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Research Article

SYNTHESIS, PHOTOCHEMICAL PROBE AND ANTIMICROBIAL EFFECTS OF NOVEL NORFLOXACIN ANALOGUES

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ABSTRACT

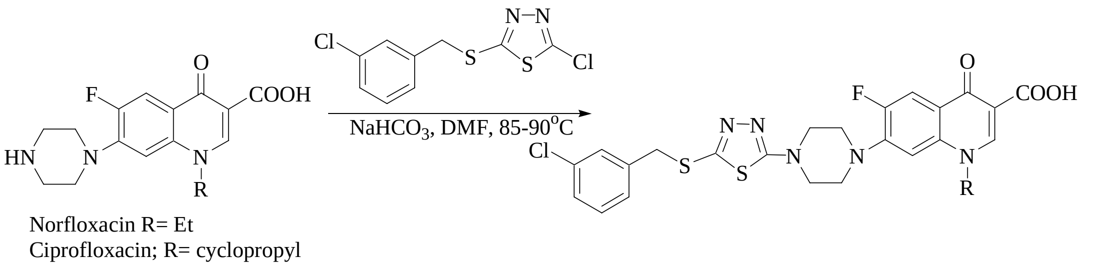
The emerging resistance to antimicrobial drugs demands the synthesis of new remedies for microbial infections. Attempts have been made to prepare new compounds by modifications in the quinolone structure. An important method for the synthesis of new quinolones is using Vilsmeier approach, but has its own limitations. In an effort to synthesize norfloxacin analogues, only 7-bromo-6-*N*-benzyl piperazinyl-4-oxoquinoline-3-carboxylic acid was isolated using Vilsmeier approach at high temperature. On the other hand, *N,N'*-bis-(4-fluoro-3-nitrophenyl)oxalamide and *N,N'*-bis-(3-chloro-4-fluorophenyl)malonamide were obtained under reverse Vilsmeier approach. Structures of the products have been established from their elemental analysis and spectral measurements. Correlation results showed that lipophilicity, molecular mass and electronic factors might influence the activity. The synthesized compounds were evaluated for their antimicrobial effects including: Gram +ve bacteria: *Bacillus Subtilis* and *Staphylacoccus Aureus*; Gram -ve bacteria *Escherichia Coli* and *Klebsiella Pneumonia* and Fungi: *Candida Albicans* and *Aspergillus Funigates* compared with standard drugs, like Nalidixic acid and Nystatin. These compounds were also studied for their potential use in the inhibition of vitiligo.

Keywords: Norfloxacin analogues, Physicochemical parameters, Photochemical probe, Vitiligo, Antimicrobial activity

INTRODUCTION

The Structure Activity Relationship (SAR) for the quinolone skeleton 1-alkyl-1,4-dihydro-4-oxo -quinoline-3-carboxylic acid studies revealed that the 6-halogen atom, especially the 6-fluorine, is responsible for the potency as represented by the binding capacity with DNA gyrase and topoisomerase IV. It is clear that chemical modifications at C-7 are suitable for controlling of the pharmacokinetic properties. *N*-piperazinyl derivatives of fluoroquinolones were introduced and demonstrated for various biological activities that possess broad-spectrum activity. Furthermore, it is clear that the neutral species of fluoroquinolones are more lipophilic than Zwitterionic form. So, factors that can affect *N*-protonation like steric and electronic effect or charge density can also affect lipophilicity.

Procopiou *et al.* prepared a series of asymmetrical 1,4-disubstituted piperazines as a novel class of Non-Brain-Penetrant Histamine H3 Receptor Antagonists. In addition, Foroumadi *et al.* synthesized a modified norfloxacin via heteroarylation of norfloxacin on *N*-piperazinyl position (Scheme 1).



Scheme 1: Norfloxacin modification

The antibacterial activity of these modified norfloxacin depends not only on the bicyclic heteroaromatic pharmacophore but also on the nature of the peripheral substitutions and their spatial relationship such as solubility, thermal stability, hydrolysis and a possibility to form a Zwitter ion. Meth-Cohn reported an important method to for the synthesis of quinolones using reverse Vilsmeier approach. In this study we attempted to synthesize a novel norfloxacin analogues using modified Vilsmeier approach and conduct preliminary investigations for the evaluation of their physicochemical properties, photochemical probe and antimicrobial effects.

MATERIALS AND METHODS

Electrothermal 9100 (fisher Scientific, US) was used to determine melting points or ranges. Infrared (IR) spectra were recorded on a Unicam Research Series 2000 FTIR using KBr disks. 1H NMR (300MHz) and 13C NMR (75 MHz) spectra's were recorded in DMSO or CDCl3 on a Bruker AVANCE 300. Mass spectrometry was performs on an Esquire 3000 plus or Bruker ApexII, for low and high resolution. Elemental analysis was performed on (Perkin Elmer series II 2400). GCMS was performed on Shimadzu GC-17A and QP-5000 Mass Spectrometer. Chemicals and solvents were supplied from Sigma-Aldrich, US and UK.

Nutrient Agar, MacConkey Agar and Sabouraud Dextrose Agar were obtained from GLP.

Preparation of 3-bromo-4-fluoronitrobenzene (3)

A mixture of nitric acid and sulphuric acid (1:1, 25 ml: 25ml) was stirred at 5 °C. A solution of 2-fluorobromobenzene (25 g, 0.143 mol) in methanol (30 ml) was added to mixture with stirring gradually over a period of 20-30 min; After complete addition, the temperature was raised gradually to 70 °C for 1 h. After cooling, the reaction mixture was poured into cold water (20 ml) and the immediate cream solid precipitate collected by filtration.

