Synthesis, Biological evaluation and Comparative Study of Some Cinnoline Derivatives

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Abstract

In medicinal chemistry nitrogen containing heterocycles are the most important compounds which show various biological activities. The cinnoline are nitrogenous derivatives and found to elicit many pharmacological actions like anti-hypertensive, antithrombotic, antihistamine, antileukemic, CNS activity, anti tumor, antibacterial and antiserotery activity. We planned to synthesis new series of substituted Cinnoline derivatives, and evaluated for antibacterial, antifungal and anti-inflammatory activity. The Cinnoline moiety substituted with different substituents condensed with Pyrazole, Piperazine, Imidazole, Furan and Thiophene moieties separately. The antibacterial and antifungal activities of synthesized compounds were determined by disc diffusion method. The anti-inflammatory activity of synthesized compounds was assessed by rat paw edema method. The synthesized compound exhibited moderate to good antibacterial, antifungal and anti-inflammatory activity. The substituted Cinnoline Piperazine series and Cinnoline thiophene series compound exhibited maximum antibacterial activity and antifungal activity, respectively. The substituted Cinnoline Imidazole series revealed potent anti-inflammatory activity. Further investigations are required to find out possible mechanism of action.

Keywords: Cinnoline, Antibacterial activity, Antifungal activity, Anti-inflammatory activity

1 Introduction

Cinnoline 1, 1,2-diazanaphtalene or benzo[c]-1,2-diazine (Hantsch-Widmann system), C9H6N2 is a nitrogenous organic base, obtained from certain complex diazo compounds. Their system is an isosteric relative to either quinoline or isoquinoline. Therefore, in many cases the synthesized compounds were designed as analogs of the previously obtained quinoline or isoquinoline derivatives. Cinnoline are the six membered heterocyclic compound having two hetero atoms in the ring. Cinnoline are the six membered heterocyclic compound having two hetero atoms in the ring. Cinnoline is a pale yellow solid, m.p. 24-25 °C and was first discovered by Von Richter in 1883. Researchers reported that cinnoline derivatives are found to elicit many pharmacological actions like anti-hypertensive, antithrombotic, antihistamine, antileukemic, CNS activity, anti tumor, antibacterial and antiserotery activity.1-5.

Furan is a class of organic compounds of the heterocyclic aromatic series characterized by a ring structure composed of one oxygen atom and four carbon atoms. The simplest member of the furan family is furan itself, a colourless, volatile, and somewhat toxic liquid that boils at 31.36 °C. Several other members of the furan family are produced on a large scale for use as solvents and chemical raw materials. Furan and related compound have been reported to possess various biological activities such as antihyperglycemic, analgesic, anti-inflammatory, antibacterial, antifungal, antitumor activities.6-9.

Pyrazole refers both to the class of simple aromatic ring organic compounds of the heterocyclic diazole. In medicine, derivatives of pyrazoles are used for their analgesic, anti-inflammatory, antipyretic, antiarrhythmic, tranquilizing, muscle relaxing, psychoanaleptic, anticonvulsant, monoamineoxidase inhibiting, antidiabetic and antibacterial activities.10,11.

Imidazoles is heterocyclic diazole and is found in various analgesics, anti-inflammatory, antiparasitic, anthelminic, platelet aggregation inhibitors and antiepileptic agents. Imidazole can be found in many other drugs such as...
dencarbazine, metronidazole, cimetidine, flumazenil, thyroliberin, methimazole, pilocarpine and etomidate\textsuperscript{12}.

Thiophenes are important heterocyclic compounds that are widely used as building blocks in many agrochemicals and pharmaceuticals as seen in examples such as the NSAID lornoxicam, thiophene analog of piroxicam\textsuperscript{13}.

Piperazine is an organic compound that consists of a six-membered ring containing two nitrogen atoms at opposite positions in the ring. Piperazine exists as small alkaline deliquescent crystals with a salty taste. The piperazines are a broad class of chemical compounds, many with important pharmacological properties, which contain a core piperazine functional group. Many currently notable drugs contain a piperazine ring as part of their molecular structure such as anthelmintics, antianginals and antidepressants drugs\textsuperscript{14-16}.

