SYNTHESIS OF SOME NEW ANNELATED FUSED PYRIMIDINE DERIVATIVES AND STUDY OF THEIR BIOLOGICAL ACTIVITIES

M. PHIL THESIS

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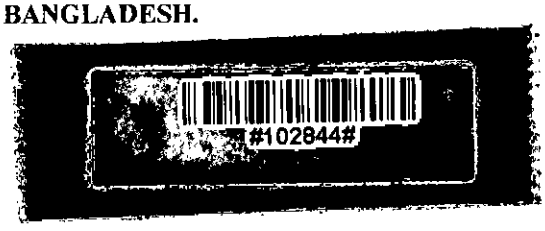

To New Generation of My Family

Saimoon, Mysha, Mahia, Samia, Tehemi ... ...

BANGLADESH UNIVERSITY OF ENGINEERING AND TECHNOLOGY, DHAKA, BANGLADESH
DEPARTMENT OF CHEMISTRY

THESIS ACCEPTANCE LETTER

The thesis titled **"Synthesis of Some New Annelated Fused Pyrimidine Derivatives and Study of Their Biological Activities"** Submitted by Morshed Ali. Roll No: 100103104F, Registration No: 0110035, Session: October'2001 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Master of Philosophy (M. Phil) on September 16, 2006.

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It is hereby declared that this thesis or any part of it has not been submitted elsewhere for the award of any degree or diploma.

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Date: 16th September 2006

.....

(MORSHED ALI)

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.....

Author

(***Morshed Ali***)

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ABBREVIATIONS

M.F.	Molecular Formula
NMR	Nuclear Magnetic Resonance
IR	Infra-red
MP.	Melting Point
BP.	Boiling Point
TLC	Thin Layer Chromatography
Toluyl	*p*-toluoylchloride
Ac	Acetyl
Ph	Phenyl
Bz	Benzoyl
^0C	Degree Celsius
Me	Methyl
Et	Ethyl
Et_3N	Triethylamine
Ph	Phenyl
DMF	*N,N*-dimethylformamide
THF	Tetrahydrofuran
DMSO	Dimethylsulfoxide
NBS	*N*-Bromosuccinimide
EMCA	Ethoxymethylene Cyanoacetate
Hrs.	Hours
Hz	Hertz
s	Singlet
d	Doublet
t	Triplet
q	Quartet
m	Multiplet

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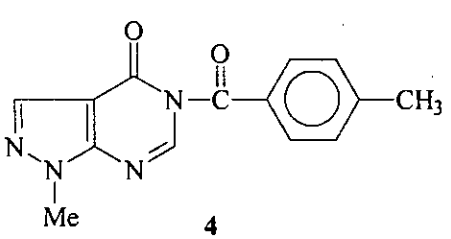
Title: Synthesis of Some New Annelated Fused Pyrimidine Derivatives and Study of their Biological Activities.

Abstract

The synthesis of the fused pyrimidine is divided into two headings: such as; annelated substrate and annelating reagent. The annelating substrate *ortho*-amino ester as ethyl-5-amino-1-methylpyrazol-4-carboxylate (**2**), which was prepared from ethyl(ethoxymethylene)cyanoacetate by using Gewald procedure and annelating reagents as 2-methylthio-2-methylthiazoline (**7**) and 2-methylthio-imidazoline (**11**), which were synthesized from ethanol amine (**5**) and diethylamine (**9**) by using Jensen & Hofmann method. The annelating substrate (**2**) was used in the synthesis of 1-methyl-4-oxo-5-(*p*-toluoyl)-pyrazolo[3,4-d]pyrimidin (**4**). The annelating substrate (**2**) was used to synthesize 1-methyl-6,7-dihydropyrazolo[3,4-d]thiazolo[1,2-a]pyrimidine-4-one (**8**) and 1-methyl-6,7-dihydropyrazolo[3,4-d]imidazo[1,2-a]pyrimidin-4(8H)-one (**12**) by one step reaction in dry acetic acid. The compounds ethyl-5-iodo-1-methylpyrazol-4-carboxylate (**13**), ethyl-5-phenylethynyl-1-methylpyrazol-4-carboxylate (**14**), ethyl-5-trimethylsilylethynyl-1-methylpyrazol-4-carboxylate (**15**), ethyl-5-propynylol-1-methylpyrazol-4-carboxylate (**16**) and ethyl-5-hexayne-1-methylpyrazol-4-carboxylate (**17**) were synthesized from annelating substrate (**2**). *In vitro* antimicrobial activity of fused primidine and 5-alkynyl pyrazol derivatives were evaluated. All the synthesized compounds demonstrated mild growth inhibition against antibiotic-susceptible standard and clinically isolated strains of gram-positive and gram-negative bacteria as well as human fungal pathogens.



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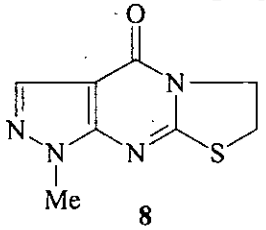

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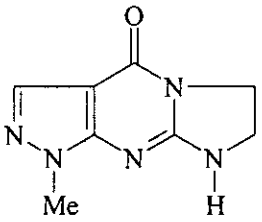

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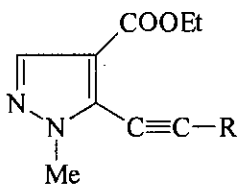


14). R = 
15). R = - $Si(CH_3)_3$
16). R = - CH_2 - OH
17). R = - $(CH_2)_3$ - CH_3

SUMMARY

Investigations incorporated in this dissertation entitled *"Synthesis of some new annelated fused pyrimidine derivatives and study of their biological activities"* have been presented into two parts. Part-I is divided into four sections and part-II is also divided into four sections. Each part has introductory section-1, in which the background biological action and the important synthetic reactions involved in the synthesis are presented. Section-2 of each part deals with the detailed methodologies and experimental procedures for the synthesis of fused pyrimidine derivatives and its biological test. Section-2 of part-I and section-3 of part- II represent the results and discussion of the synthesis of fused pyridine derivative and study of their biological activities respectively.

Part-1: Synthesis of fused pyrimidines and 5-alkynyl pyrazole derivatives.

Section-1, represents the importance of fused pyrimidine derivatives. Heterocyclic compounds containing the pyrimidine moiety are of great interest because of their occurrence in nature and their fascinating pharmaceutical and medicinal activities. Although various methods have been developed previously for the synthesis of fused pyrimidine but no report is available in the literature for the synthesis of 5-alkynyl pyrazole derivatives.

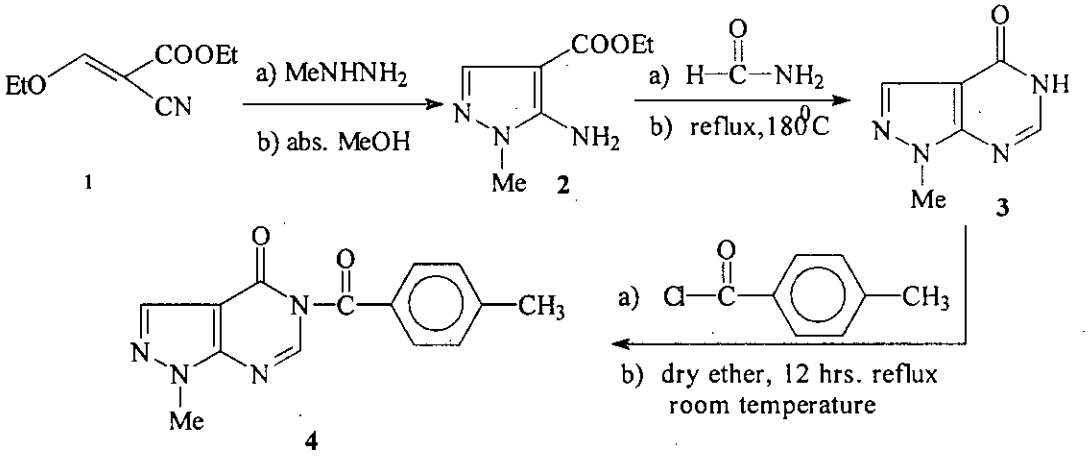
In section-2, the work described in this dissertation concerns the preparation of new annelated fused pyrimidine compounds by using the annelating reagent thiopseudourea and the *ortho*-aminoesters as a starting material due to pharmaceutical interest.

Annelating reagent as 2-methylthio-2-methylthiozoline (7) was prepared from the ethanolamine by using Jenson method[76] in 85.56% yield; bp. 70-71^{0}C (Lit.[76] b.p. 70^{0}C). When ethanolamine was treated with carbon disulfide in ethanol then the

compound 2-mercaptothiazoline (**6**) was afforded. This compound (**6**) was treated with methyl iodide to give 2-methylthio-2-methylthiazoline (**7**).

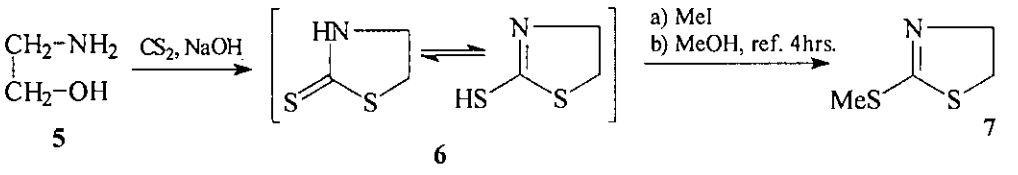
Annelating substrate as ethyl-5-amino-1-methylpyrazol-4-carboxylate (**2**) was prepared from ethyl(ethoxy methylene)cyanoacetate by using Gewald procedure[48] in 84.31% yield; mp. 90-91^{0}C.

The target compound 1-methyl-4-oxo-5-(*p*-toluoyl)pyrazolo[3,4-d]pyrimidine (**4**) was synthesized from the compound (**3**) and *p*-toluyl chloride in dry ether (**scheme-1**). 1-Methyl-4-oxo-5-(*p*-toluoyl)pyrazolo[3,4-d]pyrimidine (**4**) was prepared from 1-methylpyrazolo[3,4-d]pyrimidin-4(5H)-one (**3**) and *p*-toluoylchloride by three steps method in 64.46% yield. When *ortho*-amino ester (**2**) was treated with formamide then the compound (**3**) was formed in 68.77% yield; mp. 220-222^{0}C. *Ortho*-amino ester (**2**) which was earlier prepared.

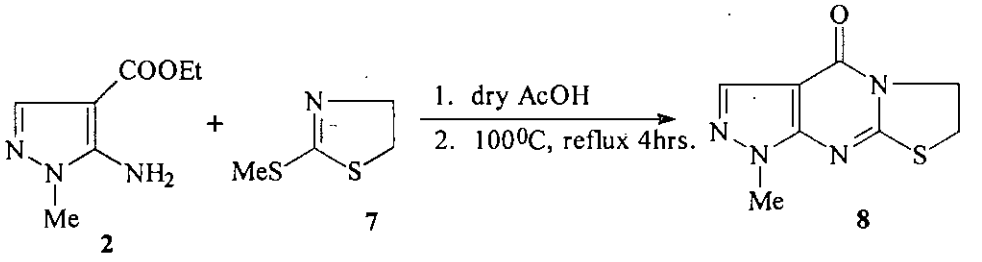


Seheme-1

The annelating substrate (**2**) was used to synthesize of fused pyrimidine derivatives (**8**) and (**12**). 1-Methyl-6,7-dihydropyrazolo[3,4-d]thiazolo[1,2-a] pyrimidin-4-one (**8**) was prepared from annelating substrates as ethyl-5-amino-1-methylpyrozol-4-carboxylate (**2**) and annelating reagent 2-methylthio-2-methylthiozoline (**7**), which was earlier prepared.

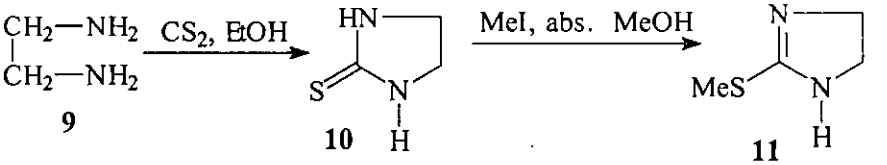


When *ortho*-aminoester (**2**) was treated with annelating reagent (7) by one step reaction in dry acetic acid then the compound (**8**) was furnished in 63.82% yield (**Scheme-2**), m.p. above 250^{0}C.

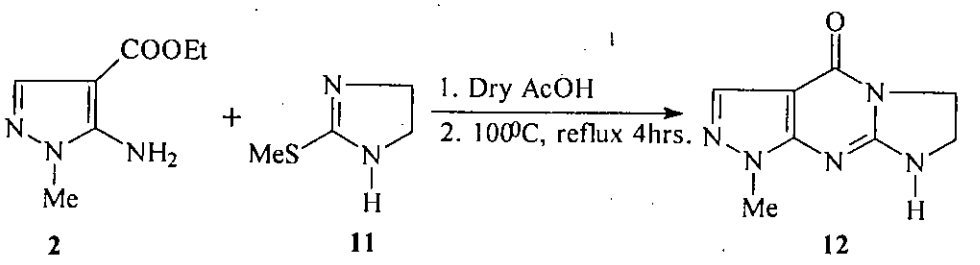


Scheme-2

And another annelating reagent 2-methylthio-imidazoline (**11**) was prepared from the ethylene diamine by two-step method in 58.63% yield, m.p. 119-121^{0}C (Lit.[81] m.p. 120-121^{0}C). When ethylene diamine was reacted with carbon disulfide in ethanol then the compound 2-imidazolinethione (**10**) was afforded. This compound (**10**) on reaction with methyliodide in absolute methanol to give 2-methylthio imidazoline (**11**).

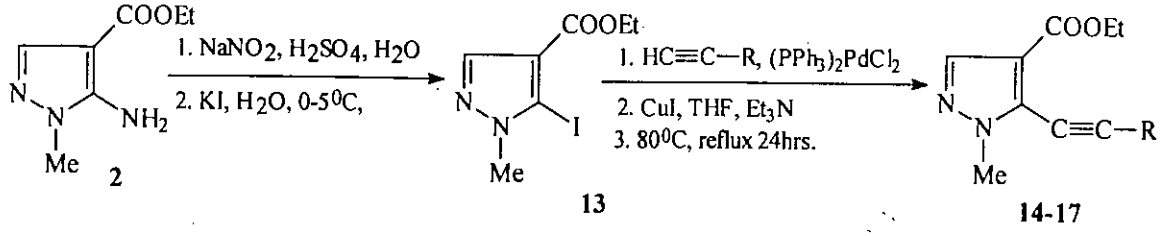


1-Methyl-6,7-dihydropyrazolo[3,4-d]imidazo[1,2-a]pyrimidine-4(8H)-one(**12**)was prepared from ortho-amino ester (**2**) and annelating reagent (**11**) which was earlier prepared. When the compound (**2**) was reacted with 2-methylthio-imidazoline (**11**) in dry acetic acid then the compound (**12**) was furnished (**Scheme-3**); mp. above 250^{0}C.



Scheme-3

In section-3, a new strategy for the regioselective synthesis of 5-alkynyl pyrazol derivatives (**14-17**) through the palladium-catalyzed condensation of ethyl-5-iodo-1-methylpyrazol-4-carboxylate with terminal alkyne (**18-21**) in DMF/THF at 80^0C-85^0C for 24 hrs. under nitrogen atmosphere in the presence of bis(triphenyl phosphene)palladium(II)chloride, copper(I)iodide, triethylamine to yield 5-alkynyl pyrazol derivatives (**18-21**) directly in excellent yields.



Scheme-4

14). R = 
15). R = -$Si(CH_3)_3$
16). R = -CH_2-OH
17). R = -$(CH_2)_3$-CH_3

The starting materials ethyl-5-iodo-1-methylpyrazol-4-carboxylate (**13**) was synthesized from *ortho*-aminoester (**2**) via. Sandmeyer iodination with potassium iodide according to the known literature procedure.

Part-II: Biological activities of synthesized compound.

In part-II, section-1 the introduction of the biological test is presented. In section-2 and section-3 the methodology and results and discussion of the biological test of the synthesized fused pyrimidine and 5-alkynyl pyrazol derivatives respectively.

All these newly synthesized products of the fused pyrimidines and 5-alkynyl pyrazol derivatives have been employed as test chemicals for determining their antibacterial and antifungal activities against a number of human and plant pathogens. Most of the compound demonstrated mild to moderate antimicrobial activity against most of the test organism. Twelve bacterial strains and four fungi strains were used to study the antibacterial and antifungal activity of the compounds at the higher concentration 200μg/disc. The results of these screening experiments are reported in detail in the part of this dissertation. Most of this compound demonstrated mild to moderate antimicrobial activity against most of the test organism. From these structures we found that the fused pyrimidine ring causes relatively better microbial growth inhibition. Among tested compounds 5-alkynyl pyrazole derivatives (**14, 15, 16** and **17**) exhibited relatively greater inhibition of growth of the microorganism. Substitution of iodine of the ring carbon, with bulkier terminal alkyne group increase in the antimicrobial activity of the compounds **14, 15** and **16** while hexyne substitution at the same place produce weakly active compound **17**.

PREFATORY NOTE

Unless otherwise mentioned the following procedures were used throughout the research.

1. Purification of Solvents ant Reagents:

The purity of solvent is extremely important in chromatographic analysis as well as for other purposes like chemical reaction. The methods by which they were purified and dried are described below:

a) Dry methanol:

A dry 500 ml round bottom flask was fitted with a double surface condenser and a calcium chloride guard tube. In the flask 1.25 g of clear dry magnesium turnings and 0.125 g of iodine were placed, followed by 20-25 ml of commercial grade methanol. The mixture were warm until the iodine has disappeared if a lively evaluation of hydrogen did not set in a further little amount of iodine was added. Heating continued until all the magnesium was converted into methanolate then added 230 ml of commercial grade methanol and refluxed the mixture for one hour. Then the suspension was distilled and stored under nitrogen atmosphere, as the "super dry" methanol exceedingly hygroscopic.

b) Ethyl alcohol:

These solvents were purified in exactly analogues manner as described with methyl alcohol.

c) Anhydrous acetone:

The acetone was heated under reflux with successive quantities of potassium permanganate until the violet color persists. It was then dried by the addition of anhydrous potassium carbonate, filtered and distilled. The distillate was collected at 55-56°C as pure solvent.

d) Chloroform:

The commercial product was contain up to 1 percent of ethyl alcohol, which was added as a stabilizer. The alcohol was removed by the following procedures:

i) The chloroform was shaken six times with about half its volume of water then dried over anhydrous calcium chloride for at least 24 hours and distilled.

ii) The chloroform was shaken three times with a small volume (5 percent) of concentrated sulphuric acid, thoroughly washed with water, dried with anhydrous potassium carbonate and distilled.

Pure chloroform had b.p. 61°C/760 mm. The solvent when free from alcohol was kept in the dark to avoid the photochemical formation of phosgene.

e) Dry acetic acid:

The commercial grade acetic acid was refluxed over phosphorus pentaoxide for 3 hours and then distilled. The distillate was collected at 116-117°C as dry acetic acid.

2. Melting Point (m.p):

Generally melting points are determined for solid and well dry compound. Melting points were recorded on Gallenkamp melting point apparatus (England) and paraffin oil bath were uncorrected.

3. Infra-red (IR) Spectra:

The infrared Infrared (IR) spectra were determined on KBr disc for films with a shimadzu FTIR spectrophotometer and the UV spectra were recorded in dry EtOH with a Shimadzu UV visible spectrophotometer at the Department of Chemistry, BUET, and Dhaka, Bangladesh.

4. Nuclear Magnetic Resonance (NMR) Spectra:

The NMR spectroscopy is very widely used for the detailed investigation of an unknown compound. With the help of this spectroscopy the structure or pattern of unknown can be set up. ^{1}H NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded in deauteriocholoroform ($CDCl_3$) & DMSO-d_6 with a Bruker DPX-400 spectrophotometer (400 MHz) using tetramethylsilane (TMS) as internal standard at the Bangladesh council of scientific and industrial research Laboratories (BCSIR), Dhaka, Bangladesh.

And also ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) were recorded in $CDCl_3$ & DMSO-d6 (TMS as internal standard) at Iwate University, Japan.

5) Evaporation:

All evaporations were conducted under reduced pressure using Buchi Rotatory Evaporator (W. Germany) with a bath temperature below 40^0C.

6. Drying:

All organic extracts were dried over anhydrous sodium sulfate (Na_2SO_4) or magnesium sulfate ($MgSO_4$) before concentration.

7. Techniques and applications of thin-layer chromatography (TLC):

The thin-layer chromatography is the most helpful method for separation, isolation, purification and identification of a mixture of organic compounds which involves an adsorbent (usually silica gel and alumina) as the stationary phase and a solvent or solvent mixture as the mobile phase. The components of the mixture migrate differentially along the TLC plates due to the differential rate of absorption on the absorbent and due to this difference in mobility of the components; they are separated from each other by the solvents. The more polar compound moves first. The mobility of the components also depends on the polarity of the solvent or solvent mixture.

To spot the plates, firstly a mark was made about half a centimeter from the bottom of each plate and the solutions of the components were then spotted with glass capillaries. To develop the chromatogram the plates were then places downward in a chromatography tank, in such a way that the spotted mark of the components remained above the solvent surface. The tank contained the developing solvent or solvent mixture and the atmosphere inside the tank would be saturated with the vapour of same solvent or solvent mixture. The plates were removed when the solvent front reached half centimeter apart from the upper edge. The plates were then allowed to dry.

If the components of the mixture were coloured, the spots were readily located. If the components were colourless the dried plate was developed with iodine vapour or UV light. For identification of the sample by TLC at least three different solvent were tried and R_f value computed and compared with each case but only the solvent conditions that gave the best results were mentioned. The ratio of the distance traveled by the solvent front was characteristic of each component and was known as R_f value, i.e.

$$R_f\ value = \frac{Distance\ traveled\ by\ the\ component}{Distance\ traveled\ by\ the\ solvent\ front}$$

8. Column chromatography:

Column chromatography has been successfully applied to separate the individual components (having different R_f values) of the mixture obtained from the reaction. This technique was also employed for purification of the product.

A cylindrical column (70 cm long and 2 cm in diameter usually a burette type) made of glass drawn out at one end and packed with glass wool or cotton at the bottom was taken the lower constricted end of the column was fitted with a stop clock for controlling the flow of the eluant. A round bottom flask fitted with a

specially made quick fit stopper and filled with the eluant was placed at the top of the column and this served as a store for eluant.

The flow of the eluant was controlled by stop cock. The column was made half filled with various types of solvents aspected. Benzene, ethyl acetate, chloroform, n-hexane etc. and slurry of silica gel in chosed solvent was then poured into it, so that the packing was compact and uniform, air bubble was avoided by making the column as quickly as possible and allowing the solvent to fall drop by drop through the stop cock of the column. At that time the mixture of the component was placed on the upper surface of the slurry silica gel and which is also covered in limited area by some amount of dry silica gel. Then the solvent mixture that is eluant was passed from the upper round bottom flask (which served as a reservoir eluant) through the column. Then the fractions were collected in a test tube about 2.3 ml at a regular interval. And the respective fractions were detected by TLC technique. The solvent used for eluation were chromatographyically pure.

Part-I

Section-1

BACKGROUND OF THE PRESENT WORK

1.1. INTRODUCTION:

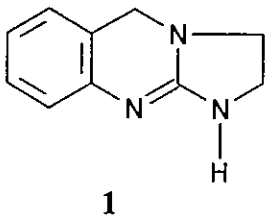
1.1.A. FUSED PYRIMIDINES AND THEIR IMPORTANCES:

Fused pyrimidines have become attractive targets for organic synthesis because of their structural diversity and biological importance.[1] For these properties they have attracted much more attention from the point of view of the medicinal chemistry.

Recent development of physiologically highly potent fused pyrimidines with interesting sedative[2], antiviral[3,4], antibacterial[2,5], antimalarial[2,5,6], antiallergic[3], anti-parasitic[8], anti-inflammatory[7], anti-HIV[5], antihypertensive agents[9,10], blood platelet aggregation inhibitors[11] and specially anticancer agents[3,4,5,6] prompted a great interest in the preparation of annelated pyrimidines. Hetero aromatic carboamines and nitriles readily undergo cyclization, which allow convenient preparation of a variety of condensed pyrimidines.[12]

With the development of isolation techniques and rapid structural elucidations by recent methods (IR, NMR, Mass, UV, etc.) a large number of fused pyrimidine have been discovered from the various sources. Many pyrimidines and fused pyrimidine derivatives have important biological activity. Many synthetic quinazolines were found to have some specific activity.

As a part of a broad investigation of structures containing an amidine moiety as potential antihypertensive agents. T. Jen and his co-workers[13] reported the synthesis of a series of imidazo-quinazolines. One of these was 1,2,3,5-tetrahydroimidazo[2,1-b] quinazoline (**1**) which was found to be particularly effective in lowering the blood pressure.



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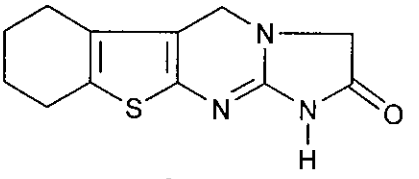
A series of novel 1,2,3,5-tetrahydroimidazol[1,2-a]thienopyrimidin-2-ones (**2**) were prepared by F. Ishikawa *et al.*[11(b)] and were found to be highly potent inhibitors of blood platelet aggregation. Structure activity relationship was indicated the essential contribution of the lactum structures and liophilic substituents on the thiophene ring to the effective interaction of the compounds with a receptor site on the platelet.



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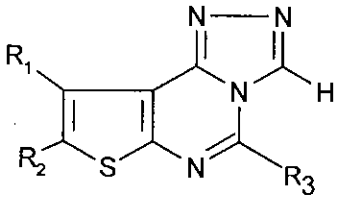
Fumiyashi *et al.*[14] also pointed out the essential contribution of the lactum structure and lipophilic substitution molecules inhibit in platelet blood aggregation. Condensed triazoles posses a variety of pharmacological activities like mitotic (**3**) hypotensive (**4**) and analgesic activity[15] (**5, 6**).



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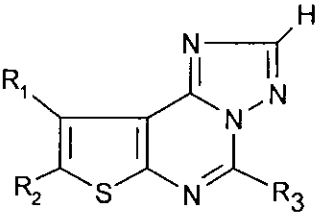

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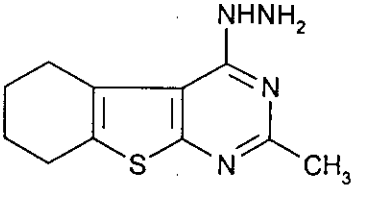

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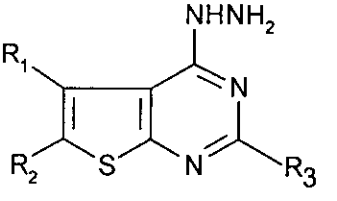
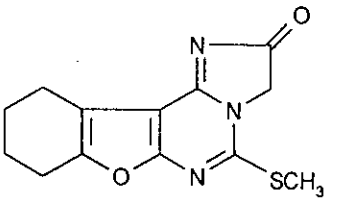


6

The tetracyclic fused pyrimidine reported by Shaifullah *et al.*[15] which was prepared from N-[bis(methylthio)methylene]amino or ethyl isothiocyanatoacetate and furonitrile in acetic acid or pyridine medium. These compounds were 5-

(methylthio)-8,9,10,11-tetrahydro-benzofuro[3,2-e]imidazo[1,2-c]pyrimidin-2(3*H*) -one (**7**) and 5-thioxo-benzofuro [3,2-e]imidazo[1,2-c]pyrimidin-2-(3*H*)-one (**8**) showed antihypertensive activity as well as antibacterial and antifungal activity.[15]



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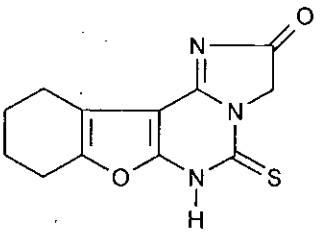
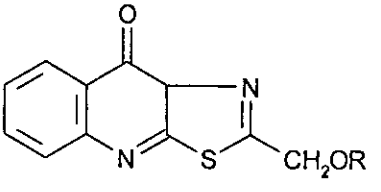


8

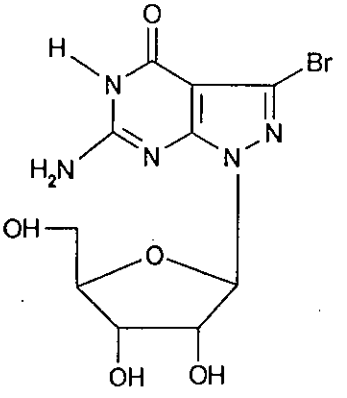
Shishoo *et al.*[16] were interested in the synthesis of triazol-thieno-pyrimidines as potential antiinflammatory compounds. In many instances, formic acid was used for the cyclization of 4-hydrazinothieno[2,3-d]pyrimidines to the corresponding triazoles.

In 1991, Nirupama Tiwari *et al.*[17] reported that 2-aryloxymethyl-1,2,4-thiadiazolo [2,2-b]quinazoline-4-ones (**9**) were evaluated for their fungicidal and herbicidal activities.



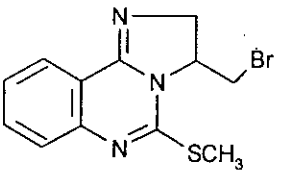
9

Ribo nucleosides compounds showed *in vitro* against certain viruses and tumor cells. The guanosins analogous (10) showed significant activity against muscle *in vitro* and to exhibit moderate antitumor activity *in vitro* against L_{R10} and P_{388} Leukemia.[18]



10

The fused pyrimidine has been reported by Chern and his co-workers[19] 3-Bromomethyl-5-methylthio-2,3-dihydroimidazo[1,2-c]quinazoline (**11**) which was synthesized from anthranilonitrile. It showed antimalarial activity.[19] The compound imidazo[1,2-c]quinazoline-2,5-(3*H*, 6*H*)dione (**12**) acted as hypertensive agent which was reported by papadopoulas *et al.*[20]



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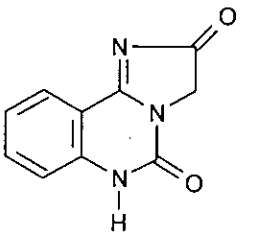
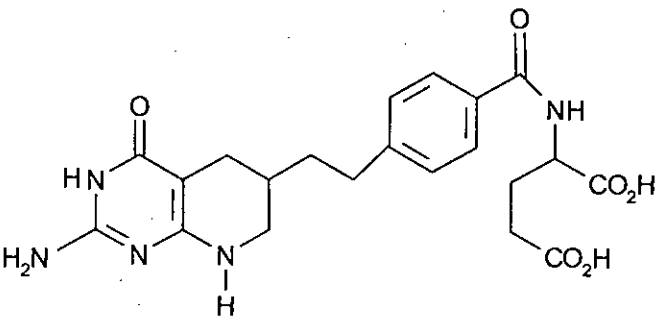


12

A detail description of the isolation, structural elucidation and synthesis of different types of fused pyrimidine and their biological activity will be given in the subsequent chapters.

1.1.B. NATURAL SOURCES OF FUSED PYRIMIDINES:

Fused pyrimidines are found in a broad variety of natural products [e.g. purines, pyrrolo-pyrimidines, pteridines], pharmaceuticals, agrochemicals and veterinary products (**13**).[21]

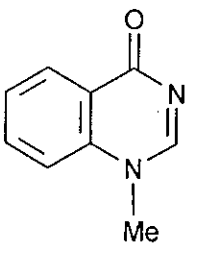


13

The fused pyrimidine rings or purine rings are present in the nucleic acids, co-enzymes etc. They were also isolated from the living cells.[22] A variety of natural

products such as purine alkaloids contain the pyrimidine ring systems. These include hypoxanthine and xanthine which occur in tea. Caffeine and theophylline are found in tea-leaves.[22] Theobromine is found in coca-beans which has a stimulating effect on the central nervous system.[22]

The quinazoline alkaloids from a small but important group of naturally occurring bases which were isolated from a number of different families in the plant kingdom. Quinazolines were found in the botanical families. Glycorine (**14**), Aborine (**15**), Glycosmicine (**16**) and Glycosminine (**17**) were isolated from the leaves of *Glycosmis arborea*.



