Medicinal Potential of Pyrazine-Substituted Cinnolo-Condensed Compounds: Synthesis, Spectral Characterization, and Biological Evaluation

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Abstract: This study reports the synthesis of a series of substituted 4(-5-amino-pyrazine) cinnoline-3-carboxamide derivatives (11a-j) via a multi-step reaction scheme. The synthesized compounds were characterized using spectroscopic techniques, including IR, ^{1}H NMR, and mass spectrometry. Biological evaluation revealed that several compounds, particularly those with chloro substitutions (11c, 11d, 11 g, and 11i), exhibited potent antimicrobial activity against both bacterial and fungal strains compared to standard drugs. These compounds also demonstrated significant anthelmintic effects against earthworms, comparable to those of the reference drug, mebendazole. Additionally, selected chloro-substituted derivatives (11c and 11i) exhibited promising anti-inflammatory activity in animal studies. Structure-activity relationship analysis indicated that halogen substitutions, especially with chloro groups, enhanced the biological activities of this class of compounds. These findings suggest that the novel pyrazine-substituted cinnoline derivatives represent a promising scaffold for developing new antimicrobial, anthelmintic, and anti-inflammatory agents. Further studies are warranted to optimize the lead compounds and evaluate their therapeutic potential.

KEYWORDS:
Pyrazine-substituted cinnoline derivatives, Anthelmintic effects, Anti-inflammatory agents, Multi-step synthesis

INTRODUCTION:

Heterocyclic compounds have been a fundamental component of medicinal chemistry for decades and play a pivotal role in drug discovery and development. These versatile molecules, characterized by cyclic structures containing one or more heteroatoms, have been instrumental in the development of a diverse array of therapeutic agents across various disease areas[1].

Among the extensive range of heterocyclic compounds, cinnoline and its derivatives have shown diverse biological activities and potential applications in pharmaceutical sciences[2,3].

Cinnoline, a benzofused heterocycle containing two nitrogen atoms, was first synthesized in 1883 by von Richter. This discovery initiated a rich history of research and exploration of the properties and potential applications of cinnoline-based compounds[4]. The structural versatility of cinnoline facilitates numerous modifications, rendering it an attractive scaffold for medicinal chemists designing and developing novel bioactive molecules. The significance of heterocyclic compounds in drug discovery is paramount and cannot be overstated. These molecules are crucial for developing antibiotics, antiviral drugs, and anticancer treatments, among numerous other therapeutic classes. The unique electronic properties and structural features of heterocycles, including cinnoline, render them invaluable scaffolds for designing bioactive molecules with enhanced efficacy and reduced side effects. This has led to the ongoing exploration of new heterocyclic systems and their derivatives in the pursuit of more effective and safer drugs[5-7].

Pyrazine is another heterocyclic compound that has emerged as a promising moiety in medicinal chemistry. The incorporation of these elements into various molecular frameworks has led to the discovery of compounds with remarkable biological activities. Pyrazine-substituted compounds have demonstrated potential in antimicrobial, antitumor, and anti-inflammatory therapies[8]. The presence of two nitrogen atoms in the pyrazine ring confers unique electronic properties that can significantly influence the interaction of the compound with biological targets. The future scope of pyrazine derivatives in drug discovery is extensive, with ongoing research focusing on optimizing their pharmacological properties and exploring their novel applications. Scientists are investigating the potential of pyrazine-based compounds for treating neurodegenerative disorders and metabolic diseases, and as diagnostic tools in medical imaging. The versatility of pyrazine as a building block in medicinal chemistry continues to drive innovations in drug design and development[9,10].

The biological activities of pyrazine-substituted cinnolo-condensed compounds are of particular interest. These hybrid molecules combine the structural features of both cinnoline and pyrazine, potentially synergizing their biological properties[6]. The fusion of these two heterocyclic systems creates a unique molecular architecture that can interact with biological targets in novel ways, thereby opening new avenues for drug discovery. Recent studies have demonstrated that these compounds exhibit a wide range of activities, including antibacterial, antifungal, and anticancer properties. The antibacterial activity of these hybrid molecules is particularly noteworthy, given the growing concern of antibiotic resistance. Some pyrazine-substituted cinnolo-condensed compounds have shown promising results against multidrug-resistant bacterial strains, offering the potential for the development of new classes of antibiotics. The dual biological evaluation of these compounds provides insights into their mechanism of action and aids in identifying lead compounds for further development. By assessing their activity against multiple biological targets, researchers can gain a more comprehensive understanding of the potential therapeutic applications of these compounds and any possible off-target effects. This approach aligns with the modern paradigm of

polypharmacology, which recognizes that many effective drugs act on multiple targets rather than just one[7-10].