Crystallization with $CHCl_3$ gave a cream shiny crystals (29.23 g, 93 % yield), mp 60-62 °C (lit. mp 58-59 °C); *v*max/cm-1 1535 and 1342 (NO2); δH (300 MHz; CDCl3) 7.29 (1H, t, *J* = 6.0 Hz, H-5), 8.24 (1H, m, H-6), 8.50 (1H, dd, *J* = 2.0 and 4.3 Hz, H-2); δC (75 MHz; CDCl3) 110.1 (d, *J* = 22.5 Hz, C-3), 117.1 (d, *J* = 22.5 Hz, C-5), 123.3 (d, *J* = 7.5 Hz, C-6), 129.6 (C-2), 144.4 (C-1), 162.9 (d, *JC-F* = 195.7 Hz, C-4); δF (MHz;CDCl3) -74.22 (s); *m/z* 221(M+, 44 %), 219 (M+, 46 %), 203 (3), 189 (17), 173 (38), 161 (14), 94 (M-Br-NO2, 100), 68 (25), 61 (7), 50 (38).

Preparation of 4-(4'-benzylpiperazin-1'-yl)- 3-bromo-1-nitrobenzene (9*)*

Under dry conditions, 3-bromo-4-fluoronitrobenzene **(3)** (5.1 g, 23 mmol) was dissolved in dry acetonitrile (2 ml) then anhydrous K2CO3 (9.6 g, 69.2 mmol) was added followed by addition of *N*-benzylpiperazine (8 g, 46 mmol) to the mixture suspension using a syringe, the temperature gradually raised to reflux for 12 h (or until the complete disappearance of the starting material). The reaction was monitored by TLC (CHCl3:petroleum ether (b.p 40-60), 50 %). The acetonitrile was removed under *vacuo*, and the resulting solid stirred in cold water (200 ml) for 20 min. The pale brown solid formed was recrystallized from CHCl3 to give bright yellow needle-like crystals of **9** (5.8 g, 81 % yield), mp 123-124 °C; [C17H18BrN3O2 Calc. C, 54.3; H, 4.8; N, 11.2; Found C, 54.5; H, 4.8; N, 11.1]; *v*max/cm-1 1580 and 1339 (NO2); δH (300 MHz; CDCl3), 2.57 (4H, m, H-3' and H-5'), 3.17 (4H, m, H-2' and H-6'), 3.56 (2H, s, Ph-CH2), 7.12 (1H, d, *J* = 9.0 Hz, H-5), 7.24 (5H, m, Ph), 8.08 (1H, dd, *J* = 2.7 and 9.0 Hz, H-6), 8.26 (1H, d, *J* = 2.7 Hz, H-2); δc (75 MHz; CDCl3) 51.1 (C-3' and C-5'), 52.8 (C-2' and C-6'), 62.4 (CH2-Ph), 116.9 (C-3), 121.1 (C-5), 124.7 (C-6), 127.5 (C-2), 129.5 (Ph), 142.3 (C-1), 156.6 (C-4); *m/z* (M+373 / 375) .

Preparation of 4-(4'-benzylpiperazin-1'-yl)-3-bromo-4-phenylamine (10)

A pale yellow oil (2.7 g, 60 % yield); vmax cm-1 3150 (NH2); δH (300 MHz; CDCl3) 2.68 (4H, s, CH2-3' and 5') and 3.01 (4H, s, CH2-2' and 6'), 3.57 (2H, s, Ph-CH2), 6.62 (1H, dd, *J* = 1.2 and 4.2 Hz, H-6), 6.94 (1H, d, *J* = 4.2 Hz, H-5), 6.97 (1H, d, *J* = 1.2 Hz, H-2), 7.37 (5H, m, Ph); δc (75 MHz; CDCl3) 52.2 (C-3' and C-5'), 53.6 (C-2' and C-6'), 63.3 (Ph-CH2), 114.9 (C-3), 120.1 (C-6), 121.1 (C-5), 121.8 (C-2), 128.4 (Ph), 142.3 (C-1), 143.4 (C-4); HRMS (ESI): Found: MH+ , 346.0908. Calc. for C17H20BrN3 : MH+ =346.0919.

Preparation of 4-(4'-benzylpiperazin-1'-yl)-3-bromoformamide (11)

Formic acid (5 ml, 0.13 mol) was added to 4-(4'-benzylpiperazin-1'-yl)-3-bromo-4-phenylamine **(12)** (5 g, 14.4 mmol) and the resulting clear solution was refluxed for 2 h, After cooling to room temperature, the reaction mixture poured into ice water (10 ml); then, extracted with CH2Cl2. (3 × 20 ml). The organic layers were combined, and dried over MgSO4. The solvent removed *in vacuo* until complete dryness to give **11** as a brown solid which was purified by column chromatography on silica, eluted with CHCl3 to give a white solid (2.94 g, 54 %), mp 73-74 °C; [C18H20BrN3O Calc. C, 57.76; H, 5.39; N, 11.23; Found C, 57.79; H, 5.41; N, 11.23]; vmax/cm-1 3320 (br, NH), 1716 (NCHO); δH (300 MHz; CDCl3) 2.68 (4H, br s, CH2-3' and 5'), 3.06 (4H, br s, CH2-2' and 6'), 3.62 (2H, s, Ph-CH2), 7.34 (6H, m, Ph+H-5), 7.48 (1H, dd, *J* = 1.2 and 4.2 Hz, H-6), 7.81 (1H, d, *J* = 1.2, H-2), 8.34 (1H, s, CHO), 8.58 (1H, s, NH); δc (75 MHz; CDCl3) 51.7 (C-3' and C-5'), 53.2 (C-2' and C-6'), 63.2 (Ph-CH2), 119.3 (C-3), 120.1 (C-5), 121.0 (C-6), 125.5 (C-2), 129.4 (C-Ph), 132.5 and 132.8 (C-1), 147.7 and 148.5 (C-4), 158.9 and 162.5 (*N*-CHO).

