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# SYNTHESIS OF MANNICH BASES OF NORFLOXACIN: CONVENTIONAL AND MICROWAVE ASSISTED SYNTHESIS

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**Keywords:**

Mannich bases, Norfloxacin, Isatin, $^{1}$H NMR, $^{13}$C NMR, Agar well diffusion method

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**ABSTRACT:** Quinolones are the organic compounds that act against the DNA-gyrase enzyme, a type II topoisomerase. Norfloxacin is one such quinolone which is characterized by a piperazinemoiety at C-7 position. This moiety plays a significant role in determining the antibacterial spectrum and potency and serves as a site amenable to significant modification. N-Mannich bases have proved to be potential prodrug candidates for amides, urea derivatives, imides and amines. It has proved to be a versatile base in current applications of organic chemistry. Mannich bases with N-4 substituted piperazine containing moieties were biologically active. We designed and synthesized new series of Mannich bases of Norfloxacin by conventional as well as microwave method by reacting them with isatin and various aromatic aldehydes. Their chemical structures have been confirmed by IR, $^{1}$H NMR and $^{13}$C NMR spectroscopy and evaluated for antibacterial activity by Agar well diffusion method. Antimicrobial evaluation was done against gram positive bacteria *B. subtilis* and gram negative bacteria *E. coli*. Among the compounds tested, 7a (i) and 7b (i) showed promising activity.

**INTRODUCTION:** Organic chemistry is the most versatile branch of chemistry which deals with the synthesis of pharmaceuticals, natural molecules and various nitrogenous biologically active compounds [1]. Mannich reaction has proved to be the most utile method for the synthesis of such compounds. There are very few methods of synthesis that involve C-C bond formation. Mannich reaction has proved to be one such beneficial reaction. Various secondary and tertiary amines are synthesized with Mannich reaction [2]. These amines could be further used for the synthesis of intermediates, natural and synthetic compounds for medicinal as well as industrial purpose [3].

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Mannich bases are usually *β*-amino carbonyl compounds. A compound with at least one hydrogen atom condenses with a non-enolizable aldehyde and a primary, secondary amines or ammonia to furnish a product known as Mannich base. Mannich reaction involves nucleophilic addition of an amine to formaldehyde [4]. Quinoline **Fig. 1** is a urea derivatives, imides nitrogen containing heterocyclic aromatic compound. Quinoline is a versatile compound possessing antibacterial, antimalarial, anthelminthic, antifungal, anti-inflammatory, cardiotonic, anti-covulsant and analgesic activity [5]. The quinolones are a class of synthetic broad spectrum antibiotics [6].

Fluoro substitution at the 6$^{th}$ position give rise to a class of antibiotics called fluoroquinolones [7]. The Fluroquinolones are 1-substituted 1, 4-dihydro-4-oxopyridine-3-carboxylic moiety. The nitrogen atom at 1$^{st}$ position is important for the antibacterial action. The carboxylic acid group at 3$^{rd}$ position binds to DNA gyrase.

The oxo pyridine group shows broad spectrum activity and is very much essential [8]. Norfloxacin **Fig. 2** [1-ethyl-6-fluoro-1, 4-dihydro- 4-oxo -7- (1-piperazinyl) -3- quinoline carboxylic acid] is a quinolone ring fused to piperazine moiety. It is a synthetic antibacterial agent used for treating common as well as complicated urinary tract infections [9]. The piperazine moiety at C-7 position plays an important role in broad spectrum activity and is an amenable site for significant modification [10].

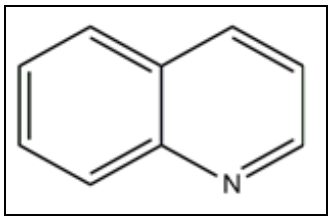



**FIG. 1: QUINOLINE**

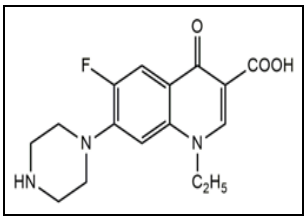



**FIG. 2: NORFLOXACIN**

Indole nucleus is found out to be of clinical significance since the alkaloids containing indole moiety have shown therapeutic effectiveness. Isatin (indole-2, 3-dione) is an indole derivative which is widely distributed in mammalian body fluids and tissues [11]. Isatins are widely used in organic synthesis for broad spectrum compounds like anti-inflammatory, antiviral, antibacterial, anti-tubercular, antidepressant, antifungal [12]. Isoniazid is a good antimicrobial compound. Due to resistance developed towards isoniazid treatment, it could be substituted with other drugs which are urea derivatives, thus maintaining the anti-tubercular action [13]. Urea and its derivatives have been found to possess better antimicrobial and antioxidant properties [14].

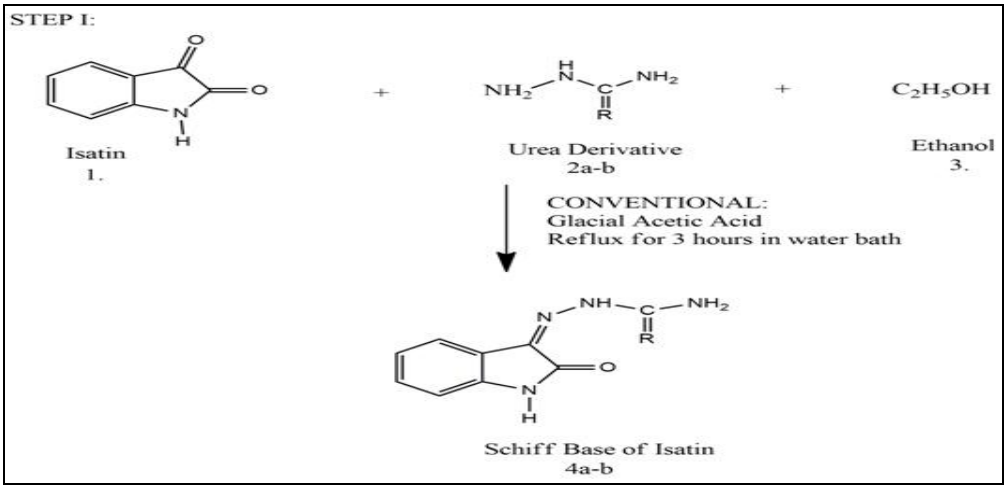
Considering the antimicrobial properties of isatin, urea derivatives (anti-tubercular) and Norfloxacin (a quinolone), we have aimed to synthesize N-Mannich bases of Norfloxacin by reacting them with various aromatic aldehydes and isatin. Microwave synthesis has also been used as the source of heating in this reaction. It has been proved to be a greener, less time consuming and good yielding method of synthesis, whereas the conventional heating is the inefficient and time-consuming [15]. By replacing N4 hydrogen of piperazine with various isatin derivatives newer moieties were synthesized by conventional as well as microwave methods.

