Chapter 2
CHAPTER 2
Syntheses of aminothiophene-dicarboxamides and dicyanothiophene-acetamides and study of their molluscicidal activity against \textit{Indoplanorbis exustus} snail

In this chapter, we have synthesized 5-amino-3-(aryl)thiophene-2,4-dicarboxamides and 4-aryl-dicyanothiopheneacetamides. These derivatives were studied for bioactivity against \textit{Indoplanorbis exustus} snail which is a serious host of parasite of genus \textit{Schistosoma}. These parasites reduce livestock productivity and also act as a source of cercarial dermatitis in human beings.

This chapter is divided into two sections \textbf{I} and \textbf{II}

\textbf{Section I}: Synthesis of 5-amino-3-(aryl)thiophene-2,4-dicarboxamide and 4-aryl-dicyanothiopheneacetamide

\textbf{Section II}: Evaluation of molluscicidal activity of above derivatives against \textit{Indoplanorbis exustus} snail

\textbf{Section I} Synthesis of 5-amino-3-(aryl)thiophene-2,4-dicarboxamide (ATDC) and 4-aryl-dicyanothiopheneacetamide (DCTA)

\textbf{2.1 Introduction}

The freshwater snail \textit{Indoplanorbis exustus} found across India [1], Southeast Asia [2], Arabia and Africa [2], Central Asia (Afghanistan) [3], is the intermediate host for parasite of genus \textit{Schistosoma nasale}, \textit{Schistosoma spindale} and \textit{Schistosoma indicum} [2]. This snail is a hermaphroditic invasive species with high fecundity and is responsible for the
transmission of several immature stages of the genus *Schistosoma* which infects cattle and causes reduction in livestock productivity, also causes cercarial dermatitis among rural workers, particularly in India [4]. In spite of its long history and wide geographical range, it is thought that *Indoplanorbis* includes only a single species. Schistosomiasis is an endemic parasitic disease in tropical and subtropical regions that affect over 200 million people worldwide and is second only to malaria having adverse effect on social and economic development of the countries [5]. The control of snail intermediate host is an effective means of reducing the disease transmission. The early use of molluscicides in schistosomiasis control was reviewed by Duncan et al. [6, 7]. Quinolone derivatives [8], quinoline alkaloids [9] were prepared and tested as molluscicides previously. Thiophene in particular has been investigated by Royer et al. [10] and reported the effectiveness of some mono and polyhalogenated thiophene carboxanilides against *Biomphalaria glabrata* snail. Also other thiophene containing compounds have been evaluated [11]. On this context, in this chapter, we presented the syntheses and molluscicidal activity of thiophene derivatives which showed high molluscicidal activity at ppm concentrations. The results obtained showed a significant ($P < 0.05$) molluscicidal activity with $LC_{50} = 0.6043$ ppm and $LC_{50} = 0.6511$ ppm. Though these compounds are very effective molluscicides, may have more adverse effect to the environment. However, this work is only at the preliminary stage until we find some more suitable effective thiophene moieties. Then we can enhance our study to evaluate the biodegradability as well as its effect on other living organisms. Unsubstituted analogue of thiophene 4 has been reported in literature in Chapter 1 while its derivatives are still unknown which showed very good molluscicidal activity explained in this chapter.
2.1.1 Literature updates of various heterocyclic compounds having molluscicidal activity

In literature many workers have synthesized various classes of heterocyclic compounds and tested for their molluscicidal activity as;

1) M. Abbas et al. [12] has synthesized and evaluated molluscicidal and larvicidal activities of some novel enaminones derived from 4-hydroxyquinolinones. (Scheme 1 and 2)

\[ \text{Scheme 1} \]

\[ \text{Scheme 2} \]

The LC\textsubscript{50} results revealed that the most active compounds against \textit{B. Alexandrina} snails are in sequence \( 2 > 5 > 8 \) and vice a versa against \textit{L. Natalensis} snails.
2) Our labmate [13] have synthesized quinolon-3-azoles 4 and tested against *Machrochlamys indica* snails which showed good molluscidal activity. The pyrazoline and isoxazoline 4 derivatives showed good molluscidal activity with LC$_{50}$ = 0.7876 and LC$_{50}$ = 0.7765 respectively. (Scheme 3)

![Scheme 3](image)

