Synthesis and Evaluation of Anti-inflammatory Activity of Some Cinnoline Derivatives-4-(2-amino-thiophene) Cinnoline-3-Carboxamide

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Abstract

A prolonged increase in pro-inflammatory cytokines, TNF-α and IL-6 occurs in inflammatory diseases. Although cinnoline belongs to a family of fairly well known heterocycles, the interest in the study of its derivatives continues. Cinnoline compounds demonstrate interesting bioactivity and many research papers have discussed the biological property, structure-activity relationship, and applications in medicinal science. A new series of substituted Cinnoline derivatives condensed with Thiophene moieties (12a-j) were synthesized and their anti-inflammatory activity was evaluated. The anti-inflammatory activity was assessed by rat paw edema method. The pharmacological screening showed that many of these obtained compounds have significant anti-inflammatory activity, comparable to the standard drug (phenylbutazone). The compound 12DSDi produces maximum anti-inflammatory activity compared to other tested compound.

Keywords: Cinnoline compounds, Thiophene moieties, Anti-inflammatory.

1 Introduction

The practice of medicinal chemistry is devoted to the discovery and development of new agents for treating disease. Most of the activity is directed to new natural or synthetic organic compounds (1). Heterocyclic nucleus imparts an important role in medicinal chemistry and serves as a key template for the development of various therapeutic agents. Significant number of compounds synthesized in industrial sector each year is heterocyclic in nature.

The main objective of organic and medicinal chemistry is the synthesis, characterization and pharmacological evaluation of molecules having highly therapeutic and efficacy in nature. Now a day increasing the resistance of many organisms, we have to synthesize the more active new molecules for the treatment of various diseases or disorders.

Researchers reported that cinnoline derivatives were found to elicit many pharmacological actions like anti-hypertensive, antithrombotic, antihistamine, antileukemic, CNS activity, anti tumor, antibacterial and antisecretory activity. They are reactive by virtue of the presence of a benzene ring and the electrophillic attack takes place in this ring. Cinnolines are the six-membered heterocyclic compounds having two hetero atoms in the ring. They are also called as 1, 2- benzodiazine or benzopyridazine or 1, 2- diazanaphthalene or phenodiazine (VI)7-10.

In medicinal chemistry, thiophene derivatives have been very well known for their therapeutic applications. The simple thiophenes are stable liquids which closely resemble the corresponding benzene compounds in boiling point and even in smell. They occur in coal tar distillates. The discovery of thiophene in coal tar benzene provides one of the classic anecdotes of organic chemistry. Thiophene was discovered as a contaminant in benzene. It was observed that isatin (1Hindole-2, 3- Dione) forms a blue dye if it is mixed with sulfuric acid and crude benzene. Victor Meyer was able to isolate the substance responsible for this reaction. The compound was found to be a heterocyclic compound. Thiophene 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, the thiophene analog of piroxicam11.

We planned to synthesize new series of substituted Cinnoline derivatives condensed with Thiophene moieties. All the
synthesized compounds were screened for their anti-inflammatory activity.

2 Materials and Methods

2.1 Synthesis of Cinnoline Thiophene series

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 CH₃COONa 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.

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 (12a-j).

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

Fig 1: Synthesis of substituted Cinnoline Thiophene derivatives

2.2. 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 (IAEAC).

2.3 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) under investigation. An identification mark was made on both the hind paws just beyond tibiotorsal junction so that every time the paw was dipped in mercury column upto 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:

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

Where:
- \(V_c\) = volume of paw edema in control animals
- \(V_t\) = volume of paw edema in treated animals

2.4 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

3.1 Cinnoline Thiophene derivatives

The properties of synthesis of substituted cinnoline thiophene derivatives are displayed in table 1. The melting point of all compounds ranged from 178 to 267 °C. The percentage yields of compounds were ranged from 57.71% to 74.78%, and the compound 12DSD produces maximum yield compared to other synthesized compound.

