Synthesis of novel cyano quinolone derivatives and their antibacterial activities

Verma Vikrant
Department of Pharmaceutical Chemistry, Kharvel Subharti College of Pharmacy, Swami Vivekanand Subharti University, Meerut, 250005, INDIA
vijestsingh84@rediffmail.com

Abstract

Seven cyano 4-quinolone derivatives were synthesized 2 (A-G) and the synthesized compound’s antibacterial activity was tested. Structural elucidation of the synthesized compounds was carried out by their mass, infrared and N.M.R analysis. Antibacterial activity was evaluated against six bacterial strains e.g. Escherichia coli, Enterococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis, Salmonella enterica and Pseudomonas aeruginosa. After the analysis of the antibacterial activity, it was inferred that compounds 2C and 2G were found to be more active against Gram-negative and Gram-positive bacteria and are found to be potential candidates for the antibacterial drug. A different approach of replacing the 6th position fluorine of the quinolone ring with the cyanide group was carried out. Compound 2E was found to be the most active with MIC values of 0.0047, 0.0031, 0.0013, 0.0011, 0.0008, 0.012 respectively. This research leads to the development of cyano-4-quinolones.

Keywords: Quinolone, Antibacterial, Agar, 3-formyl-4-methoxybenzonitrile, DMFDMA, PTSA.

Introduction

There are several modes by which bacteria can be transmitted in the body like water, nutrition, air and through living vectors. There is a huge concern regarding the resistance development of bacteria during the treatment of bacteria and it necessitates the development of novel drugs. The class of quinolones drugs embodies a variety of bactericidal agents having a broad-spectrum activity which makes them suitable for the management of a variety of diseases in different parts of the body. 

Nalidixic acid was the first drug developed in the class of quinolones which was found to have antibacterial action, therefore new modifications were made at position 7 and position 6 by piperazine and fluorine, hence different attempts were already made to affect the half-life of drug and plasma protein binding capacity. In the 1st position, cyclopropene, difluorobenzene and alkyl substituents were introduced to increase the potency. In the 5th position, amino, hydroxyl and methyl group substitutions were done to increase the activity against the Gram-positive bacteria. In the 6th position, fluorine substitution was carried out which leads to an increase in the activity. The 7th position substitution by piperazine ring was alkylated in some derivatives. Pyrrolidine and cyclopropyl group were used to increase the potency and activity. In the 8th position substitution by OCH₃ substituent was done to increase the activity and Gram-positive bacteria.

The main target of quinolones is the enzyme topoisomerase IV in Gram-positive bacteria and topoisomerase II in gram-negative bacteria. It is mainly due to the formation of a complex with the DNA enzyme of bacteria. Quinolones produce its activity which results in cell death due to inhibition of DNA replication. As there is increasing resistance found in quinolones against the bacteria, there is an urgent need for the development of novel quinolones, Cyanide derivatives of quinolones are not prepared. This research is mainly based on the development of the novel cyanide derivatives which can decrease the resistance against the bacteria. Although quinolones have a wide variety of pharmacological actions, their antibacterial activity is mainly focused in this present research work. All the derivatives obtained are tested for antibacterial activity and were analyzed with the help of Infrared spectrometry, ¹³C-NMR, ¹H-NMR and mass spectrometry.

There is still a huge research potential for newer substituents at the 6th and 2nd positions. In the 2nd position, different modifications can be done and in position 6th also the fluorine can be substituted with cyanide to give remarkable antibacterial activity. These two positions are the main focus of this research study. To achieve a better antibacterial activity, cyanide substitution was done and some substitution on the piperazine ring was already done by the previous researchers. Even the copper chelated quinolones were synthesized in the presence of N-donor co-ligands.

Previously 4-quinolones were mainly synthesized by old-fashioned methodologies like Camps cyclization, Gould-Jacobs cyclization, Niementowski reaction and Conrad-Limpach cyclization, but these methods utilized strong bases. Recently Shi et al synthesized quinolone derivatives with the help of n-butyl, n-methyl, ethyl and benzyl substitution. The reaction of cyclopropenones and Nitrosouanlines yielded quinolones in which AgNT₂ was utilized for its significant role in N-N bond scission. Microwave aided synthesis of quinolone complexed with Ru(II) was carried out by Liu et al. Two novel complexes were synthesized: levofoxacin (LOFX) [Ru(dmbpy)₂(LOFLX)Cl₂]COCl₂ and [Ru(bpy)₂(LOFLX)Cl₂]Cl₂.
2C10(d)(dmbmpy=4,4-dimethyl-2,2'-bipyridine, bpy= 2,2'-bipyridine). This complexing increases the binding affinity and hence increases the efficiency of cancer therapy.12

