SYNTHESIS OF 2-METHYL-4-QUINOLONE-3-ACETIC ACIDS WITH POTENTIAL ANTIBACTERIAL ACTIVITY

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ABSTRACT

A number of quinolone-3-acetic acids were synthesized by cyclocondensation of substituted anilines with diethyl acetylsuccinate in the presence of phosphorous pentoxide and followed by base hydrolysis of the resultant esters to form respective acids. All synthesized compounds were found to exhibit antibacterial activities against a range of gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*) and gram-negative bacteria (*Shigella sonnei*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*) by broth dilution method. All the compounds exhibited antibacterial activities comparable to fluoroquinolones and in some cases even better activity was found. These findings suggest a great potential of these compounds for screening and use as antibacterial compounds for further studies with a battery of bacteria.

KEY WORDS: 2-methyl-4-quinolone-3-acetic acid, 4-oxo-2-methylquinoline-3-acetic acids, antibacterial activity, gram positive, gram negative bacterial strains.

INTRODUCTION

Quinolones comprises a large and well known class of synthetic antimicrobials that are proved to be effective in the treatment of many types of infectious diseases specially those caused by bacteria¹. A number of quinolones antimicrobial agents have been available for the treatment of urinary tract infections in humans for many years. The therapeutic utility of such drugs is in danger due to rapid development of resistance by the microorganisms^{2,3,4}.

Over the last two decades, research on 4-quinolone-3-carboxylic acids and their derivatives has led to the discovery of a family fluoroquinolones (e.g., 6-fluoro-7-piperazinyl-4-quinolone-3-carboxylic acid) which are active against gram-negative and gram-positive bacteria⁵. Although number of fluoroquinolones have been synthesized⁶ and reported, the most notable ones in medicine include ciprofloxacin (1), levofloxacin (2), moxifloxacin (3), ofloxacin (4) and sparfloxacin (5). Their bactericidal action is due to inhibition of DNA gyrases (type II and IV topoisomerase) which are involved in DNA replication. The mechanism of action of quinolones is mainly by the inhibition of bacterial gyrase, an enzyme involved in DNA replication, recombination and repair⁷. By interfering with gyrase, quinolones arrest bacterial cell growth. It is still under discussion whether the effectiveness of fluoroquinolones for the treatment of respiratory disorders is similar to that of other antibiotic classes or not⁸.

Some of the serious adverse effects that occur more commonly with fluoroquinolones than other antibiotic drug classes include CNS and tendon toxicity⁹. The serious events may occur during therapeutic use at therapeutic dose levels or with acute overdose. At therapeutic doses, may include: CNS toxicity, cardiovascular toxicity, tendon / articular toxicity, and, rarely, hepatic toxicity¹⁰.

Bacterial resistance against quinolones and fluoroquinolones exhibits plasmids mediated mechanism¹¹. Resistance to quinolones drug can be evolved rapidly by gene mutation, even during a treatment. Numerous microbes, including *Staphylococcus aureus*, *enterococci* and *Streptococcus pyogenes* have already exhibited resistance against antibiotics including ciprofloxacin¹².

In figure-1 a group of quinolones have been presented. Ciprofloxacin (1) is most active against *Pseudomonas aeruginosa*¹³ in comparison to other fluoroquinolones. Third-generation quinolones retain expanded gram-negative and atypical intracellular activity but have improved gram-positive coverage. Moxifloxacin (3), gatifloxacin (6), ofloxacin (4) and gemifloxacin (7) have exceptional activity against *legionella*, *chlamydia*, *mycoplasma*, and *ureaplasma* species. Intracellular respiratory pathogens such as *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, and *Legionella pneumophila* are significantly susceptible to fluoroquinolones. These antibiotics are also active

as antituberculous agents and are successfully used for the treatment of resistant tuberculosis¹⁴.

Figure 1

In the present study, a series of quinoloneacetic acids were synthesized and their antibacterial activities have been determined by micro dilution method.

EXPERIMENTAL

Synthesis

All chemicals were purchased from Sigma Aldrich (United Kingdom) and Merck (Pakistan). Some inorganic chemicals were purchased from Scharlau (France). The solvents were purchased from Fluka (Germany).

IR spectra were recorded on a Perkin-Elmer 1600 FT-IR spectrometer using KBr discs and Nujol mull was used in some cases. Bruker AVANCE Nuclear Magnetic Resonance Spectrophotometer 300 and 400 MHz was used for ¹H and 100 MHz for ¹³CNMR for compound analysis. Bruker Esquire 3000 + ion trap with ESI ionization and FAB⁺-MS spectra were used for measurement of m/z ratio. Perkin Elmer 2400-CHN analyzer and Leco CHNS analyzer CHNS-932 leco corp USA were used for microanalysis. The melting points were determined on a Gallenkamp melting point apparatus.

