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SYNTHESIS AND ANTIMICROBIAL EVALUATION OF UNIQUE 3-CARBOXAMIDE DERIVATIVES OF MOXIFLOXACIN WITH SELECTED H₂ INHIBITOR

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ABSTRACT

The purpose of this study was to control infectious diseases by discovering new antibiotic compounds. To achieve this goal, series of carboxamide derivatives of moxifloxacin has been synthesized. The structures of synthesized derivatives have been established through spectroscopic method that was UV, IR, H¹NMR and Mass-spectra. In present work, we focused on carboxylic group (C-3 position) of drug for derivatization. Screening of their antimicrobial activities have been systematically carried out against various Gram-positive, Gram-negative organisms and fungi in comparison with parent drugs. Statistical analysis has been carried out by SPSS and results were analysed by applying one way ANOVA. The biological data shows that the prepared derivatives have good activities against *B. subtilis* and *P. aeruginosa*. Furthermore, all derivatives showed excellent result against *F. solani* and *T. rubrum*. So, we can conclude that,

the developed novel compounds have higher antifungal and antibacterial activities as related to standard drug.

Keywords: Moxifloxacin, carboxamide derivatives, biological evaluation, one-way ANOVA

INTRODUCTION

Fluoroquinolones (FQs) are totally synthesized, broad spectrum antibacterial agents. The studied drug, Moxifloxacin (MFX) is a synthetic 8-methoxyquinolone, fourth generation agents of FQ antibacterial agent. It was discovered in 1999 [1], 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* [2, 3], *M. pneumoniae*, or *C. pneumonia* [2-4], *S. pyogenes*, methicillin-susceptible *S. aureus*, *E. coli*, *E. cloacae* [5]). The bactericidal action of MFX is due to the inhibition of the topoisomerase II and topoisomerase IV [6-8].

Literature study revealed that a foundation of structural biology and bioorganic chemistry is the peptide bond (amide bond), the link between consecutive amino acids in a protein [9, 10], a chemically carboxamide formed between amine and carboxylic acid by dehydration reaction [11]. It is also present in various drugs; however, many methods for synthesis of this important group have been developed in the past century [12], that is by

direct thermal condensation reaction [13, 14], acid halide [15-17] acid anhydrides [18-22], activated amides [23], acyl azides [24] and activated esters [25].

Mostly FQs illustrate minor adverse effects among which photosensitivity is most common. This adverse reaction is terminated by co-administration of H_2 receptor antagonist [26]. Deppermann et al., also reported the interaction of H_2 receptor antagonist and FQs [27].

In present work, our group have synthesized a series of moxifloxacin derivatives with different H_2 receptor antagonist (famotidine, ranitidine, and cimetidine) by amide formation focused C-3 positions of MFX, like other quinolones, C-3 position of moxifloxacin is essential for gyrase binding and bacterial transport. The bulky bicycloamine substituent at C-7 position prevents active efflux and is associated with the *Nor A* or *pmr A* genes as seen in certain Gram-positive bacteria [28].

The biological assay of these newly synthesized and characterized analogues and MFX was carried out against three fungi (*F. solani*, *T. rubrum* and *C. albican*).

Furthermore, the bactericidal screening of analogues was also carried against seven Gram negative (*Klebsiella pneumoniae*, *Salmonella typhi*, *Proteus mirabilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella flexneri* and *Citrobacter species*) and four Gram positive bacteria (*Streptococcus features*, *Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus luteus*).

MATERIAL AND METHODS

Materials

The MFX was the kind of gift from GETZ pharma, other reagents and solvents used in this study were of analytical grade, purchased from Merck, Germany and used without further purification.

The melting point of prepared analogues were taken from Gallenkamp apparatus. IR of derivatives were verified from Shimadzu prestige-21 200 VCE spectrophotometer with KBr pellets ($400\text{--}4000\text{ cm}^{-1}$). Bruker/XWIN-NMR spectrophotometer was used to obtained ^1H NMR spectra of analogues. The derivatives were dissolved for ^1H NMR study in CDCl_3 and methanol. TMS work as internal standard. Mass-spectra were taken by JEOL MS Route. The aluminum TLC plates were used for thin layer chromatography (TLC) and iodine vapors was used as detector.

