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SYNTHESIS AND EVALUATION OF ANTHELMINTIC ACTIVITY OF SOME SUBSTITUTED CINNOLOTHIOPHENE DERIVATIVES

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ABSTRACT

Cinnoline are the six membered heterocyclic compound found to elicit many pharmacological actions like anti-hypertensive, antithrombotic, antihistamine, antileukemic, CNS activity, anti tumor, antibacterial and antisecretory activity. Similarly thiophene moiety is well known for their therapeutic values. Study was aimed to synthesize some cinnoline derivatives from substituted anilines and these substituted cinnolines are further condensed with thiophene moiety and further evaluated these substituted cinnolothiophene derivatives for biological activity.

KEY WORDS: Cinnoline, Aniline, Anthelmintic activity.

INTRODUCTION

The approach to the practice of medicinal chemistry has developed extensively involving synthesis of new organic compounds based on modification of naturally available compounds of biological interest. Cinnolines are the six-membered heterocyclic compounds having two hetero atoms in the ring. They are also called as 1,2- benzodiazines or benzopyridazine or 1,2- diazanaphthalene or phenodiazine[1]. V. Von. Richter (1883) prepared cinnoline derivatives by the diazotization of o-aminoaryl propiolic acids(1) or o-amino aryl acetylenes followed by hydration and cyclization. Thiophene is a heterocyclic compound with the formula C_4H_4S. Consisting of a flat five-membered ring, it is aromatic as indicated by its extensive substitution reactions. Related to thiophene are benzothiophene and dibenzothiophene, containing the thiophene ring fused with one and two benzene rings, respectively. Compounds analogous to thiophene include furan (C_4H_4O) and pyrrole (C_4H_4NH). Thiophenes are classically prepared by the reaction of 1,4-diketones, diesters, or dicarboxylates with sulfiding reagents such as P_4S_{10}. Specialized thiophenes can be synthesized similarly using Lawesson's reagent as the sulfiding agent, or via the Gewald

reaction, which involves the condensation of two esters in the presence of elemental sulfur. Thiophenes are important heterocyclic compounds that are widely used as building blocks in many agrochemicals and pharmaceuticals. The benzene ring of a biologically active compound may often be replaced by a thiophene without loss of activity. This is seen in examples such as the NSAID lornoxicam, the thiophene analog of piroxicam.

MATERIALS AND METHOD

IR spectra (λ_{max} in cm^{-1}) were recorded on Perkin-Elmer infrared-283 FTIR spectrometer. ^1H spectra were recorded on Bruker DRX-400 (400 MHZ, FT NMR) spectrometer using tetramethylsilane as internal standard and the chemical shifts are reported in δ units.

EXPERIMENTAL

Preparation of substituted hydrazono (cyano) acetamide: 4(a -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 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_3COONa 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.

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

To the anhydrous $Alcl_3$ (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.

Preparation of substituted 4-(-amino-2-methyl thiophene-)-cinnoline-3-carboxamide 7(a-j): The substituted 4-amino cinnoline-3-carboxamide **5(a-j)** and 2-chloromethyl

thiophene **(6)** in DMF will be refluxed for 2hrs, and poured in to crushed ice. The precipitate obtained will be filtered, dried and recrystallized in methanol.

