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Microwave assisted synthesis of some novel benzimidazole substituted Fluoroquinolones and their antimicrobial evaluation

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Abstract:
We have synthesized a series of Mannich bases of ciprofloxacin & norfloxacin with various benzimidazoles. The compounds were confirmed by physical parameters (solubility, melting point), chromatographic methods (TLC) and at last spectroscopic methods (IR, NMR). Since our titled compounds are known to possess antimicrobial activity, the compounds were screened for their antibacterial and antifungal activity by cup-plate method. All the benzimidazole substituted norfloxacin derivatives (NF_1, NF2 & NF_3) & ciprofloxacin derivatives (CF_1, CF2 & CF_3) showed significant activities compared to the standards norfloxacin & ciprofloxacin and significant activity against *Candida albicans* at 50, 100 mcg/ml. The benzimidazoles showed mild antibacterial activities and significant antifungal activities.

Keywords: Fluoroquinolones, ciprofloxacin, norfloxacin, benzimidazoles & antimicrobial.

Introduction:

Nowadays, heating and driving chemical reactions by microwave energy is a crucial chemical reaction by microwave energy is a crucial matter in our scientific community. The groups of Gedye and Geigure / majetich in 1986 first reported on the use of microwave heating to accelerate organic chemical transformations. Since the last few years, high speed microwave synthesis is being practiced owing to its vitality in organic synthesis procedures [1]. Benzimidazole nucleus was reported to have significant antibacterial, antifungal activities [2,3]. The second generation of fluoroquinolones such as norfloxacin & ciprofloxacin are known as a major class of antibacterial agents and widely used to treat patients with infections. In recent years, and due to the increasing of resistance of many infections by gram negative and gram positive bacteria to these quinolones, several studies described various modifications in the fluroquinolone ring.

It has been observed from literature that piperazine group is although beneficial is not essential for low MIC or for IC_{50} against the target enzyme [4-7]. Since benzimidazoles are possessing broad spectrum antibacterial activity, we are planning to incorporate these into the piperazine ring of the fluoroquinolones via Mannich reaction and to screen antibacterial and antifungal activities for these compounds.

Materials and Methods:

Synthesis of fluoroquinolone derivatives involved following steps: The starting materials 1H-benzo (d) imidazole, 2 ethyl-1H- benzo (d) imidazole & 2 propyl- 1H-benzo (d) imidazoles were prepared according to the literature method by reacting o-phenylene diamine and different acids. Mannich bases of fluoroquinolones were prepared by condensing the active hydrogen atom of benzimidazole derivatives with formaldehyde and the secondary amino function (piperazine moiety) of fluoroquinolones. The synthetic work was done by using CATALYST Scientific Microwave Synthesis System.TLC were performed to monitor the reactions and to determine the purity of the products. The melting points of the synthesized compounds were determined in open capillaries using Veego VMP-1 Apparatus and expressed in ° C and are uncorrected. The IR spectrum of compounds was recorded on Shimadzu FT-IR spectrometer using KBr pellet technique and is expressed in cm^{-1}. ^{1}H-NMR spectra was recorded on Bruker DRX-300 (300 MHz FT-NMR) using $CDCl_3$ as solvent and tetramethylsilane as internal standard.

Synthesis of substituted benzimidazoles

A reaction mixture of o-phenylene diamine (0.01 mol) and appropriate acid (0.01 mol) was subjected to microwave irradiation at 350W for 25 minutes. Completion of the reaction was monitored by TLC. The reaction mixture was cooled and basified by addition of sodium hydroxide solution. The separated benzimidazole was filtered and washed with ice-cold water. It was recrystallized from boiling water [8].

TLC: Chloroform: Methanol (9.5: 0.5)

Synthesis of benzimidazoles substituted fluoroquinolones via Mannich reaction

To a stirred suspension of appropriate benzimidazole derivatives (0.02 mol) in ethanol were added ciprofloxacin & norfloxacin (0.02 mol) and 37% formaldehyde (0.5 ml) and irradiated in a microwave oven at an intensity of 80% with 3-5 mins. The completion of reaction was checked by TLC. The reaction time varied from 1.5-3 minutes. The solution obtained after the completion of reaction was kept at 0^0 C for 30 minutes and the resulting mixture was recrystallized from the mixture of DMF & Water [9].

