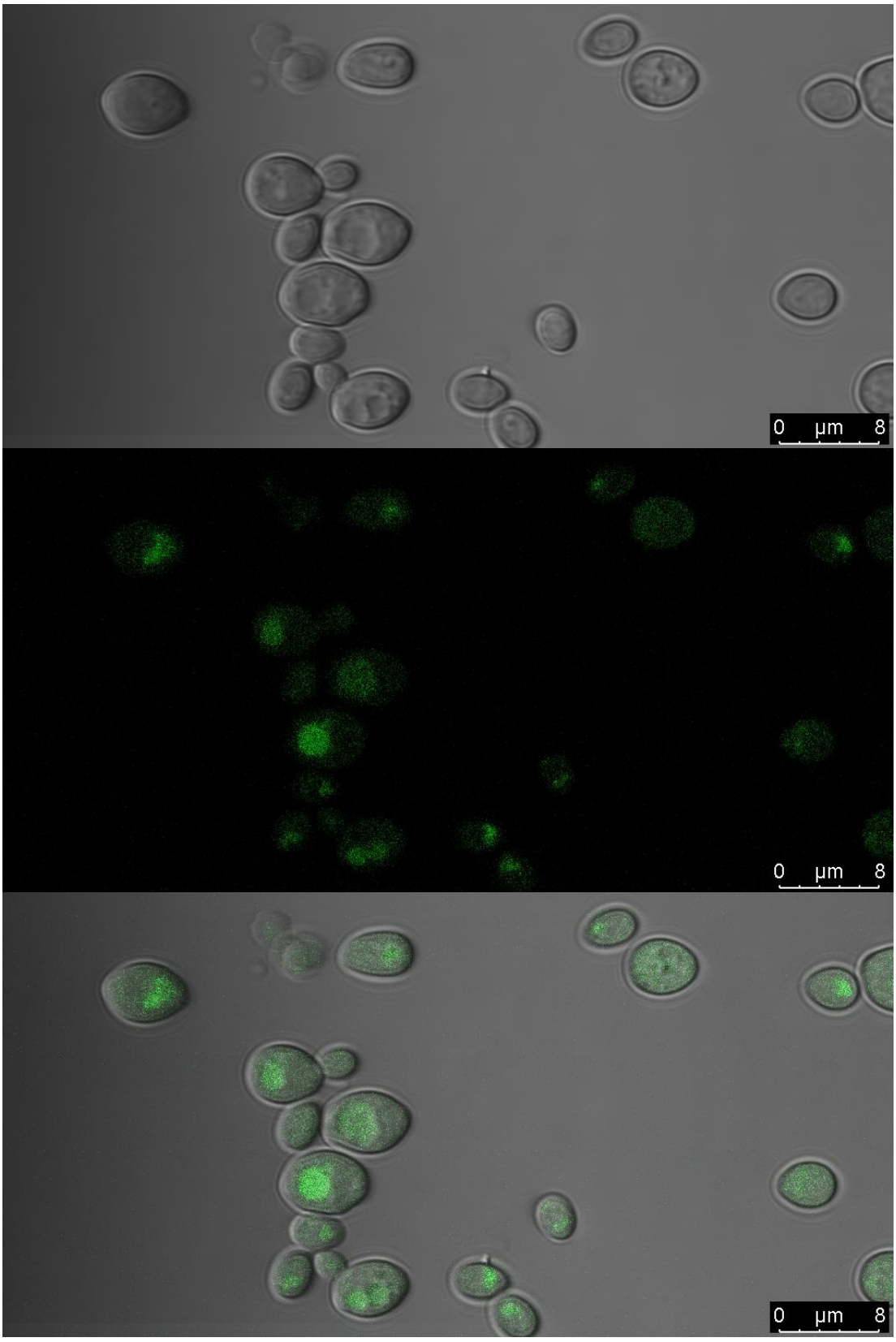

Fig.S11-B. Staining of the DSY1050 strain with **1-KeP** imaged by confocal microscopy (excitation at 405 nm, emission 600 - 700 nm signal collected). Top: bright field; Middle: luminescence; Bottom: merged.