The synthesized compounds were evaluated for antiplasmodial activity.19 Biswas et al1 synthesized quinolone derivatives from pyridines with the aid of Rhodium and this is utilized for the detection of nitroaromatic compounds, here oxidative annulation cyclization takes place. This reaction strategy can also be used for the synthesis of 4-quinolones. 4-quinolones was synthesised utilizing dimethylformamide, o-xylene, PTSA and 1-(2-cyclopropylamino-phenyl)ethanone in a one-pot synthesis2, this reaction methodology is utilized in the present research work with some modifications. By the utilization of this methodology, the derivatives were synthesized and it is a very simple method.2 The sixth position is still untouched for the research, in other positions mainly 1,5,7 and 8 different substitutions have already been carried out.

Material and Methods

All the chemicals and reagents utilized in the synthesis of derivatives were of synthetic grade and were purchased from Sigma Aldrich. All the reactions were monitored with the aid of Thin-layer chromatography (TLC) which utilizes silica. The solvents utilized for TLC determination were methanol and dichloromethane. Determination of melting points was carried out with the help of the melting point apparatus purchased from Labline, utilizing capillary tubes.

The infrared spectrum was taken with the aid of Perkin-Elmer RXI FT-IR spectrometer and the values are expressed in cm⁻¹. The IR spectra were recorded in the region 4000-400 cm⁻¹. The mass spectrum was taken with the help of ESI-ToF mass spectrometer. Bruker (Advance II) spectrometer was utilized to record the ¹³C and ¹H-NMR spectra and tetramethylsilane (TMS) was used as reference. The coupling constant (J) was expressed by Hz and chemical shift (δ) values were expressed by ppm. Compounds 1A to 1G were used as a starting material for the synthesis of derivatives and were purchased from Sigma Aldrich.

Preparation of 1-methyl-4-oxo-1,2,3,4-tetrahydroquinoline-6-carbonitrile(2A): Primarily 3-formyl-4-methoxybenzonitrile(4mg) was treated with sulphuric acid(5ml) and dimethylamine(1A)(25ml) leading to the formation of 4-(dimethylamino)-3-formylbenzonitrile. The synthesis of quinolones was carried out with the help of DMFDMDA (3ml) and 4-(dimethylamino)-3-formylbenzonitrile(15ml) and the reaction was catalyzed with the aid of ortho-xylene(15ml) and PTSA with temperature maintained at 130°C for 12 hours and then allowed to cool. In this reaction, catalysis occurred with the adjusted reaction conditions, the cyclization can also be achieved in DMFDMDA with other compounds.

Preparation of 1-cyclopropyl-4-oxo-2-(thiazol-5-yl)-1,2,3,4-tetrahydroquinoline-6-carbonitrile(2B): Primarily 3-formyl-4-methoxybenzonitrile (4mg) was treated with sulphuric acid (5ml) and N-Benzylcyclopropyamine (1B) (25ml) leading to the formation of 4-(benzyl(cyclopropyl)amino)-3-formylbenzonitrile. The synthesis of quinolones was carried out with the help of DMFDMDA(3ml) and 4-(benzyl(cyclopropyl)amino)-3-formylbenzonitrile (15ml) and the reaction catalyzed with the aid of ortho-xylene(15ml) and PTSA with temperature maintained at 130°C for 12 hours and then allowed to cool. In this reaction, cyclization occurred with the adjusted reaction conditions, the cyclization can also be achieved in DMFDMDA with other compounds.

Yield: 91%; m.p.:191-193°C; IR (KBr, cm⁻¹) νmax: 3373.90 (:=C-H), 1477.72 (NH), 1695.48(C=O), 1158 cm. -1(C=N); ¹H NMR (CDCl₃): 7.96 (d, J = 2.2 Hz, 1H), 7.61 (dd, J = 7.9, 2.2 Hz, 1H), 7.04 (d, J = 7.9 Hz, 1H), 3.69 – 3.66 (m, 2H), 3.08 – 3.04 (m, 2H), 2.99 (s, 2H). 13C NMR (125 MHz, DMSO-d₆): δ194.72, 147.02, 137.13, 131.62, 121.51, 119.01, 112.27, 105.80, 50.20, 39.60, 36.14. C₁₁H₁₀N₂O₂ exact mass 186.0793, found 187.0.

