Synthesis, electrochemistry, spectroscopic investigations and cytotoxicity of ferrocene-based 2-(methylthio)-5-(pyridin-2-ylmethylene)-imidazol-4H-ones and their copper complexes. New ligands with a redox-active fragment capable of intramolecular reduction of coordinated copper(II) to copper(I)

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Supplementary Information

1. Experimental

1.1 Materials and methods

All reagents were obtained from Sigma Aldrich and used without further purification. NMR measurements were carried out on Brucker-Avance 400 MHz spectrometer in DMSO and $CDCl_3$ using TMS as an internal reference. HRMS spectra were obtained with Orbitrap Elite (Thermo Scientific) mass spectrometer. Ionization were carried out by ESI mod with ±3.5 kV, capillary temperature 275ºC. Mass spectra were recorded with an analyzer Orbitrap, resolution 480000. DMSO and diisooctyl phthalate (m/z 157.03515 and 413.26623) in positive mode and dodecyl sulfate (m/z 265.14790) in negative mode were used as internal standards. IR spectra in nujol were recorded on Perkin-Elmer 1430 spectrophotometer, EPR spectra were recorded on Varian E-3 X-band radiospectrometer at 77K in a capillary with an inner diameter of 1mm. Melting points were determined in a block with an open capillary; uncorrected values of melting points are given.

Ethyl isothiocyanoacetate was synthesized according to described procedue [1]. 3-Azidoethyl-5-((Z)-2-pyridylmethylene)-2-thioxotetrahydro-4H-imidazol-4-one and 5-(Z)-3-(2-azidoethyl)-2-(methylthio)-5-(pyridin-2-ylmethylene)-1H-imidazol-4H-one were synthesized according to procedures reported previously by our group with small modifications [2].

1.1 3-(2-Azidoethyl)-5-((Z)-2-pyridylmethylene)-2-thioxotetrahydro-4H-imidazol-4-one (1)

To a solution of 2-chloroethylamine hydrochloride (2 g, 17.2 mmol) in 20 ml of distilled water sodium azide (1.12 g, 17.2 mmol) was added with stirring. Stirring was continued at 80°C for 20 hours, then after cooling to ambient temperature pellets of sodium hydroxide (1.36 g, 34 mmol) were added. The reaction mixture was extracted

with diethyl ether (3x30 ml). The organic layers were combined and dried over anhydrous sodium sulfate. The residue (azidoethylamine) was dissolved in ether. Then an excess of ethyl isothiocyanoacetate (2.61 g, 18 mmol) was added dropwise to this solution. The reaction mixture was stirred overnight, and then ether was evaporated under reduced pressure. Residue was dissolved in 2% KOH solution in ethanol, than pyridine 2-carboxaldehyde 1.84 g (1.63 ml, 17.2 mmol) was added dropwise to this solution. After stirring for 3h at room temperature the reaction mixture was diluted with the same volume of water and acidified to pH 6 with 1M hydrochloric acid to form yellow precipitate. This precipitate was separated filtered, washed with water, ethanol and diethyl ether. 3.31 g (70%) of compound **1** was obtained.

M.p. = 131 ^{o}C (dec.)

^{1}H-NMR (DMSO-d6, δ, ppm): 11.47 (s, 1H, NH), 8,66 (d, 1H, J=4,70 Hz, H_{α}-Py), 7.73 (td, 1H, J_1=7.83 Hz, J_2=1,57 Hz, H_{γ}-Py), 7.40 (d, 1H, J=7,83 Hz, $H_{\beta'}$-Py), 7.25 (ddd, 1H, J_1=7,83 Hz, J_2=4,70 Hz J_3=1,57 Hz, Hβ-Py), 6.56 (s, 1H, -CH=), 4.13 (t, 2H, J=5.87 Hz, CH2CH2N3), 3.65 (t, 2H, J=6.26 Hz, $CH_2CH_2N_3$).

IR, cm^{-1}: 3340 (NH), 2102 (N_3), 1720 (C=O), 1614 (C=C).

Elemental anal. $C_{11}H_{10}N_6OS$: calculated C 48.17%, H 3.67%, N 30.64%, S 11.69%; found C 47.96%, H 3.72%, N 30.20%, S 11.96%.

1.2 5-(Z)-3-(2-azidoethyl)-2-(methylthio)-5-(pyridin-2-ylmethylene)-1H-imidazol-4H-one (2)

100 mg (0.36 mmol) of 3-2-azidoethyl-5-((Z)-2-pyridylmethylene)-2-thioxotetrahydro-4H-imidazol-4-one **2a** was suspended in a mixture of 2 ml of water and 2 ml of EtOH. 24 mg (0.43 mmol) of potassium hydroxide was added to this suspension to form clear red solution, then 104 mg (0.74 mmol) methyl iodide was added dropwise, and reaction mixture was stirred for 10 minutes. The reaction mixture was placed in a freezer for 12 h, the precipitate formed was separated by filtration, dried and washed with 10% aqueous solution of potassium hydroxide, water and small amounts of ice-cold ethyl alcohol and diethyl ether. As a result, 71 mg (72%) of 5-(Z)-3-(2-azidoethyl)-2-(methylthio)-5-(pyridin-2-ylmethylene)-1Himidazol-4H-one **2** was obtained as a pale yellow powder.

^{1}H-NMR (DMSO-d6, δ, ppm): 8.80 (d, 1H, J=8.02 Hz, H_{α}-Py), 8.70 (d, 1H, J=4.70 Hz, H_{β}-Py), 7.77 (td, 1H, J_1=7.82 Hz, J_2=1.56 Hz H_{γ}-Py), 7.24 (dd, 1H, J_1=4.89 Hz, J_2=1.57 Hz $H_{\beta'}$-Py,), 7.17 (s, 1H, -CH=), 3.28 (t, 2H, J=6.06 Hz, $CH_2\underline{CH_2}N_3$), 3.61 (t, 2H, J=6.06 Hz, $\underline{CH_2}CH_2N_3$), 2.80 (s, 3H, -CH_3).

IR, cm^{-1}: 3320 (NH), 2120 (N_3), 1730 (C=O), 1665 (C=C).

Elemental anal. $C_{11}H_{10}N_6OS$: calculated C 49.99%, H 4.19%, N 29.15%, S 11.12%; found C 50.02%, H 4.17%, N 29.16%, S 11.10%.

1.3 Synthesis of ethynylferrocene (3)

1.3.1 3-Ferrocenyl-1,1-dimethyl-2-propynol

A modified procedure [3]. In a 250 ml flat-bottom flask were placed by turns: ferrocene (5.13 g; 27.6 mmol), dry dichloromethane (18 ml), glacial acetic acid (100 ml), anhydrous $FeCl_3$ (1.12 g; 6.9 mmol), finely grinded $Cu(OAc)_2 \cdot H_2O$ (0.92 g; 4.6 mmol) and 2-methyl-3-butin-2-ol (2.59 ml; 2.25 g; 26.7 mmol). The reaction mixture was stirred at ambient temperature for 1 h, then was refluxed for 1.5 h (boiling point of the mixture 75°C) and cooled to ambient temperature. After cooling, new portions of $Cu(OAc)_2 \cdot H_2O$ (1.84 g; 9.2 mmol) and 2-methyl-3-butin-2-ol (2.59 ml; 2.25 g; 26.7 mmol) were added and stirring was continued at ambient temperature within 19.5 h in open flask (an access of air is essential for reaction).

On completion of the stirring, the reaction mixture was poured into 400 ml of water and ferrocene containing components were extracted with dichloromethane. The organic extract was washed with water to pH 6.5-7.0, dried over Na_2SO_4 and solvent was removed on rotor evaporator.

The residue was transferred to a chromatographic column (35-mm diameter) consisting of 200 ml of silica gel. The target compound was eluted with a mixture of ether and benzene 1:9. Two frontal yellow bands were discarded. Third, orange-red band gave 2.25 g (30%) of 3-ferrocenyl-1,1-dimethyl-2-propynole, m. p. 93-94° (after crystallization from heptane), cf. [3]: m. p. 93-94°.

^{1}H NMR ($CDCl_3$, δ, ppm): 1.58 (s, 6H, $(CH_3)_2$); 2.43 (br. s., 1H, OH); 4.16 (m, 2H, C_5H_4); 4.18 (s, 5H , C_5H_5); 4.38 (m, 2H , C_5H_4), cf [3].

^{13}C NMR ($CDCl_3$, δ, ppm): 31.60 (CH_3) ; 64.73 (C_5H_4); 65.56 (C-OH); 68.44 (C_5H_4); 69.81 (C_5H_5); 71.22 (C_5H_4); 80.36 and 90.20 (C≡C), cf [3].

1.3.2 Ethynylferrocene (3)

0.031 g of 60% sodium hydride suspension in mineral oil (corresponds to 0.019 g or 0.738 mmol of NaH) was placed in 50 ml Claisen flask and washed with dry toluene. Then dry toluene (27.8 ml) and 3-ferrocenyl-1,1-dimethyl-2-propynole (0.9 g; 3.38 mmol) were added and flask was equipped with a condenser.

By gradual raise of heating bath temperature the reaction mixture was brought to boil and distillation of toluene started (bath temperature 140°C). Distillation of toluene was

continued till a conversion of 3-ferrocenyl-1,1-dimethyl-2-propynole achieved 100% (monitoring by TLC).

The reaction mixture was cooled to ambient temperature and poured into 150 ml of saturated NaHCO3 solution. Toluene layer was separated and water layer was extracted with benzene (3 portions of 30 ml). Combined organic layers were washed with brine (2 portions of 50 ml) and dried over Na2SO4. A removal of the solvent gave 0.7858 g of red-orange oil.

This oil was dissolved in petroleum ether (40/70^{o}C) and subjected to flash-chromatography on column (diameter 45 mm) consisting of 25 ml of silica gel. Removal of petroleum ether gave 0.6 g (81%) of ethynylferrocene as an orange oil which gradually crystallized at +4^{o}C, m. p. 51-54^{o}C, cf.: [4] m. p. 55-56^{o}C.: Vol. pl. 55-56 °C.

