doi.org/10.36721/PJPS.2022.35.5.REG.1437-1443.1

Synthesis, characterization and assessment of antioxidant and antibacterial potential of dihydroquinoline-3-carboxylic acid and their metal derivatives

Gul Muhammad[1,3], Asia Naz*[1], Fouzia Haider[2], Almas Kanwal[3], Nisar Ahmed Shahwani[3], Faria Khurshid[5], Ghulam Farooq[4], Muhammad Shoaib[1], Toqeer Ahmed Rao[6], Mohammad Arsalan[8] and Sobia Iftikhar Ali[7]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Pakistan
[2]Department of Microbiology, University of Karachi, Karachi, Pakistan
[3]Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Health Sciences, University of Balochistan, Quetta, Pakistan
[4]ICCBS, HEJ, University of Karachi, Karachi, Pakistan
[5]Department of Pharmacology, Faculty of Pharmacy and Health Sciences, University of Balochistan, Quetta, Pakistan
[6]Department of Botany, Federal Urdu University of Arts, Sciences and Technology, Karachi, Pakistan
[7]Department of Pharmaceutical Chemistry, Institute of Pharmaceutical Sciences, Jinnah Sindh Medial University, Karachi, Pakistan
[8]Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan

Abstract: Metal complexes of drug are used to inhibit growth of pathogenic microorganisms and reduces drug resistance. Moxifloxacin is a dihydroquinoline-3-carboxylic acid 4th generation fluoroquinolone antibiotic that has tendency to bind with metal ions. In current study four moxifloxacin-metal complexes i.e. Moxifloxacin-sliver (Moxi-Ag), Moxifloxacin-rhodium (Moxi-Rh), Moxifloxacin-titanium (Moxi-Ti) and Moxifloxacin-rubidium (Moxi-Rb) have been synthesized and evaluated for antibacterial activities against resistant microorganisms along with antioxidant effects. The structure elucidation was carried out using FTIR, ^{1}H- NMR, and UV-Vis spectroscopy. Agar well diffusion method and DPPH (1, 1- dipheny1-2-picrylhydrazyl) methods were used to study the antibacterial and antioxidant activity respectively. Both ^{1}H NMR and FTIR spectra clearly showed that Moxi-metal complexes are formed due to change in their carboxyl stretching band in IR, H-2 and H-5 peak position in ^{1}H NMR. All the Moxi-metal complexes showed distinguished antibacterial effects against both *Gram-negative* and *Gram-positive* bacteria as compared to drug which was found resistant against many microorganisms. Moxi-Rb and Moxi-Ag metal complexes showed higher antioxidant activity (IC_{50} values range from 8.26 - 9.19 µg/ml) than Moxi-Ti and Moxi-Rh metal complexes (IC_{50} range from 11.23 - 14.65 µg/ml).

Keywords: Moxifloxacin**,** antibacterial activity, drug resistance, antioxidant activity, metal derivatives of dihydroquinolone-3- carboxylate.

INTRODUCTION

The health system of world is severally and rapidly affected by resistant bacteria nowadays, the efficacy of many antibiotics is at dangerous stage, previously these antibiotics used to save the lives of hundreds of peoples around the globe. The misuse, overuse of existing antibiotics, decrease in drug development due to rising regulatory capital requirements are the main issues behind the rise in the crisis of antibiotic resistance (Blair *et al*., 2015, Ventola, 2015). In the past decades, only few new antibiotics i.e. tigecycline from group of lipopeptides and tedizolid from group of oxazolidinones have been developed and approved by FDA which showed narrow spectrum activity only against *Gram-positive* bacteria (Luepke *et al*., 2017). Broad spectrum dihydroquinoline-3-carboxylic acid fluoroquinolones such as gemifloxacin, moxifloxacin and gatifloxacin became available for general use in 2004 (Andriole, 2005, Kondaiah, *et al*., 2017). Moxifloxacin is chemically [7 - [(4*aS*,7*aS*) - 1,2,3,4,4*a*,5,7,7*a* -octahydropyrrolo [3,4-b]pyridine – 6 - yl] -1-cyclopropyl - 6-fluoro - 8-methoxy - 4-oxoquinoline – 3 - carboxylic acid; hydrochloride] (Kondaiah *et al*., 2017).

In last decade many microorganisms showed resistance to moxifloxacin (Luepke *et al*., 2017, Murray *et al*., 2017). There are two strategies commonly used to overcome drug resistance hurdles; new drug development and to prepare modified drug delivery system. For new drug development at least ten years and 10 million dollars are required which is an expensive and time consuming process (Fernandes and Martens, 2017). Generally, the preparation of new drug delivery system of existing antibiotics is more appropriate approach to circumvent the drug resistance issues (Kondaiah *et al*., 2017, Skuredina *et al*., 2017). There are several drug delivery systems based on organic and inorganic materials which are physically or chemically conjugated with drugs (Cuprys, *et al*., 2018, Refaat *et al*., 2016, Skuredina *et al*., 2017).

