Transit Met Chem (2017) 42:503–508
DOI 10.1007/s11243-017-0154-8

A copper(II) complex of a semicarbazone: crystal structure, spectroscopic analysis and cytotoxicity against human cancer cell lines

Claudia C. Gatto[1] · Patrícia M. Miguel[1] · Carolane M. Almeida[1] · Pedro H. O. Santiago[1] · Carlos R. K. Paier[2] · Claudia Pessoa[2,3]

Received: 20 March 2017 / Accepted: 24 May 2017 / Published online: 31 May 2017

Abstract A Cu(II) complex of 2-hydroxyacetophenone *N*(4)-phenyl semicarbazone (H_2L) has been prepared, and its crystal structure is reported. The single-crystal X-ray study reveals a dimeric structure with a distorted square planar coordination geometry around each copper atom, with an *ONO*-donor ligand and μ_2-oxo group located between the two copper atoms. The secondary coordination sphere of Cu(II) is six-coordinate, with a NO_3^- ion and methanol solvent molecule making weak interactions. Supramolecular interactions involving the adjacent complexes were investigated. The complex $[Cu(HL)]_2(CH_3OH)_2(NO_3)_2$ was also characterized by physicochemical and spectroscopic methods. The biological activity of the free semicarbazone and its Cu(II) complex were evaluated by cytotoxicity assays against human cancer cell lines.

Introduction

In recent years, there has been a rapid expansion of research into less toxic metal-based antitumor drugs for use in medicine [1–3]. Metal complexes are beginning to have a major impact in the development of novel compounds with biological activity, although there has so for been limited understanding of their molecular mechanisms of action [4]. Several transition metal ions play important roles in living organisms, and copper is an essential trace metal for both animals and plants. Several types of copper-based complexes have been extensively investigated as potential antitumor agents [5]. It has been established that copper(II) is generally chelated by N, O, S and halide donor atoms when complexed with mono- or polydentate Schiff bases [5–8]. These Cu(II) derivatives are generally stable complexes, with coordination numbers that vary from four to six. In general, the biological activity of a metal complex depends on many factors, including the oxidation state, geometry, and the number and types of ligands [9, 10].

Previous studies of semicarbazones have revealed the biological properties of these compounds [11], indicating their medicinal potential and pharmacological properties [12, 13]. Semicarbazones are also very promising ligands, possessing versatile structures that exhibit keto-enol tautomerism and are capable of coordination in neutral, mono- or dianionic forms, with wide variation in their modes of bonding [14–17].

In continuation of our interest in the study of semicarbazones and new copper(II) complexes [18, 19], here we describe the synthesis, crystal structure, spectroscopy and potential anticancer activity of a new copper(II) complex of a semicarbazone ligand. The complex $[Cu(HL)]_2(CH_3OH)_2(NO_3)_2$ (where H_2L is 2-hydroxyacetophenone *N*(4)-phenyl semicarbazone) was synthesized and characterized by single-crystal X-ray diffraction analysis, revealing supramolecular interactions. Moreover, a detailed assignment of the infrared and UV–Vis spectra of both the free semicarbazone and its complex have been performed, as well as cytotoxicity assays against human cancer cell lines (ovarian, colon and central nervous system).

✉ Claudia C. Gatto
ccgatto@gmail.com

[1] Laboratory of Inorganic Synthesis and Crystallography, University of Brasilia (IQ-UnB), Campus Universitário Darcy Ribeiro, P.O. Box 4478, Brasília, DF CEP 70904970, Brazil

[2] Laboratory of Experimental Oncology, Center for Drug Research and Development, Faculty of Medicine, University of Ceará, Fortaleza, CE CEP 60430275, Brazil

[3] Oswaldo Cruz Foundation – Ceará (FIOCRUZ), Fortaleza, CE CEP 60180900, Brazil

Experimental

Materials and methods

All reagents and solvents were obtained from commercial sources. Elemental analyses were obtained with a PerkinElmer/Series II 2400 analyzer. FT-IR spectra were recorded from KBr pellets (4000–400 cm^{-1}) using an FT-IR Varian 640 spectrometer. UV–Vis spectra were collected on a Varian–Cary spectrophotometer, and the concentration used for all analyses was 10 μM. 2-Hydroxyacetophenone *N*(4)-phenyl semicarbazone (H_2L) was prepared according to a reported methodology [20].