This study focuses on the synthesis, spectral characterization, and dual biological evaluation of novel pyrazine-substituted cinnolo-condensed compounds. The synthetic strategies employed to create these hybrid molecules often involve multi-step processes that require careful optimization to achieve good yields and purity. Modern synthetic methodologies, including microwave-assisted synthesis and flow chemistry, are increasingly being applied to streamline the production of complex heterocyclic systems. The spectral characterization of the synthesized compounds is crucial for confirming their structure and purity. Techniques like nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry, and X-ray crystallography elucidate structures, and bond in between structure and activity. Advanced spectroscopic methods, including two-dimensional (2D) NMR techniques, are often employed to fully characterize these complex molecules. This study seeks to enhance the understanding of heterocyclic chemistry and its role in drug discovery by investigating the medicinal potential of these hybrid molecules. It involves the design and synthesis of a series of compounds, their detailed spectral analysis, and the assessment of their biological activities against selected targets. This multidisciplinary approach synthesizes elements of synthetic organic chemistry, analytical chemistry, and biological screening to provide a comprehensive understanding of the potential of these novel compounds. The findings presented in this study may facilitate the novel therapeutic agents for improved safety profiles. The structure-activity relationships identified herein can inform future medicinal chemistry endeavours, enabling the rational design of next-generation compounds with optimized properties. Furthermore, the insights gained from this study may extend beyond the specific compounds studied, potentially informing broader strategies in heterocyclic chemistry and drug discovery[11-15].

In conclusion, the exploration of pyrazine-substituted cinnolo-condensed compounds represents a promising frontier in the field of medicinal chemistry. By leveraging the unique properties of these hybrid heterocyclic systems, researchers have opened new possibilities for addressing unmet medical needs and developing innovative therapeutic solutions. As this field continues to evolve, it has the potential to significantly impact drug discovery and development.

EXPERIMENTAL METHODOLOGY

The methodology used for the synthesis of substituted condensed cinnolo pyrazine series is as follows **(figure 1):**

1. Preparation of substituted hydrazono (cyano) acetamide: (4a -j)

[R: a = o-NO_2**, b** = p-NO_2**, c** = p- Cl**, d** = p- Br**, e** = 3,4-di-nitro**, f** = 2-Me, **g** = 3- Chloro**, h** = 2- Fluoro**, i** = 2,3 di Chloro**, j** = 3- Nitro **]**

The substituted aniline (0.195 mol) was dissolved in a mixture of conc. HCl (7.5 ml) and water (7.5 ml) and cooled to 0-5°C in an ice bath. To this, a cold saturated solution of sodium nitrite (0.19 mol) was added slowly. Soon after the addition, fumes of nitrous acid were liberated, and a pinch of sulfamic acid/thiourea was added and stirred until the fumes ceased. The diazonium salt thus formed was filtered into a cooled solution of cyanoacetamide (0.195 mol) in water (350 ml),10 gm CH_3COONa, and 15 ml alcohol. The mixture was stirred for 6 h at room temperature, and the resulting solid was collected and recrystallized from methanol.

2. Substituted aniline 4-amino cinnoline 3-carboxamide: (5a -j)

Chlorobenzene (150 ml) was added to anhydrous $AlCl_3$ (0.111 mol), and nitrogen gas passed for half an hour. This mixture was added to the substituted phenyl hydrazono cyano acetamide, and nitrogen was bubbled through it for 10 min. The mixture was then refluxed for 2 h. The solution was cooled, and dilute HCl (20 ml) was added. It was then heated in a water bath, cooled, filtered, washed twice with dilute NaOH solution, and filtered. The product was recrystallized from methanol and water (10:1).

3. Preparation of substituted 4-(-5-amino-pyrazine)-cinnoline-3-carboxamide (11a-j): The substituted 4-amino cinnoline-3-carboxamide **(5a-j)** and 2-chloro pyrazine in DMF were refluxed for 2 hours and poured into crushed ice. The prepared precipitate was then obtained filtered, dried, and recrystallized in methanol.

BIOLOGICAL EVALUATION

1. Anti-bacterial activity studies

A. Disk diffusion method

1. Prepare Mueller-Hinton agar plates and allow to solidify.
2. Prepare bacterial suspensions of test organisms (e.g. *S. aureus*, *E. coli*) adjusted to 0.5 McFarland standard.
3. Inoculate the agar plates with the bacterial suspensions using a sterile swab to create a lawn of growth.
4. Prepare stock solutions of the cinnoline derivative compounds at 1 mg/mL concentration in DMSO.
5. Soak sterile 6 mm filter paper discs in the compound solutions for 1 minute.
6. Place the impregnated discs on the inoculated agar plates using sterile forceps. Include discs with standard antibiotics as positive controls and DMSO as negative control.
7. Incubate at 37°C for 18-24 hours.
8. Measure the diameter of inhibition zones around each disc in mm.
9. Interpret results by comparing zone sizes to standard breakpoints. Larger zones indicate greater antibacterial activity.
10. Repeated the assay in triplicate and calculated the mean zone diameters for each compound tested.

This method allows for screening of multiple cinnoline derivatives against different bacterial strains to assess their antibacterial potential. The zone sizes provide a quantitative measure of activity that can be used to compare compounds[16-19].

B. MIC (Minimum Inhibitory Concentration)

2. Antifungal Activity Studies

A. Disk diffusion method

1. Prepare Sabouraud Dextrose Agar (SDA) plates and allow them to solidify.
2. Inoculate the fungal strains onto the SDA plates using a sterile swab to create a uniform lawn.
3. Prepare stock solutions of the cinnoline compounds in DMSO at desired concentrations.
4. Soak sterile filter paper disks (6 mm diameter) with the prepared cinnoline compound solutions.
5. Allow the disks to dry completely at room temperature.
6. Place the impregnated disks onto the inoculated SDA plates using sterile forceps.
7. Include positive control disks with standard antifungal agents and negative control disks with DMSO.
8. Incubate the plates at 28°C for 24-48 hours, depending on the fungal species.
9. Measure the diameter of the inhibition zones around each disk in millimeters.
10. Record and analyze the results, comparing the antifungal activity of the cinnoline compounds to the controls.
11. Repeat the experiment in triplicate to ensure reproducibility.
12. Calculate the mean inhibition zone diameters and standard deviations for each compound tested[20-23].