Preparation of 1-cyclopropyl-4-oxo-2-phenyl-1,2,3,4-tetrahydroquinoline-6-carbonitrile(2B): Primarily 3-formyl-4-methoxybenzonitrile (4mg) was treated with sulphuric acid (5ml) and N-Benzylcyclopropyamine (1B) (25ml) leading to the formation of 4-(benzyl(cyclopropyl)amino)-3-formylbenzonitrile. The synthesis of quinolones was carried out with the help of DMFDMDA(3ml) and 4-(benzyl(cyclopropyl)amino)-3-formylbenzonitrile (15ml) and the reaction catalyzed with the aid of ortho-xylene(15ml) and PTSA with temperature maintained at 130°C for 12 hours and then allowed to cool. In this reaction, cyclization occurred with the adjusted reaction conditions, the cyclization can also be achieved in DMFDMDA with other compounds.

Yield: 89%; m.p.:163-165°C; IR (KBr, cm⁻¹) νmax: 3372.63 (=C-H), 1478.81(N-H), 1602.40(C=O),1144(C-N):7.97 (d, J = 2.2 Hz, 1H), 7.65 (dd, J = 8.4, 2.2 Hz, 1H), 7.33 – 7.25 (m, 3H), 7.09 (d, J = 8.4 Hz, 1H), 5.01 (t, J = 7.2 Hz, 1H), 3.69 – 3.63 (m, 1H), 3.32 (dd, J = 15.5, 7.2 Hz, 1H), 3.07 (dd, J = 15.5, 7.2 Hz, 1H), 1.29 – 1.23 (m, 2H), 1.03 – 0.98 (m, 2H). 13C NMR (125 MHz, DMSO-d₆): δ192.82, 145.32, 141.08, 137.28, 131.70,125.24, 121.39, 119.00, 114.36, 105.63, 61.39,43.04, 36.14. C₁₁H₁₀N₂O₂ exact mass 288.126, found 289.1.

Preparation of 1-cyclopropyl-4-oxo-2-(thiazol-5-yl)-1,2,3,4-tetrahydroquinoline-6-carbonitrile(2C): Primarily 3-formyl-4-methoxybenzonitrile(4mg) was treated with sulphuric acid (5ml) and N-(1,3-Thiazol-5-ylmethyl) cyclopropanamine(1C) (25ml) leading to the formation of 4-(cyclopropyl(thiazol-5-ylmethyl)amino)-3-formylbenzonitrile. The synthesis of quinolones was carried out with the help of DMFDMDA(3ml) and 4-(cyclopropyl(thiazol-5-ylmethyl)amino)-3-formylbenzonitrile (15ml) and the reaction catalyzed with the aid of ortho-xylene(15ml) and PTSA with temperature maintained at 130°C for 12 hours and then allowed to cool. In this reaction, cyclization occurred with the adjusted reaction conditions, the cyclization can also be achieved in DMFDMDA with other compounds.

Yield: 88%; m.p.:151-153°C; IR (KBr, cm⁻¹) νmax: 3150.62 (=C-H), 1511.82(N-H), 1261.89(C-N)726(C=S):8.82 (d, J = 1.6 Hz, 1H), 7.97 (d, J = 2.2 Hz, 1H), 7.90 – 7.89 (m, 1H), 7.65 (dd, J = 8.4, 2.2 Hz, 1H), 7.09 (d, J = 8.4 Hz, 1H), 5.06
Preparation of 1-cyclopropyl-2-((methylamino)methyl)-4-oxo-1,2,3,4-tetrahydroquinoline-6-carbonitrile (2F): Primarily 3-formyl-4-methoxybenzonitrile (4mg) was treated with sulphuric acid (5ml) and N-Methyl-N'-cyclopropyl ethylenediamine (1F) (25ml) leading to the formation of 4-((cyclopropyl)(methylamino)-3-formylbenzonitrile. The synthesis of quinolones was carried out with the help of DMFDMA (3ml) and 4-((cyclopropyl)(methylamino)-3-formylbenzonitrile (15ml) and the reaction was catalyzed with aid of ortho-xylene (15ml) and PTSA with temperature maintained at 130°C for 12 hours and then allowed to cool. In this reaction, cyclization occurred with the adjusted reaction conditions, the cyclization can also be achieved in DMFDA with other compounds.

