Synthesis, characterization, antimicrobial and enzyme inhibitory studies of moxifloxacin with aromatic carboxylic acids

Mahwish Akhtar[1], Najma Sultana[2], Mohammad Saeed Arayne[3], Tanveer Ahmed Siddiqi[4] and Ajmal Khan[5]

[1]Dow College of Pharmacy, Dow University of Health Sciences, Karachi, Pakistan
[2#]Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi
[3#]Department of Chemistry, University of Karachi, Karachi, Pakistan
[4]Department of Mathematics, University of Karachi, Karachi, Pakistan
[5]HEJ Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan

Abstract: A series of carboxamide derivatives of moxifloxacin has been synthesized. The synthesized derivatives has been characterization by using spectroscopic techniques such as UV-Vis, IR, H^1NMR and Mass spectra, which suggested that incoming group has occupied azabicylo groups of selected moxifloxacin at 7^{th} position. Antimicrobial screening has been systematically carried out against various gram-positive, Gram-negatives and fungi in comparison with parent drug. Enzymatic assay were also performed. The results obtained were statistically analyzed by one way ANOVA. The antimicrobial results reveals that the synthesized derivative of moxifloxacin possess good activities against *B. subtilis, F. solani, T. rubrum and P. aeruginosa* concluding that derivatives are more potent antimicrobial agents as compared to parent drug. While compound B1 solely possess mild enzymatic activity against urease whereas, no other compounds is active against both urease and carbonic anhydrase.

Keywords: Moxifloxacin, carboxamide derivatives, biological evaluation, enzymatic activities, ANOVA.

INTRODUCTION

Nowadays, the resistance of antibiotic agents is an alarming issue, all over the world. For this modification purpose fluoroquinolones are compound of interest because of their high potency against several bacteria. During the recent years, many scientists had modified C3 and C7 positions of fluoroquinolones and synthesized number of derivatives of ciprofloxacin, norfloxacin, levofloxacin, garenoxacin, ofloxacin, gatifloxacin and moxifloxacin and screened against different Gram-positive and Gram-negative bacteria (Casal *et al*., 2017; Huang *et al*., 2016; Huang *et al*., 2015; Garza *et al*., 2017; Hu *et al*., 2018).

Moxifloxacin (MFX) is a synthetic 8-methoxyquinolone, fourth generation agents of FQ antibacterial agent. It was discovered in 1999 (Ronald AR *et al*., 2003), by introducing an azabicyclo at C-7, which is related with significantly improved Gram-negative and positive activity (*S. pneumoniae, H. influenzae, M. catarrhalis, H. parainfluenzae, S. aureus* and *K. pneumoniae, M. pneumoniae*, or *C. pneumonia, S. pyogenes*, methicillin-susceptible *S. aureus, E. coli, E. cloacae* (Xu *et al*., 2018). The bactericidal action of MFX is due to the inhibition of topoisomerase II and IV (Towle *et al*., 2018).

In present work, we have synthesized a series of moxifloxacin, MFX, (fig. 1) derivatives with different aromatic carboxylic acids by focusing C-7 position. Structure elucidation was carried out through IR, 1HNMR and mass spectroscopy. Screening of antibacterial activities of these derivatives have been systematically carried out against numerous Gram +ve and Gram -ve organisms and compared with parent drug. Some of these derivatives have shown good antibacterial activity as compared to parent drug.

MATERIALS AND METHODS

Getz pharmaceuticals, Karachi, Gifted moxifloxacin hydrochloride and other analytical grade reagents and solvents were purchase from Merck, Darmstadt, Germany laboratory supplies. All glass-wares used were of Pyrex grade, washed with chromic acid than rinse with freshly prepared de-ionized water.