## MATERIALS AND METHODS:

**General:** All Chemicals were obtained from LOBA Chemicals, Mumbai. All glassware was of Borosilicate grade. Melting Points were determined in open capillaries and are uncorrected. The purity of the compounds was ascertained by TLC on silica gel-G plate. The synthetic work was done by using catalyst Scientific Microwave Synthesis System. Characterization of synthesized compounds was done by spectral studies. IR spectra were done on SHIMADZU Spectrophotometer. NMR spectra were recorded on BRUKE AVANCE II 400 NMR SPECTROMETER instrument, operating at 400MHz for $^{1}$H NMR and 100 MHz for $^{13}$C NMR in DMSO with TMS as internal standard. The experiments were performed in the year 2016-2017 at V. E. S. College of Pharmacy.

**Synthesis of Newer Derivatives of Quinolone Class of Agents (Mannich Bases):**

**Step 1: Synthesis of Schiff's Base of Isatin (Conventional Method):**




**SCHEME 1**

**Procedure:** Urea derivatives (0.1mol) were dissolved in ethanol in a 500 ml round bottom flask. To this mixture, isatin (0.1mol) was added. Few drops of glacial acetic acid were also added to this solution. The whole content was refluxed for 3-4 h on a water bath with occasional shaking. The completion of reaction was monitored by Thin Layer Chromatography (TLC) (hexane: ethyl acetate 1:1). The flask was cooled to room temperature and kept in ice cold condition for 1 h. The solid mass was filtered off under suction, air dried and recrystallized from ethanol-water mixture **Table 1**. The structures of the synthesized compounds 4 (a-b) were confirmed by their melting points, IR spectra and $^1$H NMR spectra.

**TABLE 1: PHYSICAL DATA OF SCHIFF BASES OF ISATIN (CONVENTIONAL METHOD)**

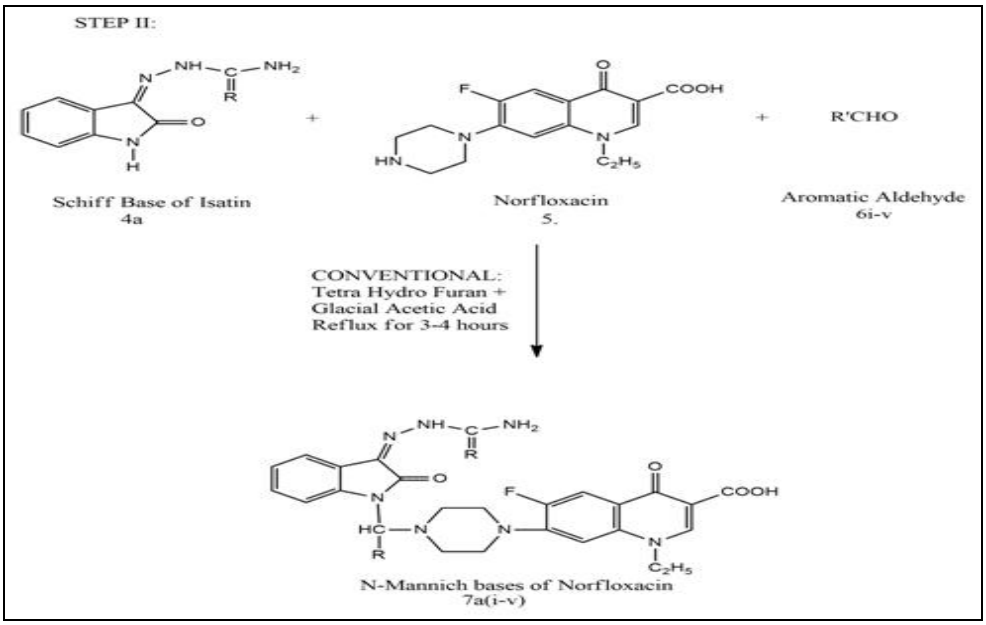
| Compd. no. | R | Molecular Formula | Yield (%) | M.P. (°C) | $R_f$ value |
|---|---|---|---|---|---|
| 4a | O | $C_9H_8N_4O_2$ | 75% | 244 °C | 0.92 |
| 4b | S | $C_9H_8N_4OS$ | 77% | 240°C | 0.91 |

*Mobile Phase Solvents: *n*-hexane: ethyl acetate (1: 1], DMSO: Dimethyl Sulfoxide

**2- (2-oxoindolin-3- ylidene) hydrazinecarboxamide 4(a):** IR (KBr, $\nu_{max}$, cm$^{-1}$): 3468.13 (NH str.), 1620(C=N str.), 1685.84 (amide, urea -C=O str.), 1730.46 (*β*-lactam -C=O str.), 1570.11 (NH bend), 744.55, 783.13 (aromatic C-H, out of the plane, bending). $^1$H NMR (δ ppm): 7.01 (1H, s, hydrazid NH), 6.01 (2H, s, urea $NH_2$), 7.87 (1H, d, aromatic CH), 7.85 (1H, d, aromatic CH), 7.55 (1H, t, aromatic CH), 7.26 (1H, t, aromatic CH), 8.01 (1H, s, secondary amide H).

