Biointerface Research in Applied Chemistry
Platinum Open Access Journal (ISSN: 2069-5837)

Article
Volume 11, Issue 4, 2021, 12447 - 12453
https://doi.org/10.33263/BRIAC114.1244712453

Synthesis, Characterization, and Biological Studies of *In Vitro* Antibacterial, and Cytotoxic Activities of Norfloxacin Based Square Pyramidal Copper(II) Complexes

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Received: 8.12.2020; Revised: 3.01.2021; Accepted: 4.01.2021; Published: 6.01.2021

Abstract: The substantial increase in copper-containing complexes' research to treat bacterial infections encouraged us to synthesize fluoroquinolone drug norfloxacin (NFLH) based copper complexes. In this work, we presented the synthesis of mixed ligand Cu complexes with the basic structural formula [Cu(NFL)(A_1)Cl], where An is isatin-based ligands. The characterization of compounds by Mass, FT-IR, UV-Vis spectroscopy, magnetic measurement, and conductivity measurement revealed the neutral nature and square pyramidal geometry of all the copper compounds. The biological screening of compounds displayed the potent bacteriostatic activity of copper compounds compare with the standard drug against four medically significant pathogens. The in vitro cytotoxic activity of copper compounds was checked on artemia cyst. The biological studies data are promising for their application as medicinally important compounds for future perspective.

Keywords: fluoroquinolone drug; spectroscopy; bacteriostatic activity; cytotoxicity.

1. Introduction

The success of cisplatin has influenced the anticancer drug discovery methodologies and open up a new era for bioinorganic researchers to study and develop metal-based drugs [1-3]. The medicinal role of metal-based compounds has been widely explored [4, 5]. In metal-based drugs, the metal-ligands space configuration allows the molecule to interact with a definite molecular target, enriched by modifications of the ligand structure. Also, the metal complexes are involved in biological redox chemistry and can interact with bio-molecules. The notable point is the practice of copper-based biologically active complexes for these studies [6-13]. Copper is involved in numerous physiological cellular processes and cytotoxic; hence the copper-based compounds were found promising particularly [14, 15]. A recent study found that copper complex may selectively inhibit tumor cell growth and induce apoptosis through the mitochondrial apoptotic pathway [16], down-regulation of anti-apoptosis proteins [17], or via accumulation of ROS [18, 19]. Recent studies indicated that copper-based Schiff base compounds exhibited better antitumor and antibacterial activities than ligands. The copper complexes are also known to possess good antibacterial activity [20-23].

Norfloxacin is a xenobiotic broad-spectrum bactericidal quinolone monocarboxylic acid, which functions as a DNA synthesis inhibitor through binding with DNA gyrase. The binding of norfloxacin and subsequent alteration in the normal function of DNA gyrase has been creating enormous interest among medicinal researchers to explore antibacterial and antitumor activities of norfloxacin based compounds [24, 25]. So, we synthesized norfloxacin based mixed ligand copper complexes with isatin ligands. The neutral Cu(II) complexes were characterized by mass spectrometry, FT-IR, electronic spectroscopy, conductivity, and magnetic moment measurement. Also, the drug-based compounds were studied for antibacterial and cytotoxic activities.

