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SYNTHESIS, CHARACTERIZATION, IN-VITRO BACTERIAL, FUNGAL AND ENZYMATIC ACTIVITIES OF C-7 MODIFIED GEMIFLOXACIN ANALOGUES

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ABSTRACT

The design and synthesis of new Gemifloxacin C-7 modified analogues is described here with selected NSAIDs , which were characterized through FT-IR, ^{1}HNMR and Mass spectroscopy having high lipophilic character Log **P** values (4.58-4.94) in comparison with GMFX (0.80). Most of them have altered antibacterial and antifungal activity, while compound ***4a*** and ***3a*** unveils increased activity against few Gram negatives and Gram positive bacterial strains in comparison with parent molecule (gemifloxacin). These results were further analyzed through one-way ANOVA by using SPSS ver. 20.Compound **1a, 2a, 4a** and **5a** showed higher enzymatic activity against urease than standard and parent molecule. However, only **5a** produced mild inhibitory activity against α-chymotrypsin.

Key words: Gemifloxacin, synthesis, analogues, microbial activities, enzymatic activities

INTRODUCTION

Fluoroquinolones, also used as chemotherapeutic agents for the management and cure of numerous infections in both community and hospital settings, have gathered great consideration of researchers because of their exceptional potency and safety [**1**]. These antibiotics exerts their actions by inhibiting type-II and IV

topoisomerases and DNA gyrases, leading to the torrent of events causing bacterial cell death [**2**].

Recent research concerning fluoroquinolones is mainly focused on the modifications at the C-7 position, characteristic of the potency, spectrum, and safety of analogues. This has led to the series of active analogues like ciprofloxacin (CPFX), levofloxacin (LVFX), gemifloxacin (GMFX) **(Figure 1)**, moxifloxacin (MXFX) and so on have been successfully used as an antibiotics [**3**]. Therefore, continuous efforts have been directed to synthesize and discover newer members of quinolones that can provide improved pharmacokinetic and antibacterial activity [**4**].

Fluoroquinolones (FQ) are prescribed in many infections including urinary tract and respiratory tract infection [**5**]. They are also frequently co-administered with anti-inflammatory agents such as nonsteroidal anti-inflammatory drugs (NSAIDs) [**6**]. It has been reported that simultaneous administration of both drugs (FQ + NSAIDs) may alter the bioavailability of fluoroquinolones causing convulsions in some patients after prolonged use [**7-9**].

Therefore, in the light of previous work, our group has studied the in-vitro interaction studies on RP-HLPC prior to synthesizing the analogue to study the possible interactions among FQ and some selected and commonly used NSAIDs. The work concluded the possible interaction among the studied drug causing the decreased level of FQ (Gemifloxacin and sparfloxacin.) [**10, 11**].

Therefore, we have here used NSAIDs (ibuprofen, diclofenac Na, mefenamic acid, flurbiprofen and meloxicam) as reagents for derivatives synthesis with GMFX at position 7. These derivatives act as potent antibacterial and antifungal agents comparable or higher than parent drug.

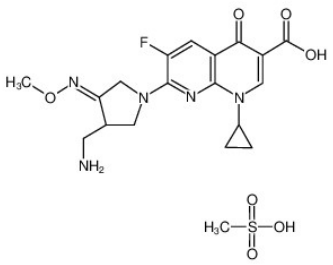


Figure 1: Gemifloxacin mesylate

MATERIAL AND METHODS

Gemifloxacin mesylate was gifted by Pharm Evo (Pvt) Ltd. and other analytical grade reagents and solvents were purchase from Merck, Darmstadt, Germany laboratory supplies which don't require further purification. All glass-wares were of Pyrex quality.

Melting points of derivatives were determined on 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 using TMS as an internal standard. Mass spectra were obtained by JEOL MS Route for derivatives of gemifloxacin. Thin layer chromatography (TLC) was performed on HSF-254 TLC plate and compounds were envisaged under UV lamp.

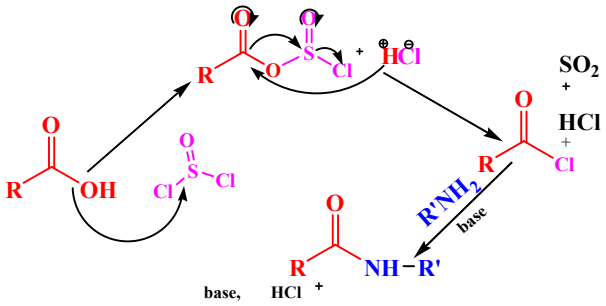
Recovery of gemifloxacin

The 25g gemifloxacin mesylate was dissolved in water and add excess aqueous solution of 5% Na_2CO_3. Neutral, pure precipitate of gemifloxacin was collected by filtration and dry at room temperature. The weight of dry pure gemfloxacin was 18.9g (91%).

