BANGLADESH UNIVERSITY OF ENGINEERING & TECHNOLOGY, DHAKA, BANGLADESH.

SYNTHESIS AND PHARMACOLOGICAL STUDIES OF SUBSTITUTED 1, 3, 4 - THIADIAZOLINE DERIVATIVES

A
DISSERTATION SUBMITTED
IN PARTIAL FULFILMENT OF THE REQUIRMENTS
FOR THE DEGREE OF MASTERS OF PHILOSOPHY
IN
CHEMISTRY

SUBMITTED
BY

Examination Roll-9603108 F
Registration No. 961808

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BANGLADESH UNIVERSITY OF ENGINEERING & TECHNOLOGY, DHAKA -1000, BANGLADESH, DEPARTMENT OF CHEMISTRY

THESIS ACCEPTANCE LETTER

We hereby recommend thesis entitled "Synthesis and Pharmacological studies of substituted 1, 3, 4 - thiadiazoline derivatives", presented by MD. SHOFIUR RAHMAN (Roll NO. 9603108F, Reg. NO. 961808, Session : 1995 - 96 - 97) to accept as partial fulfillment of the requirements for the degree of Master of Philosophy (M. Phil.) in Chemistry, on the 15th March, 2001.

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Author

DECLARATION

I hereby declare that the whole of the work of this thesis has been carried out by myself in the Organic Research Laboratory of the Department of Chemistry, under the supervision of Dr. Md. Abdur Rashid, Associate Professor in Chemistry, BUET, Dhaka, during the Period starting from December 6, 1998 to February 10, 2001. I, further, declare that this work has not been submitted in part or full any where else for a degree or diploma. Any source of information in connection with this thesis has been duly acknowledged and all quotation has been marked by quotation marks.

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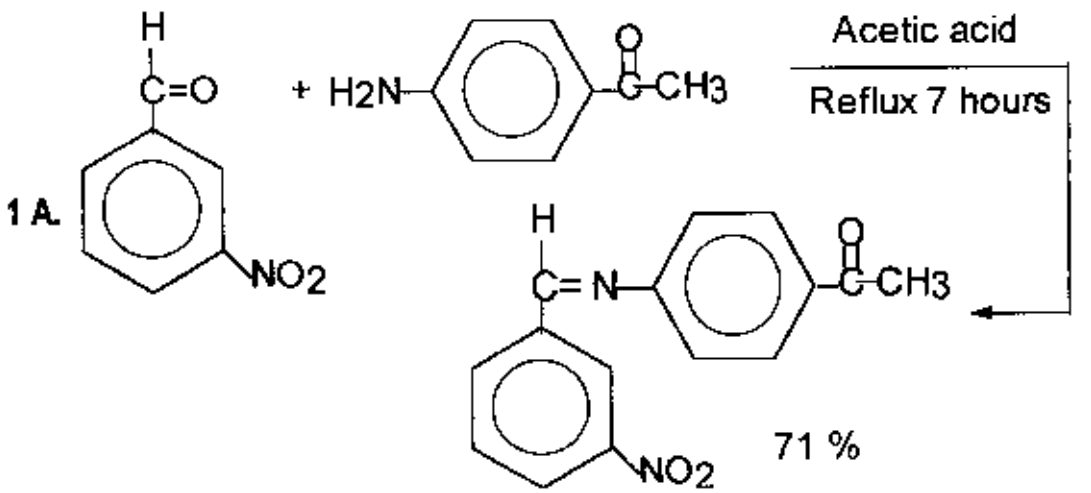
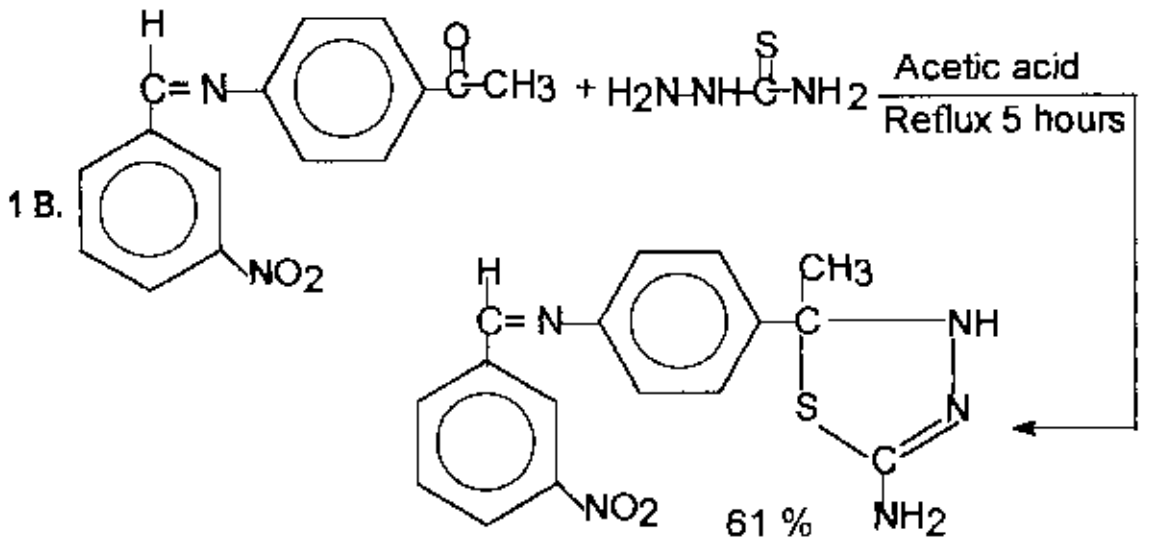
Session 1995-96-97

C ONTENTS

ABSTRACT

Some substituted 1, 3, 4 - thiadiazoline derivatives have been synthesized and characterized by the elemental analysis and with spectral evidences. The experimental results are described in detail in the experimental section. The synthesized products are briefly described in the following Scheme:





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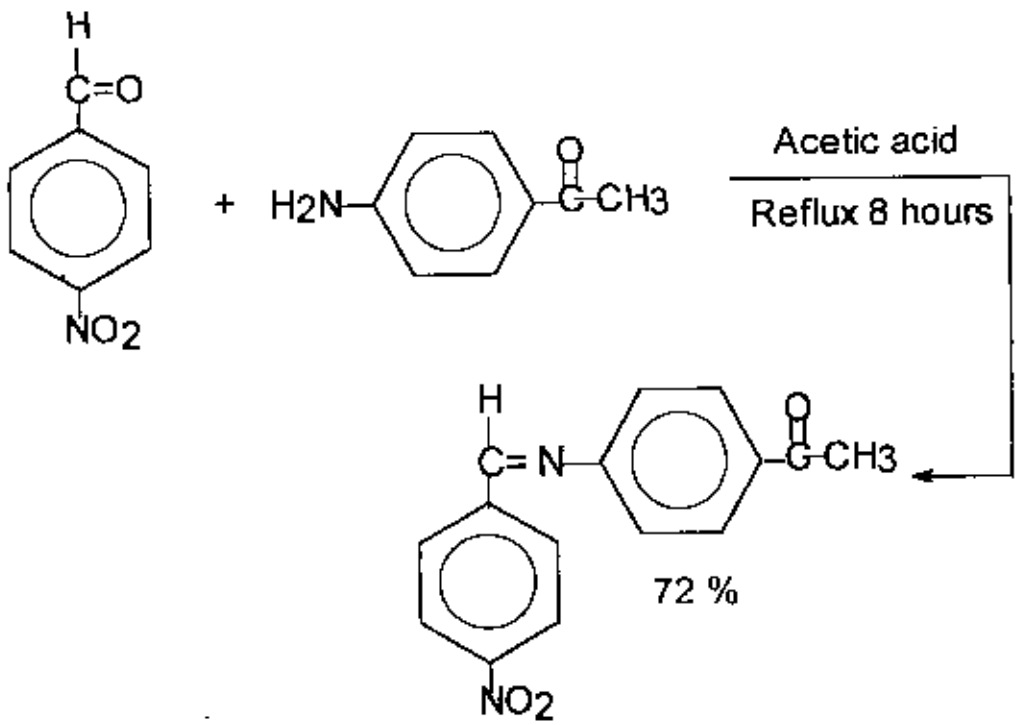
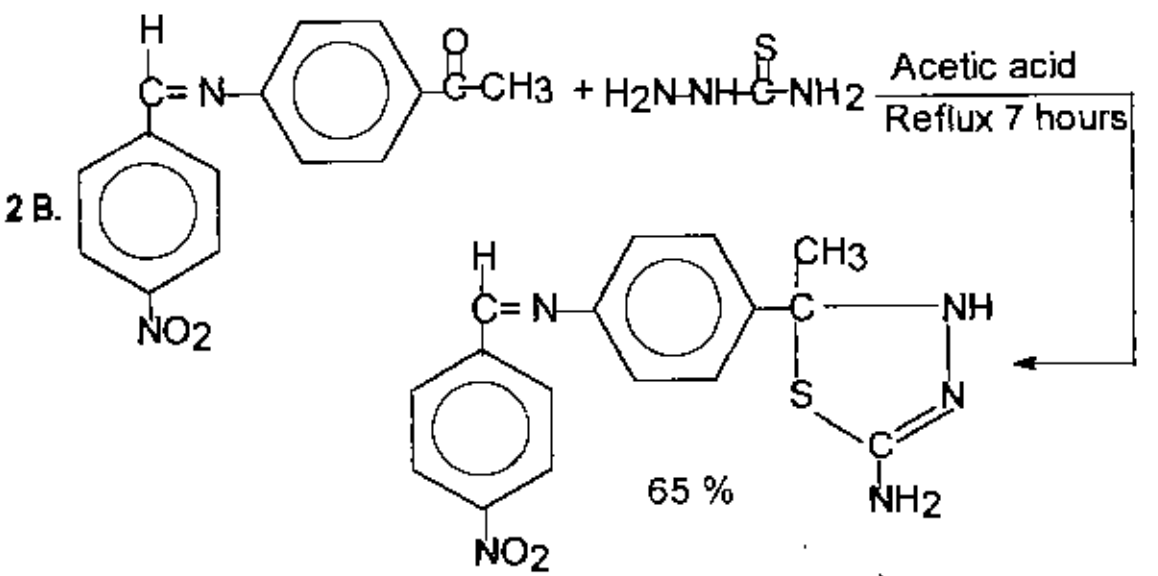
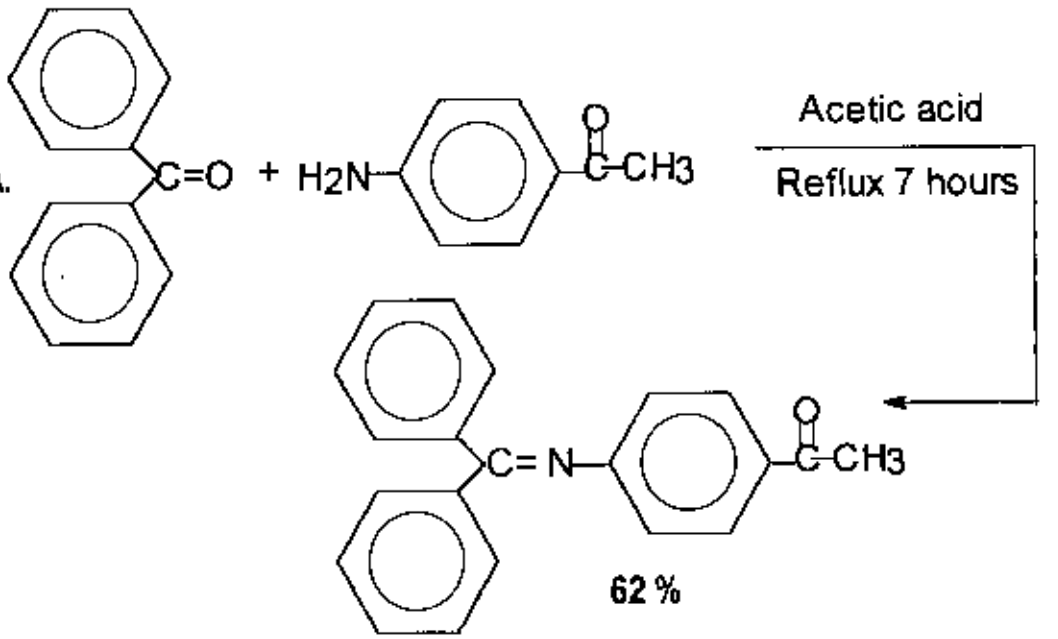

H
C=O
NO2
+
H2N
O
C-CH3
Acetic acid
Reflux 8 hours
H
C= N
O
C-CH3
NO2
72 %

2 B.

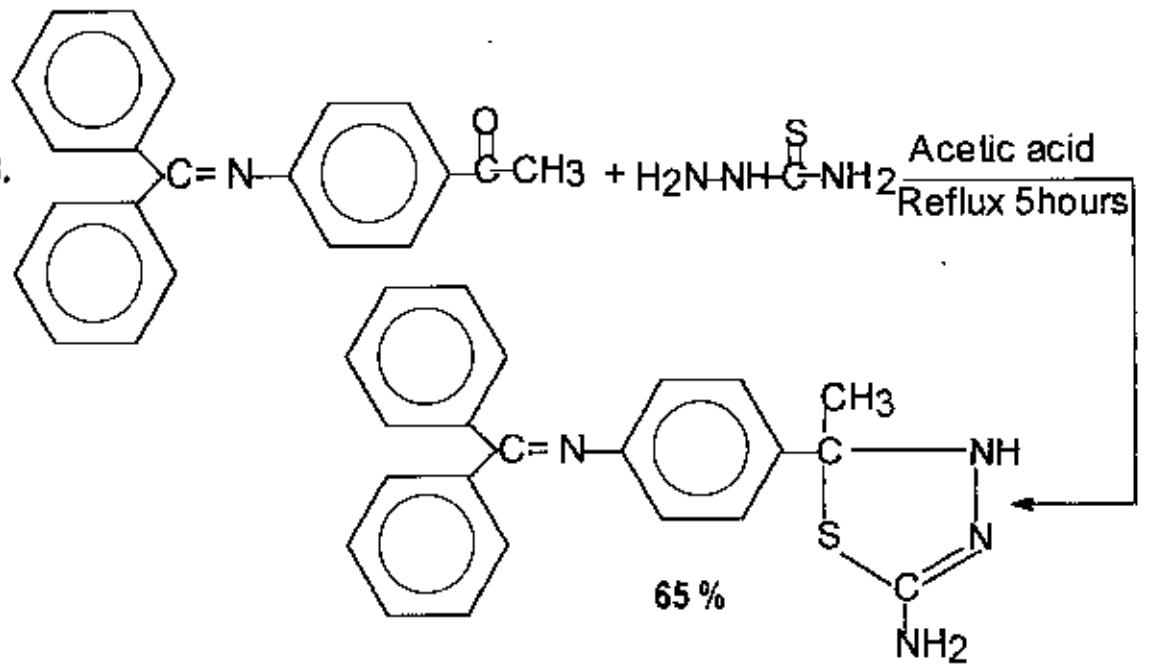

H
C= N
O
C-CH3
NO2
+ H2N-NH-C-NH2
S
Acetic acid
Reflux 7 hours
H
C= N
NO2
CH3
C
NH
S
N
C
NH2
65 %

3 A.

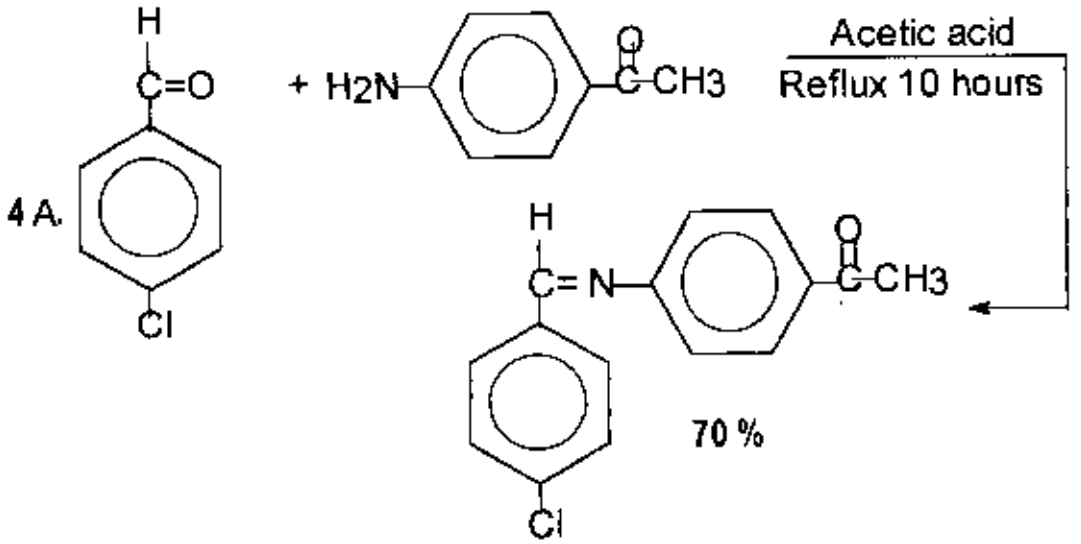

C=O
+ H2N
O
C-CH3
Acetic acid
Reflux 7 hours
C= N
O
C-CH3

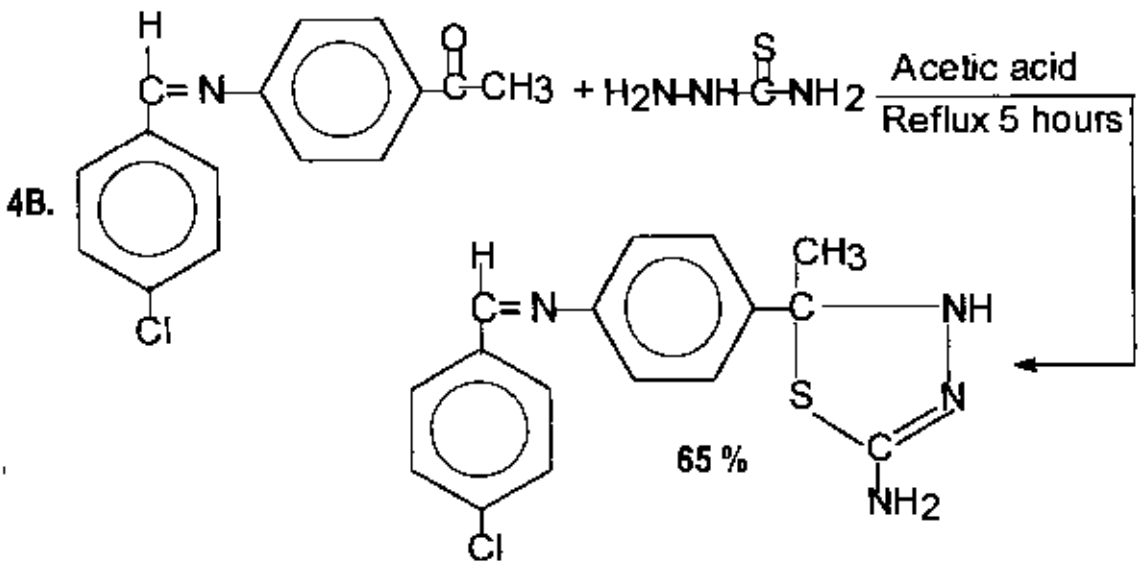
62 %

3 B.


C= N
O
C-CH3
+ H2N-NH-C-NH2
S
Acetic acid
Reflux 5hours
C= N
CH3
C
NH
S
N
C
NH2

65 %





Finally antibacterial activities have been tested on these compounds and are described in chapter 4.

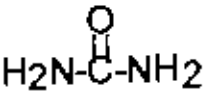
CHAPTER -1

INTRODUCTION

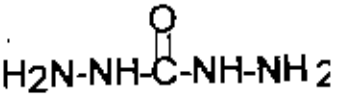
INTRODUCTION

1.1 GENERAL REMARKS AND HISTORICAL BACKGROUND

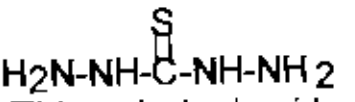
Carbohydrazide and its thioanalogue i.e. thiocarbohydrazide and thiosemicarbazide are very closely related to urea. These compounds are most directly associated with the foundation of synthetic organic chemistry.



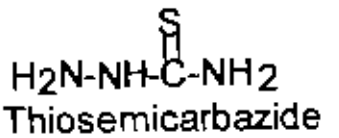
Urea
1



Carbohydrazide
2

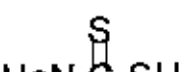


Thiocarbohydrazide
3



Thiosemicarbazide
4

Carbohydrazide and thiosemicarbazide are the hydrazine derivatives of carbonic and thiocarbonic acids respectively. Moreover, these hydrazides have a very close relation with thiocarbamic acids (**5**) and thiocarbazic acids (**6**) as well as with the aminoguanidines (**7-10**).



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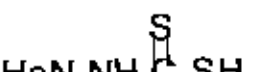

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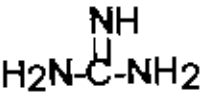

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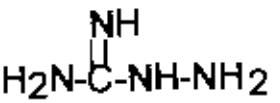

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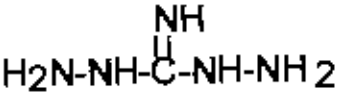

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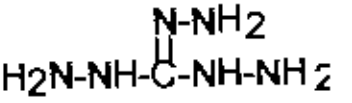

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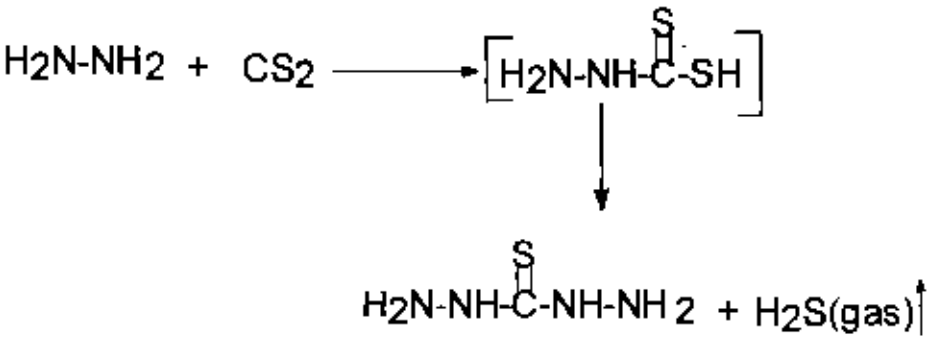
Thiosemicarbazide is a white crystalline solid. It can be recrystallized from water. It is soluble in water, ethanol, methanol and sparingly soluble in ethylacetate.

The discovery of hydrazine was a prerequisite for that of carbohydrazide and thiocarbohydrazide. A. W. Hoffmann had prepared diphenylhydrazine in 1863 and E. Fischer began his classical researches on phenylhydrazine in 1875. Curtius *et al.*[1, 2] described the results of hydrazinolysis of carbonic acid derivatives partly in 1894 and fully in 1895. In the course of their work, they synthesized the hydrazine salt of dithiocarbazic acid ($NH_2NHCSSH$: NH_2NH_2) by the condensation of carbon disulfide with hydrazine. It was not until 1908 R. Stolle[3] formerly Curtius's associates obtained thiocarbohydrazide by the hydrazinolysis of dithiocarbazic acid.



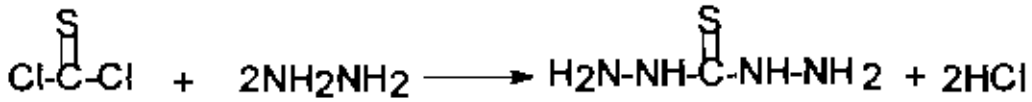
Over the years, interest in the chemistry of thiocarbohydrazide and thiosemicarbazide have steadily increased. The major studies were undertaken by Wilson and his co-workers and Guha *et al*[4]. They included the more notable contributions of Audrieth *et al*.[5-6], who carefully reinvestigated and improved thiocarbohydrazide synthesis of Sandstrom *et al*.[7], where the main interests were the use of these nitrogenous compounds in heterocyclic synthesis.

1.2 PREPARATION OF THIOCARBOHYDRAZIDES

Thiocarbohydrazides can be prepared by the following methods:

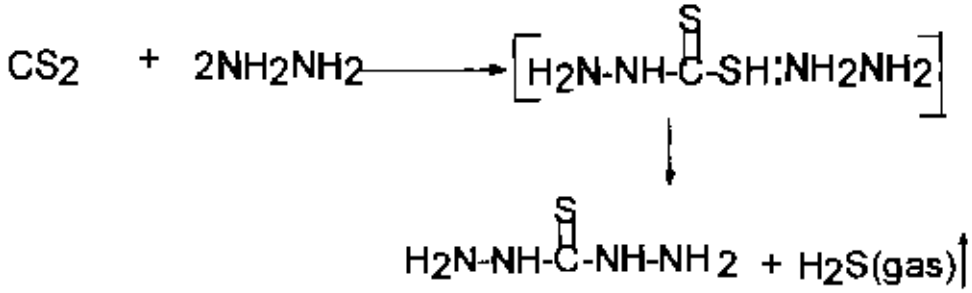
(1) Hydrazinolysis of thiophosgene[3]

Like its oxygen analog, thiophosgene readily undergoes hydrazinolysis in ethereal or in aqueous media that furnishes thiocarbohydrazide in satisfactory yield.



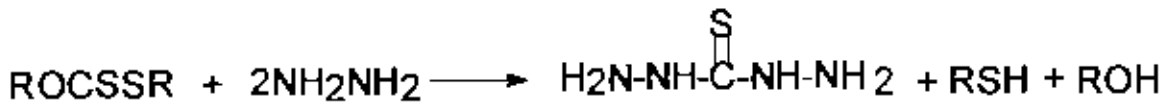
(2) Hydrazinolysis of carbondisulfide

The reaction of hydrazine with carbondisulfide is the cheapest, easiest and the most useful method for the large scale preparation of thiocarbohydrazide.



(3) Hydrazinolysis of diethylxanthate

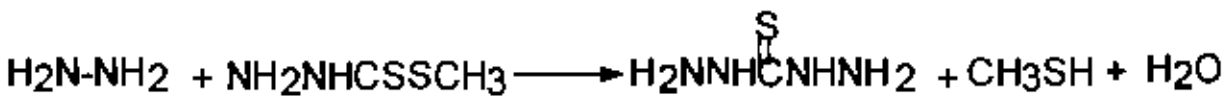
The hydrazinolysis of diethylxanthate is a possible route to thiocarbohydrazide.



As a solvent, ethanol has been found to be unsuitable[4] but in an aqueous media at room temperature promotes the production of thiocarbohydrazide to high yield. At slightly higher temperature 4 - amino - 3 - hydrazino - 5 - mercapto-1, 2, 4 - triazole[8] is formed as a by product.

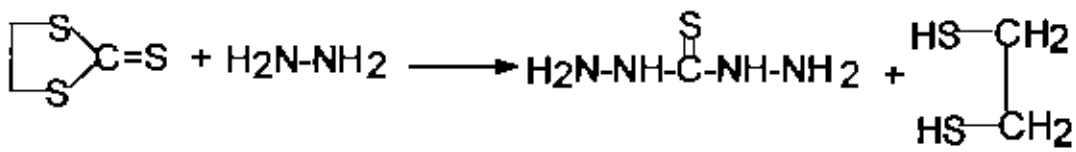
(4) Hydrazinolysis of methyl dithiocarbazinate

The hydrazinolysis of methyl dithiocarbazinate in boiling ethanol produces thiocarbohydrazide in 65% yield.[5]



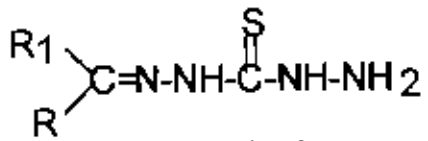
(5) Hydrazinolysis of dialkyl trithiocarbonates

Hydrazine and dialkyltrithiocarbonates presence of hanol to give thiocarbohydrazide in good yield with the elimination of alkanethiol[7]. Cyclictrithiocarbonates[9] are also used as a starting material for the preparation of thiocarbohydrazide. Treatment of ethylene trithiocarbonate with 2 moles of hydrazine hydrate in boiling ethanol yields pure thiocarbohydrazide in 75% yield.



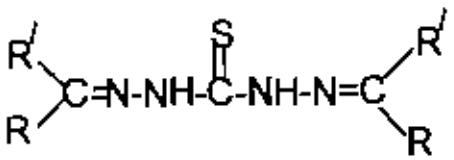
1.3 Condensation of thiocarbohydrazide with carbonyl compounds

Both hydrazine groups of thiocarbohydrazide display normal reactivity towards carbonyl compounds and give crystalline mono and dihydrazones. Generally, the mono addition products are formed very rapidly than diadducts. The mono adducts are obtained only under specially controlled condition.



Thiocarbohydrazone

11

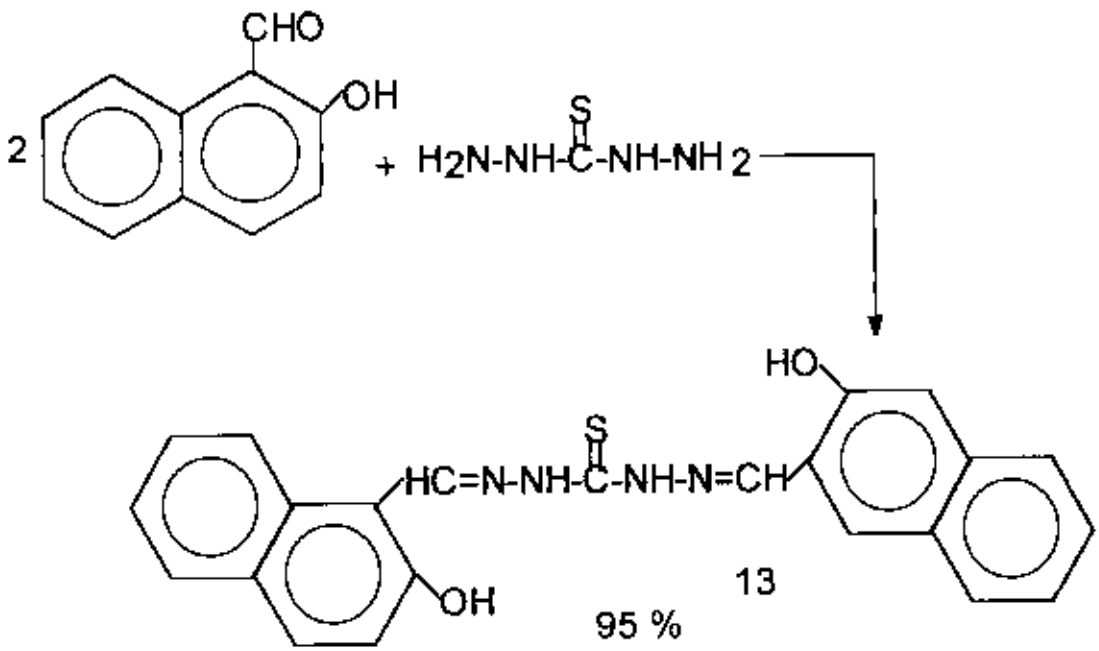


Dithiocarbohydrazone

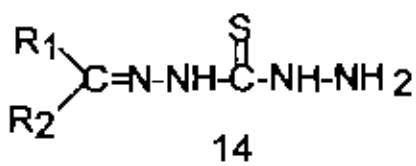
12

A. Mono and Dithiocarbohydrazone

Thiocarbohydrazide reacts readily with two molar equivalents of aldehydes and ketones to yield the corresponding 1,5-bis-thiocarbohydrazones.[4,10,11-15] These compounds are usually highly crystalline. In certain cases, there is a distinct difference in reactivity of the first and second hydrazine groups of thiocarbohydrazide towards carbonyl compounds. The hydrazones derived from acetone, acetophenone and dibenzylketone are formed only after prolonged boiling using an excess of ketone[12]. The dithiocarbohydrazone[16] has been prepared by condensing the aldehyde in two molar equivalents with thiocarbohydrazide in presence of concentrated hydrochloric acid.



An effective method for the preparation of mono thiocarbohydrazone is based on the Stolle's method and has been further developed by Sandstorm,[17] in which an excess amount of aldehydes or ketones in ethanol is treated with a solution of thiocarbohydrazide in 1N acetic acid and the products are separated quickly on cooling.