Vilsmeier reaction of 4-(4'-benzylpiperazin-1'-yl)-3-bromoformanilide (9) and formation of compound 12

In dry atmosphere, a solution of 4-(4'-benzylpiperazin-1'-yl)-3-bromoformamide **11** (1 g, 2.7 mmol) in POCl3 (5 ml) was stirred for 15 min at 25 °C. A solution of methyl malonyl chloride (1.12 g, 8.5 mmol) in POCl3 (2 ml) was gradually added to the reaction mixture the oil bath temperature was gradually raised to 130-140 °C and the reaction was continued for 12 h. Column chromatography on the resulting residue (CHCl3:MeOH, 90:10) gave 6-(4'-benzylpiperazin-1'-yl)-7-bromo-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid **(12)** R*f* = 0.12. Re-crystallized from EtOH to produce a yellow solid (0.3 g, 15 % yield); mp 285-286 °C; vmax/cm-1 3525 (carboxylic OH), 1699 (carboxylic C=O), 1611 (COO- st as), 1462 (COO- st sy); δH (600 MHz; DMSO-*d6*) 3.13 (8H, br s, piperazine), 4.19 (2H, s, Ph-CH2), 7.45 (5H, m, Ph), 7.83 (1H, s, H-8), 8.15 (1H, s, H-5), 8.87 (1H, s, H-2), 15.21 (1H, br s, NH); δc 51.21 (piperazine), 60.0 (CH2), 107.4 (C-3), 115.0 (C-8), 124.4 (C-5), 124.6 (C-7), 126.1 (C-10), 128.6 and 130.6 (Ph), 136.1 (C-9), 147.3 (C-2), 166.1 (C-6), 177.3 (CO2H), 206.5 (C=O); HRMS (ESI): Found: MH+, 442.0764. Calc. for C17H20BrN3 : MH+ =442.0761.

Solid phase synthesis with 4-fluoro-3-bromo-1-nitrobenzene

Loading the piperazine to Merrifield resin

General resin preparation. The Merrifield resin **1** (5 g) was suspension in dry DMF (20 ml) for 6-12 h. The resin had a gel like appearance with double its original volume.

To the resin suspension, a molar excess of free piperazine (5 g), pyridine (2 ml) or K2CO3 (3 g), and stirred at 80 °C for 24 h. the cold resin was then filtered and washed with water (2 × 20 ml) and MeOH (2 × 20 ml), then dried *in vacuo* for a minimum of 24 h or until a constant weight was achieved (5.6 g); *v*max /cm-1 3441 (NH); (Found; C, 85.1; H, 10.4; N, 2.9 %).

Preparation of 3-bromo-4-(resin-supported benzylpiperazine)-1-nitrobenzene (4)

3-Bromo-4-fluoro-1-nitrobenzene 3 (2 g) was stirred in dry DMF (10 ml) and anhydrous K2CO3 (3 g) were added to the suspended piperazine-Merrifield resin **2** (3 g) and the reaction was continuo at 50 °C for 24 h. the cold resin was filtered then washed with water (2 × 20 ml) and MeOH (4 ×10 ml). The solid dried under *vacuo* for 24 h or until constant weight (4.6 g); *v*max /cm-1 1509 and 1339 (NO2).

Preparation of 3-bromo-4-(4'-resin-supported benzylpiperazino)-1-aniline (5)

3-Bromo-4-(4'-resin-supported benzylpiperazine)-1-nitrobenzene **4** (2 g) was suspended in dry DMF (10 ml) for 12 h. An excess of stannous chloride (5 g), EtOH (5 ml) was added to the resin. The resulting reaction mixture was stirred at 50 °C for 8 h. At this time, the resin color changed from yellow to pale yellow. The cold resin was filtered and washed with water (4 × 20 ml). The resin was stirred in a solution of NaHCO3 (20 % w/v, 20 ml), filtered, washed several times with water and dried to give a yellow resin (1.8 g); *v*max/cm-1 3360 (NH2).

Preparation of 3-bromo-4-(4'-resin-supported benzylpiperazino)-1-formamide (6)

The resin supported amine **5** (1 g) was suspended in dry DMF (10 ml) for 12 h before the addition of formic acid (5 ml). The reaction suspension was stirred and heated at 50 °C for 2 h. the cooled reaction mixture was filtered and washed with water (4 × 10 ml), NaHCO3 solution (30 %w/v, 20 ml) and MeOH (2 × 10 ml) to give derivatized resin **6** (1.2 g); *v*max /cm-1 3362 cm-1 (NH), 1721 cm-1 (C=O).

Preparation of resin supported 7-bromo-6-piperazino-4-oxo-3-quinolone carboxylic acid (7)

3-Bromo-4-(4'-resin-supported benzylpiperazino)-1-formamide **(6)** (1 g) was suspended in dry DMF (10 ml) for 12 h. Phosphorus oxychloride (POCl3, 5 ml) was added to the suspended resin and the mixture was stirred for 30 min at 25 °C. A solution of methyl malonyl chloride (1.32 g, 9.6 mmol) in POCl3 (2 ml) was gradually added to the reaction mixture with. When the addition was complete, the temperature was gradually raised to 100 °C for 24 h. After cooling, the reaction mixture was added gradually and carefully to ice (20 ml) then stirred for a further 20 min. The solution was basified using NaOH (10 % w/v, 5 ml) and refluxed for a further 30 min. The resin was filtered and washed with water (2 × 10 ml), MeOH (2 × 10 ml) and dried *in vacuo* to constant weight (1.2 g); νmax/cm-1 1719 cm-1 (C=O).

Preparation of *N*-(2-fluoro-5-nitrophenyl)piperazine (13)

Note The *N,N*-bis-(2-chloroethyl)ammonium chloride is very toxic and must handle with care only in fuming hood.

