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Synthesis Characterization and Antimicrobial Activities of Azithromycin Metal Complexes

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Abstract

Azithromycin is a well-established antimicrobial agent which has been widely prescribed for the treatment of respiratory tract infections owing to its high efficacy and safety. Various essential metal complexes of azithromycin were synthesized and characterized by techniques as UV, FT-IR, NMR, atomic absorption and elemental analysis. Spectroscopic studies of complexes suggested that the $-N(CH_3)_2$ and hydroxyl group of desosamine sugar moiety present in azithromycin has been involved in complexation i.e., azithromycin ligand (L) behaves bidentately for complexation with different metal ions such as Mg (II), Ca (II), Cr (III), Mn (II), Fe (III), Co (II), Ni (II), Cu (II), Zn (II) and Cd (II). These complexes were then subjected to *in-vitro* antibacterial and antifungal studies against several Gram positive, Gram negative bacteria and fungi. ANOVA studies illustrates that all the tested complexes exhibited significantly mild to moderate antibacterial activity against all bacterial strains and highly significant against fungus *C. albican*.

Keywords: Azithromycin; Metal complexes; Spectroscopic techniques; Antibacterial; Antifungal studies; ANOVA

Introduction

The interaction of metal ions with drugs has been recognized internationally as an important area for research [1] and also evident by huge financial support by National Institute of Health (NIH), USA program [2] and two European Union Cost Collaborative programs. Metals due to their variable oxidation states, number and types of coordinated ligands, and coordinative geometry after complexation can provide variety of properties.

On the other side, the ligands can not only control the reactivity of the metal, but also play critical roles in determining the nature of interactions involved in the recognition of biological target sites, such as DNA, enzymes and protein receptors. These variables provide enormous potential diversity for the design of metallodrugs [3]. Synthesized metal complexes might prove to have altered therapeutic activity or may have toxic effects. Therefore it is worth emphasizing point to synthesize these complexes and determine their biological activity. Most widely prescribed drugs in chemotherapy are metal-based drugs (Platinum drugs) and there are ranges of iron, copper, cobalt, gold, molybdenum complexes possessing potent anti-cancerous activity [4,5]. Metal complexes have proved potent antimicrobial agents and are in common day-to-day use in medicine such as silver bandages [6] for treatment of burns, zinc antiseptic creams, and metal clusters as anti-HIV drugs.

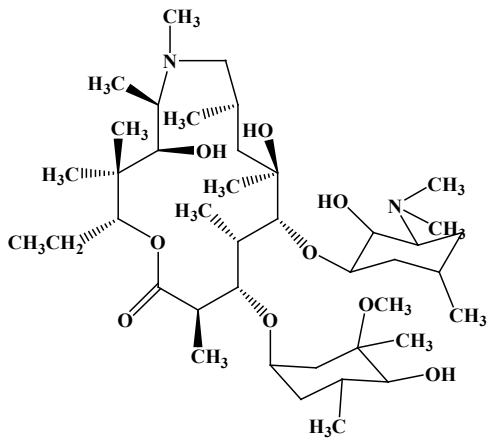

Figure 1: Chemical structure of azithromycin.

Therefore, the potential for further development of metal-based drugs and treatments as antimicrobial agents is enormous and have great importance with the evolution of drug-resistant bacteria and threats from a range of viral diseases. Numerous clinical trials for the usage of metals in therapeutics have been carried out worldwide for assessing metal based drug's efficacy in a wide diversity of human problems, including malaria, upper respiratory tract infections, urinary tract infections, sinusitis infections, vaginal yeast infections, ENT infections, cuts and fungal skin infections and even for sexually transmitted diseases like gonorrhea etc. proving it to be an antibiotic alternative at a convenient dosage [2,3,7,8].

Macrolide antibiotic azithromycin (Figure 1), the first representative of the azalide class, is entrenched antimicrobial agent that has been widely prescribed for the treatment of Respiratory Tract Infections (RTI's) owing to its high efficacy and safety [9,10]. Azithromycin contains methyl substituted nitrogen in the 15-membered macrolide aglycone ring and has greater stability than macrolide antibiotics in the presence of acids, leading to good absorption in the digestive tract [11]. Macrolide antibiotics exert their antibacterial activity by binding to ribosomal 23S RNA and thus blocking the bacterial protein synthesis [12]. The lactone ring in azithromycin, substituted with a number of hydroxyl and amine functional groups is involved in interaction with metal ions [13]. The bioavailability of azithromycin is affected by the coadministration of medications containing multivalent cations,

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aluminum and magnesium containing antacids and decreases the bioavailability by approximately 24%, but has no effect on the area under the plasma concentration-time curve. Oral azithromycin should be administered at least one hour before or two hours after aluminum and magnesium containing antacids [14]. Work done by Sher, et al. on interaction of azithromycin with copper(II) ion [13] and Rjoob, et al. also supports the interaction of azithromycin with iron sulfate and iron perchlorate salts for the characterization of complexes that can be formed after interaction [15].