^{1}H NMR ($CDCl_3$, δ, ppm): 2.70 (s, 1H, HC≡C); 4.18 (m, 2H, C_5H_4); 4.20 (s, 2H, C_5H_5); 4.44 (m, 2H, C_5H_4), cf. [5].

1.4 Synthesis of 4-Ferrocenylbutyn-1 (4)

1.4.1 1-Ferrocenyl-3-butin-1-ol

In a Schlenk flask were placed: ferrocene-carboxaldehyde (1 g; 4.69 mmol), zink dust (1.1 g; 16.47 mmol) and THF (3.2 ml). The flask was cooled with running water (10^{o}C) and 1.06 ml (1.66 g; 13.93 mmol) of 3-bromopropyne was added with stirring in argon purge. Saturated water solution of ammonium chloride (2.4 ml) was added by drops within 30 min with good stirring and cooling (13^{o}C). Then stirring was continued at ambient temperature. The reaction was monitored with 1H NMR.

After 5 h stirring was stopped and a precipitate was separated with Shott filter and extracted with 100 ml of dichloromethane. A clay-like mass remaining on the filter was suspended in 50 ml of 5% HCl and the obtained suspension was extracted with dichloromethane (2 portions of 25 ml). All dichloromethane extracts were combined, washed with saturated solution of $NaHCO_3$ (2 portions of 25 ml), 100 ml of brine and dried over Na_2SO_4. A removal of solvent gave 1.37 g of red-orange oil.

This oil was transferred to chromatographic column (diameter 35 mm) consisting of 130 ml of silica gel. An orangey band was eluted with petroleum ether (40/70^{o}). This band was discarded. Then with a mixture of petroleum ether (40/70^{o}) and ethyl acetate 4:1 second – orange band was eluted which gave 1.02 g (85%) of 1-ferrocenyl-3-butin-1-ol as red-orange oil.

^{1}H NMR ($CDCl_3$, δ, ppm): 2.08 s (1H, HC≡C)); 2.28 bs (1H, OH); 2.61 m (2H, CH_2); 4.18 m (2H, C_5H_4); 4.21 s (5H, C_5H_5); 4.25 m (1H, C_5H_4); 4.31 m (1H, C_5H_4); 4.55 m (1H, CH).

^{13}C NMR ($CDCl_3$, δ, ppm): 28.29 (CH_2); 65.90 (CHOH); 68.19 (C_5H_4); 68.30 (C_5H_4); 68.56 (C_5H_5); 70.75 (C_5H_4); 81.23 (C≡C); 91.86 (C≡C).

ESI(+) HRMS. Found: 254.0387. Calculated for $C_{14}H_{14}FeO$: 254.0395.

1.3.2 4-Ferrocenylbutyn-1 (4)

A solution of anhydrous aluminium chloride (1.4 g; 10.5 mmol) in abs. ether (21.5 ml) was added by drops with stirring to a cold (0^o) suspension of lithium aluminum hydride (0.4 g; 10.5 mmol) in abs. ether (10 ml). On completion of the addition cold bath was removed and a solution of 1-ferrocenyl-3-butyn-1-ol (2.59 g; 19.2 mmol) in abs. ether (40 ml) was added by drops within 10 min. The reaction mixture, dirty green in the start, gradually became orangey during the addition of 1-ferrocenyl-3-butyn-1-ol. Stirring was continued for 15 min and then 25 ml of cold (1-2^o) water was added (hydrogen evolution!). Ether layer was separated, washed with saturated solution of $NaHCO_3$ and ether was removed on rotor evaporator.

The residual red-orange oil was transferred to chromatographic column (diameter 35 mm) consisting of 150 ml of silica gel. With a mixture of petroleum ether and benzene 1:1 two bands were eluted. Fist band was discarded. Wide second orange band gave 2.13 g (85%) of 4-ferrocenyl-1-butyne as red-orange oil.

^{1}H NMR ($CDCl_3$, δ, ppm): 2.00 (s, 1H, HC≡C)); 2.40 (m, 2H, CH_2); 2.59 (m, 2H, CH_2); 4.07 (m, 2H , C_5H_4); 4.12 (m, 7H , C_5H_5 + C_5H_4).

^{13}C NMR ($CDCl_3$, δ, ppm): 20.23 (CH_2); 29.01 (CH_2); 67.32 (C_5H_4); 68.01 (C_5H_4); 68.49 (C_5H_5); 68.59 (C_5H_4); 84.30 and 87.28 (C≡C).

ESI(+) HRMS. Found: 238.0438. Calculated for $C_{14}H_{14}Fe$: 238.0446.

1.5 General procedure for click reaction of alkinyl ferrocenes

To a vigorously stirring solution of alkyne **3** or **4** in CH_2Cl_2 10mol% $CuSO_4$•$5H_2O$ and 20mol% sodium ascorbate in 200 mkL of distilled water were added under inert atmosphere (Argon). After color change of the aqueous droplets, containing the catalyst, to a dark brown, solution of azide **2** in CH_2Cl_2 was added dropwise to the reaction mixture. Stirring at room temperature was continued until the azide spot on the TLC of

the reaction mixture disappeared. The resulting product was separated by centrifugation, washed with a small amount of diethyl ether and dried in air.

1.5.1 (Z)-1-(2-(4-ferrocenyl-1-H-1,2,3-triazol-1-yl)-2-(methylthio)-4-(pyridin-2-ylmethylidene)-1H-imidazole-5(4H)-one (5)

To a solution of 100 mg (0.48 mmol) ethynylferrocene **3** in 5 ml CH_2Cl_2, under argon atmosphere 12 mg $CuSO_4 \bullet 5H_2O$ in 200 mkL of distilled water and 20 mg sodium ascorbate in 200 mkL of distilled water were added. Then the solution of 138 mg (0.48 mmol) of 5-(Z)-3-(2-azidoethyl)-2-(methylthio)-5-(pyridin-2-ylmethylene)-1H-imidazol-4H-one (**2**) in 2.5 ml of CH_2Cl_2 was added dropwise. The reaction mixture was stirred for 24 hours, after which it was processed according to the general procedure to give 220 mg (92%) of product **5** as a gray-yellow powder.

^{1}H NMR (DMSO-d_6, δ, ppm): 8.73 (d, 1H, J=8.83 Hz, H_α-Py), 8.60 (m, 1H, H_γ-Py), 8.15 (s, 1H, H-triazole), 7.86 (t, 1H, J=7.83 Hz, $H_{\beta'}$-Py), 7.33 (m, 1H, H_β-Py), 6.69 (s, 1H,-CH=), 4.62 (m, 4H, Cp-C), 4.24 (t, 2H, -CH_2-), 4.01 (m, 5H, Cp), 3.36 (t, 2H, -CH_2-), 2.67 (s, 3H, S-CH_3).

IR, cm^{-1}: 3340 (NH), 2100 (N3), 1723 (C=O), 1611 (C=C).

ESI(+) HRMS: calculated: 499.1003 ($C_{24}H_{23}FeN_6OS^+$), found: 499.1001 ($C_{24}H_{23}FeN_6OS^+$).

1.5.2 (Z)-1-(2-(4-ethylferrocenyl-1-H-1,2,3-triazol-1-yl)-2-(methylthio)-4-(pyridin-2-ylmethylidene)-1H-imidazole-5-(4H)-one (6)

To a solution of 100 mg (0.42 mmol) 4-Ferrocenylbutyn-1 **4** in 5 ml of CH_2Cl_2, under argon atmosphere 10.5 mg $CuSO_4 \bullet 5H_2O$ in 200 mkL of distilled water and 17.5 mg sodium ascorbate in 200 mkL of distilled water were added. Then the solution of 121 mg (0.42 mmol) of 5-(Z)-3-(2-azidoethyl)-2-(methylthio)-5-(pyridin-2-ylmethylene)-1H-imidazol-4H-one (**2**) in 2.5 ml of CH_2Cl_2 was added dropwise. The reaction mixture was stirred for 24 hours, after which it was processed according to the general procedure. The product was purified by column chromatography on silica gel with CH_2Cl_2: MeOH (10: 1). As a result, 199 mg (90%) of product **6** was obtained as a bright yellow powder.

^{1}H-NMR (DMSO-d_6, δ, ppm): 8.74 (d, 1H, J=7.83 Hz, H_α-Py), 8.62 (d, 1H, J=4.50 Hz, H_γ-Py), 7.87 (td, 1H, J_1=7.24 Hz, J_2=1.57 Hz, $H_{\beta'}$-Py), 7.83 (s, 1H, H-triazole), 7.33 (m, 1H, H_β-Py), 6.72 (s, 1H,-CH=), 4.57 (t, 2H, Cp_2Fe-CH_2-), 4.05 (s, 5H, Cp), 3.97 (m, 4H,

Cp-CH_2-), 3.35 (t, 2H, -CH_2-), 2.76 (t, 2H,-CH_2-), 2.64 (s, 3H, S-CH_3), 2.53 (t, 2H, -CH_2-).

ESI(+) HRMS: calculated: 527.1272 ($C_{26}H_{27}FeN_6OS^+$), found: 527.1269 ($C_{26}H_{27}FeN_6OS^+$).

1.6 Reaction of (Z)-1-(2-(4-ferrocenyl-1-H-1,2,3-triazol-1-yl)-2-(methylthio)-4-(pyridin-2-ylmethylidene)-1H-imidazole-5(4H)-one (5) with $Cu(ClO_4)_2$ *$6H_2O$ (copper complex (7))

To a stirred suspension of 15 mg (30 mkmol) of (Z)-1-(2-(4-ferrocenyl-1-H-1,2,3-triazol-1-yl)-2-(methylthio)-4-(pyridin-2-ylmethylidene)-1H-imidazole-5(4H)-one **5** in 1 ml of acetonitrile under argon atmosphere the solution of 8.1 mg (30 mkmol) copper(II) perchlorate hexahydrate in 1 ml of acetonitrile was added. After formation of clear dark brown solution the reaction mixture was stirred for 30 minutes and dried under reduced pressure. Complex **7** was isolated as dark green powder (unstable on air). Yield: 18.9 mg (100%).

ESI(+) HRMS: calculated: 561.0221 ($C_{24}H_{22}CuFeN_6OS^+$), found: 561.0221 ($C_{24}H_{22}CuFeN_6OS^+$), 280.5108 ($C_{24}H_{22}CuFeN_6OS^{2+}$).

