Co(II) Complexes Based on the *bis*-Pyrazol-*s*-Triazine Pincer Ligand: Synthesis, X-ray Structure Studies and Cytotoxic Evaluation

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Physicochemical characterization

Chemicals were purchased from the Sigma-Aldrich Company (Chemie GmbH, 82024 Taufkirchen, Germany). CHN analyses were performed using a Perkin Elmer 2400 Elemental Analyzer (PerkinElmer, Inc.940 Winter Street, and Waltham, MA, USA). The Co content was determined using a Shimadzu atomic absorption spectrophotometer (AA-7000 series, Shimadzu, Ltd, Japan). An Alpha Bruker spectrophotometer (Billerica, Massachusetts, USA) was used to measure the FTIR spectra in KBr pellets (**Figure S1**).

Crystal structure determination

For X-ray diffraction analyses, suitable crystals were selected and data collection was performed on a Bruker diffractometer equipped with graphite monochromatic Mo-Kα radiation capability at 296 K. The structures were solved by direct methods using SHELXT-2018 [33] and refined by full-matrix least-squares methods on F^2 using SHELXL-2018 [34] from within the WINGX [35] suite of software. Bruker APEX2 [36] was used for data collection, and molecular diagrams were created using MERCURY [37]. Hirshfeld calculations were performed using the Crystal Explorer 17.5 program [38].

Synthesis of L

Ligand **L** was synthesized following the reported method [24], and the spectral data were in good agreement with the reported values (**Figure S3**).

Synthesis of complexes 2-4

2.2.1. $[Co(L)(H_2O)_2Cl]Cl$; (**2**) and $[Co(L)(H_2O)_3](ClO_4)_2.H_2O$; (**3**)

A 10 mL methanolic solution of **L** (~0.299 g, 1 mmol) was mixed with a 5 mL methanolic solution of $CoCl_2$ (0.130 g, 1 mmol) or $Co(ClO_4)_2.6H_2O$ (0.366 g, 1 mmol). Block pink crystals of **2** and plate-like pink crystals of **3** were obtained after six and ten days of slow evaporation, respectively.

Yield: $C_{14}H_{21}Cl_2CoN_7O_3$ (**2**) 81%. Anal. Calc. C, 36.15; H, 4.55; N, 21.08%. Found: C, 36.37; H, 4.51; N, 20.93%. IR (KBr, cm^{-1}): 3383, 3081, 1617, 1574, 1545.

Yield: $C_{14}H_{25}Cl_2CoN_7O_{13}$ (**3**) 83%. Anal. Calc. C, 26.72; H, 4.00; N, 15.58%. Found: C, 26.55; H, 3.97; N, 15.46%. IR (KBr, cm^{-1}): 3386, 3167, 3085, 1620, 1594(Sh), 1575, 1544, 1145, 1115, 1086.

2.2.2. [$Co(L)(NO_3)_2$]; (**4**)

A 10 mL methanolic solution of **L** (~0.299 g, 1 mmol) was mixed with $Co(NO_3)_2.6H_2O$ (0.291 g, 1 mmol) in 5 mL methanol. After two days, block pink crystals of **4** were obtained as a major product.

Yield: $C_{14}H_{17}CoN_9O_7$ (**4**) 76 %. Anal. Calc. C, 34.87; H, 3.55; N, 26.14%. Found: C, 34.69; H, 3.58; N, 26.01%. IR (KBr, cm^{-1}): 3166, 3085, 1620, 1594, 1575, 1545, 1384, 1361.

Evaluation of Cytotoxic Effects [S1, S2]

Cell line Propagation

The cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50µg/mL Gentamycin. All cells were maintained at 37ºC in a humidified atmosphere with 5% CO_2 and were subcultured two times per week.

Cytotoxicity evaluation using viability assays

For cytotoxicity assays, the cells were seeded in 96-well plates at a cell concentration of 1×10^4 cells per well in 100 µL of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Serial two-fold dilutions of the tested chemical compound were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37ºC in a humidified incubator with 5% CO_2 for a period of 24 h. Three wells were used for each concentration of the test sample. Control cells were incubated without the test sample and with or without DMSO. The small percentage of DMSO present in the wells (maximum 0.1%) was found to not affect the experiment. After incubation of the cells at 37°C for 24 h, the viable cell yields were determined through a colorimetric method.