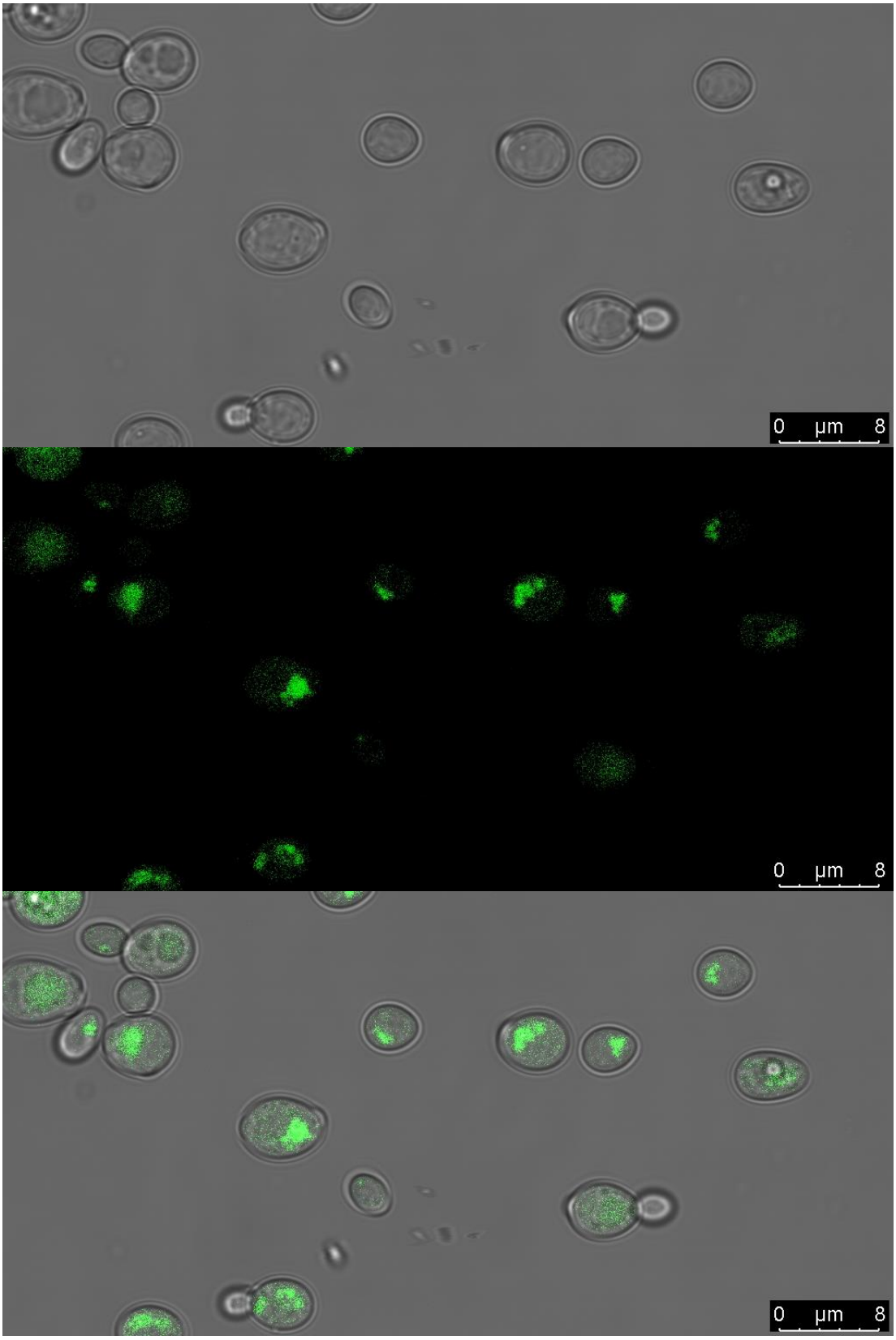

Fig.S11-C. Staining of the DSY1050 strain with **1-KeP** imaged by confocal microscopy (excitation at 405 nm, emission 600 - 700 nm signal collected). Top: bright field; Middle: luminescence; Bottom: merged.

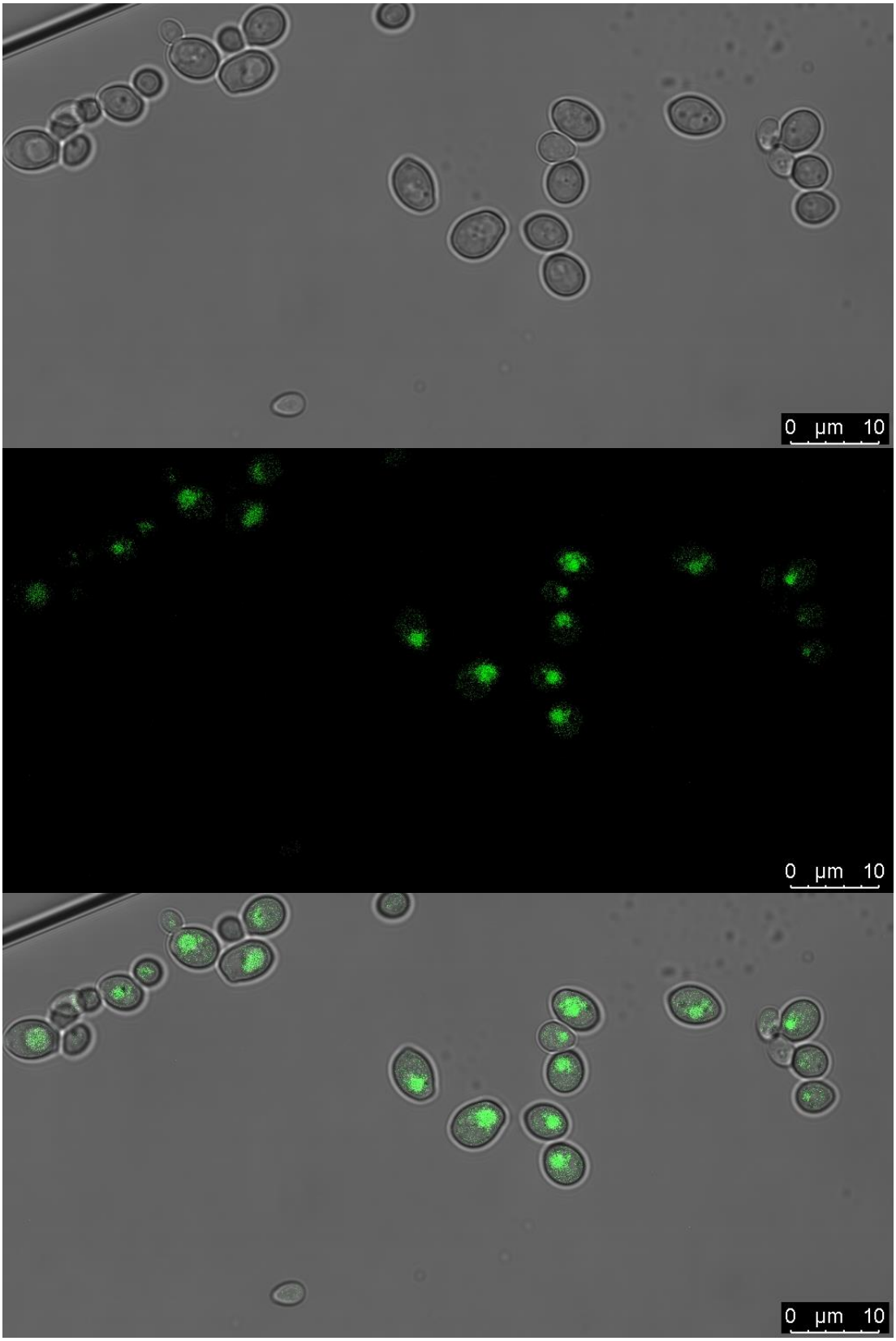

Fig.S12-A. Staining of the DSY1050 strain with **2-KeP** imaged by confocal microscopy (excitation at 405 nm, emission 600 - 700 nm signal collected). Top: bright field; Middle: luminescence; Bottom: merged.