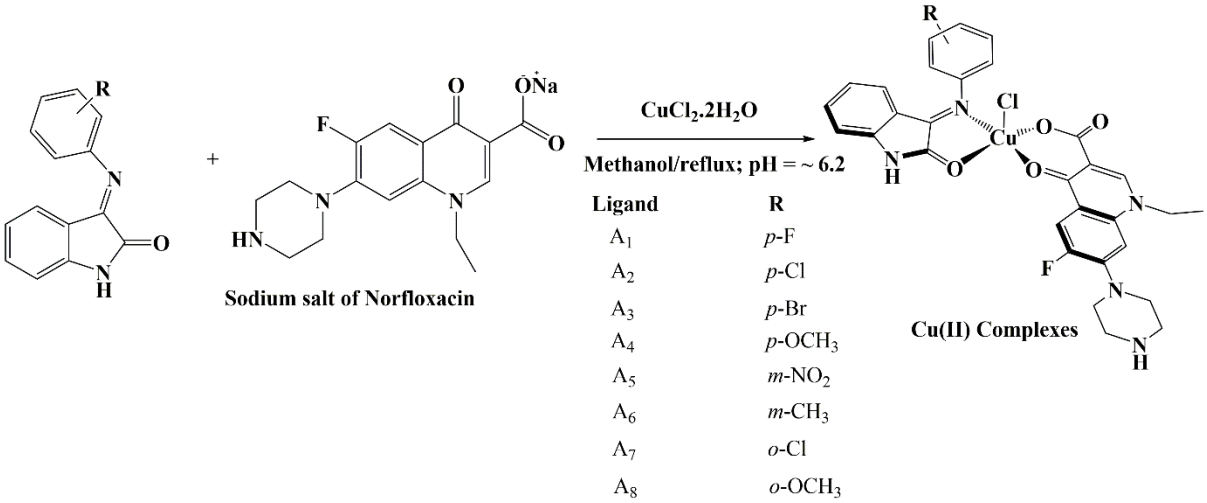
2. Materials and Methods

2.1. Chemicals and materials.

Analytical grade chemicals and solvents were used for all the studies. Isatin, *p*-chloro aniline, *m*-chloro aniline, *o*-chloro aniline, *p*-bromo aniline, *m*-nitro aniline, *p*-nitro aniline, *p*-hydroxybenzoic acid, *p*-methoxy aniline, and *p*-toluidine were procured from Sigma Aldrich (India). IR spectra were obtained on an FT-IR Shimadzu instrument in the range 4000–400cm$^{-1,}$ and the sample was prepared as KBr pellets. The electronic spectra were obtained on a UV-160A, Shimadzu (Japan) UV-visible spectrophotometer. The Gouy's balance was used for magnetic moment measurement.

2.2. Synthesis.

The ligands (isatin derivatives) were synthesized according to the reported procedure [26]. The copper complexes [Cu(NFL)(A^n)Cl] were synthesized by stirring $CuCl_2{\cdot}2H_2O$ (1.5 mmol) and ligand (A_n) (1.5 mmol) solution in an RBF for 10 minutes. This is followed by adding the norfloxacin (NFLH) solution (1.5 mmol, methanolic solution neutralized by sodium methoxide) at pH ~6.2. The reaction mixture was refluxed on a steam bath for 1.5 h. The completion of the reaction was monitored on TLC. The reaction mass was concentrated under vacuum, filtered, and washed by dichloromethane. The air drying of reaction mass yielded an amorphous product of light brown color (Scheme 1).



Scheme 1. Reaction scheme for synthesis of metal complexes.

2.3. In vitro antibacterial assay.

The bacteriostatic activity of the metal salt, norfloxacin (standard), and complexes were screened *in vitro* against two Gram$^{(+ve)}$: *Bacillus subtilis*, *Staphylococcus aureus* microorganisms; and two Gram$^{(-ve)}$: *Escherichia coli*, *Serratia marcescens*; in terms of MIC (minimum inhibitory concentration) by using reported procedure [27]. The double dilution method was used to incubate bacterial culture in Luria Broth at 37 oC, resulting in turbid solution after 18 h. The clear solution is an indication of the inhibitory effect shown by the compounds under study.

2.4. Brine shrimp lethality assay.

According to the literature, the assay was performed in which the hatching of *artemia cyst* eggs in artificial seawater for 2 days produced nauplii. A stock solution of complex (concentration 10 mg/10 mL DMSO) was transferred to vials so that the final concentration of complexes in solution remains 2, 4, 8, 12, 16, and 20 µg mL^{-1}. The 10 nauplii were added to a total volume of 2.5 mL per vial with seawater. The mortality rate of nauplii was determined after 24 h. The log [complex] was plotted against the %mortality of nauplii, which gives the LC_{50} value [28].

3. Results and Discussion

3.1. Characterization.

The copper compounds were characterized by diverse spectrometric (Mass, FT-IR, electronic spectra) and analytical (magnetic moment and conductivity measurement) techniques. The Physico-chemical parameters are provided in Table 1. The estimation of metal content was performed by the spectrophotometric method [29]. The spectrophotometry titrimetric data supports the calculated copper percentage. The norfloxacin pyridine ring ν(C=O) band shifts from 1730 cm^{-1} to 1615-1632 cm^{-1} in the infrared spectra of complexes, which agrees to the pyridine carbonyl oxygen participation as coordinating atom (Table 2). The difference between the asymmetric and symmetric stretching frequency of –COO (Δν) for the complexes is obtained ~200 cm^{-1}. The difference of about 200 cm^{-1} indicates unidentate coordination of copper with the carboxylic oxygen atom. The additional bands in the range 542-552 cm^{-1} and 515-523 cm^{-1} in the infrared spectra of complexes are assigned to ν(M-N) and ν(M-O), respectively. The mass spectrum of complex-1 displayed a molecular ion peak at 686.7 m/z (Figure 1).