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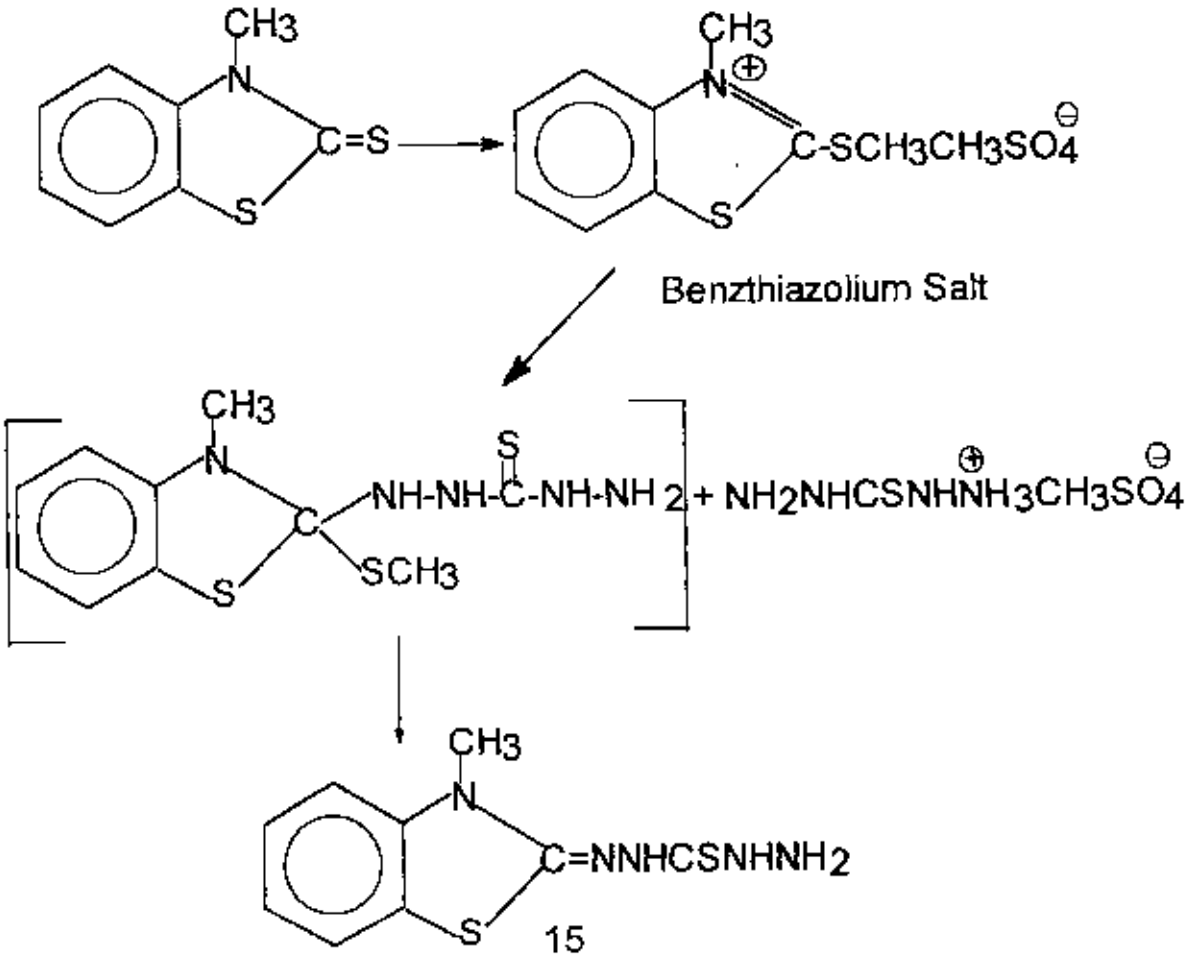
$R_1=R_2=CH_3$, $R_1=Ph$, $R_2=CH_3$, $R_1R_2=PhCH_2$

A novel route to monothiocarbohydrazone is the treatment of benzthiazolium salt (obtained by quarternization of the benzthiol) with thiocarbohydrazide[18] in aqueous solution at 80°C. Nucleophilic

displacement of the methylthiol group yields 1- (3-methylbenzthiazol-2-ylidene) thiocar-bohydrazide as shown in scheme 1.

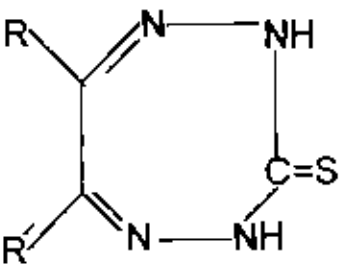


Scheme 1

Recently a suitable method for the mono thiocarbohydrazone has been developed by Duddeck *et al*[19]. In this method condensation of the carbonyl compound with the thiocarbohydrazide in unimolar ratio in presence of trace amount of concentrated hydrochloric acid yield the monothio-carbohydrazone in high yields (80-86 %).

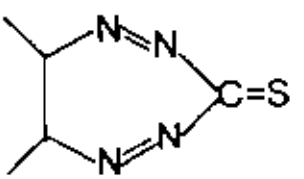
B. Condensation with ortho-diketones

De *et al*[20] frist reported the condensation of thiocarbohydrazide with cyclic ortho diketones. The condensation reaction may be subdivided into two groups. First one is initiated by the formation of mono and the other is 1, 5-dihydrazones. Thus, thiocarbohydrazide reacts with one molar equivalent of benzil, acenapthaquinone, camphorquinone or alloxane in acetic acid to afford the products formulated as seven membered rings (**A**).



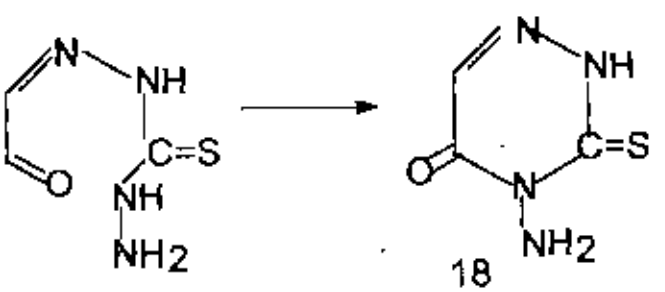
A 16

There were no adequate evidence in favour of the suggested structure. The products were insoluble in base. The absence of thioureido group (-NHCSNH-) in A indicated structure B to be better agreement.



B 17

The general tendency of thiocarbohydrazide is to yield *N*-amino compounds in ring closures e.g., the condensation with α-ketocarboxylic acids afford triazines.



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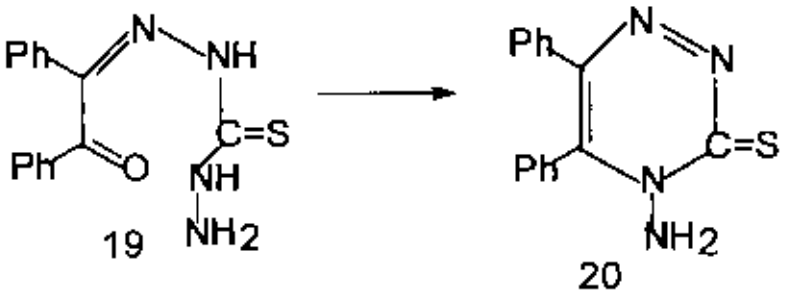
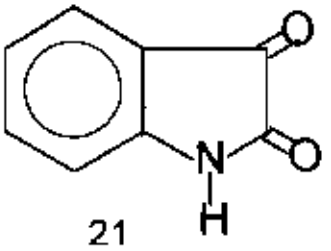
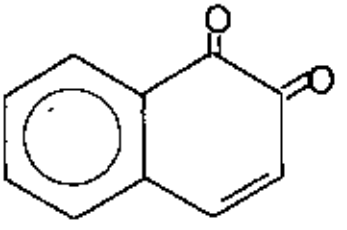
It would seem feasible that the condensation with α-diketones proceeds in fact analogously, yielding the alkali insoluble triazines by the way of forming intermediates shown below:



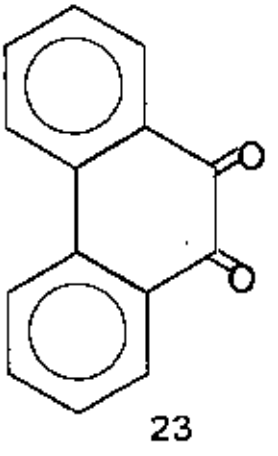
In the second group of reactions, thiocarbohydrazide condenses with an excess of the orthodiketones such as isatin, β-napthaquinone and phenanthraquinone to produce dihydrazones[20].



Isatin

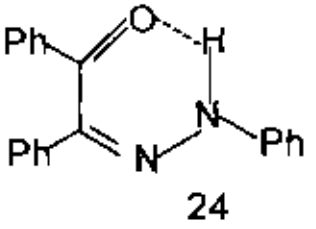


β-napthaquinone

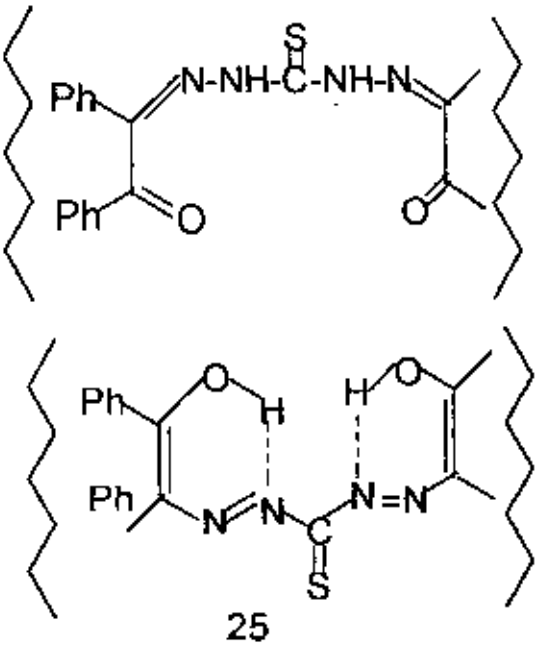


Phenanthraquinone

Furthermore, benzil mono phenyl hydrazone shows intensive resistance to acetylation, even under drastic conditions. This observation again suggests the existence of hydrogen bond iu the structure of the type as



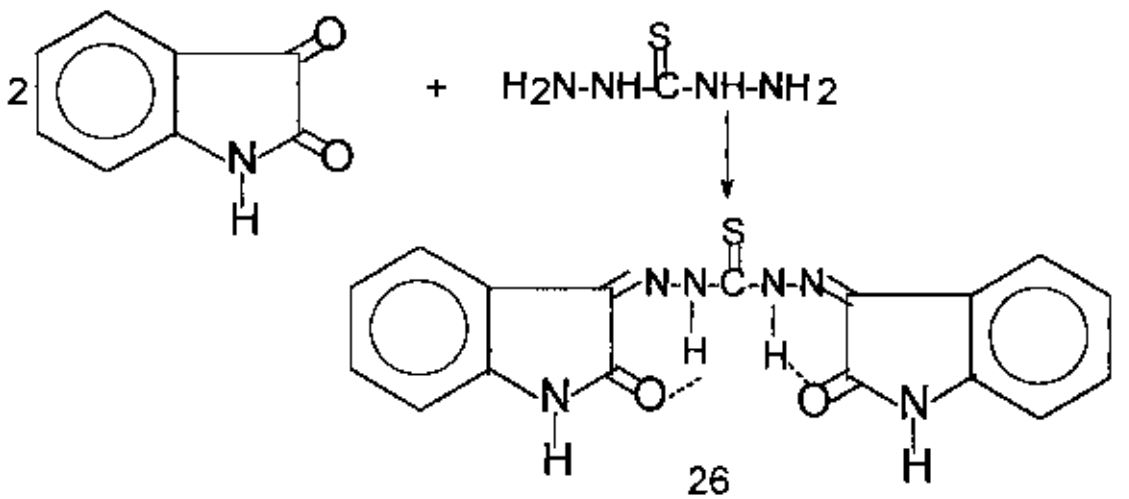
In the light of this evidence the hydrazones could be written as,



25

These structures can be accounted due to the insolubility in alkali for the chelation of their hydroxyl groups.

Isatin may show exceptional behaviour in this respect since only its 3-carbonyl group reacts normally to form hydrazones, the other being an amide function does not react[21]. Thus, isatin dihydrazones may be represented as the following with "reverse" hydrogen bonding.

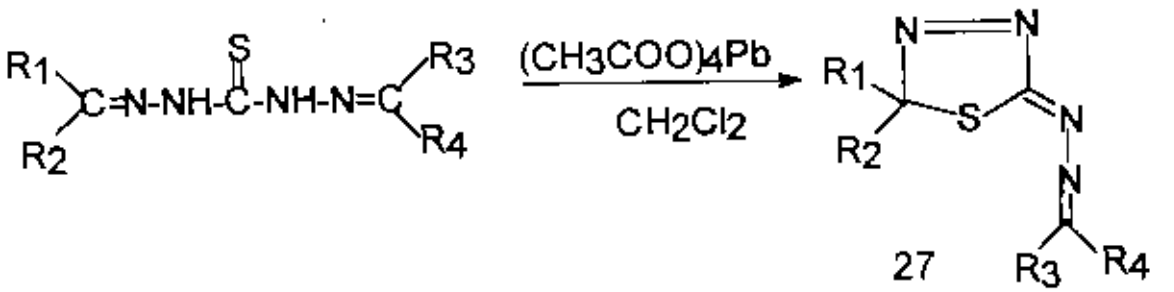


26

But no structural proof of this compound is available. Other reactions of diketones with thiocarbohydrazide are also reported[20].

1.4 Oxidative cyclization of thiocarbohydrazone

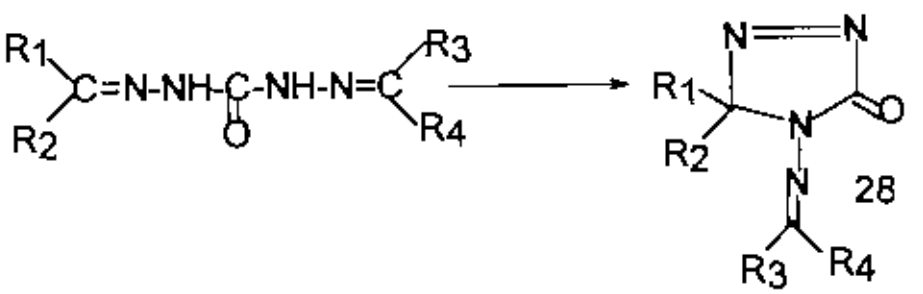
Thiocarbohydrazooc can be easily cyclized by different means to heterocyclic compounds. Dithiocarbohydrazone derived from ketones are oxidatively cyclized by lead tetraacetate in methylene chloride to 2-alkyl- (or aryl) dienehydrazone-5, 5- dialkyl (or aryl) Δ^3 - 1, 3, 4-oxa(thia) diazolines in moderate to good yields[22].



27

The alkyl compounds are formed rapidly at 0°C but the aryl analogs are produced more slowly and in poor yields, probably due to both steric and polar efects[23].

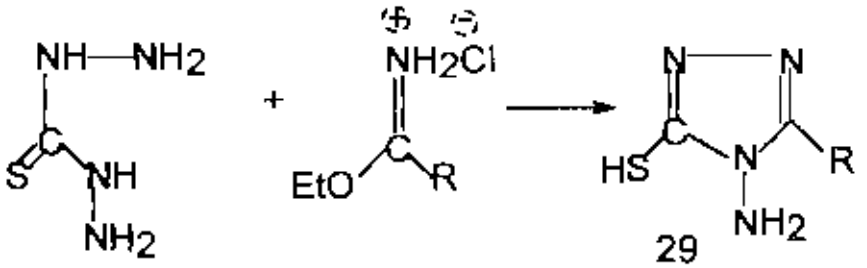
A provisional report by the same authors had formulated the oxidation products of diketocarbohydrazones as 4-ketimino-Δ^1-1,2,4-triazoline-3-ones.



28

1.5 Heterocyclic compounds derived from thiocarbohydrazide

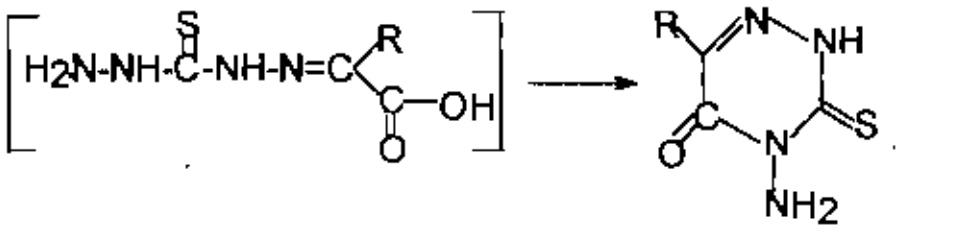
The condensation of thiocarbohydrazide with iminoethers appears to have been reported as only one instance in the patent literature[24]. A boiling suspension of thiocarbohydrazide and iminoethers affords heptadecylimino-3 - hepta - decyl - 5 - mercapto - 1, 2, 4 - triazole in moderate yield.



29

$R=C_{17}H_{35}$

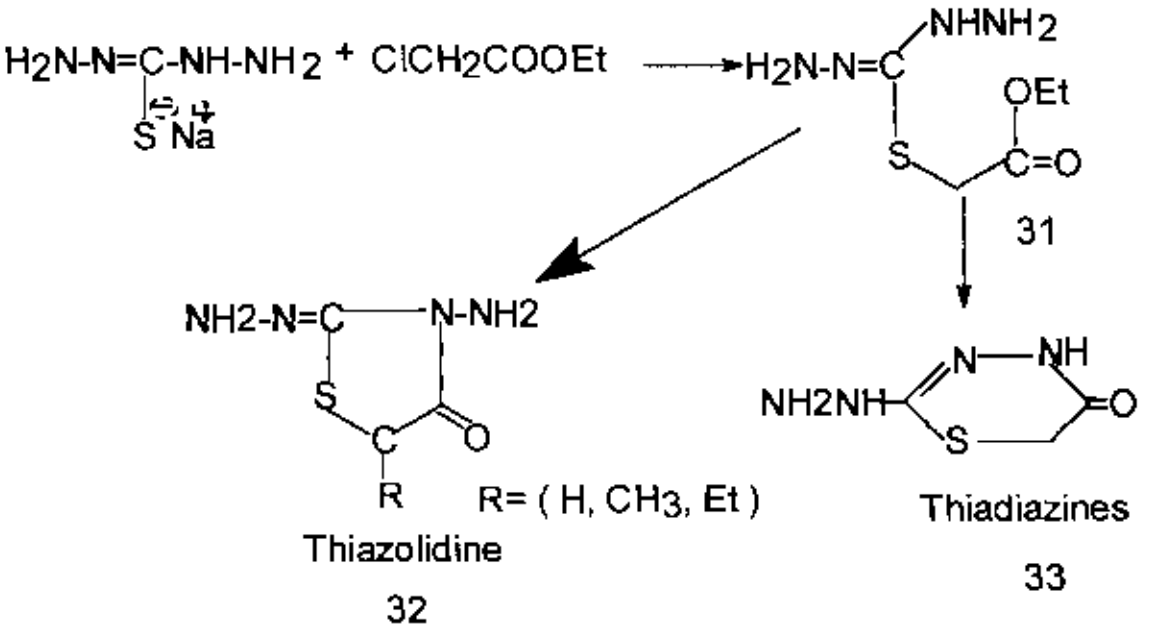
The condensation of thiocarbohydrazide with α-ketocarboxylic acids differs markedly from that of carboxylic acids in yielding 4-amino-1, 2, 4-triazines[25-26] instead of 4-amino-1, 2, 4-triazol.



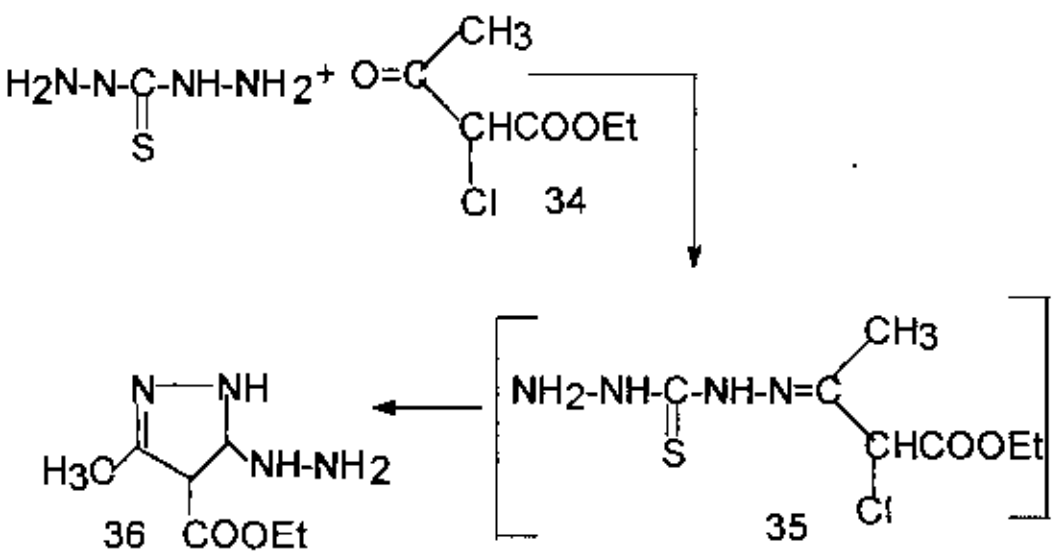
4-amino-1, 2, 4-triazine

30

The interaction of this carbohydrazide and α-halocarboxylic ester in alkaline media gives heterocyclic products that have been formulated as thiadiazines by Gnha *et al*[27] bnt as thiazolidine derivatives by Stephen *et al*[28] in 1928 .



The initial stage of the interaction of thiocarbohydrazide with α-chloro-β-keto esters appears to be formation of hydrazones to 5-hydrazinopyrazoles[29] via cyclization and loss of sulphur atom.



Thiocarbohydrazide reacts with dimethyl trithiocarbonate in alkaline solution to form 1-dithiomethoxycarbonyl as a crystalline solid in high yield which is readily cyclized in ethanolic hydrochloric acid[30] to mercapto-5-methyl-thio-1, 3, 4 - thiadiazoline in high yield.

1 . 6 Importance of thiocarbohydrazide

Thiocarbohydrazide is useful in analytical chemistry for the identification and estimation of both organic and inorganic compounds[31-35]. Thiocarbohydrazide precipitates aldehydes and ketones, quantitatively giving derivatives having sharp melting point which are suitable for the identification purposes and in gravimetric analysis.

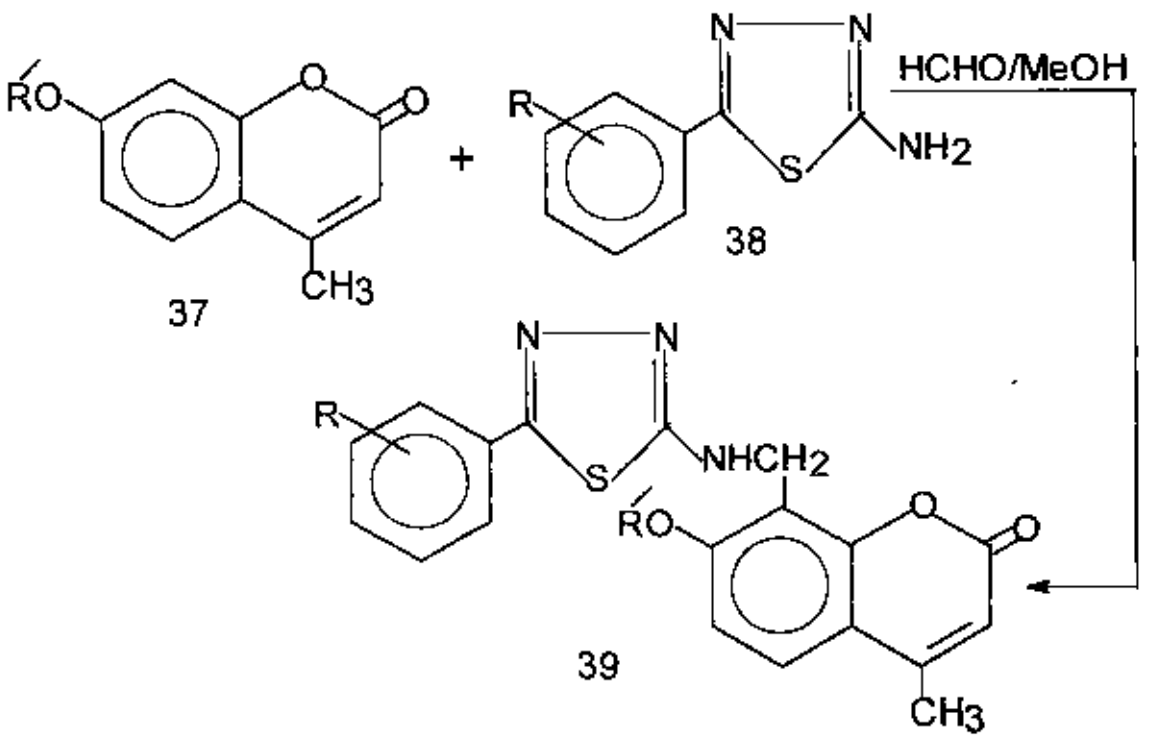
Thiocarbohydrazides are useful as an osmiophilic reageuts for demonstrating the presence of aldehyde-contaiuing macromolecules, originating from iodic acid oxidation of tissue and of lipid containing membranes in osmium tetraoxide fixed tissue[34-35]. Thiocarbohydrazide causes the irreversible inhibition of purified recrystallized catalase preparation (OX liver) in the presence of hydrogen peroxide[36]. The degradation of serotonin by ceruloplasmin is severely inhibited by thiocarbohydrazide ***in vitro***[37]. Thiocarbohydrazides are carbonyl trapping agents, diamine oxidase inhibitor and inhibitors of enzyme systems and produce convulsious after a latent period of one hour[38-40]. The convulsant activity of thiocarbohydrazide at a single unit level has been assayed using the giant neurons of ***Aplysia Californica*** under carefully controlled conditions[41].

Thiocarbohydrazide is active ***in vitro*** against tubercle bacilli(strain $H_{37}RV$, concentration 10^{-5}), against ***Micrococcus Pyogenes*** var aureus (1mg/ml, corresponding to 1.02 μg / ml of penicillin) and against ***Escherichia Coli*** (1mg / ml, corresponding to 1.02 μg / ml of Chloramphenicol)[42]. However, its tuberculastatic activity is not applicable ***in vivo***. Activity is also exhibited ***in vitro*** against ***mycrobacterium tuberculosis*** (BCG strain)[17]. Thiocarbohydrazide exhibits toxicity behaviour towards the house-fly comparable to that of DDT[43].

1.7 Heterocyclic compounds Containing Nitrogen and Sulphur and their Importances

The synthesis of heterocyclic compounds containing sulphur and nitrogen and their Pharmacological studies have been carrying out for the last two decades and at present the chemists pharmacists and microbiologists have diverted their attention towards the biological applications.

Giri *et al*[44] synthesized 8 - [(5' - aryl-1', 3', 4'-thiadiazol-2'-yl)aminomethyl] -7-hydroxy/acetoxy-4-methylcoumarins from the Mannich reaction on 7-hydroxy/acetoxy - 4 - methylcoumarin (37) with 2- amino - 5 - aryl -1, 3, 4 -thiadiazols (**38**).



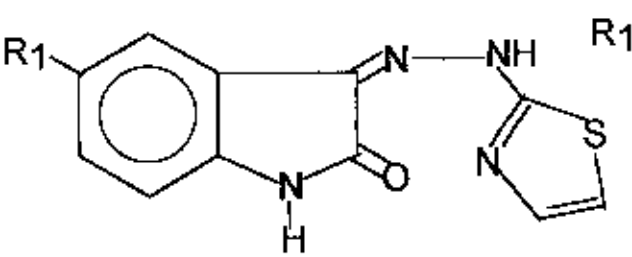
The antifungal activity of compounds of the type (39) have been evaluated against ***Aspergillus niger*** and ***Helminthosporium oryzae*** at three different

concentrations (1000, 100 and 10 ppm) by agar growth technique. The number of replication in each case was three. The plates have been in cubated at room temperature for 7 days. No remarkable morphological change was observed in the developing fungi. A commercial fungicide Dithane M - 45 have also been tested under similar conditions for comparison. The percentage inhibition of the mycelial growth or spore germination have been calculated by the following equation

$$\% \text{ Inhibition} = \frac{(C-T)100}{C}$$

where C and T are the average diameters (in mm) of the fungal colony in control and in treated plates respectively. The fungicidal data of the tested compounds indicated that they were moderately to fairly active at 1000 ppm concentration and their activity decreases with dilution. The compounds containing a chloro group have greater toxicity probably due to lipophilic character of the chloro group which in turn favours its permeation through the lipoid layer of the fungal membrane.

Dahiya ***et al***[45] reported the synthesis of thiazolotriazinoindoline type compounds (**40, 41**). These compounds have beeo tested for their antifungal and antibacterial activities.



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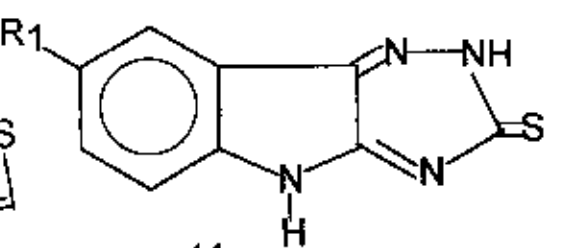
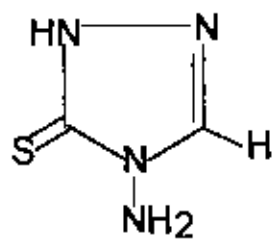


41

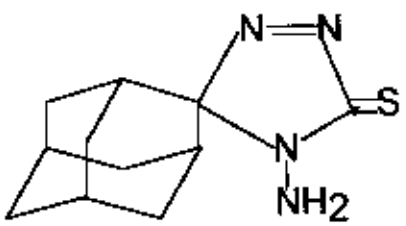
$R_1 = OCH_3, Cl$

For antifungal assay, the method of Aytoun *et al*[46] was employed using ***Aspergillus flavus, Fusarium oxysporum*** and ***Sclerotium oryzae*** as test fungi. For antibacterial activity they employed the Rideal-Walker[47] serial drop dilution method. The test organisms were 24 hrs old cultures of ***Staphylococcus aureus*** and ***Escherichia coli.*** None of the compounds screened was found to posses activity against both the bacteria at 1:500 dilution.

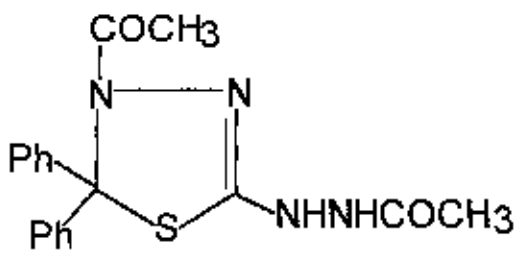
Saha *et al.*[48] reported an improved synthesis of this carbohydrazide and some ketones monothiocarbohydrazones with high yields. Some triazines (**42-43**) and thiadiazoline derivatives (**44** - **45**) have been prepared by various cyclizing agents.



