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ORIGINAL RESEARCH PAPER

SYNTHESIS, CHARACTERIZATION AND EVALUATION OF NOVEL QUINOLINE MOLECULES AS ANTIBACTERIAL AGENTS

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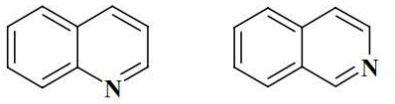
Abstract: The objective of the present investigation was to synthesize novel quinoline derivatives for antibacterial activity. Five compounds **SK1-5** were synthesized and these compounds were tested yield (%), solubility, retention factor (R_f) and melting point (°C). The compounds were evaluated for antibacterial activity using zone of inhibition method at four different concentrations. The products were insoluble in water, methanol and DMSO while they were soluble in chloroform and were obtained in 67 - 76 % yield. The antibacterial action against both gram-positive and gram-negative bacteria was exhibited by the compounds. The compounds were found to be possessing better inhibitory action against the gram-negative bacteria in comparison to gram positive bacteria. The zone of inhibition exhibited by **SK3** was highest among all compound and it was equally effective in both gram-negative and positive bacteria.

Keywords: *Conrad-Limpach, epichlorohydrin, ethyl acetoacetate, disc diffusion, zone of inhibition*

INTRODUCTION

Heterocyclic compounds occur widely in nature and in a variety of non-naturally occurring material. A significant part of large number of compounds such as alkaloids, antibiotics, essential amino acids, vitamins, hemoglobin, the hormones, synthetic drugs and dyes composed of heterocyclic ring systems and have significant importance in for human and animal health.

Quinoline is the benzo condensed heterocycles containing one nitrogen atom. Quinoline (Figure 1) is a building block of naturally occurring alkaloids isolated from a number of families of the plant species. The heterocycle has been known to be used as cure for diseases since long via several alkaloidal molecules like Chimanine [1], Cryptolepine [2], Streptonigrin [3].



QUINOLINE ISOQUINOLINE

Figure 1. *Isomeric quinolines*

The quinoline nucleus is simple to be synthesized and several methods have been reported that yield quinoline in high amount using not very harsh reaction conditions [4 – 9].

Infectious disease has always an adverse effect to human beings as it is a pathological condition which is caused specific infectious agents which can be virus, bacterium, fungi or protozoan that affects the body parts or tissues, characterized by a group of signs and symptoms. These pathogens are responsible for at least 13 million infections and

1.5 million deaths globally per year, primarily in those with some compromised immune function [10]. Thus, the development of novel antimicrobial agents with low resistance and high efficacy is a priority [11].

The objective of present study was to synthesize new quinoline containing molecules and evaluate their antibacterial activity.

MATERIALS AND METHODS

Synthesis grade 4-Bromoaniline, ethylacetoacetate, 4-chloroaniline, 4-nitroaniline and 4-methylaniline were obtained from Loba Chemie (India); premix nutrient broth and nutrient agar powder were purchased from Microgen (India). All chemical and reagents were used without any processing or purification. Melting point were determined on BioTechnics (BTI-34) melting point apparatus in open capillaries and are reported without correction. Spectral characterization was obtained on Bruker Alpha, Germany (FTIR) and Bruker Avance, Germany (^{1}H NMR) spectrophotometer.

Synthetic methodology

The steps adopted in the synthesis of the quinoline derivatives are depicted in the below (Figure 2) which is adopted from the reports by Ramesh and Sreenivasulu [12] and Mishra [13].

