SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL QUINOLINE-4-CARBOXYLIC ACID DERIVATIVES BASED ON DOEBNER HYDROGEN TRANSFER REACTION

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Abstract:
Quinoline derivatives are a group of compounds known to possess a wide range of biological activities. A series of new 2-aryl-quinoline-4-carboxylic acid derivatives were synthesized starting from aniline, different aromatic aldehydes, and pyruvic acid followed by the Doebner reaction. The purity and identities of the synthesized compounds were elucidated through spectroscopic techniques. All of the newly synthesized compounds were characterized by IR and ^{1}H-NMR. The spectral data were interpreted and correlated with the target structures. The synthesized compounds were tested for their antibacterial activity against two Gram +ve strains (*Staphylococcus aureus*, *Bacillus subtilis*) and two Gram – ve strains (*Klebsiella pneumoniae*, *Escherichia coli*) by Kirby - Bauer Disc Diffusion Method (Zone of inhibition). Among the compounds tested for antimicrobial activity, Furan-2-yl, 2-hydroxyphenyl and 3-methoxy-4-hydroxy phenyl substituted at C_2 showed significant activity against Gram +ve and Gram – ve bacteria. Compounds substituted with pyridin-2-yl and 4-nitrophenyl at C_2 displayed remarkable activity against both Gram +ve and Gram – ve bacteria.

Keywords: 2-aryl-quinoline-4-carboxylic acid; Quinoline synthesis; Doebner reaction; antibacterial activity by the Kirby - Bauer Disc Diffusion Method.

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1. Introduction

The discovery and introduction of antibiotics into clinical use was the greatest medical achievement of the twentieth century[1] that undeniably improved human and animal health significantly. However, antibiotic resistance has become one of the serious global public health challenges of the 21st century. Currently, it has been common to see people being affected and dying from untreatable infections caused by multidrug resistance (MDR) germs globally, including the developed nations. The widespread and incorrect use of antibiotics is thought to be the main cause of various resistance mechanisms in bacteria that lead to MDR. Antimicrobial resistance mechanisms[2] fall into four main categories: limiting uptake of a drug, modifying a drug target, inactivating a drug, and active drug efflux. Because of the differences in their morphology, there is variation in the types of mechanisms used by Gram-negative bacteria versus Gram-positive bacteria. Gram-negative bacteria make use of all four main mechanisms, whereas Gram-positive bacteria less commonly use limiting the uptake of a drug. However, this problem has not been completely resolved. Therefore, the development of novel and effective chemotherapeutic agents are still in demand to overcome these problems.

Generally, quinolines are a structurally varied group of compounds, mainly comprising quinoline nuclei, found in various natural and synthetic products that exhibit a broad range of biological activities. Quinolines and their derivatives exhibited anti-inflammatory[3], antibacterial[4], antifungal[5], antimalarial[6], antiallergy[7], antitumor[8], antituberculosis[9], and antihypertensive[10] activities. Although quinoline-4-carboxylic acid derivatives exhibit various bioactivities, the research of new quinoline-4-carboxylic acid derivatives manifesting antibacterial activities has gained attention gradually. Furthermore, the presence of an aryl ring at the second position of quinoline-4-carboxylic acid derivatives exhibits good antibacterial activity for the target compound and plays a significant role in the development of new antibacterials and they are very suitable for further modifications to obtain more effective antibacterial agents.

Keeping in view, the wide range of pharmaceutical activities of quinoline-4-carboxylic acid, in this report, the introduction of aryl and heteroaryl substituents in the molecule not only improved their pharmacological response but also their physicochemical properties. Thus, a series of 2 -aryl / heteroaryl 4- carboxylic acid quinoline derivative compounds (1a-1e) were designed, synthesized, and evaluated for antibacterial activities.

2. Materials and methods:

2.1. Chemistry

All chemicals used were of commercially available reagent grade and were used without further purification. The major chemicals were purchased from Aldrich Chemical Corporation. The melting points were determined in open capillary tubes and were uncorrected. The reaction's progress was monitored by Aluminum sheets precoated with silica gel plates of 0.25 mm thickness. Spots were visualized by using either a UV lamp or iodine.