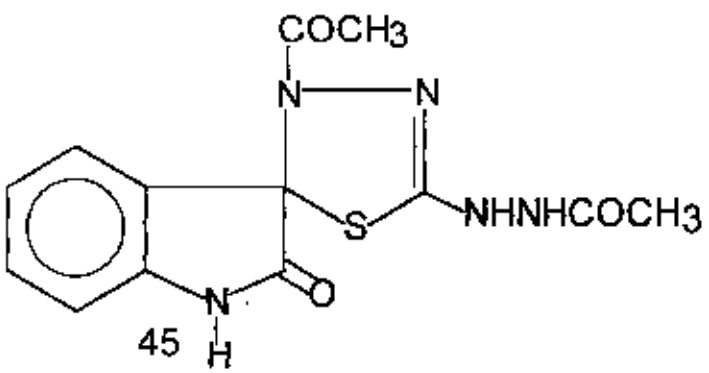
42



43



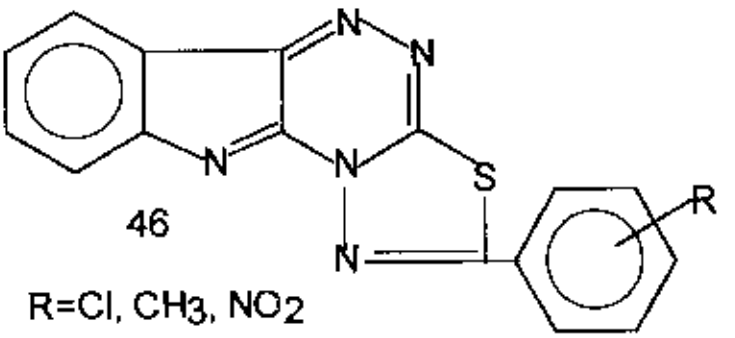
44



45

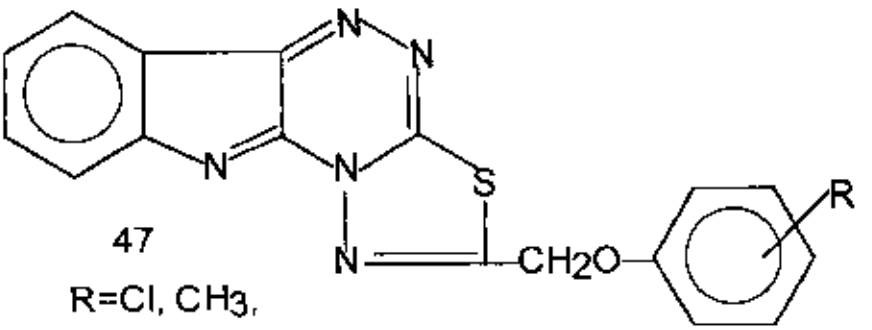
The pharmacological results have been described as antimicrobial activities of the compounds and are quantitatively determined against different microorganism by the zone inhibition technique. All the compounds showed antibacterial activities against ***Shigella sonnei***, ***Shigella shiga***, ***Shigella Boydii***, ***Staphylococcus aureus***, ***Pseudomonas aesuginosa***. For pharmacological studies, male mice CFW (Swiss Wester) BR were used and a control group of six mice were injected intraperitoneally with 0.9% normal saline, compounds were administered as a suspension with 0.9% normal saline with volume of 0.01 ml / gm body weight. Locomotion tests for spontaneous motor activity and amphetamine induced hyperactivity were done. The effects of the compounds on pentobarbitone sleeping time, body temperature, neurotoxicity, antinociceptive activity and metabolic changes were also induced. The results reveal that the compounds reduces the spontaneous motor activity and the same trend was also obtained in the locomotion test for amphetamine induced hyperactivity where the compound antagonized the effect of amphetamine. It significantly reduces the time taken for initiation of sleep and increases sleeping time. These results showed the characteristic similarity to a compound with classical central nervous system depressant activity, especially like serotonin with the neuronal mechanisms responsible for controlling sleep. The compound is devoid of any neurotoxicity at a dose of 20 mg / kg body weight which brings an extra plus point in the pharmacological findings.

Some 2 - substituted- 1, 3, 4-thiadiazolo[2, 3-C]-1, 2, 4-triazino [5, 6-b] indoles (**46, 47, 48**) have been synthesized by Tiwari *et al.*[49] via cyclization of isatin - 3 - (5 - substituted - 1, 3, 4 - thiadiazol - 2 - yl) hydrazones with concentrated H_2SO_4. All the compounds have been assayed for their fungicidal activity as described.



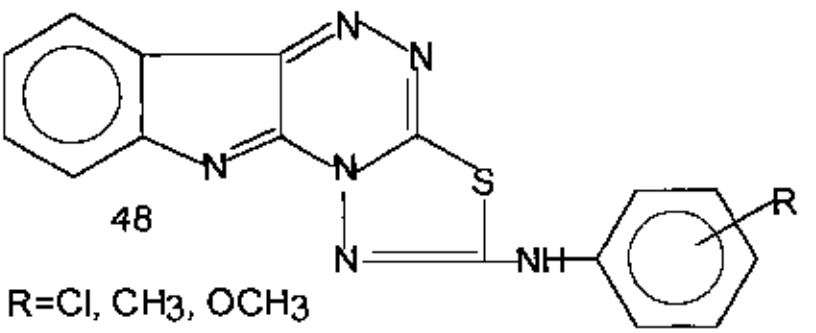
46

R=Cl, CH_3, NO_2



47

R=Cl, CH_3,



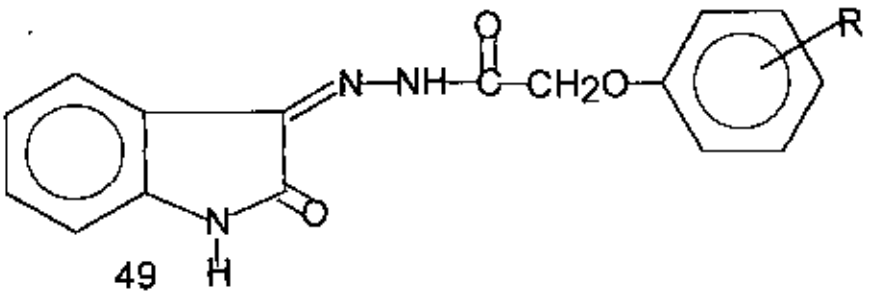
48

R=Cl, CH_3, OCH_3

The compounds were screened for their antifungal activity against ***Pyricularia oryzae***, ***Rhizoctoria Solanai***, ***Pseudoperonospora Cubensis*** and ***Phytophthora infestans*** at 1000 ppm, 100 ppm and 10 ppm concentrations respectively. The results were tested and compared with commercial fungicide carbendazim under similar condition.

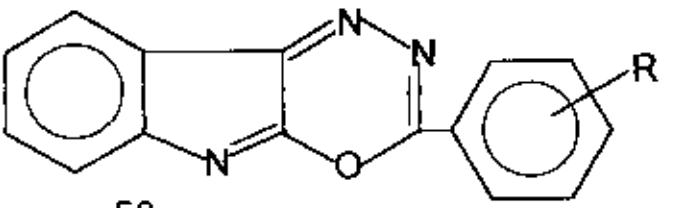
Tiwari ***et al.***[50] also reported the pesticidal interest on fused heterocycles and guided by the observation that sometimes the fusion of two or more heterocyclic nuclei enhances the biological profile many-fold than its parent nuclei. They synthesized the compounds bearing 1, 3, 4–thiadiazole, 1, 2, 4–triazine and indole moieties in a single molecular framework and evaluated their fungicidal activity.

Bharati ***et al.***[51] reported the synthesis and biological activities of novel heterocycles such as [1, 3, 4] oxadiazoloquinazolones[52], [1, 2, 4] - triazolo thiazoles [1, 3, 4] - thiadiazoloquinazolones[53], 1, 3, 4-thiadiazolotriazines [1, 2, 4] triazoloquinazolones and [1, 3, 4] oxadiazolyl-2-azetidenones[54]. They also reported the convenient synthesis of fused heterocyclic system [1, 3, 4] oxadiazino [5, 6, b] indoles and a novel spiro heterocyclic, spiro - [3H-indole - 3, 2'- thiazolidine] - 2, 2' (1H) - diones. Furthermore several 1' - (substituted aryloxyactamido) - spiro [3H-indole-3, 2' - thiazolidine] - 3, 2' (IH) - diones (**49**) and 2-aryl - [1, 3, 4] oxadiazino [5, 6, b] indoles (**50**) have beeo synthesized and screened for their antifungal activity against ***Helminthosporium oryzae*** and ***Aspergellus flavus*** (**51**).



49

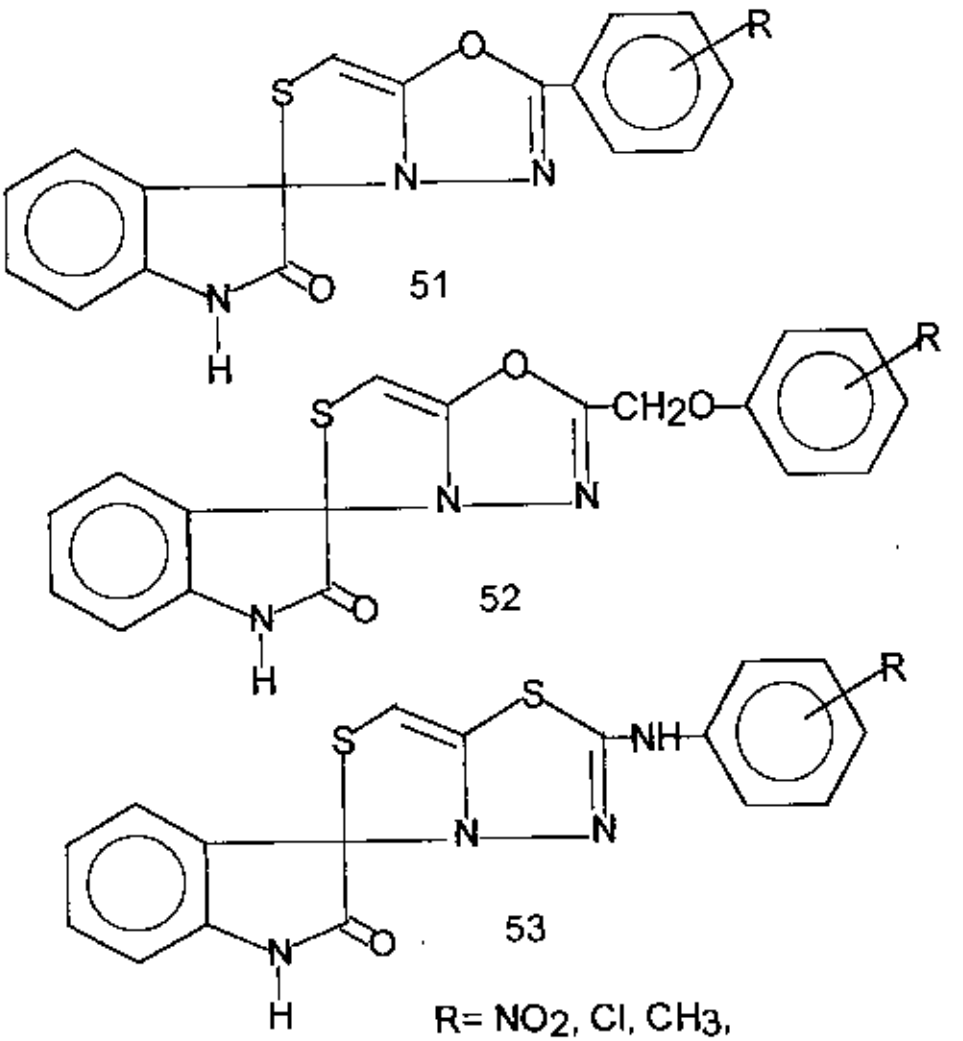
R= H, - Cl, - CH_3, - OCH_3



50

The key compounds isatin-β-(aryloxyacetylhydrazones) and isatin-β-(arylhydrazones) were prepared by condensation of isatin with aryloxyacetylhydrazines and arylhydrazines respectively.

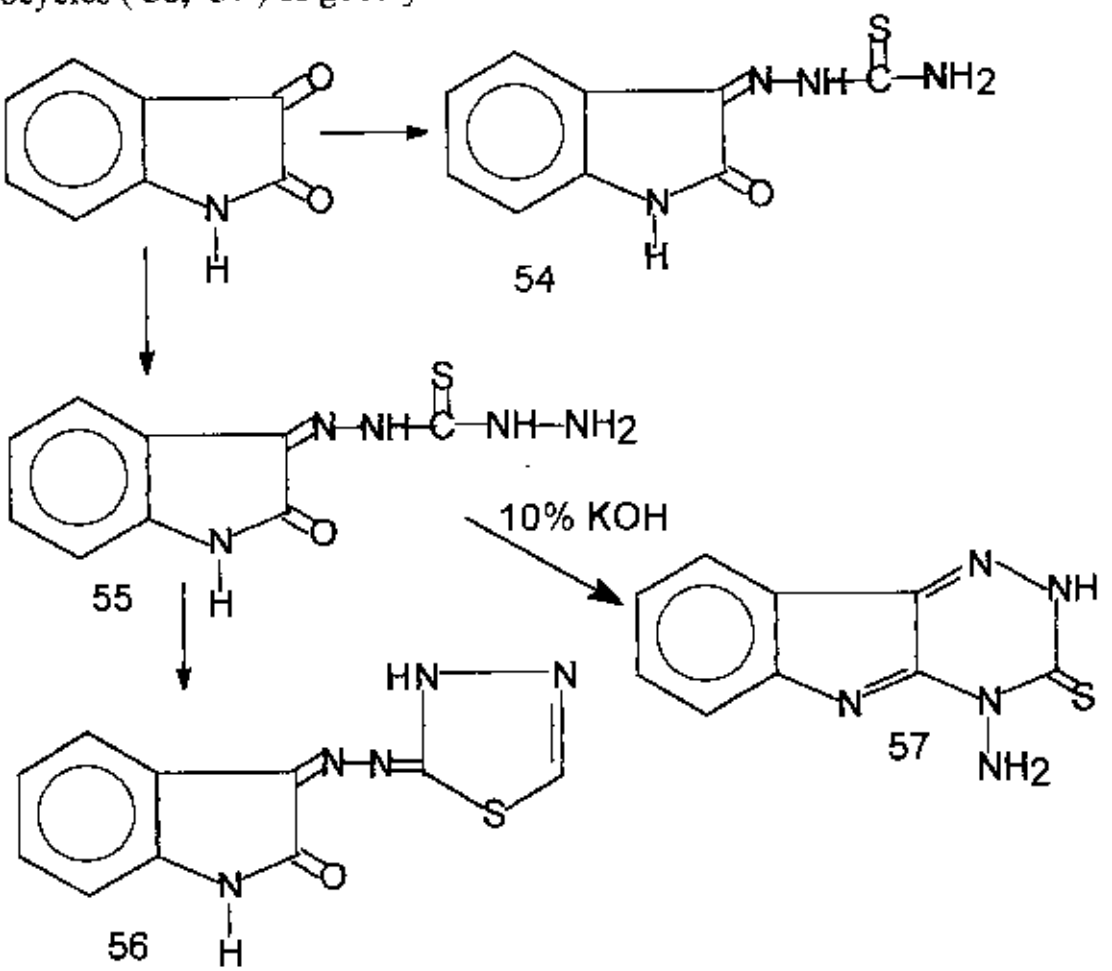
A simple and convenient synthesis of 2′ – substituted spiro-indoline-3′, 5′– [5H] [1, 3, 4] oxa/thiadiazolo [3, 2-C] thiazol-2 ones (**51, 52** and **53**) have been reported by Kumud *et al.*[55]. In continuation of their work on fused heterocycles[53,56-57] and in view of the activities exhibited by 1, 3, 4 - oxadiazole, 1, 3 - thiazole and indole rings, they thought of interest to fuse these rings together with the hope of getting compounds with improved biological activities. They have developed an efficient method of synthesis (**51, 52**) and (**53**) with a view to evaluating them for their biological activities.



Condensation of arylhydrazides / aryloxyacetohydrazides and thiosemicarba -zides with isatin in methanol furnished the corresponding hydrazones which were converted into spiro compounds with mercaptoacetic acid. The cyclodehydration of the spiro compounds with concentrated H_2SO_4 furnished the compounds (**51, 52**) and (**53**).

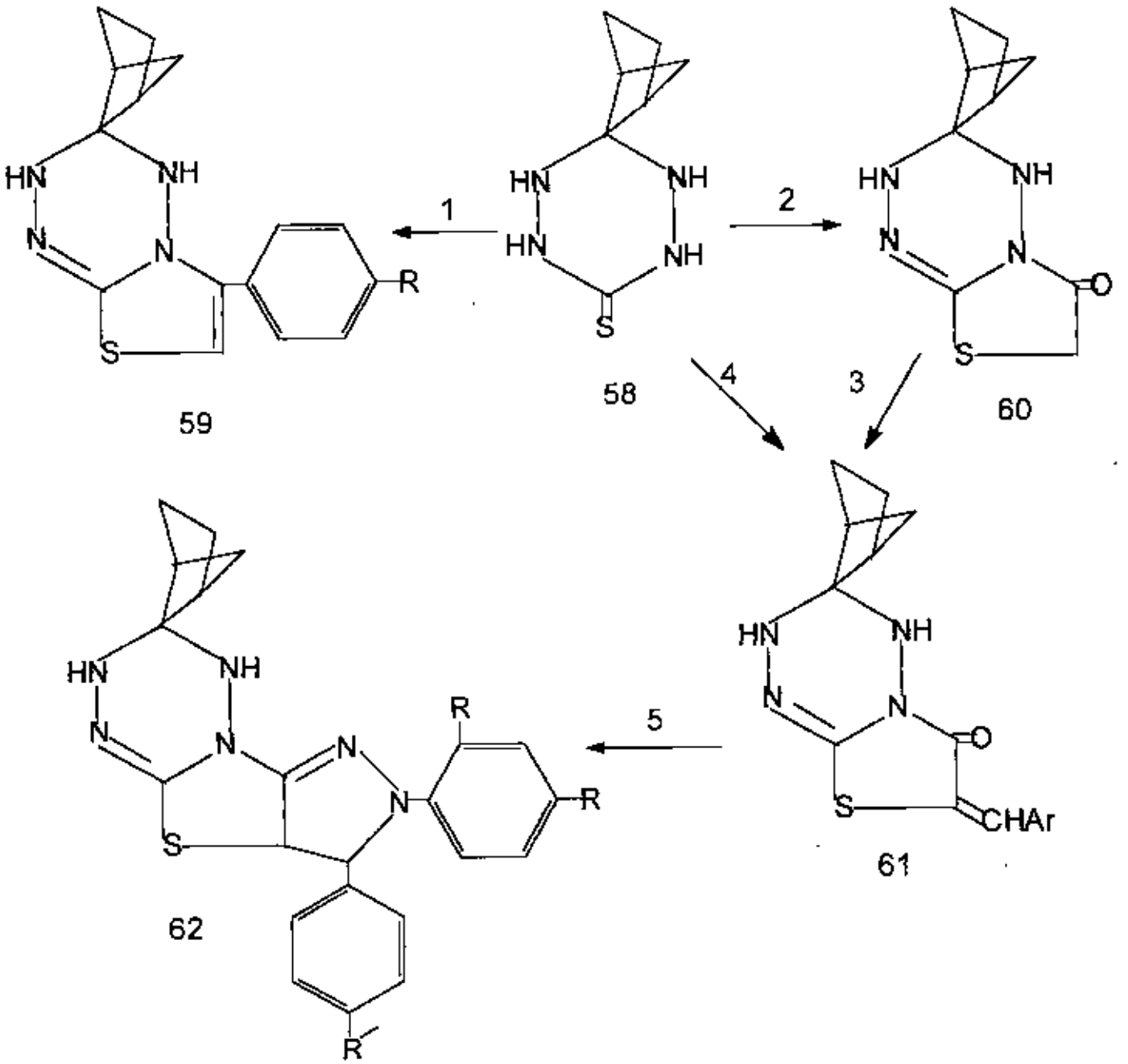
Compounds (**51, 52**) and (**53**) were screened for their fungicidal activity against ***Aspergillus niger, Helminthosporium oryzae*** and ***Aspergillus flavus*** at 1000, 100 and 10 ppm concentrations. Carbendazim, a commercial fungicide, was also tested for comparison. It was also notable that these compounds have – CH_3 group and Chloro function on the phenyl ring which probably enhances the fungitoxicity.

Laila ***et al.***[58] reported their systematic study on thiosemicarbazones and thiocarbohydrazones and on its antibacterial properties. They extended their study on isatin series and synthesized isatin thiosemicarbazone and thiocarbohydrazone (**54, 55**). Cyclization of (**54, 55**) led to the heterocycles (**56, 57**) of good yields.



The antibacterial activity of (**54**) and (**55**) were measured against Mycobacterium tuberculosis in Lowenstein-Jensen media at concentrations of 300, 200 and 100 μg/ml. Compound (**56**) was found to be 100% sensitive to the microorganisms whereas (**54**) was 50% sensitive.

The synthesis of spiro [bicyclo [2, 2, 1] heptane - 2, 3′ (4′ H)-[2 H] - thiazolo [3, 2-b] -s- tetrazin] - 6′ (7′ H) -one (**60**) and 6′ aryl spiro [bicyclo [2, 2, 1] heptane - 2′, 3′- (4′ H) - [2H]-thiazolo [3, 2-b]-s-tetrazines] (**59**) have been accomplished from the reaction of 1′, 2′, 4′, 5′-tetrahydro-spiro [bicyclo (2, 2, 1) heptane - 2, 3′ - s - tetrazin] - 6′- thione (**58**) with chloroacetic acid and α-haloketones respectively by Mohan *et al.*[59]. 7′-Arylidenespiro [bicyclo [2, 2, 1] heptane - 2′, 3′- (4′ H) - [2H] - thiazolo [3, 2- b] - s - tetrazines] - 6′ (7′ H) - ones (**61**) have been prepared either by the condensation of (**60**) with aldehydes or in a single step by the reaction of (**58**) with ethylchloroacetate and aldehydes in the presence of pyridine and piperidine. Condensation of (**61**) with phenyl hydrazine in the presence of anhydrous sodium acetate furnishes 7′, 8′-diaryl-trans - 8′, 8′a - dihydrospiro [bicyclo [2, 2, 1] heptane - 2, 3′ (4′ H) pyrazolo (3′, 4′, 4, 5) thiazolo [3, 2-b]-s-tetrazin] (**62**).



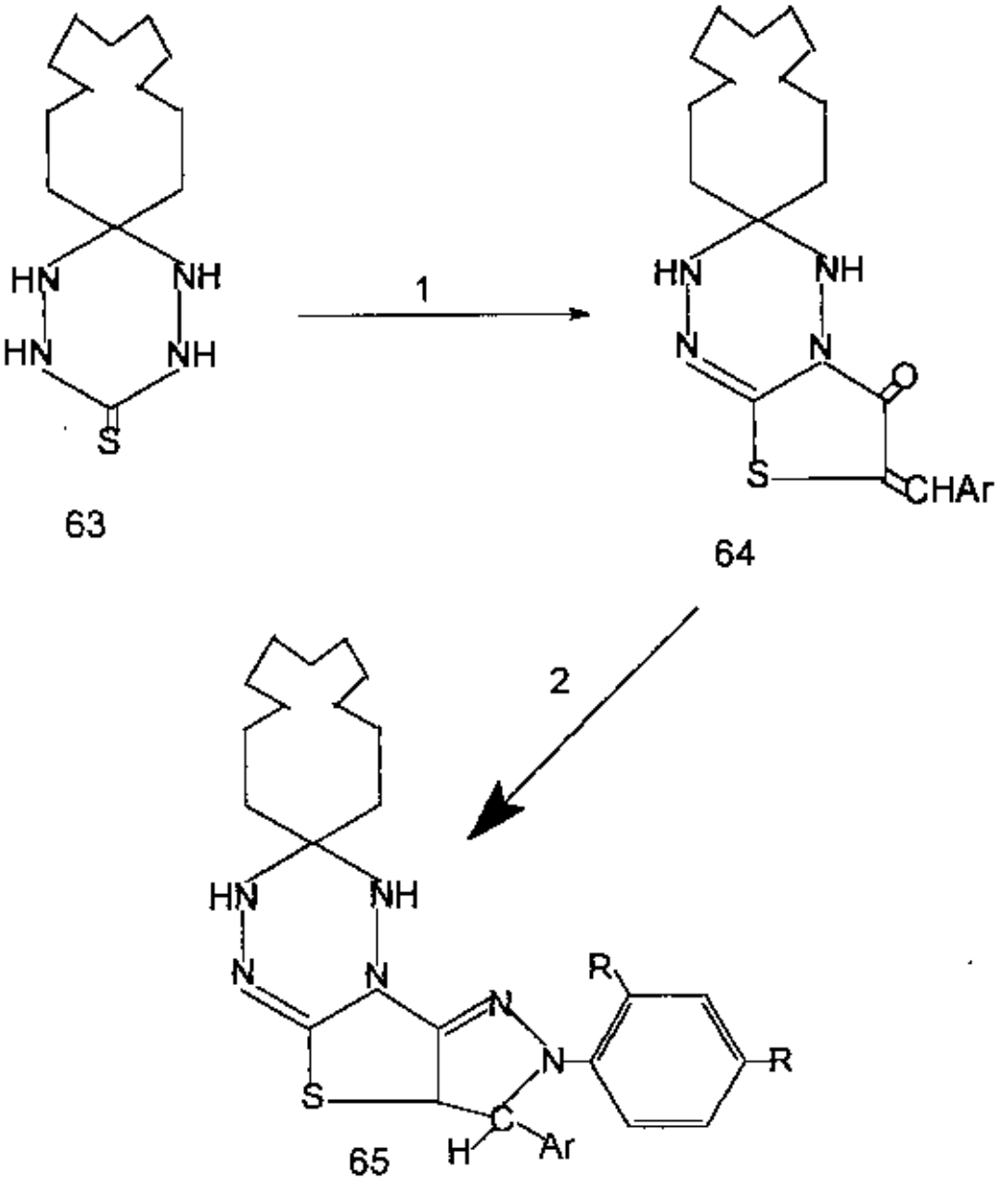
a, R=H ; b, R=NO_2

1. $RCOCH_2Br$, 2. ClCH2COOH, NaOAc, 3. ArCHO, NaOAc,
4. $ClCH_2COOEt$, Pyridine, ArCHO, Piperidine, 5. $ArNHNH_2$

The compounds (**60, 61a,** Ar = p- ClPh), (**61b** Ar = m-NO_2Ph-), (**62a,** R'Cl ; R=H), (**62b,** R'- OCH_3 ; R = NO_2), (**59a** R-Br) and (**59b,** R-Cl) have been evaluated as ***in vitro*** antimicrobial activity against the gram positive ***Staphylococcus aureus***, gram negative ***Escherichia Coli and Pseudomonas aeruginosa*** and the fungus ***Candida albicans*** using neat samples and serial plate dilution method[60]. The minimum inhibitory concentration (MIC) values of the compounds (**60, 61a** and **61b**) against ***Candida albicans*** were found to be 500 and 250 µg/ml respectively, where as the MIC values for (**60, 61a** and **62a**) against ***E. Coli*** and ***Candida albicans*** were found to be 250 and 500 µg/ml respectively. The compounds **61a** and **61b** showed active against ***C. albicans*** and ***E. Coli*** when tested as neat samples. The author also reported[61-63] the synthesis and biological activity on the bridge head nitrogen heterocyclic system.

Jag Mohan ***et al.***[64] reported the synthesis of a novel bridgehead nitrogen spiro heterocyclic system derived from 13, 14, 16, 17-tetraazaspiro (5, 11) heptadecane-15-thione (**63**) and the biological activity associated with the derivatives of this system. The required 13, 14, 16, 17-tetraazaspiro [5, 11]-heptadecane-15-thione (**63**) have been synthesized by the reaction of cyclododecanone with thiocarbohydrazide following the method of Lamon[65]. Treatment of (**63**) with ethylchloroacetate and aldehydes in the presence of pyridine and piperidine afforded 7'-arylidene-6' (7' H)-Oxospiro [cyclo-dodecane-1, 3' (4 H)- [2 H]- thiazolo [3, 2 - b] - s - tetrazines] (**64**). The structures (**64**) were supported by the appearance of a band at 1690-1710 cm^{-1} in their IR spectra. Condensation of (**64**) with arylhydrazines yielded in one step cyclized products 3', 3'a-trans-dihydro-2',3'-diarylspiro

[cyclododecane-1′, 7′ (8′ H)-[6 H]-pyrazolo [3′, 4′, 4, 5] thiazolo [3, 2-b]-s-tetrazin] (**65**).



63

64

65

a, R=NO_2 ; b, R=H

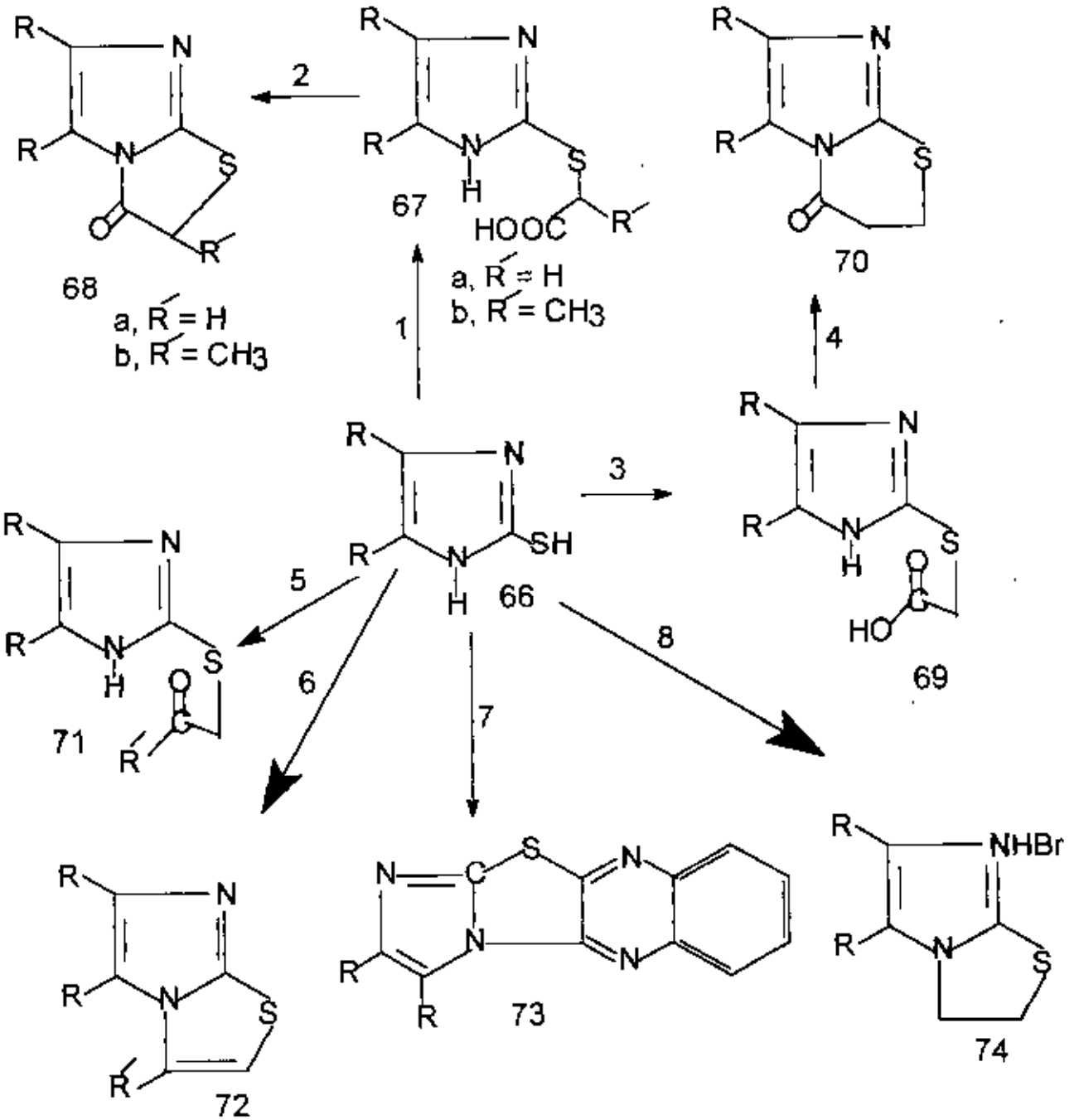
1. $ClCH_2COOEt$, Pyridine, ArCHO, Piperidine

2. $ArNHNH_2$

The compounds (**65a**, Ar = *P*-Cl Ph) and (**65b** *P* - $OCH_3C_6H_4$) have been evaluated for their antimicrobial activity against the gram positive ***Staphylococcus aureus***, gram negative ***Escherichre Coli*** and ***Pseudomonas aeruginosa*** and the fungus ***Candida albicans***. Neat samples and serial plate dilution method were used. The minimum inhibitory concentrations (MIC) of compounds (**65a**), (**65b**) against ***E. Coli*** were found to be 62.5 and 125 µg/ml respectively. These compounds (**65a** and **65b**) were found to be active against ***C. albicans*** when tested as neat samples.