TLC: Chloroform: Methanol (9.5: 0.5)

1H-benzo[d]imidazoles (B_1)

IR (KBr) cm^{-1}: 3061.83, 3113.21 (Ar C-H str), 1458, 1477.52(C-Nstr), 1300.07 (tertiary amine) 3061.83 (N-H str), ^{1}H-NMR (300 MHz, CDCl3) δ (ppm) : 5 (s, 1H, NH), 8.08 (s, 1H, N-CH), 7.2-7.7 (m, Ar-H),

2 ethyl-1H-benzo (d) imidazoles (B_2)

IR (KBr cm^{-1}): 1425.44 (C-Nstr), 1271.13 (tertiary amine), 3053.42 (N-H str). ^{1}H-NMR (300 MHz, CDCl3) δ (ppm) :
5 (s, 1H, NH), 8.08 (s, 1H, N-CH), 7.7 (m, Ar-H), 1.2(d, 3H, CH_3), 1.1(t, 2H, CH_2)

2-propyl-1H-benzo[d]imidazoles (B_3)

IR (KBr cm^{-1}): 3053.42 (N-H str), 2964.69 (C=C str), 1421.58 (C-N str), ^{1}H-NMR (300 MHz, CDCl3) δ (ppm) : 5 (s, 1H, NH), 8.08 (s, 1H, N-CH), 7.7 (m, Ar-H), 1.1 (m, 5H, methyl), 1.2 (t, 3H, ethyl)

7-(4-((1H-benzo[d]imidazol-1-yl) meth yl) piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1, 4-dihy droquinoline-3-carboxylic acid (CF_1)

IR (KBr cm^{-1}): 3012.91, 3097.78 (Ar C-H str), 3369.75 (OH str) 1708.99 (C=O str), 1626.05 (C=Nstr), 1494.88 (C-N str) ^{1}H-NMR (300 MHz, CDCl3) δ (ppm) : 14.9 (s, 1H, OH), 8.6 (s, 1H, N-CH), 7.9 (m, 5H, Ar-H), 7.5 (m, 3H, Ar-H), 4.5 (s, 2H, N-CH_2-N), 3.2 (m, 8H, piperazine), 1.2 (d, 5H, cyclopropyl).

1-cyclopropyl-7-(4-((2-ethyl-1H-benzo[d]imidazol-1-yl)methyl)piperazin-1-yl)-6-fluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (CF_2)

IR (KBr cm^{-1}): 3354.32 (OH str), 3091.99 (Ar C-H str),1720.56 (C=O str), 1627.97 (C=Nstr),1496.81 (C-N str),1435.09 (alkane C-H bending) ^{1}H-NMR (300 MHz, CDCl3) δ (ppm) : 14.9 (s, 1H, OH), 8.6 (s, 1H, N-CH), 7.9 (m,5H, Ar-H), 7.6 (m,3H,Ar-H), 4.5 (s,2H,N-CH_2-N), 3.1- 3.4 (m, 8H, piperazine),1.4 (d,5H,cyclopropyl), 1.2(d, 3H, CH_3), 1.1(t,2H, CH_2)

4-cyclopropyl-6-(4-((2-propyl-1H-benzo[d]imidazol-1-yl) methyl) piperazin-1-yl)-7-fluoro-1-oxo-1, 4-dihydronaphthalene-2-carboxylic aci d (CF_3)

IR (KBr cm^{-1}): 3095.85 (Ar-H str), 3348.54 (OH str), 1705.13(C=O str), 1627.97 (C=Nstr), 1498.74 (C-N str), 1433.16 (alkane C-H bending), ^{1}H-NMR (300 MHz, CDCl3) δ (ppm) :
14.9 (s, 1H, OH), 8.6 (s, 1H, N-CH), 7.9 (m,5H, Ar-H), 7.6 (m,3H,Ar-H), 4.5 (s,2H,N-CH_2-N), 3.1- 3.6 (m, 8H, piperazine),1.3 (d, 5H,cyclopropyl), 1.1(m, 5H, methyl), 1.2(t,3H, ethyl)