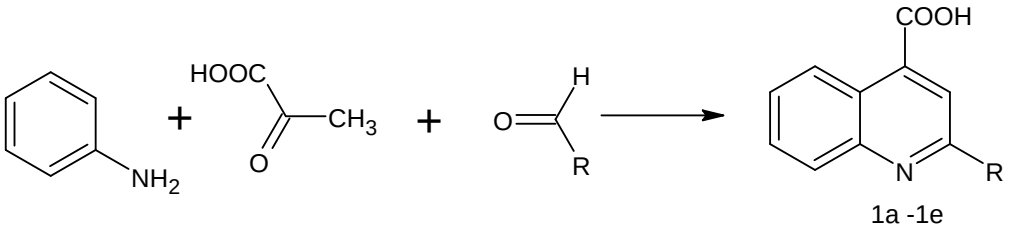
Infrared (IR) spectra were recorded as a KBr disk using Shimadzu FT-IR. The data are given in (cm^{-1}). ^{1}HNMR spectra were determined in DMSO-d6 and recorded on Bruker ^{1}HNMR spectrophotometer (500 MHz). The chemical shifts are expressed as δ values (ppm) relative to the internal standard, tetramethylsilane (TMS). Signals are indicated by the following abbreviations: s = singlet, bs = broad singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, and m = multiplet. The *J* constants were given in (Hz). Elemental analysis was performed by using chem sketch software.

2.1.1. General Procedure for the Synthesis of 2-Aryl substituted Quinoline 4-carboxylic acid (1a-1d):

Placed aromatic aldehyde (0.01 mol of different aldehydes such as furfuraldehyde, salicylaldehyde, vanillin, p-nitro benzaldehyde, and pyridine 2 carbaldehyde), 1.5ml pyruvic acid, and added 20ml of ethanol. Heated the mixture to the boiling point on a water bath and added slowly with frequent shaking a solution of 0.01 mol of aniline in 20ml of ethanol. The addition usually occupied about 1hr. Refluxed the mixture on a water

bath for 3-5hr, and allowed it to stand overnight. Filtered the crude product at the pump and washed the crystals with a little ether. Recrystallized from ethanol.

Scheme 1



aniline pyruvic acid aromatic aldehyde 2-Aryl substituted quinoline-4-carboxylic acid

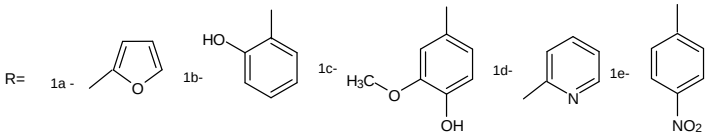
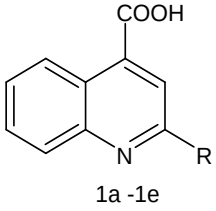
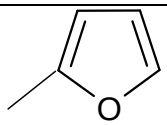
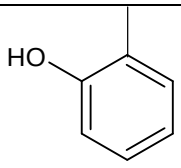
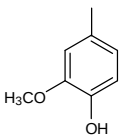
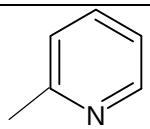
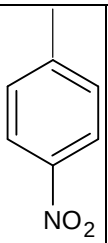


Table: 1. Physical characterization of 2 -aryl / heteroaryl 4- carboxylic acid quinoline derivative compounds (1a-1e)

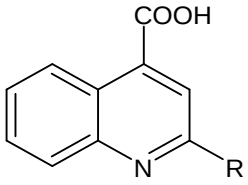


S.No	Compounds (R)	Color	% Yield W/W	Melting point (^{0}C)	Rf value	Molecular Formula	Molecular Weight	Composition%			
								C	H	O	N
1a		Yellow	65.00	252-254	0.42	$C_{14}H_9NO_3$	239.23	70.29	3.79	20.06	5.86
1b		Yellow	52.14	198-202	0.40	$C_{16}H_{11}NO_3$	265.26	72.45	4.18	8.09	5.28
1c		Yellow	49.43	201-204	0.39	$C_{17}H_{13}NO_4$	295.28	69.15	4.44	21.67	4.44
1d		Yellow	44.54	196-200	0.42	$C_{15}H_{10}N_2O_2$	250.25	71.99	4.03	12.79	11.19

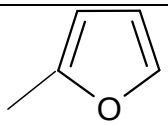
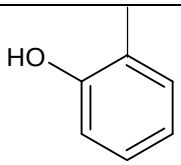
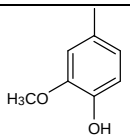
1e		Yellow	44.24	252-255	0.48	$C_{16}H_{10}N_2O_4$	294.26	65.31	3.43	21.75	9.52

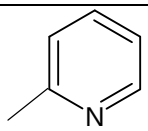
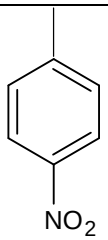
Mobile phase - methanol: dichloro methane (0.1:5)