Hence, we aimed to synthesis new series of substituted Cinnoline with different substituents condensed with Pyrazole, Piperazine, Imidazole, Furan and Thiophene moieties separately, and evaluated for antibacterial, antifungal and anti-inflammatory activity.

2 Materials and Methods

2.1 Synthesis of Cinnoline derivatives

2.1.1 Preparation of substituted hydrazono (cyano) acetamide (4a-j)

\[R = a = o-NO_2, \quad b = p-NO_2, \quad c = p-Cl, \quad d = p-Br, \quad e = 3,4-di-nitro, \quad f = 2-Me, \quad g = 3-Chloro, \quad h = 2-Fluoro, \quad i = 2.3 dI-Chloro, \quad j = 3-Nitro\]

The substituted aniline (0.195 mole) was dissolved in a mixture of conc HCl (7.5ml) and water (7.5ml) and cooled to 0° to 5° c in an ice bath. To this a cold saturated solution of sodium nitrite (0.19mole) was added slowly. Soon after the addition, the fumes of nitrous acid were liberated; a pinch of sulphamic acid / thiourea was added, stirred till the fumes were ceased. The diazonium salt thus formed was filtered in to a cooled solution of cyano acetamide (0.195 mole) in water (350ml),10 gm CH\textsubscript{3}COONa and 15 ml alcohol. The mixture was kept for stirring up to 6 hrs at room temperature; the solid was collected and recrystallized from methanol.

2.1.2 Synthesis of substituted aniline 4-amino cinnoline 3-carboxamide (5a-j)

To the anhydrous AlCl\textsubscript{3} (0.111mole) the chlorobenzene 150ml was added and nitrogen gas was passed for half an hour. This mixture was added to the substituted phenyl hydrazono cyano acetamide then nitrogen was passed for 10 min, the mixture was then refluxed for 2hrs. It was cooled, dilute HCl (20ml) was added to it. It was then heated on water bath cooled, filtered, washed twice with dilute NaOH solution and filtered. The product was recrystallized from methanol, water 10:1.

2.1.2.1 Preparation of substituted 4-(1-amino- piperazine) -cinnoline -3-carboxamide

11 (a – j): The substituted 4-amino cinnoline-3-carboxamide (5a-j) and 2-chloro piperazine in DMF was refluxed for 2hrs, and poured in to crushed ice. The precipitate obtained was filtered, dried and recrystallized in methanol.

2.1.2.2 Preparation of substituted 4-(2-amino- thiophene) -cinnoline -3-carboxamide

12 (a – j): The substituted 4-amino cinnoline-3-carboxamide (5a-j) and 2-chloro thiophene in DMF was refluxed for 2hrs, and poured in to crushed ice. The precipitate obtained was filtered, dried and recrystallized in methanol.

2.1.2.3 Preparation of substituted 4-(2-amino-furan) -cinnoline -3-carboxamide

13 (a – j): The substituted 4-amino cinnoline-3-carboxamide (5a-j) and 2-chloro furan in DMF was refluxed for 2hrs, and poured in to crushed ice. The precipitate obtained was filtered, dried and recrystallized in methanol.

2.1.2.4 Preparation of substituted 4-(5-amino-pyrazole) -cinnoline -3-carboxamide

14 (a – j): The substituted 4-amino cinnoline-3-carboxamide (5a-j) and 2-chloro pyrazole in DMF was refluxed for 2hrs, and poured in to crushed ice. The precipitate obtained was filtered, dried and recrystallized in methanol.

2.1.2.5 Preparation of substituted 4-(5-amino-Imidazole) -cinnoline -3-carboxamide

15 (a – j): The substituted 4-amino cinnoline-3-carboxamide (5a-j) and 2-chloro imidazole in DMF was refluxed for 2hrs, and poured in to crushed ice. The precipitate obtained was filtered, dried and recrystallized in methanol.

The methodology used for the Synthesis of Substituted Cinnoline derivatives series is as follows in figure 1.