**Corresponding author:* e-mail: asia.naz@uok.edu.pk

However, the inorganic conjugation or complexation is more easy, economical and less time consuming method (Ali *et al*., 2017, Elshafie *et al*., 2019).

The moxifloxacin–imidazole-metal complexes, Ti, Y, Pd and Ce metal complexes have been synthesized and evaluated for antibacterial activities (Issa *et al*., 1995, Sadeek *et al*., 2011, Soayed *et al*., 2013). This study is aimed to synthesis moxifloxacin metal complexes to combat bacterial resistance and restore drug broad spectrum activity and anti-oxidant potential.

The aim and objective of this research is to introduce more effective antibacterial and antioxidant derivatives then the original parent drug.

MATERIALS AND METHODS

Sigma-Aldrich Limited were used for purchasing of standard drug (moxifloxacin) and all metals (Ag, Rh, Ti and Rb). All the chemicals and reagents were of analytical grade and no need of further purification before use.

Synthesis of drug-metal complexation

The moxifloxacin (1mmole) and metal ions (0.5mmole) were dissolved separately in methanol. The solutions were mixed and refluxed for 8 -12hours at 60^0C with continuous stirring. The resultant product was concentrated by evaporation and was precipitated using chilled chloroform. The precipitates were recrystallized.

Moxifloxacin spectral characterization

^{1}H NMR (D_2O) µg/ml : δ 8.57 (s, 1H; H-2, -CH), 6.89 (d, 1H, H-5, -CH, J=13.8), 4.67 (s, 2H, H-15, -CH_2), 4.06 (m, 2H, H-13, N-CH_2), δ 3.91 (d, H, H-14', -CH, J=4.2), 3.70 (m, 3H, H-10 & 10', CH-CH_2), δ3.50 (s, 3H,- OCH_3), δ 2.76 (d , 1H, --NH-, J= 3.3), 1.88 (t, 4H, H-11 & H-12, -CH_2-CH_2--), δ1.22 (q, 1H, cyclopropane, - CH), 1.09 (m, 2H, cyclopropane, CH_2), 0.91 (q, 2H, cyclopropane, - CH). IR (cm-1, KBr): 3530 (carboxylic's O-H stretching), 3156 (C-H stretching of aromatic ring), 2978, 2800 (C-H stretching of aliphatic chain), 1709 (carboxylic's C=O stretching), 1620 (N-H bending), 1592, 1533 (C-C stretching of aromatic ring), 1490 (C=C stretching of aromatic ring), 1422, 1323 (C-H bending of aliphatic chain), 1245 (Stretching of C-F bond), 1184 (Stretching of C-O bond), 1166 (Stretching of C-N bond), 1111 (Stretching of C-C single bond), 1016, 991 (bending of phenyl's C-H bond). UV/Vis (nm. 190.00 to 500.00): 195, 292, 332.

Moxifloxacin-silver spectral characterization

^{1}H NMR (D_2O) µg/ml : δ 8.67 (s, 1H; H-2, -CH), 7.81 (q, 1H, H-5, -CH), 4.70 (s, 2H, H-15, -CH_2), 4.09 (s, 2H, H-13, N-CH_2), δ 3.74 (d, H, H-14', -CH, J= 8.1), 3.65 (s, 1H, H-10', -CH), 3.33 (t, , 1H, --NH-), δ3.26 (s, 3H,- OCH_3), 2.884 (s, 2H, H-10, --CH_2), δ2.72 (s, 2H,H-11,N-CH_2), δ 1.25 (q, 4H, cyclopropane, -CH_2-CH_2--), 1.07 (s, 1H, cyclopropane, - CH) 1.03 (s, 2H, H-12, CH_2). IR (KBr, cm-1): 3300 (carboxylic's O-H stretching), 3000 (C-H stretching of aromatic ring), 2930, 2850 (C-H stretching of aliphatic chain), 1690 (carboxylic's C=O stretching), 1600 (N-H bending), 1505 (C-C stretching of aromatic ring), 1420 (C=C stretching of aromatic ring), 1350 (C-H bending of aliphatic chain), 1250 (C-F stretching), 1160 (C-N stretching), 1105 (C-C stretching), 1030, 810 (phenyl's C-H bending). UV/Vis (nm.190.00 to 500.00): 199, 291 and 330.