The melting points were obtained using Galleukamp apparatus. Infrared spectra were recorded in KBr pellets on Shimadzu prestige-21 200 VCE Spectrophotometer in the region of 400-4000 cm-1. 1H-NMR spectra were obtained by Bruker/XWIN-NMR spectrophotometer, the compounds were dissolved in $CDCl_3$ and methanol and an internal standard was TMS. Mass spectra were obtained by JEOL MS Route for derivatives of moxifloxacin. CHN analysis was performed on Elemental analyzer Carlo Erba 1106 for metal complexes. The TLC plate was used to performed thin layer chromatography (TLC) and compounds analyzed by iodine vapors.

*Corresponding author: e-mail: mahwish.akhtar@duhs.edu.pk #Retired Professor

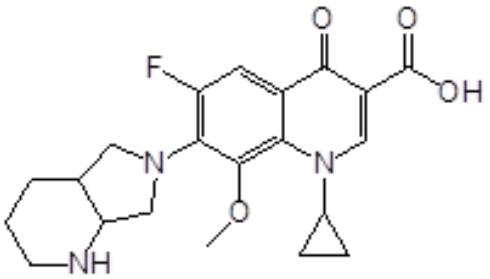
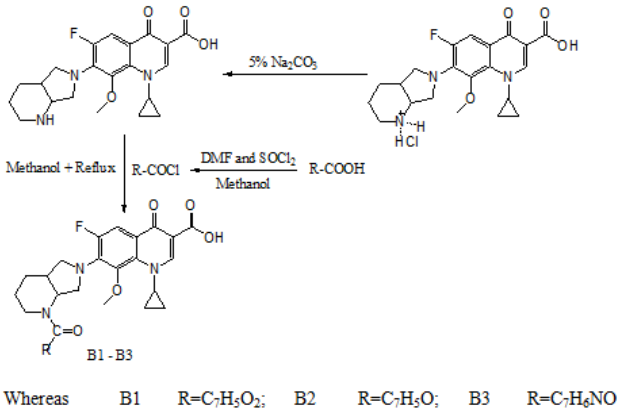


Fig. 1: Moxifloxacin

Recovery of moxifloxacin

A solution of moxifloxacin (20g) was treated with excess of 5% $NaHCO_3$. Pure neutral moxifloxacin was precipitate out and collect by filtration and left it till dry at room temperature. Weight of dry pure moxifloxacin was 17.8g that was 89 %. It was use as starting material for derivative synthesis without further purification.



Scheme 1: Synthesis of carboxamide of B1-B3

Synthesis of compound B1 to B3

Designed derivatives were synthesized, as outlined in scheme 1. Carboxylic acids of selected reagents were treated with DMF and thionyl chloride in methanol, at room temperature for 24 hours, to have analogous acid chlorides. These acyl-chlorides were then used to synthesize the related carboxamide bond following Schotten-Baumann reaction **(**Jursic BS *et al*., 1993) with MFX by refluxing at 80°C. Reactions were periodically monitored, every 30 min, through TLC (solvent system; Methanol: butanol: ammonia (v:v:v), 1:5:2), till the confirmed completion. The products were then crystallized to give derivative. These new 7-carboxamide derivatives of MFX were evaluated by physical parameters like percent yield, color, solubility and melting points.

Antimicrobial activities (in vitro)

The derivatives of MFX were evaluated against 4 Gram +ve, 7 Gram -ve bacteria and three fungi by disk susceptibility technique at concentration of 5, 10, 20 and 40 $\mu gmL^{-1.}$ Nutrient agar was prepared and set in Petri dishes. By the help of cotton swab, bacterial culture was spread on the agar surface. The loaded discs of MFX and its derivatives were placed onto the agar surface, streaked with bacterial culture then incubated at 36ºC ± 1ºC, for 24h using placebo paper discs (disc with water) as a positive control. Against each organism and each concentration, three repeated trials were conducted (n=3).

Statistical investigation was conducted for data interpretation included mean values, standard deviation and one-way ANOVA with 95% level of significance. Significant differences between individual means were identified using Dunnett's test. Same method was followed for determining antifungal activities. Discs of derivative were located on SDS medium plates with fungal culture, incubated at room temperature for 48 hours. ZOI were judiciously analyzed by using Vernier caliper.

