

SUPPLEMENTARY MATERIAL TO

Synthesis, antioxidant, antimicrobial and antifungal activity of carbohydrazones

ALEKSANDRA R. BOŽIĆ,¹ NENAD R. FILIPOVIĆ,² IRENA T. NOVAKOVIĆ,³ SNEŽANA K. BJELOGRLIĆ,⁴ JASMINA B. NIKOLIĆ,⁵ SAŠA Ž. DRMANIĆ⁵ and ALEKSANDAR D. MARINKOVIĆ⁵

¹Belgrade Polytechnic, Brankova 17, 11000 Belgrade, Serbia, ²Faculty of Agriculture, University of Belgrade, Nemanjina 6, Belgrade, Serbia, ³Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Njegoševa 12, Belgrade, Serbia, ⁴National Cancer Research Center of Serbia, Pasterova 14, Belgrade, Serbia, ⁵Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, Belgrade, Serbia

SPECTRAL DATA FOR COMPOUNDS **a1-a14**

*Salicylaldehyde carbohydrazones (a1)*¹. White solid (solvent used for crystallization: ethanol). Yield: 66 %; m.p. 180-181°C (lit m.p. -); Anal. Calcd. for C₈H₉N₄O₂ (*M_w* = 245.24 g mol⁻¹): C, 49.74; H, 4.70; N, 29.00 %; Found: C, 49.68; H, 4.66; N, 28.93 %; IR (KBr, cm⁻¹): 3353 (OH), 3282 (NH₂), 3096 (NH), 1680 (C=O), 1640 (C=N).

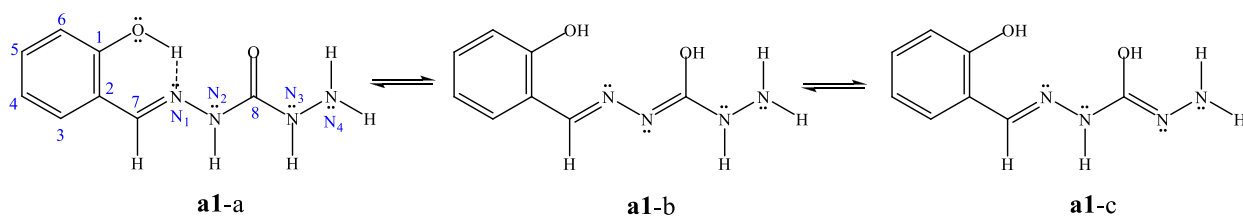


Fig. S1. Equilibrium of tautomeric forms and geometrical isomers of **a1** with numeration of the atom of interest.

2-pyridylaldehyde carbohydrazones (a2). White solid (acetonitrile). Yield: 67 %; m.p. 173-174 °C. Anal. Calcd. for C₇H₉N₅O (*M_w* = 179.18 g mol⁻¹): C, 46.92; H, 5.06; N, 39.09 %, Found: C, 46.88; H, 5.01; N, 39.11 %; IR (KBr, cm⁻¹): 3313 (NH₂), 3208 (NH), 1678 (C=O), 1635 (C=N); ¹H NMR (500 MHz, DMSO-*d*₆, δ / ppm) 4.11 (2H, s, H₂-N₅), 7.31 (1H, ddd, H-C₅, ³*J*_{5,4} = 7.5 Hz, ³*J*_{5,6} = 4.9 Hz), 7.78 (1H, td, H-C₄, ³*J*_{4,3} = 7.9 Hz, ³*J*_{4,5} = 7.5 Hz, ⁴*J*_{4,6} = 1.5 Hz), 7.89 (1H, s, H-C₇), 8.284-8.105 (2H, br.m.ovlp., H-C₃, H-N₄, ³*J*_{3,4} = 7.9 Hz), 8.51 (1H, ddd, H-C₆, ³*J*_{6,5} = 4.9 Hz, ⁴*J*_{6,4} = 1.5 Hz), 10.64 (1H, s, H-N₃); ¹³C NMR (126 MHz, DMSO-*d*₆, δ / ppm) 119.85

(C₃), 123.69 (C₅), 136.47 (C₄), 140.59 (C₇), 149.09 (C₆), 153.77 (C₂), 156.85 (C₈); ¹⁵N NMR (derived from 2D HMBC, δ / ppm): 51.1 (N₅), 99.7 (N₄), 153.6 (N₃), 312.2 (N₁), 326.0 (N₂).

