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-nitrophenoxy)malonamide 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: Staphylococcus, Staphylococcus Aureus; Gram -ve bacteria Escherichia Coli and Klebsiella Pneumonia and Fungi: Candida Albicans and Aspergillus Fungigates 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-oxoquinoline-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).

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 were recorded in DMSO or CDCB on a Bruker AVANCE 300. Mass spectrometry was performed on an Esquire 3000 plus or Bruker Apex II, 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.
Crytalization with CHCl₃ gave a cream shiny crystals (29.2 g, 93 % yield), mp 60-62 °C (lit. mp 58-59 °C); vmax/cm-1 1353 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, d, J = 2.0 and 4.3 Hz, H-2); δC (75 MHz; CDCl₃) 110.1 (d, J = 22.5 Hz, C-3), 117.7 (d, J = 22.5 Hz, C-5), 123.3 (d, δ = 7.5 Hz, C-6) 129.6 (C-2), 144.4 (C-1), 162.9 (d, J= FC = 195.7 Hz, C-4); δF (MeF-CDCl₃) -74.22 (s); m/z 221(M++4, 44%), 219 (M+2, 46%), 203 (M), 189 (17), 173 (38), 161 (14), 94 (M-Br-N=O), 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 acetone (2 ml) then anhydrous K2CO₃ (9.6 g, 69.2 mmol) was added followed by addition of N-benzylpiperazine (8, 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 (bp 40-60), 50 %).

The acetone 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; δ1H (300 MHz; CDCl₃) 7.74-7.67 (m, 6H), 7.29 (1H, t, J = 7.2 Hz, H-2), δ2C (75 MHz; CDCl₃) 132.5 and 132.8 (C-1), 147.7 and 148.5 (C-4), 158.9 and 159.9 (C-3 and C-5) ppm. Recrystallized from EtOH to constant weight was achieved (5.1 g); vmax/cm-1 1352 (carboxylic OH), 1699 (carboxylic C=O), 1611 (NO₂-st as), 1462 (COO-st sy); δH (600 MHz; DMSO-d₆) 3.13 (3H, br s, piperazine), 4.19 (2H, s, Ph-CH₂), 7.45 (5H, s, MeH), 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), 60.00 (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) ppm. HRMS (EI): Found: M+, +442.0764; Calcd: for C17H20BrN₃; M+ = +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 suspended 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 x 20 ml) and MeOH (2 x 20 ml), then dried in vacuo for a minimum of 24 h or until a constant weight was achieved (5.6 g); vmax/cm-1 3441 (NH); (Found 4, 85.1; H, 10.4; N, 2.9 %).

Preparation of 3-bromo-4-(resin-supported benzylpiperazin-1-yl)-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 continued at 50 °C for 24 h, the cold resin was then filtered and washed with water (2 x 20 ml) and MeOH (4 x 10 ml). The solid dried under vacuo for 24 h or until constant weight (4.6 g); vmax/cm-1 1509 and 1339 (NO₂).

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

3-Bromo-4-(4'-resin-supported benzylpiperazin-1-yl)-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 reacting 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 x 20 ml) and MeOH (4 x 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); vmax/cm-1 3360 (NH2).

Preparation of 3-bromo-4-(4'-resin-supported benzylpiperazin-1-yl)-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 coiled reaction mixture was filtered and washed with water (4 x 10 ml). NaHCO₃ solution (30 %w/v, 20 ml) and MeOH (2 x 10 ml) to give derivatized resin 6 (1.2 g); vmax/cm-1 3362 cm-1 (NH), 1721 cm-1 (C=O).

Preparation of resin supported 7-bromo-6-piperazin-4-oxide-3-quinoline carboxylic acid (7)

3-Bromo-4-(4'-resin-supported benzylpiperazin-1-yl)-1-formamide (6) (1 g) was suspended in dry DMF (10 ml) for 12 h. Phosphorus oxychloride (POCl₃, 5 ml) was added to the resin suspension 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 2.4 h. After 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 x 10 ml), MeOH (2 x 10 ml)
and dried in vacuo to constant weight (12 g). v max/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 diethyl glycol monomethylether (1 ml) was heated under dry nitrogen at 150 °C for 24 h. The reaction was monitored by TLC (ethyl acetate:CHCl3, 80:20), product Rf = 0.42, the dark solid of N-(2-fluoro-5-nitrophenyl)piperazine 13 (0.87 g, 60 %); mp 216-217 °C; v max/cm-1 3386 (NH), 1719 (C=O); δ (75 MHz, δ C (75 MHz,; DMSO-d6)) 41.3 (C-5'), 46.9 (C-2'), 111.2 (d, J = 5.3 Hz, C-6), 117.8 (d, J = 24 Hz, C-3), 119.2 (d, J = 10 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-(benzoylaminomethyl)-2-fluoro-5-nitrobenzene (9)

Yellow shiny crystal of 9 (4 g, 92 %); mp 108-109 °C; v max/cm-1 3080 (NH) 1684 (NH-CHO), 1620 (C=O). In the synthetic sequence, the Merrifield resin (Scheme 2). The procedure, in general, yielded a mixture of byproducts to UV light (λmax 366 nm) for 3 hours before the incubation.