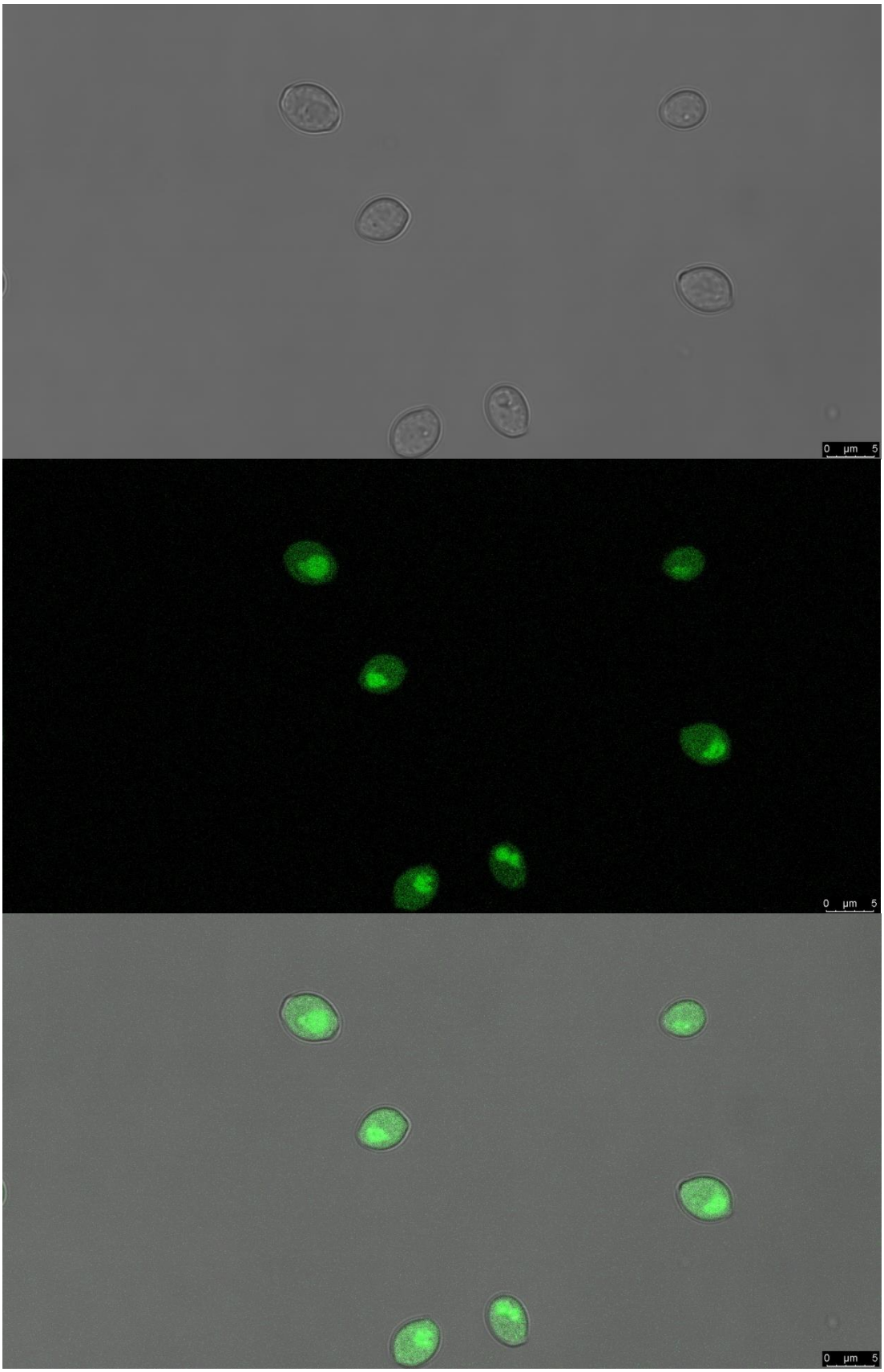

Fig.S12-B. Staining of the DSY1050 strain with **2-KeP** imaged by confocal microscopy (excitation at 405 nm, emission 600 - 700 nm signal collected). Top: bright field; Middle: luminescence; Bottom: merged.