Enzymatic analysis

Urease assay

A solution of reaction mixtures containing 25µL of enzyme which was jack bean urease, a mixture of buffers (55 µL), urea (100mM) and prepared derivatives (concentration was 5 µL; 0.5 mM) was incubated at 30°C for 15 min in 96-well plates. Urease activity was analyzed through indophenols technique, published by wetherburn in 1967, in which ammonia production was measured. 45 µL of phenol reagent (0.005% w/v sodium nitroprussside, 1% w/v phenol) and 70µL of alkali reagent (0.1% active chloride NaOCl and 0.5% w/v NaOH) were added to individually well. After 50 mins, the absorbance was caculated by microplater reader (Molecular Device, USA) which was at 630 nm. The final volume of solution was 200 µL (n=3) and pH of entire assays was 6.8. Rates of change in absorbance were calculated by softMax Pro software (molecular Device, USA). Percentage inhibitions were measured from formula $100-(OD_{testwell}/OD_{control}) \times 100$. The internal standard of urease was thiourea. (Khan KM *et al*., 2004).

Carbonic anhydrase assay

The yellow compound of 4-nitrophenol was synthesized by the hydrolyzed a colorless compound (4-NPA) and acetate which was evaluated in HEPES buffer, pH ranging from 7.2-7.9 at 25-28ºC. 140µL of the HEPES-Tris solution was present in reaction tube for each sample, 20µL of freshly prepared aqueous solution of purified bovine erythrocyte CA-II (0.1-0.2mg/2000µL of deionized water for 96-well), Fluka biochemical. 20µL of formerly ready solution (1%) of target derivative dissolved in DMSO, 20µL of substrate 4-PNA was prepared in ethanol with concentration of 0.6-0.8mM. The reaction was initiated by addition of 4-PNA after 15min incubation of target derivative, the derivatives were screened at least 3 times at all selected concentrations. The reaction was initiated, the plate was placed in a SPECTRA max 340 spectrophotometer and the amount of