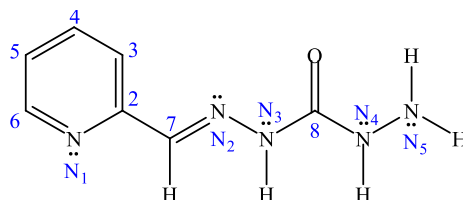


Fig. S2. (*E*)-isomer of compound **a2** with numeration of atom of interest

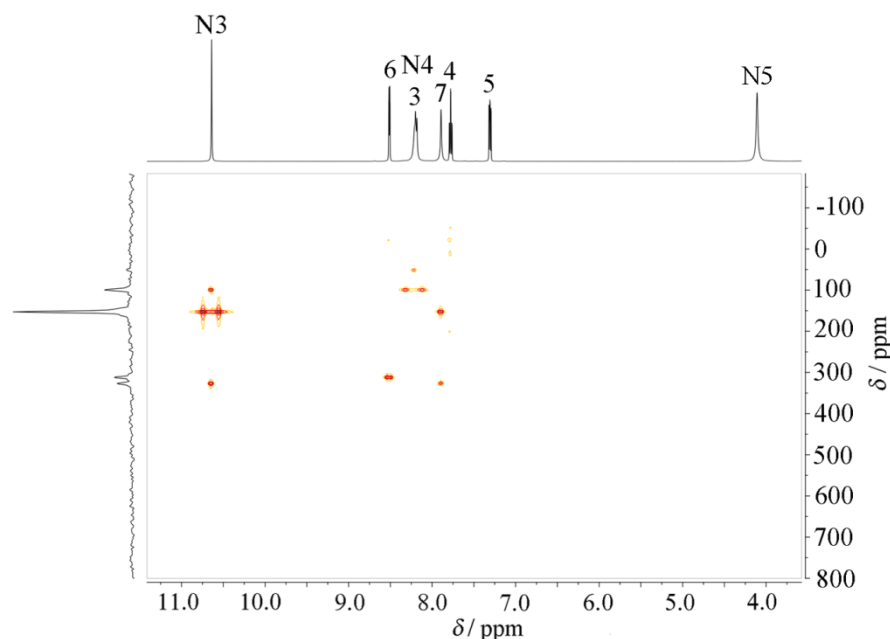
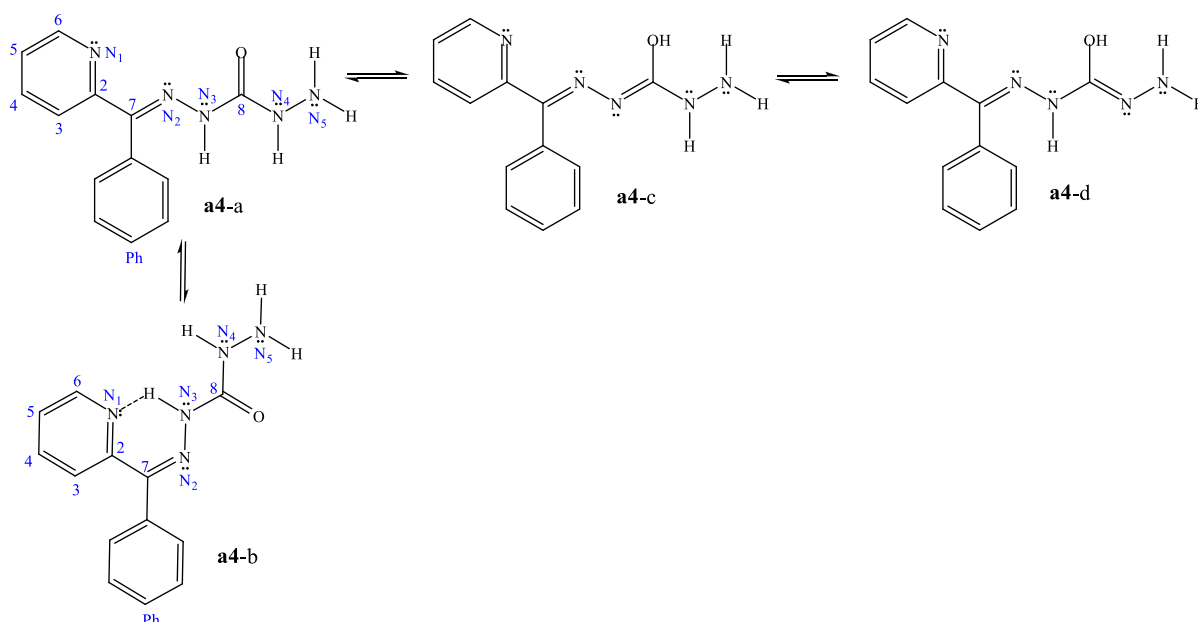