2.2 Antibacterial activity

The extracts were subjected to antibacterial activity using modified disc diffusion method. Mueller Hinton Agar was used to culture the bacteria (\textit{Bacillus subtilis}, \textit{Staphylococcus aureus}, \textit{Escherichia coli} and \textit{Pseudomonas aeruginosa}). The bacterial suspension was spread uniformly on the solid agar medium using cotton swab. Sterile Watmann filter paper disc with diameter of 6 mm was impregnated with 10 µl of cinnoline derivatives compound (25 mg/10ml) and placed on the upper layer of inoculated agar medium. The standard 6mm disc of Norfloxacin (10 µg/disc) were used as positive control whereas 10 µl of DMSO as negative control. The seeded agar plate were dried for 15 minutes and incubated at 37° C for 24 hours. The antibacterial activity was assessed by measuring the diameter of inhibition zone.
2.3 Antifungal activity

The extracts were tested for antibacterial activity using modified disc diffusion method. Sabourad agar was used to culture the fungi (Candida albicans and Aspergillus niger). The fungal suspension was spread uniformly on the solid agar medium using cotton swab. Sterile Watmann filter paper disc with diameter of 6 mm was impregnated with 10 µl of cinnoline derivatives compound (25 mg/10ml) and placed on the upper layer of inoculated agar medium. The standard 6 mm disc of Fluconazole (30 µg/disc) were used as positive control whereas 10 µl of DMSO as negative control. The seeded agar plate were dried for 15 minutes and incubated at 37 °C for 72 hours. The antifungal activity was assessed by measuring the diameter of inhibition zone²⁰⁻²².

2.4 Animals

Albino rats of either sex weighing 150-200 grams were used for the present study. They were fed with standard pellet diet and water ad libitum. All animals were acclimatized for at least one week before the experimental session. All the experimental procedures were done following the guidelines of the Institutional Animals Ethics Committee (IEAC).

2.5 Anti-inflammatory activity

The anti-inflammatory activity was assessed by rat paw edema method wherein the procedure of plethysmographic measurement of edema produced by planter injection of 1% w/v formalin in the hind paw of the rat was followed.

Albino rats of either sex weighing 150-200 grams were used and divided into groups containing six rats in each group. First group served as control, second group was used for standard drug phenylbutazone (100 mg/kg body weight) and the remaining groups served for compounds (100 mg/kg body weight).
weight) under investigation. An identification mark was made on both the hind paws just beyond tibiotarsal junction so that every time the paw was dipped in mercury column up to a fixed mark to ensure constant paw volume. Immediately after 30 minutes of drug administration, 0.1 ml of 1% w/v formalin was injected in the planter region of left paw of the rats. The right paw was used as reference for non inflamed paw for comparison. The paw volume of all the test animals was measured after 2nd and 4th hours of drug administration. The percentage of increase in edema over the initial reading was also calculated. The increase in edema of animals treated with standard test compounds were compared with the increase in the edema of untreated control animal with the corresponding intervals of 2nd and 4th hours. Thus the percentage inhibition of edema at known intervals in treated animals was calculated as given below:

\[
\text{Percentage inhibition} = \frac{V_c - V_t}{V_c} \times 100
\]

\(V_c\) = volume of paw edema in control animals

\(V_t\) = volume of paw edema in treated animals

2.6 Statistical analysis

The results are expressed as mean ± SEM of six independent experiments. Statistical significance between the groups was evaluated by one-way analysis of variance (ANOVA) followed by Dunet’s test. A P < 0.05 value was considered as statistically significant.

3 Results and Discussions

3.1 Cinnoline derivatives

4 (a – j) was prepared by diazotization of substituted aniline and followed interaction with cyanoacetamide through the Japp-Klingemann reaction. 5 (a – j) was prepared by Substituting phenyl hydrazono (cyano) acetamide voluntarily undergoes intra molecular friedelcrafts reaction in chlorobenzene in presence of AlCl₃ leading to substituted 4-amino cinnoline-3-carboxamide.

The substituted cinnoline piparazine derivatives (11a-j), cinnoline thiophene derivatives (12a-j) cinnoline furan derivatives (13a-j) cinnoline pyrazole derivatives (14a-j) and cinnoline imidazole derivatives (15a-j) were obtained with good yield.