B. Determination of MIC using the tube dilution technique

1. Prepare a stock solution of the cinnoline derivative at a known concentration.
2. Create a series of test tubes containing sterile nutrient broth.
3. Perform serial dilutions of the cinnoline derivative in the test tubes, typically using two-fold dilutions.
4. Prepare a standardized bacterial inoculum (usually 10^5 - 10^6 CFU/mL) from a fresh culture.
5. Add equal volumes of the bacterial inoculum to each test tube containing the diluted cinnoline derivative.
6. Include positive control (broth + bacteria, no cinnoline derivative) and negative control (broth only) tubes.
7. Incubate all tubes at appropriate temperature (usually 35-37°C) for 16-20 hours.
8. After incubation, visually inspect tubes for bacterial growth (turbidity).
9. Determine the MIC as the lowest concentration of cinnoline derivative that completely inhibits visible bacterial growth.
10. Confirm results by plating samples from clear tubes onto agar plates to verify absence of viable bacteria.
11. Record MIC values and repeat the experiment in triplicate for statistical validity.

12. Analyse data and report MIC values in µg/mL or mg/L for each tested cinnoline derivative[24,25].

3. Anti-helminthic Activity

The procedure for evaluating the antihelminthic activity of cinnoline derivative compounds involves a series of steps designed to assess their effectiveness against adult helminths. Initially, adult helminths such as Ascaris suum or Haemonchus contortus are obtained from infected animals or laboratory cultures. A stock solution of the cinnoline derivative compound is prepared at a known concentration, followed by the creation of a series of petri dishes or multi-well plates containing a suitable physiological solution for maintaining helminth viability. Serial dilutions of the cinnoline derivative are performed in these containers, and a predetermined number of adult worms (usually 5-10) are placed into each. Positive control (known antihelminthic drug) and negative control (physiological solution only) groups are included for comparison. The containers are then incubated at an appropriate temperature, typically 37°C, for 24-48 hours. Throughout the incubation period, the worms are observed at regular intervals for motility, mortality, and any morphological changes. The number of dead or immobilized worms in each container is recorded, and the percentage of worm mortality or paralysis is calculated for each concentration of the cinnoline derivative. LC50 (lethal concentration that kills 50% of the worms) and/or EC50 (effective concentration that paralyzes 50% of the worms) values are determined. Additionally, microscopic examination of treated worms is conducted to assess any structural damage or changes. To ensure reliability and reproducibility, the experiment is typically performed in triplicate[26,27].

Data analysis

The data were subjected to analysis of variance (ANOVA) as per statistical methods using the SPSS (1996) software package.

RESULTS

Substitutes Cinnoline Pyrazine Series (11a – 11j)

Preliminary Analysis of the sample:

Thin-layer chromatography (TLC) is commonly used for the qualitative description of the complexity and composition of chemical mixtures.

Application of the sample on TLC plates:

- The sample was applied to the chromatogram by repeated “spotting” above 1-2 cm from one end of the plate with a capillary tube.
- The most important precaution was not to apply spots below the level of the top of the solvent system in the developing chamber.

Development of solvent systems:

A development chamber was used to develop the chromatogram. Ethyl acetate and alcohol chloroform (1:2:1) solvent system was used for running TLC of these compounds.

Visualization of the chromatogram:

After development, the TLC plates were dried and then exposed to iodine vapors in a chamber, as chromatograms of many synthetic products were frequently observed by iodine vapors. The Rf value was noted. The purity of all the synthesized compounds, including intermediates, was checked by TLC on silica gel G plates (Merck India Ltd). All compounds showed only a single spot, indicating the completion of the reaction and the purity of the obtained product.

RESULTS OF SCHEME 11(a –j)

Figure 2

The synthesis of substituted cinnoline pyrazine derivatives by the method described above resulted in products with good yields **(Figure 3).**

Table I. Physical data of substituted 4(-5-amino-pyrazine) cinnoline-3-carboxamide derivatives: 11(a–j)

Sl. No.	**Comp. No**	**Physical nature**	**M.P(°C)**	**Yield (%)**
8-Nitro-4(-5-amino-Pyrazine) cinnoline-3-carboxamide	**11DSD$_a$**	Dark Yellow crystals	204-206°C	75.43%
6-Nitro-4(-5-amino-Pyrazine) cinnoline-3-carboxamide	**11DSD$_b$**	Reddish brown crystals	114-116°C	51.44%
6-Chloro-4(-5-amino-Pyrazine) cinnoline-3-carboxamide	**11DSD$_c$**	Pale Yellow crystals	196-188°C	64.23%
6-Bromo-4(-5-amino-Pyrazine) cinnoline-3-carboxamide	**11DSD$_d$**	Light orange crystals	178-180°C	42.56%
6,7-dinitro-4(-5-amino-Pyrazine) cinnoline-3-carboxamide	**11DSD$_e$**	Off white crystals	167-169°C	53.12%
8-Methyl-4(-5-amino-Pyrazine) cinnoline-3-carboxamide	**11DSD$_f$**	Dark green crystals	192-194°C	32.45%
7-Chloro-4(-5-amino-Pyrazine) cinnoline-3-carboxamide	**11DSD$_g$**	Golden brown crystals	175-177°C	67.34%
8-Fluoro-4(-5-amino-Pyrazine) cinnoline-3-carboxamide	**11DSD$_h$**	Light brown crystals	184-185°C	71.65%
7,8- Dichloro-4(-5-amino-Pyrazine) cinnoline-3-carboxamide	**11DSD$_i$**	Reddish white crystals	177-179°C	45.32%