A mixture of 2-fluoro-5-nitroaniline (1 g, 6.4 mmol) and *N,N*-bis-(2-chloroethyl)ammonium chloride (1.3 g, 7.0 mmol) in diethylene glycol monomethyl ether (1 ml) was heated under dry nitrogen at 150 ºC for 24 h. The reaction was monitored by TLC (ethyl acetate:$CHCl_3$, 80:20), product R*f* = 0.42. the dark solid of *N*-(2-fluoro-5-nitrophenyl)piperazine 13 (0.87 g, 60 %); mp 216-217 °C; νmax/cm-1 3386 (NH), 1522 and 1346 (NO_2); δH (300 MHz; DMSO-*d6*) 3.26 (4H, m, CH_2-3' and CH_2-5'), 3.40 (4H, m, CH_2-2' and CH_2-6'), 7.49 (1H, dd, *J* = 9.0 Hz and 12 Hz, H-3), 7.85 (1H, dd, *J* = 3 and 9 Hz, H-6), 7.94 (1H, m, H-4), 9.59 (1H, br s, NH); δC (75 Hz; DMSO-*d6*) 43.0 (C-3' and C-5', 47.0 (C-2' and C-6'), 115.2 (d, *J* = 5.3 Hz, C-6), 117.8 (d, *J* = 24 Hz, C-3), 119.2 (d, *J* = 10.5 Hz, C-4), 139.9 (d, *J* = 9.8 Hz, C-1), 144.9 (C-5), 158.8 (d, *J* = 257 Hz, C-2).

Preparation of 1-(benzoylpiperazinyl)-2-fluoro-5-nitrobenzene (9)

Yellow shiny crystal of **9** (4 g, 92 %); mp 108-109 °C; (Calc. for $C_{18}H_{18}FN_3O_2$: C, 66.0; H, 5.5; N, 12.8. Found: C, 66.1; H, 5.5; N, 12.7); νmax/cm-1 1694 (CO-N), 1508 (NO_2), 1347 (NO_2); δH (300 MHz,; DMSO-*d6*) 2.94 (4H, s, $CH_2$2', CH_2-6'), 3.49 (2H, s, CH_2-3'), 3.71 (2H, s, CH_2-5'), 7.46 (6H, m, Ph + H-3), 7.81 (1H, dd, *J* = 3 and 7.5 Hz, H-6), 7.92 (1H, m, H-4); δC (75 MHz,; DMSO-*d6*) 41.3 (C-5'), 46.9 (C-3'), 49.6 (C-2' and C-6'), 115.2 (d, *J* = 5.2 Hz, C-6), 117.7 (d, *J* = 23.0 Hz, C-3), 118.8 (d, *J* = 9.5 Hz, C-4), 128.9 (Ph), 135.9 (C-8), 140.7 (d, *J* = 10 Hz, C-1), 144.9 (C-5), 158.9 (d, *J* = 254 Hz, C-2) 169.6 (C=O).

Preparation of 1-(benzoylpiperazinyl)-2-fluoroaniline (10)

A white powdered solid (**10**) (2.14 g, 78 %); mp 89-90 °C; (Calc. for $C_{17}H_{18}FN_3O$: C, 68.2; H, 6.1; N, 14.0. Found: C, 68.2; H, 6.05; N, 14.0); νmax/cm-1 3450 and 3350 (NH_2), 1724 (CO-N); δH (300 MHz,; DMSO-*d6*) 2.94 (4H, s, CH_2-2' and CH_2-6'), 3.48 (2H, s, CH_2-3'), 3.73 (2H, s, CH_2-5'), 4.84 (2H, br s, NH_2), 6.13 (1H, m, H-4), 6.25 (1H, dd, *J* = 2.4 and 7.5 Hz, H-6), 6.77 (1H, dd, *J* = 8.7 and 12.6 Hz, H-3), 7.45 (5H, m, Ph); δC (75 MHz,; DMSO-*d6*) 50.17 (2CH_2), 50.22 (2CH_2), 105.5 (C-6), 107.6 (d, *J* = 7.1 Hz, C-4), 116.3 (d, *J* = 21.2 Hz, C-3), 128.9 (Ph), 140.0 (d, *J* = 9.5 Hz, C-1), 145.9 (C-5), 147.7 (d, *J* = 230 Hz, C-2), 169.5 (C=O).

Preparation of 1-(benzoylpiperazinyl)-2-fluoroformanilide (11)

White crystals of **11** (0.62 g, 56 %); mp 189-191 °C; (Calc. for $C_{18}H_{18}FN_3O_2$: C, 66.0; H, 5.5; N, 12.8. Found: C, 66.0; H, 5.5; N, 12.8); νmax/cm-1 3080 (NH) 1684 (NH-CHO), 1620 (CO-Ph); δH (300 MHz,; DMSO-*d6*) 3.02 (4H, s, H-2', H-6'), 3.55 (2H, s, H-3'), 3.74 (2H, s, H-5'), 7.11 (1H, dd, *J* = 8.7 and *J* = 12.0 Hz, H-3), 7.19 (1H, ddd, *J* = 8.7, 2.7 and 1.5 Hz, H-4), 7.35 (1H, dd, *J* = 2.7 and *J* = 5.4 Hz, H-6), 7.46 (5H, m, CO-Ph), 8.25 (1H, d, *J* = 1.8 Hz, CHO), 10.12 (1H, br s, NH); δC (75 MHz,; DMSO-*d6*) 50.2 and 50.22 (piperazine-C), 111.1 (C-6), 113.7 (d, *J* = 7.5 Hz, C-4), 116.5 (d, *J* = 21.0 Hz, C-3), 128.9 (Ph), 136.3 (C-5), 140.0 (d, *J* = 9.0 Hz, C-1), 153.1 (d, *J* = 240 Hz, C-2), 159.9 (CHO), 169.6 (C=O).