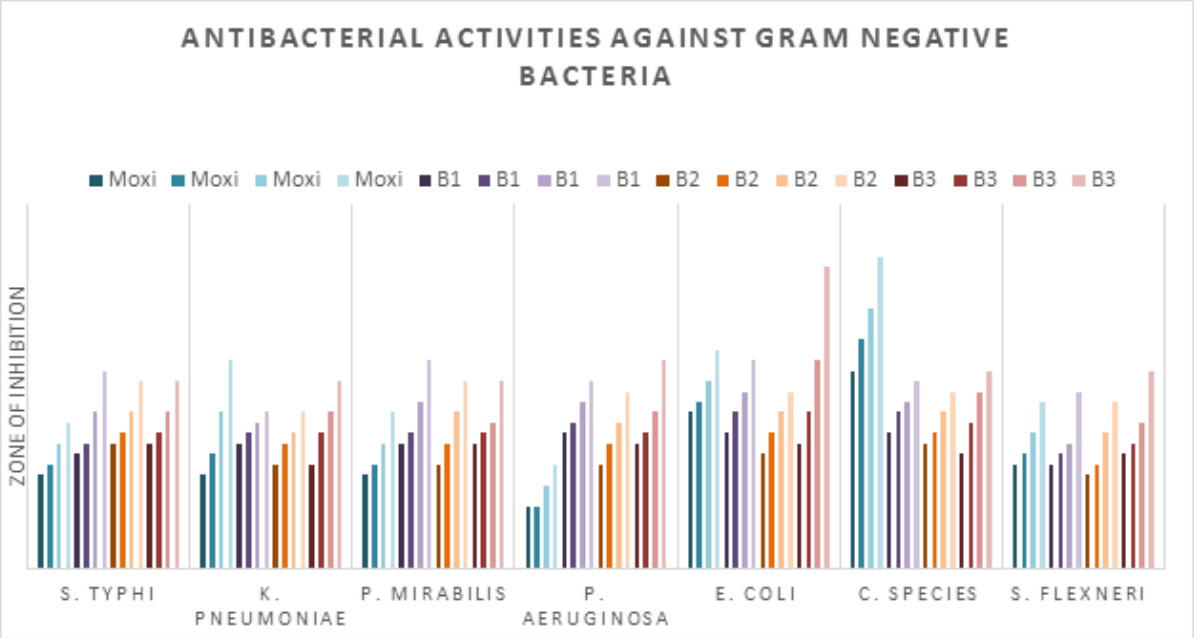


Fig. 2: Antibacterial activities against gram negative bacteria

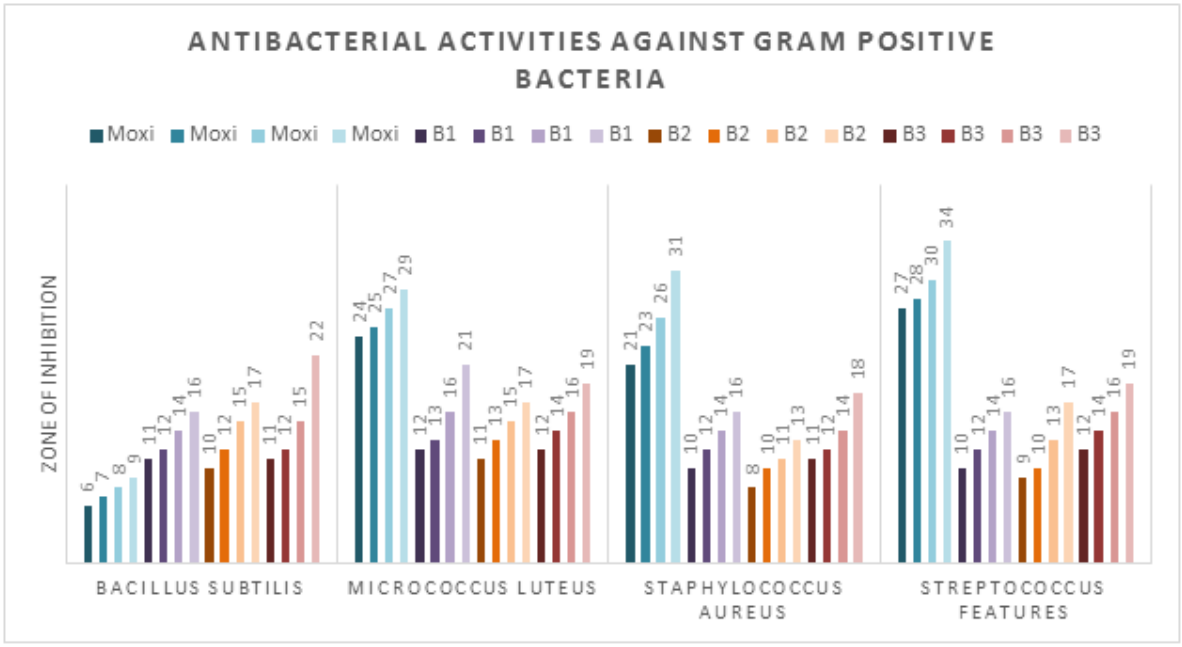


Fig. 3: Antibacterial activities against gram positive bacteria

reaction product formed was monitored at 1 min interval for 30mins at 400nm (Ho *et al*., 2003; Bayram *et al*., 2008).

RESULTS

All physicochemical and spectroscopic studies were studied and confirmed the synthesis of analogues. Their antibacterial, antifungal and enzymatic activities were also presented in the form of graphs (fig. 2 - 4).