7-Nitro-1H-Cinnoline-4(-5-amino-Pyrazine) cinnoline-3-carboxamide	**11DSD$_j$**	Dark orange Crystals	180-182°C	73.29%

RESULTS OF BIOLOGICAL EVALUATION (SCHEME - 11A-J)

1.1 Anti-bacterial activity studies

1.1. (A) Disk Diffusion Method

Table II (A) Data for anti-bacterial activities of synthesized compounds

Sl.No.	**Compound No.**	**Diameter of zone of inhibition (mm)**			
		P. aeruginosa	***E. coli***	***B.subtilis***	***S. aureus***
01	11DSDa	12	13	14	12
02	11DSDb	15	11	17	15
03	11DSDc	20	20	20	21
04	11DSDd	19	19	19	20
05	11DSDe	14	14	12	17
06	11DSDf	15	12	13	11
07	11DSDg	19	21	18	19
08	11DSDh	17	18	15	13
09	11DSDi	20	19	19	20
10	11DSDj	15	13	16	11
11 12	Norfloxacin (10µg) THF	21 0	23 0	24 0	22 0

All the synthesized substituted cinnoline Pyrazine derivatives were tested at 50µg level and shown moderate to good anti-bacterial activity, among the tested compounds **11DSDc, 11DSDd, 11DSDg and 11DSDi** showed significant activity while other compounds showed moderate activity in comparison with the standard drug norfloxacin.

1.1. (B) MIC (Minimum Inhibitory Concentration)

Table III. (B) Data of MIC for anti-bacterial activity

S.No.	Compd.No. ↓	*S.aureus*						*B.subtilis*						*E.coli*						*P.aeruglnosa*					
	Dilution ®	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
1	11DSDa	-	-	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+	-	-	-	+	+	+
2	11DSDb	-	-	-	+	+	+	-	-	-	+	+	+	-	-	+	+	+	+	-	-	-	+	+	+
3	11DSDc	-	-	-	-	+	+	-	-	-	+	+	+	-	-	-	-	+	+	-	-	-	-	+	+
4	11DSDd	-	-	-	+	+	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	+	+
5	11DSDe	-	-	+	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+
6	11DSDf	-	-	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	+	-	-	-	+	+	+
7	11DSDg	-	-	-	-	+	+	-	-	-	+	+	+	-	-	-	-	+	+	-	-	-	+	+	+
8	11DSDh	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	-	+	+	-	-	-	+	+	+
9	11DSDi	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	+	+	+	-	-	-	-	+	+
10	11DSDj	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	+	+	+	+
11	Norfloxacin	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	+	+	+
12	+ve control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13	-ve control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

'+' indicates the presence of growth and '–' indicates the absence of growth.

Table IV: The concentrations of the derivatives at different dilutions are as follows

Dilution	1	2	3	4	5	6
Conc. mg/ml	1000	500	250	125	62.5	31.25

The derivatives **11DSDc, 11DSDd, 11DSDg**, **and 11DSDi** exhibited higher toxicity, warranting further toxicity studies.

1.2 Antifungal Activity Studies

1.2 (A) Disk diffusion method

Table V: Data for antifungal activity of synthesized compounds

Sl. No.	***Compound No.***	**Diameter of zone of inhibition (mm)**	
		C. albicans	***A. niger***
1.	11DSDa	12	14
2.	11DSDb	15	16
3.	11DSDc	19	20
4.	11DSDd	20	20
5.	11DSDe	13	14
6.	11DSDf	18	14
7.	11DSDg	21	21
8.	11DSDh	18	17
9.	11DSDi	21	21
10.	11DSDj	13	15
11.	Griseofulvin (25µg)	23	24
12.	THF	0	0

All Substituted Cinnoline Pyrazine derivatives were tested at 50µg level. The antifungal activity studies revealed that the synthesized compounds exhibited moderate to good antifungal activity. Among the tested compounds, 11DSDc, 11DSDd, 11DSDg, and 11DSDi exhibited good activity against *C. albicans* and *A. niger*. Other compounds showed weak antifungal activity compared to the standard drug, griseofulvin.

1.2 (B) MIC (Minimum Inhibitory Concentration)

Table VI: Data of MIC for antifungal activity.