Preparation of *N,N'*-bis-(4-fluoro-3-nitrophenyl)oxalamide 13

Under anhydrous conditions, 3-chloro-4-fluoroformanilide (2 g, 10.86 mmol) was dissolved in dry $CHCl_3$ (20 ml), then $(COCl)_2$ (2 ml) was added gradually over 30 min., (a vigorous reaction was observed). The resulting reaction mixture was heated to 40 °C for 30 min. The reaction flask was removed from the oil bath, methyl malonyl chloride (1.78 g, 13.03 mmol) in $CHCl_3$ (2 ml) was added gradually to the Vilsmeier reagent over 30 min. the reaction was continued at 40 °C for 3 h. The reaction mixture was concentrated *in vacuo*, The resulting yellow solid was re-crystallized from $CHCl_3$ to give yellow crystals of *N,N'*-bis-(4-fluoro-3-nitrophenyl)oxalamide **(13)** (0.62 g, 16 %); mp 109-111; °C; (Calc. for $C_{14}H_8F_2N_4O_6$: C, 45.9; H, 2.2; N, 15.3. Found: C, 45.9; H, 2.2; N, 15.3); νmax/cm-1 3275 (NH), 1673 (NCO); 1H NMR δH (300 MHz; DMSO-*d6*) 7.64 (2H, t, *J* = 9.0 Hz, H-5 and H-5'), 8.23 (2H, m, H-6 and H-6'), 8.80 (2H, dd, *J* = 0.8 and 1.5 Hz, H-2 and H-2'), 11.40 (2H, s, 2NH); δc(75 MHz; $CDCl_3$) 120.1 (C-2 and C-2'), 121.7 (d, *J* = 22.5 Hz, C-5 and C-5'), 130.9 (d, *J* = 7.5 Hz, C-6 and C-6'), 137.2 (C-1 and C-1'), 139.1 (d, *J* = 7.5 Hz, C-3 and C-3'), 154.1 (d, *J* = 262.5 Hz, C-4 and C-4'), 161.2 (C=O).

Preparation of *N,N'*-bis-(3-chloro-4-fluorophenyl)malonamide (14)

The resulting yellow solid was re-crystallized from $CHCl_3$ to give shiny needle-like crystals of compound **14** *N,N'*-bis-(3-chloro-4-fluorophenyl)malonamide (0.72 g, 18 %); mp 201-202 °C; (Calc. for $C_{15}H_{10}Cl_2F_2N_2O_2$: C, 50.2; H, 2.8; N, 7.8. Found: C, 50.2; H, 2.8; N, 7.8); νmax/cm-1 3281 (br, NH), 1677 (C=O), 1497 (NH), 811 (Cl-C=O); δH (300 MHz; DMSO-*d6*) 7.38 (2H, t, *J* = 9.0 Hz, H-5 and H-5'), 7.48 (2H, ddd, *J* = 2.4, 4.5 and 9.0 Hz, H-6 and H-6'), 7.93 (2H, dd, *J* = 2.4 and 2.7 Hz, H-2 and H-2'), 10.39 (2H, s, 2NH); δC (75 MHz,; DMSO-*d6*) 46.3 (CH_2), 117.5 (d, *J* = 21.7 Hz, C-5 and C-5'), 119.7 (d, *J* = 18 Hz, C-3 and C-3'), 119.9 (d, *J* = 7.5 Hz, C-6 and C-6'), 121.0 (C-2 and C-2'), 136.6 (C-1 and C-1'), 153.7 (d, *J* = 240 Hz, C-4 and C-4'), 165.9(C=O).

Thermal stability of highly bio-active compounds

The thermal behaviors for the bioactive compound **12** was investigated by thermogravimetric technique and indicated by the DTG peaks at 177 and 270 °C.

Antimicrobial assay

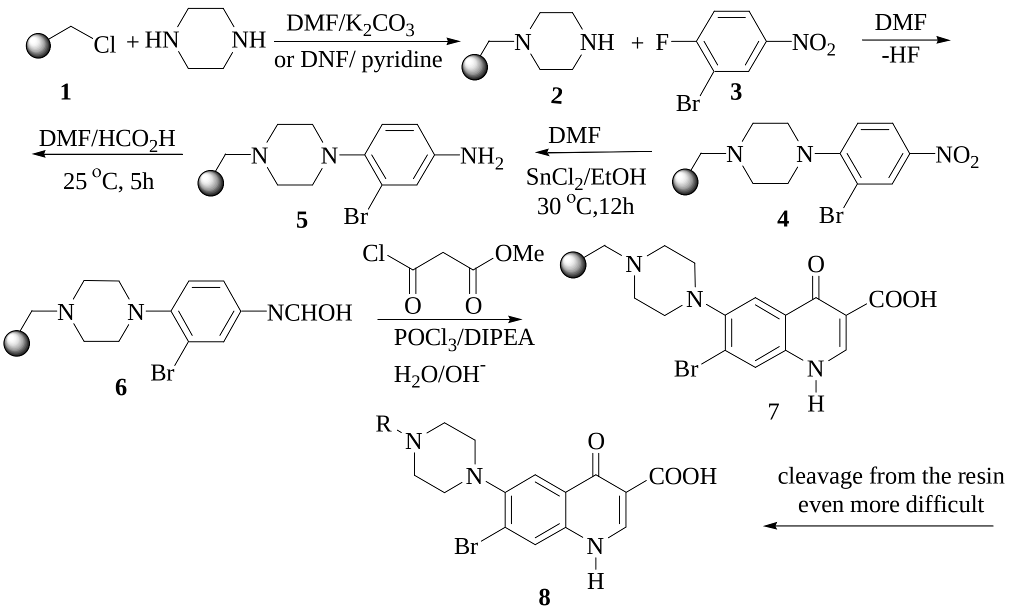
Some synthesized compounds **9, 10, 11, 13** and **12** were evaluated for their antimicrobial effects by Agar diffusion disk method using Nutrient Agar, MacConkey Agar and Sabouraud Dextrose Agar. The potentialities of these compounds were estimated against some important and representative microbes like: Gram +ve: *Bacillus Subtilis* (*B.S.); Staphylacoccus Aureus* (*S.A.*), Gram -ve: *Escherichia Coli* (*E.C.*); *Klebsiella Pneumonia* (*K.P.*) and Fungi *Candida Albicans* (*C.A.*) and *Aspergillus Funigates* (*A.F.*). The pre-sterilized filter paper disks (6 mm diameter) were impregnated with 30, 40 and 50µg of the compound, dissolved in DMF as solvent, which has no effect on either bacteria or fungi. These disks were implanted on different sets of agar plates containing the microbes. The agar plates were then incubated for 24 hours at 37 °C for bacteria and for 7 days at 28 °C for fungi. Nalidixic acid and Nystain were used as reference antibiotics.