Spectral Data B1

1-cyclopropyl-6-fluoro-7-(1-(2-hydroxybenzoyl)-octahydropyrrolo[3,4-b]pyridin-6-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ($C_{28}H_{31}FN_3O_6$)

Percent yield: 66%, molecular weight: 521.54, melting point: 124°C; IR (KBr) ν_{max}: 3238, 1705, 1660, 1581, 1294, 1249, 1207; 1H-NMR (400MHz- $CDCl_3$): δ:0.82-1.17 (m, 4H, cyclopropane), 2.62-3.25 (s, azobicyclo moiety), 3.48 (s, 3H, methoxy), 4.01 (m, 1H, cyclopropane), 7.2 (s, $CDCl_3$), 7.48(d, 1H, J = 0.046 of

ring), 7.63-7.67 (m, benzyl ring), 8.70 (s, 1H, H2 position), 14.46 (s, 1H of carboxylic acid); EIMS: m/z (rel. abundance %):521 (M^{+1} 1.28), 400 (base peak 100), 357 (36.4), 231 (6.2), 205 (8.9), 163 (11.2), 122 (4.58), 96 (31.8), 94 (5.13), 78 (4.12), 68 (20.14).

B2

7-(1-benzoyl-octahydropyrrolo[3,4-b]pyridin-6-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ($C_{28}H_{28}FN_3O_5$)

Percent yield: 75%, molecular weight:505.26, melting point: 180°C; IR (KBr) ν_{max}: 3515, 3473, 1705, 1685, 1674, 1656, 1651, 1560; 1H-NMR (400MHz- $CDCl_3$): δ: 0.82-1.17 (m, 4H, cyclopropane), 2.62-3.25 (s, azobicyclo moiety), 3.48 (s, 3H, methoxy), 4.01 (m, 1H, cyclopropane), 7.2 (s, $CDCl_3$), 7.48(d, 1H, J = 0.046 of ring), 7.56-8.06 (m, benzyl ring), 8.71 (s, 1H, H2 position), 14.46 (s, 1H of carboxylic acid); EIMS: m/z (rel. abundance %):505(M^{+1} 1.28), 401 (20.95), 357 (7.91), 231 (7.46), 205 (5.75), 163 (3.72), 106 (3.55), 96 (42.8), 92 (71.95), 78 (3.31), 68 (20.85).

B3

7-(1-(2-aminobenzoyl)-octahydropyrrolo[3,4-b]pyridin-6-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ($C_{28}H_{29}FN_4O_5$)

Percent yield: 78%, molecular weight:520.55, melting point: 112°C; IR (KBr) ν_{max}: 3493, 3381, 1705, 1680-1666, 1485, 1301, 1249, 1113, 914, 765; 1H-NMR (400MHz- $CDCl_3$): δ:0.82-1.17 (m, 4H, cyclopropane), 2.62-3.25 (s, azobicyclo moiety), 3.48 (s, 3H, methoxy), 4.01 (m, 1H, cyclopropane), 7.2 (s, $CDCl_3$), 7.48(d, 1H, J = 0.046 of ring), 6.51-7.68 (m, benzyl ring), 7.75 (d, 2H, amine, J = 0.0197), 8.67 (s, 1H, H2 position), 14.46 (s, 1H of carboxylic acid); EIMS: m/z (rel. abundance %):520(M^{+1} 1.28), 400 (base peak 100), 357 (26.8), 231 (8.12), 205 (4.2), 163 (11.4), 121 (4.26), 96 (22.1), 93 (3.2), 78 (15.7), 68 (5.05).

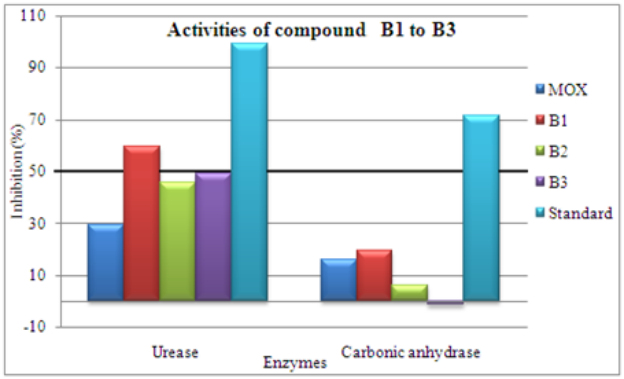


Fig. 5: Graphical representation of enzymatic inhibition B1 to B3

Antimicrobial activity

Zone of inhibition (ZOI) in diameter in millimeter (n=3), at 4 concentrations (5, 10, 20 and 40 μgmL^{-1}) of each derivatives were analyzed. One-way analysis of variance (ANOVA) was applied to identify significance differences between the ZOI of all derivatives and standard (fig. 2 & 3).