Therefore, as part of our continuous efforts focused on the *in vitro* activity of cephalosporins in presence of essential and trace elements [16-18], fluoroquinolone interactions with essential and trace elements [19-24] and macrolides including clarithromycin and erythromycin synergism [25,26] and roxithromycin antagonism [27] with essential and trace elements impelled us to study the antibacterial and antifungal activities of newly synthesized essential metal complexes of azithromycin. These complexes were synthesized as later are already present in our body and food or may be co-administered along with azithromycin as part of multivitamin combinations. The stoichiometry's of the complexes were determined by conductometric titrations prior to synthesis. One-way analysis of variance (ANOVA) studies were conducted to check the differences between the zone of inhibitions of all synthesized complexes and reference standard. Post hoc Dunnett's test [31] was applied to the data and differences were considered.

Materials and Methods

Materials and instruments

Azithromycin sample was gifted by Platinum Pharmaceuticals (PVT) Ltd. While, the hydrated metal salts ($MgCl_2.6H_2O$, $CaCl_2.2H_2O$, $CrCl_3.6H_2O$, $MnCl_2.H_2O$, $FeCl_3.6H_2O$, $CoCl_2.6H_2O$, $NiCl_2.6H_2O$, $CuCl_2.2H_2O$, $ZnCl_2$, $CdCl_2.H_2O$), other solvents and reagents of analytical grade were purchased from Merck Marker (PVT) LTD. FT-IR and ^{1}H-NMR spectra were recorded on Prestige-21 Shimadzu FT-IR instrument. The samples were scanned in the form of KBr pellets. ^{1}H-NMR instrument was Bruker AMX 400 MHz. Chemical shifts were reported in ppm using tetramethylsilane (TMS) as an internal standard. CHN analysis was done on elemental analyzer Carlo Erba 1106. Atomic absorption studies were carried out by Perkin-Elmer "AAnalyst 700" atomic absorption spectrometer using "Analyst" software and conductometric titrations were carried on Vernier Lab Pro™. Data acquisition and analysis was carried out by using Logger pro 3.2 software. Chlorine was determined by titration with $Hg(NO_3)_2$.

Conductometric titration

For conductometric titration, metal chloride solutions of 1 mM were prepared and titrated with 1 mM ligand solution at 25°C [17]. Results obtained were then extrapolated to determine the stoichiometric ratio.

Synthesis of azithromycin metal complexes

Metal complexes were synthesized by mixing a hot methanolic solution of ligand (1 mM) with 0.5 mM solution of metallic chlorides in the ratio of 2:1. The reaction mixture was continuously refluxed on a water bath for 3.0-3.5 h at 60°C [19]. The solutions were then filtered and left for crystallization at room temperature for two to three weeks. In each case, a fine solid product was obtained which was washed, dried and their melting points were noted. These were than subjected for spectroscopic and microbial studies after their characterization. Unfortunately X-ray diffraction analysis could not be performed as we were not able to obtain appropriate mono crystals

Antimicrobial activity

The synthesized metal complexes were screened for their antibacterial activity against Gram-positive organisms as *Bacillus subtilis*, *Micrococcus luteus, Staphylococcus aureus,* and *Streptococcus features* and Gram-negative organisms which include *Salmonella typhi, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Escherichia coli, Citrobacter* and *Shigella flexneri*. A disk diffusion assay was employed for the evaluation of antibacterial activity [28,29], 6mm filter paper discs were impregnated with 5, 10 and 20 ppm dilutions and were allowed to remain at 37°C till complete diluents evaporation and kept under refrigeration before antibacterial activity was assessed [28]. They were grown routinely overnight in a nutrient broth (Merck) at 37°C.

The antibacterial discs of drug and drug based metal complexes were applied over each of the culture plates previously seeded with the 0.5 McFarland turbidity cultures of the test bacteria and then incubated at 37°C for 18-24 h. The release of drugs into the surrounding agar medium shown by growth inhibition of microorganisms was evaluated. The growth inhibitory effect was determined by measuring the zone of growth inhibition around the disk.