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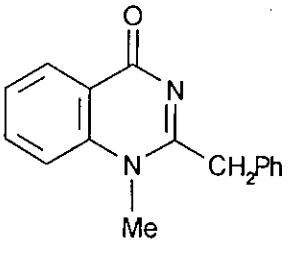

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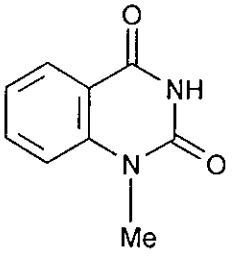

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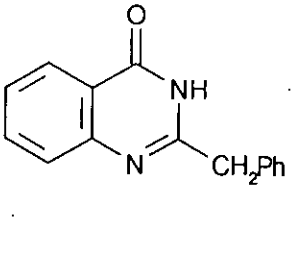
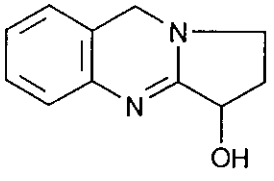


17

l-Vasicine (**18**) isolated from *Adhatoda vasica* and vasicinone (**19**) was isolated from *Pegnum harmala* and *Galega officinalis.* The vasicine group has bronchodilator activity.[23]



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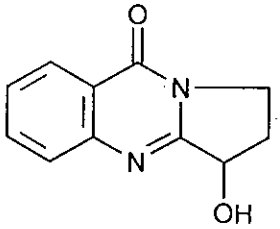
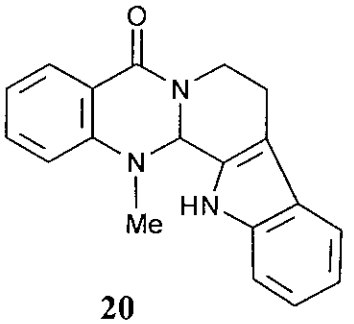


19

The evadomine (**20**) was isolated[23] from the dired fruits of *Evodia rutaecarapa* and it exhibits hypotensive action. The other alkaloids febrifugine (**21**) was isolated from *Dichora febrifuga* which was high antimalarial activity.



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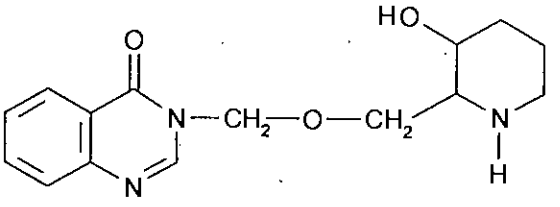
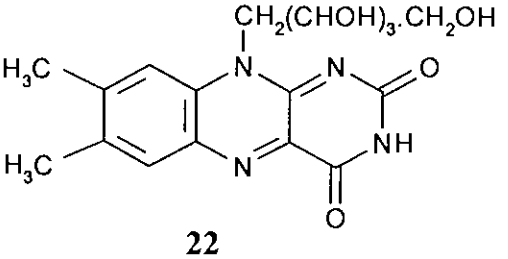


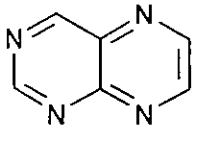
21

The fused pyrimidine rings are also present in vitamin riboflavin B_2 (**22**). The best sources of vitamin B_2 are yeast, green vegetables, egg, milk, meat, fish, etc.[24]



22

Folic acid contains a pteridine (**23**) nucleus which is a fused pyrimidine. The pterines are pigments of butterfly-wings, waps etc. They were first isolated from butterfly-wings. Some pterins are also found to be in man, e.g. Xanthopterin (**24**) and biopterin (**25**).



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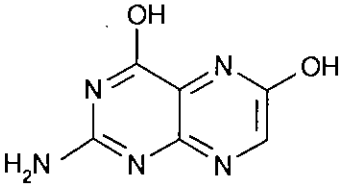

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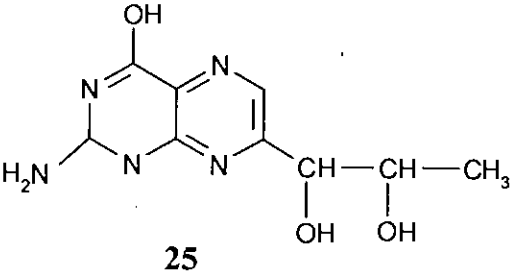
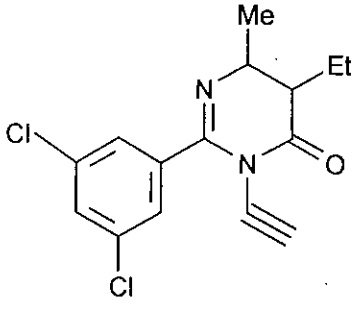


25

1.1.C. VARIOUS FUSED PYRIMIDINES:

The synthesis and design of new herbicides is a continuing challenge in agricultural chemistry because of the persistent problem of resistance development and as a result of economic and environmental pressures to find compounds with different modes of action.

Edward C. Taylor *et al.*[25] reported the novel synthesis of promising new herbicide 2-(2,6-dichloro-4-pyrilyl)-3-propargyl-5-ethyl-6-methyl-4-(3*H*)-pyrimidinone (**26**). Bis[quinazoline-4-one-2-yl]-1,3-phenylene (**27**) and its 3-N-substituted derivatives were prepared by S.A. Shiba *et al.*[26] from the corresponding bis[3,1-benzoxazin-4-one-2-yl]-1,3-phenyline as precursor. Quinazoline (**27**) was converted into several derivatives such as bis[quinazoline-4-thioxo-2-yl] etc. These types of compounds show activity against Gram-positive and Gram-negative bacteria.



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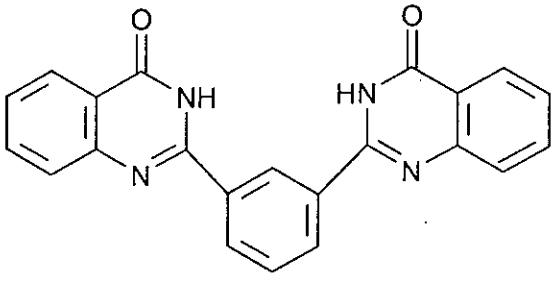
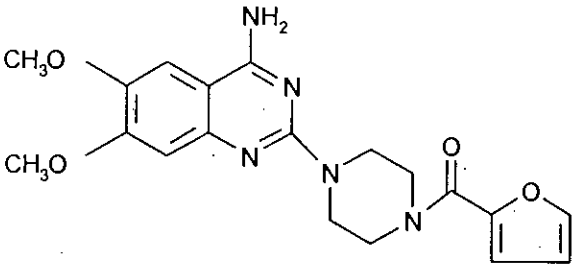


27

Hypertension is a serious risk factor for cerebrovascular disease and heart disease in developed countries. Although there are several antihypertensive drugs clinically available, due to the different origin and pathology of hypertension, it is difficult to control all types of hypertension through the use of only one drug. Varieties of antihypertensive agents contain pyrimidine and fused pyrimidine ring systems.

For example prazosin (**28**)[27] a 2-substituted quinazoline derivative has been proven effective in the clinic, acting as a α-adrenoceptor antagonist.[27] Other 3-

substituted quinazolines such as SGB-1534 (**29**)[28] and ketanserin (**30**)[29] have been found to have antihypertensive activities medicated via-α-adrenoceptor and serotonic receptor antagonism respectively.



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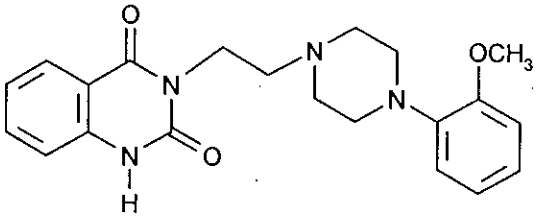

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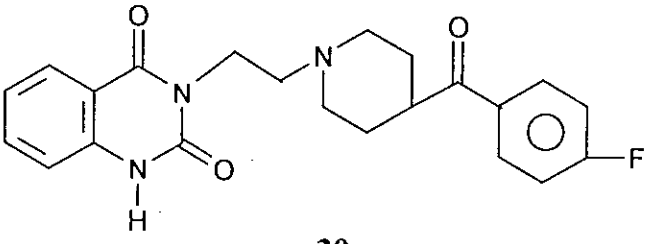
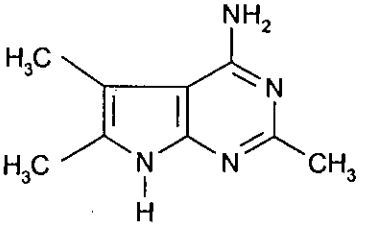


30

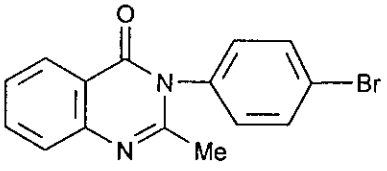
Compounds containing a fused pyrimidine ring represent a broad class of compound which have received considerable attention over the past years due to their wide range of biological activity with the development of clinically useful anticancer (5-fluorouracil) and antiviral drugs.[30] 2-Substituted pyrolo[2,3 d]pyrimidin-4-amines (**31**) obtained with proven anti-phlogisic and anticonvulsant properties.[31]



31

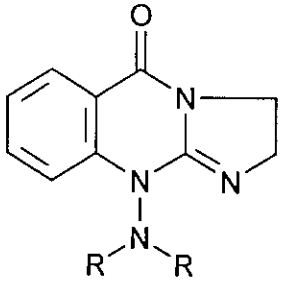
Methanqualone was superior to sodium phenobarbitione as an anticonvulsant against methrazol-induced seizures and of forty compounds showed for oral

anticonvulsant activity against Leptazo induced convulsions in mice. 3-*p*-Bromophenyl-3,4-dihydro-2-methyl-4-oxoquinazoline (**32**) B.D.H. was one quarter as active as phenytoin against Leptazol and eight times more active than Troxidone against electroshocle induced convulsions.[32]



32

Malecha and his co-workers reported[33] that imidaz[2,1-b]quinazolin-5(3*H*)-one (**33**) was synthesized from 1-(2-flurobenzyl)-2-methylthio-2-imidazoline (**34**)[33] which was used as potentially selective tracheal smooth muscle relaxants.[33]



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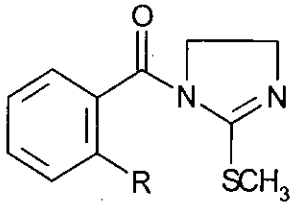
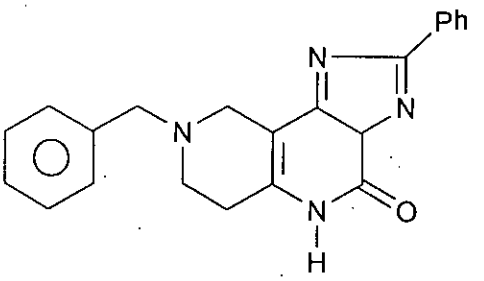


34

The best compound in view of chemical and biological evaluation, solubility in water, potency of the platelet aggregation inhibition, antithrombic drug with little effect on the cardiovascular system was 7-piperidino-1,2,3,4-tetrahydro-imidazo [2,1-b]quinazolin-2-one (**35**) 7,8,9,10-tetrahydro-2-phenyl-9(phenylmethyl) pyrido [3,4-e][1,2,4]triazol[1,5-c]quinazolin-5-(6*H*)-one (**36**) reported by Fumiyoshi and his co-workers.[34]

Schlecker and his co-workers[35] reported that the triazoloquinazoline (**37**) acted as a nervous system agent and showed little effected on other bacteria, fungi and viruses.



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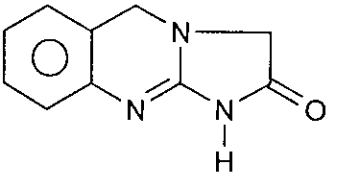
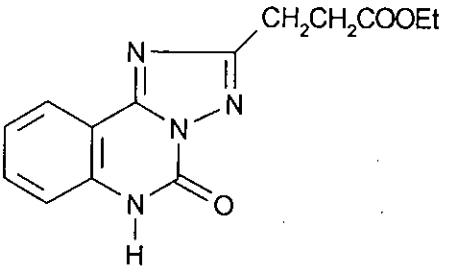


35

A. Rosowsky and his co-workers[36] reported the synthesis of 2,4-diamino-5,6,7,8-tetrahydrothianaptheno[2,3-d]pyrimidine (**38**) which acts as antifolates and anti-malarial. It was obtained from the aminonitrile.



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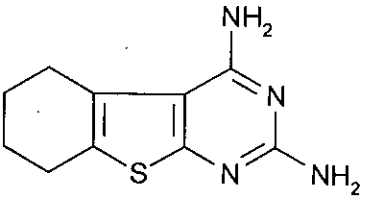
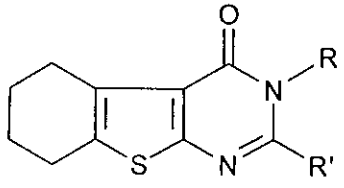


38

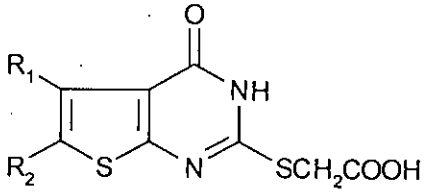
M.M.El-Enamy *et al.*[37] reported that substituted thieno[2,3-d]pyrimidones (**39**) have microbial activity. 2-Marcapto-3,4-dihydrothieno[2,3-d]pyrimidine-4-one (**40**) showed hypocholest-erolemic and antitussive properties which was reported by P. Sukumaran and his co-workers.[38]



R = $NHSO_2C_6H_5$

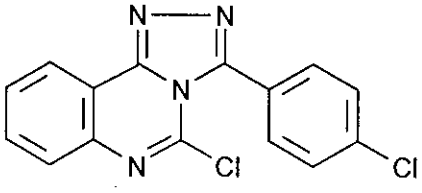
R' = Me

39



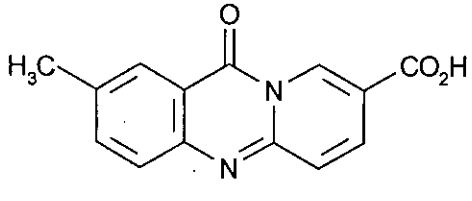
40

R.A. Glennon and his co-workers[39] revealed that triazoloquinazolines such as 3-aryl-1,2,4-triazolo[4,3-c]quinazolines (**41**) posses antiinflammatory activity.



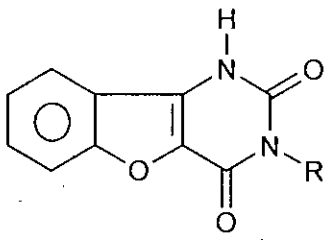
41

A great deal of synthetic work has been directed toward the discovery of more potent and orally active compounds. A series of 2-substituted-pyrido[2,1-b]-quinazolin-8-carboxylic acids (**42**) are used as orally active antiallergy agent which were reported by J. W. Tilley and his co-workers.[40] The compound (**42**) was synthesized from the reaction of the appropriate anthranilic acids with 6-chloronicotinic acid in 38% yield.



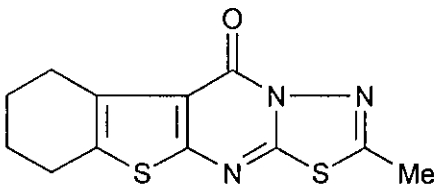
42

V.M. Patil and his co-workers[41] reported a new antialergic compound 3-amino-1,2,3,4-tetrahydro-2,4-dioxobenzofuro[3,2-d]pyrimidine (**43**).

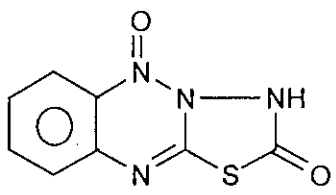


43

The compound 6,7,8,9-tetrahydro-2-methyl-10*H*[1]benzothieno[2,3-d][1,3,4]thiadiazolo[3,2-a]pyrimidin-10-one (**44**) and 2,3-dihydro-5*H*-[1,3,4]thiadiazolo[2,3-b]quinazolin-2,5-dione (**45**)[42] showed antiinflammatory and analgesic activities.

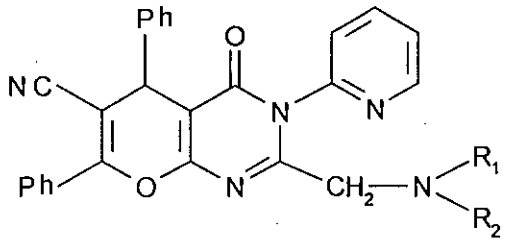


44



45

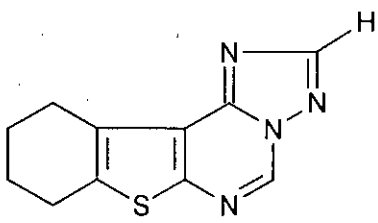
A.A. Fadda and his co-workers[42] reported the compound 2-(N,N-substituted amino-methyl)-6-cyano-4(3H)-oxo-5,7-diphenyl-3-(2-pyridal)-pyrazo-(3,2-b)pyrimidine (**46**), which is known to have diverse pharmacological properties including antibacterial, antiviral and antiallergic.



46(a). $R_1 = R_2 = CH_3$

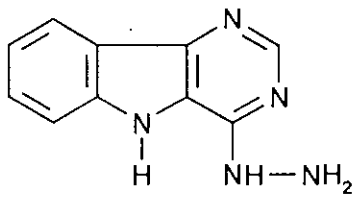
46(b). $R_1 = R_2 = C_2H_5$

C.J. Shishoo and his co-workers[16] reported the synthesis of triazolotheino-pyrimidines (**47**) which was found to be potential antiinflammatory compounds.

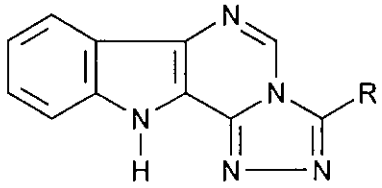


47

A. Monge and his co-workers[43] reported the synthesis of 4-hydrazino-5*H*-pyrimido[5,4-b]indole (**48**) and some related compounds (**49**) which are structural analogous of antihypertensive agent.



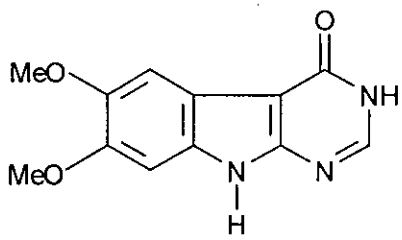
48



R = H , CH_3

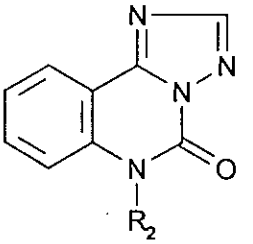
49

B. Venugopalon *et al.*[44] reported the synthesis of 4-oxo-pyrimido[4,5-b]indole (**50**) which has antihypertensive properties. *Ortho*-aminoesters serve as a good synthon for the construction of a pyrimidine ring.

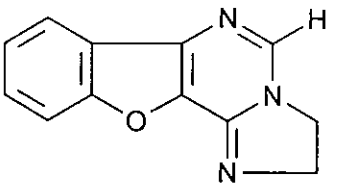


50

The fused pyrimidine [1,2,4]triazolo[1,5-c]quinazoline (**51**) exhibited the anticonvulsant, muscle relaxant, anxilytic and sedative properties and benzofuro[2,3-e] imidazo[1,2-c]pyrimidine (**52**) showed antidepressant activity and antihypertensive agent.[45]



51



52

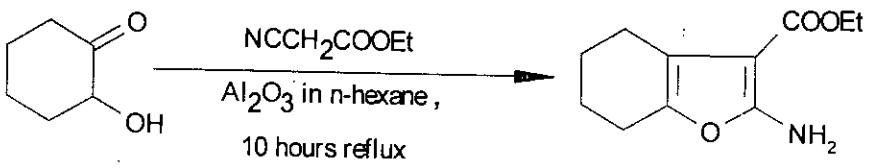
For more detail description of pyrimidine and fused pyrimidine will be discussed in the next chapters.

1.1.D. SYNTHESIS OF GEWALD PRODUCTS: *ORTHO*-AMINOESTERS:

Pyrimidine and fused pyrimidine compounds bearing *ortho*-aminoester moieties were useful substrates for the preparation of various condensed heterocyclic (pyrimidine and fused pyrimidine) ring systems i.e. for synthesizing a group of new pyrimidine compounds and a variety of new heterocyclic condensed pyrimidine parent systems belonging to the class of annealated pyrimidine systems. Important starting materials of this type are *ortho*-aminoesters derived from furan, indole, thiophene, oxazole, pyrazole, isoxazole, thiazole and anthranilate fused to new heterocyclic pyrimidine derivatives or to new heterocyclic pyrimidine parent systems belonging to new heterocyclic condensed pyrimidine parent systems (e.g. some novel tri and tetracyclic systems obtained by annelation of imidazol, pyrimido, thiazolo and thiazino moieties).

Synthesis of ethyl 2-amino-4,5,6,7-tetrahydrobenzofuran-3-carboxylate (54):

Reaction of adipoin (**53**) with ethylcyanoacetate in n-hexane containing basic alumina (Al_2O_3) as catalyst under reflux 10 hrs. formed ethyl 2-amino-4,5,6,7-tetrahy-drobenzofuran-3-carboxylate (**54**)[46] in yield 57%.

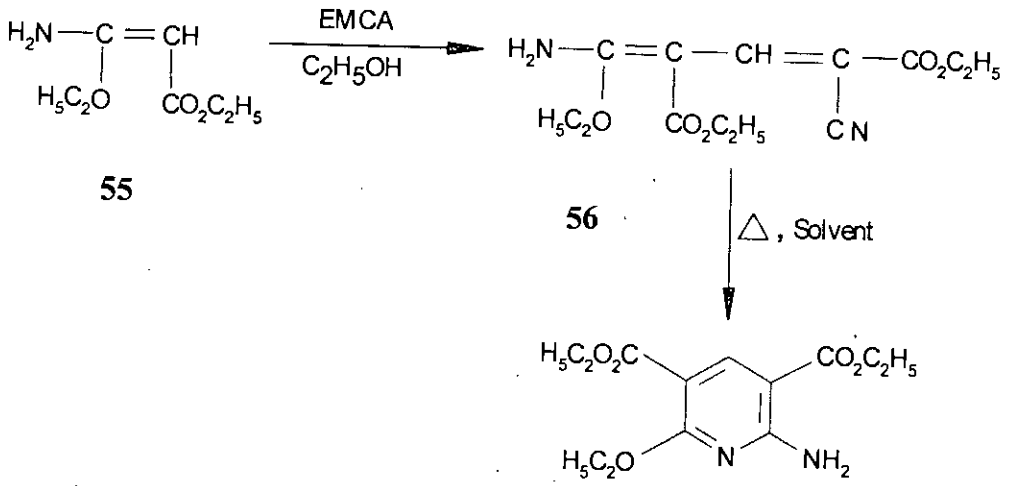


53 **54**

Scheme-1

Synthesis of ethyl 2-aminopyridine-3-carboxylate derivatives (57):

M.J. Cocco *et al.*[47] obtained dienaminoesters (**56**) by reacting compounds of 3-ethoxy-3-iminopropanenitrile (**55**) or their corres-ponding amidines with an equivalent of ethoxy methylene cyanoacetate (EMCA) in alcohol solution at room temperature in good yields. The adducts undergo intramolecular rearrangement easily when refluxed in dimethyl sulpoxide 2:1 solution or when treated with sodium ethoxide in ethanol at room temperature, to yield the ethyl 2-aminopyridine-3-carboxylate derivatives (**57**).

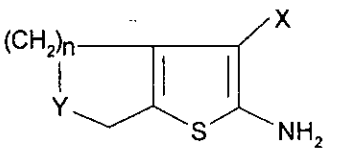


55 **56** **57**

Scheme-2

Synthesis of Gewald products: ortho-aminoesters of thiophene:

The direct one-step base catalyzed condensation of ketones with malonitrile or ethylcyanoacetate and sulfur has been described by Gewald and co-workers[48] served as the basis for the synthesis of a homologous series of ethyl 2-amino-cyclo-alkano-thiophene-3-carboxylate (**58, 59**) starting from cyclohexanone, cyclopentanone respectively. Similarly 4-methylcyclohexanone, its aza-analogue 1-methyl-4-piperidone and thiopyranone[49] were converted into the corresponding *ortho*-aminoesters **58-60** (**Scheme-3**).



Scheme-3

58. X = COOEt, Y = CH_2, n = 1

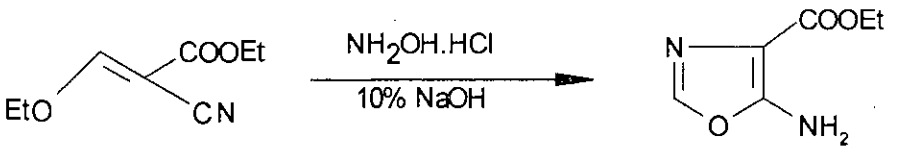
59. X = COOEt, Y = CH_2, n = 2

60. X = COOEt, Y = NMe, n = 2

Ortho-aminoester (**59**) is also a useful intermediate, the preparation and its application will be discussed in experimentals and results and discussion chapters.

Synthesis of ethyl 1-methyl-5-aminopyrazole-4-carboxylate (62):

Ethyl 1-methyl-5-aminopyrazole-4-carboxylate (**62**) was prepared[50] from ethyl (ethoxy methylene) cyanoacetate (**61**) with hydroxylamine hydrochloride in 10% NaOH.

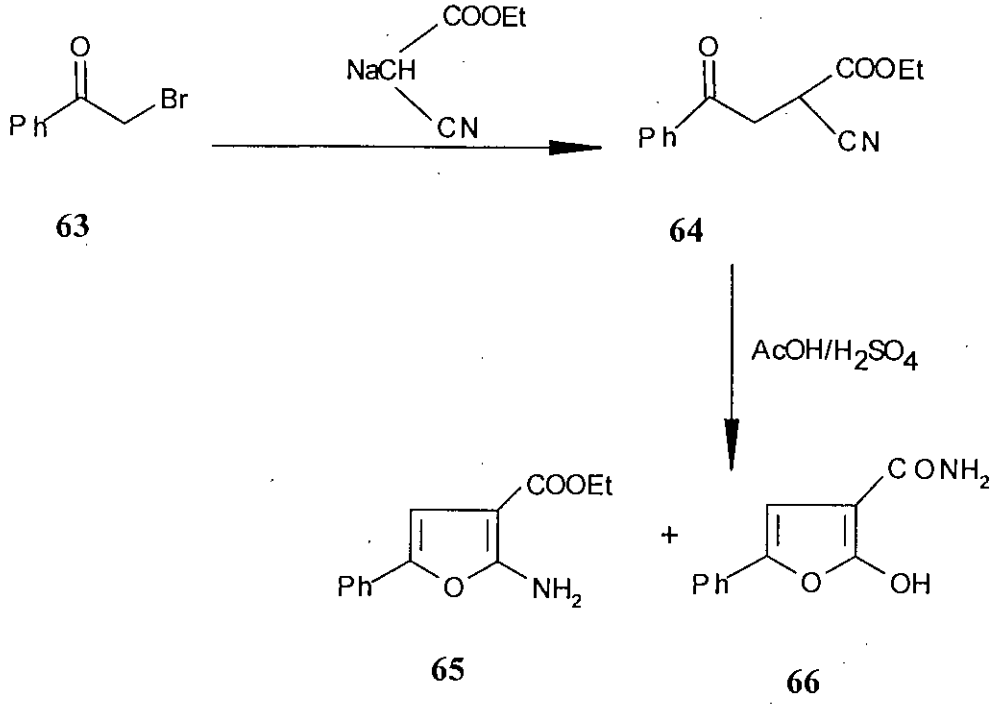


60 **62**

Scheme-4

Synthesis of ethyl 2-phenyl-5-aminofuran-4-carboxylate (64):

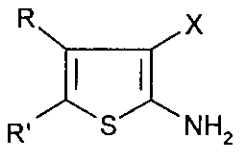
ω-Bromo acetophenone (**63**) reacted with sodium salt of ethylcyanoacetate to form ethyl phenyl cyanoacetate (**64**) which was treated with acetic acid or sulphuric acid to form a mixture of ethyl 2-phenyl-5-aminofuran-4-carboxylate (**64**)[51] and compound (**65**) reported by Fathy *et al.*[51]



63 **64** **65** **66**

Scheme-5

Again, the direct one-step base catalyzed condensation of ketones with malonitrile or ethyl cyanoacetate and sulfur has been described by Gewald and co-workers[52] served as the basis for the synthesis of a homologous series of 2-amino-4,5-dimethylthiophene-3-carbonitrile (**67**) and ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate (**68**), starting from butanone. Similarly, acetophenone, its aza-analogue ethyl 2-amino-5-phenylthiophene-3-carboxylate (**69**) and *ortho*-aminoamide (**70, 71**) and *ortho*-aminoesters **72-78** (**scheme-6**).



Scheme-6

67.	$X = CN$,	$R = CH_3$,	$R' = CH_3$
68.	$X = CO_2C_2H_5$,	$R = CH_3$,	$R' = CH_3$
69.	$X = CO_2C_2H_5$,	$R = H$,	$R' = C_6H_5$
70.	$X = CONH_2$,	$R = H$,	$R' = C_6H_5$
71.	$X = CONH_2$,	$R = CH_3$,	$R' = C_6H_5$
72.	$X = CO_2C_2H_5$,	$R = C_2H_5$,	$R' = CH_3$
73.	$X = CO_2C_2H_5$,	$R = CH_3$,	$R' = C_2H_5$
74.	$X = CO_2C_2H_5$,	$R = H$,	$R' = CH_3$
75.	$X = CO_2C_2H_5$,	$R = H$,	$R' = C_2H_5$
76.	$X = CO_2C_2H_5$,	$R = C_6H_5$,	$R' = H$
77.	$X = CO_2C_2H_5$,	$R = C_6H_5$,	$R' = CH_3$
78.	$X = CO_2C_2H_5$,	$R = CH_3$,	$R' = C_6H_5$

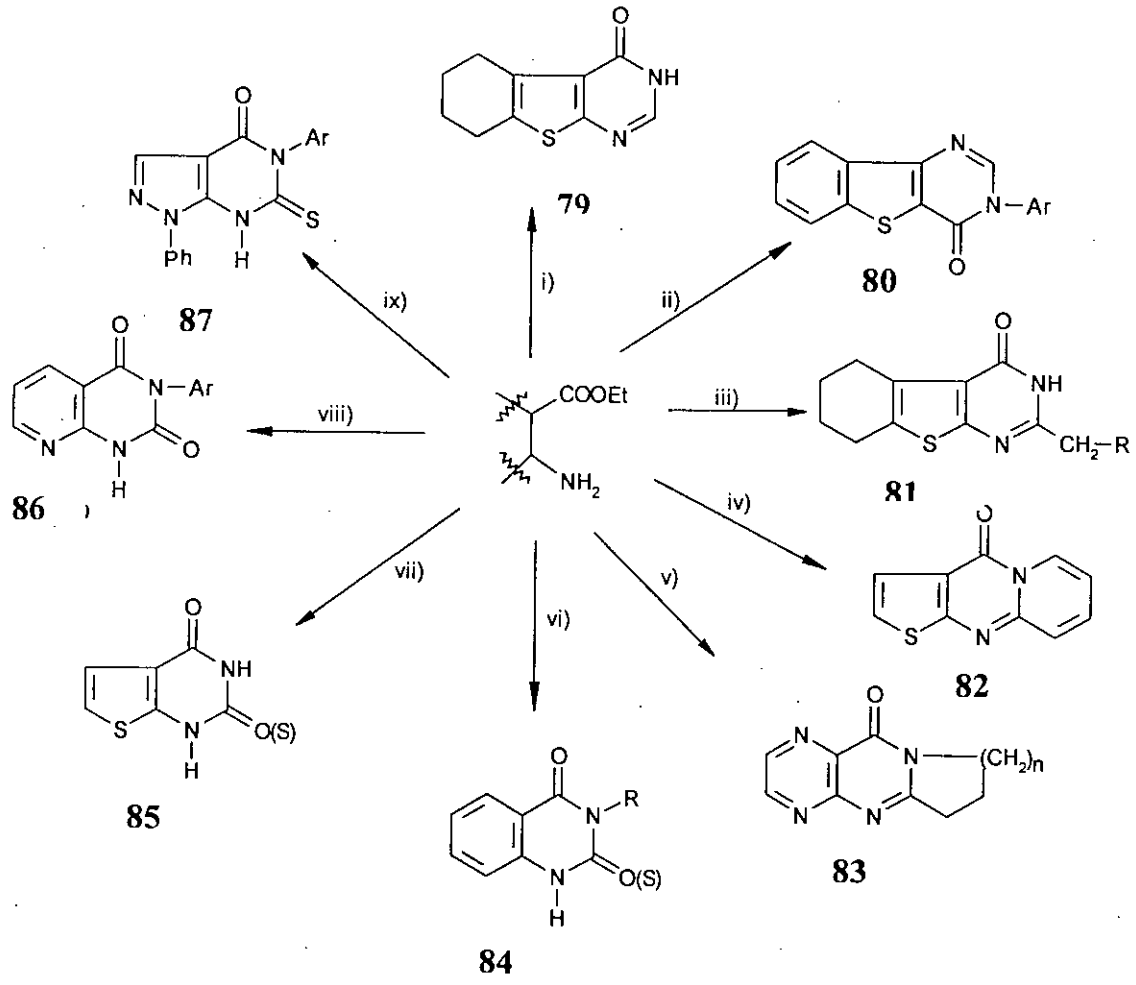
Ortho-aminoester (**68**) is also a useful intermediate, the preparation and its application will be discussed in experimental, results and discussion chapters.