Table 1. Physical characterization data of complexes.

Complexes	Empirical formula	Molecular weight	%Cu		m.p. /^{0}C	μ_{eff} /B.M.	Molar conductivity/ µS cm^{-1}
			Theoretical	Experimental			
[Cu(NFL)(A^1)Cl] (1)	$C_{32}H_{32}ClCuF_2N_5O_4$	687.62	9.24	8.99	246	1.79	24.0
[Cu(NFL)(A^2)Cl] (2)	$C_{32}H_{32}Cl_2CuFN_5O_4$	704.08	9.03	8.71	248	1.80	23.6
[Cu(NFL)(A^3)Cl] (3)	$C_{32}H_{32}BrClCuFN_5O_4$	748.53	8.49	8.26	>250	1.85	23.8
[Cu(NFL)(A^4)Cl] (4)	$C_{33}H_{35}Cl_2CuFN_5O_5$	699.66	9.08	8.78	>250	1.82	22.5
[Cu(NFL)(A^5)Cl] (5)	$C_{32}H_{32}ClCuFN_6O_6$	714.63	8.89	8.57	241	1.78	23.5
[Cu(NFL)(A^6)Cl] (6)	$C_{33}H_{35}ClCuFN_5O_4$	683.66	9.29	9.02	245	1.76	22.9
[Cu(NFL)(A^7)Cl] (7)	$C_{32}H_{32}Cl_2CuFN_5O_4$	704.08	9.03	8.75	242	1.86	21.8
/[Cu(NFL)(A^8)Cl] (8)	$C_{33}H_{35}Cl_2CuFN_5O_5$	699.66	9.08	8.84	>250	1.89	21.4

The different m/z observed at 368.1 and 318 are assigned to norfloxacin and isatin ligands fragments, respectively. The μ_{eff} (magnetic moment value) of complexes is observed in a 1.76-1.89 BM (Table 1), demonstrating an unpaired electron to the Cu^{+2} in complexes.

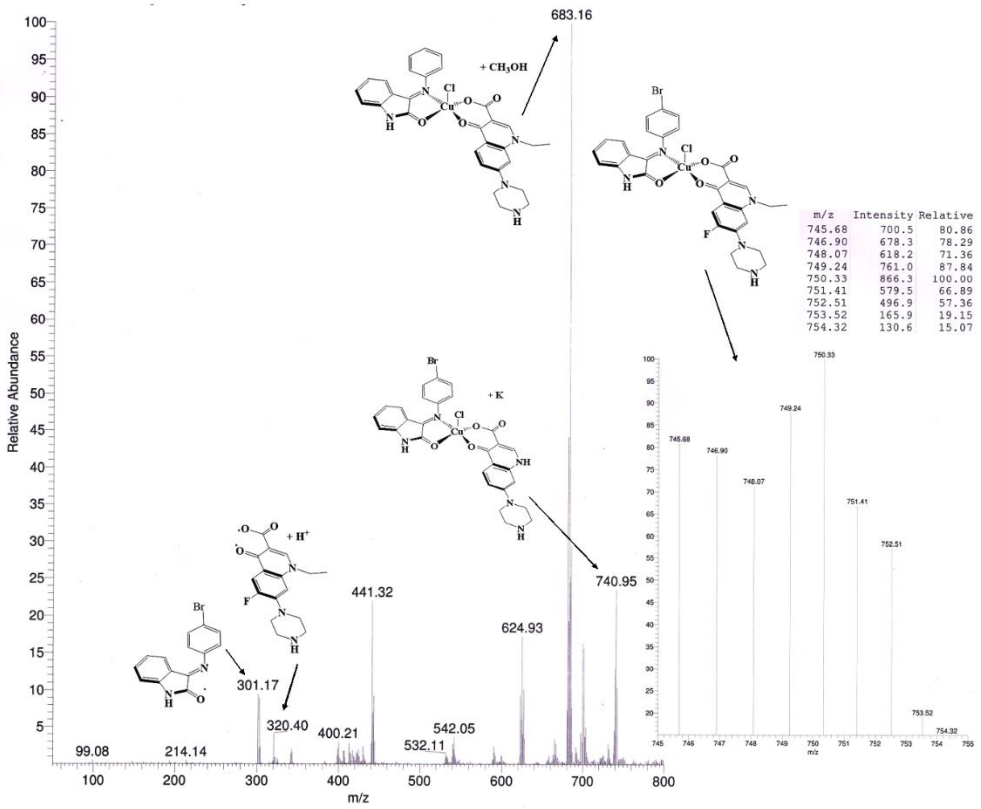


Figure 1. Mass spectra of complex -1.