Antifungal activity

Same procedure was repeated for antifungal activity (as done for antibacterial activity) against series of fungi (*C.albicans, F.solani, T.rubrub, A.parasitieus, A.effusis and S.cervicis).* Dilutions were made in same manner for soaking discs as before. Sabraoud dextrose agar was then prepared and autoclaved at 121°C for 15 minutes, cooled and then poured in Petri dishes. Streaking was done in same way as done for antibacterial activity and dishes then incubated for 48 hrs at 37°C. Finally the zones of inhibition were carefully measured.

Statistical study

One-way analysis of variance (ANOVA) studies were conducted by using Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) software with the level of significance chosen at $p \leq 0.05$ (any values lesser than 0.05 were measured significant).

Results and Discussion

Conductometric titration

The stoichiometry of the complexes in methanolic solution was determined by conductometric titration [30] at 298 K. The conductance value was measured during the titration of 0.1 mM metal ion solution against a 0.1 mM azithromycin solution (40 mL). The conductance was corrected for dilution by means of the following equation, assuming that conductivity is a linear function of dilution:

$$\Omega_{corr} = \Omega_{obs} (v_1 + v_2) / v_1$$

Where Ω is the electrolytic conductivity, v_1 is the initial volume and v_2 is the volume of the metal solution added. The drug to metal ratio was determined by plotting a graph of corrected conductivity versus the volume of titrant added and the end point was determined shown in Figure 2. The general trend of the conductograms is a steady increase of the conductance values of the solution after each addition of the metal up to equivalence point where a sudden change in the slope occurs [30]. This behavior is recognized to the formation of ion-pair in solution because of complexation reaction. The electrical conductance of the complexes point out that M:L ratio is 1:2 in all complexes.

From the conductance measurement data an attempt was made to synthesize azithromycin-metal complexes in the ratio of 2:1 (L:M) using

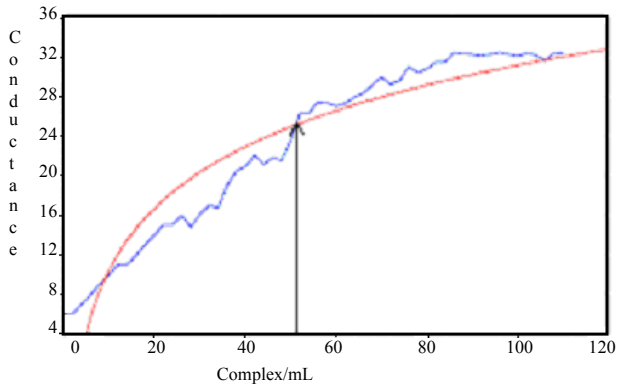

Figure 2: Conductometric titration curve.

Derivatives	M.P (°C)	State	Color	% Yield
Azithromycin	115	crystalline	white	45
$[Mg(Azi)_2(H_2O)_2]Cl_2$	130	crystalline	white	35
$[Ca(Azi)_2(H_2O)_2]Cl_2$	150	crystalline	white	55
$[Cr(AziI)_2 (H_2O)_2]2Cl.2H_2O$	140	crystalline	light green	45
$[Mn (Azi)_2(H_2O)_2].4H_2O$	168	powder	white	42
$[Fe(Azi)_2 (H_2O)_2]2Cl_2.2H_2O$	166	crystalline	white	51
$[Co (Azi)_2(H_2O)_2]$	139	crystalline	white	45
$[Ni (Azi)_2(H_2O)_2]Cl_2.2H_2O$	134	powder	white	58
$[Zn (Azi)_2(H_2O)_2].2H_2O$	160	crystalline	purple	40
$[Zn (Azi)_2(H_2O)_2].2H_2O$	148	powder	white	60
$[Cd (Azi)_2(H_2O)_2] Cl_2.4H_2O$	170	crystalline	light pink	52

Table 1: Physiochemical parameters of azithromycin and azithromycin– complexes.

methanol as solvent. Their physical parameters as color, melting points and % yields of are given in Table 1. These complexes are insoluble in water, slightly soluble in chloroform but completely soluble in methanol and DMSO. Techniques as IR, NMR and elemental analysis were used to characterize these complexes.

Infrared absorption studies

The assignments of IR bands were made by comparing the spectra of the complexes with azithromycin. In the reported spectra of azithromycin, there were two very strong absorption peaks at 1750 cm^{-1} and 1652 cm^{-1} due to lactone and ketonic carbonyl groups, respectively [9]. The absorption peaks between 1000 and 1250 cm^{-1} are due to the ethers and amine functions. The CH_2 bending is evident by peaks between 1340 and 1460 cm^{-1} and alkane stretching peaks appeared among 2800-2980 cm^{-1}. Hydrogen bonded OH and water molecule appeared as bands between 3350 and 3650 cm^{-1} with peak maxima at 3550 cm^{-1}.