ANOVA showed significant differences between all prepared compounds against *M. luteus*, *S. aureus* and *S. features*. Dunnett's test analysis provided that antibacterial activities of all compounds were significantly decreased ($p<0.001$) against all selected concentrations.

Enzymatic activity

The enzymatic activity of the synthesized compounds has been analyzed against carbonic anhydrase and urease. The results showed that compound B1 had mild activity against urease (IC_{50} = 392) although remaining of the derivatives were inactive while all derivatives were inactive against carbonic anhydrase (fig. 5).

DISCUSSION

Spectroscopic studies

The 7-carboxamide derivatives have been characterized by physical and spectroscopic technique such as UV, IR, NMR and Mass spectrometry. IR spectra of target derivatives reveals the removal of NH peak at 3534, 3481 cm^{-1} proving that the NH group of diazabicyclo amine moiety of MFX was involved in derivative synthesis with acyl-chloride. IR spectra of the target derivatives showed new characteristic bands at 3238-3871 & 1651-1680cm^{-1} for NH and C=O stretching bands (Czock *et al*., 2006) respectively. These changes prove the synthesis of amide bond between amine of drug and acyl group of reagents. In case of compound B1, OH stretching peak appeared in region of 3238cm^{-1} (Culley *et al*., 2001) which was merged with OH peak of drug. The NH stretching of compound B3 appeared in the region of 3493-3381 cm^{-1} (Culley *et al*., 2001).

In ^{1}HNMR spectra of desired amides, a prominent singlet peak of NH of amide appeared at 8.71-8.67 ppm. Aromatic amides also produced aromatic signals at 6.51-8.06 ppm. All the prepared amides were further confirmed by mass spectra and elemental analysis. Mass-spectra of target derivatives showed isotropic peak at m/z value in relative spectra.

Antibacterial activity

All data are existing as zones of inhibition (ZOI) in diameter in millimeter (n=3) and collected at 4 concentrations (5, 10, 20 and 40 μgmL^{-1}). One-way analysis of variance (ANOVA) was applied to identify significance differences between the ZOI of all derivatives and standard (fig. 2 & 3). Dunnett's test was used and differences were measured significant at $p \leq 0.05$. ANOVA presented significant differences between derivatives and MFX (standard) against *S. typhi*, *K.*