MALDI: m/z = 218 ($C_{24}H_{22}FeN_6OS^{2+}$) 100%, 499 ($C_{24}H_{22}FeN_6OS^+$) 10%.

1.7 Reaction of (Z)-1-(2-(4-ethylferrocenyl-1-H-1,2,3-triazol-1-yl)-2-(methylthio)-4-(pyridin-2-ylmethylidene)-1H-imidazole-5-(4H)-one (6) with $Cu(ClO_4)_2$ *$6H_2O$ (copper complex (8))

To a stirred solution of 15 mg (28.5 mkmol) (Z)-1-(2-(4-ethylferrocenyl-1-H-1,2,3-triazol-1-yl)-2-(methylthio)-4-(pyridin-2-ylmethylidene)-1H-imidazole-5-(4H)-one **5** in 2 ml of acetonitrile under argon atmosphere was added solution of 7.7 mg (28.5 mkmol) copper(II) perchlorate hexahydrate in 1 ml of acetonitrile. After color change from orange to dark brown the reaction mixture was stirred for 30 minutes and dried under reduced pressure. Complex **8** was isolated as dark red powder (unstable on air). Yield: 18.8 mg (100%).

ESI(+) HRMS: calculated: 589.0523 ($C_{26}H_{26}CuFeN_6OS^+$), found: 589.0527 ($C_{24}H_{22}CuFeN_6OS^+$), 294.5263 ($C_{26}H_{26}CuFeN_6OS^{2+}$).

MALDI: m/z = 233 ($C_{26}H_{26}FeN_6OS^{2+}$) 100%, 525 ($C_{26}H_{26}FeN_6OS^+$) 8%.

1.8 Electrochemical measurements

Cyclic voltammetric experiments were carried out using a IPC Pro M potentiostat using cyclic voltammetry (CV) and rotating disk electrode (RDE) techniques. Glass-carbon (d = 2 mm) disks were used as the working electrodes, 0.05 M Bu_4NClO_4 solution

in CH_3CN or DMF served as the supporting electrolyte, and Ag/AgCl/KCl(satur.) was used as the reference electrode. The numbers of electrons transferred in each steps of the redox processes were determined in RDE experiments comparing the magnitude of the wave current with the current of single-electron oxidation of ferrocene, taken in equal concentration. The potential scan rates were 200 mV s^{-1}. All measurements were carried out under argon. The samples were dissolved in the pre-deaerated solvent. Dimethylformamide (high purity grade) was purified by refluxing followed by successive vacuum distillation over anhydrous $CuSO_4$ and CaH_2.

1.9 XANES spectroscopy

The samples were prepared to measure the X-ray absorption spectra by mixing and grinding 30 mg of dry cellulose powder in a mortar with 10 mg of the sample, followed by compression of the tablets from the resulting mixture. Samples of the comparison for measurement were also pressed into tablets with dry powder.

The spectra were measured using a Rigaku R-XAS Looper X-ray spectrometer equipped with an X-ray tube as the radiation source (cathode and anode material-tungsten). All measurements were made in the geometry of "passing". As an incident radiation intensity detector, an argon-filled ionization chamber (300 mbar) was used, and a scintillation detector was used to detect the transmitted radiation. The size of the X-ray beam on the sample was 10x3 mm^2. For each of the samples, the spectrum was measured in 10 passes with subsequent averaging, for reference samples in 4 passes. Approximate time for the measurement of each sample was 15 hours.

Measurements of the XAS spectra behind the K-edge of copper were carried out using a crystal-monochromator Ge (440) (second-order reflection of the Ge (220) crystal) in the 8775 - 9500 eV range. The energy resolution was 0.7 eV. Copper compounds, containing Cu ions with different charge states: Cu(foil), Cu_2O, CuO, $Cu(NO_3)_2$, $Cu(CH_3COO)_2$, $CuCl_2$, $CuSO_4$ were used as reference samples.

Measurements of the XAS spectra behind the K-edge of iron were carried out using a Ge crystal (311) monochromator in the range 6900-7600 eV. The energy resolution was 1.4 eV. As the reference samples, iron compounds, containing Fe ions with different charge states were used: FeO, α-Fe_2O_3, Fe_3O_4.

1.10 EPR spectroscopy

EPR spectra were recorded on a Varian E-3 EPR spectrometer at the boiling point of liquid nitrogen (77.4 K). Equimolar solutions of copper(II) chloride dihydrate and organic

ligand **6** were mixed just before the measurement, sealed in a capillary and frozen to the boiling point of liquid nitrogen. After recording the EPR spectrum, the capillary was removed from the spectrometer, heated to room temperature and held for 0, 5, 10, 20, 50, 90, 720 minutes before recording the next spectrum, respectively. By decreasing the integral intensity of the signal of paramagnetic nuclei, a kinetic curve was plotted and processed by methods of mathematical analysis to calculate the effective constants of the reaction.

1.11 Cytotoxicity assay (MTT assay)

The measurements were carried out using the standard MTT method [6 (main article)]. 2500 cells per well for MCF7, HEK293T and A549 cell lines or 4000 cells per well for VA13 were plated out in 135 µl of DMEM-F12 media (Gibco) in 96-well plate and incubated in the 5% CO_2 incubator for first 16 h without treating. Then 15 µl of media-DMSO solutions of tested substances to the cells (final DMSO concentrations in the media were 1% or less) and treated cells 72 h with 50 nM -100 µM (eight dilutions) of our substances (triplicate each). The MTT reagent then was added to cells up to final concentration of 0.5 g/l (10X stock solution in PBS was used) and incubated for 2 h at 37ºC in the incubator, under an atmosphere of 5% CO_2. The MTT solution was then discarded and 140 µl of DMSO was added. The plates were swayed on a shaker (60 rpm) to solubilize the formazan. The absorbance was measured using a microplate reader (VICTOR X5 Plate Reader) at a wavelength of 565 nm (in order to measure formazan concentration). The results were used to construct a dose-response graph and to estimate CC50 value (GraphPad Software, Inc.).

All measurements were reproduced in two bio-replicas to averaging the obtained data and calculating the IC50 values.

1.12 Inhibition of telomerase activity

To evaluate telomerase activity, the TRAP method [6] of telomeric repeating amplification using PCR-RTQ was used. 28 µl of TRAP mixtures were prepared, containing TRAP buffer (20 mM of HEPES-KOH; 1.5 mM MgCl2; 63 mM KCl; 1mM EGTA; 0.1 mg/ml of bovine serum albumin; 0.005% v/v polyoxyethylene (20) sorbitan monolaurate); 20 µM dNTP; 1.6 µM TS oligonucleotide (AATCCGTCGAGCAGAGTT). 1 µl of inhibitor solution was added in sample probes. Control probes didn't contain inhibitor at this stage. Then cellular extract from 1000 cells were added to the sample and control probes and reaction mixtures were incubated for 30 min at 25°C. Finally samples were placed in ice.

At the second stage inhibitor solution was added to the control probes. Then two units of Taq DNA polymerase (Fermentas), up to 0.17x Sybr I (Invitrogen) and 0.1 μg of ACX oligonucleotide (GCGCGGCTTACCCTTACCCTTACCCTTACCCTAACC) were added in all probes. 1 μl of inhibitor solution was added to PCR controls. PCR was performed according to the following scheme: 35 s (94°C), 35 s (50°C), 90 s (72°C) (29 cycles, CFX96 (Biorad)). Telomerase inhibition was calculated, based on ΔCt.

1.13 DNA cleavage assay

The mixtures (15 μl), containing 150 ng pUC18 plasmid DNA, 20 mM Hepes-KOH (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid with KOH) pH 7.8, and 2-200 μM of ligand **5, 6** or complex **7, 8** were prepared. Mixtures were incubated at 37˚C 4 hours and analyzed in 1% agarose gel [7]. Gel densitometry was estimated with ImageQuant program.

1.14 Cytosol/Pellet distributionin 4T1 cells investigated by AAS

A monolayer (80%) of 4T1 cells (mouse mammary carcinoma) was grown in RPMI medium (10% FBS, 1% glutamine) on the T-25 (eppendorth) mattress. Exact amount of coordination compound 7,8 was dissolved in DMSO and then diluted with medium (RPMI with 5% FBS and 1% glutamine) to a concentration of 10 mkM of coordination compound 7, 10.7 mkM of coordination compound 8. The cell monolayer was incubated with prepared solutions of 4 ml samples for 24 hours in a CO_2 incubator at 37°C.

After 24 hours, the medium was drained, the cells were washed twice with PBS (10 mM, pH=7.4) and harvested by trypsinization. The cell suspension was centrifuged at 2500 rpm 5 min on Beckman. The harvested cells were resuspended in 1 ml of PBS and their concentration determined by the TC20 automated cell counter (Biorad). The samples were further studied by differential centrifugation. Samples were centrifuged on a centrifuge Optima L-90K Ultracentrifuge, Beckman Coulter, USA, 100,000g and 4C. To measure the amount of copper, an atomic emission spectrometer with an Agilent 4200 MP-AES microwave plasma, 324,754 and 327,395 nm radiation wavelengths was used, an average of two intensity values for two wavelengths was used, a nitric acid solution with concentration, similar concentration in the measured samples.

1.15 Quantification of apoptosis by Annexin V and PI double staining for ligand 6 and coordination compound 8.

Apoptotic rates were determined by flow cytometry using an Annexin V/PI apoptosis kit. Briefly, A549 cells were seeded at a density of $1x10^6$ cells per well in six-well plates overnight and then treated with 5 or 20 μmol/l of ligand **6,** 50 or 100 μmol/l of complex **8** for 24 h. A total of $1x10^6$ cells were collected by centrifugation and washed twice with cold phosphate-buffered saline (PBS). Annexin V-FITC/PI staining was performed according to the manufacturer's instructions, the cells were analyzed using a FACScan flow cytometer BD Biosciences (San Jose, CA, USA). and data were examined using CellQuest™ software (BD Biosciences, Franklin Lakes, NJ, USA). At least three independent experiments were performed.

2. Additional graphic information

Fig. S1. The color of the ferrocenyl derivatives (on the left - when ferrocene is mixed with $CuCl_2*2H_2O$ (1: 1) in CH_3CN, right - in DMF).