Fig. S3. 2D ¹⁵N-HMBC spectrum of **a2** in DMSO-*d*₆ recorded at 298 K (abscissa ¹H; ordinate ¹⁵N NMR chemical shift)

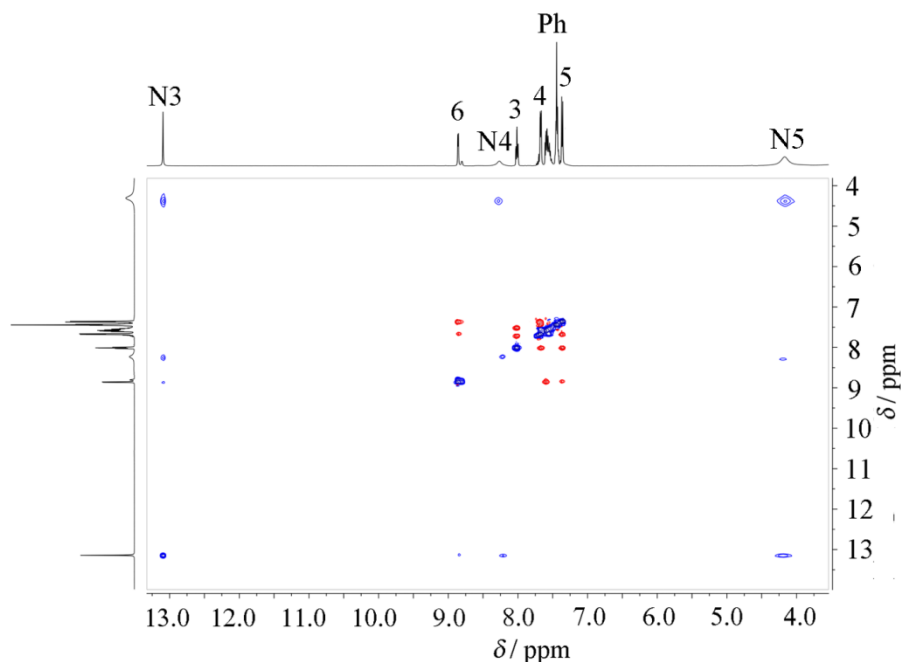
*Methyl 2-pyridyl ketone carbonohydrazone (a3)*². White solid (ethanol). Yield: 72,0 %; m.p. 203 °C. (lit m.p. 202-203°C); Anal. Calcd. for C₈H₁₁N₅O (*M*_w = 193.21 g mol⁻¹): C, 47.73; H, 5.74; N, 36.25 %, Found: C, 47.61; H, 5.82; N, 36.18 %; IR (KBr, cm⁻¹): 3308 (NH₂), 3197 (NH), 1674 (C=O), 1631 (C=N).