3.2 Antibacterial activity

The synthesized compounds were ready to display antibacterial activity. Antibacterial activities were observed for all heterocyclic compounds using strains of bacteria such as Bacillus subtilis, Staphylococcus aureous, Escherichia coli and Pseudomonas aeruginosa. The potency of the test compounds are displayed in figure 2. 8-Nitro, 6-Nitro, 6, 7 Dinitro & 7- Nitro Substituted compounds were Partially active in all the series. 6-Chloro Substituted compounds were found to be highest & equally potent in all five series. 6-Bromo substituted compounds are optimum potent in all series except 6-Bromo cinnolo piparazine compound which is most potent near to standard drug. 8-Methyl Substituted Compounds are partial potent in all series except in piparazine & imidazole series where they perform more than optimum. 7-Chloro Substituted compounds were found most potent in three series viz. piparazine, thiophene and Pyrazole. 8- Fluoro Substituted Compounds were found most active/potent in only piparazine series.

The outcomes suggested that among all the Compounds 6-Chloro substituted compounds in all series were found most potent in comparison to standard drug. In General halogen substituted compounds were found to be most active followed by methyl substituted and lastly nitro substituted in all the series.

3.3 Antifungal activity

The synthesized compounds were ready to display antifungal activity. Antifungal activities were observed for all heterocyclic compounds using strains of bacteria such as Candida albicans and Aspergillus niger. The potency of the test compounds are displayed in figure 3. 8-Nitro, 6-Nitro, 6, 7 Dinitro & 7- Nitro Substituted Compounds were partially active in all the series. 6-Chloro Substituted compounds were found to be highest & equally potent in all five series. But found highly potent in Substituted furan series. 6-Bromo substituted compounds are optimum potent in all series except 6-Bromo cinnolopyrazole compound which is most potent near to standard drug. 8-Methyl Substituted compounds are partially potent in all series except in imidazole series where they perform more than optimum. 7-Chloro Substituted compounds were found most potent in three series viz. piparazine, thiophene and pyrazole, found as potent as 6-Chloro substituted compounds. 8- Fluoro Substituted Compounds were found most active/potent in only piparazine series.

The findings concluded that among all the Compounds 6-Chloro substituted compounds in all series were found most potent in comparison to standard drug. In all the compounds the most potent antifungal agent was 7-Chloro Substituted Cinnolothiophene & 6-Chloro Substituted Cinnolofuran derivative. In General halogen substituted compounds were found to be most active followed by methyl substituted and lastly nitro substituted in all the series.

3.4 Anti-inflammatory activity

The anti-inflammatory activity was carried out by the rat paw edema method. In all the five substituted cinnoline series, the compounds which are halogen mainly substituted were showed potent anti-inflammatory activity than other compounds (Figure 4). If we compare all the series then found they are almost similarly potent however most potent was substituted cinnoline imidazole series followed by substituted Cinnoline pyrazole series and substituted thiophene series. It was also assumed
that compounds require some improvement in absorption properties because if we see the graph (Figure 4) then we found that their %inhibition is slow in first two hours but when they gets absorbed their %inflammation potency increases, as after 4 hours ratio of %inflammation is high.

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**Fig 2:** Comparative antibacterial activity of the Cinnoline derivatives

**Fig 3:** Comparative antifungal activity of the Cinnoline derivatives

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4 Conclusions

The substituted cinnoline piperazine derivatives (11a-j), cinnoline thiophene derivatives (12a-j) cinnoline furan derivatives (13a-j) cinnoline pyrazole derivatives (14a-j) and cinnoline imidazole derivatives (15a-j) were obtained with good yield. The findings of antibacterial study showed on comparing all the series then found they were almost similar potent however most antibacterial potent was substituted Cinnoline Piperazine Series followed by imidazole series. The outcomes of antifungal activity demonstrated that on comparing all the series then found they are almost similar potent however most antifungal potent was substituted Cinnoline thiophene series followed by substituted Cinnoline pyrazole series. The anti-inflammatory activity showed that on comparing all the series then found they were almost similar potent however most potent was substituted cinnoline imidazole series followed by substituted Cinnoline pyrazole series and substituted thiophene series. Further, it would be interesting to obtain the possible mechanism of action.

5 Conflicts of Interests

We have not declared any conflict of interest.

6 Author’s contributions

PM, AM and VS designed the experimental work and performed; AS carried out literature review of this study. Authors read and approved the final manuscript.

7 References


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