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Research article

Synthesis, Characterization, Antibacterial and Antifungal Studies of Azithromycin with NSAIDS.

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Abstract

Azithromycin or 1-oxa-6 azacyclopentadecan-15-one, 13- [(2-6-dideoxy – 3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl),oxy]-2- ethyl-3,4-10- trihydroxy-3, 5, 6, 8, 10, 12, 14-heptamethyl-11- [[3,4,6- trideoxy-3-(dimethylamino) - β-D-xylo-hexopyranosyl]oxy] is a 15-membered semi synthetic derivative of erythromycin, also known as azalides. It is more active against Gram-negative bacteria and less active against Gram-positive bacteria than other macrolides. Derivatives of Azithromycin have been synthesized by reactions with various NSAID's. These synthesized analogues were confirmed by spectroscopic technique such as UV, IR, NMR and Mass Spectroscopy in which the incoming groups occupy either amino, hydroxyl or keto group of the drug. Their antibacterial and antifungal activities against various organisms were performed and compared with that of the parent drug.

Keywords: Azithromycin, Macrolides, NSAIDs, Spectroscopy.

Introduction

Azithromycin or [1-oxa-6 azacyclopentadecan-15-one ,13- [(2-6-dideoxy – 3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl) oxy]- 2- ethyl - 3, 4- 10- trihydroxy-3, 5, 6, 8, 10, 12, 14-heptamethyl-11- [[3,4,6- trideoxy-3-(dimethylamino) - β-D-xylo-hexopyranosyl]oxy]- [2R (2R*,3S* ,4R* ,5R* ,8R* ,10R* ,11R* ,12S* , 13S*,

14R*) **[1]** semi synthetic derivative of erythromycin A **[2, 3]**. It was first approved in 1991 as a newer macrolide to overcome some of the shortcomings of erythromycin such as intolerance, pharmacokinetics, and limited antimicrobial spectrum **[4].** Azithromycin has 15-membered ring, which is derived from the insertion of an amino group into the lactone ring C-60.94%, H-9.69%, N-3.74%, O-25.63%b **[5]**. The dibasic nature of azithromycin results in enhanced acid stability **[6, 7]**. It is stable at gastric pH and has an absolute bioavailability of approximately 37 percent following oral administration **[8].** It binds to the 50 S ribosomal subunits of susceptible bacteria and suppresses protein synthesis. Azithromycin appears to bind to the same receptor as erythromycin. It is also used to treat bacterial upper and lower respiratory tract infections, skin and skin structure infections, and sexually transmitted diseases. Azithromycin represents a significant improvement in the treatment of selected community-acquired infections **[9, 10].**

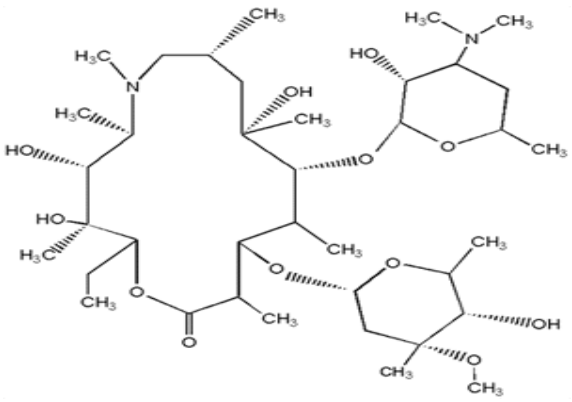

Figure 1: Azithromycin

The azalide azithromycin and the ketolide ABT-773, which were derived by modifications of erythromycin, exhibit elevated activity against a number of penicillin- and macrolide-resistant pathogenic bacteria **[11]**. Azithromycin is used to treat bacterial infections in many different parts of the body but most often used to treat respiratory infections in children and adults. It is active against Gram negative pathogens such as *Haemophilus influenzae* as well as some of the *Enterobacteriaceae* such as *Escherichia coli and Salmonella*. Azithromycin **[12]** is official in the United States Pharmacopoeia, and it is assayed by the high performance liquid chromatographic method. Literature survey reveals that azithromycin is estimated in pharmaceuticals and biological fluids by spectrophotometric **[13]**, HPLC **[14], [15]** and microbiological methods **[16].**

Azithromycin exerts significant bactericidal actions on microbial flora isolated from
oral infectious foci, and it was proven to be clinically effective when administered to patients with dental infections. **[17]** Therefore, combined treatments with non-steroidal anti-inflammatory drugs and antibiotics may offer significant benefits in the prevention of pain and infections associated with oral surgery. **[18].**It was observed that, upon combined treatment with piroxicam and azithromycin, the macrolide antibiotic may interfere with the therapeutic action of piroxicam on the recovery of oral tissues from surgical procedures. **[19].**these changes and the

formation of new compound may interfere with the antibacterial activity of azithromycin as well as analgesic effects of NSAID's.