Phenyl 2-pyridyl ketone carbonohydrazone (a4). White solid (ethanol). Yield: 84 %; m.p. 203-205 °C; Anal. Calcd. for C₁₃H₁₃N₅O (*M*_w = 255.11 g mol⁻¹): C, 61.17; H, 5.13; N, 27.43 %, Found: C, 61.02; H, 4.98; N, 27.15 %; IR (KBr, cm⁻¹): 3304 (NH₂), 3215 (NH), 1674 (C=O), 1623 (C=N); ¹H NMR (500 MHz, DMSO-*d*₆, δ / ppm) 4.15 (2H, s, H₂-N₅), 7.36 (1H, dt, H-C₅, ³*J*_{5,4} = 7.5 Hz, ³*J*_{5,6} = 4.8 Hz), 7.40-7.60 (5H, m, Ph), 7.68 (1H, td, H-C₄, ³*J*_{4,3} = 7.8 Hz, ³*J*_{4,5} = 7.2 Hz), 8.01 (1H, dd, H-C₃, ³*J*_{3,4} = 7.8 Hz); 8.26 (1H, s, H-N₄), 8.85 (1H, dd, H-C₆, ³*J*_{6,5} = 4.8

46 Hz), 13.20 (1H, s, H-N₃); ¹³C NMR (126 MHz, DMSO-*d*₆, δ / ppm) 121.57 (C₃), 123.88 (C₅),
 47 124.84 (Ph), 125.95 (Ph), 128.27 (Ph), 128.93(Ph), 129.14 (Ph), 138.18 (C₄), 143.23 (C₇), 148.90
 48 (C₆), 151.40 (C₂), 157.21 (C₈); ¹⁵N NMR (derived from 2D HMBC, δ / ppm): 57.62 (N₅),
 49 100.07 (N₄), 155.1 (N₃), 306.33 (N₁), 315.85 (N₂).



50
 51 Fig. S4. Equilibrium of tautomeric forms (**a4-a**, **a4-c** and **a4-d**) and geometrical isomers (**a4-a** and **a4-b**)
 52 of compound **a4** with numeration of the atom of interest.



53
 54 Fig. S5. 2D NOESY of **a4** in DMSO-*d*₆ recorded at 298 K (abscissa and ordinate ¹H NMR chemical shift)

55

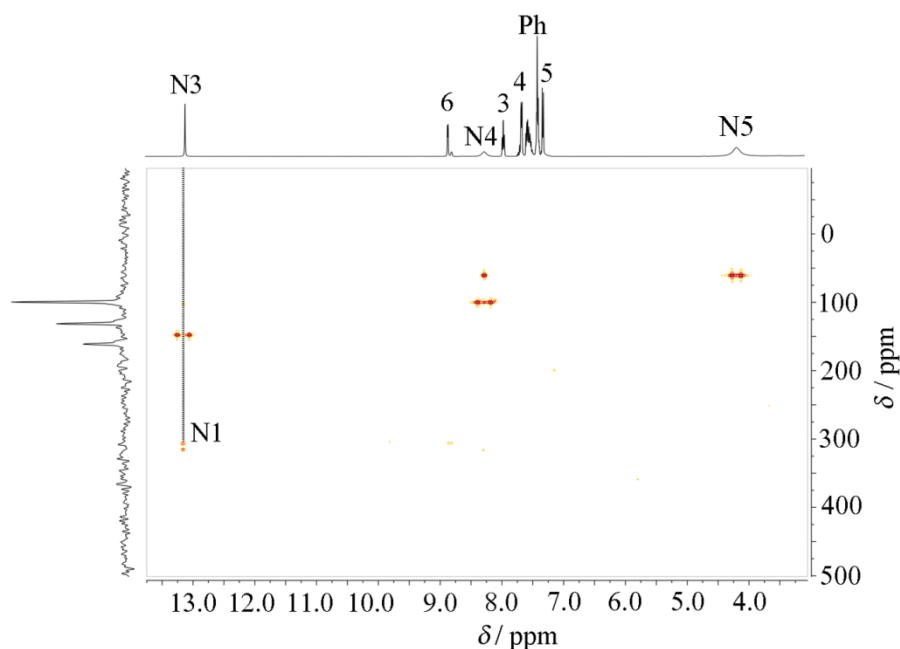


Fig. S6. 2D ^{15}N -HMBC of **a4** in DMSO- d_6 at 298 K (abscissa ^1H ; ordinate ^{15}N NMR chemical shift)

