SYNTHESIS OF SOME NEW SUBSTITUTED ISATIN 1, 3, 4-THIADIAZOLINE DERIVATIVES AND THEIR PHARMACOLOGICAL STUDIES

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DEDICATED
TO MY BELOVED
PARENTS
This work was carried out in Organic Research Laboratory of the Department of Chemistry, Bangladesh University of Engineering and Technology (BUET), Dhaka during the period of May 1996 to November 1997.

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ABSTRACT

Recently isatin-1,3,4-thiadiazoline acyl derivatives have proved to be potential anticancer agent.
According to Sandmeyer Synthesis of isatin, treatment of acidic solution of primary aromatic amine with aqueous solution of chloral hydrate and hydroxylamine hydrochloride in presence of Na₂SO₄ yields oximinoacetanilide. This product on cyclization with conc. H₂SO₄ or polyphosphoric acid (PPA) gives isatin:

![Chemical structure of isatin synthesis]

All possible attempts of getting isatin structure (A-C) from different types of 1º aromatic amines by modified as well as standard procedure of Sandmeyer failed:

![Chemical structures A, B, and C]

Finally a series of disubstituted isatin derivatives (2A - 6A) were synthesized for anticancer screening tests:
All the synthesized compounds have been characterized spectroscopically.
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CHAPTER ONE
INTRODUCTION
1. INTRODUCTION

1.1. GENERAL REMARKS AND HISTORICAL BACKGROUND OF ISATIN

Isatin and Indole: Both Isatin and Indole have identical chemical structures. Indole comprises a benzene ring fused to the pyrole nucleus where the numbering begins with nitrogen and proceeds around the ring as indicated below:

![Indole and Isatin Structures](image)

Position 2 and 3 are often called α and β respectively. Indole is a structural moiety of important indole alkaloids. The orthoquinine type indole containing two carbonyl groups in the hetero ring is known as isatin. Numbering of isatin is similar to that of Indole. As a dioxo indole derivative, the chemical name of isatin is indole-2,3-dione (2).

Erdmann \(^3,4\) and Laurent \(^5,6\) independently obtained isatin (2) from the oxidation of trans indigo in 1840 and 1842 respectively. Trans indigo (6) was obtained by the oxidation of indoxyl (5) which was a fermentation product of indican glucoside (3), a dye isolated from the plant Isatin Tinctoria. The overall reaction steps are shown in the following (scheme-1).

Instead of natural product, Baeyer\(^7\) synthesized isatin in the laboratory from phenyl acetic acid. The success of the last venture spurred onwards the work on indigo (6) which was first prepared commercially in 1878 by Baeyer, through different route\(^8\), in 1897 according to the scheme-2.

This is one of the earliest synthesis in organic chemistry and it may be taken as a landmark in the history of chemistry.
Phenylacetic acid on nitration followed by reduction with zinc / hydrochloric acid leads to cyclized product, lactam. This lactam on treatment with nitrous acid gives the oxime which on reduction affords aminolactam. The aminolactam on oxidation with ferric chloride yields isatin (2). Laboratory synthesized isatin was then converted into trans indigo (6). Another route to trans indigo was from aniline as shown in scheme-2.

Isatin dissolves in alkali and gives the salt of an acid, isatinic acid (7).

This is the quick realization that isatinic acid was o-amino phenyl formic acid and that isatin was its lactam (2,3-diketo-2,3-dihydro indole).
Scheme 2
1.2 General properties of Isatin
1.2.1 Physical properties:

Isatin is a red shining crystalline solid of melting point, 200°-201°C.

It may be recrystallized from water, alcohol or glacial acetic acid. It is soluble in water, ethanol, chloroform, etc. Isatin is also soluble in conc. HCl or H₂SO₄.

Salt formation:

Isatin forms sodium or potassium salt of isatin with NaOH or KOH. Silver salt of isatin was found by the action of silver acetate in alcohol. Perchloric acid produces perchlorate of isatin. Isatin undergoes salt formation with mercurous and mercuric form of the metal mercury. But the actual structure of all the metal salts of isatin either N-metal or O-metal is unknown.

The most plausible representation for isatin anion is a hybrid of the two charged species (8,9) with a smaller contribution from the quinoid structure (10).

Due to the various canonical forms the structure of the metal complex is very difficult to characterise.

1.2.2 Tautomerism and its evidences:

Isatin and dioxindoles display tautomerism. Isatin is a ketonic lactam of the structure (2). Tautomeric possibility of isatin was early represented in the structure (2) and (2a).
The term $\psi$-isatin$^{16}$ being applied to the lactam form 2 and isatin to the lactim form 2a. This is an example of the amido-imidol tautomerism:

$$-\text{NH} - \text{C} = 0 \leftrightarrow -\text{N} = \text{C} - \text{OH}$$

amido-imidol tautomerism

Jullian et-al$^{17}$ showed that dioxindole also exhibits the following tautomerism.

Keto-enol tautomerism of dioxindole.

Evidences of tautomerism: Isatin reacts as the lactam form (8) towards most of the reagents, e.g. the N-atom of isatin is readily alkylated or acetylated when it remains in salt form$^{18}$.

But the silver salt of isatin yields $\alpha$-alkyl derivative indicated the lactim (2a) form of isatin.
Further evidence of the lactim structure 2a was observed from the reaction of isatin with PCl₅ in benzene which gave isatin α-chloride at warm condition and 3, 3-dichloro isatin at cold condition.

1.3 PREPARATION OF SUBSTITUTED ISATINS AND BIS-ISATINS

Isatin was the raw material of antimalarial drugs which was then very important drug. So, the industrial scale production was of great importance. The various methods have been developed for the preparation of isatin and its derivatives. The most important and general method was developed by Sandmeyer in Germany. Besides the Sandmeyer method, other methods have been developed. A brief account is given below:

1. **Sandmeyer Method**

Thiocarbanilide was the starting material of the early method which was treated with lead carbonate and hydrogen cyanide to convert nitrile anilide. Treatment of the later with ammonium-sulfide yields thioamide (11). The subsequent cyclization with sulphuric acid followed by hydrolysis, produced Isatin (2). This work was reported in 1903.
Later on, in 1919 Sandmeyer synthesized isatin from aniline. Treatment of the solution of aniline in concentrated hydrochloric acid with aqueous solution of chloral hydrate and hydroxylamine hydrochloride in presence of sodium sulphate produces crystals of oximinoacetanilide which on treatment with conc. sulfuric acid yields isatin by ring closure according to scheme-4.

Scheme-4
Using Sandmeyer method mono-substituted (o-, m- and p-) anilines can be converted in their corresponding isatins. Ortho- and para-substituted anilines give 7-substituted and 5-substituted isatins respectively whereas the meta substituted anilines give two positional isomeric isatins (a and b) as shown in scheme-5.

Para-substituted aniline

Ortho-substituted aniline

Meta-substituted aniline

Scheme-5

For m-substituted aniline, the major isomeric product (a or b) is determined by electronic and steric effect. Recently Islam et al. obtained 4 and 6-chloroisatin in a ratio of ca. 1:1, and the products were separated by column chromatography.
Although ortho-halo (Cl, Br, I)\textsuperscript{25-27} anilines give their corresponding 7-halo isatin but ortho-fluoro-aniline has been reported\textsuperscript{28} not to give the corresponding isatin due to strong inductive effect of fluorine atom. Similarly m-nitro aniline\textsuperscript{23} gave negative result although isonitrosoacetanilide obtained in high yield. So it is a matter of great interest whether 2-aminopyridine will undergo Sandmeyer reaction or not as pyridine is less active than benzene ring.

In a recent study by Islam et al.\textsuperscript{29} it was observed that 7-azaisatin was not possible to get by Sandmeyer reaction.

First Morsh and Schulze\textsuperscript{30} used m- and p-phenylenediamines for Sandmeyer method and obtained mono cyclization products whereas \( \alpha \)-phenylenediamine yielded hydroxyquinoxaline by intramolecular cyclization as follows:
Later on Z. Allan\textsuperscript{31} successfully applied Sandmeyer reaction to prepare bis-dioxopyrrole benzene (14) from 2, 6-diamino toluene (13) and m-phenylenediamin according to the scheme 8 A.

\begin{align*}
X = H, CH_3 \quad 13
\end{align*}

**Scheme-8A**

2. **Oxidative Halogenation Method:** Isatin can be prepared by oxidative halogenation of a 3-(lower alkylthio) oxindole, followed by hydrolysis of the 3-halo-3-(lower alkylthio) oxindole formed\textsuperscript{32-33}.

\begin{align*}
\text{Scheme- 8B}
\end{align*}
Thus 5-carbethoxy-3-(methylthio) oxindole (15) and N-chloro succinamide (NCS) in CH$_2$Cl$_2$ were stirred 24 hours at room temperature to give 3-chloro-5-carbethoxy-3-(methylthio) oxindole (15') to which red mercury oxide (HgO) and BF$_3$. Et$_2$O in 50% aqueous THF were added and stirred at room temperature for 1 hour to give (16) of 56% yield.

3. **From Oxindoles**: Oxindoles are prepared from anilides with α-halo-carbonyl chloride by means Friedel-Crafts$^{34}$ reaction, (a) reaction$^{35}$ of α-nitro-phenylacetic acid (b) and also the base catalyzed cyclization of N-acetylphenyl hydrazides$^{36}$.

![Scheme-9](image)

Isatin 3' and 4' obtained from the redox reaction of oxindoles 1' and 2' with bis-nitrone (17) respectively. These reactions are progressed via some intermediate. Finally 3' and 4' are formed via H-transfer from 6' and 2,2'-diphenyl-3,3-bisindol (9') also obtained as reducing form.
Oxidation of synthesized oxindoles\textsuperscript{37} with indolic binitrone (17) gives isatin as follows:

\begin{align*}
\text{R} = \text{H} & \quad 1' \\
= \text{CH}_3 & \quad 2' \\
\text{Scheme-10}
\end{align*}

Some intermediates

\begin{align*}
\text{R} = \text{H} & \quad 1' \\
= \text{CH}_3 & \quad 2' \\
\text{Scheme-10}
\end{align*}
(4) **N-alkylated isatin with the help of oxalyl chloride:**

This is an alternative method of isatin formation. In this method a mono anilide is formed by an aniline with oxalyl chloride\(^3\) (18).

\[ \text{R} \text{NH} \quad \text{ClO}_{2} \quad \text{NH} \quad \text{Cl} \]

\[ \rightarrow \]

\[ \text{R} \text{NH} \quad \text{Cl} \quad \text{Cl} \quad \text{O} \quad \text{N} \quad \text{Cl} \quad \text{O} \quad \text{Cl} \quad \text{AlCl}_3 \]

\[ \text{R} = \text{H} \quad \text{R} = \text{CH}_3 \]

**Scheme-11**

The 1st step is simply an addition reaction but 2nd step is an intramolecular Friedel-Crafts reaction on a monoanilide.

(5) **Heller Synthesis:** Treatment of \(\alpha\)-hydroxyaminocyano mandelate\(^3\) (19) with conc. HCl to give (20) which undergoes hydrolysis to yield \(\alpha\)-aminophenyl pyruvic acid. Finally the later on gives isatin 2 by intramolecular water elimination (scheme-12).

(6) **Claisen-Shadwell\(^4\) Synthesis:** \(\alpha\)-Nitro-benzoyl chloride reacts with sodium cyanide to give \(\alpha\)-NO\(_2\) benzoyl cyanide (21). Hydrolysis of compound, 21 gives \(\alpha\)-NO\(_2\) phenylpyruvic acid (21'). Ferrous sulphate ammonia reduction of compound, 21' gives \(\alpha\)-aminophenyl pyruvic acid under warm condition which on elimination of water molecule gives isatin (2).
When thiooxanilide (22) is treated with conc. H₂SO₄, isatin (2) is formed.

From Azalactone: High substituted isatin can be obtain when 2-nitro-3-methyl-4-hydroxyl azalactone is treated with aq. sodium hydroxide.

