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Synthesis and Antimicrobial Activity of Some New Coumarin Incorporated Pyridine-3-carbonitrile Derivatives

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Authors' contributions

This work was carried out in collaboration between all authors. Author AK and PK designed the study, performed the statistical analysis, and wrote the protocol. Author HS wrote the first draft of the manuscript. Author PN helped in antimicrobial study. Author JPJ managed the literature searches. Authors DSS and SP helped in drafting the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Drug resistance causes serious difficulties in the routine therapy for curing common microbial infections. Thus it is very essential to develop new antimicrobial agents which can offer alternative treatments. The development of potent and effective antimicrobial agents is of utmost importance to overcome the emerging multidrug resistance strains of bacteria and fungi. The technique involves Knoevenagel reaction between substituted salicylaldehyde and ethyl acetoacetate in presence of piperidine as catalyst to give 3-acetyl coumarin. The intermediate coumarinyl chalcones was synthesized by condensing with various substituted benzaldehyde in presence of ethanolic KOH. The final synthesized pyridine-3-carbonitrile derivative was prepared upon refluxing with coumarinyl chalcones with malononitrile in presence of ammonium acetate. All the newly synthesized

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compounds were assigned on the basis of IR, ^{1}H NMR and mass spectral data. The finalsynthesized compounds were screened for their antibacterial activity tube dilution method. Most of the compounds showed promising MIC by tube dilution method as compared to standard Cephalosporin.

Keywords: Coumarin; chalcone; pyridine; antibacterial activity.

1. INTRODUCTION

Drug-resistant infections kill more than 700,000 people each year and the number could increase to 10 million per year by 2050 in the world. The menace of antimicrobial resistance could force up to 24million people into extreme poverty. The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agent, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection. [1-3] According to Centers for Disease Control and Prevention (CDC) report, each year more than 2.8 million antibiotic infections occur and more than 35,000 people die as a result of these infections in the United States alone. In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. Therefore, there is need to search new infection-fighting strategies to control microbial infections. [4] One of the four guidelines recommended by CDC to combat antibiotic resistance is promoting the development of new antibiotics and new diagnostic tests for dealing with drug-resistant bacteria. *Staphylococcus aureus* and its drug-resistant variants are among the most common bacterial pathogens. Methicillin-resistant *S. aureus* (MRSA) causes ten-fold more infections than all multidrug resistant Gram-negative bacteria. A special pathogenic feature of *S aureus* is its ability to survive on abiotic and biotic surfaces in a slimy extracellular matrix called biofilm. The bacteria comprising biofilms are more resistant to antibiotics and the host immune system by a variety of mechanisms [5].

Coumarins owe their class name to 'Coumarou', the vernacular name of the tonka bean (DipteryxodorataWild, Fabaceae), from which coumarin itself was isolated in 1820. Coumarin (2*H*-1-Benzopyran-2-one) derivatives belong to one of the most widespread classes of natural compounds. They have been also found to exhibit antitumour, antioxidant, anti-inflammatory, antimicrobial [6] and antidiabetic activities. Antibacterial activities of naturally occurring coumarins have been extensively investigated [7-9]. In addition, renewed interest for coumarin-containing antibiotics such as novobiocin, clorobiocin and coumermycinA produced by a number of Streptomyces species has been aroused by the discovery that these antibiotics are potent inhibitors of bacterial DNA gyrase and topoisomerase IV [10]. The coumarin compounds recently identified as protease and integrase inhibitors have given new impetus in studying the antimicrobial profile [11-12]. Therefore, it is worthwhile to study antimicrobial effect of newly synthesized coumarin derivatives.The antibacterial activity of coumarin and other 45 coumarin derivatives have been tested against strains of *Bacillus cereus* MIP 96016, *Escherichia coli* ATCC 25922,*Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923. Coumarin has fungicidal properties as well. These biological activities make coumarin compounds more attractive and testing as novel therapeutic compounds. Coumarin compounds are a large class of quite important lactones with a fused structure of benzene ring and α-pyrone, and virtually contain conjugated system with rich electron and good charge-transport properties. This kind of rigid fused ring endues coumarin-based derivatives with wide potential applications in the fields of bromatology, material and supramolecular chemistry as well as medicinal chemistry. Specially, coumarin compounds as medicinal drugs have been increasingly attracting special interest due to their potential outstanding contributions in the prevention and treatment of diseases, and the related researches and developments have become an extremely attractive highlight [13]. Coumarin compounds can be synthesized by Pechmann, Knoevenagel, Perkin, Witty condensation methods. Chalcones are versatile molecules used for the synthesis of different heterocyclic compounds like thiazines, pyrazole, oxazine etc. Pyridines and their benzo derivatives contain one nitrogen atom in a six membered aromatic ring. Pyridyl compounds have received interest of organic chemists in recent years owing to their wide spectrum of