Synthesis of $[Cu(HL)]_2(CH_3OH)_2(NO_3)_2$

A solution of $Cu(NO_3)_2{\cdot}3H_2O$ (21 mg, 0.1 mmol) in MeOH (5 mL) was added to a stirred solution of H_2L (25 mg, 0.1 mmol) in water (5 mL). The mixture was heated at reflux for 3 h. Dark green crystals suitable for X-ray diffraction were obtained directly from the mother liquor upon standing at room temperature for several days. Yield 268 mg (63%). Melting Point: 309 °C. Anal. calcd for $C_{16}H_{18}N_4O_6Cu$: C, 45.12; H, 4.26; N, 13.16; Found: C, 45.17; H, 4.16; N, 13.43%. Selected IR bands (KBr, ν/cm^{-1}): ν(OH) 3442, ν(N–H) 3274, ν(C=O) 1630; δ(N–H) 1596; ν(C=N) 1576; ν(N–O) 1384; ν(N–N) 1113.

Crystal and data collection

Crystal structure for $C_{16}H_{18}N_4O_6Cu$; $M = 425.88$; triclinic, space group $P-1$; $a = 9.684(8)$ Å, $b = 9.816(9)$ Å, $c = 9.857(9)$ Å; $\alpha = 93.291(6)^\circ$, $\beta = 105.246(6)^\circ$, $\gamma = 95.758(6)^\circ$; $V = 896.04(14)$ $Å^3$; $Z = 2$; $Dc = 1.58$ g cm^{-1}; $F(000) = 438$; GooF = 1.00; $T = 293(2)$ K; green block, size 0.41 × 0.26 × 0.16 mm; 4416 independent measured reflections, refinement based on F^2 to give R_1 $[F^2 > 4\sigma(F^2)] = 0.048$; $w_2 = 0.092$ for 17,954 observed reflections and 276 parameters. We obtained a large value of Rint in the crystallographic data, due to the crystal quality. The X-ray diffraction data were collected on a Bruker CCD SMART APEX II single-crystal diffractometer with Mo Kα radiation (0.71073 Å). SADABS [21] was used to scale the data and perform the multi-scan absorption correction. The structure was solved by direct methods using SHELXS-97 [22] and subsequent Fourier-difference map analyses yielded the positions of the non-hydrogen atoms. The refinement was performed using SHELXL-2016 [23]. Molecular graphics were generated with the ORTEP [24], POV-Ray and DIAMOND [25] programs.

Biological activity

Compounds were tested for cytotoxic activity against human cancer cell lines obtained from the National Cancer Institute, NCI (Bethesda, MD, USA). Peripheral blood mononuclear cells (PBMC) were isolated from the heparinized blood of healthy, non-smoker donors who had not taken any medication at least 15 days prior to sampling, using a standard method of density-gradient centrifugation on Histopaque-1077 (Sigma-Aldrich). All cancer cell lines and PBMC were maintained in RPMI 1640 medium, at 37 °C and 5% CO_2 under a humidified atmosphere. All culture media were supplemented with 20% (PBMC) or 10% (cancer cells) fetal bovine serum, 2 mM L-glutamine, 100 IU/mL penicillin and 100 μg/mL streptomycin. PBMC cultures were also supplemented with 2% phytohemagglutinin. In the cytotoxicity assays, cells were plated in 96-well plates (0.1×10^6 cells/well for cancer cells and 1.0×10^6 cells/well for PBMC). All tested compounds were dissolved in DMSO. The final concentration of DMSO in the culture medium was kept constant (0.1% v/v). Doxorubicin (0.001-1.10 μM) was used as a positive control and negative control groups received equal amounts of the vehicle (DMSO). The cell viability was determined through the reduction of the yellow dye 3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) to a blue formazan product, as described by Mosmann [26]. At the end of the incubation time (72 h), the plates were centrifuged and the medium was replaced with fresh medium (200 μL) containing MTT 0.5 mg/mL. After 3 h, the formazan product was dissolved in DMSO (150 μL) and the absorbance was measured at 550 nm in a multiplate reader (Spectra Count, Packard, Ontario, Canada). The drug effect was quantified as the percentage of mean absorbance of the control assays. All cell treatments were carried out with three replicates. Control groups (negative and positive) received the same amount of DMSO (0.1%). Doxorubicin was used as positive control (0.005–5.0 μg/mL). All compounds were diluted in DMSO to a final concentration of 5 μg/mL (stock solution).