Chemistry

The novel GMFX derivatives **1a**–**5a** described herein were synthesized after reacting carboxylic acid halogenide or acyl halide with an alcohol/phenol (scheme. 1) through targeted synthesis of derivatives [**12-14**]. To prepare analogous acid chlorides, selected reagents were treated with DMF and thionyl chloride in methanol, for 24 hours at room temperature which were then reacted to prepare the related carboxamide bond through Schotten-Baumann pathway **[15]** with GMFX by refluxing in methanol at 80 °C.

The completion of synthesis was determined by taking TLC of reaction with an interval of 30 min, using ethyl acetate: methanol: 25% ammonia, (8:4.5:3, v/v/v) as solvent system. The reaction mixture was then left to crystallize to give dried derivative.



Scheme 1: Synthesis mechanism of analogues

Lipophilicity

The lipophilic character of these newly synthesized derivatives compound **1a**–**5a** and the GMFX is determined inby studying their Log P values as calculated by using Chem office professional 17.0 suite software, CambridgeSoft. Remarkable improvement in the lipophilic character of these derivatives **1a**–**5a** has been evident by change in Log P values (4.58-4.94) in comparison with GMFX (0.80) (statistically significant at $p < 0.001$ using **t** test).

Compound 1a: (Z)-*N*-((1-8-cyclopropyl-3-fluoro-6-(hydroxymethyl)-5-oxo-5, 8-dihydro-1, 8-napyhyridine-2-yl)-4-(methoxyimino)-4, 5-dihydro-1*H*-pyrrol-3-yl) methyl) -2 -(4-isobutylphenyl) propanamide: ($C_{31}H_{36}FN_5O_5$),

577.65 (g/mol), 120ºC, White, DMSO, methanol, chloroform, 0.62 (CH_3OH/NH_3 /$C_4H_9O_6$ 0.8:2.0:5.2). IR (KBr) ν_{max}; 3535, 2946, 2892, 1730, 1653, 1573, 1506, 1338, 1247, 1149, 991, 943. ^{1}H-NMR ($CDCl_3$), δ: 1.30–1.03 (m, 4H, CH_2CHCH_2), δ: 3.22 (s, 3H, NH_3 +), δ: 3.55 (m, 1H, CH_2CHCH_2), δ: 3.84 (m, 1H, pyrrolidine CH_2), δ: 3.93 (s, 3H, OCH_3), δ: 4.46 (m, 1H, pyrrolidine CH_2), δ: 4.58 (s, 2H, pyrrolidine CH_2), 7.53-7.95 (m, benzyl ring), δ: 6.86-7.01(d, 1H, J = 0.018, amide), δ: 4.36-4.37 (m, 1H, pyrrolidine CH_2) EIMS: m/z (rel. abd %)577 [M^{+1}] (0.52), 390 (2.48), 345 (1.02), 316 (2.13), 273 (3.02), 204 (0.17), 190 (100) base peak, 176 (11.73), 164.

Compound 2a: (Z)-1-cyclopropyl-7-(3-((2-(2-(2, 6-dichlorophenylamino) phenyl) propanamido) methyl) -4- (methoxyimino) pyrrolidin-1-yl)-6-fluoro-4-oxo-1,4-dihydro-1,8-napthyridine-3-carboxylic acid. ($C_{32}H_{29}Cl_2FN_6O_5$), 667.16 (g/mol), 170ºC, off white, DMSO, methanol, chloroform, yield 82% R_f value (F_{256}silica gel) 0.59 (CH_3OH/NH_3 /$C_4H_9O_6$ 0.8:2.0:5.2). IR (KBr) ν_{max}3437, 2959, 2868, 1718, 1652, 1573, 1452, 1323, 1228, 1180, 933. ^{1}H-NMR ($CDCl_3$) δ: 1.30–1.03 (m, 4H, CH_2CHCH_2), δ: 3.25 (s, 3H, NH_3 +), δ: 3.61 (m, 1H, CH_2CHCH_2), δ: 3.84 (m, 1H, pyrrolidine CH_2), δ: 3.93 (s, 3H, OCH_3), δ: 4.46 (m, 1H, pyrrolidine CH_2), δ: 4.58 (s, 2H, pyrrolidine CH_2), 7.62-7.81 (m, benzyl ring), δ: 7.66-7.98(d, 1H, J = 0.026, amide), δ: 4.36-4.37 (m, 1H, pyrrolidine CH_2) EIMS: m/z (rel. abd %)668 [M^{+1}] (1.02), 390 (1.16), 345 (0.59), 316 (1.73), 279 (1.13), 273 (0.29), 250 (2.32), 215 (1.19), 204 (38.95), 180 (2.72), 164 (24.72), 96 (100) base peak, 79(7.55).