Table 2. IR spectra data.

Complexes	ν(C=O) pyridone /cm^{-1}	ν(COO)$_{as}$ /cm^{-1}	ν(COO)$_{s}$ /cm^{-1}	Δν /cm^{-1}	ν(M-N) /cm^{-1}	ν(M-O) /cm^{-1}
Norfloxacin	1730	1642	1336	306		-
1	1632	1561	1356	205	548	523
2	1622	1572	1371	201	552	522
3	1628	1560	1358	202	543	518
4	1625	1569	1372	197	546	520
5	1620	1572	1363	209	542	516
6	1615	1570	1365	205	551	517
7	1627	1568	1368	200	550	522
8	1621	1562	1363	199	548	515

3.2. In vitro antibacterial activity.

The drug resistance acquired among pathogenic bacteria is a matter of concern globally. This problem has stimulated the research to discover antibacterial agents that may have an alternate mechanism of action. The metal chelation of the drug could be a synthetic alternative for such a purpose. So we studied the bacteriostatic activity of norfloxacin based Cu(II) complexes in terms of MIC against two gram(+ve), and two gram(-ve) pathogens. In vitro antibacterial activity results show that all the metal complexes have exceptionally well potency than metal salt. Some complexes have more potency than norfloxacin (standard) against all tested pathogens (Table 3). Among all, the complex 1 bearing electron-withdrawing F atom

shows very good potency against all the microorganisms. Tweedy's chelation theory may be applicable for such a higher potency of metal complexes, which explains that chelation results in π- electrons delocalization over the whole ring. The sharing of Cu^{+2} charge with the orbital of ligand (orbital overlap) reduces the polarity, which increases the lipophilic nature of complexes [30]. This facilitates the complex's penetration into lipid membranes and results in improved potency.

Table 3. Biological activities data of metal complexes.

Compounds	Antibacterial activity (MIC, µM)				Cytotoxic activity (LC_{50}, µM)
	Gram-positive		Gram-negative		
	S. aureus	*B. subtilis*	*S. marcescens*	*E. coli*	
$CuCl_2 \cdot 2H_2O$	2698.0	2815.0	2756.0	3402.0	-
Norfloxacin	2.5	2.5	4.1	2.8	-
1	2.4	2.1	3.9	2.5	7.2
2	2.5	2.2	4.2	2.5	7.9
3	2.7	2.8	4.5	2.6	8.9
4	2.9	3.0	4.5	2.7	10.5
5	2.6	2.4	4.0	2.6	8.2
6	3.0	3.2	5.0	3.5	11.1
7	2.5	2.3	4.2	2.5	8.1
8	3.5	3.2	6.0	4.0	11.5

3.3. Cytotoxic activity.

Brine shrimp lethality bioassay cytotoxicity test is extensively used for screening of toxicity of a wide range of natural and synthetic materials. It's a preliminary toxicity assay for further investigations on higher animal models [31]. The cytotoxicity data (LC_{50} values) are revealed in Table 3. The LC_{50} data demonstrates the potent cytotoxic nature of complexes. Complexes 1, 2, 5, and 7 are more cytotoxic than rest complexes. Complexes 6 and 8 are the least cytotoxic in nature.

4. Conclusions

The norfloxacin based Cu(II) complexes of isatin derivatives were synthesized and characterized by various analytical and spectroscopic techniques. The shift in norfloxacin infrared frequencies recommends the pyridine ring and COO functional group's oxygen atom as the coordinating atoms. The presence of an additional ν(M-N) and ν(M-O) infrared bands proposes coordination of amide O atom and C=N N atoms of isatin ligand as the coordinating units. The magnetic moment estimation recommends the presence of an unpaired electron with Cu^{+2} ion, and conductivity estimation suggests the non-electrolytic nature of complexes. The UV-vis. spectrophotometry proposes the square pyramidal geometry of ligands around the metal ion. The complexes were screened for antibacterial and cytotoxicity. The MIC data reveal that some complexes are more potent bacteriostatic agents than standard drug norfloxacin, with an increase in lipophilicity due to chelation could be a probable reason, which increases the permeability of complexes. The LC_{50} values evaluated by brine shrimp lethality bioassay proposes metal complexes potent cytotoxic nature.

Funding

This research received no external funding.

Acknowledgments

The authors are thankful to the authority of C. U. Shah University for providing the necessary research facility and giving permission to carry out the research work.

Conflicts of Interest

The authors declare no conflict of interest.

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