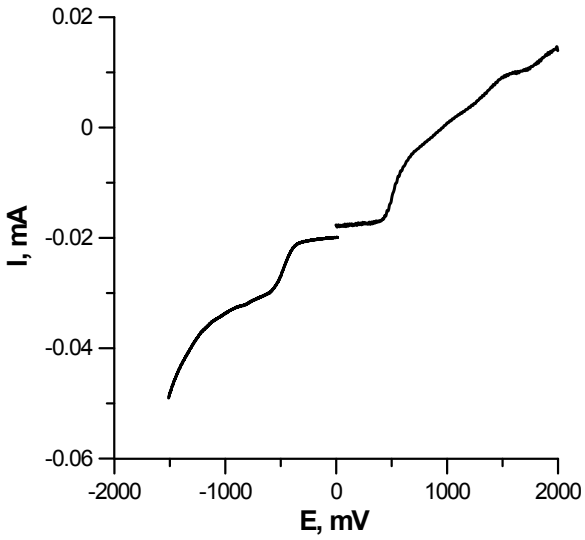

Fig. S2. RDE curves of a ligand **6** mixture with $Cu^{II}(ClO_4)_2 \cdot 6H_2O$ in CH_3CN (10^{-4} M, GC electrode, 0.1 M Bu_4NClO_4).

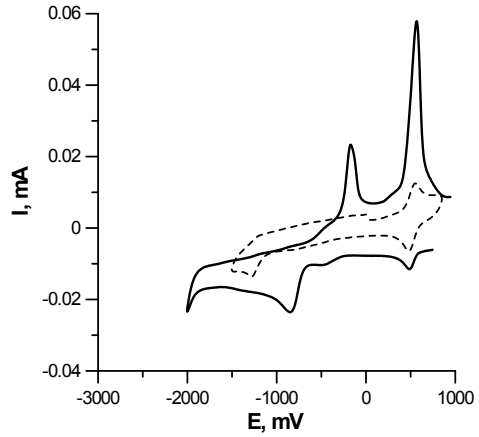

Fig. S3. CVA curves of a ligand **5** mixture with $Cu^{II}(ClO_4)_2 \cdot 6H_2O$ (solid line) and free ligand **5** (dotted line) in CH_3CN (10^{-4} M, GC electrode, 0.1 M Bu_4NClO_4).

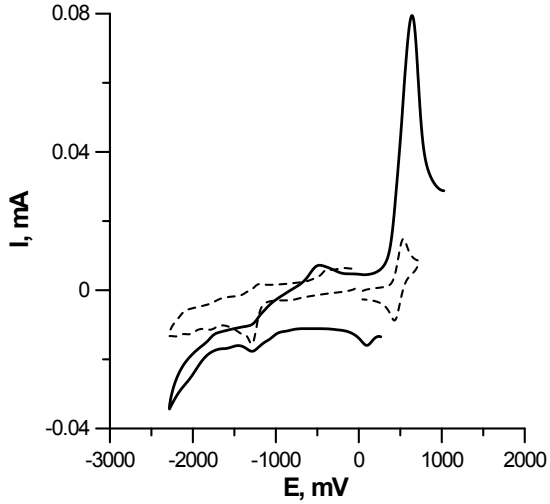

Fig. S4. CVA curves of a ligand **6** mixture with $Cu^{II}Cl_2 \cdot 2H_2O$ (solid line) and ligand **6** (dotted line) in DMF (10^{-4} M, GC electrode, 0.1 M Bu_4NClO_4).

Calf thymus DNA intercalation

Double-stranded DNA is a classic target for antitumor drugs. Intercalation of DNA affects the cellular mitosis, reduces metabolism of tumor cells and is one of the most common mechanisms of cytotoxic action, widely described both for the clinical use of doxorubicin [39] and for coordination compounds of copper [(a) Sangeetha S., Murali M., Int. J. Biol. Macromol. 2018. 107. Part B. 2501-2511; (b) Azarkish M., Akbari A., Sedaghat T., Simpson J., J. Mol. Struct. 2017. **1156.** 34-42; (c) Maheswari P.U., Palaniandavar M., J. Inorg. Biochem. 2004. **98.** 219–230]. Hence, the interaction between DNA and metal complexes is important for understanding the mechanism. To estimate the ability of **7**, **8** to intercalate DNA, the mode and propensity for binding of ferrocene ligands **5**, **6** and corresponding binuclear Cu(I)/Fe(III) complexes to CT-DNA were studied with fluorescence emission using ethidium bromide (EB) [Shahabadi N., Hakimi M., Morovati T., Fatahi N.. Nucleosides Nucleotides Nucleic Acids

2017. **36**. 497-510]. Figure S5-1 shows the fluorescence intensity of DNA conjugate with ethidium bromide vs the concentration of the coordination compound **8**.

The fluorescence intensity of the DNA conjugate with ethidium bromide does not decrease with increase of concentration of compounds **7** and **8**, which leads to the conclusion that intercalation the displacement of ethidium bromide into the solution does not occur (Figure S5-2).

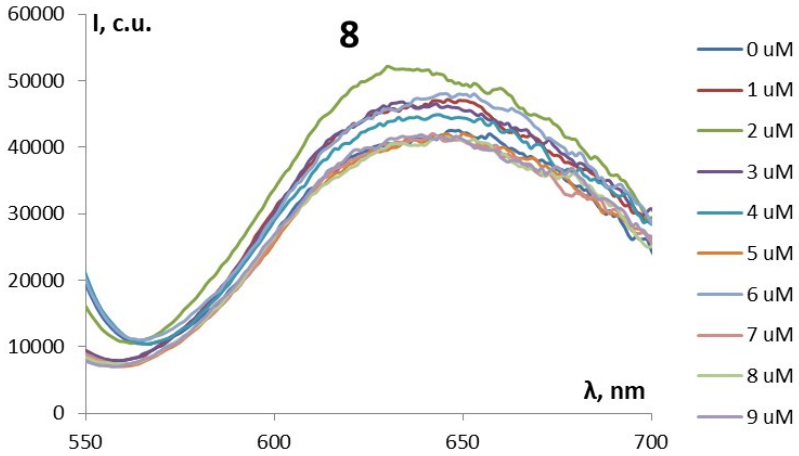

Fig. S5-1. Changes in the fluorescence spectra of the DNA conjugate with ethidium bromide in the presence of increasing concentrations of coordination compounds **8**

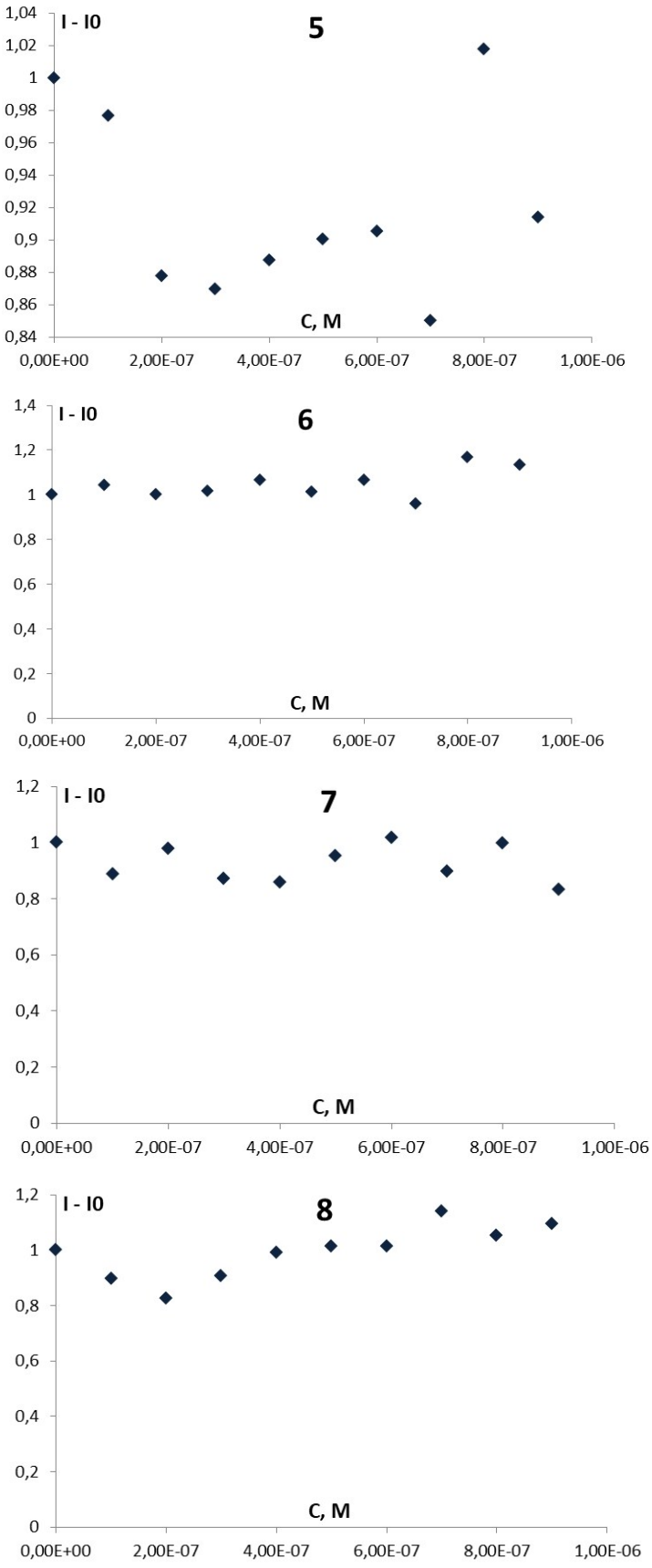

Fig. S5-2. Linearized by the Stern-Volmer method values of fluorescence intensity of the DNA conjugate with ethidium bromide in the presence of increasing concentrations of coordination compounds **7**, **8**.

