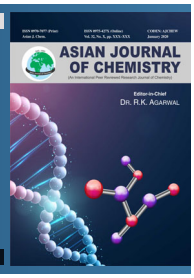
Asian Journal of Chemistry; Vol. 32, No. 3 (2020), 641-645

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2020.22349



Synthesis, Structure and Antioxidant Activity of Mixed Ruthenium(III) Benzoyl Pyridine Complex

E. JAYANTHI[1,*], M. ANUSUYA[1,2], R. ANUSUYA[3], K. THENMOZHI[3], A. NAGAVENI[1] and N.S.P. BHUVANESH[4]

[1]Department of Chemistry, Kongunadu Arts and Science College, Coimbatore-641029, India
[2]Department of Chemistry, Nallamuthu Gounder Mahalingam College, Pollachi-642001, India
[3]Department of Botany, Kongunadu Arts and Science College, Coimbatore-641029, India
[4]Department of Chemistry, Texas A&M University, College Station, TX 77843, U.S.A.

*Corresponding author: Fax: +91 422 2644452; Tel.: +91 422 2642095; E-mail: jayakumar.jayanthi@gmail.com

Received: 21 July 2019; *Accepted*: 10 November 2019; *Published online*: 31 January 2019; AJC-19772

A new ruthenium arsine complex was prepared by reacting equimolar ratio of $[RuBr_3(AsPh_3)_3]$ and 2-benzoyl pyridine. It was characterized by microanalysis, FT-IR and single crystal X-ray diffraction studies. X-ray diffraction data showed the distorted octahedral geometry of the complex. The pyridine nitrogen and carbonyl oxygen of the ligand coordinated with the metal center. Antioxidant activity of the complex was analyzed using different assays, which manifested significant activity. It has been found that a newly synthesized complex possessed better antioxidant activity than the ligand and precursor complex.

Keywords: Antioxidant, Ruthenium arsine complex, 2-Benzoyl pyridine.

INTRODUCTION

Heterocycles have provided a platform for the rapid exchange of research in the areas of organic, pharmaceutical and analytical chemistry [1,2]. Heterocyclic compounds such as pyridine, 2,2′-bipyridine and related molecules are good ligands due to the presence of at least one ring nitrogen atom with a localized pair of electrons [3]. The successful application of heterocyclic compounds has led to the formation of series of novel compounds with a wide range of physical, chemical and biological properties, spanning a broad spectrum of reactivity and stability [4,5]. 8-Hydroxyquinoline has been found to be non-carcinogenic and employed for *in vitro* assays as well as genetic toxicity [6,7].

Pyridine heterocycles are versatile compounds of great importance in organometallics, coordination, analytical and various other fields [8]. These can form stable metal complexes with almost all transition metals and show good antimicrobial, antiamoebic [9], antileishmanial [10], antimalarial [11], anticancer [12] and antifungal efficiency. A class of drugs with pyridine moiety has been reported as potent HIV-1 inhibitors [13], protein tyrosine kinase inhibitors, protozoal-retroviral co-infections [14] and therapeutic drugs for the inflammatory diseases [15].

Metal ions play a vital role in a large number of biological processes and it is well-known that chelation of metal ions with organic ligand acts synergistically to increase their biological activities [16]. Further, many organic compounds used in medicine do not have a purely organic mode of action and require traces of metal ions directly or indirectly for activation or biotransformation [17]. Medicinal applications of ruthenium complexes have shown great promise as metallo-drugs, two ruthenium complexes, NAMI-A and KP1019, have entered in preclinical and clinical trials. Among these compounds, there are species with potential antioxidant activity [18], antimicrobial activity [19], antibacterial activity, anti-cancer activity, anti-parasitic [20], gastric, neural-protection activity and cardio-vascular activity. There are many studies for the interactions of ruthenium complexes with DNA and RNA [21].