Table IR & ^{1}H NMR spectrum data of 2 -aryl / heteroaryl 4- carboxylic acid quinoline derivative compounds (1a-1e)



1a -1e

S. No	Compounds (R)	IR frequency region(cm^{-1})	^{1}H NMR (δ ppm)
1a		3309.85 (O-H Stretching) 3155.54 (Aromatic C-H Stretching) 1651.07 (C=O Stretching) 1604.07 (Aromatic C-N Stretching) 1465.90 (Aromatic C-C Stretching) 1211.30 (Asymmetrical C-O-C Stretching)	6.77 (1H, dd,), 7.56 (1H, dd),7.79 (1H, ddd), 7.97 (1H, ddd), 8.06-8.20 (3H, m),8.84 (1H, d).
1b		3320.54 (O-H Stretching) 3186.33 (Aromatic C-H Stretching) 1717.21 (C=O Stretching) 1686.74 (Aromatic C-N Stretching) 1530.59 (Aromatic C-C Stretching)	7.08 (1H, ddd), 7.30-7.74 (4H, m) 7.80 (1H, td), 7.99 (1H, ddd), 8.19 (1H, ddt), 8.48 (1H, d),9.95(1H, s)
1c		3295.27 (O-H Stretching) 2970.38 (Aromatic C-H Stretching) 1706.15 (C=O Stretching) 1567.24 (Aromatic C-N Stretching) 1598.26 (Aromatic C-C Stretching) 1249.87 (Asymmetrical C-O-C	δ 2.27 (3H, s) 2.43 (3H, s), 7.08 (1H, ddd), 7.30-7.74 (4H, m) 7.80 (1H, td), 7.99 (1H, ddd), 8.19 (1H, ddt), 8.48 (1H, d).

		Stretching)	
1d		3225.73 (O-H Stretching) 3115.31 (Aromatic C-H Stretching) 1722.97 (C=O Stretching) 1649.84 (Aromatic C-N Stretching) 1605.48 (Aromatic C-C Stretching)	7.36 (1H, ddd), 7.70-7.94 (3H, m) 8.06-8.22 (3H, m) 8.73-8.85 (2H, m) 8.79 (1H,d).
1e		3354.13 (O-H Stretching) 3124.63 (Aromatic C-H Stretching) 1687.58 (C=O Stretching) 1534.94 (N-O Stretching) 1490.33 (Aromatic C-N Stretching) 1420.51 (Aromatic C-C Stretching)	7.74 (1H, ddd), 7.89-8.05 (3H, m) 8.07-8.26 (3H, m), 8.61 (1H, ddt), 8.79 (1H, d).

In vitro anti-microbial screening

The antibacterial activity of synthesized compounds was carried out by the Kirby - Bauer Disc Diffusion Method[11]. The antibacterial activity was tested against both Gram +ve (*Staphylococcus aureus, Bacillus subtilis)* and Gram – ve (*Klebsiella pneumoniae, Escherichia coli*) bacteria. Nutrient agar (1g of beef extract, 1g peptone, 0.5 g NaCl dissolved in 100 ml of double distilled water) was used to cultivate bacteria. The media was autoclaved and cooled. The media was poured into the petri dish and kept for 30 minutes for solidification. After 30 minutes, the fresh overnight cultures of inoculum of four different strains were spread onto solidified nutrient agar plates. Sterile paper discs made of Whatman filter paper of 5 mm diameter were dipped in synthesized compounds at different concentrations of 20µg and a standard solution (20µg) was placed in each plate. Ciprofloxacin was used as standard. The cultured agar plates were incubated at 37°C for 24 h. The Zone of inhibition was calculated by measuring the diameters of the inhibition zone around the well.