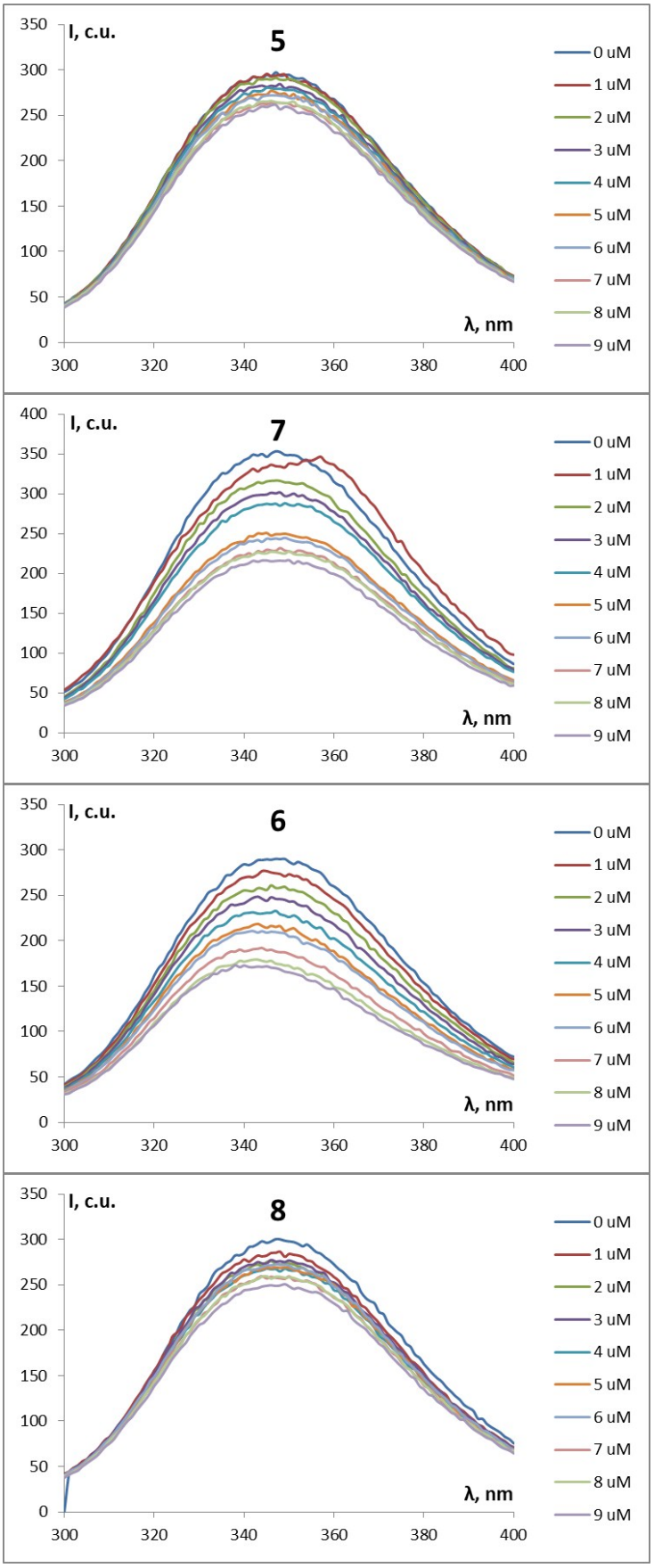

Fig. S6. Changes in the fluorescence spectra of the BSA in the presence of increasing concentrations of compounds **5**, **6**, **7**, **8**.

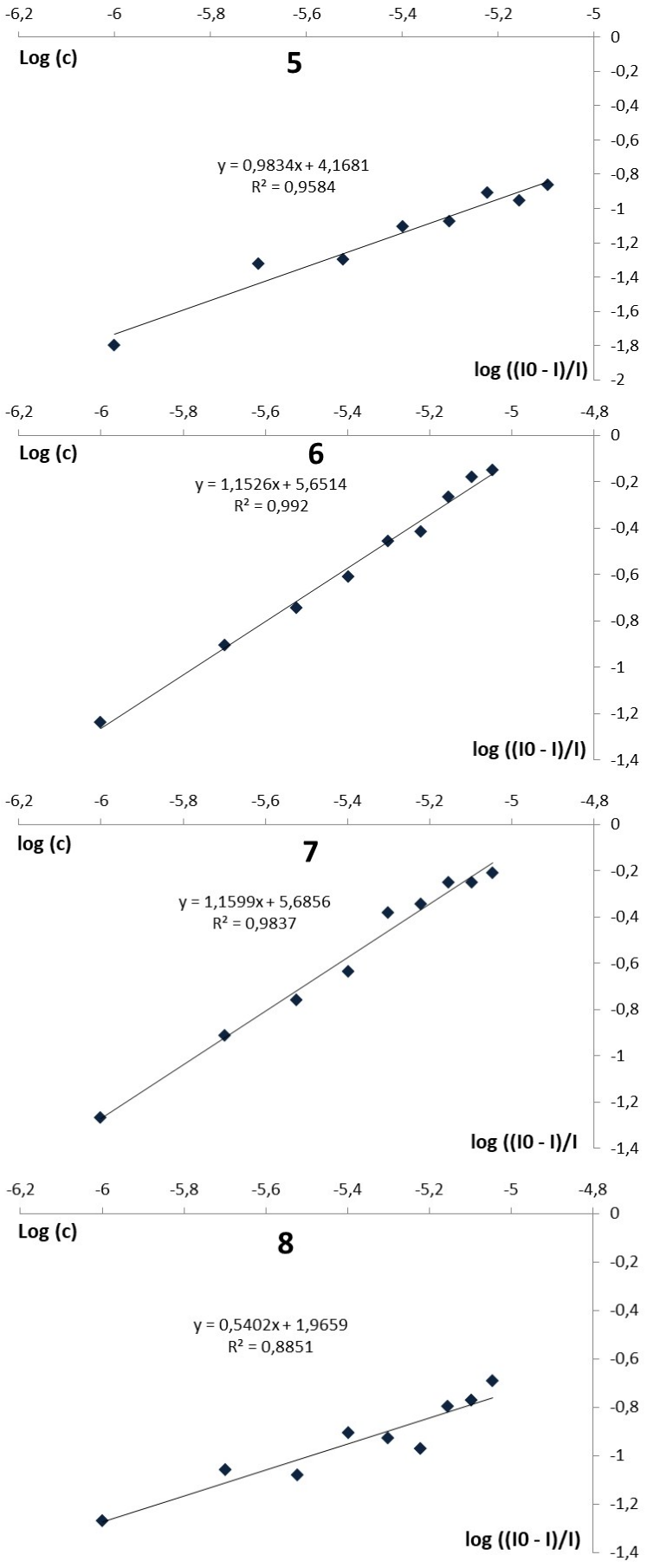

Fig. S7. Sketchard graphs for compounds **5**, **6**, **7**, **8**.

The concentration stability constants of the conjugate of coordination compounds **7**, **8** with bovine serum albumin, number of binding sites, respectively, were determined from the slope tangent and the free term of the straight line, approximating the experimental points in double inverse logarithmic coordinates.

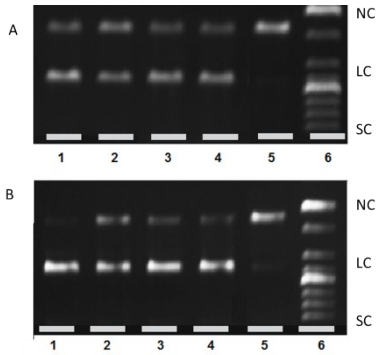

Fig. S8 Agarose gel electrophoresis of φX174 DNA treated with increasing concentrations of complex **5** and coordination compound **7** (A) or complex **6** and coordination compound (B). Lanes 1–6 represent the DNA cleavage using 2/ 10 50, 250 mcM of ligand **5**(**6**) (line 1-4) and 250 mcM complex **7**(**8**) with 20 lM (in base pairs) of φX174 DNA. Incubation time was 2 h at 37 ◦C for all reactions. Lane 6 is the DNA control without the copper complexes.

NMR spectra:

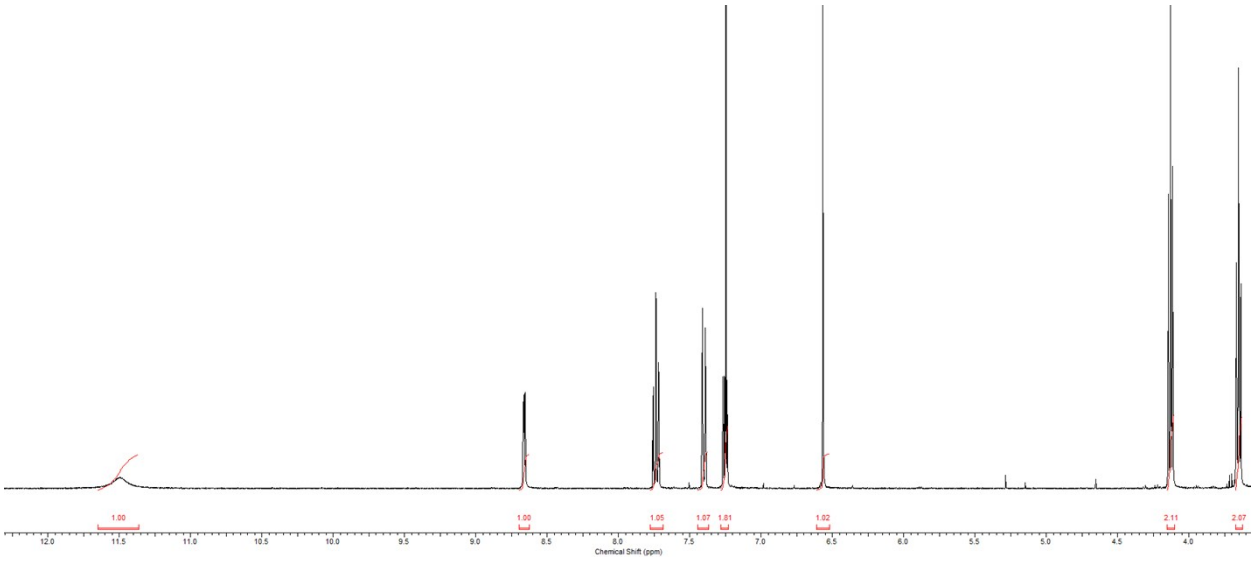

Fig. S9. ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm) of 3-(2-Azidoethyl)-5-((Z)-2-pyridylmethylene)-2-thioxotetrahydro-4H-imidazol-4-one (**1**)