The synthesis of various Ru(III) complexes containing tertiary phosphines or arsines have been carried out and possessed good biological and catalytic applications [22,23]. A few of these complexes have been used as good starting materials for the synthesis of new complexes [24,25]. However, inspite of extensive works, only a few Ru(III) complexes with tertiary monoarsines as one of the ligands have been reported in the literature [26]. There is no report which correlates exactly the

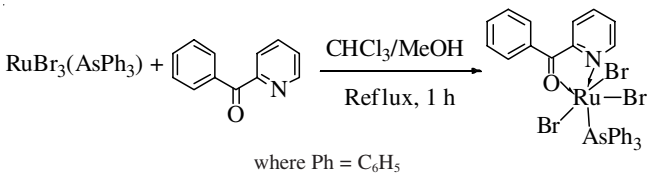
co-ligand triphenyl arsine Ru(III) complex with 2-benzoyl pyridine as one of the ligand. So, we herein interested in synthesizing Ru(III) complex with 2-benzoyl pyridine moiety. Numerous ruthenium containing complexes were reported as potent antioxidants like ruthenium-polypyridyl complexes, ruthenium thiosemicarbazone/ semicarbazone complexes [27]. Few benzoyl and bipyridyl derivatives were also reported to possess remarkable antioxidant activity [28], so we interested in knowing the potential of present novel crystalline compound′s antioxidant activity.

EXPERIMENTAL

All chemicals used were of analytical reagent grade (AR) and of the highest purity available. $RuCl_3 \cdot 3H_2O$ was purchased from Loba Chemie Pvt. Ltd., Mumbai, India. Triphenylarsine and 2-benzoyl pyridine were purchased from Sigma-Aldrich. Commercial solvents were procured from reputed suppliers and used without further purification. All other chemicals and reagents utilized for synthesis and antioxidant studies were purchased from reputed suppliers.

The starting complex $[RuBr_3(AsPh_3)_3]$ was synthesized according to the literature procedure [29]. Elemental analyses were performed on a Vario EL III CHNS analyser. IR spectra of the ligand and its complex were recorded using KBr pellets on a Nicolet Avatar instrument in the frequency range 4000-400 cm^{-1}. Melting points were recorded using Raaga apparatus and are uncorrected.

Synthesis of mixed Ru(III) 2-benzoyl pyridine complex: The complex was synthesized by refluxing $[RuBr_3(AsPh_3)_3]$ (0.1 mM; 0.126 g) in 20 mL of chloroform and 2-benzoyl pyridine ligand (0.1 mM; 0.018 g) in methanol (20 mL). After the addition of ligand, the solution changed into dark brown colour and then into dark blue. The reaction mixture was refluxed for 1 h. Microcrystals of the complex precipitated on slow evaporation was filtered, washed several times with petroleum ether and then dried. It was dissolved in 1:1 $CHCl_3$/MeOH solution and kept at room temperature. Upon slow evaporation, brownish violet sugar like crystals of the complex was obtained after one week time (**Scheme-I**). Yield: 45 %. Colour: brownish violet; m.p.: 191 ºC; m.w.: 800.22. Elemental analysis calcd. (found) % of $C_{30}H_{24}NOAsRuBr_3$: C, 45.03 (45.01); H, 3.04 (3.02); N, 1.75 (1.76). IR (KBr, ν_{max}, cm^{-1}): 1582 & 1507 (C=N-N=C); 1362 (enolic C-O); 1084 (N-N).



where Ph = C_6H_5

Scheme-I: Synthesis of mixed ruthenium(III) benzoyl pyridine complex

X-ray crystallographic measurements: The X-ray diffraction intensities of complex was collected at 110 K with a Mo sealed X-ray tube (K = 0.70173 Å) using a BRUKER APEX2 X-ray (three-circle) diffractometer equipped with graphite monochromator. Integrated intensity information for each reflection was obtained by reduction of data frames with APEX2. The integration method employed a three dimensional profiling algorithm and all data were corrected for Lorentz and polarization factors, as well as for crystal decay effects. SADABS was employed to correct the data for absorption effects. All the non-hydrogen atoms were refined anisotropically and the hydrogen atoms were positioned geometrically and refined as riding model. OLEX2 [30] was employed for the structure plots. Systematic reflection conditions and statistical tests of the data suggested the space group P-1. A solution was obtained readily using SHELXTL (XS) [31]. Hydrogen atoms were placed in idealized positions and were set riding on the respective parent atoms. The structure was refined (weighted least squares refinement on F^2) to convergence. Olex2 was employed for the final data presentation and structure plots.