Therefore, purpose of our present work is to study the interactions in the NSAID's-Azithromycin system as well as to determine and characterize the specie formed, if any. On the basis of this study we have performed this experiment with many NSAID's. The integrated analysis is based on the spectroscopic methods i.e. IR, NMR and Mass spectroscopy to attain the reliable information. Antibacterial and Antifungal activities of these derivatives have been carried out against various organisms and results were compared with that of the parent drug. Some of these derivatives have shown good activity.

Materials and methods

Materials and reagents

Azithromycin was a kind gift from Platinum Pharmaceuticals (Karachi) while solvents and chemicals of analytical grade were purchased from the market. All solutions were freshly prepared.

Instruments

The melting points were taken on an electro thermal melting point apparatus (Gallenkamp) in open capillary tubes and were found uncorrected. TLC spots were detected by UV lamp. Infrared spectra were recorded in KBr pellets on Shimadzu 470 instrument. ^{1}H NMR spectra were obtained by using Bruker /XWIN NMR spectrometer with TMS as internal standard. Complexes were dissolved in $CDCl_3$, D_2O or MeOH for NMR. An elemental analysis is done by Carlo Erba Strumentazione Elemental analyzer-MOD 1106 instrument.

Method for preparation:

The derivatives were synthesized by refluxing Azithromycin and NSAID's (Naproxin sodium, Ibuprofen, Tiaprofenic acid, Diclofenic sodium, Meloxicam, Mefenamic acid) in MeOH in the ratio of 2:1. First clear solution of each reactant was made then mixed together and heated for 15 minutes on hot plate and finally refluxed on water bath at $80^{o}C$. Purity of all synthesized derivatives were checked by TLC on precoated silica gel plates utilizing methanol/chloroform as developing solvent system in ratio(1:1v/v) and spots were detected either in UV lamp or by exposure to iodine vapors in a tightly closed chamber. Moreover their melting points and solubility's were noted.

Microbiological screening:

The susceptibility of certain bacterial and fungal strains towards azithromycin and its derivatives were evaluated by measuring the size of zone of inhibition diameter **[20]**. The results are shown in table.

Antibacterial studies:

The antibacterial screening of derivatives was performed by the conventional cylinder-plate method **[18]** for the determination of minimal inhibitory concentrations (MICs). MIC values for all compounds were determined in comparison with azithromycin on a panel of sensitive and resistant Gram-positive bacterial strains (*Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus,* and *Streptococcus features*), and on Gram-negative strains (*Salmonella typhi, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Escherichia coli, Citrobacter* and *Shigella flexneri)* **[19].**

The solutions for soaking discs were made of different dilutions including 5, 10 and 20ppm by simple dilution method using water/DMSO as solvent. To ensure that the solvent had no effect on bacterial growth, a control test was performed with test medium supplemented with methanol at the same dilutions as used in the experiment.

Nutrient agar used for antibacterial activity was prepared and then autoclaved at 121^{o}C for 15 minutes, cooled and then poured in Petri dishes. Streaking is done with the help of sterile cotton swab, soaked discs of derivative solutions were placed in them and the dishes were incubated for 24 hrs at 37^{o}C. The zones were carefully measured with the help of Vernier caliper.

Antifungal studies:

The synthesized derivatives were screened for their antifungal activity against a series of fingi, (*Candida albicans, F.solani, T.rubrub, A.parasitieus, A.effusis and S.cervicis)* by the conventional cylinder-plate method **[21]**. Discs of different concentration (5, 10, 20ppm) were used. Sabrouad dextrose agar was prepared and then autoclaved at 121^{o}C for 15 minutes, cooled and then poured in Petri dishes. Streaking is done with the help of sterile cotton swab, soaked discs of derivative solutions were placed in them and the dishes were incubated for 48 hrs at 37^{o}C. The zones were carefully measured with the help of Vernier calliper.