**2-(2 –oxoindolin- 3- ylidene) hydrazinecarbothio amide (4b):** IR (KBr, $\nu_{max}$, cm$^{-1}$): 3468.13 (NH str.), 1620.26 (C=N str.), 1570.11 (NH bend), 1685.84 (amide, -C=O str.), 1207.48 (C=S str.), 700-900 (aromatic C-H, out of the plane, bending). $^1$H NMR (δ ppm): 7.00 (1H, s, hydrazid NH), 8.52 (2H, s, urea $NH_2$), 7.90 (1H, d, aromatic CH), 7.82 (1H, d, aromatic CH), 7.53 (1H, t, aromatic CH), 7.22 (1H, t, aromatic CH), 8.00 (1H, s, secondary amide H).

**Step II: Synthesis of N-Mannich Bases of Isatin with Norfloxacin (Conventional Route):**

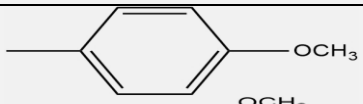
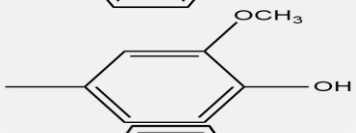
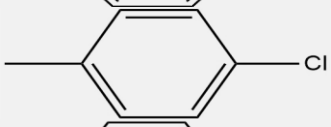
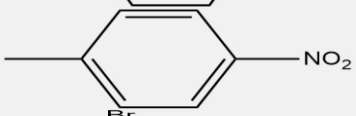



**SCHEME 2**

**Procedure:**

**Conventional Method:** A solution of Norfloxacin (2 mol) in glacial acetic acid was added to slurry of isatin derivatives (4a and 4b) and various aldehydes (1 mol) in little amount of tetrahydrofuran. The reaction mixture was refluxed for 7-8 h. TLC control was performed to assess the completion of the reaction as indicated by complete disappearance of Norfloxacin in the reaction mixture. The reaction mixture was concentrated to half of its volume and precipitated thus obtained was recrystallized from DMF-water mixture **Table 2**.

**TABLE 2: PHYSICAL CHARACTERISTICS OF N-NORFLOXACIN MANNICH BASES (CONVENTIONAL METHOD)**

| Compd. no. | R | R' | Formula | Yield (%) | M.P. (ºC) | $R_f$ value |
|---|---|---|---|---|---|---|
| 7a(i) | O |  | $C_{33}H_{31}N_7O_6F$ | 95% | 235 | 0.95 |
| 7a(ii) | O |  | $C_{33}H_{31}N_7O_7F$ | 69% | 252 | 0.88 |
| 7a(iii) | O |  | $C_{32}H_{28}N_7O_5ClF$ | 70% | 258 | 0.93 |
| 7a(iv) | O |  | $C_{32}H_{28}N_8O_7F$ | 75% | 268 | 0.91 |
| 7a(v) | O |  | $C_{32}H_{28}N_7O_5BrF$ | 73% | 256 | 0.81 |

*Mobile Phase Solvents: ammonia: acetonitrile [1: 1], DMSO: Dimethyl Sulfoxide

**7-(4-((3 - (2-carbamoylhydrazono)-2-oxoindolin-1-yl) (4 methoxy phenyl) methyl) piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid [7a (i)]:** IR (KBr, $\nu_{max}$, $cm^{-1}$): 3230.77 (NH str.), 1614.42 (C=N str.), 1571.99 (N-H bend), 1774.65 (β-lactam C=O str.), 1645.28 (amide, urea -C=O str.), 1703.14 (COOH group C=O str.), 3057.17 (COOH group OH str.), 1300.02 (C-N str.), 1242.16 (C-F str.), 1265.30 (O-$CH_3$ str.), 700-900 (aromatic C-H, out of the plane, bending). $^1$H NMR (δ ppm): 11.01 (1H, s, carboxylic acid OH), 7.01 (1H, s, hydrazid NH), 6.10 (2H, s, urea $NH_2$), 3.00 (2H, t, methylene $CH_2$), 2.77 (2H, t, methylene $CH_2$), 7.91 (1H, d, aromatic CH), 7.87 (1H, d, aromatic CH), 6.01 (1H, s, methine CH), 1.23 (3H, t, methyl $CH_3$), 8.18 (1H, s, aromatic CH), 6.89 (1H, d, aromatic CH), 6.50 (1H, s, aromatic CH), 7.30 (1H, d, aromatic CH), 7.58 (1H, t, aromatic CH), 7.26 (1H, t, aromatic CH), 3.87 (3H, s, methyl $CH_3$), 4.57 (2H, q, methylene $CH_2$), 8.92 (1H, s, ethylene H). Analytical calculated % ($C_{33}H_{32}FN_7O_6$): C 61.77, H 5.03, F 2.96, N 15.28, O 14.96 found %: C 61 H 5.06 F 2.80 N 15.73 O 14.99.

