2012

Synthesis, Characterization and Biological Studies of Mononuclear Copper(II) Complexes with Ciprofloxacin and N, O Donor Ligands

Mohan N. Patel

Chintan R. Patel,

Hardik N. Joshi
Sheridan College, hardik.joshi@sheridancollege.ca

Inorganic Chemistry Communications 27 (2013) 51–55

Contents lists available at SciVerse ScienceDirect

Inorganic Chemistry Communications

journal homepage: www.elsevier.com/locate/inoche

Synthesis, characterization and biological studies of mononuclear copper(II) complexes with ciprofloxacin and N, O donor ligands

Mohan N. Patel *, Chintan R. Patel, Hardik N. Joshi

Department of Chemistry, Sardar Patel University, Vallabh Vidyanagar-388 120, Gujarat, India

ARTICLE INFO

Article history:
Received 4 September 2012
Accepted 11 October 2012
Available online 17 October 2012

Keywords:
Copper(II) complexes
MIC
DNA interaction
DNA cleavage activity
Cytotoxicity
Enzymatic behaviour

ABSTRACT

The mixed ligand Cu(II) complexes of ciprofloxacin and N, O donor Schiff bases have been prepared and characterized by physicochemical techniques. In these complexes, ciprofloxacin acts as bidentate deprotonated ligand bound to the metal through the pyridone oxygen and one carboxylate oxygen. The antimicrobial activity of the complexes has been tested on three Gram(−ve) and two Gram(+ve) microorganisms in terms of minimum inhibitory concentration (MIC) and colony forming unit (CFU). The interaction study of the complexes with Herring Sperm DNA (HS-DNA) has been performed by absorption titration and viscosity measurement. The DNA cleavage activity has been carried out by gel electrophoresis experiment using supercoiled form of pUC19 DNA. Potential cytotoxic effect of complexes has been investigated by brine shrimp lethality assay method. The complexes have been also screened for their enzymatic behaviour in terms of IC_{50} value.

Copper(II) complexes with diverse drugs have been the subject of a large number of research studies [1,2], most probably due to the biological role of copper(II) and its synergetic activity with the drug [3]. It was already reported that some quinolone-metal complexes exert biological activity against various microorganisms or have some other positive effects in the treatment of certain diseases [4,5]. The antifungal and antibacterial properties of a range of copper(II) complexes have been evaluated against several pathogenic fungi and bacteria [6,7].

Quinolones, a commonly used term for the quinolonecarboxylic acids or 4-quinolones, are a group of synthetic antibacterial agents containing a 4-oxo-1,4-dihydroquinoline skeleton [8]. Ciprofloxacin (1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid) is a member of this large family and is used for the treatment of certain diseases caused by various Gram(−ve) and Gram(+ve) microorganisms [9]. They are extremely useful for the treatment of a variety of infections, including urinary tract infections, soft tissue infections, respiratory infections, bone-joint infections, typhoid fever, sexually transmitted diseases, prostatitis, community-acquired pneumonia, acute bronchitis and sinusitis [10]. In general, quinolones can act as antibacterial drugs that effectively inhibit DNA replication and are commonly used as treatment for many infections [10]. In literature, complexes of ciprofloxacin with diverse metal ions such as copper(II), vanadium(IV), magnesium(II), uranium(VI), manganese(II), iron(III), cobalt(II), nickel(II), molybdenum(II) and europium(III) have been reported and explored for their biological activities, because of its biological relevance [11–18]. A large number of mixed ligand copper(II) complexes have been shown to exhibit superoxide dismutase activity [19] and pharmacological activity [1]. This activity depends on the Cu(II)/Cu(I) redox process, which is related to the flexibility of the geometric transformation around the metal centres [20].

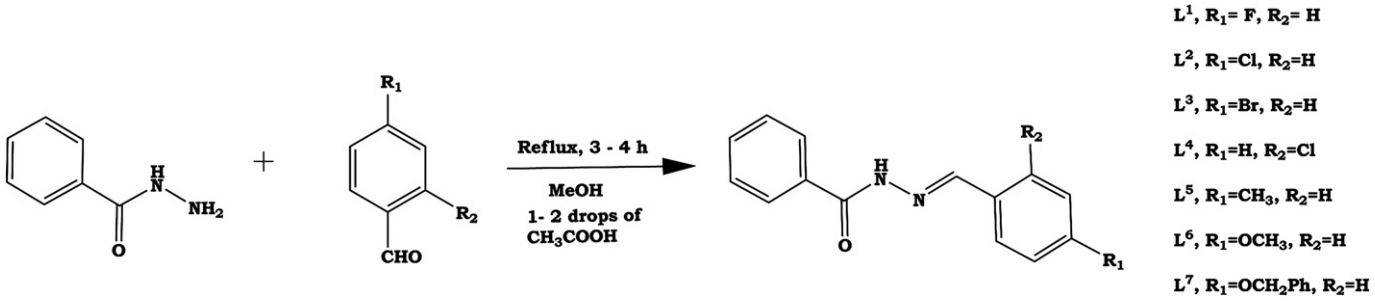
In continuation of our previous work [21], herein we report mononuclear mixed ligand copper(II) complexes of ciprofloxacin and N, O donor Schiff bases. The synthesized complexes were characterized by physicochemical and spectroscopic techniques and explored for their biological activity such as; antimicrobial activity, DNA interaction study, SOD mimics activity and cytotoxicity.