General Procedure

For synthesis of quinolone-3-acetic acids the method of Avetisyan *et al.*¹⁵ was adopted. A mixture of diethyl acetylsuccinate (2.16 g, 0.01 mol) and an arylamine (0.01 mol) was left at room temperature in a desiccator charged

with phosphorous (V) oxide. The resulting ethyl 2-(ethoxycarbonylmethyl)-3-(arylamino)crotonate (**8**) was washed with dilute hydrochloric acid followed by water and dried. Ethyl 2-(ethoxycarbonylmethyl)-3-(arylamino)crotonate (**8**) was added into preheated (250 °C) diphenyl ether. The reaction contents were heated at this for 30 minutes and cooled. Hexanes (25 mL) were added to reaction mixture. The precipitates were filtered and again washed with hexane. The precipitates of resultant ester (**9**) was suspended in aqueous KOH (10%, 25 mL) and heated at reflux for 2 hours, cooled and acidified with HCl to pH 5. The precipitates of acids (**10a-g**) were filtered off, washed with water and recrystallised from methanol.

2-Methyl-4-quinolone-3-acetic acid (10a): Yield: 57 %; m.p. 297-8 °C (lit.¹⁵ 299 °C); Anal. Calcd. for C₁₂H₁₁NO₃: C, 66.5; H, 5.20; N, 6.60; Found %: C, 66.36; H, 5.07; N, 6.45; IR $\nu_{\max}$ (KBr/cm⁻¹) 3260, 1730, 1440; ¹HNMR (400 MHz, DMSO-*d*₆) δ : 2.36 (3H, s, CH₃), 2.49 (2H, s, CH₂), 3.44 (1H, s, NH), 7.28 (1H, m, 6-H), 7.53 (1H, d, J = 8.1 Hz, 8-H), 7.61 (1H, m, 7-H), 8.06 (1H, dd, J_{5,6} = 8.0 Hz, J_{5,7} = 1.0 Hz, 5-H), 11.65 (1H, s, COOH); ¹³CNMR (100 MHz, DMSO-*d*₆) δ : 18, 27, 111, 118, 121, 123, 124, 131, 140, 147, 172, 174; MS (*m/z*, relative abundance, %): 217 (M, 11), 199 (98), 173 (100), 156 (12), 144 (12), 130 (12), 115 (12), 102 (37), 92 (12), 85 (17)

6-Chloro-2-methyl-4-quinolone-3-acetic acid (10b): Yield: 57 %; m.p. 301 °C; Anal. Calcd. for C₁₂H₁₀ClNO₃: C, 57.2; H, 4.01; N, 5.57; Found %: C, 57.1; H, 4.08; N, 5.52; IR $\nu_{\max}$ (KBr/cm⁻¹) 3260, 2870, 1700; ¹HNMR (300 MHz, DMSO-*d*₆) δ : 2.35 (3H, s, CH₃), 3.31 (2H, s, CH₂), 7.55 (1H, d, J_{7,8} = 8.8 Hz, 8-H), 7.62 (1H, dd, J_{7,8} = 8.8 Hz, J_{5,7} = 2.3 Hz, 7-H), 7.97 (1H, d, J_{5,7} = 2.3 Hz, 5-H), 12.00 (1H, s, COOH); ¹³CNMR (100 MHz, DMSO-*d*₆) δ : 17, 30, 113, 115, 121, 127, 128, 133, 138, 143, 146, 170; MS (*m/z*, relative abundance, %): 253 (M+2, 8), 252 (M+1, 3), 251 (M, 30), 234 (60), 221 (4), 207 (100), 190 (5), 178 (29), 143 (15), 115 (12), 100 (12)

2-Methylbenzo[g]quinoline-4(1H)-one-3-acetic acid (10c): Yield: 73 %; m.p. 222-4 °C; Anal. Calcd. for C₁₆H₁₇NO₃: C, 70.83; H, 6.32; N, 5.16; Found %: C, 70.79; H, 6.36; N, 5.12; IR $\nu_{\max}$ (KBr/cm⁻¹) 3300, 2860, 1785, 1640; ¹HNMR (400 MHz, DMSO-*d*₆) δ : 2.54 (3H, s, CH₃), 3.57 (2H, s, CH₂), 7.73 (4H, m, 6-H, 7-H, 8-H, 9-H), 8.07 (1H, m, 10-H), 8.29 (1H, s, NH), 8.91 (1H, m, 5-H), 11.34 (1H, s, COOH); ¹³CNMR (100 MHz, DMSO-*d*₆) δ : 28, 56, 113, 114, 121, 124, 128, 129, 138, 147, 165, 170; MS (*m/z*, relative abundance, %): 267 (M, 35), 232 (25.6), 214 (3), 188 (100), 160 (67), 145 (30), 128 (27), 115 (20), 91 (10)