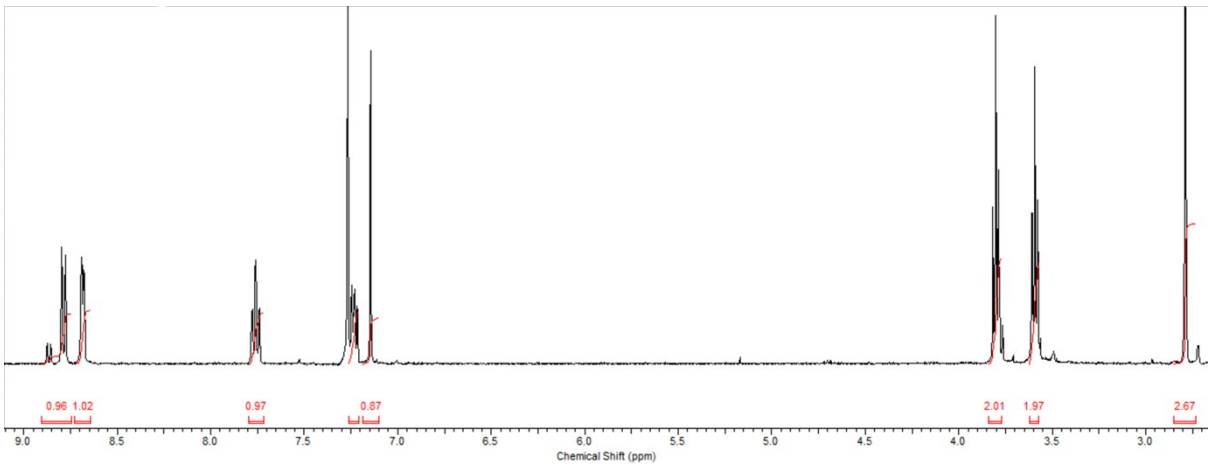

Fig. S10. ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm) of 5-(Z)-3-(2-azidoethyl)-2-(methylthio)-5-(pyridin-2-ylmethylene)-1H-imidazol-4H-one (**2)**

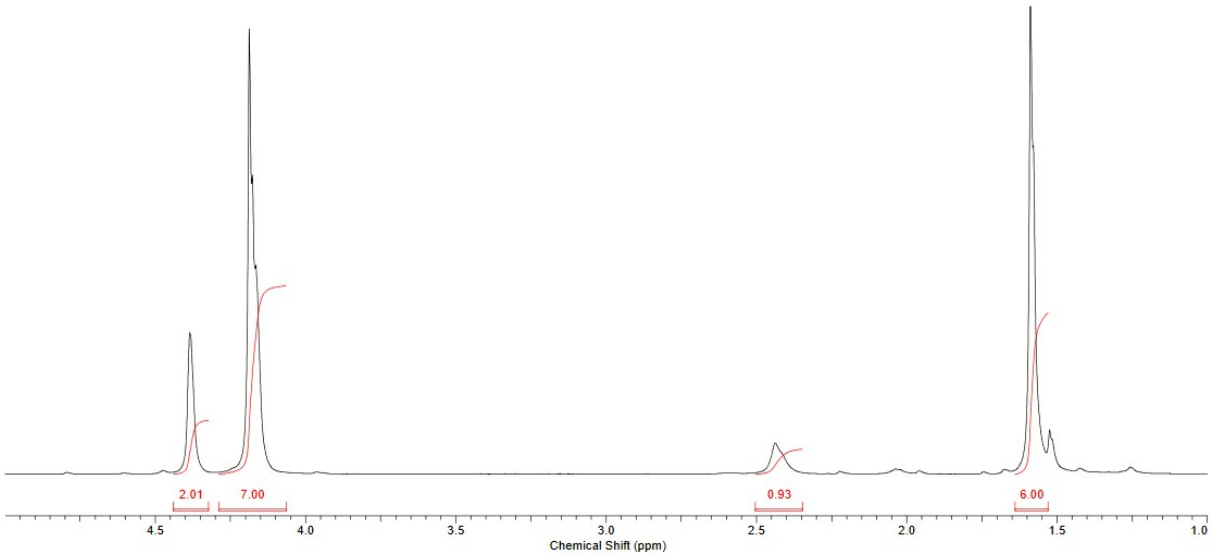

Fig. S11. ^{1}H NMR (400 MHz, CDCl3, δ, ppm) of 3-Ferrocenyl-1,1-dimethyl-2-propynol

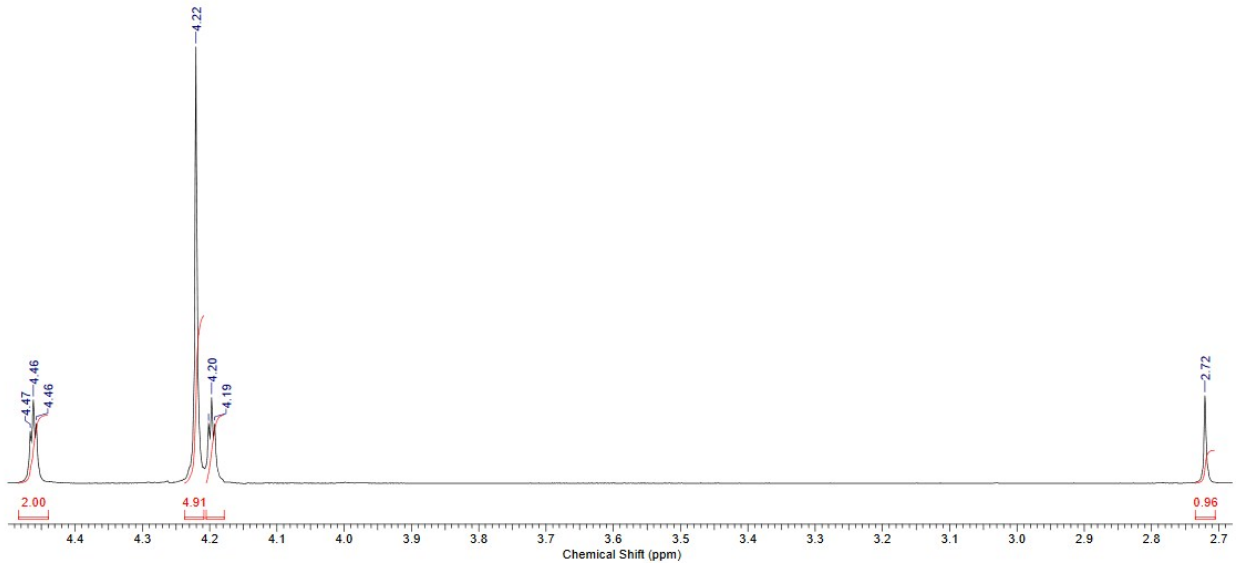

Fig. S12. ^{1}H NMR (400 MHz, CDCl3, δ, ppm) of Ethynylferrocene (**3**).

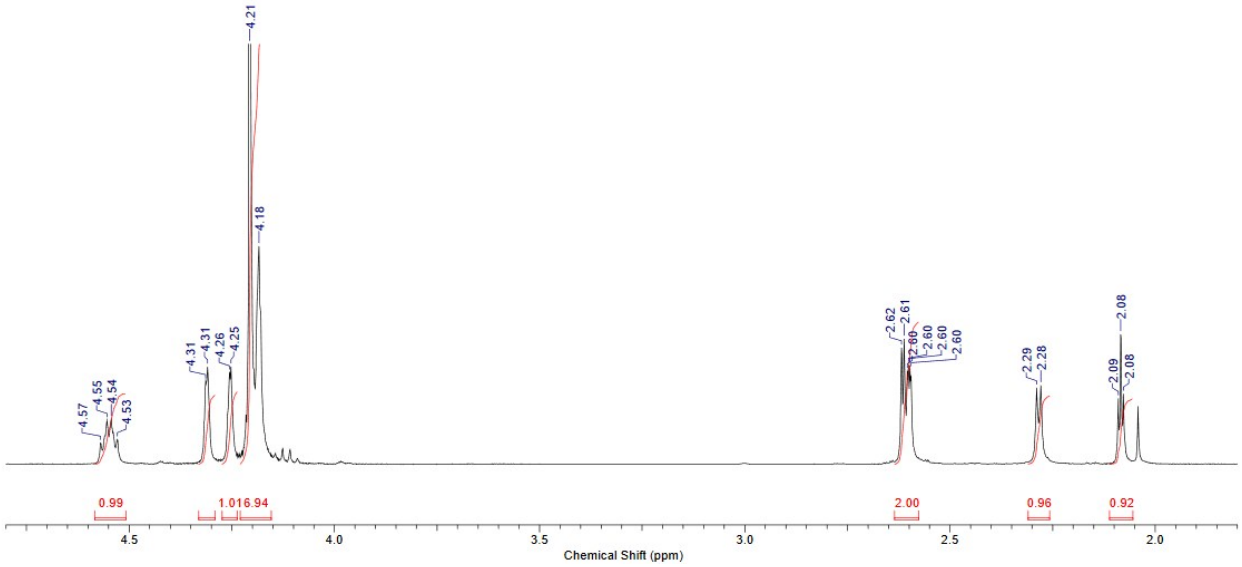

Fig. S13. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm) of 1-Ferrocenyl-3-butin-1-ol.