The synthetic compound Bayluscide [14] is an ethanol amine salt of niclosamide 5 and yurumin 6 were used as a molluscicide in many parts of the world. But major demerit of Bayluscide as a synthetic molluscicide is its toxicity to fish. (Structure 1)

![Structure 1](image)

3) Recently, Fadda *et al.* [15] has reported the molluscidal activity of new thiophene, thiazole and pyrazole derivatives. Such chemical compounds reduce oxygen consumption level in glycolysis pathway which consequently causes death of snails [16].
4) *E. M. El-Telbani et al.* [17] has successfully synthesized and evaluated molluscicidal activities of some new pyrazole, isoxazole, pyridine, pyrimidine, 1,4-thiazine and 1,3,4-thiadiazine derivatives incorporating benzofuran moiety.

2.2 Present Work

Since scientists have been extensively studied the syntheses of various new class of heterocyclic compounds and evaluated their molluscicidal and larvicidal activities, encouraged us to synthesize new class of thiophene derivatives and their functional group
interconversions (FGIs) to study bio-activity against *Indoplanorbis exustus* snail which carries *schistosoma* species.

**A retrosynthetic Approach**

### 2.2.1 Synthesis of 5-amino-3-(aryl)thiophene-2,4-dicarbonitrile, 23a-c

Syntheses of 5-amino-3-(aryl)thiophene-2,4-dicarbonitrile 23a-c can be achieved by *in situ* condensation of 3-(aryl)-3-oxo-propionitrile 24a-c, malononitrile 25 and elemental sulphur (S₈) as depicted in following retrosynthetic approach. Synthesis can be done as discussed (Chapter 1, 1.2.1) (Scheme 6)

![Scheme 6](image)

**Scheme 6**

### 2.2.2 Syntheses of N-(4-(aryl)-3,5-dicyanothiophen-2-yl)acetamide, 29a-c

Syntheses of N-(4-(4-chlorophenyl)-3,5-dicyanothiophen-2-yl)acetamide, 29a-c could be synthesized by treatment of compound 23a-c with acetic anhydride. (Scheme 7)

![Scheme 7](image)

**Scheme 7**
2.2.3 Syntheses of 5-amino-3-(aryl)thiophene-2,4-dicarboxamide, 30a-c

Syntheses of dicarboxamide compound 5-amino-3-(4-chlorophenyl)thiophene-2,4-dicarboxamide 30a-c could be achieved by acid hydrolysis of compound 23a-c. (Scheme 8)

\[ \text{Scheme 8} \]

2.2.4 Syntheses of 5-(4-chlorophenyl)-3,4-dihydro-2-alkyl-4-oxothieno[2,3-d]pyrimidine-6-carboxamide, 36a-b

Syntheses of 5-(4-chlorophenyl)-3,4-dihydro-2-alkyl-4-oxothieno[2,3-d] pyrimidine-6-carboxamide 36a-b derivatives could be achieved by treatment of compound 30 with aromatic aldehydes and acid chlorides respectively. (Chapter 1, 1.2.7) (Scheme 9)

\[ \text{Scheme 9} \]
2.3 Results and Discussion

2.3.1 Syntheses of 5-amino-3-(aryl)thiophene-2,4-dicarbonitrile, 23a-c

![Scheme 6](image)

The dicyano compounds 23a-c were successfully prepared by one pot reaction of 3-(aryl)-3-oxo-propionitrile 24a-c, malononitrile 25 and elemental sulphur (S₈) in pyridine at 55°C provided stochiometric yield i.e. 85-90% yield. (Experiment No. 1, Page 67) Structural assignment of these compounds were performed by IR, ¹H NMR, mass spectrometric and elemental analysis. The IR spectrum of this compound 23b showed absorption peaks at 3398, 3327 cm⁻¹ for NH₂, 2206 cm⁻¹ for CN and 1618 cm⁻¹ for Ar-C=CH functional groups. The ¹H NMR in DMSO-d₆ showed three aromatic protons as singlets at δ 8.19-8.26, 8.48 assigned for two NH₂ protons. (Spectrum No. 1 and 2, Page 55). The mass spectral analysis showed the molecular ion peak at m/z = 361 and the elemental analysis was in agreement with the molecular formula C₁₄H₁₃F₂N₂S.