physiological activity. Cyanopyridine derivatives have attracted considerable attention in view of their great therapeutic importance as antifungal, antibacterial [14], antiepileptic, anti-tubercular, analgesic, insecticidal, antiallergic and anti-inflammatory activities. Among the heterocyclic-substituted coumarins, pyridine-substituted coumarins constitute an elite class of compounds as they exhibit important biological activities, such as CNS depressant, antifungal, antibacterial, [15] anti-tubercular. Certain bipyridinyl moieties are reported to possess important biological properties, such as antimicrobial[16], antioxidant, cardiotonic, DNA interacting and cytotoxic. It is reported in the literature that when pyridine ring is coupled with coumarins, the biological activity gets enhanced many fold. The challenges of antibacterial research are significant and a good start towards the development of new class of hybrid antimicrobials along with the aid of computer aided drug design may deliver new antimicrobials to the clinic. Based on the above observation it is worthwhile to prepare newer compounds for their antimicrobial activity.

2. MATERIALS AND METHODS

All chemicals that were used are of analytical grade: Salicylaldehyde, ethyl acetoacetate, piperidine, malononitrile, ammonium acetate, ethanol, substituted benzaldehyde and sodium hydroxide**.** Melting points were determined by open capillary method and are uncorrected. Purity of the intermediates and final compounds were monitored by thin layer chromatography (TLC) using silica gel G plates as given in Table 1.The spots were visualized under UV light. n-hexane: Ethylacetate (5:5) was used as solvent for running the TLC of these compounds. All IR spectra were recorded in Alpha Bruker using ATR method. ^{1}H NMR spectra were recorded at 400MHz Bruker Avance II NMR Spectrometer. Mass spectrum was recorded on GC-MS Perkin Elmer Clarus 680 Spectrometer obtained by electro impact ionization method.

2.1 Acetyl Coumarin

A mixture of salicylaldehyde (0.05 mol) and ethylacetoacetate was added to 250ml conical flask. It was then condensed by adding sufficient piperidine dropwise with stirring in ice cold condition. The reaction mixture was then kept overnight in refrigerator. The solid lumps were broken in cold ethanol. The resulting yellow colored solid mass was then filtered and washed with cold ethanol to remove the excess piperidine. It was then recrystallized from ethanol to give white needle shaped crystals.

2.2 Coumarinyl Chalcones

A mixture of 3 acetyl coumarin (0.01 mol) and substituted benzaldehyde (0.01 mol) in 20 ml ethanol were stirred for 10 hrs in presence of 20% NaOH (4-5 ml).The completion of the reaction was monitored by TLC. The reaction mixture was then poured into crushed ice and acidified with 2N HCl with stirring. The product obtained was filtered, washed with water and recrystallized from ethanol.

2.3 Pyridine-3-carbonitrie Derivative (CP1-CP6)

A mixture of chalcone (0.05 moles) and malononitrile in ethanol (20 ml) was refluxed for 4-5 hours in the presence of ammonium acetate. Thereaction mixture was then cooled and added to crushed ice. The product obtained was filtered, dried and recrystallized from ethanol as given in Fig. 1.

2.4 Minimum Inhibitory Concentration

The broth dilution test is one of the standard method for determining the level of resistance to an antibiotic. Serial dilutions of the antibiotic are made in a liquid medium which is inoculated with a standardized number of organisms and incubated for a prescribed time. The lowest concentration of antibiotic preventing appearance of turbidity is considered to be the minimal inhibitory concentration (MIC). After preparation of different concentrations of the test compound in nutrient broth (by using the broth dilution method), we inoculate them with the test organism. The MIC is determined after incubation by choosing the lowest concentration in which no growth occurs. The MIC and the zone of inhibition are inversely correlated. In other words, the more susceptible the microorganism is to the antimicrobial agent, the lower the MIC and the larger the zone of inhibition. Conversely, the more resistant the microorganism, the higher the MIC and the smaller the zone of inhibition. The method gives information on the storage of standard antibiotic powder, preparation of stock antibiotic solution, media, preparation of inocula, incubation condition and reading and interpretation of results [16].