3.2 Anti-inflammatory activity

The anti-inflammatory activity was carried out by the rat paw edema method. It is concluded that substituted cinnoline thiophene derivatives can be synthesized successfully in the laboratory. The results from anti-inflammatory activity showed that some of the substituted cinnoline thiophene derivatives possess good anti-inflammatory activity and some compounds...
showed moderate to good activity when compared to the standard drug phenylbutazone (Table 2).

Table 1: Physical data of substituted 4-(2-amino-thiophene) cinnoline-3-carboxamide derivatives

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Com. No</th>
<th>Physical nature</th>
<th>MP (°C)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-Nitro-4-(2-amino-2-thiophene) cinnoline-3-carboxamide</td>
<td>12DSDa</td>
<td>Dark Yellow crystals</td>
<td>184-185</td>
<td>74.78%</td>
</tr>
<tr>
<td>6-nitro- 4-(2-amino thiophene)cinnoline-3-carboxamide</td>
<td>12DSDb</td>
<td>Pale Yellow crystals</td>
<td>188-189</td>
<td>67.54%</td>
</tr>
<tr>
<td>6-chloro-4-(2-amino- thiophene) cinnoline-3-carboxamide</td>
<td>12DSDc</td>
<td>Greenish yellow</td>
<td>246-248</td>
<td>60.87%</td>
</tr>
<tr>
<td>6-bromo-4-(2-amino- thiophene) cinnoline-3-carboxamide</td>
<td>12DSDd</td>
<td>Brown crystals</td>
<td>220-222</td>
<td>64.34%</td>
</tr>
<tr>
<td>6,7 di nitro- 4-(2-amino- thiophene) cinnoline-3-carboxamide</td>
<td>12DSE</td>
<td>Greenish yellow</td>
<td>203-204</td>
<td>61.83%</td>
</tr>
<tr>
<td>8-methyl- 4-(2-amino-2- thiophene) cinnoline-3-carboxamide</td>
<td>12DSF</td>
<td>White crystals</td>
<td>221-222</td>
<td>67.40%</td>
</tr>
<tr>
<td>7 chloro- 4-(2-amino- thiophene) cinnoline-3-carboxamide</td>
<td>12DSDg</td>
<td>Yellow Brown crystals</td>
<td>226-228</td>
<td>64.80%</td>
</tr>
<tr>
<td>8-Fluoro- 4-(2-amino- thiophene) cinnoline-3-carboxamide</td>
<td>12DSDh</td>
<td>Greenish yellow crystals</td>
<td>198-200</td>
<td>60.81%</td>
</tr>
<tr>
<td>7,8-Di-chloro-4-(2-amino- thiophene) cinnoline-3-carboxamide</td>
<td>12DSDi</td>
<td>Creamish white cryst</td>
<td>265-267</td>
<td>70.12%</td>
</tr>
<tr>
<td>7- Nitro- 4-(2-amino-thiophene) cinnoline-3-carboxamide</td>
<td>12DSDj</td>
<td>Pale yellow Crystals</td>
<td>178-180</td>
<td>57.71%</td>
</tr>
</tbody>
</table>