6-Nitro-2-methyl-4-quinolone-3-acetic acid (10d): Yield: 25%; m.p. 269-271 °C; Anal. Calcd. for C₁₂H₁₀N₂O₅: C, 54.97; H, 3.84; N, 10.68; Found %: C, 54.92; H, 3.78; N, 10.70; IR $\nu_{\max}$ (KBr/cm⁻¹) 3300, 3180, 1740, 1630; ¹HNMR (400 MHz, DMSO-*d*₆) δ : 2.49 (3H, s, CH₃), 3.28 (2H, s, CH₂), 7.56 (1H, d, J_{7,8} = 8.8 Hz, 8-H), 7.66 (1H, dd, J_{7,8} = 8.8 Hz, J_{5,7} = 2.3 Hz, 7-H), 7.99 (1H, s, NH), 8.02 (1H, d, J_{5,7} = 2.3 Hz, 5-H), 12.37 (1H, s, COOH); ¹³CNMR (100 MHz, DMSO-*d*₆) δ : 12, 32, 112, 114, 120, 128, 129, 133, 137, 146, 148, 171; MS (*m/z*, relative abundance, %): 262 (M, 43), 247 (23), 234 (68), 211 (25), 194 (15), 160 (18), 144 (25), 134 (100), 118 (33), 106 (26), 90 (23)

6-Bromo-2-methyl-4-quinolone-3-acetic acid (10e): Yield: 39 %; m.p. > 300 °C; Anal. Calcd. for C₁₂H₁₀BrNO₃: C, 48.67; H, 3.40; N, 4.21; Found %: C, 48.36; H, 3.01; N, 4.45; IR $\nu_{\max}$ (KBr/cm⁻¹) 3200, 2965, 1730; ¹HNMR (400 MHz, DMSO-*d*₆) δ : 2.36 (3H, s, CH₃), 3.30 (2H, s, CH₂), 7.45 (1H, d, J_{7,8} = 8.6 Hz, 8-H), 7.56 (1H, s, NH), 7.75 (1H, dd, J_{7,8} = 8.6 Hz, J_{5,7} = 2.24 Hz, 7-H), 8.13 (1H, d, J_{5,7} = 2.24 Hz, 5-H), 12.01 (1H, s, COOH); ¹³CNMR (100 MHz, DMSO-*d*₆) δ : 19, 26, 111, 118, 121, 126, 127, 130, 141, 146, 172, 186; MS (*m/z*, relative abundance, %): 297 (M+2, 27), 295 (M, 27), 278 (10), 266 (10), 186 (12), 172 (21), 136 (15), 101 (12), 75 (17)

2,8-Dimethyl-6-nitro-4-quinolone-3-acetic acids (10f): Yield: 40%; m.p. 278-9 °C; Anal. Calcd. for C₁₃H₁₂N₂O₅: C, 56.52; H, 4.38; N, 10.14; Found %: C, 56.53; H, 4.28; N, 10.12; IR $\nu_{\max}$ (KBr/cm⁻¹) 3200, 2760, 1700, 1680; ¹HNMR (300 MHz, DMSO-*d*₆) δ : 2.3 (3H, s, CH₃), 2.4 (3H, s, CH₃), 7.65 (1H, d, J_{5,7} = 2.3 Hz, 7-H), 8.02 (1H, d, J_{5,7} = 2.3 Hz, 5-H), 7.99 (1H, s, NH), 12.02 (1H, s, COOH); ¹³CNMR (100 MHz, DMSO-*d*₆) δ : 14, 17, 31, 112, 119, 121, 124, 129, 135, 138, 148, 147, 172; MS (*m/z*, relative abundance, %): 276 (M, 26), 257 (32), 233 (4), 211 (9), 196 (100), 183 (82), 168 (22), 153 (44), 138 (12), 127 (23), 111 (30), 97 (50), 83 (57), 71 (64), 57 (40)

