Synthesis, characterization and pharmacological evaluation of cinnoline (thiophene) derivatives

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Abstract
The review of literature showed that cinnoline derivatives were found to elicit many pharmacological actions like anti-hypertensive, antithrombotic, antihistamine, antileukemic, CNS activity, anti-tumor, antibacterial and antisecretory activity.

In the substituted Cinnoline thiophene series, the compounds which are halogen mainly Chloro, Bromo and Fluoro Substituted were showed potent antibacterial, anti-inflammatory and anti-fungal activity than other compounds. Especially Chloro Substituted Compounds Showed more potent antimicrobial activity and anti-inflammatory activity among all the substituted cinnoline thiophene compounds.

Keywords: cinnoline, thiophene, anti-inflammatory activity.

Introduction

Cinnolines: Cinnoline is a pale yellow solid, m.p. 24-25 °C and was first discovered by Von Richter in 1883. He also prepared a cinnoline derivative from 2-aminophenylpropionic acid via intramolecular cyclization of the diazonium salt. The review of literature showed that cinnoline derivatives were found to elicit many pharmacological actions like anti-hypertensive, antithrombotic, antihistamine, antileukemic, CNS activity, anti-tumor, antibacterial and antisecretory activity.

Cinnolines are reactive by virtue of the presence of a benzene ring and the electrophilic 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-benzodiazepine or benzopyridazine or 1, 2-dizanaphthalene or phenothiazine. (VI)

The main approach for the synthesis of cinnoline is electrophilic attack by diazonium cation on carbon – carbon center of unsaturation as given below.

![Synthesis Diagram](image)

Thiophene
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 piroxicam.
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Objectives
2. Characterization of newly synthesized compounds by analytical and spectral methods viz., IR spectra, NMR spectra and Mass spectra.
3. Anti-inflammatory activity of some of the synthesized compounds.

Review of Literature
Narayana et al., (2006) Studied antibacterial and antifungal studies on some new acetyl cinnolines and cinnolinyl thiazole derivatives. They are reported antibacterial and antifungal activity.
Iradyan MA et al. (2007) [26] reported potent anti-tumor activity in some amino imidazole compounds.
Saxena et al., (2009) Synthesis, characterization and biological activities of substituted cinnoline culphonamides. They are reported that these derivatives particularly halogen substituted cinnoline derivatives showed potent antimicrobial activity.
Dutta et al., (2010) [19] Synthesized a series of 2-substituted-4,5-diphenyl imidazoles by refluxing benzil with different substituted aldehydes and screened for anthelmintic activity. The compounds showed significant anthelmintic activity compared to the standard drugs.
Patel et al., (2011) Studied synthesis and microbial evaluation of pyrazoline derivatives. They are reported that anti-microbial activities.
Priyadarsini et al., (2012) prepared new substituted pyrazoles from o-hydroxyacetophenone and cinnamic acids as starting material through 1, 3-diketones as intermediates. These intermediates on reaction with hydrazines in alkaline media produce pyrazoles. The antimicrobial activity of synthesized pyrazoles. In the most cases having Chloro substitution on the styryl ring was found to be more efficient.
Chaudhary et al., (2014) Studied synthesis and biological screening of some cinnoline derivatives. They are reported that newly synthesized compounds were screened for their anti-inflammatory and antibacterial activity.

Material and Method
The Methodology Used for the Synthesis of Substituted Cinnoline imidazole Series is as follows:
The synthesis of substituted cinnoline Imidazole derivatives by the described above method remitted in products with good yield.

Results of Scheme

The synthesis of substituted cinnoline thiophene derivatives by the described above method remitted in products with good yield.

Table 1.0: 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>M.P (°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 °C</td>
<td>73.78%</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 °C</td>
<td>67.12%</td>
</tr>
<tr>
<td>6-chloro-4(-2-amino- thiophene) cinnoline-3-carboxamide</td>
<td>12DSDc</td>
<td>Greenish yellow</td>
<td>246-248 °C</td>
<td>59.87%</td>
</tr>
<tr>
<td>6-bromo-4(-2-amino- thiophene) cinnoline-3-carboxamide</td>
<td>12DSDd</td>
<td>Brown crystals</td>
<td>220-222 °C</td>
<td>64.34%</td>
</tr>
<tr>
<td>6,7 di nitro-4(-2-amino- thiophene) cinnoline-3-carboxamide</td>
<td>12DSDe</td>
<td>Greenish yellow</td>
<td>203-204 °C</td>
<td>61.83%</td>
</tr>
<tr>
<td>8-methyl-4(-2-amino-2-thiophene) cinnoline-3-carboxamide</td>
<td>12DSDf</td>
<td>White crystals</td>
<td>221-222 °C</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 °C</td>
<td>64.80%</td>
</tr>
<tr>
<td>8-Fluoro-4(-2-amino-thiophene) cinnoline-3-carboxamide</td>
<td>12DSDh</td>
<td>Greenish yellow</td>
<td>198-200 °C</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 °C</td>
<td>71.12%</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 °C</td>
<td>58.71%</td>
</tr>
</tbody>
</table>

Methodology for Anti-inflammatory

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. The method described by Wilhelm and Domenoz as modified by Sisodia and Rao was used for measuring the paw volume. Suspension of phenylbutazone containing 40 mg/ml of drug was prepared in 2% gum acacia and used as standard drug. Suspensions of test compounds at a concentration of 40 mg/ml were also prepared in 2% gum acacia. The dose concentration of both standard drug and the test compounds was 100 mg/kg body weight. 1% w/v of formalin solution prepared and 0.1 ml of it in each case was injected in the planter region of left hind paw of albino rats.