Compound 3a: (Z)-1-cyclopropyl-7-(3-((2-(2, 3-dichlorophenylamino) benzamido) methyl)-4- (methoxyimino) pyrrolidin-1-yl)-6-fluoro-4-oxo-1, 4-dihydro-1, 8-napthyridine-3-carboxylic acid.($C_{33}H_{33}FN_6O_5$) 612.65 (g/mol), 155ºC, Yellow, DMSO, methanol, chloroform, Yield (70 %), R_f value (F_{256}silica gel), UV lamp. 0.64 (CH_3OH/NH_3 /$C_4H_9O_6$ 0.8:2.0:5.2). IR (KBr) ν_{max} 3313, 2972, 2860, 1729, 1654, 1597, 1571, 1469, 1257, 1091, 954. ^{1}H-NMR ($CDCl_3$) δ: 1.30–1.03 (m, 4H, CH_2CHCH_2), δ: 3.19 (s, 3H, NH_3 +), δ: 3.54 (m, 1H, CH_2CHCH_2), δ: 3.84 (m, 1H, pyrrolidine CH_2), δ: 3.93 (s, 3H, OCH_3), δ: 4.46 (m, 1H, pyrrolidine CH_2), δ: 4.58 (s, 2H, pyrrolidine CH_2), 7.59-7.99 (m, benzyl ring), δ: 6.66-6.83(d, 1H, J = 0.013, amide), δ: 4.36-4.37 (m, 1H, pyrrolidine CH_2) EIMS: m/z (rel. abd %) 613 [M^{+1}] (0.27), 390 (1.78), 345 (2.07), 316 (1.17), 273 (0.98), 224 (100) base peak, 204 (53.28), 196 (9.48), 182 (12.22),

164 (4.26), 105 (11.39), 96 (36.09), 91 (36.54) 79(80.58).

Compound 4a: (Z)-1-cyclopropyl-7-(3-((2-(2,3-dichlorophenylamino)benzamido)methyl)-4-(methoxyimino)pyrrolidin-1-yl)-6-fluoro-4-oxo-1,4-oxo-3 quinoline carboxamide (4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide). ($C_{32}H_{29}FN_8O_7S_2$): 720.75 (g/mol), 168ºC, White, DMSO, methanol, chloroform, Yield (75 %), R_f value (F_{256}silica gel), UV lamp. 0.32 (CH_3OH/NH_3 /$C_4H_9O_6$ 0.8:2.0:5.2). IR (KBr) ν_{max} 3434, 2940, 2871, 1708, 1643, 1552, 1516, 1463, 1350, 1176, 941. ^{1}H-NMR ($CDCl_3$) δ: 1.30–1.03 (m, 4H, CH_2CHCH_2), δ: 3.31 (s, 3H, NH_3 +), δ: 3.46 (m, 1H, CH_2CHCH_2), δ: 3.84 (m, 1H, pyrrolidine CH_2), δ: 3.93 (s, 3H, OCH_3), δ: 4.46 (m, 1H, pyrrolidine CH_2), δ: 4.58 (s, 2H, pyrrolidine CH_2), 7.51-7.9 (m, benzyl ring), δ: 6.61-6.79(d, 1H, J = 0.018, amide), δ: 4.36-4.37 (m, 1H, pyrrolidine CH_2) EIMS: m/z (rel. abd %) 722 [M^{+1}] (0.28), 373 (1.62), 350(21.23), 345 (2.15), 316 (2.31), 273 (0.63), 204 (15.12), 141 (100) base peak, 113 (1.22), 79(68.23).

