Asian Journal of Chemistry; Vol. 34, No. 12 (2022), 3369-3373

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2022.23788

Cytotoxic Activity of Ruthenium-Pyridyl Triazole Complexes against Human Cancer Cell

MD. MAHBUBUL ALAM*, BELAL AHMED and MD. SHAHADAT H. CHOWDHURY

Department of Chemistry, Faculty of Physical Sciences, Shahjalal University of Science and Technology, Sylhet-3114, Bangladesh

*Corresponding author: Tel: +880 713850, Ext-698; E-mail: mahbubana-che@sust.edu

Received: 17 June 2022; *Accepted*: 3 November 2022; *Published online*: 25 November 2022; AJC-21068

[RuCl(η^6-*p*-cymene)(ligand **1**)] (complex **1**) was synthesized by simple mixing of 2-(1-((pyridine-2-yl)methyl)-1*H*-1,2,3-triazol-4-yl)ethyl sodium sulfate (ligand **1**) with [$RuCl_2$(η^6-*p*-cymene)]$_2$ in methanol. The microcrystals of the reported complex were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR spectroscopic techniques and elemental analysis. The cytotoxic activity of the ruthenium complex wass tested against human cancer cells A375, A431, BxPC3, A549, HCT-15, LoVo cancer cell and human nontumor cell (human embryonic kidney HEK2) by the MTT assay method. The synthesized ruthenium complex exhibits a limited cytotoxic effect on human cancer cells and also reveals a dose-depended antimetastatic property against LoVo cancer cell.

Keywords: Ruthenium, Triazole ligand, Cytotoxicity, Antimetastatis, MTT assay.

INTRODUCTION

Synthesis of triazoles especially 1,2,3-triazole containing molecules has been considered because of their important biological activities [1]. The interesting properties within 1,2,3-triazoles have made them into pharmaceutically vital molecules. The triazole molecules reveal high dipole moment, the ability of hydrogen bonding and π-stacking interactions [2,3]. However, the heterocyclic molecules containing triazole moiety have expanded the range of therapeutically interesting drugs *e.g.*, anti-inflammatory, sedatives, antimicrobial and antifungal activity. In addition, several important triazole-based drugs such as fluconazole, rilmazafone, trapidil, rufinamide, ribavirin are used for clinical treaments [4-6].

High coordination numbers, suitable geometries, redox activities and thermodynamic and kinetic affinities of metal complexes to ligands have given a good mechanism of biological action. Generally, the nature of metals and ligands plays a significant role in the biological activities of metal complexes [7,8]. In 1967, Rosenberg *et al.* [9] identified the electrogenerated platinum(II) species that stop the growth of *Escherichia coli*. This observation has been encouraged inorganic chemists to test the anticancer activity of cisplatin, (*cis*-[$Pt(NH_3)_2Cl_2$]). In 1978, cisplatin have been reached for clinical approval and became the most used anticancer drug [10]. Moreover, various metal complexes have been prepared and screened for antitumor activities. Among them, only two complexes, namely carboplatin and oxaliplatin have been approved for the clinical trial. While these complexes are active against a number of cancer cells, but their uses are limited due to their side effects [11]. These complexes damage DNA, kill the cell by crosslinking with DNA and disrupted the replication and transaction. The crosslinking ability is not selective only for cancer cells but also for healthy cells. For these reasons, the use of these drugs is bad like a disease [12]. This fact encourages us to prepare the non-platinum metal complexes, which may expand the activities of metal-anticancer drugs.

Ruthenium metal may be the most promising alternative preference, since its complexes have particular biochemical properties and can easily accumulate in neoplastic cells [13-15]. The first property seems to correlate with its ability to interact with transferrin. It has been assumed that Ru-transferrin complexes are effectively transported into neoplastic tissues, which contain high transferrin receptors, low oxygen content and high acidity, such as in hypoxic tumors. NAMI-A and KP1019 tested Ru complexes have appeared for clinical trials [16]. Both complexes have a similar structure with Ru^{3+} cation and are coordinated with chloride and heterocyclic ligands. However, the mode of action for these Ru drugs is different. KP1019 was used as a potential primarily anticancer drug, while