Moxifloxacin-rhodium spectral characterization

^{1}H NMR (D_2O) µg/ml : δ 8.81 (s, 1H; H-2, -CH), 7.47 (s, 1H, H-5, -CH), 4.69 (s, 2H, H-15, –CH_2), 4.13 (t, 2H, H-13, N-CH_2), 3.92 (s, 1H, H-14', --CH), δ 3.82 (t, 2H, H-10, -CH_2), 3.66 (d, 1H, H-10' , --CH, J= 9.6), δ3.55 (s, 3H, -OCH_3), 3.36 (d, 2H,H-12, --CH_2, J= 9.0), δ2.81 (s, 1H, --NH-), 1.87 (d, 4H, cyclopropane, CH_2-CH_2, J= 18.6), 1.23 (s, 1H, cyclopropane, - CH), 1.09 (s, 2H, H-11, CH_2). IR (KBr, cm^{-1}): 3370 (carboxylic's O-H stretching), 3020 (C–H stretching of aromatic ring), 2940 (C-H stretching of aliphatic chain), 1710, 1700 (carboxylic's C=O stretching), 1610, 1590 (N-H bending), 1520 (C–C stretching of aromatic ring), 1445 (C=C stretching of aromatic ring), 1320, 1370 (C-H bending of aliphatic chain), 1210, 1240 (C–F stretching), 1180 (C-O stretching), 1120 (C-N stretching), 1105 (C-C stretching), 1005, 980, 910, 830 (phenyl's C-H bending). UV/Vis (nm.190.00 to 500.00): 196, 298, and 328.

Moxifloxacin-titanium spectral characterization

^{1}H NMR (D_2O) µg/ml : δ 8.37 (s, 1H; H-2, -CH), 6.69 (d, 1H, H-5, -CH, J=13.8), 4.47 (s, 2H, H–15, -CH_2), 4.26 (m, 2H, H-13, N-CH_2), δ 3.71 (d, H, H-14', -CH, J=4.2), 3.50 (m, 3H, H-10 & 10', CH-CH_2), δ3.30 (s, 3H,-OCH_3), δ 2.56 (d , 1H, --NH-, J= 3.3), 1.68 (t, 4H, H-11 & H-12, -CH_2-CH_2-), δ1.02 (q, 1H, cyclopropane, - CH), 0.89 (m, 2H, cyclopropane, CH_2), 0.71 (q, 2H, cyclopropane, - CH). IR (KBr, cm^{-1}): 3300 (carboxylic's O–H stretching), 3005 (C-H stretching of aromatic ring), 2940, 2860 (C-H stretching of aliphatic chain), 1715 (carboxylic's C=O stretching), 1620 (N-H bending), 1520 (C-C stretching of aromatic ring), 1460 (C=C stretching of aromatic ring), 1310, 1350 (C-H bending of aliphatic chain), 1280 (C-F stretching), 1185 (C-O stretching), 1160 (C-N stretching), 1100 (C-C stretching), 1030, 1002, 955, 870, 805, 730 (phenyl's C-H bending). UV/VIS (nm.190.00 to 500.00): 193, 294, and 325.

Moxifloxacin-rubidium spectral characterization

^{1}H NMR (D_2O)µg/ml : δ 8.51 (s, 1H; H-2, -CH), 7.49 (s, 1H, H-5, -CH), 4.49 (s, 2H, H-15, -CH_2), 4.23 (t, 2H, H-13, N-CH_2), 3.62 (s, 1H, H-14', --CH), δ 3.52 (t, 2H, H-10, -CH_2), 3.36 (d, 1H, H-10', --CH, J= 9.6), δ3.25 (s, 3H, -OCH_3), 3.06 (d, 2H,H-12, --CH_2, J= 9.0), δ2.85 (s, 1H, --NH–), 1.88 (d, 4H, cyclopropane, CH_2-CH_2, J= 18.6), 1.33 (s, 1H, cyclopropane, - CH), 1.06 (s, 2H, H-11, CH_2). IR (KBr, cm^{-1}): 3320 (carboxylic's O-H stretching), 3000

(C–H stretching of aromatic ring), 2930, 2870 (C-H stretching of aliphatic chain), 1710 (carboxylic's C=O stretching), 1620 (N-H bending), 1510 (C-C stretching of aromatic ring), 1450 (C=C stretching of aromatic ring), 1330, 1350 (C-H bending of aliphatic chain), 1210, 1280 (C–F stretching), 1180 (C-O stretching), 1130 (C-N stretching), 1100 (C-C stretching), 1030, 970, 905, 815, 720 (phenyl's C-H bending). UV/Vis (nm. 190.00 to 500.00): 200, 294, and 333.

Antibacterial effects

Antibacterial effects of moxifloxacin and their metal complexes were evaluated by agar well diffusion method(Singh and Tafida, 2014). The suspension of bacteria was first inoculated (freshly grown culture) by using sterile saline (5ml) and turbidity was maintained optically equivalent to 0.5 McFarland standard. This suspension was then evenly swabbed onto labeled Mueller Hinton agar plates. The plates were allowed to dry for 5 minutes before punching wells with the help of sterile metallic borer (7mm diameter). Each of the holes was filled with different dilutions. The plates were then incubated at 37°c for 24 hours. The diameters of zones of inhibition were measured in mm and the mean value for each organism was recorded. Growth inhibition was calculated with reference to the positive control moxifloxacin. The mean and standard deviation is done by using IBM SPSS statistics 21 premium.