Azithromycin is more likely to form complexes, with metals as it possess number of lone pair rich sites and amine substituted lactone ring [13]. In metal complexes of azithromycin, some very prominent peak shifting has been observed along with change in intensities of several important peaks indicating azithromycin has undergone complexation reaction with metals as shown in Figures 3A and 3B. In azithromycin chromium complex, the aliphatic amine stretch at 1100 cm^{-1} diminished to a significant extent than in azithromycin while the peak of 1200 cm^{-1} was shifted to 1160 cm^{-1}. The intensity of OH band decreased considerably and its maxima shifted from 3550 cm^{-1} to 3500 cm^{-1}. In azithromycin calcium complex same results were observed, i-e the OH absorption bands were shifted to 3400 cm^{-1} as shown in Figure 3A. The aliphatic amine stretch diminished to a significant extent that was present at 1100 cm^{-1} in azithromycin while the peak of 1200 cm^{-1} shifted to 1160 cm^{-1}. The same results were observed in azithromycin-copper, cobalt, cadmium, manganese, magnesium, ferric, zinc, nickel and calcium complex. In the light of these observations, it can be fairly concluded that the N $(CH_3)_2$ group of desosamine and hydroxyl group, have been utilized in the complex formation. The anionic part is out side the sphere and is represented by a dotted line.

NMR studies

The ^{1}HNMR spectra of azithromycin is harmonized with reported spectra which showed the resonances at δ_H 3.352 (3 H, s), 2.316 (3 H, s) and 2.288 (6 H, s) assigned to the 3"-OCH_3 absorption of cladinose, the 9a-NCH_3 group of the 15-membered aglycone ring, and the 3'-$N(CH_3)_2$ group of desosamine, respectively [9]. The same chemical shifts (3.352, 2.316 and 2.287) are also observed in the ^{1}H NMR spectra of metal containing compounds but the signals of 9a-NCH_3 group of the 15-membered aglycone ring were found less intensive. Additionally, the intense signal at 2.927 ppm observed in the spectrum of azithromycin is absent in the spectra of coordinative compounds of Cu(II), Mn(II), Fe(III), Cr(III), Co(II), Ni(II), and Ca(II) and much obscure in case of Mg(II), Zn(II) and Cd(II). Moreover, in the region δ_H 3.00-3.20, a triplet in the spectra of the coordinative compounds is observed instead of a quadruplet seen in the spectrum of azithromycin. It confirmed that the hydroxyl group and 9a-NCH_3 group of the 15-membered aglycone ring of azithromycin are bonded to metal ions. On the basis of the

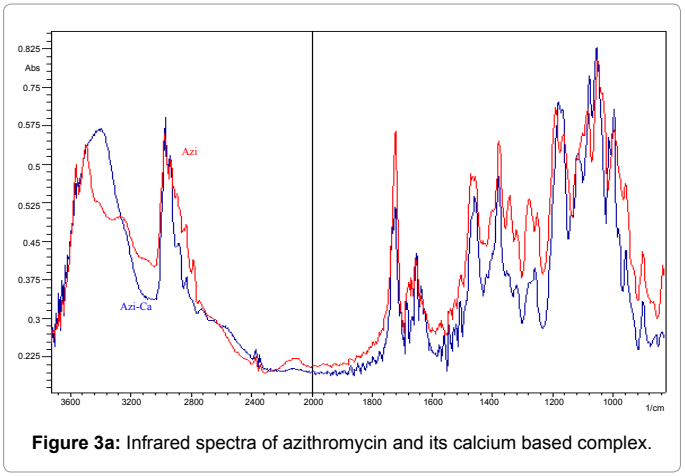

Figure 3a: Infrared spectra of azithromycin and its calcium based complex.

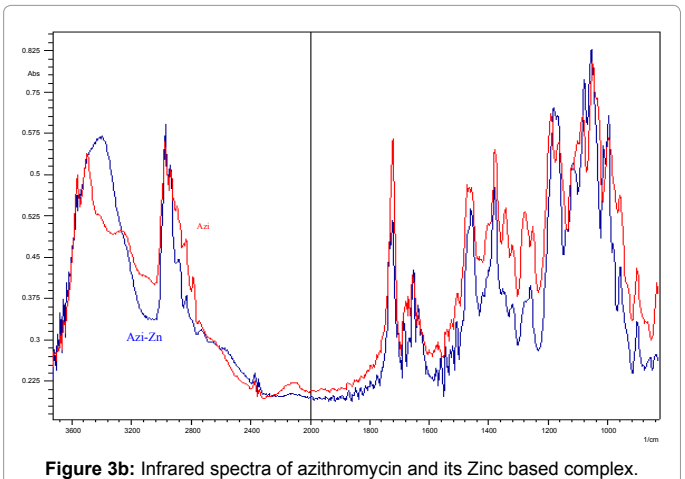

Figure 3b: Infrared spectra of azithromycin and its Zinc based complex.