2.3.2 Syntheses of N-(4-(aryl)-3,5-dicyanothiophen-2-yl)acetamide, 29a-c
Scheme 7

\[
\text{Ar-CN-SN-H}_2 \xrightarrow{\text{Ac}_2O, \text{Conc. H}_2\text{SO}_4} \text{Ar-CN-SN-O} \\
\text{rt, 1 h} \\
85-90\%
\]

Spectrum No. 1: \(^1\text{H NMR (DMSO-\text{d}_6)}\) spectrum of 5-Amino-3-(3,5-bis(trifluoromethyI)phenyl)thiophene-2,4-dicarbonitrile, 23b

Spectrum No. 2: MS of 5-Amino-3-(3,5-bis(trifluoromethyI)phenyl)thiophene-2,4-dicarbonitrile, 23b
Treatment of compound 23a-c with acetic anhydride in presence of catalytic amount of Conc. H₂SO₄ at room temperature (rt) afforded \( N-(4\)-(aryl)-3,5-dicyanothiophen-2-yl)acetamide 29a-c as an off-white solid with 85-90% yield. (Experiment No. 2, Page no. 69) The product obtained need not require any purification and its structure was established on the basis of IR, \(^1\)H NMR, mass spectrometric and elemental analysis. Compound 29b exhibited –NH and –CN stretching frequencies at 3261, 2216 cm\(^{-1}\) and amide CO at 1703 cm\(^{-1}\). The \(^1\)H-NMR in DMSO-\(d_6\) clearly showed singlet at \(\delta 2.33\) for CH\(_3\) protons and broad singlet for NH proton much downfield at \(\delta 12.73\). (Spectrum No. 3, Page 57) Mass spectrometric analysis of acetamide 29b showed the molecular ion peak at \(m/z = 402\) and the elemental analysis was in agreement with the molecular formula \(C_{16}H_{17}F_6N_3OS\).
### 2.3.3 Syntheses of 5-amino-3-(aryl)thiophene-2,4-dicarboxamide, 30a-c

Dicarboxamide compound 30a-c was easily synthesized by acid hydrolysis of dicyanoo compound 23a-c using Conc. H$_2$SO$_4$ at room temperature in 90-94% yield. *(Experiment No. 3, Page no. 70)* This compound was well characterized by IR, $^1$H NMR, mass spectrometric and elemental analysis. Compound exhibited amide NH$_2$ and amide free
NH₂ stretching frequencies at 3364, 3308, 3284 cm⁻¹ and amide CO at 1656, 1672 cm⁻¹. The ¹H-NMR in DMSO-d₆ clearly showed broad singlets at δ 4.67, 4.98 for amide NH₂ protons, broad singlet for free NH₂ proton at δ 8.03 and three aromatic protons as singlets at δ 6.87-7.95. (Spectrum No. 4, Page 58) Mass spectrometric analysis of dicarboxamide 30b showed the molecular ion peak at m/z = 398 (M+1) and the elemental analysis was in agreement with the molecular formula C₁₄H₉F₆N₃O₂S.

2.3.4 Syntheses of 5-(4-chlorophenyl)-3,4-dihydro-2-alkyl-4-oxothieno[2,3-d]pyrimidine-6-carboxamide, 36a-b
This part of result and discussion is already mentioned in [Chapter 1, Page No. 41 (Scheme 24)] (Experiment No. 10, Page no. 41)

**Section II** Evaluation of molluscicidal activity of above derivatives against

*Indoplanorbis exustus* snail

### 2.1.1 Introduction

**2.1 Molluscicidal activity**

Scistosomiasis is the most prevalent tropical parasitic disease of humans after malaria especially in developing countries with estimation that at least that at least 200 million people in 74 countries are infected and 600 million are at a risk of infection. Although, the current strategy for morbidity control is largely based on chemotherapy [18], control of the snail host utilizing various molluscicides to stop transmission cycle is considered a very important role which leads to break evolutionary lifecycle of *scistosoma* parasites via destructing its intermediate host. [19]
Material and method used for recording the Mortality and LC₅₀ value

The *Indoplanorbis exustus* snails were collected Godavari river in Nashik district (MS, India). The name confirmed from our institute’s expertise from Zoology department Nashik (M.S, India). These snails were first acclimatized for three weeks in laboratory conditions and examined to ensure that they were free from parasitic infection. Molluscicidal effect of the new compounds was evaluated by inclusion of an inert solvent to help the dissolution of samples. The compounds 23a-c, 29a-c, 30a-c and 36a-b were separately dissolved in a small amount of DMF (~0.5 mL) and added to dechlorinated water to obtain a series of concentrations ranging from 0.02 to 40 ppm of each compound under investigation. Then, 10 snails having same size were selected and used in each experiment and maintained in the tested solution under laboratory condition at room temperature for 24 h. Each experiment was repeated three times and the mean number of killed snails counted by for each concentration as shown in Tables 1 and 2. Snails were considered dead if they remained motionless. A control condition was taken by placing
10 snails in dechlorinated water containing DMF (~ 0.5 mL) which showed no harmful effect. These bioassays are in accordance with the WHO guidelines and Finney’s method [20, 21]. Compounds 23a-c and 36a-b did not showed any activity at all against these snails.