NAMI-A acted as antimetastatic agents. Sadler *et al*. [17] reported new Ru-arene complexes, which have cytotoxic effects on various human cancer cells [17]. Based on the reported complexes, only a few ruthenium-triazole complexes have been used for anticancer agents. Tornøe *et al.* [18] reported the Ru-triazole ligand complexes and tested them against human epithelial cells (HEp-2) and human lung cancer cells (A-549). These metal complexes have exhibited good cytotoxic activities. The potentiality of these complexes has been significantly influenced by the nature of ligands. The introduction of water-soluble parts into triazole ligands might have the positive effects to leave the non-chelating ligand (-Cl) and make them available site for DNA. The above reasons have been led to the synthesis of Ru-water soluble triazole ligands as an anticancer drug. Recently, the triazole ligands for homogeneous catalysis have been reported by our research group [19-23].

In the current work, the synthesis of water-soluble triazole ligand and their ruthenium complex as well as the anticancer and antimetastatic properties of the Ru complex are discussed. A431 (human cervical carcinoma cell), A549 (human lung cancer cell), BxPC3 (pancreatic cancer cell) and HCT-15 (colon cancer cell) are used as target cell lines.

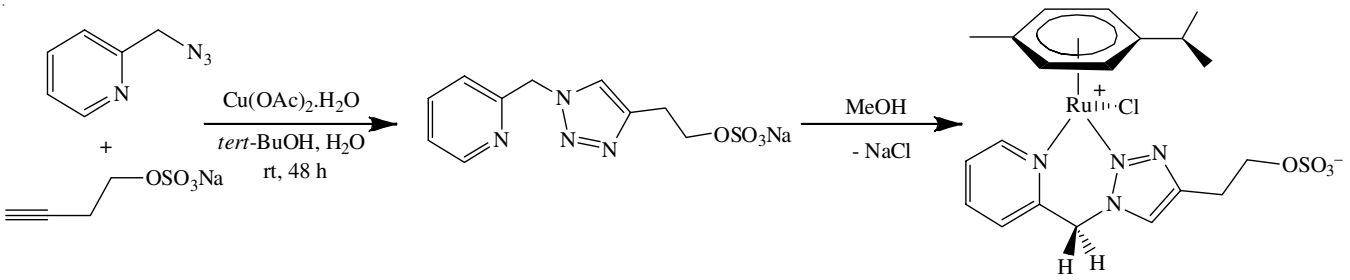
EXPERIMENTAL

Synthesis of ruthenium complex: A mixture of 0.12 g (0.39 mmol) 2-(1-((pyridine-2-yl)methyl)-1*H*-1,2,3-triazol-4-yl)ethyl sodium sulfate (ligand **1**) and 0.12 g (0.20 mmol) $[RuCl_2(\eta^6\text{-}p\text{-cymene})]_2$ was stirred in 20 mL methanol for three days at room temperature. After filtration, the filtrate was evaporated by vacuum evaporation and obtained yellow crystalline powder. Then, the product was dissolved in boiled EtOH (10 mL) and the solution was left overnight at room temperature. Finally, yellow microcrystals of [RuCl(*p*-cymene)(ligand **1**)] complex were collected [21,23] (**Scheme-I**) and isolated by hot-cold recrystallization of crystalline powder using ethanol. IR (KBr, ν_{max}, cm^{-1}): 2947 (*p*-cym, -C-H *str.*), 1603 (Py C=N, *str.*), 1005 (C-O *str.*), 773 (C-H bend.). Elemental anal. of calcd. (found) % for $C_{20}H_{25}N_4O_4SClRu$: C, 43.36 (43.11); H, 4.55 (4.55); N, 10.11 (9.89). ^{1}H NMR (CD_3OD, 300 MHz, δ ppm): 9.11 (doublet, 1H(1), *J* = 5.0 Hz), 8.33 (singlet, 1H(7)), 8.06 (triplet of doublet, 1H(3), *J* = 7.7, 1.25 Hz), 7.77 (doublet, 1H(4), *J* = 7.3 Hz), 7.59 (triplet, 1H(2), *J* = 6.7 Hz), 6.13-6.07 (multiplet, CH^a/CH Ar, 2H), 5.97 (doublet, 1H Ar, *J* = 6.0 Hz), 5.87 (doublet, 1H Ar, *J* = 6.1 Hz), 5.81 (doublet, 1H Ar, *J* = 6.2 Hz), 5.62 (doublet, $1H^b$, *J* = 15.8 Hz), 4.34-4.20 (multiplet, 2H(10)), 3.19-3.04 (multiplet, 2H(9)), 2.97-2.88 (multiplet, 1H(13)), 2.01 (s, 3H(18)), 1.32 (doublet, 6H(11,12), *J* = 6.9 Hz). ^{13}C NMR (CD_3OD, 300 MHz, δ ppm): 159.48 (C1), 154.66 (C5), 149.68 (C7), 141.78 (C3), 130.10 (C4), 127.39 (C8 or C2), 127.28 (C8 or C2), 107.87 (*p*-cym), 102.59 (*p*-cym), 89.02 (*p*-cym), 86.32 (*p*-cym), 85.64 (*p*-cym), 84.88 (*p*-cym), 67.11 (C10), 55.38 (C6), 32.18 (*p*-cymCH), 27.16 (C9), 22.57 ($CHCH_3$), 22.43 ($CHCH_3$), 18.30 (*p*-cymCH_3).