For the synthesis of fused pyrimidines from *ortho*-aminoesters and *ortho*-aminonitriles many procedures are published, some of them are describe in next chapters.

1.1.E: METHODS OF SYNTHESIS OF FUSED PYRIMIDINES:

For the synthesis of fused pyrimidines from *ortho*-aminoesters and *ortho*-aminonitriles many procedures are published, some of them are outlined in below:

SYNTHESIS OF FUSED PYRIMIDINES FROM ORTHO-AMINOESTERS:



Scheme-7

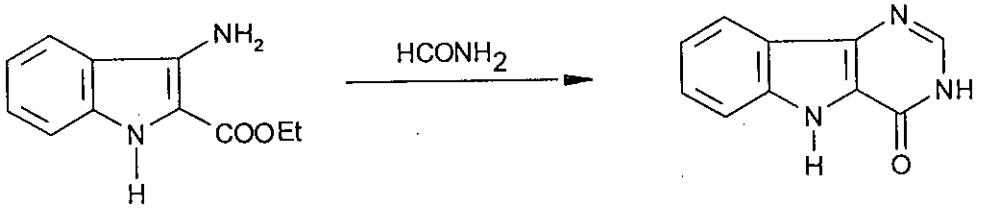
i) $HCONH_2$, Δ; ii) $ArNH_2$, $HC(OEt)_3$, decalin, Δ; iii) RCH_2CN, HCl-dioxane; iv) Iminodichloride or lactum, $POCl_3$; v) Lactim ether; vi) RNCO or RNCS, Pyridine, Δ; vii) $CO(NH_2)_2$ or $CS(NH_2)_2$, Δ; viii) $ArCON_3$, DMF; ix) ArNH-CSSMe, NaOH, DMF, Δ.

For cyclization of *ortho*-aminoesters the ring-closing reagents were usually formamide, urea, lactams, lactim, ethers, iminodichlorides, nitriles or isocyanates (**Scheme-7**).

Reaction of ethyl 2-amino-4,5,6,7-tetrahydro-benzo[b]thiohenc-3-carboxylate with formamide gave [1]benzothieno[2,3-d]pyrimidin-4(3*H*)-one (**79**)[2]. A mixture of a primary arylamine, an *ortho*-ester and an *ortho*-aminoester reacted on heating in declain to form a 3-N-arylpyrimidinone ring (**80**).[53] Nitriles (including cyanamides) and amidines caused ring closure and relatively mild conditions, but only the lower alkyl amidines were effective. Chloroacetonitrile reacted with two moles of *ortho*-aminoester to benzothieno[2,3-d]pyrimidine-4-one (**81**).[14] Iminochlorides, a cyclic or acyclic chloroimine or a heterocycle containing a reactive halogen atom cyclize an *ortho*-aminoester to give a doubly fused pyrimidine type (**82**)[53(a)]. In the presence of phosphorus oxychloride, cyclic amides (lactams) also reacted and led to (**82**)[53(b)]. The reaction of 2-amino-3-methoxy carbonyl-pyrazine with lactim ethers led in one step to the polynuclear heterocyclic pteridines (**83**).[54] The NH-CO(S) group appeared in ring (**84**)[55], when *ortho*-aminoesters reacted with isocyanates or isothiocyanates. A ureido or thioureido group, either formed *in situ* from an amine or present in the substrate, reacted with an ester group to give a pyrimidinedione or thioxo-one (**85**).[56] A 3-substituted ureide (**86**)[57], can also be prepared by reaction of an amine and an azide. In alkaline solution an aminoester reacted with a dithiocarbamate to give a thioxo-pyrimidinone (**87**)[58] in good yield.

SYNTHESIS OF FUSED PYRIMIDINES FROM SOME OTHER EDUCTS ANALOGOUS TO ORTHO-AMINOESTERS:

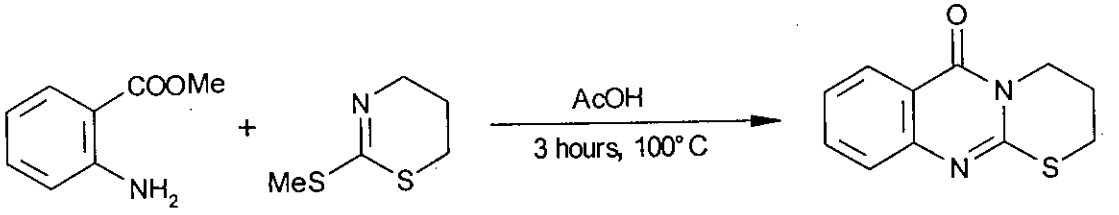
Ethyl 3-aminoindole-2-carboxylate (**88**) in formamide was warmed (**Scheme-8**) with stirring under nitrogen at about 220^{0}C for 2 hours, the product was collected 5*H*-pyrimido[5,4-b]indole-4-one (**89**) reported by Monge *et al.*[59]



88 **89**

Scheme-8

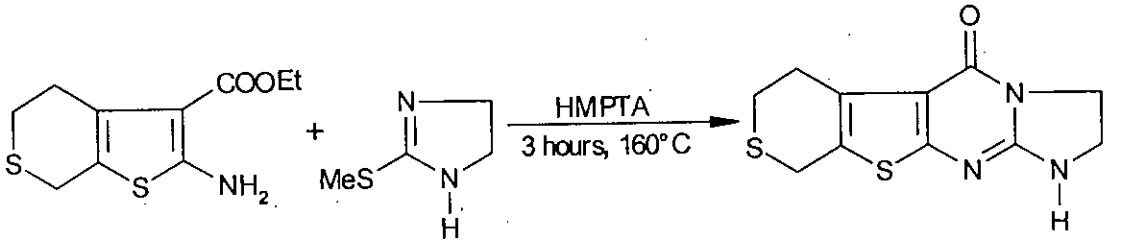
Methyl anthranilate (**91**) reacted with 5,6-dihydro-2-methylthio-4*H*-1,3-thiazine (**92**) in dry acetic acid to form 3,4-dihydro-2*H*,6*H*-[1,3]thiazino[2,3-b] quinazolin-6-one (**93**) published by F. Sauter *et al.*[60] in **Scheme-9.**



91 **92** **93**

Scheme-9

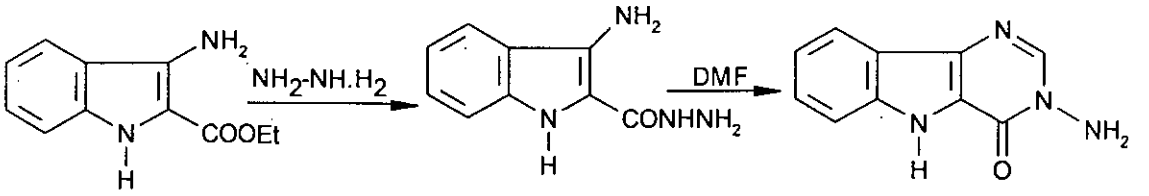
J. Frohlich *et al.*[61] also reported ethyl 2-amino-4,7-dihydro-5*H*-thieno[2,3-c] thiopyran-3-carboxylale (**94**) reacted with 2-methylthio-2-imidazoline (**95**) in hexamethylphosphoric triamide (HMPTA) at 160^{0}C for three hours afforded hexahydro-5*H*-imidazo[1,2-a]thiopyrano-[4′,3′:4,5]thieno[2,3-d]pyrimidin-5-one (**96**) (**Scheme-10**).



94 **95** **96**

Scheme-10

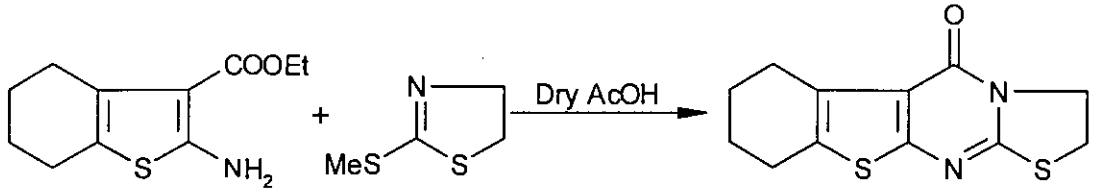
A. Monge and his co-workers[62] reported that the ethyl 3-aminoindole-2-carboxylate (**97**) reacted with hydrazine hydrate afforded 3-aminoindole-2-carbohydrazide (**98**). Compound (**98**) boiling in N,N-dimethyl formamide for 10 hours to give 3-amino-5*H*-pyrimido[5,4-b]indol-4-one (**99**) (**scheme-11**).



97 **98** **99**

Scheme-11

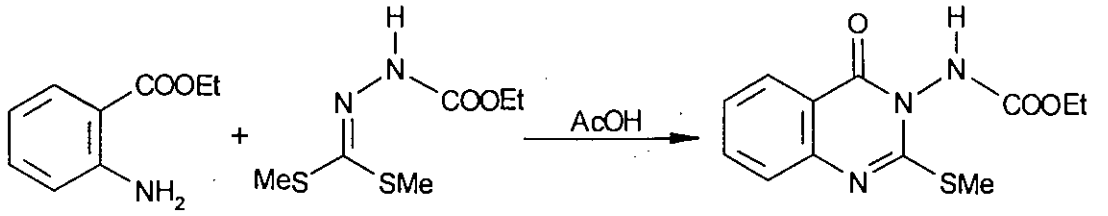
Shifullah and his co-workers[63] reported that ethyl 2-amino-4,5,6,7-tetrahydroben-zo[b]thiophene-carboxylate (**77**) reacted with imino-thioether-2-methylthio-2-thiazoline (**100**) in dry acetic acid to give 2,3,6,7,8,9-hexahydro-5*H*-[1]benzo-thieno[2,3-d]thiazolo[3,2-a]pyrimidin-5-one (**101**) (**scheme-12**).



77 **100** **101**

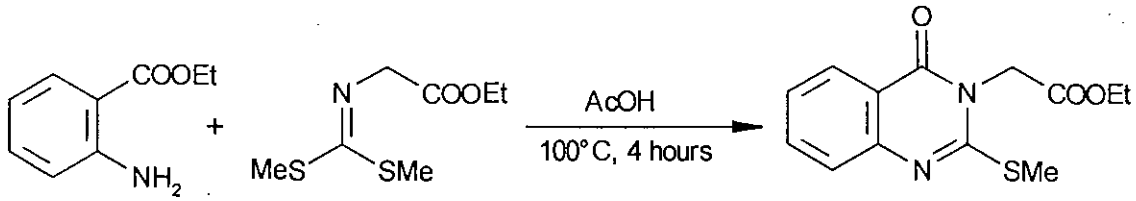
Scheme-12

K. Blasl for the first time reported[64] that aza reagent N-[bis(methylthio) methylene]hydrazine carboxylic acid ethylester (**102**) reacted with ethyl anthra-nilate (**103**) in acetic acid to afford the annelated pyrimidine product (**104**) in 70% yield, showing in **scheme-13.**



102 **103** **Scheme-13** **104**

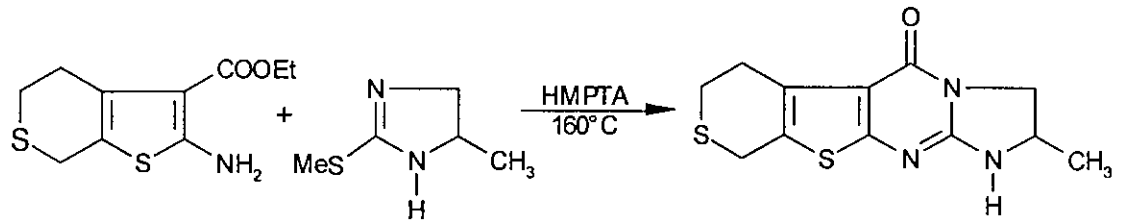
F. Sauter and his co-workers[65] reported that ethyl anthranilate (**105**) reacted with N-[bis(methylthio)-methylene]glycine-ethylester (**106**) formed 3,4-dihydro-2-(methylthio)-4-oxo-quinazoline-3-acetic acid ethyl ester (**107**) (**Scheme-14**).



105 **106** **107**

Scheme-14

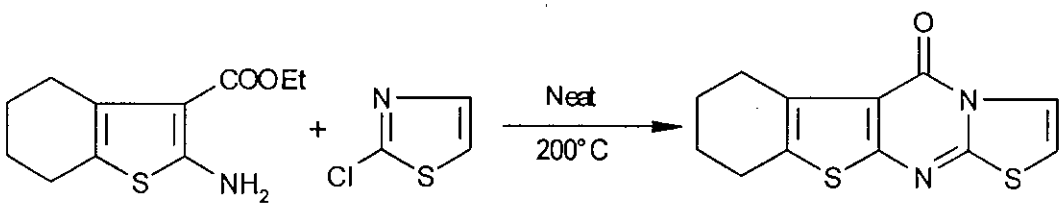
K.M.M. Rahman *et al.*[66] reported the synthesis of 1,2,3,6,7,9-hexahydro-5*H*-imidazo[1,2-a]thiopyrano[4′,3′:4,5]thieno[2,3-d]pyrimidin-5-one (**109**) from 2-amino-4,7-dihy-dro-5*H*-thieno[2,3-c]thiopyran-3-carboxylate (**94**) and 5-methyl-2-methylthio-imidazoline (**108**) in 64% yield shown in **scheme-15.**



94 **108** **109**

Scheme-15

Mannhas *et al.* reported[67] that ethyl 2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-carboxylate (**76**) reacted with iminochloride 2-chlorothiazole (**110**) afforded 2,3,6,9-tetrahydro-5*H*,7*H*-thiazolo[2,3-a]thiopyrano[4′,3′:4,5]thieno[2,3-d]pyrimi-din-5-one (**111**) as shown in **Scheme-16.**



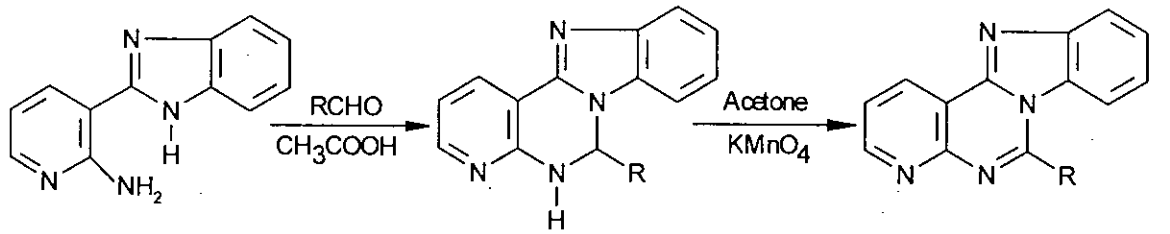
76 **110** **111**

Scheme-16

Synthesis of our target pyrimidine system will be discussed experimental, results and discussion part.

1.1.F: SYNTHESIS OF FUSED PYRIMIDINES BY VARIOUS METHOD:

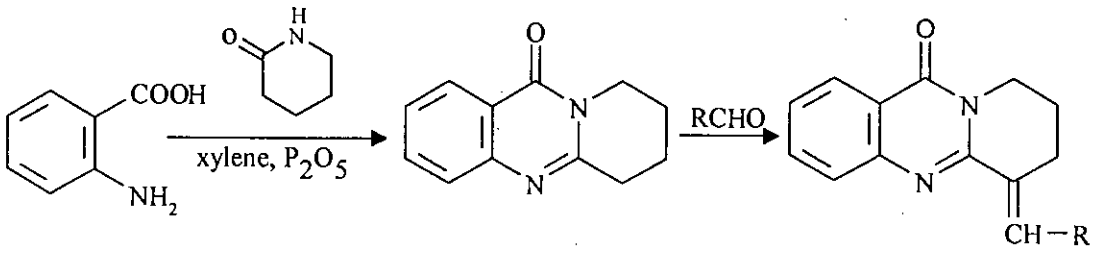
The starting compound 2-(2-amino-3-pyridyl)-benzimidazole (**112**) was obtained by the condensation of O-phenylenediamine with 2-amino-nicotinoaldehyde in the presence of ethanol and nitrobenzene. Condensation of (**112**) with aromatic aldehyde in alcoholic acetic acid led to the formation of either 6-aryl-5,6-dihydropyrido[2′,3′:4,5]pyrimido[1,6-a]benzimidazole (**113**). The oxidation of (**113**) with $KMnO_4$ in acetone resulted in the formation of fully aromatic 6-arylpyrido[2′,3′:4,5]pyrimido[1,6-a]benzimidazoles (**114**) reported by K. Vijayendar Reddy[68] showing in **scheme-17.**



112 **113** **114**

Scheme-17

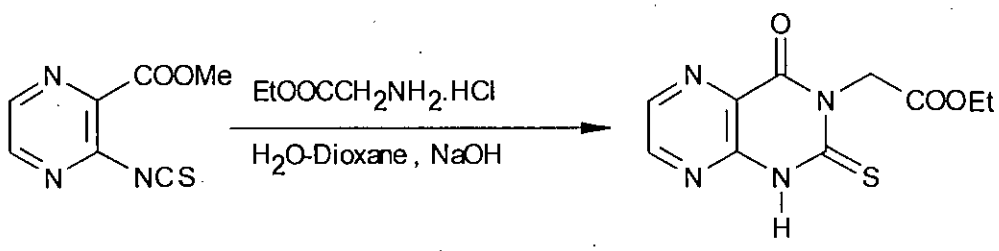
M.P. Jain *et al.*[68] reported the synthesis of tetrahydro[2,1-b]quinazolin-10(*H*)-one (**117**). The parent compound tetrahydropyridoquinazoline (**116**) was synthesized by the condensation of anthranilic acid (**115**) with 2-piperidone in xylene in the presence of phosphorus pentaoxide (**116**) on condensation with various aldehyde in xyline at 140^{0}C for 15-50 hours gave the (**117**). When R = C_6H_5 the product was obtained in 65% yield.



115 **116** **117**

Scheme-18

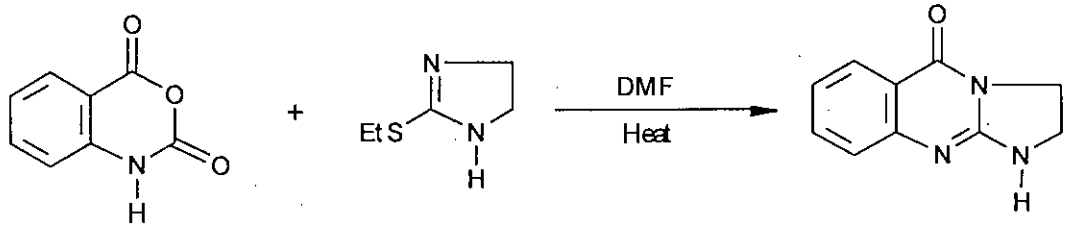
For the pteridine, a fused pyrimidine, synthesis Urleb described[69] the reaction of methyl-3-isothiocyanato-2-pyrazine carboxylate (**118**) with ethyl glycinate-hydrochloride in water-dioxane and sodium hydroxide leading to 3-(2-ethoxy-carbonyl-methyl)-2-thioxo-1,2-dihydro-4(3*H*)-pterdinone (**119**) as shown in **Scheme-19.**



118 **119**

Scheme-19

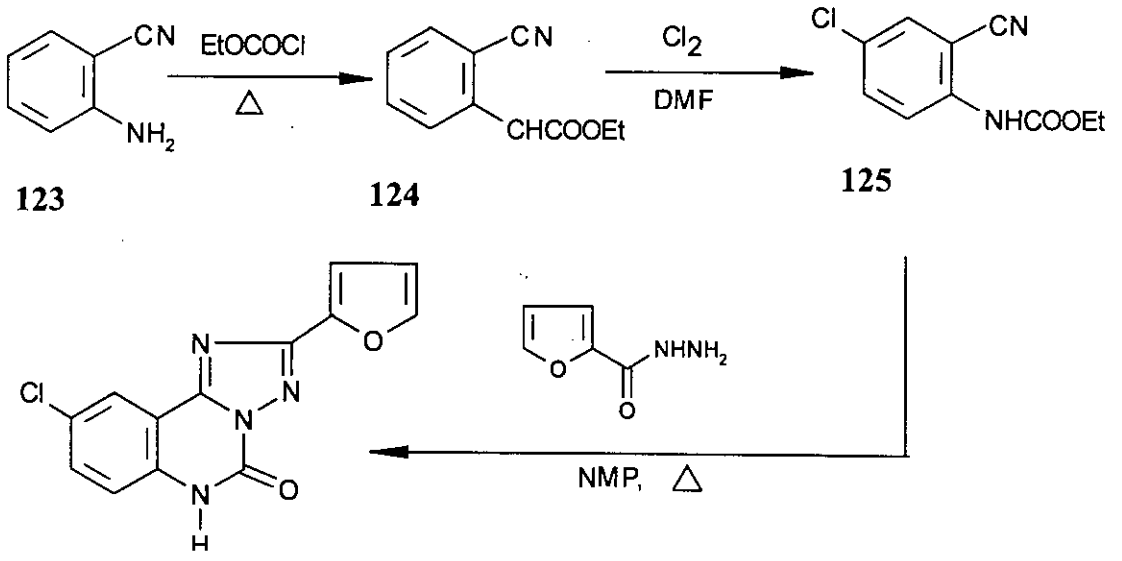
E. Zeigler and W. Steiger reported[70] isotoic anhydride (**120**) and 2-ethyl mercapto-2-imidazoline (**121**) in DMF at high temperature to give 1,2,3,5-tetrahydro-imidazo[2,1-b]quinazolin-5-one (**122**) in good yield showing in **Scheme-20.**



120 **121** **122**

Scheme-20

Anthranilonitrile (**123**) reacted with ethylchloroformate yielded ethyl N-(2-cyanophenyl)carbamate (**124**) which was then reacted with chlorine gas in N,N-dimethyl-formamide afforded ethyl N-(4-chloro-2-cyano-phenyl)carbamate (**195**). Compound (**125**) and 2-furoic acid hydrazide reacted in 1-methyl-2-pyrrolidinone (NMP) under nitrogen atmosphere to give 9-chloro-2-(2-furanyl)-5,6-dihdro[1,2,4]triazo[1,5-c]quinazolin-5(6*H*)-one (**126**). This procedure by Karl O. Gelotte and his co-workers[71] showing in **Scheme-21.**

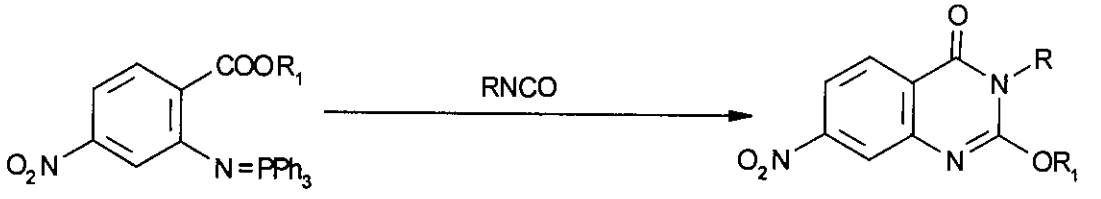


123 **124** **125**

126

Scheme-21

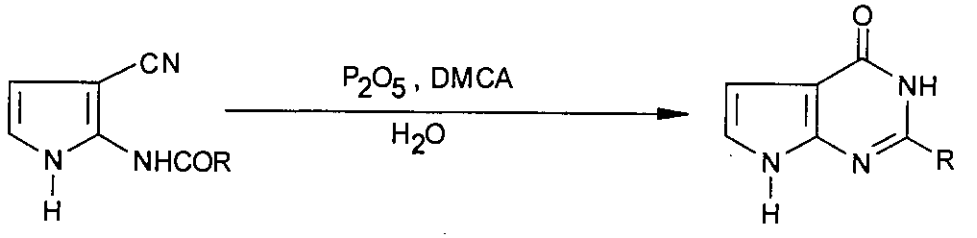
A sequence employed by Wamhoff and co-workers[72] (shown in **Scheme-22**) used the intermediate iminophosphorane (**127**), which was readily obtained from *ortho*-aminoesters by treatment with the triphenyl-phosphine, triethylamine and hexachloromethane in dry acetonitrile. Aza-wittig-type reaction of iminophosphorane with several isocyanates in acetonitrile led to quinazoline derivatives (**128**).



127 **128**

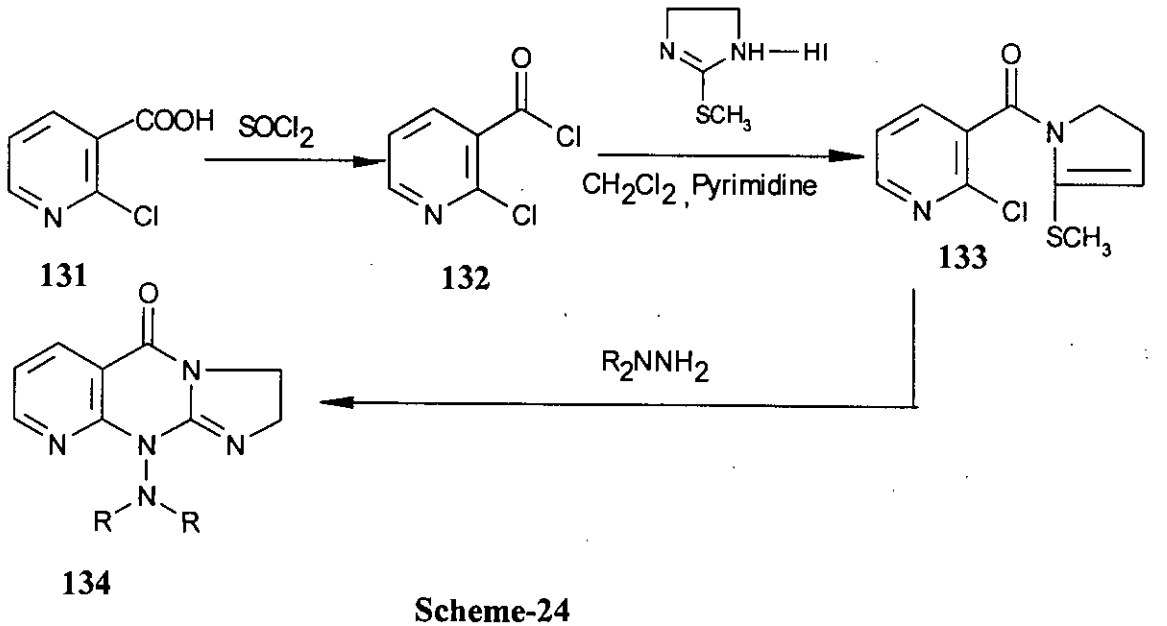
Scheme-22

The deazahypoxanthine (**130**) was obtained by a one step cyclzation of the corresponding 2-acylamino-3-cyano-pyrrole derivatives (**129**). The reaction was carried out by heating 2-acylamino-3-cyanopyrroles (**129**) in a mixture phosphoruspentaoxide, *N,N*-dimethylcyclohexamine (DMCA) and water reported by Girgis *et al.*[73] (**Scheme-23**).



129 **Scheme-23** **130**

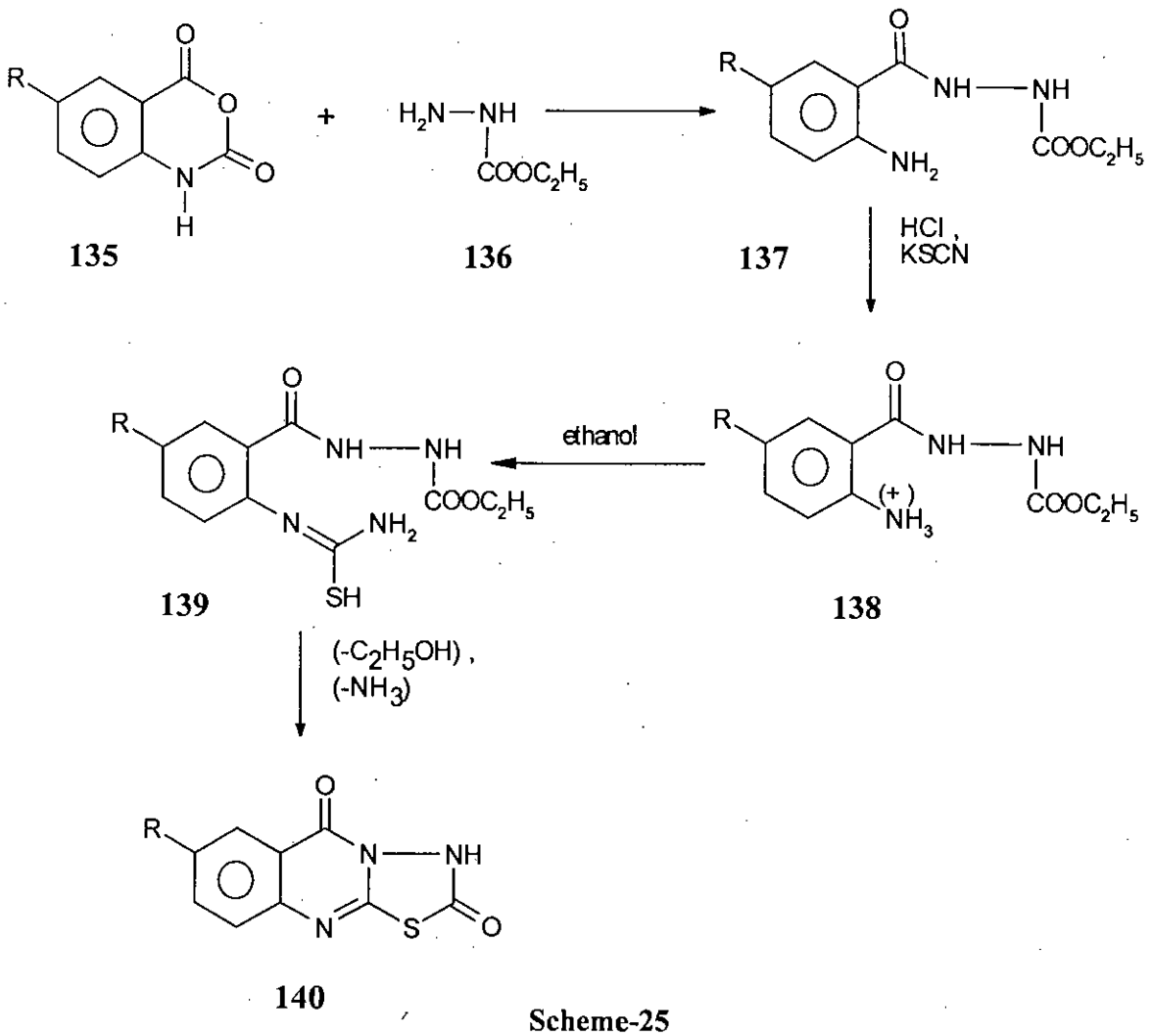
2-Chloropyridine-3-carboxylic acid (**131**) was concerted into 2,10-dihydro-10(4-morpholinyl)imidazo[1,2-a]pyrido[2,3-d]pyrimidin-5(3*H*)-one (**134**) described by Norton P. Peet and his co-workers[74]. This reaction reported that the 2-chloropyridin-3-carboxylic acid (**131**) and thionyl chloride was reluxed for 3 hours and the resulting solution was concentrated to dryness. The resulting oil was twice diluted with methylene chloride and reflux to give of 2-chloropyridine-3-carbonyl chloride (**132**). The acid chloride was added to a mixture of 2-methyl thio-2-imidazoline hydroiodide and triethylamine and new mixture was reflux for 16 hrs. to give the compound (**133**). Finally the compound (**133**) and N-amino-morpholine was heated in an oil bath at 160^{0}C for 1 hour afforded 2,10-dihydro-10-(4-morpholinyl)imidazo[1,2-a]pyrido[2,3-d]pyrimidin-5(3*H*)-one (**134**) showing in **Scheme-24.**



Scheme-24

This method was described by H.K. Gakhar and his co-workers[75] that isotoic anhydride (**135**) was condensed with carbethoxy hydrazine (**136**) to obtain ethyl 2-(2-amino-benzyl)hydrazine carboxylate (**137**) which on treatment with KSCN in

pressure of HCl in cold gave the amino thiocyanate (**138**). The compound (**138**) on refluxing in ethanol for 30 minutes gave the compound (**139**) which was then refluxed and converted into 2,3-dihydro-5*H*-[1,3,4]thiadiazo[1,3-b]quinazolin-2,5-dione (**140**) in good yield (**Scheme-25**).