**Finney’s method:**

**a) Bioassays**

Quantal response data was obtained using bioassay and each unit in the bioassay was the entity that receives the treatment. In assays each snail is individually treated the unit was the individual snail. When a group of snails were treated by solution, the group (not individuals) is unit. For experimental precision each unit must be a constant, for instance the snails were obtained from the same place, same age, stage, sex, same nutrition and rearing conditions. In bioassay batches of snails were exposed to a range of doses of poison. The size of each batch is often determined by practical considerations. The larger number per batch will have more accuracy. However there was little advantage in exceeding 30 to 50 per batch unless the population was heterogeneous (Busvin, 1971) [25]. For rice planthoppers experimental batches of 10 to 15 in 4 or 5 batches of a total of 40 to 65 standardized units will often suffice. Selection of snails units to each batch was best done in a randomized manner. In selecting the doses or concentrations of the poison for the experiment it was best to space them evenly over the mortality range. Since toxicity was related to the logarithm of dose, the dose range in a geometric series was preferred, such as 2, 4, 8, 6, 32 or 1, 3, 9, 27. The control batches were exposed to the same treatments, except for the inclusion of the poison which means control snails need to be treated with the solvent used to dilute the solutions. Replications were best done on
different days within a short period assuming that the day-to-day variability was not a source of error. Within each replicate the order in which treatment doses are used should be from the lowest to the highest.

b) Correction for control mortality – the Abbott formula

In bioassays, it was common to expect a proportion of the snails in the control batches to die during the experiment due to natural causes or the control treatment with the solvent. To correct this Abbott formula was often used. The formula attributed to Abbott [22] had in fact been used earlier by Tattersfield and Morris [23] is usually in the form

\[
P = \frac{Po-Pc}{100-Pc} \times 100
\]

Where P is the corrected mortality, Po is the observed mortality and Pc is the control mortality, all expressed in percentages.

c) Probit analysis – a statistical method in bioassays

The statistical theory and techniques using probit analysis for analyzing data from dosequantal response experiments were developed by D.J. Finney in 1971 and details are also discussed by Finney in 1978 and Robertson et. al. 2007 [24]. Data obtained from the bioassays are generally in percent response (mortality or affected) at the corresponding doses (or concentrations). When the percent responses were plotted against the doses, an S shape curve is obtained. This was because toxicity was better related to the logarithm of the dose thus in the analysis the dose variable was normally transformed into the logarithmic scale. The usual way to estimate LD₅₀ is from a regression line relating log dose to a transformed percentage response [25] and the usual transformation used were probits. Transformation of percent response to probits is available in Appendix A and can also be calculated by using a microcomputer. The critical LD₅₀ values may be estimated
from probits and log doses in several ways. The simplest is by graphical methods. Another is by using standard computation using a calculator. Step by step calculations are also available in Busvine 1971. The faster and more accurate way is using computer program or software. Several statistical packages like SAS, SPSS have probit analyses options. In this book we focus our attention on using the POLO software [26] and further refined by LeOra software in 2004.

d) Relative potency

The toxicities of two or more molluscicides were compared on the basis of potency or the reciprocal of an equitoxic dose [25]. For valid comparison the dose- mortality lines for the molluscicides should be parallel. Otherwise the relative potency will vary with the mortality used. If two regression lines were written as

\[ Y_1 = a_1 + b x_1 \] and \[ Y_2 = a_2 + b x_2 \] When the slopes are similar, b is common and at the equitoxic dose \( Y_1 = Y_2 \) and hence \( a_1 + b x_1 = a_2 + b x_2 \)

\[ X_1 + X_2 = \frac{a_2 - a_1}{b} = M \]