Results and discussion

Crystal structure description

The crystal structure determination of $[Cu(HL)]_2(CH_3OH)_2(NO_3)_2$ revealed a dinuclear centrosymmetric copper(II) complex. An anisotropic thermal displacement ellipsoids representation of the complex is illustrated in

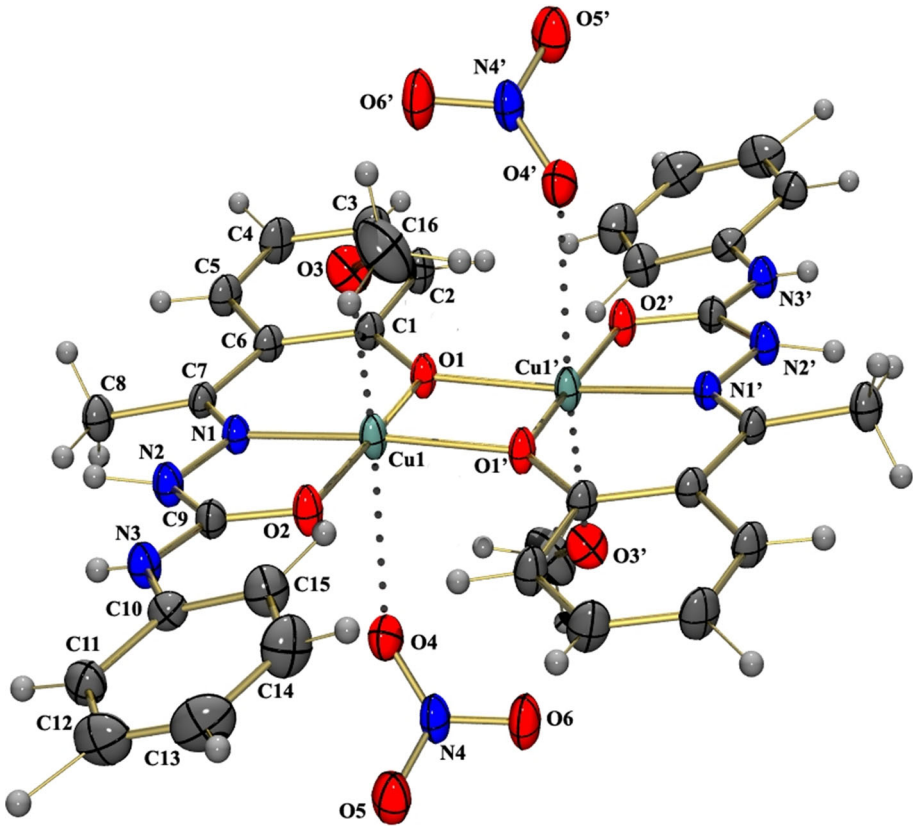

Fig. 1 Molecular structures of $[Cu(HL)]_2(CH_3OH)_2(NO_3)_2$ with crystallographic labeling. The thermal ellipsoids are drawn at 30% probability level

Fig. 1. The bond lengths and angles (listed in Table 1) are in good agreement with those reported for other copper(II) semicarbazone complexes [27, 28]. The semicarbazone acts as a monodeprotonated ligand, coordinated in the *E* isomer form, which chelates the copper atom through the phenoxo oxygen O1, imine nitrogen N1 and carbonyl oxygen O2. An interesting feature of the complex is its μ_2-oxo ligand, which is located between two copper atoms, in contrast to the behavior with other metal ions [15, 29]. The ligand is able to connect two copper atoms by the formation of Cu–O–Cu bridges via the phenolato functional group, with Cu1–O1 bond lengths of 1.907(2) and 1.988(2) Å. This results in a Cu–Cu' distance of 2.973(8) Å (symmetry code: (′) $-x + 2, -y + 1, -z + 1$), which is long enough to preclude any direct copper–copper interactions [28, 30]. Each copper(II) atom is bonded to the semicarbazone ligand through of the atoms O1, N1, O2, and O1′, such that the coordination environment around the metal is best described as distorted square planar. The secondary coordination sphere of copper(II) is weakly six-coordinated by means of a NO_3^- ion and methanol solvent molecule. The O3 atom from methanol presents a Cu1···O3 distance of 2.318(4) Å. A weak interaction between the copper and O4 of the nitrate counter ion is also observed, with a Cu1···O4 distance of 2.792(14) Å. Due to the expected Jahn–Teller effects, copper(II) binds only weakly to the NO_3^- and MeOH, and these weak interactions have an insignificant effect on the coordination geometry of the cationic copper(II) complex.