Antioxidant investigation

Mix 2ml freshly prepared DPPH radical (0.1mM) methanolic solution with 2ml methanolic solution of drug and metal derivatives of dihydroquinolone-3- carboxylate (moxi-Ag, moxi-Rh, moxi-Ti and moxi-Rb) of different concentrations (50, 100 and 200µg/ml). Shake the prepared mixture vigorously and then allowed the mixture to stand for 30 minutes at room temperature. Use same conditions for both DPPH redical (blank) and standard compound (ascorbic acid). The UV/Visible spectro-photometer was used to record the absorbance at 517 nm in triplicate. All the blank, standard and sample's absorbance was used to calculate the DPPH scavenging effect in percentage by using following formula;

$$\text{DPPH scavenging effect (\%)} = \frac{A_o - A_1}{A_o} \times 100$$

Where: Ao = absorbance of blank, A_1= absorbance of test.

STATICAL ANALYSIS

The data was presented in mean ± S.D for continues variables, the difference between the activities were analyzed by IC_{50}. All analysis were done my using Microsoft Office (Excel).

RESULTS

Metal derivatives of dihydroquinoline-3-carboxylate i.e. moxi-Ag, moxi-Rh, moxi-Ti and moxi-Rb were synthesized by a reported method (Gul Muhammad, 2019) and different spectroscopic techniques were used for characterization.

UV/Visible spectroscopic studies

The UV-Visible spectrum of moxifloxacin showed an electronic transition at 292 nm which was observed between 291 and 299nm for its metal complexes manifesting the electronic charge between drug and metal ions. Bands appeared at the region of 291, 298, 294 and 299 nm owing to electronic transitions (L→M) for [Ag $(MOX)_2$] [Rh $(MOX)_2$] [Ti $(MOX)_2$] [Rb (MOX)] drug-metal complexes respectively (Kondaiah, Bhagavanth Reddy *et al*., 2017).

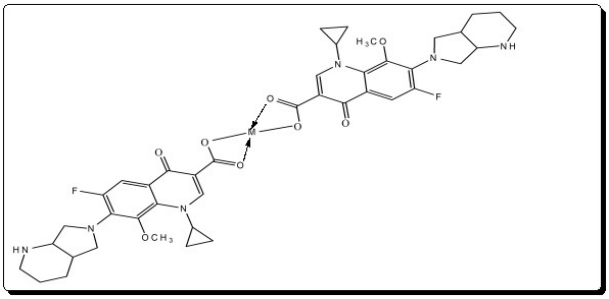

Fig. 1: Structure of moxi-metal complex

UV/Visible spectroscopic studies

The UV-Visible spectrum of moxifloxacin showed an electronic transition at 292 nm which was observed between 291 and 299nm for its metal complexes manifesting the electronic charge between drug and metal ions. Bands appeared at the region of 291, 298, 294 and 299 nm owing to electronic transitions (L→M) for [Ag $(MOX)_2$] [Rh $(MOX)_2$] [Ti $(MOX)_2$] [Rb (MOX)] drug-metal complexes respectively (Kondaiah, Bhagavanth Reddy *et al*., 2017).

Table 1: Melting point of all metals and drug-metal complexes (^oC)

	Ag	Rh	Ti	Rb
Metal	212	1100	1843	682
Drug-Complex	310	260	186	252

FTIR spectroscopic studies

The mode of binding of moxifloxacin with metal ion was through the carbonyl group. The carboxyl stretching was observed at 3530 cm^{-1} in the FTIR spectrum of drug however spectra of drug-metal complexes showed the carboxyl band at 200-300cm^{-1} below then 3530cm^{-1} owing to bonding of metal ion with oxygen of carboxylate(Kunze, Neda *et al*., 2002, Maftei, Fodor *et al*., 2013). The one extra band is also seen between 1600-1620 cm^{-1} for all moxifloxacin complexes in FTIR spectrum which confirm the drug- metal chemical conjugation (Imran, Iqbal *et al*., 2007, Tulkens, Arvis *et al*., 2012).

Table 2: Antibacterial effect of moxifloxacin (zone of inhibition in mm)

Dilution (µg/ml)	0.19	0.38	0.77	1.55	3.125	6.25
Strains						
S. aureus	-	-	10± 0.1	13± 0.13	20± 0.14	23± 0.15
S. epidermidis	-	-	-	13± 0.13	16± 0.13	20± 0.14
S. faecalis	-	-	10± 0.11	12± 0.132	14± 0.19	16± 0.14
S. pyogenes	-	-	12± 0.121	16± 0.145	18± 0.173	20± 0.144
C. diphtheria	-	-	-	-	-	-
P. aeruginosa	-	-	-	-	-	-
Enterobacter	-	-	-	-	12± 0.119	16± 0.142
K. pneumonia	-	-	-	-	16± 0.139	19± 0.143
S. typhi	-	-	-	-	-	-