Mohn *et al.*[66] reported the synthesis of 5, 6-di-p-(tolyl)imidazol [2, 1-b] thiazol-3 (2H)-one (**68a**), its 2-methyl analogue (**68b**), 7H-2, 3-bis-p-(tolyl) imidazo [2, 1-b] [1, 3] thiazin-5 (6 H)-one (**70**) and 3-substituted –5, 6-di-(p - tolyl) imidazo [2, 1-b] thiazoles (**72**) in two steps; whereas 3-methyl - 5, 6 - di - p [tolyl] imidazo [2, 1 - b] thiazole hydrochloride (**72c**), 2, 3-di - p - (tolyl) imidazo [2′, 1; 2, 3] thiazole [4, 5 - b] quinoxaline (**73**) and 2, 3-dihydro 5, 6-di - (p - tolyl) inidazo [2, 1- b] thiazole hydrobromide 5, 6 - di - p - (tolyl) imidazo [2, 1- b] thiazole hydrobronide (**74**) in one step only starting from 2 - mercapto - 4, 5 - 6 - di - (p - tolyl) imidazol (**66**). Compounds (**68a, 68b, 72** R′= $PClC_6H_4$-) (**73**) and (**74**) have been evaluated for their antibacterial activity against ***Staphylococcus aureus***, ***Escherichia Coli*** and ***Pseudomonas aeruginosa*** and antifungal activity against ***Candida albicans*** by serial plate dilution method[60].

The minimum inhibitory concentration (MIC) of the compound (**74**) against ***Staphylococcus aureus*** and ***Candida albicans*** were found to be 250 µg/ml whereas against ***Pseudomonas aeruginosa*** it was 1000 µg/ml.

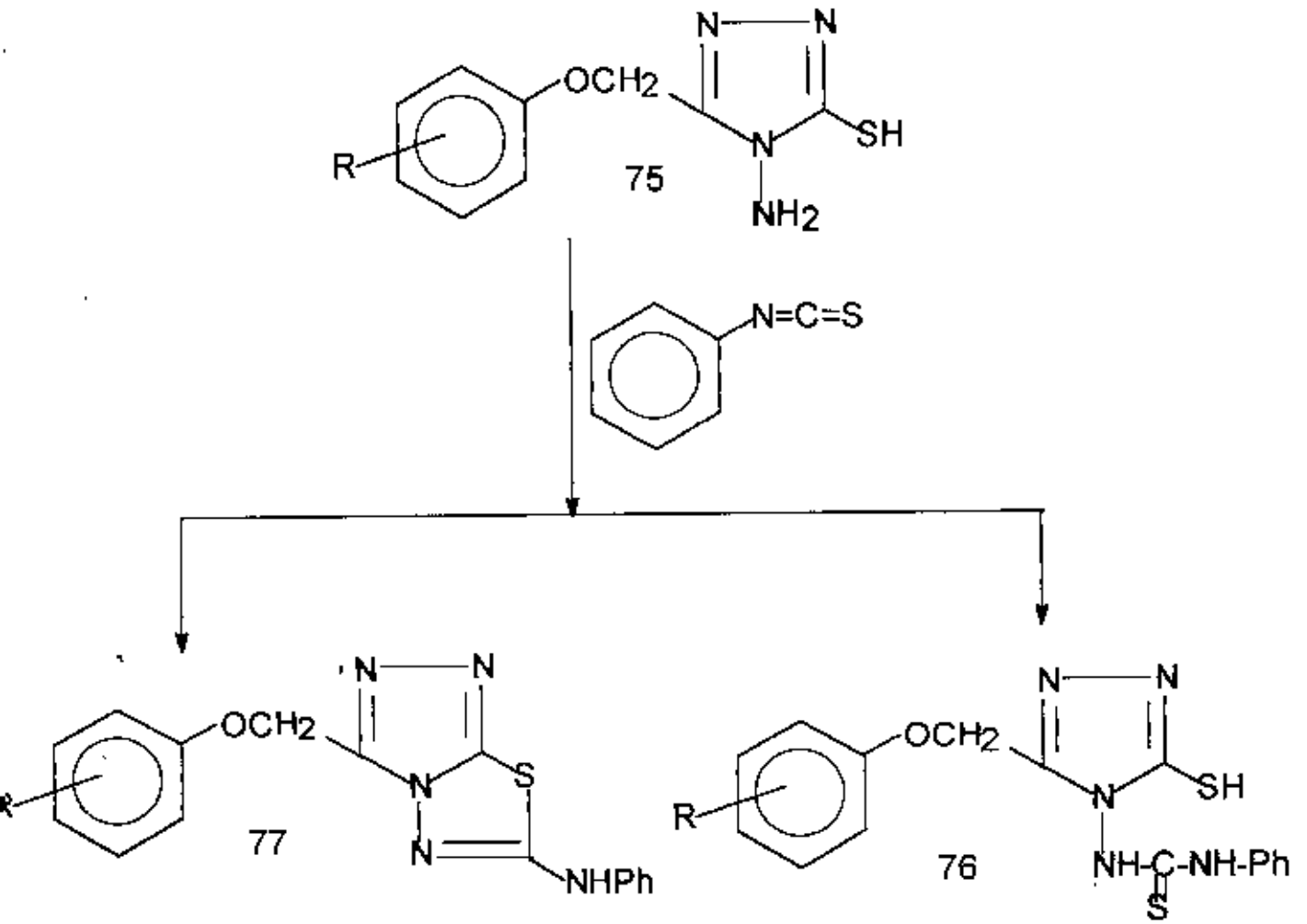


(1) R-CHX-COOH, NaOAc; (2 & 4) Ac_2O, Pyridine;
(3) $BrCH_2CH_2$-COOH, NaOAc; (5) RCH_2COBr, K_2CO_3;
(6) PPA; (7) 2, 3-dithioquinoxaline, NaOAc; (8) $BrCH_2CH_2Br$

The MIC of the compound (**73**) may be > 1000 μg/ml and showed activity against ***Candida albicans*** , when tested as neat sample and may be used for local application in form of powder or ointment provided further studies indicate absence of toxicity following local application.

Mishra ***et al***[67] reported the synthesis of 6 - arylamino - 3 - aryloxymethyl - s - triazolo [3, 4 - b] [1, 3, 4] thiadiazoles by the condensation of 4-amino-3-aryloxymethyl - 5 - mercapto - 1. 2. 4-triazoles with phenyl isothiocyanate. The synthon (**75**) reacted smoothly with phenyl isothiocyanate (**76**) in excellent yields (80 - 85%). Cyclodehydro sulphurization of (**76**) by heating in DMF furnished the compound (**77**). The same compounds were also prepared in one step without isolating the thioureas (**76**). Thus heating of (**75**) with phenylisothiocyanate in DMF at reflux temperature for 24 hours furnished the required compounds (**77**).

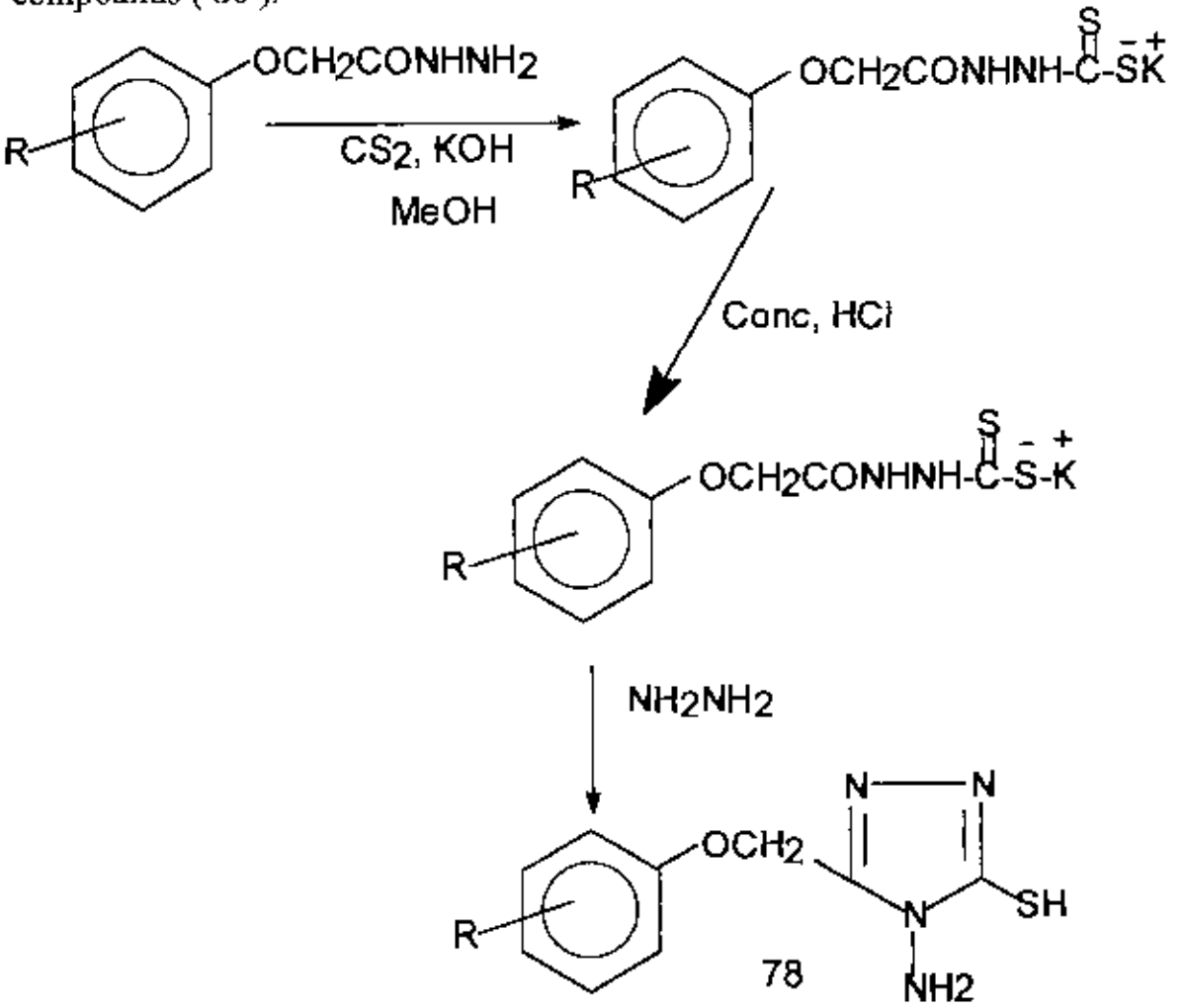
All the synthesized compounds have been evaluated for their antifungal activity against ***Aspergillus niger*** at three different concentration (10, 100, and 1000 ppm). The results were compared with commercial fungicide bavistin (carbendazin). Compound (**76c**) showed activity comparable to that of bavistin.



(76) and (77) a, R = 2-CH_3; b, R = 3-CH_3
c, R = 4-CH_3; d, R = 2, 4 - (CH_3)2

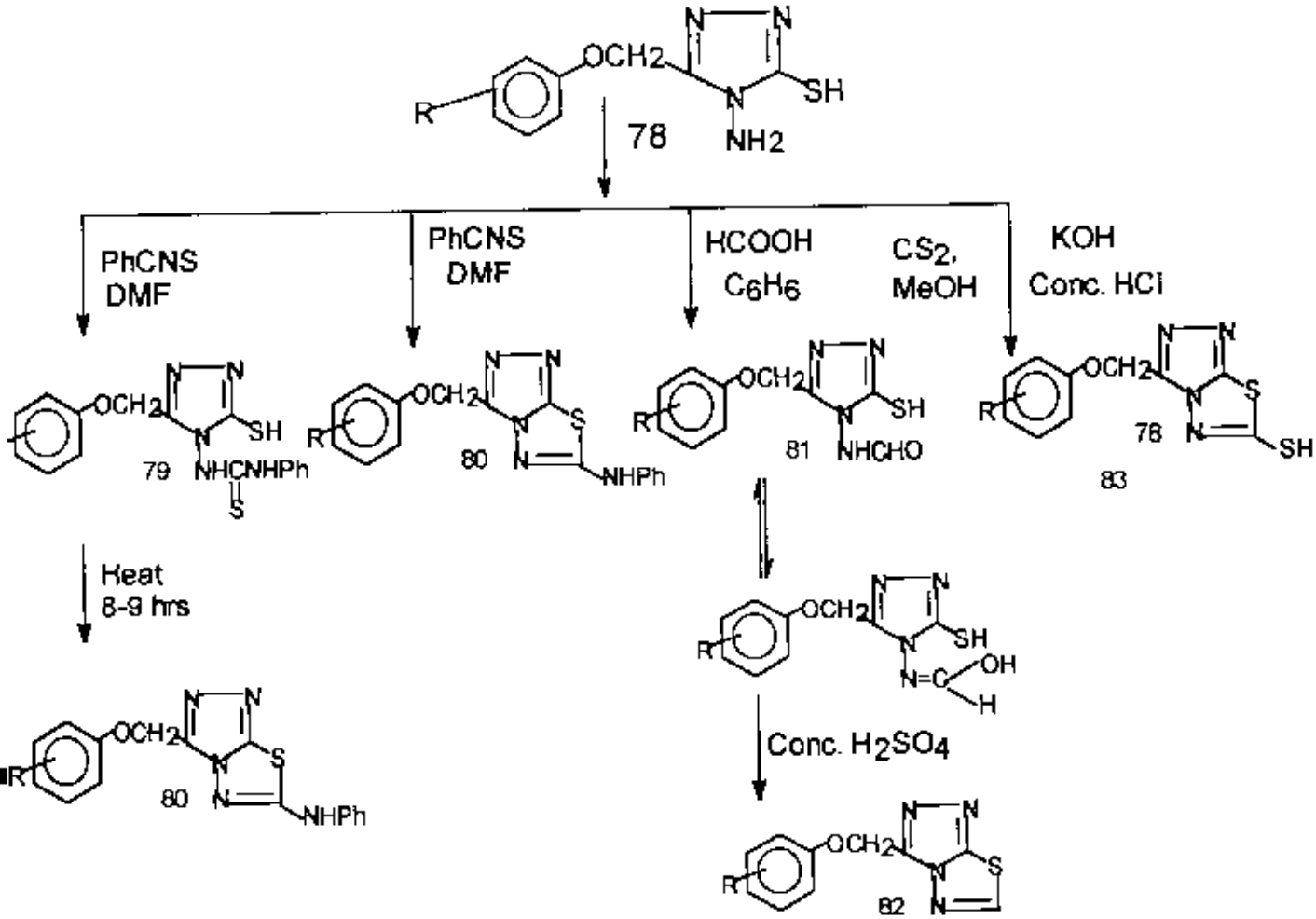
The methods they reported for the preparation of s-triazolo [3, 4 - b] [1, 3, 4] thiadiazoles consists of (**a**) dehydrative ring closure of 4-acylamino-s-triazoles - 5 - thiols and cyclization of 3 - substituted 4 - amino - 5 -mercapto - s - triazols with cyanogen bromide or carboxylic acids (**b**) ring closure of 1, 3, 4-thiadiazol - 2 - ylhydrazines with CS_2 or cyanogen bromide. Several 3-aryloxymethyl - 1, 2, 4 - triazoles (**79** and **81**) and 3-aryloxymethyl - 6 -

substituted - 1, 2, 4 - triazolo [3, 4 - b] - 1, 3, 4 - thiadiazoles (**80, 82** and **83**) have been synthesized by Bano *et al.*[68]. They reported this synthon on refluxing with phenyliso thiocyanate for 30 min in DMF, furnished the corresponding phenylthioureas (**79**) in excellent yields (80-85%). Cyclodehydrosulphurization of (**79**) by heating in DMF furnished the compounds (**80**).



These compounds were also prepared in a single pot without isolating the thioureas (**79**) by heating (**78**) with phenyliso-thiocyanate in DMF for 24 hour. The synthon (**78**) on refluxing with formic acid in benzene for 30 min furnished the compounds (**81**) which on cyclodehydration with conc. H_2SO_4 furnished the compounds (**82**). Synthon (**78**) when refluxed with

carbon disulfide and KOH in methanol produced the potassium salt of (**78**) which on acidification with dil. HCl furnished compound (**83**).



The compounds were screened for their antifungal activity against ***Aspergillus flavus*** and ***Aspergillus niger*** at 1000, 100 and 10 ppm concentrations. The results were compared with the standard fungicide Dithane M-45 tested under similar conditions. Compound (**83**) showed activity nearly comparable to that of Dithane M-45. Bano ***et al.***[69] also reported several 3-substituted-6-arylamino-1, 2, 4-triazolo [3, 4 - b] –1, 3, 4-thiadiazoles and screened for their antifungal activity against ***Helminthosporum oryzae*** and ***Cephalosporium sacchari***.

Aim of the Project

Heterocyclic compounds containing C = N moiety plays an important role as common denominator for various biological activities such as antibacterial, fungicidal and herbicidal that discussed so far. Like wise 1, 3, 4 - thiadiazoline ring is associated by virtue of incorporating toxophoric N - C = S linkage. On this point of view, the object of the research is to synthesize substituted 1, 3, 4 - thiadiazoline derivatives and to characterize them by physical constants and spectral evidences. After characterization pharmacological test will be conducted on them. The aim of the project is as following.

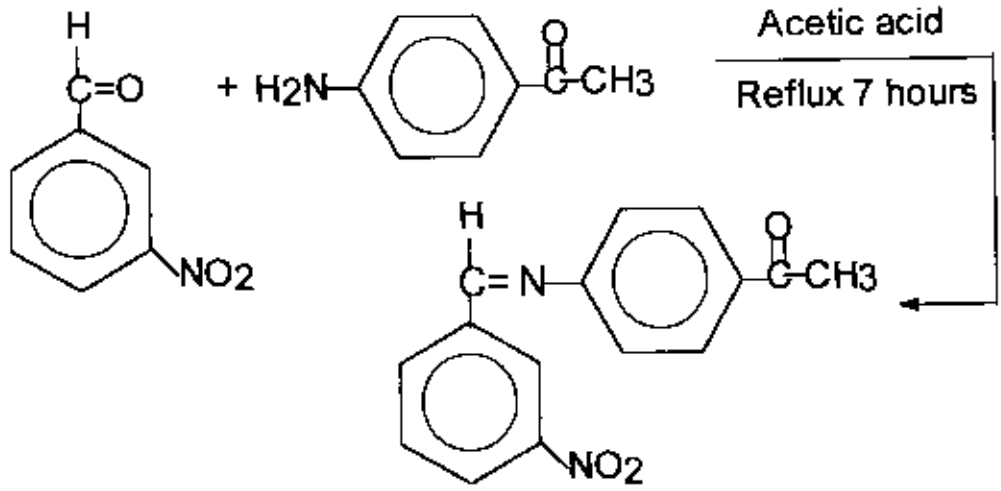
1. First step involves formation of imino compounds
2. Second step Schiff base formation with thiosemicarbazide
3. One pot cyclization of thiosemicarbazone
4. Purification by different techniques
5. Characterization by physical constants and spectroscopic methods
6. Biological test.

CHAPTER - 2

EXPERIMENTAL

1 A. SYNTHESIS OF 4 (*m*-NITROBENZIMINO) ACETOPHENONE

Reaction involved



Procedure

A mixture of *m*-nitrobenzaldehyde (0.453 gm ; 3 m mol) and 4- aminoacetophenone (0.405 gm ; 3 m mol) was refluxed in glacial acetic acid (10 ml) for 7 hours. The progress of the reaction was monitored by TLC. The reaction mixture was then cooled to room temperature and acetic acid was removed by rotary evaporator under reduced pressure. The resulting solid mass was recrystallized several times from ethylacetate. The desired compound was isolated as a reddish yellow crystal, yield 71%, m. p. 175-177^{0}C.

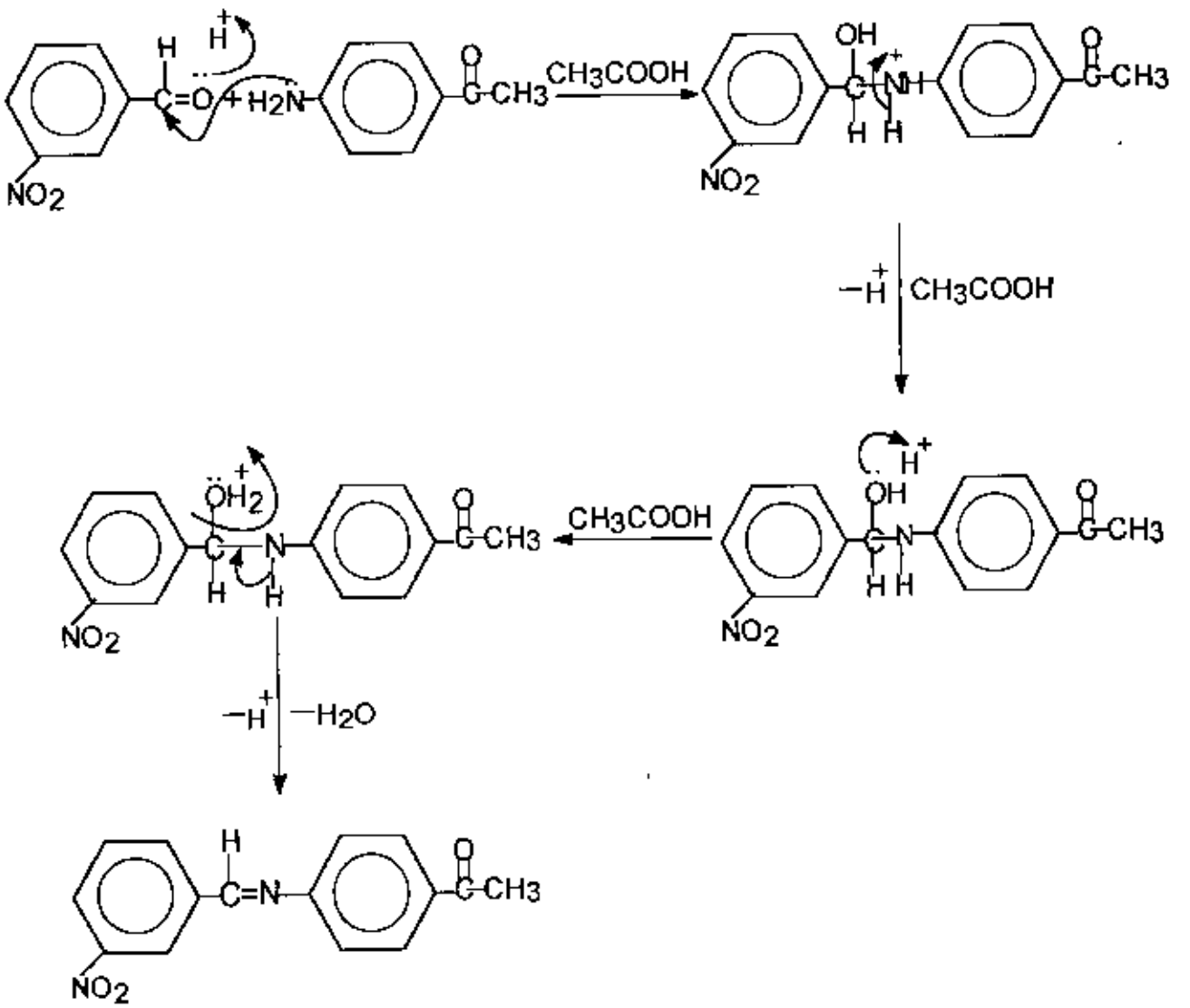
^{1}H NMR ($CDCl_3$ / TMS)

δ: 1.57 (s, 3H, CH_3), 6.65 (d, 1H, N=C-H), 7.83-7.71(m, aromatic).

Elemental analysis

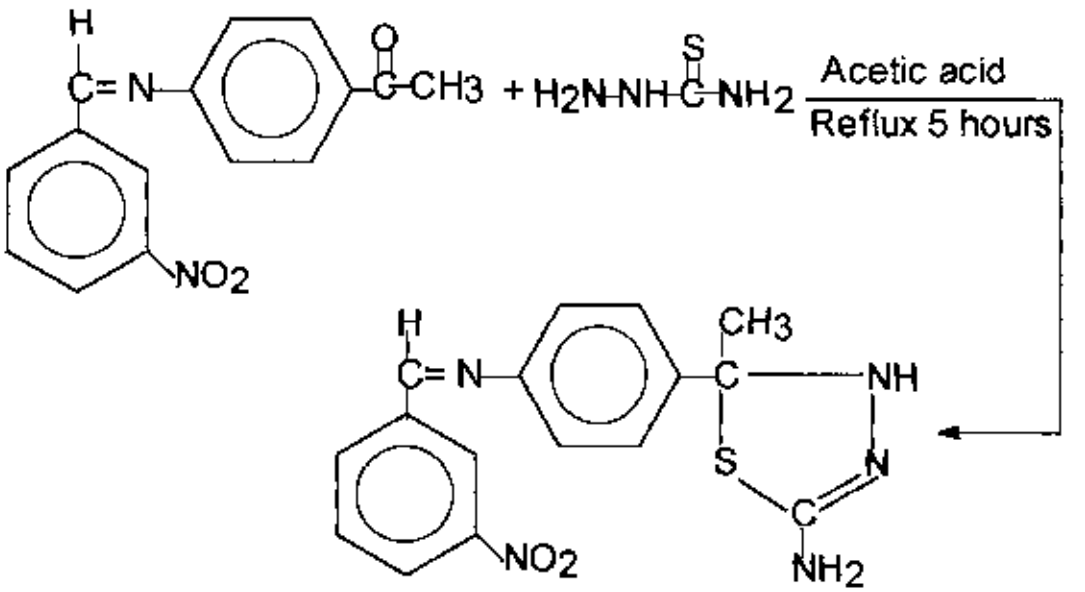
Found: C, 67.16 ; H, 4.51 ; N, 10.44; O, 17.89. $C_{15}H_{12}N_2O_3$.

Reaction Mechanism



1 B. SYNTHESIS OF 5-METHYL-5-[4′(*m* - NITROBENZIMINO)] PHENYL-2-AMINO-Δ^2-1,3,4-THIADIAZOLINE

Reaction involved



Procedure

A mixture of 4 (*m*-nitrobenzimino) acetophenone (1.340 gm; 5 m mol) and thiosemicarbazide (0.455 gm ; 5m mol) was refluxed in glacial acetic acid (4 ml) for 5 hours. The progress of the reaction was monitored by TLC. The reaction mixture was then cooled to room temperature and acetic acid was removed by rotary evaporator under reduced pressure. The resulting solid mass was recrystallized several times from ethylacetate. The desired compound was isolated as yellow crystal, yield 61%, m. p. 145^{0}C.

IR Spectrum

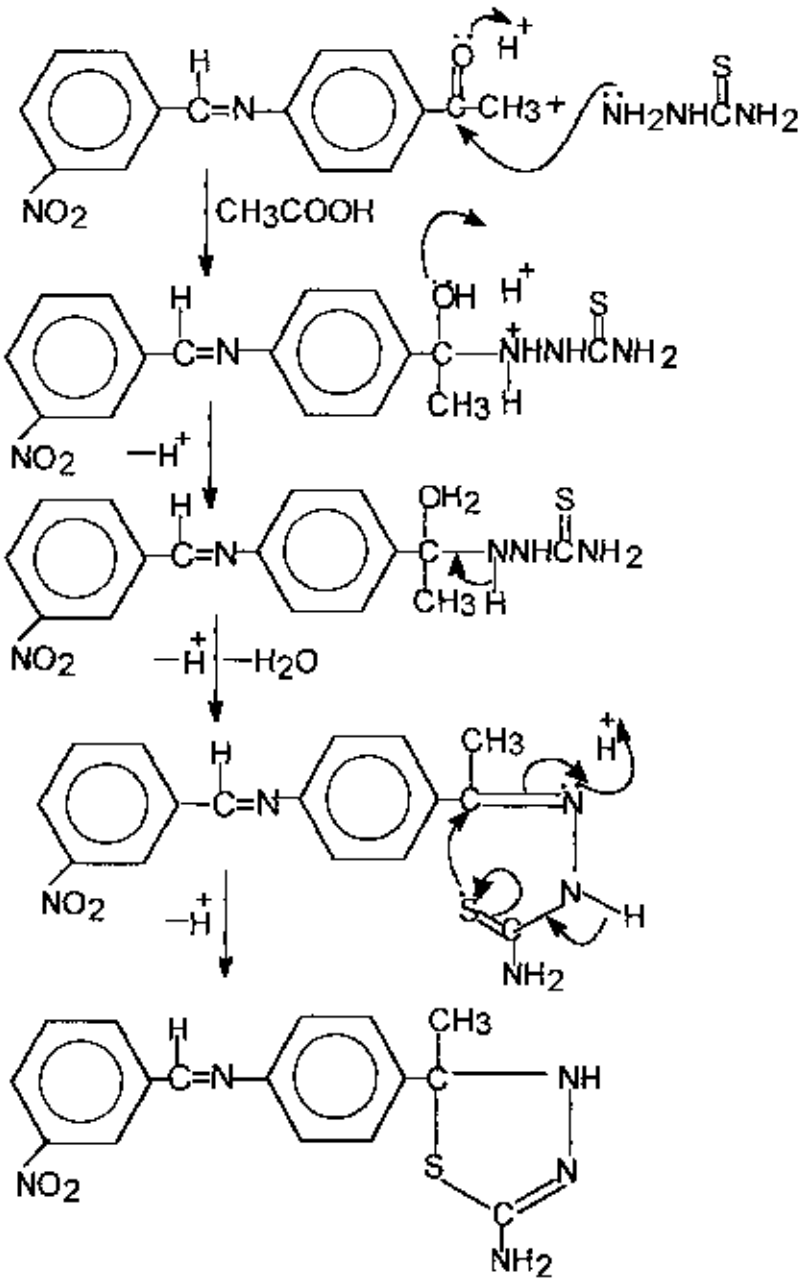
ν_{max} (KBr) cm^{-1} : 3310-3069 (NH, C-H aromatic, H-C=N), 1664 (C=N), 1647 (C =N), 1634 (C =C). 1599 (C=C), 1516 (C = C), 1341(O - N = O).

^{1}H NMR ($CDCl_3$ / TMS)

δ: 1.57 (s, 3H, CH_3), 2.24 (2H, NH_2), 6.65 (d, 1H, = C - H), 7.96-7.26(m, aromatic), 8.2 (1H, NH).

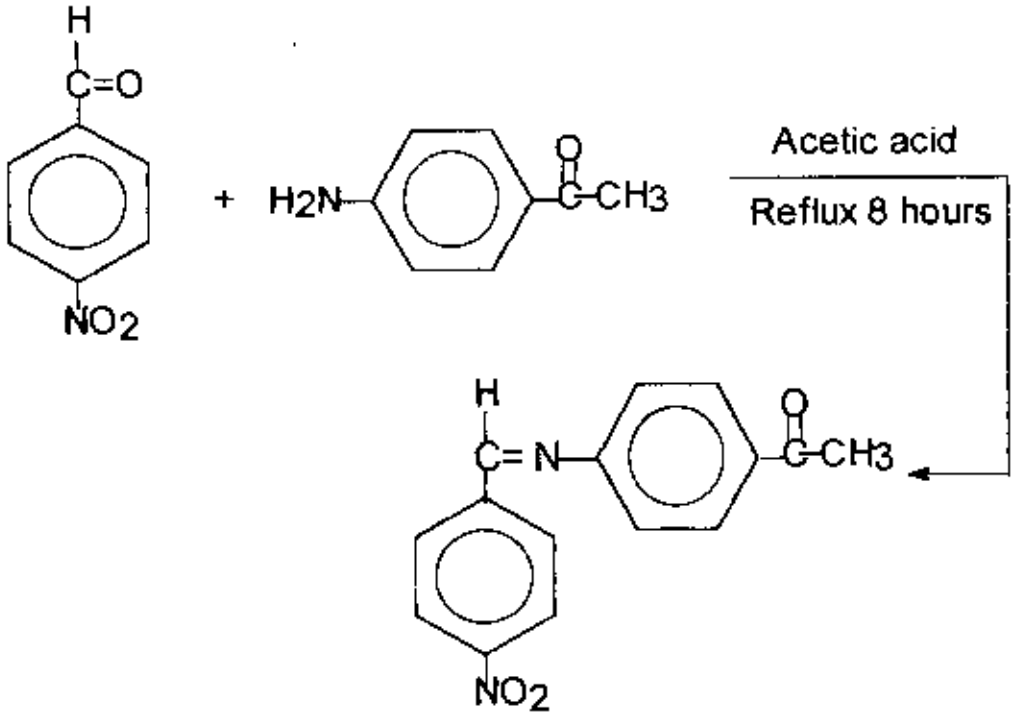
Elemental analysis

Found: C, 56.29; H, 4.43; N, 20.51; O, 9.37; S, 9.39. $C_{16}H_{15}N_5O_2S$.