Cell cultures: Human lung (A549), pancreatic (BxPC3), melanoma (A375) and colon (HCT-15) carcinoma cell lines were collected from American Type Culture Collection (ATCC, Rockville, MD). Nontumor HEK293 cell was collected from the European Collection of Cell Cultures (ECACC, Salisbury, UK). Human cervical carcinoma cell A431 was provided by Prof. F. Zunino (Istituto Nazionale dei Tumori, Milan, Italy). LoVo human colon-carcinoma cell line was kindly providedby Prof. F. Majone of Padova University, Italy. Cell lines were maintained in the logarithmic phase at 37 ºC in a 5% CO_2 atmosphere using the following culture media containing 10% fetal calf serum (Euroclone, Milan, Italy), antibiotics (50 units/mL penicillin and 50 µg/mL streptomycin) and L-glutamine (2 mM): (i) F-12 HAM'S (Sigma Chemical Co.) for LoVo and A549 cells, (ii) DMEM for A375 cells and (iii) RPMI-1640 medium (Euroclone) for A431, HCT-15 and BxPC3 cells.

Complex **1** was dissolved in DMSO and then a calculated amount of drug solution was added into the cell growth medium to get a final solvent with the concentration of 0.5%, which had no detectable effect on cell killing. Cisplatin was dissolved in 0.9% NaCl solution. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and cisplatin were obtained from Sigma Chemical Co, St. Louis, USA.

MTT assay: The growth inhibitory effect against tumor cells was assessed by MTT assay [24]. Depending upon the cell growth characteristics, (3-8) × 10^3 cells/well were seeded in 96-well microplates in a 100 µL growth medium. After 24 h, fresh media containing the tested compound at the proper concentration was placed. Triplicate cultures were settled for individual treatment. In each well, a 10 µL MTT saline solution of 5 ppm was added. After, 5 h of incubation, a solution (0.01 M HCl) of sodium dodecylsulfate (SDS) solution was added. After overnight incubation, cell growth inhibition was detected by measuring the absorbance of each well at 570 nm using a Bio-Rad 680 microplate reader. Mean absorbance for each drug dose was expressed as a percentage of the control untreated well absorbance and plotted *vs*. drug concentration. IC_{50} values, the drug concentrations that reduce the mean absorbance at 570 nm to 50% of those in the untreated control wells, were



Scheme-I: Synthetic route of [RuCl(η⁶-*p*-cymene)(ligand **1**)] complex

TABLE-1
CYTOTOXIC ACTIVITY IN TERMS OF IC_{50} (μM) ± S.D. FOR THE TESTED COMPOUNDS AT 72 h ON THE HUMAN CANCER CELL LINES, A549, A431, A375, BxPC3 AND HCT-15

Compound	IC_{50} (μM) ± S.D.				
	A549	A431	A375	BxPC3	HCT-15
Complex **1**	126.76 ± 5.99	113.42 ± 6.11	32.98 ± 4.22	49.43 ± 3.13	35.65 ± 2.38
Cisplatin	9.98 ± 2.86	1.65 ± 0.51	3.11 ± 0.98	11.13 ± 2.36	11.32 ± 1.51

calculated by the four-parameter logistic (4-PL) model. The evaluation was based on at least four individual experiments.