Result and Discussion

Derivatives of azithromycin were prepared by refluxing of NSAID's with azithromycin. These derivatives were prepared by reaction of single compound with parent drug at different targeted sites. Table 1 and 2, explains Physical Parameters and spectroscopic details of the newly synthesized compounds.

Table 1: Physical Parameters

Compounds	**Melting Points^{0} C**	**State**	**Color**	**Solubility**			**% Yield**
				methanol	**DMSO**	**chloroform**	
Naproxen	135	crystalline	white	++	++	+	43
Ibuprofen	80	crystalline	white	++	+++	+	59
Tiaprofenic acid	100	powder	off white	+++	+++	+	20
Diclofenac sodium	80	powder	white	++	++	+	23
Meloxicam	140	crystalline	yellow	++	++	+	70
Mefanamic acid	100	flakes	white	++	+++	+	80
Azithromycin	115	crystalline	white	+++	+++	+	-

Table 2: IR Spectroscopic data

Compounds	N-H stretch	OH	C-H Aliphatic stretching	C=O	C-O	O-CH_3	CH_3
Naproxen	-	3400	2750	1730	1600	-	1325-1375
Ibuprofen	-	3400	2950. 2850. 2800	1725	1625	-	-
Tiaprofenic acid	-	3400	2975	1720	1625	-	-
Diclofenac sodium	-	3400	2950. 2900. 2750	1725	1600	-	1300-1325
Meloxicam	-	3400	2975. 2775	1720	1650	1425	1375
Mefanamic acid	-	3400	2950. 2750	1725	1625	1500-1575	-
Azithromycin	3500	-	2850-3000	1728-1735	1650	-	1315

The characteristic absorption in the IR spectra of the derivatives is listed in Tables **2**. In general, in the IR spectra of Azithromycin, shows N-H stretching band which is indicative for the presence of primary amine is no more in that for the derivatives .Where as, presence of bonded hydrogen is indicated by the appearance of O-H peak at 3400 in all of the compounds synthesized. Bands in the region of 1300-1800, assigned to the C=O, C=N, C-O vibrations were observed. The reported peak of carbonyl group in azithromycin occurs at 1728 cm^{-1} where as in azithromycin reference this peak appears at 1725-1735 cm^{-1} as a sharp and medium peak where as in derivatives there is no such prominent peak shifts but change is observed in almost all of the compound with respect to the intensity, sharpness, broadness and presence.

Further characterization of the derivatives synthesized was accompanied by NMR measurements. The ^1H NMR spectra of azithromycin shows well defined resonance at δ ppm 3.352(3H,s),2.316(3H,s) and 2.288(6H,s)assigned to 3"-OCH_3 absorbance of cladinose, the 9a N-CH_3 group of 15 membered aglycone ring and the $N(CH_3)_2$ group of desosamine, respectively.

Microbiological screening:

All data were presented as zone of inhibition diameter in mm. One way analysis of variance (ANOVA) was carried out to check any differences between the zone of inhibitions of all prepared derivatives and standard. Post hoc Dunnett's test was applied to the data and differences were considered significant at $p \leq 0.05$. ANOVA show significance differences between all prepared derivatives with azithromycin.

Against Gram negatives and yeast

ANOVA showed significance differences between all prepared derivatives with azithromycin against P. *mirabilis* at 5 µg ($F=1491.128$, $p< 0.001$), 10 µg ($F=1336.135$, $p< 0.001$) and 20 µg ($F=1174.385$, $p< 0.001$), Dunnett's test analyzed that against *P. mirabilis* all compounds were significantly decreased ($p<0.001$). At 5µg, the order of inhibition was Mef >Dic> Mel >Nap> Ibu and their percent zone of inhibitions were 16.96, 17.94, 26.87, 27.14 and 44.2 %, respectively. At 10µg the order of inhibition was Mef > Dic>Nap> Ibu> Mel and their percent zone of

inhibitions were 22.73, 23.15, 30.67, 30.63 and 30.74 %, respectively. At 20 µg the order of inhibition was Mel > Ibu>Dic> Mef >Nap and their percent zone of inhibitions were 26.33, 26.67, 26.77, 27.01 and 33.3%, respectively.