Both isatin (2) and nuclear substituted isatins have been synthesized by treating substituted imide chlorides of oxalic acid with H₂SO₄ and subsequent hydrolysis.
10. 0-Nitrophenyl propiolic acid\(^{44}\) (24) reacts with alkali to amino compound which undergoes hydrolysis and subsequent cyclization and decarboxylation gives isatin (2).

\[ \text{alkali} \quad \xrightarrow{\text{H}_2\text{O}} \quad \text{alkali} \]

\[
\begin{align*}
\text{alkali} & \quad \xrightarrow{\text{H}_2\text{O}} \\
\text{alkali} & \quad \xrightarrow{\text{H}_2\text{O}} \\
\text{alkali} & \quad \xrightarrow{\text{H}_2\text{O}}
\end{align*}
\]

\textbf{Scheme-17}

11. The most valuable method for synthesis many isatins is condensation of anilines with oxomalonic ester. Hydrolysis and subsequent decarboxylation of the dioxoester in open atmospheric air gives\(^{45}\) isatin (2).

\[
\begin{align*}
\text{H} \quad & \quad \xrightarrow{\text{CO}_2\text{C}_2\text{H}_5} \\
\text{H} \quad & \quad \xrightarrow{\text{CO}_2\text{C}_2\text{H}_5} \\
\text{H} \quad & \quad \xrightarrow{\text{CO}_2\text{C}_2\text{H}_5}
\end{align*}
\]

\textbf{Scheme-18}

12. Treatment of aromatic amine with dichloro acetic acid (2:1 molar ratio) leads to intermediate A (anilino oxindole\(^{46,47}\)). Upon oxidation of the latter anilide, B is formed. Hydrolysis of B yielded isatin (2).

\[
\begin{align*}
\text{2} & \quad \xrightarrow{\text{Cl}_2\text{CHCOOH}} \\
\text{2} & \quad \xrightarrow{\text{Cl}_2\text{CHCOOH}} \\
\text{2} & \quad \xrightarrow{\text{Cl}_2\text{CHCOOH}}
\end{align*}
\]

\textbf{Scheme-19}
1.4 CHEMICAL PROPERTIES OF ISATIN AND ITS DERIVATIVES:

Chemically isatin possesses both an amide and a keto group present in a five membered heterocyclic ring having fused with an aromatic nucleus. So, usually isatin displays the characteristics reactions of keto group very easily. In special circumstances both the keto and amide groups are involved in chemical reactions. Typical examples are shown below.

1.4.1 Condensation of isatin with thiosemicarbazide and thiocarbohydrazide forming schiff base :

Carbonyl group at C-3 of isatin (2) and nuclear substituted isatins is undisturbed from tautomerism\textsuperscript{48}. C-3 carbonyl group of isatin reacts with a large variety of ammonia derivatives to give many Schiff bases.

(i) Thiosemicarbazide reacts with isatin (2) to yield their corresponding thiosemicarbazones\textsuperscript{49-51}(25).

![Reaction diagram](image)

Reaction of hydrazines and amines with isatins :

(i) Shudhakar et al\textsuperscript{52} recently observed that condensation of 2-methyl-1, 8-naphththyridine-3-carboxylic acid hydrazide (26) with substituted isatins (2) to gives
their corresponding hydrazones (27), which on treatment with mercapto acetic acid in DMF in the presence of anhy. ZnCl₂ afforded the compounds (28). But upon conc. H₂SO₄ treatment of 27 cyclodehydration undergoes to yield the desired compound (29).

\[
\begin{align*}
R & = H & 5-\text{Me} & 7-\text{Me} & 5-\text{Cl} & 5-\text{Br} \\
a & & b & c & d & e
\end{align*}
\]

Scheme-20
Shailendra et al very recently synthesized some important 2-substituted 1,3,4-thiadiazolo[2,3-c]1,2,4-triazino[5,6-b]indoles (30 & 31) by the reaction of isatin with various hydrazides followed by cyclization with conc. H₂SO₄.

Scheme-21

\[
\begin{array}{c}
\text{R'} = H, 2-Cl, 4-CH₃, 2-OH, 4-NO₂ \\
a, b, c, d, e
\end{array}
\]
1.4.2. Reaction of Isatin with Aromatic Amines:

(a) Although isatin shows amido-imidol tautomerism but it reacts with o-diamines to give indolo quinoxaline or indophenazine \(^5^4\) (32) as follows:

(b) Islam et al \(^5^5\) synthesized some quinoxalines (33) from substituted isatins with o-phenylene diamine with suitable method.

(c) A novel heterocyclic \(^5^6\) system indolo \([2', 3': 5, 6] [1, 2, 4] triazino [2, 3- a] benzimidazoles (35) were obtained by condensation of diamino benzimidazoles (34) with isatin 2'.
(d) Indophenazines (37) were prepared by the reaction of isatins with natural flavones (36) at refluxing condition with AcOH.

\[
\begin{align*}
R_2^1 & \quad R_2^2 & \quad R_3 \\
36 & \quad a & \quad Me & \quad H & \quad H \\
 & \quad b & \quad Cl & \quad Cl & \quad NO_2 \\
 & \quad c & \quad Cl & \quad Br & \quad NO_2 \\
37 & 
\end{align*}
\]

N-alkylation of isatins and quinoxalines: Quinoxalines or indophenazines can be methylated at 1-position (N-Me) with dimethyl sulphate in ethanolic solution of sodium hydroxide at heating conditions. Two isomeric N-methyl products are obtained (38 and 39).

But recently a new method is developed by Ola et al. in 1990 for the methylation of isatins and indophenazines. In this method dimethyl oxalate is used in presence of a very strong base.
Oxalates are well suited synthetic reagent not only for N- but also O- and S- alkylation and often display in interesting resonoselectivity. Benzylation of isatin can also carry out with dibenzyloxalate in presence of tri-tert. potassium butoxide.

\[
\text{PhCH}_2\text{O-C} + \text{PhCH}_2\text{O-C} \xrightarrow{(\text{CH}_3)_3\text{CO}_2} \text{PhCH}_2\text{O-C}_x\text{PhCH}_2\text{O-C}
\]

Isatin reacts with thiosemicarbazide afforded isatin thiosemicarbazones which undergoes a beautiful intracyclization to give the 3 - (R - subst.) - 1, 2, 4 - triazacarbazoles (40) of greater than 75% yield.

\[
\text{R} = \text{OH, SH}
\]

\[
\text{X} = \text{O, S}
\]

1.4.3 Hydrazones and metal complexes of isatins:

(a) Although direct isatin metal complex is scarecly observed but isatin - platinum complex formation is reported. Isatin combines with tetrakis [triphenyl phosphine] platinum to give a platinum - isatin complexes of probable structure (41).
Very recently further direct isatin-metal complexes are found in literature. Jain et al.\textsuperscript{64} in 1990 prepared some metal isatin complex. According to Jain when MCl\textsubscript{4} (M=Sn, Ti, Th) mixed with isatin in a suitable solvent (dry THF, dry ether in N\textsubscript{2} atmosphere) to give the metal isatin complex. Structure of these complexes is a matter of discussions. But Sn and Ti making their complexes with isatin at the (IV) state.

**Hydrazones:** Isatins are very quickly react with aryl-hydrazide to give their corresponding hydrazones\textsuperscript{65,66} of (42) and (43)\textsuperscript{67}.

\[
\text{H}_2\text{N} - \text{NH} - \text{Ar} + \text{Isatin} \rightarrow \text{Hydrazone} (42)
\]

\[
\text{H}_2\text{N} - \text{NH} - \text{CO} - \text{Ar} + \text{Isatin} \rightarrow \text{Hydrazone} (43)
\]

**Metal complex of isatin phenyl hydrazones:**

Isatin -3-phenyl hydrazones combined with metal chloride to form the metal isatin complexes. O- and N- atom of isatin acts as donor atom. As a result isatin acts as bidentate ligand and hence of metal chelates\textsuperscript{68,69} are formed as (44) and (45).

\[M = \text{Cu, Zn, Cd}\]

\[M = \text{Hg, Mn, Co}\]
1.4.4 Oxidation Reduction Reactions of Isatin:

(a) Isatin (2) undergoes ring expansion with CrO₃. This reaction is carried out in CrO₃, NaOH, HNO₂, and NH₄OH with isatin. Primarily benzotriazinone (46) is formed which is converted into benzoxazinedione (47) that is isatoic anhydride (47).

\[
\text{\text{\includegraphics[width=\textwidth]{oxo_1.png}}}
\]

But Kolbe⁴³ observed that chromic acid oxidation of isatin (2) in AcOH solution yields isatoic anhydride (47) and it can also be synthesized from anthranilic acid and carbonyl chloride⁷¹.

\[
\text{\text{\includegraphics[width=\textwidth]{oxo_2.png}}}
\]

(b) Bayer-Villiger Oxidation:

Bayer Villiger⁷² Oxidation of isatins 4° with H₂O₂ in AcOH gives 70 - 90% isatoic anhydrides (48), whereas oxidation of 4° with disulphuric acid in conc. H₂SO₄ to give benzoxazinediones (49) in an excellent yield. Each of the isomeric products 48 and 49 is uncontaminated by the other isomer.

\[
R \quad R^1 \quad R^2 \quad R^3 \\
H \quad H \quad H \quad H \\
Me \quad F \quad F \quad Cl \\
Cl \quad Me \quad Me \\
Br \quad OMe \\
NO₂ \quad CF₃
\]
Reduction of Isatin:

(a) Wolf Kishner Reduction: Reduction of 1-(2, 6 dichloro) phenyl isatin with hydrazine and sodium ethoxide at 130° - 140°C to give ring opening compound (50) called 2-(2',6'-dichloro anilino) phenyl acetic acid.

\[
\text{N}_2\text{H}_4, \text{NaOEt} \xrightarrow{130-140^\circ C} \text{NH} \quad 50
\]

Recently Crestini and Saladino\(^7^3\) prepared 2-oxindoles as like as Wolf - Kishner reduction of isatin derivatives by hydrazine hydride directly.

\[
\text{NH}_2\cdot \text{NH}_2\cdot \text{H}_2\text{O} \xrightarrow{R} \text{N} \quad \text{2-Oxindoles}
\]

Very recently it is reported that irradiation of 1-alkyl isatins in degassed alcohol afford 1-alkyl-3-hydroxyindole (A) and 1-alkyl oxindoles (B), as a chemoselective products:

\[
\begin{align*}
\text{h} & \xrightarrow{\lambda} \text{A} + \text{B} \\
\end{align*}
\]

(b) Reduction with amalgam: Isatin is reduced into dioxindole (51) by means of Na-Hg or zinc and acetic acid.\(^7^4, 7^5\)

\[
\text{Na-Hg} \xrightarrow{\text{Zn}} \text{Zn} \quad 2 \quad 51
\]
1.4.5 Formation some spiro compounds of isatins:

a) 5-Choro isatin (52) reacts with p-Cl-benzyl chloride to give N-substituted isatin (53) which is reduced by trimethylecyanosilane followed by hydrolysis to give (54). Compound (54) is converted into (55) with ClSO₂NCO and then cyclocondensation in basic medium yielded the important spiro molecule (56).

\[
\text{Formation of Compound (56):}
\]

b) p-Methoxy aniline and isatin gives a Schiff base (57). Compound, 57 undergoes condensation and subsequent elimination of one molecule water to give (58) which react with substituted hydrazine and hence the product (59) is obtained. Compound, 59 is converted into spiro molecule (60) by cyclocondensation with chloroacetyl chloride or mercapto acetic acid.

Kumud et al\textsuperscript{77} very recently (1993) synthesized 2'-Substituted Spiro [indoline - 3, 5' (5H)] [1, 3, 4] oxathiadiazolo [3, 2-thiazole] - 2'-ones, e.g., 62: molecule, 62 has been achieved by cyclocondensation to 3'-substituted spiro [indoline -3, 2 - thiazolidene] - 2', 4'-diones e.g., 61 with conc. H\textsubscript{2}SO\textsubscript{4}.
Cyclocondensation of some isatin-β-thiosemicarbazones with suitable solvent gives the following type of spiro compounds in cyclocondensation:

\[ R = \text{Ac, Me, H} \]
\[ R^1 = \text{H, Ph} \]
\[ R^2 = \text{H, Br} \]

Spiro [indoline-3,2'(3H)] [1,3,4] thiadiazoline-2-ones

\[ X = \text{Cl, SCH}_2; \ R = \text{Ph, 2-HOC}_6\text{H}_4, 4-\text{ClC}_6\text{H}_4, 4-\text{Me}_2\text{NC}_6\text{H}_4, 4-\text{NO}_2\text{C}_6\text{H}_4 \]
Very recently Islam et al. prepared some spiro molecules (64) from isatin thiocarbohydrazones with Ac₂O or benzoyl chloride at reflux conditions. Islam et al. still working on this project for screening test of the synthesized spiro 1, 3, 4 thiadiazoline compounds against carcinoma and malignant cancer.

\[
\begin{align*}
\text{R} & \quad \text{N-N} & \quad \text{R} \\
\text{O} & \quad \text{S} & \quad \text{OCH₃} \\
\text{C} & \quad \text{COCH₃/Ph} \\
\end{align*}
\]

\[\text{S-Br.} \]

\[\text{S-COOH •} \]

\[\text{7-CI .} \]

\[\text{R= 5-COOH, 5-Br, 7-Cl, 5-OCH₃} \]

1.4.6 Electrophilic substitution reaction in the benzene ring of isatin:
Aromatic benzene nucleus of isatin undergoes electrophilic substitution in a suitable condition. Normally substitution occurs at 5- and 7-positions but not at 4- and 6- positions. This can be explained by resonance of benzene nucleus as follows:

From the canonical structures it is clear that positions 5- and 7- are relatively enriched of electrons and are sensitive toward electrophiles while the positions 4 and 6 become positively charged, so electrophile (E⁺) do not attack these positions.