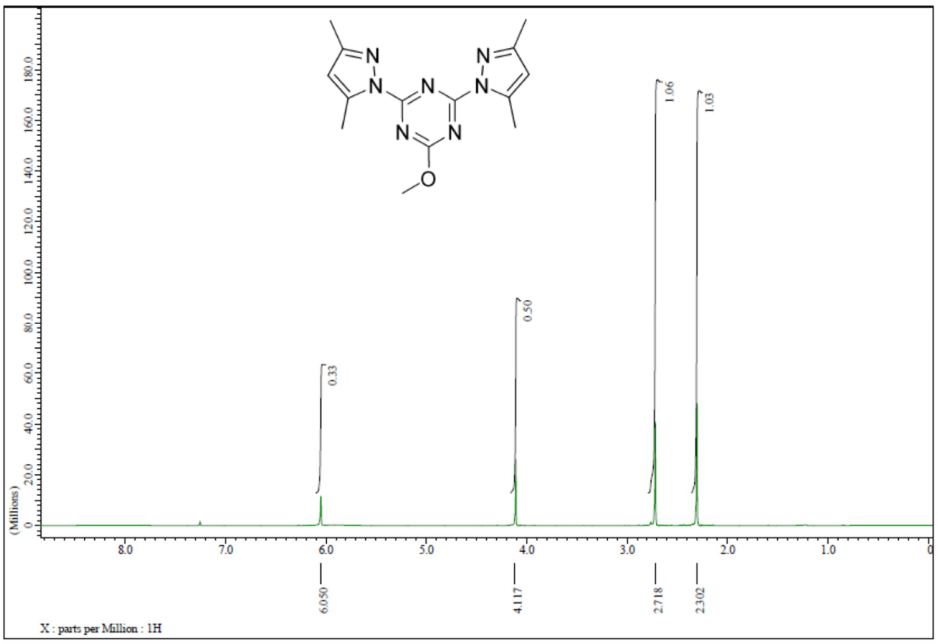
In brief, after the end of the incubation period, media were aspirated, and crystal violet solution (1%) was added to each well for at least 30 minutes. The stains were removed and the plates were rinsed using tap water until all excess staining was removed. Glacial acetic acid (30%) was added to all wells and mixed thoroughly; then, the absorbance values of the plates were measured after gentle shaking on a Microplate reader (TECAN, Inc.), using a test wavelength of 490 nm. All results were corrected for background absorbance detected in wells without added stains. Treated samples were compared with a

cell control absent of the tested compounds. All experiments were carried out in triplicates. The cell cytotoxic effect of each tested compound was calculated**.** The optical density was measured with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage viability was calculated as [(ODt/ODc)]x100%, where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The relationship between surviving cells and drug concentration was plotted to derive the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration (IC_{50}), i.e., the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose–response curve for each concentration using GraphPad Prism software (San Diego, CA. USA).

References:

[S1] Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods,* **1983**, *65,* 55-63.

[S2] Gomha, S.M.; Riyadh, S.M.; Mahmmoud, E.A. and Elaasser, M.M. Synthesis and Anticancer Activities of Thiazoles, 1,3-Thiazines, and Thiazolidine Using Chitosan-Grafted-Poly(vinylpyridine) as Basic Catalyst. *Heterocycles*, **2015**, *91*,1227-1243.


(Millions)
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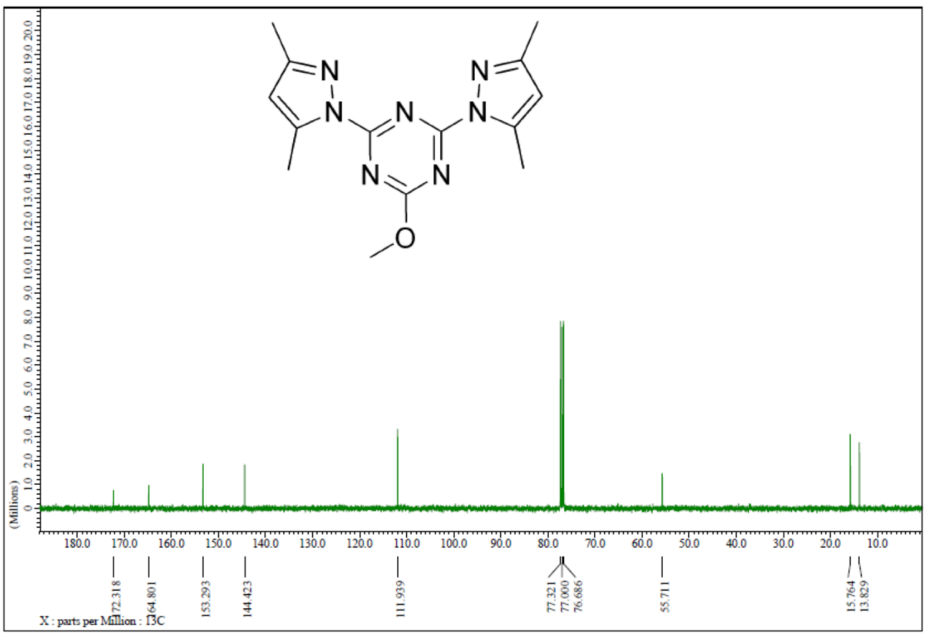
1.06
1.03
6.050
4.117
2.718
2.302
X : parts per Million : 1H