\( M \) is thus the difference in position of the two slopes and its anti-logarithm is the potency ratio. PoloPlus computes the potency ratio and its fiducial limits (at \( p = 0.95 \)).
Table 4 Molluscidal activity of ATDC 29a-c against *Indoplanorbis exustus*, (10 snail’s concentration) after 24 h exposure at ambient temperature 24 ± 1°C

<table>
<thead>
<tr>
<th>Cpd</th>
<th>Dose (ppm)</th>
<th>% mortality</th>
<th>LC50 (ppm ± S.E.)</th>
<th>Regression Eq&quot; Y = a + bx</th>
<th>Heterogeneity</th>
<th>Variance</th>
<th>Fiducial Limit</th>
</tr>
</thead>
<tbody>
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<td>Control</td>
<td>0</td>
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<td>10</td>
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<td></td>
</tr>
<tr>
<td><strong>29a</strong></td>
<td>20</td>
<td>20</td>
<td>0.6043 ± 0.4078</td>
<td>Y = 3.3001 - 0.7791(x)</td>
<td>3.063</td>
<td>0.1663</td>
<td>m1 = -1.2005</td>
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<tr>
<td>30</td>
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<tr>
<td><strong>29b</strong></td>
<td>20</td>
<td>30</td>
<td>0.7506 ± 0.3089</td>
<td>Y = 2.0403 - 2.0418(x)</td>
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<tr>
<td><strong>29c</strong></td>
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<td>40</td>
<td>0.6067 ± 0.5028</td>
<td>Y = 1.8094 - 2.3115(x)</td>
<td>3.045</td>
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</table>

The data are the average of three replicates and significant at P < 0.05
### Table 5 Molluscicidal activity of DCTA 30a-c against *Indoplanorbis exustus* (10 snail's concentration) after 24 h exposure at ambient temperature 24 ± 1°C

<table>
<thead>
<tr>
<th>Cpd</th>
<th>Dose in ppm</th>
<th>% Mortality</th>
<th>LC₅₀ (ppm ± S.E.)</th>
<th>Regression Eq (^a)</th>
<th>Heterogeneity</th>
<th>Variance</th>
<th>Fiducial Limit</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>(Y = a + bx)</td>
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<td><strong>30a</strong></td>
<td>0.04</td>
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<td>0.6511 ± 0.0786</td>
<td>(Y = 5.0131 - 2.6986(x))</td>
<td>2.2224</td>
<td>0.0061</td>
<td>(m_1 = -1.6597)</td>
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<td>0.06</td>
<td>60</td>
<td>2.6986(x)</td>
<td>(Y = 4.6902 - 4.193(x))</td>
<td>2.3725</td>
<td>0.0118</td>
<td>(m_2 = -1.9677)</td>
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<td></td>
<td>0.08</td>
<td>80</td>
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<td>(Y = 4.8324 - 0.7971(x))</td>
<td>2.1042</td>
<td>0.0156</td>
<td>(m_3 = -2.1275)</td>
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<td><strong>30b</strong></td>
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<td>0.7403 ± 0.1087</td>
<td>(Y = 4.6902 - 4.193(x))</td>
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<td>4.193(x)</td>
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<td>2.1042</td>
<td>0.0156</td>
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<td><strong>30c</strong></td>
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<td>0.7628 ± 0.1250</td>
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<td>0.0061</td>
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<td>2.3725</td>
<td>0.0118</td>
<td>(m_3 = -1.9677)</td>
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The data are the average of three replicates and significant at \(P < 0.05\)
2.3.11 Results and Discussion

These snails had been recently considered as one of the most serious pests in Nashik District (MS, India). The toxicity of compounds 23a-c, 29a-c, 30a-c and 36a-b against *I. exustus* snails was evaluated and results with calculated half lethal dose (LC$_{50}$) in ppm (Table 4 and Table 5). Compounds 29a and 30a exhibited the highest toxic action with LC$_{50}$ = 0.6043 ppm and LC$_{50}$ = 0.6511 ppm respectively. In the series of ATDC 29a-c and DCTA 30a-c, compound 29a showed higher molluscicidal activity with LC$_{50}$ value (0.6043 ppm) at 10-40 ppm concentration while, compound 30a showed highest molluscicidal activity with LC$_{50}$ value (0.6511 ppm) at 0.02-0.08 ppm concentration. The activity at this minimal amount in compound 30a may be due to the presence of carboxanilide and two nitrile functionalities attached to the thiophene ring. It was also observed that the presence of chlorine (-Cl) substituent may enhance the biological activity more than the difluoro (-diF) and bis-trifluoromethyl (-bisCF$_3$) groups (Tables 4 and 5 entries) attached to the phenyl ring. Similarly, we have evaluated the toxicity of compounds 23a-c and 36a-b, which did not show any toxic action at all against *I. exustus* snail. During the exposure period, the effusion of the gelatinous material was observed from snails under influence of the tested compounds. This fact may reveal the mode of their toxic action is through the physical interference of cell membranes, resulting in hindering their functions and cause of subsequent death. Compounds 29a and 30a (LC$_{50}$ = 0.6043 ppm and LC$_{50}$ = 0.6511 ppm resp.) seemed to be promising and after some modifications will be considered in future study. Therefore, control measures are necessary. The manufacturing of these compounds is inexpensive, hence can be considered as further molluscicides because of their higher activity at ppm concentration.