Table 3: Antibacterial effect of sliver-moxifloxacin complex (zone of inhibition in mm± SEM)

Dilution (µg/ml)	0.19	0.38	0.77	1.55	3.125	6.25
Strains						
S. aureus	18± 0.14	20±0.149	24±0.152	26±0.157	28± 0.158	32±0.161
S. epidermidis	18± 0.113	20± 0.142	24± 0.149	27± 0.152	30± 0.157	32±0.158
S. faecalis	14± 0.11	16± 0.121	18± 0.13	20± 0.137	21± 0.141	26±0.149
S. pyogenes	22± 0.143	26± 0.149	30± 0.145	34± 0.155	-	-
C. diphtheria	18± 0.121	20± 0.141	22± 0.14	24± 0.141	26± 0.149	28±0.149
P. aeruginosa	-	-	12± 0.112	16± 0.117	18± 0.119	20± .122
Enterobacter	14± 0.107	18± 0.11	20± 0.14	22± 0.121	24± 0.122	26±0.129
K. pneumonia	12± 0.103	16± 0.1	18± 0.109	20± 0.112	24± 0.117	26± 0.12
S. typhi	-	-	13± 0.106	16± 0.11	18± 0.117	20± 0.12

Table 4: Antibacterial effect of rubidium-moxifloxacin complex (zone of inhibition in mm± SEM)

Dilution (µg/ml)	0.19	0.38	0.77	1.55	3.125	6.25
Strains						
S. aureus	12±0.101	16±0.102	20±0.112	23±0.119	27±0.121	30±0.12
S. epidermidis	10± 0.1	12±0.102	16± .102	19 0.11	22±.117	26±0.127
S. faecalis	12±0.101	14±0.105	16±0.112	20±0.118	22±0.122	24±0.125
S. pyogenes	14±0.103	18± 0.11	16± 0.12	20± .124	22±0.129	24±0.131
C. diphtheria	22±0.117	26± 0.12	28±0.123	30±0.129	Clear	Clear
P. aeruginosa	-	-	-	10± 0.1	14± 0.11	18±0.117
Enterobacter	12±0.101	18±0.105	20± 0.11	22±0.116	28± 0.12	32±.125
K. pneumonia	-	11± 0.1	14± .107	20±0.011	22± .117	24± 0.12
S. typhi	-	-	-	11±0.101	18±0.105	20±0.118

All the metal derivatives of dihydroquinolone-3-carboxylate were investigated for both their potential antibacterial and antioxidant effects. The antibacterial effects of metal derivatives of dihydroquinolone-3-carboxylate according to zone of inhibition were shown in (table 2). The percentage scavenging activity of derivatives of dihydroquinolone-3- carboxylate on DPPH radical and IC_{50} values were shown in fig-1 and table-8 respectively.

^{1}H NMR spectroscopic studies

The D_2O solvent was used for recording ^{1}HNMR spectra of drug and its metal complexes. The moxifloxacin spectra speculates the two H-2 and H-5 aromatic protons; a singlet at δ 8.57 µg/ml for H-2 proton and another singlet at δ 6.89 µg/ml for H-5 proton. A multiplet for [2H, N–4′-CH2] was appeared at δ 4.06µg/ml. The H-14′ protons were observed as doublet signal at (δ 3.91µg/ml), i.e., [1H,-CH)], H-10 and H-10′ protons were observed as multiplet signal at (δ 3.70 µg/ml), i.e., [3H, (N-CH_2-CH--)], a singlet of 3H of OCH_3 group appeared at δ 3.50 µg/ml. The spectral band of [1H, NH] was shown at δ 2.76µg/ml , a triplet for [4H (2H-11, 2H-12 ′)] was seen at δ 1.88µg/ml, a quartet is appeared at δ 1.22µg/ml due to [H, (cyclopropane -CH--)], a multiplet at [2H (δ 1.09µg/ml)] due to [2H (Cyclopropane -$CH_{2'}$)] and a quartet at [2H (δ 0.91µg/ml)] due to [2H (Cyclopropane -$CH_{2'}$)].

Table 5: Antibacterial effect of titanium-moxifloxacin complex (zone of inhibition in mm± SEM)

Dilution (µg/ml)	0.19	0.38	0.77	1.55	3.125	6.25
Strains						
S. aureus	19±0.107	20±0.11	26±0.117	30±0.122	32±0.127	34±0.128
S. epidermidis	18±0.107	20±0.109	24±0.112	26±0.118	30± 0.12	32±0.129
S. faecalis	-	12±0.102	16 0108	22±0.112	24±0.118	26±0.12
S. pyogenes	-	-	-	-	-	-
C. diphtheria	22± 0.11	24±0.115	26±0.119	32 0123	Clear	Clear
P. aeruginosa	-	14±0.106	16± 0.11	20± .115	24±0.119	26±0.123
Enterobacter	18±0.107	20±0.111	26± .117	28± 0.12	30±0.122	32±0.125
K. pneumonia	18±0.102	20±0.105	22± 0.11	26±0.116	30± 0.12	32±0.125
S. typhi	-	12±0.101	14±0.109	16± 0.11	24± 0.16	26±0.122