(a) Halogenation: Chlorination of isatin 2 gives 5-chloro-isatin (65) and 5, 7-dichloroisatin. Bromination behaves similarly.
(b) **Nitration:**

Nitration of Isatin (2) with KNO₃ in a conc. H₂SO₄ solution, or with HNO₃ and H₂SO₄ has been shown to yield 5-nitroisatin (67).

![Nitration Reaction Diagram](image)

1.4.7 Nucleophilic Addition reaction of Isatin with Grignard Reagent:

The reactions of the two carbonyl groups of isatin are typical of such functions. Although the α-carbonyl (2) is part of the amide group does not enter into the usual reactions of a ketonic function, there are cases in which isatin behaves as an α-dicarbonyl compound, with both groups taking part in a reaction. For example, with the Grignard reagent isatin normally yields the 3-alkyl or aryl-3-hydroxy oxindoles. For example N-methyl isatin and C₆H₅MgBr in equimolar proportions give 1-methyl-3-phenyl-dioxindole (68).

![Grignard Reaction Diagram](image)

Compound, 68 will react further with the Grignard reagent, producing presumably the intermediate diol (69). The product actually isolated on hydrolysis of Grignard complex with H₂SO₄ as a mixture of the isomeric indoxyl (70) and oxindole (71) derivatives formed by Wagner rearrangement.
1.4.8 Some Important Reaction of Isatins with Diazomethane as well as oxosteroids:

a) **With oxo-steroids:** Isatin (2) condensed with 5α-cholestan 3-one (72) in EtOH containing N, N diethylamine to give a macromolecule, indolyl cholestan-3-one (73).
b) **With diazomethane:** i) By trapping\(^9\) with MeOCC \(\equiv\) CCOOMe it was shown that treatment of 1-substituted isatins with MeOH free diazomethane yielded aldolic 1:1 adducts (diazoldoles) i.e. substitute -3- hydroxy -3-(diazomethyl) - 2-indolinones (74).

\[
\begin{align*}
R = \text{CH}_3, \text{C}_2\text{H}_5, \text{Ph}, P-\text{Cl}-\text{C}_6\text{H}_4
\end{align*}
\]

ii) The ring expansion reaction of cyclic ketones (isatin) with diazomethane has its counterpart in isatin chemistry\(^9\). Thus isatin reacts with diazomethane yields both the epoxide (75) as a simple ketone addition product, or the rearranged 2,3-dihydroxy -quinoline (76), usually obtained as an ether (77).

### 1.5 ECONOMICAL IMPORTANCES OF ISATIN AND ITS DERIVATIVES

1.5.1. **Anti inflammatory Agents:** Marfat et al\(^9\) produced some pharmacological agent i.e. antiinflammatory compounds from isatins. These compounds are effective on some virus and bacteria.
1.5.2 As Chemotherapeutic and Pharmacological Agents:

The indole nucleus plays an important role as common denominator for various biological activities. Indole itself has been found to possess fungicidal, bactericidal, herbicidal activities. 1, 3, 4-thiadiazole ring is associated with broad spectrum of biological activities by virtue of incorporation toxophoric N=C-S linkage. A triazino-thiadiazole system may be viewed as a cyclic analogue of very important toxophore i.e. thiosemicarbazide which often displays diverse biological activities.

i) On the basis of above discussion Sailendra et al. in 1994 prepared following some important compounds (78-80) from isatin and heterocyclic aromatic hydrazines. All the compounds show the tremendous antifungal activity.
ii) **Biological active compounds**: The vivo anti influenza virus and anti vaccinia virus activity of 156 biol. active compounds were detected. Several drug doses (DL-noformocin, NSC 72942) were tested against that virus. Isatin -β-thiosemicarbazone (NCS 721) also showed as reproducible activity against vaccinia virus.

\[
\text{Isatin-β-thiosemicarbazone}
\]

iii) In 1995 Sudhakar et al synthesized some important biologically active compounds from isatin and substituted napthyridine as follows (81-84).

\[
\begin{align*}
\text{R} & = 5\text{-CH}_3 & 7\text{-CH}_3 & 5\text{-Cl} & 7\text{-Cl} & 5\text{-Br} & 6\text{-Br} \\
\end{align*}
\]
Recently, much interest has been focussed on the synthesis and biological activities of substituted 8-naphthyridines\(^{101, 32, 33}\) (84). Moreover, compounds having 4-thiazolidinone moiety are reported to be useful antimicrobial\(^{102}\) antitubercular\(^{103}\), and hypnotic agents. Further 1, 2, 3-oxadiazino\(^{104}\) and indole derivatives\(^{105-106}\) have gained prominence because of their potential pharmaceutical values. Prompted by these observations and in contribution of their work on the synthesis of 1,8-naphthyridines\(^{107-110}\) they report herein the synthesis of novel spiro heterocyclic, viz- spiro - [3H - indole - 3, 2 - thiaidiazoline] -2, 4' (1H) diones and a fused heterocyclic system, [1,3, 4] oxadiazino [5, 6-b] indoles containing 8-naphthyridine moiety.

All the title compounds (81-83) were screened against various bacteria. Compounds 81a, 81c, 81f and 83e showed moderate antibacterial activity. Other compounds exhibited very weak antibacterial activity.

iv) As Anticancer Reagents: Following compounds (85) and (86) were reported\(^{111}\) for the suppression of carcinoma (cancer).

\[
\begin{align*}
\text{R} = & \, H; \, \text{Ph} \\
\text{R}' = & \, \text{CH}_3; \, \text{CH}_2\text{Ph;} \, \text{CH}_2\text{CO}_2\text{Et}
\end{align*}
\]

These compounds display immunosuppressive and anticancerous activities.

Very recently Islam et al\(^{79}\) collaboration with National Cancer Institute (NCI) of U.S.A. observed that acylated 1,3, 4-thiaidiazoline derivatives (87) of isatin show potential anticancerous activity against a number of cancer cells.
v) **As antidiabetes**: The following spiro compounds (88) can inhibited the enzymetic activity of aldose reductase and hence it is used as antidiabetes\(^{112}\).

\[
\begin{align*}
\text{Anticancer drug} & \quad 87 \\
\text{Antidiabetes} & \quad 88
\end{align*}
\]

vi) **Anti Ulcer Agent**: Highly N-substituted β-thiosemicarbazides of the following types compounds (89) are highly active against ulcer\(^{113-114}\).

\[
\begin{align*}
\text{Antidiabetes} & \quad 88 \\
\text{89}
\end{align*}
\]

vii) **Antivirustatic compounds**: Some N-cyclopropane derivatives\(^{115}\) of isatin and its β-thiocarbohydrazones display antivirus activity. Following compounds (90) and (91) are widely used very sincerely for virus infection.
viii) Some broad-spectrum chemotherapeutic agents:

The title compounds\textsuperscript{116} (92 and 93) were prepared from Isatins and aromatic amines followed Mannich reaction. Compunds, 92 and 93 inhibited the growth of a large variety of infectious virus.

Some indole derivatives of benzoquinone nucleus synthesized by Kita et al\textsuperscript{117} these compounds inhibited human cancer KB cell.

Some indian workers\textsuperscript{118-120} prepared following compounds. They show screening effect against some harmful microorganisms.
1.5.3. Physiological Effect of Isatin:

![Chemical Structure of Isatin]

The effect of various doses of isatin 2 on electroencephalogram (EEG) manifestations of vigilance groups of rhythmic discharges and the incidence of epileptic pathological activities was studied in repeated chronic experiments in rats. Isatin (0.1 - 6 mg) perfusion caused a dose dependent decrease in the amplitude of ventricular contraction and the CARDIAC OUTPUT\textsuperscript{121} of in situ frog heart. This effect was not blocked by atropine, isatin had no effect on guineapig or dog heart in doses upto 10 mg and 100 mg respectively. Isatin had little effect on blood pressure of cats and dogs.

1.5.4. Enzyme like molecules:

Isatin possesses an apparent enzymelike activity in the dehydrogenation of amino acids. Thus, heating $\alpha$-aminophenylacetic acid with isatin in aqueous solution converts the amino acid into benzaldehyde in good yields\textsuperscript{122}. Other reports\textsuperscript{123} confirm activity and the following mechanism has been suggested\textsuperscript{124} in which either atmospheric oxygen or methylene blue serves as the H-acceptor, isatin being reduced reversibly in intermediate step to isatide.

\[
\begin{align*}
\text{R-C-COOH} + 2\text{H}_2\text{O} & \rightarrow \text{R-C-COOH} \\
\text{methylene blue} & \rightarrow \text{RCHO + CO}_2 + \text{NH}_3
\end{align*}
\]
1.5.5 As Dyeing Agents:

i. **Disperse dyes based on isatins**: Title compounds\(^1\)\(^\text{25}\) (94) are described that give yellow green to orange shades on polyester fibers. 94, dyed polyester fiber a yellow shade that was fast to washing, perspiration, solvents and rubbing.

![Disperse dye structure](image1)

ii. A large number of dyes have synthesized from isatin. Isatin and piperidine combine with each other to form two products (95 and 96) i.e. isatin mono-piperidine and isatin dipiperidine\(^1\)\(^\text{26}\) respectively. Compounds, 96 can be converted into the product (97). Isatin blue\(^1\)\(^\text{27}\) is a powerful dying agent.

![Isatin blue structure](image2)

iii. Isatins precursor indigo\(^1\)\(^\text{28}\) condenses with phenyl acetylchloride to Ciba lake red B (95) and indigo condenses with malonic ester to give the red violet ethyl indigo malonate (98). Ethyl phenyl acetate condenses with indigo to yield the dye (99).

![Red violet Indigo structure](image3)
iv. A familiar blue dye indophenin (100) is formed when isatin is treated with conc. \( \text{H}_2\text{SO}_4 \).

1.6 AIM OF THE PRESENT WORK

From the above discussions it is clear that isatin (indole-2, 3-dione) and its different kinds of derivatives are very important classes of organic compound which is pharmacologically active. The schiff base of isatin show their activity in the medicinal and in agrochemical fields. For example isatin thiosemicarbazones display fungicidal, insecticidal and also the pesticidal activities. Spiro heterocyclic compounds of isatin are obtained from the cyclocondensation of isatin thiosemicarbazones and thiocarbohydrazones which are very important as chemotherapeutic agents. For example isatin - 1, 3, 4-thiadiazoline acyl derivatives are obtained from the isatin thiocarbohydrazones by cyclocondensation with acetic anhydride. These molecules show anticancerous activity especially against leukemia (blood cancer) and
breast cancer also. Some Schiff bases and spiro compounds of isatins also exhibit pharmacological screening effect on malaria and diabetes. So the present work aims at

a. The synthesis of disubstituted mono isatins from aniline and other 1°-aromatic diamines (e.g. 4-methyl-2-nitroaniline and 4-chloro-2-nitroaniline).

b. The synthesis of isatins from benzidine, 1-naphthylamine.

c. The synthesis of Schiff bases from all isatins with thiosemicarbazide.

d. The synthesis of spiro-1,3,4-thiadiazoline acyl derivatives from the isatin thiosemicarbazones with acetic anhydride.

e. The synthesis of spiro-1,3,4-triazole-2-thiol derivatives from the isatin thiosemicarbazones with potassium hydroxide and finally to get expanded ring with 1,2-dibromoethane.

f. Finding out the optimum reaction condition to establish a suitable method regarding the cyclized products.

g. The study of pharmacological screening of all the synthesized compounds, especially to test antibacterial, antifungal and anticancer activities as well as to find out the Structure-Activity-Relationship (SAR).
CHAPTER TWO
SYNTHESIS
CHAPTER-2: SYNTHESIS

2.1. LIST OF THE SYNTHESIZED COMPOUNDS

(A) Oximinoacetanilides:

(B) Substituted isatins:

(C) Schiff bases:
(D) 1,2,4 - Triazino-3-thione:

(E) Ring Expansion of Compound 4 A:

(F) 1,3,4 - Thiadiazoline derivative:

(G) Attempted synthesis of:

(i) 

(ii) 

(iii) 

41
2.2 SYNTHESIS PLAN:

(i) The substituted isatins may be synthesized by the standard method of Sandmeyer from substituted aniline with chloral hydrate and hydroxylamine hydrochloride according to the scheme 2.1.

\[
\begin{align*}
\text{Mono-amine} & \quad \text{PPA} = \text{Poly Phosphoric Acid} \\
\text{x} = \text{Cl, CH}_3
\end{align*}
\]

The first step involves the preparation of isoacetanilide from the reaction of -NH\_2 and chloral hydrate, NH\_2-OH.HCl in presence of Na\_2SO\_4. The second step is the cyclization with Conc. H\_2SO\_4 or polyphosphoric acid. This step is critical as this step depends upon the nature of the substituent (x) in the benzene ring. The condition for cyclization is (a) the ortho-position with respect to the -NH\_2 must be free (b) the electron density at this position should be sufficiently enough.