2.4 Conclusion

The polysubstituted thiophene derivatives having potential molluscicidal activity can be obtained by simple and convenient method of preparation having satisfactory yields. The series of aminothiophene-dicarboxamides 29a-c and dicyanothiophene-acetamides 30a-c showed significantly potent activities against *I. exustus* snails. In addition, the infection rate and prepatent period of *I. exustus* snails were remarkably controlled on exposure to the tested *N*(4-(aryl)-3,5-dicyanothiophen-2-yl)acetamide, 29a-c and 5-amino-3-(aryl)-thiophene-2,4-dicarboxamide derivatives 30a-c.

2.5 Experimental Section

2.5.1 Experiment No. 1

Synthesis of *N*-amino-(4-aryl)thiophene-2,4-dicarbonitrile, 23a-c

These compounds were synthesized by the known literature method [16-19, 22] and recrystallized from appropriate solvents. (EJMC paper references number)

\[
\text{General Procedure}
\]

3-(Aryl)-3-oxo-propionitrile 24a-c (0.01 mol) and malononitrile 25 (0.01 mol) and elemental sulphur (0.012 mol) were heated together in pyridine (40 ml) at 55 °C for 8 h. After completion, excess pyridine was concentrated under reduced pressure and solid
obtained was stirred in methanol (20 ml) for 0.5 h at room temperature. This solid was filtered, dried under vacuum and need not any further purification.

**5-Amino-3-(4-chlorophenyl)thiophene-2,4-dicarbonitrile, 23a**

This compound was recrystallized in (EtOH: DMF, 8:2) obtained as yellow amorphous solid; Yield 2.33 g (90%); m.p.: 292-294 °C; IR (KBr): 3383, 3304, 2198, 1630 cm\(^{-1}\); \(^1\)H NMR: (DMSO-\(d_6\)): \(\delta\) 7.57 (d, \(J = 8.4\) Hz, 2H, Ar-H), 7.62 (d, \(J = 8.4\) Hz, 2H, Ar-H), 8.37 (bs, 2H, NH\(_2\)); \(^13\)C NMR: (DMSO-\(d_6\)): \(\delta\) 85.7, 85.8, 114.9, 115.1, 129.5, 130.5, 135.2, 150.9, 198.3; MS (70 eV): \(m/z = 259\) (M\(^+\), 33), 261 (M+2, 11).

Analysis Calculated for C\(_{12}\)H\(_6\)ClN\(_3\)S (259.71): Calcd: C, 55.50 %; H, 2.33 %; N, 16.18 %

Found: C, 55.38 %; H, 2.15 %; N, 16.32 %

**5-Amino-3-(3,5-bis(trifluoromethyl)phenyl)thiophene-2,4-dicarbonitrile, 23b**

This compound was obtained as colourless solid; Yield 3.00 g (85%); m.p. 230-231 °C; IR (KBr): 3398, 3327, 2206, 1618 cm\(^{-1}\); \(^1\)H NMR: (DMSO-\(d_6\)): \(\delta\) 8.19 (s, 2H, Ar), 8.26 (s, 1H, Ar-H), 8.48 (bs, 2H, NH\(_2\)); MS (70 eV): \(m/z = 361\) (M\(^+\), 80).