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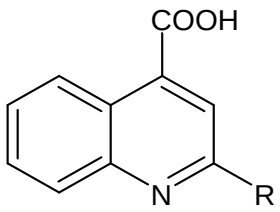
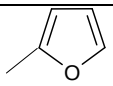
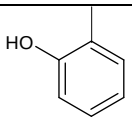
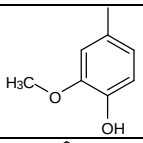
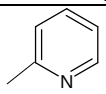
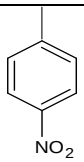
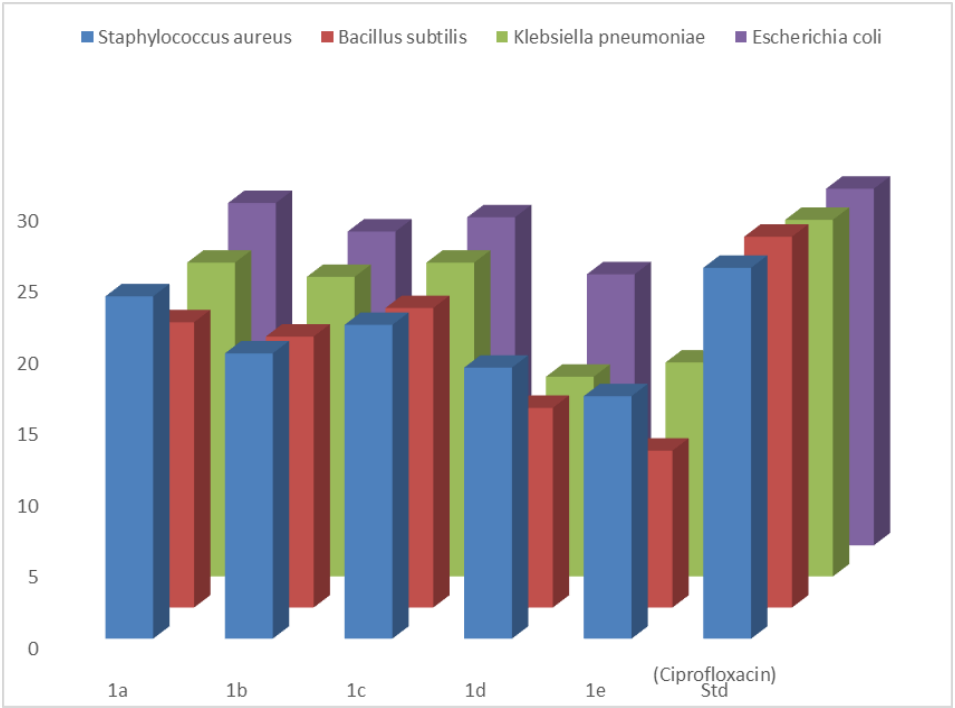
Table:3 Anti-microbial activity of synthesized 2-aryl / heteroaryl 4-carboxylic acid quinoline derivatives (1a-1e):



1a -1e

S.NO	**COMPOUND**	**Zone of inhibition (MIC) in mm**			
		Gram +ve		Gram – ve	
		S. aureus	*B. subtilis*	*K. pneumoniae*	*E. coli*
1a		24	20	22	24
1b		20	19	21	22
1c		22	21	22	23
1d		19	14	14	19
1e		17	11	15	18
Standard	Ciprofloxacin	26		25	



All the compounds were tested for their antimicrobial activity furan-2-yl (**1a),** 2-hydroxyphenyl (1b), and 3-methoxy phenyl (1c) substituted at C_2 showed significant activity against Gram +ve (*Staphylococcus aureus, Bacillus subtilis)* and Gram – ve

(*Klebsiella pneumoniae, Escherichia coli*) bacteria. pyridine-2-yl (1d), 4-nitrophenyl (1e) substituted at C_2 displayed remarkable activity against both Gram +ve (*Staphylococcus aureus, Bacillus subtilis)* and Gram – ve (*Klebsiella pneumoniae, Escherichia coli*) using Ciprofloxacin as the reference.

Results and discussion:

Quinolines play an important role in organic chemistry, as exemplified by their extensive application as biologically and pharmacologically active compounds. However, current methods available to access quinoline compounds employ harsh reaction conditions and expensive starting materials, hazardous reagents (10 M hydrochloric acid), tedious isolation procedures, side products, and varying product yields. Consequently, a need to develop a simple and environmentally friendly route to synthesize quinoline derivatives via the Doebner reaction is essential.

The synthesis of the desired compounds was accomplished according to the representation **Scheme 1.** The final compounds of 2-aryl / heteroaryl 4-carboxylic acid quinoline derivatives were obtained from the Doebner reaction. Using this approach, three-component coupling, the addition of an aniline, aromatic/ heteroaryl aldehydes, and α keto acid (pyruvic acid) by refluxing with ethanol under various reaction times affording the best results. Using the optimized reaction conditions, a series of substituted quinoline derivatives were synthesized in moderate to good yields (43.2-65%W/W) in 3-5 hours. Compound (**1a)** 2-(furan-2-yl) quinoline-4-carboxylic acid and Compound **(1b**) 2-(2-hydroxyphenyl) quinoline-4-carboxylic acid afforded very good yield. Compound (**1c**) 2-(3-methoxy-4-methylphenyl) quinoline-4-carboxylic acid, Compound (**1d**)2-(pyridin-2-yl) quinoline-4-carboxylic acid and Compound (**1e)** 2-(4-nitrophenyl) quinoline-4-carboxylic acid were moderate in their yield.