Schiff base N′-(4-fluorobenzylidene)benzohydrazide (L^1) was prepared by condensation of 4-fluoro benzaldehyde and benzohydrazide. A solution of 4-fluoro benzaldehyde (10 mmol) was added to a solution of benzohydrazide (10 mmol). The resulting solution was refluxed for 4–5 h on water bath after addition of 1–2 drops of acetic acid. On cooling the solution at room temperature product was separated, which was filtered and washed with methanol. The product was recrystallized from hot methanol.

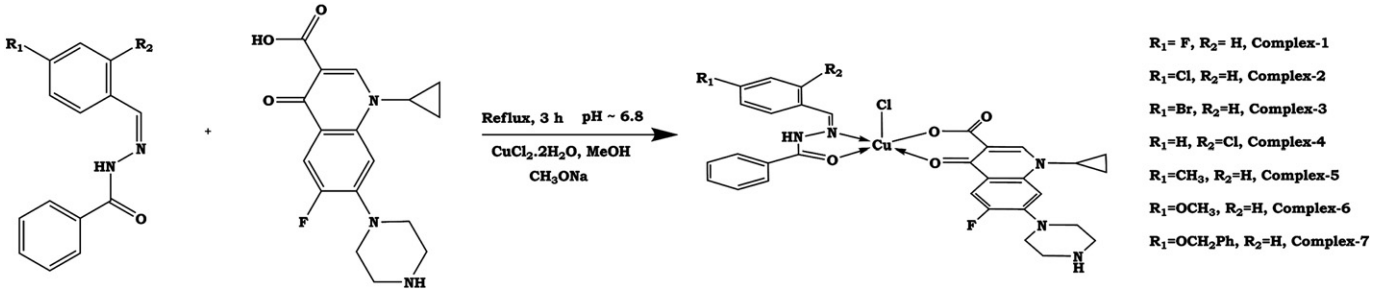
Similarly Schiff bases N′-(4-chlorobenzylidene)benzohydrazide (L^2), N′-(4-bromorobenzylidene)benzohydrazide (L^3), N′-(2-chloro benzylidene)benzohydrazide (L^4), N′-(4-methylbenzylidene)benzohydrazide (L^5), N′-(4-methoxybenzylidene)benzohydrazide (L^6) and N′-(4-(benzyloxy)benzylidene)benzohydrazide (L^7) were prepared by condensation of different substituted aldehydes and benzohydrazides. Schiff bases (L^1–L^7) were characterized by elemental analysis, ^1H and ^{13}C NMR spectroscopy. The reaction scheme used for the preparation of Schiff bases and proposed structures are

* Corresponding author. Tel.: +91 2692 226856x218.
E-mail address: jeenen@gmail.com (M.N. Patel).

1387-7003/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.inoche.2012.10.018



Scheme 1. Proposed structure and reaction scheme for the preparation of Schiff bases.



Scheme 2. General synthesis and proposed structures of complexes.

shown in Scheme 1. Physicochemical parameter data of ligands L^1–L^7 are shown in the supplementary material.

Methanolic solution of $CuCl_2 \cdot 2H_2O$ (1 mmol) was added to a methanolic solution of neutral bidentate ligand (L^1–L^7) (1 mmol), followed by addition of previously prepared methanolic solution of ciprofloxacin in the presence of CH_3ONa (1 mmol). The pH of reaction mixture was adjusted at ~6.8 using dilute solution of CH_3ONa. The resulting solution was refluxed for 3 h on a water bath, followed by concentrating it to half of its volume. A fine amorphous product obtained was washed with chloroform and dried in vacuum desiccator. The proposed structure of complexes is shown in Scheme 2. Physico-chemical parameters and microanalysis data of the synthesized complexes are in good agreement with proposed structure and are shown in the supplementary material.

Visible emission spectra of the copper(II) complexes i.e. d^9 system were recorded in DMSO. Complexes exhibited the only broad peak at λ_{max} = ~660 nm, which was attributed to *d–d* transition, in which Cu(II) atom was in distorted square pyramidal environment [22]. The λ_{max} value observed for the synthesized complexes 1 to 7 are 667 [23], 655 [24], 665 [23], 673 [25], 650 [24], 662 [26] and 672