135 **136** **137** **138** **139** **140**

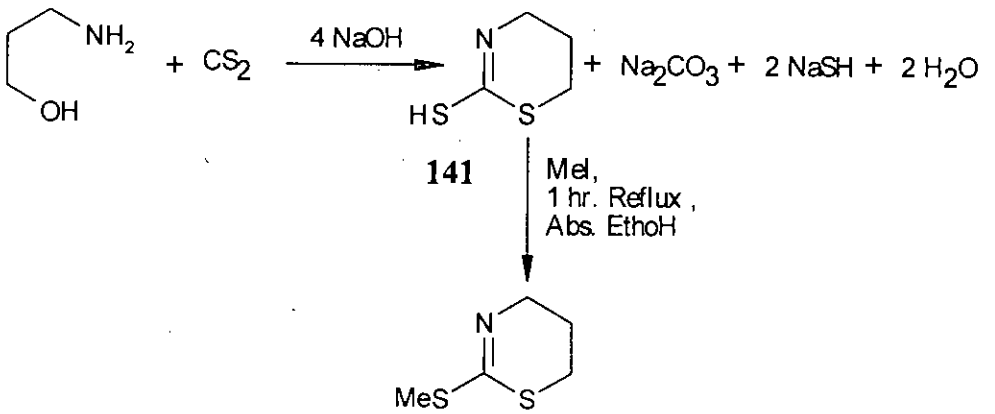
Scheme-25

1.1.G: SYNTHESIS OF ANNELATING REAGENTS:

Synthesis of 5, 6-dihydro-2-methylthio-4H-1, 3-thiazine (142):

(142) was synthesized by following methods:

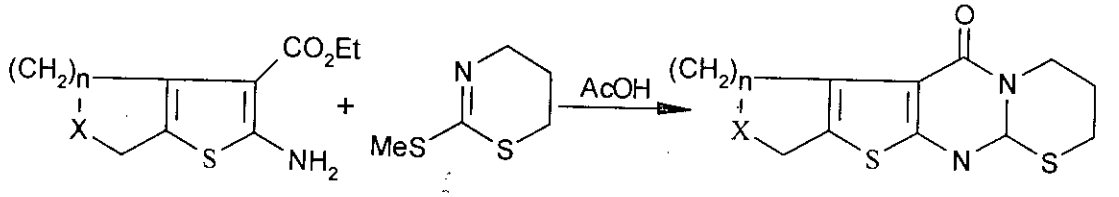
Jansens method[76] was used to prepare tetrahydro-1,3-thiazine-2-thione (**141**) in 62% yield. Thione (**141**) was methylated[77] with methyl iodide in dry methanol by heating under reflux for one hour. The solution was cooled and diluted with ether, the yellow crystalline hydroiodide salt was removed (86% yield, m.p.: 155-157^{0}C). The hydroiodide salt was neutralized with triethylamine, extracted with chloroform, dried and the solvent was evaporated to give the free base of (**142**) as yellow oil.



142

The iminothioether-5,6-dihydro-2-methylthio-4*H*-1,3-thiazine (**142**) is also a versatile reagent for the preparation of pyrimidine derivatives. The compound (**142**) reacted with *ortho*-aminoesters in a one-pot synthesis to give double annelated products of quinazolino-thiazine, thieno-pyrimido-thiazine and thiopyrano-thieno-pyrimido-thiazine derivatives. Compound (**143**) is a derivative of new heterocylic system; it was smoothly prepared from *ortho*-aminoester and thiazine (**142**) in dry acetic acid. Compounds (**143a, 143b, 143c**) were synthesized in a similar manner.

Thus the following compounds were obtained 3,4-dihydro-2*H*,6*H*-[1,3]-thiazino[2,3-b]quinazolin-6-one (**143**); 3,4,7,8,9,10-hexahydro-2*H*,6*H*-9-methyl[1] benzothieno[2′,3′:4,5]pyrimido[2,1-b][1,3]thiazin-6-one (**143a**), 3,4,8,9,10,11-hexahydro-2*H*,6*H*,7*H*-cyclohepta[4,5]thieno[2′,3′:4,5]pyrimido[2,1-b][1,3]thiazin-6-one (**143b**) and 3,4,8,9-tetrahydro-2*H*,6*H*,7*H*-cyclopenta[4,5]thieno[2′,3′:4,5] pyrimido[2,1-b][1,3]thiazin-6-one (**143c**).



142 **143**

143 a: X=MeCH; n=2

143 b: X=CH_2; n=2

143 c: X=CH_2; n=2

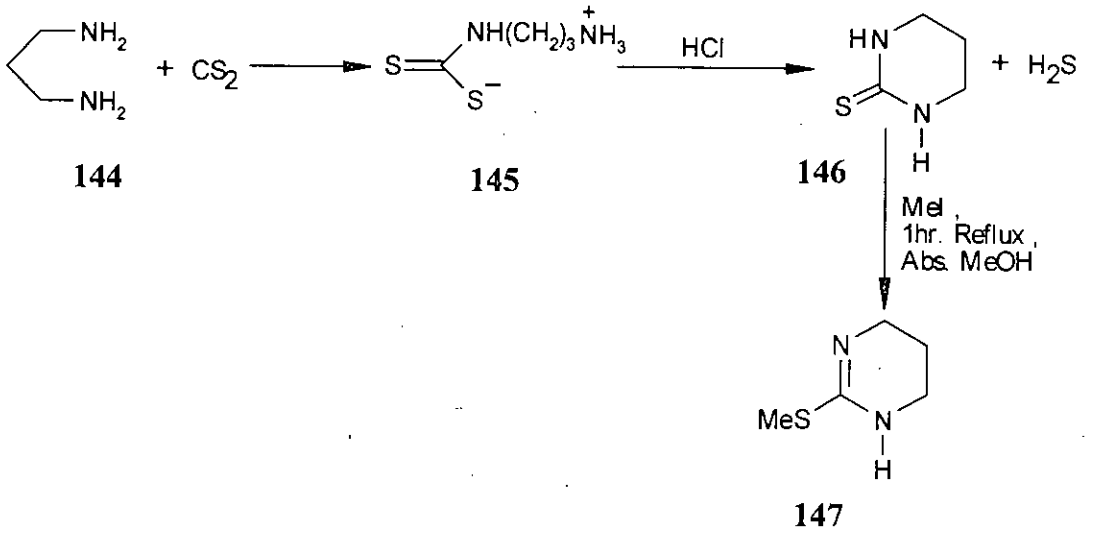
Thus the synthesis outlined in general scheme furnished a facile and efficient route for the synthesis of thiazino-pyrimidine derivatives.

Synthesis of 2-methylthio-1,4,5,6-tetrahydropyrimidine (147):

2-methylthio-1,2,4,6-tetrahydropyrimidine (**147**) was prepared for the first time by Brown *et al.*[78] from 1,4,5,6-tetrahydro-pyrimidine by heating with sulfur. Exactly the same procedure[79] as for 2-imidazolidine thione was used for the preparation at 1,4,5,6-tetrahydropyrimidin-2-thione (**146**), in that case propylenediamine (**144**) has been used in place of ethylene diamine. The yield was 80% of colourless product, m.p.: 207-208^{0}C (after recrystalisation from water).

Methylation of thione (**146**) with MeI in dry methanol for one hour under reflux resulted in 2-methylthio-1,4,5,6-tetrahydro-pyrimidinehydroiodide salt (**145**). This was neutralized with 50% NaOH solution, extracted with $CHCl_3$, dried over

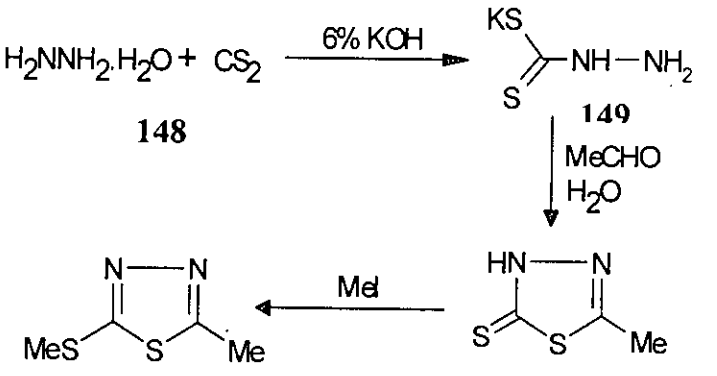
Na_2SO_4 and the solvent evaporated to leave white crystals of iodide free 2-methylthio-1,2,4,6-tetrahydropyrimidine (**147**).



144 **145** **146** **147**

Synthesis of 2-methylthio-5-methyl-1,3,4-thiadiazole (151):

2-Methylthio-5-methyl-1,3,4-thiadiazole (**151**) was synthesized from 2-thioxo-5-methyl-1,3,4-thiadiazole (**150**) by methylation, thiadiazole (**150**) was prepared from an aqueous solution of the potassium dithiocarbazate (**149**) with an acetaldehyde-water mixture in analogy to literature method.[80] Treatment of hydrazine hydrate with carbon disulfide and 6% potassium hydroxide gave the potassium dithiocarbazate (**149**).



148 **149** **151** **150**

As described in the introduction that various fused pyrimidines posses different biological and pharmaceutical activities. From the above discussion, it is conclusion that pyrimidine and fused pyrimidine derivatives have a great value for the medicinal chemistry as well as agrochemicals, veternary products and industrial purposes. Some of them are used to control the microbial pathogen of human body and other animals as medicine.

For more detail description of pyrimidine and fused pyrimidine will be discussed in the next chapters.

Part-I

Section-2

PRESENT WORK:

"SYNTHESIS OF SOME NEW ANNELATED FUSED PYRIMIDINE DERIVATIVES AND STUDY OF THEIR BIOLOGICAL ACTIVITIES"

1.2.0. PRESENT WORK:

Synthesis of some new annelated fused pyrimidine derivatives and study their biological activities.

1.2.1. Rationale:

Fused pyrimidines and their derivatives are the most important compounds in organic chemistry. Heterocyclic compounds containing the pyrimidine skeleton have generated considerable interest in recent years as reflected by recent articles dealing with their synthesis and emphasizing their biological and medicinal properties. The fused pyrimidines exhibited broad spectrum biological activities.[1] Recent development of physiologically highly potent fused pyrimidines with interesting sedative,[2] antiviral[3,4] antibacterial[2,5] antimalarial[2,5,6], antiallergic[3], antiparasitic[8], anti-inflammatory,[7] analgesic,[7] anti-HIV[5], radio protective effects[2], antagonists,[9] antihypertensive agents,[9,10] blood platelet aggregation inhibitors[11] and specially anticancer agents[3,4,5,6] prompted a great interest in this field and to find out facile routes for the synthesis of these molecules in useful yields. Pyrimidine and fused pyrimidine derivatives have a great value for the medicinal chemistry as well as agrochemicals, veterinary products and industrial purposes. Some of them are used to control the microbial pathogen of human body and other animals as medicine.

Various methods for the synthesis of fused pyrimidine systems have been developed and employed successfully in heterocyclic chemistry as described in sec.-1. Pyrimidine and fused pyrimidine compounds bearing *o*-aminoester moieties were useful substrates for the preparation of various condensed heterocyclic ring systems i.e. for synthesizing a group of new pyrimidine compounds and a variety of new heterocyclic condensed pyrimidine parent systems belonging to the class of annealated pyrimidine systems. In view of the extensive natural occurrence and biological importance of fused pyrimidine

extensive natural occurrence and biological importance of fused pyrimidine derivatives we planned to develop a general and facile method for the synthesis of fused pyrimidines. We were interested in developing methods for the synthesis of a novel series of linear or angular *tetra-*, *penta-* and *hexa-* heterocyclic ring system.

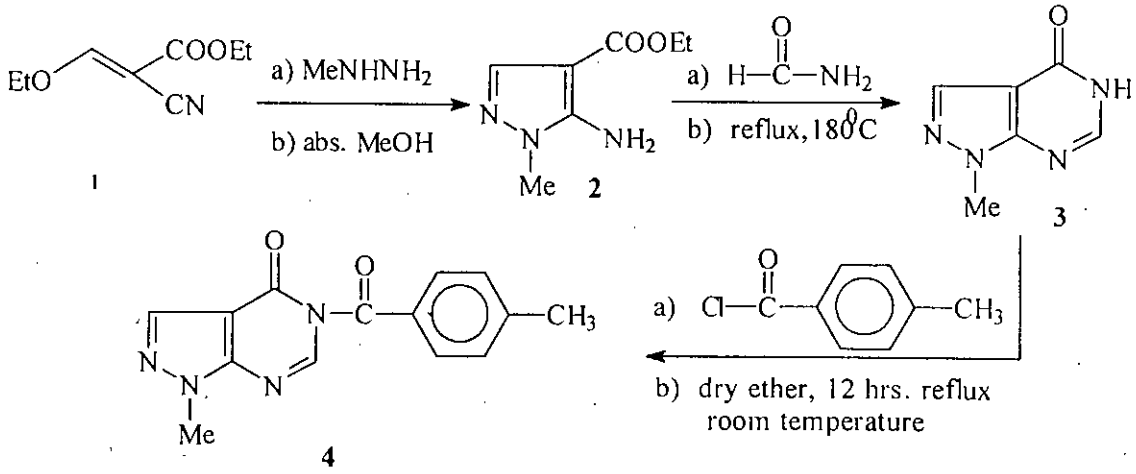
In our present studies, for the synthesis of new fused pyrimidine parent systems, we planned to use *o*-aminoester (which was prepared by Gewald method[48]) and annelating reagents (which was synthesized by standard procedure[76,81]) as the starting materials. These would be reacted with suitable reagents to develop new synthetic compounds. It was also planned to test the synthesized fused pyrimidine derivatives for biological and physiological activities.

1.2.1. RESULTS AND DISCUSSION:

As already mentioned in the introduction, various methods for the synthesis of fused pyrimidine systems have been developed and employed successfully in heterocyclic chemistry. In the present work we synthesized some fused pyrimidine systems which are described below.

1.2.1.A: *Preparation of 1-methyl-4-oxo-5-(p-toluoyl)pyrozolo [3,4-d] pyrimidine (4):*

The target compound 1-methyl-4-oxo-5-(*p*-toluoyl)pyrozolo[3,4-d] pyrimidin (**4**) was synthesized in a three-step path way as shown in **scheme-1**. The starting material ethyl-5-amino-1-methylpyrazol-4-carboxylate (**2**) was prepared from ethyl(ethoxymethylene)cyanoacetate by Gewald method[48], which was reported by the following way:



Seheme-1

Synthesis of ethyl-5-amino-1-methylpyrazol-4-carboxylate (2):

The *o*-amino substrate (**2**) was synthesized by using Gewald Procedure[48], which is given below.

Methyl hydrazine was added dropwise to a solution of ethyl(ethoxymethylene) cyanoacetate (**1**) in absolute methanol and the temperature of the mixture maintained below 60°C. After one hour stirring at room temperature of the mixture, the white precipitate was collected by filtration, washed with water and recrystallized from ethanol to afford ethyl-5-amino-1-methylpyrazol-4-carboxylate (**2**), 76.25% yield as white crystals, m.p. 98-100°C.

In the UV spectrum (**Fig. No. 1**) the λ_{max} values were found in the range of 254, 226 and 205 nm. IR spectrum (**Figure No. 2**) of this compound (**2**) showed absorption bands at 3401.2 cm^{-1} and 3283.6 cm^{-1}, which confirmed the presence of $-NH_2$ group and at 1678.9 cm^{-1} for C=O group stretching in the molecule.

Its ^1H NMR spectrum (**Fig. No. 3**) showed two-proton singlet at δ 5.35 indicated the presence of $-NH_2$ proton, three-proton quartet at δ 4.25 for $-CH_2$, three-proton singlet at δ 3.61 for –NMe group and three-proton triplet at δ 1.33 for $-CH_3$ in the molecule.

The ^{13}C NMR spectrum (**Fig. No. 4 & 5**) showed signals at δ 164.3 (C=O), 149.23, 138.9, 95.8, 59.3, 33.9 and 14.3 respectively. The presence of seven number of carbon atoms was in good agreement with structure assigned for (**2**).

Synthesis of 1-methylpyrazolo[3,4-d]pyrimidin-4(5H)-one (3):

The *o*-amino ester (**2**) was prepared by adopting the same procedure as used by Gewald method[48]. The reaction was done by stirring of (**2**) with formamide under refluxed at 180°C for 4 hrs. The progress of the reaction was checked by TLC (ethyl acetate: n-hexane, 1: 3, v/v), which showed conversion of the starting material into product. The mixture was poured into ice water and stirred for one hour. The precipitated was collected by filtration and recrystallized from ethanol to afford (**3**) in 68.77% yield, m.p. above 250°C.

In the UV spectrum (**Fig. No. 6**) the λ_{max} value was found in the range of 253.0 nm. In its IR spectrum (**Figure No. 7**) showed the absorption bands at 3147.6 cm^{-1}, 3096.5 cm^{-1} and 1675.1 cm^{-1}, which correspond to –NH, –CH and C=O stretchings respectively.

The ^{1}H NMR spectrum (**Fig. No. 8**) exhibited signals at δ 8.05 one proton singlet for -CH, another –CH proton singlet at δ 8.01, three-proton singlet at δ 3.89 for –NCH_3 and one-proton singlet at δ 2.50 for -NH group in the molecule.

The structure of compound (**3**) was also confirmed by ^{13}C NMR spectrum, which showed signals at δ 166.13 (C=O), 157.18, 144.8, 132.02, 114.17 and 36.23.

1-methyl-4-oxo-5-(p-toluoyl)pyrozolo [3,4-d] pyrimidine (4):

The compound (4) was synthesized in the following way:

When compound (**3**) was refluxed with toluoyl chloride in ether at 30°C for 6 hrs, which yielded substitute novel product (**4**). The progress of the reaction was monitored by TLC (ethyl acetate: n-hexane, 1: 5, v/v). After complete the reaction, TLC examination was showed one faster moving major product. The solution was

evaporated to dryness under reduced pressure to furnish (**4**), 84.25% yield as white crystals, m.p. 220-222°C.

The structural assignment was done by spectroscopic analysis. In the UV spectrum (**Fig. No. 10**) the λ_{max} values were found in the range of 263.0, 220.0 and 204.0 nm.

The IR spectrum (**Fig. No. 11**) of this compound showed absorption bands at 1758.0cm^{-1} and 2978.0cm^{-1} corresponding to carbonyl group (C=O) and –CH stretching but absence of –NH stretching band.

The ^{1}H NMR spectrum (**Fig. No. 12**) provided a two-proton doublet at δ_{H} 7.97 for Ar-H (d, Ar-H, J=8.1Hz), a two-proton doublet at 7.92 for aromatic group Ar-H (d, Ar-H, J=8.1Hz), a one-proton singlet at δ 7.58 for -CH, a three-proton singlet δ 3.60 for $-NCH_3$ and a three-proton singlet at δ 2.48 for $-CH_3$ respectively. All this NMR data is in consistent with the structure of target compound (**4**).

The structure of compound was further confirmed by ^{13}C NMR data (**Fig. No. 13**). The spectrum showed the signals at δ 173.61 (C=O), 164.11 (C=O), 151.53, 149.25, 136.63, 127.78, 127.60, 127.11, 126.77, 95.32, 59.12, 57.08, 42.93 and 28.89 respectively. The ^{13}C NMR spectrum indicated the presence of thirteen carbons in the molecule corresponding to the molecular formula $C_{13}H_{12}N_4O_2$, thereby suggesting the formation of a compound (**4**).

On the basis of complete analysis of the IR, ^{1}H NMR and ^{13}C NMR spectra, the structure of this compound was accorded as 1-methyl-4-oxo-5-(*p*-toluoyl)pyrozolo [3,4-d]pyrimidine (**4**).

1.2.2.B. *Preparation of 1-methyl-6,7-dihydropyrozolo[3,4-d]thiazolo[1,2-a]pyrimidin-4-one (8):*

The synthesis of the fused pyrimidine is divided into two headings.

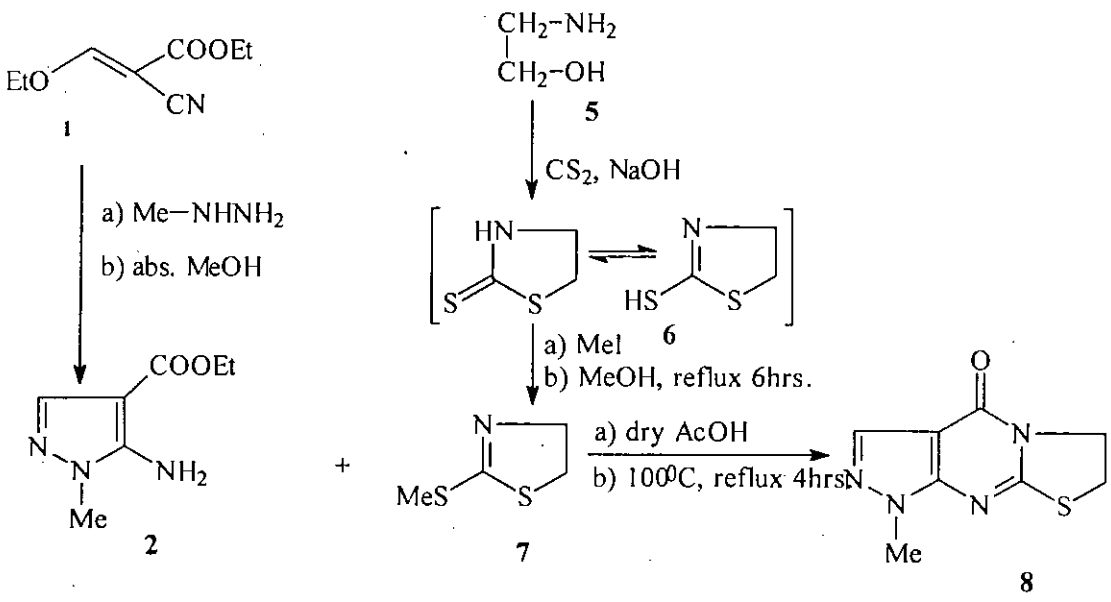
I) Synthesis of annelating substrate:

Ethyl-5-amino-1-methylpyrazol-4-carboxylate (2).

II) Synthesis of annelating reagent:

2-Methylthio-2-thiazoline (7).

In this approach we used annelated substrate as ethyl-5-amino-1-methylpyrazol-4-carboxylate (**2**) and annelating reagent as 2-methylthio-2-thiazoline (**7**) for the synthesis of fused pyrimidine 1-methyl-6,7-dihydropyrozolo-[3,4-d] thiazolo [1,2-a] pyrimidin-4-one (**8**) by one-step reaction in dry acetic acid medium.



Scheme-2

For more detail description of these reaction sequence is discussed in below:

I). Synthesis of annelating substrate:

Ethyl-5-amino-1-methylpyrazol-4-carboxylate (2)

This compound (**2**) was synthesized from ethyl(ethoxymethylene)cyanoacetate (**1**) by using Gewald method[48] which is reported in **scheme-1**.

II). Synthesis of annelating reagent:

2-Methylthio-2-thiazoline (7).

This compound (7) was prepared from 2-mercaptothiazoline (**6**) by using Hofmann method[81].

Synthesis of 2-mercaptothiazoline (6):

To a solution of ethanolamine, water, sodium hydroxide and was added carbon disulfide dropwise. The temperature of the reaction mixture cooled to 35°C. And the reaction mixture was warmed to keep it at 45°C. After refluxing for 7 hrs. the reaction mixture was heated when a residue of carbon disulfide has boiled off at 100°C. The progress of the reaction was checked by TLC (chloroform : methanol, 13: 1, v/v). The largely precipitate was collected by filtration, washed with conc. HCl and recrystallized from water to give (**7**) as yellow crystals in 82.43% yield, m.p. 102-104°C.

In the UV spectrum (**Fig. No. 14**) the λ_{max} values were found in the range of 240.0 nm. The IR spectrum (**Fig. No. 15**) of this compound showed following characteristic peaks: 3133cm^{-1} (-NH str.) and 1296cm^{-1} (C=S str.) respectively.

Its ^{1}H NMR spectrum (**Fig. No. 16**) showed two-proton triplet at δ 3.98 (as t, 2H, CH_2, J = 7.97Hz) and δ 3.55 (as t, 2H, CH_2, J = 7.97Hz) indicated for two methylene (-CH_2) groups at 4- and 5- position in the compound (**6**). The one-proton singlet at δ 8.16 is due to –SH proton or –NH proton which is involved in phototropic tautomerism as shown (**scheme-2**) in the molecule.

The structure of compound (**6**) was also confirmed by ^{13}C NMR spectrum, which showed signals at δ_C 174.85 (C-2), 44.24 (C-4) and 32.18 (C-5) respectively. The rest of the C-3 atoms corresponded to the molecular formula $C_3H_5NS_2$.

2-Methylthio-2-thiazoline (7):

The compound (**6**) was methylated by standard procedure[76,81]. To the mixture of compound (**6**) in absolute methanol, was added methyl iodide dropwise. The mixture was heated under reflux for 1 hrs. The suspension was cooled and diluted with ether. The crystalline hydroxide salts was decomposed with 15% NaOH solution and extracted with chloroform. The solvent was evaporated under reduced pressure to give yellow syrup in 56% yield; b.p. 70°C.

In the UV spectrum (**Fig. No. 18**) the λ_{max} value was found at 253.0 nm. The IR spectrum (**Fig. No. 19**) of this compound showed absorption bands at 2931.6 cm^{-1} (CH str.), 1255.0 cm^{-1} (C=S str.) and 1564.5 cm^{-1}(C=C str.) respectively.

The ^1H NMR spectrum (**Fig. No. 20**) showed a three-proton singlet at δ 2.52 for –SMe group, a two-proton triplet at δ 4.20 and δ 3.99 were observed for two methylene (-CH_2) group, which indicated the formation of (**7**).

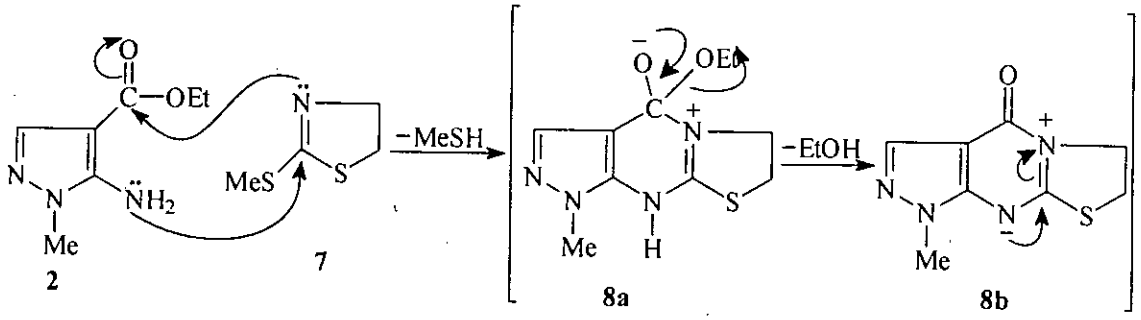
The ^{13}C NMR spectrum showed signals at δ_C 165.47 (C=N), 55.85, 44.31 and 14.83 (-SMe) respectively.

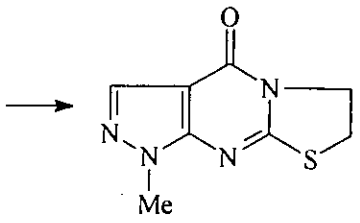
Preparation of 1-methyl-6,7-dihydropyrozolo [3,4-d] thiazolo [1,2-a[pyrimidin-4-one (8):

Annelating reagent 2-methylthio-2-thiazoline (7) with *o*-amino ester, ethyl -5-amino-1-methylpyrozol-4-carboxylate (**2**) in dry acetic acid was heated under reflux for 4 hrs. The progress of the reaction was checked by TLC (chloroform : methanol, 13: 1, v/v). The precipitate was filtered, washed with water and recrystallized from ethanol to give (**8**) as yellow crystals in 76.5% yield; m.p. above 250°C.

Reaction mechanism:

The mechanism for the condensation of *o*-amino ester, ethyl-5-amino-1-methylpyrazol-4-carboxylate (**2**) and annelating reagent as 2-methylthio-2-thiazoline (7) for the synthesis of fused pyrimidine, 1-methyl-6,7-dihydropyrazolo [3,4-d]thiazolo[1,2-a]pyrimidin-4-one (**8**) may be shown in below:





Mechanism-1

8

The mechanism of this reaction probably involved in the initial nucleophilic addition of the amino group of *o*-aminoester (**2**) to the electron deficient carbon of the thiazoline, 2-methylthio-2-thiazoline (**7**) to form the intermediate (**8a**) which eliminates the marcapto group (-SH) from the intermediate (**8**b). Ring closure occurs by nucleophilic attack of thiazoline nitrogen atom to the sp^2 carbon of the carboxylate followed by an elimination of ethanol to give (**8**) via. an intermolecular cyclization.

In the UV spectrum (**Fig. No. 22**) the λ_{max} value was found in the range of 268.0 nm. The IR spectrum (**Fig. No. 23**) of this compound showed the following characteristic peaks: 3096.5 cm^{-1} (CH str.), 1676.0 cm^{-1} (C=O str.), 1598.9 cm^{-1} (-CN) and 1544 cm^{-1} (C=C str.) respectively.

Its ^1H NMR spectrum (**Fig. No. 24 & 25**) exhibited a one-proton singlet at δ 7.76 for –CH, a two-proton triplet at δ 4.14 (t, 2H, J = 7.4Hz) and δ 3.27 (t, 2H, J = 7.4Hz) were due to two methylene ($-CH_2$) groups and a three-proton singlet at δ 2.47 for $-NCH_3$ group in the molecule. Disappearance of –NH peak from the ^1H NMR (**Fig. No. 24**) indicated the formation of a new thiazoline ring. This assignment is in complete agreement with the structure of the compound (**8**).

The ^{13}C NMR spectrum of the compound showed the signals at δ 164.4 (C=O), 140.8, 136.8, 115.2, 72.1, 44.5, 42.9 and 35.8 respectively. So, the ^{13}C NMR spectrum indicated the presence of eight carbons in the molecule corresponding to the molecular formula $C_8H_8N_4S$, thereby suggesting the formation of a compound (**8**).

Complete analysis of the IR, ^1H NMR and ^{13}C NMR spectrum of this compound was in complete agreement with the structure accorded to it as Synthesis of 1-methyl-6,7-dihydropyrazolo [3,4-d]thiazolo[1,2-a]pyrimidin-4-one (**8**)

1.2.3.C: *Preparation of 1-methyl-4-oxazolo-6,7-dihydropyrozolo[3,4-d] imidazo [1,2-a]pyrimidin-4(8H)-one (12):*

The synthesis of the fused pyrimidine is divided into two headings.

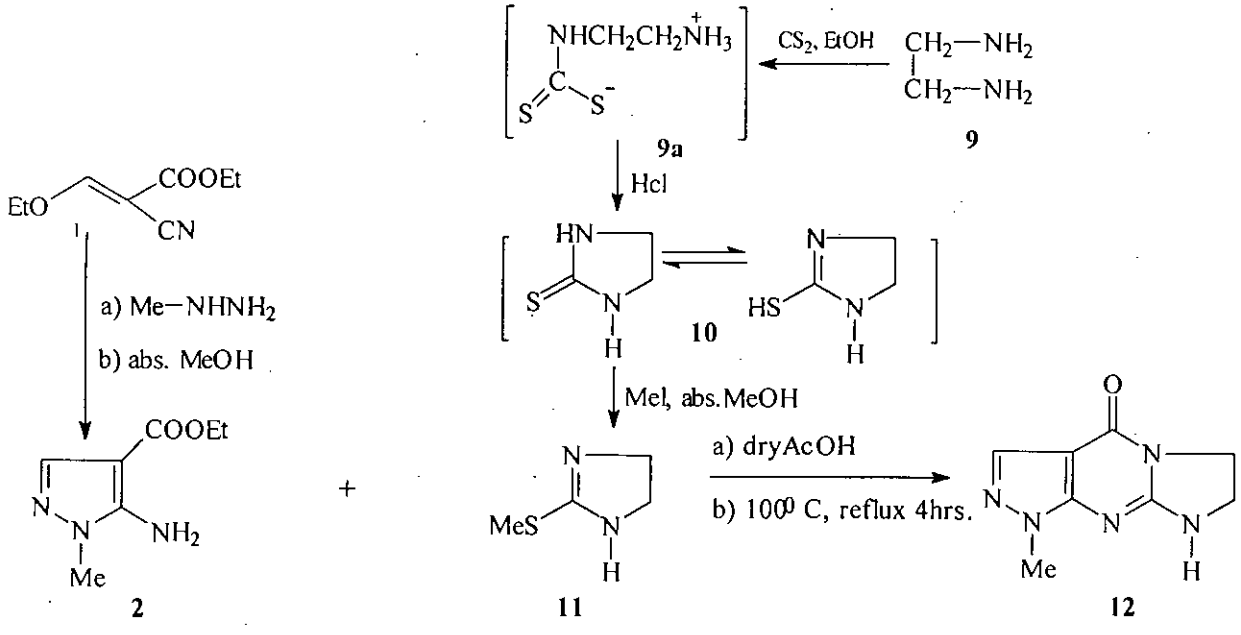
I) Synthesis of annelating substrate:

Ethyl-5-amino-1-methylpyrazol-4-carboxylate (2)

II) Synthesis of annelating reagent:

2-Methylthio-imidazoline (11).