Sl.No.	**Compound No. ↓**	**Presence or absence of growth**											
		C. albicans						***A. niger***					
	Dilution→	**1**	**2**	*3*	**4**	**5**	**6**	**1**	**2**	*3*	**4**	**5**	**6**
01	$11DSD_a$	-	-	+	+	+	+	-	-	+	+	+	+
02	$11DSD_b$	-	-	-	+	+	+	-	-	-	+	+	+
03	$11DSD_c$	-	-	-	-	+	+	-	-	-	-	+	+
04	$11DSD_d$	-	-	-	+	+	+	-	-	-	+	+	+
05	$11DSD_e$	-	-	+	+	+	+	-	-	-	-	+	+
06	$11DSD_f$	-	-	-	+	+	+	-	-	+	+	+	+
07	$11DSD_g$	-	-	-	-	+	+	-	-	-	-	+	+

08	$11DSD_h$	-	-	-	+	+	+	-	-	-	+	+	+
09	$11DSD_i$	-	-	-	-	+	+	-	-	-	-	+	+
10	$11DSD_j$	-	-	+	+	+	+	-	-	-	+	+	+
11	+ve control	+	+	+	+	+	+	+	+	+	+	+	+
12	-ve control	-	-	-	-	-	-	-	-	-	-	-	-

'+' Indicate presence of growth and '–' indicate absence of growth

All the Synthesized compounds exhibited antifungal activity to a certain extent. Among the tested compounds**, 11DSDc, 11DSDg**, and **11DSDi** exhibited good antifungal activity against *C. albicans* and *A. niger*. The other compounds exhibited moderate activity.

RESULTS

Anthelmintic Assay:

The anthelmintic assay was performed as per the method described by Garg and Atal (1963)66 with minor modifications. The assay was performed on adult Indian earthworms (*Pheretima posthuma*) because of their anatomical and physiological resemblance to the intestinal roundworms of humans and animals. Five earthworms of similar sizes were placed in a Petri plate of 4 inches diameter containing 50 ml of suspension of the test standard drug mebendazole at room temperature. Another set of five earthworms was kept as a control in a 50 ml suspension of distilled water and 12.5% Tween 80. Fifty milliliters of each test compound suspension were added to separate Petri plates containing five earthworms each. The time required for paralysis and death of the worms was recorded. The death time was ascertained by placing the earthworms in warm water at 50°C, which stimulated the movement if the worm was alive.

Table VII: Anthelmintic activity of substituted Cinnoline Pyrazine derivatives

Test Samples	**Concentration (mg)**	**Mean paralysing time (min) ±S.E.**	**Mean death time (min) ± S.E.**
Control	-	No effect	No effect
Mebendazole	100	4.12±0.16	5.30±0.40
11DSDa	100	4.34±0.43**	5.48±0.21*
11DSDb	100	4.23±0.48**	5.60±0.62**
11DSDc	100	4.15±0.21**	5.34±0.42**
11DSDd	100	4.40±0.35**	5.37±0.12**
11DSDe	100	4.31±0.32**	5.56±0.63**
11DSDf	100	4.33±0.58**	5.44±0.57**
11DSDg	100	4.17±0.74***	5.37±0.26***
11DSDh	100	4.45±0.20**	6.02±0.74**
11DSDi	100	4.14±0.24**	5.36±0.35***
11DSDj	100	4.56±0.50**	5.58±0.45**

'S.E.' represents Standard Error. Values are significantly different from the reference standard (mebendazole) *p<0.05; ** p< 0.01, *** p< 0.001.

The evaluation of the anthelmintic activity of substituted cinnoline pyrazine derivatives exhibited potent anthelmintic activity against earthworms *(Pheretima posthuma)*. The three derivatives **(11DSDc, 11DSDg, and 11DSDi)** demonstrated significant activity, which was almost like that of the reference drug (mebendazole), whereas the other derivatives showed partial activity **(Figure 4)**.

In the substituted Cinnoline Pyrazine series, the compounds with halogens, mainly chloro-substituted compounds, showed potent antibacterial, anti-inflammatory, and antifungal activities compared to other compounds. In particular, the chloro-substituted compounds exhibited more potent antimicrobial and anti-helminthic activities among all the substituted cinnoline pyrazine compounds.

Anti-Inflammatory Activity

The Anti-inflammatory activity is due to the approval of restricted usage of less number of The Animal Ethics Committee was performed on Two Compounds (11c & 11i), mainly chloro-substituted Cinnoline Pyrazine Compounds, as these compounds were found to be more potent and active among all the compounds of this series during antimicrobial and anti-helminthic activities.

Table VII: Anti-inflammatory Activity of mainly Chloro Sunstituted Cinnolo Pyrazine Compounds

Compound	Substitution	Dose Mg/kg	Mean value (+S.E) of edema at different intervals		Percentage inhibition at Different intervals	
			2nd Hour	**4th hour**	**2nd hr**	**4th hr**
11DSDc	6- Chloro	100	1.14 (±0.001)	0.90 (±0.003)	34.95	45.45
11DSDi	7,8 –DiChloro	100	1.16 (±0.002)	1.01 (±0.001)	36.64	40.00
Phenyl butazone	Standard	100	1.01 (±.001)	0.88 (±0.002)	42.35	46.6

Both **11DSDc and 11DSDi** the Synthesized compounds exhibited anti-inflammatory activity to a certain extent compared to the standard drug phenylbutazone. (**Figure 5)**

RESULTS CHARACTERIZATION

Characterization requires the identification of the molecular framework, the nature of the functional groups that are present, their location within the skeletal structure, and the establishment of any stereochemical relationships that might exist.

The characterization of organic compounds has been revolutionized by the progressive adoption of a wide range of spectroscopic techniques that are now available. These have been applied extensively in the preparative section to confirm the structures of the expected products. The same was applied in the present work to confirm the structures of the newly synthesized compounds.

In the present work, the representative products were characterized by their infrared (IR) spectra, proton magnetic resonance (PMR) spectra, and mass spectra. Some intermediates were characterized by measuring their melting points and comparing them with literature values, wherever possible.