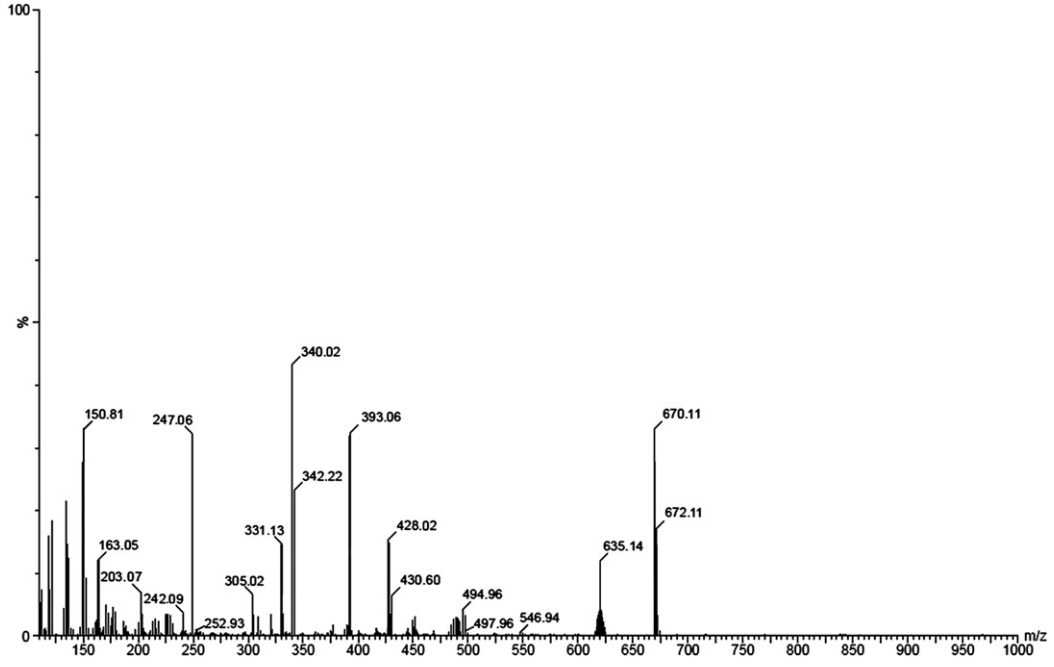


Fig. 1. LC–MS spectrum of complex [Cu(CPF)(L^1)Cl].

Table 1
Antimicrobial activities of CPFH, copper(II) salt and complexes in terms of minimum inhibitory concentration (MIC) (μM).

Compound	Gram positive		Gram negative		
	S. aureus	*B. subtilis*	*S. marcescens*	*P. aeruginosa*	*E. coli*
$CuCl_2 \cdot 2H_2O$	2698.0	2815.0	2756.0	2404.0	3402.0
CPFH	1.52	1.04	1.52	1.32	1.36
[Cu(CPF)L^1Cl] (1)	0.58	0.29	0.39	0.45	0.51
[Cu(CPF)L^2Cl] (2)	0.63	0.34	0.43	0.51	0.58
[Cu(CPF)L^3Cl] (3)	0.69	0.41	0.51	0.58	0.67
[Cu(CPF)L^4Cl] (4)	0.76	0.47	0.53	0.63	0.73
[Cu(CPF)L^5Cl] (5)	1.50	1.01	1.50	1.30	1.29
[Cu(CPF)L^6Cl] (6)	1.53	1.04	1.54	1.34	1.35
[Cu(CPF)L^7Cl] (7)	1.55	1.05	1.55	1.36	1.36

[26] nm respectively, which are similar to the complexes reported in the literature. The possibility of trigonal bipyramidal geometry at the metal centre was ruled out because the peak of λ_{max} greater than 800 nm along with the shoulder at ~660 nm was not observed in the case of the synthesized complexes [27].

The magnetic moment measurement for any geometry in copper(II) complexes results in the range of 1.76–1.86 BM, which is very close to spin-only value i.e. 1.73 BM. The observed values in our case were very close to the spin-only values for single unpaired electron, and confirm the copper in +2 state with d^9 configuration ($t_{2g}^6 e_g^3$) [28]. The copper content in the complex is determined by spectrophotometric titration technique [29]. The calculated result from the equivalent endpoint reveals the metallic content of the complex. Spectrophotometric titration curves and table for equivalent endpoint determination of the complexes are shown in the supplementary material.

Ring mode vibrations of the ligands are affected by coordination to Cu(II), corresponding to a shift in energy for bands in the 1700–400 cm^{-1} region of the IR spectra. In the IR spectra, bands at 1708 and 1624 cm^{-1} in case of ciprofloxacin correspond to $\nu(C{=}O)_{carb}$ and $\nu(C{=}O)_p$ respectively. The peak at 1708 cm^{-1} corresponding to $\nu(C{=}O)_{carb}$ of ciprofloxacin is disappeared on complexation with metal ion and replaced by $\nu(COO)_{as}$ = ~1580 cm^{-1} and $\nu(COO)_s$ = ~1350 cm^{-1} respectively [30]. Unidentate nature for the carboxylato group of ciprofloxacin is proved by frequency separation of ~220 cm^{-1} ($\Delta\nu = \nu COO_{as} - \nu COO_s$) [31]. Deprotonation of the hydroxyl group of ciprofloxacin is indicated by disappearance of band at 3519 cm^{-1} from spectra due to hydrogen bonding [30]. The band at 1624 cm^{-1} responsible for $\nu(C{=}O)_p$ in ciprofloxacin is observed between 1620 and 1635 cm^{-1} in case of complexes [18],