2 A. SYNTHESIS OF 4(*p* -NITROBENZIMINO) ACETOPHENONE

Reaction involved



Procedure

A mixture of *p*- nitrobenzaldehyde (0.604 gm ; 4 m mol) and 4 - amino-acetophenone (0.540 gm ; 4 m mol) was refluxed in glacial acetic acid (10 ml) for 8 hours. The progress of the reaction was monitored by TLC. The reaction mixture was then cooled to room temperature and acetic acid was removed by rotary evaporator under reduced pressure. The resulting solid mass was recrystallized several times from ethylacetate. The desired compound was isolated as a reddish yellow crystal, yield 72%, m. p. 105-106 ^{0}C.

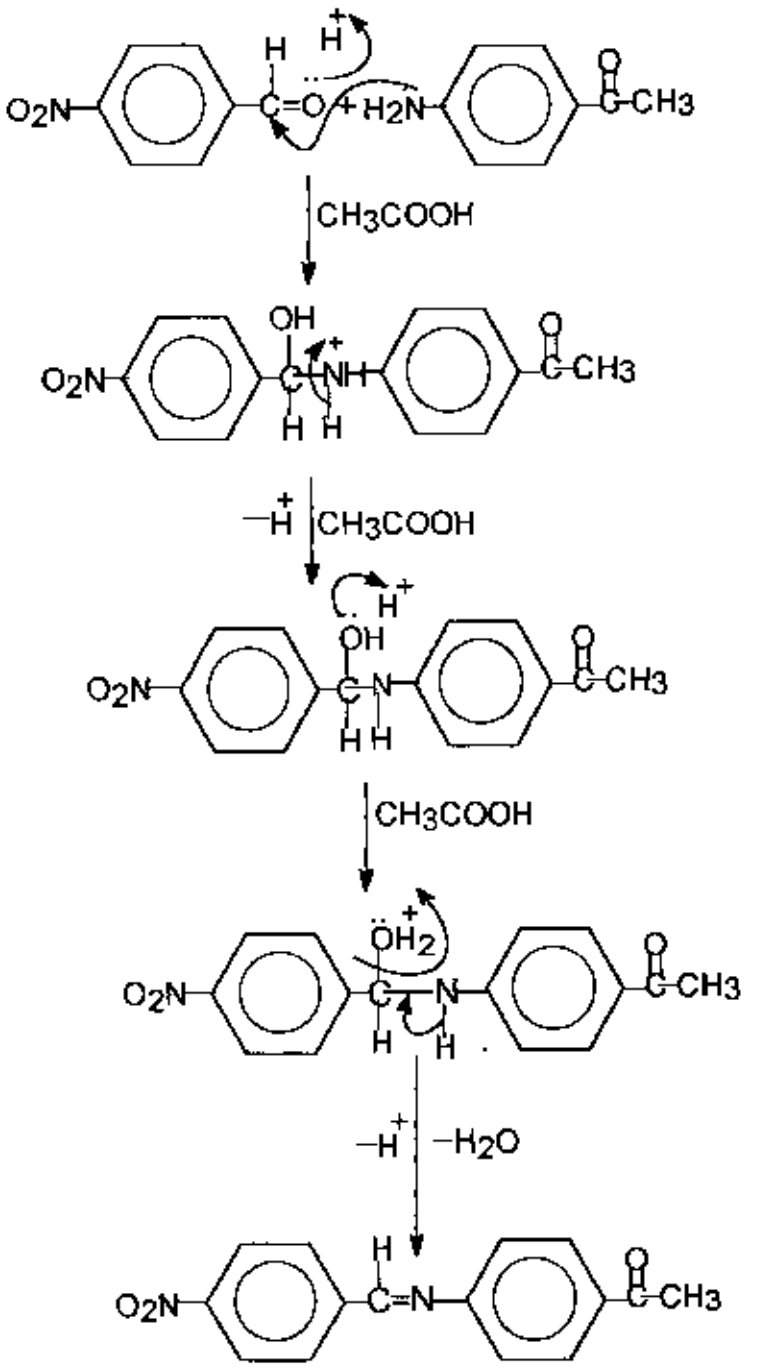
^{1}H NMR ($CDCl_3$ / TMS)

δ: 1.57(S, 3H, CH_3), 6.72(m, 1H, H-C=N), 7.98-7.59 (m, aromatic).

Elemental analysis

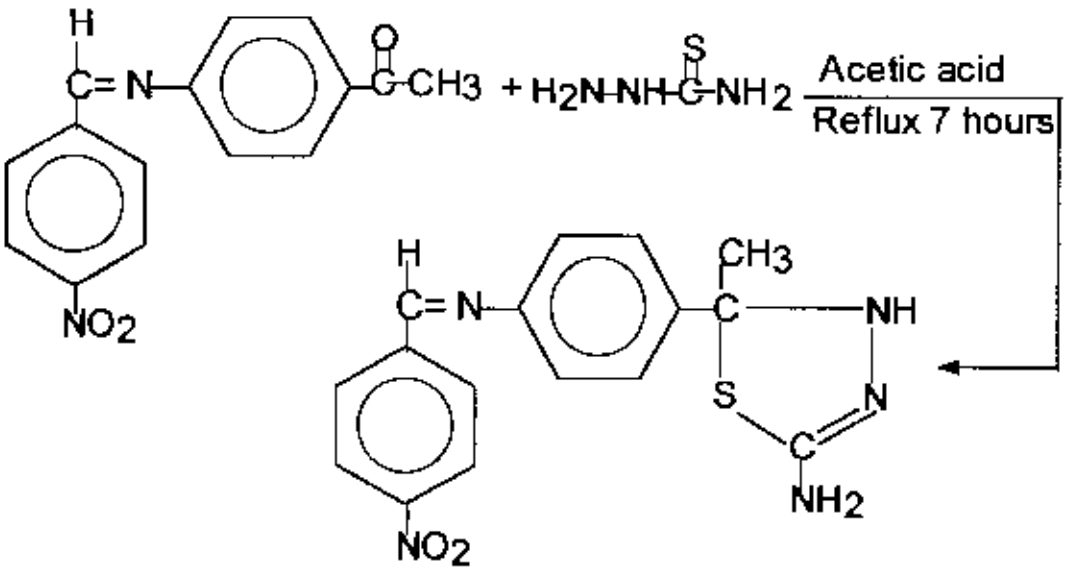
Found: C, 67.16; H, 4.51; N, 10.44; O, 17.89. $C_{15}H_{12}N_2O_3$.

Reaction Mechanism:



2 B. SYNTHESIS OF 5-METHYL-5-[4′(*p* - NITROBENZIMINO)] PHENYL - 2 - AMINO - Δ^2 -1, 3, 4-THIADIAZOLINE

Reaction involved



Procedure

A mixture of 4 (*p* - nitrobenzimino)acetophenone (1.340 gm ; 5 m mol) and thiosemicarbazide (0.455 gm; 5 m mol) was refluxed in glacial acetic acid (5 ml) for 7 hours. The progress of the reaction was monitored by TLC. The reaction mixture was then cooled to room temperature and acetic acid was removed by rotary evaporator under reduced pressure. The resulting solid mass was recrystallized several times from ethylacetate. The desired compound was isolated as yellow crystal, yield 65%, m. p. 140^{0}C.

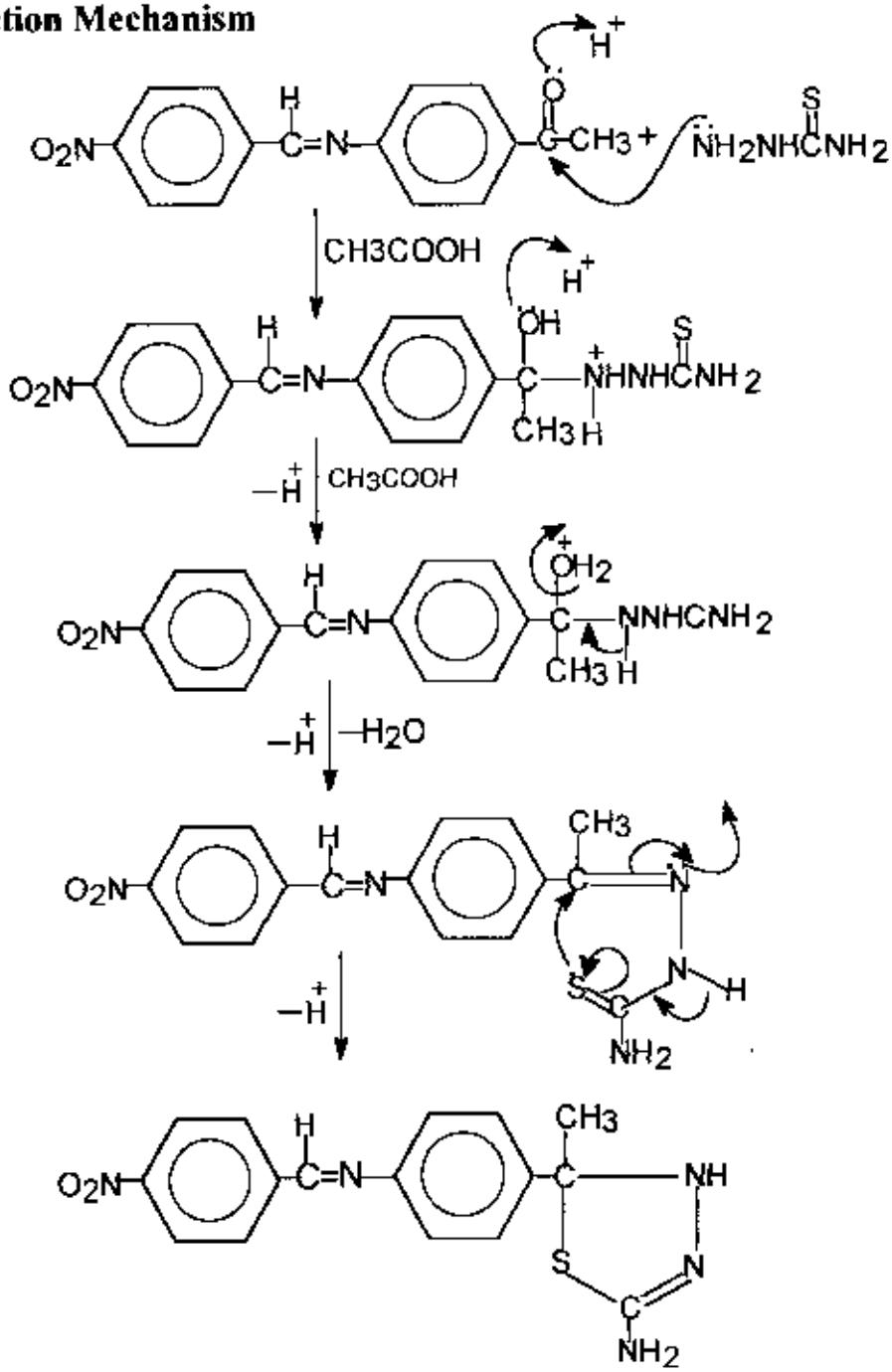
^{1}H NMR ($CDCl_3$ / TMS)

δ: 1.58 (s, 3H, CH_3), 2.24 (2H, NH_2), 6.73-6.69 (m, 1H, H - C = N), 7.96-7.66 (m, aromatic), 8.25 (1H, NH).

Elemental analysis

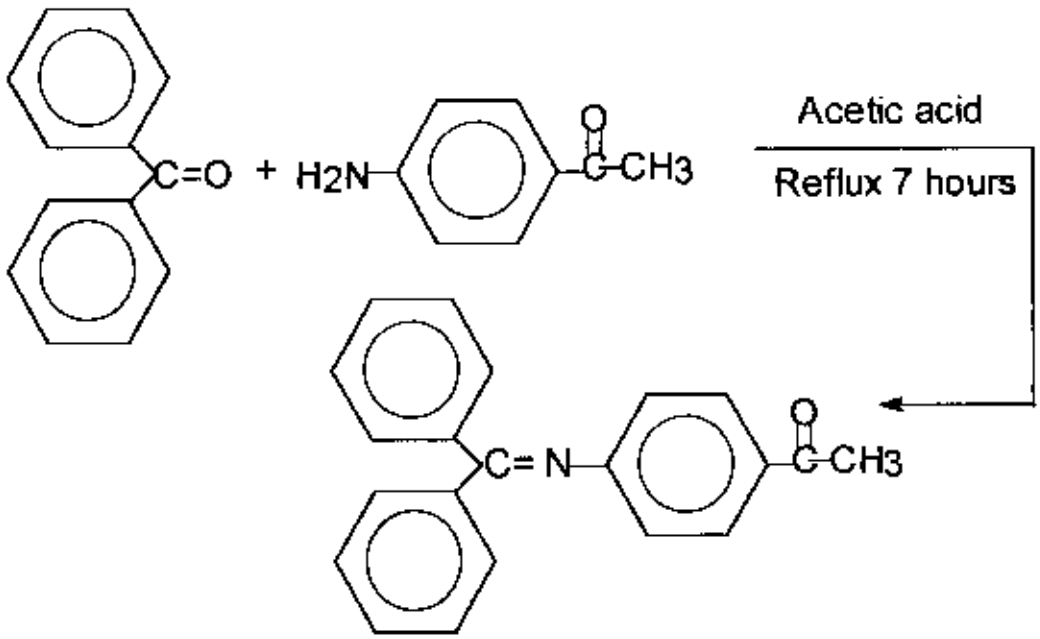
Found: C, 56.29; H, 4.43; N, 20.51; O, 9.37; S, 9.39. $C_{16}H_{15}N_5O_2S$.

Reaction Mechanism



3 A. SYNTHESIS OF 4 (DIPHENYLIMINO) ACETOPHENONE

Reaction involved



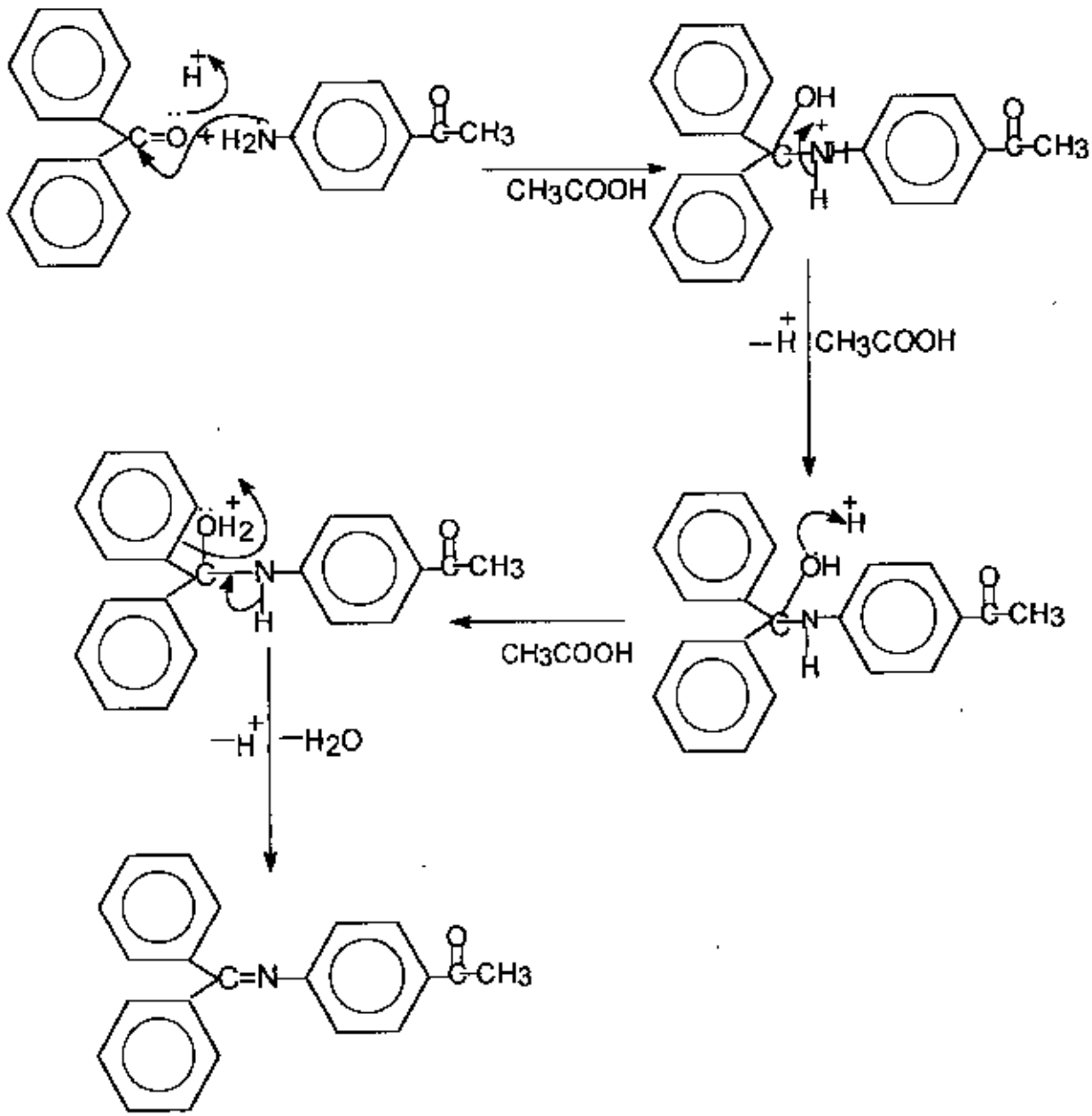
Procedure

A mixture of benzophenone (0.362 gm ; 2 m mol) and 4-aminoacetophenone (0.270 gm ; 2 m mol) was refluxed in glacial acetic acid (5 ml) for 7 hours. The progress of the reaction was monitored by TLC. The reaction mixture was then cooled to room temperature and acetic acid was removed by rotary evaporator under reduced pressure. The resulting solid mass was recrystallized several times from ethylacetate. The desired compound was isolated as a greyish crystal, yield 62%, m. p. 85-87^{0}C.

Elemental analysis

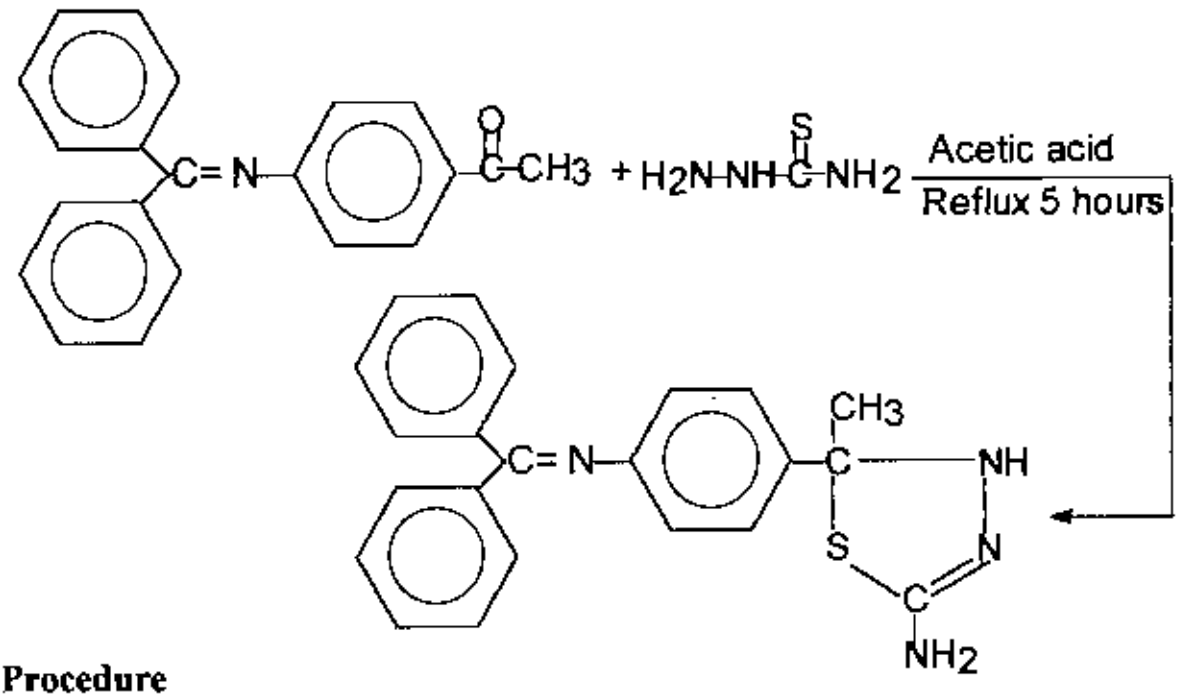
Found: C, 84.25 ; H, 5.72 ; N, 4.68 ; O, 5.34. $C_{21}H_{17}NO$.

Reaction Mechanism



3 B. SYNTHESIS OF 5-METHYL-5-[4' (DIPHENYLIMINO)] PHENYL-2-AMINO - Δ^2 - 1, 3, 4 - THIADIAZOLINE

Reaction involved



Procedure

4 (Diphenylimino) acetophenone (1.196 gm ; 4 m mol) and thiosemicarbazide (0.364 mg ; 4 m mol) was refluxed in glacial acetic acid (5 ml) for 5 hours. The progress of the reaction was monitored by TLC. The reaction mixture was then cooled to room temperature and acetic acid was removed by rotary evaporator under reduced pressure. The resulting solid mass was recrystallized several times from ethylacetate. The desired compound was isolated as a greyish crystal, yield 65%, m. p. 90^0C.

IR Spectrum

ν_{max} (KBr) cm^{-1} : 3360 - 3209 (b, NH), 3109 - 3101 (b, C - H, aromatic), 3116 (H - C = N), 2975 (H - C, aliphatic), 1674 (C = N), 1602 (C = C), 1590 (C = C), 1490 (C = C).

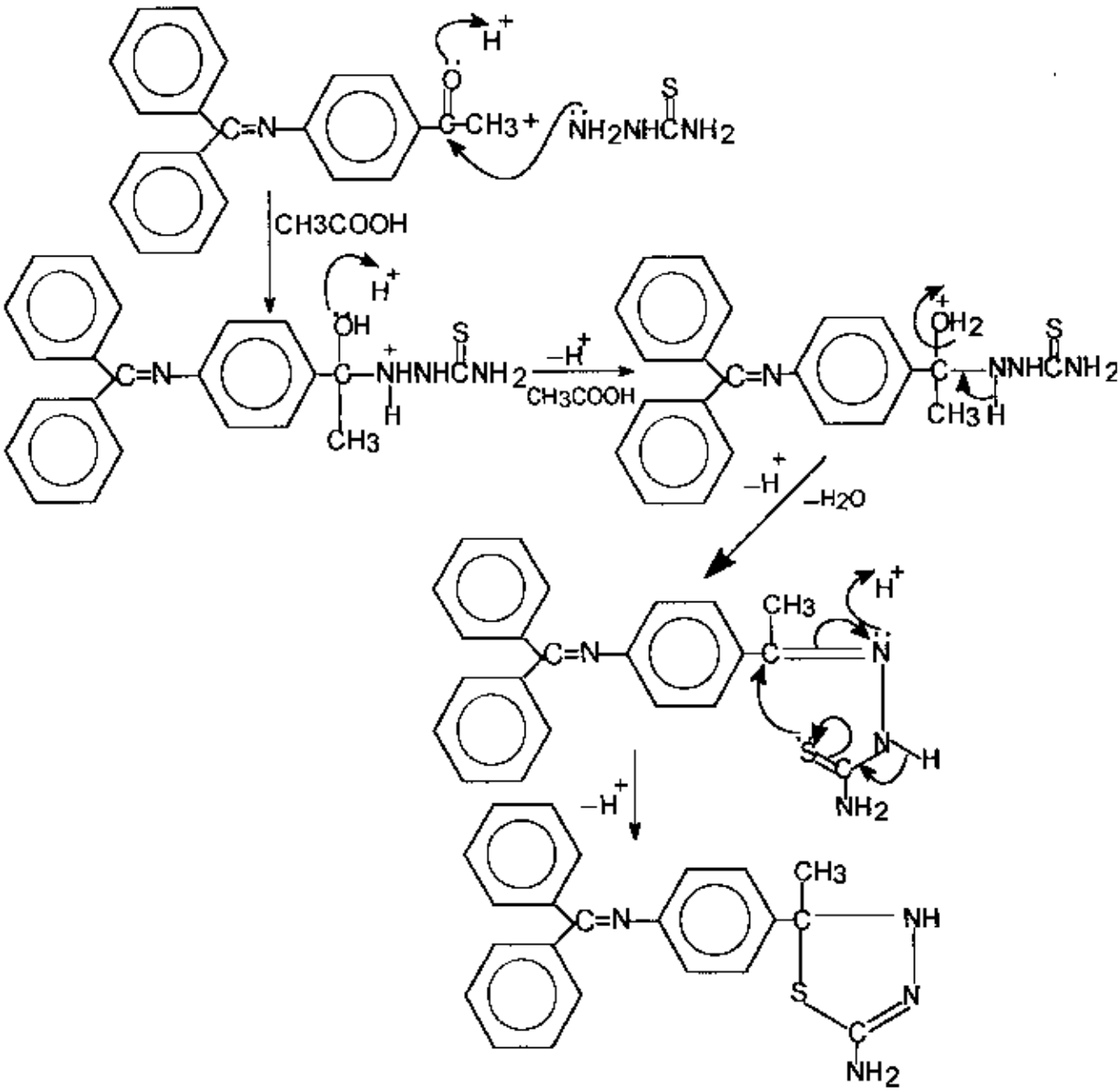
^{1}H NMR ($CDCl_3$ / TMS)

δ: 1.57 (s, 3H, CH_3), 2.20 (2H, NH_2), 7.66 - 7.56 (m, aromatic), 7.96 (1H, NH).

Elemental analysis

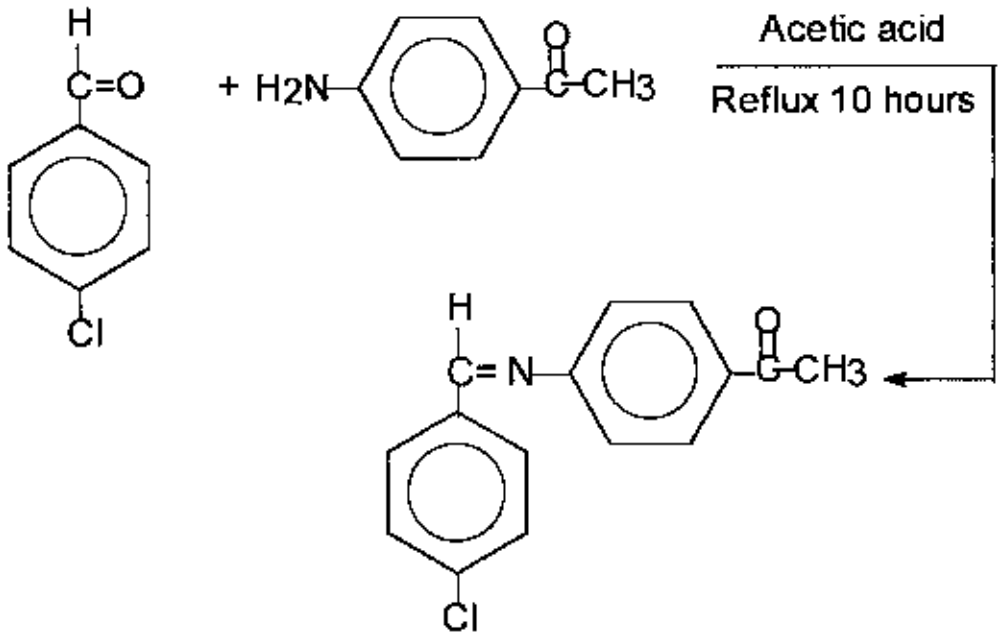
Found: C, 70.94 ; H, 5.41 ; N, 15.04 ; S, 8.61. $C_{22}H_{20}N_4S$.

Reaction Mechanism



4 A. SYNTHESIS OF 4 (*p*-CHLOROBENZIMINO) ACETOPHENONE

Reaction involved



Procedure

A mixture of *p*-chlorobenzaldehyde (0.562 gm ; 4 m mol) and 4-aminoaceto -phenone (0.540 gm ; 4 m mol) was refluxed in glacial acetic acid (10 ml) for 10 hours. The progress of the reaction was monitored by TLC. The reaction mixture was then cooled to room temperature and acetic acid was removed by rotary evaporator under reduced pressure. The resulting solid mass was recrystallized several times from ethylacetate. The desired compound was isolated as a reddish crystal, yield 70%, m. p. 125 ^{0}C.

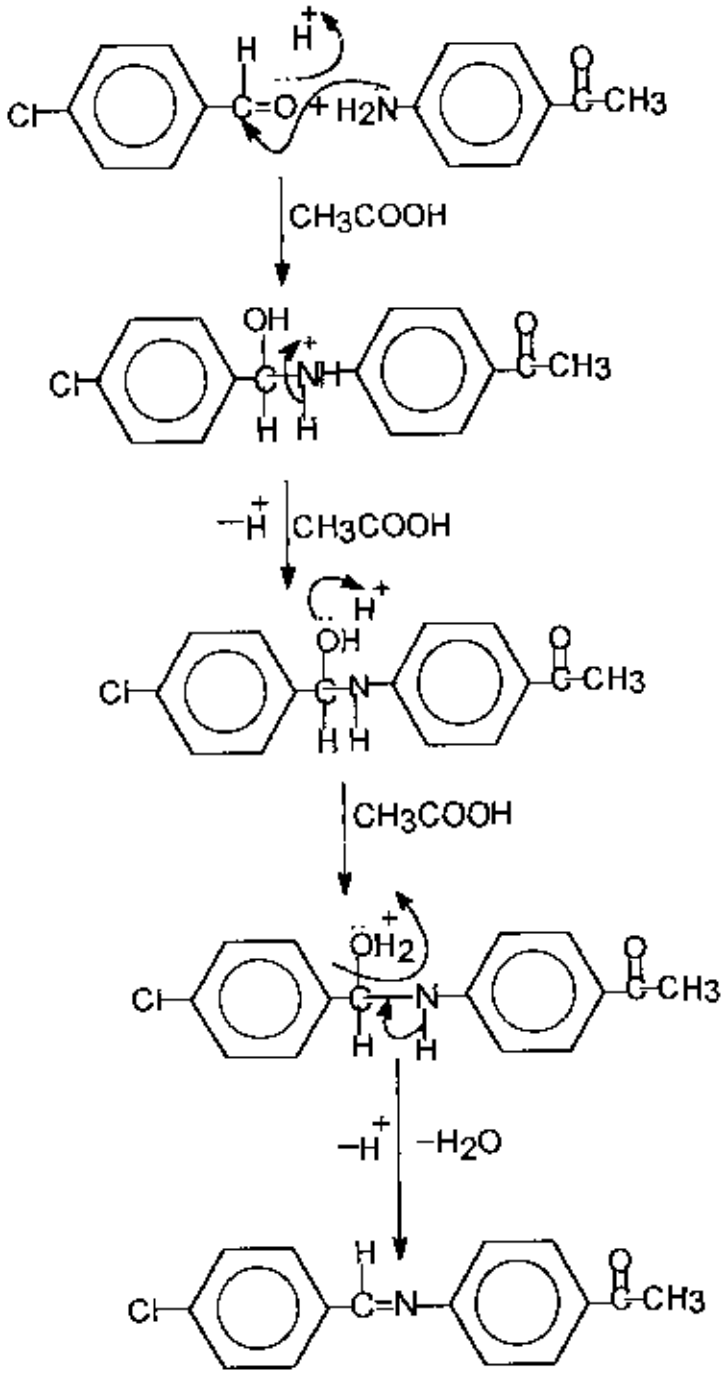
^{1}H NMR ($CDCl_3$ / TMS)

δ: 1.7 (b, 3H, CH_3), 6.6 (d, 1H, H - C =N), 7.5-7.2 (m, aromatic).