7-(4-((1H-benzo[d]imidazol-1-yl) meth yl) piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylic aci d (NF_1)

IR (KBr cm^{-1}): 3041.84 (ArC-H str), 1710.92(C=O str), 1627.97 (C=Nstr),

1479.45(C-N str), 1465.95 alkane C-H bend), ^{1}H-NMR (300 MHz, CDCl3) δ (ppm) : 14.9 (s, 1H, OH), 8.6 (s, 1H, N-CH), 7.9 (m, 5H, Ar-H), 7.6 (m, 3H, Ar-H), 4.5 (s, 2H, N-CH_2-N), 3.1- 3.6 (m, 8H, piperazine), 1.1(m, 5H, methyl), 1.2 (t,3H, ethyl)

4-ethyl-7-fluoro-1-oxo-6-(4-((2-ethyl-1H-benzo[d]imidazol-1-yl)methyl)piperazin-1-yl) -1, 4-di hydronaphthalene-2-carboxylic acid (NF_3)

IR (KBr cm^{-1}): 3340.82 (OH str), 3047.63 (Ar-H str), 1716.70(C=O str), 1627.97 (C=Nstr), 1483.31 (C-N str), 1452.45 (alkane C-H bend) ^{1}H-NMR (300 MHz, CDCl3) δ (ppm) : 14.9 (s, 1H, OH), 8.6 (s, 1H, N-CH), 7.9 (m, 5H, Ar-H), 7.6 (m, 3H, Ar-H), 4.5 (s, 2H, N-CH_2-N), 3.1- 3.6 (m, 8H, piperazine), 1.1(m, 5H, methyl), 1.2(t, 3H, ethyl)

1-ethyl-6-fluoro-4-oxo-7-(4-((2-propyl-1H-benzo[d]imidazol-1-yl)methyl)piperazin-1-yl)-1, 4-d ihydroquinoline-3-carboxylic acid (NF_3)

IR (KBr cm^{-1}): 3053.42 (Ar-C-H str), 1739.85, 1732.13(C=O str), 1444.73 (alkane C-H bend), 1627.97 (C=Nstr), 1479.45 (C-N str), ^{1}H-NMR (300 MHz, CDCl3) δ (ppm) : 14.9 (s, 1H, OH), 8.6 (s, 1H, N-CH), 7.9 (m, 5H, Ar-H), 7.6 (m, 3H, Ar-H), 4.5 (s, 2H, N-CH_2-N), 3.1- 3.6 (m, 8H, piperazine),

Antimicrobial activity:

All the synthesized compounds were screened for their antibacterial activity against *B.subtilis, K.pneumoniae & P.aeruginosa* by using disc diffusion method [10]. Bacteria were cultured in nutrient agar medium and used as inoculum for study. The test compounds were dissolved in N, N dimethyl formamide (DMF) to obtain a solution of 25, 50,100µg/ml concentration. The data are given in Table-1. The compounds were also screened for their in vitro antifungal activities against *A. Niger*, *C.albicans* & *R.nigricans.* The inhibitory activities were compared with the commercial fungicide griseofulvin. The compounds exhibited varying degree of activity. The data are given in Table-2.

Results and discussion:

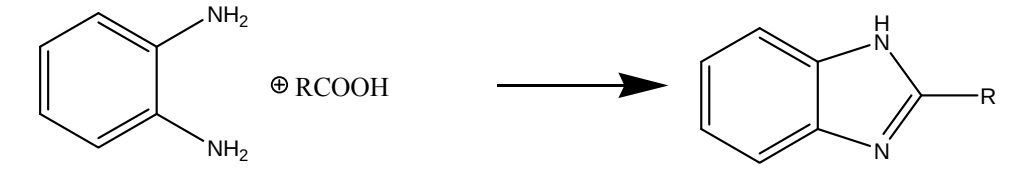
The purity of the synthesized compounds was checked by performing thin layer chromatography and determining melting points. IR and ^{1}HNMR spectra were consistent with the assigned structures. Since our titled compounds are known to possess antimicrobial activity, the compounds were screened for their antibacterial and antifungal activity by cup-plate method. Among the B series (B_1, B_2, and B_3) tested for antibacterial activity, B_2 showed good antibacterial activity against both gram negative bacterial species. The inhibition zones of B_2 ranged from 12.3 to 19.3 mm. But it was found to have moderate antibacterial activity against gram positive bacteria.