Antioxidant activity

DPPH radical scavenging activity: The hydrogen donating capacity was assessed using stable DPPH$^{\bullet}$ method [32]. Briefly, a solution of 0.1 mM DPPH$^{\bullet}$ was prepared using methanol. The samples (50-250 µg/mL) were mixed with 5.0 mL of DPPH$^{\bullet}$ solution. Reaction mixture was shaken, incubated at 27 ºC for 20 min and the absorbance was measured at 517 nm. Results were compared with the activity of BHA. DPPH$^{\bullet}$ (%) discolouration of the sample was calculated using the formula:
DPPH radical scavenging activity (%) = [(Control OD - Sample OD) / Control OD] × 100.

Antioxidant activities of the extracts were expressed as IC_{50}, the values were calculated from the linear regression of the percentage antioxidant activity *versus* concentration of the extracts. A lower IC_{50} value indicates greater antioxidant activity.

Trolox equivalent antioxidant capacity (TEAC) assay: Antioxidant activity was performed using an improved ABTS$^{\bullet+}$ method proposed by Siddhuraju and Manian [33]. The ABTS radical cation (ABTS$^{\bullet+}$) was generated by a reaction of 7 mM ABTS$^{\bullet+}$ and 2.45 mM potassium persulfate and the mixture was incubated for 12-16 h at room temperature in dark. Prior to assay, the solution was diluted in ethanol (about 1:89 v/v) and equilibrated to obtain an absorbance of 0.700 ± 0.02 at 734 nm. A 10 µL/mL of sample was added to 1.0 mL of diluted ABTS$^{\bullet+}$ solution. After 30 min of incubation, absorbance was read at 734 nm. Trolox was used as a reference material.

Chelating ability for ferrous ions: Ferrous ion chelating potential for the ligand and complexes were assessed according to the literature method [34]. The reaction was initiated with the sequential addition of 250 µg of the sample extract, 0.25 mL of 1 mM $FeSO_4$ solution, 1.0 mL of 0.2 M Tris-HCl buffer (pH 7.4), 1.0 mL of 2,2′-bipyridyl solution, 0.4 mL of 10 % hydroxylamine hydrochloride and 2.0 mL of ethanol. The final volume was made up to 5.0 mL with deionized water and the absorbance was determined at 522 nm. EDTA was used to benchmark the chelating abilities. Lower absorbance of the reaction mixture indicated higher ferrous ion chelating ability.

Superoxide radical scavenging activity: Superoxide radicals were generated by the modified method of Beauchamp and Fridovich [35]. The assay was based on the capacity of the sample to inhibit formation by scavenging superoxide radicals generated by riboflavin-light-NBT in the system. Each 3 mL reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 20 mg riboflavin, 12 mM EDTA, 0.1 mg NBT and various concentrations (200-1000 µg) of the compounds. Reaction

was initiated by illuminating the reaction mixture with sample for 90 s. Immediately after illumination the absorbance was measured at 590 nm. The entire reaction assembly was enclosed in a box lined with aluminum foil. Identical tubes with reaction mixture kept in dark served as blank.

RESULTS AND DISCUSSION

Synthesis of metal complexes of heterocyclic ligands are of great importance due to their versatile coordination behaviour, enhanced biological, biomolecular interaction and cytotoxicity activity when compared to free ligands. Thus, a new stable mixed ruthenium(III) benzoyl pyridine complex is synthesized using triphenylarsine as co-ligand in methanol and chloroform medium with 45 % yield of the brownish violet compound. The complex is soluble in common organic solvents like methanol, ethanol, DMSO, *etc*. and was characterized by elemental analysis, IR spectral and single crystal X-ray diffraction techniques.

The IR spectrum of ligand showed two bands at 1665 and 1585 cm^{-1} due to stretching frequencies of ν(C=O) and ν(C=N), respectively of 2-benzoyl pyridine. The strong band appeared at 1593 cm^{-1} in the infrared spectrum of complex was assigned to ν(C=O) stretching frequency. Another strong band at 1480 cm^{-1} was assigned for pyridine C=N bond. The reduction in both carbonyl and C=N stretching frequencies of the complex when compared to ligand might be due to the complexation of ligand to ruthenium through carbonyl oxygen and pyridine nitrogen. This reduces the bond length and results in the reduction of stretching frequency. The analytical data and IR characteristics are in good agreement with the proposed structure of complex given in **Scheme-I**.

Crystal structure of complex: To further confirm the structure of the complex, single crystal X-ray diffraction data of complex was collected. Data collection conditions and the parameters of refinement process of the complex are given in Table-1. The ORTEP diagram of complex with atom numbering scheme is given in Fig. 1. The selected geometric parameters are also given in Table-2.