Table 6: Antibacterial effect of rhodium-moxifloxacin complex (zone of inhibition in mm± SEM)

Dilution (µg/ml)	0.19	0.38	0.77	1.55	3.125	6.25
Strains						
S. aureus	-	12± 0.1	14±0.104	18±0.107	20± 0.11	26± 0.12
S. epidermidis	-	-	16±0.107	18± 0.11	26±0.117	30±0.125
S. faecalis	-	-	-	-	14±0.102	16± 0.11
S. pyogenes	10±0.101	12±0.103	16±0.109	20±0.112	28± .119	30±0.123
C. diphtheria	-	12±0.101	16±.105	22± 0.11	26±0.113	30±0.119
P. aeruginosa	-	-	12±0.101	14±0.107	16± 0.11	18±0.117
Enterobacter	11±0.103	12±0.109	14±0.11	18±0.115	20 ± 0.12	22±0.122
K. pneumonia	-	12±0.1	14±0.105	16±0.109	20± 0.11	22± .115
S. typhi	-	-	10±0.101	14±0.105	16± 0.11	18±0.113

- = no activity

Table 7: The antibacterial strength of moxi-metal complexes

Stains	Metal moxifloxacin complex strength
S. aureus	Ti > Ag > Rb > Rh.
S. epidermidis	Ag, Ti > Rb, Rh.
S. faecalis	Ag > Rb > Ti > Rh.
S. pyogenes	Ag > Rb > Rh.
C. diphtheria	Ti, Rb > Ag > Rh.
P. aeruginosa	Ti > Ag, Rh > Rb.
Enterobacter	Ti > Ag > Rb > Rh.
K. pneumonia	Ti > Ag > Rh > Rb.
S. typhi	Ti > Ag > Rh >Rb.

Table 8: Antioxidant activities of moxi-metal complexes

S. No.	Code	IC_{50}±SEM[a](µg/ml)
1.	Moxi-Ag	9.19 ± 0.26
2.	Moxi-Rh	14.65 ± 0.26
3.	Moxi-Ti	11.23 ± 0.22
4.	Moxi-Rb	8.26 ± 0.31
5.	Moxi	121 ± 2.31
6.	Standard	1.65 ± 0.16

Upon metal complexation, the spectra of moxifloxacin-metal complexes speculate remarkable changes. In moxi-metal complexes, the signals of H-2 proton shifts towards δ= 8.67, 8.81, 8.37 & 8.51µg/ml while the signals of H-5 proton were sifted at δ 7.81, 7.47, 6.69 and 7.49µg/ml , respectively, for Ag, Rh, Ti and Rb complexes (Ali, Abd-

Elzaher *et al*., 2013, Chiririwa and Muzenda, 2014, Efthimiadou, Sanakis *et al*., 2006). The H-2 and H-5 shift confirms the complex formation and difference in the configuration of complexes. The physicochemical properties of the metal complexes are shown in table 1.

DISCUSSION

Antibacterial activity

Antibiotic resistance is one of the major problems in treating bacterial infections. The treatment of such resistant bacterial infections relies on the new drug development and drug delivery system which can be effective against a wide range of *Gram-positive* and *Gram-negative* bacteria. This study deals with moxi-metal complexes synthesis and antimicrobial testing against wide range of microorganism. The moxifloxacin was proved completely resistant against *S. typhi, Diphtheria* and an opportunist bacteria *Pseudomonas aeruginosa* as shown in table 2 showed while moderate antibacterial activity for other *Gram-negative* and *Gram-positive* bacteria. The four moxi-metal complexes showed spectacular broad spectrum activity as shown in table 2-6. However, Ag and Ti moxi complexes were found superior to Rh and Rb moxi-metal complexes. The moxi-Ag complex (table 3) showed potent activity against *S. pyogenes*, *S. typhi, C. diphtheria* and *P. aeruginosa* having MIC; 0.19, 0.77, 0.19 and 0.77 µg/ml respectively. The moxi-Rb complex also showed similar potent efficacy against *C. diphtheria, S. typhi* and *P. aeruginosa* having MIC; 0.19, 1.55 and 1.55 µg/ml respectively. The moxi-Ti complex was found to be most effective against *C. diphtheria, S. typhi* and *P. aeruginosa* having MIC; 0.19, 0.38 and 0.38µg/ml as shown in table 5. The moxi-Rh complex was found most potent against *Enterobacter* at 0.19 µg/ml concentration which was 65 fold more potent than of moxifloxacin. It also possesses potent antibacterial effect against *S. typhi, C. diphtheria* and *P. aeruginosa* with MIC; 0.77, 0.38 and 0.38µg/ml respectively when moxifloxacin was resistant against these bacteria. It showed that metal drug delivery system sensitizes drug to bacteria owing different route of penetration. The activity of moxi-metal complexes against the *Salmonella typhi* was found significant and order of activity of compound is as moxi- Ti >Ag >Rh >Rb. Emergence of drug resistant strains of *S. typhi*, classified as extensively drug resistance (XDR) typhoid, resistant against chloramphenicol, ampicillin and trimethoprim sulfamethoxazole along with third-generation cephalosporin and fluoroquinolones are at rise (Klemm, Shakoor *et al*., 2018) with multiple cases reported in various parts of Pakistan. In this connection the discovery of highly effective moxi-metal complexes, against not only *S. typhi* but also against other multidrug resistant organisms is quite promising. While existing drug therapies are exhausting, these compounds could prove to be novel effective treatment options.

