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SYNTHESIS AND EVALUATION OF NOVEL PRODRUGS OF 1-ETHYL-6, 8-DIFLUORO-1, 4-DIHYDRO-7-(3-METHYL-1-PIPERAZINYL)-4-OXO QUINOLONE-3-CARBOXYLIC ACID

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ABSTRACT

The synthesis of *1-ethyl-6, 8-difluoro-1, 4-dihydro-7-(3-methyl-1-piperazinyl)-4-oxo quinolone-3-carboxylic acid* based Mannich bases (**4a-j**) using various secondary amines and formaldehyde in absolute ethanol is described. The target compounds were screened for their antimicrobial activity in gram positive cocci, gram negative bacteria and fungus by using serial dilution method.

KEYWORDS: Lomefloxacin, Mannich bases,prodrugs, antimicrobial agents.

INTRODUCTION

Lomefloxacin ($C_{17}H_{19}N_3O_3F_2$) is a difluorinated quinolone (**Figure 1**), IUPAC name *1-ethyl-6,8-difluoro-1,4-dihydro-7-(3-methyl-1-piperazinyl)-4-oxo quinolone-3-carboxylic acid*, with a longer elimination half-life (7-8 hours) compared to other members of this class of antimicrobial agents.[1, 2] This agent has been approved majorly for two indications namely acute bacterial exacerbations of chronic bronchitis[3] caused by *H. influenza* or *M.catarrhalis*, but it is not effective against Streptococcus pneumonia and it is also used for prophylaxis of infection following transurethral surgery.

Lomefloxacin showed a high in vitro activity against variety of bacterial species, including the enteropathogenic bacteria. Due to the presence of fluorine moiety at 8-position it exhibits the highest phototoxicity among the members of this class [4]. The antimicrobial activity of this agent is attributed to its inhibition of DNA gyrase, a type II topoisomerase [5]. Mannich reaction has been utilized for over 60 years as an important tool to obtain compounds with favourable pharmacological actions. The chemistry of Mannich bases [6] was first studied by Carl Mannich.N-Mannich bases are simply prepared by heating formaldehyde, the amine or amine hydrochloride and the active hydrogen containing compound in water, ethanol, methanol or dioxane [7].

According to the literature, N-Mannich bases have been proposed as potentially useful prodrug candidates for –NH acidic compounds such as amides, imides and hydantoins.N-Mannich bases of tetracyclines,[8] sulphonamides,benoxazinones have been proposed as potentially useful prodrug. Lomefloxacin possess both carboxylic acid group and a free –NH group of the piperazine moiety. Modification can be made at the carboxylic acid group and the piperazine nucleus which may result in the alteration of the physiochemical parameters and change in the biological activity of the drug.This has prompted to prepare the N-Mannich bases of Lomefloxacin.

METHODS AND MATERIALS

Preparation of *1-ethyl-6,8-difluoro-1,4-dihydro-7-(3-methyl-1-piperazinyl)-4-oxo quinolone-3-carboxylic acid* based Mannich bases:1-Ethyl-6,8-difluoro-1,4-dihydro-7-(3-methyl-1-piperazinyl)-4-oxo quinolone-3-carboxylic acid based Mannich bases (**4a-j**) were prepared by reacting Lomefloxacin (1 mole), various secondary amines (**3a-j**) (1 mole) and formaldehyde (1.5 to 2 moles) in 50 ml of absolute ethanol.This was then refluxed for 8 to 12 hours at 70-75°C (**Figure 2**). The resulting solution was refrigerated overnight, and the precipitate obtained was washed with absolute ethanol, filtered, dried and was recrystallized using hot rectified spirit. The purity of the synthesized compounds (**4a-j**) was confirmed by TLC analysis on silica gel G plates using Chloroform and water with one drop of glacial acetic acid as the mobile phase. Iodine vapours or UV Cabinet was used for visualizing the plates. Melting points of the compounds were determined using VEEGO and are uncorrected. The IR spectrum of the target compounds was taken in KBr pellets by Perkin Elmer 841 Spectrophotometer. The ^{1}H-NMR Spectroscopy was recorded on Varian EM 360 in D_2O as solvent.