Albino rats of either sex weighing 150-200 grams were used and divided into groups of six.

Albino rats in each group. First group served as control, second group was used for standard drug phenylbutazone and the remaining groups served for compounds 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 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 comparision. 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.

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

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

Data analysis

The data were subjected to analysis of variance (ANOVA) as per statistical methods using SPSS (1996) software package.
## Result of Anti-inflammatory Activity

<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>12DSDa</td>
<td>8 - Nitro</td>
<td>100</td>
<td>1.41 (±0.0064) 1.31 (±0.0002)</td>
<td>16.11 15.08</td>
</tr>
<tr>
<td>12DSDb</td>
<td>6- Nitro</td>
<td>100</td>
<td>1.48 (±0.015) 1.38 (±0.0002)</td>
<td>12.90 12.87</td>
</tr>
<tr>
<td>12DSDc</td>
<td>6- Chloro</td>
<td>100</td>
<td>1.11 (±0.001) 1.01 (±0.003)</td>
<td>35.26 42.16</td>
</tr>
<tr>
<td>12DSDd</td>
<td>6-Bromo</td>
<td>100</td>
<td>1.31 (±0.032) 1.40 (±0.003)</td>
<td>26.12 17.30</td>
</tr>
<tr>
<td>12DSDe</td>
<td>6-7 di nitro</td>
<td>100</td>
<td>1.59 (±0.015) 1.43 (±0.026)</td>
<td>19.15 13.94</td>
</tr>
<tr>
<td>12DSDf</td>
<td>8- Methyl</td>
<td>100</td>
<td>1.60 (±0.601) 1.59 (±0.005)</td>
<td>18.00 19.15</td>
</tr>
<tr>
<td>12DSDg</td>
<td>7 – Chloro</td>
<td>100</td>
<td>1.14 (±0.002) 1.02 (±0.001)</td>
<td>32.61 40.00</td>
</tr>
<tr>
<td>12DSDh</td>
<td>8-Fluoro</td>
<td>100</td>
<td>1.32 (±0.001) 1.09 (±0.006)</td>
<td>26.08 38.08</td>
</tr>
<tr>
<td>12DSDi</td>
<td>7,8- iChloro</td>
<td>100</td>
<td>1.10 (±0.003) 1.00 (±0.001)</td>
<td>36.51 44.18</td>
</tr>
<tr>
<td>12DSDj</td>
<td>7- Nitro</td>
<td>100</td>
<td>1.53 (±0.005) 1.38 (±0.004)</td>
<td>11.90 12.87</td>
</tr>
<tr>
<td>Phenyl butazone</td>
<td>Standard</td>
<td>100</td>
<td>1.01 (±0.001) 0.88 (±0.002)</td>
<td>42.35 46.6</td>
</tr>
</tbody>
</table>

All the Synthesized compounds have shown anti-inflammatory activity to a certain extent as compared to standard drug Phenylbutazone. Among the tested compounds 12DSDc, 12DSDd, 12DSDg, 12DSDh and 12DSDi have shown good activity by formalin induced rat paw edema method.

### Result and Discussion

In the substituted Cinnoline thiophene series, the compounds which are halogen mainly Chloro, Bromo and Fluoro Substituted were showed potent antibacterial, anti-inflammatory and anti-fungal activity than other compounds. Especially Chloro Substituted Compounds Showed more potent antimicrobial activity and anti-inflammatory activity among all the substituted cinnoline thiophene compounds.
Results Characterization

The characterization requires the identification of molecular frame work, the nature of functional groups that are present and their location within the skeletal structure and finally the establishment of any stereo chemical relationships, which might exist.

The problem of characterization of organic compounds has been revolutionized by the progressive adoption of the wide range of spectroscopic techniques, which are now available. These have been applied extensively in the preparative section to confirm the structure of the expected products. The same were applied in present work to confirm the structure of 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 point and comparing with literature value, wherever possible.

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

The PMR spectra were recorded on JEOL-JMS D-300 (300 MHz) NMR spectro meter. All spectra were obtained in Deuterated Methanol and chemical shift values are reported as values in ppm relative to TMS (δ = 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 is given in figure 12.1 to 12.3 for the representative compounds.

Spectral Features

12DSDi : 7,8-Di-chloro-4-(2-amino-thiophene) cinnoline-3-carboxamide

IR (KBr) in cm⁻¹ (Figure-12.1)
Peak at 3481.7 cm⁻¹ corresponds to NH stretching
Peak at 3361.4 cm⁻¹ corresponds to asymmetric NH₂ group.
Peak at 3106.9 cm⁻¹ corresponds to CH stretching.
Peak at 1631.9 cm⁻¹ corresponds to C = O stretching.
Peak at 1505.2 cm⁻¹ corresponds to aromatic C = C stretching.
Peak at 997.00 cm⁻¹ corresponds to C = S stretching.
Peak at 840 - 1113 cm⁻¹ corresponds to thiophene

H¹-NMR δ in ppm (figure.12.2)
δ 7.9 – 8.1 (2H, d, of cinnolines)
δ 7.5 – 7.67 (3H, m, Thiophene)
δ 13.60 (1H, s, of NH)
δ 10.6 – 10.7 (2H, s, of CONH₂)

Mass in m/z (Figure-12.3)
Molecular ion peak at m/z = 341 mHz is because of molecular formula C₁₃H₁₀Cl₂N₃SO. Base peak is at m/z = 160 mHz. Fragment ion peak is observed at m/z = 327 because of C₇H₄Cl₂N₂O m/z = 241 because of C₅H₄Cl₂S m/z = 185 because of C₃H₆S₂.