Table 1 Selected bond distances (Å) and bond angles (°) for the complex $[Cu(HL)]_2(CH_3OH)_2(NO_3)_2$

Cu(1)–O(1)	1.907(2)	N(1)–Cu(1)–O(1)	92.40(11)
Cu(1)–O(2)	1.950(2)	O(2)–Cu(1)–N(1)	82.68(11)
Cu(1)–O(3)	2.318(4)	O(1)–Cu(1)–O(1)[a]	80.52(10)
Cu(1)–O(1)[a]	1.988(2)	O(2)–Cu(1)–O(1)[a]	103.90(10)
Cu(1)–N(1)	1.951(3)	O(1)–Cu(1)–O(2)	174.16(13)
Cu(1)–Cu(1)[a]	2.973(8)	N(1)–Cu(1)–O(1)[a]	169.47(14)

Symmetry transformations: (a) $-x + 2, -y+1, -z + 1$

The observed deviations from 90° of the bond angles with distortions from the ideal square planar symmetry are due to the 5- and 6-membered chelate rings and the two 4-membered $\{Cu_2O_2\}$ rings in the central part of the molecule. This results in X–Cu–Y angles between neighboring donor atoms that range from 80.52(10) to 103.90(10)°. The mean deviation of CuO_2N plane [Cu1–O1–N1–O2] is 0.0238 Å. A variation of the system π distances of H_2L is observed from the bond lengths in the backbone of the ligand upon coordination to the metal. Spectroscopic studies and the crystal structure of the

Table 2 Hydrogen bonding interactions (Å and °) for compound $[Cu(HL)]_2(CH_3OH)_2(NO_3)_2$

D–H···A	d(D–H)	d(H···A)	d(D···A)	(D–H···A)
N(3)–H(3A)···N(4)[a]	0.94(7)	2.61(7)	3.498(7)	158(4)
N(3)–H(3A)···O(4)[a]	0.94(7)	1.89(7)	2.818(6)	173(6)
N(2)–H(2A)···O(5)[a]	0.93(7)	1.93(7)	2.859(6)	173(4)
O(3)–H(3B)···O(6)[b]	0.70(5)	2.10(5)	2.749(5)	155(6)

Symmetry transformations: (a) $-x + 3, -y + 1, -z + 1$; (b) $-x + 2, -y + 1, -z + 1$

complex indicate protonation of the nitrogen atom N2 and the formation of a hydrogen bond. The packing architecture revealed by single-crystal X-ray diffraction analysis showed the association of the NO_3^- anion and cationic complex stabilized by a hydrogen bonding network within the crystal packing. Interestingly, the three oxygen atoms from NO_3^- form hydrogen bonds with donor–acceptor distances between 2.749(5) and 2.859(6) Å (Table 2). These observed interactions contribute to the formation of the supramolecular structure, as shown in Fig. 2.