Among the norfloxacin derivatives tested for antibacterial activity, norfloxacin showed good antibacterial activity against gram negative bacteria but it has showed moderate activity against gram positive bacteria. NF_3 was found to have good antibacterial activity against gram positive bacteria and moderate activity against gram negative bacteria. Among the ciprofloxacin series CF_1 showed good antibacterial activity against gram negative bacteria but it was found to have moderate activity against gram positive bacteria (Table 1). CF_2 showed good antimicrobial activity against both gram positive and gram negative bacterial strains when compared to CF standard.

All thé titled compounds were tested for their antifungal activity with three fungal strains (*Aspergillus niger, Rhizopus nigricans, Candida albicans*) by using cup plate method (Table 3). All the fungal strains tested for assay showed susceptible to all tested compounds, except *Candida albicans.*

SCHEME

STEP 1

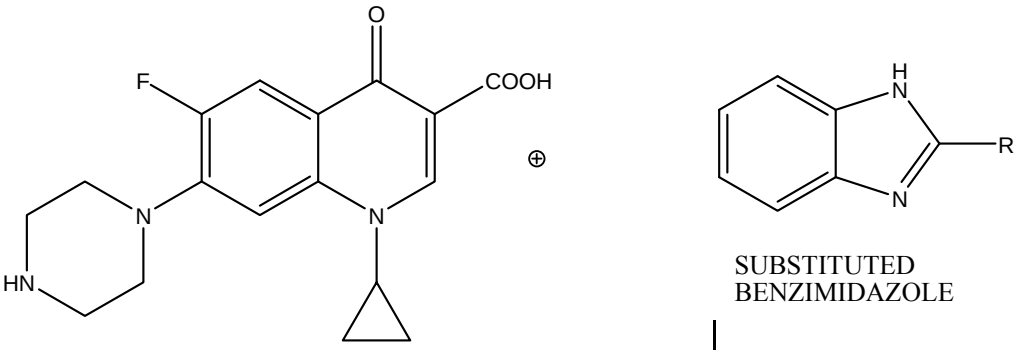


0-PHENYLENE DIAMINE

SUBSTITUTED ACIDS

SUBSTITUTED BENZIMIDAZOLE B_1-B_3

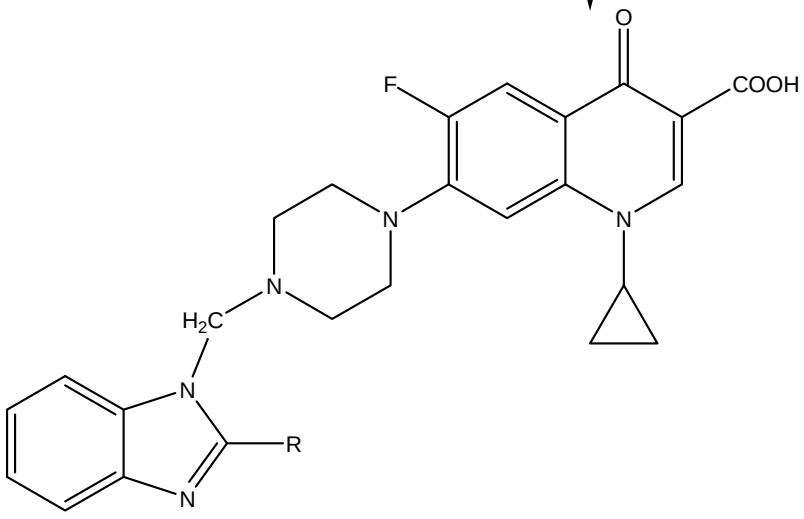
STEP 2



CIPROFLOXACIN

SUBSTITUTED BENZIMIDAZOLE

HCHO



MANNICH BASE (CF_1-CF_3)