By fulfilling the above mentioned conditions bis-isatin may be synthesized from diamines like phenylenediamines, benzidine etc.
(ii) Thiosemicarbazones (Schiff base) may be prepared by simple condensation reaction between substituted isatin and bis isatin with thiosemicarbazide refluxing with methanol in presence of 2-3 drops conc. HCl or glacial acetic acid (Scheme 2.2).

(iii) Isatin thiosemicarbazone may be converted into cyclized product, [1,2,4-triazino-3-thione] by treating the Schiff base with 4% KOH solution (Scheme 2.3).
(iv) Ring extension of 1,2,4-triazino-3-thione may be achieved by refluxing the thione with 1,2-dibromoethane in DMF (Scheme 2.4).

![Scheme 2.4](image)

(v) Thiosemicarbazones may be converted into its important spiro-products by refluxing the Schiff base with acetic anhydride (Scheme 2.5).

![Scheme 2.5](image)

So, it is planned to synthesize a series of substituted isatins (mono and bis) derivatives using the above synthetic pathways.
2.3.1 SYNTHESIS OF OXIMINOACETANILIDE (1A) FROM META-CHLORO-P-TOLUIDINE.

The 3-chloro-4-methyl-oximinoacetanilide was synthesized according to the standard method. The crude product was obtained as an off-white solid and crystallization from methanol yielded white crystalline solid compound 1A, yield 85%, m.p. 170°-173°C and was found to be homogeneous on tlc plate with Rf 0.58 (P.E: EA, 1:1).

\[
\begin{align*}
\text{NH}_2\text{OH} \cdot \text{HCl} & \quad \text{CONC. HCl} \\
\text{Na}_2\text{SO}_4 \cdot \text{H}_2\text{O} & \quad \text{Cl}_3\text{C} \cdot \text{CH(OH)}_2
\end{align*}
\]

\[
\text{Cl} \quad \text{H}_3\text{C} \quad \text{NH}_2
\]

\[
\text{Cl} \quad \text{H}_3\text{C} \quad \begin{array}{c}
\text{HC} \equiv \text{N} \begin{array}{c}
\text{OH}
\end{array}
\end{array}
\]

\[
\begin{array}{c}
\text{Cl} \\
1\text{A}
\end{array}
\]

Compound 1A was characterized with the help of spectral analysis.

The IR spectrum of the compound 1A (Fig.-1) shows a strong band at 3324 cm\(^{-1}\) is due to V\(_{\text{O-H}}\). The weak band at 3252 cm\(^{-1}\) indicates the presence of V\(_{\text{N-H}}\). The aromatic V\(_{\text{C-H}}\) appears at 3052 cm\(^{-1}\). The aliphatic V\(_{\text{C-H}}\) may be assigned at 2920 cm\(^{-1}\). The sharp band at 1656 cm\(^{-1}\) corresponds to V\(_{\text{C=O}}\) (amide). The sharp band at 1600 cm\(^{-1}\) may be ascertained for V\(_{\text{C=N}}\). The bands at 1540, 1496, 1256, 804 and 712 cm\(^{-1}\) are due to V\(_{\text{C=C}}\) (aromatic), \(\delta_{\text{C-H}}\) (aliphatic), \(\delta_{\text{O-H}}\), \(\delta_{\text{C-H}}\) (aromatic) and V\(_{\text{C-Cl}}\) respectively.
Fig-1: IR spectrum of compound 1A.
In IH-NMR spectrum of the compound 1A [Fig. 2 (a,b,c)] a singlet appearing at $\delta 2.32$ is assigned for the three protons of methyl group attached to the benzene ring. The doublet at $\delta 7.8$ is assigned to $H_d$ as it couples with $H_c$ (at meta position) having $4J_{meta} = 2$ Hz. The aromatic proton $H_b$ appears as doublet at $\delta 7.22$ and it is due to ortho coupling with $H_c$, $3J_{ortho} = 7$ Hz. The rest aromatic proton $H_c$ is coupled with its neighbouring proton $H_b$ into doublet at $\delta 7.42$ which is further split up into doublets with meta proton $H_d$ having coupling constant $4J_{meta} = 2$ Hz. The sharp singlet at $\delta 7.58$ is due to CH proton (Hf) attached to CO and $= N-OH$. Due to use of deuterated methanol (CD$_3$OD) no signal for NH and N-OH is observed because of proton-deuteron exchange reaction.

In mass spectrum of the compound 1A (Fig. 3) the molecular ion peak appears at m/z 212/214 (3:1) correspond to the molecular formula C$_9$H$_9$N$_2$O$_2$ Cl with isotopic pattern of chlorine atom, while the base peak at m/z 141/143 (3:1) is due to loss of C$_2$HNO$_2$ from the molecular ion peak by the McLafferty Rearrangement$^{130}$. The mass fragmentation pattern is shown in the scheme-2.6.
Fig-2(a): $^1$H-NMR spectrum of compound 1A
Fig-3: Mass spectrum of compound 1A
Scheme 2.6: Mass Fragmentation of Compound 1A.
2.3.2 SYNTHESIS OF 5-METHYL-4-CHLORO ISATIN (2A) AND 6-CHLORO-5-METHYL ISATIN (2B)

A mixture of 6-chloro-5-methyl isatin (2B) and 5-methyl-4-chloroisatin (2A) was obtained by Sandmeyer reaction from the compound 1A using conc. H₂SO₄ according to the standard procedure²².

The crude product was obtained as a yellow orange powder solid, yield 84%. It was found as a mixture of the two products on the tlc plate (PE: EA, 2:1) with Rf 0.65 and Rf 0.33. The crude product (500 mg) on fractionation over a silica gel column chromatography (pet. ether : Ethyl acetate, 2:1) yielded pure components of 2A and 2B.
2.3.3 First Fraction: Isolation and characterization of compound 2B

The fast moving fraction obtained as yellowish orange coloured component was minor compound and characterized as 6-chloro-5-methyl isatin (2B), yield ca. 28%, m.p. 256-258°C and was found to be homogeneous on tlc plate with $R_f$ 0.65 (PE: EA, 2:1).

**Compound 2B was characterized spectroscopically:**

In IR spectrum of the compound 2B (Fig-4) a sharp band appearing at 3275 cm$^{-1}$ is due to $\nu_{N\cdot H}$. The aromatic $\nu_{C\cdot H}$ shows a weak band at 3100 cm$^{-1}$ The bands at 1772, 1734 and 1653 cm$^{-1}$ may be assigned to $\nu_{C=O}$ (lactam), $\nu_{C=O}$ (keto) and $\nu_{C=C}$ (aromatic) respectively.

![2B](image)

In $^1$H-NMR spectrum of the compound 2B (Fig.-5) one aromatic proton $H_a$ (ortho position with respect to chlorine atom) appearing at $\delta$7.53 i.e. higher $\delta$-value because proton $H_a$ experiences a para-magnetic shift due inductive effect of chlorine atom. The rest aromatic proton $H_b$ shows a singlet at $\delta$6.95. A broad singlet centred at $\delta$7.85 may be assigned to one NH (d) proton of lactam. Three protons (c) of methyl group attached to the benzene ring appear as a singlet at $\delta$2.35.
Fig-5: 1H-NMR spectrum of compound 2B
2.3.4 Second Fraction: Isolation and characterization of compound 2A

The deep red coloured compound eluted as second fraction was the major product and it was characterized as 5-methyl-4-chloro isatin (2A), yield 72%, m.p. 240-243°C and was found to be homogeneous on tlc plate (PE : EA. 2:1) with Rf 0.33.

Compound 2A was characterized with the help of spectral analysis.

The IR spectrum of the compound 2A (Fig-6) shows a sharp band at 3292 cm\(^{-1}\) due to the VN-H. The aromatic \(\nu_{\text{C-H}}\) appear at 2980 cm\(^{-1}\) as a weak band while the band at 2956 corresponded to \(\nu_{\text{C-H}}\) (aliphatic). The carbonyl stretching, \(\nu_{\text{C=O}}\) of the lactam may be assigned to 1752 cm\(^{-1}\) while the \(\nu_{\text{C=O}}\) (keto) appearing at 1726 cm\(^{-1}\). A sharp band is observed at 1612 cm\(^{-1}\) due to \(\nu_{\text{C=C}}\) (aromatic). The \(\nu_{\text{C-Cl}}\) appears at 676 cm\(^{-1}\).

\[
\text{Cl} \quad \text{C} \quad \text{H}_3 \quad \text{C} \quad \text{H}^2 \quad \text{N}\quad \text{H}
\]

\[
\text{O} \\
\text{O}
\]

In \(^1\text{H}-\text{NMR}\) spectrum of the compound 2A [Fig - 7 (a,b)] a doublet at \(\delta 7.5\) is due to one aromatic proton \(H_a\) coupled with ortho proton \(H_a\) (3J\text{ortho} = 8 Hz) and each line is further split up by fine coupling with methyl protons having coupling constant 4J = 1 Hz. The other doublet at \(\delta 6.8\) is due to \(H_a\) having coupling constant 3J\text{ortho} = 8 Hz shown in the above picture (2A). Three protons \(H_a\) of the methyl group attached to the benzene ring is observed at \(\delta 2.32\) as a singlet. In this spectrum again no signal for NH of the lactam was observed as CD\(_3\)OD was used as solvent.
Fig. 6: IR spectrum of compound 2A.
Fig. 7(a): 1H-NMR spectrum of compound 2A
Fig-7(b): $^1$H-NMR spectrum of compound 2A
In mass spectrum (Fig-8) the molecular ion peak of the compound 2A appears at m/z 195/197 (3:1) due to the molecular formula C_9H_6NO_2Cl with characteristic isotopic pattern of the chlorine atom. The base peak appearing at m/z 167/169 (3:1) is due to loss of C=O group from the molecular ion peak. The other prominent peaks at m/z 140/142, 124/126, 77 are consistent with the proposed structure. The molecular ion peak, base peak and other fragmentation pattern of both the mass spectra of compound 2A as well as 2B are identical. The mass fragmentation pattern is shown in the scheme-2.7.

The proposed structures of compounds 2A and 2B are further supported by their corresponding 13C-NMR spectrum (Fig-9 and Fig. 10 respectively) in the following way.

<table>
<thead>
<tr>
<th>δ13C-value</th>
<th>Carbon assigned</th>
<th>δ13C-value</th>
<th>Carbon assigned</th>
</tr>
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<td>18.48</td>
<td>C - 9</td>
<td>19.62</td>
<td>C - 9</td>
</tr>
<tr>
<td>111.72</td>
<td>C - 8</td>
<td>113.00</td>
<td>C - 4</td>
</tr>
<tr>
<td>116.52</td>
<td>C - 7</td>
<td>127.55</td>
<td>C - 8</td>
</tr>
<tr>
<td>127.95</td>
<td>C - 3</td>
<td>134.00</td>
<td>C - 7</td>
</tr>
<tr>
<td>133.01</td>
<td>C - 4</td>
<td>145.80</td>
<td>C - 6</td>
</tr>
<tr>
<td>133.80</td>
<td>C - 5</td>
<td>197.60</td>
<td>C - 1</td>
</tr>
<tr>
<td>140.94</td>
<td>C - 6</td>
<td>216.58</td>
<td>C - 2</td>
</tr>
<tr>
<td>151.44</td>
<td>C - 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>182.58</td>
<td>C - 2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In the case of compound 2B δ13C signals for C-3 and C-4 could not be assigned properly.
Fig-8: Mass spectrum of compound 2A
Scheme-2.7: Mass Fragmentation of Compound 2A & 2B.
Fig-9: $^{13}$C-NMR spectrum of compound 2B
Fig-10: $^{13}$C-NMR spectrum of compound 2A
2.3.4a Explanation of the compound 2A as a major product between two isomeric isatins (2A and 2B):

Normally the electronic effect of methyl group at the ring closure position - 2 and 6-(indicated by arrow) are equal and hence it has no influence on the product ratio (2A and 2B). So the effect of chlorine substituent is of prime importance in determining the product ratio.