Infra-red (FT-IR) spectroscopy measures the vibrations of the molecules. Each functional group or structural characteristic of a molecule has a unique vibrational frequency that can be used for its identification in a sample. When the effects of all the different functional groups are taken together, the results are a unique molecular "fingerprint region" that can be used to confirm the identity of the sample.

The FT-IR in the KBr spectrum of compound (1a-1e) exhibited absorption bands in the region between 3354.13 and 3295.27 cm^{-1} (the broadband belongs to the OH stretching) and 1722.97 and 1651.07 cm^{-1} (hydroxy carbonyl stretching). The absorption bands are between 3186.33 and 2970.38 cm^{-1} (aromatic C-H Stretching). The absorption bands are between 1649.48 and 1420.51 cm^{-1} (aromatic C-C Stretching). The intense bands are between 1686.74 and 1490.33 cm^{-1} (aromatic C=N Stretching).

The absorption band at 1534.94 bands is caused by the valency vibration of N-O Stretching. The intense bands between 1249.87 and 1211.30 cm^{-1} (aromatic C-O-C Stretching) are caused by the valency vibration of the C-O-C bond of the methoxy and furan group. The results are summarized in Table: 1.

For ^1H NMR, spectra of the synthesized compounds were found to be consistence with the suggested structures. Furthermore, the number of integrated protons in the spectra matched the expected number of aromatic protons in each case. The spectra showed a broad singlet at δ 14 ppm integrated to one proton most likely assigning to the most deshielded acidic COOH proton. Compound 1c spectra displayed a singlet at δ 2.27 ppm and 2.43 ppm (for the remaining) integrated to three protons corresponding to the $–CH_3$ and $–OCH_3$ protons, respectively. A highly deshielded singlet at 9.95 for compound 1b, corresponding to phenolic OH was observed.

Antimicrobial activity:

Bacterial resistance to existing antibiotics is a worldwide health issue, particularly affecting immunocompromised patients. Without effective antibacterial agents, several medical procedures could endanger the lives of patients by increasing the risk of microbial infections. The basic structure of quinoline derivatives has inspired research to develop new antibacterial agents with improved bio-availability and antibacterial properties.

Quinolones are a special structural class of antimicrobial agents. It is characterized by 2 -

aryl / heteroaryl 4- quinoline derivatives. Extensive SAR has been established on this nucleus and resulted in several currently marketed synthetic antimicrobial agents like ciprofloxacin, ofloxacin, and sparfloxacin, etc. Synthesized 2-aryl / heteroaryl 4- carboxylic acid quinoline derivative compounds 1a-1e have been screened in vitro for their antibacterial activity against Gram +ve (*Staphylococcus aureus, Bacillus subtilis)* and Gram – ve (*Klebsiella pneumoniae, Escherichia coli*) bacteria at 20 µg/ml concentration by the Kirby -Bauer Disc Diffusion Method using dimethylsulfoxide as a solvent and using Ciprofloxacin as a reference. After 24 h of incubation at 37º ±1, the antimicrobial activity was determined by measuring the Zones of inhibition in mm. The results are summarized in Table: 3.

New quinoline-based analogs were synthesized and evaluated for their antibacterial activity. The synthesized compounds' antibacterial activity is mentioned in Table 2. Furan-2-yl (**1a),** 2-hydroxyphenyl (1b), 3-methoxy phenyl (1c), pyridine-2-yl (1d), 4-nitrophenyl (1e) substituted at C_2 in a portion of the A-ring were so to have significant antibacterial activity against active against Gram +ve (*Staphylococcus aureus, Bacillus subtilis)* and Gram – ve (*Klebsiella pneumoniae, Escherichia coli*) bacteria.

CONCLUSION:

Quinoline and its derivatives are known for their wide spectrum of pharmacological activities, several methods have been developed from time to time for their synthesis by conventional, homogeneous, and heterogeneous without catalyzed methods, with catalyzed methods, microwave-assisted, solvent-free conditions, solvent conditions, and many more.

These derivatives synthesized from Doebner reaction are 2-aryl / heteroaryl 4-4-carboxylic acid quinoline derivatives (1a-1e) with an increase in their antibacterial activity. These quinoline derivatives are very useful to researchers working in this field, and it would help them to develop in future new synthetic methods for potent quinoline derivatives with good or enhanced biological activities.

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