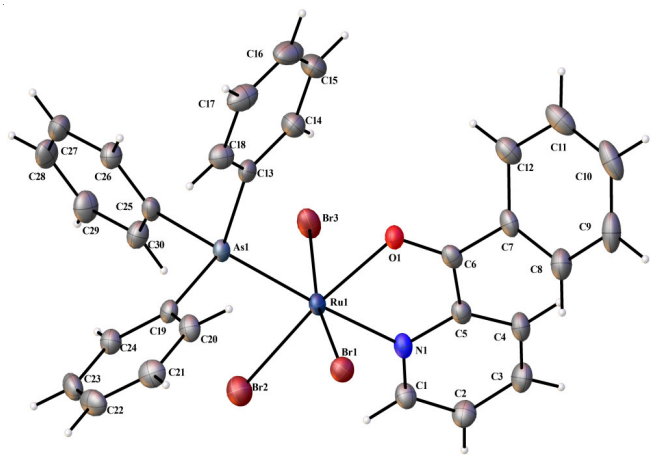


Fig. 1. ORTEP diagram of complex with the atom numbering scheme

Asymmetric unit of complex crystallizes in space group P-1. The ORTEP diagram of the complex reveals that Ru(III) ion is in a distorted octahedral geometry. Basal plane is occupied by carbonyl oxygen and pyridine nitrogen atoms from 2-benzoyl pyridine moiety, arsine of triphenylarsine and one bromide [Br(2)] ion. The axial positions were occupied by two bromide [Br(1) and Br(3)] ions revealed by the elongation of Br1 and Br3 bond lengths approximately 0.02135 Å (mean) when compared to bromide present in the basal plane. The charge on Ru(III) centre is balanced by three bromide ions. The bond angles which are acute and obtuse not equal to 90° and 180° confirmed the distortion in the octahedral geometry. Bond lengths are comparable to that of the complexes reported in the literature.

TABLE-1
EXPERIMENTAL DATA FOR
CRYSTALLOGRAPHIC ANALYSES

CCDC deposition no.	897080
Empirical formula	$C_{30}H_{24}NOAsRuBr_3$
Formula weight	830.22
Temperature (K)	110.15
Wavelength (Å)	0.71073
Crystal system	Triclinic
Space group	P-1
Unit cell dimensions	
a (Å)	11.389(3)
b (Å)	11.596(3)
c (Å)	12.749(4)
α (°)	69.642(3)
β (°)	75.704(3)
γ (°)	66.891(3)
Volume ($Å^3$)	1439.8(7)
Z	2
Density (calculated) (Mg/m^3)	1.915
Abs. coefficient (mm^{-1})	5.872
F(000)	802
Reflections collected	15564
Independent reflections	6453 [R(int) = 0.0239]
Goodness-of-fit on F^2	1.031
Final R indices I>2σ(I)]	R1 = 0.0565, wR2 = 0.0920
R indices (all data)	R1 = 0.1187, wR2 = 0.1136

TABLE-2
SELECTED BOND LENGTHS (Å) AND
BOND ANGLES (°) FOR COMPLEX

Bond lengths	(Å)	Bond angle	(°)
Ru(1)-N(1)	2.088(3)	N(1)-Ru(1)-O(1)	77.05(11)
Ru(1)-O(1)	2.132(3)	N(1)-Ru(1)-As(1)	176.46(9)
Ru(1)-As(1)	2.4375(7)	O(1)-Ru(1)-As(1)	99.41(8)
Ru(1)-Br(2)	2.4533(7)	N(1)-Ru(1)-Br(2)	96.38(9)
Ru(1)-Br(1)	2.4714(7)	O(1)-Ru(1)-Br(2)	173.14(7)
Ru(1)-Br(3)	2.4779(8)	As(1)-Ru(1)-Br(2)	87.15(3)
		N(1)-Ru(1)-Br(1)	89.96(9)
		O(1)-Ru(1)-Br(1)	82.33(7)
		As(1)-Ru(1)-Br(1)	89.37(2)
		Br(2)-Ru(1)-Br(1)	95.83(2)
		N(1)-Ru(1)-Br(3)	87.76(9)
		O(1)-Ru(1)-Br(3)	86.92(7)
		As(1)-Ru(1)-Br(3)	92.262(19)
		Br(2)-Ru(1)-Br(3)	94.86(2)

Antioxidant activity: The antioxidant activity of free ligand, precursor complex and new benzoyl pyridine complex were evaluated in a dose dependent manner in a series of *in vitro* assays involving DPPH radical scavenging activity, Trolox equivalent antioxidant capacity (TEAC) assay, chelating ability for ferrous ions and superoxide radical scavenging activity in a dose dependent manner.