Elemental analysis

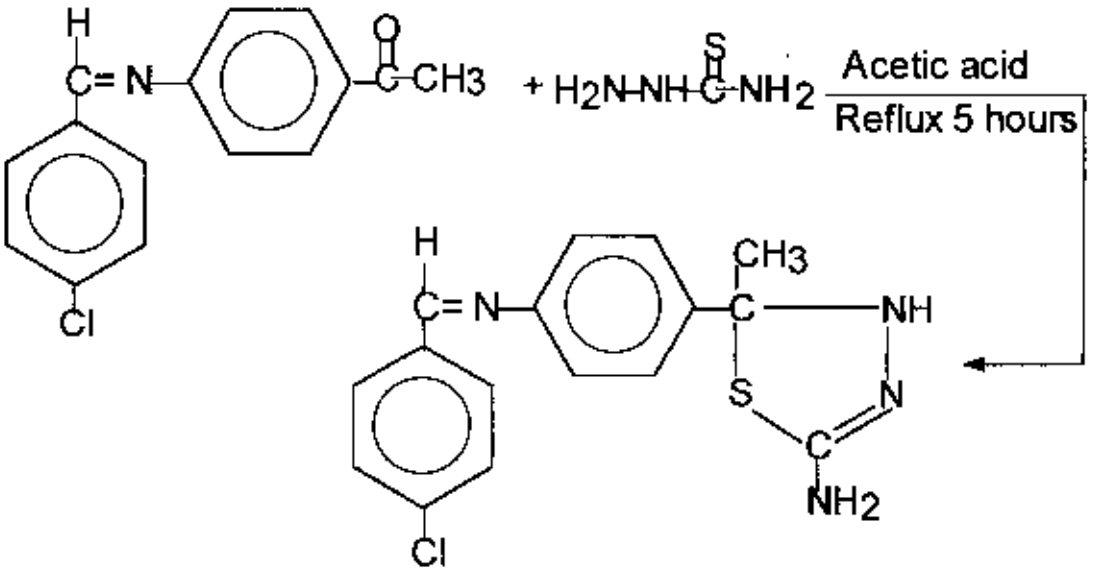
Found: C, 81.06; H, 5.44; N, 6.30; O, 7.20; Cl, 15.95. $C_{15}H_{12}NOCl$.

Reaction Mechanism:



4 B. SYNTHESIS OF 5-METHYL-5-[4′(*p* - CHLOROBENZIMINO)] PHENYL-2-AMINO-Δ^2-1, 3, 4-THIADIAZOLINE

Reaction involved



Procedure

A mixture of 4 (*p*-chlorobenzimino) acetophenone (1.030 gm ; 4 m mol) and thiosemicarbazide (0.364 gm, 4 m mol) was refluxed in glacial acetic acid (7 ml) for 5 hours. The progress of the reaction was monitored by TLC. The reaction mixture was then cooled to room temperature and acetic acid was removed by rotary evaporator under reduced pressure. The resulting solid mass was recrystallized several times from ethylacetate. The desired compound was isolated as reddish crystal, yield 65%, m. p. 140-142^{0}C.

IR Spectrum

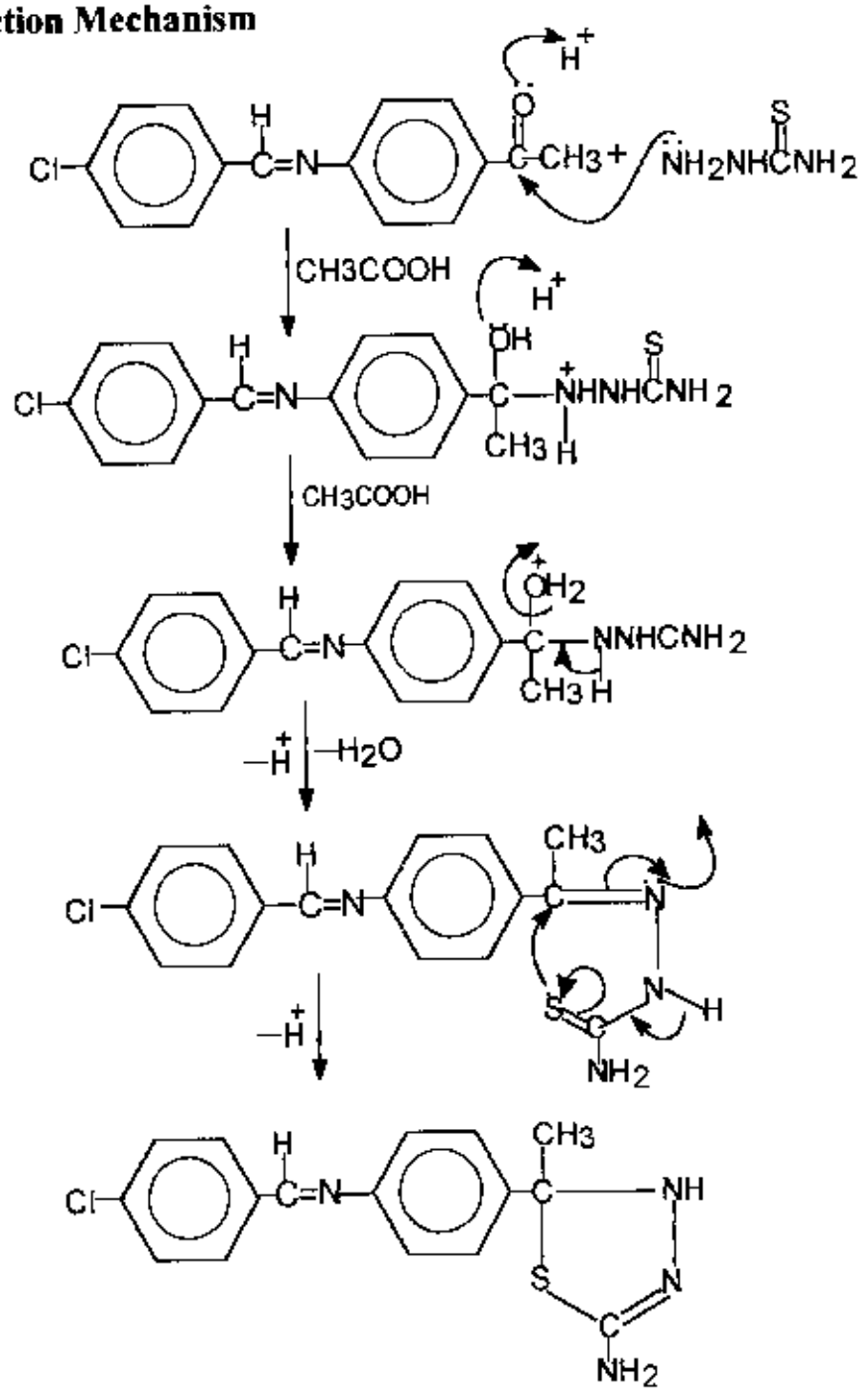
ν_{max} (KBr)cm^{-1} : 3347 - 3317 (b, NH), 3053 (aromatic C - H,), 3190 (H -C = N), 2971 (aliphatic C - H), 1664 (C=N), 1655 (C = N), 1594 (C = C), 1529 (C=C), 1490(C = C), 819 (C - Cl).

^{1}H NMR ($CDCl_3$ / TMS)

δ: 1.62 (s, 3H, CH_3), 2.27 (2H, NH_2), 6.6 (m, 1H, H - C = N),

7.61 - 7.26 (m, aromatic), 7.92 (1H, NH).

Reaction Mechanism

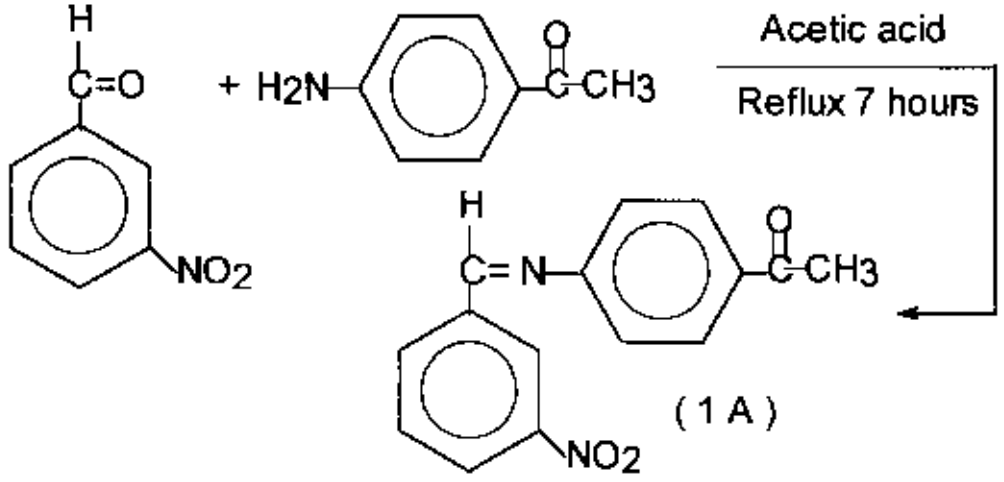


CHAPTER -3

RESULTS AND DISCUSSION

3.1 Synthesis and characterization of 4 (*m*-nitrobenzimino) acetophenone (IA)

A mixture of *m* - nitrobenzaldehyde (0.453 mg ; 3 m mol) and *p* - amino-acetophenone (0.405 mg ; 3 m mol) was refluxed for 7 hours in glacial acetic acid (10 ml). The progress of the reaction was followed by TLC. The reaction mixture was then cooled to room temperature and acetic acid was removed by rotary evaporator under reduced pressure. The resulting solid mass was then recrystallized several times from ethylacetate, as reddish yellow crystal (IA), yields 71%, m. p. 175-77^0, R_f 0.68 in pet - ether : ethylacetate (2 : 1) solvent system.



(1 A)

The ^{1}H NMR spectrum (300 MHz, $CDCl_3$, Fig. 1) with a singlet at δ 1.57 integrating for three protons were ascribable to methyl protons. A doublet at δ 6.65 could be assigned for one imino proton. A bunch of signals within δ 7.83 – 7.71 were indicative for aromatic protons.

Elemental analysis (Table : 1) of the compound (IA) were found as C, 67.16% ; H, 4.51% ; N, 10.44% ; O, 17.89%, which indicates the molecular formula $C_{15}H_{12}N_2O_3$. Therefore, the ^{1}H NMR spectrum and elemental analysis of the compound confirmed (IA) to be 4 (*m*-nitrobenzimino) acetophenone.

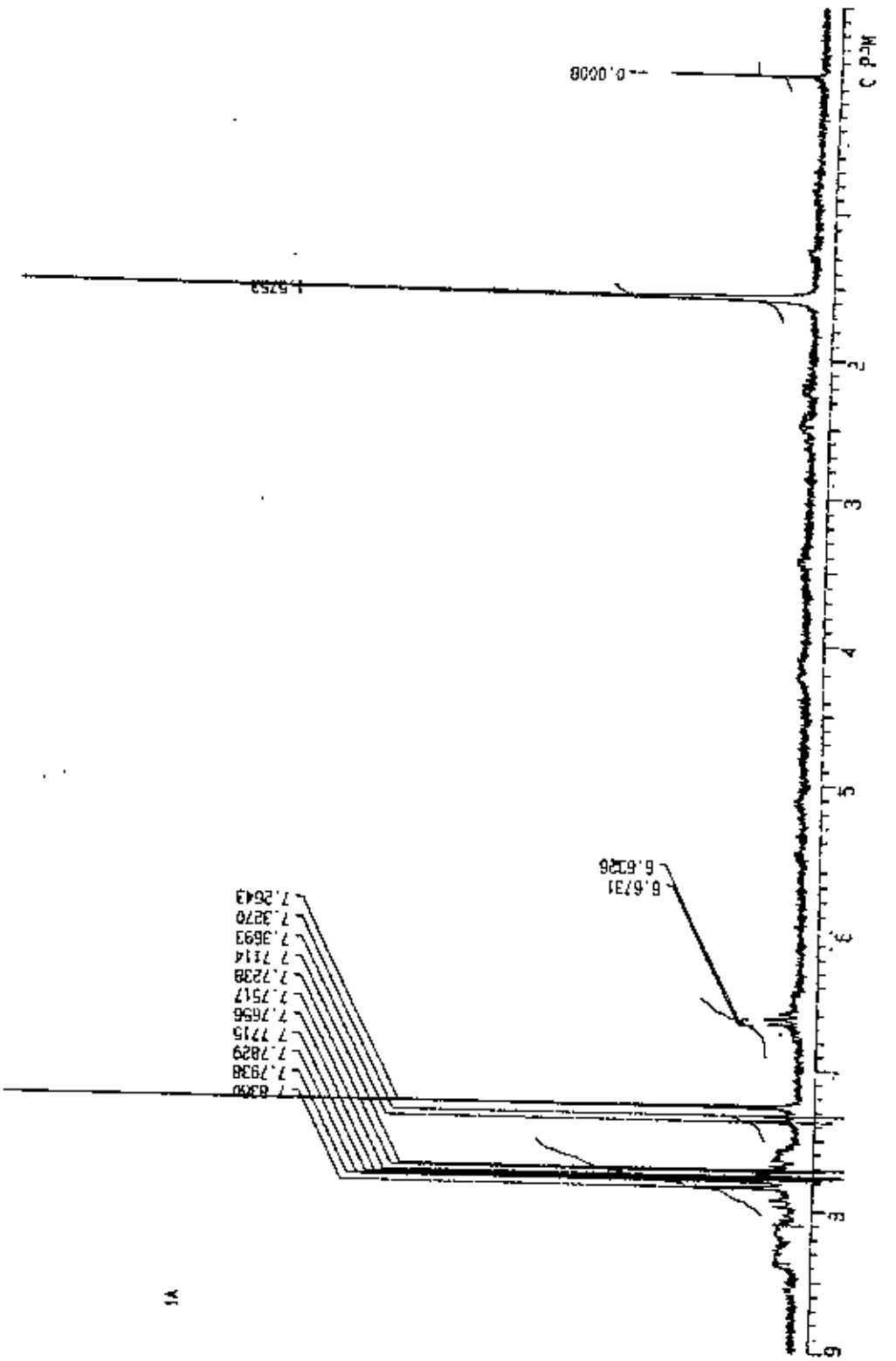


Figure 1 : ^{1}H NMR Spectrum of the compound 1A

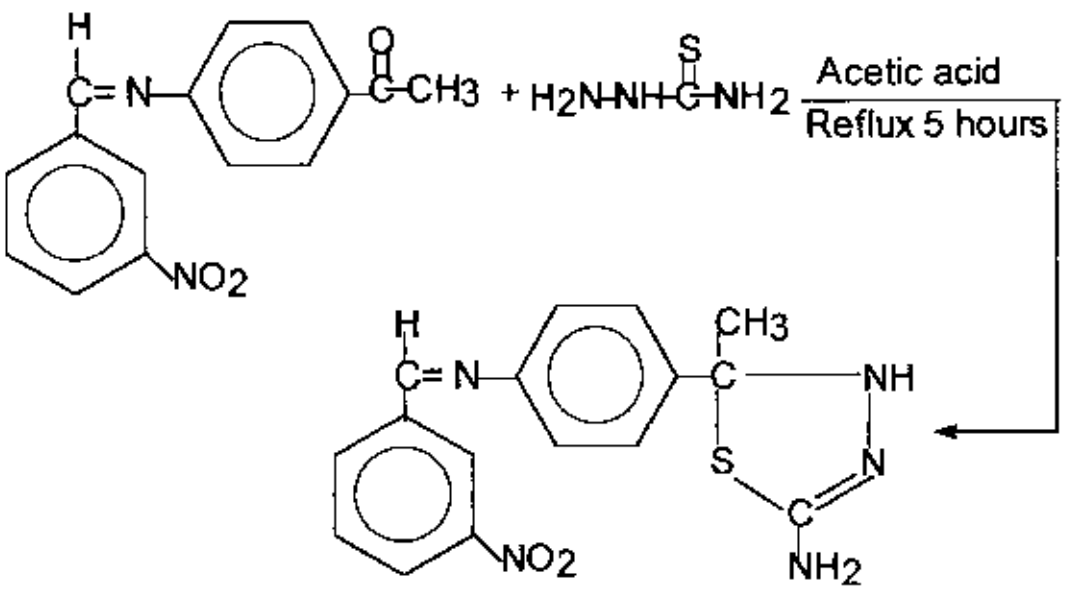
Sample	分析量	C			H			N		
	(mg)	予測値	測定値	Δ%C	予測値	測定値	Δ%H	予測値	測定値	Δ%N
Acetoanilide	2.125	71.09	71.16	0.07	6.71	6.78	0.07	10.36	10.38	0.02
1a	2.130	67.16	66.52	−0.64	4.51	4.42	−0.09	10.44	10.29	−0.15
1b	2.337	56.29	39.36	−16.93	4.43	4.96	0.53	20.51	22.79	2.28
2a	2.162	67.16	66.12	−1.04	4.51	3.73	−0.78	10.44	10.20	−0.24
2b	2.233	56.29	57.16	0.87	4.43	4.50	0.07	20.51	15.19	−5.32
3a	2.132	84.25	72.48	−11.77	5.72	6.38	0.66	4.68	9.21	4.53
3b	2.173	70.94	54.79	−16.15	5.40	5.91	0.51	15.04	15.92	0.88
4a	2.088	81.06	68.46	−12.60	5.44	4.25	−1.19	6.30	4.89	−1.41
4b	2.047	61.72	46.70	−15.02	4.86	4.02	−0.84	17.99	18.35	0.36

1a C	12.011	15	180.165	67.16
H	1.008	12	12.096	4.51
N	14.0067	2	28.0134	10.44
O	15.999	3	47.997	17.89
S	32.06	0	0	0.00
MW			268.2714	100.00

Table 1 : Elemental analysis of the compound 1A

3.2 Synthesis and characterization of 5-methyl-5 [4′ (*m*-nitrobenz-imino)] phenyl-2-amino - Δ^2-1, 3, 4 - thiadiazoline (IB)

An equimolecular mixture of 4 (*m*-nitrobenzimino) acetophenone (1.340 gm ; 5 m mol) and thiosemicarbazide (0.455 gm ; 5 m mol) was refluxed for 5 hours in glacial acetic acid (4 ml). The progress of the reaction was monitored by TLC. The reaction mixture was then cooled to room temperature and acetic acid was removed by rotary evaporator under reduced pressure. The resulting solid mass was recrystallized several times from ethylacetate as yellow crystal (**IB**) yield 61 %, m. p. 145^{0}C, R_f 0.60 in pet-ether : ethylacetate (2 : 1) solvent system.



(1 B)

Its IR spectrum (Fig : 2) showed a broad absorption band at 3310 - 3069 cm^{-1} indicative of N - H, H - C = N and aromatic C - H stretching together. The two absorption bands at 1664 cm^{-1} and 1647 cm^{-1} were due to the two C = N stretching. The sharp distinction bands at 1634, 1599 and 1516 cm^{-1} were indicative for aromatic C = C bonds. The weak band at 1341 cm^{-1} was

observed for N = O bond but the absence of C = S bond in the spectrum was suggestive for the cyclization of thiosemicarbazone.

The ^{1}H NMR spectrum (300 MHz, $CDCl_3$, Fig. 3) with a singlet at δ 1.57 integrating for three protons were assignable to methyl group. A singlet at δ 2.24 was ascribable for two NH_2 protons. A doublet centered at δ 6.65 (J = 0.04) integrating for one proton was attributable to imino group. A broad multiplet ranging from δ 7.96 - 7.26 were ascribable for aromatic protons. The down field resonance at δ 8.2 was thought to be due to the NH proton.

Elemental analysis (Table 2) were found to be C, 56.29 ; H, 4.43, N, 20.51 ; O, 9.37 ; S, 9.39, which were in full agreement for the molecular formula $C_{16}H_{15}N_5O_2S$.

Therefore, the IR spectrum, ^{1}H NMR spectrum and elemental analysis of the compound confirmed (**1B**) to be 5-methyl – 5 [4′ (*m* - nitrobenzimino)] phenyl - 2 - amino - Δ^2- 1, 3, 4 - thiadiazoline.

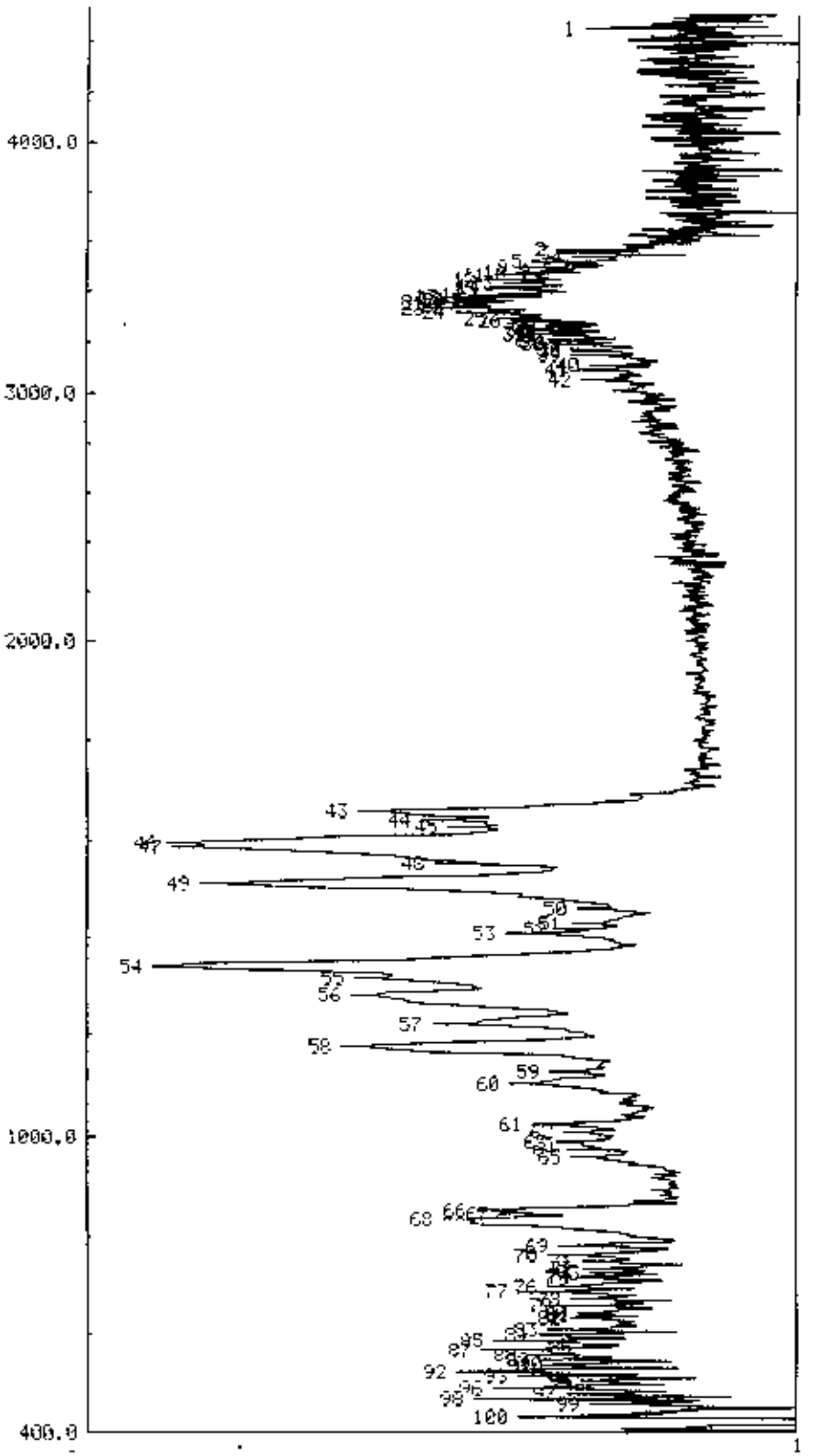


Figure 2 : IR Spectrum of the compound 1B

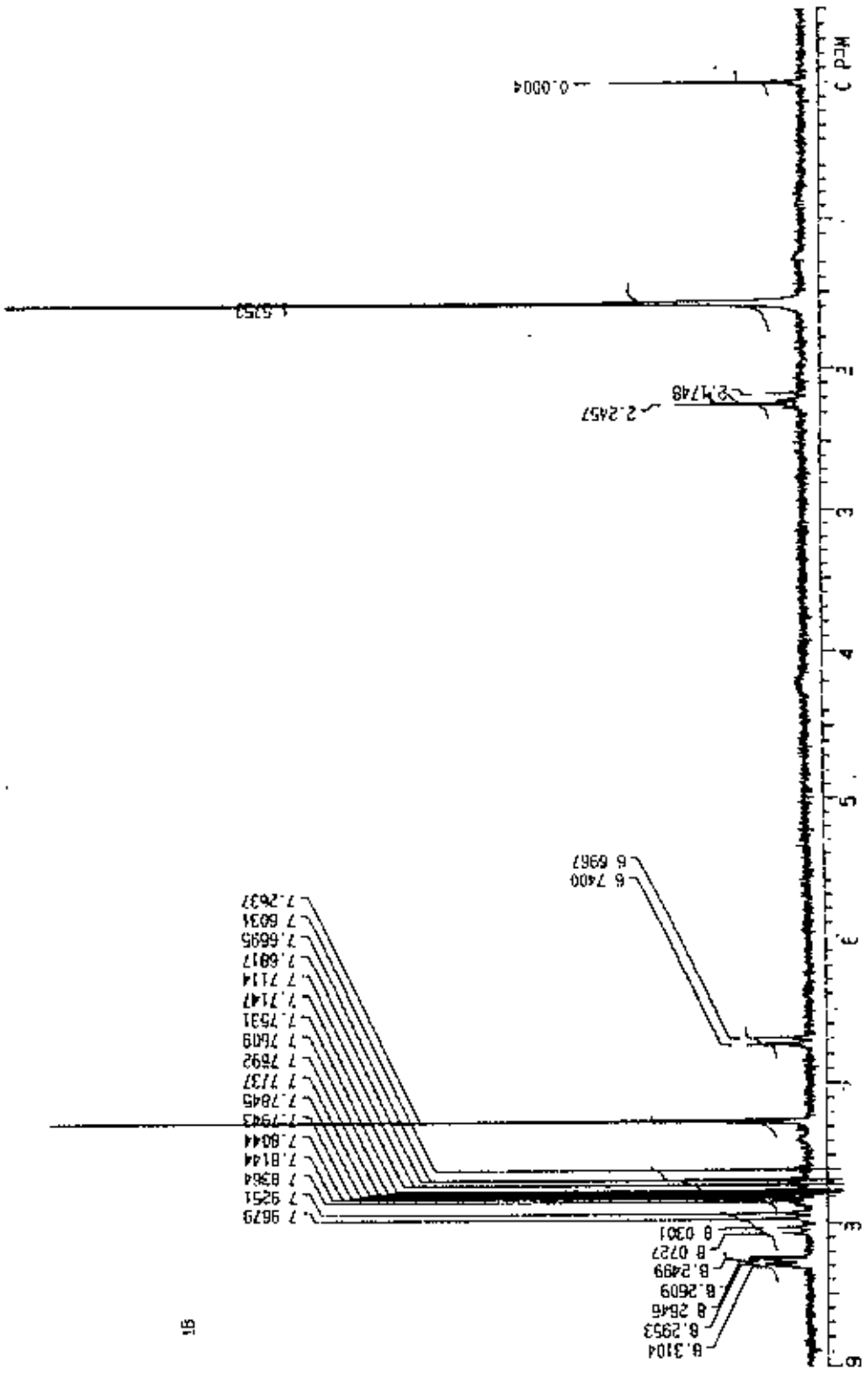


Figure 3 : ^{1}H NMR Spectrum of the compound 1B

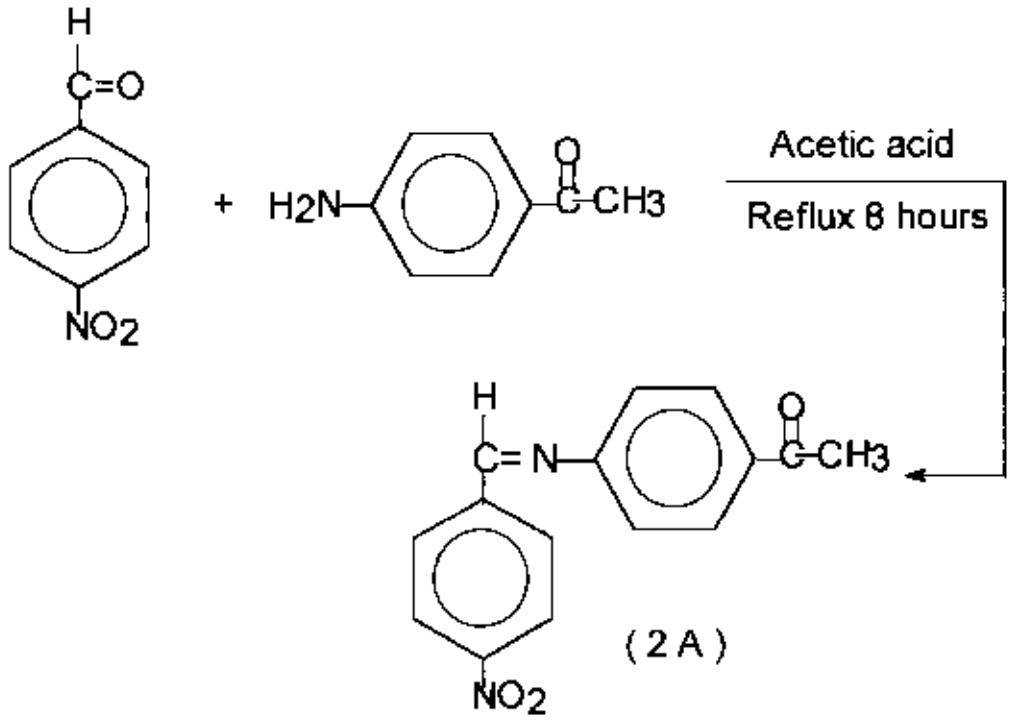
Sample	分析量	C			H			N		
	(mg)	予測値	測定値	Δ%C	予測値	測定値	Δ%H	予測値	測定値	Δ%N
Acetoanilide	2.125	71.09	71.16	0.07	6.71	6.78	0.07	10.36	10.38	0.02
1a	2.130	67.16	66.52	−0.64	4.51	4.42	−0.09	10.44	10.29	−0.15
1b	2.337	56.29	39.36	−16.93	4.43	4.96	0.53	20.51	22.79	2.28
2a	2.162	67.16	66.12	−1.04	4.51	3.73	−0.78	10.44	10.20	−0.24
2b	2.233	56.29	57.16	0.87	4.43	4.50	0.07	20.51	15.19	−5.32
3a	2.132	84.25	72.48	−11.77	5.72	6.38	0.66	4.68	9.21	4.53
3b	2.173	70.94	54.79	−16.15	5.40	5.91	0.51	15.04	15.92	0.88
4a	2.088	81.06	68.46	−12.60	5.44	4.25	−1.19	6.30	4.89	−1.41
4b	2.047	61.72	46.70	−15.02	4.86	4.02	−0.84	17.99	18.35	0.36

The elemental analysis of the compound 1B showed almost similar to theoretical value for H and N but variation was observed in case of C. This type of variation may be occurred in case of nitrogenous compound staying for a long time. So, we can give the calculated value for the componnd 1B.

Table 2 : Elemental analysis of the compound 1B

3.3 Synthesis and characterization of 4 (*p*-nitrobenzimino) acetophenone (2A)

A mixture of *p* - nitrobenzaldehyde (0.604 gm ; 4 m mol) and 4-amino-acetophenone (0.540 gm ; 4 m mol) was refluxed for 8 hours in acetic acid. The reaction mixture was cooled to room temperature and acetic acid was removed by rotary evaporator under reduced pressure. The resulting solid mass was recystallized several times from ethylacetate as reddish yellow crystal (**2A**), yield 72%, m. p. 105-106^{0}C, R_f 0.67 in ethylacetate : pet - ether (1 : 2) solvent system.



Its ^{1}H NMR spectrum (300 MHz, $CDCl_3$, Fig. 4) with a sharp singlet at δ 1.57 integrating for three protons were ascribable for methyl group. The multipet at δ 6.72 was assigned for one imino proton. The multiplet was due to the long range coupling of ortho proton of aromatic ring. A broad multiplet ranging from δ 7.98 - 7.59 were attributable for aromatic protons.

The elemental analysis (Table - 3) of the compounced (**2A**) were found as C, 67.16; H, 4.51; N, 10.44 ; O, 17.89 ; which were indicative for molecular formula $C_{15}H_{12}N_2O_3$.