In this approach annelated substrate was used as ethyl-5-amino-1-methylpyrazol-4-carboxylate (**2**) and annelating reagent as 2-methylthio-imidazoline (**11**) for the synthesis of fused pyrimidine 1-methyl-4-oxazolo-6,7-dihydropyrozolo[3,4-d]imidazo[1,2-a]pyridine-4(8H)-one (**12**) by one-step reaction in dry acetic acid medium.



Scheme-3

I) Synthesis of annelating substrate:

Ethyl-5-amino-1-methylpyrazol-4-carboxylate (2)

This compound (2) was synthesized from ethyl(ethoxy methylene)cyanoacetate (1) by using Gewald method[48] which is reported **scheme-1**.

II) Synthesis of annelating reagent:

2-methylthio-imidazoline (11).

This compound (**11**) was synthesized from 2-imidazoline thione (**10**) by using Hofmann method[81].

Synthesis of 2-imidazolinethione (10):

Carbon disulfide was added dropwise to a solution of ethylene diamine, rectified spirit and water with occasional shaking for two hours and then refluxed on a water bath for one hrs. Concentrated HCl was then added and further refluxed for 9-10 hrs. The resulting solid, on cooling, was filtered, washed with cold acetone and crystallized from ethanol to give (**10**) as white crystals in 47% yield; m.p. 155-156^{0}C.

In the UV spectrum (**Fig. No. 26**) the λ_{max} value was found in the range of 251.0 nm. The IR spectrum (**Fig. No. 27**) of this compound (**10**) showed absorption bands at 3248.9cm^{-1} and 2879.5cm^{-1} corresponding to –NH and –CH stretching respectively.

Its ^{1}H NMR spectrum (**Fig. No. 28**) showed a two-proton singlet at δ 6.00 for two -NH group and a four-proton multiplet at δ 3.76 was observed for two methylene (-CH_2) groups in the molecule.

The ^{13}C NMR spectrum showed singlet at δ 183.36 and at δ 44.24 for C-4 and C-5 which indicated the formation of (**10**).

Synthesis of 2-methylthio-imidazoline (11):

The reaction was performed by stirring compound (**10**) and methyl iodide in absolute methanol for 2 hrs. Methanol was removed under reduced pressure and this was neutralized with 15% sodium hydroxide solution, extracted with chloroform, dried over sodium sulfate and the solvent evaporated to give (**11**) as white crystals in 58.63% yield; mp. 119-121^{0}C.

In the UV spectrum (**Fig. No. 29**) the λ_{max} value was found in the range of 245.6 nm. The IR spectrum (**Fig. No. 30**) of this compound showed the following characteristic peaks: 3392.6cm^{-1} (-NH str.), 3149.5 (-CH str.) and 1603.7cm^{-1} (-CN) respectively.

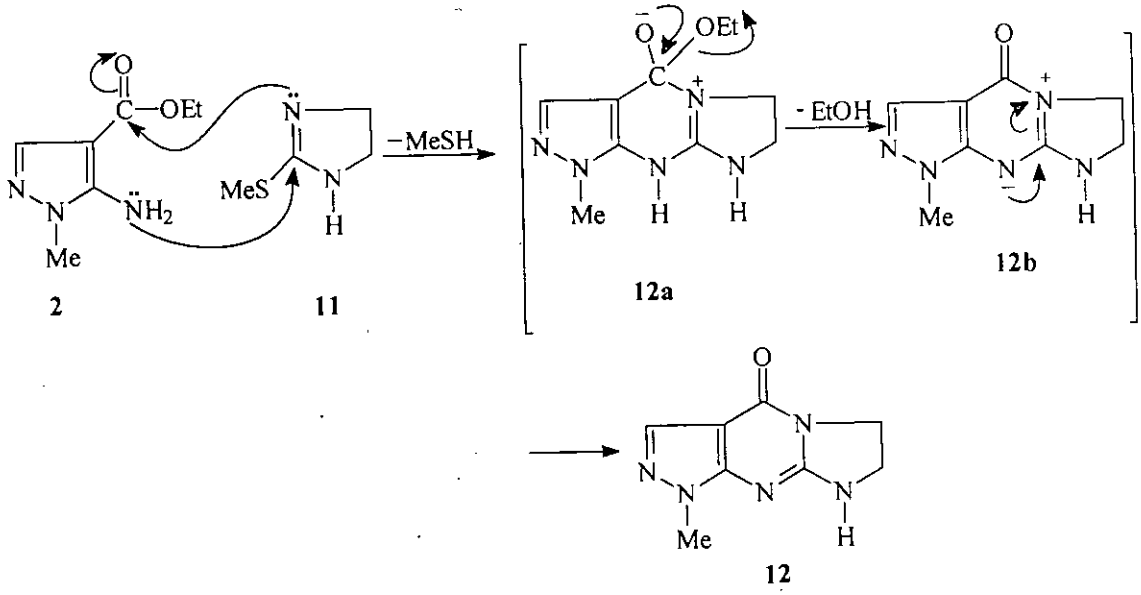
The ^{1}H NMR spectrum (**Fig. No. 31**) showed a one-proton singlet at δ 5.98 for -NH group, a three-proton singlet at δ 2.50 for –SMe group and a four-proton multiplet at δ 3.65 were observed for two methylene (-CH_2) group, which indicated the formation of (**11**).

The structure of 2-methylthio-imidazoline (**11**) was also confirmed by ^{13}C NMR spectrum. The spectrum exhibits the signal at δ 164.85 for C-2, δ 13.25 for –SMe, 50.15 for C-4 and C-5 respectively. The spectrum displayed the presence of four carbon atoms corresponding to its molecular formula ($C_4H_8N_2S$).

Preparation of 1-methyl-4-oxazolo-6,7-dihydropyrozolo[3,4-d] imidazo[1,2-a] pyrimidin-4(8H)-one (12)

Annelated substrate, ethyl-5-amino-1-methylpyrazol-4-carboxylate (**2**) was heated with annelating reagent, 2-methylthio-imidazoline (**11**) in presence of dry acetic acid under reflux for 4 hrs. The reaction mixture was filtered and the solid mass was obtained from methanol to furnish new tricyclic fused pyrimidine compound (**12**) in 65.46% yield; mp. > 250°C.

Reaction mechanism:



The mechanism of this reaction probably involved in the initial nucleophilic addition of the amino group of *o*-aminoester (**2**) to the electron deficient carbon of the imidazoline, 2-methylthio-imidazoline (**11**) to form the intermediate (**12a**) which eliminates the marcapto group (-SH) from the intermediate (**12b**). Ring closure occured by nucleophilic attack of imidazoline nitrogen atom to the sp^2 carbon of the carboxylate followed by an elimination of ethanol to give compound 1-methyl-4-oxazolo-6,7-dihydropyrozolo[3,4-d]imidazo[1,2-a]pyrimidine-4(8H)-one (**12**).

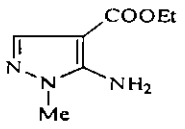
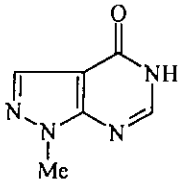
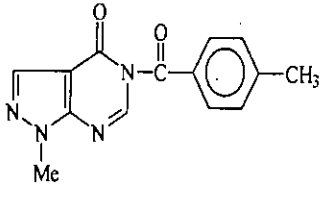
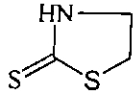
The structure of the compound (**12**) was established by spectral data. In the UV spectrum (**Fig. No. 32**) the λ_{max} value was found in the range of 275.0 and 208 nm. The IR spectrum (**Fig. No. 33**) of this compound (**10**) showed absorption bands at 3433.1cm^{-1}, 3196.8cm^{-1} and 1692.4cm^{-1}corresponding to –NH, –CH and C=O stretching respectively.

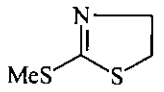
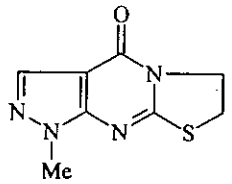
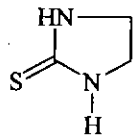
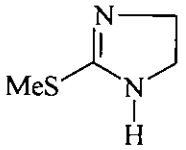
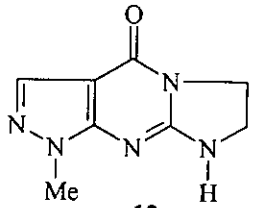
In its ^{1}H NMR spectrum (**Fig. No. 34**) a one-proton singlet at δ 7.76 for –CH, a one-proton singlet at δ -NH, a two-proton triplet at δ 4.15 (t, 2H, CH_2, *J*=7.8Hz), and δ 3.25 (t, 2H, CH_2, *J*=7.8Hz) due to two methylene (-CH_2) groups and a three-proton singlet at δ 3.03 for –NCH_3 group were observed (**12**). The absents of the –NH_2 group and –COOEt group in the spectrum also indicated the formation of the annelating ring. It was suggested by the mechanism in **scheme-3**.

Again ^{13}C NMR spectrum displayed singlet at δ 165.40 (C=0), 137.32, 125.12, 123.86, 121.58, 96.34, 59.12, 39.32, 39.16, 36.39 and 34.37, which indicated of the formation a new pyrimidine ring (**12**).

Thus it is a efficient and facile method for the preparation of tetracyclic fused pyrimidine 1-methyl-4-oxazolo-6,7-dihydropyrozolo[3,4-d]imidazo[1,2-a]pyridine -4(8H)-one (**12**).

1.2.3.D. Comparison of some spectral data of heterocyclic derivatives:

Compound	IR (cm^{-1})	^1H NMR (δ_H)	^{13}C NMR (δ_C)	UV nm
 2	3401.2 (γ_{NH}) 3283.6 (γ_{NH}) 2985.6 (γ_{CH}) 1678.9 (γ_{CO})	7.59 (s, 1H, CH), 5.35-5.20 (br s, 2H, NH_2) 4.25 (q, 2H, CH_2, J=7.1 Hz), 3.61 (s, 3H, NCH_3), 1.33 (t, 3H, CH_3, J=7.1 Hz).	164.31 (C=O), 149.24, 138.86, 95.76, 59.29, 33.86, 14.25.	254.0 226.0
 3	3147.8 (γ_{NH}) 3096.5 (γ_{CH}) 1675.1 (γ_{CO})	δ_H 8.05 (s, 1H, CH), 8.00 (s, 1H, NH), 3.88 (s, 3H, NCH_3), 2.58 (S, 1H, CH).	166.13 (C=O), 157.18, 144.8, 132.02, 114.17, 36.23, 33.09.	253
 4	2978.6 (γ_{CH}) 1758.0 (γ_{CO}) 1733.9 (γ_{CO})	7.97 (d, 2H, Ar-H, J=8.0Hz), 7.92 (d, 2H, Ar- H, J=8.0Hz), 7.58 (s, 1H, CH), 5.47 (s, 1H, CH), 3.60 (s, 3H, NCH_3), 2.48 (s, 3H, Ar-CH_3).	173.61 (C=O), 164.11 (C=O), 151.53, 149.25, 136.63, 127.78, 127.60, 127.11, 126.77, 95.32, 59.12, 57.08, 42.93, 28.89.	263.0 220.0
 6	3133.1 (γ_{NH}) 1296.1 (γ_{CS}) 1507.3 (γ_{CN})	8.16 (s, 1H, SH), 3.98 (t, 2H, H_2, J=7.97Hz), 3.55 (t, 2H, CH_2, J=7.97Hz).	174.85 (-CSH), 44.24, 32.18.	240 207

Compound	IR (cm^{-1})	^{1}H NMR (δ_H)	^{13}C NMR (δ_C)	UV nm
 7	2931.6 (γ_{CH}) 2852.5 (γ_{CH}) 1564.2 (γ_{CN})	4.21 (t, 2H, CH_2, J = 7.8Hz), 3.39 (t, 2H, CH_2, J = 7.8Hz), 2.52 (s, 3H, CH_3).	165.47 (-CN), 55.85, 44.31, 14.83	253.0
 8	3096.5 (γ_{CH}) 2898.6 (γ_{CH}) 1676.9 (γ_{CO})	7.76 (s, 1H, CH), 4.14 (t, 2H, CH_2, J = 7.4Hz), 3.27 (t, 2H, CH_2, J = 7.4Hz), 2.47 (s, 3H, NCH_3).	164.40 (C=0), 140.8, 136.8, 115.2, 72.1, 44.0, 42.9, 35.84.	268.0
 10	3248.9 (γ_{NH}) 2879.5 (γ_{CH})	6.0 (s, 2H, 2× NH), 3.76 (m, 4H, 2× CH_2).	183.36 (C-2), 44.24 (C-4 and C-5).	245.6
 11	3392.6 (γ_{NH}) 3149.5 (γ_{CH}) 1582.5 (γ_{CN})	5.98 (s, 1H, NH), 3.65 (m, 4H, 2× CH_2), 2.50 (s, 3H, SCH_3).	164.85 (C-2), 50.13 (C-4 and C-5),	251.6
 12	3433.1 (γ_{NH}) 2985.6 (γ_{CH}) 1692.4 (γ_{CO})	7.76 (s, 1H, CH), 6.98 (s, 1H, NH), 4.15 (t, 2H, CH_2, J = 7.8Hz), 3.25 (t, 2H, CH_2, J = 7.8Hz), 3.03 (s, 3H, NCH_3).	165.40 (C=0), 137.32, 25.12, 110.25, 72.05, 39.32, 34.37.	275.0

1.2.3. Conclusion:

A convenient, general and facile method for the synthesis of fused pyrimidine from the reaction of *o*-amino substrate with annelating reagent was developed.

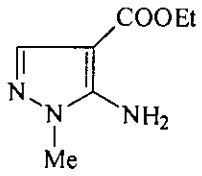
The most important features of the synthesis are that readily available, inexpensive starting materials were used at relatively mild reaction conditions and relatively good yields were obtained. Also, no toxic and hazardous compounds were produced by this procedure. A variety of fused heterocyclic ring could be introduced at the suitable positions by this procedure. Through this methodology biologically and medicinally important fused pyrimidines might be synthesized.

1.2.4: EXPERIMENTAL:

1.2.4.A. SYNTHESIS OF 1-METHYL-4-OXO-5-(*p*-TOLUOYL)PYRAZOLO [3,4-d]PYRIMIDINE (4):

2.5.1. (i) Preparation of ethyl-5-amino-1-methylpyrozolo-4-carboxylate (2):

To a solution of ethyl(ethoxymethylene)cyanoacetate (**1**) (10g, 59.10 mmol) in 35 ml absolute ethanol, slight heat was produced during the addition. The resulting solution was refluxed with stirring for 1 hour, after which the solvent was evaporated under reduced pressure. The resulting solid, after washing with ether and crystallization from water to give (**2**) white crystals. The yield was 84.31%; mp. 90-91^{0}C.



2

M.F.: $C_7H_{11}N_3O_2$.

UV (EtOH): 254.0 and 226.0 nm.

IR (KBr): ν_{max} 3401.2, 3283.6, 3114.8, 2985.6, 1678.9, 1561.6 and 1385cm^{-1}.

^{1}H NMR (400MHz, $CDCl_3$): δ_H 7.59 (s, 1H, CH), 5.35-5.20 (br s, 2H, NH_2), 4.25 (q, 2H, CH_2, J = 7.1 Hz), 3.61 (s, 3H, NCH_3), 1.33 (t, 3H, CH_3, J = 7.1 Hz).

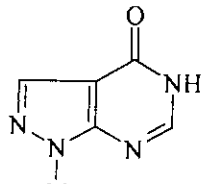
^{13}C NMR (100MHz, $CDCl_3$): δ_C 164.31 (C=O), 149.24, 138.86, 95.76, 59.29, 33.86, 14.25.

Anal. Calcd. for $C_7H_{11}N_3O_2$: C, 49.52 H, 6.41 N, 24.65% Found: C, 49.48 H, 6.38 N, 24.70%.

(ii) Preparation of 1-methyl-pyrozolo[3,4-d]pyrimidin-4(5H)-one (3):

A solution of *o*-amino ester (**2**) (1g, 5.92 mmol) in 4 ml formamide was refluxed at 180^{0}C for 4 hrs. The progress of the reaction was checked by the TLC (n-hexane: ethyl acetate, 1:2, v/v). TLC of the reaction mixture showed that the reactant was completely converted to the product.

The R_f value was 0.35. The mixture was poured into ice-water and stirred for one hour. The precipitated product collected by filtration and recrystallized from ethanol to give (**3**) white crystals. The yield was 0.68g (68.77%); mp. > 250^{0}C.



3

M.F.: $C_6H_6N_4O$.

UV (EtOH): 253nm.

IR (KBr): ν_{max} 3147.8, 3096.5, 2897.0, 1675.1 and 1396.4 cm^{-1}.

^{1}H NMR (400MHz, DMSO-d_6): δ_H 8.05 (s, 1H, CH), 8.00 (s, 1H, NH), 3.88 (s, 3H, NCH_3), 2.58 (S, 1H, CH).

^{13}C NMR (100MHz, DMSO): δ_C 166.13 (C=O), 157.18, 144.8, 132.02, 114.17, 36.23, 33.09.

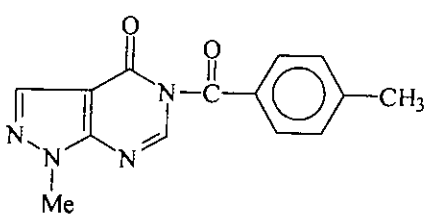
(iii) Preparation of 1-methyl-4-oxo-5-(p-toluoyl)pyrozolo[3,4-d]pyrimidin (4):

To a solution of (**2**) (0.25g, 1.67 mmol), *p*-toluoyl chloride (0.32g, 1.67mmol) and diethyl ether (6 ml) were added. Then the mixture was stirred reflux for about 6 hrs. The progress of the reaction was monitored by the TLC (n-hexane: ethyl acetate, 1:3, v/v). After complete the reaction, TLC showed that the reactant was completely converted to the product.

The R_f value was 0.61. The mixture was evaporated to dryness under reduced pressure in vacuum evaporator. The resulting solid was recrystallized from ethanol to give (**4**) as a white crystal. The yield was 64.46%; mp. 220^{0}C-222^{0}C.

M.F.: $C_{14}H_{12}N_4O_2$.

UV (EtOH): 263.0 and 220.0 nm.



4

IR (KBr): ν_{max} 2978.6, 1758.0, 1733.9, 1539.1, 1347.2 and 1188cm^{-1}.

^{1}H NMR (400MHz, DMSO): δ_H 7.97 (d, 2H, Ar-H, *J*=8.0Hz), 7.92 (d, 2H, Ar-H, *J*=8.0Hz), 7.58 (s, 1H, CH), 5.47 (s, 1H, CH), 3.60 (s, 3H, NCH_3), 2.48 (s, 3H, Ar-CH_3).

^{13}C NMR (100MHz, DMSO): δ_C 173.61 (C=O), 164.11 (C=O), 151.53, 149.25, 136.63, 127.78, 127.60, 127.11, 126.77, 95.32, 59.12, 57.08, 42.93, 28.89.

1.2.4.B. SYNTHESIS OF 1-METHYL-4-OXO-6,7-DIHYDROPYROZOLO[3, 4-d] THIAZOLO[1,2-a]PYRIMIDIN-4-ONE (8).

2.4.2.(i) Preparation of ethyl-5-amino-1-methylpyrozolo-4-carboxylate (2):

This compound (**2**) was synthesized from ethyl(ethoxy methylene)cyanoacetate (**1**) by using Gewald method[48] which was reported in previous scheme.

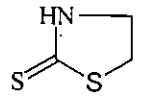
(ii) Preparation of 2-mercaptothiazoline (6):

A mixture of water (26.29 ml), 3.66 g ethanolamine (**5**), 9.61 g sodium hydroxide was cooled to 30^{0}C with constant stirring and 12g of carbon disulfide was added and the mixture was cooled in ice water to prevent the carbon disulfide from refluxing too vigorously. The reaction mixture was warmed to 45^{0}C. After refluxing for seven hours the reaction mixture was heated more strongly and when a residue of carbon disulfide has boiled off the temperature rises to100^{0}C. Where it was kept for three hours. The progress of the reaction was checked by TLC (chloroform : methanol 13:1; v/v, R_f = 0.62), which showed conversion of the starting material into one faster moving product.

Upon cooling to room temperature the 2-mercaptothiazoline is largely deposited as a solid, the remainder being precipitated by the addition of 200 ml of concentrated

hydrochloric acid. The product was filtered off, washed with water and dried. The yield was 5.90 g (83%); mp. 102-104^{0}C.

M.F.: C_3H_5NS.



UV (EtOH): 240 nm.

6

IR (KBr): ν_{max} 3133.1, 1507.3, 1296.1 and 1050.2cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 8.16 (s, 1H, SH), 3.98 (t, 2H, CH_2, *J*=7.97Hz), 3.55 (t, 2H, CH_2, *J*=7.97Hz).

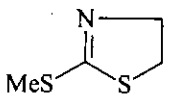
^{13}C NMR (100 MHz, $CDCl_3$): δ_C 174.85 (-CSH), 44.24, 32.18.

(iii) Preparation of 2-methylthio-2-thiazoline (7):

To a solution of 2-mercaptothiazoline (**6**) (6 g, 50.4 mmol), methyl iodide (7.15 gm, 50.4 mmol) and absolute methanol (30.249 ml) was heated under reflux for 1.5 hrs. The solvent was removed to give the product hydroiodide as a white crystalline mass. The progress of the reaction was monitored by TLC (chloroform : methanol 13:1, v/v, R_f = 0.54), which showed conversion of the starting material into one faster moving product with complete disappearance of starting material.

Methanol was removed under reduced pressure and the solid white hydroiodide salt neutralized with 15% NaOH (2.4 ml). Then it was stirred for 2 hrs., extracted with chloroform (30 ml × 4), dried over sodium sulfate and solvent solution was evaporated to dryness under reduced pressure in vacuum evaporator to give (**7**) as a syrup. The yield was 6g (46%); bp. 70^{0}C.

M.F.: C_4H_5NS.



UV(EtOH): 253 nm.

7

IR(KBr): ν_{max} 2931.6, 2852.5, 1564.2, 1303.8, 1001.0, 920.0 and 727.1cm^{-1}.

^{1}H NMR (400MHz, $CDCl_3$): δ_H 4.21 (t, 2H, CH_2, *J*=7.8Hz), 3.39 (t, 2H, CH_2,

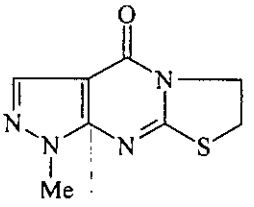
J=7.8Hz), 2.52 (s, 3H, CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 165.47 (-CN), 55.85, 44.31, 14.83.

2.4.2.[(i)+(iii)]= Preparation of 1-methyl-6,7-dihydropyrozolo [3,4-d]thiazolo [1,2-a] pyrimidin-4-one (8):

A solution of *ortho*-aminoester (**2**) (0.675 g, 3 mmol), and 2-methylthio-2-thiazoline (**7**) (0.522 g, 4.5 mmol) in dry acetic acid (6 ml) was heated under reflux for 4 hours. The progress of the reaction was monitored by TLC (chloroform : methanol 13:1, v/v, R_f = 0.86), which showed the conversion of the starting material to product.

The R_f value was 0.17. After cooling to room temperature, crushed ice (35 g) was added and the mixture was stirred for one hour. The precipitated was collected and crystallised from methanol to give (**8**) as red crystals. Yield: 0.45 g (63.82%); mp. > 250^{0}C.



8

M.F.: $C_8H_8N_4OS$.

UV (EtOH): 268 0nm.

IR (KBr): ν_{max} 3096.5, 3039.6, 2898.6, 1676.9, 1598.9, 1396.4 and 927.3cm^{-1}.

^{1}H NMR (400MHz, $CDCl_3$): δ_H 7.76 (s, 1H, CH), 4.14 (t, 2H, CH_2, *J*=7.4Hz), 3.27 (t, 2H, CH_2, *J*=7.4Hz), 2.47 (s, 3H, NCH_3).

^{13}H NMR (100MHz, $CDCl_3$): δ_C 164.40 (C=0), 140.8, 136.8, 115.2, 72.1, 44.0, 42.9, 35.84.

1.2.4.C. SYNTHESIS OF 1-METHYL-6,7-DIHYDROPYRAZOLO[3,4-d] IMIDAZO[1,2-a]PYRIMIDIN-4(8H)-ONE (12).

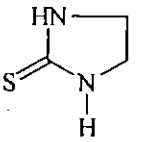
2.4.3.(i) Preparation of ethyl-5-amino-1-methylpyrozolo-4-carboxylate (2):

This compound (2) was synthesized from ethyl(ethoxy methylene)cyanoacetate (1) by using Gewald method[48] which was reported in previous **scheme-1**.

(ii) Preparation of 2-imidazoline thione (10):

To a mixture of ethylene diamine (9) (5 g, 83.33 mmol), rectified spirit (100 ml) and water (100 ml), carbon disulfide (3.95 ml, 83.33 mmol) was added dropwise with occasional shaking for 2 hrs. and refluxed on a water-bath for 1 hour. Concentrated HCl (15 ml) was then added and further refluxed for 9-10 hours.

The progress of the reaction was checked by the TLC (acetone: chloroform, 1:2, v/v). The R_f value was 0.67. The resulting solid on cooling was filtered, washed with cold acetone (80 ml) and recrystallized from ethanol to give (**10**) as white crystal. The yield was 4 g (47%); m.p.: 155-156^{0}C.



10

M.F.: $C_3H_6N_2S$.
UV (EtOH): 245.6nm.

IR (KBr): ν_{max} 3248.9, 2879.5, 2570.9, 1498.6, 1274.9, 1195.8 and 920.0cm^{-1}.

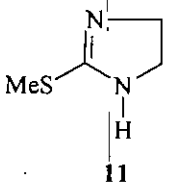
^{1}H NMR (400 MHz, $CDCl_3$): δ_H 6.0 (s, 2H, 2× NH), 3.76 (m, 4H, 2× CH_2).

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 183.36 (C-2), 44.24 (C-4 and C-5).

(iii) Preparation of 2-methylthio-imidazoline (11):

To a solution of 2-imidazoline thione (**10**) (3 g, 29.41 mmol), methyl iodide (1.85 ml, 29.41 mmol) in absolute methanol (18 ml) and it was refluxed for 2 hrs. with stirring. The progress of the reaction was checked by TLC (acetone: chloroform, 1:1, v/v). TLC of the reaction mixture showed the reactant was completely converted to the product.

The R_f value was 0.35. Methanol was removed under reduced pressure and the solid white hydroiodide salt neutralized with 15% NaOH (2.4 ml). Then it was stirred for 2 hrs., extracted with chloroform (30 ml×4), dried over sodium sulfate and the solvent was evaporated to dryness under reduced pressure under vacuum evaporator to give (**11**) as white crystals, 1.5 g (65.75%); mp. 119-121^{0}C.



11

M.F.: $C_4H_8N_2S$.

UV (EtOH): 251.6 nm.

IR (KBr): ν_{max} 3392.6, 3149.5, 1603.7, 1582 and 1107.1 cm^{-1}.

^{1}H NMR (400MHz, $CDCl_3$): δ_H 5.98 (s, 1H, NH), 3.65 (m, 4H, 2× CH_2), 2.50 (s, 3H, SCH_3).

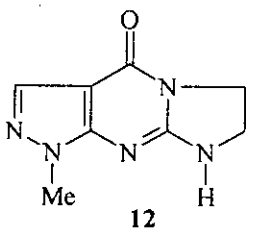
^{13}C NMR (100MHz, $CDCl_3$): δ_C 164.85 (C-2), 50.13 (C-4 and C-5), 13.25 (-SMe).

2.4.3.[(i)+(iii)]= Preparation of 1-methyl-6,7-dihydropyrozolo[3,4-d]imidazo [1,2-a]pyridine-4(8H)-one (12):

A solution of *ortho*-aminoester (**2**) (0.675 g, 3 mmol) was treated with 2-methylthio-imidazoline (**11**) (0.522 g, 4.5 mmol) at 160^{0}C under nitrogen atmosphere for 1 hours. The progress of the reaction was monitored by TLC (n-hexane : ethyl acetate, v/v, R_f = 0.54), which showed conversion of the starting material into one faster moving product with complete disappearance of starting material.

After cooling to room temperature, crushed ice (35 g) was added and the mixture was stirred for one hour. The precipitated was collected and crystallized from methanol to give (**12**) as red crystals.

Yield: 0.56 g (70.45%); mp. > 250^{0}C.



12

M.F.: $C_8H_9N_5O$.

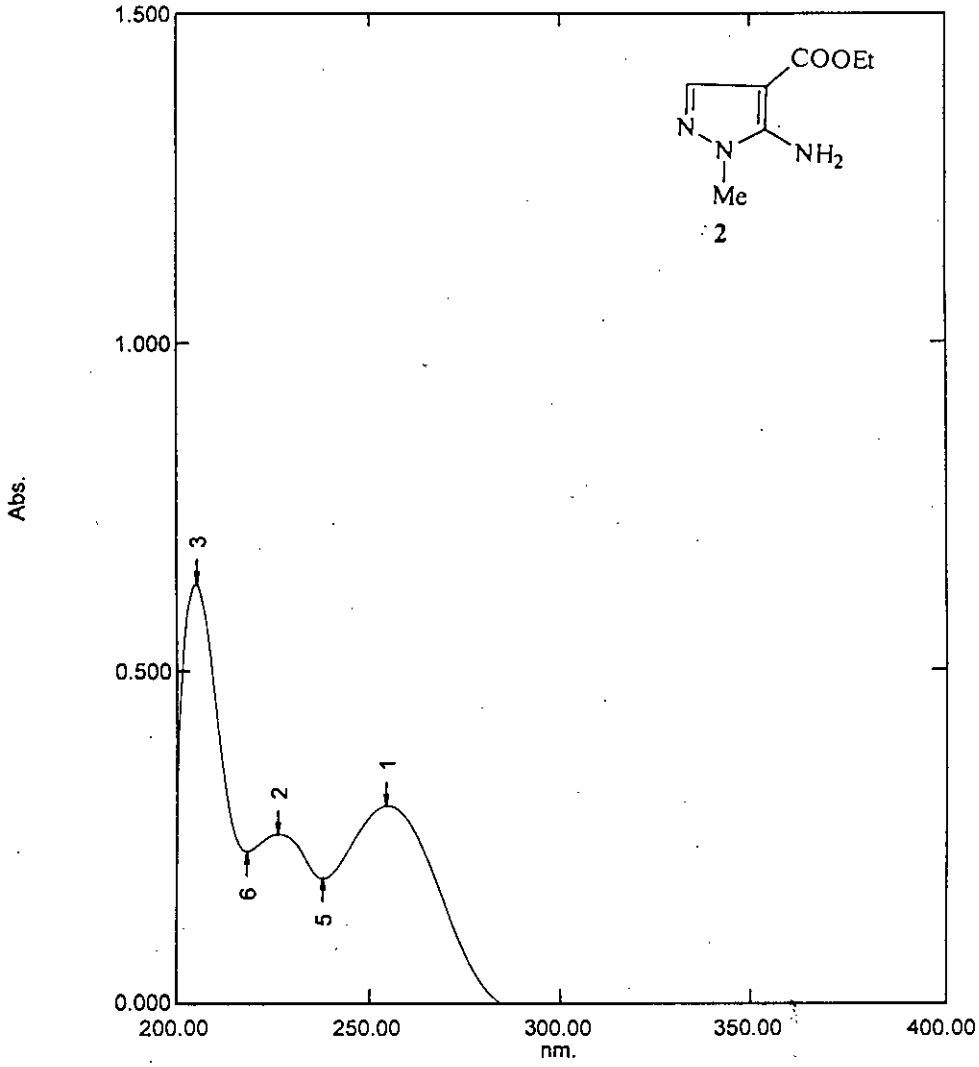
UV (EtOH): 275 nm.

IR (KBr): ν_{max} 3433.1, 3284.5, 3196.8, 2985.6, 1692.4, 1501.5 and 1105cm^{-1}.

^{1}H NMR (400MHz, CDCl3): δ_H 7.76 (s, 1H, CH), 6.98 (s, 1H, NH), 4.15 (t, 2H, CH_2, J=7.8Hz), 3.25 (t, 2H, CH_2, J=7.8Hz), 3.03 (s, 3H, NCH_3).

^{13}C NMR (100MHz, CDCl3): δ_C 165.40 (C=0), 137.32, 125.12, 110.25, 72.05, 39.32, 34.37.