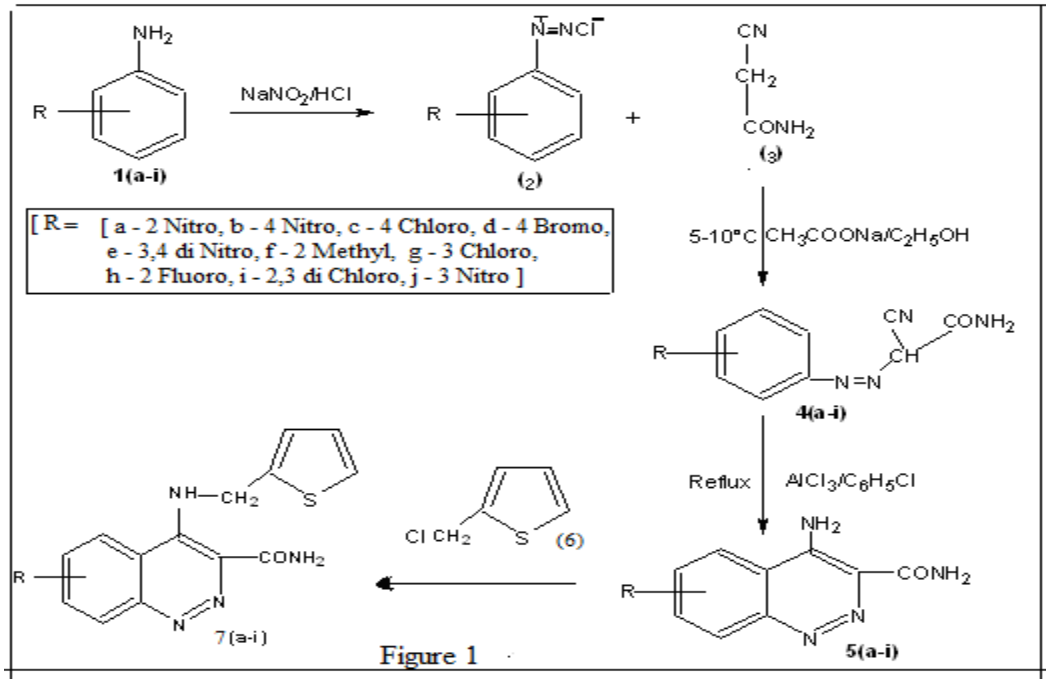

Figure 1

Table : 1.0 Physical & Analytical data of substituted 4-(-amino-2-methyl thiophene) Cinnoline-3-Carboxamide derivatives: (7a –j)

Com. No.	Physical nature	M.P(°C)	Yield (%)
7_a	Dark Yellow Crystals	185°C	73.78%
7_b	Pale Yellow Crystals	189°C	67.12%
7_c	Greenish Yellow	248°C	59.87%
7_d	Brown Crystals	222°C	64.34%
7_e	Greenish Yellow Crystals	204°C	61.83%
7_f	White Crystals	222°C	67.40%
7_g	Yellow Brown Crystals	228°C	64.80%
7_h	Grenish Yellow Crystals	200°C	60.81%
7_i	Creamish White Crystals	267°C	71.12%
7_j	Pale Yellow Crystals	180°C	58.71%

All compounds gave corrected CHN analysis.

7f : IR (KBr) cm^{-1}, 1675 (C=O), 3234 (NH), 1457-587, 1024 (thiophene), 736 (C-Cl) : PMR: (CDCL3), 6.74, (s,2H, NH_2), 7.11-8.22 (s, H, thiazole C_6-H, thiazole, 7H, Ar-H).

7g : IR (KBr): 650 (C=O), 3234 (NH) (NH_2), 1500, 1321 (-NO_2), 1606, 1569, 1024 (thiophene), PMR ($CDCl_3$)6.38, (s, 2H, NH_2), 6.84 (s, H, NH), 6.88 (s, H, C_5-H), 11-8.27 (6H, Ar-H).

METHODOLOGY FOR BIOLOGICAL ACTIVITY

Antihelmintic Activity Studies

Literature Survey revealed that there is no report available regarding anthelmintic activity of substituted cinnolothiophene derivatives. In the present work the substituted cinnolothiophene derivatives were evaluated for anthelmintic activity by following method.

Worms Collection and Authentication

Indian earthworms (Pheretima posthuma) were collected from the waterlogged areas of soil and identified at Indian Veterinary Research Institute, Izatnagar and were utilized for in-vitro anthelminthic assay as per standard protocol (Garg and Atal, 1963).[6]

Preparation of Test Sample

Suspensions of samples were prepared by triturating the samples with 12.5% tween 80 and distilled water and the resultant mixture stirred using a mechanical stirrer for 30 minutes. The resulting suspensions were used for the activity studies. The suspensions were diluted to contain 100 mg in 50 ml of the test samples. Standard drug mebendazole was also be prepared with the same concentration in a similar way.