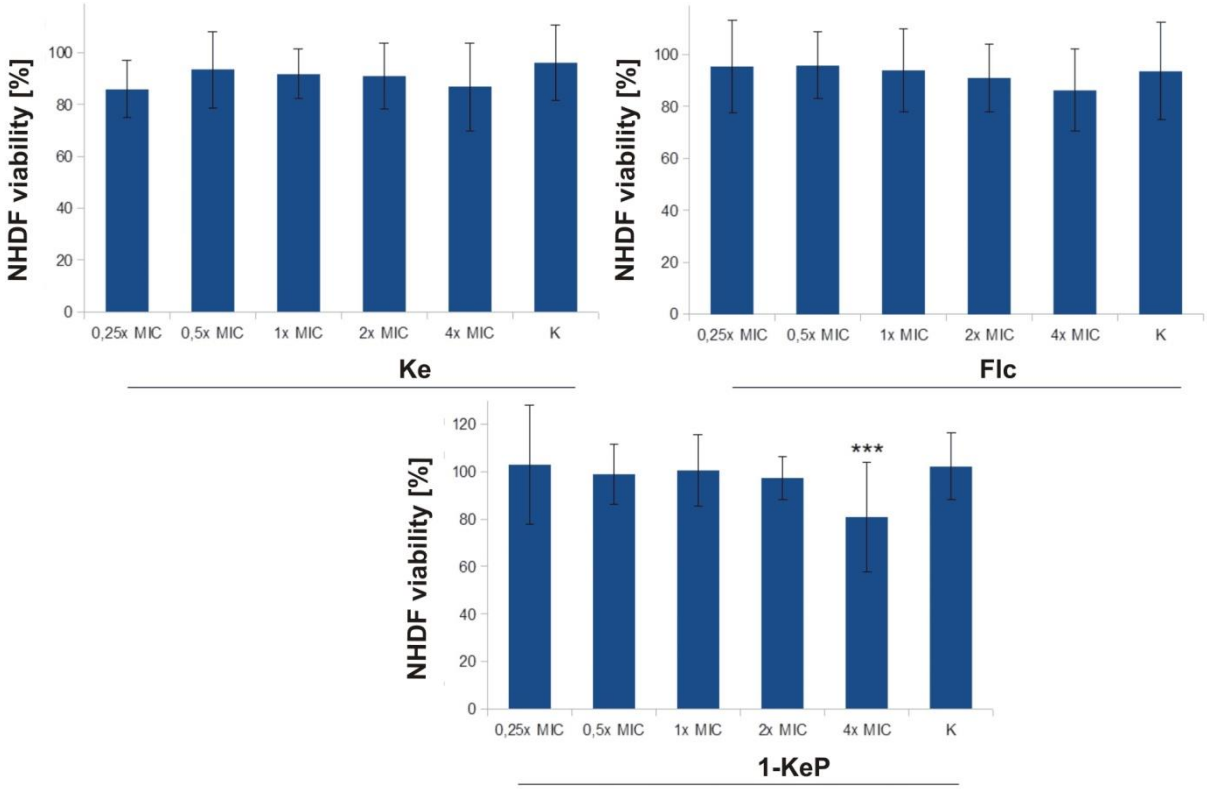

Fig.S13 Proliferation rate of NHDF cells measured by MTT assay. NHDF cells were treated with 0.25; 0.5; 1; 2 and 4 x MIC_{50} concentrations of **Ke**, **Flc** and **1-KeP** for 72 h, incubated with MTT and the number of viable cells measured spectrophotometrically at 540 nm. The bars represent the means ± SD of triplicate values for three independent experiments. ***P<0.001.

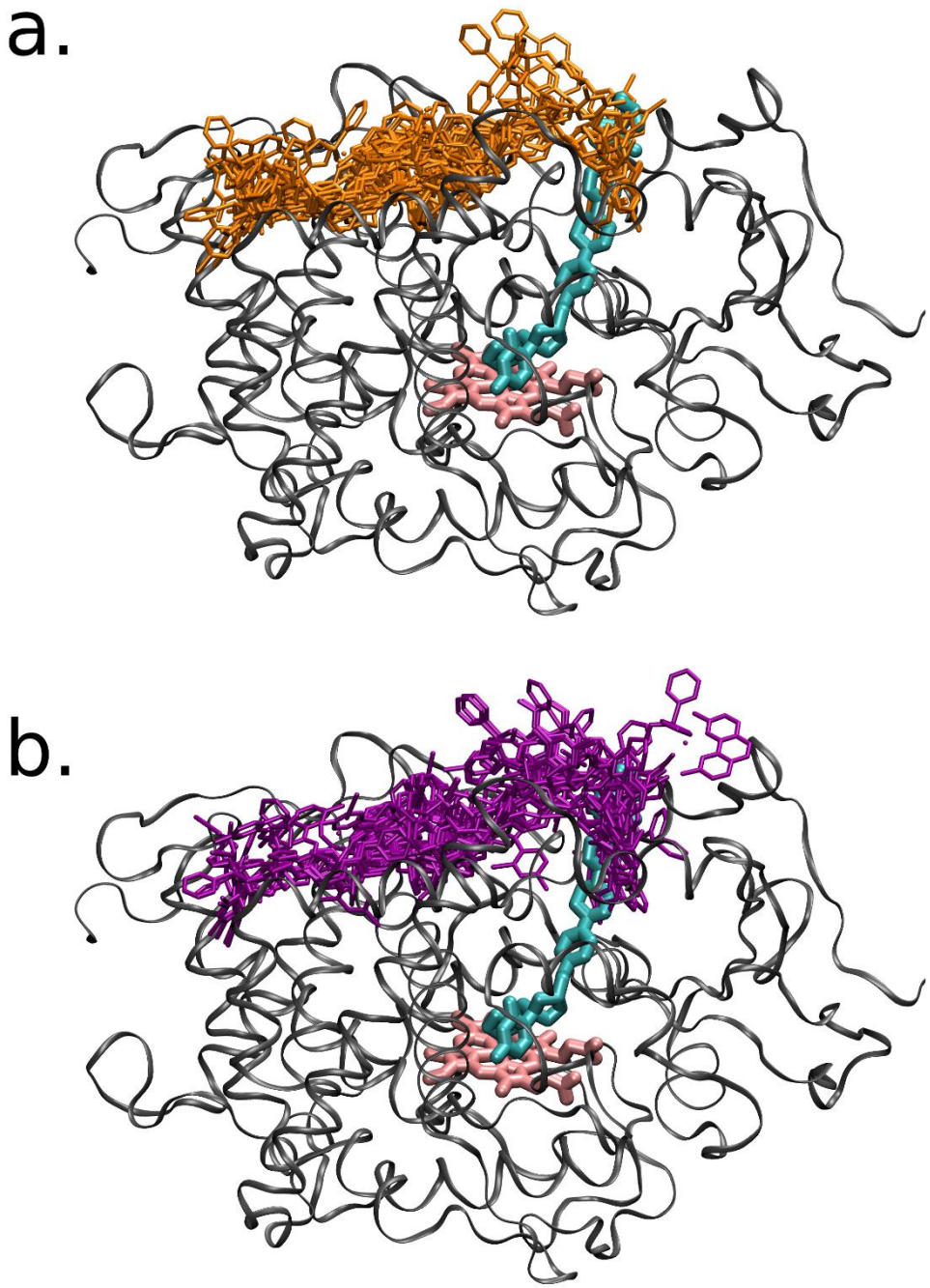

Fig.S14 Docking of a) **1-KeP** (orange) and b) **2-KeP** (purple) to the CYP51 of *C. albicans* (grey ribbons). Experimental positions of the heme cofactor (pink) and the inhibitor – posaconazole (cyan) – are taken from the 5FSA PDB deposit. Docking ensembles of 27 conformations are shown for each ligand. The large molecules of the complexes **1-KeP** and **2-KeP** are not able to enter the binding cavity accessible to the posaconazole molecule; in particular, they are not able to interact directly with the heme cofactor. They are, instead, located on the protein surface, possibly obstructing the entrance to the binding cavity.