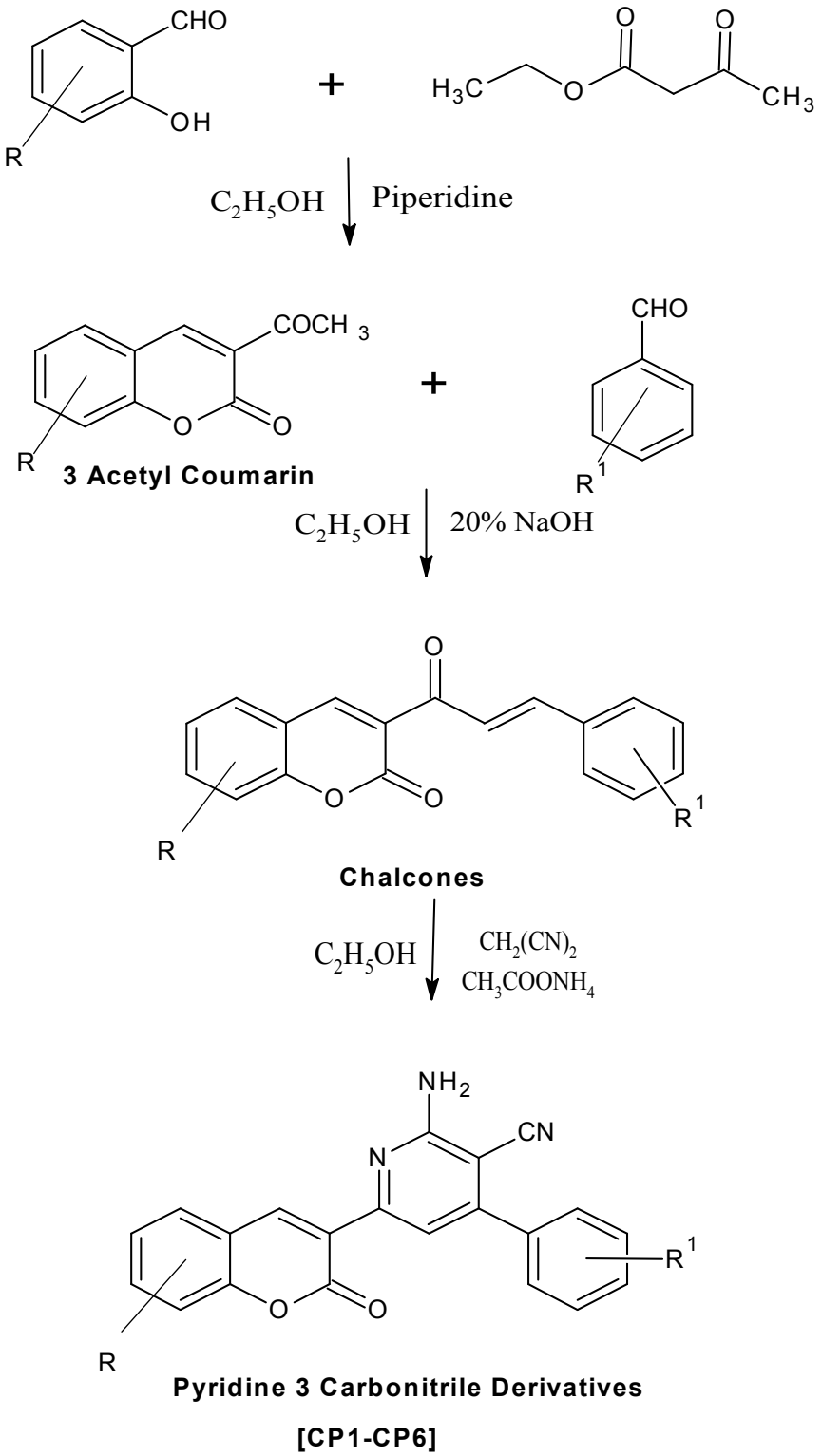


Fig. 1.Scheme for synthesis of pyridine-3-carbonitrile derivatives (CP1-CP6)

2.5 Procedure

Double concentration of the nutrient broth were prepared. Distribute each 2.5 ml into 8 test tubes and label them A1 to A8. Distribute 2.5 ml in two test tubes and label them as positive control and negative control. Prepare drug stock solution of 2000 µg/ml by dissolving the drug in water. From this stock solution the following dilutions were prepared; 2.5 ml of the stock solution diluted to 25 ml with water to give 200 µg/ml. Serial dilution of the same was performed to give 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml and 6.25 µg/ml respectively. Add 2.5 ml each double concentration nutrient broth to 2.5 ml of the above dilutions so that the concentration further gets halved. i.e., 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml and 3.12 µg/ml respectively. Add 2.5 ml of water to positive control and negative control tube and mix well. Mix all the tubes well close with nonabsorbent cotton plugs and sterilize by autoclaving 15 lbs./sq. in (121°C) for 15 min. Cool the tubes to room temperature and inoculate all the tubes with one loopful of the test organism *E.coli*, except in the negative control tube. Incubate all the tube at 37°C for 48 hrs and observe the turbidity.