Anthelmintic Assay

The anthelmintic assay was carried out as per the method of Garg and Atal, (1963)[66] with minor modifications. The assay was performed on adult Indian earthworm (Pheretima posthuma) due to it anatomical and physiological resemblance with the intestinal roundworm of man and animal. 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 were kept as control in 50 ml suspension of distilled water and 12.5% tween 80. 50 ml each of suspension of the test compounds were added into separate Petri plates containing five earthworms in each. The time required for the paralysis and death of the worms were noted. The death time was ascertained by placing the earthworms in warm water at 50^{o}C, which stimulated the movement if the worm will alive.

RESULTS AND DISCUSSION

The synthesis of substituted cinnolothiophene derivatives by the described method, has resulted in products with good yield and Analysis of spectral data by IR, NMR and MASS spectroscopy method revealed the successful formation of substituted cinnoline thiophene derivatives (Table 1). Purity of all the compounds is proved by checking their melting point,

which gave sharp melting point, also by carrying out their TLC where all the compounds gave single spot. Halogen Substituted Compounds Showed potent Ant-helmintic activity in comparison to other compounds**.**

Figure 2 : Anthelmintic Activity of Substituted Cinnolothiophene Derivatives

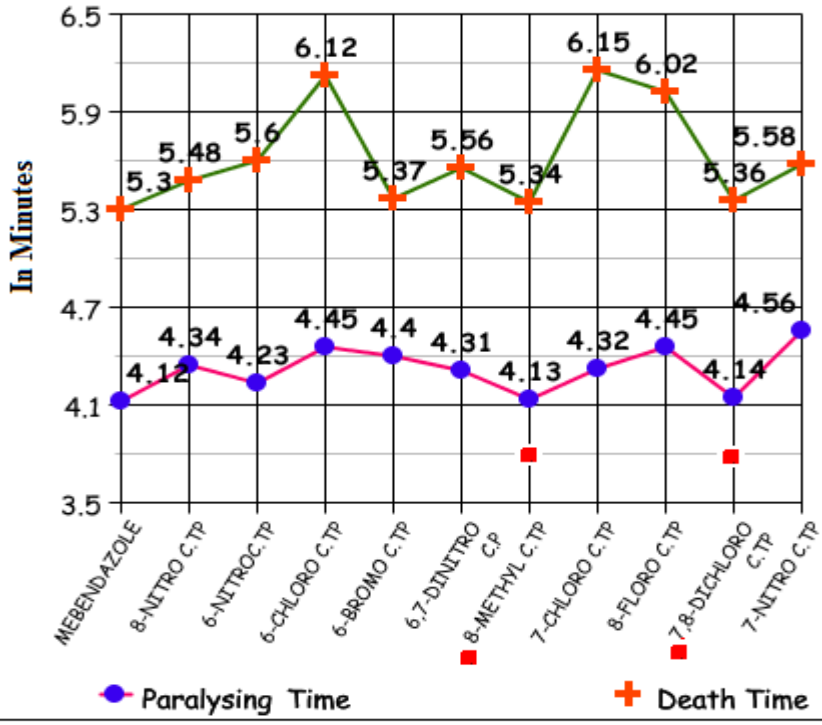

Table : 2 : Anthelmintic activity of substituted Cinnolothiophene derivatives

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
7a	100	4.34±0.43**	5.48±0.21*
7b	100	4.23±0.48**	5.60±0.62**
7c	100	4.45±0.21**	6.12±0.42**
7d	100	4.40±0.35**	5.37±0.12**
7e	100	4.31±0.32**	5.56±0.63**
7f	100	4.13±0.58**	5.34±0.57**
7g	100	4.32±0.74***	6.15±0.26***
7h	100	4.45±0.20**	6.02±0.74**
7i	100	4.14±0.24**	5.36±0.35***
7j	100	4.56±0.50**	5.58±0.45**

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

Anthelmintic Activity: If we compare anthelmintic results among all the compounds of substituted cinnolothiophene series then we can say that only Methyl and Dichloro substituted compounds showed potent activity and majority of compounds showed partial activity. The most compounds showed delayed paralyzing time in comparison to standard drug but even less death time when compared to standard drug, this might be due to the reason that these derivatives required more time for absorption and once when they absorbed they produced anthelmintic action rapidly due to this they require more time for paralyzing the worms rather than death. Further, it would be suggested that these derivatives require improvement in their physicochemical properties in order to get good absorption properties and then further evaluated for shorter paralyzing time in worms.

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