Preparation of 1-phenyl-2-fluoro-2-hydroxyaniline (10)

A white powder solid (10) (2.14 g, 78 %); mp 189-191 °C; v max/cm-1 1719 (C=O); δ (75 MHz, δ C (75 MHz,; CDCl3)) 120.1 (C-2 and C-2'), 121.7 (d, J = 22.5 Hz, C-5 and C-5'), 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-fluorobenzoyl)piperazine (14)

The resulting yellow solid was re-crystallized from CHCl3 to give yellow shiny needle-like crystals of compound 14. v max/cm-1 3386 (NH), 1719 (C=O); δ (75 MHz, δ C (75 MHz,; CDCl3)) 41.3 (C-5'), 46.9 (C-2'), 111.2 (d, J = 5.3 Hz, C-6), 117.8 (d, J = 24 Hz, C-3), 119.2 (d, J = 10 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 N,N-bis-(3-chloro-4-fluorophenyl)piperazine (11)

A white powder solid (11) (2.14 g, 78 %); mp 189-191 °C; v max/cm-1 3080 (NH) 1684 (NH-CHO), 1620 (C=O). In the synthetic sequence, the Merrifield resin (Scheme 2). The procedure, in general, yielded a mixture of byproducts to UV light (λmax 366 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 solid dichloromethane (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 malonate chloride (1.78 g, 12.03 mmol) in CH2Cl2 (3 ml) was added gradually to the Vigreux 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 CHCl3 to give yellow crystals of N,N-bis-(3-chloro-4-fluorophenyl)piperazine (11) (0.62 g, 16 %); mp 109-111 °C; v max/cm-1 3386 (NH), 1719 (C=O). In the synthetic sequence, the Merrifield resin (Scheme 2). The procedure, in general, yielded a mixture of byproducts in very low quantities and TLC and GCMS were used for assessment of the recovered cleavage products.

and dried in vacuo to constant weight (12 g). v max/cm-1 1719 cm-1 (C=O).
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 (13) and N,N'-bis-(3-chloro-4-fluorophenyl)malonamide (14) were obtained under reverse 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 monoaclylation 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 inhibitor.

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 physiobiological pH values by decreasing their protonation resulting in enhancement of absorption through bio-membranes. It is clear that the neutral species of halo-quinolone s 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 diagram]

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

a) Solid state

b) Solution state
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.

Photochemical probe agents

The melanocytes successfully treated vitiligo patients by PUVA therapy. Increasing use of PUVA-BMP could be responsible for a type of skin-cancer21. 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 4: Various physico-chemical properties of highly bioactive compounds

<table>
<thead>
<tr>
<th>Compd. No.</th>
<th>MIC at 30 μg/disc</th>
<th>Melting Point</th>
<th>Solubility in water (20 °C), μg/L</th>
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<tr>
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<td>B.S.</td>
<td>S.A.</td>
<td>E.C.</td>
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<td>Na</td>
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Table 1: The Preliminary Screening of Antimicrobial activity of the new synthesized compounds

<table>
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<tr>
<th>Compounds/DMF</th>
<th>Microorganisms / Inhibition Zone (mm)</th>
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<td>50μg/ml disk</td>
<td>Gram +ve Bacteria</td>
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<td>13</td>
<td>6</td>
</tr>
<tr>
<td>Na</td>
<td>32</td>
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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 Staphylococcus Aureus (S.A); b) Escherichia Coli (E.C) and Klebsiella Pneumonia (K.P); c) Candida Albicans (C.A) and Aspergillus Fungigates (A.F).
Table 2: MIC of the active biological compounds towards Bacteria

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<tr>
<th>Compd. No.</th>
<th>Inhibition Zones (µg/mm)</th>
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Highly active: (IZ) ≥ 12 mm; Moderately active: (IZ) 9–12 mm; Slightly active (IZ) ≥ 6–9 mm

CONCLUSION

Novel norfloxicin analogues were synthesized 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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REFERENCE