When the electronegative chlorine atom pulls electron towards itself from the benzene nucleus due to inductive effect, a partial positive charge is created in the nucleus at C-2 and C-6 as shown in the structure A. When the positive charge develops at 6-position, a canonical form is possible in which all the \( \pi \)-orbitals are in parallel form (B).
In this situation orbitals are more symmetric and this form (B) is more stable compared to (C). Therefore the suitable position of ring closure is at position 2(C) instead of position 6(b). So electrophilic substitution i.e. ring closure goes faster at position 2 than position 6. As a result the red product (2A) is found to be major product. Here it seems that the steric effect of chlorine atom does not play a role.

It is to be mentioned here that Baker et al\textsuperscript{131} also reported the isolation of the above mentioned products (2A and 2B) by Sandmeyer method. Their purification method\textsuperscript{132} was different. They treated the crude isatin mixture with NaOH solution which resulted the opening of the ring. Then the reaction mixture was treated slowly with HCl which caused cyclization again at different pH. The red isomer, major fraction precipitated out (pH, 8-4.5) first. As the pH lowered (more acid was added) the yellow product came out (pH, 4-2) as shown in the scheme- 2.8.

The ratio between the major and minor product was 2.57:1. Other findings are also consistent with fact that the red isomer forms as the major component.

2.3.5 SYNTHESIS OF 5-METHYL-4-CHLORO ISATIN-3-THIOSEMICARBAZONE (3A).

The refluxing of unimolecular ratio of 5-methyl-4-chloro isatin and thiosemicarbazide in methanol with a few drops of concentrated hydrochloric acid for three hours and the usual work-up\textsuperscript{133} afforded pale yellow powder of 3A. The product is recrystallized from methanol having m.p. 265-268\(^\circ\)C and yield 68.4\%. This product was homogeneous on the tlc plate \(R_f\) 0.32 (Pet-ether: Ethylacetate, 1:1).
Scheme-2.8
The characterization of 3A was accomplished with the help of spectral data.

In IR spectrum (Fig. 11) of compound 3A, the absorption band at 3427 cm\(^{-1}\) and 3219 cm\(^{-1}\) may be assigned to NH\(_2\) group and NH group respectively. The stretching frequency of C-H (aromatic) is due to the band at 3163 cm\(^{-1}\) whereas the band at 2855 cm\(^{-1}\) corresponds to the stretching frequency of C-H (aliphatic). The strong band at 1736 cm\(^{-1}\) is due to \(\nu\text{C} = \text{N}\). The carbonyl stretching, \(\nu\text{C} = \text{O}\) of the lactam may be assigned at 1691 cm\(^{-1}\). The band at 1603 cm\(^{-1}\) and 1142 cm\(^{-1}\) may be ascertained for the \(\nu\text{C} = \text{C}\) and \(\nu\text{C} = \text{S}\) respectively. The \(\nu\text{C} = \text{Cl}\) exhibits a sharp band at 717 cm\(^{-1}\).

In \(^1\text{H-NMR}\)-spectrum of the compound 3A (Fig. 12), two protons of NH\(_2\)(a) group attached to the S=C-NH at the side chain of lactam ring show a singlet at \(\delta 9.1\). The singlet at \(\delta 8.8\) is safely assigned to the one NH(c) proton of the lactam ring. A sharp singlet at \(\delta 8.4\) is due to the one NH (b) proton. Two aromatic protons appear as two doublets at \(\delta 7.3\) (e) and \(\delta 6.9\) (d). Three protons of the methyl group attached to the benzene ring centred at \(\delta 2.35\) as a singlet.
Fig. 11: IR spectrum of compound 3A.
Fig-12: $^1$H-NMR spectrum of compound 3A
The mass spectrum of the compound 3A (Fig. 13) shows the molecular ion peak at m/z 268/270 having to the molecular formula C_{10}H_{9}N_{4}OSCl with characteristic isotopic pattern of chlorine atom. The base peak appears at m/z 60 is due to \( H_{3}NC = S \). The other fragmentation pattern shown in the following scheme (2.9) is consistent with the structure.

The mechanism of the formation of compound 3A can be shown by following scheme (2.10).

**2.3.6 SYNTHESIS OF 9-CHLORO-8-METHYL-5H-2,3-DIHYDRO[1,2,4]TRIAZINO [5,6-b] INDOLE-3-THIONE, (4A).**

Refluxing of 5-methyl-4-chloro isatin-3-thiosemicarbazone (3A) with 4% aqueous KOH solution for four hours and the usual work-up gave the crude product. The crude product on purification followed by recrystallization from methanol afforded compound 4A as a yellowish-red crystalline solid, yield 83%, m.p. > 300°C, Rf: 0.54 (pet-ether : Ethyl acetate, 1:1)
Fig-13: Mass spectrum of compound 3A
Scheme-2.9: Mass Fragmentation of Compound 3A.
Scheme - 2.10:  Mechanism of the Formation of Compound 3A
The characterization of compound 4A was accomplished with help of spectral analysis.

In IR-spectrum (Fig.-14) of compound 4A, the weak band at 3320 cm\(^{-1}\) indicates the presence of NH group. The sharp band at 2995 cm\(^{-1}\) and 2895 cm\(^{-1}\) may be due to \(\nu_{C-H}\) (aromatic) and the aliphatic \(\nu_{C-H}\) respectively. Bands at 1690 cm\(^{-1}\), 1610 cm\(^{-1}\) and 1151 cm\(^{-1}\) correspond to \(\nu_{C=N}, \nu_{C=C}\) and \(\nu_{C=S}\) respectively. The band at 725 cm\(^{-1}\) is due to \(\nu_{C-Cl}\).

In \(^1\)H-NMR spectrum [Fig. 15(a, b, c, d)] of compound 4A, the broad singlet at \(\delta 14.7-14.6\) is assigned to two NH (c&d) proton. The two doublets at \(\delta 7.6\) and \(\delta 7.21\) may be assigned at two aromatic protons (a) and (b) respectively. Three protons of methyl group attached to the benzene ring shows a sharp singlet at \(\delta 2.45\).

In the mass spectrum of the compound 4A (Fig. 16), the molecular ion peak and also as the base peak is found to occur at \(m/z\) 250/252 corresponding to the molecular formula \(C_{10}H_{7}N_{4}SCl\) with characteristic isotopic pattern of chlorine atom. Other prominent peaks at \(m/z\) 234, 222,194, 187 etc. are consistent with the proposed structure. The mass fragmentation pattern is shown in the scheme 2.11.

The mechanism of the formation of compound 4A can be explained by following way (Scheme- 2.12).
Fig-14: IR spectrum of compound 4A.
Fig. 15(a): 1H-NMR Spectrum of Compound 4A
Fig. 15(b): 1H-NMR Spectrum of Compound 4A
Fig. 15(d): $^1$H-NMR Spectrum of Compound 4A
Fig-16: Mass spectrum of compound 4A
Scheme-2.11: Mass Fragmentation of Compound 4A.
2.3.7 SYNTHESIS OF 9-CHLORO-8-METHYL-1,2-DIHYDRO THIAZOLO [2',3':3,4] [1,2,4] TRIAZINO [5,6-b] INDOLE, (SA).

Compound 4A and 1,2-dibromo ethane in DMF were heated under reflux on a hot plate for 4 hours. The reaction mixture was cooled to room temperature and poured into water. The mixture was neutralized with 5% aqueous sodium carbonate solution to get 5A as solid. It was filtered and dried in a desiccator. The crude product was purified by preparative tlc on silica gel plate (Pet ether : ethylacetate, 1:3). The pure compound was found to be pink crystalline solid, m.p. 245-250°C (decom.), yield 39.2%. Rf 0.36 (Pet-ether: ethylacetate, 1:3).

Compound 5A was characterized with the help of spectral analysis.

In IR spectrum (Fig. 17), the band at 2953 cm⁻¹ corresponds to the presence of a aromatic \( \nu_{C-H} \) stretching. The band at 2924 cm⁻¹ is due to \( \nu_{C-H} \) (aliphatic) stretching where as \( \nu_{C=C} \), \( \nu_{C=N} \), \( \nu_{C=N} \) and \( \nu_{C=S} \) appeared at 1545, 1460, 1377 and 1225 cm⁻¹ respectively. The band at 721 cm⁻¹ is due to \( \nu_{C-Cl} \).
Fig-17: IR spectrum of compound 5A.
In $^1$H-NMR spectrum [Fig. 18(a, b, c, d)] of compound 5A, two sharp doublets at $\delta 7.62$ and $\delta 7.12$ correspond to two aromatic protons (d) and (c) respectively. The two triplets at $\delta 4.92$ and $\delta 3.78$ with two protons each are assigned safely to $\text{CH}_2\text{N}$ (b) and $\text{CH}_2\text{S}$ (a). The other singlet at $\delta 2.46$ indicates the presence of methyl group attached to the benzene ring.

In mass spectrum (Fig. 19) the molecular ion peak appeared as a base peak at $m/z$ 276/278 which corresponds to the molecular formula C$_{12}$H$_9$N$_4$SCI. The fragmentation pattern shown in the scheme-2.13 is consistent with the structure 5A.

Formation of compound 5A may be shown by the following mechanism (Scheme- 2.14).

![Scheme- 2.14: Mechanism of the Formation of Compound 5A](image-url)
Fig-18: 1H-NMR spectrum of compound 5A
Fig. 18(a): $^1$H-NMR Spectrum of Compound 5A
Fig. 18(b): $^1$H-NMR Spectrum of Compound 5A
Fig. 18(c): $^1$H-NMR Spectrum of Compound 5A
Fig-19: Mass spectrum of compound 5A
Scheme-2.13: Mass Fragmentation of Compound 5A.
2.3.8 SYNTHESIS OF SUBSTITUTED ISATIN-1,3,4-THIADIAZOLINE DERIVATIVE (6A) FROM THE SCHIFF BASE (3A).

The refluxing of 5-methyl-4-chloro isatin-3-thiosemicarbazone (3A) with freshly distilled acetic anhydride for five hours and usual work-up gave the reddish yellow solid mass. This crude product was recrystallized from CHCl₃ to get an orange red crystalline solid of the compound 6A, yield 49% m.p. 156-158°C and was found to be homogeneous on tlc plate with Rf 0.28 (pet-ether : ethylacetate, 2: 1).

![Chemical structure of 3A and 6A](image)

The characterization of 6A was done with the help of spectroscopic data.

The IR spectrum of the compound 6A (Fig.-20) shows a sharp band at 3405 cm⁻¹ corresponds to the presence of a NH group. The weak bands 3050 and 2945 cm⁻¹ may be assigned for
\( \nu_{C=H} \) aromatic and \( \nu_{C-H} \) aliphatic respectively. The \( \nu_{C=O} \) of lactam shows weak band at 1760 cm\(^{-1}\) but the acetyl \( \nu_{C=O} \) appears as a sharp band at 1730 cm\(^{-1}\). A weak band at 1690 cm\(^{-1}\) is due to the \( \nu_{C=N} \). The sharp band at 1615 cm\(^{-1}\) indicates the presence of \( \nu_{C=C} \) (aromatic). A sharp band at 760 cm\(^{-1}\) is safely assigned for the aromatic \( \delta_{C\equiv H} \) whereas \( \nu_{C-Cl} \) appears at 710 cm\(^{-1}\).

In \( ^1H\)-NMR spectrum of the compound 6A (Fig. 21) one proton of the indole ring (NH,b) appears as a sharp singlet at \( \delta 8.35 \). Two doublets at \( \delta 7.35 \) and \( \delta 6.95 \) are due to aromatic protons H\( d \) and H\( e \) respectively. Nine protons (e) of the three acetyl groups (3 x COCH\(_3\)) may be assigned as a singlet at \( \delta 2.65 \). Three protons (a) of the methyl group attached to the benzene ring is exhibited a singlet at \( \delta 2.15 \).

In mass spectrum (Fig. 22) of compound 6A, the molecular ion peak appears at m/z 394 which corresponds to the molecular formula C\(_{16}\)H\(_{15}\)O\(_4\)N\(_4\)SCl whereas the base peak observed in lower mass at m/z 43 which is due to CH\(_3\)C\(^+\) = 0 (acyl ion). The other prominent peaks at m/z 353, 323, 306, 250, 223, 180, 89 etc. are agreement with the proposed structure of the compound 6A. The mass fragmentation pattern is shown in the scheme- 2.15.

The formation of the compound 6A follows the following mechanism in the scheme- 2.16.