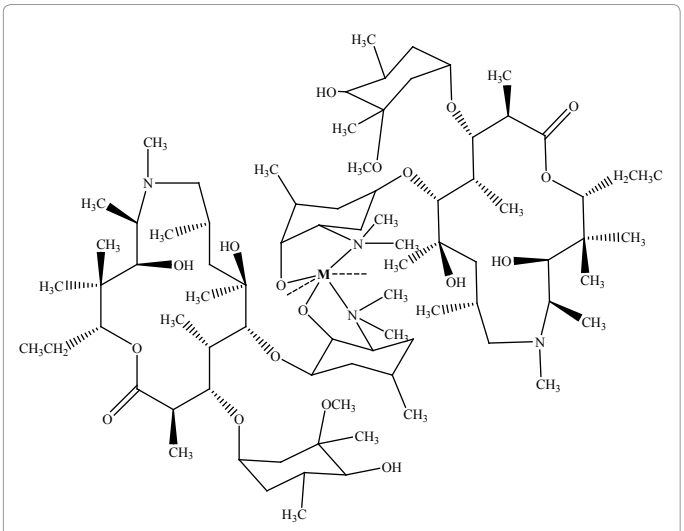

Figure 4: Proposed structures of the azithromycin-metal complexes.

Compound	C% Found(Calc)	H% Found(Calc)	N% Found(Calc)	Metal% Found(Calc)
Azithromycin	61.36 (61.39)	9.73 (9.78)	3.62 (3.67)	-
$[Mg(Azi)_2(H_2O)_2]Cl_2$	56.52 (56.60)	9.30 (9.31)	3.36 (3.38)	1.45 (1.47)
$[Ca(Azi)_2(H_2O)_2]Cl_2$	55.99 (56.06)	9.04 (9.05)	3.30 (3.35)	2.39 (2.40)
$[Cr(Azil)_2 (H2O)_2]2Cl_2.2H_2O$	54.48 (54.50)	9.40 (9.30)	3.22 (3.26)	4.04 (4.12)
$[Mn (Azi)_2(H_2O)_2].4H_2O$	56.71 (56.74)	9.45 (9.42)	3.34 (3.39)	3.30 (3.33)
$[Fe(Azi)_2 (H_2O)_2]2Cl_2.2H_2O$	54.32 (54.38)	9.00 (9.01)	3.20 (3.25)	3.20 (3.24)
$[Co (Azi)_2(H_2O)_2]$	57.80 (57.87)	9.32 (9.34)	3.42 (3.46)	6.41 (6.44)
$[Ni (Azi)_2(H_2O)_2]Cl_2.2H_2O$	54.20 (54.29)	9.00 (8.99)	3.24 (3.25)	3.39 (3.40)
$[Zn (Azi)_2(H_2O)_2].2H_2O$	57.11 (57.07)	9.35 (9.33)	3.42 (3.41)	3.40 (3.41)
$[Zn (Azi)_2(H_2O)_2].2H_2O$	57.42 (57.64)	9.50 (9.30)	3.49 (3.45)	4.01 (4.02)
$[Cd (Azi)_2(H_2O)_2] Cl_2.4H_2O$	52.49 (52.65)	8.70 (8.72)	3.05 (3.15)	6.33 (6.32)

Table 2: Elemental analysis of azithromycin and its metal complexes.

above mentioned data and literature data concerning the structure and stereochemical configuration of azithromycin, the purposed structure of ML_2 is shown in Figure 4.

Elemental analysis

The structure of the complexes suggested form the elemental analyses agree well with their proposed formulae as given in Table 2. The found values of elemental analysis agree well with calculated percentages of CHN data are in a well agreement with each other and prove the molecular formulas of the complexes.

Biological study

The results of Minimal Inhibitory Concentrations (MIC) in µg/mL concentration of Azithromycin metal complexes against various Gram positive and Gram negative microorganisms are given in Table 3. The results of the disk diffusion assay method for each bacterial and fungal strain are shown in Table 4. All data are presented as zone of inhibition, diameter in mm. One way analysis of variance (ANOVA) was carried out to check the differences between the zone of inhibitions of all synthesized complexes and standard. Post hoc Dunnett's test [31] was applied to the data and differences were considered significant at $p \leq 0.05$. ANOVA showed the significance differences between all synthesized complexes with comparison to azithromycin.