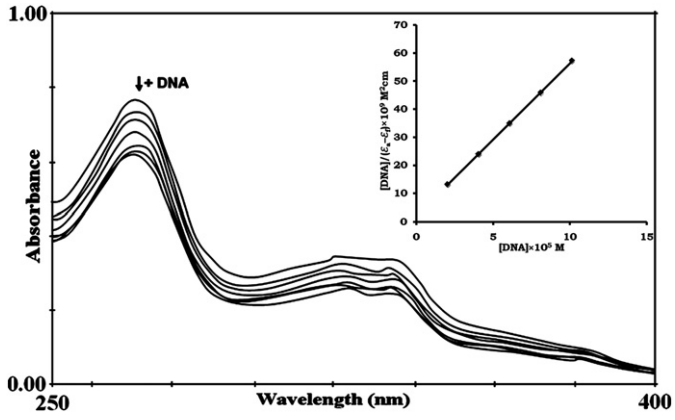


Fig. 2. Electronic absorption spectra of [Cu(CPF)(L^1)Cl] in phosphate buffer (Na_2HPO_4/NaH_2PO_4, pH 7.2) in the absence and presence of increasing amount of DNA. The [Cu] complex = 10 μM; [DNA] = 0–150 μM. The incubation period is 10 min at room temperature, Inset: Plot of [DNA]/(ε_a–ε_f) versus [DNA]. Arrow shows the absorbance change upon increasing DNA concentrations.

Table 2
Binding constant (K_b), IC_{50} and LC_{50} values of the synthesized complexes.

Complex	K_b (M^{-1})	IC_{50} (μM)	LC_{50} (μM)
[Cu(CPF)L^1Cl] (1)	2.68×10^5	0.64	7.76
[Cu(CPF)L^2Cl] (2)	2.55×10^5	0.82	10.00
[Cu(CPF)L^3Cl] (3)	2.35×10^5	0.87	12.59
[Cu(CPF)L^4Cl] (4)	2.20×10^5	1.02	14.45
[Cu(CPF)L^5Cl] (5)	0.70×10^5	2.19	39.81
[Cu(CPF)L^6Cl] (6)	0.44×10^5	2.50	47.86
[Cu(CPF)L^7Cl] (7)	0.32×10^5	2.68	58.88

and these data are further supported by observation of $\nu(M-O)$ at ~540–575 cm^{-1} [32] and $\nu(M-N)$ at ~490–535 cm^{-1} [33]. Significant wave numbers are given in the supplementary material.

The structure of complexes was further confirmed by molecular ion peak $[M]^+$ at 670.11 *m/z* and [M + 2] at 672.11 *m/z* in the LC–MS spectrum of complex 1 (Fig. 1). Some other fragments are observed at *m/z* = 672.11, 428.02, 393.06, 340.02, 342.22, 331.13, 305.02, 247.06, 203.07, 163.05 and 242.09, respectively. The proposed mass fragmentation pattern for complex 1 is shown in the supplementary material.

The antibacterial activity of the Cu(II) salt, ciprofloxacin and its complexes were tested against two Gram(+ve) *Staphylococcus aureus*, *Bacillus subtilis* and three Gram(−ve) *Serratia marcescens*, *Escherichia coli* and *Pseudomonas aeruginosa* organisms using double dilution method and represented in Table 1. Data reveals that, complexes 1, 2, 3 and 4 are more potent against all microorganisms than other complexes i.e. 5, 6 and 7, ciprofloxacin and copper(II) salt. An acceptable reason for this increase in bactericidal activity may be considered in the light of Overtone's concept [34] and chelation theory [35] or may be due to the effect of the metal ion on the normal cell process.

In addition to our study regarding bactericidal activity in terms of CFU/mL of the metal complexes against same microorganisms, a decrease in number of colonies with increasing the concentration of complexes is revealed. The CFU/mL for different microorganisms against complexes is shown in the supplementary material.

Fig. 2 shows the absorption spectra of complex 1 in the presence of increasing amounts of HS-DNA at room temperature. Complex binding with DNA through intercalation usually results in hypochromism and bathochromism, due to intercalative mode involving a strong stacking interaction between an aromatic chromophore and the base pairs of DNA [36]. To compare the binding strength of complexes quantitatively,

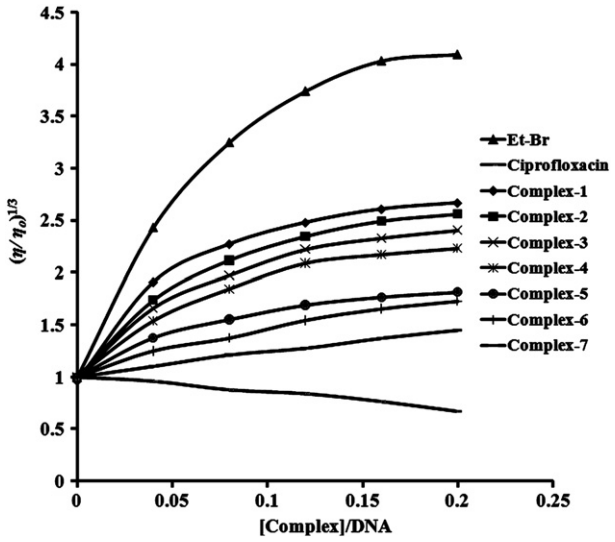


Fig. 3. Effect of increasing amount of EtBr, CPFH and complexes on the relative viscosity of herring sperm DNA at 37 ± 0.1 °C.