Yield: 90%; m.p.: 117-119°C; IR (KBr, cm⁻¹) v₉max: 3458.57 (=C=O), 1663.97(C=O), 1281.66(C=N) 2923.65(C=N): 7.95 (d, J = 2.2 Hz, 1H), 7.64 (dd, J = 8.4, 2.2 Hz, 1H), 7.07 (d, J = 8.4 Hz, 1H), 4.23 (ddd, J = 7.0, 5.0, 1.9 Hz, 1H), 3.66 (p, J = 6.0 Hz, 1H), 3.20 (dd, J = 15.8, 6.9 Hz, 1H), 3.00 – 2.92 (m, 2H), 2.83 (d, J = 5.3 Hz, 1H), 2.72 (dt, J = 11.9, 5.2 Hz, 1H), 2.41 (d, J = 5.2 Hz, 3H), 1.22 (d, J = 6.0 Hz, 1H), 1.01 – 0.92 (m, 2H). 13C NMR (125 MHz, DMSO-d₆): δ 193.90, 144.36, 137.29, 131.79, 131.29, 129.53, 119.90, 113.78, 105.57, 54.74, 39.99, 36.62, 20.78, 9.30, C₅H₄N₂O exact mass 255.13, found 256.

Preparation of 2-(3-chlorophenyl)-1-cyclopropyl-4-oxo-1,2,3,4-tetrahydroquinoline-6-carbonitrile (2G): Primarily 3-formyl-4-methoxybenzonitrile (4mg) was treated with sulphuric acid (5ml) and (3-Chlorobenzyl)cyclopropylamine hydrochloride (1G) (25ml) leading to the formation of 4-(((3-chlorobenzyl)(cyclopropyl)amino)-3-formylbenzonitrile. The synthesis of quinolones was carried out with the help of DMFDA (3ml) and 4-(((3-chlorobenzyl)(cyclopropyl)amino)-3-formylbenzonitrile (15ml) and the reaction was catalyzed with aid of ortho-xylene (15ml) and PTSA with temperature maintained at 130°C for 12 hours and then allowed to cool. In this reaction, cyclization occurred with the adjusted reaction conditions, the cyclization can also be achieved in DMFDA with other compounds.

Yield: 93%; m.p.: 153-155°C; IR (KBr, cm⁻¹) v₉max: 2815 (=C=H), 1623.32(C=C), 1521.35(C=N), 2899.93(C=O), 1271.73(C=N); 7.97 (d, J = 2.2 Hz, 1H), 7.65 (dd, J = 8.4, 2.2 Hz, 1H), 7.39 – 7.21 (m, 4H), 7.09 (d, J = 8.4 Hz, 1H), 5.09 (td, J = 7.1, 0.8 Hz, 1H), 3.66 (d, J = 5.9 Hz, 1H), 3.34 (dd, J = 15.6, 7.1 Hz, 1H), 3.09 (dd, J = 15.6, 7.1 Hz, 1H), 1.26 (ddd, J = 8.7, 7.4, 5.7 Hz, 2H), 1.05 – 0.94 (m, 2H). 13C NMR (125 MHz, DMSO-d₆): δ 192.80, 145.34, 140.39, 137.28, 134.07, 134.30, 129.52, 128.40, 128.13, 126.31, 120.20, 114.36, 105.63, 61.15, 43.01, 36.41, 9.04, C₁₀H₁₅ClN₂O exact mass 322.08, found 323.03.
Figure 1: Synthesis of novel quinolone derivatives 2A-2G

1-methyl-4-oxo-1,2,3,4-tetrahydroquinoline-6-carbonitrile (2A-2G)

Figure 2: $^1$HNMR spectra obtained for derivative 2A

Figure 3: $^1$HNMR spectra obtained for derivative 2B
Figure 4: $^1$HNMR spectra obtained for derivative 2C