The IR spectra were recorded using a NICOLETT-IMPACT-400FT-IR SPECTRO PHOTOMETER using a thin film supported on KBr pellets.

The PMR spectra were recorded on a JEOL-JMS D-300 (300 MHz) NMR spectrometer. All spectra were obtained in Deuterated Methanol and chemical shift values are reported as values in ppm relative to TMS ($\delta = 0$) as internal standard.

Mass spectra were recorded on JEOL SX102 MS System operating at 70 ev.
The IR, NMR, and MASS spectra of one Compound from each Series are shown in figure 11.1–11.3 for the representative compounds.

C.No. 11DSD$_i$ _ 7,8-Di-chloro-4(-5-amino- Pyrazine) cinnoline-3-carboxamide

Figure 6

- **IR (KBr) in cm $^{-1}$**
  Peak at 3481.8 cm^{-1} corresponds to NH stretching.
  Peak at 3363.1 cm^{-1} corresponds to the asymmetric NH_2 group.
  Peak at 3221.8 cm^{-1} corresponds to CH stretching.
  Peak at 1633.0 cm^{-1} corresponds to C = O stretching.
  Peak at 1475.3 cm^{-1} corresponds to the aromatic C = C stretching.
  Peak at 1725.5 cm^{-1} corresponds to C = N stretching.
  Peak at 1111 - 1633 cm^{-1} corresponds to Pyrazine.
- **H^1-NMR δ in ppm**
  δ 7.35 – 7.57 (2H, d, of cinnolines)
  δ 6.45 – 6.61 (2H, d, Pyrazine)
  δ 13.60 (1H, s, of NH)
  δ 10.60 – 10.78 (2H, s, of $CONH_2$)
  δ 13.00 (1H, s, Pyrazine)

- **Mass in m/z (figure 11.3)**
  Molecular ion peak at m/z = 335 mHz is because of molecular formula $C_{13}H_8Cl_2N_6O$. The base peak was at m/z = 152 mHz. Fragment ion peak is observed at

m/z = 256 because of $C_{11}H_6Cl_2NS$, m/z = 242 because of $C_9H_4Cl_2N_3O$, and m/z = 97 because of $C_4H_8N_3$.

CONCLUSION

It has been concluded that this study has demonstrated the successful synthesis and characterization of novel pyrazine-substituted cinnolo-condensed compounds, highlighting their potential as promising candidates for medicinal applications. Through a comprehensive approach involving synthesis, spectral characterization, and dual biological evaluation, we have gained valuable insights into the structure-activity relationships and therapeutic potential of these compounds.

The synthesized compounds exhibited notable antimicrobial and antioxidant properties, with several derivatives showing particularly promising results. Compound 11DSDi demonstrated the most potent antimicrobial activity against a range of pathogenic microorganisms, and further optimization of these lead compounds could yield even more effective therapeutic agents.

Spectral characterization techniques, including NMR, IR, and mass spectrometry, provided crucial structural information, confirming the successful synthesis and purity of the target compounds. The elucidation of these structural details will facilitate future structure-activity relationship studies and rational drug design efforts.

The dual biological evaluation approach employed in this study offers a comprehensive assessment of the compounds' potential therapeutic applications. The observed antimicrobial and antioxidant activities suggest that these pyrazine-substituted cinnolo-condensed compounds may have broader applications in treating various diseases associated with oxidative stress and microbial infections.

While these results are promising, further investigations are warranted to fully elucidate the mechanisms of action, toxicity profiles, and in vivo efficacy of these compounds. Additionally, exploring structure-activity relationships and conducting molecular docking studies could provide valuable insights for optimizing these compounds and developing more potent derivatives.

In conclusion, this research contributes to the growing body of knowledge on pyrazine-substituted cinnolo-condensed compounds and their medicinal potential. The findings presented here lay the groundwork for future studies aimed at developing novel therapeutic agents based on this promising class of compounds, potentially addressing the urgent need for new antimicrobial and antioxidant drugs in clinical practice.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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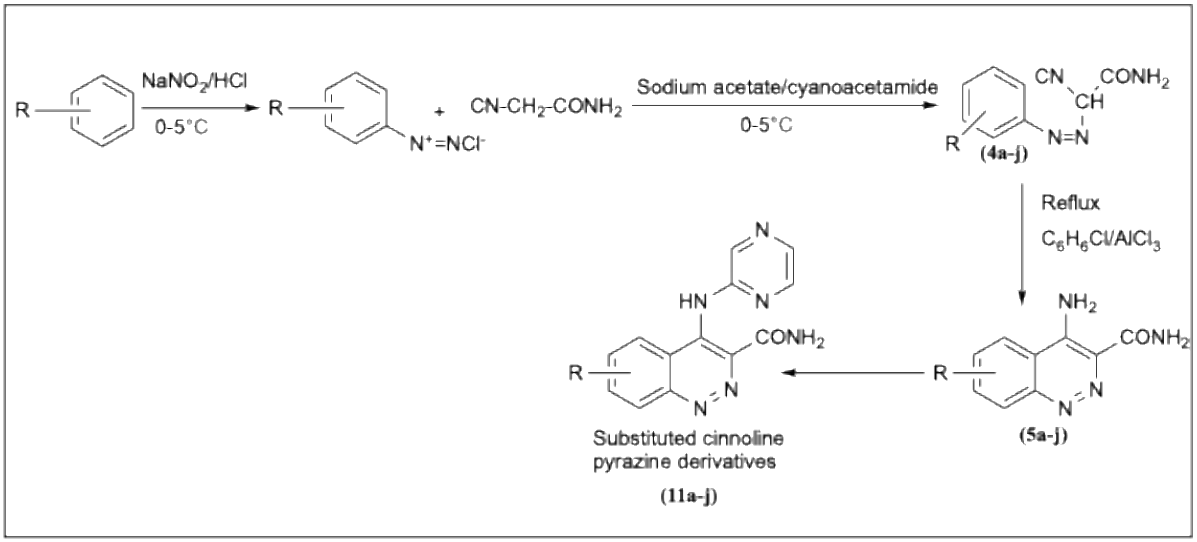

