Synthesis and Biological evaluation of Novel Nicotinonitrile derivatives derived from N-Substituted Coumarinyl Chalcones.

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Abstract: This work included synthesis of several new pyridine derivatives (3a-c) by cyclization of Coumarinyl Chalcones (2a-c) with malononitrile in presence of ammonium acetate. Prepared compounds were diagnosed using Melting point, TLC, FT-IR spectroscopy and ¹H-NMR. The synthesized pyridines (3a-c) were screened for their antibacterial and antifungal activity against Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella sp., and Candida albicans. Respectively by diffusion method compounds 3a, 3b and 3c showed good antibacterial activity and by comparison with the medicine Ampicillin. Compounds 3a, 3b and 3c showed moderate antifungal activity compared to the standard drug fluconazole.

Keywords: 2H-chromen-2-one, chalcone, pyridine

1. Introduction

Antimicrobial resistance (AMR) has protruded like one from the leading Health problems from the 21st century that intimidate the efficacious forbidding while treatment from an ever-increasing range from infections give rise to through bacteria, parasites, viruses while fungi no longer susceptible till The usual medications utilized till treat them. Abundant decades in accordance with the first patients were treated for antibiotics , As bacterial infection became a threat again, this crisis has been studied and diagnosed due to the misuse of medicines. Therefore, new drugs have been manufactured to reduce these risks and to provide economic benefits. [1-3]. Coumarin and its derivatives are a compound with high biological activity [4]. Coumarin (1,2H-chromen-2-one or 2H-1-benzopyran-2-one) is a bicyclic heterocycle compound, depending from benzene while 2-pyrone rings. Coumarins are likewise a wide class from natural while organic compounds have proven to be frequently used during pharmacological activities [5], such as anti-inflammatory, antioxidant, hepatoprotective, antithrombotic, antiviral,
antimicrobial, antituberculosis, anti carcinogenic, antihyperlipidemic, monoamine oxidase-B inhibitors and anticholinesterase activities [6-9].

The Pyridine ring is a heterocyclic compound that has great importance over the years [10]. And the ring of pyridine is considered one of the most important compounds that are used in and among medical drugs, cyanopyridines (nicotinonitriles) for diverse alkyl while when the aryl group exists for have antihypertensive [11] antiinflammatory, analgesic, antipyretic properties [12] antimicrobial [13,14], cardiotonic [15] while anticancer activity [16]. Through deeming the up facts while their raising importance during pharmaceutical while biological domain, it was considered from benefit till synthesize several The new prepared chemical compounds included two compounds that have a high effectiveness against bacteria and fungi, by measuring the biological activity of them. The compound included the merging of the Pyridines ring with Coumarinyl Chalcones while till probe how this collection could influence the biological activity. Hence the synthesized compounds were evaluated until their antimicrobial while compared with standard drugs.

![Chemical Structures and Diagrams](image-url)

**Figure 1.** Schematic illustrating the pathway for the synthesis of substituted Nicotinonitrile derivatives.
2. Experimental

Melting points were located through open capillary method while were uncorrected. The purity from the compounds has been tracked through thin layer chromatography (TLC). The spots were visualized by the exposure to iodine vapors. The Fourier-transform infrared spectroscopy spectra (KBr Tablet) were within record within a Pye Unicam Sp-3-300 while a Shimadzu Fourier-transform infrared spectroscopy 8101 PC infrared Optical spectrometer. The \(^1\)H NMR spectra were located for JEOL-JNM-LA 400 while 500 MHz Optical spectrometer. Chemical transformations were represented within the \(\delta\) (ppm) gauge utilizing TMS like the level source.

2.1- 3-acetyl-6-bromo-2H-chromen-2-on [17] (1):

A mixture of 5-bromosalicylaldehyde (2.02gm.,0.01 mole), piperidine (1.00 ml.), and ethyl acetoacetate (1.3g.,0.01 mole) during ethanol (20 ml.) is reflux untill 6 hrs. the completion of The reaction was tracked by TLC. The solid formed was filtrated off, and Where after the compound is deposited, the crystallization is returned with ethanol , pale yellow precipitate, yield 91%, M.P (221-223) °C. The FTIR spectral information displayed assimilation within (1735cm\(^{-1}\), belonging to \(\text{C}=\text{O}, \text{ketone}\), (1676cm\(^{-1}\), Belonging to \(\text{C}=\text{O}, \text{coumarin}\), (1604,1545 cm\(^{-1}\), for \(\text{C}=C, \text{Ar.}\), (3043 cm\(^{-1}\), for \(\text{C}-\text{H}, \text{Ar.}\), (2924 cm\(^{-1}\), for \(\text{C}-\text{H}, \text{alph.}\) and (837 cm\(^{-1}\), for \(\text{C}-\text{Br}\)). \(^1\)H-NMR spectra information displayed signal within 2.3 (s,3H,CH\(_3\)) and 7-8.5(m,4H,Ar-H and C=CH).