**7-(4-((3- (2-carbamoylhydrazono)-2- oxoindolin-1- yl) (4- hydroxy-3- methoxyphenyl) methyl) piperazin-1- yl)-1- ethyl-6- fluoro- 4-oxo-1, 4-dihydroquinoline-3-carboxylic acid [7a(ii)]:** IR (KBr, $\nu_{max}$, $cm^{-1}$): 1614.42 (C=N str.), 1597.06 (N-H bend), 1724.36 (*β*-lactam C=O str.), 1687.71 (amide, urea -C=O str.), 3468.01 (NH str.), 1708.93 (COOH group C=O str.), 3059.10 (COOH group OH str.), 1330.88 (C-N str.), 1213.23 (C-F str.), 3035.96 (phenol OH str.), 1201.65 (O-$CH_3$ str.), 700-900 (aromatic C-H, out of the plane, bending). $^1$H NMR (δ ppm): 4.58 (1H, s, aromatic C-OH), 11.89 (1H, s, carboxylic acid OH), 7.01 (1H, s, hydrazid NH), 6.01 (2H, s, urea $NH_2$), 3.29 (1H, t, methylene $CH_2$), 2.93 (2H, t, methylene $CH_2$), 7.88 (1H, d, aromatic CH), 7.87 (1H, d, aromatic CH), 8.14 (1H, s, aromatic CH), 1.24 (3H, t, methyl $CH_3$), 6.91 (1H, s, aromatic CH), 6.89 (1H, d, aromatic CH), 6.10 (1H, s, aromatic CH), 6.92 (1H, d, aromatic CH), 7.58 (1H, t, aromatic CH), 7.25 (1H, t, aromatic CH), 3.88 (3H, s, methyl $CH_3$), 6.01 (1H, s, methine CH), 4.56 (2H, q, methylene $CH_2$). Analytical calculated % ($C_{33}H_{32}FN_7O_7$): C 60.27, H 4.90, F 2.89, N 14.91, O 17.03 found %: C 60.34 H 4.56, F: 2.78, N 14.80, O 17.52.

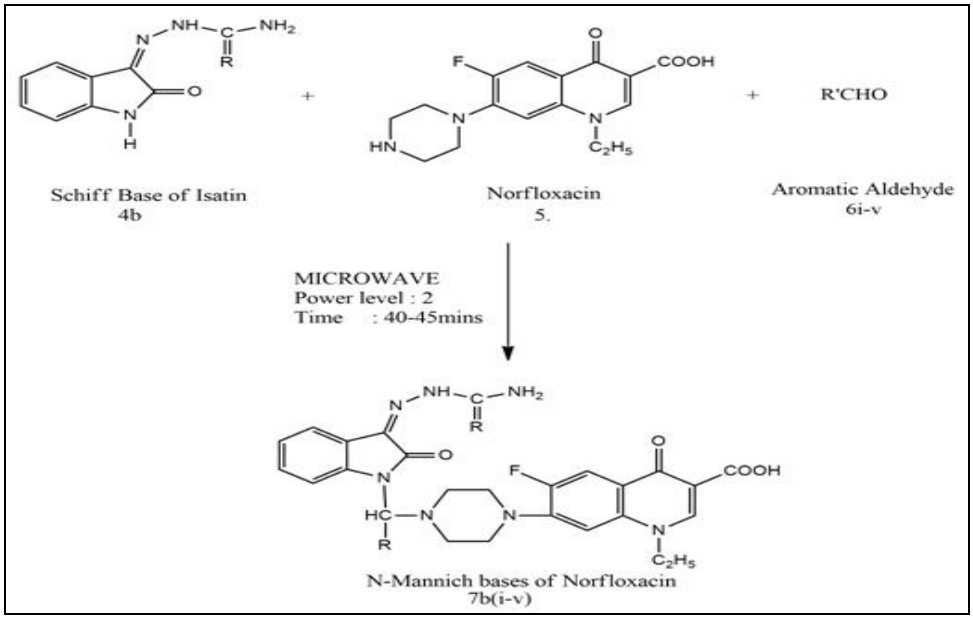
**7-(4 -( (3-(2-carbamoylhydrazono)-2-oxoindolin-1-yl) (4-chlorophenyl)methyl)piperazin-1- yl)-1-ethyl-6- fluoro-4 –oxo -1, 4-dihydroquinoline-3-carboxylic acid [7a(iii)]:** IR (KBr, $\nu_{max}$, $cm^{-1}$): 1618.28 (C=N str.), 1514.12 (N-H bend), 1785.04 (*β*-lactam C=O str.), 1664.20 (amide, urea -C=O str.), 3321.42 (NH str.), 1708.93 (COOH group C=O str.), 3199.91 (COOH group OH str.), 1330.88 (C-N str.), 1143.79 (C-F str.), 767.67 (C-Cl str.), 700-900 (aromatic C-H, out of the plane, bending). $^1$H NMR (δ ppm): 11.05 (1H, s, carboxylic acid OH), 7.11 (1H, s, hydrazid NH), 6.12 (2H, s, urea $NH_2$), 3.03 (2H, t, methylene

$CH_2$), 2.80 (2H, t, methylene $CH_2$), 7.88 (1H, d, aromatic CH), 7.52 (1H, d, aromatic CH), 7.34 (1H, d, aromatic CH), 8.01 (1H, s, aromatic CH), 6.13 (1H, s, aromatic CH), 7.32 (1H, d, aromatic CH), 7.50 (1H, t, aromatic CH), 7.21 (1H, t, aromatic CH), 6.21 (1H, s, methine CH), 4.59 (2H, q, methylene $CH_2$), 1.47 (3H, t, methyl $CH_3$), 8.90 (1H, s, ethylene H). Analytical calculated % ($C_{32}H_{29}ClFN_7O_5$): C 59.49, H 4.52, Cl: 5.49, F: 2.94, N 15.18, O 12.38 found %: C 59.37, H 4.60, Cl 5.90, F 2.57, N 15.67, O 11.89.