Antioxidant investigation

It is emergent requirement to synthesize new compounds which possess protection capabilities from oxidative damage by neutralizing the free radicals. Natural and synthetic antioxidants by blocking substrates causing oxidation reduce the risk of chronic diseases (Odeyemi, Afolayan *et al*., 2017). Different *in-vitro* methods used to analyze compounds capacity to capture redicals and inhibit their formation to indicate antioxidant capabilities of compound, but DPPH scavenging method is most desired method as compared to other methods because of convenience, reliability and speed. The DPPH is colorless in reduced form after receiving hydrogen or electron from antioxidant become scavenged (Mishra, Ojha *et al*., 2012). The results shown in table 8 that there is direct relationship of concentration and percent inhibition, the maximum inhibition was found between 15-20 µg/ml (Biswas, Haldar *et al*., 2010). Due to substitution at carbonyl group with metal groups and presence of different metal ions potentiates the scavenging effect. The prepared metal complexes showed modest to decent antioxidant activities as compared with standard ascorbic acid.

CONCLUSION

This study reveals that Ag, Rh, Ti and Rb chemically bind to moxifloxacin via carboxylate group and metal complexes were found bidentate. All four complexes have publicized spectacular potent antibacterial effects against wide range of bacteria especially against *S. typhi, Pseudomonas aeruginosa* and *Diphtheria* which were resistant to moxifloxacin. The moxi–Ag and moxi-Ti complexes were more potent to moxi-Rh & moxi-Rb complexes. The synthesized metal complexes showed moderate to decent antioxidant activities as compared with standard ascorbic acid.

REFERENCES

Ali HRH, Ali R, Batakoushy HA and Derayea SM (2017). Spectroscopic analysis and antibacterial evaluation of certain third generation cephalosporins through metal complexation. *Ana. Chem. Lett. TCAL.,*7(4): 445-457.

Ali KA, Abd-Elzaher MM and Mahmoud K (2013). Synthesis and anticancer properties of silver (I) complexes containing 2, 6-Bis (substituted) pyridine derivatives. *Int. J. Med. Chem.,* **5**(1): 39.

Andriole VT (2005). The quinolones: past, present, and future. *Clin. Infec. Dis.,***41**(Suppl. 2): 113-119.

Biswas M, Haldar PK and Ghosh AK (2010). Antioxidant and free-radical-scavenging effects of fruits of *Dregea volubilis*. *J. Nat. Sci. Biol. Med.,* **1**(1): 29.

Blair JM, Webber MA, Baylay AJ, Ogbolu DO and Piddock LJ (2015). Molecular mechanisms of antibiotic resistance. *Nat. Rev. Microbiol.,* **13**(1):42-51.

Chiririwa H and Muzenda E (2014). Synthesis, characterization of gold (III) complexes and an *in vitro* evaluation of their cytotoxic properties. *WCECS.* **2**: 1-4.

Cuprys A, Pulicharla R, Brar SK, Drogui P, Verma M and Surampalli RY (2018). Fluoroquinolones metal complexation and its environmental impacts. *Coord. Chem. Rev*. **376**(12): 46-61.

Demir S, Gelincik A, Akdeniz N, Aktas-Cetin E, Olgac M, Unal D, Ertek B, Coskun R, Colakoğlu B and Deniz G (2017). Usefulness of *in vivo* and *in vitro* diagnostic tests in the diagnosis of hypersensitivity reactions to quinolones and in the evaluation of cross-reactivity: A comprehensive study including the latest quinolone gemifloxacin. *Allergy, Asthma Allergy Immunol.*. **9**(4): 347-359.

Efthimiadou EK, Sanakis Y, Raptopoulou CP, Karaliota A, Katsaros N and Psomas G (2006). Crystal structure, spectroscopic, and biological study of the copper (II) complex with third-generation quinolone antibiotic sparfloxacin. *Bioorg. Med. Chem. Lett.,* **16**(14): 3864-3867.

Elshafie HS, Sakr SH, Sadeek SA and Camele I (2019). Biological investigations and spectroscopic studies of new moxifloxacin/glycine-metal complexes. *Chem. Biodivers,* **16**(3): e1800633.