In addition, similar anti microbial assay was performed for the biologically highly active compounds **9, 10, 11, 13** and **12** after exposure of the Petri dishes containing microorganisms and the test compounds to UV light (λ366 nm) for 3 hours before the incubation.

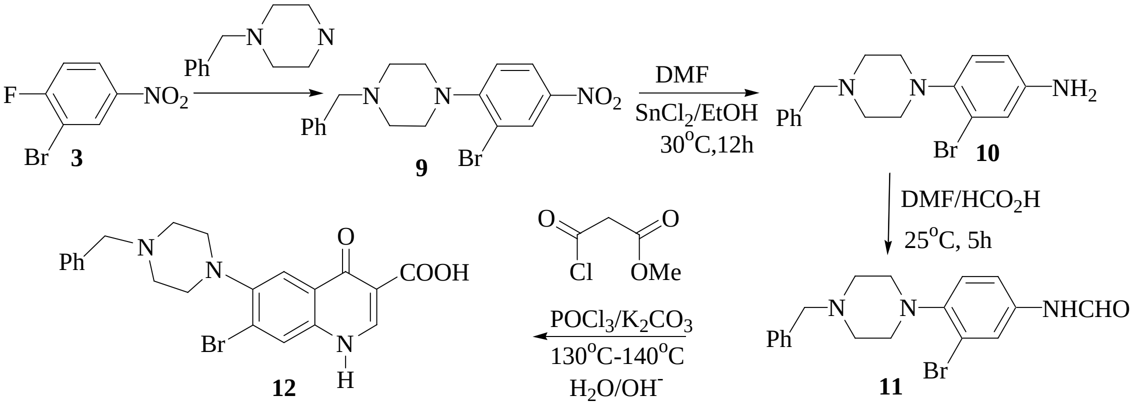
RESULTS AND DISCUSSION

Solid phase Synthesis of Norfloxacin Analogues

In the synthetic sequence, the Merrifield resin **(1)** was first suspended in dry DMF and to this suspension was added an excess of piperazine (2-3 equivalents) in pyridine or anhydrous K_2CO_3 (6-8 equivalents). The reaction mixture was continued at 40 °C for 24 hours then piperazine resin (**2**) was obtained, filtered, washed with CH_2Cl_2 and dried. Compound **2** was re-suspended in DMF and reacted with 3-bromo-4-fluoro nitrobenzene **(3)** to give the 4-piperazine resin supported-3-bromo-1-nitro benzene (**4**) (not the expected 3-piperazine resin supported-4-fluoro-1-nitrobenzene) which on reduction with $SnCl_2$-EtOH yielded the 3-bromo-4-(4'-resin supported benzyl piperazinyl)-1-aniline **(5)** and then by treatment with an excess of formic acid at room temperature for 12 hours produced the corresponded 3-bromo-4-(4'-resin supported benzyl piperazinyl)-1-formanild **(6)**. The dry resin supported formanilide **6** when reacted with Phosphorus oxychloride or Oxalyl chloride and methyl malonyl chloride **(7)** under reverse Vilsmeier conditions mainly gave the resin-supported quinolone, 6-fluoro-7-piperazino-4-oxo-3-quinolone carboxylic acid, **(8)** (Scheme 2). The procedure, in general, yielded a mixture of by products in very low quantities and TLC and GCMS were used for assessment of the recovered cleavage products.



Scheme 2



Scheme 3

Solution phase Synthesis of novel norfloxacin analogues

In the present study novel norfloxacin analogues were synthesized using basically the Vilsmeier method with some modifications. The 7-bromo-6-*N*-benzyl piperazinyl-4-oxoquinoline-3-carboxylic acid **(12)** was isolated at high temperature (130-140 °C), scheme 3. On the other hand, bis-compounds *N,N'*-bis-(4-fluoro-3-nitrophenyl)oxalamide and *N,N'*-bis-(3-chloro-4-fluorophenyl)malonamide **(13)** and **(14)** were obtained under reveres Vilsmeier approach. The formation of these two novel *N,N'*-bis-(aryl) compounds **13, 14** instead of norfloxacin analogue targets could be due to a type of interaction between oxalyl chloride with methyl malonyl chloride followed by monoacylation of anilidimide which hinders the formation of norfloxacin analogues via a second interaction with other anilidimide molecule (Scheme 4). Recently, non-fluorinated *N,N'*-bis-aryl derivative was reported as an HIV-1 integrase inhibition.