Compound *5a:* (Z)-1-cyclopropyl-7-(3-((2-(2-fluorobiphenyl-4-yl) propanoic acid) methyl)-4- (methoxyimino) pyrrolidin-1-yl)-6-fluoro-4-oxo-1, 4-dihydro-1, 8-napthyridine-3-carboxylic acid. ($C_{33}H_{31}F_2N_5O_5$), 615.63 (g/mol), 149ºC, White, DMSO, methanol, chloroform, Yield (80 %). R_f value (F_{256}silica gel). 0.75 (CH_3OH/NH_3 /$C_4H_9O_6$ 0.8:2.0:5.2), IR(KBr)ν_{max}3448, 2946, 2632, 1714, 1651, 1577, 1475, 1348, 1209, 1132, 923., ^{1}H-NMR ($CDCl_3$), δ: 1.30–1.03 (m, 4H, CH_2CHCH_2), δ: 3.24 (s, 3H, NH_3 +), δ: 3.57 (m, 1H, CH_2CHCH_2), δ: 3.84 (m, 1H, pyrrolidine CH_2), δ: 3.93 (s, 3H, OCH_3), δ: 4.46 (m, 1H, pyrrolidine CH_2), δ: 4.58 (s, 2H, pyrrolidine CH_2), 8.21-8.91 (m, benzyl ring), δ: 6.43-6.52(d, 1H, J = 0.021, amide), δ: 4.36-4.37 (m, 1H, pyrrolidine CH_2), EIMS: m/z (rel. abd %) 616 [M^{+1}] (0.32), 390 (2.12), 345 (1.01), 316 (0.93), 273 (1.51), 227 (7.59), 204 (16.10), 199 (100) base peak, 185 (12.87), 171 (9.05), 164 (2.06), 96 (2.17), 95 (1.32), 79(14.31).

Antimicrobial activities

The targeted designed compounds **1a–5a** were evaluated for their in vitro antibacterial activity against selected organisms using standard disk diffusion method [**16]** on freshly prepared nutrient agar recommended by the National Committee on Clinical Laboratory Standards [**17**, **18**]. Recommended concentrations of derivatives and standard were 5, 10 and 20 μgmL^{-1}. The

standard organismstrains were uniformly spread by streak plate method then antimicrobial discs soaked and dried with compound **1a-5a** and gemifloxacin were placed on the agar already streaked with organism using positive control discs soaked and dried in water. Incubated at 36ºC ± 1ºC, for 24hrs. Three replicate trials were conducted against each organism at each concentration. Statistical analytical parameters like standard mean, standard deviation and investigation of significant differences were determined for data interpretation though one-way ANOVA with 0.95 level of significance. Significant differences between individual means were identified using Dunnett's test.

Antifungal activity of derivatives were achieved by using SDS medium, discs were placed on plates already streaked with fungal culture, incubated at room temperature, for two days. Zones of inhibition were measured using Vernier caliper and the results were analyzed through one-way ANOVA **[17, 18]**.

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 test compounds (5 µL; 0.5 mM concentration) was incubated at 30°C for 15 min in 96-well plates. Urease activity was determined by indophenols method, defined by wetherburn in 1967, in which ammonia production was measured. Phenol reagent 45 µL (phenol:sodium nitroprusside, 1%: 0.005%, w/v) and alkali reagent 70 µL (NaOH: active chloride NaOCl, 0.5 %: 0.1%, w/v) added to each wellin plate. The absorbance was measured by microplater reader (Molecular Device, USA) which was at 630 nm after 50 min 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 calculated from the formula $100-(OD_{testwell}/OD_{control}) \times 100$. Thiourea was used as the standard inhibitor of urease [**19, 20**].

Alpha chymotrypsin assay

Cannel's method was used to study the inhibitory effects of all newly synthesized compounds, absorbance were determined at 410 nm [**21**].

RESULT AND DISCUSSION

Spectral studies

Infrared absorption studies

In FT-IR spectra of derivatives ***1a-3a*** *and* ***5a*****,** changes occurred at 1651-1653cm^{-1} attributed to $\upsilon(C=O)_{carb}$, which was originally observed at 1641-1633 cm^{-1} in

gemifloxacin, indicating formation of amide bond between parent drug and reagents. Upon bonding, N-H stretching vibration at 2941 cm^{-1} in GMFX disappeared confirming that GMFX reacted with NSAIDs via substitution at NH_2 of drug to form these derivatives via carboxy group of reagents. Exception was observed in case of derivative ***4a***. It showed band of ester stretching at 1734 cm^{-1}. C=O, C-O groups of carboxylic acid, at position 3. The absorption bands associated with other functional groups appeared in the expected regions.

^{1}H NMR studies

NMR spectra of the desired amides, additional aromatic ring signals at δ: 7.53-8.91 ppm and singlet peak was visible at δ: 6.43-7.98 ppm indicating the formation of C-NH amide bond. All prepared amides were further confirmed by mass spectra.