REFERENCES

1 Eds. A. Casini, A. Vessières, S. M. Meier-Menches, Metal-based Anticancer Agents, Royal Society of Chemistry, **2019**, ISBN 978-1-78801-406-9 https://doi.org/10.1039/9781788016452

2 C. Santini, M. Pellei, V. Gandin, M. Porchia, F. Tisato, C. Marzano, Advances in Copper Complexes as Anticancer Agents, *Chem. Rev.*, 2014, **114**, 815-862 https://doi.org/10.1021/cr400135x1uyh

3 L. Colina-Vegas, W. Villarreal, M. Navarro, Copper(I)–phosphine complexes: a promising approach in the search for antitumor agents, Ed.: M. S. Balakrishna, Copper(I) Chemistry of Phosphines, Functionalized Phosphines and Phosphorus Heterocycles, Elsevier, 2019, 109-143, ISBN 9780128150528, https://doi.org/10.1016/B978-0-12-815052-8.00005-1

4 C., Marzano, F., Tisato, M., Porchia, M., Pellei, V. Gandin, Phosphine copper(I) complexes as anticancer agents: biological characterization. Part II, Ed.: M. S. Balakrishna, Copper(I) Chemistry of Phosphines, Functionalized Phosphines and Phosphorus Heterocycles Elsevier, **2019**, 83-107, ISBN 9780128150528, https://doi.org/10.1016/B978-0-12-815052-8.00004-X

5 Ed: L. Martinez, The evolving threat of antimicrobial resistance – Options for actions, World Health Organization, **2012**, ISBN 978-92-4-15031-1

6 M. C. Fisher, N. J. Hawkins, D. Sanglard, S. J. Gurr, Worldwide emergence of resistance to antifungal drugs challenges human health and food security., *Science*, 2018, **360**, 739-742 https://doi.org/10.1126/science.aap7999

7 L. Krishnasamy, S. Krishnakumar, G. Kumaramanickavel, C. Saikumar, Molecular mechanisms of antifungal drug resistance in Candida species., *J. Clin. Diagnostic Res.*, 2018, **12**, DE01–DE06 https://doi.org/10.7860/JCDR/2018/36218.11961

8 M. B., Lohse, M., Gulati, A. D., Johnson, C. J. Nobile, Development and regulation of single-and multi-species Candida albicans biofilms., *Nat. Rev. Microbiol.*, 2018, **16**, 19–31 https://doi.org/10.1038/nrmicro.2017.107

9 S. G. Whaley, E. L. Berkow, J. M. Rybak, A. T. Nishimoto, K. S. Barker, P. D. Rogers, Azole Antifungal Resistance in Candida albicans and Emerging Non-albicans Candida Species*., Front Microbiol.*, 2017, **12**, A2173 https://doi.org/10.3389/fmicb.2016.02173

10 K. T. Gunsalus, C. A. Kumamoto, Transcriptional Profiling of Candida albicans in the Host., *Methods Mol. Biol.*, 2016, **1356**, 17-29 https://doi.org/10.1007/978-1-4939-3052-4_2

11 T. P. Salci, M. Negri, A. K. R. Abadio, T. I. E. Svidzinski, É. S. Kioshima, Targeting Candida spp. to develop antifungal agents., *Drug Discov. Today*, 2018, **23**, 802–814 https://doi.org/10.1016/j.drudis.2018.01.003

12 L. Rodriguez, B. Bustamante, L. Huaroto, C. Agurto, R. Illescas, R. Ramirez, A. Diaz, J. Hidalgo, A multi-centric study of Candida bloodstream infection in Lima-Callao, Peru: species distribution, antifungal resistance and clinical outcomes. *PLoS One*, 2017, **12**, e0175172 https://doi.org/10.1371/journal.pone.0175172

13 A. Levina, D. C. Crans, P. A. Lay, Speciation of Metal Drugs, Supplements and Toxins in Media and Bodily Fluids Controls In-Vitro Activities, *Coord. Chem. Rev.* 2017, **352**, 473-498 http://doi.org/10.1016/j.ccr.2017.01.002

14 B N. Cioffi, L. Torsi, N. Ditaranto, G. Tantillo, L. Ghibelli, Luigia Sabbatini, Teresa Bleve-Zacheo, Maria D'Alessio, P. G. Zambonin, Enrico Traversa, Copper Nanoparticle/Polymer Composites with Antifungal and Bacteriostatic Properties, *Chem. Mater.* 2005, **17**, 5255-5262 https://doi.org/10.1021/cm0505244

15 Y. Wei, S. Chen, B. Kowalczyk, S. Huda, T. P. Gray, B. A. Grzybowski, Synthesis of Stable, Low-Dispersity Copper Nanoparticles and Nanorods and Their Antifungal and Catalytic Properties, *J. Phys. Chem. C* 2010, **114**, 15612–15616 https://doi.org/10.1021/jp1055683

16 M. E. Helsel, E. J. White, S. Z. A. Razvi, B. Alies, K. J. Franz, Chemical and functional properties of metal chelators that mobilize copper to elicit fungal killing of Cryptococcus neoformans, *Metallomics* 2017, **9**, 69–81. https://doi.org/10.1039/c6mt00172f

17 S. Shams, B. Ali, M. Afzal, I. Kazmi, F. A. Al-Abbasi, F. Anwar, Antifungal effect of Gatifloxacin and copper ions combination, *J. Antibiot.* 2014, **67**, 499–504 https://doi.org/10.1038/ja.2014.35

18 E. W. Hunsaker, K. J. Franz, Copper potentiates azole antifungal activity in a way that does not involve complex formation, *Dalton Trans.* 2019, **48**, 9654-9662 https://doi.org/10.1039/C9DT00642G

19 E. W. Hunsaker, K. J. Franz, Candida albicans reprioritizes metal handling during fluconazole stress, *Metallomics* 2019, **11**, 2020-2032 https://doi.org/10.1039/C9MT00228F