Antibacterial study

Post hoc Dunnett's test analyzed that against *P. mirabilis* all complexes have significantly decreased ($p<0.001$) activity except $Co(Azi)_2$ complex which showed significant increase ($p<0.001$) in antibacterial activity. Significant differences also existed between all synthesized complexes with azithromycin against S. *typhi* however only $Ni(Azi)_2$ complex showed significant ($p<0.001$) increased antibacterial activity. Significant decrease ($p<0.001$) was exhibited by all complexes against *E. coli* and *P. aeruginosa* except $Cr(Azi)_2$ which showed significant increase ($p<0.001$) against *P. aeruginosa*. Azithromycin based $Mg(Azi)_2$, $Cr(Azi)_2$, $Fe(Azi)_2$, $Cd(Azi)_2$ and $Zn(Azi)_2$ complexes were shown to have significantly ($p<0.001$) increased antibacterial activity against *K. pneumonia*. These complexes $Ni(Azi)_2$ and $Ca(Azi)_2$ also showed significant increase ($p<0.001$) at 10 and 20 µg concentration against *S. flexneri* while, remaining complexes have significant ($p<0.001$) decrease in activity. Post hoc Dunnett's test analysis also assures that all synthesized metal complexes showed significant decrease ($p<0.001$) in activity at all concentration levels against *Citrobacter, S. features* and *M. luteus*. Only $Zn(Azi)_2$, $Fe(Azi)_2$ and $Ca(Azi)_2$ complexes exhibits significant increase ($p<0.001$) in activity against *B. subtilis*. Against *S. aureus* all the complexes showed significant decrease ($p<0.001$) except $Co(Azi)_2$ which showed significant increase ($p<0.001$) in activity. Microbiological screening of all synthesized complexes revealed that against *P. mirabilis* $Co(Azi)_2$ complex and against *S.typhi* $Mn(Azi)_2$, $Fe(Azi)_2$ and $Cu(Azi)_2$ complexes exhibit enhanced activity

Compound	Minimum Inhibitory Concentration (MIC, µg/mL)											
	BS	CT	MRSA	PA	ML	SF	PM	CA	EC	SF!	KP	ST
Azi	2	8	8	10	8	8	9	4	2	4	4	2
Azi – Mg	5	4	8	4	5	4	8	5	6	3	5	3
Azi – Ca	2	2	6	5	8	4	5	6	5	3	6	4
Azi – Cr	2	2	8	4	4	5	4	4	3	3	7	2
Azi – Mn	6	1	4	6	4	8	4	6	5	4	4	8
Azi – Fe	1	6	4	4	8	12	14	13	7	6	9	7
Azi – Co	6	2	4	4	5	4	7	5	7	8	7	2
Azi – Ni	3	4	4	4	4	7	7	7	6	5	7	5
Azi – Cu	2	4	6	4	3	4	7	4	6	5	3	4
Azi – Zn	4	1	6	3	2	5	5	5	6	5	2	7
Azi – Cd	8	3	4	8	5	6	5	4	4	9	2	5

BS: *Bacillus subtilis*; CT: *Citrobacterium;* MRSA: Methicillin-Resistant *Staphylococcus aureus;* PA: *Pseudomonas aeruginosa*; ML: *Micrococcus luteus*; SF: *Streptococcus features*; PM: *Proteus mirabilis*; CA: *Candida albicans*; EC: *Escherichia coli*; SF!: *Shigella flexenri*; KP: *Klebsiella pneumoniae*; ST: *Shigella typhi*

Table 3: The minimal inhibitory concentrations (MIC, µg/mL) of Azithromycin metal complexes against various Gram positive and Gram negative microorganisms

Compound	ST	BS	CT	MRSA	PA	ML	SF	PM	CA	EC	SF!	KP
Azi	22	-	30	30	20	35	20	30	20	22	24	22
Azi – Mg	22	-	30	25	18	-	20	22	20	20	15	20
Azi – Ca	25	30	20	22	24	22	24	21	20	25	20	22
Azi – Cr	25	30	20	23	-	22	20	18	20	-	25	-
Azi – Mn	28	-	28	26	-	-	17	20	15	21	14	20
Azi – Fe	22	25	30	20	-	22	25	29	30	22	25	25
Azi – Co	22	-	30	35	25	25	20	38	23	20	22	19
Azi – Ni	20	30	18	23	21	24	22	20	18	30	22	21
Azi – Cu	22	-	25	-	25	22	20	22	-	16	20	22
Azi – Zn	28	30	20	20	-	22	25	25	20	22	23	18
Azi – Cd	20	-	26	-	19	-	25	28	-	20	22	25