**7-(4-((3- (2-carbamoylhydrazono)-2- oxoindolin-1-yl) (4-nitrophenyl) methyl) piperazin-1-yl)-1-ethyl- 6- fluoro-4-oxo-1, 4- dihydroquinoline-3-carboxylic acid [7a (iv)]:** IR (KBr, $\nu_{max}$, $cm^{-1}$): 1612.49 (C=N str.), 1512.19 (N-H bend), 1782.56 (*β*-lactam C=O str.), 1672.28 (amide, urea -C=O str.), 3446.79 (NH str.), 1720.26 (COOH group C=O str.), 3057.17 (COOH group OH str.), 1332.81 (C-N str.), 1143.79 (C-F str.), 1303.88 (N-O str.), 700-900 (aromatic C-H, out of the plane, bending). $^1$H NMR (δ ppm): 11.01 (1H, s, carboxylic acid OH), 7.01 (1H, s, hydrazid NH), 6.06 (2H, s, urea $NH_2$), 3.02 (2H, t, methylene $CH_2$), 2.80 (2H, t, methylene $CH_2$), 7.88 (1H, d, aromatic CH), 7.80 (1H, d, aromatic CH), 8.19 (1H, s, aromatic CH), 6.18 (1H, s, aromatic CH), 8.20 (1H, d, aromatic CH), 7.62 (1H, d, aromatic CH), 7.54 (1H, t, aromatic CH), 7.50 (1H, t, aromatic CH), 6.07 (1H, s, methine CH), 4.56 (2H, q, methylene $CH_2$), 1.55 (3H, t, methyl $CH_3$), 9.32 (1H, s, ethylene H). Analytical calculated % ($C_{32}H_{29}FN_8O_7$): C 58.53, H 4.45, F 2.89, N 17.07, O 17.06 found %: C 58.32, H 4.68, F 2.45, N 17.10, O 17.45.

**7- (4- ((2 -bromophenyl) (3- (2-carbamoyl hydrazono)-2-oxoindolin-1-yl) methyl)piperazin-1-yl) -1- ethyl- 6- fluoro -4- oxo-1, 4-dihydro quinoline-3-carboxylic acid [7a(v)]:** IR(KBr, $\nu_{max}$, $cm^{-1}$): 1618.28 (C=N str.), 1514.12 (N-H bend), 1785.04 (*β*-lactam C=O str.), 1664.20 (amide, urea -C=O str.), 3321.42 (NH str.), 1708.93 (COOH group C=O str.), 3070.68 (COOH group OH str.), 1330.88 (C-N str.), 1249.87 (C-F str.), 590.22 (C-Br str.), 700-900 (aromatic C-H, out of the plane, bending). $^1$H NMR (δ ppm): 11.79 (1H, s, carboxylic acid OH), 7.01 (1H, s, hydrazid NH), 6.00 (2H, s, urea $NH_2$), 3.00 (2H, t, methylene $CH_2$), 2.92 (2H, t, methylene $CH_2$), 7.91 (1H, d, aromatic CH), 7.87 (1H, d, aromatic CH), 8.18 (1H, s, aromatic CH), 6.02 (1H, s, aromatic CH), 7.30 (1H, d, aromatic CH), 7.58 (1H, t, aromatic CH), 7.26 (1H, t, aromatic CH), 7.08 (1H, d, aromatic CH), 7.10 (1H, t, aromatic CH), 7.27 (1H, t, aromatic CH), 6.01 (1H, s, methine CH), 4.57 (2H, q, methylene $CH_2$), 8.98 (1H, s, ethylene H), 1.23 (3H, t, methyl $CH_3$). Analytical calculated % ($C_{32}H_{29}BrFN_7O_5$): C 55.66, H 4.23, Br 11.57, F 2.75, N 14.20, O 11.59 found %: C 55.66, H 4.34, Br 11.60, F 2.40, N 14.21, O 11.79.

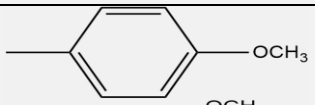
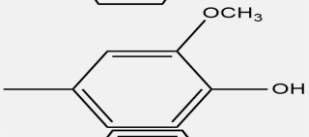
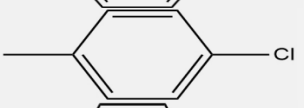
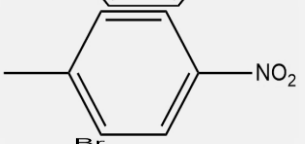
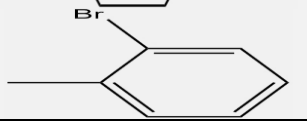
**Step II: Synthesis of N-Mannich Bases of Isatin with Norfloxacin (Greener Route):**




**SCHEME 3**

**Greener Method:** Norfloxacin (2 mol) was added in glacial acetic acid and stirred till it completely dissolved. Slurry of isatin derivatives (4a and 4b) along with various aldehydes (1 mol) was made in a little amount of tetrahydrofuran. The later was added to the solution of Norfloxacin and stirred for about 10 min. The microwave synthesis was performed by irradiating the reaction mixture under the microwave at the power level 2 (170 W, 20% POWER). The completion of reaction was monitored by Thin Layer Chromatography (TLC) (ammonia: acetonitrile 1:1). The precipitate thus obtained was recrystallized from DMF-water mixture **Table 3**.

**TABLE 3: PHYSICAL CHARACTERISTICS OF N-NORFLOXACIN MANNICH BASES (GREENER METHOD)**

| Compd. no. | R | R' | Molecular Formula | Yield (%) | M.P. (ºC) | $R_f$ value |
|---|---|---|---|---|---|---|
| 7b(i) | S |  | $C_{33}H_{31}N_7O_5SF$ | 93% | 268 | 0.61 |
| 7b(ii) | S |  | $C_{33}H_{31}N_7O_6SF$ | 90% | 274 | 0.65 |
| 7b(iii) | S |  | $C_{32}H_{28}N_7O_4SClF$ | 97% | 278 | 0.68 |
| 7b(iv) | S |  | $C_{32}H_{28}N_8O_6SF$ | 90% | 252 | 0.71 |
| 7b(v) | S |  | $C_{32}H_{28}N_7O_4SBrF$ | 92% | 250 | 0.86 |