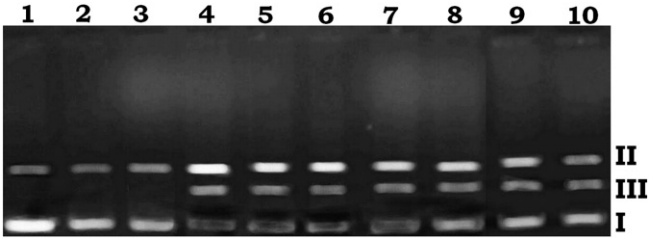


Fig. 4. Photogenic view of cleavage of pUC19 DNA (300 μg/mL) with a series of copper(II) complexes (200 μM) using 1% agarose gel containing 0.5 μg/mL ethidium bromide. All reactions were incubated in TE buffer (pH 8) in a final volume of 15 μL, for 24 h at 37 °C. Lane 1, DNA control; lane 2, $CuCl_2 \cdot 2H_2O$; lane 3, ciprofloxacin; lane 4, [Cu(CPF)(L^1)Cl]; lane 5, [Cu(CPF)(L^2)Cl]; lane 6, [Cu(CPF)(L^3)Cl]; lane 7, [Cu(CPF)(L^4)Cl]; lane 8, [Cu(CPF)(L^5)Cl]; lane 9, [Cu(CPF)(L^6)Cl], lane 10, [Cu(CPF)(L^7)Cl].

Table 3
Complex mediated DNA cleavage data by gel electrophoresis.

Lane no.	Compound	Form I SC	Form II OC	Form III L	% Cleavage
1	Control	82	18	–	–
2	$CuCl_2 \cdot 2H_2O$	76	24	–	7.31
3	Ciprofloxacin	61	39	–	25.6
4	[Cu(CPF)L^1Cl] (1)	11	62	27	86.6
5	[Cu(CPF)L^2Cl] (2)	14	61	25	82.9
6	[Cu(CPF)L^3Cl] (3)	17	60	23	79.3
7	[Cu(CPF)L^4Cl] (4)	19	57	24	76.8
8	[Cu(CPF)L^5Cl] (5)	36	44	20	56.1
9	[Cu(CPF)L^6Cl] (6)	40	37	22	51.2
10	[Cu(CPF)L^7Cl] (7)	48	27	25	41.5

the intrinsic binding constants K_b of the complexes were determined by monitoring the changes of absorbance with increasing concentration of HS-DNA. From the plot of [DNA]/(ε_b–ε_f) vs. [DNA] (inset in Fig. 2), the K_b value of complexes was determined and was found in the range of $0.32–2.68 \times 10^5$ (Table 2). This K_b value is much lower than that of classical intercalator (ethidium bromide), but higher than reported [Cu^{II}(phen)(bnp)H_2O] [37], [$CuL^1(ClO_4)_2$] [38], [$Zn(erx)_2(H_2O)_2$] [39], and [Ni(sf)$_2$(bipyam)] [40] complexes.

The effects of complexes, ethidium bromide (EB) and ciprofloxacin (CPFH) on the viscosity of rod-like DNA are shown in Fig. 3. In our case increasing the amounts of complexes, the relative viscosity of DNA increases steadily, hence complexes bind to DNA via intercalation mode [41] and the increase in relative viscosity is of same magnitude reported by different research groups [42]. Out of all the synthesized complexes complex 1 binds more strongly than the other.

The cleavage of plasmid pUC19 DNA was monitored by gel electrophoresis to investigate the ability of the copper(II) complexes to serve as metallonucleases [43]. When pUC19 DNA is subjected to gel electrophoresis, Form I shows the fastest migration compared to Forms II and III as shown in Fig. 4. Form II migrates very slowly prior to its relaxed structure, whereas Form III migrates somewhere between the positions of Form I and Form II. These clearly show that the relative binding efficacy of complexes to DNA is much higher than the binding efficacy of metal salt or ciprofloxacin (Table 3).

The superoxide radicals ($O_2^{\bullet -}$) were generated in vitro using a non-enzymatic (NBT/NADH/PMS) system and determined spectrophotometrically by measuring absorbance at 560 nm and plotted to have a straight line (Fig. 5). Percent inhibition of the reduction of NBT plotted against the concentration of the complex (Fig. 5). The superoxide scavenging data shown in Table 2 are in the range of 0.64–2.68 μM. Results show that complexes exhibit greater scavenging activity toward superoxide radicals, which may be accredited to the redox potential of the Cu(II) complex which depends on the geometry at the metal centre.

The in vitro lethality test has been carried out using brine shrimp eggs i.e. *Artemia cysts*. The shrimp lethality assay is considered as a useful tool for preliminary assessment of toxicity. Brine shrimp lethality bioassay is a development in the assay procedure of bioactive compound. All the synthesized compounds were screened for their cytotoxicity (brine shrimp bioassay) using the protocol of Meyer et al. [44]. The mortality rate of brine shrimp nauplii was found to increase with increasing the concentration of the complexes. Results for the lethality were noted in terms of deaths of larvae. A plot of Log of sample's concentration versus percentage of mortality showed a linear correlation. From the graph, the LC_{50} values of the samples were calculated and found in the range of 7.76–58.88 μM (Table 2).