General method for derivative synthesis

The amide is directly formed from amine and carboxylic acid by condensation method at very high temperature ($160\text{--}180^\circ\text{C}$) [29], which are generally unsuitable for other functionalities. Therefore, it is necessary to activate acid first, by converting to corresponding halide or acetate than allow amino group to attack on acyl carbon of acid and formed amide group [30, 31].

Synthesis of the target derivatives was carried out 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 prepare analogous acid chlorides. These acyl-chlorides were used directly to prepare the related carboxamide bond through Schotten-Baumann reaction with MFX in refluxing methanol at 80°C . Designed procedure is applicable for synthesis of target carboxamide derivative in high yield by introducing variety of substituents such as salicylic acid, benzoic acid and 2-aminobenzoic acid into amine group of diaza-bicyclo-amine moiety of moxifloxacin at position C-7. Reactions were periodically observed, every half an hour, by thin layer chromatography the produced solvent system was MeOH/ammonia/butanol, 1:2:5, till the completion of reaction. The reaction mixture

was transferred in flask. The reaction mixture could crystallize. The new 7-carboxamide derivatives of MFX were evaluated by physical parameters like solubility, percentage yield, melting points and colour. The solubility of all the derivatives were checked in water, dimethyl sulfoxide, chloroform and methanol. The derivatives were characterized by spectroscopic techniques (IR, $^1\text{H-NMR}$) and mass spectrometry. These derivatives act as potent antibacterial and antifungal agents comparable or higher than parent drug.

Antimicrobial activities (in vitro)

The derivatives of MFX were analyzed against four seven Gram negative, Gram positive bacteria and three fungi by disk diffusion method published by Bauer et al., [32] and recommended by the FDA [33]. Activities of analogues and standard were performed in four concentrations (5, 10, 20 and $40\ \mu\text{g mL}^{-1}$). Nutrient agar was prepared; autoclaved at 121°C for 15 mins then poured in dry sterile Petri dishes, cooled and set. The bacterial inoculum was uniformly spread using sterile cotton swab on agar surface. Discs loaded with analogues and standard were load on the surface of the agar with culture and incubated at $36^\circ\text{C} \pm 1^\circ\text{C}$, for 24h while the water paper discs were used as a positive control. Three replicate trials were

directed against each culture for each concentration. Statistical parameters were data analysis that were mean, SD (standard deviation) and one-way ANOVA with 95% level of significance. Significant differences between individual means were identified using Dunnett's test. Similar procedures were followed for antifungal study with SDS medium plates. They were incubated at room temperature, for 48 hours. Zones of inhibition were carefully measured using Vernier caliper.

Spectral Data.

H1

1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-[(4aS,7aS)-octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl] 4-oxo-3 quinoline 3 carboxamide derivatives (2-cyano- 1-methyl- 3-(2-[(5-methyl- 1H-imidazol- 4-yl)methylthio]ethyl)guanidine)

($\text{C}_{31}\text{H}_{38}\text{FN}_9\text{O}_3\text{S}$)

Percent yield: 71%, molecular weight: 635.76, melting point: 100°C ; IR (KBr) ν_{max} : 3504, 3389, 3305, 2939, 2152, 1717, 1683, 1649, 1580, 1367, 1256; $^1\text{H-NMR}$ (400MHz- CDCl_3): δ : 0.82-1.17 (m, 4H, cyclopropane), 1.79 (s, 1H, secondary amine), 2.07 (s, 1H, NH), 2.44 (s, 3H, CH_3), 2.62-3.25 (s, azobicyclo moiety), 3.17 (s, 3H, CH_3), 3.48 (s, 3H, methoxy), 3.15-3.55 (m, 4H, CH_2), 4.01 (m, 1H, cyclopropane), 7.2 (s,

CDCl₃), 7.43 (s, 2H, NH), 7.48(d, 1H, J = 0.046 of ring), 8.61 (s, 1H, H2 position); EIMS: m/z (rel. abundance %): 636 (M⁺ 0.82), 400 (11.18), 357 (2.98), 251 (2.29), 205 (2.78), 163 (2.33), 157 (10.28), 117 (10.60), 96 (100), 95(84.19), 68 (27.94).