*Mobile Phase Solvents: ammonia: acetonitrile [1: 1], DMSO: Dimethyl Sulfoxide

**7- (4- ((3- (2- carbamothioyl hydrazono) -2- oxoindolin -1- yl) (4-methoxyphenyl) methyl) piperazin-1 -yl)-1- ethyl-6- fluoro-4- oxo-1, 4-dihydroquinoline-3-carboxylic acid [7b (i)]:** IR (KBr, $\nu_{max}$, $cm^{-1}$): 1624.06 (C=N str.), 1556.55 (N-H bend), 1716.65 (amide, -C=O str.), 1207.44 (C=S str.), 3406.29 (NH str.), 1703.14 (COOH group C=O str.), 3053.32 (COOH group OH str.), 1305.81 (C-N str.), 1386.82 (C-F str.), 1253.73 (O-$CH_3$ str.), 700-900 (aromatic C-H, out of the plane, bending). $^1$H NMR (δ ppm): 11.02 (1H, s, carboxylic acid OH), 7.03 (1H, s, hydrazid NH), 8.20 (2H, s, amine $NH_2$), 3.29 (2H, t, methylene $CH_2$), 2.90 (2H, t, methylene $CH_2$), 7.91 (1H, d, aromatic CH), 7.87 (1H, d, aromatic CH), 8.20 (1H, s, aromatic CH), 6.91 (1H, d, aromatic CH), 6.07 (1H, s, aromatic CH), 7.40 (1H, d, aromatic CH), 7.60 (1H, t, aromatic CH), 7.27 (1H, t, aromatic CH), 3.85 (3H, s, methyl $CH_3$), 6.11 (1H, s, methine CH), 1.45 (3H, t, methyl $CH_3$), 4.60 (2H, q, methylene $CH_2$), 9.04 (1H, s, ethylene H). Analytical calculated % ($C_{33}H_{32}FN_7O_5S$): C 60.26, H 4.90, F 2.89, N 14.91, O 12.16, S 4.88 found %: C 60.25, H 4.90, F 2.86, N 14.89, O 12.18, S 4.92.

**7- (4- ((3- (2- carbamothioyl hydrazono) -2- oxoindolin-1-yl) (4-hydroxy- 3- methoxyphenyl) methyl) piperazin-1 -yl)- 1- ethyl-6-fluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylicacid [7b(ii)]:** IR (KBr, $\nu_{max}$, $cm^{-1}$): 1627.92 (C=N str.), 1552.70 (N-H bend), 1751.61 (amide, -C=O str.), 1207.44 (C=S str.), 3435.22 (NH str.), 1710.86 (COOH group C=O str.), 3053.32 (COOH group OH str.), 1305.81 (C-N str.), 1143.79(C-F str.), 1265.30 (O-$CH_3$ str.), 1382.96 (phenol OH str.), 700-900 (aromatic C-H, out of the plane, bending). $^1$H NMR (δ ppm): 4.56 (1H, s, aromatic C-OH), 11.81 (1H, s, carboxylic acid OH), 7.03 (1H, s, hydrazid NH), 8.50 (2H, s, amine $NH_2$), 2.99 (2H, t, methylene $CH_2$), 2.78 (2H, t, methylene $CH_2$), 7.88 (1H, d, aromatic CH), 7.91 (1H, d, aromatic CH), 7.95 (1H, s, aromatic CH), 6.92 (1H, s, aromatic CH), 6.89 (1H, d, aromatic CH), 6.02 (1H, s, aromatic CH), 6.91 (1H, d, aromatic CH), 7.60 (1H, t, aromatic CH), 7.25 (1H, t, aromatic CH), 3.79 (3H, s, methyl $CH_3$), 6.02 (1H, s, methine CH), 4.58 (2H, q, methylene $CH_2$), 1.46 (3H, t, methyl $CH_3$), 8.72 (1H, s, ethylene H). Analytical calculated % ($C_{33}H_{32}FN_7O_6S$): C 58.83, H 4.79, F 2.82, N 14.55,

O 14.25, S 4.76 found %: C 58.85, H 4.75, F 2.84, N 14.58, O 14.21, S 4.77.

**7- (4- ((3- (2-carbamothioyl hydrazono)-2-oxoindolin-1 -yl) (4- chlorophenyl) methyl) piperazin-1-yl) -1- ethyl-6- fluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid [7b (iii)]:** IR (KBr, $\nu_{max}$, $cm^{-1}$): 1620.21 (C=N str.), 1514.12 (N-H bend), 1775.34 (amide, -C=O str.), 1203.58 (C=S str.), 3008.95 (NH str.), 1730.15 (COOH group C=O str.), 3180.62 (COOH group OH str.), 1330.88 (C-N str.), 1228.66 (C-F str.), 750.31 (C-Cl str.), 700-900 (aromatic C-H, out of the plane, bending). $^1$H NMR (δ ppm): 10.9 (1H, s, carboxylic acid OH), 7.10 (1H, s, hydrazid NH), 8.51 (2H, s, amine $NH_2$), 3.02 (2H, t, methylene $CH_2$), 2.81 (2H, t, methylene $CH_2$), 7.84 (1H, d, aromatic CH), 7.82 (1H, d, aromatic CH), 7.34(1H, d, aromatic CH), 8.12 (1H, s, aromatic CH), 6.32 (1H, s, aromatic CH), 7.33 (1H, d, aromatic CH), 7.51 (1H, t, aromatic CH), 7.21 (1H, t, aromatic CH), 6.31 (1H, s, methine CH), 4.52 (2H, q, methylene $CH_2$), 1.47 (3H, t, methyl $CH_3$), 9.09 (1H, s, ethylene H).

Analytical calculated % ($C_{32}H_{29}ClFN_7O_4S$): C 58.05, H 4.41, Cl 5.35, F 2.87, N 14.81, O 9.67, S 4.84 found %: C 58.07, H 4.39, Cl 5.32, F 2.84, N 14.84, O 9.65, S 4.89.