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***1-Ethyl-6,8-difluoro-1,4-dihydro-7-[3-methyl-4-(dimethylaminomethyl)-1-piperazinyl]-4-oxo quinolone-3-carboxylic acid* (4a):** IR (KBr) cm^{-1};3200 (N-H stretching sec. amine), 1700 (C=O, carboxylic acid), 1280 (C-N stretching, aromatic), 1330 (C-F stretching), 1030 (C-N stretching, aliphatic), 3160 (C-H Stretching aliphatic), 1660 (C=O, quinone). ^{1}H NMR (D_2O) (δ, ppm); 1.3 (m, 3H, CH_3), 1.5 (m, 2H, piperazinyl CH_2), 2.7 (m, 2H, aromatic N-CH_2), 2.0 (m, 2H, piperazinyl CH_2), 2.5 (s, 3H, N-CH_3), 3.5 (s, 2H, N-CH_2-N), 7.8 (s, 1H, Ar-H), 8.5-8.8 (s, 1H, aromatic N-CH=).

***1-Ethyl-6,8-difluoro-1,4-dihydro-7-[3-methyl-4-(diethylaminomethyl)-1-piperazinyl]-4-oxo quinolone-3-carboxylic acid* (4b):** IR (KBr) cm^{-1}; 3210 (N-H stretching, sec. amine), 1710 (C=O, carboxylic acid), 1300 (C-N stretching, aromatic), 1330 (C-F stretching), 1040 (C-N stretching, aliphatic), 3150 (C-H stretching aliphatic), 1680 (C=O, quinone). ^{1}H NMR (D_2O) (δ, ppm); (m, 3H, N-CH_2CH_3), 2.75-2.85 (m, 2H, aromatic N-CH_2), 3.0-3.1(m, 2H, piperazinyl CH_2), 4.5 (s, 2H, N-CH_2-N), 7.75 (s, 1H, Ar-H), 8.8 (s, 1H, aromatic N-CH=).

***1-Ethyl-6,8-difluoro-1,4-dihydro-7-[3-methyl-4-(dipropylaminomethyl)-1-piperazinyl]-4-oxo quinolone-3-carboxylic acid* (4c):** IR (KBr) cm^{-1}; 3540 (N-H stretching, sec. amine), 1720 (C=O, carboxylic acid), 1110 (propyl), 1300 (C-N stretching, aromatic), 1030 (C-N stretching, aliphatic), 1330 (C-F stretching), 3100 (C-H stretching aliphatic), 1680 (C=O, quinone). ^{1}H NMR (D_2O) (δ, ppm); 0.7 (m,3H, CH_3), 1.15 (m, 3H, piperazinyl CH_3), 1.4 (m, 2H, CH_2), 1.5 (m, 3H, N-CH_2-CH_3), 2.45 (m, 2H, N-CH_2), 2.65 (m, 2H, aromatic N-CH_2), 3.05-3.15 (m, 2H, piperazinyl CH_2), 4.5 (s, 2H, N-CH_2-N), 7.8 (s, 1H, Ar-H), 8.8 (s, 1H, aromatic N-CH=).

***1-Ethyl-6,8-difluoro-1,4-dihydro-7-[3-methyl-4-(dibutylaminomethyl)-1-piperazinyl]-4-oxo quinolone-3-carboxylic acid* (4d):**IR (KBr) cm^{-1}; 3210 (N-H stretching, sec. amine), 1720 (C=O, carboxylic acid), 1200 & 1230 (C-N stretching, aromatic), 1010 (C-N stretching, aliphatic), 1320 (C-F stretching) 3010 (C-H stretching aliphatic), 1680 (C=O, quinone). ^{1}H NMR (D_2O) (δ, ppm); 0.75 (m, 3H, CH_3), 1.15 (m, 3H, piperazinyl CH_3), 1.3-1.45 (m, 2H, CH_2), 1.55 (m, 3H, N-CH_2-CH_3), 2.4 (m, 2H, N-CH_2), 4.5 (s, 2H, N-CH_2-N), 7.8 (s, 1H, Ar-H), 8.8 (s, 1H, aromatic N-CH=).