From Figs. 2-5, it is clear that the DPPH scavenging activity of all the compounds including new complex are lesser compared to the value of standard. But $O_2^{\bullet-}$ scavenging activity of the newly synthesized complex is greater than the ligand, precursor and the standard. Further, ferrous ion chelating ability and $ABTS^{\bullet+}$ scavenging activity of the newly synthesized complex is significantly higher than that of ligand and precursor. Thus, synthesized complex has the potential to act as an antioxidant.

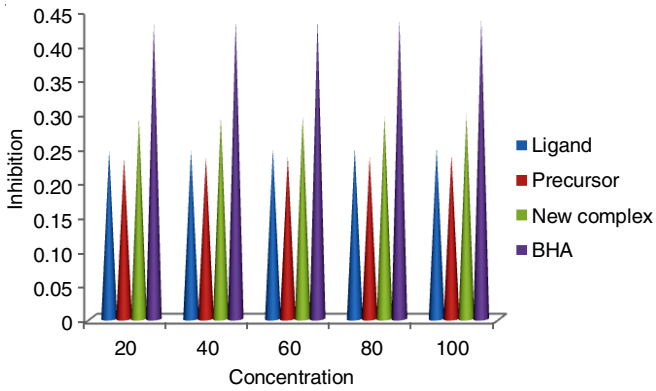


Fig. 2. DPPH activities of different compounds

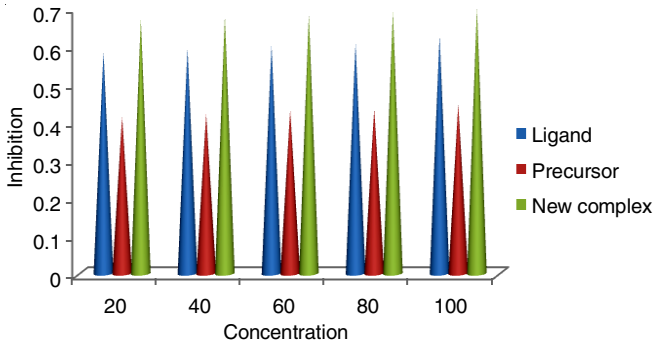


Fig. 3. ABTS ability in ethanolic extract of different compounds

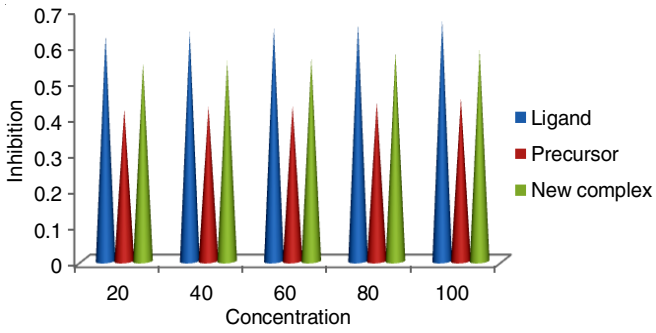


Fig. 4. Ferrous ion chelating ability of different compounds

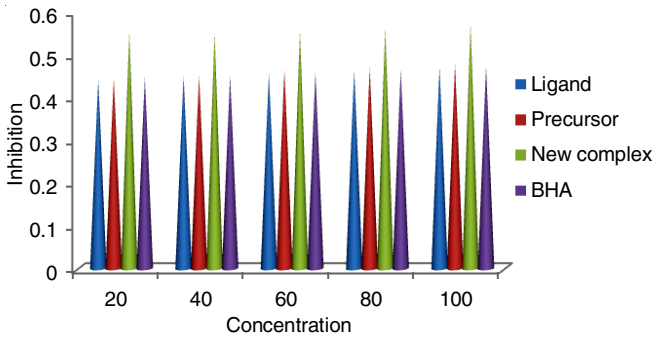


Fig. 5. Superoxide ability of different compounds

Conclusion

A new ruthenium complex is synthesized by using tribromotristriphenylarsine ruthenium(III) as starting complex and 2-benzoyl pyridine as neutral bidentate ligand in 1:1 methanol and chloroform mixture leading to the formation of complex. The complex is obtained as crystalline powder from the reaction mixture on slow evaporation, which was washed with petroleum ether and recrystallized from chloroform and methanol, characterized by using microanalysis, FT-IR and single crystal X-ray diffraction studies. The analytical data and IR characteristics are in good agreement with the structure of complex. X-Ray diffraction data showed the presence of neutral oxygen and nitrogen atoms from benzoyl pyridine moiety, arsine of triphenylarsine and one bromine (Br2) atom in the basal pane and two bromine atoms in apical positions completed the distorted octahedral geometry of the complex. The synthesized complex acted as better antioxidant scavenger than the ligand and the precursor.

Supplementary materials