Therefore, the ^1H NMR spectrum and elemental analysis of the compound 2A confirmed the desired structure and it was named as 4 (*p* - nitrobenz-imino) acetophenone.

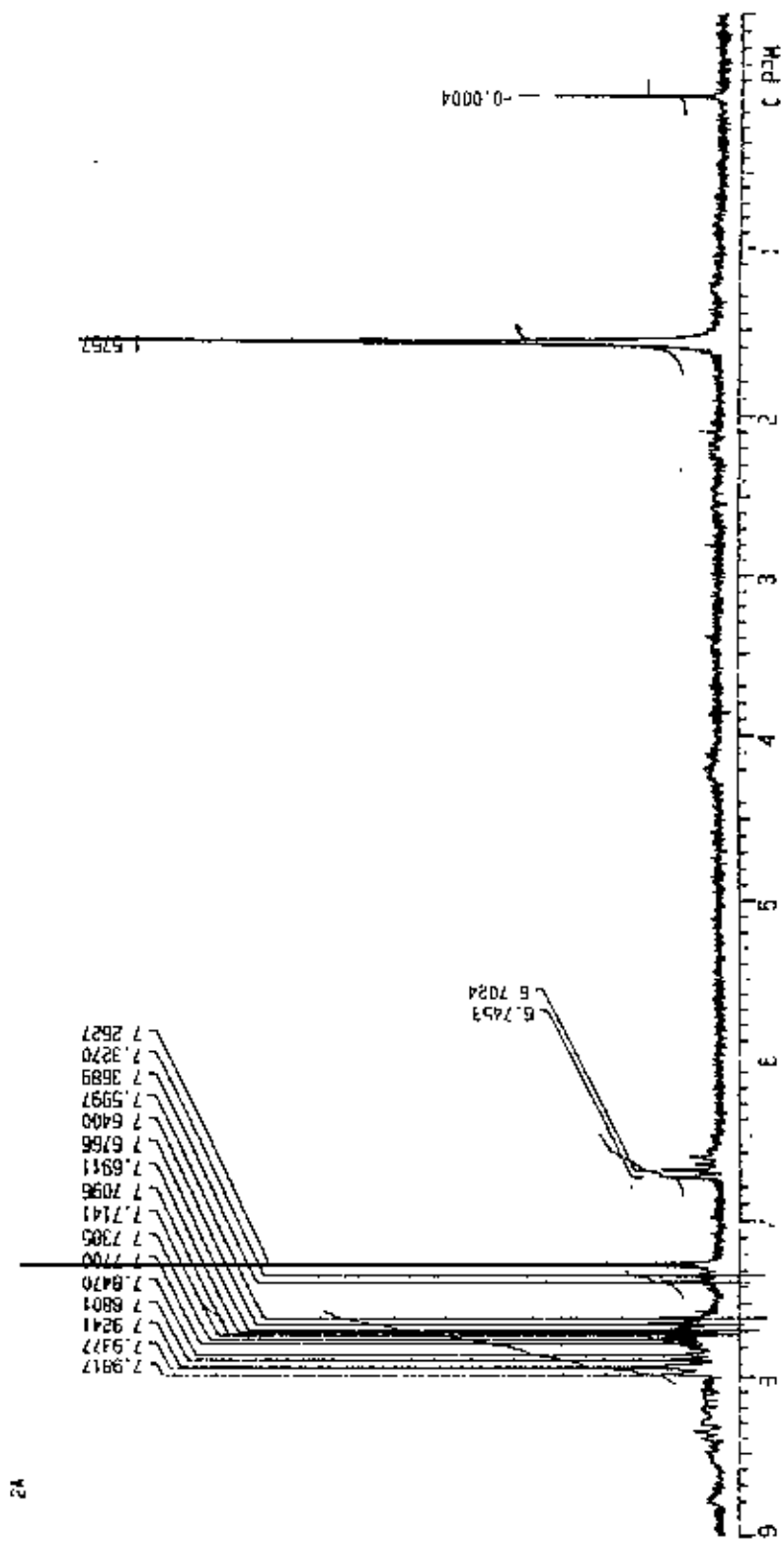


Figure 4 : ^{1}H NMR Spectrum of the compound 2A

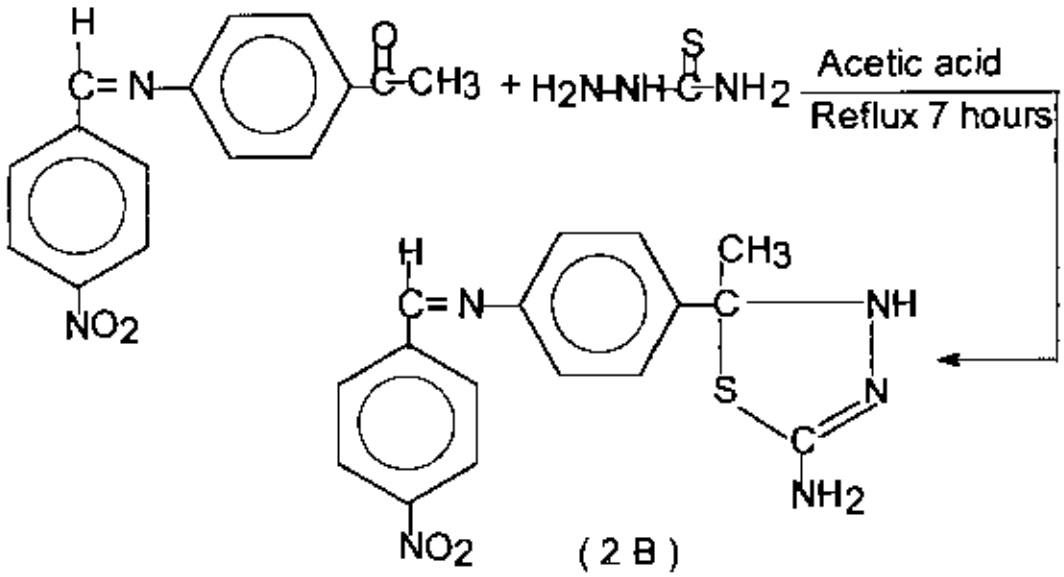
Sample	分析量	C			H			N		
	(mg)	予測値	測定値	Δ%C	予測値	測定値	Δ%H	予測値	測定値	Δ%N
Acetoanilide	2.125	71.09	71.16	0.07	6.71	6.78	0.07	10.36	10.38	0.02
1a	2.130	67.16	66.52	−0.64	4.51	4.42	−0.09	10.44	10.29	−0.15
1b	2.337	56.29	39.36	−16.93	4.43	4.96	0.53	20.51	22.79	2.28
2a	2.162	67.16	66.12	−1.04	4.51	3.73	−0.78	10.44	10.20	−0.24
2b	2.233	56.29	57.16	0.87	4.43	4.50	0.07	20.51	15.19	−5.32
3a	2.132	84.25	72.48	−11.77	5.72	6.38	0.66	4.68	9.21	4.53
3b	2.173	70.94	54.79	−16.15	5.40	5.91	0.51	15.04	15.92	0.88
4a	2.088	81.06	68.46	−12.60	5.44	4.25	−1.19	6.30	4.89	−1.41
4b	2.047	61.72	46.70	−15.02	4.86	4.02	−0.84	17.99	18.35	0.36

2a	C	12.011	15	180.165	67.16
	H	1.008	12	12.096	4.51
	N	14.0067	2	28.0134	10.44
	O	15.999	3	47.997	17.89
	S	32.06	0	0	0.00
	MW			268.2714	100.00

Table 3 : Elemental analysis of the compound 2A

3.4 Synthesis and characterization of 5-methyl -5 - [4′ (*p*-nitrobenz-imino)] phenyl - 2 - amino - Δ^2-1, 3, 4 - thiadiazoline (2B)

A mixture of 4 (*p* - nitrobenzimino) acetophenone (1.340 gm ; 5 m mol) and thiosemicarbazide (0.455 gm ; 5 m mol) was refluxed for 7 hours in glacial acetic acid (5ml). The reaction mixture was cooled to room temperature and acetic acid was removed by rotary evaporator under reduced pressure and the resulting solid mass was recrystallized several times from ethylacetate as yellow crystal (**2B**), yield 68%, m. p. 140^{0}C, R_f 0.65 in ethylacetate : pet- ether (2 : 1) solvent system.



(2 B)

Its ^{1}H NMR spectrum (300 MHz, $CDCl_3$, Fig. 5) showed sharp singlet at δ 1.58 integrating for three protons assignable for methyl group. A broad peak at δ 2.24 was ascribable for two proton of the NH_2 group. The multiplet centered at δ 6.70 was attributable for one imino proton. The multiplet was

due to the long range ortho coupling of aromatic ring. The peak at δ 8.25 was assignable for one NH proton. A broad multiplet ranging from δ 7.96 - 7.66 was attributable for aromatic protons.

The elemental analysis (Table 4) of the compound **2B** were found to be C, 56.29 ; H : 4.43 ; N, 20.51 ; O, 9.37 ; S, 9.39, which were suggestive for the molecular formula $C_{16}H_{13}N_5O_2S$.

The ^1H NMR spectrum and elemental analysis confirmed compound (**2B**) to be 5 - methyl - 5 - [4′ (*p* - nitrobenzimino)] phenyl - 2 - amino - Δ^2 1, 3, 4- thiadiazoline.

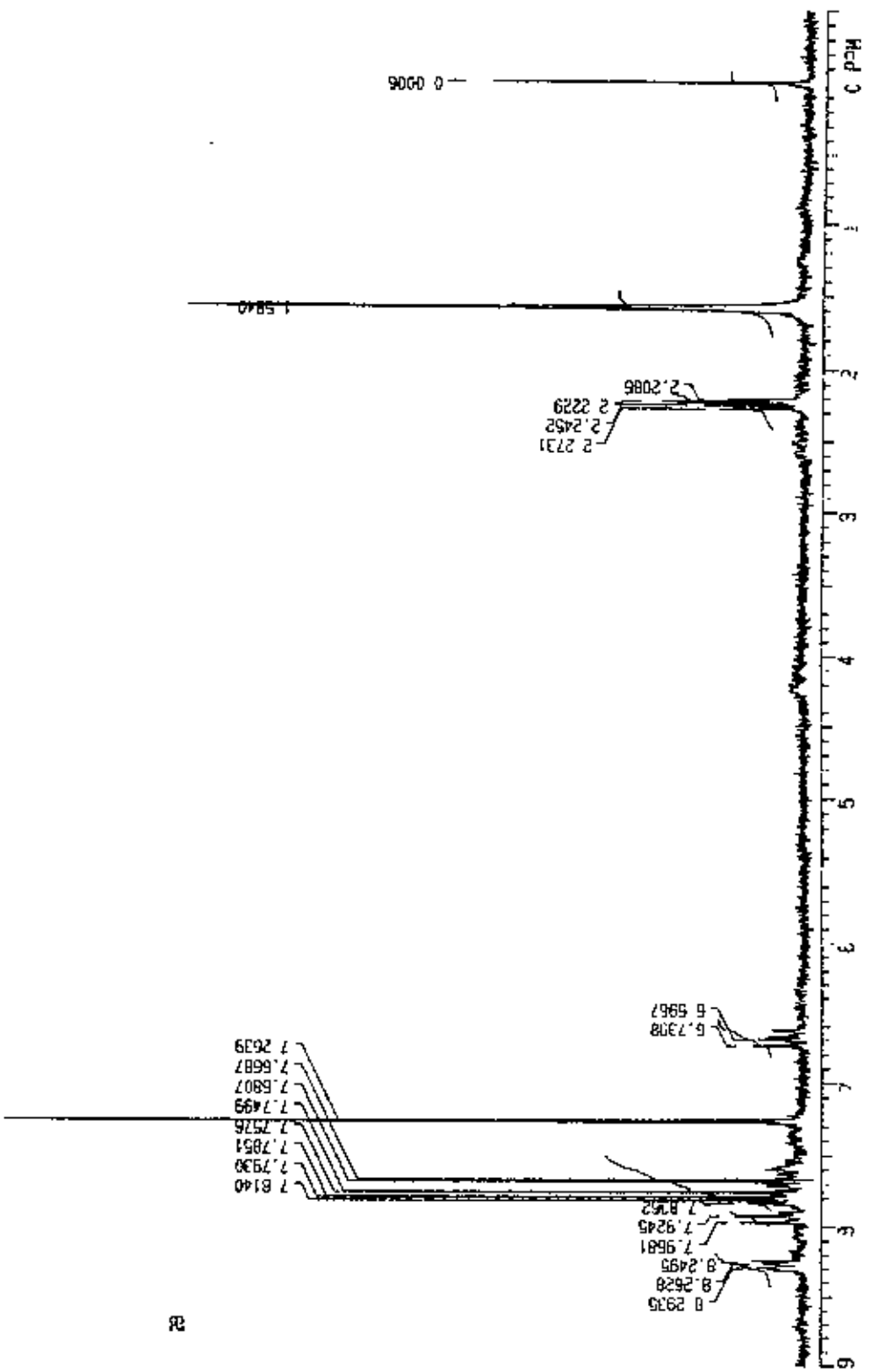


Figure 5 : ^{1}H NMR Spectrum of the compound 2B

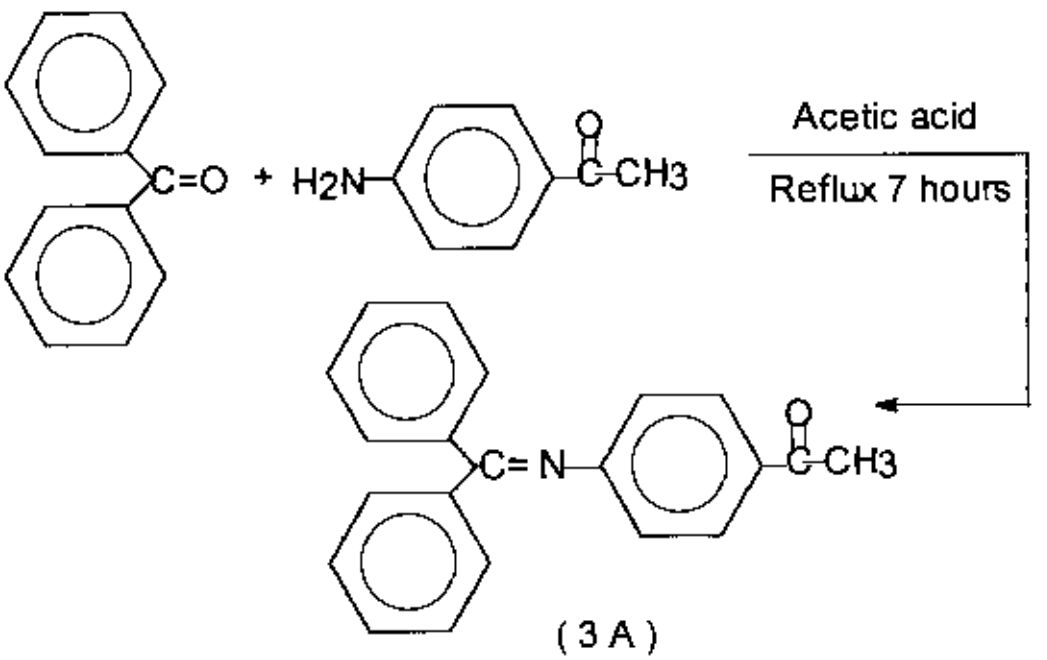
Sample	分析量	C			H			N		
	(mg)	予測値	測定値	Δ%C	予測値	測定値	Δ%H	予測値	測定値	Δ%N
Acetoanilide	2.125	71.09	71.16	0.07	6.71	6.78	0.07	10.36	10.38	0.02
1a	2.130	67.16	66.52	−0.64	4.51	4.42	−0.09	10.44	10.29	−0.15
1b	2.337	56.29	39.36	−16.93	4.43	4.96	0.53	20.51	22.79	2.28
2a	2.162	67.16	66.12	−1.04	4.51	3.73	−0.78	10.44	10.20	−0.24
2b	2.233	56.29	57.16	0.87	4.43	4.50	0.07	20.51	15.19	−5.32
3a	2.132	84.25	72.48	−11.77	5.72	6.38	0.66	4.68	9.21	4.53
3b	2.173	70.94	54.79	−16.15	5.40	5.91	0.51	15.04	15.92	0.88
4a	2.088	81.06	68.46	−12.60	5.44	4.25	−1.19	6.30	4.89	−1.41
4b	2.047	61.72	46.70	−15.02	4.86	4.02	−0.84	17.99	18.35	0.36

The elemental analysis of the compound 2B showed almost similar to theoretical value for H and N but variation was observed in case of C. This type of variation may be occurred in case of nitrogenous compound staying for a long time and it was observed by the fading of colour. So, we can give the calculated value for the compound 2B.

Table 4 : Elemental analysis of the compound 2B

3.5 Synthesis and characterization 4 (diphenylimino) acetophenone (3A)

An equimolecular mixture of benzophenone (0.362gm ; 2 m mole) and 4 - aminoacetophenone (0.270 gm ; 2 m mole) was refluxed for 7 hours in glacial acetic acid. The reaction mixture was cooled to room temperature and the acetic acid was removed by the rotary evaporator under reduced pressure and the resulting solid mass was recrystallized several times from ethylacetate as grayish crystal (**3A**), yield 62 %, m. p. 85-87^{0}C. R_f 0.71 in ethylacetate : pet - ether (1 : 2) solvent system.



(3 A)

Its elemental analysis (Table : 5) were found to be C, 84.25 ; H, 5.72 ; N, 4.68; O, 5.34, which were suggestive for the structure named as 4 (diphenyl- imino) acetophenone.

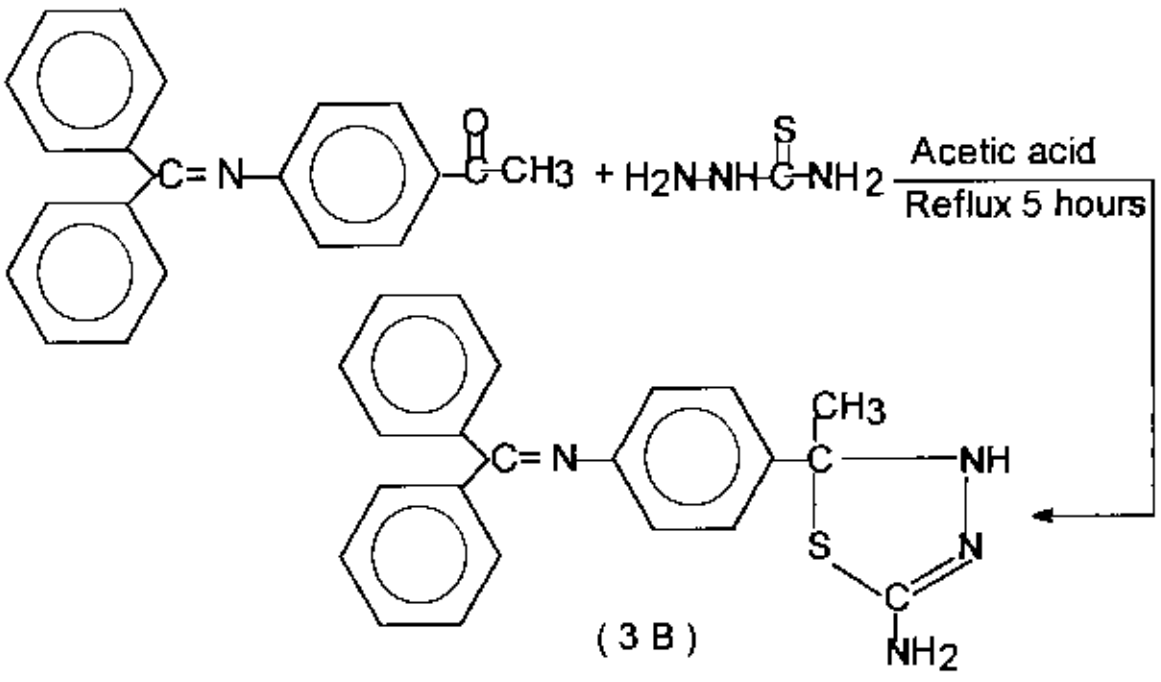
Sample	分析量	C			H			N		
	(mg)	予測値	測定値	Δ%C	予測値	測定値	Δ%H	予測値	測定値	Δ%N
Acetoanilide	2.125	71.09	71.16	0.07	6.71	6.78	0.07	10.36	10.38	0.02
1a	2.130	67.16	66.52	−0.64	4.51	4.42	−0.09	10.44	10.29	−0.15
1b	2.337	56.29	39.36	−16.93	4.43	4.96	−0.53	20.51	22.79	2.28
2a	2.162	67.16	66.12	−1.04	4.51	3.73	−0.78	10.44	10.20	−0.24
2b	2.233	56.29	57.16	0.87	4.43	4.50	0.07	20.51	15.19	−5.32
3a	2.132	84.25	72.48	−11.77	5.72	6.38	0.66	4.68	9.21	4.53
3b	2.173	70.94	54.79	−16.15	5.40	5.91	0.51	15.04	15.92	0.88
4a	2.088	81.06	68.46	−12.60	5.44	4.25	−1.19	6.30	4.89	−1.41
4b	2.047	61.72	46.70	−15.02	4.86	4.02	−0.84	17.99	18.35	0.36

The elemental analysis of the compound 3A was found similar to that of theoretical value for H but variation were observed in case of C and N. This type of variation may be occurred due to the presence of moisture sensitive C=N moiety in the compound. So, we can give the calculated value for the compound 3A.

Table 5 : Elemental analysis of the compound 3A

3.6 Synthesis and characterization of 5-methyl - 5 - [4' (diphenyl-imino)| phenyl - 2 - amino - Δ^2-1, 3, 4 thiadiazoline (3B)

An equimolecular mixture of 4 (diphenylimino) acetophenone (1.196gm ; 4 m mmol) and thiosemicarbazide (0.364gm ; 4 m mol) was refluxed for 5 hours in acetic acid (5ml). The reaction mixture was cooled to room temperature and acetic acid was removed by the rotary evaporator under reduced pressure and the resulting solid mass was recrystallized several times from ethylacetate as grayish crystal, yield 65%. m.p. 90^0C, R_f 0.68 in pet - ether : ethyl acetate (2 : 1) solvent system.



(3 B)

Its IR spectrum (Fig : 6) showed a broad absorption band ranging from 3360 - 3209 cm^{-1} indicative of NH_2 or NH groups. The absorption band at 3090 -3116 cm^{-1} were C - H stretchiug for aromatic C - H and immo C - H. Very weak absorption band was observed at 2975 cm^{-1} for aliphatic C - H stretching. The bands at 1674 cm^{-1} were suggestive of C = N moiety.

The next sharp consecutive bands at 1602, 1590 at 1490 cm^{-1} were ascribable for the C = C bond of aromatic ring.

The ^1H NMR spectrum (300 MH_3, $CDCl_3$, Fig : 7) showed a broad singlet at δ 1.57 integrating three protons were suggestive for methyl group. A singlet at δ 2.20 integrating two protons were attributable for NH_2 moiety. A broad multiplet at δ 7.66 - 7.56 were assignable for aromatic protons. A peak at δ 7.96 integrating one proton was ascribable for NH proton.

Elemental analysis (Table : 6) were found to be C, 70.94 ; H, 5.41 ; N, 15.04 ; S, 8.61. Which were suggestive for the molecular formula $C_{22}H_{20}N_4S$.

Therefore, IR spectrum, ^1H NMR spectrum and elemental analysis were expressed harmony for the strueture named as 5 - methyl - 5 - [4' (diphyl-imino)] phenyl - 2 - amino - Δ^2 1, 3, 4 - thiadiazoline.

Sample	分析量	C			H			N		
	(mg)	予測値	測定値	Δ%C	予測値	測定値	Δ%H	予測値	測定値	Δ%N
Acetoanilide	2.125	71.09	71.16	0.07	6.71	6.78	0.07	10.36	10.38	0.02
1a	2.130	67.16	66.52	−0.64	4.51	4.42	−0.09	10.44	10.29	−0.15
1b	2.337	56.29	39.36	−16.93	4.43	4.96	0.53	20.51	22.79	2.28
2a	2.162	67.16	66.12	−1.04	4.51	3.73	−0.78	10.44	10.20	−0.24
2b	2.233	56.29	57.16	0.87	4.43	4.50	0.07	20.51	15.19	−5.32
3a	2.132	84.25	72.48	−11.77	5.72	6.38	0.66	4.68	9.21	4.53
3b	2.173	70.94	54.79	−16.15	5.40	5.91	0.51	15.04	15.92	0.88
4a	2.088	81.06	68 46	−12.60	5.44	4.25	−1.19	6.30	4.89	−1.41
4b	2.047	61.72	46.70	−15.02	4.86	4.02	−0.84	17.99	18.35	0.36

The elemental analysis of the compound 3B showed almost similar for that of theoretical value for H and N but variation was observed in case of C. This type of variation may be occurred in case of nitrogenous compound staying for a long time. So, we can give the theoretical value for the compound 3B.

Table 6 : Elemental analysis of the compound 3B

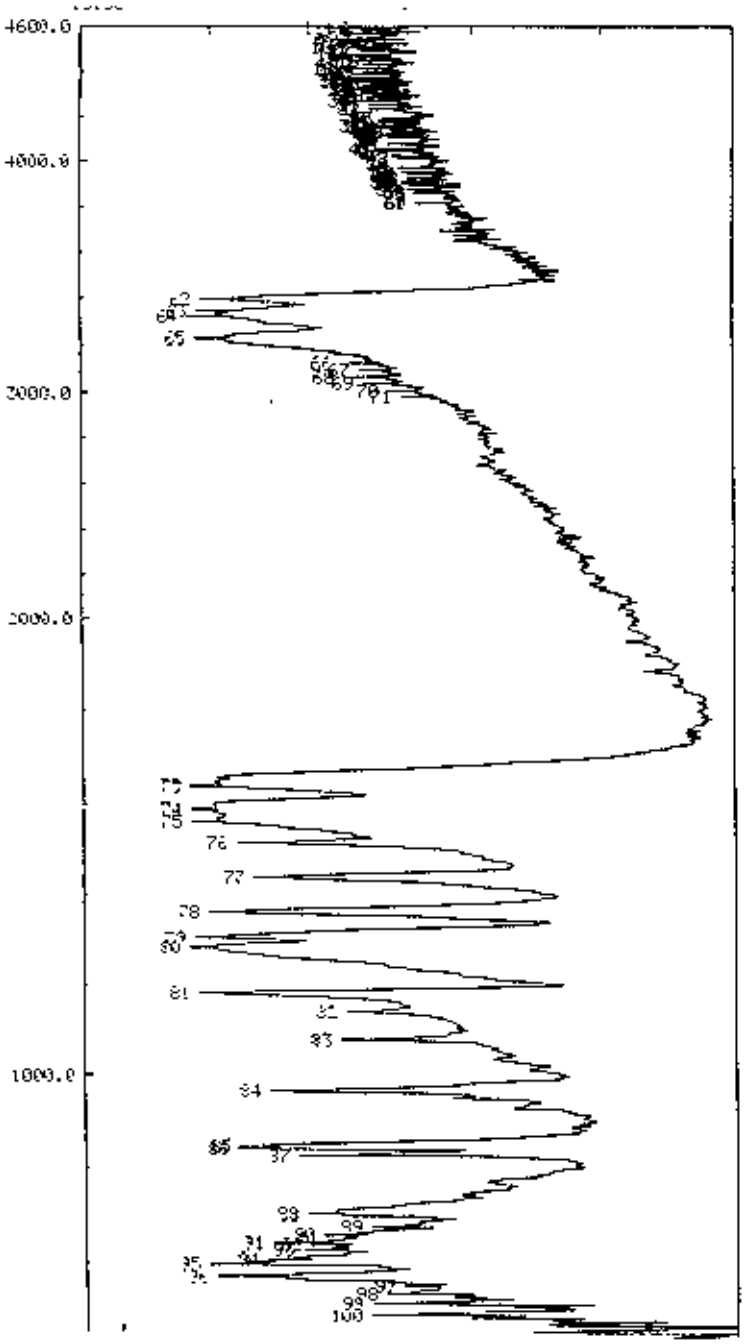


Figure 6 : IR Spectrum of the compound 3B

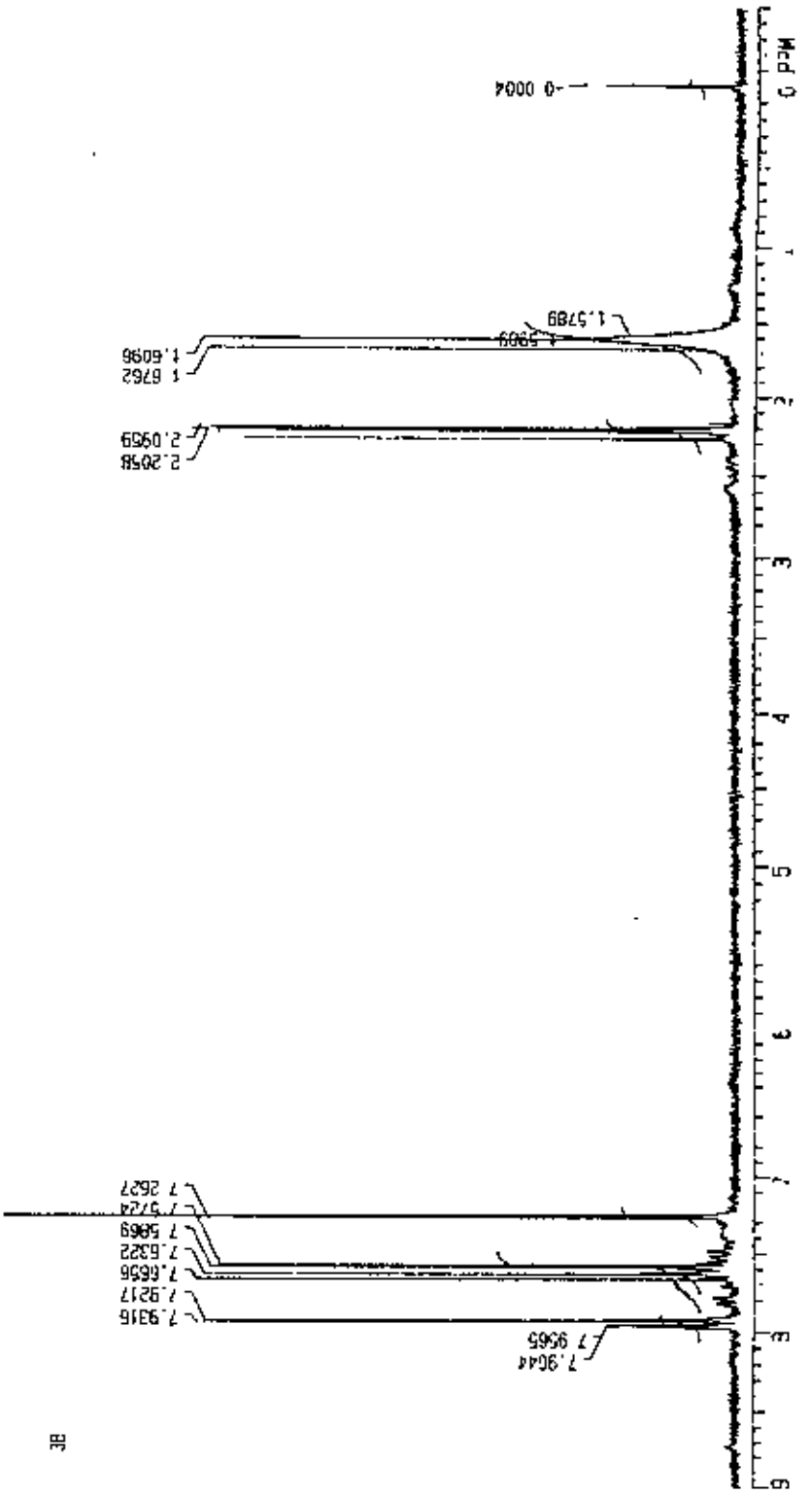
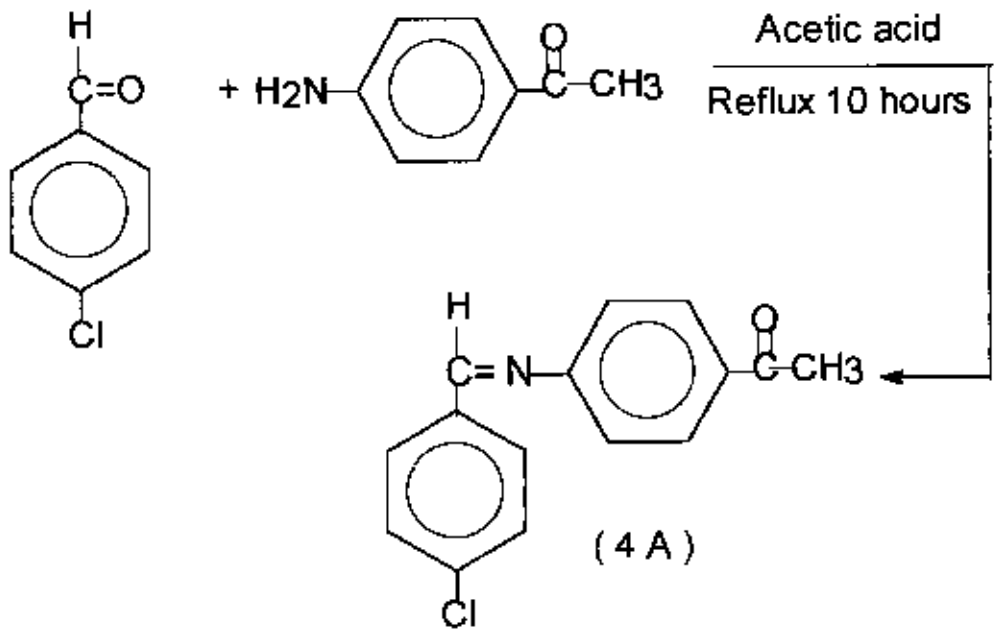


Figure 7 : ^{1}H NMR Spectrum of the compound 3B

3.7 Synthesis and characterization of 4 (*p*-chlorobenzimino) acetophenone (4A)

A mixture of *p* - chlorobenzaldeylde (0.562g ; 4 m mol) and 4 aminoacto-phenone (0.540 gm ; 4 m mol) was refluxed for 10 hours in acetic acid (10ml). The reaction mixture was cooled to room temperature and acetic acid was removed by rotary evaporator under reduced pressure and the resulting solid mass was recrystallized several times from ethylacetate as reddish crystal solid, yield 70 %, m. p. 125^{0}C, R_f 0.65 in ethylacetate : pet - ether (1 : 2) solvent system.