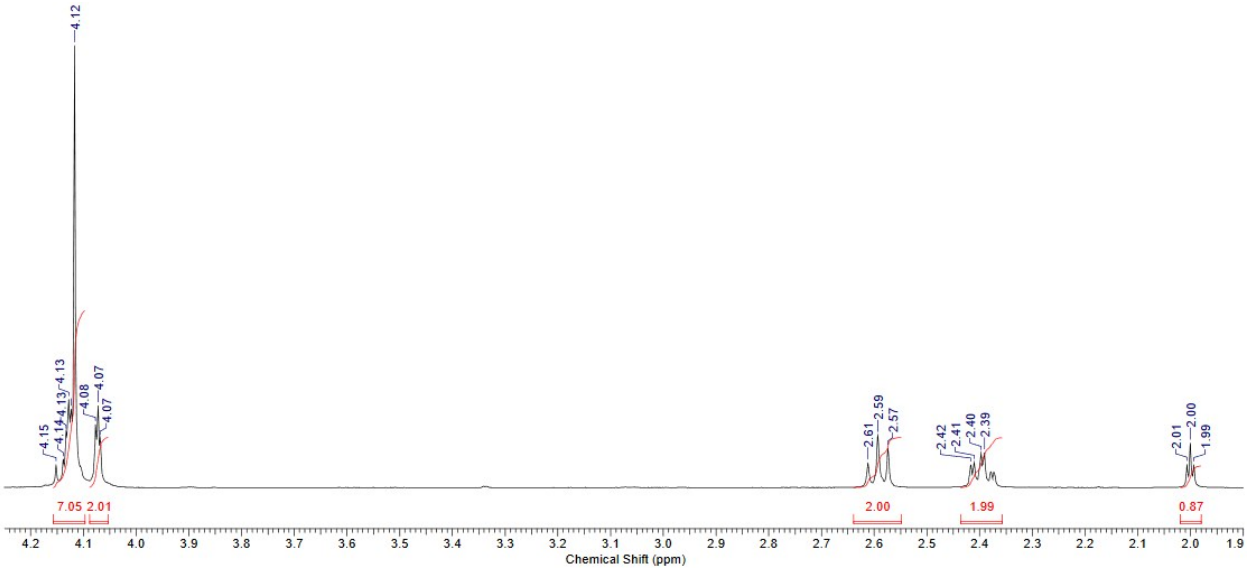

Fig. S14. ^{1}H NMR (400 MHz, chloroform-d, δ, ppm) of 4-Ferrocenylbutyn-1 (**4**)

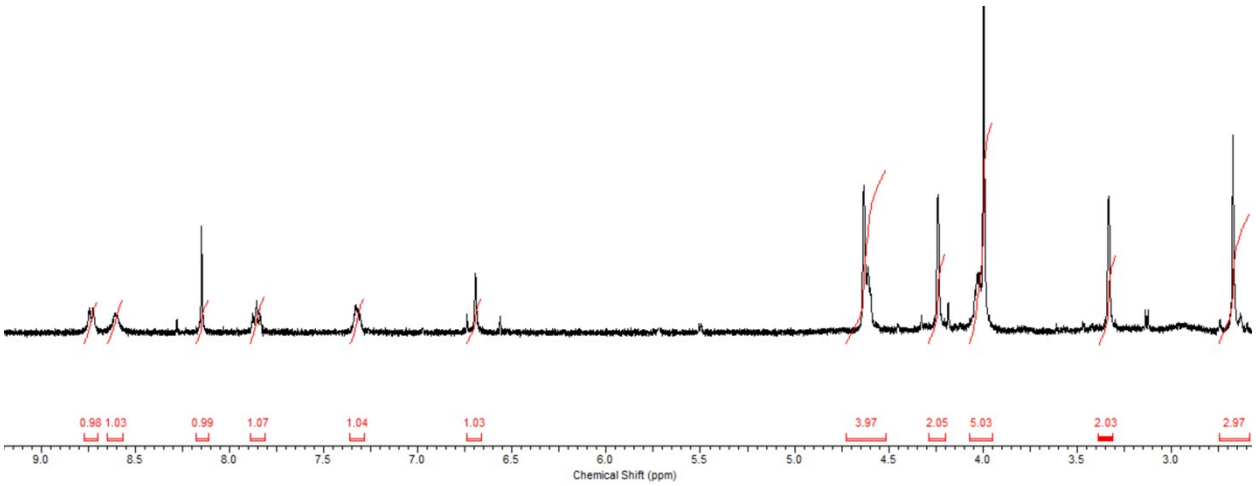

Fig. S15. ^{1}H-NMR (400 MHz, DMSO-d6, δ, ppm) of (Z)-1-(2-(4-ferrocenyl-1-H-1,2,3-triazol-1-yl)-2-(methylthio)-4-(pyridin-2-ylmethylidene)-1H-imidazole-5(4H)-one (**5**)

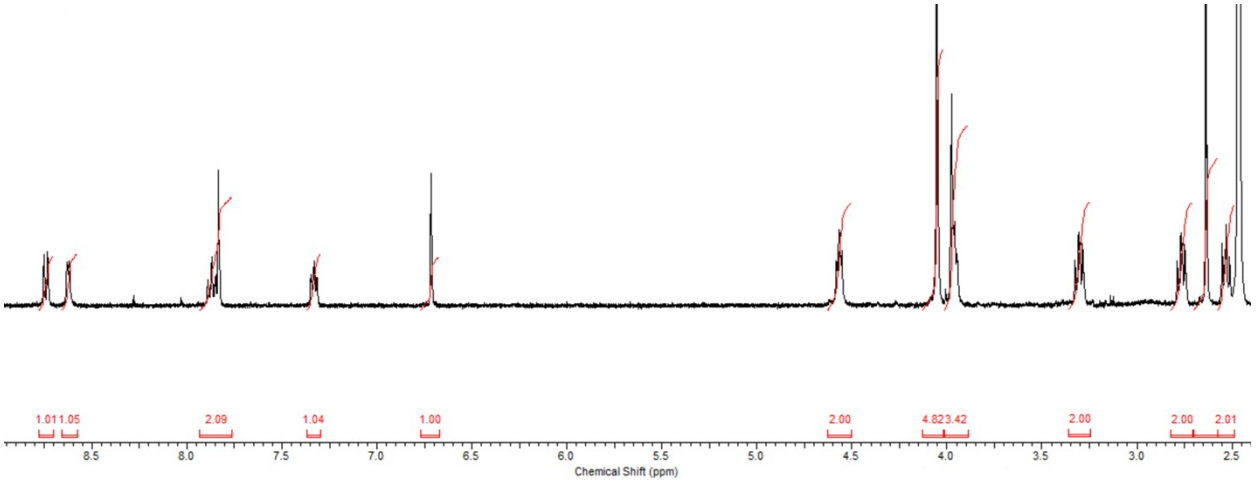

Fig.S16. ^{1}H-NMR (400 MHz, DMSO-d6, δ, ppm) of (Z)-1-(2-(4-ethylferrocenyl-1-H-1,2,3-triazol-1-yl)-2-(methylthio)-4-(pyridin-2-ylmethylidene)-1H-imidazole-5-(4H)-one (**6**).

IR spectra (nujol):

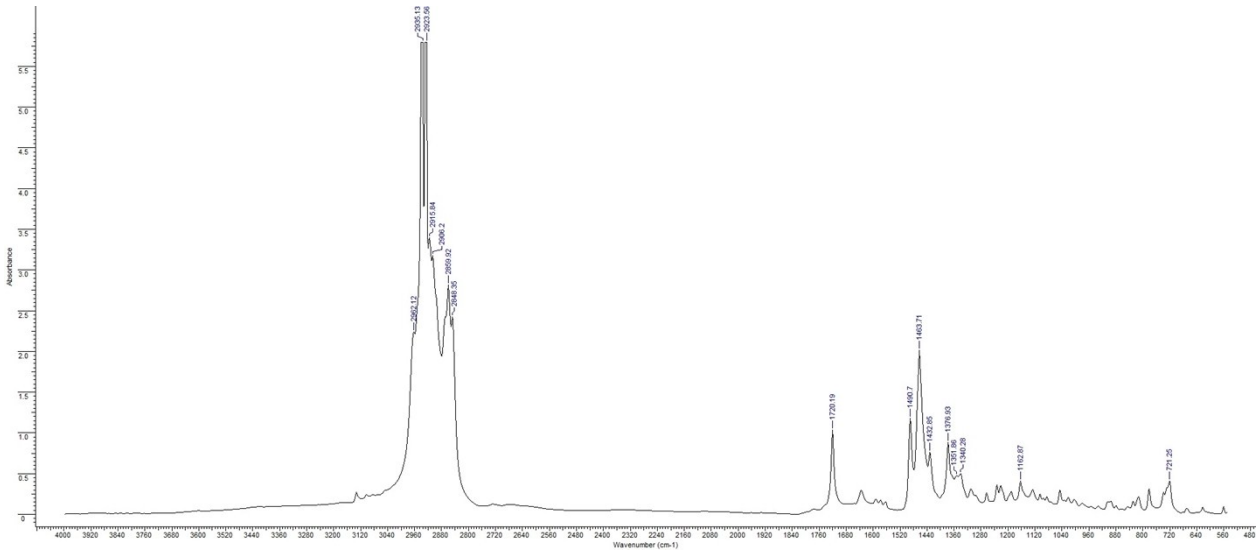

Fig.S17. IR of (Z)-1-(2-(4-ferrocenyl-1-H-1,2,3-triazol-1-yl)-2-(methylthio)-4-(pyridin-2-ylmethylidene)-1H-imidazole-5(4H)-one (**5**).