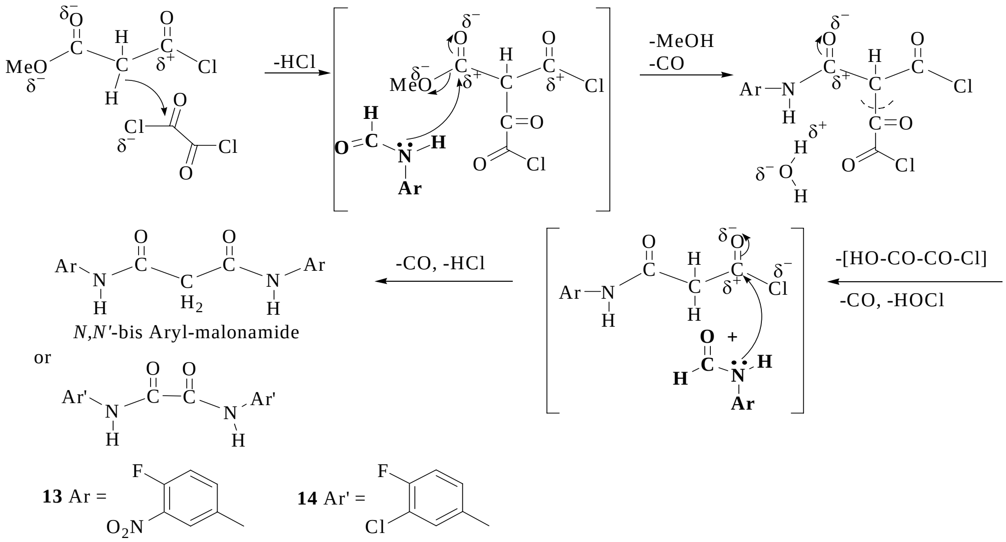
Physiochemical properties:

Lipophilicity

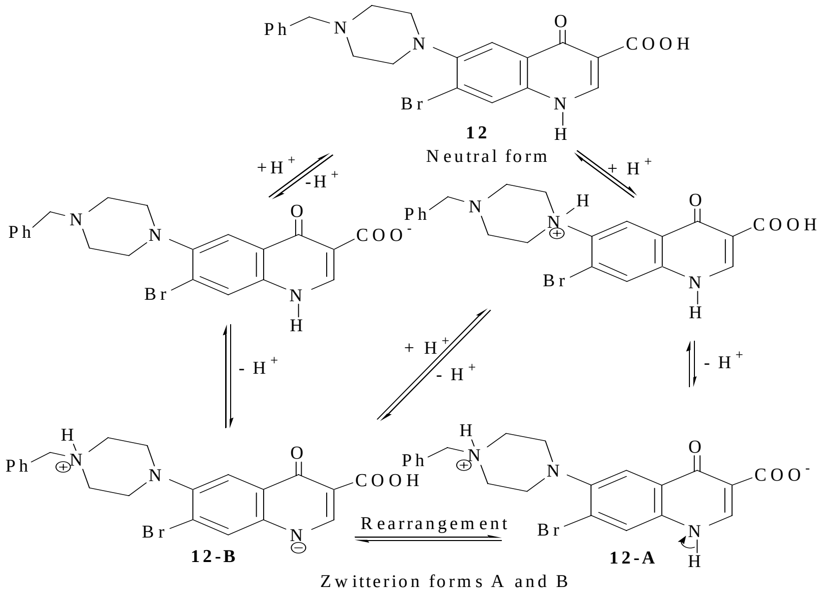
The lipophilic and Zwitterionic form of the obtained compounds, as well as steric and electronic effects or charge density, play an important role for chemical and biocidal activities. *N*-Mannich base functional group can increase the lipophilicity of the tested compounds for example **12** at physicobiological pH values by decreasing their protonation resulting in enhancement of absorption through bio-membranes. It is clear that the neutral species of halo-quinolones are more lipophilic than Zwitter ionic form. In addition, steric and electronic effects or molecular charge density, can affect lipophilicity (Scheme 5).

Fourier transforms infrared spectroscopy

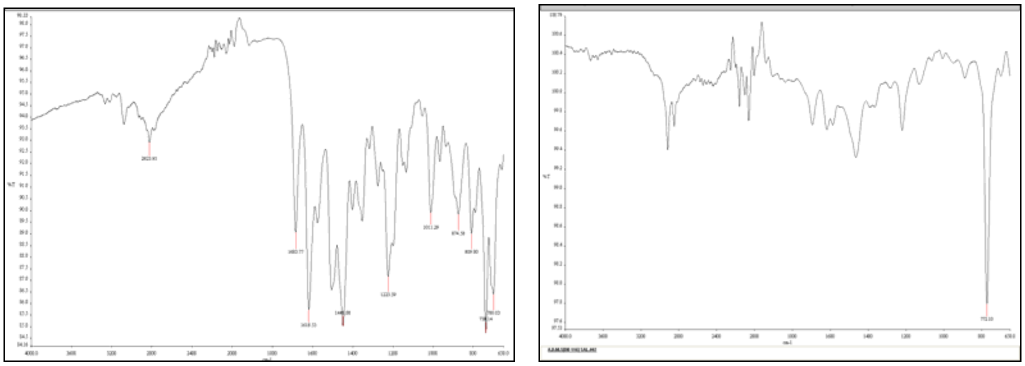
Generally, Fourier Transforms Infrared Spectroscopy (FT-IR) studies of the obtained compounds in both the solid and solution (CHCl3) states showed lack of some characteristic bands in the solution state, for example compound **12**. This effect may be due to a type of intramolecular and/ or intermolecular H-bonding between functional group of the tested compounds and a functional group in the solvent used, which possibly act similar to the functional groups of the organisms leading to inhibition their vital activities and death. The results of the Fourier Transform Infrared Spectroscopy are given in Fig. 1 and Fig 2.