STEP 3

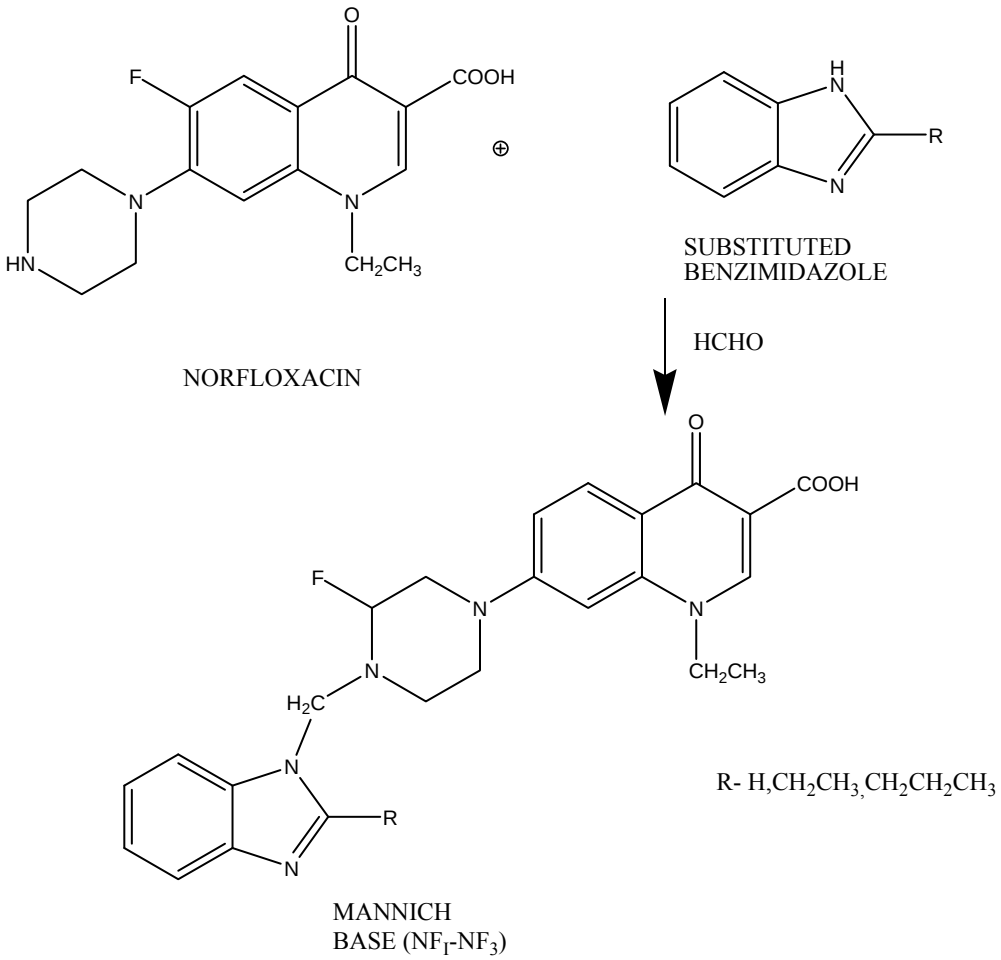


Table 1: Physical parameters of synthesized compounds

Compound No.	Molecular weight	Molecular formula	Melting Point (^{0}C)	% Yield	Rf values
B_1	118.14	$C_7H_6N_2$	198-200	48	0.14
B_2	146.14	$C_9H_{13}N_2$	213-215	46	0.16
B_3	160.14	$C_{10}H_{12}N_2$	180-184	44	0.22
CF_1	460.50	$C_{26}H_{25}FN_4O_3$	210-212	50	0.16
CF_2	488.50	$C_{28}H_{29}FN_4O_3$	200-205	47	0.19
CF_3	502.50	$C_{29}H_{31}FN_4O_3$	190-192	52	0.20
NF_1	448.49	$C_{25}H_{25}FN_4O_3$	275-278	55	0.20
NF_2	476.49	$C_{27}H_{29}FN_4O_3$	198-200	48	0.15
NF_3	490.49	$C_{28}H_{31}FN_4O_3$	280-282	55	0.19

Table2: Antibacterial activity of synthesized compounds (Zone of inhibition in mm)