(Millions)
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164.801
153.293
144.423
111.939
77.321
77.000
76.686
55.711
15.764
13.829
X : parts per Million : 13C

Figure S1. ^{1}H and ^{13}C NMR spectra of the ligand (**L**). Chemical shifts are reported in parts per million (ppm).

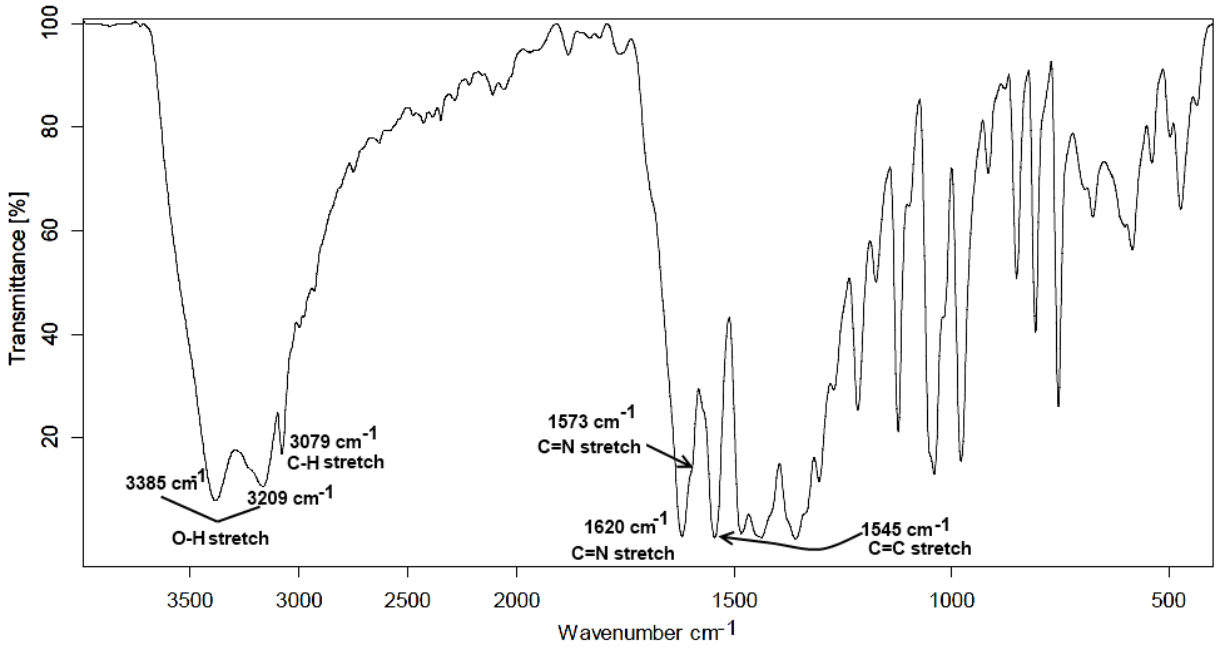

Figure S2. FTIR spectra of complex **1**.