ANOVA showed significance differences between all prepared derivatives with azithromycin against S. *typi* at 5µg (F=8143.149, p<0.001), 10 µg (F=9466.626, p< 0.001) and 20 µg (F=13843.762, p< 0.001), Dunnett's test analyzed that against S. *typi*, all derivatives showed significant decrease (p<0.001) in activities at 5, 10 and 20 µg concentration. At 5µg the order of inhibition was Nap>Mef>Dic>Ibu>Mel and their percent zone of inhibitions were 0.33, 10.76, 11.19 22.16 and 23 %, respectively. At 10µg the order of inhibition was Nap>Mef >Dic>Ibu>Mel and their percent zone of inhibitions were-0.8, 0.4, 9.82, 10.46 and 20.41%, respectively. At 20µg the order of inhibition was Mef >Nap>Ibu>Mel>Tia>Dic and their percent zone of inhibitions were -0.6, -0.15, 8.31, 8.58 and 9.31%, respectively.

Organism	***P. mirabilis***			**S. *typi***		
Concentrations	5	10	20	5	10	20
Azi (mean±S.D)	22.2±0.17	26.29±0.16	30.28±0.17	18.29±0.12	20.23±0.17	22.21±0.13
Ibu (mean±S.D) %Z.I	12.39±0.13* 44.2	18.21±0.13* 30.74	22.2±0.22* 26.67	16.24±0.18* 11.19	18.12±0.11* 10.46	20.37±0.11* 8.31
Dic (mean±S.D) %Z.I	18.22±0.19* 17.94	20.2±0.06* 23.15	22.18±0.11* 26.77	16.32±0.21* 0.76	18.25±0.19* 9.82	20.14±0.13* 9.31
Mef (mean±S.D) %Z.I	18.43±0.08* 16.96	20.31±0.13* 22.73	22.1±0.09* 27.01	18.23±0.12 0.33	20.4±0.1 -0.8	22.34±0.11 -0.6
Mlx (mean±S.D) %Z.I	16.23±0.19* 26.87	18.24±0.13* 0.63	22.31±0.17* 26.33	14.24±0.11* 22.16	16.1±0.12* 20.41	20.3±0.15* 8.58
Nap (mean±S.D) %Z.I	16.17±0.12* 7.14	18.23±0.13* 30.63	20.2±0.12* 33.3	18.25±0.04 0.23	20.15±0.06 0.4	22.24±0.14 -0.15
ANOVA (P<0.001), df = 6, 14.						
F- value	1324.57	1701.70	1463.33	327.84	362.58	241.69
* indicates significance and -ve sign shows increase in activity.						

ANOVA showed significance differences between all prepared derivatives with azithromycin against E. *coli* at 5 µg (F=471.175, p< 0.001), 10 µg (F=461.717, p< 0.001) and 20 µg (F=390.995, p< 0.001), Dunnett's test reveals that significant decrease (p<0.001) was exhibited by all derivatives against *E. coli*; At 5µg the order of inhibition was Mel > Mef > Ibu> Nap > Dic and their percent zone of inhibitions were -0.14, 0.26, 10.83, 11.26 and 22.28 %, respectively. At 10µg the order of inhibition was Mef > Mel > Dic > Nap>Ibu and their percent zone of inhibitions

were -0.74, -0.2, 9.77, 10.23 and 29.57 %, respectively. At 20µg the order of inhibition was Mef >Mel > Dic> Ibu>Nap and their percent zone of inhibitions were 0.25, 0.3, 0.36, 8.62 and 8.72 %, respectively.

ANOVA showed significance differences between all prepared derivatives with azithromycin against P. *aeruginosa* at 5 µg (F=459.571, p< 0.001), 10 µg (F=146.117, p< 0.001) and 20 µg (F=112.548, p< 0.001), Dunnett's test reveals that antibacterial activity of all derivatives against *P. aeruginosa* were significantly decreased (p<0.001). At 5µg the order of inhibition was Mef > Mel > Ibu>Dic> Nap and their percent zone of inhibitions were 0.07, 7.33, 7.34, 12.51 and 3.45 %, respectively. At 10 µg the order of inhibition was Mef >Ibu>Dic> Nap > Mel and their percent zone of inhibitions were 0.03, 0.62, 0.9, 1.07 and 1.23 %, respectively. At 20 the order of inhibition was µg Nap >Mel > Ibu> Mef >Dic and the order of their percent zone of inhibitions were -10.45, -5.51, -5.27, 0.05 and 0.09 %, respectively.