H2

1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-[(4aS,7aS)-octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl] 4-oxo-3 quinoline 3 carboxamide derivatives (3-([2-(diaminomethyleneamino)thiazol-4-yl]methylthio)-N'-sulfamoylpropanimidamide) (C₂₉H₃₇FN₁₀O₄S)

Percent yield: 56%, molecular weight: 704.86, melting point: 120 °C; IR (KBr) ν_{max}: 3412-3244, 2946, 1649, 1542, 1458, 1325, 1291, 1138 ; 1H-NMR (400MHz-CDCl₃): δ:0.82-1.17 (m, 4H, cyclopropane), 1.79 (s, 1H, secondary amine), 2.07 (s, 1H, NH), 2.44 (s, 3H, CH₃), 2.62-3.25 (s, azobicyclo moiety), 3.17 (s, 3H, CH₃), 3.48 (s, 3H, methoxy), 2.57-3.87 (m, 4H, CH₂), 3.91 (m, 1H, cyclopropane), 3.96 (s, 7H, NH), 7.2 (s, CDCl₃), 7.61 (d, 1H, J = 0.046 of ring), 8.63 (s, 1H, H2 position); EIMS: m/z (rel. abundance %): 720 (M⁺ 0.58), 400 (11.51), 357 (3.64), 336 (2.81), 260 (2.99), 231 (2.08), 216 (2.29), 205 (2.58), 188

(77.52), 163 (2.14), 96 (100), 68 (54.05), 63 (23.31).

H3

1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-[(4aS,7aS)-octahydro-6H-pyrrolo[3,4-b] pyridin-6-yl] 4-oxo-3 quinoline 3 carboxamide derivatives (N-(2-[(5-(dimethylaminomethyl) furan-2-yl)methylthio]ethyl)- N-methyl- 2-nitroethene- 1,1-diamine) (C₃₃H₄₂FN₇O₆S)

Percent yield: 61%, molecular weight: 683.79, melting point: 130 °C; IR (KBr) ν_{max}: 3473, 1710, 1698, 1653, 1569, 1397, 1355, 1317, 1184 ; 1H-NMR (400MHz-CDCl₃): δ:0.82-1.17 (m, 4H, cyclopropane), 1.79 (s, 1H, secondary amine), 2.07 (s, 1H, NH), 2.44 (s, 3H, CH₃), 2.62-3.25 (s, azobicyclo moiety), 3.17 (s, 3H, CH₃), 3.48 (s, 3H, methoxy), 2.57-3.87 (m, 4H, CH₂), 3.91 (m, 1H, cyclopropane), 4.09 (s, 2H, NH), 7.2 (s, CDCl₃), 7.56 (d, 1H, J = 0.046 of ring), 8.60 (s, 1H, H2 position); EIMS: m/z (rel. abundance %): 697 (M⁺ 0.62), 400 (24.28), 357 (8.43), 313 (10.21), 284 (2.63), 238 (3.01), 231 (9.24), 205 (10.40), 184 (3.56), 163 (4.80), 96 (100), 68 (51.43).

RESULTS AND DISCUSSION

Physical data of all synthesized analogues of MFX was presented in table 1.

Spectroscopic studies

IR analysis

Carboxylic group of MFX was involved in derivative synthesis which was proved by IR spectroscopy. Spectra of the prepared derivatives showed C=O and NH stretching bands at 1683–1649 and 3504–3244 cm^{-1} [9, 10] respectively; which confirmed the formation of amide bond between reagents. C=O, C-O groups of carboxylic acid showed band at 1705 and 1317 cm^{-1} [10] which disappeared in target derivatives, this fact proved the involvement of carboxylic carbon at C-3. Some amine groups were present in new derivatives which also appeared in the region 3529–3298 cm^{-1} [9]. In compounds H1, a strong, high intensity peak of cyano group appeared at 2152 cm^{-1} [34].

¹HNMR analysis

It has been observed that the spectra of all derivatives showed no peak for acidic proton at δ : 14 ppm which supports the FTIR analysis and suggests the utilization of the carboxylic moiety in amide formation. The H_2 protons of all derivatives were slightly shifted from 8.7 to 8.6–8.63. These statements proved the participation of carbonyl group at C-3 position of parent molecule.