**7- (4- ((3- (2- carbamothioylhydrazono) -2-oxoindolin-1 -yl) (4- nitrophenyl) methyl) piperazin-1-yl) -1- ethyl-6- fluoro- 4-oxo-1, 4-dihydroquinoline-3-carboxylic acid [7b (iv)]:** IR (KBr, $\nu_{max}$, $cm^{-1}$): 1612.49 (C=N str.), 1570.35 (N-H bend), 1775.30 (amide, -C=O str.), 1195.87 (C=S str.), 3269.34 (NH str.), 1701.22 (COOH group C=O str.), 3161.33 (COOH group OH str.), 1328.95 (C-N str.), 1269.16 (C-F str.), 1382.96 (N-O str.), 700-900 (aromatic C-H, out of the plane, bending). $^1$H NMR (δ ppm): 11.09 (1H, s, carboxylic acid OH), 7.00 (1H, s, hydrazid NH), 8.49 (2H, s, amine $NH_2$), 3.01 (2H, t, methylene $CH_2$), 2.80 (2H, t, methylene $CH_2$), 7.87 (1H, d, aromatic CH), 7.78 (1H, d, aromatic CH), 8.18 (1H, s, aromatic CH), 6.59 (1H, s, aromatic CH), 8.17 (1H, d, aromatic CH), 7.73 (1H, d, aromatic CH), 7.58 (1H, t, aromatic CH),7.52 (1H, t, aromatic CH), 6.58 (1H, s, methine CH), 4.79 (2H, q, methylene $CH_2$), 1.49 (3H, t, methyl $CH_3$), 9.63 (1H, s, ethylene H). Analytical calculated % ($C_{32}H_{29}FN_8O_6S$): C 57.14, H 4.35, F 2.82, N 16.66, O 14.27, S 4.77 found %: C 57.18, H 4.33, F 2.86, N 16.69, O 14.30, S 4.64.

**7- (4- ((2-bromophenyl) (3- (2- carbamothioy lhydrazono)-2-oxoindolin-1-yl)methyl)piperazin-1-yl) -1- ethyl-6- fluoro-4 -oxo-1, 4-dihydro quinoline-3-carboxylic acid [7b(v)]:** IR (KBr, $\nu_{max}$, $cm^{-1}$): 1624.06 (C=N str.), 1530.26 (N-H bend), 1658.78 (amide, -C=O str.), 1203.58 (C=S str.), 3412.08 (NH str.), 1701.22 (COOH group C=O str.), 3047.53 (COOH group OH str.), 1300.02 (C-N str.), 1386.82 (C-F str.), 700-900 (aromatic C-H, out of the plane, bending). $^1$H NMR (δ ppm): 11.80 (1H, s, carboxylic acid OH), 6.90 (1H, s, hydrazid NH), 8.20 (2H, s, amine $NH_2$), 3.29 (2H, t, methylene $CH_2$), 2.79 (2H, t, methylene $CH_2$), 7.98 (1H, d, aromatic CH), 7.82 (1H, d, aromatic CH), 8.00 (1H, s, aromatic CH), 5.99 (1H, s, aromatic CH), 7.48 (1H, d, aromatic CH), 7.50 (1H, t, aromatic CH), 7.29 (1H, t, aromatic CH), 7.01 (1H, d, aromatic CH), 7.03 (1H, t, aromatic CH), 7.23 (1H, t, aromatic CH), 6.00 (1H, s, methine CH), 4.29 (2H, q, methylene $CH_2$), 1.60 (3H, t, methyl $CH_3$), 9.20 (1H, s, ethylene H). Analytical calculated % ($C_{32}H_{29}BrFN_7O_4S$): C 54.39, H 4.14, Br 11.31, F 2.69, N 13.88, O 9.06, S 4.54 found %: C 54.39, H 4.14, Br 11.30, F 2.65, N 13.85, O 9.10, S 4.57.

**Antibacterial Activity:** All the synthesized Norfloxacin derivatives 7a (i-v) and 7b (i-v) were characterized and screened for their antimicrobial activities. The zone of inhibition was measured against *B. subtilis* (gram positive) and *E. coli* (gram negative). The difference in anti-bacterial activities of the synthesised compounds was studied by the Agar well diffusion method. A clearing zone around the disc indicates the inhibitory activity of the compound on the organism. Four different concentrations were selected (25 µg/ml, 50 µg/ml, 100 µg/ml and 200µg/ml) using DMSO as negative control and Norfloxacin as positive control.

**Agar Well Diffusion Method:** The antibacterial activity of the synthesized compounds was evaluated *in-vitro.* Antibacterial activity of different concentrations (25 µg, 50 µg, 100 µg and 200 µg) of test compounds were tested against gram positive bacteria *B. subtilis* and gram negative bacteria *E. coli*. The inoculated sterilized nutrient

agar media was poured into petri dishes and allowed to solidify. 6 mm wells were made on the agar surface, into each of these wells, 30 µl of the test compound with different concentrations /reference standard / control was added by using a micropipette. Norfloxacin was used as standard reference and DMSO was used as a control (solvent) which did not possess any inhibition zone. The plates were incubated at 37 ºC for 24 h for bacterial activity. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in three different fixed directions in all 3 replicates and the average values were tabulated. The inhibition zones were calculated and recorded.

**RESULTS AND DISCUSSION:** The synthesized Norfloxacin derivatives 7a (i-v) and 7b (i-v) were evaluated for their *in-vitro* antimicrobial activities against the test pathogens *E. coli* and *B. subtilis.*

The synthesized compound 7a (i) exhibits potent antibacterial activity against gram negative and gram positive bacteria, compared with reference standards. The minimum inhibitory concentration (MIC) value was noticeable with 25 µg/ml for gram positive bacteria *B. subtilis.* The MIC value was observed with 25 µg/ml for gram negative bacteria *E. coli.*

The compound 7a (ii) exhibits equipotent antimicrobial activity against gram negative and gram positive bacteria compared with reference standards. The MIC value was noticeable with 25µg/ml for gram positive bacteria *B. subtilis.* The MIC value was noticeable with 25 µg/ml for gram negative bacteria *E. coli.*