CCDC deposition No. 897080 contains the supplementary crystallographic data for the complex.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. R. Dua, S. Shrivastava, S.K. Sonwane and S.K. Srivastava, *Adv. Biol. Res. (Faisalabad)*, **5**, 120 (2011).
2. L. Mercs and M. Albrecht, *Chem. Soc. Rev.*, **39**, 1903 (2010); https://doi.org/10.1039/b902238b
3. C. Kaes, A. Katz and M.W. Hosseini, *Chem. Rev.*, **100**, 3553 (2000); https://doi.org/10.1021/cr990376z
4. F.W. Bergstrom, *Chem. Rev.*, **35**, 77 (1944); https://doi.org/10.1021/cr60111a001
5. P. Sathyadevi, P. Krishnamoorthy, E. Jayanthi, R.R. Butorac, A.H. Cowley and N. Dharmaraj, *Inorg. Chim. Acta*, **384**, 83 (2012); https://doi.org/10.1016/j.ica.2011.11.033
6. T.K. Köprülü, S. Ökten, S. Tekin and O. Çakmak, *J. Biochem. Mol. Toxicol.*, **33**, e22260 (2019); https://doi.org/10.1002/jbt.22260
7. S. Li, H. Zhang and J. Liu, *Trans. Nonferr. Met. Soc. China*, **17**, 318 (2007); https://doi.org/10.1016/S1003-6326(07)60092-2
8. M.N. Hopkinson, C. Richter, M. Schedler and F. Glorius, *Nature*, **510**, 485 (2014); https://doi.org/10.1038/nature13384
9. S. Singh, N. Bharti and P.P. Mohapatra, *Chem. Rev.*, **109**, 1900 (2009); https://doi.org/10.1021/cr068217k
10. F.A.K. Khan, Z. Zaheer, J.N. Sangshetti, R.H. Patil and M. Farooqui, *Bioorg. Med. Chem. Lett.*, **27**, 567 (2017); https://doi.org/10.1016/j.bmcl.2016.12.018
11. N.T. Huy, D.T. Uyen, A. Maeda, D.T.X. Trang, T. Oida, S. Harada and K. Kamei, *Antimicrob. Agents Chemother.*, **51**, 350 (2007); https://doi.org/10.1128/AAC.00985-06
12. M. Gras, B. Therrien, G. Suss-Fink, A. Casini, F. Edafe and P.J. Dyson, *J. Organomet. Chem.*, **695**, 1119 (2010); https://doi.org/10.1016/j.jorganchem.2010.01.020
13. M.S.A. Gill, S.S. Hassan and N. Ahemad, *Eur. J. Med. Chem.*, **179**, 423 (2019); https://doi.org/10.1016/j.ejmech.2019.06.058
14. M.A. Fakhfakh, A. Fournet, E. Prina, J.-F. Mouscadet, X. Franck, R. Hocquemiller and B. Figadère, *Bioorg. Med. Chem.*, **11**, 5013 (2003); https://doi.org/10.1016/j.bmc.2003.09.007