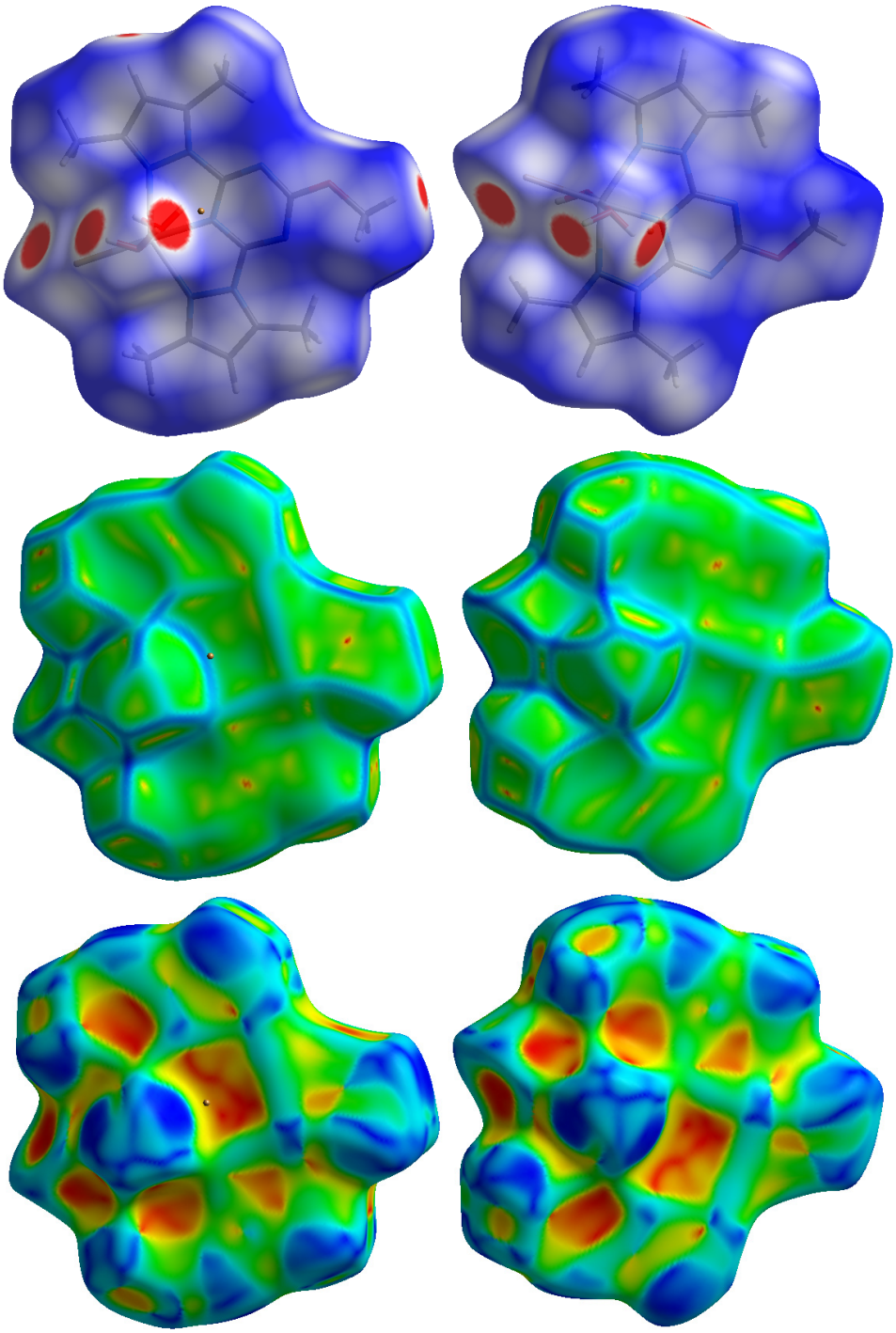

Figure S3. Hirshfeld maps for the [Co(L)(H_2O)$_2$Br]Br complex.

Table S1. Evaluation of the cytotoxicity of **1** against the A-549 cell line.

Sample conc. (µM)	Viability %	Inhibitory %	S.D. (±)
902	17.54	82.46	2.32
451	39.76	60.24	3.18
226	67.41	32.59	3.97
113	89.52	10.48	2.06
56	98.16	1.84	1.08
28	100	0	
14	100	0	
7	100	0	
0	100	0	

Table S2. Evaluation of the cytotoxicity of **2** against the A-549 cell line.

Sample conc. (µM)	Viability %	Inhibitory %	S.D. (±)
1075	19.05	80.95	2.19
537	42.63	57.37	3.15
269	81.37	18.63	2.69
134	98.04	1.96	0.68
67	100	0	
34	100	0	
17	100	0	
8	100	0	
0	100	0	

Table S3. Evaluation of the cytotoxicity of **3** against the A-549 cell line.

Sample conc. (µM)	Viability %	Inhibitory %	S.D. (±)
795	37.84	62.16	3.12
397	86.03	13.97	3.69
199	97.96	2.04	1.02
99	100	0	
50	100	0	
25	100	0	
12	100	0	
6	100	0	
0	100	0	

Table S4. Evaluation of the cytotoxicity of **4** against the A-549 cell line.