*2-Quinolinealdehyde carbohydrazone (a5)*³. White solid (methanol). Yield: 56 %; m.p. 183 °C. (lit m.p. 183°C); Anal. Calcd. for $\text{C}_{11}\text{H}_{11}\text{N}_5\text{O}$ ($M_w = 229.24 \text{ g mol}^{-1}$): C, 57.63; H, 4.84; N, 30.55 %, Found: C, 57.58; H, 4.62; N, 30.69 %; IR (KBr, cm^{-1}): 3297 (NH_2), 3188 (NH), 1679 (C=O), 1638 (C=N).

*8-Hydroxy-2-quinolinealdehyde carbohydrazone (a6)*³. Yellow solid (methanol). Yield: 72 %; m.p. 214-215 °C. (lit m.p. 214-215°C). Anal. Calcd. for $\text{C}_{11}\text{H}_{11}\text{N}_5\text{O}_2$ ($M_w = 245.24 \text{ g mol}^{-1}$): C, 53.83; H, 4.525; N, 28.56 %, Found: C, 53.66; H, 4.68; N, 28.74 %; IR (KBr, cm^{-1}): 3371 (OH), 3335 (NH_2), 3198 (NH), 1696 (C=O), 1600 (C=N).

*8-Quinolinealdehyde carbohydrazone (a7)*³. Yellow solid (methanol). Yield: 64 %; m.p. 185 °C. (lit m.p. 185°C); Anal. Calcd. for $\text{C}_{11}\text{H}_{11}\text{N}_5\text{O}$ ($M_w = 229.24 \text{ g mol}^{-1}$): C, 57.63; H, 4.84; N, 30.55 %, Found: C, 57.71; H, 4.78; N, 30.62 %; IR (KBr, cm^{-1}): 3316 (NH_2), 3200 (NH), 1681 (C=O), 1621 (C=N).

*bis(Salcylaldehyde) carbohydrazones (a8)*⁴. Yellow cristal (ethanol). Yield: 78 %; m.p. 219°C (lit m.p. 216°C); Anal. Calcd. for $\text{C}_{15}\text{H}_{14}\text{N}_4\text{O}_3$ ($M_w = 298.11 \text{ g mol}^{-1}$): C, 60.35; H, 4.74; N, 18.79 %, Found: C, 60.22; H, 4.62; N, 18.93 %; IR (KBr, cm^{-1}): 3344 (OH), 3284 (NH), 1704 (C=O), 1622 (C=N).

*bis(2-pyridylaldehyd) carbohydrazones (a9)*⁵. White solid (ethanol). Yield: 88 %; m.p. 185°C (lit m.p. 190-191°C); Anal. Calcd. for C₁₃H₁₂N₆O (*M*_w = 268.11 g mol⁻¹): C, 58.20; H, 4.51; N, 31.33 %, Found: C, 58.12; H, 4.88; N, 30.98 %; IR (KBr, cm⁻¹): 3201 (NH), 1696 (C=O), 1604 (C=N).

*bis(Methyl 2-pyridyl ketone) carbonohydrazone (a10)*². White solid (ethanol). Yield: 88,0 %; m.p.(decomp.) 187 °C (lit M.p.(decomp.) 186°C); Anal. Calcd. for C₁₅H₁₆N₆O (*M*_w = 296.14 g mol⁻¹): C, 60.80; H, 5.44; N, 28.36 %, Found: C, 60.61; H, 5.31; N, 28.62 %; IR (KBr, cm⁻¹): 3206 (NH), 1698 (C=O), 1611 (C=N).