2.5.1 Negative control

In this no growth is expected. It confirms that the medium is sterile.

2.5.2 Positive control

In this the growth of the inoculated organism is expected. This indicates that (a) The nutrient of medium supports the growth of organism that has been inoculated. (b) Inoculation of live organisms.

3. RESULTS

3.1 Spectral Data

2-amino-4-[4-(dimethylamino)phenyl]-6-(2-oxo-2*H*-1-benzopyran-3-yl)pyridine-3-carbonitrile (CP1)

IR (cm^{-1}): 1516(Ar C=C str), 1608 (C=O str of δ lactone), 3322 (NH_2 str), 2202 (C≡N str), 1256 (C-O str), 1174 (C-N str)
^{1}H NMR (400 MHz, DMSO-d_6):δ 6.878-7.859 (m, 9H, Ar-H), 4.331 (s, 2H, NH_2)
Mass (m/z): 382 (M^+)

2-amino-4-(3,5-dichlorophenyl)-6-(2-oxo-2*H*-1-benzopyran-3-yl)pyridine-3-carbonitrile (CP2)

IR (cm^{-1}):1512 (Ar C=C str), 1556 (C=O str of δ lactone), 3334 (NH_2 str), 1286 (C-O str), 1330 (C-N str), 1647(C=N str), 816 (C-Cl str)
^{1}H NMR (400 MHz, DMSO-d_6):δ 6.978-7.859 (m, 8H, Ar-H), 4.335 (s, 2H, NH_2)
Mass (m/z): 408 (M^+)

2-amino-4-(4-fluorophenyl)-6-(2-oxo-2*H*-1-benzopyran-3-yl)pyridine-3-carbonitrile (CP3)

IR (cm^{-1}):1508 (Ar C=C str), 3567 (NH_2str), 1045 (C-O str), 1272 (C-N str), 1649 (C=N str), 1323(C-F str)
^{1}H NMR (400 MHz, DMSO-d_6):δ 6.987-7.652 (m, 9H, Ar-H), 4.289 (s, 2H, NH_2)
Mass (m/z): 357 (M^+)

2-amino-4-(anthracen-2-yl)-6-(2-oxo-2*H*-chromen-3-yl)nicotinonitrile (CP4)

IR in cm^{-1}:1518 (Ar C=C str), 1541 (C=O str of δ lactone), 3368 (NH_2 str), 1158 (C-O str), 1663(C=N str).
^{1}H NMR (400 MHz, DMSO-d_6):δ7.121-7.992 (m, 14H, Ar-H), 4.225 (s, 2H, NH_2)
Mass (m/z): 439 (M^+)

2-amino-4-(4-bromophenyl)-6-(6-nitro-2-oxo-2*H*-1-benzopyran-3-yl)pyridine-3-carbonitrile (CP5)

IR in cm^{-1}:1506 (Ar C=C str), 1175 (C-O str), 1125 (C-N str), 1674(C=N str), 629 (C-Br str)
^{1}H NMR (400 MHz, DMSO-d_6):δ 6.859-7.823(m, 8H, Ar-H), 4.128 (s, 2H, NH_2)
Mass (m/z): 463(M^+)

2-amino-4-(4-hydroxyphenyl)-6-(6-nitro-2-oxo-2*H*-1-benzopyran-3-yl)pyridine-3-carbonitrile (CP6)

IR in cm^{-1}:1504 (Ar C=C str), 1653(C=O str of δ lactone), 3329 (NH_2 str), 1078 (C-O str), 1647(C=N str), 3385(O-H str phenolic)
^{1}H NMR (400 MHz, DMSO-d_6):δ 6.775-7.673 (m, 8H, Ar-H), 3.331 (s, 2H, NH_2)
Mass (m/z): 400 (M^+)