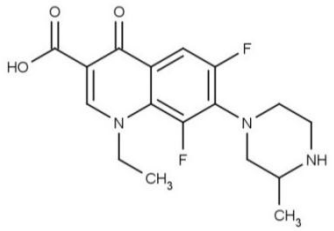


Figure 1: The structure of Lomefloxacin

***1-Ethyl-6,8-difluoro-1,4-dihydro-7-[3-methyl-4-(dipentylaminomethyl)-1-piperazinyl]-4-oxo quinolone-3-carboxylic acid* (4e):** IR (KBr) cm^{-1}; 3400 (N-H stretching, sec. amine), 1720 (C=O, carboxylic acid), 1230 (C-N stretching, aromatic), 1010 (C-N stretching, aliphatic), 1330 (C-F stretching), 3010 (C-H stretching aliphatic), 1650 &1680 (C=O, quinone). ^{1}H NMR (D_2O) (δ, ppm); 0.95 (m, 3H, CH_3), 1.15 (m, 3H, piperazinyl CH_3), 1.25-1.5 (m, 2H, CH_2), 2.4 (m, 2H, N-CH_2), 2.75-2.85 (m, 2H, aromatic N-CH_2), 3.1-3.2 (m, 2H, piperazinyl CH_2), 4.5 (s, 2H, N-CH_2-N), 7.8 (s, 1H, ArH), 8.8 (s, 1H, aromatic N-CH=).

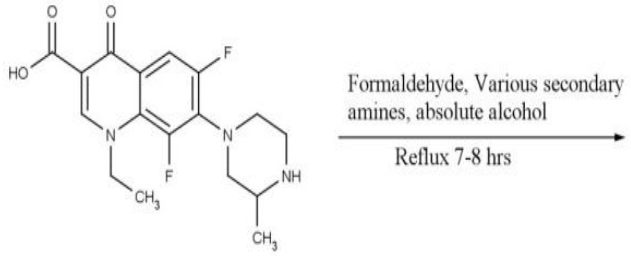


Figure 2: The synthetic scheme for the compounds for 4a-4j.

***1-Ethyl-6,8-difluoro-1,4-dihydro-7-[3-methyl-4-(diphenylaminomethyl)-1-piperazinyl]-4-oxo quinolone-3-carboxylic acid* (4f):** IR (KBr) cm^{-1}; 3100 (N-H stretching, sec. amine), 1730 (C=O, carboxylic acid), 1420 (C-N stretching, aromatic), 1300 (C-F stretching), 1100 & 1000 (diphenyl), 1030 (C-N stretching, aliphatic), 1640 (C=O, quinone). ^1H NMR (D_2O) (δ, ppm); 1.15 (m, 3H, piperazinyl CH_3), 1.5 (m, 3H, N-CH_2CH_3), 2.75 (m, 2H, aromatic N-CH_2), 3.0-3.1 (m, 2H, piperazinyl CH_2), 4.5 (s, 2H, N-CH_2-N), 7.0-7.15 (m, 5H, Ar-H [phenyl]), 7.8 (s, 1H, ArH), 8.8 (s, 1H, aromatic N-CH=).

Table 1: Physicochemical data of target compounds

Compounds	Molecular Weight	Melting Point (°C)	Percent yield (%)	CLog P	Appearance
Lomefloxacin	351	239-240	--	2.00	Yellow
4a	421	160-161	70	2.52	Yellow
4b	449	176-180	60	3.23	Yellowish brown
4c	477	181-184	72.4	4.27	Yellowish white
4d	505	175-176	65	5.16	Whitish yellow
4e	533	178-181	71	6.05	Light yellow
4f	545	185-187	57.8	5.96	Yellow
4g	573	189-190	52.5	5.97	White
4h	463	196-198	68.8	2.30	Light yellow
4i	447	201-205	70.5	2.92	Yellow
4j	461	214-217	72	3.37	Light yellow