Scheme 4: A possible formation of *N,N'*-bis-Aryl malonamide instead of norfloxacin analogues



Scheme 4



a) Solid state **b) Solution stat**

Fig. 1: FT-IR spectrum of 12 in a) solid and b) solution states for compound 12

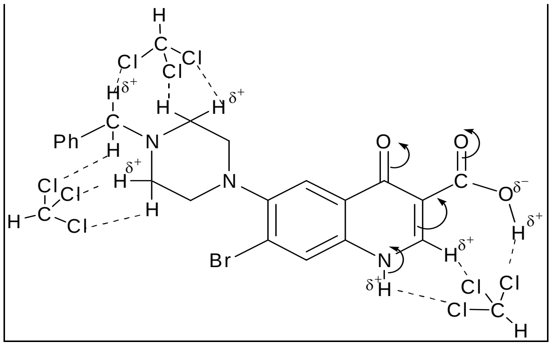


Fig 2: The interaction between compound 12 with CHCl3

Other physico-chemical properties of highly bioactive compounds

The physico-chemical properties of highly bioactive pure tested compounds are demonstrated such as:

(a) Melting points: Differ according to the type of solvent from which crystals are obtained for example compound **10** had approximately 87 °C for pure crystallized from cyclohexane, and 90 °C from chloroform.

(b) Solubility in water: Pure compound **10**, for example gave approximately 200 µg/L while compound **13** showed 350 µg/L at 20 °C.

c) pka: Pure tested compounds at pH 5.7 and 9 at 24 °C showed different types of protons, in quinolone the -COOH and NH, while in the formylamino derivative, -COOH, -CHO and NH. This data indicated that tested compounds **9, 10, 11, 13** and **12** have a very low rate of hydrolysis because of it is stability in suspension concentration under normal conditions Table 4.

Table 4: Various physico-chemical properties of highly bioactive compounds

Compd. No.	**MIC at 30 µg/disc**				**Melting Point**		**Solubility[c] in water (20 °C), µg/L**
	B.S	***S.A***	***E.C***	***K.P***	**CHCl3[a]**	**Cy-hexane[b]**	
9	6	10	10	8	90	87	200
10	6	10	10	9	191	186	300
11	6	10	6	10	74	70	90
13	6	10	6	9	111	107	350
12	15	10	12	12	285	-	400
Ny	6	6	6	6	-	-	-
Na	32	30	30	32	-	-	-

a) Crystals cyclisation from CHCl3; b) Crystals cyclisation from cyclohexane.

c) Hydrolysis characteristics at pH 5.7 and 9 and at 24 °C, the tested compounds have a very low rate of hydrolysis, which is due to consider stable in suspension concentrations under normal condition

Anti microbial assay

Some synthesized compounds, like **9. 10, 11 13** and **12** were evaluated by agar diffusion disk method against some pathogenic bacteria and fungi like: Gram +ve: *Bacillus Subtilis* (B.S.); *Staphylacoccus Aureus* (S.A.), Gram -ve: *Escherichia Coli* (E.C.); *Klebsiella Pneumonia (*K.P.) and Fungi *Candida Albicans* (C.A.) and *Aspergillus Funigates* (A.F.). The potentialities of the tested compounds are given in Table 1, 2.

Photochemical probe agents

The melanocytes successfully treated vitiligo patients by PUVA therapy. Increasing use of PUVA-8MP could be responsible for a type of skin-cancer[21]. Thus, some antibiotics like Nalidixic acid and Nystatin are now used to control the vitiligo symptoms. Preliminary Screening of compounds **9, 10, 11, 13** and **12** using UV (λ366 nm) light, showed no significant effect when exposed to UV light Table 3.

Table 1: The Preliminary Screening of Antimicrobial activity of the new synthesized compounds

Compounds/DMF 50µg/ml disk	**Microorganisms / Inhibition Zone (mm)**					
	Gram +ve Bacteria[a]		**Gram –ve Bacteria[b]**		**Fungi[c]**	
	B.S.	***S.A.***	***E.C.***	***K.P.***	***C.A.***	***A.F.***
9	9	8	8	10	10	6
10	18	16	15	14	10	6
11	13	11	12	13	9	6
13	15	14	15	13	10	6
12	20	19	18	17	10	10
Ny.	6	6	6	10	10	32
Na.	32	30	30	22	6	6

Ny = Nystatin, manufactured by Pasteur Lab., Egypt. NS 100 units (100 µg/disk)

Na = Nalidixic acid, 30 µg/disk, Bioanalize, Egypt. a) *Bacillus Subtilis (B.S)* and *Stphylacoccus Aureus (S.A);* b) *Escherichia Coli (E.C)* and *Klebsiella Pneumonia (K.P);* c) *Candida Albicans (C.A)* and *Aspergillus Funigates (A.F).*

Table 2: MIC of the active biological compounds towards Bacteria

Compd. No.	**Inhibition Zones (µg /mm)**											
	B.S.			***S.A.***			***E.C.***			***K.P.***		
	50	**40**	**30**	**50**	**40**	**30**	**50**	**40**	**30**	**50**	**40**	**30**
9	18	12	6	16	14	10	15	12	10	14	12	8
10	16	14	6	16	12	10	14	13	10	14	11	9
11	13	10	6	11	10	10	12	10	6	13	11	10
13	15	12	6	14	12	10	15	12	6	13	11	9
12	20	18	15	19	16	10	18	16	12	17	14	12

Table 3: Preliminary Screening using UV (λ366 nm) light, conc. 50 µg/disk

Compd. No.	**Microorganisms (Inhibition Zones in mm)**					
	+ve Bacteria		**-ve Bacteria**		**Fungi**	
	B.S.	***S.A.***	***E.C***	***K.P.***	***C.A.***	***A.F.***
9	18	16	15	14	No change	No change
10	18	17	16	18	No change	No change
11	14	14	12	14	No change	No change
13	17	16	17	16	No change	No change
12	24	21	21	21	No change	No change

Highly active: (IZ) ≥ 12 mm; Moderately active: (IZ) ≥ 9-12 mm; Slightly active (IZ) ≥ 6-9 mm

CONCLUSION

Novel norfloxacin analogues were synthesis using modified Vilsmeier approach on both solution and solid phase. Preliminary investigations for the evaluation of their physicochemical properties, photochemical probe and antimicrobial effects showed a significant effect on selected bacteria in comparisons with Nalidixic acid and Nystatin drugs.

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