20 A. Bykowska, R. Starosta, J. Jezierska, M. Jeżowska-Bojczuk, Coordination versatility of phosphine derivatives of fluoroquinolones. New Cu^{I} and Cu^{II} complexes and their interactions with DNA., *RSC Advances*, 2015, *5*, 80804-80815 https://doi.org/10.1039/c5ra07483e

21 R. Starosta, U. K. Komarnicka, M. Puchalska, Luminescent copper(I) (pseudo)halide complexes with neocuproine and a novel bulky tris(aminomethyl)phosphine derived from 2-piperazinopyridine., *J. Lumin.*, 2013, **143**, 137-144 https://doi.org/10.1016/j.jlumin.2013.04.050

22 R. Starosta, U. K. Komarnicka, M. Puchalska, Solid state luminescence of CuI and CuNCS complexes with phenanthrolines and a new tris(aminomethyl)phosphine derived from N-methyl-2-phenylethanamine., *J. Lumin.*, 2014, **145**, 430-437 https://doi.org/10.1016/j.jlumin.2013.07.015

23 U. K. Komarnicka, R. Starosta, A. Kyzioł, M. Jeżowska-Bojczuk, Copper(I) complexes with phosphine derived from sparfloxacin. Part I – structures, spectroscopic properties and cytotoxicity., *Dalton Trans.*, 2015, **44**, 12688-12699 https://doi.org/10.1039/c5dt01146a

24 U. K. Komarnicka, R. Starosta, M. Płotek, R. F. M. de Almeida, M. Jeżowska-Bojczuk, A. Kyzioł, Copper(I) complexes with phosphine derived from sparfloxacin. Part II: a first insight into the cytotoxic action mode., *Dalton Trans.*, 2016, **45**, 5052-5063 https://doi.org/10.1039/C5DT04011F

25 V. Gandin, F. Tisato, A. Dolmella, M. Pellei, C. Santini, M. Giorgetti, C. Marzano, M. Porchia, In Vitro and in Vivo Anticancer Activity of Copper(I) Complexes with Homoscorpionate Tridentate Tris(pyrazolyl)borate and Auxiliary Monodentate Phosphine Ligands, *J. Med. Chem.* 2014, **57**, 4745-4760 https://doi.org/10.1021/jm500279x

26 U. K. Komarnicka, S. Kozieł, R. Starosta, A. Kyzioł, Selective Cu(I) complex with phosphine-peptide (SarGly) conjugate contra breast cancer: synthesis, spectroscopic characterization and insight into cytotoxic action, *J. Inorg. Biochem.* 2018, **186**, 162-175 https://doi.org/10.1016/j.jinorgbio.2018.06.009

27 M Patra, T. Joshi, V. Pierroz, K. Ingram, M. Kaiser, S. Ferrari, B. Spingler, J. Keiser, G. Gasser, DMSO-Mediated Ligand Dissociation: Renaissance for Biological Activity of N-Heterocyclic-[Ru(η6-arene)Cl2] Drug Candidates, *Chem. Eur. J.* 2013, **19**, 14768-14772 https://doi.org/10.1002/chem.201303341

28 A. Kobayashi, K. Komatsu, H. Ohara, W. Kamada, Y. Chishina, K. Tsuge, H.-C. Chang, M. Kato, Photo- and Vapor-Controlled Luminescence of Rhombic Dicopper(I) Complexes Containing Dimethyl Sulfoxide, *Inorg. Chem.* 2013, **52**, 13188−13198 https://doi.org/10.1021/ic402104q

29 V. Patel, B. Liaw, W. Oh, The role of ketoconazole in current prostate cancer care., *Nature Rev. Urol.*, 2018, **15**, 643–651 https://doi.org/10.1038/s41585-018-0077-y

30 P. Contreras, E. Altieri, C. Liberman, A. Gac, A. Rojas, A. Ibarra, M. Ravanal, M. Seron-Ferre, Adrenal rest tumor of the liver causing Cushing's syndrome: treatment with ketoconazole preceding an apparent surgical cure., *J. Clin. Endocrinol. Metab.*, 1985, **60**, 21-28 https://doi.org/10.1210/jcem-60-1-21

31 Committee for Medicinal Products for Human Use (CHMP), Assessment report Ketoconazole HRA, Procedure No. EMEA/H/C/003906/0000 https://www.ema.europa.eu/documents/assessment-report/ketoconazole-hra-epar-public-assessment-report_en.pdf (accessed Mar. 20. 2020)

32 B. C. Das, A. V. Madhukumar, J. Anguiano, S. Kim, M., Sinz, T. A. Zvyaga, E. C. Power, C. R. Ganellin, S. Mani, Synthesis of novel ketoconazole derivatives as inhibitors of the human Pregnane X Receptor (PXR, NR1I2, also termed SXR, PAR)., *Bioorg. Med. Chem. Let.*, 2008, **18**, 3974-3977 https://doi.org/10.1016/j.bmcl.2008.06.018

33 S. ManiBhasar, C. Das, Ketoconazole-derivative antagonists of human pregnane x receptor. U.S. Patent 8669260, **2011**

34 P. Pirson, B. Leclef, A. Trouet, Activity of ketoconazole derivatives against Leishmania mexicana amazonensis within mouse peritoneal macrophages., *Ann. Trop. Med. Parasitol.*, 1990, **84**, 133-139 https://doi.org/10.1080/00034983.1990.11812446

35 M. S. Rieber, A. Anzellotti, R. A. Sánchez-Delgado, M. Rieber, Tumor apoptosis induced by ruthenium(II)-ketoconazole is enhanced in nonsusceptible carcinoma by monoclonal antibody to EGF receptor., *Int. J. Cancer*, 2004, **112**, 376–384 https://doi.org/10.1002/ijc.20415

36 E. Robles-Escajeda, A. Martínez, A. Varela-Ramirez, R. A. Sánchez-Delgado, R. J. Aguilera, Analysis of the cytotoxic effects of ruthenium – ketoconazole and ruthenium – clotrimazole complexes on cancer cells., *Cell Biol. Toxic.*, 2013, **29**, 431-443 https://doi.org/10.1007/s10565-013-9264-z