Fernandes P and Martens E (2017). Antibiotics in late clinical development. *Biochem. Pharmacol.,* **133**(6): 152-163.

Gul MK (2019). Synthesis, characterization and biological activities of bioinorganic complexes of moxifloxacin. *Int. J. Adv. Res.,* **7**(8): 8.

Imran M, Iqbal J, Iqbal S and Ijaz N (2007). *In vitro* antibacterial studies of ciprofloxacin-imines and their complexes with Cu (II), Ni (II), Co (II), and Zn (II). *Turk. J. Biol.,* **31**(2): 67-72.

Issa YM, El-Hawary WF, Abdel-Salam HA and Issa RM (1995). Separation and structural studies of manganese (II), cobalt (II), nickel (II), copper (II) and cadmium (II) complexes of benzilic and mandelic esters. *Transit. Met. Chem.,* **20**(5): 423-425.

Klemm EJ, Shakoor S, Page AJ, Qamar FN, Judge K, Saeed DK, Wong VK, Dallman TJ, Nair S and Baker S (2018). Emergence of an extensively drug-resistant *Salmonella enterica* serovar Typhi clone harboring a promiscuous plasmid encoding resistance to fluoroquinolones and third-generation cephalosporins. *MBio,* **9**(1): e00105-00118.

Kondaiah S, Bhagavanth Reddy G, Rajesh D and Vijayakumr B (2017). Synthesis, characterization and biological activity of moxifloxacin-cu (II) metal complex. *IJETSR.,* **4**(12): 1138-1145.

Kunze C, Neda I, Freytag M, Jones PG and Schmutzler R (2002). Mono-and binuclear rhodium and platinum complexes of 1, 3, 5-trimethyl-1, 3, 5-triaza-2σ3λ3-phosphorin-4, 6-dionyloxy-substituted calix [4] arenes. *Z. Anorg. Allg. Chem.,* **628**(3): 545-552.

Luepke KH, Suda KJ, Boucher H, Russo RL, Bonney MW, Hunt TD and Mohr III JF (2017). Past, present, and future of antibacterial economics: increasing bacterial resistance, limited antibiotic pipeline, and societal implications. *Pharmacotherapy: J. Human. Pharmacol. Drug Ther.,* **37**(1): 71-84.

Maftei CV, Fodor E, Jones PG, Franz MH, Kelter G, Fiebig H and Neda I (2013). Synthesis and characterization of novel bioactive 1, 2, 4-oxadiazole natural product analogs bearing the N-phenylmaleimide and N-phenylsuccinimide moieties. *Beilstein J. Org. Chem.,* **9**(1): 2202-2215.

Mishra K, Ojha H and Chaudhury NK (2012). Estimation of antiradical properties of antioxidants using DPPH assay: A critical review and results. *Food Chem.,* **130**(4): 1036-1043.

Murray GL, Bradshaw CS, Bissessor M, Danielewski J, Garland SM, Jensen JS, Fairley CK and Tabrizi SN (2017). Increasing macrolide and fluoroquinolone resistance in *Mycoplasma genitalium*. *Emerg. Infect. Dis.,* **23**(5): 809.

Odeyemi S, Afolayan A and Bradley G (2017). Phytochemical analysis and anti-oxidant activities of *Albuca bracteata* Jacq. and *Albuca setosa* Jacq bulb extracts used for the management of diabetes in the Eastern Cape, South Africa. *Asian Pac. J. Trop. Biomed.* **7**(6): 577-584.

Refaat HM, El-Badway HA and Morgan SM (2016). Molecular docking, geometrical structure, potentiometric and thermodynamic studies of moxifloxacin and its metal complexes. *J. Mol. Liq.,* **220**(8): 802-812.

Sadeek SA, El-Shwiniy WH and El-Attar MS (2011). Synthesis, characterization and antimicrobial investigation of some moxifloxacin metal complexes. *Spectrochimica Acta Part A: Mol Biomol Spectrosc.,* **84**(1): 99-110.

Singh K and Tafida G (2014). Antibacterial activity of *Moringa oleifera* (Lam) leaves extracts against some selected bacteria. *Int. J. Pharm. Pharm. Sci.,* **6**(9): 52-54.

Skuredina A, Le-Deygen I, Uporov I and Kudryashova E (2017). A study of the physicochemical properties and structure of moxifloxacin complex with methyl-β-cyclodextrin. *J. Colloid. Sci.,***79**(5): 668-676.

Soayed AA, Refaat HM and El-Din DAN (2013). Metal complexes of moxifloxacin–imidazole mixed ligands: Characterization and biological studies. *ISO4.* **406**(9): 230-240.

Tulkens PM, Arvis P and Kruesmann F (2012). Moxifloxacin safety. *Drugs in R&D.,* **12**(2): 71-100.

Ventola CL (2015). The antibiotic resistance crisis: Part 1: Causes and threats. *Pharm Ther.,* **40**(4): 277.