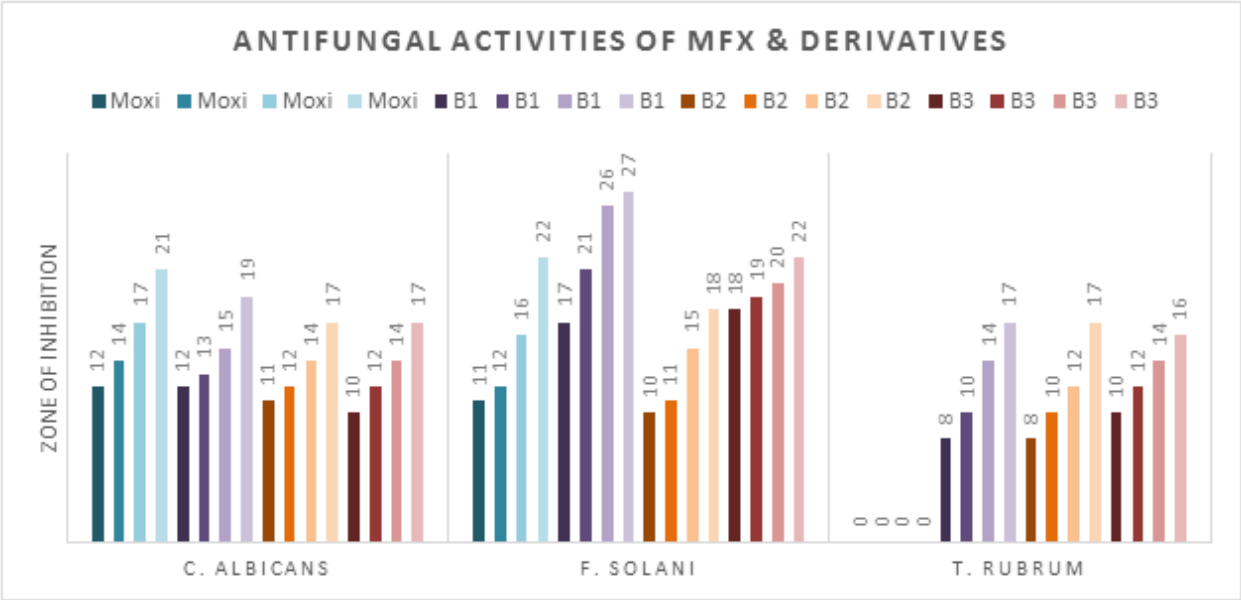


Fig. 4: Antifungal activities of MFX & derivatives

pneumonia, *P. aeruginosa* and *P. mirabilis*. Dunnett's test studied that all derivatives increased significantly ($p<0.001$) in their activities. Significant differences were also seen against *E. coli*, Citrobacter species. Post hoc test provided that all compound had significant difference in decreasing behavior ($p<0.001$). Significant differences were also detected between all prepared compounds and standard against *B. subtilis*. Dunnett's test provided that antibacterial activities of derivatives were significantly increased ($p<0.001$) with high percent inhibitions.

ANOVA showed significant differences between all prepared compounds against *M. luteus*, *S. aureus* and *S. features*. Dunnett's test analysis provided that antibacterial activities of all compounds were significantly decreased ($p<0.001$) in all selected concentrations.

Antifungal activities

ANOVA revealed significant difference between all derivatives and MFX against *C. albicans* in $5\mu gmL^{-1}$. Dunnett's test studied that derivative B2 was significantly decreased ($p<0.001$) where as B1 and B3 were insignificant in term of antifungal activity against *F. solani*. Dunnett's test investigated that derivatives B1 and B3 were significantly increased ($p<0.001$) while B2 was significantly decreased ($p<0.001$). Significant difference was produced, as result of ANOVA, between all prepared compounds against *T. rubrum*. Post hoc test analyzed that all compounds produced increased significant difference ($p<0.001$) in antifungal activities (fig. 4).

Enzymatic activity

In this assay, the IC_{50} values for inhibition of carbonic anhydrase and urease activity of a series of compounds (B1-B3) were determined. Acetazolamide and thiourea were used as standard for carbonic anhydrase and urease, respectively. B1 showed mild activity against urease (IC_{50} = 392) although remaining of the derivatives were inactive. All prepared derivatives were inactive against carbonic anhydrase (fig. 5).

CONCLUSION

We have designed and synthesized new, novel, derivatives of MFX by simple modification at C-7 position, characterized by UV, IR, ^{1}HNMR and mass spectrometry that proved involvement of the amine group of diazabicyclo moiety of drug in derivatives. It has been concluded that the novel derivatives acts as more potent antibacterial agents as compared to clinically used quinolone against various Gram negative and Gram positive bacteria including *S. typhi*, *P. mirabilis*, *P. aeruginosa*, *S. flexneri*, *B. subtilis*. Our findings suggested that these C-7 modified derivatives also possessed high potent antifungal activity as compared to MFX against *F. solani* and *T. rubrum*. In terms of SAR, the antibacterial and antifungal profile of drug was altered and improved by the aromatic acid attachment and hydroxyl and amino variation to ring via amide linkage at C-7 diazabicyclo moiety of the moxifloxacin molecule.

REFERENCES

Bauer AW, Kirby WMM, Sherris JC and Turck M (1966). Antibiotic susceptibility testing by a standerized single disk method; *Am. J. Clin. Pathol.*, **45**: 493-496.