Tumor cell invasion assays: Transwell embedded with 8 μm pores (Corning) were covered with 100 μL Matrigel (Becton Dickinson), which was diluted 1:4 in ice-cold F-12 HAM'S and allowed to gel at 37 ºC. LoVo cells were starved in a medium without fetal bovine serum overnight and then 1×10^5 cells resuspended in 100 μL of serum-free medium, containing the compound to be tested at the appropriate concentration, were added to the upper chamber and cultured for 24 h. The non-migratory cells on the upper surface of the membrane were removed and the cells were stained in 0.1% crystal violet and counted.

RESULTS AND DISCUSSION

Yellow crystalline powder [RuCl (η^6-*p*-cymene)(ligand **1**)] was obtained from the reaction of an equivalent amount of ligand **1** with $[RuCl_2(\eta^6\text{-}p\text{-cymene})]_2$. The synthesized complex was characterized by FT-IR, elemental analysis, ^1H & ^{13}C NMR spectroscopic methods. The characteristics IR frequencies for ruthenium complex were observed at *ca.* 2947 (*p*-cym, -C-H stretching), 1603 (Py C=N, *str.*), 1005 (C-O *str.*), 773 (C-H bend.) cm^{-1}. The shifting of C=N stretching frequency from 1650 to 1603 cm^{-1} indicates the bonding of Ru-ligand in the complex.

From ^1H NMR (Fig. 1a, CD_3OD, 298 K) spectra for ligand **1** and complex **1**, it has been shown that the singlet peak for H(7) atom and the doublet peak for H(1) atom are shifted downfield region compared to those for free ligand ($\Delta\delta = 0.57$ and 0.33 ppm), The shifting is matched well with the reported spectra data of metal-triazole complexes [21-26]. The formation of Ru-complex is also confirmed by the ^{13}C NMR spectrum (Fig. 1b) in which the resonance of C(7) and C(1) atoms occur at lower fields compared to those for free ligand **1** ($\Delta\delta s = 5.13$ and 4.80 ppm) [25,27].

Cytotoxicity studies: The cytotoxicity properties for complex **1** against several human cancer cells *e.g.* cervical (A431), lung (A549), pancreatic (BxPC3), colon (HCT-15) and melanoma (A375) cancer were tested by MTT method. The IC_{50} values (Table-1) were obtained after 72 h of exposure. The cytotoxicity of cisplatin was compared under the same conditions. The tested complex showed a limited antitumor activity, with higher IC_{50} values than those recorded with cisplatin. Cytotoxicity of the complex was also estimated against HEK293 (human nontumor) cell line. As reported in Table-2, the IC_{50} value of complex **1** against HEK293 cells was 200 times lower than that registered after the treatment with cisplatin. Interestingly, the SI value of the synthesized ruthenium complex was significantly higher (about 2 times) than that calculated with cisplatin. Complex **1** was also tested against human colon cancer LoVo cells, endowed with high metastatic potential (Fig. 2). The invasion potential of LoVo treated and untreated cells was assayed through the matrigel barrier in the transwell inserts test. The addition of complex **1** accordingly reduced the invasion potential of LoVo cells in a dose-dependent manner. Notably, at 25 μM Ru1 (complex **1**) determined a cell invasion inhibition which was rather similar to that obtained with Vandetanib (5 μM).

TABLE-2
CYTOTOXIC ACTIVITY AGAINST THE HUMAN NONTUMOR CELL, HEK293

Compound	IC_{50} (μM) ± S.D. (HEK293)	S.I.
Complex **1**	456.22 ± 3.23	6.1
Cisplatin	21.11 ± 2.17	2.8