Table 3 UV–Vis data (in different solvents) for H_2L and $[Cu(HL)]_2(CH_3OH)_2(NO_3)_2$

	$\pi \rightarrow \pi^*$[a]	Logε	$n \rightarrow \pi^*$[a]	Logε	LMCT[a]	Logε
Ligand						
DMF	281	4.58	316	4.32	–	–
H_2O	278	4.48	308	4.16	–	–
MeOH	228	4.36	278	4.04	354	3.88
Complex						
DMF[b]	–	–	319	4.86	374	4.93
H_2O[b]	–	–	279	4.90	355	4.53
MeOH						

(a) Absorption band values in nm. (b) Wavelengths and logε values of the complexes spectra were not determined due to the absorption region of the solvent

Spectroscopic analyses

The FT-IR spectrum of $[Cu(HL)]_2(CH_3OH)_2(NO_3)_2$ was compared with that of free H_2L. In the IR spectrum of the complex, a strong ν(OH) absorption was observed at 3442 cm^{-1} and a ν(NH) absorption at 3274 cm^{-1}. In the

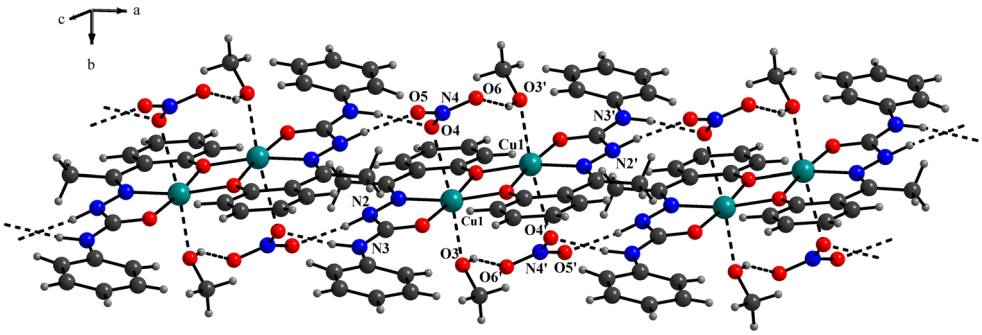

Fig. 2 Hydrogen-bonding network of $[Cu(HL)]_2(CH_3OH)_2(NO_3)_2$ with hydrogen bonds indicated by *dashed lines*

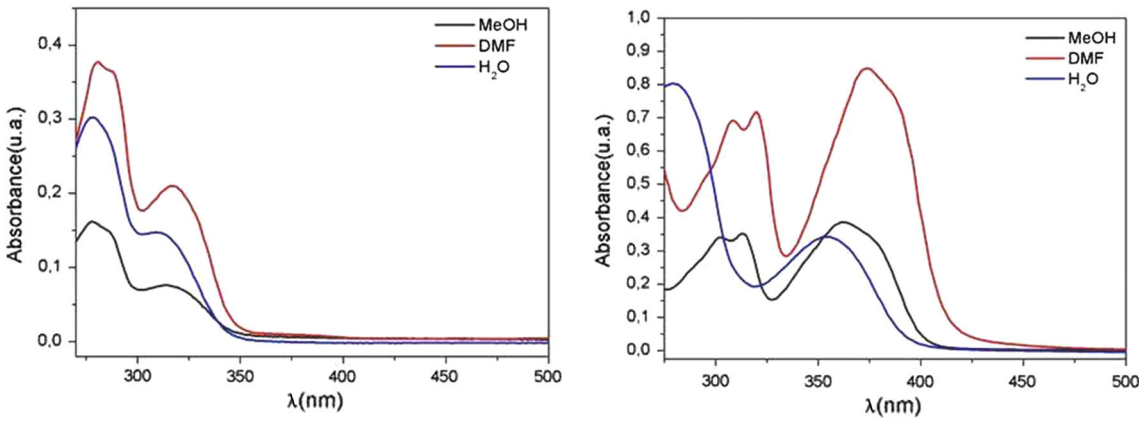

Fig. 3 UV–Vis spectra of H_2L (*left*) and $[Cu(HL)]_2(CH_3OH)_2(NO_3)_2$ (*right*) in different solvents (concentration 10 μM)

Table 4 Percentage of cellular growth inhibition (GI%) performed by the compounds H_2L and $[Cu(HL)]_2(CH_3OH)_2(NO_3)_2$ against three cancer cell lines (mean ± standard error of the mean)

Compound	OVCAR8	HCT-116	SF-295
H_2L	39.49% (10.36%)	40.86% (8.03%)	53.14% (4.74%)
$[Cu(HL)]_2(CH_3OH)_2(NO_3)_2$	100% (0.17%)	100% (0.00%)	73.93% (41.11%)