Bayram E, Senturk M, Kufrevioglu OI and Supuran CT (2008). *In vitro* inhibition of salicylic acid derivatives on human cytosolic carbonic anhydrase isozymes I and II; Bioorg. Med. Chem., **16**: 9101-9105.

Casal JJ and Asís SE (2017). Natural and synthetic quinoline derivatives as antituberculosis agents. *Austin Tuberc. Res. Treat.,* **2**(1): 1007-1010.

Culley CM, Lacy MK, Klutman N and Edwards B (2001) Moxifloxacin: Clinical efficacy and safety. *American J. Health-Syst. Pharm.,* **58**: 379-388.

Czock D, Hüsig-Linde C, Langhoff A, Schöpke T, Hafer C, de Groot K, Swoboda S, Kuse E, Haller H, Fliser D, Keller F and Kielstein JT (2006). Pharmacokinetics of moxifloxacin and levofloxacin in intensive care unit patients who have acute renal failure and undergo extended daily dialysis. *Clin. J. Am. Soc. Nephrol.,* **1**: 1263-1268.

Garza I, Wallace MJ, Fernando D, Singh A, Lee RE, Gerding JS, Franklin C and Yendapally S (2017). Synthesis and evaluation of thiazolidine amide and N-thiazolyl amide fluoroquinolone derivatives. *Arch. Pharm. Chem. Life Sci.,* **350**: e1700029

Ho YT, Purohit A, Vicker N, Newman SP, Robinson JJ, Leese MP, Ganeshapillai D, Woo LWL, Potter BVL and Reeda MJ (2003). Inhibition of carbonic anhydrase II by steroidal and non-steroidal sulphamates; *Biochem. Biophys. Res. Commun.,* **305**: 909-914.

Hu,YQ, Fan J and Song XF (2018). Design, Synthesis and *In vitro* Anti-mycobacterial Activity of Propylene-1H-1,2,3-triazole-4-methylene-tethered Isatin-moxifloxacin Hybrids. *J. Heterocyclic Chem.,* **55**: 246.

Huang J, Wang M, Wang B, Wu Z, Liu M, Feng L, Zhang J, Li X, Yang Y and Lu Y (2016), Synthesis, antimycobacterial and antibacterial activity, of 1-(6-amino-3,5-difluoropyridin-2-yl)fluoroquinolone, derivatives containing an oxime functional moiety. *Bioorg. Med. Chem. Lett.,* **26**: 2262-2267.

Huang X, Bao Y, Zhu S, Zhang X, Lan S and Wang T (2015). Synthesis and biological evaluation of levofloxacin core-based derivatives with potent antibacterial activity against resistant Gram-positive pathogens. *Bioorg. Med. Chem. Lett.,* **25**: 3928-3932.

Jursic BS and Zdravkovski Z (1993). A simple preparation of amides from acids and amines by heating of their mixture. *Synth. Commun.,* **23**: 2761-2770.

Khan KM, Iqbal S, Lodhi MA, Maharvi GM, Zia-Ullah, Choudhary MI, Atta Ur R and Perveen S (2004). Biscoumarine: New class of urease inhibitors; economical synthesis and activity. *Bioorg. Med. Chem.,* **12**: 1963-1968.

Towle TR, Kulkarni CA, Oppegard LM, Williams BP, Picha TA, Hiasa H and Kerns RJ (2018). Design, synthesis, and evaluation of novel N-1 fluoroquinolone derivatives: Probing for binding contact with the active site tyrosine of gyrase. *Bioorg. Med. Chem. Lett.,* **28**(10): 1903-1910.

Xu Z, Song XF, Fan J and Lv ZS (2018) Design, Synthesis, and *in vitro* Anti mycobacterial Evaluation of Propylene 1H 1,2,3 triazole 4 methylene tethered (Thio) semicarbazone isatin moxifloxacin Hybrids. *J. Heterocyclic Chem.,* **55**: 77-82.