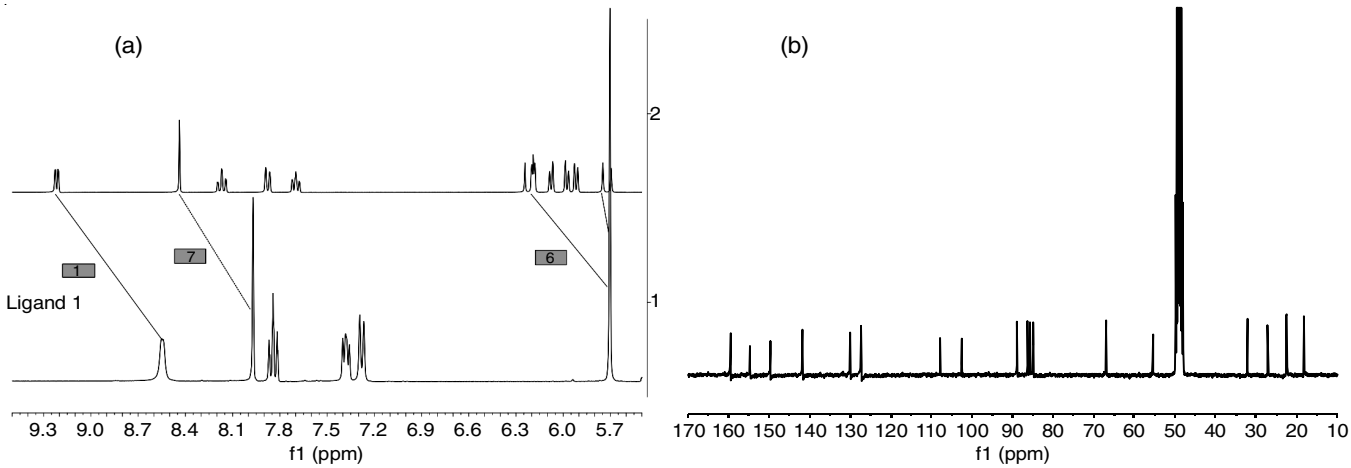


Fig. 1. (a) Comparison between ^1H NMR spectra of complex **1** and ligand **1** in CD_3OD; (b) ^{13}C NMR spectrum of complex **1** in CD_3OD

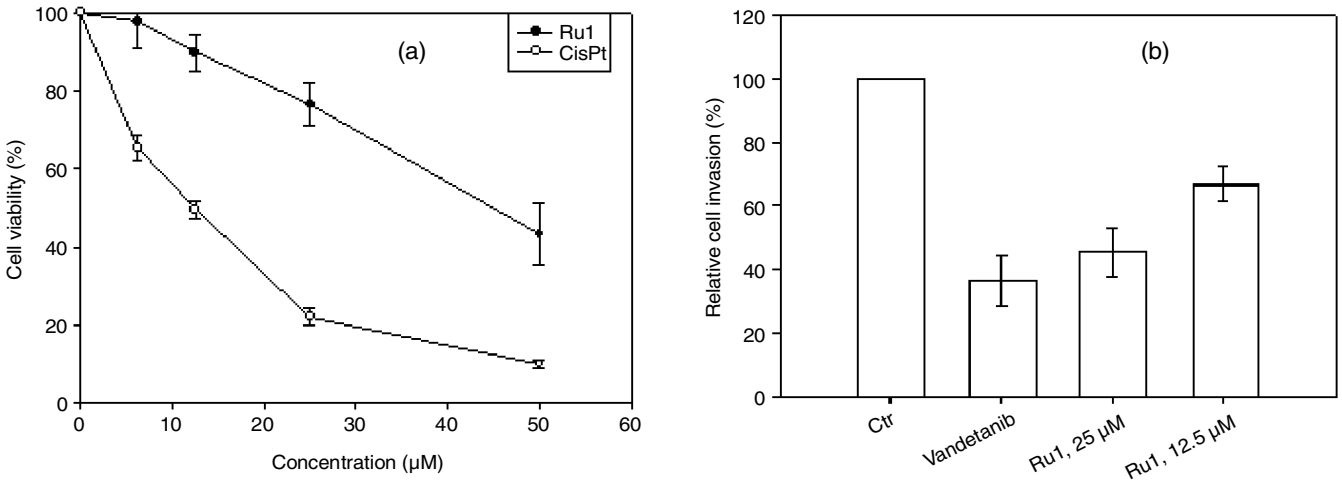


Fig. 2. Cytotoxicity (a) and (b) antimetastatic potential against LoVo cells. The treatment of cells (5×10^4 mL^{-1}) was carried out for 72 h with tested compounds (concentration of compound increasing in PEG400). Cytotoxicity was checked by MTT test. The calculation of IC_{50} values was carried out by the 4-PL model ($p < 0.05$). Error bars represent standard deviation, where Ru1 = complex **1**, CisPt = cisplatin and Ctr = Chemotherapy resistance test