Copper(II) complexes of ciprofloxacin bind to DNA ingeniously but distinctly different. The antibacterial activity of ciprofloxacin is changed upon coordination with copper(II) ion. Hypochromism and bathochromism of band in absorption titration, and increase in relative viscosity of DNA suggest that all complexes bind with DNA via classical intercalative mode. Complexation of drug with metal ion enhances their DNA cleavage ability. As the electro negativity of the substituted group on neutral bidentate N, O donor ligands increases,

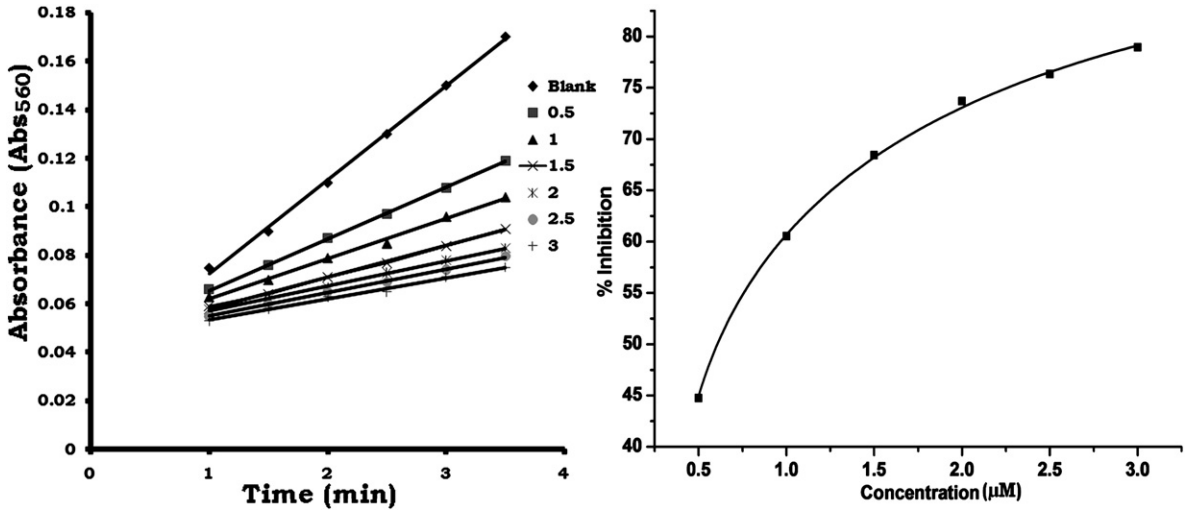


Fig. 5. Plot of absorbance (Abs_{560}) as a function of time (t) to determine % inhibition of formazan formation at various concentrations of complex 1 (0.25 μm to 3 μm) as a function of time and the plot of percentage of inhibiting NBT reduction with an increase in the concentration of complex 1.

the antimicrobial activity, DNA interacting behaviours and superoxide dismutase activity become more and more acute. Among all the complexes, complex 1 is more active compared to ciprofloxacin and copper(II) salt. The reason behind the increase in potency of drug is its coordination with copper(II) ion. The ligand, which can facilitate the stabilization of bonding between metal centre and oxygen radical anion favours enhancement in enzymatic behaviour. Results suggests that the synthesised complexes can be kept forward for their in vivo nuclease, antibacterial and enzymatic behaviour.

Acknowledgements

Authors thank the Head, Department of Chemistry, Sardar Patel University, India for making it convenient to work in a laboratory and U.G.C. for providing financial support under the "UGC Research Fellowship in Science for Meritorious Students" scheme.

Appendix A. Supplementary material

Materials, instrumental details, physicochemical parameters of ligands and complexes, characteristic absorption bands of IR spectra, determination of copper by spectrophotometric titration, proposed mass fragmentation pattern of complex 1, CFU/mL for complexes against different microorganisms, method for determination of MIC, absorption titration, viscosity measurement, IC_{50} and LC_{50} values are embedded in the supplementary material. Supplementary data associated with this article can be found, in the online version at http://dx.doi.org/10.1016/j.inoche.2012.10.018.