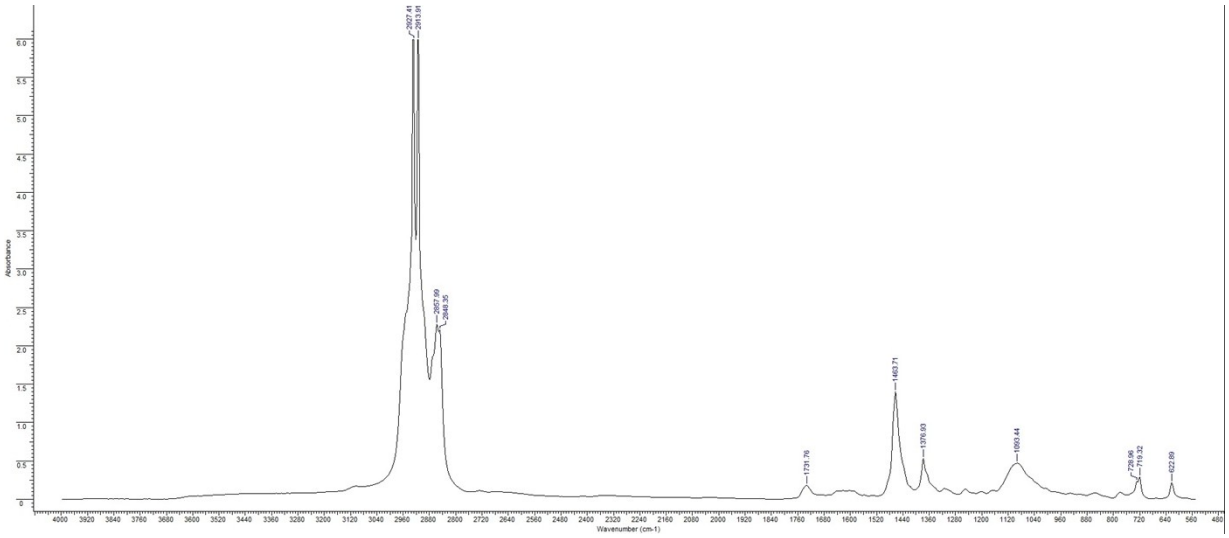

Fig.S18. IR of (Z)-1-(2-(4-ferrocenyl-1-H-1,2,3-triazol-1-yl)-2-(methylthio)-4-(pyridin-2-ylmethylidene)-1H-imidazole-5(4H)-one copper complex (**7**)

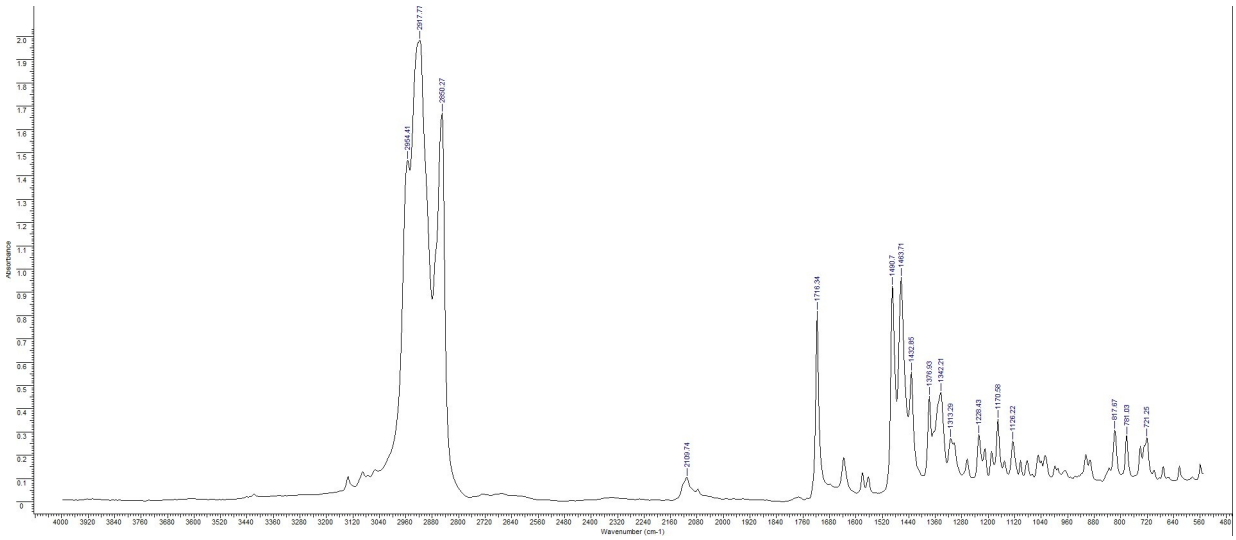

Fig.S19. IR of (Z)-1-(2-(4-ethylferrocenyl-1-H-1,2,3-triazol-1-yl)-2-(methylthio)-4-(pyridin-2-ylmethylidene)-1H-imidazole-5-(4H)-one (**6**)

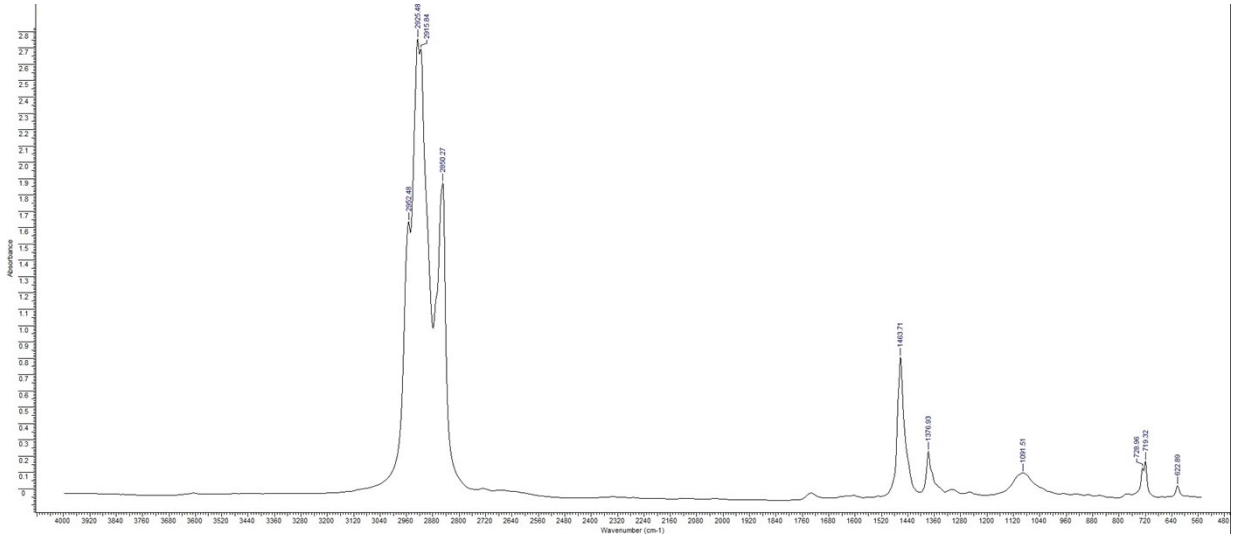

Fig.S20. IR of (Z)-1-(2-(4-ethylferrocenyl-1-H-1,2,3-triazol-1-yl)-2-(methylthio)-4-(pyridin-2-ylmethylidene)-1H-imidazole-5-(4H)-one copper complex (**8**)

EPR modeling:

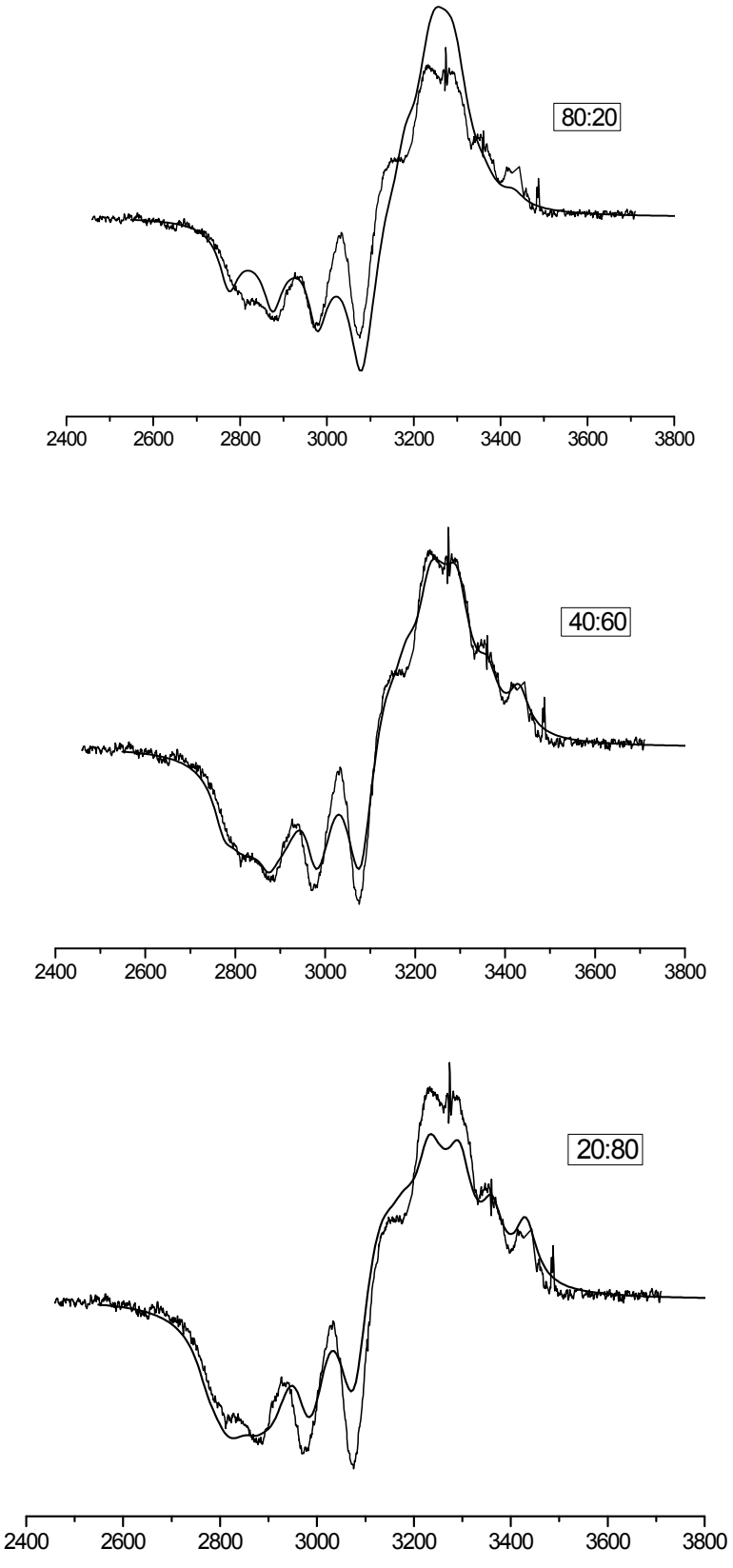

Fig.S21. The experimental EPR spectrum of the solution of coordination compound **8**, and sum of the the simulated EPR spectra of isomeric coordination compounds **8a** (pyramidal copper surroundings) and **8b** (octahedral copper surroundings) in different molar ratio (**8b**:**8a**).

S.I. Notes and references

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