Organism	***E. coli***			***P. aeruginosa***		
Concentrations	5	10	20	5	10	20
Azi (mean±S.D)	18.22±0.16	20.17±0.16	22.26±0.11	16.41±0.09	18.37±0.06	20.21±0.15
Ibu (mean±S.D) %Z.I	16.24±0.2* 10.83	18.11±0.06* 10.23	20.34±0.14* 8.62	15.2±0.2* 7.34	18.26±0.1 40.62	21.28±0.13* -5.77
Dic (mean±S.D) %Z.I	14.16±0.16* 22.28	18.24±0.16* 9.57	22.2±0.14 0.25	14.35±0.11* 12.5	18.21±0.2 20.9	20.19±0.17 0.09
Mef (mean±S.D) %Z.I	18.17±0.19 0.26	20.32±0.12 -0.74	22.34±0.22 -0.36	16.39±0.09 0.07	18.37±0.13 0.03	20.2±0.22 0.05
Mlx (mean±S.D) %Z.I	18.24±0.21 -0.14	20.17±0.11 -0.02	22.19±0.01 0.3	15.2±0.01* 7.33	18.15±0.05 0.03	21.33±0.25* -5.51
Nap (mean±S.D) %Z.I	16.17±0.14* 11.26	18.2±0.03* 9.77	20.32±0.19* 8.72	14.2±0.16* 13.45	18.18±0.2 11.07	22.32±0.05* -10.45
ANOVA (P<0.001), df = 6, 14.						
F- value	246.58	219.10	139.07	178.28	1.46	64.03
* indicates significance and -ve sign shows increase in activity.						

ANOVA showed significance differences between all prepared derivatives with azithromycin against K. *pneumonia* at 5 µg (F=435.936, p< 0.001), 10 µg (F=538.993, p< 0.001) and 20 µg (F=548.511, p< 0.001), Dunnett's test reveals that against K. *pneumonia,* almost all derivatives were significantly decreased (p<0.001). At 5 µg while at 10 and 20 µg Dic was found significantly increased (p<0.001); at 5 µg the order of inhibition was Dic> Mef > Nap> Mel >Ibu and their percent zone of inhibitions were -0.45, 10.77, 10.94, 11.01 and 22.38 %, respectively. At 10µg the order of inhibition was Dic>Nap> Mel > Mef >Ibu and their percent zone of inhibitions were 9.6, -0.57, -0.53,

9.64 and 10.05 %, respectively. At 20 µg the order of inhibition was Dic>Nap> Ibu> Mel > Mef and their percent zone of inhibitions were -9.89, -8.75, -0.6, -0.44, and 9.07 %, respectively.

ANOVA showed significance differences between all prepared derivatives with azithromycin against S. *flexneri* at 5 µg (F=6281.517, p< 0.001), 10 µg (F=19519.773, p< 0.001) and 20 µg (F=13129.221, p< 0.001), Dunnett's test reveals that significant decrease (p<0.001) was observed against *S. flexneri.* At 5 µg the order of inhibition was Mef> Dic >Ibu > Mel > Nap and their percent zone of inhibitions were 9.82, 10.02, 19.96, 30.67 and 40.01 %, respectively. At 10µg g the order of inhibition was Mef > Dic > Ibu > Nap >Mel and and their percent zone of inhibitions were -0.18, 9.42, 18.39, 26.78 and 26.92 %, respectively. At 20µg the order of inhibition was Mef >Nap> Dic > Ibu > Mel and their percent zone of inhibitions were -7.79, 8.03, 8.41, 16.43 and 24.47 %, respectively.