Analysis Calculated for C\(_{14}\)H\(_5\)F\(_6\)N\(_3\)S (361.27): Calcd: C, 46.54 %; H, 1.40 %; N, 11.63 %

Found: C, 46.45 %; H, 1.36 %; N, 11.70 %

**5-Amino-3-(2,4-difluorophenyl)thiophene-2,4-dicarbonitrile, 23c**

This compound was recrystallized in (EtOH: DMF, 8:2) obtained as brown solid; Yield 2.22 g (85%); m.p. 244-246 °C; IR (KBr): 3387, 3292, 2204, 1629 cm\(^{-1}\); \(^1\)H NMR: (DMSO-\(d_6\)): \(\delta\) 7.27-7.34 (m, 1H, Ar-H), 7.49-7.68 (m, 2H, Ar-H), 8.43 (bs, 2H, NH\(_2\)); MS (70 eV): \(m/z = 260\) (M-1, 40).

Analysis calculated for C\(_{12}\)H\(_3\)F\(_2\)N\(_3\)S (261.25): Calcd: C, 55.17 %; H, 1.93 %; N, 16.08 %

Found: C, 55.09 %; H, 1.99 %; N, 16.22 %
2.5.2 Experiment No. 2

Syntheses of N-(4-(aryl)-3,5-dicyanothiophen-2-yl)acetamide, 29a-c

![Scheme 7](image)

**Procedure**

To compound 23a-c (0.01 mol) and acetic anhydride (5 mL), a drop of conc. H$_2$SO$_4$ was added and then stirred at room temperature for 1-1.5 h (TLC check hexane: ethylacetate, 2:1). Resulting reaction mixture was poured over crushed ice and stirred overnight. The separated solid was filtered, washed with cold water, dried in vacuum and purified by column chromatography (Hexane: ethyl acetate, 2:1).

**N-(3,5-dicyano-4-(4-chlorophenyl)thiophen-2-yl)acetamide, 29a**

This compound was obtained as pale yellow amorphous solid; Yield 2.72 g (90%); m.p. 298-300 °C; IR (KBr): 3267, 2216, 1703, 1625 cm$^{-1}$; $^1$H NMR: (DMSO-$d_6$): $\delta$ 2.31 (s, 3H, CH$_3$), 7.64 (d, $J$ = 8.7 Hz, 2H, Ar-H), 7.73 (d, $J$ = 8.7 Hz, 2H, Ar-H), 12.63 (s, 1H, NH); $^{13}$C NMR (DMSO-$d_6$): $\delta$ 22.3, 92.7, 96.4, 112.9, 113.7, 129.0, 129.4, 130.3, 135.0, 147.8, 153.1, 170.2; MS (70 eV): $m/z$ = 301 (M$^+$, 20), 303 (M+2, 6.66).

Analysis Calculated for C$_{14}$H$_8$CIN$_3$O$_3$ (301.75): Calcd: C, 55.72 %; H, 2.67 %; N, 13.93 %

Found: C, 55.66 %; H, 2.52 %; N, 13.75 %

**N-(3,5-dicyano-4-(3,5-bis(trifluoromethyl)phenyl)thiophen-2-yl)acetamide, 29b**

This compound was recrystallized in hexane: ethyl acetate, 2:1 obtained as white amorphous solid; Yield 3.55 g (88%); m.p. 272-274 °C; IR (KBr): 3261, 3227, 2216, 1703, 1622; $^1$H
NMR: (DMSO-d$_6$): $\delta$ 2.33 (s, 3H, CH$_3$), 8.35-8.38 (m, 3H, Ar-H), 12.73 (s, 1H, NH; MS (70 eV): $m/z = 402$ (M-2, 100).

Analysis Calculated for C$_{16}$H$_7$F$_6$N$_3$OS (403.30): Calcd: C, 47.65 %; H, 1.75 %; N, 10.42%  
Found: C, 47.52 %; H, 1.64 %; N, 10.38 %

**N-(3,5-dicyano-4-(2,4-difluorophenyl)thiophen-2-yl)acetamide, 29c**

Colourless amorphous solid; Yield 2.57 g (85%);  
m.p. 310-312 °C; IR (KBr): 3267, 3211, 2216, 1699, 1618 cm$^{-1}$; $^1$H NMR: (DMSO-d$_6$): $\delta$ 2.31 (s, 3H, CH$_3$), 7.33-7.75 (m, 3H, Ar-H), 12.67 (s, 1H, NH); MS (70 eV): $m/z = 302$ (M-1, 70).