Mean ± Standard Error of the Mean of at least three independent experiments

Table 5 Cytotoxic activity of Doxorubicin (reference drug) and the complex $[Cu(HL)]_2(CH_3OH)_2(NO_3)_2$, expressed as the inhibition concentration that causes a 50% decrease in cell growth (IC_{50}), in μmol L^{-1} (95% CI), against cancer cell lines

Compound	OVCAR-8	SF-295	HCT-116	HL-60
$[Cu(HL)]_2(CH_3OH)_2(NO_3)_2$	3.076	0.603	1.85	0.722
	(0.47–20.29)	(0.21–1.68)	(0.67–5.146)	(0.30–1.71)
Doxorubicin	0.55	0.48	0.02	0.03
	(0.40–0.90)	(0.37–0.52)	(0.01–0.03)	(0.01–0.05)

72-h exposure and the data obtained by nonlinear regression for all cell lines from three independent experiments

IR spectrum of the free semicarbazone, two peaks were observed at 1646 and 1597 cm^{-1}, which can be attributed to *ν*(C=O) and *ν*(C=N), respectively, and the metal complex exhibited a *ν*(C=O) and *ν*(C=N) absorption at 1630 and 1576 cm^{-1}, respectively [31]. The magnitude was less than for the free ligand, indicating that the C=O and C=N groups are coordinated to the metal. The spectrum of the complex also includes a strong ν(NO) absorption at 1384 cm^{-1}. A new peak at 453 or 521 cm^{-1} in the spectra of the complex could be attributed to the Cu–O vibration. Therefore, the IR data for the complex indicate coordination of the semicarbazone ligand through O1, N1 and O2 as the chelating atoms, in accordance with the X-ray crystal structure determination.

UV–Vis experiments were conducted at room temperature in three different solvents (water, methanol and *N*,*N*-dimethylformamide) for both the free ligand and its copper complex (Fig. 3; Table 3). The spectrum of H_2L shows absorption bands in the range of 308–316 nm, assigned to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions, by virtue of their large values of molar extinction coefficients (logε values between 4.04 and 4.32) [32]. The absorption bands are displaced to lower wavelengths in the spectrum of the complex, indicating coordination of the copper(II) center to the semicarbazone ligand, which is also indicated by the appearance of a ligand–metal charge transfer band in the region of 355–374 nm in the spectrum of the complex. No d–d transitions were observed in the spectrum of the complex; such bands are expected to have low values of molar extinction coefficients, and the electronic spectra were obtained at low concentration [31, 33].

Biological activity

The free semicarbazone H_2L and $[Cu(HL)]_2(CH_3OH)_2(NO_3)_2$ were both tested against four cancer cell lines: OVCAR-8, HCT-116, SF-295 and HL-60. We first investigated the antiproliferative effects of these compounds (Table 4). Cells treated with DMSO were used as controls. In all tests, the complex was more active than the free semicarbazone. In fact, the copper(II) complex was highly cytostatic to OVCAR8, HCT-116 and SF-295 cancer cells, resulting in growth-inhibitory activities of 100, 100 and 73.93%, respectively. However, free H_2L induced less than 50% inhibition and had no effect after 24 h of treatment. We found that the complex has very good potency, comparable to Doxorubicin, with IC_{50} values of 0.603 and 0.722 μM to SF-295 and HL-60 cells, respectively (Table 5).

Conclusions

In this paper, we have described a new dimeric copper(II) complex of a semicarbazone ligand. The biological results show that the complex exhibits more potent inhibition of tumor cell growth than the free ligand. More generally, copper(II) complexes of semicarbazone ligands may have a great potential as novel anticancer drugs. Further studies should also provide a better understanding of the cytotoxic potential and selectivity against tumor and normal cells, as well as tumor regression in animal models.

Supplementary information

Crystallographic data for the structures in this work have been deposited at the Cambridge Crystallographic Data Centre, CCDC 1056358. Copies of the available material can be obtained free of charge by application to the Director, CCDC, 12 Union Road, Cambridge CH2 1EZ, UK (Fax: +44 1223 336033; E-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk.

Acknowledgements We gratefully acknowledge the financial support of FINEP/CTINFRA, CNPq, CAPES, FAPDF and FUNCAP.