Organism	**K. *pneumonia***			***S. flexneri***		
Concentrations	5	10	20	5	10	20
Azi (mean±S.D)	18.17±0.12	20.26±0.18	22.20±0.12	20.32±0.07	22.21±0.11	24.29±0.23
Ibu (mean±S.D) %Z.I	14.1±0.07* 22.38	18.23±0.21* 10.05	22.33±0.04 -0.6	16.26±0.21* 19.96	18.13±0.04* 18.39	20.3±0.12* 16.43
Dic (mean±S.D) %Z.I	18.25±0.16 -0.45	22.21±0.12* -9.6	24.4±0.09* -9.89	18.28±0.07* 10.02	20.12±0.09* 9.42	2.25±0.2* 8.41
Mef (mean±S.D) %Z.I	16.21±0.22* 10.77	18.31±0.16* 9.64	20.19±0.11* 9.07	18.32±0.25* 9.82	22.25±0.08 - 0.18	26.18±0.02* -7.79
Mlx (mean±S.D) %Z.I	16.17±0.18* 11.01	20.37±0.04 -0.53	22.3±0.16 -0.44	14.09±0.09* 30.67	16.23±0.05* 26.92	18.35±0.11* 24.47
Nap (mean±S.D) %Z.I	16.18±0.24* 10.94	20.38±0.02 -0.57	24.14±0.08* -8.75	12.19±0.25* 40.01	16.26±0.11* 26.78	22.34±0.16* 8.03
ANOVA (P<0.001), df = 6, 14.						
F- value	199.76	390.19	536.84	845.37	1750.19	756.24
* indicates significance and -ve sign shows increase in activity.						

ANOVA showed significance differences between all prepared derivatives with azithromycin against *Citrobacter* at 5 µg (F=2920.120, p< 0.001), 10 µg (F=2525.611, p< 0.001) and 20 µg (F=2428.673, p< 0.001), Dunnett's test reveals that every derivative showed significant decrease (p<0.001) at all concentrations against *Citrobacter*; at 5µg the order of inhibition was Ibu > Dic > Mef > Mel > Nap and their percent zone of inhibitions were 30.01, 30.44, 37.37, 38.08 and 53.49 %, respectively. At 10 µg the order of inhibition was Ibu> Mel > Mef > Dic > Nap and their percent zone of inhibitions were 28.61, 28.92, 31.96, 35.64, and 45.97 %, respectively. At 20µg the order of inhibition was Ibu> Mel> Mef > Dic > Nap and their percent zone of inhibitions were 26.31, 26.53, 29.31, 33, and 39.49 %, respectively.

ANOVA showed significance differences between all prepared derivatives with azithromycin against C. *albicans* at 5 µg (F=584.127, p< 0.001), 10 µg (F=356.356, p< 0.001) and 20 µg (F=287.906, p< 0.001), Dunnett's test reveals that activity of derivatives against *C. albicans* was found to be significantly increased (p<0.001) except Ibu and Nap at concentrations 5 and 10 µg, whereas, Ibu at 20 µg which showed significant decrease (p<0.001). At 5µg the order of inhibition was Mef > Nap > Dic > Ibu >Mel and their percent zone of inhibitions were -13.03,-12.41, -6.43, 11.72 and 3.64 %, respectively. At 10µg the order of inhibition was Mef > Nap > Dic > Ibu > Mel and their percent zone of inhibitions were -11.42, -10.72, -6.27, 11.21 and 11.112 %, respectively. At 20µg the order of inhibition was Mef > Nap > Dic > Mel> Ibu and their percent zone of inhibitions were -10.33, -9.4, -4.47, -0.33 and 9.22 %, respectively.

Organism	***Citrobacter***			***C. albicans***		
Concentrations	5	10	20	5	10	20
Azi (mean±S.D)	26.25±0.04	28.31±0.03	30.21±0.11	16.2±0.05	18.26±0.17	20.31±0.16
Ibu (mean±S.D) %Z.I	18.37±0.13* 30.01	20.13±0.17* 28.92	22.25±0.19* 26.31	14.3±0.15* 11.7	16.21±0.1* 11.21	18.43±0.04* 9.22
Dic (mean±S.D) %Z.I	16.44±0.04* 37.37	18.19±0.15* 35.75	20.23±0.1* 33	17.23±0.17* -6.43	19.4±0.05* -6.27	21.21±0.17* -4.47
Mef (mean±S.D) %Z.I	17.27±0.18* 34.23	19.26±0.21* 31.96	21.35±0.15* 29.31	18.3±0.16* -13.03	20.34±0.08* -11.42	22.4±0.09* -10.33
Mlx (mean±S.D) %Z.I	18.26±0.11* 30.44	20.21±0.22* 28.61	22.19±0.24* 26.53	12.36±0.05* 23.64	16.23±0.2* 11.1	20.37±0.05 -0.33
Nap (mean±S.D) %Z.I	12.21±0.17* 53.49	15.3±0.02* 45.97	18.27±0.07* 39.49	18.2±0.2* -12.41	20.21±0.16* -10.77	22.21±0.12* -9.4
ANOVA (P<0.001), df = 6, 14.						
F- value	3194.81	1869.03	2011.80	696.78	504.71	441.76
* indicates significance and -ve sign shows increase in activity.						