2.2 General procedure

3.2.1- 6-bromo-3-(3-(4-substituted phenyl)acryloyl)-2H-chromen-2-one (2a-c)

A solution from compound (1) (13.35gm., 0.05 mole) in ethanol (10 ml), proper aldehydes (4-bromobenzaldehyde, 4-N,N-dimethylaminobenzaldehyde and 5-bromosalicylaldehyde) (0.05 mole) was added during the being from (4-5) drops from piperidine for persistent stirring untill 1 hr. The solution was heated untill 3 hrs., The reaction was tracked monitored through TLC and The solution is cooled. The product was filtered off, and recrystallized from appropriate solvent.

3.2.2 6-bromo-3-(3-(4-bromophenyl)acryloyl)-2H-chromen-2-one (2a)

Red precipitate, yield 65%, M.P (204-206) °C. The FTIR spectral information displayed absorption within (1726 cm\(^{-1}\), belonging to \(\text{C}=\text{O}, \text{chalcone}\), (1672 cm\(^{-1}\), belonging to \(\text{C}=\text{O}, \text{coumarin}\), (1604,1546cm\(^{-1}\), belonging to \(\text{C}=\text{C}, \text{Ar.}\), (3043 cm\(^{-1}\), for \(\text{C}-\text{H}, \text{Ar.}\), (2924 cm\(^{-1}\), for \(\text{C}-\text{H}, \text{alph.}\) and (833 cm\(^{-1}\), for \(\text{C}-\text{Br}\)).

3.2.3- 6-bromo-3-(3-(4-(dimethylamino)phenyl)acryloyl)-2H-chromen-2-one (2b)

Yellow precipitate, yield 71%, M.P (206-209) °C. The FTIR spectral information displayed absorption within (1726 cm\(^{-1}\), belonging to \(\text{C}=\text{O}, \text{chalcone}\), (1670 cm\(^{-1}\), belonging to \(\text{C}=\text{O}, \text{coumarin}\), (1600,1558 cm\(^{-1}\), for \(\text{C}=\text{C}, \text{Ar.}\), (3063 cm\(^{-1}\), for \(\text{C}-\text{H}, \text{Ar.}\) and (833 cm\(^{-1}\), for \(\text{C}-\text{Br}\)).

2.2.4- 6-bromo-3-(3-(5-bromo-2-hydroxyphenyl)acryloyl)-2H-chromen-2-one (2c)

Yellow precipitate, yield 78%, M.P (211-214) °C. The FTIR spectral information displayed absorption within (1710 cm\(^{-1}\), belonging to \(\text{C}=\text{O}, \text{chalcone}\), (1672 cm\(^{-1}\), belonging to \(\text{C}=\text{O}, \text{coumarin}\), (1600,1558 cm\(^{-1}\), for \(\text{C}=\text{C}, \text{Ar.}\), (3063 cm\(^{-1}\), for \(\text{C}-\text{H}, \text{Ar.}\) and (833 cm\(^{-1}\), for \(\text{C}-\text{Br}\)).
coumarin), (1606,1546 cm\(^{-1}\), belonging to \(\nu C=\text{C}, \text{Ar.}\)), (3041 cm\(^{-1}\), belonging to \(\nu C-H, \text{Ar.}\)) and (833 cm\(^{-1}\), belonging to \(\nu C-\text{Br}\)).

2.3 General procedure

2.3.1- 2-amino-6-(6-bromo-2-oxo-2H-chromen-3-yl)-4-(4-substituted phenyl)nicotinonitrile (3a-c)

A solution of Coumarinyl Chalcones (2a-c) (0.005 mole) during ethanol (10 ml.),malononitrile (0.005 mole), ammonium acetate (,0.04 mole) have been added [13]. The reaction mixture was refluxed until 2hrs., the completion from the reaction has been monitored by TLC. Be the precipitate was filtered off then washing with water, dried while recrystallized from ethanol.

2.3.2 - 2-amino-6-(6-bromo-2-oxo-2H-chromen-3-yl)-4-(4-bromophenyl)nicotinonitrile (3a)

Pale yellow precipitate, yield 63%, M.P (266-268) °C. Fourier-transform infrared spectroscopy spectral information displayed absorption within (1666 cm\(^{-1}\), belonging to \(\nu C=O\), coumarin), (1606,1541 cm\(^{-1}\), for \(\nu C=\text{C}, \text{Ar.}\)), (3057 cm\(^{-1}\), for \(\nu C-H, \text{Ar.}\)), (2214 cm\(^{-1}\), for \(\nu CN\) ),(3444 , 3360 for \(\nu NH_2\)) and (831 cm\(^{-1}\), belonging to \(\nu C-\text{Br}\)). \(^1\)H-NMR spectra information displayed signal at 6.8 (s,2H,NH\(_2\)) while 7.3-8.9(m,9H,Ar-H and C=CH).