S-2



Wavelength	Abs.
254	0.297
226	0.253
205	0.630

Fig. 1: UV Spectrum of Compound **2**

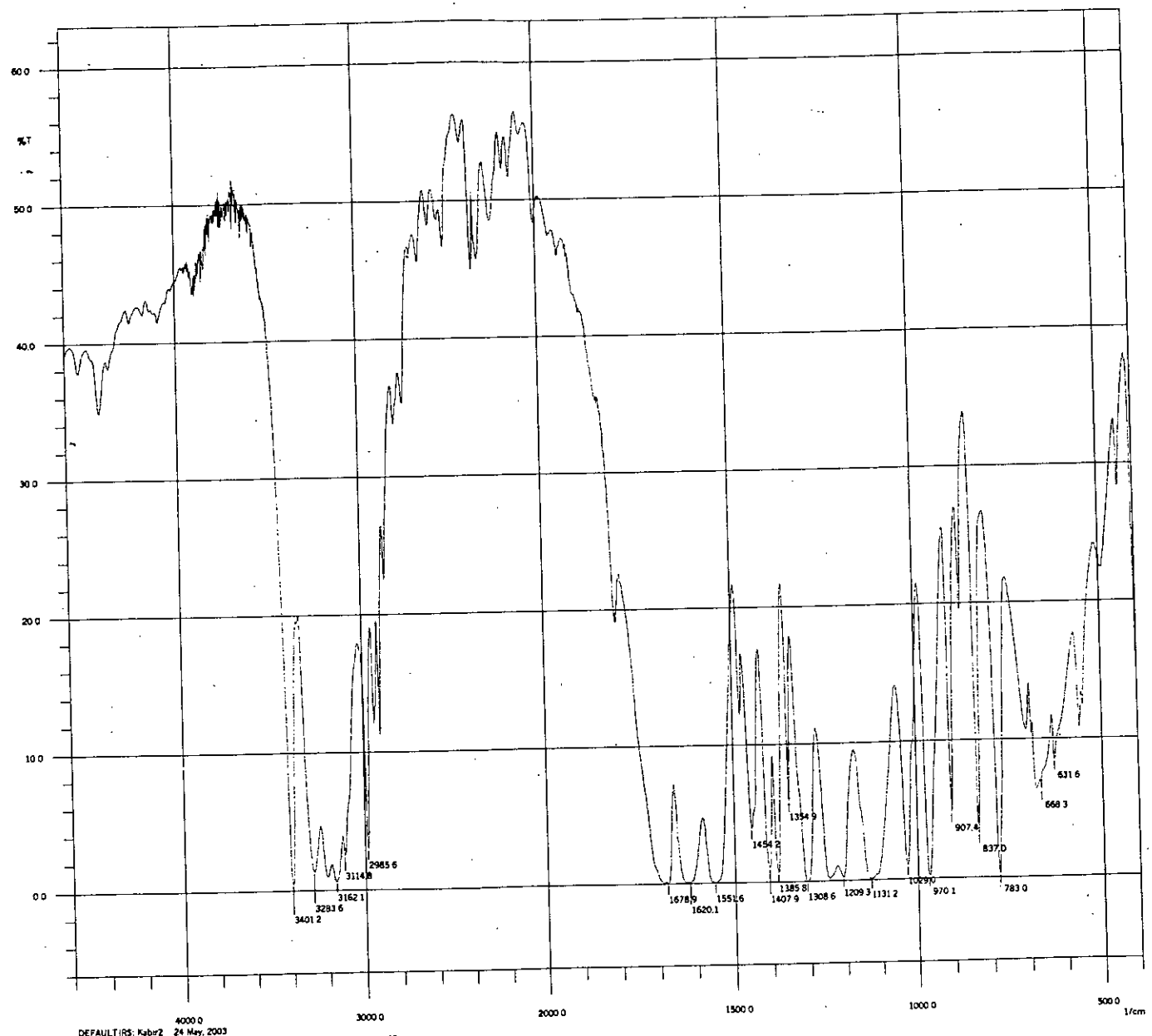
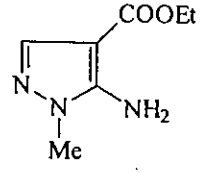


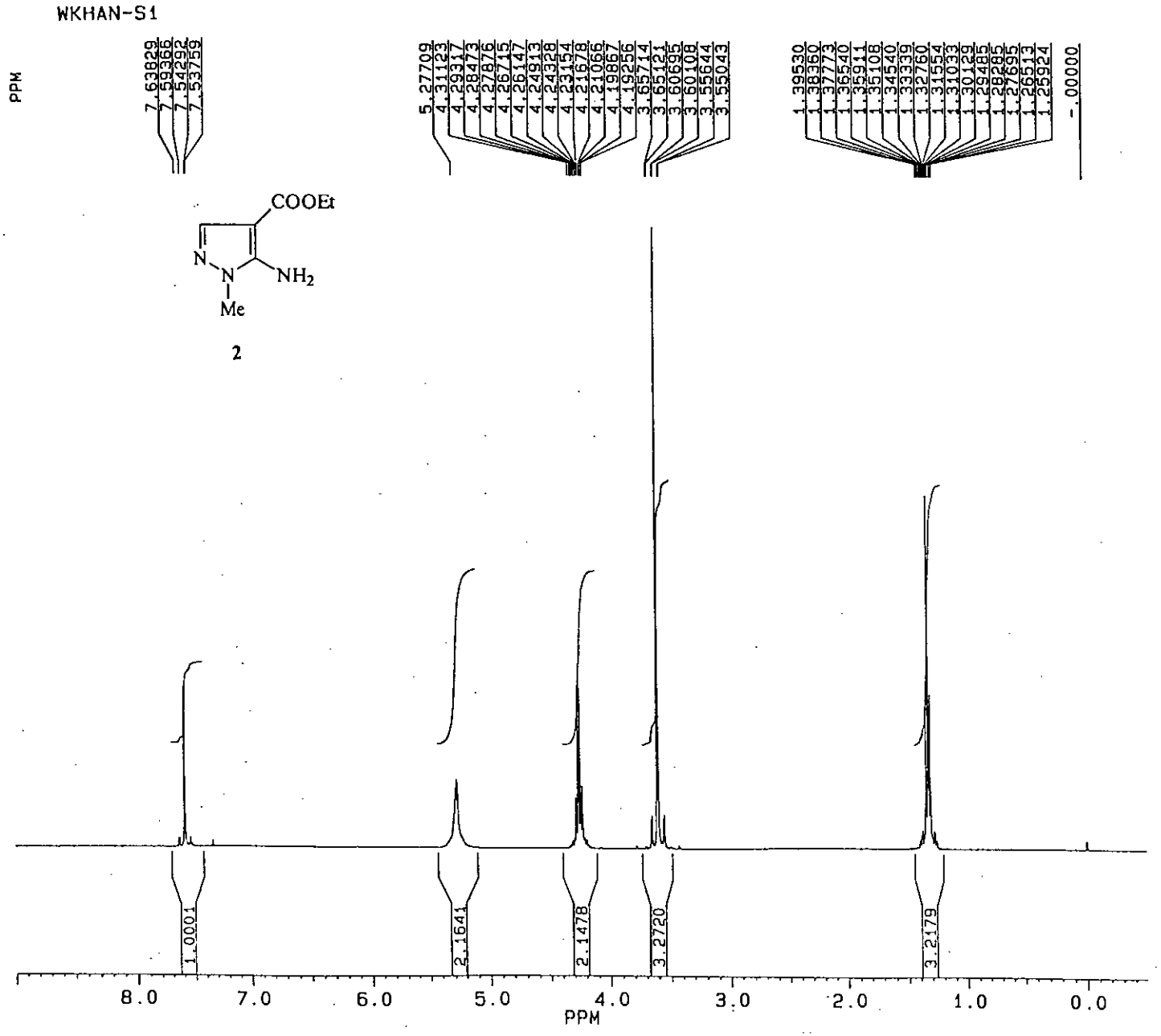
Fig. 2: IR Spectrum of Compound **2**

Peaktable of DEFAULT.IRS, 22 Peaks
Threshhold: 10, Noise: 2, No Range Selection

No.	Pos. (1/cm)	Inten. (%T)
1	631.6	8.4746
2	668.3	6.3197
3	783.0	0.2798
4	837.0	3.1947
5	907.4	4.7510
6	970.1	0.1838
7	1029.0	1.0086
8	1131.2	0.0758
9	1209.3	0.2382
10	1308.6	0.0245
11	1354.9	5.8567
12	1385.8	0.6829
13	1407.9	1.0370
14	1454.2	3.9049
15	1551.6	0.0331
16	1620.1	0.0777
17	1678.9	0.0232
18	2985.6	2.9426
19	3114.8	2.5138
20	3162.1	0.6264
21	3283.6	1.3269
22	3401.2	0.4610



2



KSMDKHAN.001
DATE 13-1-4

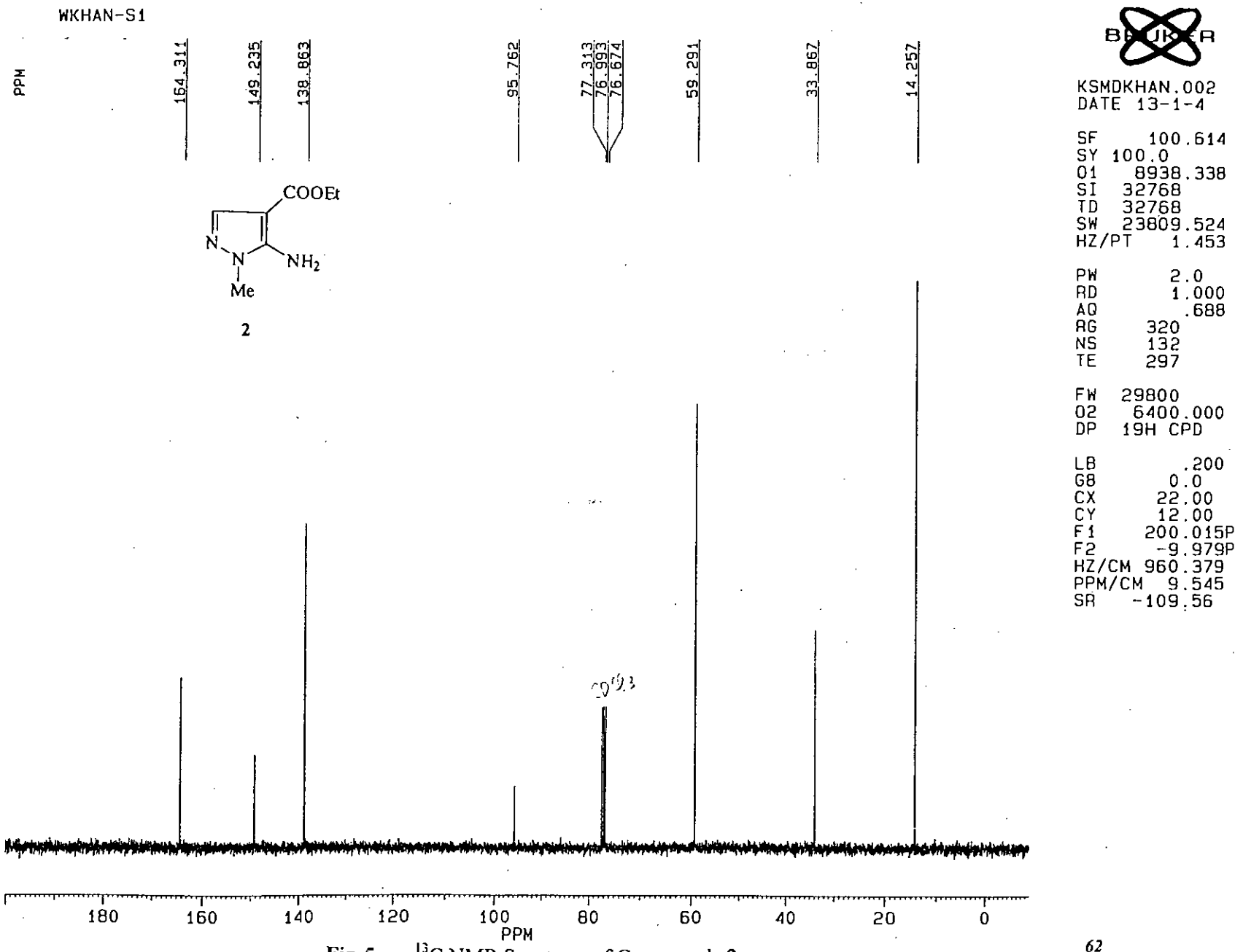
SF 400.134
SY 230.0
O1 6395.963
SI 32768
TD 32768
SW 8064.516
HZ/PT .492

PW 6.2
RD 2.000
AQ 2.032
RG 2
NS 8
TE 297

FW 10100
O2 20.000
DP 63L P0

LB .200
GB 0.0
CX 22.00
CY 12.00
F1 9.001P
F2 -.498P
HZ/CM 172.769
PPM/CM .432
SR 4363.59

Fig. 3: ^{1}H NMR Spectrum of Compound **2**



KSMDKHAN.002
DATE 13-1-4

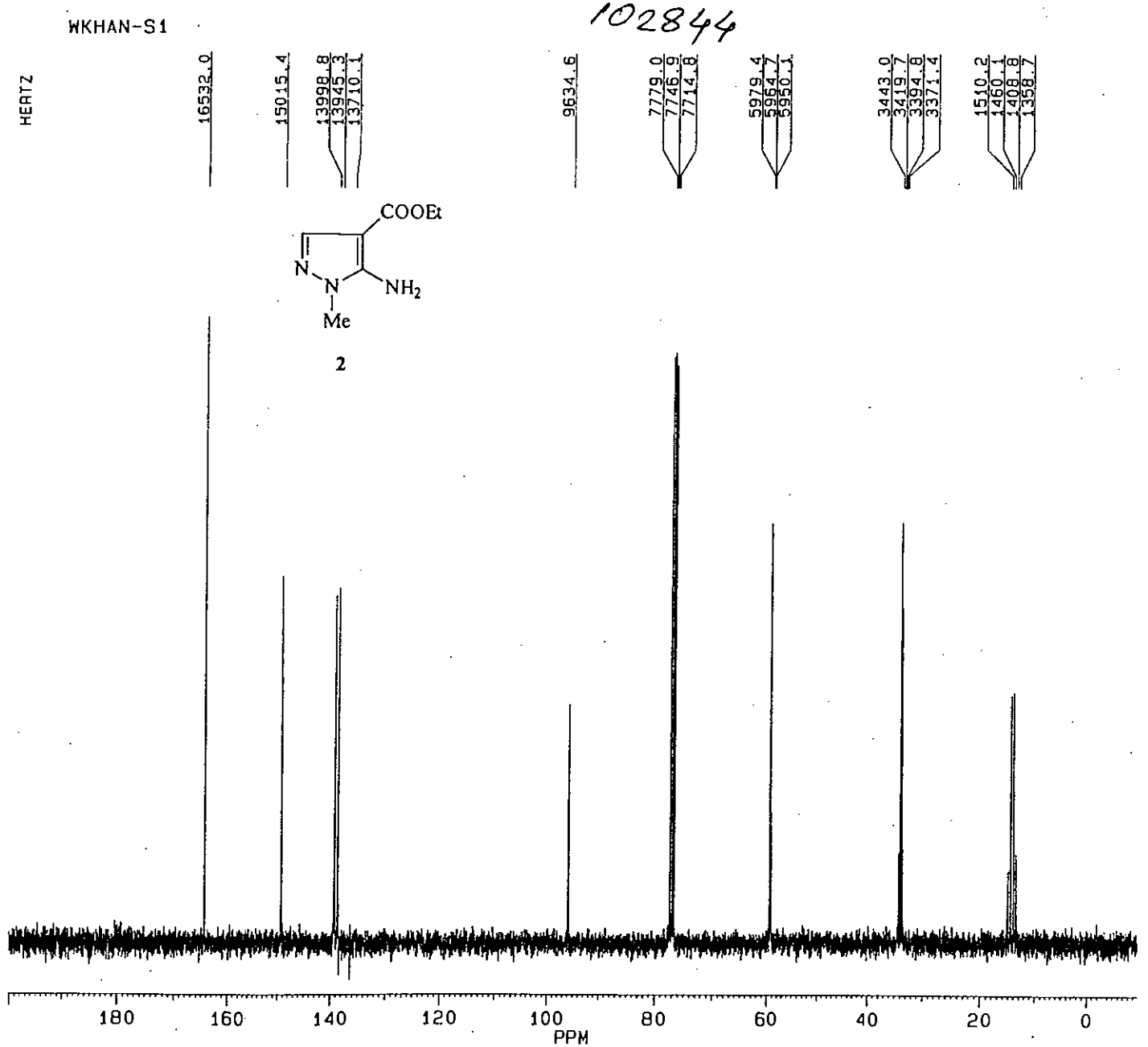
SF 100.614
SY 100.0
O1 8938.338
SI 32768
TD 32768
SW 23809.524
HZ/PT 1.453

PW 2.0
RD 1.000
AQ .688
RG 320
NS 132
TE 297

FW 29800
O2 6400.000
DP 19H CPD

LB .200
GB 0.0
CX 22.00
CY 12.00
F1 200.015P
F2 -9.979P
HZ/CM 960.379
PPM/CM 9.545
SR -109.56

Fig. 5: ^{13}C NMR Spectrum of Compound 2



BRUKER

KSMDKHAN.003
DATE 13-1-4

SF	100.614
SY	100.0
O1	8938.338
SI	32768
TD	32768
SW	23809.524
HZ/PT	1.453
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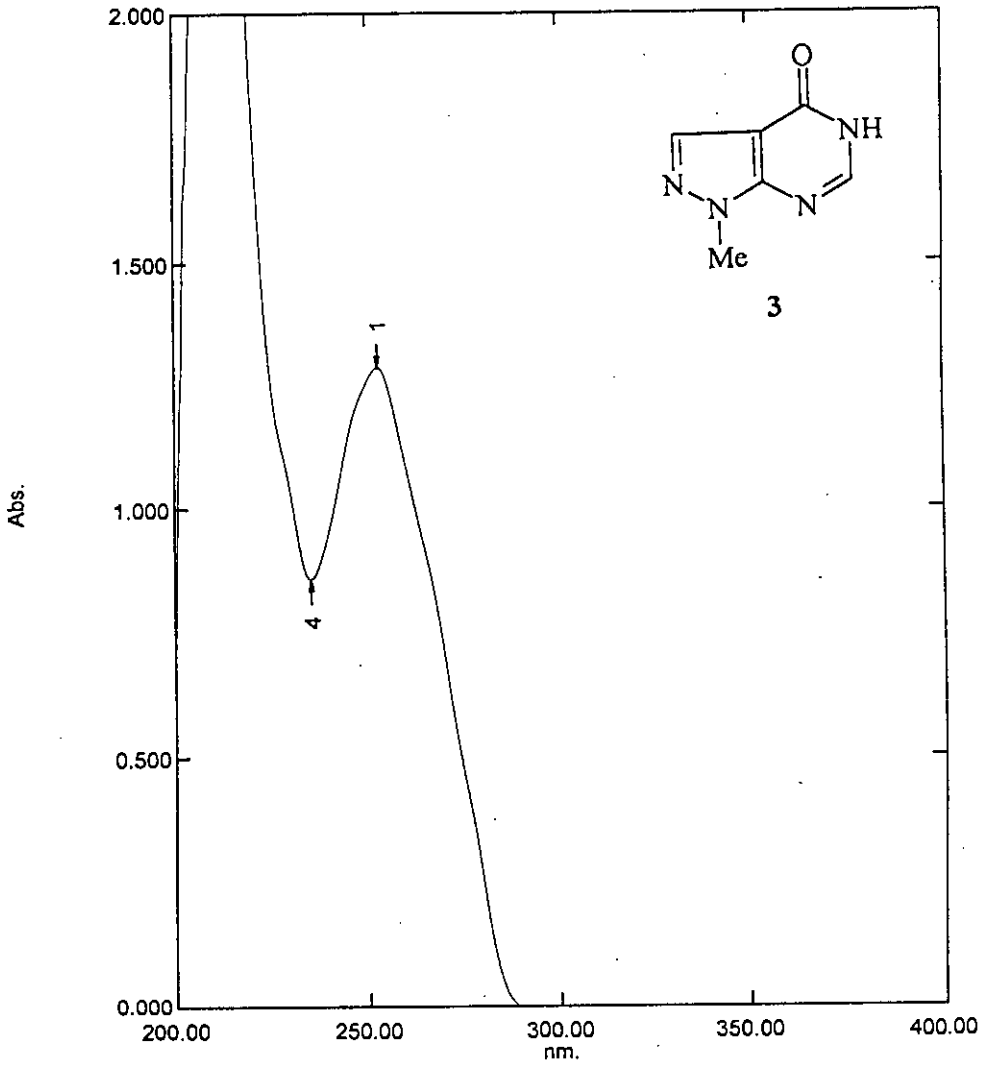
RD	1.000
AQ	.688
RG	320
NS	518
TE	297
FW	29800
O2	6400.000
DP	19H CW
LB	.200
GB	0.0
CX	22.00
CY	12.00
F1	200.001P
F2	-9.993P
HZ/CM	960.379
PPM/CM	9.545
SR	-109.56

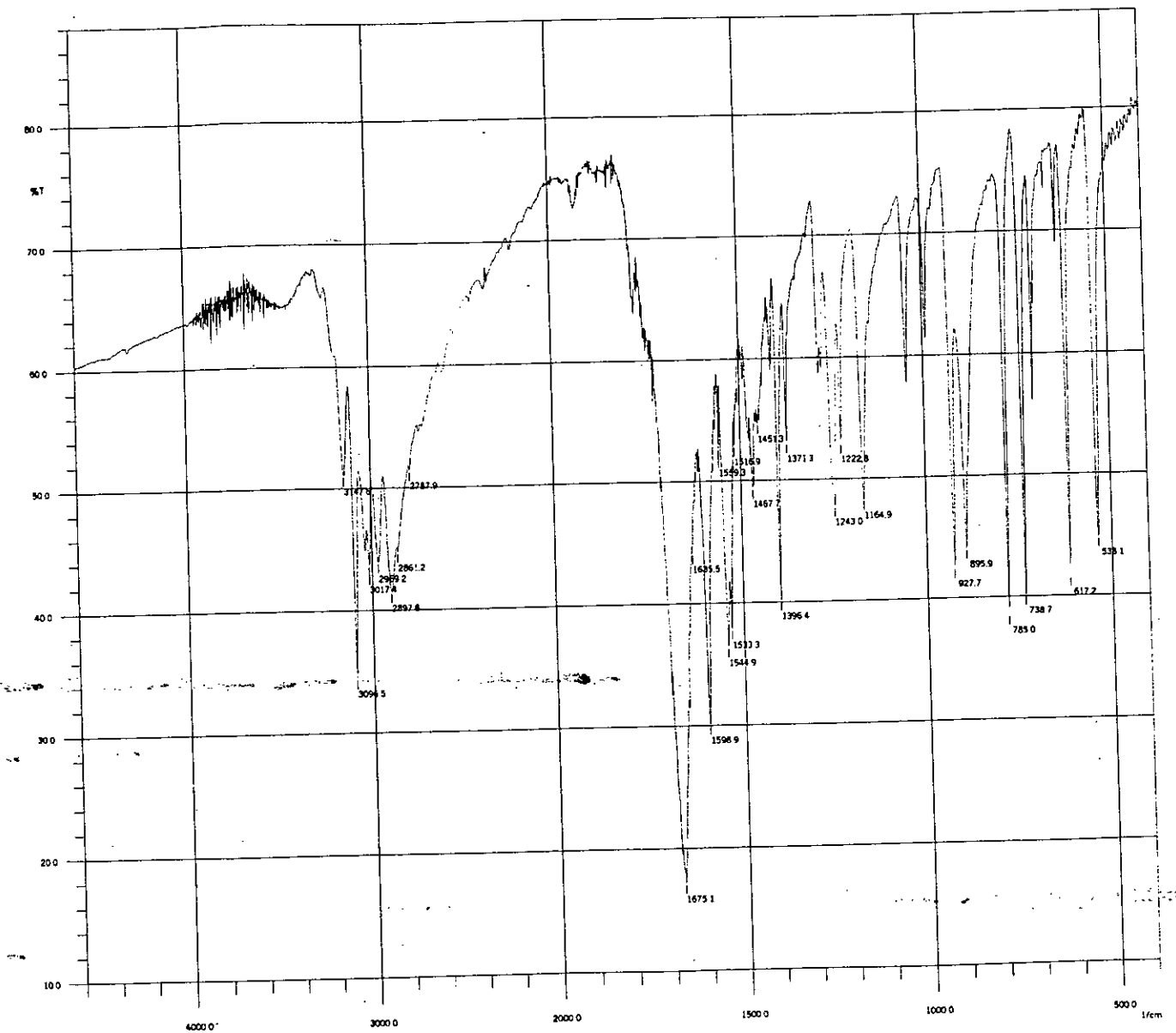
Fig. 4: ^{13}C NMR Spectrum of Compound 2

S-3



Wavelength	Abs.
253	1.285

Fig. 6: UV Spectrum of Compound 3

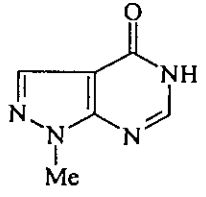


DEFAULT.IRS: S-11, April 05, 2004

Data:	03/06/2004	Time:	13:27:56	NScans:	45
Type:	HYPER IR	User:	SHIMADZU	Detector:	standard
Abscissa:	1/cm	Ordinate:	%T	Apodization:	Happ
Min:	400.20	Max:	4599.91	Range:	1/cm
Ndp:	4356	Data Interval:	0.96434	Resolution:	2.0
Gain:	auto	Aperture:	auto	Mirror Speed:	2.8(low)

Peaktable of DEFAULT.IRS, 27 Peaks
Threshhold: 55, Noise: 2, No Range Selection

No.	Pos. (1/cm)	Inten. (%T)
1	538.1	44.832
2	617.2	41.612
3	738.7	40.147
4	785.0	38.540
5	895.9	44.040
6	927.7	42.476
7	1164.9	48.310
8	1222.8	52.966
9	1243.0	47.800
10	1371.3	53.062
11	1396.4	40.156
12	1451.3	54.727
13	1467.7	49.396
14	1516.9	52.876
15	1533.3	37.858
16	1544.9	36.417
17	1559.3	52.676
18	1598.9	30.092
19	1635.5	44.125
20	1675.1	17.187
21	2787.9	51.450
22	2861.2	44.767
23	2897.8	41.509
24	2969.2	43.947
25	3017.4	43.761
26	3096.5	34.483
27	3147.6	51.065



3

Fig. 7: IR Spectrum of Compound 3

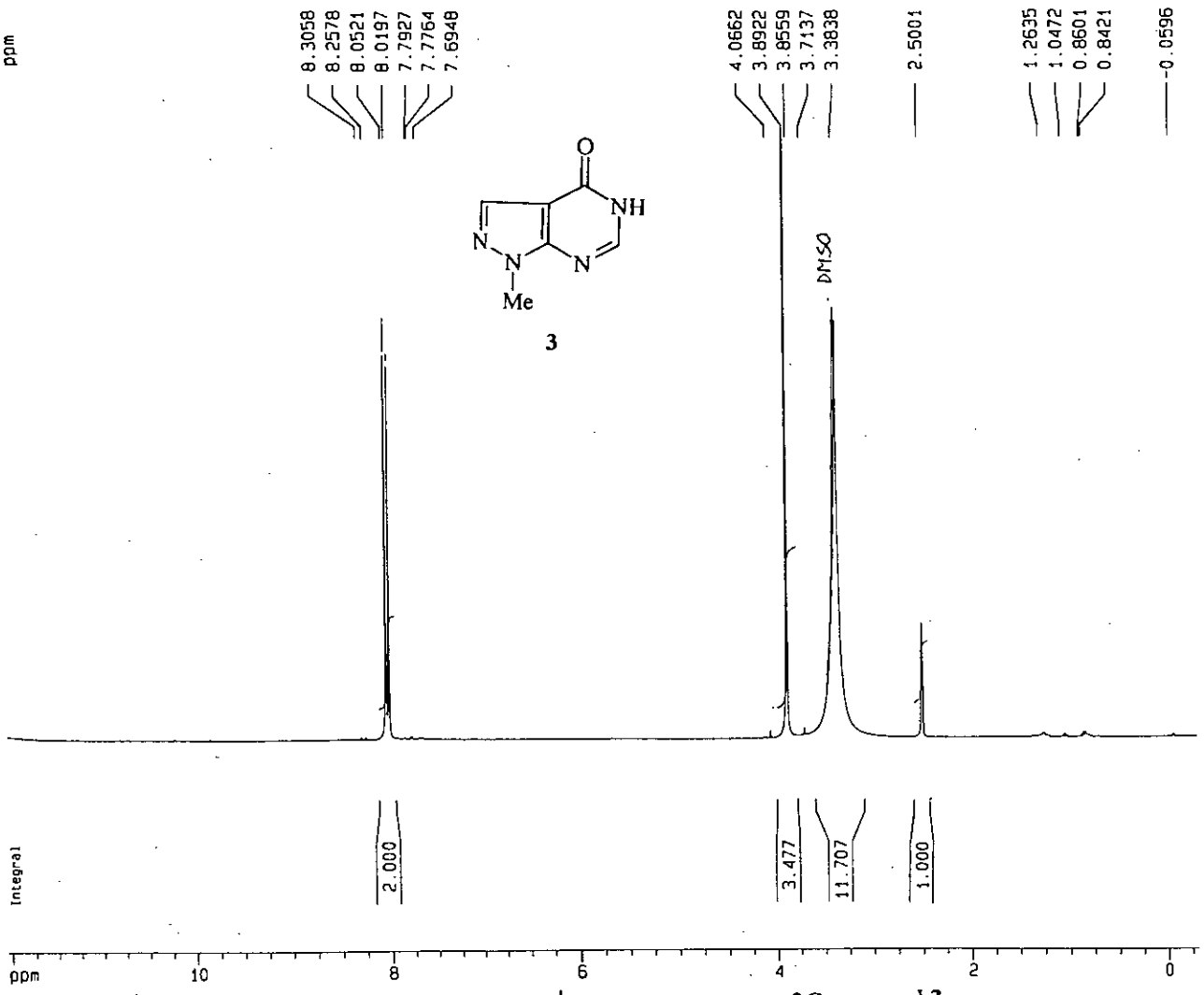


Fig. 8: ^{1}H NMR Spectrum of Compound **3**

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Current Data Parameters
NAME                  A2020
EXPNO                     1
PROCNO                    1

F2 - Acquisition Parameters
Date_              20050807
Time                 10.32
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PROBHD     5 mm Multinuc
PULPROG               zg30
TD                   32768
SOLVENT              Aceton
NS                      128
OS                        2
SWH               6410.256 Hz
FIDRES            0.195625 Hz
AQ                2.5559540 sec
RG                      128
DW                 78.000 usec
DE                  6.00 usec
TE                  310.0 K
D1                1.00000000 sec

============ CHANNEL f1 =============
NUC1                    1H
P1                   8.30 usec
PL1                 -6.00 dB
SFO1          400.1428010 MHz

F2 - Processing parameters
SI                   32768
SF            400.1400019 MHz
WDW                     EM
SSB                      0
LB                   0.30 Hz
GB                       0
PC                    1.40

1D NMR plot parameters
CX                  20.00 cm
F1P                 12.048 ppm
F1                 4820.93 Hz
F2P                 -0.305 ppm
F2                 -122.00 Hz
PPMCM              0.61765 ppm/cm
HZCM               247.14650 Hz/cm
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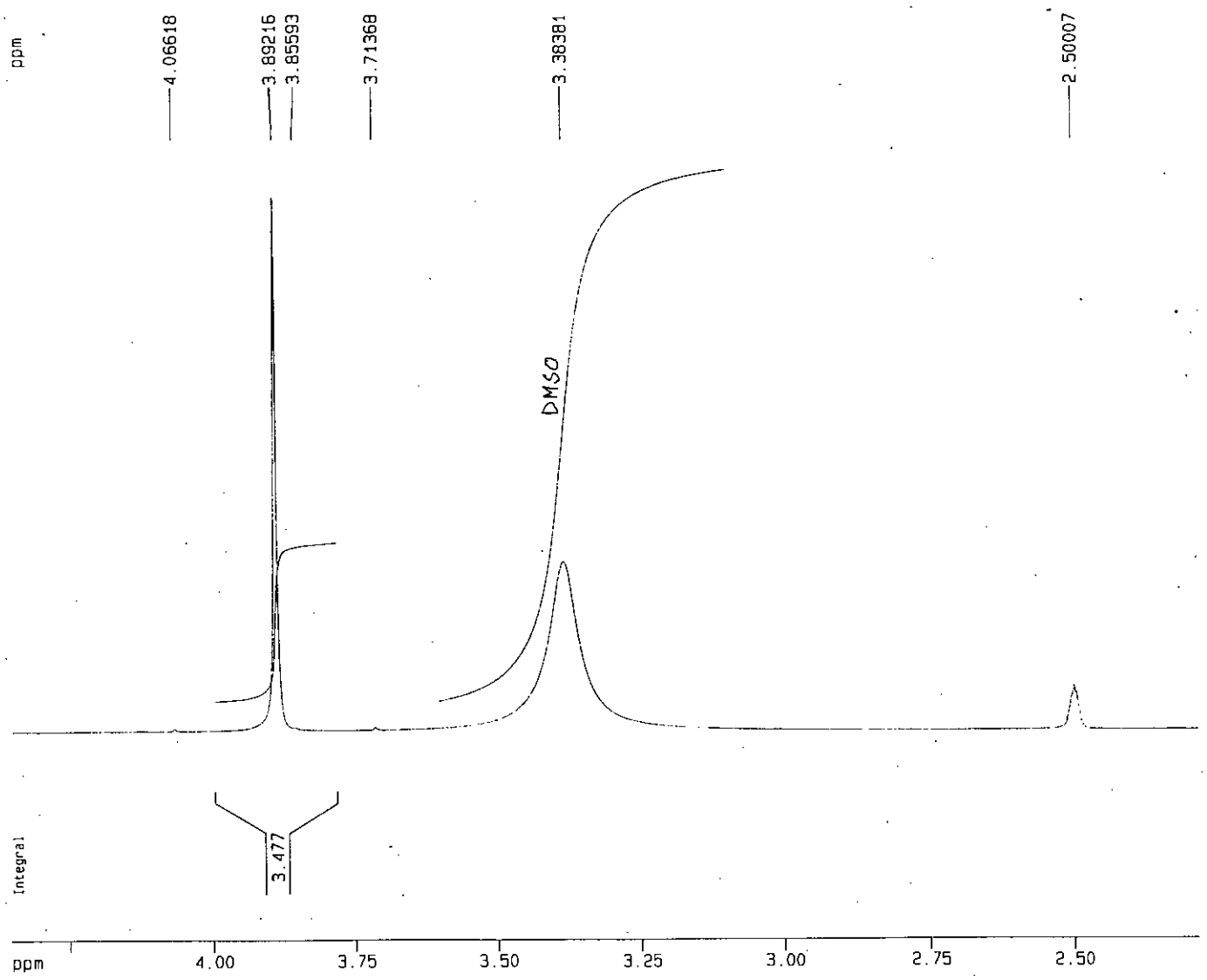