Analysis Calculated for C$_{14}$H$_7$F$_2$N$_3$OS (303.29): Calcd: C, 55.44 %; H, 2.33 %; N, 13.85 %  
Found: C, 55.58 %; H, 2.26 %; N, 13.71 %

### 2.5.3 Experiment No. 3

**Syntheses of 5-amino-3-(aryl)thiophene-2,4-dicarboxamide, 30a-c**

![Scheme 8](image)

**General Procedure**

Compound 23a-c (0.01 mol) was stirred in conc. H$_2$SO$_4$ (10 mL) at room temperature for about 4-6 h (TLC check hexane: ethylacetate, 1:1). The reaction mass was then added over (250 mL) crushed ice and neutralized with saturated NaHCO$_3$ (30 mL) solution. The crude solid separated was filtered, washed with water, dried and recrystallized or purified.
by either appropriate solvent or from column chromatography (Hexane: ethyl acetate 6:4).

5-Amino-3-(4-chlorophenyl)thiophene-2,4-dicarboxamide, 30a

This compound was recrystallized in EtOH obtained as pale yellow crystals; Yield 2.65 g (90%); m.p. 200-202 °C; IR (KBr): 3475, 3345, 3327, 3306, 3252, 3169, 1668, 1672, 1564, 1479, 1384 cm\(^{-1}\); \(^1\)H NMR (DMSO-\(d_6\)): \(\delta\) 4.82 (bs, 2H, amide NH\(_2\)), 6.84 (bs, 2H, amide NH\(_2\)), 7.38 (d, \(J = 8.3\) Hz, 2H, Ar-H), 7.56 (d, \(J = 8.3\) Hz, 2H, Ar-H), 7.69 (bs, 2H, NH\(_2\)); \(^1^3\)C NMR (DMSO-\(d_6\)): \(\delta\) 109.3, 114.7, 129.2, 131.1, 133.6, 134.3, 139.0, 162.9, 163.0, 166.8; MS (70 eV): \(m/z = 296\) (M\(^+\) 1, 50), 298 (M\(^+\)2, 15).

Analysis Calculated for C\(_{12}\)H\(_8\)ClN\(_3\)O\(_2\)S (295.74): Calcd: C, 48.73 %; H, 3.41%; N, 14.21 %

Found: C, 48.82 %; H, 3.62 %; N, 14.12 %

5-Amino-3-(3,5-bis(trifluoromethyl)phenyl)thiophene-2,4-dicarboxamide, 30b

This compound was obtained as colourless amorphous solid; Yield 3.65 g (92%); m.p. 190-192 °C; IR (KBr): 3497, 3364, 3308, 3284, 1656 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\)): \(\delta\) 4.67 (bs, 2H, amide NH\(_2\)), 4.98 (bs, 2H, amide NH\(_2\)), 6.87-7.95 (m, 3H, Ar-H), 8.03 (bs, 2H, NH\(_2\)); MS (70 eV): \(m/z = 398\) (M\(^+\) 100).

Analysis Calculated for C\(_{14}\)H\(_8\)F\(_6\)N\(_3\)O\(_2\)S (397.30): Calcd: C, 42.32 %; H, 2.28 %; N, 10.58 %

Found: C, 42.20 %; H, 2.34 %; N, 10.41 %

5-Amino-3-(2,4-difluorophenyl)thiophene-2,4-dicarboxamide, 30c

This compound was obtained as green amorphous solid; Yield 2.80 g (94%); m.p. 264-265 °C; IR (KBr): 3484, 3357 for (NH\(_2\)), 3312, 3289, 1662 cm\(^{-1}\); \(^1\)H NMR (DMSO-\(d_6\)): \(\delta\) 6.15 (bs, 2H, amide NH\(_2\)), 6.38 (bs, 2H, amide NH\(_2\)), 7.22-7.52 (m, 3H, Ar-H), 12.06 (bs, 2H, NH\(_2\)); MS (70 eV): \(m/z = 297\) (M\(^+\), 50).
Analysis Calculated for C_{14}H_{9}F_{6}N_{3}O_{2}S (297.28): Calcd: C, 48.48%; H, 3.05%; N, 14.13%

Found: C, 48.31%; H, 3.16%; N, 14.24%

Experiment No. 4

2.5.4 Syntheses of 5-(4-chlorophenyl)-3,4-dihydro-2-alkyl-4-oxothieno[2,3-d]
pyrimidine-6-carboxamide, 36a-b

The procedure and analytical data of compounds 36a-b were already presented
[Chapter 1, Page No. 41]
2.6 References

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