Figure 1: Synthetic Reaction Scheme

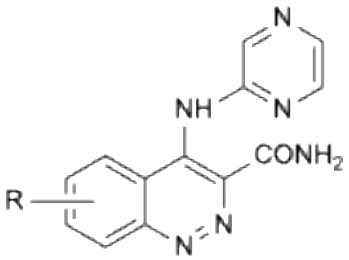

Figure 2: Substituted Cinnoline Pyrazine Derivatives 11(a –j)

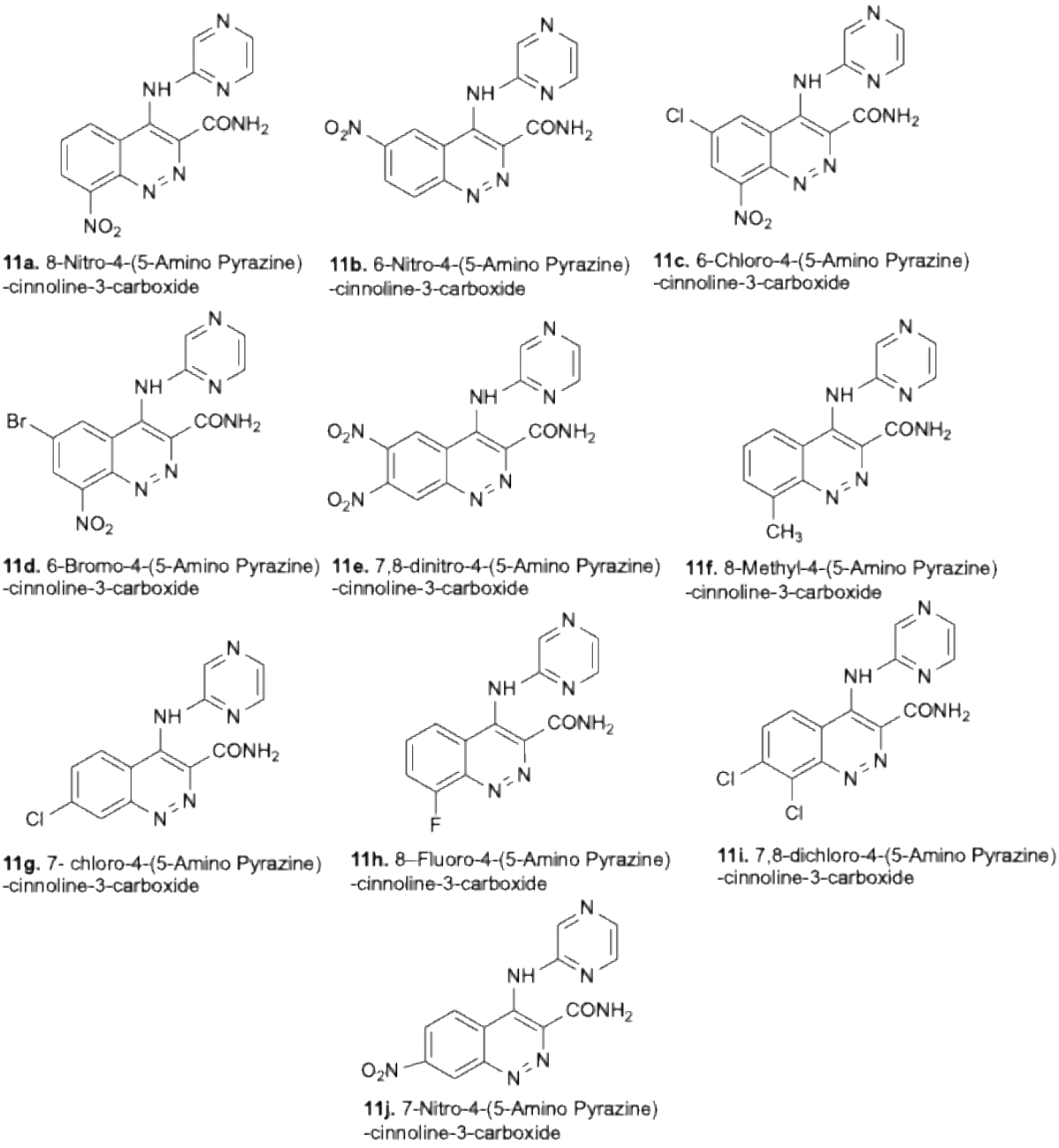

Figure 3: Structures of substituted 4(-5-amino-pyrazINE) cinnoline-3-carboxamide derivatives