Current Data Parameters

NAME	A2020
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

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Time	10.32
INSTRUM	dpx400
PROBHD	5 mm Multinuc
PULPROG	zg30
TD	32768
SOLVENT	DMSO
NS	128
DS	2
SWH	6410.256 Hz
FIDRES	0.195625 Hz
AQ	2.5559540 sec
RG	128
DW	78.000 usec
DE	6.00 usec
TE	310.0 K
D1	1.00000000 sec

========== CHANNEL f1 ==========

NUC1	1H
P1	8.30 usec
PL1	-6.00 dB
SFO1	400.1428010 MHz

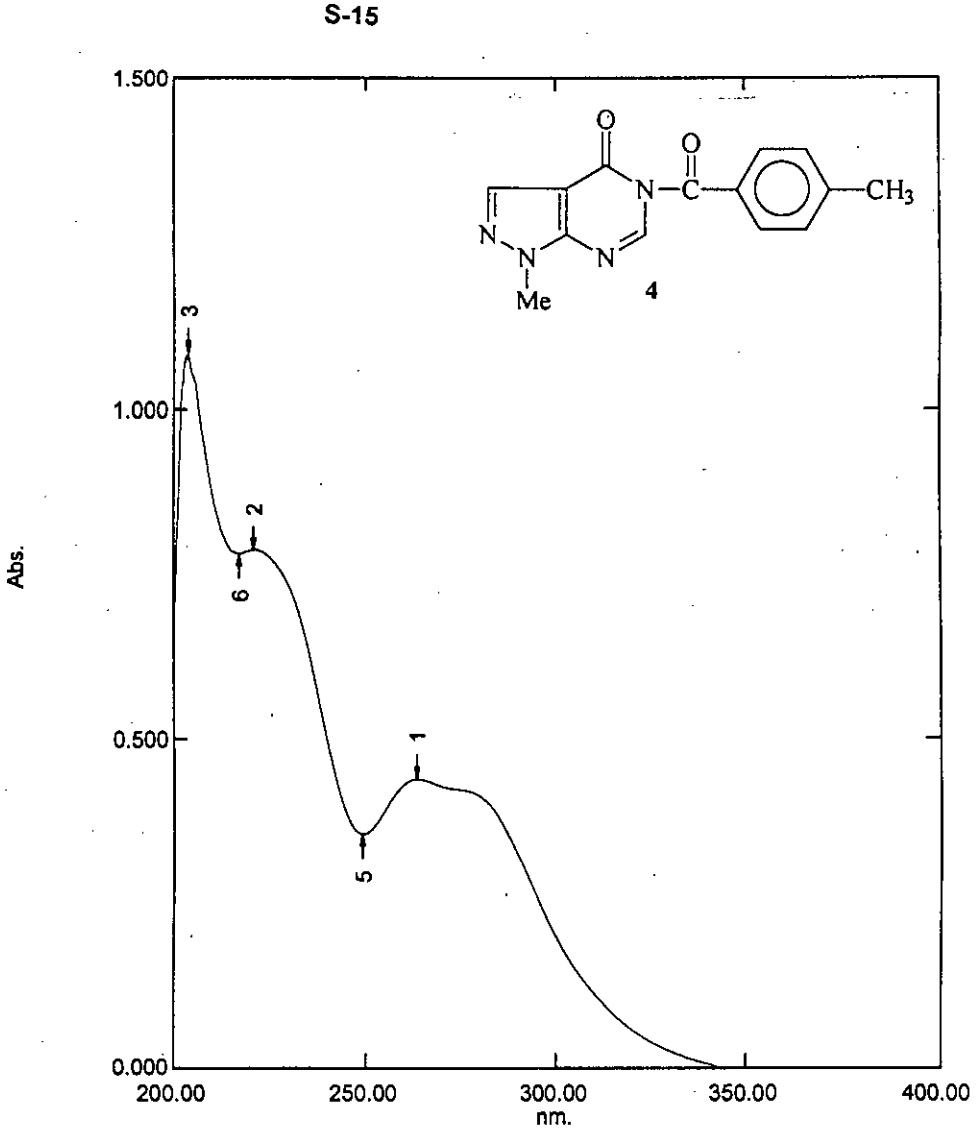
F2 - Processing parameters

SI	32768
SF	400.1400019 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.40

1D NMR plot parameters

CX	20.00 cm
F1P	4.350 ppm
F1	1740.59 Hz
F2P	2.284 ppm
F2	913.77 Hz
PPMCM	0.10332 ppm/cm
HZCM	41.34114 Hz/cm

Fig. 9: ^{1}H NMR Spectrum of Compound **3** (Expansion)



Wavelength	Abs.
263	0.440
220	0.787
204	1.081

Fig. 10: UV Spectrum of Compound **4**

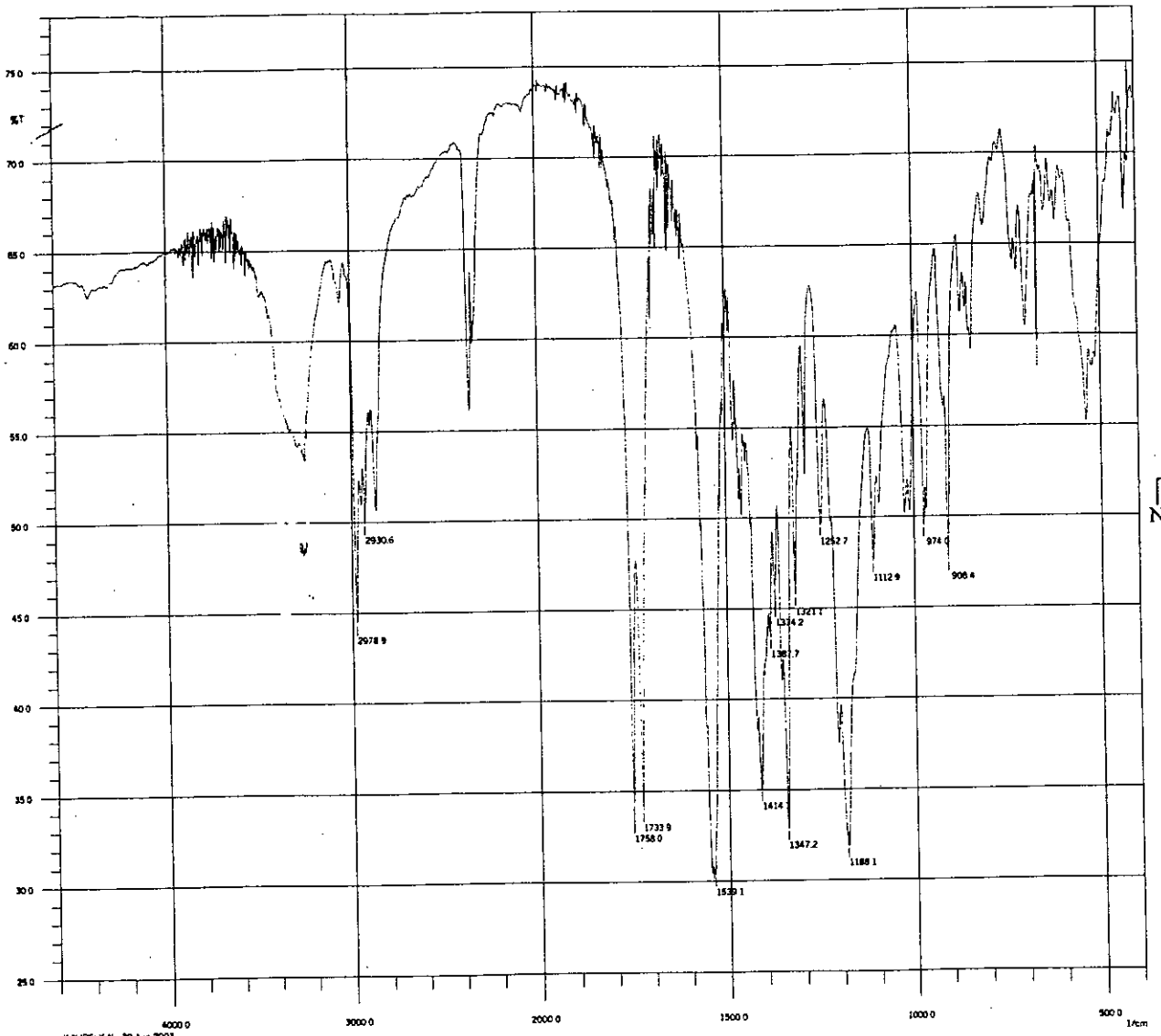
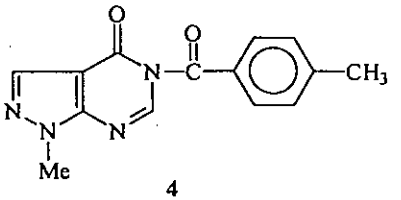


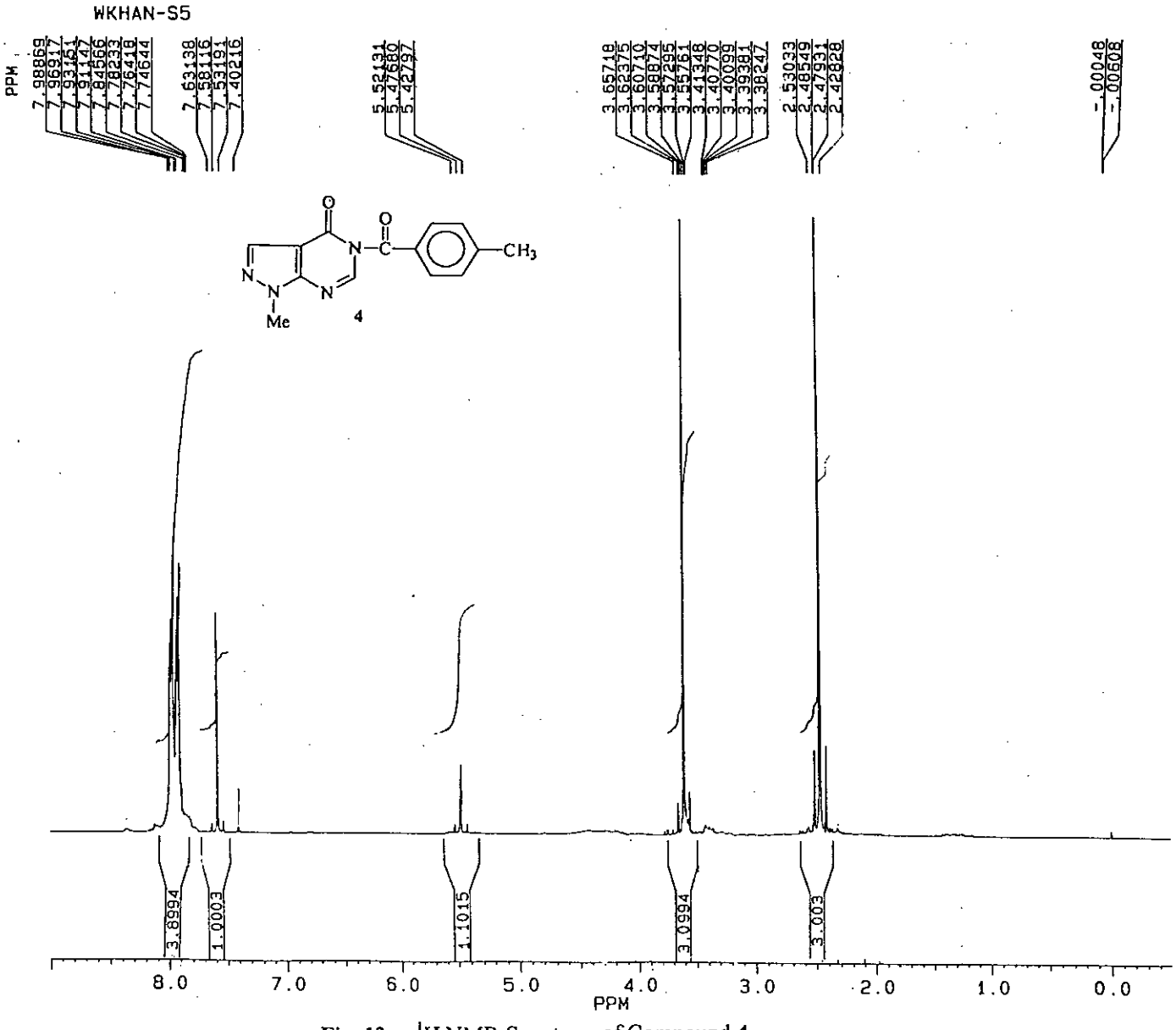
Fig. 11: IR Spectrum of Compound 4

Peaktable of K-N.IRS, 17 Peaks
Threshhold: 50, Noise: 2, No Range Selection

No.	Pos. (1/cm)	Inten. (%T)
1	908.4	47.557
2	974.0	49.492
3	1112.9	47.536
4	1188.1	31.865
5	1252.7	49.584
6	1321.1	45.723
7	1347.2	32.869
8	1374.2	46.209
9	1387.7	43.379
10	1414.7	35.033
11	1539.1	30.321
12	1733.9	33.906
13	1758.0	34.301
14	2930.6	49.927
15	2978.9	44.392
16	3286.5	44.054
17	3369.4	38.796



4



KSMDKHAN.001
DATE 14-1-4

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O1	6395.963
SI	32768
TD	32768
SW	8064.516
HZ/PT	.492
PW	6.2
RD	2.000
AQ	2.032
RG	1
NS	8
TE	297
FW	10100
O2	20.000
DP	63L PO
LB	.200
GB	0.0
CX	22.00
CY	12.00
F1	9.001P
F2	-.498P
HZ/CM	172.769
PPM/CM	.432
SR	4338.00

Fig. 12: ^{1}H NMR Spectrum of Compound **4**

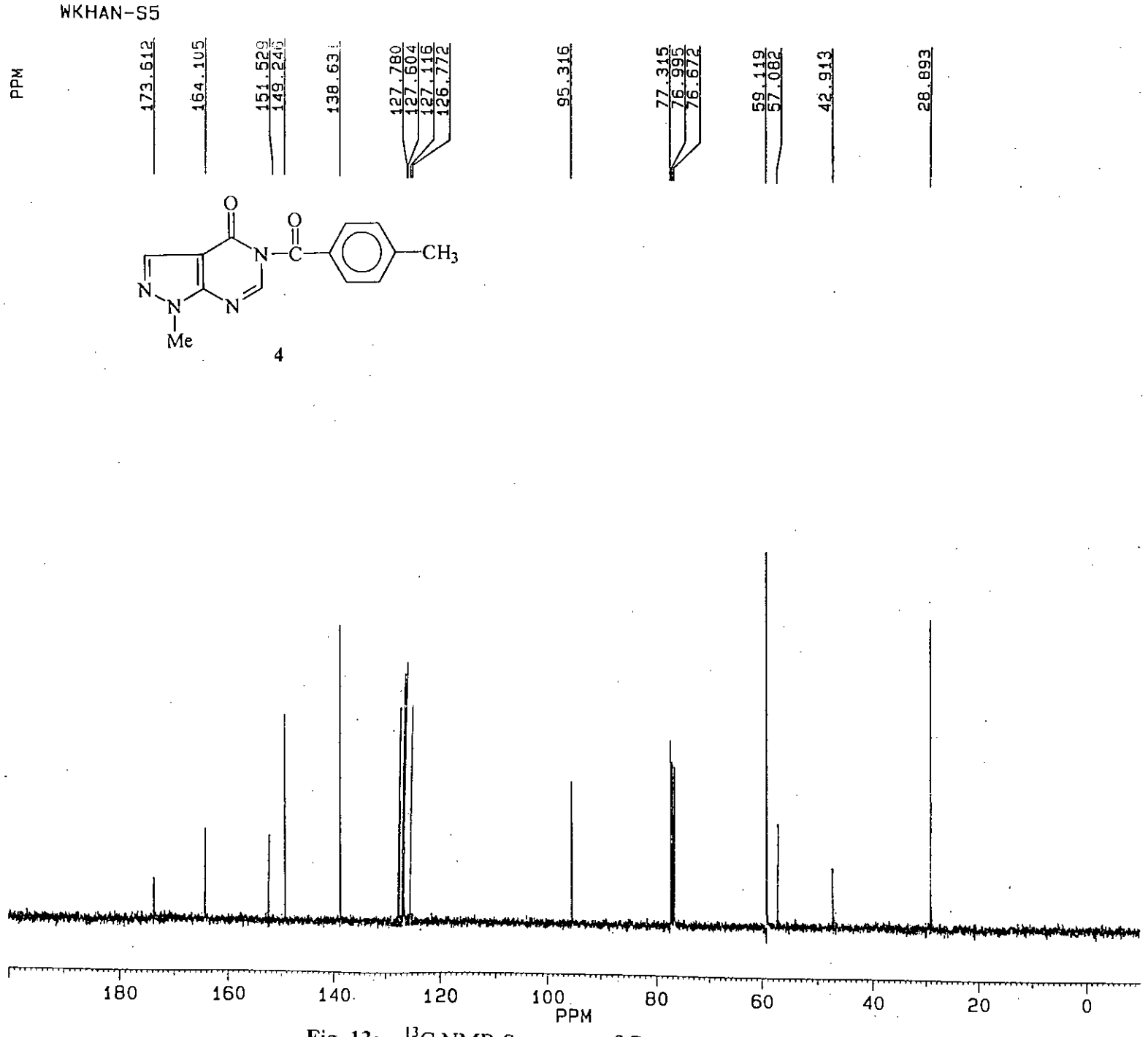
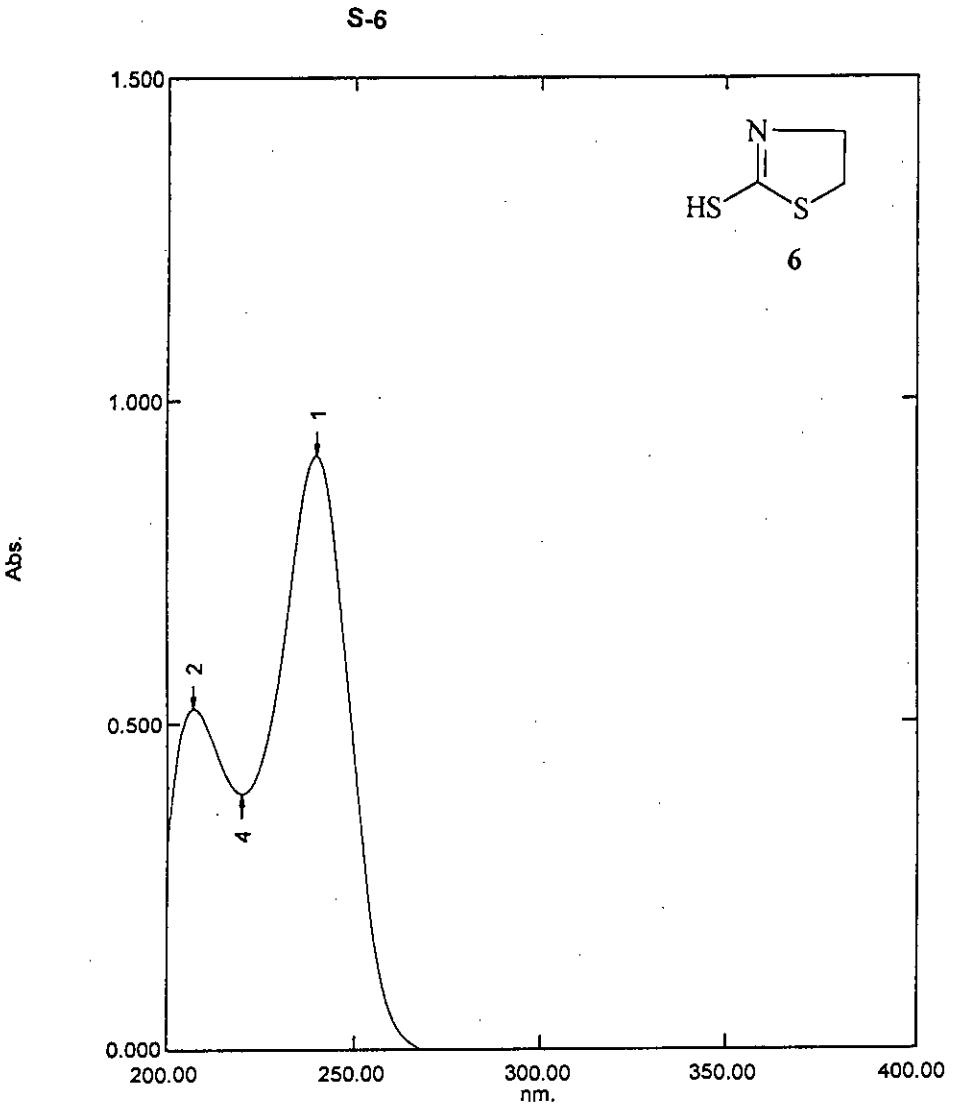


Fig. 13: ^{13}C NMR Spectrum of Compound 4



Wavelength	Abs.
240	0.913
207	0.525

Fig. 14: UV Spectrum of Compound **6**

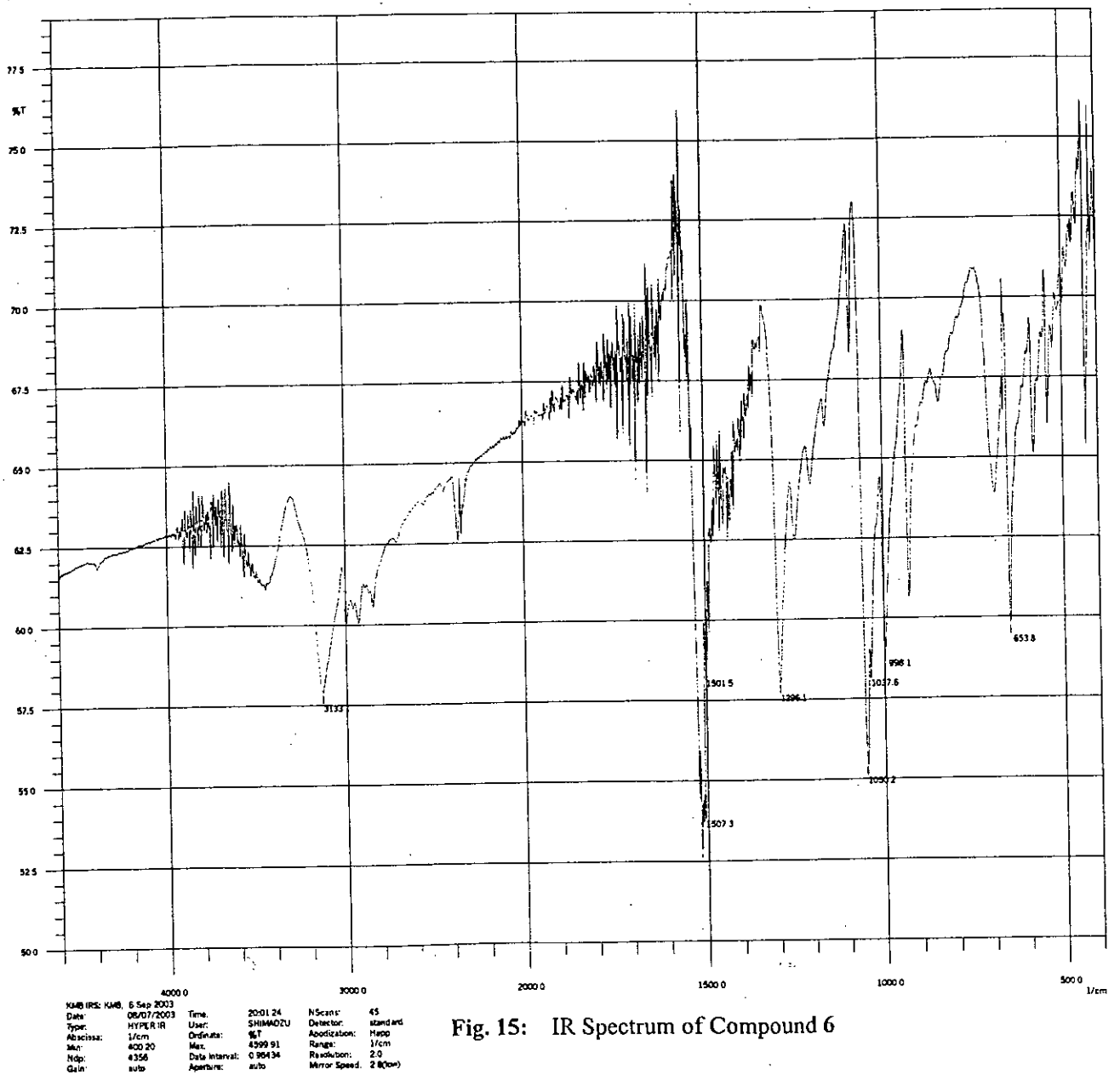
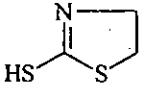


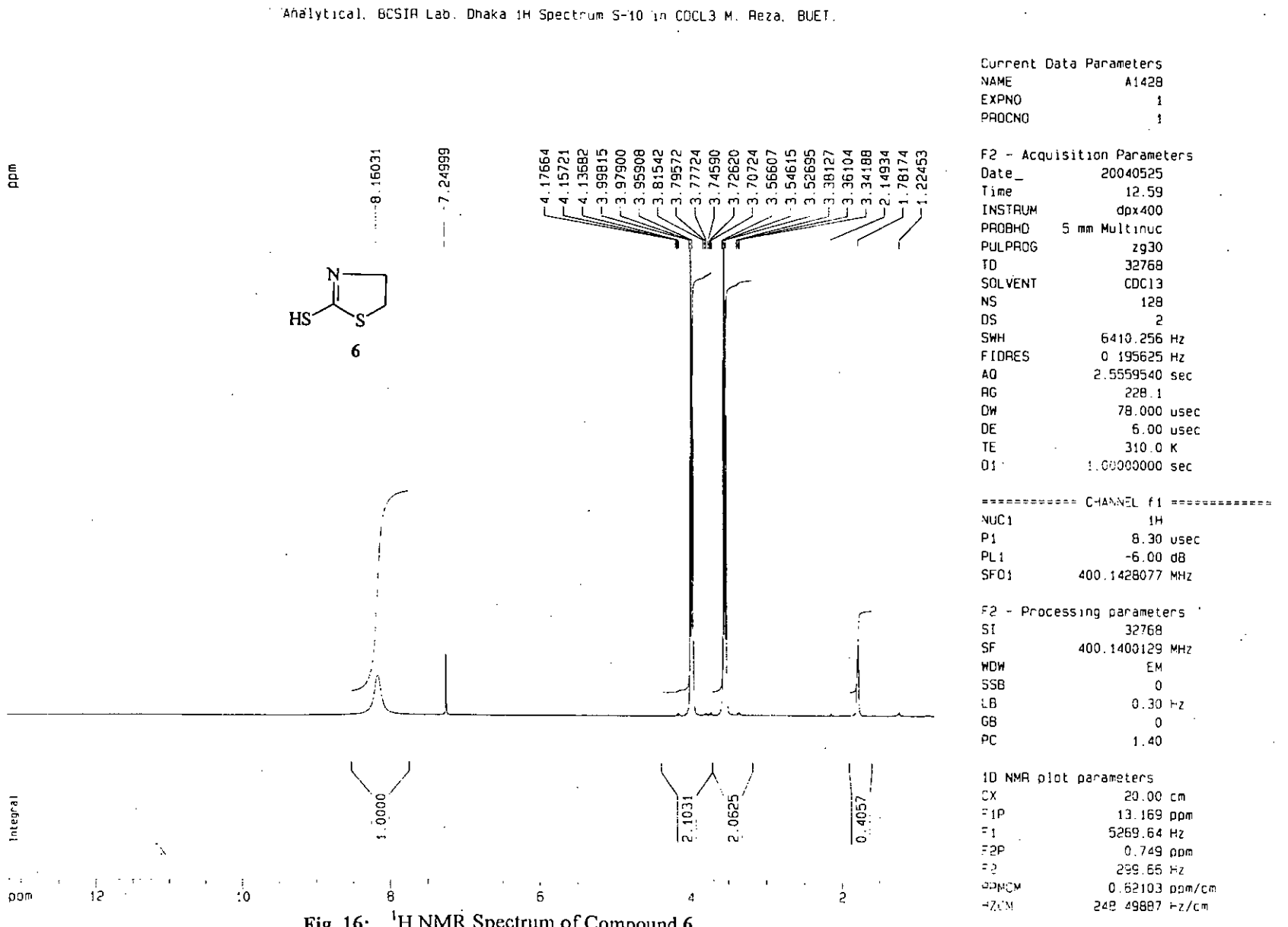
Fig. 15: IR Spectrum of Compound 6

Peaktable of KM8.IRS, 8 Peaks
Threshhold: 60, Noise: 2, No Range Selection

No.	Pos. (1/cm)	Inten. (%T)
1	653.8	59.784
2	998.1	59.079
3	1037.6	58.465
4	1050.2	55.440
5	1296.1	58.043
6	1501.5	58.525
7	1507.3	54.154
8	3133.1	57.895



6



Current Data Parameters

NAME	A1428
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20040525
Time	12.59
INSTRUM	dpx400
PROBHD	5 mm Multinuc
PULPROG	zg30
TD	32768
SOLVENT	CDCl3
NS	128
DS	2
SWH	6410.256 Hz
FIDRES	0.195625 Hz
AQ	2.5559540 sec
RG	228.1
DW	78.000 usec
DE	6.00 usec
TE	310.0 K
D1	1.00000000 sec