References

[1] M. Kato, Y. Muto, Factors affecting the magnetic properties of dimeric copper(II) complexes, Coord. Chem. Rev. 92 (1988) 45–83.
[2] J.E. Weder, C.T. Dillon, T.W. Hambley, B.J. Kennedy, P.A. Lay, J.R. Biffin, H.L. Regtop, N.M. Davies, Copper complexes of non-steroidal anti-inflammatory drugs: an opportunity yet to be realized, Coord. Chem. Rev. 232 (2002) 95–126.
[3] J.R.J. Sorenson, 6 Copper complexes offer a physiological approach to treatment of chronic diseases, Prog. Med. Chem. 26 (1989) 437–568.
[4] F. Gao, P. Yang, J. Xie, H. Wang, Synthesis, characterization and antibacterial activity of novel Fe(III), Co(II), and Zn(II) complexes with norfloxacin, J. Inorg. Biochem. 60 (1995) 61–67.
[5] I. Turel, A. Šonc, M. Zupančič, K. Sepčić, T. Turk, Biological activity of some magnesium(II) complexes of quinolones, Metal-Based Drugs 7 (2000) 101–104.
[6] A.M. Ramadan, Structural and biological aspects of copper (II) complexes with 2-methyl-3-amino-(3H)-quinazolin-4-one, J. Inorg. Biochem. 65 (1997) 183–189.
[7] M. Melnik, M. Auderova, M. Holko, Copper(II) carboxylates and their antimicrobial effect, Inorg. Chim. Acta 67 (1982) 117–120.
[8] A. Albert, The Physico-chemical Basis of Therapy: Selective Toxicity, sixth ed. Chapman & Hall, London, 1979.
[9] In: J.E.F. Reynolds (Ed.), The Extra Pharmacopeia, thirtieth ed., The Pharmaceutical Press, London, 1993.
[10] H.C. Neu, Ciprofloxacin: an overview and prospective appraisal, Am. J. Med. 82 (1987) 395–404.
[11] I. Turel, A. Golobic, A. Klavzar, B. Pihlar, P. Buglyo, E. Tolis, D. Rehder, K. Sepcic, Interactions of oxovanadium(IV) and the quinolone family member—ciprofloxacin, J. Inorg. Biochem. 95 (2003) 199–207.
[12] P. Drevensek, T. Zupancic, B. Pihlar, R. Jerala, U. Kolitsch, A. Plaper, I. Turel, Mixed-valence Cu(II)/Cu(I) complex of quinolone ciprofloxacin isolated by a hydrothermal reaction in the presence of l-histidine: comparison of biological activities of various copper–ciprofloxacin compounds, J. Inorg. Biochem. 99 (2005) 432–442.
[13] I. Turel, P. Zivec, A. Pevec, S. Tempelaar, G. Psomas, Compounds of antibacterial agent ciprofloxacin and magnesium—crystal structures and molecular modeling calculations, Eur. J. Inorg. Chem. 23 (2008) 3718–3727.
[14] P. Drevensek, N.P. Ulrih, A. Majerle, I. Turel, Synthesis, characterization and DNA binding of magnesium–ciprofloxacin (cfH) complex $[Mg(cf)_2]\cdot 2.5H_2O$, J. Inorg. Biochem. 100 (2006) 1705–1713.
[15] G. Psomas, A. Tarushi, E.K. Efthimiadou, Synthesis, characterization and DNA-binding of the mononuclear dioxouranium(VI) complex with ciprofloxacin, Polyhedron 27 (2008) 133–138.
[16] G. Psomas, Mononuclear metal complexes with ciprofloxacin: synthesis, characterization and DNA-binding properties, J. Inorg. Biochem. 102 (2008) 1798–1811.
[17] D. Curman, P. Zivec, I. Leban, I. Turel, A. Polishchuk, K.D. Klika, E. Karaseva, V. Karasev, Spectral properties of Eu(III) compound with antibacterial agent ciprofloxacin (cfqH). Crystal structure of $[Eu(cfqH)(cfq)(H_2O)_4]Cl_2\cdot 4.55H_2O$, Polyhedron 27 (2008) 1489–1496.
[18] I. Turel, The interactions of metal ions with quinolone antibacterial agents, Coord. Chem. Rev. 232 (2002) 27–48.
[19] A.L. Abuhijleh, Ternary copper(II) complexes of the anticonvulsant drug valproate with diimines as superoxide dismutase mimics, J. Inorg. Biochem. 68 (1997) 167–175.
[20] J.C. Casanova, G. Alzuci, J. Borras, J. Latorre, M. Sanau, S.G. Granda, Coordination behavior of sulfathiazole. Crystal structure of $[Cu(sulfathiazole)(py)_3Cl]$ superoxide dismutase activity, J. Inorg. Biochem. 60 (1995) 219–230.
[21] M.N. Patel, D.S. Gandhi, P.A. Parmar, SOD mimic activity, DNA binding and in-vitro antibacterial studies of drug based copper(II) complexes, Inorg. Chem. Commun. 13 (5) (2010) 618–621.
[22] M.F. Iskander, L. EL–Sayed, N.M.H. Salem, R.W. Warner, Synthesis, characterization and magnetochemical studies of dicopper(II) complexes derived from bis(N-salicylidene) dicarboxylic acid dihydrazides, J. Coord. Chem. 58 (2) (2005) 125–139.
[23] J. Hernandez-Gil, L. Perello, R. Ortiz, G. Alzuet, M. Gonzalez-Alvarez, M. Liu-Gonzalez, Synthesis, structure and biological properties of several binary and ternary complexes of copper(II) with ciprofloxacin and 1,10 phenanthroline, Polyhedron 28 (2009) 138–144.
[24] D.K. Saha, U. Sandbhor, K. Shirisha, S. Padhye, D. Deobagkar, C.E. Ansond, A.K. Powell, A novel mixed-ligand antimycobacterial dimeric copper complex of ciprofloxacin and phenanthroline, Bioorg. Med. Chem. Lett. 14 (2004) 3027–3032.