Against Gram positives

ANOVA showed significance differences between all prepared derivatives with azithromycin against *M. luteus* at 5 µg (F=5496.21, p< 0.001), 10 µg (F=3981.98, p< 0.001), and 20 µg (F=3919.37, p< 0.001), Dunnett's test analysis reveals that all derivatives showed significant decrease (p< 0.001) against *M. luteus* at all concentrations. At 5µg the order of inhibition was Ibu> Dic> Mel>Mef> Nap and their percent zone of inhibitions were 35.09, 42.16, 49.45, 49.73 and 59.87 %, respectively. At 10µg the order of inhibition was Dic>Ibu> Mef>Mel> Nap and their percent zone of inhibitions were 33.29, 33.87, 39.89, 46.8 and 56.14 %, respectively. At 20µg the order of inhibition

was Dic> Mef>Ibu> Mel> Nap and their percent zone of inhibitions were 24.59, 30.83, 31.07, 43.15 and 52.53 %, respectively.

ANOVA showed significance differences between all prepared derivatives with azithromycin against *B. subtilis* at 5µg (F=4336.85, p<0.001), 10 µg (F=54264.98, p< 0.001) and 20 µg (F=8509.81, p< 0.001), Dunnett's test analysis reveals against *B. subtilis*, all derivatives showed significant increase (p<0.001). At 5, 10 and 20µg the order of inhibition was Mef>Ibu> Dic> Mel> Nap> Tia and their percent zone of inhibitions were at 5 µg -8131.77, -8121.11, -7196.26, and -7183.48, at 10 µg, -5428.13, -5403.61, -4827.42, -4856.64 and -4299.27. Whereas, at 20 µg -9075.89, -9055.89, -8257.7, -8233.55 and -7373.96 %, respectively.

Organism	***M. luteus***			***B. subtilis***		
Concentrations	5	10	20	5	10	20
Azi (mean±S.D)	28.19±0.15	30.33±0.21	32.25±0.23	0.22±0.15	0.37±0.09	0.24±0.07
Ibu (mean±S.D) %Z.I	18.3±0.09* 35.09	20.05±0.05* 33.87	22.23±0.11* 31.07	18.27±0.17* -8121.11	20.27±0.23* -5428.11	22.29±0.2* -9055.89
Dic (mean±S.D) %Z.I	16.3±0.17* 42.16	20.23±0.12* 33.29	24.32±0.15* 24.59	16.21±0.11* -7196.26	18.15±0.23* -4827.42	20.35±0.05* -8257.7
Mef (mean±S.D) %Z.I	14.17±0.1* 49.73	18.23±0.13* 39.89	22.31±0.07* 30.83	18.29±0.18* -8131.77	20.36±0.16* -5482.13	22.34±0.19* -9075.89
Mlx (mean±S.D) %Z.I	14.25±0.14* 49.54	16.13±0.17* 46.8	18.33±0.13* 43.15	16.18±0.25* -7183.48	18.26±0.17* -4856.64	20.29±0.12* -8233.55
Nap (mean±S.D) %Z.I	11.31±0.15* 59.87	13.3±0.25* 6.14	15.31±0.15* 52.53	14.09±0.15* -6424.98	16.2±0.2* -4299.27	18.2±0.22* -7373.96
ANOVA (P<0.001), df = 6, 14.						
F- value	5496.21	3981.98	3919.37	4336.85	54264.98	8509.81
* indicates significance and -ve sign shows increase in activity.						

ANOVA showed significance differences between all prepared derivatives with azithromycin against, *S. features at* 5 µg (F=433.718, p< 0.001), 10 µg (F=512.419, p< 0.001) and 20 µg (F=1192.478, p< 0.001), Dunnett's test analysis reveals that against *S. features*, all derivatives showed significant increase (p<0.001) except Nap which was found to be insignificant; at 5 µg the order of inhibition was Ibu> Mef> Dic>Mel> Nap and their percent zone of inhibitions were -23.55, -22.48, -11.4, -10.81 and 0.32 %, respectively. At 10 µg the order of inhibition was Mef> Ibu> Dic> Mel> Nap and their percent zone of inhibitions were -22.2, -21.93, -21.73, -11.23 and 0.4 %, respectively. At 20 µg the order of inhibition was Dic> Mef> Ibu> Mel> Nap and their percent zone of inhibitions were 20 µg -29.97, -20.35, -20.08, -9.34 and 0.31 %, respectively.