BS: *Bacillus subtilis*; CT: *Citrobacterium;* MRSA: Methicillin-Resistant *Staphylococcus aureus;* PA: *Pseudomonas aeruginosa*; ML: *Micrococcus luteus*; SF: *Streptococcus features*; PM: *Proteus mirabilis*; CA: *Candida albicans*; EC: *Escherichia coli*; SF!: *Shigella flexenri*; KP: *Klebsiella pneumoniae*; ST: *Shigella typhi*; (-): Inactive

Table 4: Antimicrobial activity of Azithromycin metal complexes (20 µg/8 mm disc), as compared to azithromycin.

in comparison to parent compound. While against *E.coli*, $Fe(Azi)_2$ complex and against *P. aureogenosa* $Ca(Azi)_2$, $Cr(Azi)_2$, $Fe(Azi)_2$, $Co(Azi)_2$ and $Ni(Azi)_2$ complexes showed greater activity. Against *K. pneumonea* $Cr(Azi)_2$, $Fe(Azi)_2$ and $Cd(Azi)_2$ while against *S. flexneri* $Ni(Azi)_2$ complexes exhibits increased activity whereas, other metal-Azi complexes have activities equivalent or less than azithromycin (Azi). Against *Citrobacter* all complexes exhibits activities equivalent to azithromycin. Against Gram positives as *M. luteus*, *S. faetures* and *S. aureus* all complexes showed slight increased or equivalent activity in comparison to azithromycin. While against *B. subtilus* $Ca(Azi)_2$, $Cr(Azi)_2$, $Fe(Azi)_2$, $Ni(Azi)_2$ and $Zn(Azi)_2$ have increased activity whereas, azithromycin didn't show any activity.

Antifungal study

Azithromycin was not used as antifungal agent before, to the best of our knowledge; it is tested as antifungal agent for the first time along with its synthesized complexes. Surprisingly, all of the complexes and azithromycin showed excellent activity only against *C. albican* and did not exhibit any activity against *F.solani, T. rubrub, A. purasiticus*, *A. effuris* and *S. cervicis*. Post hoc ANOVA studies reveals that $Mg(Azi)_2$, $Co(Azi)_2$, $Fe(Azi)_2$, $Zn(Azi)_2$ and $Ca(Azi)_2$ complexes showed significant increase ($p<0.001$) in antifungal activity against *C. albicans* in comparison to azithromycin.

Conclusion

The azithromycin (ligand, L) here, shows a bidentate behavior and was found that $N(CH_3)_2$ group of desosamine and hydroxyl group of azithromycin underwent complexation with selected metals. Among all synthesized drug based metal complexes, $Ca(Azi)_2$, $Fe(Azi)_2$, $Co(Azi)_2$, $Ni(Azi)_2$ and $Zn(Azi)_2$ complexes exhibits increased activity against both Gram negative and Gram positive organisms. All the other tested complexes exhibited mild to moderate antibacterial activity while, $Mg(Azi)_2$ and $Co(Azi)_2$ possess enhanced antifungal activity in comparison to azithromycin. Therefore, it can be concluded that azithromycin can be used as antifungal agent along with synthesized complexes.