Complex **1** elicited dose-dependent cytotoxicity that was, however, lower than that of cisplatin. Based on the dose-response curve, 25 and 12.5 μM were selected as non-toxic doses to be employed to assess tumor cell invasion ability (Fig. 2b). Vandetanib, a good tyrosine kinase inhibitor endowed with significant antimetastatic potential, has been used as a positive control.

Conclusion

[RuCl (η^6-*p*-cymene)(ligand **1**)] complex (where ligand **1** = 2-(1-((pyridine-2-yl)methyl)-1*H*-1,2,3-triazol-4-yl)ethyl sodium sulfate) was synthesized by the hot solution evaporation method and characterized by FT-IR and NMR spectroscopic techniques. The ligand shows mononucleated properties toward the ruthenium metal to form a neutral complex. The cytotoxic activities of the sythesized complex **1** on human cancer cells A375, A431, BxPC3, A549 and HCT-15 showed a limited *in vitro* antitumor activity, with IC_{50} values up to one order of magnitude higher than cisplatin. Cytotoxicity of the complex **1** against a human nontumor embryonic kidney HEK293 cells (human noncancerous cells in rapid proliferation) is 200 times lower than cisplatin after treatment. The selectivity index value for the complex was also remarkably higher (about 2 times) than that for calculated with cisplatin.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Alberto scrivanti and Dr. Beghetto Valentina, Ca' Foscari University of Venice, Italy, for their kind help for the cytotoxic evaluations. The authors are thanks to Dr. M. Razaul Karim, Shahjalal University of Science and Technology, Sylhet, Bangladesh for his kind and valuable suggestions about this work.

CONFLICT OF INTEREST

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

REFERENCES

1. B.R. Nemallapudi, D.R. Guda, N. Ummadi, B. Avula, G.V. Zyryanov, C.S. Reddy and S. Gundala, *Polycycl. Arom. Comp.*, **42**, 3874 (2022); https://doi.org/10.1080/10406638.2020.1866038
2. S. Haider, M.S. Alam and H. Hamid, *Inflammat. Cell Signal.*, **2014**, e95 (2014).
3. C. Sheng and W. Zhang, *Curr. Med. Chem.*, **18**, 733 (2011); https://doi.org/10.2174/092986711794480113
4. S. Kumar, S.L. Khokra and A. Yadav, *Futur. J. Pharm. Sci.*, **7**, 106 (2021); https://doi.org/10.1186/s43094-021-00241-3
5. S. Haider, M.S. Alam, H. Hamid, S. Shafi, A. Nargotra, P. Mahajan, S. Nazreen, A.M. Kalle, C. Kharbanda, Y. Ali, A. Alam and A.K. Panda, *Eur. J. Med. Chem.*, **70**, 579 (2013); https://doi.org/10.1016/j.ejmech.2013.10.032
6. S. Shafi, M. Mahboob Alam, N. Mulakayala, C. Mulakayala, G. Vanaja, A.M. Kalle, R. Pallu and M.S. Alam, *Eur. J. Med. Chem.*, **49**, 324 (2012); https://doi.org/10.1016/j.ejmech.2012.01.032
7. Z. Guo and P.J. Sadler, *Angew. Chem. Int. Ed.*, **38**, 1512 (1999); https://doi.org/10.1002/(SICI)1521-3773(19990601)38:11<1512::AID-ANIE1512>3.0.CO;2-Y
8. M. Moustakas, *Materials*, **14**, 549 (2021); https://doi.org/10.3390/ma14030549
9. B. Rosenberg, E. Renshaw, L. Vancamp, J. Hartwick and J. Drobnik, *J. Bacteriol.*, **93**, 716 (1967); https://doi.org/10.1128/jb.93.2.716-721.1967
10. S. Dasari and P.B. Tchounwou, *Eur. J. Pharmacol.*, **740**, 364 (2014); https://doi.org/10.1016/j.ejphar.2014.07.025
11. T. Akman, L. Akman, O. Erbas, M.C. Terek, D. Taskiran and A. Ozsaran, *BioMed. Int. Res.*, **2015**, 167235 (2015); https://doi.org/10.1155/2015/167235
12. B.S. Howerton, D.K. Heidary and E.C. Glazer, *J. Am. Chem. Soc.*, **134**, 8324 (2012); https://doi.org/10.1021/ja3009677
13. F.P. Rodrigues, Z.A. Carneiro, P. Mascharak, C. Curti and R.S. da Silva, *Coord. Chem. Rev.*, **306**, 701 (2016); https://doi.org/10.1016/j.ccr.2015.03.028
14. W.L. Kwong, K.Y. Lam, C.N. Lok, Y.T. Lai, P.Y. Lee and C.M. Che, *Angew. Chem. Int. Ed.*, **128**, 13722 (2016); https://doi.org/10.1002/ange.201608094
15. M. Montani, G.V.B. Pazmay, A. Hysi, G. Lupidi, R. Pettinari, V. Gambini, M. Tilio, F. Marchetti, C. Pettinari, S. Ferraro, M. Iezzi, C. Marchini and A. Amici, *Pharmacol. Res.*, **107**, 282 (2016); https://doi.org/10.1016/j.phrs.2016.03.032
16. A. Levina, A. Mitra and P.A. Lay, *Metallomics*, **1**, 458 (2009); https://doi.org/10.1039/b904071d