Mass Fragmentation

Mass fragmentation patterns of derivatives **1a-5a** assure the formation as given in scheme **II.**

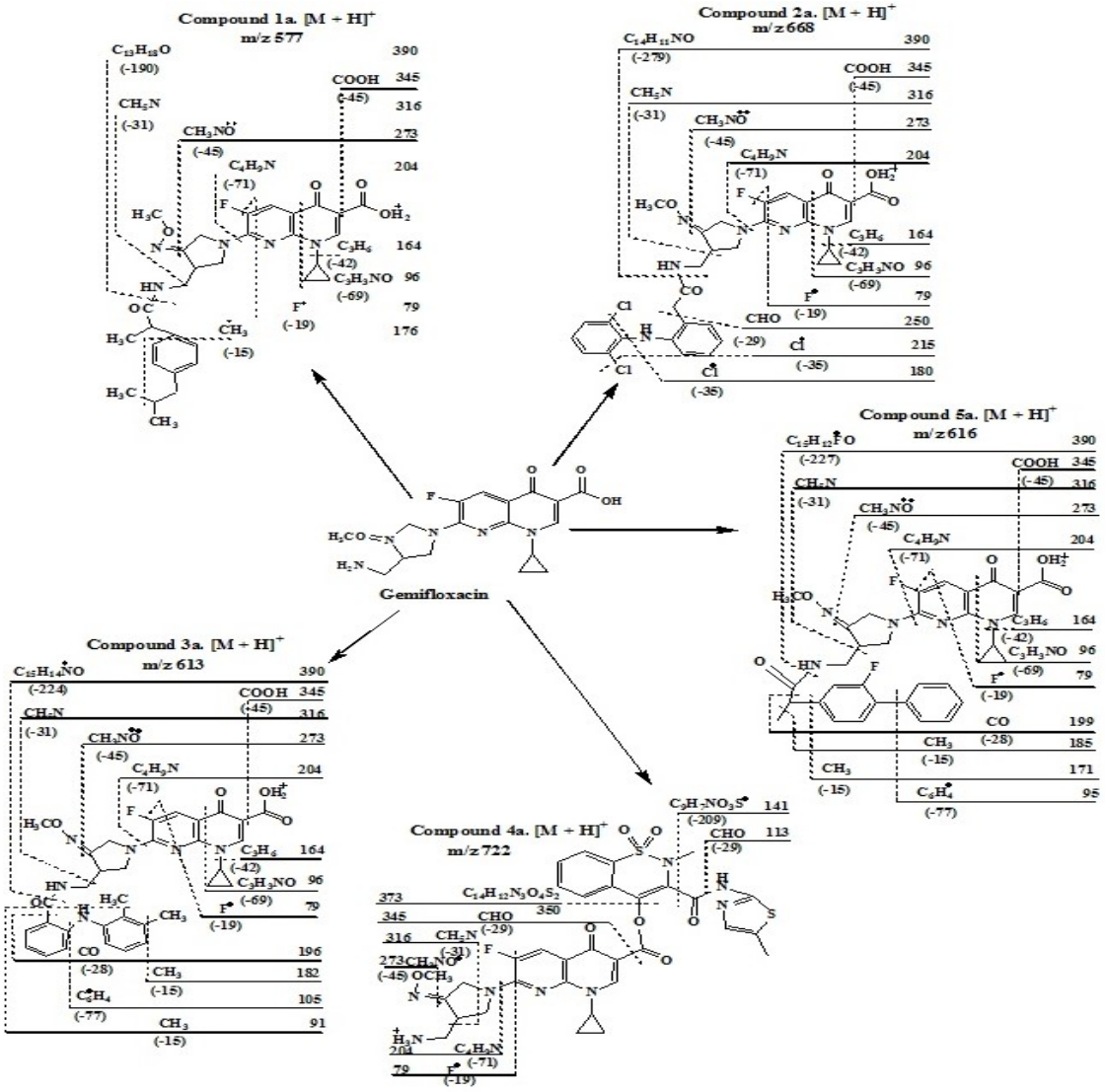
Microbiological screening of synthesized derivatives 1a-5a

In-vitro antimicrobial activities of these newly synthesized compounds **1a-5a**were analyzed for against selected strains of organisms. The zone of inhibition of each compound was determined along with the standard drugs GMFX for comparative studies. The data obtained suggested that compounds **1a**–**5a** have considerable less activity against majority of the tested Gram-positive and Gram-negative strains than GMFX with few exceptions. Compound **4a**possess activity more than the parent molecule against *S.typhi*, whereas have mild to moderate activity againt *S. flexneri, P. aureogenosa,* and *S.features*. While, compound **3a** have good activity against *B.subtilis* and *S.aureus.* These results were further analyzed through applying ANOVA and Dunnet's test having significant level of $p< 0.001$ at all concentrations **(Tables 1-6)**.