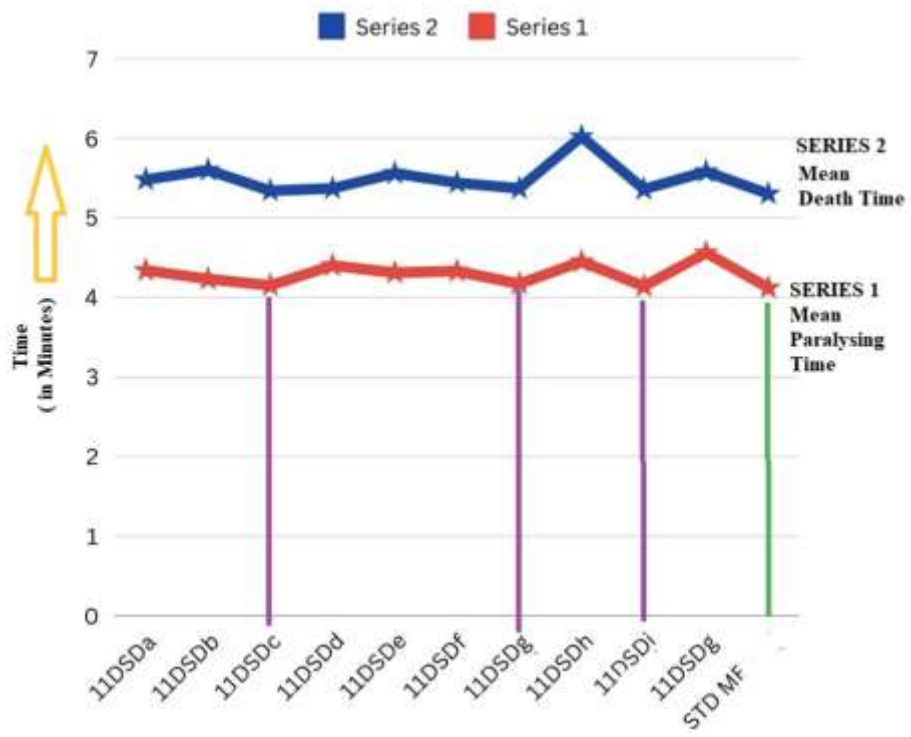

Figure 4: Comparison of Anti-helminthic activity of substituted cinnoline pyrazine compounds

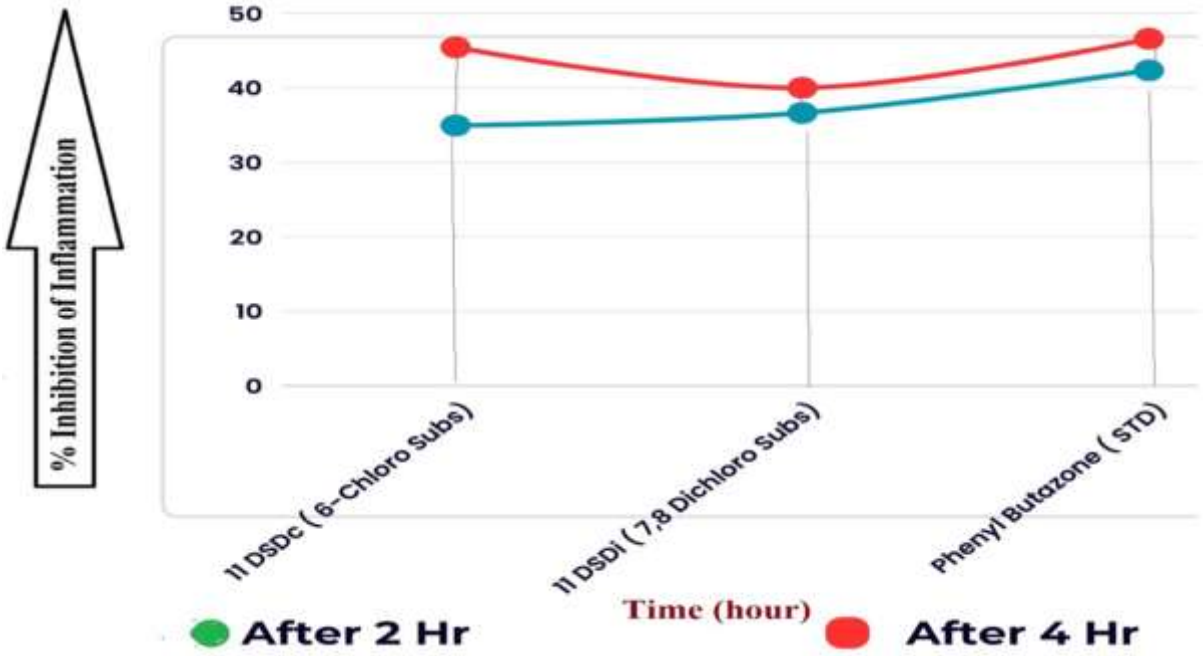

Figure 5: Comparative % inhibition of inflammation

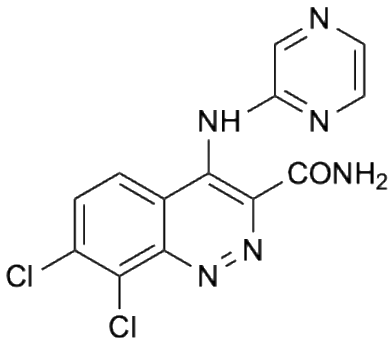

Figure 6: C.No. 11DSD$_i$ _ 7,8-Di-chloro-4(-5-amino- Pyrazine) cinnoline-3-carboxamide