Figure 5: $^{13}$C NMR spectra obtained for derivative 2A

Figure 6: $^{13}$C NMR spectra obtained for derivative 2B
Antibacterial Activity: The antibacterial activity was carried out on the novel synthesized compounds and the agar dilution method was utilized for the calculation of minimum inhibitory concentration (MIC). The obtained MICs (µg/ml) are given in table 3 and the standard drugs utilized for the antibacterial activity were ciprofloxacin, ofloxacin and lomefloxacin,\textsuperscript{1,4,9,10} Standard drugs and the novel synthesized derivatives were diluted with the aid of Mueller-Hinton agar, mainly two-fold dilutions were made. The synthesized compound (20mg) was dissolved in a suitable solvent in which it is soluble. In this study, DMSO was the solvent of choice in which all the derivatives were found soluble. The dissolved derivatives are further diluted with the aid of water (10ml). When the colonies were kept overnight in media of Mueller-Hinton agar embodying 0.85% saline, the inoculum of bacteria was prepared which was then set to a cell density corresponding to 0.5 McFarland standard by fine-tuning to 600nm photometrically \textsuperscript{6,8,14,20}

Results and Discussion
The synthetic scheme for the novel derivatives is given in figure 1. 3-formyl-4-methoxybenzonitrile was utilized as a starting material which was protonated with the help of sulphuric acid to give an intermediate. In this step, protonation of the methoxy group takes place. The intermediate was then treated with 1A to 1G to give intermediate which was then treated with DMFDMA and the reaction was catalyzed with the aid of ortho-xylene(15ml) and PTSA with temperature maintained at 130 °C for 12 hours and then allowed to cool to give 2A to 2G. Physical parameters of novel quinolone synthesized compounds and starting material and final synthesized compounds are given in table 1 and table 2. The reaction scheme is given in figure 1.

All the synthesized compounds were characterized with the aid of thin-layer chromatography (TLC) which incorporated plates which are made of silica gel. The synthesized compounds were evaluated by \textsuperscript{1}H NMR, \textsuperscript{13}C NMR and mass spectroscopy and their \textsuperscript{1}H NMR spectra are given in figure 2 to figure 4, \textsuperscript{13}C NMR spectra are given in figure 5 to 7.

The testing of the synthesized derivatives 2 (A-G) was carried out utilizing a wide variety of bacteria including Escherichia coli, Enterococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis, Salmonella enterica and Pseudomonas aeruginosa. After analyzing the MICs values, it is inferred that compound 2A exhibits good activity against Escherichia coli and compound 2B exhibits the most potent activity against Salmonella enterica. Compounds 2C and 2G exhibit good activity against both Gram-negative and Gram-positive bacteria. Only compound 2D was found to be less active than the reference drug ofloxacin but more active than lomefloxacin. As far as compound 2G is concerned, its activity was found better than reference quinolones against Pseudomonas aeruginosa and Staphylococcus aureus having MIC values of 0.0026 µg/ml and 0.0028 µg/ml. The antibacterial activity of the novel synthesized derivatives and reference drugs are given in table 3.

Compound 2E was found to be more potent against Staphylococci aureus and its resistant forms with MIC values of 0.0008 µg/ml and in comparison with the reference drug having MIC values of 0.012- 0.64µg/ml, it was found to have better activity. After analysis of all the synthesized compounds, it is inferred that there is improvement in the activity against Gram-positive bacteria and Gram-negative bacteria compared to the reference drugs. Compound 2E exhibited the most potent activity against E.coli, P.aeruginosa, E.faecalis, S.aureus, S.epidermidis and S.enterica and is found more potent than the reference drug.

Conclusion
Some novel 4-quinolone derivatives bearing a cyanide group at the 6th position were synthesized, which is a revolutionary
step in the synthesis of quinolones. Structural modification mainly at the 6th and 2nd positions was carried out in this research. A total of seven derivatives were synthesized and their antibacterial activity was evaluated. All synthesized compounds were found efficient in antibacterial activity. However, three derivatives (2A, 2E, 2G) were found more efficient in antibacterial activity.

Compound 2E was found most potent as compared to all the reference drugs. It leads to the development of cyano-quinolones which are effective against both Gram-negative and Gram-positive bacteria.

Acknowledgement
The author is thankful to the Shobhit University, Meerut for laboratory and instrument facilities.

References
22. Shi Y., Xing H., Huang T., Liu X., Chen J., Guo X., Li G.B. and Wu Y., Divergent C-H activation synthesis of chalcones,
quinolones and indoles, *Chemical Communications*, 56(10), 1585–1588 (2020)


(Received 15th May 2023, accepted 18th July 2023)