The Compound **4a** possess the antifungal activity equivalent than that of the parent GMFX against *C. albican* while all other compounds were found to have significantly decreased ($p<0.001$) activity.

Enzymatic activity

Enzymatic inhibitory effects of newly synthesized derivatives against urease and α-chymotrypsin were also determined in **Figure 2**. Result explains that only compound 1a, 2a, 4a and 5a showed activity higher than standard against urease i.e (thiourea). However, only *5a* produced mild inhibitory activity against α-chymotrypsin (chymostatin).



Scheme II: Mass fragmentation patterns of compound *1a-5a*

Table 1: Percent zone of inhibitions (ZI) of derivatives 1a- 5a

Organism	*M. luteus*			*B. subtilis*		
Concentration	**5 µgmL^{-1}**	**10 µgmL^{-1}**	**20 µgmL^{-1}**	**5 µgmL^{-1}**	**10 µgmL^{-1}**	**20 µgmL^{-1}**
GMFX	**14.33±0.09**	**15.32±0.09**	**18.41±0.05**	**16.01±0.01**	**18.27±0.22**	**22.21±0.21**
1a	**7.15±0.13* 50.1**	**11.39±0.06* 25.65**	**14.3±0.24* 22.32**	**12.15±0.22* 24.11**	**16.16±0.05 *11.55**	**18.2±0.14* 18.05**
2a	**1.25±0.13* 91.28**	**13.17±0.15* 14.03**	**14.22±0.06* 22.76**	**14.21±0.19* 11.24**	**17.22±0.2* 5.75**	**20.32±0.15* 8.51**
3a	**8.18±0.19* 42.92**	**12.29±0.21* 19.78**	**16.15±0.11* 12.28**	**14.2±0.14* 11.31**	**19.17±0.16* -4.93**	**28.23±0.12* -27.1**
4a	**17.13±0.1* -19.54**	**1.16±0.09* 2.43**	**16.31±0.12* 11.41**	**14.29±0.18* 10.74**	**19.19±0.22* -5.04**	**22.24±0.17 - 0.14**
5a	**6.28±0.13* 56.18**	**12.39±0.08* 19.13**	**14.24±0.22* 22.65**	**13.19±0.2* 17.61**	**17.19±0.2* 5.91**	**20.25±0.12* 8.82**
	ANOVA (P<0.001), df = 5 , 12					
F- value	**3116.43**	**580.69**	**366.37**	**168.11**	**113.63**	**1491.38**

mean±S.D, % ZI * indicates significance and -ve sign shows increase in activity

Table 2: Percent zone of inhibitions (ZI) of derivatives 1a- 5a

Organism	*S. aureus*			*S. features*		
Concentration	5 µgmL^{-1}	10 µgmL^{-1}	20 µgmL^{-1}	5 µgmL^{-1}	10 µgmL^{-1}	20 µgmL^{-1}
GMFX	12.36±0.14	14.25±0.22	16.2±0.12	10.18±0.18	13.14±0.15	16.33±0.06
1a	9.03±0.01* 26.94	11.25±0.21* 21.05	15.16±0.1* 6.42	9.35±0.25* 8.15	12.42±0.02* 5.48	15.2±0.23* 6.92
2a	10.19±0.2* 17.56	12.42±0.1* 12.84	14.31±0.06* 11.67	10.22±0.12 -0.39	13.36±0.09 -1.67	14.17±0.12* 13.23
3a	10.27±0.21* 16.91	13.25±0.07* 7.02	16.31±0.2 `- 0.68	9.32±0.05* 8.54	10.24±0.13* 22.07	14.39±0.05* 11.88
4a	10.14±0.04* 17.96	13.35±0.17* 6.32	15.29±0.13* 5.62	1.27±0.22 87.52	13.19±0.23 -0.38	16.28±0.19 0.31
5a	0±0 100	0±0 100	0±0 100	9.3±0.1* 8.64	10.29±0.15* 21.69	13.28±0.18* 18.68
ANOVA (P<0.001), df = 5 , 12						
F- value	3152.79	3814.18	8222.99	25.58	310.35	189.98