2.3.3 - 2-amino-6-(6-bromo-2-oxo-2H-chromen-3-yl)-4-(4-(dimethylaminophenyl) nicotinonitrile (3b)

Pale yellow precipitate, yield 69%, M.P (244-247) °C. Fourier-transform infrared spectroscopy spectral information displayed absorption within (1651 cm\(^{-1}\), belonging to \(\nu C=O\), coumarin), (1597,1548 cm\(^{-1}\), for \(\nu C=\text{C}, \text{Ar.}\)), (3178 cm\(^{-1}\), for \(\nu C-H, \text{Ar.}\)), (2922, 2852 cm\(^{-1}\), for \(\nu C-H, \text{Alph.}\)), (2208 cm\(^{-1}\), for \(\nu CN\) ),(3458, 3346 for \(\nu NH_2\)) and (802 cm\(^{-1}\), belonging to \(\nu C-\text{Br}\)). \(^1\)H-NMR spectra information displayed signal at 3 (s,6H,2CH\(_3\)), 6.7 (s,2H,NH\(_2\))  while 7.1-8.5(m,9H,Ar-H and C=CH).

2.3.4-2-amino-6-(6-bromo-2-oxo-2H-chromen-3-yl)-4-(5-bromo-2-hydroxyphenyl) nicotinonitrile (3c)

Pale yellow precipitate, yield 71%, M.P (220-223) °C. Fourier-transform infrared spectroscopy spectral information displayed absorption within (1653 cm\(^{-1}\), belonging to \(\nu C=O\), coumarin), (1602,1556 cm\(^{-1}\), for \(\nu C=\text{C}, \text{Ar.}\)), (3053cm\(^{-1}\), for \(\nu C-H, \text{Ar.}\)), (2200 cm\(^{-1}\), for \(\nu CN\) ),(3444 , 3225 for \(\nu NH_2\)) and (812 cm\(^{-1}\), belonging to \(\nu C-\text{Br}\)). \(^1\)H-NMR information displayed showed signal at 6.9 (s,2H,NH\(_2\)), 7-8.6(m,8H,Ar-H and C=CH) and 9(s,1H,OH).
Figure 2. Fourier-transform infrared spectroscopy spectrum from compound (1).

Figure 3. Fourier-transform infrared spectroscopy spectrum from compound (2a).
2.4 Biological activity assay

Antibacterial activity from the Nicotinonitrile derivatives compounds [3a, 3b and 3c] have been tried for four types of bacteria, *(Staphylococcus aureus, Staphylococcus epidermidis)* gram positive while *(E. coli, Klebsiella sp)* gram negative utilizing nutrient agar waist viawell diffusion method [18]. Prepared derivatives were tried during different concentrations 1μg while 10 μg during 100 ml from DMSO. The results have been installed within MIC (minimal inhibitory concentration), while these prepared compounds showed more high biological activity.
than the drug (Ampicillin while fluconazole). The results of the biological activity are shown during Table 2.

**Figure 6.** Inhibition zone of the compound (3a) on the gram positive bacteria (*S.aureus* at conc. 10 µg/mL).

**Figure 7.** Inhibition zone of the compound (3c) on the gram negative bacteria (*Klebsiella sp.* at conc. 10 µg/mL).
Figure 8. Inhibition zone of the compound (3a-c) on the *Candida albicans* at conc. 10 and 1 µg/mL).

Table 1: Physical properties from synthesized derivatives.

| No. | M.F              | M.W gm/mol | M.P °C | Yield | Rf (7:3) Hexane.: Eth.acetate | Color  |
|-----|------------------|------------|--------|-------|-------------------------------|--------|
| 1   | C_{11}H_{7}O_{3}Br | 267        | 221-223| 91    | -                             | pale yellow |
| 2a  | C_{18}H_{10}O_{3}Br | 434        | 204-206| 65    | 0.76                          | red     |
| 2b  | C_{20}H_{6}NO_{3}Br | 408        | 206-209| 71    | 0.7                           | yellow  |
| 2c  | C_{18}H_{10}O_{4}Br | 450        | 211-214| 78    | 0.68                          | yellow  |
| 3a  | C_{21}H_{11}N_{3}O_{2}Br | 497   | 266-268| 63    | 0.6                           | pale yellow |
| 3b  | C_{23}H_{17}N_{4}O_{2}Br | 461   | 244-247| 69    | 0.56                          | pale yellow |
| 3c  | C_{21}H_{11}N_{3}O_{3}Br | 513   | 220-223| 71    | 0.62                          | pale yellow |