37 T. Gagini, L. Colina-Vegas, W. Villarreal, L. P. Borba-Santos, P. C. de Souza, A. A. Batista, F. M. Kneip, W. de Souza, S. Rozental, L. A. S. Costa, M. Navarro, Metal–azole fungistatic drug complexes as anti-Sporothrix spp. Agents., *New J. Chem.*, 2018, **42**, 13641-13650 https://doi.org/10.1039/C8NJ01544A

38 R. F. M. de Almeida, F. C. Santos, K. Marycz, M. Alicka, A. Krasowska, J. Suchodolski, J. J. Panek, A. Jezierska, R. Starosta, New diphenylphosphane derivatives of ketoconazole are promising antifungal agents., *Sci. Rep.*, 2019, **9**, 16214 https://doi.org/10.1038/s41598-019-52525-7

39 D. S. Loose, P. B. Kan, M. A. Hirst, R. A. Marcus, D. Feldman, Ketoconazole blocks adrenal steroidogenesis by inhibiting cytochrome P450-dependent enzymes., *J. Clin. Invest.*, 1983, **71**, 1495–1499 https://doi.org/10.1172/JCI110903

40 A. Bykowska, R. Starosta, A. Brzuszkiewicz, B. Bażanów, M. Florek, N. Jackulak, J. Król, J. Grzesiak, K. Kaliński, M. Jeżowska-Bojczuk, Synthesis, properties and biological activity of a novel phosphine ligand derived from ciprofloxacin., *Polyhedron*, 2013, **60**, 23-29 https://doi.org/10.1016/j.poly.2013.04.059

41 A. Bykowska, R. Starosta, U. K. Komarnicka, Z. Ciunik, A. Kyzioł, K. Guz-Regner, G., Bugla-Płoskońska, M. Jeżowska-Bojczuk, Phosphine derivatives of ciprofloxacin and norfloxacin, a new class of potential therapeutic agents., *New J. Chem.*, 2014 **38**, 1062-1071 https://doi.org/10.1039/c3nj01243c

42 U. K. Komarnicka, R. Starosta, A. Kyzioł, M. Płotek, M. Puchalska, M. Jeżowska-Bojczuk, New copper(I) complexes bearing lomefloxacin motif: spectroscopic properties, in vitro cytotoxicity and interactions with DNA and human serum albumin., *J. Inorg. Biochem.*, 2016, **165**, 25-35 https://doi.org/10.1016/j.jinorgbio.2016.09.015

43 B. R. James, R. J. P. Williams, 383. The oxidation–reduction potentials of some copper complexes, *J. Chem. Soc.* **1961**, 2007-2019 https://doi.org/10.1039/JR9610002007

44 C. E. McCusker, F. N. Castellano, Design of a Long-Lifetime, Earth-Abundant, Aqueous Compatible Cu(I) Photosensitizer Using Cooperative Steric Effects, *Inorg. Chem.* 2013, **52**, 8114-8120 https://doi.org/10.1021/ic401213p

45 R. Starosta, A. Bykowska, A. Kyzioł, M. Płotek, M. Florek, J. Król, M. Jeżowska-Bojczuk, Copper(I) (pseudo)halide complexes with neocuproine and aminomethylphosphines derived from morpholine and thiomorpholine - in vitro cytotoxic and antimicrobial activity and the interactions with DNA and serum albumins., *Chem. Biol. Drug Des.*, 2013, **82**, 579-586 https://doi.org/10.1111/cbdd.12187

46 R. Starosta, M. Puchalska, J. Cybińska, M. Barys, A. V. Mudring, Structures, electronic properties and solid state luminescence of Cu(I) iodide complexes with 2,9-dimethyl-1,10-phenanthroline and aliphatic aminomethylphosphines or triphenylphosphine., *Dalton Trans.*, 2011, **40**, 2459-2468 https://doi.org/10.1039/c0dt01284j

47 R. Starosta, U. K. Komarnicka, M. Puchalska, M. Barys, Solid state luminescence of copper(I) (pseudo)halide complexes with neocuproine and aminomethylphosphanes derived from morpholine and thiomorpholine., *New J. Chem.*, 2012, **36**, 1673-1683 https://doi.org/10.1039/C2NJ40229G

48 F. Jalali, A. Afshoon, Spectrofluorimetric Study and Detection of Ketoconazole in the Presence of β-cyclodextrin., *J. Fluoresc.*, 2008, **18**, 219–225 https://doi.org/10.1007/s10895-007-0265-2

49 F. Jalali, A. Afshoon, M. Shamsipur, Micellar-enhanced spectrofluorimetric determination of ketoconazole in cetyltrimethylammonium bromide medium. *Chem Anal.* 2007, **52**, 115–123 EID: 2-s2.0-34047274959

50 P. Y. Khashaba, K. M. Emara, A. M. Mohamed, Analysis of some antifungal drugs by spectrophotometric and spectrofluorimetric methods in different pharmaceutical dosage forms., *J. Pharm. Biomed. Anal.*, 2000, **22**, 363–376 https://doi.org/10.1016/S0731-7085(99)00280-0

51 A. El-Bayoumi, A. A. El-Shanawany, M. E. El-Sadek, A. Abd-ElSattar, Synchronous spectrofluorimetric determination of famotidine, fluconazole and ketoconazole in bulk powder and in pharmaceutical dosage forms., *Spect. Lett.*, 1997, **30**, 25–46 https://doi.org/10.1080/00387019708002587

52 R. Starosta, R.F.M. de Almeida, Luminescence properties of the antifungal agent ketoconazole and its diphenylphosphane derivatives, *J. Lumin.*, 2020, **220**, 116956 https://doi.org/10.1016/j.jlumin.2019.116956

53 B. Kobin, L. Grubert, S. Blumstengel, F. Henneberger, S. Hecht., Vacuum-Processable Ladder-type Oligophenylenes for Organic-Inorganic Hybrid structures: Synthesis, Optical and Electrochemical Properties upon Increasing Planarization as Well as Thin Film Growth., *J. Mat. Chem.*, 2012, **22**, 4383–4390 http://doi.org/10.1039/C2JM15868J