2,6-Dimethyl-4-quinolone-3-acetic acid (10g): Yield: 31%; m.p. 248 °C; Anal. Calcd. for C₁₃H₁₃NO₃: C, 67.52; H, 5.67; N, 6.06; Found %: C, 67.56; H, 5.61; N, 6.05; IR $\nu_{\max}$ (KBr/cm⁻¹) 3270, 2550, 1760, 1620; ¹HNMR (300 MHz, DMSO-*d*₆) δ : 2.22 (3H, s, CH₃), 2.32 (3H, s, CH₃), 3.24 (2H, s, CH₂), 6.9 (1H, d, J_{7,8} = 7.8 Hz, 8-H), 7.84 (2H, dd, J_{7,8} = 7.8 Hz, J_{5,7} = 1.8 Hz, 7-H), 7.98 (1H, d, J = 1.8 Hz, 5-H), 8.6 (1H, s, NH), 11.0 (1H, s, COOH); ¹³CNMR (100 MHz, DMSO-*d*₆) δ : 16, 17, 30, 113, 115, 120, 124, 127, 130, 139, 147, 154, 169; MS (*m/z*, relative abundance, %): 231 (M, 59), 227 (29), 206 (100), 175 (20), 160 (100), 102 (12), 69 (26)

Antibacterial activity

Antibacterial activity was performed in sterile 96-wells microplates under aseptic environments. The method is based on the principle that microbial cell number increases as the microbial growth proceeds in the log phase of growth which results in increased absorbance of broth medium^{16,17}. Four gram-negative (*Shigella sonnei*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*) and two gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*) were included in the study. The organisms were maintained on stock culture agar. The test samples (20 µg/well) with suitable solvent and dilution were pipetted into wells. Overnight maintained fresh bacterial culture after suitable dilution with fresh nutrient broth was poured into wells (180 µl). The initial absorbance of the culture was strictly maintained between 0.12-0.19 at 540 nm. The total volume in each well was kept to 200 µl. The incubation was done at 37°C for 16-24 hours with lid on the microplate. After shaking, the absorbance was measured at 540 nm using Synergy HT BioTek® 50 microplate reader, before and after incubation and the difference was noted as an index of bacterial growth. The percent inhibition was calculated using the formula: Inhibition (%) = 100 (X - Y) / X where X is absorbance in control with bacterial culture and Y is absorbance in test sample. Results are mean of triplicate (n = 3, mean ± sem). Ciprofloxacin (**1**) and moxifloxacin (**3**) were taken as standard. Minimum inhibitory concentration (MIC) was measured with suitable dilutions (5-30 µg/well) and results were calculated using EZ-Fit5 Perrella Scientific Inc. Amherst USA software, and data expressed as MIC₅₀. The mean and standard error of mean (sem) were calculated by using Excell software by descriptive statistical methods.

RESULTS AND DISCUSSION

For the synthesis of quinolone-3-acetic acids simple condensation of substituted arylamines with diethyl acetosuccinate was adopted by placing this mixture overnight at room temperature in dessicator charged with P₂O₅ the resultant oils of ethyl 2-(ethoxycarbonylmethyl)-3-(arylamino)crotonate (**8**) were heated with diphenyl ether at 250 °C to carry out cyclization and the esters obtained hydrolyzed *in situ* with aqueous sodium hydroxide followed by acidification with dilute hydrochloric acid resulted in respective acids (Fig. 2). The method was simple and easy to handle. The formation of products was confirmed with the help of IR, ¹HNMR, ¹³CNMR, mass and microanalysis. Infrared spectral values of compounds carbonyl peaks corresponding to lactone and carboxylic C=O stretching bands can be observed in the region of 1700-1650 cm⁻¹. Peak at 3100-3000 cm⁻¹ is indicative of OH and N-H stretching while C=C signal is around 1680-1475 cm⁻¹ describes quinolone-3-acetic acid to some extent. In compound **10d** and **10f** N-O stretching 1660 cm⁻¹ is also important. Stretching of C=C is also clearly seen at 3200-2850 cm⁻¹.

In proton NMR values are in the range δ 7.5-8.5 corresponding the N-H proton in all quinolone-3-acetic acids. The presence of acetic acid moiety in all these compounds can be confirmed by the appearance of signals integrating to protons of the methylene attached to carboxylic functionality in the region of δ 2.8-3.4 in ¹HNMRs. Proton NMR in dimethyl sulfoxide solvent also clearly showed signal at δ 11.34-12.37. Aromatic protons were in the range δ 6.92-8.06 in all of the quinoloneacetic acids confirmed the aromatic ring. In compound **10d** multiplet at δ 7.73 proved that there are more than aromatic ring in it.