mean±S.D, % ZI * indicates significance and -ve sign shows increase in activity

Table 3: Percent zone of inhibitions (ZI) of derivatives 1a- 5a

Organism	*P. mirabilis*			S. *typhi*		
Concentration	5 µgmL^{-1}	10 µgmL^{-1}	20 µgmL^{-1}	5 µgmL^{-1}	10 µgmL^{-1}	20 µgmL^{-1}
GMFX	15.24±0.05	16.3±0.128	20.38±0.045	12.18±0.17	14.22±0.13	17.27±0.22
1a	10.22±0.07* 32.94	12.27±0.12* 24.72	14.18±0.11* 30.42	8.36±0.02* 31.36	11.43±0.03* 19.62	14.2±0.17* 17.78
2a	9.25±0.18 *39.3	12.36±0.16* 24.17	15.43±0.05* 24.92	9.2±0.2* 24.47	12.18±0.11* 14.35	15.11±0.09* 12.51
3a	9.28±0.14* 39.11	11.28±0.23* 0.8	15.17±0.13* 25.56	9.25±0.23* 24.06	11.26±0.22* 20.82	14.44±0.06* 16.39
4a	10.41±0.07* 31.69	13.25±0.09* 18.17	15.29±0.11* 24.98	10.25±0.18* 15.85	13.26±0.09* 46.75	20.37±0.11* -17.95
5a	10.27±0.24* 32.61	12.16±0.16* 25.4	15.26±0.17* 25.12	8.31±0.01* 31.77	10.29±0.02* 27.64	14.22±0.13* 17.66
ANOVA (P<0.001), df = 5 , 12						
F- value	756.78	431.58	1123.33	244.50	415.72	908.43

mean±S.D, % ZI * indicates significance and -ve sign shows increase in activity

Table 4: Percent zone of inhibitions (ZI) of derivatives 1a- 5a

Organism	*E. coli*			*P. aeruginosa*		
Concentration	5 µgmL^{-1}	10 µgmL^{-1}	20 µgmL^{-1}	5 µgmL^{-1}	10 µgmL^{-1}	20 µgmL^{-1}
GMFX	15.12±0.1	17.25±0.2	20.42±0.1	16.19±0.08	20.17±0.14	25.19±0.17
1a	11.27±0.18* 25.46	15.34±0.19* 11.07	17.19±0.21* 5.82	10.09±0.02* 37.68	16.37±0.1* 18.84	20.26±0.2* 19.57
2a	11.25±0.17* 25.6	15.14±0.12* 12.23	16.1±0.06* 21.16	1.32±0.25* 91.85	15.23±0.05* 24.49	20.14±0.06* 20.05
3a	8.27±0* 45.3	13.15±0.08* 23.77	15.08±0.07* 26.15	8.29±0.13* 48.8	10.29±0.15* 48.98	15.36±0.1* 39.02
4a	8.16±0.12* 46.3	14.28±0.09* 17.22	16.24±0.03* 20.47	1.37±0.12* 91.54	16.31±0.18* 19.14	20.24±0.11* 9.65
5a	10.23±0.16* 32.34	12.27±0.2* 28.87	14.25±0.15* 0.22	15.11±0.08* 6.67	14.23±0.18* 29.45	20.29±0.09* 19.45
ANOVA (P<0.001), df = 5 , 12						
F- value	1073.09	381.95	1082.34	1678.68	1607.81	1683.29

mean±S.D, % ZI * indicates significance and -ve sign shows increase in activity

Table 5: Percent zone of inhibitions (ZI) of derivatives 1a- 5a

Organism	*K.pneumonia*			*S. flexneri*		
Concentration	5 µgmL^{-1}	10 µgmL^{-1}	20 µgmL^{-1}	5 µgmL^{-1}	10 µgmL^{-1}	20 µgmL^{-1}
GMFX	1.2±0.06	12.16±0.12	16.11±0.12	10.39±0.02	14.25±0.15	17.08±0.13
1a	8.14±0.09* -578.33	10.24±0.08* 15.79	12.25±0.14* 23.96	9.24±0.17 11.07	12.2±0.25* 14.39	15.31±0.15 1.36
2a	8.21±0.16* -584.17	10.19±0.12* 16.2	14.26±0.02* 11.487	1.16±0.14 88.84	14.2±0.2 0.35	16.11±0.05* 35.68
3a	7.07±0.02* -489.17	9.34±0.1* 23.19	12.31±0.18* 23.59	10.25±0.06 21.35	12.3±0.08* 13.68	14.36±0.09* 15.93
4a	8.28±0.1* -590	12.27±0.25 - 0.9	14.07±0.08* 12.66	13.14±0.0* -26.74	15.33±0.27* -7.58	17.35±0.18* -1.58
5a	7.26±0.12* -505	1.23±0.05* 89.88	12.39±0.06* 23.09	10.3±30.14 0.58	13.23±0.04* 27.16	16.16±0.1* -580.09
ANOVA (P<0.001), df = 5 , 12						
F- value	361.79	234.77	544.96	427.57	134.13	247.41