References

1. van Rijt SH, Sadler PJ (2009) Drug Discov Today 14:1089–1097
2. Tiekink ERT (2008) Inflammopharmacology 16:138–142
3. Medici S, Peana M, Nurchi VM, Lachowicz JI, Crisponi G, Zoroddu MA (2015) Coord Chem Rev 284:329–350
4. Guo Z, Sadler PJ (1999) Angew Chem Int Ed 38:1512–1531
5. Santini C, Pellei M, Gandin V, Porchia M, Tisato F, Marzano C (2014) Chem Rev 114:815–862
6. Marzano C, Pellei M, Tisato F, Santini C (2009) Med Chem 9:185–211
7. Mohamed Subarkhan M, Prabhu RN, Raj Kumar R, Ramesh R (2016) RSC Adv 6:25082–25093
8. Qin Q-P, Liu Y-C, Wang H-L, Qin J-L, Cheng F-J, Tang S-F, Liang H (2015) Metallomics 7:1124–1136
9. Tardito S, Marchio L (2009) Curr Med Chem 16:1325–1348
10. Denoyer D, Masaldan S, La Fontaine S, Cater MA (2015) Metallomics 7:1459–1476
11. Beraldo H, Gambinob D (2004) Med Chem 4:31–39
12. Ali SMH, Yan Y-K, Lee PPF, Khong KZX, Sk MA, Lim KH, Klejevskaja B, Vilar R (2014) Dalton Trans 43:1449–1459
13. Chen H, Ma X-Q, Lv Y-Y, Jia L, Xu J, Wang Y, Ge Z-J (2016) J. Mol. Struct. 1109:146–153
14. Casas JS, García-Tasende MS, Sordo J (2000) J Coord Chem Rev 209:197–261
15. Sreekanth A, Kala UL, Nayar CR, Prathapachandra Kurup MR (2004) Polyhedron 23:41–47
16. Casas JS, Castro-Vidal E, García-Tasende MS, Sánchez A, Sordo J, Touceda Á, Vázquez-López EM (2011) Eur J Inorg Chem 2011:868–878
17. Kurup MRP, Varghese B, Sithambaresan M, Krishnan S, Sheeja SR, Suresh E (2011) Polyhedron 30:70–78
18. Tiago FS, Santiago PHO, Amaral MMP, Martins JBL, Gatto CC (2016) J Coord Chem 69:330–342
19. Neto BAD, Viana BFL, Rodrigues TS, Lalli PM, Eberlin MN, da Silva WA, de Oliveira HCB, Gatto CC (2013) Dalton Trans 42:11497–11506
20. Annie CF, Sithambaresan M, Kurup MRP (2015) Acta Crystallogr Sect E 71:427–430
21. Sheldrick GM (1997) SADABS, program for empirical absorption correction of area detector data. University of Göttingen, Germany
22. Sheldrick GM (1997) SHELXS-97 program for crystal structure resolution University of Göttingen. Göttingen, Germany
23. Sheldrick G (2015) Acta Crystallogr Sect C 71(1):3–8
24. Farrugia LJ (2012) J Appl Cryst 45:849–854
25. Brandenburg K (2004) Crystal Impact GbR. Bonn, Germany
26. Mosmann T (1983) J Immunol Methods 65:55–63
27. Lee PF, Yang C-T, Fan D, Vittal JJ, Ranford JD (2003) Polyhedron 22:2781–2786
28. Salem NMH, Rashad AR, El Sayed L, Foro S, Haase W, Iskander MF (2015) Inorg Chim Acta 432:231–242
29. Pal I, Dutta S, Basuli F, Goverdhan S, Peng S-M, Lee G-H, Bhattacharya S (2003) Inorg Chem 42:4338–4345
30. Vafazadeh R, Esteghamat-Panah R, Willis AC, Hill AF (2012) Polyhedron 48:51–57
31. Kala UL, Suma S, Kurup MRP, Krishnan S, John RP (2007) Polyhedron 26:1427–1435
32. Shaabani B, Khandar AA, Mahmoudi F, Maestro MA, Balula SS, Cunha-Silva L (2013) Polyhedron 57:118–126
33. Patel RN (2010) J Coord Chem 63:1207–1219