*bis(Phenyl 2-pyridyl ketone) carbonohydrazone (a11)*². White solid (ethanol). Yield: 91,0 %. M.p. 223 °C (lit M.p. 225-226°C). Anal. Calcd. for C₂₅H₂₀N₆O (*M*_w = 420.17 g mol⁻¹): C, 71.41; H, 4.79; N, 19.99 %, Found: C, 71.16; H, 4.82; N, 20.04 %. IR (KBr, cm⁻¹): 3177 (NH), 1702 (C=O), 1612 (C=N).

*bis(2-Quinolinealdehyde) carbohydrazone (a12)*³. White solid (DMF/methanol mixture 1 : 9 v/v). Yield 78 %; m.p. 162-164 °C (lit m.p. 162-164°C); Anal. Calcd. for C₂₁H₁₆N₆O (*M*_w = 368.14 g mol⁻¹): C, 68.47; H, 4.38; N, 22.81 %, Found: C, 68.81; H, 4.80; N, 22.56 %; IR (KBr, cm⁻¹): 3392 (NH), 1708 (C=O), 1630 (C=N).

*bis(8-Hydroxy-2-quinolinealdehyde) carbohydrazone (a13)*³. Yellow solid (DMF/methanol mixture 1 : 9 v/v). Yield: 66 %; m.p. 248-249 °C (lit m.p. 248-249°C); Anal. Calcd. for C₂₁H₁₆N₆O₃ (*M*_w = 400.39 g mol⁻¹): C, 62.99; H, 4.03; N, 20.99 %, Found: C, 62.84; H, 4.11; N, 21.22 %; IR (KBr, cm⁻¹): 3408 (OH), 3116 (NH), 1684 (C=O), 1601 (C=N).

*bis(8-Quinolinealdehyde) carbohydrazone (a14)*³. Yellow solid (methanol). Yield: 54 %; m.p. 219-220 °C (lit m.p. 219-220°C); Anal. Calcd. for C₂₁H₁₆N₆O (*M*_w = 368.14 g mol⁻¹): C, 68.47; H, 4.38; N, 22.81 %, Found: C, 68.32; H, 4.91; N, 22.73 %; IR (KBr, cm⁻¹): 3331 (NH), 1707 (C=O), 1614 (C=N).

100

101

102

103

104

105

TABLE SI. *In vitro* antifungal activity of the compounds tested by the well-diffusion agar assay expressed as the diameter (mm) of the inhibition zone

Tested compound	<i>C. albicans</i>	<i>S. cerevisiae</i>	<i>A. brasiliensis</i>
a1	/	/	/
a2	/	/	/
a3	/	/	/
a4	/	/	/
a5	10	14	12
a6	12	14	12
a7	12	16	16
a8	/	/	/
a9	/	/	/
a10	/	/	/
a11	/	/	/
a12	/	/	/
a13	12	10	10
a14	14	10	10
Nystatin	34	56	32

REFERENCES

1. P. Novak, T. Jednačak, J. Parlov Vuković, K. Zangger, M. Rubčić, N. Galić, T. Hrenar, *Croat. Chem. Acta* **85** (2012) 451.
2. A. Bacchi, M. Carcelli, P. Pelagatti, C. Pelizzi, G. Pelizzi, **75** (1999) 123.
3. A. Božić, A. Marinković, S. Bjelogrić, T. R. Todorović, I. N. Cvijetić, I. Novaković, C. D. Muller, N. R. Filipović, *RSC Adv.* **6** (2016) 104763.
4. D. Maity, A. K. Manna, D. Karthigeyan, T. K. Kundu, S. K. Pati, T. Govindaraju, *Chem. - A Eur. J.* **17** (2011) 11152.
5. A. Hoque, Md. Najbul Basu, G. Das, *Cryst. Growth Des.* **14** (2014) 6.