Antibacterial activity

All data are presented as zone of inhibition in diameter (mm) and collected at four different concentrations that are 5, 10, 20 and 40

$\mu\text{g mL}^{-1}$ (n=3). One-way analysis of variance (ANOVA) was carried out to check differences between the zone of inhibitions of all prepared derivatives with standard that was MFX as well as number of potent fluoroquinolones such as sparfloxacin (SFX), Gemifloxacin (GEFX) and Gatifloxacin (GAFX). Dunnett's test was applied to the data and differences were considered significant at $p \leq 0.05$ (figure 1-6). ANOVA showed significant differences in antibacterial activity between derivatives and selected fluoroquinolones against all Gram-negative bacteria in all prepared concentrations. Dunnett's test analyzed that all analogues were increased significantly ($p < 0.001$) in all concentrations against all selected Gram-negative cultures except *E. coli*, *C. species* and *S. flexneri*. In case of *E. coli*, *C. species* and *S. flexneri* microbes the synthesized analogues were significantly increased as compare to MFX, SFX, GEFX but significantly decreased as compared to GAFX.

Similarly, one-way analysis of variances was also applied for checked differences between synthesized analogues and selected fluoroquinolones. ANOVA showed significant difference between all prepared compounds and standard against some common Gram-positive organisms like *B.*

subtilis, *M. luteus*, *S. aureus* and *S. features* in four dilutions. Dunnett's test analyzed that antibacterial activities of all analogues were significantly better ($p < 0.001$) with high percent inhibitions as compared to all fluoroquinolones except MFX. The prepared analogues were significantly increased against *B. subtilis* and significantly decreased against remaining microbes.

Antifungal activity

Like antibacterial activities, the antifungal screening was also performed. The results

were compared by the help of statistical parameters that was one-way ANOVA. The ANOVA discovered significant differences between all prepared derivatives and selected fluoroquinolones against some fungi that were *C. albicans*, *F. solani* and *T. rubrum*. Dunnett's test analyzed that antifungal activity of all analogues were significantly increased against all selected fungi as compared to FQs but insignificant difference seen in case of MFX against *C. albicans*.

Scheme 1: Synthesis of MFX derivatives

Table 1: Physical properties of derivatives H1 – H3

Comp.	Molecular Formula	Molecular Weight	M. P.	Percent Yield	UV absorbance (methanol)	State/Color	Solubility
H1	$\text{C}_{31}\text{H}_{38}\text{FN}_9\text{O}_3\text{S}$	635.76	100 °C	71%	294 nm	solid/ Yellow	Water, Methanol, Ethanol, DMSO
H2	$\text{C}_{29}\text{H}_{37}\text{FN}_{10}\text{O}_4\text{S}$	704.86	120 °C	56%	307 nm	solid/ Yellow	Water, Methanol, Ethanol, DMSO
H3	$\text{C}_{33}\text{H}_{42}\text{FN}_7\text{O}_6\text{S}$	638.79	130 °C	61%	289nm	solid/ Yellow	Water, Methanol, Ethanol, DMSO

Figure 4: Graphical representation of zone of inhibitions against Gram negative bacteria

Figure 5: Graphical representation of zone of inhibitions against Gram negative bacteria

Figure 6: Graphical representation of zone of inhibitions against fungi

CONCLUSION

Present study reports synthesis, characterization and biological activities of new C-3 derivatives. Our results revealed that all compounds (H1 to H3) showed significant activity against almost all selected Gram-positive, Gram negative organisms as well as fungi; they are equally or more potent than parent drug. However, the prepared compounds showed reduced activity against *Citrobacter species*, *M. luteus*, *S. aureus*, *S. features* and some concentrations of *C. albicans*. Among all prepared compounds H3 showed excellent activities against *E. coli* (13 %), *P. aeruginosa* (157 %), *Proteus mirabilis* (51 %), *K. pneumoniae* (119 %), *B. subtilis* (153 %), *C. albicans* (24 %) and *F. solani* (215 %) and it showed good to moderate activities against rest of organisms. Compound H2 showed good activity 54 % against *S. typhi* and 35 % against *S. Flexneri* and moderate activities against other organisms while the third compound H1 among this class produced better results against bacteria and fungi. We can conclude that compound H3 was best antibacterial and antifungal among all compounds.

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