**TABLE 4: ACTIVITY OF THE COMPOUNDS AT 25 µg/ml, 50 µg/ml, 100 µg/ml AND 200 µg/ml**

| S. no. | Compound code | Gram positive (*B. subtilis*) | | | | Gram negative (*E. coli*) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 25 µg/ml | 50 µg/ml | 100 µg/ml | 200 µg/ml | 25 µg/ml | 50 µg/ml | 100 µg/ml | 200 µg/ml |
| 1 | 7a(i) | 17 | 18 | 20 | 22 | 16 | 17 | 19 | 20 |
| 2 | 7a(ii) | 9 | 12 | 15 | 18 | 10 | 15 | 18 | 20 |
| 3 | 7a(iii) | - | - | 8 | 11 | 8 | 11 | 13 | 16 |
| 4 | 7a(iv) | 8 | 10 | 13 | 15 | - | 10 | 13 | 16 |
| 5 | 7a(v) | - | - | - | 10 | - | 9 | 10 | 12 |
| 6 | 7b(i) | 10 | 12 | 15 | 18 | 10 | 14 | 18 | 20 |
| 7 | 7b(ii) | 9 | 11 | 15 | 18 | 9 | 11 | 14 | 17 |
| 8 | 7b(iii) | - | - | 9 | 13 | - | 8 | 10 | 13 |
| 9 | 7b(iv) | - | 9 | 11 | 14 | - | 9 | 12 | 15 |
| 10 | 7b(v) | - | - | 10 | 14 | - | 9 | 12 | 16 |
| 11 | Norfloxacin (N) | 9 | 13 | 16 | 20 | 12 | 15 | 20 | 22 |
| 12 | DMSO (control) | - | | | | | | | |

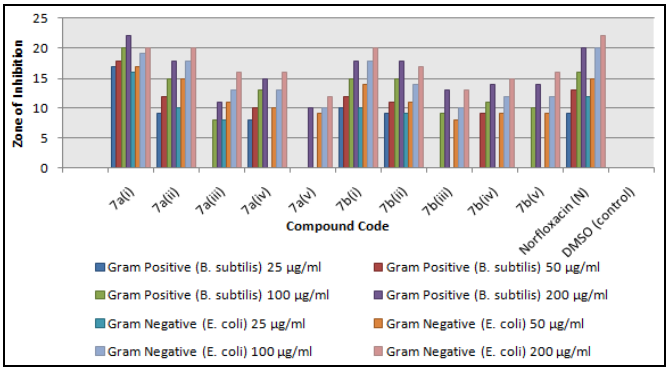



**FIG. 3: GRAPHICAL REPRESENTATION FOR COMPARISON OF ANTI-BACTERIAL ACTIVITY**

The synthesized compound 7a (iii), exhibits zone of inhibition against gram negative and gram positive bacteria. The MIC value was noticeable with 100µg/ml for gram positive bacteria *B. subtilis.* The MIC value was noticeable with 25 µg/ml for gram negative bacteria *E. coli.*

The compound 7a (iii) showed mild activity against gram positive bacteria as compared with reference standard.

The synthesized compound 7a (iv) exhibits potential antimicrobial activity against gram negative and gram positive bacteria. The MIC value was noticeable with 25 µg/ml for gram positive bacteria *B. subtilis.* The MIC value was noticeable with 50 µg/ml for gram negative bacteria *E. coli.*

The compound 7a (v) showed mild activity against gram positive and gram negative bacteria compared with reference standard. The MIC value was noticeable with 200 µg/ml for gram positive bacteria *B. subtilis.* The MIC value was noticeable with 50 µg/ml for gram negative bacteria *E. coli.*

The synthesized compound 7b (i) exhibits potent antibacterial activity against gram negative and gram positive bacteria, compared with reference standards. The MIC value was noticeable with 25µg/ml for gram positive bacteria *B. subtilis.* The MIC value was noticeable with 25 µg/ml for gram negative bacteria *E. coli.*

The synthesized compound 7b (ii) exhibits potent antibacterial activity against gram negative and gram positive bacteria, compared with reference standards. The MIC value was noticeable with 25µg/ml for gram positive bacteria *B. subtilis.* The MIC value was noticeable with 25 µg/ml for gram negative bacteria *E. coli.*

The synthesized compound 7b (iii) exhibits potential antimicrobial activity against gram negative and gram positive bacteria. The MIC value was noticeable with 100 µg/ml for gram positive bacteria *B. subtilis.* The MIC value was noticeable with 50 µg/ml for gram negative bacteria *E. coli.*

The synthesized compound 7b (iv) exhibits zone of inhibition against gram negative and gram positive bacteria. The MIC value was noticeable with 50µg/ml for gram positive bacteria *B. subtilis.* The MIC value was noticeable with 50µg/ml for gram negative bacteria *E. coli.*

The synthesized compound 7b (v) exhibits zone of inhibition against gram negative and gram positive bacteria. The MIC value was noticeable with 100µg/ml for gram positive bacteria *B. subtilis.* The MIC value was noticeable with 50 µg/ml for gram negative bacteria *E. coli.*

**CONCLUSION:** The Mannich reaction has proven to be one of the most basic and useful methods for the synthesis of nitrogenous compounds. As the quinolones have been found out to be promising class of antibiotics, it was chosen for the present study. It was important to consider substitution of the quinolone compounds. The piperazine moiety of Norfloxacin at C-7 position plays a significant role in the antibacterial spectrum and potency and represents a site amenable to significant modification. Based on the above mentioned information, our present study was aimed to synthesize Mannich bases of Norfloxacin and to evaluate their anti-bacterial activity because more effective and affordable drugs are very much needed. The synthesised compounds were preceded for physical characterization. Compounds synthesized by microwave method showed better yield and the method was time efficient and less solvent consuming as compared to conventional synthesis. The IR and NMR spectroscopy confirm the anticipated structure. The compounds thus synthesized were evaluated for the anti-bacterial activity. It was seen that the synthesised Mannich bases have good activity towards the bacterial strains which were used for the study. Compounds 7a (i), 7a (ii), 7b (i), and 7b (ii) have shown better activity. The overall results conclude that the compounds were synthesised successfully which have good anti-bacterial activity.

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**CONFLICT OF INTEREST:** The authors declare no conflicts of interest.

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