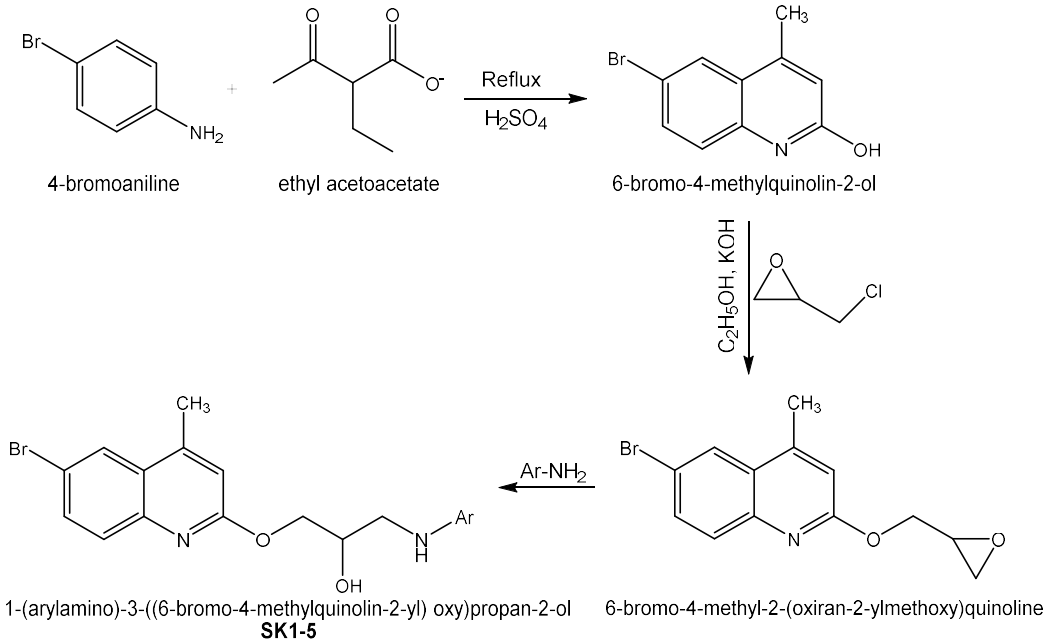


Figure 2. *Synthetic path for quinolone derivatives*

Synthesis of 6-bromo-4-methylquinolin-2-ol

The synthesis of 6-bromo-4-methylquinolin-2-ol was performed by using 4-bromoaniline and ethyl acetoacetate. To 0.1 mole of 4-bromoaniline was added 0.1 mole ethyl acetoacetate. To the mixture, 5 mL concentrated sulfuric acid was added. A white solid precipitate started forming in the reaction vessel. The reaction mixture was then refluxed at 135 °C. The white precipitate slowly started to disappear. The mixture was refluxed till the completion of the reaction, which was monitored by TLC. The reaction mixture was cooled overnight to obtain the product, which was filtered, dried and recrystallized from ethanol.

Synthesis of 6-bromo-4-methyl-2-(oxiran-2-ylmethoxy) quinolone

To 0.1 mole of epichlorohydrin was added 0.1 mole of the 6-bromo-4-methylquinolin-2-ol obtained from step 1, in the presence of 15 mL of 10 % alcoholic potassium hydroxide. The reaction mixture was refluxed until the completion of the reaction. On cooling, the product separated which was filtered, dried and recrystallized from ethanol.

General method for synthesis of 1-(arylamino)-3-((6-bromo-4-methylquinolin-2-yl) oxy)propan-2-ol (SK1-5)

To 0.01 mole of the 6-bromo-4-methyl-2-(oxiran-2-ylmethoxy) quinolone obtained from the step 2 was added 0.01 mole of an appropriate arylamine in the presence of

20 mL of alcoholic potassium hydroxide. The reaction mixture was refluxed until the completion of the reaction (monitored by TLC using chloroform:methanol (40:60 v/v)). On cooling, the product separated which was filtered, dried and recrystallized from ethanol to give the corresponding final product.