(4 A)

Its ^{1}H NMR spectrum (300 MHz, $CDCl_3$, Fig : 8) showed a broad signal at δ 1.7 integrating three proton were ascribable for methyl moicty. The doublet at δ 6.6 integrating one proton were attributable for imino proton. The doublet were due to the long range coupling of ortho proton of the

aromatic ring. The multiplet ranging at δ 7.5-7 were assignable for aromatic proton.

Elemental analysis (Table 7) were found to be C, 69.90 ; H, 4.66 ; N, 5.43 ; O, 6.21; Cl, 13.80 ; which were suggestive for the molecular formula $C_{15}H_{12}NOCl$.

The ^1H NMR spectrum and elemental analysis confirmed (**4A**) to be the desired structure named as 4 (*p*-chlorobenzimino) aceptophenone.

Sample	分析量	C			H			N		
	(mg)	予測値	測定値	Δ%C	予測値	測定値	Δ%H	予測値	測定値	Δ%N
Acetoanilide	2.125	71.09	71.16	0.07	6.71	6.78	0 07	10.36	10.38	0.02
1a	2.130	67.16	66.52	−0.64	4.51	4.42	−0.09	10.44	10.29	−0.15
1b	2.337	56.29	39.36	−16.93	4.43	4.96	0.53	20.51	22.79	2.28
2a	2.162	67.16	66.12	−1.04	4.51	3.73	−0.78	10.44	10.20	−0.24
2b	2.233	56.29	57.16	0.87	4.43	4.50	0 07	20.51	15.19	−5.32
3a	2.132	84.25	72.48	−11.77	5.72	6.38	0.66	4.68	9.21	4.53
3b	2.173	70.94	54.79	−16.15	5.40	5.91	0.51	15.04	15.92	0.88
4a	2.088	81.06	68.46	−12 60	5.44	4.25	−1.19	6.30	4.89	−1.41
4b	2.047	61.72	46.70	−15.02	4.86	4.02	−0.84	17.99	18.35	0.36

The elemental analysis of the compound 4A showed almost similar to that of calculated value for H and N but variation was observed for C. This type of variation may be occurred in case of nitrogenons compound staying for a long time and it was noticed by the fading of colour. Therefore, we can give the theoretical value of the compound 4A.

Table 7 : Elemental analysis of the compound 4A

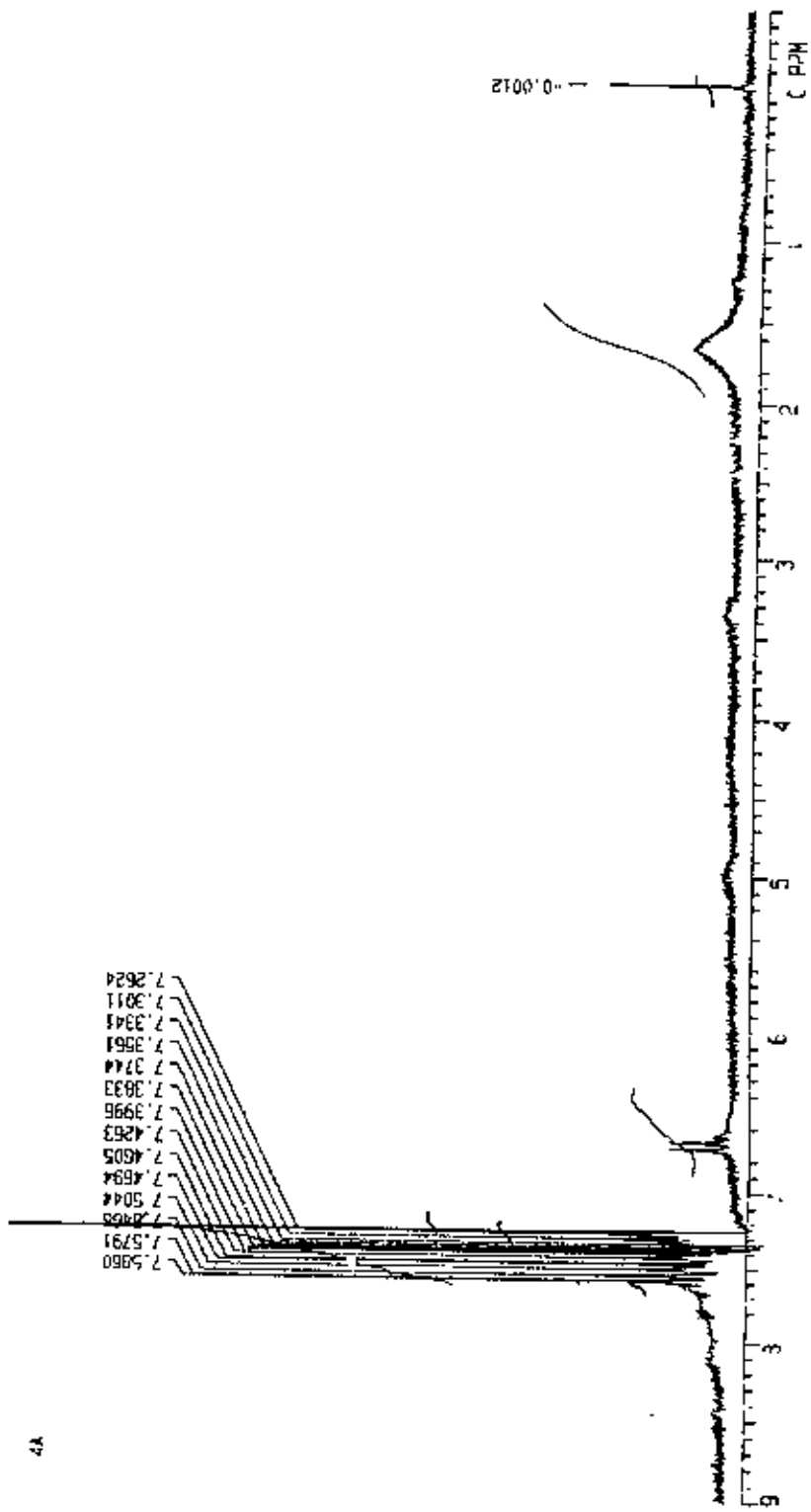
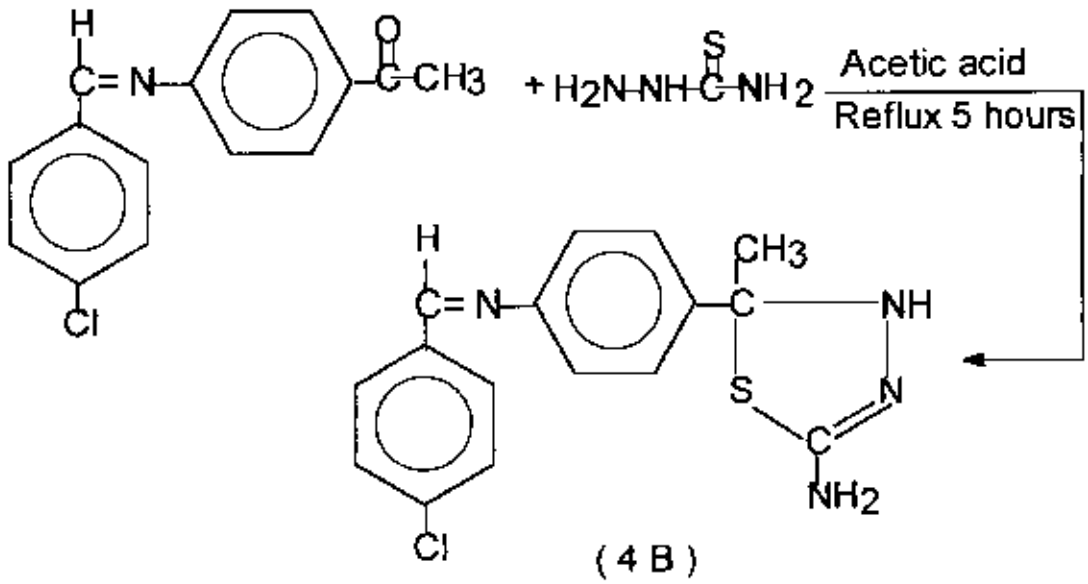


Figure 8 : ^{1}H NMR Spectrum of the compound 4A

3.8 Synthesis and characterizations of 5 - methyl - 5 - [4′ (*p* - chloro - benzimino)] phenyl - 2 - amino - Δ^2 - 1, 3, 4 - thiadiazoline (4B)

An equimolecular mixture of 4 (*p*-chlorobenzimino) acetophenone (1.030 gm; 4 m mol) and thiosemicarbazide (0.364 gm ; 4 m mol) was refluxed for 5 hours in glacial acetic acid (7 ml). The reaction mixture was cooled to room temperature and acetic acid was removed by rotary evaporator under reduced pressure. The resulting solid mass was recrystallized several times from ethylacetate furnished reddish colored crystal, yield 65%, m. p. 140-142^{0}C, R_f 0 56 in ethylacetate : pet - ether (1 : 2) solvent system.



(4 B)

Its IR spectrum (Fig : 9) showed a broad absorption band at 3347-3317 cm^{-1} were ascribable for NH group. The weak absorption band at 3190 cm^{-1} was attributed for C - H stretching of imino group. A weak band also showed at 3053 cm^{-1} were suggestive for C - H stretching of aromatic moiety. Another

weak band at 2971 cm^{-1} were indicative for C-H stretching of aliphatic moiety. The two sharp bands at 1464 and 1655 cm^{-1} were suggestive for two C = N bonds. The aromatic C = C bonds were assigned from the band at 1594, 1529 and 1490 cm^{-1}. The C - Cl bending vibration was observed at 819 cm^{-1}. But IR showed no C = S stretching absorption.

The ^1H NMR spectrum (300 MH_3, $CDCl_3$, Fig : 10) showed sharp singlet at δ 1.62 integrating three protons for methyl group. Two protons were attributed for NH_2 group at δ 2.27. A multiplet of one proton was ascribable for imino group at δ 6.6 . The multiplet was observed due to the long range ortho coupling of aromatic ring. A broad multiplet were suggestive for aromatic proton at δ 7.61 - 7.26 . One proton integration at δ 7.92 was assignable for NH group.

Therefore, based on the IR spectrum and ^1H NMR spectrum confirmed **(4B)** to be the desired compound named as 5 - methyl - 5 - [4' (*p* - chlorobenz-imino)] phenly - 2 - amino - Δ^2 1, 3, 4 - thiadiazoline.

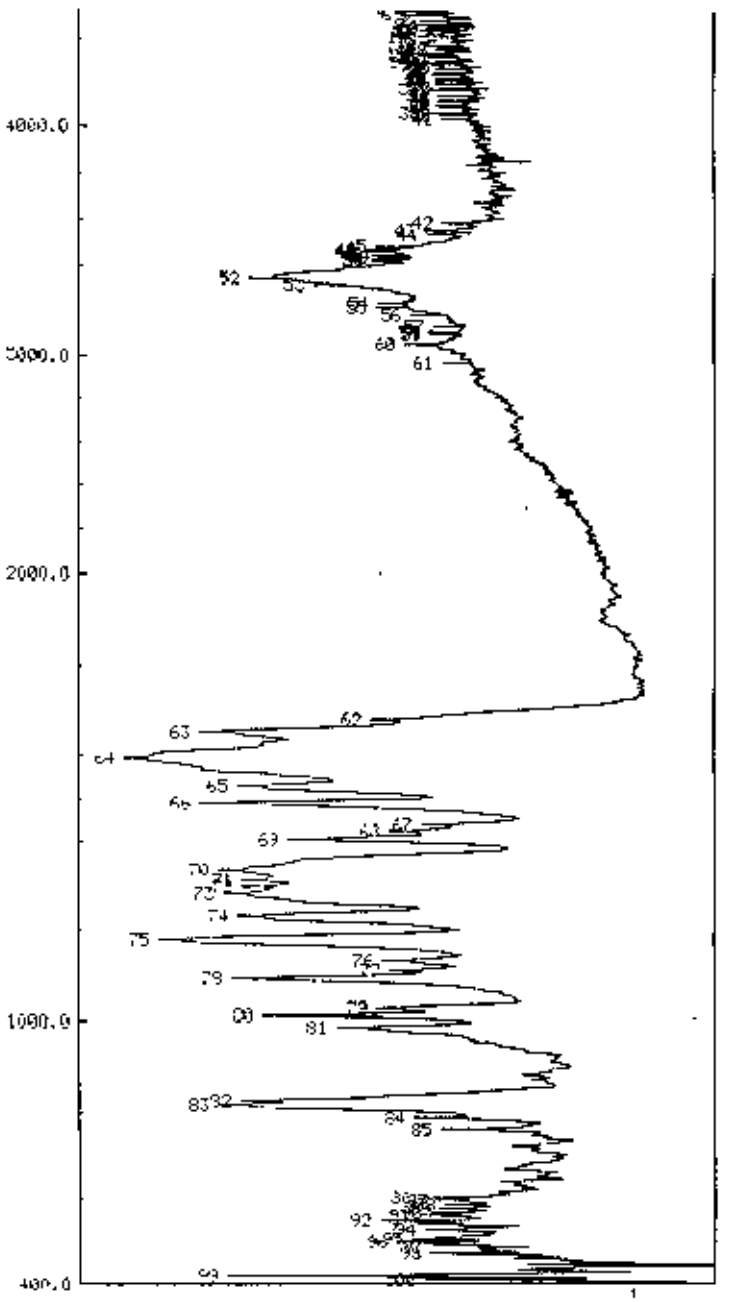


Figure 9 : IR Spectrum of the compound 4B

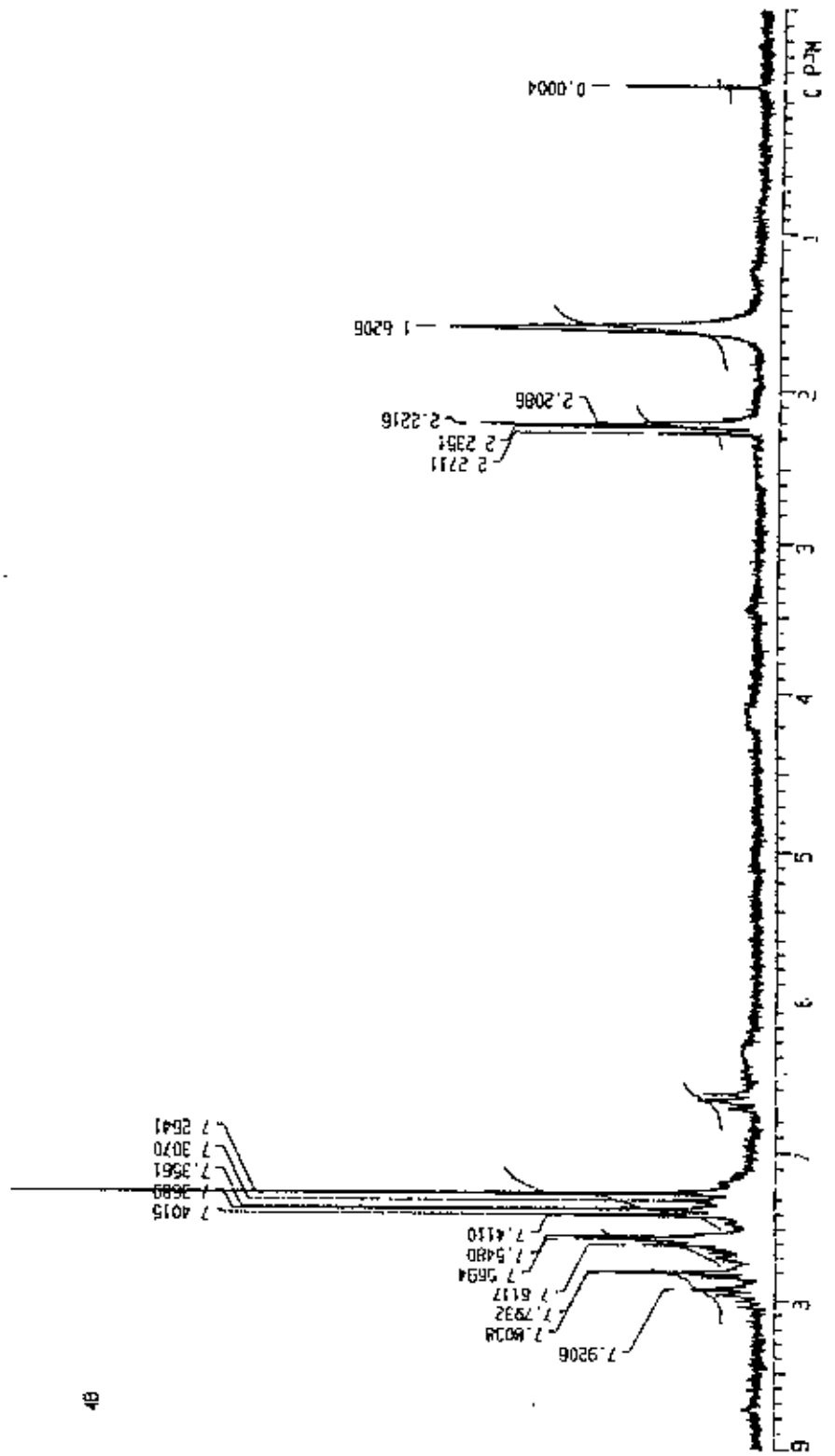


Figure 10 : ^{1}H NMR Spectrum of the compound 4B

CHAPTER - 4

PHARMACOLOGICAL STUDIES

ANTIBACTERIAL SCREENING

4.1 Introduction :

To ascertain antibacterial spectrum of an agent against various types of pathogenic organisms, antimicrobial screening is necessary. The susceptibility of microorganisms to antimicrobial agents can be measured ***in vitro*** by a number of techniques among which the disc diffusion method. using different concentrations of the agents absorbed on sterile filter paper disc, is widely acceptable for the preliminary evaluation of antimicrobial activity.

Disc diffusion technique is essentially a qualitative or semiquantitative test indicating the sensitivity or resistance of microorganism to the test material. However, no distinction between bacteriostatic and bactericidal activity can be measured by this method

4.2 Principle:

Dried and sterile filter paper discs (4-5 mm diameter) containing the test material are placed on nutrient agar plates seeded with test organism. These plates are then kept at low temperature (4°C) to allow maximum diffusion. A number of events take place simultaneously including

(a) The dried discs absorb water from the agar medium and the material under test is dissolved.

(b) The test material diffuses from the discs to the surrounding media. The diffusion takes place according to the physical law that controls the diffusion of molecules through agar gel.

(c) There is a gradual change nf test material concentration in the agar surrounding each disc. The plates are then kept in an incubator (37°C) for 12-18 hrs to allow the growth of the organisms. If the test material has any antimicrobial activity, it will inhibit the growth of microorganism giving a clear, distinct zone called zone of inhibition. The antibacterial activity of the test agent is determined by measuring the diameter of the zone if inhibition in the term of mm.

In the present study, the antibacterial activity of 5-methyl-5-[4'(p-chlrobenzimino)]phenyl-2-amino-Δ^2-1, 3. 4-thiadiazoline were investigated.

4.3 Apparatus and Reagents:

(1) Filter paper discs

(2) Nutrient agar media

(3) Petridishes

(4) Inoculating loop

(5) Sterile cotton

(6) Sterile forceps

(7) Micropipette

(8) Methanol

(9) Test tube

4.4 Microorganisms used for the activity test :

Both grams positive and gram negative bacterial strains were taken for the test. The strains used for this investigation are listed in the Table 8:

These bacterial strains were supplied in pure form.

Table 8 : List of Test Bacteria

Gram Positive	Gram Negative
1. ***Bacillus Subtills***	1. ***Shigella Sonnei***
2. ***Bacillus Cereus***	2. ***Shigella Shiga***
3. ***Streptococuss Faecelis***	3. ***Shigella Boydii***

The section criteria of the bacterial strains as test organism in their is their established potential in causing infections diseases in humans.

4.5 Culture Media:

Nutrient agar media is used to demonstrate the antibacterial activity and to make subculture of the tests organisms.

Composition of the Nutrient agar media

Ingredients	Amounts
Bactopeptone	0.5 gm
Sodium Chloride	0.5 gm
Bacto yeast extract	1.0 gm
Bacto Agar	2.0 gm
Distilled Water	100 ml
p^H	7.2 ± 0.1 at 25°C

4.6 The activity test:

Sample impregnated discs and standard antibiotic discs were placed gently on the solidified agar plates, freshly seeded wit h the test organisms with the help of a sterile forceps to assure complete contact with media surface. The spatial arrangement of the discs were such that the discs were not closer than 15 mm to the edge of the plate and for enough apart to prevent overlapping in the zones of inhibition. The plates were then inverted and kept in a refrigerator for about 24 hrs at 4°C. This is sufficient time for the material to diffuse to a considerable area of the media. Finally, the plates were incubated at 37°C for 12-18 hrs. After incubation, the antibacterial activity of the test agent was determined by measuring the diameter of inhibition zones in term of millimeter with a transparent scale.

4.7 Results of antibacterial activity test:

The thiadiazoline derivative showed significant antibacterial activity against most of the test organism. The result of the antibacterial activity. measured in term of zone of inhibition is shown in Table 9. The compound was used in two concentrations 400 µg/disc and 600 µg/disc. The zone of inhibition varied from 7 mm to 15 mm at a concentration of 400 µg/dise and from 8 mm to 18 mm at concentration of 600 µg/disc. The compouuds showed maximum activity against ***Bacillus Subtills, Bacillus Cereus, Shigella Shiga*** and moderate activity against ***Streptococcus faccalis***

Table 9 : *In Vitro* antibaeterial activity of thiadiazoline derivative

Bacterial Strains	Inhibition Zone (mm)		
	Thiadiazoline derivative		Doxycylin
	400 µg/disc	600 µg/disc	300 µg/disc
Gram Positive			
Bacillus Subtills	15	18	35
Bacillus Cereus	13	16	37
Streptococuss Faccealis	11	13	30
Gram Negative			
Shigella Sonnei	10	11	35
Shigella Shiga	13	15	35
Shigella Boydii	7	8	36

CHAPTER - 5

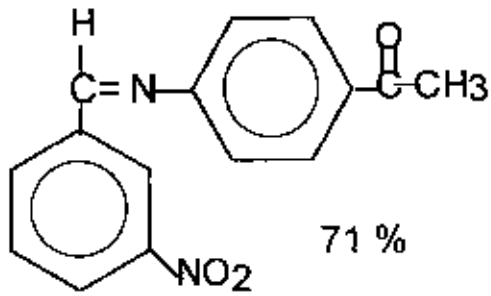
SUMMARY

SUMMARY

The work done in this dissertation may be summarized as follows :

1 A . 4 (*m* - Nitrobenzimino) acetophenone :

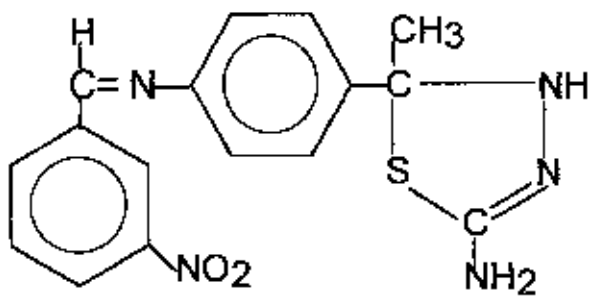
4 (*m* - nitrobenzimino) acetophenone was synthesized by the condensation of equimolar mixture of *m* - nitrobenzaldehyde and 4 - aminoacetophenone.



71 %

1 B . 5 - methyl - 5 [4' (*m* - nitrobenzimino)] phenyl-2-amino - Δ^2-1, 3, 4 - thiadiazoline :

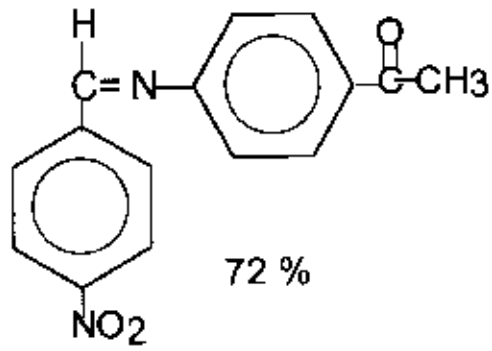
Condensation of equimolar mixture of 4 (*m* - nitrobenzimino) acetophenone and thiosemicarbazide afforded 5 - methyl-5 [4' (*m* - nitrobenzimino)] phenyl-2-amino - Δ^2 - 1, 3, 4 - thiadiazoline of moderate yield.



61 %

2A. 4 (*p* - Nitrobenzimino) acetophenone :

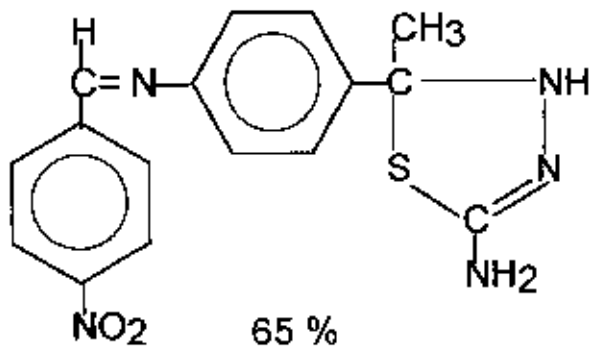
4 (*p* - nitrobenzimino) acetophenone was synthesized from the condensation of equimolar mixture of *p* - nitrobenzaldehyde and 4 amino-acetophenone



72 %

2B . 5 - methyl - 5 - [4' (*p* - nitrobenzimino)] phenyl - 2 - amino - Δ^2 - 1, 3, 4 - thiadiazoline :

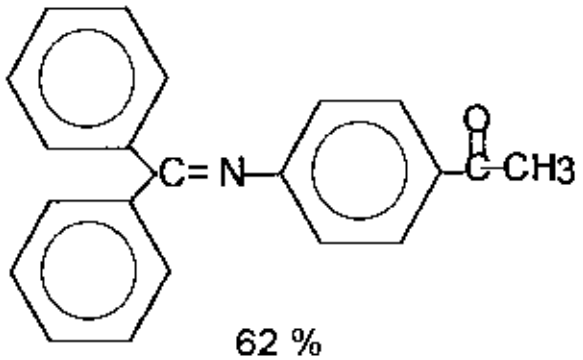
Equimolecular condensation of 4 (*p* - nitrobenzimino) acetophenone and thiosemicarbazide afforded 5 - methyl - 5 - [4' (*p* - nitrobenzimino)] phenyl - 2 - amino - Δ^2 - 1, 3, 4 - thiadiazoline.



65 %

3A . 4 (Diphenylimino) acetophenone :

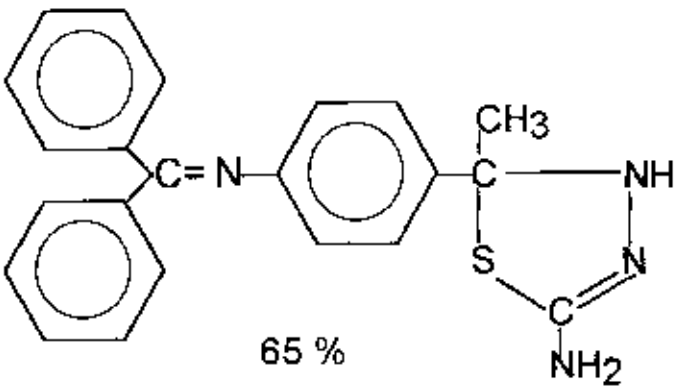
4 (Diphenylimino) acetophenone was synthesized from the equimolecular condensation of benzophenone and 4 - aminoacetophenone.



62 %

3 B . 5 - methyl - 5 - [4' (diphenylimino)] phenyl - 2 - amino - Δ^2-1, 3, 4 - thiadiazoline :

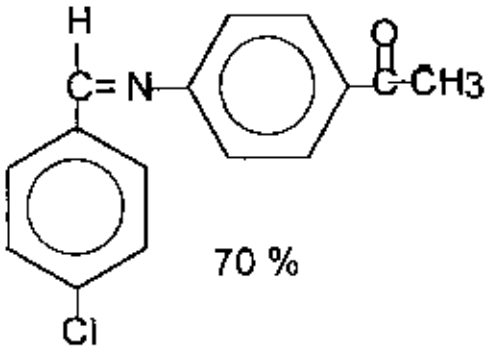
Condensation of equimolecular mixture of 4 (diphenylimino) acetophenone and thiosemcarbazide yielded 5 - methyl - 5 - [4' (diphenylimino)] phenyl - 2 - amino - Δ^2 - 1, 3, 4 - thiadiazoline of good yield.



65 %

4 A . 4 (*p* - Chlorobenzimino) acetophenone :

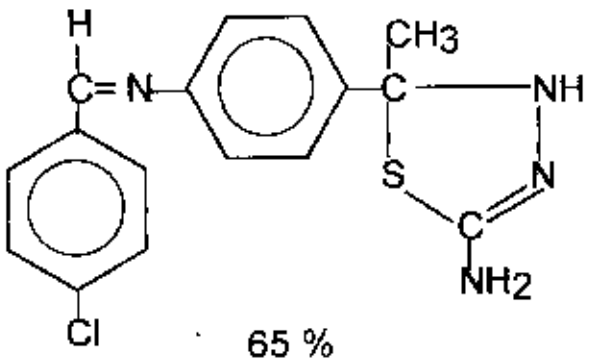
4 (*p* - chlorobenzimino) acetophenone was synthesized from the equimolar condensation of *p* - chlorobenzaldeylde and 4 aminoactophenone.



70 %

4 B . 5 - metbyl - 5 - [4′ (*p* - chlorobenzimino)] pbenyl - 2 - amino - Δ^2 - 1, 3, 4 - thiadiazoline :

Condensation of equimolar mixture of 4 (*p* - chlorobenzimino) acetophenone and thiosemicarbazide yielded 5 - methyl - 5 - [4′ (*p* - chlorobenz-imino)] phenyl - 2 - amino - Δ^2 - 1, 3, 4 - thiadiazoline.



65 %

5 . Pharmacological investigations have been carried out on all the synthesized thiadiazoline derivatives which are described in the chapter 4 .

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