¹³CNMR signal especially in the region of δ 165-174 corresponding to carboxylic carbons and δ 35-38 indicate the CH₂ also supported the structure described. Signals in the region δ 110-160 is proving the existence of aromatic ring in all of these compounds signals corresponding to carboxylic carbon at about δ 168-174.

Mass spectrum of compounds (**10a-g**) was found to be in close association with the calculated values. All compound followed nitrogen rule that confirmed the presence of nitrogen in all of the quinolone-3-acetic acids. Sharp melting point was providing the information about all of these compounds. CHNS technique found values in complete association to the calculated values. The formation of products was finally confirmed with the help of all techniques including IR, ¹HNMR, ¹³CNMR, mass and microanalysis.

Antibacterial activities of compounds were determined by using standard method as given in experimental section. All compounds exhibited 70-95% antibacterial activity against the test gram-positive and gram-negative microorganisms (data not shown). These studies were quantified by calculating MIC₅₀ (minimum inhibitory concentration to kill 50% bacterial population, µg/mL) values using suitable dilutions (5-30 µg/well) of the test compounds along with the positive standards ciprofloxacin and moxifloxacin and negative

controls. Data is given in Table 1. Lower the MIC₅₀ value, higher is the antibacterial activity.

Figure 2

6-Bromo-2-methyl-4-quinolone-3-acetic acid (**10e**) was the most active compound against *S. sonnei*, *S. typhi* and *P. aeruginosa*. It also showed moderate to good antibacterial activity against other bacteria (Table 1).

6-Chloro-2-methyl-4-quinolone-3-acetic acid (**10b**) was active against *E. coli* but 2,8-dimethyl-6-nitro-4-quinolone-3-acetic acid (**10f**) was highly good against *S. typhi*, *P. aeruginosa*, *S. aureus* and *B. subtilis* and 2,6-dimethyl-4-quinolone-3-acetic acid (**10g**) showed moderate activity against *S. sonnei*, *P. aeruginosa*, *S. aureus*, *B. subtilis* and very good against *S. typhi* demonstrated MIC₅₀ values very close to the standards. Compounds **10a**, **10c** and **10d** did not exert appreciable effects on all six gram-positive and gram-negative bacteria. It was observed that more polar group attached to benzene ring exhibited more antibacterial activity especially halogenated compound were seemed to be more active against different types of bacterial strains. On other hand alkyl group does not have an appreciable effect on the activity of quinolone-3-acetic acids.

CONCLUSIONS

Various quinolones have been found with established antibacterial activity. In this study, a series of quinolone acetic acid derivatives were synthesized and characterized with the aim to develop some new quinolones with potential antibacterial activity. All these compounds showed varying degree of activity from moderate to good against both gram-positive and gram-negative bacteria as compared to the standards.

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Table 1: MIC₅₀ (μg/mL) values of compounds (mean ± sem, n = 3). Minimum inhibitory concentration (MIC) was measured with suitable dilutions (5-30 μg/well) of the test compounds. Results calculated using EZ-Fit5 Perrella Scientific Inc. Amherst USA software, and data expressed as MIC₅₀.

Compounds	<i>E. coli</i> (G -ive)	<i>S. sonnei</i> (G -ive)	<i>S. typhi</i> (G-ive)	<i>P. aeruginosa</i> (G-ive)	<i>S. aureus</i> (G +ive)	<i>B. subtilis</i> (G +ive)
10a	11.03±0.51	12.68±0.42	11.26±0.52	12.22±0.40	12.66±0.50	13.64±0.45
10b	10.76±0.32	12.59±0.51	11.38±0.41	12.15±0.23	12.08±0.43	12.28±0.42
10c	12.47±0.51	13.28±0.26	11.79±0.44	11.33±0.31	11.19±0.41	10.63±0.52
10d	12.92±0.41	11.75±0.37	11.35±0.38	11.41±0.25	11.20±0.35	13.64±0.55
10e	8.97±0.21	10.09±0.69	10.09±0.34	9.21±0.34	9.81±0.37	12.15±0.35
10f	11.76±0.45	12.61±0.32	10.13±0.49	10.63±0.35	10.36±0.45	10.39±0.52
10g	12.19±0.42	10.89±0.41	10.11±0.46	10.35±0.29	10.19±0.49	10.36±0.47
Ciprofloxacin	8.21±0.02	9.31±0.08	10.12±0.21	9.42±0.11	9.42±0.11	8.36±0.13
Moxifloxacin	11.54±0.12	10.23±0.12	10.33±0.52	10.11±0.28	11.66±0.24	11.54±0.43

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