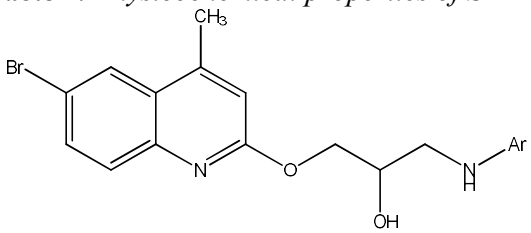
Antibacterial study

The antibacterial study was carried out to determine the zone of inhibition using *Escherichia coli*, and *Staphylococcus aureus* as the test organisms. The lyophilized cultures were revived by adding 0.3 mL of nutrient broth to the culture ampoules to obtain a suspension of the bacteria. The test samples were prepared by dissolving the synthesized quinolines in appropriate solvent to obtain the solutions of 25, 50, 75 & 100 $\mu g \cdot mL^{-1}$ concentration. A few drops of the bacterial suspension were injected onto the surface of pre-poured, 3 mm-thick nutritional agar plates. The cup and plate method was used to screen for antibacterial activity [14].
Wells were dug in the agar plate at equal distances, and 200 µL of the test compound were poured into each well. The plates were incubated at 37 ± 0.1 °C for 24 hours and the zone of inhibition was measured.
Norfloxacin (25 $\mu g \cdot mL^{-1}$) was used the standard antibacterial compound during the antibacterial study.

RESULTS AND DISCUSSION

The synthesis of the quinolines **SK1-5** was achieved by Conrad-Limpach synthesis. The synthesis of quinoline from *β*-keto esters and aromatic amines constitutes the basic Conrad-Limpach synthesis. Using 2-bromoaniline as the amine and condensing it with ethyl acetoacetate leads to the formation of desired bromo-substituted quinoline nucleus. At low temperature the amine condenses with the ester at the keto group to give an anil, which catalyzes to quinolinone on heating. On the other hand, at higher temperature, the initial product of the condensation is the anilide, which is formed by the condensation of the amine at the ester function. The anilide undergoes ring closure on heating, or in the presence of an acid, and on subsequent oxidation yields 6-bromo-4-methylquinolin-2-ol. The epoxy-propyloxy derivative of 6-bromo-4-methylquinolin-2-ol was synthesized by the treatment of the 6-bromo-4-methylquinolin-2-ol, which was obtained from step 1 with epichlorohydrin, in the presence of alcoholic potassium hydroxide. The final product, i.e., the amino propyloxy derivative of the quinoline was synthesized by the reaction of the product obtained from the step 2 by an appropriate arylamine, in the presence of alcoholic potassium hydroxide.
The products were insoluble in water, methanol and DMSO while they were soluble in chloroform and were obtained in 67 - 76 % yield [15].
The physicochemical properties of the synthesized molecules are presented in Table 1.

Table 1. *Physicochemical properties of* ***SK1-5***



Compound code	Arylamine used	Color	R_f value	Melting point [°C]	Yield [%]
SK1	4-Methylaniline	Off-white	0.59	134-136	72
SK2	4-Nitroaniline	Off-white	0.63	184-186	76
SK3	Aniline	Off-white	0.67	113-115	67
SK4	4-Chloroaniline	Pink-white	0.51	130-133	71
SK5	4-Bromoaniline	Pink-white	0.64	159-161	73

The structural identification of the compounds was done by ^{1}H NMR and FTIR spectroscopy (Table 2).

Table 2. *Spectral characters of* ***SK1-5***

Compound code	IUPAC Name	^{1}H NMR	FT-IR	Mass (calculated)
SK1	1-((6-bromo-4-methylquinolin-2-yl) oxy)-3-(p-tolylamino) propan-2-ol	6.61-8.10 (Aromatic Protons (C-H), 2.35, 2.59 (CH_3), 4.08, 4.33 (CH_2), 3.20, 3.25 Proton alpha to NH, 4.76 (N-H), 3.86 (OH)	3030.61 (Ar C-H stretch), 1603.33 (Ar C-C/Ar C=C stretch), 897.10 (C-N stretch), 570.68 (C-Br stretch)	401.30
SK2	1-((6-bromo-4-methylquinolin-2-yl) oxy)-3-((4-nitrophenyl) amino)propan-2-ol	6.61-8.10 (Aromatic Protons (C-H), 2.35, 2.59 (CH_3), 4.08, 4.33 (CH_2), 5.26 (N-H), 3.86 (OH)	3413.55 (N-H stretch), 3028.61, 3102.68 (Ar C-H stretch), 1586.89 (Ar C-C/Ar C=C stretch), 852.45, 897.35 (C-N stretch), 561.61 (C-Br stretch)	432.27
SK3	1-((6-bromo-4-methylquinolin-2-yl) oxy)-3-(phenylamino) propan-2-ol	6.61-8.10 (Aromatic Protons (C-H), 2.35, 2.59 (CH_3), 4.08, 4.33 (CH_2), 3.20, 4.41 (N-H), 3.86 (OH)	3026.05 (Ar C-H stretch), 1589.72 (Ar C-C/Ar C=C stretch), 871.65 (C-N stretch), 571.74 (C-Br stretch)	387.27
SK4	1-((6-bromo-4-methylquinolin-2-yl) oxy)-3-((4-chlorophenyl) amino)propan-2-ol	6.58-8.10 (Aromatic Protons (C-H), 2.59 (CH_3), 4.08, 4.33 (CH_2), 3.20, 3.25 Proton alpha to NH, 4.76 (N-H), 3.86 (OH)	3365.77 (N-H stretch), 3024.82, 3103.07 (Ar C-H stretch), 1578.14 (Ar C-C/Ar C=C stretch), 1082.39 (C-Cl stretch), 815.82 (C-N stretch), 566.62 (C-Br stretch)	421.72