Com B	.subtilis Conc (µg/ml) ±S.E			K.pneumoniae Conc(µg/ml) ±S.E			P.aeruginosa Conc (µg/ml) ±S.E		
Con	25	50	100	25	50	100	25	50	100
B_1	12.3 ± 0.33	13.6 ±0.33	17.3 ± 0.33	11 ±0.00	14.6 ±0.33	17.6 ±0.33	11.6 ±0.33	13.3 ±0.33	15.3 ±0.33
B_2	13.3 ±0.33	12.3 ±0.33	17.6 ±0.33	12.3 ±0.33	15.3 ±0.33	19.3 ±0.33	11.6 ±0.33	13.6 ±0.33	15.3 ±0.33
B_3	10.6 ±0.33	14.3 ±0.66	15.3 ±0.33	13 ±0.57	15.6 ±0.66	17.6 ±0.88	11.3 ±0.33	35.6 ±0.33	44.6 ±0.33
NF	42 ±0.57	44.3 ±0.33	46.3 ±0.33	35.6 ±0.33	38.3 ±0.33	43.6 ±0.33	33 ±0.57	37.6 ±0.88	43 ±0.57
NF_1	32.3 ±0.33	34.6 ±0.33	40 ±0.57	35.0 ±0.00	36.6 ±0.33	38.3 ±0.66	25.3 ±0.33	28.3 ±0.33	32.3 ±0.33
NF_2	31.3 ±0.33	33 ±0.57	36.6 ±0.33	24.6 ±0.33	25.3 ±0.33	29.6 ±0.33	26 ±0.00	36.3 ±0.33	39.3 ±0.33
NF_3	40 ±0.57	41.6 ±0.33	43.3 ±0.88	35.3 ±0.33	35.6 ±0.66	38.3 ±0.33	33.6 ±0.33	14.6 ±0.33	16.3 ±0.33
CF	32.3 ±0.33	37.6 ±0.33	42 ±0.57	33.6 ±0.33	35.3 ±0.33	40 ±0.57	33.6 ±0.33	39.3 ±0.33	43 ±0.57
CF_1	37.6 ±0.33	39.3 ±0.33	41 ±0.57	35.6 ±0.33	38.6 ±0.33	45.6 ±0.33	30 ±0.57	40.3 ±0.33	44.6 ±0.33
CF_2	34.6 ±0.33	41.3 ±0.66	44.6 ±0.33	34 ±0.00	42.3 ±0.66	46 ±0.57	35 ±0.00	31.6 ±0.88	43.3 ±0.88
CF_3	33.3 ±0.33	36.6 ±0.33	38.6 ±0.33	28.6 ±0.33	38.6 ±0.66	38.3 ±0.66	30.6 ±0.33	29.6 ±0.33	35.3 ±0.33

Table 3: Antifungal activity of synthesized compounds (Zone of inhibition in mm)

Com	*C.albicans* Conc (µg/ml) ±S.E			*R.nigricans* Conc(µg/ml) ±S.E			*A.niger* Conc (µg/ml) ±S.E		
Con	25	50	100	25	50	100)	25	50	100
B_1	-	-	48±0.00	-	-	-	-	-	-
B_2	-	-	50±0.00	-	-	-	-	-	-
B_3	-	-	46±0.00	-	-	-	-	-	-
NF	-	-	17.6±0.33	-	-	-	-	-	-
NF_1	-	-	15.3±0.33	-	-	-	-	-	-
NF_2	-	-	18.3±0.33	-	-	-	-	-	-
NF_3	-	-	22.3±0.88	-	-	-	-	-	-
CF	-	-	36.6±0.15	-	-	-	-	-	-
CF_1	-	-	70±0.00	-	-	-	-	-	-
CF_2	-	-	50±0.00	-	-	-	-	-	-
CF_3	-	-	66±0.00	-	-	-	-	-	-

Among the B series (B_1, B_2, and B^3) tested, 100µg/ml of B_2 showed good antifungal activity with zone of inhibition of 50±0.00mm. Among the norfloxacin derivatives, NF_3 showed good antifungal activity in the concentration of 100 µg/ml with zone of inhibition of 22.3±0.88mm. Among the ciprofloxacin derivatives tested for antifungal activity, CF_1 showed good antifungal activity against *Candida albicans* with zone of inhibition of 70±0.00mm (Table 2)

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