[25] D.K. Saha, S. Patitungkho, S. Padhye, D.N. Deobagkar, A. Ozarkar, M.M. Bhadbhade, R.G. Gonnade, Metalloantitubercular compounds Part 3: synthesis, crystal structure, spectroscopy, electrochemistry and antimycobacterial activity of the copper(II) ciproploxacin (cfH) complex and its phenanthroline adduct, Transit. Met. Chem. 30 (2005) 334–340.
[26] P. Ruız, R. Ortiz, L. Perello, G. Alzuet, M. Gonzalez-Alvarez, M. Liu-Gonzalez, F. Sanz-Ruız, Synthesis, structure, and nuclease properties of several binary and ternary complexes of copper(II) with norfloxacin and 1,10 phenantroline, J. Inorg. Biochem. 101 (2007) 831–840.
[27] L.Y. Wang, Q.Y. Chen, J. Huang, K. Wang, C.J. Feng, Z.R. Gen, Synthesis, characterization, and bioactivities of copper complexes with N-substituted di(picolyl) amines, Transit. Met. Chem. 34 (2009) 337–345.
[28] B.N. Figgis, J. Lewis, in: J. Lewis, R.G. Wilkins (Eds.), Modern Coordination Chemistry: Principles and Methods, Interscience, New York, 1960, p. 400, (Chapter 6).
[29] R.A. Day Jr., A.L. Underwood, Quantitative Analysis, sixth ed. Prentice-Hall of India PVT LTD, 2006.
[30] K. Nakamoto, Infrared and Raman Spectra of Inorganic and Coordination Compounds, fourth ed. A Wiley Interscience Publication, New York, 1986.
[31] Z.H. Chohan, C.T. Supuran, A. Scozzafava, Metal binding and antibacterial activity of ciprofloxacin complexes, J. Enzyme Inhib. Med. Chem. 20 (2005) 303–307.
[32] I. Turel, I. Leban, N. Bukovec, Crystal structure and characterization of the bismuth(III) compound with quinolone family member (ciprofloxacin). Antibacterial study, J. Inorg. Biochem. 66 (1997) 241–245.
[33] M. Shebl, Synthesis, spectral studies, and antimicrobial activity of binary and ternary Cu(II), Ni(II), and Fe(III) complexes of new hexadentate Schiff bases derived from 4,6-diacetylresorcinol and amino acids, J. Coord. Chem. 62 (19) (2009) 3217–3231.
[34] Y. Anjaneyula, R.P. Rao, Preparation, characterization and antimicrobial activity studies on some ternary complexes of Cu(II) with acetylacetone and various salicylic acids, Synth. React. Inorg. Met.-Org. Chem. 16 (1986) 257.
[35] T. Rosu, E. Pahontu, C. Maxim, R. Georgescu, N. Stanica, G.L. Almajan, A. Gulea, Synthesis, characterization and antibacterial activity of some new complexes of Cu(II), Ni(II), VO(II), Mn(II) with Schiff base derived from 4-amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one, Polyhedron 29 (2010) 757–766.
[36] B. Peng, H. Chao, B. Sun, H. Li, F. Gao, L.-N. Ji, DNA interactions of a functionalized ruthenium(II) mixed-polypyridyl complex $[Ru(bpy)_2ppd]^{2+}$, J. Inorg. Biochem. 100 (2006) 1487–1494.
[37] L.S. Kumar, K.S. Prasad, H.D. Revanasiddappa, Synthesis, characterization, antioxidant, antimicrobial, DNA binding and cleavage studies of mononuclear Cu(II) and Co(II) complexes of 3-hydroxy-N′-(2-hydroxybenzylidene)-2-naphthohydrazide, Eur. J. Chem. 2 (3) (2011) 394–403.
[38] J. Liu, H. Zhang, C. Chen, H. Deng, T. Lu, L. Ji, Interaction of macrocyclic copper(ii) complexes with calf thymus DNA: effects of the side chains of the ligands on the DNA-binding behaviours, Dalton Trans. (2003) 114–119.
[39] A. Tarushi, C.P. Raptopoulou, V. Psycharis, A. Terzis, G. Psomas, D.P. Kessissoglou, Zinc(II) complexes of the second-generation quinolone antibacterial drug enrofloxacin: Structure and DNA or albumin interaction, Bioorg. Med. Chem. 18 (2010) 2678–2685.
[40] K.C. Skyrianou, F. Perdih, A.N. Papadopoulos, I. Turel, D.P. Kessissoglou, G. Psomas, Nickel–quinolones interaction, J. Inorg. Biochem. 105 (2011) 1273–1285.
[41] S. Satyanaryana, J.C. Daborusak, J.B. Chaires, Tris(phenanthroline)ruthenium(II) enantiomer interactions with DNA: mode and specificity of binding, Biochemistry 32 (10) (1993) 2573–2584.
[42] Y.J. Liu, X.Y. Guan, X.Y. Wei, L.X. He, W.J. Mei, J.H. Yao, Ruthenium(II) complexes containing 2,9-dimethyl-1,10-phenanthroline and 4,4′-dimethyl-2,2′-bipyridine as ancillary ligands: synthesis, characterization and DNA-binding, Transit. Met. Chem. 33 (2008) 289–294.
[43] R.P. Hertzberg, P.B. Dervan, Cleavage of double helical DNA by methidium-propyl-EDTA-iron(II), J. Am. Chem. Soc. 104 (1982) 313–315.
[44] B.N. Meyer, N.R. Ferrigni, J.E. Putnam, J.B. Jacobsen, D.E. Nicholsand, J.L. Mclaughlin, Brine Shrimp: A Convenient General Bioassay for Active Plant Constituents, Planta. Med. 45 (1982) 31–34.