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References

1. Rehman W, Baloch MK, Badshah A (2008) Synthesis, spectral characterization and bio-analysis of some organotin(IV) complexes. Eur J Med Chem 43: 2380-2385.
2. Gordon Research Conferences (2000) Metals in Medicine: Targets, Diagnostics, and Therapeutics. June 28-29, 2000. Natcher Conference Center National Institutes of Health, Bethesda, Maryland, USA.
3. Kostova I (2010) Metal-containing drugs and novel coordination complexes in therapeutic anticancer applications-part II. Anticancer Agents Med Chem 10: 352-353.
4. Kelland L (2007) The resurgence of platinum-based cancer chemotherapy. Nat Rev Cancer 7: 573-584.
5. Hambley TW (2007) Developing new metal-based therapeutics: challenges and opportunities. Dalton Trans: 4929-4937.
6. Caldwell MD (1990) Topical wound therapy-an historical perspective. J Trauma 30: 116-122.
7. Moues CM, Heule F, Legerstee R (2009) Topical Negative Pressure in Wound Care Effectiveness and guidelines for clinical application. Ostomy and wound management 55: 16-32.
8. Rafique S, Idrees M, Nasim A, Akbar H, Athar A (2010) Transition metal complexes as potential therapeutic agents. Biotechnology and Molecular Biology Reviews 5: 38-45.
9. Bukvić Krajacić M, Novak P, Cindrić M, Brajsa K, Dumić M, et al. (2007) Azithromycin-sulfonamide conjugates as inhibitors of resistant Streptococcus pyogene. Eur J Med Chem 42: 138-145.
10. Schonfeld W, Mutak S (2002) Macrolide Antibiotics. 73-95. Birkhauser Verlag Basel, Switzerland.
11. (2006) Physician Desk Reference, Medical economic company Inc., 64th edition, USA.
12. Riedel KD, Wildfeuer A, Laufen H, Zimmermann T (1992) Equivalence of a high-performance liquid chromatographic assay and a bioassay of azithromycin in human serum samples. J Chromatogr 576: 358-362.
13. Sher A, Rau H, Greiner G, Haubold W (1996) Spectroscopic and polarographic investigations of copper(II)-azithromycin interactions under equilibrium conditions. International Journal of Pharmaceutics 133: 237-244.
14. http://www.pharmaselect.cz/soubory/F/USP_DI_2005.pdf
15. El-Rjoob AW, Al-Mustafa J, Taha Z, Abous M (2008) Spectroscopic and conductometric investigation of the interaction of azithromycin with iron (II) ion. Jordan Journal of Chemistry 3: 199-209.
16. Arayne MS, Sultana N, Rafiq K (2001) *In vitro* activity of ceftizoxime and ceftazidime in presence of essential and trace elements. Pak J Pharm Sci 14: 57-64.
17. Arayne MS, Sultana N, Khanum F, Ali MA (2002) Antibacterial studies of cefixime copper, zinc and cadmium complexes. Pak J Pharm Sci 15: 1-8.
18. Sultana N, Arayne MS, Afzal M (2003) Synthesis and antibacterial activity of cephradine metal complexes: part I complexes with magnesium, calcium, chromium and manganese. Pak J Pharm Sci 16: 59-72.
19. Sultana N, Arayne MS, Sharif S (2004) Levofloxacin interactions with essential and trace elements. Pak J Pharm Sci 17: 67-76.
20. Arayne S, Sultana N, Haroon U, Mesaik MA (2009) Synthesis, characterization, antibacterial and anti-inflammatory activities of enoxacin metal complexes. Bioinorganic Chemistry and Applications 914105.
21. Sultana N, Naz A, Arayne MS, Mesaik MA (2010) Synthesis, characterization, antibacterial, antifungal and immunomodulating activities of gatifloxacin–metal complexes. Journal of Molecular Structure 969: 17-24.
22. Sultana N, Arayne MS, Gul S, Shamim S (2010) Sparfloxacin–metal complexes as antifungal agents-Their synthesis, characterization and antimicrobial activities. Journal of Molecular Structure 291: 285-291.
23. Sultana N, Arayne MS, Rizvi SBS, Haroon U, Mesaik MA (2013) Synthesis, spectroscopic and biological evaluation of some levofloxacin metal complexes. Medicinal Chemistry Research 22: 1371-1377.
24. Sultana N, Humza E, Arayne MS, Haroon U (2011) Effect of metal ions on the *in vitro* availability of enoxacin, it's *in vivo* implications, kinetic and antibacterial studies. Quimica Nova 4: 186-189.
25. Sultana N, Arayne MS, Sabri R (2002) Clarithromycin synergism with essential and trace elements. Pak J Pharm Sci 15: 43-54.
26. Sultana N, Arayne MS, Sabri R (2005) Erythromycin synergism with essential and trace elements. Pak J Pharm Sci 18: 35-39.
27. Arayne MS, Sultana N, Sabri R (2004) Roxithromycin antagonism with essential and trace elements. Pak J Pharm Sci 17: 65-75.
28. World Health Organization (2003) Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Importance in the Developing World 103-162.
29. Akinyemi KO, Oladapo O, Okwara CE, Ibe CC, Fasure KA (2005) Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for antimethicillin resistant *staphylococcus aureus* activity. BMC Complementary and Alternative Medicine 5: 1472-1483.
30. Lingane JJ (1958) Electro analytical Chemistry, 2nd edition, Interscience Publishers, New York, USA.
31. Mazumder UK, Gupta M, Bhattacharya S, Karki SS, Rathinasamy S, et al. (2004) Antineoplastic and antibacterial activity of some mononuclear Ru(II) complexes. J Enzyme Inhib Med Chem 19: 185-192.