Table 2. Antimicrobial Activity from the some Synthesized compounds (3a-c).

| Comp. No. | Gram positive | Gram negative | Candida albicans |  |
|-----------|---------------|---------------|------------------|---|
|           | S. aureus     | Streptococcus epidermidis | E. coli | Klebsiella sp. |  |
| 10 µg/mL  | 1 µg/mL       | 10 µg/mL      | 1 µg/mL         | 10 µg/mL | 1 µg/mL |
| 3a        | 10            | -             | -               | -       | 21      | 18      |
| 3b        | -             | 11            | 10              | -       | 16      | 20      |
| 3c        | -             | -             | 8               | 20      | 23      | 19      |
| Ampicillin| 12            | 11            | 12              | 19      | 17      | -       |
3. Results and Discussion

3.1 Chemistry

6-bromo-3-(3-(4-substituted phenyl)acryloyl)-2H-chromen-2-one (2a-c) in general contain an enone (an α,β-unsaturated carbonyl) be effective nucleophilic center. The active center in the chalcone (2a-c) compounds may interact with an electrophilic compound, thus forming the ring of pyridine (3a-c) (figure 8).

![Chemical Structure](image)

**Figure 9.** Synthetic approach of pyridine derivitives (3a-c)

2-amino-6-(6-bromo-2-oxo-2H-chromen-3-yl)-4-(4-bromophenyl)nicotinonitrile (3a-c) were synthesized approving for the reported method [19, 20] through condensation from 6-bromo-3-(3-(4-substituted phenyl)acryloyl)-2H-chromen-2-one (2a-c) with malononitrile.

The IR spectrum from Nicotinonitrile 3a indicated the being from NH\(_2\) (a medium assimilation band) during the zone 3444, 3360 cm\(^{-1}\) while CN sharp absorption within 2214 cm\(^{-1}\) during addendum till the coumarin carbonyl assimilation band within 1666 cm\(^{-1}\).

Its \(^1\)H NMR displayed within 7.3-8.8 ppm belonging to aromatic protons while C=CH, the Nicotinonitrile derivative proton manifested like a one within 6.6 ppm during addendum till NH\(_2\).
3.2 Antimicrobial activity

The during vitro antibacterial while antifungal activity from the synthesized compounds were determined via utilizing diffusion method [4,18]. The results from antibacterial while antifungal activity from newly synthesized compounds are communicated against \textit{staphylococcus aureus}, \textit{Staphylococcus epidermidis}, \textit{E. coli}, \textit{Klebsiella sp} while \textit{fungi Candida albicans}. Compound 3c displayed perfect antibacterial activity against gram –ve bacterial while compound 3b displayed perfect antibacterial activity versus gram +ve bacterial respectivley liken till the medicine used medically Ampicillin. Compound 3c displayed perfect antifungal activity against \textit{Candida albicans} compared till the the drug used medically fluconazole [21]. The outcomes from the antimicrobial activity are concise during Table 2. The antimicrobial activities from several from (3a-c) compounds were identified via the agar diffusion method. Prepared concentrations (10 and 1 µg/mL) were utilized approving till a amended Kirby-Bauer’s How to spread the dish. Concentrations were placed in sterile dishes 10 and 1 µg/disc from the prepared concentrations. Each of the prepared compounds was tested during duplicate. The Dimethyl sulfoxide was utilized like an ineffective solvent while the zone from inhibition (in mm) belonging to prepared concentrations for that you gived via the the drug used medically. Three from the 3a, 3b and 3c compounds were identified. These prepared compounds showed antifungal activity in comparison with the antifungal drug utilized (fluconazole) While other compounds showed moderate effectiveness against fungi till minimal antifungal activity [21]. Despite the explanations for the organic compounds, its effectiveness and inefficiency against fungi are not understood, but the results can be explained by the appearance and non-emergence of activity against fungi as a whole.

- Effectiveness can be explained belonging to the compounds 3a–b. During the existent study, variety electron withdrawing while electron donating groups attached till aromatic ring like the totals are related till phenyl group.
- The activity against microbes showed that the results of inhibition from all where the vehicles have different effective ranges (20–23 mm and 17–19 mm) from antifungal activities against all the tested microbial strains.
- The electron donating hydroxyl group while dimethyl amine group of phenyl ring during 3b–c It is an effective activity against fungi and becteria.
- The electron withdrawing pyridine ring during 3a Give the average activity.

Conclusion

The results of the biological activity showed that the new compounds that were prepared that include the Bromine ring have effectiveness against bacteria that include Gram-negative bacteria and Gram-positive bacteria. This activity is due to the presence of the Bordine ring and the effective groups associated with the Borden ring and the most important groups are the bromine atom associated with the compound that promoted the effectiveness of associated compounds.
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