Compound code	IUPAC Name	^{1}H NMR	FT-IR	Mass (calculated)
SK5	1-((6-bromo-4-methylquinolin-2-yl) oxy)-3-((4-bromophenyl) amino)propan-2-ol	6.57-8.10 (Aromatic Protons (C-H), 2.35, 2.59 (CH_3), 4.08, 4.33 (CH_2), 3.20, 3.25 Proton alpha to NH, 4.76 (N-H), 3.86 (OH)	3342.99 (N-H stretch), 3093.00 (Ar C-H stretch), 1587.97 (Ar C-C/Ar C=C stretch), 897.14, 809.11 (C-N stretch), 595.97 (C-Br stretch)	466.17

The proton NMR spectra presented the chemical shifts presence of protons of aromatic ring (6.5 - 8.1 ppm), amine hydrogen (4.4 - 5.3 ppm) in all compounds. The chemical shifts of the aliphatic protons (2.3 - 4.3 ppm) were found in all compounds. All the compounds exhibited the stretching vibrations for aromatic C-H (3080 - 3030 cm^{-1}), aromatic C-C/C=C (1625 - 1575 cm^{-1}), N-H (3500 - 3100 cm^{-1}), C-Br (600 - 500 cm^{-1}) and C-N (900 - 800 cm^{-1}), in the FTIR spectrum. The stretching vibrations corresponding to C-Cl (1080 - 1020 cm^{-1}) and were also found in the corresponding compounds.
The zone of inhibition was measured to assess the preliminary antibacterial activity of compounds **SK1-5** (Table 3).

Table 3. *Zone of inhibition exhibited by* ***SK1-5***

Compound code	Zone of inhibition [mm]							
	S. aureus				***E. coli***			
	25 µg	**50 µg**	**75 µg**	**100 µg**	**25 µg**	**50 µg**	**75 µg**	**100 µg**
SK1	-	-	17	**20**	-	-	14	15
SK2	-	-	17	18	-	-	18	**21**
SK3	-	-	19	**24**	-	-	**21**	**26**
SK4	-	-	17	19	-	-	18	19
SK5	-	-	16	17	-	-	15	17
Norfloxacin	**24**	-	-	-	**26**	-	-	-

The antibacterial action against both gram-positive and gram-negative bacteria was exhibited by the compounds. The compounds were found to be possessing better inhibitory action against the gram-negative bacteria in comparison to gram positive bacteria. The zone of inhibition exhibited by **SK3** was highest among all compound and it was equally effective in both gram negative and positive bacteria. This suggests the absence of any substitution on the aromatic ring of side chain was beneficial for activity.

CONCLUSIONS

The preliminary object of this research work was to synthesize novel quinoline compounds and assess their antimicrobial action. Conrad-Limpach synthesis is the easiest method to prepare the desired quinoline derivatives. The use of epichlorohydrin was efficient in preparing the side chain of the molecules. The presence of bromine on the quinoline and the absence of any substitution on the phenyl ring of the side chain

was found to be particularly beneficial for the antibacterial activity of the compounds. In future, a congeneric series would be developed and molecular modeling approached would be used for designing lead molecule and defining the structure activity relationship of the molecules.

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