ANOVA showed significance differences between all prepared derivatives with azithromycin against *S. aureus at* 5 µg (F=2711.56, p< 0.001), 10 µg (F=2563.76, p< 0.001) and 20 µg (F=1330.80, p< 0.001). Dunnett's test analysis reveals that against *S. aureus* all derivatives showed significant decrease (p< 0.001) at 5 µg the order of inhibition was Dic>Ibu> Mef> Mel> Nap and their percent zone of inhibitions were 34.36, 38.14, 38.25, 45.55 and 45.89 %, respectively. At 10 µg the order of inhibition was Dic> Mel> Mef> Ibu> Nap and their percent zone of inhibitions were 27.86, 35.24, 35.34, 35.41 and 35.07 %, respectively. At 20 µg the order of inhibition was Dic> Nap> Mef > Mel >Ibu and their percent zone of inhibitions were 23.57, 26.53, 26.78, 27.11 and 33.47 %, respectively.

Organism	***S. features***			***S. aureus***		
Concentrations	5	10	20	5	10	20
Azi (mean±S.D)	16.44±0.05	18.27±0.18	20.26±0.2	26.22±0.17	28.23±0.18	30.37±0.12
Ibu (mean±S.D) %Z.I	20.31±0.11* -23.55	22.28±0.16* -21.93	24.33±0.06* -20.08	16.22±0.16* 38.14	18.23±0.13* 35.41	20.21±0.15* 33.47
Dic (mean±S.D) %Z.I	18.31±0.19* -11.4	22.24±0.23* -21.73	26.33±0.07* -29.97	17.21±0.09* 34.36	20.36±0.19* 27.86	23.22±0.15* 23.56
Mef (mean±S.D) %Z.I	20.13±0.05* -22.48	22.33±0.13* -22.2	24.39±0.11* -20.35	16.19±0.13* 38.25	18.25±0.12* 35.34	22.24±0.22* 6.78
Mlx (mean±S.D) %Z.I	18.21±0.18* -10.81	20.33±0.16* -11.23	22.15±0.14* -9.34	14.28±0.06* 45.55	18.28±0.04* 35.24	22.14±0.14* 27.11
Nap (mean±S.D) %Z.I	16.38±0.12 0.32	18.2±0.13 0.4	20.2±0.11 0.31	14.19±0.15* 45.89	18.33±0.02* 35.07	22.32±0.11* 26.35
ANOVA (P<0.001), df = 6, 14						
F- value	433.718	512.419	1192.478	2711.56	2563.76	1330.80
* indicates significance and -ve sign shows increase in activity.						

As shown in tables 1 - 3, the *in vitro* antibacterial and antifungal activities of the Azithromycin derivatives were evaluated against series of Gram positive and Gram negative bacteria as well as against fungi and compared with the parent drug.

Almost all synthesized derivatives were found to exhibit moderate to good activity against a wide variety of Gram positive and Gram negative bacteria and against fungi. Comparison of antibacterial activity data suggests that almost all derivatives are active antimicrobial agents and most of them are more potent than parent drug.

Azithromycin itself is not used as fungicidal, before. Here we have not only tested azithromycin but also its synthesized derivatives were evaluated for antifungal activity. While, Clarithromycin and Roxithromycin belongs to same class of marolides were also studied as an antifungal shows good results against both *C.albican* and *F.solani.* Antifungal activity data reveals that none of the derivative including azithromycin (parent drug) did not show any

activity against any fungal specie; *F.solani, T.rubrub A.parasitieus, A.effusis and S.cervicis* except *C.albican i.e; yeast.*

Conclusion

Present work shows successful synthesis of azithromycin derivatives by refluxing method in good yields. Most of them show excellent antimicrobial activity as compared to parent drug (azithromycin). Derivatives were proved to have good sensitivity against both Gram positive and Gram negative. On passing toxicity tests, these derivatives may prove good candidates for clinical studies and may prove to be the potential antimicrobial agents for future.

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