15. A. Bari, D. Grenier, J. Azelmat, S.A. Syed, A.M. AlObaid and E.C. Hosten, *Chem. Biol. Drug Des.*, **94**, 1750 (2019); https://doi.org/10.1111/cbdd.13576
16. C.X. Zhang and S.J. Lippard, *Curr. Opin. Chem. Biol.*, **7**, 481 (2003); https://doi.org/10.1016/S1367-5931(03)00081-4
17. S.K. Bharti and S.K. Singh, *Pharm. Lett.*, **1**, 39 (2009).
18. A.A. Raj, R. Raghunathan , M.R. SrideviKumari and N. Raman, *Bioorg. Med. Chem*., **11**, 407 (2003); https://doi.org/10.1016/S0968-0896(02)00439-X
19. F. Li, M. Feterl, Y. Mulyana, J.M. Warner, J.G. Collins and F.R. Keene, *J. Antimicrob. Chemother.*, **67**, 2686 (2012); https://doi.org/10.1093/jac/dks291
20. D. Gambino and L. Otero, *Inorg. Chim. Acta*, **393**, 103 (2012); https://doi.org/10.1016/j.ica.2012.05.028
21. X.L. Hong, H. Li and C.H. Peng, *J. Mol. Struct.*, **990**, 197 (2011); https://doi.org/10.1016/j.molstruc.2011.01.045
22. J.M. da Silveira Carvalho, A.H. de Morais Batista, N.A.P. Nogueira, A.K.M. Holanda, J.R. de Sousa, D. Zampieri, M.J.B. Bezerra, F. Stefânio Barreto, M.O. de Moraes, A.A. Batista, A.C.S. Gondim, T. de F. Paulo, L.G. de França Lopes and E.H.S. Sousa, *New J. Chem.*, **41**, 13085 (2017); https://doi.org/10.1039/C7NJ02943H
23. E. Jayanthi, M. Anusuya, N.S.P. Bhuvanesh, K.A. Khalil and N. Dharmaraj, *J. Coord. Chem.*, **68**, 3551 (2015); https://doi.org/10.1080/00958972.2015.1077950
24. S.S. Valvassori, M.P. Cristiano, D.C. Cardoso, G.D. Santos, M.R. Martins, J. Quevedo and M.M.S. da Paula, *Neurochem. Res.*, **31**, 1457 (2006); https://doi.org/10.1007/s11064-006-9198-4
25. E. Jayanthi, S. Kalaiselvi, V.V. Padma, N.S.P. Bhuvanesh and N. Dharmaraj, *Dalton Trans.*, **45**, 1693 (2016); https://doi.org/10.1039/C5DT03849A
26. R. Prabhakaran, V. Krishnan, K. Pasumpon, D. Sukanya, E. Wendel, C. Jayabalakrishnan, H. Bertagnolli and K. Natarajan, *Appl. Organomet. Chem.*, **20**, 203 (2006); https://doi.org/10.1002/aoc.1026
27. F.E. Poynton, S.A. Bright, S. Blasco, D.C. Williams, J.M. Kelly and T. Gunnlaugsson, *Chem. Soc. Rev*., **46**, 7706 (2017); https://doi.org/10.1039/C7CS00680B
28. T.S. Kamatchi, N. Chitrapriya, S.K. Kim, F.R. Fronczek and K. Natarajan, *Eur. J. Med. Chem*., **59**, 253 (2013); https://doi.org/10.1016/j.ejmech.2012.11.024
29. K. Natarajan, R.K. Poddar and U. Agarwala, *J. Inorg. Nucl. Chem.*, **39**, 431 (1977); https://doi.org/10.1016/0022-1902(77)80056-0
30. O.V. Dolomanov, L.J. Bourhis, R.J. Gildea, J.A.K. Howard and H. Puschmann, *J. Appl. Cryst.*, **42**, 339 (2009); https://doi.org/10.1107/S0021889808042726
31. SADABS, Program for Absorption Correction of Area Detector Frames; Bruker AXS Inc., 5465 East Cheryl Parkway, Madison, USA.
32. K. Mishra, K. Ojha and N.K. Chaudhury, *Food Chem.*, **130**, 1036 (2012); https://doi.org/10.1016/j.foodchem.2011.07.127
33. R. Manian, N. Anusuya, P. Siddhuraju and S. Manian, *Food Chem.*, **107**, 1000 (2008); https://doi.org/10.1016/j.foodchem.2007.09.008
34. T. Krishnaswamy, P. Subramaniam, M. Sellamuthu and K. Krishnamoorthy, *Int. J. Green Pharm.*, **8**, 58 (2014); https://doi.org/10.4103/0973-8258.126826
35. C. Beauchamp and I. Fridovich, *Anal. Biochem.*, **44**, 276 (1971); https://doi.org/10.1016/0003-2697(71)90370-8