17. Y.K. Yan, M. Melchart, A. Habtemariam and P.J. Sadler, *Chem. Commun.*, **4764**, 4764 (2005); https://doi.org/10.1039/b508531b
18. C.W. Tornøe, C. Christensen and M. Meldal, *J. Org. Chem.*, **67**, 3057 (2002); https://doi.org/10.1021/jo011148j
19. E. Amadio, A. Scrivanti, V. Beghetto, M. Bertoldini, M.M. Alam and U. Matteoli, *RSC Adv.*, **3**, 21636 (2013); https://doi.org/10.1039/c3ra44740e
20. A. Scrivanti, V. Beghetto, M.M. Alam, S. Paganelli, P. Canton, M. Bertoldini and E. Amadio, *Inorg. Chim. Acta*, **455**, 613 (2017); https://doi.org/10.1016/j.ica.2016.05.011
21. S. Paganelli, M.M. Alam, V. Beghetto, A. Scrivanti, E. Amadio, M. Bertoldini and U. Matteoli, *Appl. Catal. A Gen.*, **503**, 20 (2015); https://doi.org/10.1016/j.apcata.2014.11.013
22. M.M. Alam, V. Beghetto, A. Scrivanti, M. Bertoldini and M. Salim, *AASCIT J. Chem.*, **6**, 1 (2020).
23. M.M. Alam, V. Beghetto, A. Scrivanti, M. Bertoldini and M.A. Arafath, *SN Appl. Sci.*, **2**, 1859 (2020); https://doi.org/10.1007/s42452-020-03711-0
24. E. Amadio, M. Bertoldini, A. Scrivanti, G. Chessa, V. Beghetto, U. Matteoli, R. Bertani and A. Dolmella, *Inorg. Chim. Acta*, **370**, 388 (2011); https://doi.org/10.1016/j.ica.2011.02.002
25. D. Urankar, B. Pinter, A. Pevec, F. De Proft, I. Turel and J. Košmrlj, *Inorg. Chem.*, **49**, 4820 (2010); https://doi.org/10.1021/ic902354e
26. E. Amadio, A. Scrivanti, G. Chessa, U. Matteoli, V. Beghetto, M. Bertoldini, M. Rancan, A. Dolmella, A. Venzo and R. Bertani, *J. Organomet. Chem.*, **716**, 193 (2012); https://doi.org/10.1016/j.jorganchem.2012.06.018
27. I. Bratsos, D. Urankar, E. Zangrando, P. Genova-Kalou, J. Košmrlj, E. Alessio and I. Turel, *Dalton Trans.*, **40**, 5188 (2011); https://doi.org/10.1039/c0dt01807d