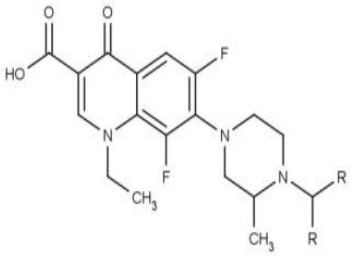
Table 2: Elemental analysis

Compounds	% Carbon	% Hydrogen	% Nitrogen
4a	58.67 (59.30)	6.6 (7.79)	13.69 (14.70)
4b	60.41 (60.90)	7.090 (8.08)	12.81 (13.91)
4c	61.93 (63.24)	7.520 (7.88)	12.04 (13.56)
4d	58.41 (62.21)	7.910 (8.04)	11.35 (12.02)
4e	64.73 (66.10)	7.899 (8.50)	10.78 (10.30)
4f	67.54 (69.10)	5.816 (4.86)	10.50 (11.59)
4g	68.69 (68.12)	5.900 (5.10)	10.01 (11.07)
4h	56.77 (58.01)	6.236 (6.41)	15.05 (16.03)
4i	58.79 (61.02)	6.458 (6.31)	15.59 (16.09)
4j	59.61 (62.40)	6.690 (7.80)	15.11 (16.80)

Values in the parentheses indicate experimentally observed values.

***1-Ethyl-6,8-difluoro-1,4-dihydro-7-[3-methyl-4 (dibenzylaminomethyl)-1-piperazinyl]-4-oxo quinolone-3-carboxylic acid* (4g):** IR (KBr) cm^{-1}; 3150 (N-H stretching, sec. amine), 1710 (C=O, carboxylic acid), 1450 (C-N stretching, aromatic), 1350 (C-F stretching), 1180 (dibenzyl), 2840 (C-H stretching aliphatic) 1620 (C=O, quinone). ^1H NMR (D_2O) (δ, ppm); 1.1 (m, 3H, piperazinyl CH_3), 1.55 (m, 3H, N-CH_2CH_3), 2.75 (m, 2H, aromatic N-CH_2), 3.15-3.25 (m, 2H, piperazinyl CH_2), 3.5 (m, 2H, benzyl CH_2), 4.5 (s, 2H, N-CH_2-N), 7.2-7.4 (m, 5H, Ar-H [benzyl]), 7.8 (s, 1H, ArH), 8.8 (s, 1H, aromatic N-CH=).

***1-Ethyl-6,8-difluoro-1,4-dihydro-7-[3-methyl-4-(morpholin-1-yl)-1-piperazinyl]-4-oxo quinolone-3-carboxylic acid* (4h):** IR (KBr) cm^{-1}; 3210 (N-H stretching, sec. amine), 1690 (C=O, carboxylic acid), 1249 (O-C, morpholinyl), 1450 (C-N stretching, aromatic), 1040 (C-N stretching, aliphatic), 2960 (C-H Stretching aliphatic), 1680 (C=O, quinone). ^1H NMR (D_2O) (δ, ppm); 1.2 (m, 3H, piperazinyl CH_3), 1.5 (m, 3H, N-CH_2CH_3), 2.5 (m, 2H, N-CH_2), 2.6-2.8 (m, 2H, aromatic N-CH_2), 3.1-3.2 (m, 2H, piperazinyl CH_2), 3.6 (m,2H, morphinyl CH_2), 4.5 (s, 2H, N-CH_2-N), 7.8 (s, 1H, ArH), 8.8 (s, 1H, aromatic N-CH=).



***1-Ethyl-6,8-difluoro-1,4-dihydro-7-[3-methyl-4-(pyrrolidin-1-yl)-1-piperazinyl]-4-oxo quinolone-3-carboxylic acid* (4i):** IR (KBr) cm^{-1}; 3432 (N-H stretching, sec. amine), 1720 (C=O, carboxylic acid), 1328 (C-N stretching, aromatic), 1280 (C-F stretching), 1090 (C-N stretching, aliphatic), 2848 (N-C pyrrolidinyl), 1628 (C=O, quinone).). ^1H NMR (D_2O) (δ, ppm); 1.15 (m, 3H, piperazinyl CH_3), 1.5 (m, 3H, N-CH_2CH_3), 1.6-1.7 (m, 2H, pyrrolidinyl CH_2), 2.7 (m, 2H, aromatic N-CH_2), 3.0-3.1 (m, 2H, piperazinyl CH_2), 4.5 (s, 2H, N-CH_2-N), 7.8 (s, 1H,ArH), 8.8 (s, 1H aromatic N-CH=).