2.3.9 SYNTHESIS OF BIS-OXIMINOACETANILIDE (1B) FROM BENZIDINE.

The bis-isonitrosoacetyl benzidine was synthesized according to the standard procedure\(^{22}\). The crude product obtained as white solid powder was recrystallized from methanol to get pure product of 1B as white crystalline solid. yield 78\%, m.p>300° C. This product was homogeneous on the plate with R\(_f\) 0.32 (PE:EA:1:3).
Fig-20: IR spectrum of compound 6A.
Fig-21: 1H-NMR spectrum of compound 6A
Fig-22: Mass spectrum of compound 6A
Scheme 2.15: Mass fragmentation of Compound 6A.
Scheme 2.16: Mechanism of the Formation of Compound 6A
Compound 1B was characterized with the help of spectral analysis.

In IR spectrum (Fig. 23) of compound 1B shows a sharp absorption band at 3476 cm\(^{-1}\) for \(\nu\text{O-H}\) group whereas the band at 3352 cm\(^{-1}\) is due to \(\nu\text{N-H}\) group. The strong band at 3096 cm\(^{-1}\) is assigned for the aromatic \(\nu\text{C-H}\) group. The weak absorption band at 1710 cm\(^{-1}\) corresponds to \(\nu\text{C=O}\) (amide). The \(\nu\text{C=N}, \nu\text{C=C}\) (aromatic) and \(\delta\text{N-H}\) appeared at 1644, 1604 and 1560 cm\(^{-1}\) respectively. The sharp band at 884 cm\(^{-1}\) is due to \(\delta\text{C-H}\) aromatic.

The \(^1\)H-NMR spectrum of compound 1B [Fig. 24(a,b,c,d)] shows a broad singlet at \(\delta\) 12.2 (a) for 2xN-OH group (a). One singlet centred at \(\delta\) 10.3 (b) is due to two protons of NH group (b). The multiplets at \(\delta\) 7.8-7.5 is due to eight aromatic protons. Two olefinic protons of 2xN=C-H (c) show a broad singlet at \(\delta\) 3.9-3.7.

In mass spectrum (Fig. 25) of the compound 1B, the molecular ion peak \((M^+)\) is at \(m/z\) 326 which also corresponds to the molecular formula C\(_{16}\)H\(_{14}\)O\(_4\)N\(_4\), while the base peak appears at \(m/z\) 236. The mass fragmentation pattern is shown in the scheme-2.17.
Fig. 23: IR spectrum of compound 1B.
Fig-24(b): 1H-NMR spectrum of compound 1B
Fig-24(c): $^1$H-NMR spectrum of compound 1B
Fig-25: Mass spectrum of compound 1B
Scheme-2.17: Mass Fragmentation of Compound 1B.
2.3.10 ATTEMPTED SYNTHESIS OF BIS BENZIDINE ISATIN

Although the compound 1B was obtained in high yields, the cyclization of the compound 1B with concentrated sulphuric acid (H₂SO₄) according to standard procedure²² yielded no expected bis-isatin, only the starting material was recovered.

\[
\begin{align*}
\text{[Diagram]} \\
\text{[Reactions]}
\end{align*}
\]

The above reaction was also carried out in polyphosphoric acid (PPA) medium but no positive result was obtained.

\[
\begin{align*}
\text{[Diagram]} \\
\text{[Reactions]}
\end{align*}
\]
2.3.11 ATTEMPTED SYNTHESIS OF 1-NAPHTYL OXIMINOACETANILIDE FROM 1-NAPHTHYLAMINE

Reaction between 1-naphthylamine, chloral hydrate and hydroxylamine hydrochloride according to standard procedure\textsuperscript{22} yielded negative result.

\[
\begin{align*}
\text{H} & \quad \text{NH}_2 \\
\text{N-OH} & \\
\text{H} & \quad \text{C} = \text{O} \\
\end{align*}
\]

The negative result may be due to solubility problem of the amine in reaction mixture. Secondly, the peripheral hydrogen atom may cause steric hindrance.

2.3.12 ATTEMPTED SYNTHESIS OF OXIMINOACETANILIDE FROM 4-CHLORO-2-NITRO ANILINE

Reaction between 4-chloro-2-nitro aniline, hydroxylamine and chloral hydrate by standard method\textsuperscript{22}, reaction does not occur. Only the starting material could be isolated from the reaction mixture.

\[
\begin{align*}
\text{Cl} & \quad \text{NO}_2 \\
\text{NH}_2 & \\
\text{N-OH} & \\
\text{H} & \quad \text{C} = \text{O} \\
\end{align*}
\]
2.3.13 ATTEMPTED SYNTHESIS OF OXIMINOACETANILIDE FROM 4-METHYL-2-NITRO ANILINE

Reaction between 4-methyl-2-nitro aniline, chloral hydrate and hydroxylamine hydrochloride according to standard procedure\textsuperscript{22}, failed to obtain the desired product. Only the 4-methyl-2-nitro aniline could be isolated from the reaction mixture.
CHAPTER THREE
EXPERIMENTAL
3.1. EXPERIMENTAL SECTION - A: General Methods.

3.1.1. Melting Points
All melting points of the synthesized compounds are uncorrected and were recorded by thin
disc method on a 'FISCHER JOHNS' electrothermal melting point apparatus. Care was
taken to ensure that the heating was at a steady rate.

3.1.2 Spectroscopic Measurements used for the characterization of the
synthesized compounds.

a) Infrared spectra (IR):
Infrared spectra were recorded as a solid which was finely ground in a small agate mortar with
a drop of liquid hydrocarbon (nujol) as a mull or a paste or as an evaporated
film(CHCl₃, CH₂Cl₂) on DR-8001 Shimadzo FT-IR spectrophotometer from the
Department of Chemistry, Jahangirnagar University, Dhaka, Bangladesh. Following
abbreviations have been used for infrared spectra: s = strong, m = moderate, w = weak, v =
variable, sh = sharp. The measuring mode was % T (Percentage transmittency). The absorption
bands/peaks were expressed in cm⁻¹ (per centimetre), and the resolution was 4 cm⁻¹.

b) Proton - Nuclear Magnetic Resonance (¹H-NMR) Spectra:
Proton NMR spectra were taken by 200 MHz spectrophotometer at the Department of
Chemistry, University of Hannover, Fed. Republic of Germany and also were measured by
300 MHz at the Central Drug Research Institute, Lucknow, India. Deuterated solvents which
were used for ¹H-NMR spectra are Dimethyl sulfoxide (DMSO-D6), Chloroform (CDCl₃).
Methanol (CD₃OD) with tetramethyl silane (TMS) as an internal standard. The chemical shifts
were given in δ- scale relative to tetramethyl silane (TMS, δ = 0). For ¹H-NMR spectra the
following abbreviations have been used. s = singlet, d = doublet, t = triplet, m = multiplet.
c) **Mass Spectroscopy (MS):**

Mass spectra were measured by a **Karatas MS 25** using a **DH 88** data system with EI technique, from the Department of Chemistry, University of Hannover, Fed. Republic of Germany and also were recorded at Central Drug Research Institute, Lucknow, India.

### 3.1.3 CHROMATOGRAPHIC TECHNIQUES USED FOR THE SEPARATION OF THE SYNTHESIZED COMPOUNDS.

a) **Thin Layer Chromatography (tlc):**

Thin layer chromatographic technique was used extensively to follow the progress of the reactions and to check the purity of the products. Glass plates (7.6 x 2.6 cm) were used to make tlc plates. The glass plates used for tlc were thoroughly cleaned and dried. Then the plates were coated with a layer of Kiessel gel 60 GF254 (Merck). This was carried out either on plates prepared from water suspension silica gel (acidic) or plates prepared from water suspension alumina (basic). Then the plates were dried in an oven for 2-3 hours and after left them in open place to attain the room temperature. The solution of the reaction mixture and the starting materials were applied thin glass capillaries at about 10 mm from the bottom of the plates. The best possible solvents system were used. The plates were then placed vertically with the spotted end placed downwards in chromatographic tanks so that the spotted mark of the compound remained above the solvent front reached 1.0 cm far from the upper edge. The plates were allowed to dry at room temperature deeping them in iodine tank. The following abbreviations were used for solvent systems. Petroleum ether (PE), Ethyl acetate (EA), Acetone (Ac) etc. The results of tlc were expressed in terms of Rf-value.

b) **Column Chromatography:**

Column chromatographic technique was extensively used for the separation of pure compounds from a reaction mixture. Sometimes crude product was purified by column chromatography. The chromatographic column was prepared by slurry method using Kiessel gel [kiessel gel
100, 70-230 mesh ASTM (Merck) as the stationary phase. The column was packed with the appropriate solvent and the slurry was poured into it so that the packing was compact and uniform. Solvent system like Pet. ether : Ethyl acetate was used as eluent at different proportions. In the case of polar compounds, the reaction mixture was dissolved in suitable polar solvents and adsorbed in small amount of Kiessel gel in a round bottomed flask. The solvent was completely removed by rotary vacuum evaporator from the Kiessel gel and that Kiessel gel with absorbed material was put on the silica gel column which was previously prepared by wet slurry fitting technique. The components of the mixtures were eluted first with least polar solvents followed by increased polar solvents.

3.1.4 Purification of the Reagents And Solvents:

Chloroform, Dimethyl sulfoxide (DMSO), Diethyl ether (Et₂O), Ethanol (EtOH), Ethyl acetate (EA), Methanol (MeOH), Petroleum ether (PE) (60°C - 80°C and 40°C - 60°C) were used as solvents during the working hour. All the solvents were procured from Merck (Germany), BDH Chem. Co. (England), Aldrich Chem. Co. (U.S.A.), Benzidine, m-chloro-p-toludine, anhydrous sodium sulphate, chloral hydrate, conc. HCl, conc. H₂SO₄, hydroxylamine hydrochloride, Methanol, acetic-anhydride, acetic acid etc. were procured from E. Marck (Germany), BDH (England), Aldrich chem. Co. (U.S.A.), Bombay Chemical Co. (India).

The solid reagents were purified by recrystallization if necessary. The solvents and liquid reagents were also purified and dried before prior use. All solution in water immiscible solvents which had been in contact with water were dried over anhydrous sodium sulphate prior to evaporation. Solvents were usually removed by means of rotary vacuum evaporator. The liquid reagents and all the solvents mentioned above were distilled before use.
3.2 EXPERIMENTAL SECTION-B

3.2.1 Sandmeyer Reaction\textsuperscript{22}:

Synthesis of Oximinoacetanilides:

Chloral hydrate (9.89 g, 0.059 mol) and water (130 ml) were placed in a round bottomed flask (1000 ml). To that solution were then added in order: i) anhydrous sodium sulphate (142.85 g, 1.05 mol) ii) a solution of substituted aromatic mono-amine (0.047 mol) in water (30 ml) to which conc. HCl (4.8 ml) had been added to complete the solution of amine and finally iii) a solution of hydroxylamine hydrochloride (0.174 mol) in water (54 ml). The flask was heated by a burner so that vigorous boiling began in about thirty to thirtyfive minutes and further heating was made to the reaction mixture to complete the reaction. During the heating period, some crystals of oximinoacetanilide were separated. On cooling the solution in running water the remainder was crystallized. Finally the reaction mixture was filtered with Buckner funnel and the crystals were dried in a desiccator.

* Synthesis of oximinoacetanilide (1A) from 4-methyl-3-chloro aniline.

Reactants Required:

1. 4-methyl-3-chloro aniline: 500 mg (3.53 m mol)
2. Chloral hydrate: 724.4 mg (4.38 m mol)
3. Conc. HCl: 2 ml
4. Hydroxylamine hydrochloride: 896.42 mg (12.90 m mol)
5. Sodium Sulphate: 10.5 g
6. Water: quantity sufficient

The crude product was obtained as off-white solid and recrystallized from methanol yielded the white crystalline solid of compound 1A, yield 85% (636.74 mg) m.p. 170\textdegree-173\textdegree C and was found to be homogeneous on tlc plate (PE:EA, 1:1) with \( R_f \) 0.58.