Sample conc. (µM)	Viability %	Inhibitory %	S.D. (±)
1037	14.29	85.71	1.13
518	31.75	68.25	2.97
259	60.38	39.62	2.46
130	84.13	15.87	1.09
65	98.79	1.21	0.37
32	100	0	
16	100	0	
8	100	0	
0	100	0	

Table S5. Evaluation of the cytotoxicity of **L** against the A-549 cell line.

Sample conc. (µM)	Viability %	Inhibitory %	S.D. (±)
1670	28.39	71.61	1.97
835	70.86	29.14	3.12
418	89.43	10.57	2.09
209	98.70	1.3	0.68
104	100	0	
52	100	0	
26	100	0	
13	100	0	
0	100	0	

Table S6. Evaluation of the cytotoxicity of **1** against the MCF-7 cell line.

Sample conc. (µM)	Viability %	Inhibitory %	S.D. (±)
902	23.95	76.05	2.71
451	48.17	51.83	3.95
226	82.93	17.07	2.86
113	97.61	2.39	0.83
56	100	0	
28	100	0	
14	100	0	
7	100	0	
0	100	0	

Table S7. Evaluation of the cytotoxicity of **2** against the MCF-7 cell line.

Sample conc. (µM)	Viability %	Inhibitory %	S.D. (±)
1075	15.27	84.73	1.49
537	38.75	61.25	2.93
269	69.41	30.59	3.15
134	89.54	10.46	1.82
67	99.23	0.77	0.69
34	100	0	
17	100	0	
8	100	0	
0	100	0	

Table S8. Evaluation of the cytotoxicity of **3** against the MCF-7 cell line.

Sample conc. (µM)	Viability %	Inhibitory %	S.D. (±)
795	35.96	64.04	3.12
397	82.37	17.63	2.69
199	98.14	1.86	1.08
99	100	0	
50	100	0	
25	100	0	
12	100	0	
6	100	0	
0	100	0	

Table S9. Evaluation of the cytotoxicity of **4** against the MCF-7 cell line.

Sample conc. (µM)	Viability %	Inhibitory %	S.D. (±)
1037	21.35	78.65	1.43
518	39.13	60.87	2.75
259	71.47	28.53	3.19
130	89.28	10.72	1.34
65	99.16	0.84	0.82
32	100	0	
16	100	0	
8	100	0	
0	100	0	

Table S10. Evaluation of the cytotoxicity of **L** against the MCF-7 cell line.

Sample conc. (µM)	Viability %	Inhibitory %	S.D. (±)
1670	20.37	79.63	1.59
835	54.29	45.71	2.37
418	81.43	18.57	1.09
209	97.16	2.84	0.88
104	100	0	
52	100	0	
26	100	0	
13	100	0	
0	100	0	

Table S11. Evaluation of the cytotoxicity of **$CoCl_2$** against the A-549 cell line [a].

Sample conc. (µM)	Viability %	Inhibitory %	S.D. (±)
3851	67.13	32.87	3.95
1925	95.20	4.8	1.42
963	99.74	0.26	0.52
481	100	0	
241	100	0	
120	100	0	
60	100	0	
30	100	0	
0	100	0	

[a]Weak inhibitory activity against lung carcinoma cells was detected.

Table S12. Evaluation of the cytotoxicity of **$Co(NO_3)_2.6H_2O$** against the A-549 cell line [a].

Sample conc. (µM)	Viability %	Inhibitory %	S.D. (±)
1718	46.91	53.09	3.75
859	89.57	10.43	2.91
430	99.40	0.6	0.68
215	100	0	
107	100	0	
54	100	0	
27	100	0	
13	100	0	
0	100	0	

[a] IC_{50} = 1655.81±69.79 µM.

Table S13. Evaluation of the cytotoxicity of **$CoCl_2$** against the MCF-7 cell line[a].

Sample conc. (µM)	Viability %	Inhibitory %	S.D. (±)
3851	82.87	17.13	1.65
1925	98.06	1.94	0.98
963	100	0	
481	100	0	
241	100	0	
120	100	0	
60	100	0	
30	100	0	
0	100	0	

[a]Weak inhibitory activity against breast carcinoma cells was detected.

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Table S14. Evaluation of the cytotoxicity of **$Co(NO_3)_2.6H_2O$** against the MCF-7 cell line [a].

Sample conc. (µM)	Viability %	Inhibitory %	S.D. (±)
1718	63.89	36.11	2.75
859	94.03	5.97	0.69
430	99.54	0.46	0.82
215	100	0	
107	100	0	
54	100	0	
27	100	0	
13	100	0	
0	100	0	

[a]Weak inhibitory activity against breast carcinoma cells was detected.