***1-Ethyl-6,8-difluoro-1,4-dihydro-7-[3-methyl-4-(piperidin-1-yl)-1-piperazinyl]-4-oxo quinolone-3-carboxylic acid (4j)*:** IR (KBr) cm^{-1}; 3230 (N-H stretching, sec. amine), 1720 (C=O, carboxylic acid), 1060 (C-N stretching, aliphatic), 1280 (C-F stretching),2960 (N-C, piperidinyl), 1650 (C=O, quinone). ^1H NMR (D_2O) (δ, ppm); 1.15 (m, 3H, piperazinyl CH_3), 1.4-1.5 (m, 3H, N-CH_2CH_3), 2.4-2.75 (m, 2H, aromatic N-CH_2), 3.0 (m, 2H, piperazinyl CH_2), 4.0-4.1 (m, 2H, piperidinyl CH_2) 4.5 (s, 2H, N-CH_2-N), .8 (s, 1H, ArH), 8.8 (s, 1H, aromatic N-CH=). The physicochemical data is summarized in Table 1, and, data obtained from elemental analysis in Table 2.

Antimicrobial Activity

The antimicrobial screening of the 1-ethyl-6,8-difluoro-1,4-dihydro-7-(3-methyl-1 piperazinyl)-4-oxo quinolone-3-carboxylic acid based Mannich bases was performed using gram positive cocci, Gram Negative bacteria and fungus.[17-19] The Serial Dilution Method was prepared and the minimum inhibitory concentration (MIC_{90}) was calculated for all the four test micro-organisms, namely *Escherichia coli* (MTCC 443), *K. pneumonia* (wild isolate), *S.aureus* (MTCC 96), and *C.albicans* (wild isolate).Stock solutions of the test compounds were prepared in 0.85% NaCl solution to get 1 mg/ml concentration. This was further diluted to get the following concentrations, 1.5µg/ml, 2µg/ml, 2.5µg/ml, 5µg/ml, and 10µg/ml. Control used in each case was 0.85% NaCl solution. The results are summarized in Table 3.

Table 3: Antimicrobial screening of the Target Compounds

Compounds	Minimum Inhibitory concentration (MIC_{90}) [µg/ml]			
	E. coli	*K. pneumonia*	*S. aureus*	*C. albicans*
Lomefloxacin	2.0	1.5	1.5	2.0
4a	5.0	2.0	2.5	5.0
4b	10.0	5.0	5.0	10.0
4c	5.0	2.0	2.0	2.5
4d	10.0	2.5	2.5	5.0
4e	5.0	5.0	5.0	5.0
4f	10.0	5.0	5.0	10.0
4g	2.0	2.5	2.5	2.0
4h	2.0	1.5	1.5	2.0
4i	2.5	5.0	2.5	5.0
4j	2.0	2.0	1.5	2.0

RESULTS AND DISCUSSION

It was observed that out of the ten compounds synthesized **4h** and **4j** were found to exhibit maximum inhibition at MIC_{90} 1.5µg/ml for*K. pneumonia* (Wild isolate) and S. aureus (MTCC 96). All the target compounds showed varying antifungal activity against *C. albicans*. All these synthesized compounds are found to exhibit poor activity, as the goal was to synthesize prodrugs which are inherently inactive or show poor activity. N-Mannich bases are found to increase the lipophilicity of the drugs for better absorption. Cleavage of the prodrug in this case is strictly pH dependent: it has been found that N-Mannich bases of amide and amine drugs show a bell shaped pH/rate profile with a high breakdown rate at pH 7.4. A drawback of the N-Mannich bases is their limited in vitro stability, raising some stability/formulation problems. The

unavoidable release of formaldehyde during decomposition is another factor that has to be taken into consideration due to its toxicity.

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