54 Z. Lu, E. Manias, D. D. Macdonald, M. Lanagan, Dielectric Relaxation in Dimethyl Sulfoxide/Water Mixtures Studied by Microwave Dielectric Relaxation Spectroscopy., *J. Phys. Chem. A*, 2009, **113**, 12207–12214 https://doi.org/10.1021/jp9059246

55 R. Jellema, J. Bulthuis, G. van der Zwan, Dielectric relaxation of acetonitrile-water mixtures. *J. Mol. Liquids*, 1997, **73–74**, 179-193 https://doi.org/10.1016/S0167-7322(97)00066-4

56 J.,Suchodolski, J. Muraszko, P. Bernat, A. Krasowska, A Crucial Role for Ergosterol in Plasma Membrane Composition, Localisation, and Activity of Cdr1p and H + -ATPase in Candida albicans., *Microorganisms*, 2019, **7**, 378 https://doi.org/10.3390/microorganisms7100378

57 T. Y. Hargrove, L. Friggeri, Z. Wawrzak, A. Qi, W. J. Hoekstra, R. J. Schotzinger, J. D. York, F. P. Guengerich, G. I. Lepesheva, Structural analyses of Candida albicans sterol 14α-demethylase complexed with azole drugs address the molecular basis of azole-mediated inhibition of fungal sterol biosynthesis., *J. Biol. Chem.*, 2017, **292**, 6728–6743 https://doi.org/10.1074/jbc.M117.778308

58 A. Krasowska, L. Chmielewska, J. Łuczyński, S. Witek, K. Sigler, Dual mechanism of the antifungal effect of new lysosomotropic agents on Saccharomyces cerevisiae RXII strain, *Cell. Mol. Biol. Lett.*, 2003, **8**, 111-120.

59 A. Krasowska, L. Chmielewska, R. Adamski, J. Łuczyński, S. Witek, K. Sigler, The sensitivity of yeast and yeast-like cells to new lysosomotropic agents, *Cell. Mol. Biol. Lett.*, 2004, **9**, 675–683.

60 C. De Duve, T. De Barsy, B. Poole, A. Trout, P. Tulkens, F. Van Hoff, Lysosomotropic agents., *Biochem. Pharmacol.*, 1974, **23**, 2495-2519 https://doi.org/10.1016/0006-2952(74)90174-9

61 Gaussian 16, Revision B.01, M. J. Frisch et al., Gaussian, Inc., Wallingford CT, 2016.

62 J.-D. Chai, M. Head-Gordon, Long-range corrected hybrid density functionals with damped atom-atom dispersion corrections, *Phys. Chem. Chem. Phys.*, 2008, **10**, 6615-6620 https://doi.org/10.1016/10.1039/B810189B

63 K. L. Schuchardt, B. T. Didier, T. Elsethagen, L. Sun, V. Gurumoorthi, J. Chase, J. Li, T. L. Windus, Basis Set Exchange: A Community Database for Computational Sciences, *J. Chem. Inf. Model.*, 2007, **47**, 1045-1052 https://doi.org/10.1021/ci600510j

64 M. N. Glukhovtsev, A. Pross, M. P. McGrath, L. Radom, Extension of Gaussian-2 (G2) theory to bromine- and iodine-containing molecules: Use of effective core potentials, *J. Chem. Phys.*, 1995, **103**, 1878-1885 https://doi.org/10.1063/1.469712

65 G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell, A. J. Olson, Autodock4 and AutoDockTools4: automated docking with selective receptor flexiblity., *J. Comput. Chem.*, 2009, **16**, 2785-2791 https://doi.org/10.1002/jcc.21256

66 O. Trott, A. J. Olson, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading., *J. Comput. Chem.*, 2010, **31**, 455-461 https://doi.org/10.1002/jcc.21334

67 W. Humphrey, A. Dalke, K. Schulten, VMD – Visual Molecular Dynamics., *J. Molec. Graph.*, 1996, **14**, 33–38 https://doi.org/10.1016/0263-7855(96)00018-5

68 W. Fonzi, M. Irwin, Isogenic strain construction and gene mapping in Candida albicans., *Genetics*, 1993, **134**, 717–728 https://doi.org/10.1046/j.1439-0507.1999.00498.x

69 P. Mukherjee, J. Chandra, Mechanism of fluconazole resistance in Candida albicans biofilms: phase-specific role of efflux pumps and membrane sterols., *Infect. Immun.*, 2003, **71**, 4333–4340 https://doi.org/10.1128/IAI.71.8.4333-4340.2003

70 D. Sanglard, K. Kuchler, F. Ischer, J. L. Pagani, M. Monod, J. Bille, Mechanisms of resistance to azole antifungal agents in Candida albicans isolates from AIDS patients involve specific multidrug transporters., *Antimicrob. Agents Chemother.*, 1995, **39**, 2378–2386 https://doi.org/10.1128/AAC.39.11.2378

71 D. Sanglard, F. Ischer, Susceptibilities of Candida albicans multidrug transporter mutants to various antifungal agents and other metabolic inhibitors., *Antimicrob. Agents Chemother.*, 1996, **40**, 2300–2305 https://doi.org/10.1128/AAC.40.10.2300

72 R. Franz, M. Ruhnke J., Morschhäuser Molecular aspects of fluconazole resistance development in Candida albicans, *Mycoses*, 1999, **42**, 453-458 https://doi.org/10.1046/j.1439-0507.1999.00498.x

73 R. Wakieć, R. Prasad, J. Morschhäuser, F. Barchiesi, E. Borowski, S. Milewski, Voriconazole and multidrug resistance in Candida albicans., *Mycoses*, 2007, **50**, 109-115 https://doi.org/10.1111/j.1439-0507.2006.01327.x

74 Reference method for broth dilution antifungal susceptibility testing of yeast. Approved Standard, 3rd ed. M27-A3 28. Clinical and Laboratory Standards Institute, Wayne, PA. 2008 ISBN 1-56238-666-2

75 J. Suchodolski, A. Krasowska, Plasma Membrane Potential of Candida albicans Measured by Di-4-ANEPPS Fluorescence Depends on Growth Phase and Regulatory Factors., *Microorganisms*, 2019, **7**, 110 https://doi.org/10.3390/microorganisms7040110