Compound 1A was characterized spectrosopically.
IR Spectrum:

\( \nu_{\text{max. kBr (cm}^{-1})}: 3324 (\text{S, } \nu_{\text{O-H}}), 3252 (\text{w, } \nu_{\text{N-H}}), 3052 (\text{w, } \nu_{\text{C-H, aromatic}}), 2920 (\text{w, } \nu_{\text{C-H aliphatic}}), 1656 (\text{Sh, } \nu_{\text{C=O, amide}}), 1600 (\text{Sh, } \nu_{\text{C=N}}), 1540 (\text{Sh, } \nu_{\text{C=C, aromatic}}), 1496 (\text{S, } \delta_{\text{C-H, aliphatic}}), 1256 (\delta_{\text{O-H}}), 804 (\text{S, } \delta_{\text{C-H, aromatic}}), 712 (\text{S, } \nu_{\text{C-Cl}}).

\[ \text{H}_3\text{C} \quad \text{Cl} \quad \text{H} \quad \text{H}_d \quad \text{Hf} \quad \text{H} \quad \text{d} \quad \text{N-H} \quad \text{C} \quad \text{N-OH} \]

\[ 1 \quad \text{A} \]

\( ^1\text{H-NMR (CD}_3\text{OD/TMS)}: \)

\( \delta: 2.32 \text{ (s, 3H, Ar-CH}_3 \text{, a)}, 7.8 \text{ (d, 1H, Ar-H, d)}, 7.22 \text{ (d, 1H, Ar-H, b)}, 7.42 \text{ (dd, 1H, Ar-H, c)}, 7.58 \text{ (s, 1H, -N=C-H, f)}. \)

Due to use of deuterated methanol as \(^1\text{H-NMR solvent no signal for NH and N-OH is observed because of proton-deuteron exchange reaction.}\)

Mass spectrum:

m/z (% relative intensities):

\[ 212/214 (M^+, 3:1, Cl^{35}, 92), 195/197(50), 167 (81), 141/143 (3:1, Cl^{35}, 100), 132 (91) \]

106(40), 77(90).

\( M^+ \) stands for \( \text{C}_9\text{H}_9\text{N}_2\text{O}_2\text{Cl} \)
SYNTHESIS OF BIS-OXIMINOACETANILIDE (1B) FROM BENZIDINE.

Reagents used (1:2 molar ratio)

1. Benzidine : 400 mg (2.1739 m mol)
2. Chloral hydrate : 902 mg (2.7265 m mol)
3. Conc. HCl : 4 ml
4. Hydroxylamine hydro chloride : 1.104 g (7.9464 m mol)
5. Anhydrous sodium sulphate : 13.04 g

Graywhite solid was found. The crude product was purified crystallization from methanol. The product was dried in a desiccator to afford white crystalline solid, yield 78% (552.78 mg), m.p. >300°C. It was soluble in methanol and found to be homogeneous on tlc plate (PE:EA, 1:3) with Rf 0.32. The compound (1B) was characterized spectroscopically.

IR Spectrum :

\[ \nu_{max. \text{, kBr (cm}^{-1})} : 3476 \text{ (Sh, } \nu_{\text{C-H}}), 3352 \text{ (Sh, } \nu_{\text{N-H}}), 3096 \text{{S, } \nu_{\text{C-H}} \text{, aromatic)} } , 1710 \text{ (w, } \nu_{\text{C=O, amide}) , 1644 \text{ (Sh, } \nu_{\text{C=N}) , 1660 \text{ (Sh, } \nu_{\text{C=C, aromatic)} , 1560 \text{ (Sh, } \delta_{\text{N-H}), 884 \text{ (Sh, } \delta_{\text{C-H, aromatic.}} }}\]

![Chemical structure of 1B](image-url)
\(^1\)H-NMR (DMSO-d\(_6\)/TMS):

\(\delta\) : 12.2 (br.s, 2H, 2x-OH,a), 10.3 (s, 2H, 2xNH,b), 7.8-7.5 (m,8H, aromatic).
3.9-3.7 (br.s,2H,2x-N=CH,c).

Mass Spectrum :
m/z (% of relative intensities)
326(M\(^+\), 1.59), 290 (3.26), 281(15.5), 263(20.8), 236(100), 210(19.17),
184(22.8), 166(6.2), 153(11.58), 127(6.73), 118(7.24), 92(4.52), 75(6.48),
M\(^+\) stands for C\(_{16}\)H\(_{14}\)N\(_4\)O\(_4\) is 326.

3.2.2 General Procedure\(^{22}\) for the preparation of substituted Isatin from oximinoacetanilide (cyclization with Conc. H\(_2\)SO\(_4\))

Concentrated sulphuric acid (quantity sufficient, sp.gr. 1.84) was warmed to 50\(^\circ\)C in a 250 ml round bottom flask, fitted with a stand by the clamp on a hot plate with an efficient mechanical stirrer. To this dry oximinoacetanilide (7.856g, 0.044 mol) prepared as above was added at such a rate to keep the temperature of the acid mixture between 60\(^\circ\)-70\(^\circ\)C but not higher. After addition was over, the reaction mixture was heated to 80\(^\circ\)C and kept at this temperature for about ten minutes. It was then cooled to room temperature and poured upon ten to twelve times of its volume of cracked ice. After standing for half an hour, the solid substituted isatin was filtered with Buckner funnel. The product was then washed several times with cold water to remove H\(_2\)SO\(_4\) and then dried in the desiccator. According to this method the following compounds were prepared and the product was so obtained was characterized by the usual spectroscopic methods.
SYNTHESIS OF 5-METHYL-4-CHLORO-ISATIN (2A) AND 6-CHLORO-5-METHYL ISATIN (2B):

The crude product (500 mg) was found to be a mixture of 5-methyl-4-chloro isatin and 6-chloro-5-methyl isatin on tlc examination. The compounds were separated by column chromatographic technique (PE:EA,2:1).

FIRST-FRACTION: Compound 2B

Yellowish orange coloured compound was characterized as 6-chloro-5-methyl isatin (2B), yield 28% (140 mg), m.p. 256°C-258°C and was found to be homogeneous on the tlc plate (PE: EA, 2:1) with Rf 0.65.

Compound 2B was characterized with the help of spectral analysis.

IR Spectrum:

\[ \nu_{\max, \text{nujol (cm}^{-1})}: 3275 (\text{sh, } \nu_{\text{N-H}}), 3100 (\omega, \nu_{\text{C-H}}), 1772 (m, \nu_{\text{C=O, lactam}}), 1734 (\omega, \nu_{\text{C=O, keto}}), 1653 (S, \nu_{\text{C=C, aromatic}}). \]

\[ \begin{array}{c}
\text{C} \\
\text{H}_3\text{C} \\
\text{H}_\text{a} \\
\text{H}_\text{b} \\
\text{N-H}_\text{d} \\
\text{O} \\
\text{O} \\
\end{array} \]

\[ \text{2B} \]

\[ \text{1H-NMR (CDCl}_3/\text{TMS)}: \]

\[ \delta: 7.85 \text{ (br. s, 1H, CO-NH,d), 7.53 (s,1H, Ar-H. a), 6.95 (s, 1H, Ar-H,b), 2.35 (s, 3H, Ar-CH}_3, c). \]
Mass spectrum:
m/z (% of relative intensities):
195/197 (M⁺ 3:1 ³⁵Cl, 39), 167/169 (3:1, ³⁵Cl, 100), 140 (39), 104(20), 77(25).
M⁺ stands for C₉H₆NO₂Cl

1³C-NMR (CDCl₃/TMS):
δ : 19.62 (C-9), 113(C-4), 127.55 (C-8),
134 (C-7), 145.80(C-6), 197.60 (C-1), 216.58 (C-2).

SECOND REACTION : COMPOUND 2A
After elution of the 2nd fraction, the deep red coloured compound was recrystallized from CHCl₃. This compound was characterized as 5-methy-J-4-chloro-isatin (2A), yield 72% (360 mg), m.p. 240-243°C and was found to be homogeneous on tlc plate (PE: EA, 2:1) with Rf 0.33.

Compound 2A was characterized with spectral data:

IR - SPECTRUM:
ν max. KBr (cm⁻¹) : 3292 (Sh, νN-H), 2980 (ω, νC-H, aromatic), 2956 (ω, νC-H, aliphatic),
1752 (Sh, νC=O, lactam), 1726 (Sh, νC=O, keto), 1612 (Sh, νC=O, aromatic), 676 (S, νC-Cl).
$^1$H-NMR (CD$_3$OD/TMS):

$\delta$: 7.5 (d, 1H, Ar-H$_b$), 6.8 (d, 1H, Ar-H$_a$) 2.32 (s, 3H, Ar-CH$_3$, c)

No signal was recorded for NH (lactam) proton due to CD$_3$OD used as solvent.

$^{13}$C-NMR (CDCl$_3$/TMS):

$\delta$: 18.48 (C-9), 111.72 (C-8), 116.52 (C-7), 127.95 (C-3), 133.01 (C-4) 140.94 (C-6), 157.44 (C-1), 182.58 (C-2), 133.80 (C-5).

Mass Spectrum:

m/z (% of relative intensities): 195/197 (M$^+$, 3:1, $^{35}$Cl, 39), 167/169 (100), 140(39), 104(20), 77(25).

M$^+$ stands for C$_9$H$_9$N$_2$O$_2$Cl is 195/197
3.2.3 Synthesis of Substituted Isatin Thiosemicarbazone from corresponding Isatin with Thiosemicarbazide (General procedure) \(^{133}\).

A three necked quick fitted flask was fitted with a dropping funnel and a spiral condenser. Thiosemicarbazide in methanol was added to a hot solution of substituted isatin (normally 1:1 molar ratio isatin) in methanol with stirring. The reaction mixture was refluxed for 2-3 hours or more (if necessary) on an oil bath with vigorous stirring. When the refluxing was completed then the reaction mixture was cooled and filtered. On the tlc examination if the product was not pure then it was recrystallized from a suitable solvent. The product was so obtained was characterized by the usual spectroscopic methods.

* SYNTHESIS OF 5-METHYL-4-CHLORO ISATIN-3-THIOSEMICARBAZONE (3A) FROM COMPOUND 2A.  
Reagents used (1:1 Molar ratio):

1. Compound 2A (subst. Isatin) : 2.56 m mol (500 mg)  
2. Thiosemicarbazide : 2.56 m mol (233.3 mg)  
3. Methanol : 30 ml  

The pale yellow crude solid was obtained and was purified by recrystallization from methanol. A pale yellow powder, yield 68.4% (470 mg), m.p. 265-268\(^\circ\)C, was found to be homogeneous on tlc plate with \(R_f\) 0.32 (PE:EA, 1:1). Compound 3A was characterized spectroscopically.

**IR Spectrum:**

\[ \nu_{\text{max. nujol}} \text{ (cm}^{-1} \text{)}: \]

- 3427 (S, \(v_{\text{N-H}}\)), 3219(S, \(v_{\text{N-H}}\)), 3163(w, \(v_{\text{C-H}}, \) aromatic),
- 2855(Sh, \(v_{\text{C-H}}, \) aliphatic), 1736(S, \(v_{\text{C=O}}, \) lactam),
- 1603(Sh, \(v_{\text{C=C}}, \) aromatic), 1142(S, \(v_{\text{C=O}}\)), 717(S, \(v_{\text{C-Cl}}\)).
$^1$H-NMR (CDCl$_3$/TMS):
$\delta$: 9.1 (s, 1xNH$_2$, a), 8.8 (s, 1H, NH$_c$ lactam), 8.4 (s, 1H, NH, b)
7.3 (d, 1H, Ar-H, e), 6.9(d, 1H, Ar-H, d), 2.35(s, 3H, Ar-CH$_3$).

Mass Spectrum:
M/Z (% of relative intensities):
268/270 (M$^+$, 3:1, $^{35}$Cl, 57.1), 251(13.4) 240(57.3), 226(11.3), 209(19.9), 194(17.5),
180(23.2), 152(35.5), 125(14.4), 117(23.4), 102(8.5), 89(16.4), 77(26.2), 60(100)
M$^+$ for C$_{10}$H$_9$N$_4$OSCl is 268/270.

3.2.4 Oxidative cyclization of substituted Isatin-3-Thiosemicarbazone by 4% aqueous KOH solution (General Procedure)$^{133}$.

Substituted Isatin-3-thiosemicarbazone was dissolved in 4% aqueous KOH solution and refluxed for 4 hours. The reaction mixture was cooled and insoluble material was removed by filtration. The filtrate on neutralization with dilute acetic acid gave a coloured solid which was washed well with distilled water and dried in a desiccator. The compound then characterized by the usual spectral analysis.
SYNTHESIS OF 9-CHLORO-8-METHYL-5H-2,3-DIHYDRO [1,2,4] TRIAZINO[5,6-b]-INDOLE-3-THIONE (4A).

Reactants used:
1. 5-methyl-4-chloro isatin-3-thiosemicarbazone (3A) : 0.92 mmol (250 mg)
2. 4% aqueous KOH solution : 40 ml.

The crude product was recrystallized from methanol and found homogeneous on the plate with Rf 0.54 (PE:EA, 1:1). A yellowish-red solid, yield 194 mg. (83%), m.p > 300°C was obtained.

IR Spectrum:
νmax, nujol (cm⁻¹): 3320 (w, νN-H), 2995 (Sh, νC-H aromatic), 2895 (Sh, νC-H aliphatic), 1690 (w, νC=N), 1610 (S, νC=O aromatic), 1151 (S, νC=S), 721 (S, νC-Cl).