4. DISCUSSION

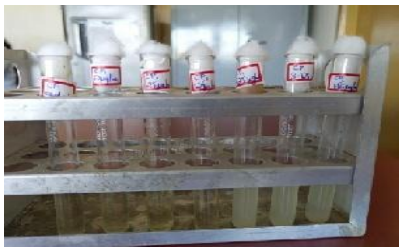
4.1 Chemistry

The yield of the synthesized pyridine-3-Carbonitrile from coumarinyl chalcones were obtained in the range of 69-95%.IR spectra

showed characteristic absorption band 1647 (C=O str of δ lactone) coumarin. The IR spectra of final compound showed characteristic absorption band at 1174 (C-N str of pyridine) and 3322, 2202 corresponding to NH_2 and C≡N group in pyridine which was absent in the intermediate coumarinyl chalcones. Similarly the ^{1}H NMR of the synthesized pyridine-3-carbonitrile derivatives showed one characteristic signal at δ 4.225 (s, 2H, NH_2) which was absent in the ^{1}H NMR spectra of substituted chalcones. Hence the formation of the coumarinyl pyridine was confirmed and further established by ^{1}H NMR and mass spectra which are in accordance with molecular formula.

4.2 Antibacterial Activity

The different Pyridine-3-Carbonitrile derivatives were evaluated for their antibacterial activity by Tube dilution method.The lowest concentration of the substance that prevents the development of visible growth was reported as the MIC value.The newly prepared derivatives have different substituents, and accordingly they can exhibit antimicrobial activity. Therefore, the antimicrobial activity of these derivatives was tested on *Escherichia coli*.The test compounds such as CP1, CP2, CP3, CP4, CP5 and CP6 showed MIC values at 12.5, 25, 25, 50, 50, 25 µg/ml respectively against *Escherichia coli*. Compound CP1 showed significant antibacterial activity with MIC of 12.5µg/ml compared to standard Cephalosporin as given in Table 2 and 3. The presence of electron donating group (dimethylamino group in Phenyl ring) in CP1 resulted in increased antibacterial activity. The presence of electron withdrawing group like fluoro and anthracene moiety in compound CP4 and CP5 resulted in decreased antibacterial activity.The presence of electron withdrawing group like Nitro in Coumarin moiety in CP5 and CP6 resulted in decreased antibacterial activity.



CP1

CP2

CP3

CP4

CP5

CP6

Fig. 2. Minimum Inhibitory concentration of compounds (CP1-CP6) against *Escherichia coli*

Table 1.Physicochemical data of pyridine-3-carbonitrile derivatives (CP1-CP6)

CompoundCode	R	R^1	Molecular formula	Molecular weight	Melting Point (^oC)	R_f value	%Yield
CP1	H	4-$N(CH_3)_2$	$C_{23}H_{18}N_4O_2$	382	250	0.62	80
CP2	H	2,4-Cl	$C_{21}H_{11}Cl_2N_3O_2$	408	268	0.72	78
CP3	H	4-F	$C_{21}H_{12}FN_3O_2$	357	296	0.52	95
CP4	H	9-Anthraldehyde	$C_{29}H_{17}N_3O_2$	439	363	0.68	85
CP5	6-NO_2	4-Br	$C_{21}H_{11}BrN_4O_4$	463	327	0.56	69
CP6	6-NO_2	4-OH	$C_{21}H_{12}N_4O_5$	400	351	0.60	94

Table 2.Antibacterial activity of pyridine-3-carbonitrile derivatives against *Escherichia coli*(CP1-CP6)

Comp Code	100µg/ml	50µg/ml	25µg/ml	12.5µg/ml	6.25µg/ml	3.12µg/ml	1.5µg/ml
CP1	X	X	X	X	✔	✔	✔
CP2	X	X	X	✔	✔	✔	✔
CP3	X	X	X	✔	✔	✔	✔
CP4	X	X	✔	✔	✔	✔	✔
CP5	X	X	✔	✔	✔	✔	✔
CP6	X	X	X	✔	✔	✔	✔

X: No growth/turbidity observed

✔: Growth/turbidity observed

Table 3.Minimum inhibitory concentration of pyridine-3-carbonitrile derivatives (CP1-CP6) by tube dilution method

Comp Code	MIC (µg/ml)
CP1	12.5
CP2	25
CP3	25
CP4	50
CP5	50
CP6	25

5. CONCLUSION

The study reports the successful synthesis of pyridine-3-carbonitrile derivatives from cyclisation of coumarinyl chalcones with moderate yields and most synthesized compounds have shown significant antibacterial activity. Indeed, these compounds possess several biological activities and their structures allow diverse substitutions and draw much interest considering the possibilities of synthesizing new antimicrobial compound.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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