mean±S.D, % ZI * indicates significance and -ve sign shows increase in activity

Table 6: Percent zone of inhibitions (ZI) of derivatives 1a- 5a

Organism	*Citrobacter species*			*C.albicans*		
Concentration	5 µgmL^{-1}	10 µgmL^{-1}	20 µgmL^{-1}	5 µgmL^{-1}	10 µgmL^{-1}	20 µgmL^{-1}
GMFX	8.18±0.2	1.36±0.06	14.18±0.13	9.31±0.15	12.35±0.07	15.25±0.14
1a	6.43±0.09* 21.39	8.38±0.1* -516.18	10.17±0.14* 28.28	6.23±0.17* 33.08	8.24±0.16* 33.28	10.2±0.12* 33.11
2a	6.32±0.27* 22.74	1.16±0.19 14.71	12.19±0.14* 14.03	8.33±0.15* 10.53	10.24±0.15* 17.09	12.18±0.12* 20.13
3a	5.08±0.03* 37.9	8.18±0.14* -501.47	10.2±0.18* 28.07	6.33±0.24* 32.01	8.9±0.01* 27.94	10.17±0.12* 33.31
4a	8.18±0.12 0	10.25±0.14 -653.68	12.34±0.25* 12.98	8.33±0.06* 10.53	10.26±0.16* 16.92	14.36±0.15* 5.84
5a	6.35±0.15* 22.37	8.11±0.1*- 496.32	10.17±0.13* 28.28	7.36±0.14* 20.95	9.15±0.13* 25.91	11.29±0.16* 25.97
ANOVA (P<0.001), df = 5 , 12						
F- value	166.34	236.17	291.54	167.54	469.30	733.54

mean±S.D, % ZI * indicates significance and -ve sign shows increase in activity

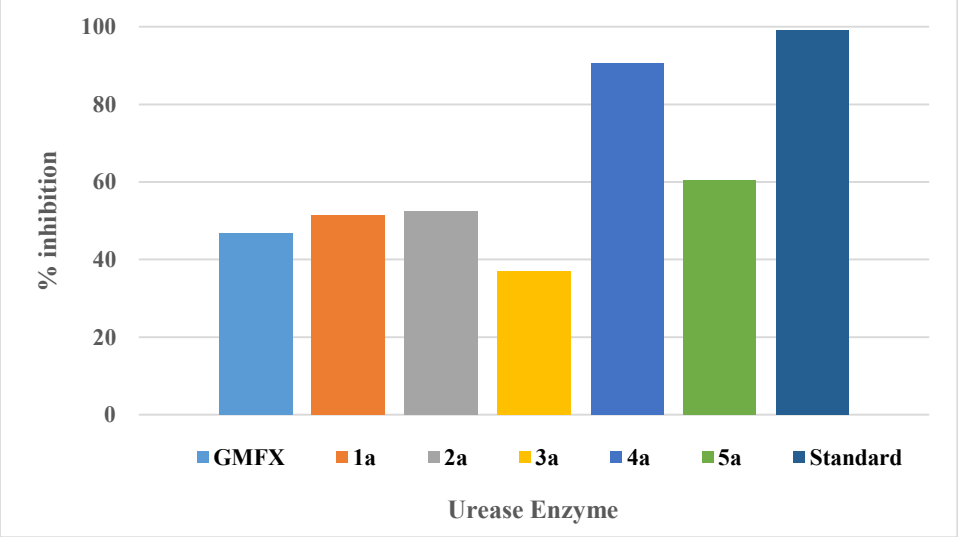


Figure 2: Activity of derivatives 1a-5a against Urease

CONCLUSION

Simple and targeted modification of gemifloxacinat C-7 position has produced newly synthesized novel derivatives of GMFX, which have been characterized by spectroscopic techniques, proving the involvement of the amine group of diazabicyclo moiety of drug in derivatives as given in scheme 1 having high yield. These compounds exhibits microbiological activity less than or equivalent to gemifloxacin except **4a** against *S. typhi* which shows increased activity than gemifloxacin. Therefore, we can conclude that after making modifications at C-7 position of the GMFX, only the compounds **4a** and **3a** exhibits increased activity against *S. typhi* and have equivalent antifungal activity. While compound **1a, 2a, 4a**and **5a** showed activity higher than GMFX against urease. However, only **5a** produced mild inhibitory activity against α-chymotrypsin**.**

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICT OF INTERESTS

Declared none.

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