Table 2: Anti-inflammatory activity of the synthesized compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Substitution</th>
<th>Dose Mg/kg</th>
<th>Mean value (±S.E.) of edema at different intervals</th>
<th>Percentage inhibition at different intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>2nd Hour</td>
<td>4th hour</td>
</tr>
<tr>
<td>Control</td>
<td>--</td>
<td>--</td>
<td>1.69±0.053</td>
<td>1.91±0.047</td>
</tr>
<tr>
<td>12DSDa</td>
<td>8-Nitro</td>
<td>100</td>
<td>1.41±0.064</td>
<td>1.47±0.002</td>
</tr>
<tr>
<td>12DSDb</td>
<td>6-Nitro</td>
<td>100</td>
<td>1.48±0.015</td>
<td>1.45±0.002</td>
</tr>
<tr>
<td>12DSDc</td>
<td>6-Chloro</td>
<td>100</td>
<td>1.11±0.001*</td>
<td>1.01±0.003*</td>
</tr>
<tr>
<td>12DSDd</td>
<td>6-Bromo</td>
<td>100</td>
<td>1.31±0.032</td>
<td>1.40±0.003*</td>
</tr>
<tr>
<td>12DSDe</td>
<td>6,7-Dinitro</td>
<td>100</td>
<td>1.59±0.015</td>
<td>1.53±0.026</td>
</tr>
<tr>
<td>12DSDf</td>
<td>8- Methyl</td>
<td>100</td>
<td>1.60±0.601</td>
<td>1.59±0.005</td>
</tr>
<tr>
<td>12DSDg</td>
<td>7-Chloro</td>
<td>100</td>
<td>1.14±0.002*</td>
<td>1.02±0.001*</td>
</tr>
<tr>
<td>12DSDh</td>
<td>8-Fluoro</td>
<td>100</td>
<td>1.32±0.001</td>
<td>1.09±0.006*</td>
</tr>
<tr>
<td>12DSDi</td>
<td>7,8-Dichloro</td>
<td>100</td>
<td>1.10±0.003*</td>
<td>1.00±0.001*</td>
</tr>
<tr>
<td>12DSDj</td>
<td>7-Nitro</td>
<td>100</td>
<td>1.48±0.005</td>
<td>1.38±0.004</td>
</tr>
<tr>
<td>Standard</td>
<td>Phenylbutazone</td>
<td>100</td>
<td>1.01±0.001*</td>
<td>0.88±0.002*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at *P<0.05 when compared with control group.

Among the tested compounds 12DSDc, 12DSDd, 12DSDg, 12DSDh and 12DSDi exhibited significant anti-inflammatory activity by formalin induced rat paw edema method. The other tested compound revealed non-significant anti-inflammatory activity. The standard drug treated animal displayed significant anti-inflammatory activity. The compound 12DSDi produces maximum anti-inflammatory activity compared to other tested compound.

4 Discussion

The melting point of synthesized compound revealed physical properties of compound. Evaluation of anti-inflammatory activity.
for substituted Cinnoline thiophene derivatives exhibits potent anti-inflammatory activity. All derivatives failed to produce significant anti-inflammatory activity, while some derivatives mainly 6-Bromo, 6-Chloro, 7-Chloro, 8-Fluoro, 7,8-Dichloro substituted derivatives not only demonstrated approximately same anti-inflammatory activity as standard drug but also show potent activity. Especially Chloro Substituted Compounds Showed more potent antimicrobial activity and anti-inflammatory activity among all the substituted cinnoline thiophene compounds. This might be due to the reason that cinnoline ring system is identical to ring system found in Phenylbutazone.

Inflammation is a normal response to infection and injury and involves the recruitment of immune systems to neutralize invading pathogens, repair injured tissues and promote wound healing. Chronic or excessive activation of the immune system is associated with an increase reactive oxygen species (ROS), prolonged activation of inducible NO synthase (iNOS) and of the release of proinflammatory cytokines. This may increase susceptibility to infections and cause inflammation. Drugs that block the action of the cytokine, tumor necrosis factor-α (TNF-α) have proved to be very effective in the treatment of inflammation[17]. The findings of study revealed that the Cinnoline thiophene derivatives inhibit the action of cytokine and TNF-α.

5 Conclusions

All the synthesized Cinnoline thiophene derivatives were evaluated with physical and Biological methods. All the compounds were subjected to anti-inflammatory activity. The compounds 12DSDe, 12DSDd, 12SDSh and 12DSDi exhibited significant anti-inflammatory activity. Further, it would be interesting to obtain the possible mechanism of action and their in vivo trial in experimental animals.

6 Conflicts of Interests

We have not declared any conflict of interest.

7 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.

8 References