'H-NMR (DMSO-d₆/TMS):
δ : 14.7-14.6 (s, 2xNH,c&d), 7.6(d,1H,a)
7.21(d,1H,b), 2.45(s,3H, Ar-CH₃).
Mass spectrum:

m/z (% of relative intensities):

250/252 (M+, 3:1, 35Cl, 100), 234(9.59), 222(20.66)
199(19.95), 187(60.95), 168(11.46), 156(19.30), 150(12.56),
127(12.44), 114 (16.84), 102(14.64), 88(13.34), 75 (15.8).

M+ stands for C_{10}H_{7}N_{4}SCl

3.2.5 Ring extension of oxidative cyclized product by 1,2-dibromoethane in DMF (General Procedure)\textsuperscript{133}.

Oxidative cyclized product (4A) and 1,2-dibromoethane in dimethyl formamide (DMF) were heated under reflux on a hot plate for 2-3 hours. The reaction mixture was cooled to room temperature and poured into water. The mixture was neutralized with 5% aqueous sodium carbonate solution to get the free base as solid which was filtered and dried in a desiccator under vacuum.

\textbf{SYNTHEISIS OF 9-CHLORO-8-METHYL-1,2-DIHYDRO THIAZOLO-[2,3-3,4] [1,2,4]-TRIAZINO [5,6-b] INDOLE, 5A.}

Reagents used:

(1) 9-chloro-8-methyl-5H-2,3-dihydro [1,2,4] triazino [5,6-b]-indole-3-thione (4A)

: 150 mg (0.6 mmol)

(2) 1,2-Dibromo ethane

: 113 mg (0.6 mmol)

(3) DMF

: 10 ml (used as solvent)

The crude product was purified by preparative tlc plate on silica gel (pet-ether: ethylacetate, 1:3) and found pink crystalline solid with yield 65 mg (39.2%), Rf 0.36 (PE: EA, 1:3), m.p 245 - 250°C (decomp).
Compound 5A was characterized with spectral data.

**IR Spectrum :**

$V_{max}$, nujol (cm$^{-1}$): 2953 (Sh, $v_{C-H}$, aromatic), 2924(Sh, $v_{C-H}$, aliphatic), 1545(S, $v_{C=C}$), 1460(Sh, $v_{C=N}$), 1377(S, $v_{C-N}$), 1225(S, $v_{C-S}$), 721(S, $v_{C-Cl}$).

![Diagram of 5A](image)

**$^1$H-NMR (DMSO-d$_6$/TMS) :**

$\delta$: 7.62(sh.d,1H,d), 7.12(sh.d,1H,c), 4.92(t,2H,b); 3.78(t,2H,a), 2.46 (s,3H,Ar-CH$_3$).

**Mass spectrum :**

$m/z$ (% of relative intensity):

276/278(M$^+$, 3:1, 35Cl, 100), 248 (4.17), 230 (9.13), 217(42.96), 203(3.04), 191(12.48), 176(33), 150(8.35), 141(15.35), 114(12.41), 99 (3.7), .88 (5.7), 75 (5.65).

M$^+$ stands for C$_{12}$H$_9$N$_4$SCl.

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3.2.6 General procedure\textsuperscript{79} for the preparation of 1,3,4-Thiadiazoline derivatives from their corresponding isatin thiosemicarbazone with acetic anhydride (AC\(_2\)O).

The refluxing of isatin thiosemicarbazone with freshly distilled acetic anhydride for 1-2-hours or more (if necessary) with vigorous stirring over a hot plate in a 250 ml round bottomed flask. After completion of the reaction the flask was cooled at room temperature. The excess acetic anhydride is removed with high vacuum pump and gave a semisolid or solid mass. The compound must be dried properly. This on purification on a silica gel column using pet-ether : acetone (10:1) as a eluant gave the cyclized product of 1,3,4-Thiadiazoline derivatives.

*SYNTHESIS OF 5-METHYL-4-CHLORO ISATIN-SPIRO-4-ACETYL-2 (DIACETYL AMINO)\(\Delta^2\)-1,3,4-THIADIAZOLINE, 6A FROM THE COMPOUND, 3A.*

Reactants used:

1. 5-methyl-4-chloro isatin (3A) : 120 mg (0.44 mmol)
2. Acetic anhydride (Ac\(_2\)O) : 10 ml

The reddish yellow solid was recrystallized from chloroform (CHCl\(_3\)) to get the orange red crystalline solid of compound 6A, yield 49\% (86 mg) m.p.156-158\(^\circ\)C and was found to be homogeneous on tlc plate (PE: EA, 2:1) with R\(_f\) 0.28.

Compound 6A was characterized spectroscopically.

IR Spectrum : 

\(\nu_{\text{max}}\) nujol (cm\(^{-1}\)):

- 3405 (Sh, \(\nu_{\text{N-H}}\)), 3050(w, \(\nu_{\text{C-H}, \text{ aromatic}}\)), 2945(w, \(\nu_{\text{C=H}, \text{ aliphatic}}\))
- 1760(w, \(\nu_{\text{C=O}, \text { lactam}}\)), 1730(Sh, \(\nu_{\text{C=O}, \text{ acyl keto}}\)), 1690(w, \(\nu_{\text{C=N}}\))
- 1615(Sh, \(\nu_{\text{C=C}, \text{ aromatic}}\)), 760(Sh, \(\delta_{\text{C-H}}\)), 710(Sh, \(\nu_{\text{C-Cl}}\)).
$^1$H-NMR (CDCl$_3$/TMS):

$\delta$ : 8.35 (s,1H,CONH, Lactam, b), 7.35(d,1H,d), 6.95(d,1H,e). 2.65(s,9H,3xCOCH$_3$,e) 2.15 (s,3H, Ar-CH$_3$,a).

Mass spectrum :
m/z % of relative intensities : 394/396 (M$^+$, 3:1, $^{35}$Cl,17.2), 353 (3.2), 323(7.1), 306(44.5), 293(41.2), 266(21), 250(31.2), 223(89.8), 208(68.8), 194(24.4) 180(90.5), 152(18.7), 125 (13.5), 116 (19.4), 89 (41.2), 43 (100).

M$^+$ stands for C$_{16}$H$_{15}$N$_4$O$_4$SCl.
CHAPTER FOUR
PHARMACOLOGICAL STUDIES
4.1 Antibacterial activities qualitative methods for bacterial sensitivity.

4.1.1 Principle

The susceptibility of the microorganism to antibacterial agents may be measured in vitro by utilizing agar diffusion technique.

4.1.2 Tests in vitro: Range of antimicrobial activity: choice of organism.

Stage I antibacterial screen includes, for example only Bacillus pumilus and Escherichia coli. These organisms are standard strains which are well adapted to laboratory conditions.

These organisms tested were predominantly from fresh clinical isolates. Individual bacterial strains in pure state were procured from the research Lab. Department of Microbiology, Dhaka University, Dhaka.

4.1.3 Preparation of the media.

Nutrient Agar:

Composition:

<table>
<thead>
<tr>
<th>Name of the ingredients:</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat extract</td>
<td>15%</td>
</tr>
<tr>
<td>Peptone from meat</td>
<td>25%</td>
</tr>
<tr>
<td>Agar agar</td>
<td>60%</td>
</tr>
<tr>
<td>Distilled water</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

Preparation of Nutrient Agar:

The above ingredients (12 gm) were taken in a flask, 100 ml distilled water added and slight heat was applied to make it a solution. The media were then autoclaved in a similar fashion (121°C, 50 lb/sq. inch). The flask was then removed from the autoclaved and placed on a water bath at 50°C. The pH of the solution was 7 ± 0.2.
4.1.4 Preparation of the culture plate:

Nutrient Agar media prepared and a 25 ml portion was poured into a petridish, kept undisturbed for 15 minutes during which fine solidification occurred when solidification was complete. 3-4 holes were made with the help of a borer. On these solid plates the cultures were spreaded through cotton stick swabs.

4.1.5 Inoculation of the compound and antimicrobial assay procedure:

A small amount of the compound was added to each hole of the petridish by using a special type of spatula. A 100 μl test chemical solution was poured into each of the holes made in the solid form and held undisturbed for about 20 minutes in order to allow sufficient time to the test material to diffuse into a considerable area of the petridish. The petridish was then incubated at 37°C for overnight or 15 hours. The antimicrobial activity was qualitatively detected by the formation of a clear zone around each hole of the petridish.

4.1.6 Results and discussion:

Antimicrobial activities were qualitatively determined against different microorganisms. These activities were measured by the zone inhibition technique expressed by average diameter and the results may be summarized as follows (Table-1).

<table>
<thead>
<tr>
<th>Organism Tested</th>
<th>Sensitivity (Growth Inhibition) of the organisms on compound test chemicals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1A</td>
</tr>
<tr>
<td>Bacillus pumilus</td>
<td>-</td>
</tr>
<tr>
<td>E. Coli</td>
<td>±</td>
</tr>
</tbody>
</table>

- : No sensitivity shown.

± : Partly sensitive (growth inhibition)
Discussion:

Results shows E. Coli may be sensitive towards oximinoacetanilide (1A). All the other compounds showed no antibacterial property towards E. Coli and B. Pumilus. One of the serious problem of this experiment faced by insolubility of the compounds in water. Hot water showed slight solubility and alcohol can not be used as solvent because alcohol itself is antibacterial. further works can be done to find out a suitable solvent for the chemicals and then testing the culture sensitivity pattern again.
CHAPTER FIVE
SUMMARY
CHAPTER-5: SUMMARY

In this thesis the work done may be summarized as follows:

1. **Oximinoacetanilides**: Two oximino acetanilides (1A and 1B) were synthesized with high yields from their corresponding aromatic anilines with chloral hydrate and hydroxylamine hydrochloride according to Sandmeyer procedure.

   ![Chemical Structure of 1A](image1)
   \[\text{1A} \rightarrow \text{HC} = \text{NOH} \quad \text{85\%}\]

   ![Chemical Structure of 1B](image2)
   \[\text{1B} \rightarrow \text{HC} = \text{NOH} \quad \text{78\%}\]

2. **Isatins**: Two isomeric substituted isatins (2A & 2B) was synthesized from the compound 1A with conc. \(\text{H}_2\text{SO}_4\).

   ![Chemical Structure of 2A](image3)
   \[\text{2A} \rightarrow \text{72\%}\]

   ![Chemical Structure of 2B](image4)
   \[\text{2B} \rightarrow \text{28\%}\]
3. Schiff base: One substituted isatin thiosemicarbazone (3A) was synthesized from the compound 2A and thiosemicarbazide with methanol at refluxing conditions.

4. By oxidative cyclization method 1,2,4-triazino-3-thione product 4A was synthesized from its corresponding thiosemicarbazone (3A) with 4% aqueous KOH solution.

5. One ring extension products (5A) has been synthesized from cyclized products 4A with 1, 2-dibromoethane in DMF.
6. **Spiro compound**: One N-acetyl-1,3,4-thiadiazoline derivative was synthesized from its corresponding thiosemicarbazone (3A) with acetic anhydride at refluxing condition.

![Spiro compound structure](image)

7. All the synthesized compounds were characterized with the help of different spectral analysis (IR, $^1$H-NMR, $^{13}$C-NMR and Mass spectra) and a lot of spectral data and the information regarding the fragmentation pattern of these compounds have been gathered in this work. Some characteristic IR band and $^1$H-NMR peaks are given below:

<table>
<thead>
<tr>
<th>IR (cm$^{-1}$)</th>
<th>$^1$H-NMR (δ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.  $\nu_{\text{C}=\text{O}}$ (lactam)</td>
<td>1691-1772</td>
</tr>
<tr>
<td>ii. $\nu_{\text{C}=\text{O}}$ (keto)</td>
<td>1656-1734</td>
</tr>
<tr>
<td>iii. $\nu_{\text{C}=$ aromatic)</td>
<td>1540-1615</td>
</tr>
<tr>
<td>iv. $\nu_{\text{C}=\text{N}}$</td>
<td>1460-1736</td>
</tr>
<tr>
<td>v.  $\nu_{\text{C}=\text{S}}$</td>
<td>1142-1151</td>
</tr>
<tr>
<td>vi. $\nu_{\text{N-H}}$</td>
<td>3219-3352</td>
</tr>
</tbody>
</table>

| i.  CO-NH                | 7.85 - 8.80      |
| ii. N-H                  | 8.4 - 14.7       |
| iii. Ar-CH$_3$           | 2.15 - 2.46      |

8. All the synthesized products have been put in pharmacological investigations (Test for antifungal and antibacterial activity). Oximinoacetanilide may have antibacterial property against E. Coli. The results of antifungal properties could not be obtained in due time. Work is in progress.
CHAPTER SIX

REFERENCES
CHAPTER 6: REFERENCES

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