========== CHANNEL f1 ==========

NUC1	1H
P1	8.30 usec
PL1	-6.00 dB
SFO1	400.1428077 MHz

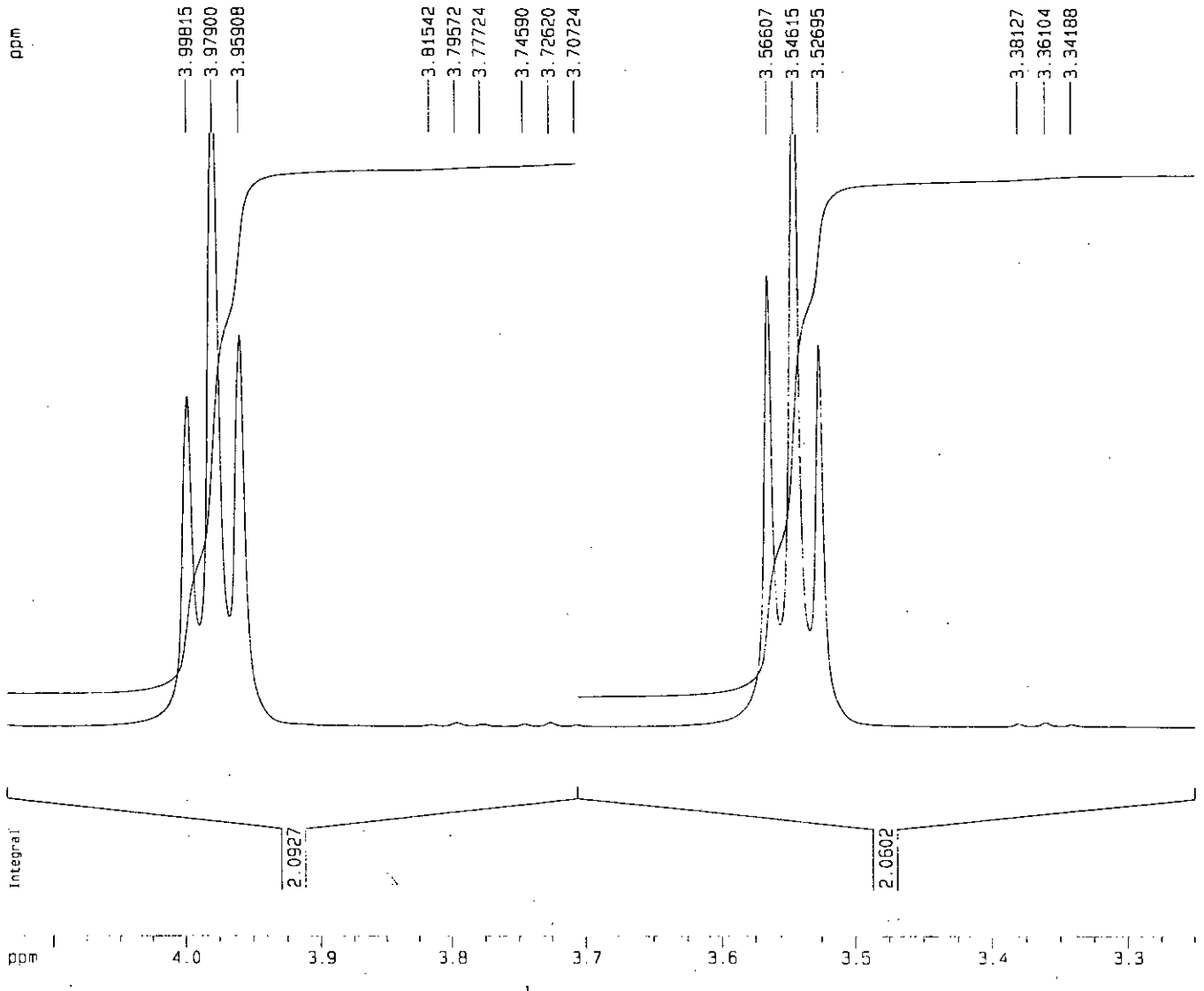
F2 - Processing parameters

SI	32768
SF	400.1400129 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.40

1D NMR plot parameters

CX	20.00 cm
F1P	13.169 ppm
F1	5269.64 Hz
F2P	0.749 ppm
F2	299.65 Hz
PPMCM	0.62103 ppm/cm
HZCM	248.49887 Hz/cm

Fig. 16: ^{1}H NMR Spectrum of Compound **6**



Current Data Parameters

NAME	A1428
EXPND	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20040525
Time	12.59
INSTRUM	dpx400
PROBHD	5 mm Multinuc
PULPROG	zg30
TD	32768
SOLVENT	CDCl3
NS	128
DS	2
SWH	6410.256 Hz
FIORES	0.195625 Hz
AQ	2.5559540 sec
RG	228.1
DW	78.000 usec
DE	6.00 usec
TE	310.0 K
D1	1.00000000 sec

========== CHANNEL f1 ==========

NUC1	1H
P1	8.30 usec
PL1	-6.00 dB
SFO1	400.1428077 MHz

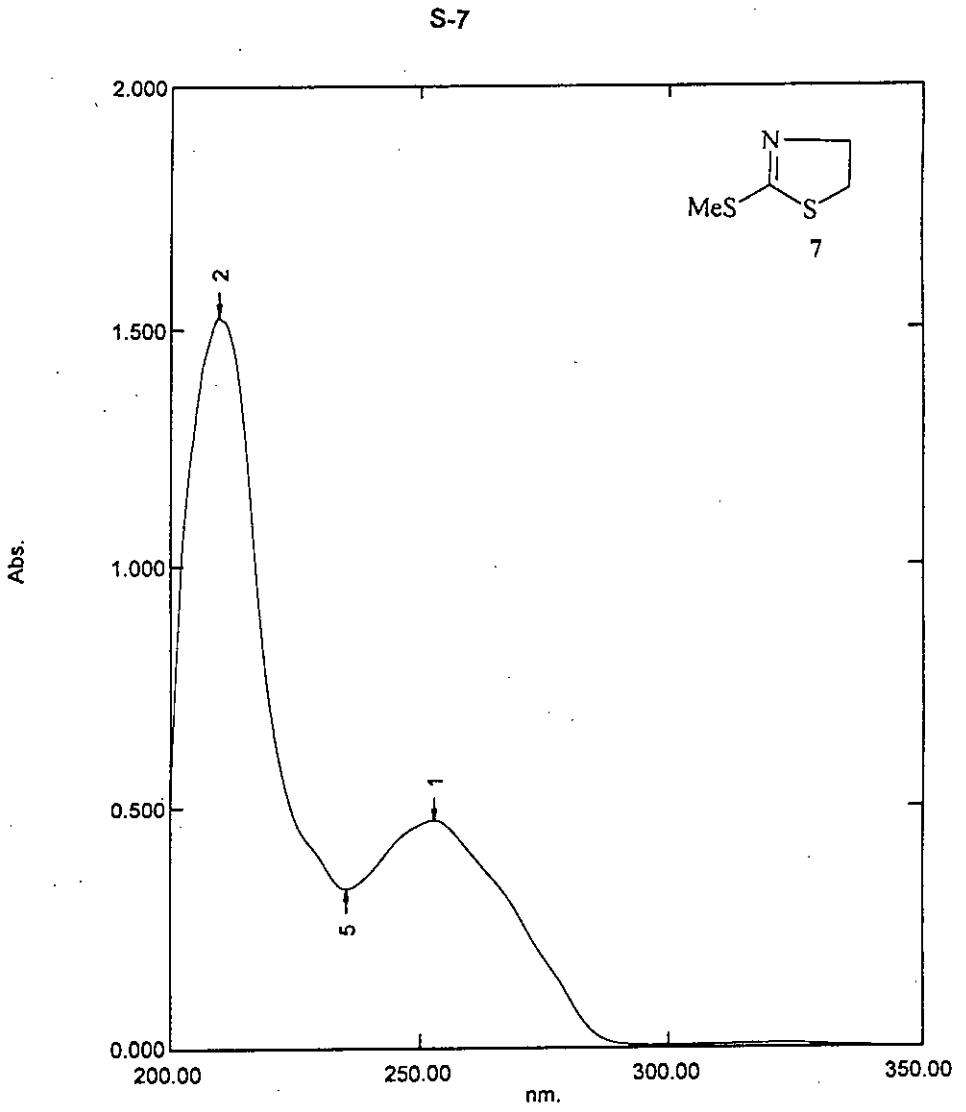
F2 - Processing parameters

SI	32768
SF	400.1400129 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.40

1D NMR plot parameters

CX	20.00 cm
F1P	4.134 ppm
F1	1654.27 Hz
F2P	3.250 ppm
F2	1300.26 Hz
PPMCM	0.04424 ppm/cm
HZCM	17.70039 Hz/cm

Fig. 17: ^{1}H NMR Spectrum of Compound **6** (Expansion)



Wavelength	Abs.
253	0.473
209	1.473

Fig. 18: UV Spectrum of Compound 7

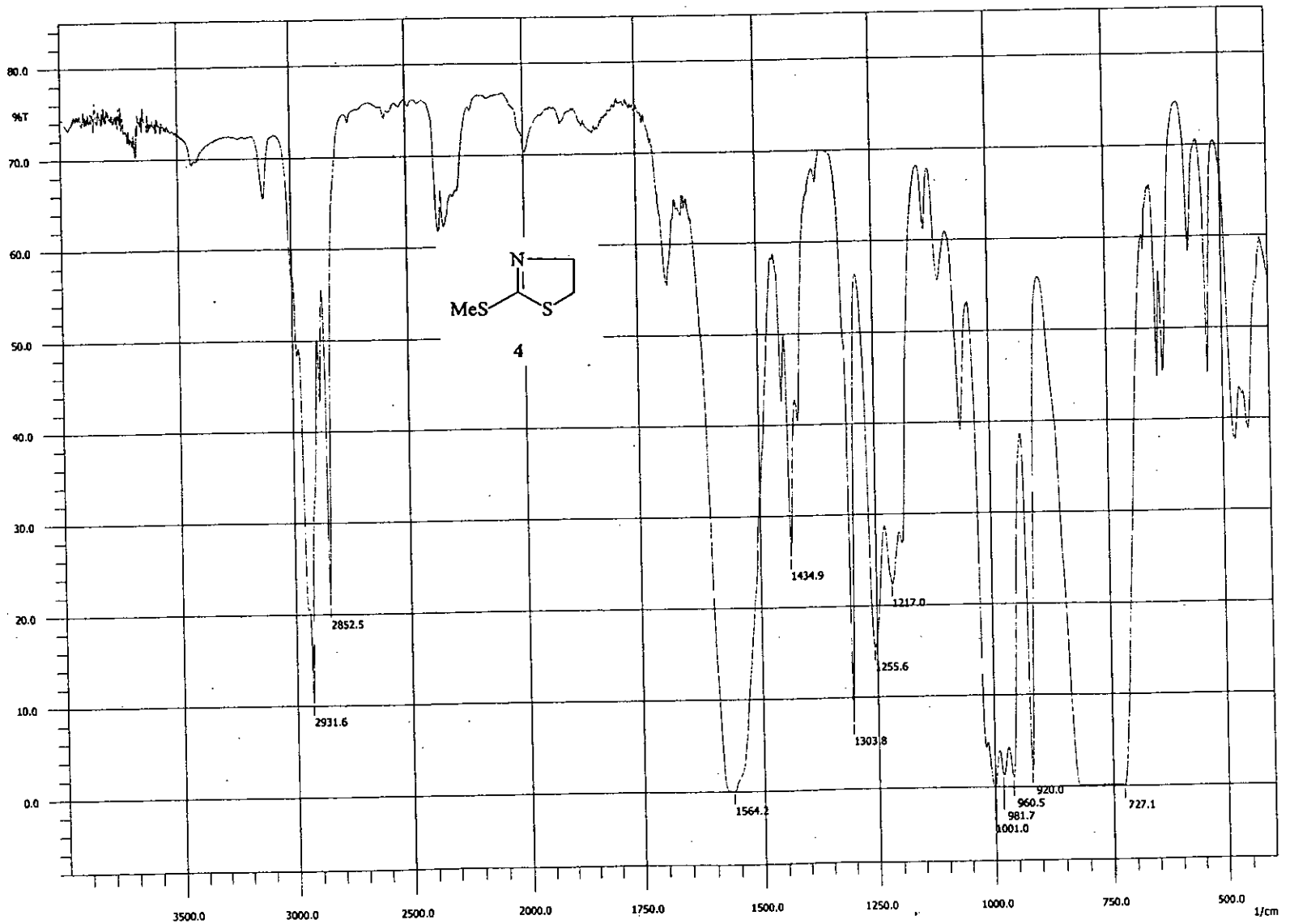


Fig. 19: IR Spectrum of Compound 7

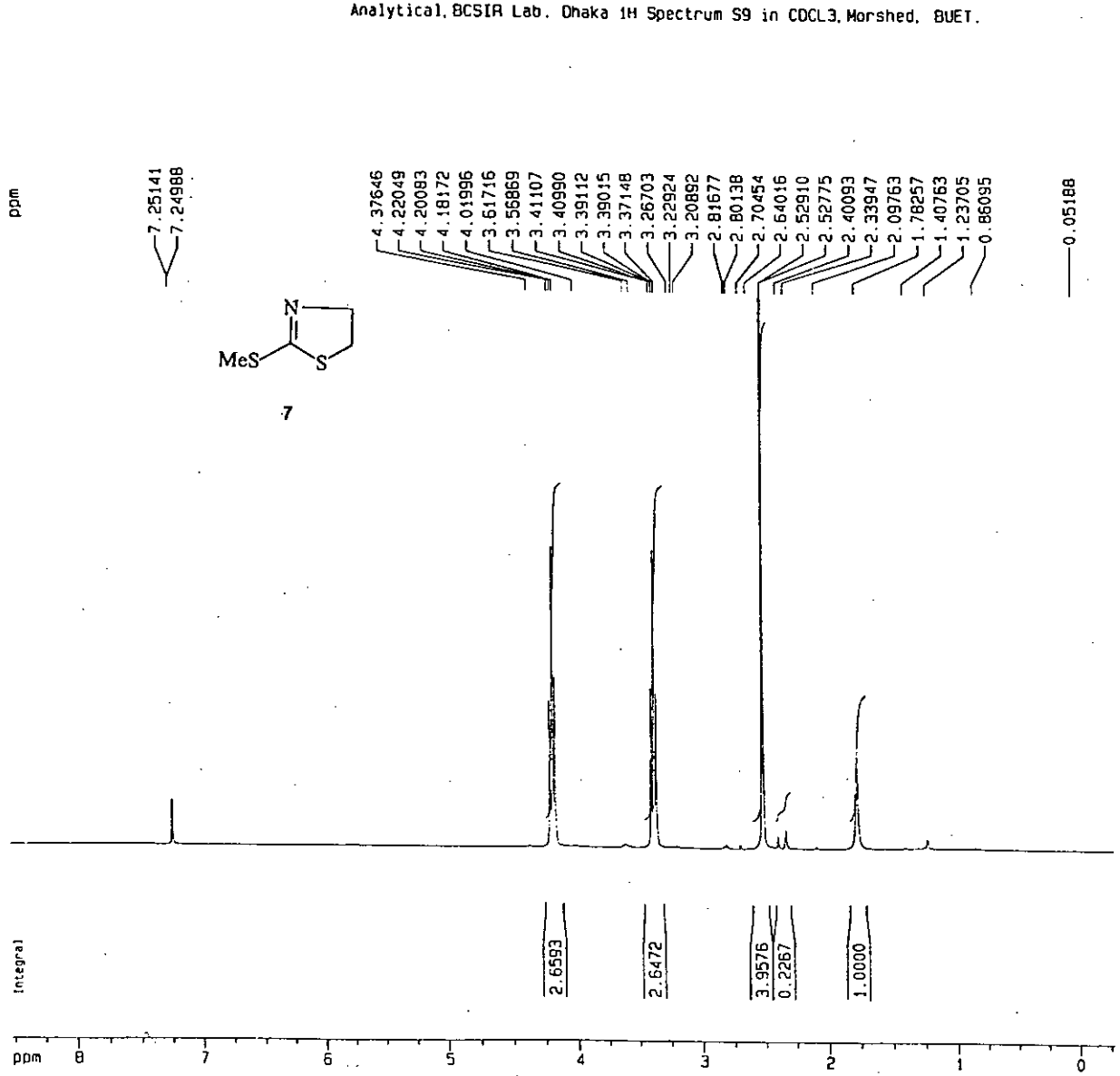


Fig. 20: ^{1}H NMR Spectrum of Compound 7

```
Current Data Parameters
NAME                  A1B14
EXPNO                     1
PROCNO                    1

F2 - Acquisition Parameters
Date_              20050517
Time                  13.52
INSTRUM               dpx400
PROBHD     5 mm Multinuc
PULPROG               zg30
TD                   32768
SOLVENT              COCl3
NS                     128
DS                       2
SWH              6410.256 Hz
FIDRES           0.195625 Hz
AQ               2.5559540 sec
RG                   287.4
DW                78.000 usec
DE                 6.00 usec
TE                310.0 K
D1             1.00000000 sec

============ CHANNEL f1 ============
NUC1                   1H
P1                 8.30 usec
PL1                -6.00 dB
SFO1          400.1428010 MHz

F2 - Processing parameters
SI                   32768
SF            400.1400125 MHz
WDW                    EM
SSB                     0
LB                 0.30 Hz
GB                      0
PC                   1.40

1D NMR plot parameters
CX                20.00 cm
F1P                8.529 ppm
F1              3412.89 Hz
F2P               -0.270 ppm
F2              -107.95 Hz
PPMCM          0.43995 ppm/cm
HZCM          176.04182 Hz/cm
```

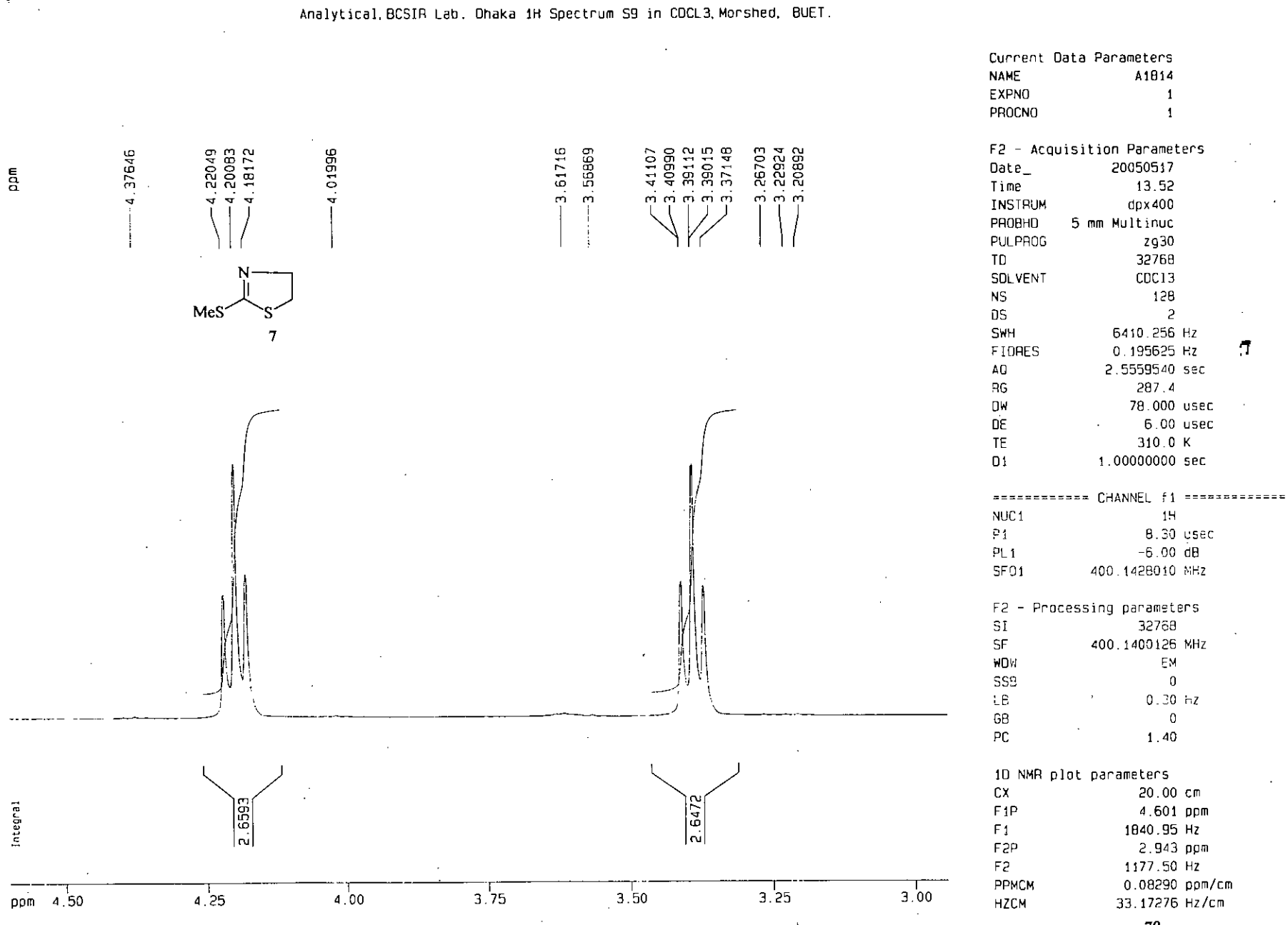
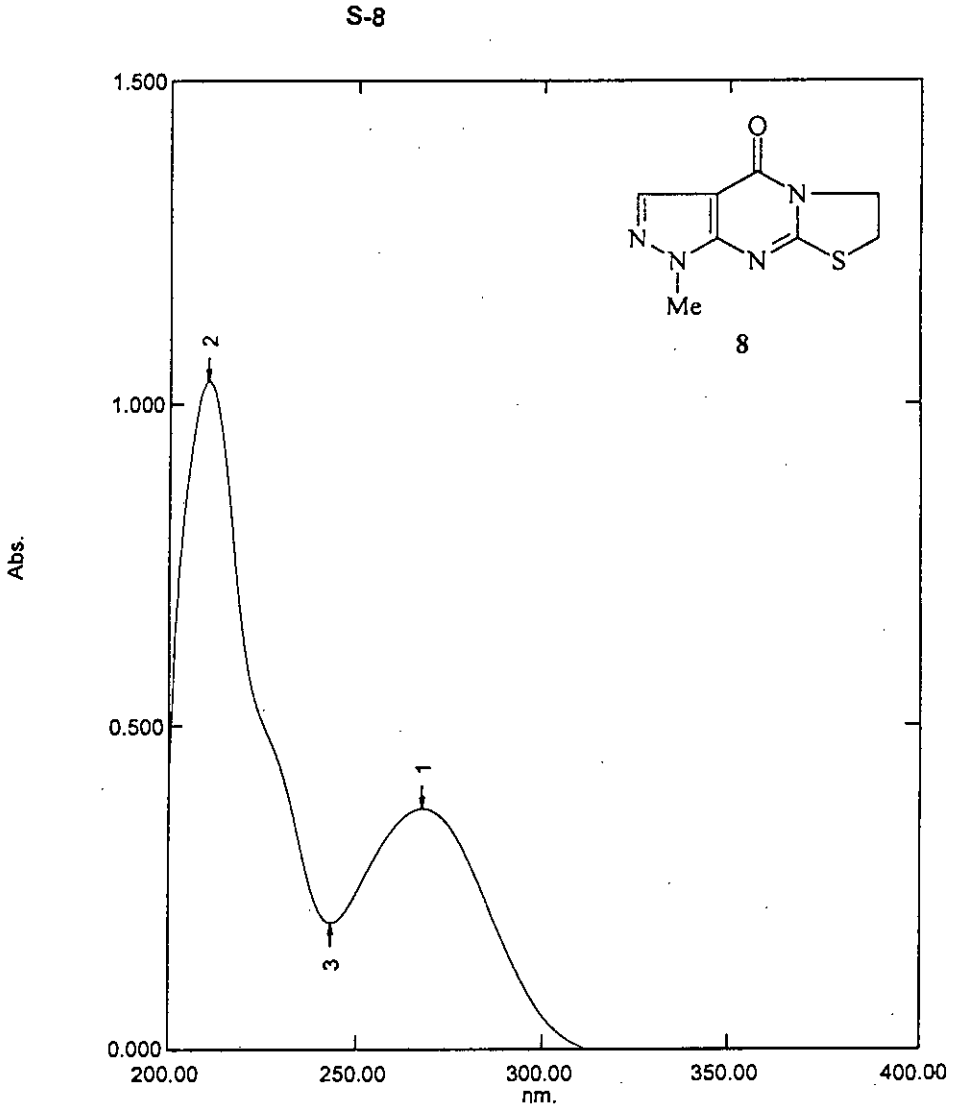
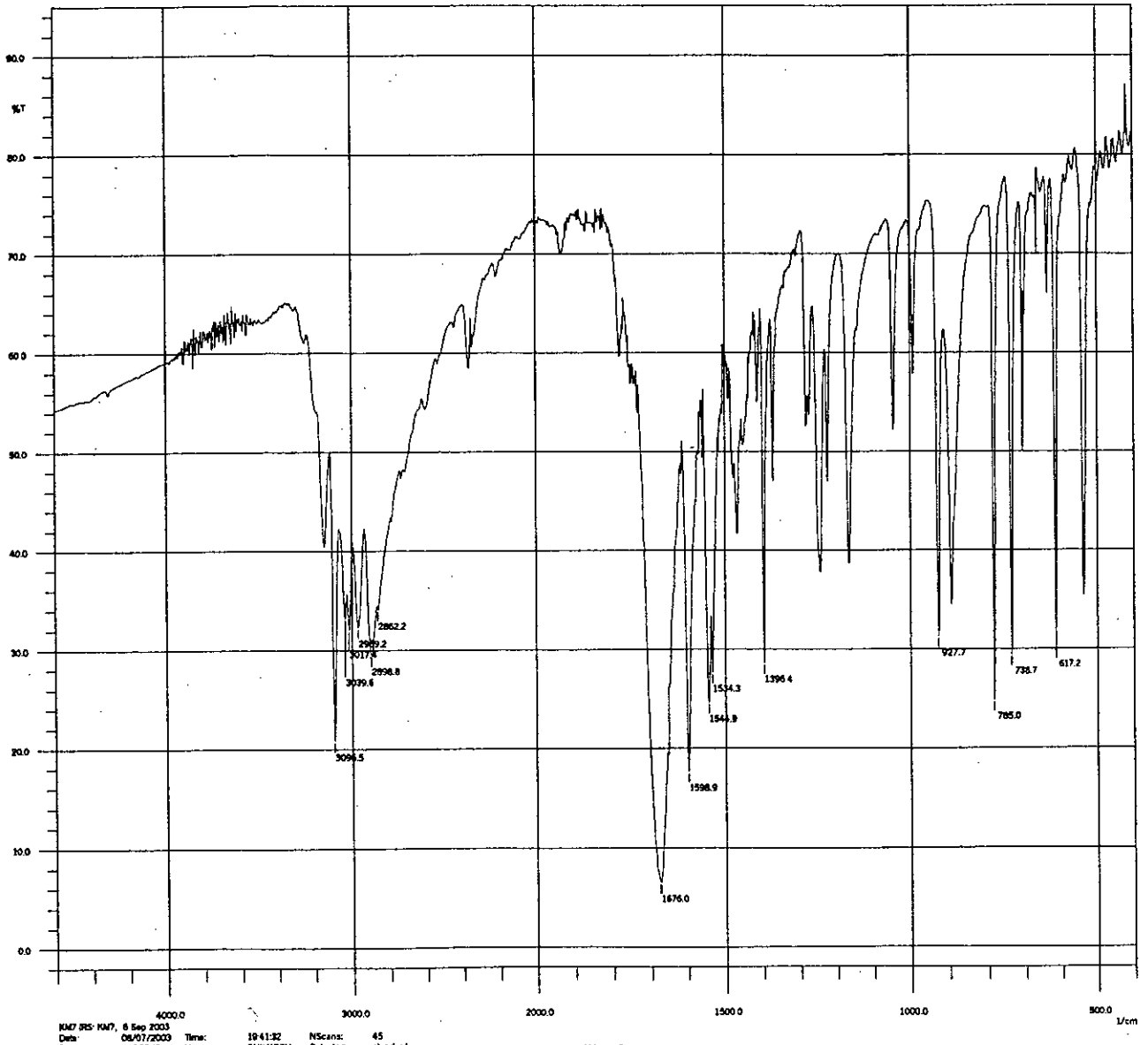


Fig. 21: ^{1}H NMR Spectrum of Compound **7** (Expansion)



Wavelength	Abs.
268	0.369
210	1.036

Fig. 22: UV Spectrum of Compound **8**



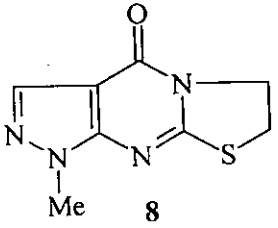
KM7.IRS: KM7, 6 Sep 2003

Data:	08/07/2003	Time:	19:41:32	NScans:	45
Type:	HYPER IR	User:	SHIMADZU	Detector:	standard
Abscissa:	1/cm	Ordinate:	%T	Apodization:	Happ
Min:	400.20	Max:	4599.91	Range:	1/cm
Ndp:	4356	Data Interval:	0.96434	Resolution:	2.0
Gain:	auto	Aperture:	auto	Mirror Speed:	2.8(low)

Fig. 23: IR Spectrum of Compound **8**

Peaktable of KM7.IRS, 15 Peaks
Threshhold: 35, Noise: 2, No Range Selection

No.	Pos. (1/cm)	Inten. (%T)
1	617.2	30.095
2	738.7	29.436
3	785.0	24.850
4	927.7	31.271
5	1396.4	28.596
6	1534.3	27.633
7	1544.9	24.626
8	1598.9	17.660
9	1676.0	6.487
10	2862.2	34.079
11	2898.8	29.502
12	2969.2	32.310
13	3017.4	32.012
14	3039.6	33.214
15	3096.5	20.869



8

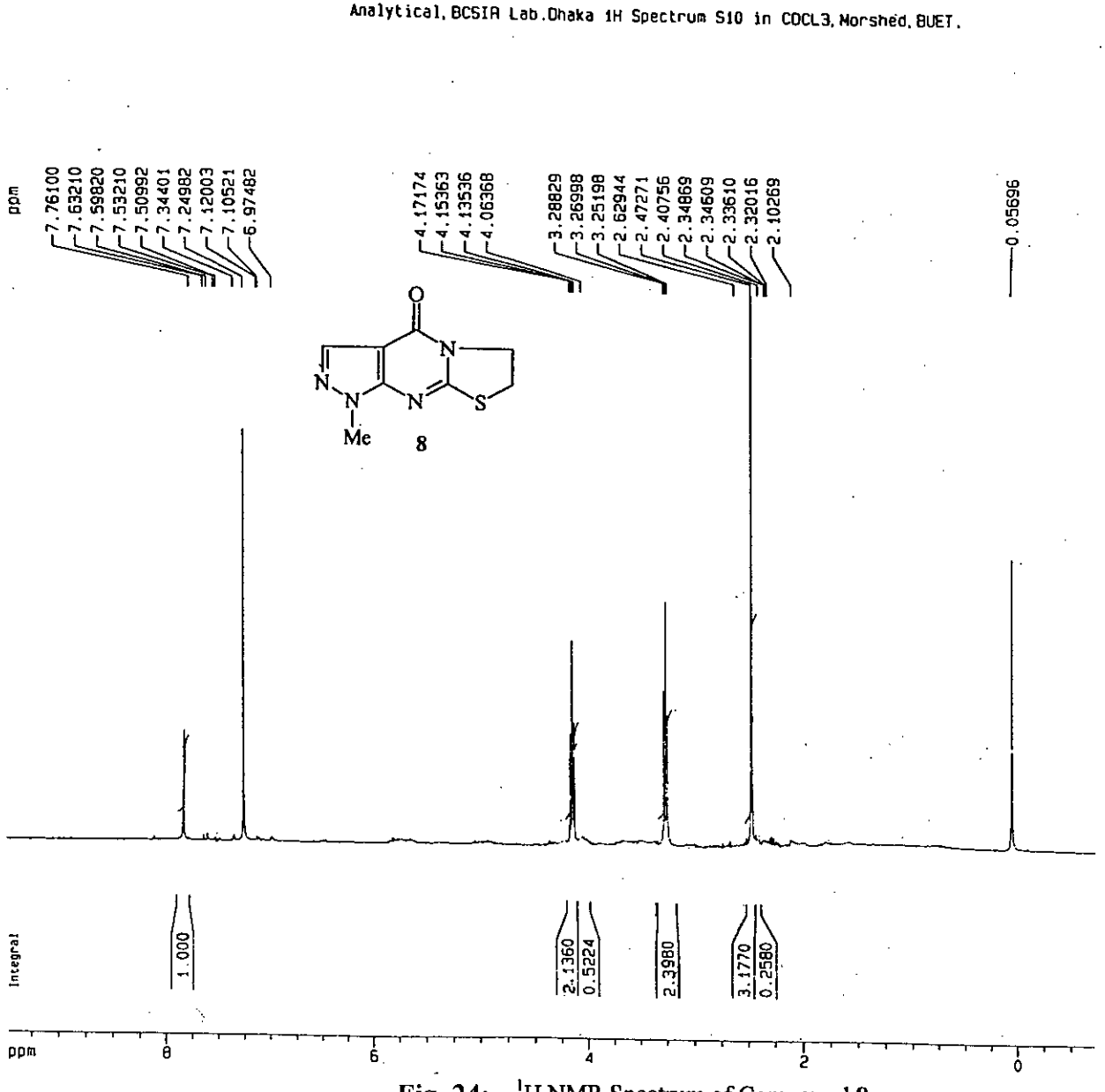


Fig. 24: ^{1}H NMR Spectrum of Compound **8**

Current Data Parameters

NAME	A1813
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20050517
Time	13.33
INSTRUM	dpx400
PROBHD	5 mm Multinuc
PULPROG	zg30
TD	32768
SOLVENT	CDCl3
NS	128
DS	2
SWH	6410.256 Hz
FIDRES	0.195625 Hz
AQ	2.5559540 sec
RG	362
DW	78.000 usec
DE	6.00 usec
TE	310.0 K
D1	1.00000000 sec

========== CHANNEL f1 ==========

NUC1	1H
P1	8.30 usec
PL1	-6.00 dB
SFO1	400.1428010 MHz

F2 - Processing parameters

SI	32768
SF	400.1400126 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.40

1D NMR plot parameters

CX	20.00 cm
F1P	9.473 ppm
F1	3790.37 Hz
F2P	-0.716 ppm
F2	-286.39 Hz
PPMCM	0.50942 ppm/cm
HZCM	203.83792 Hz/cm

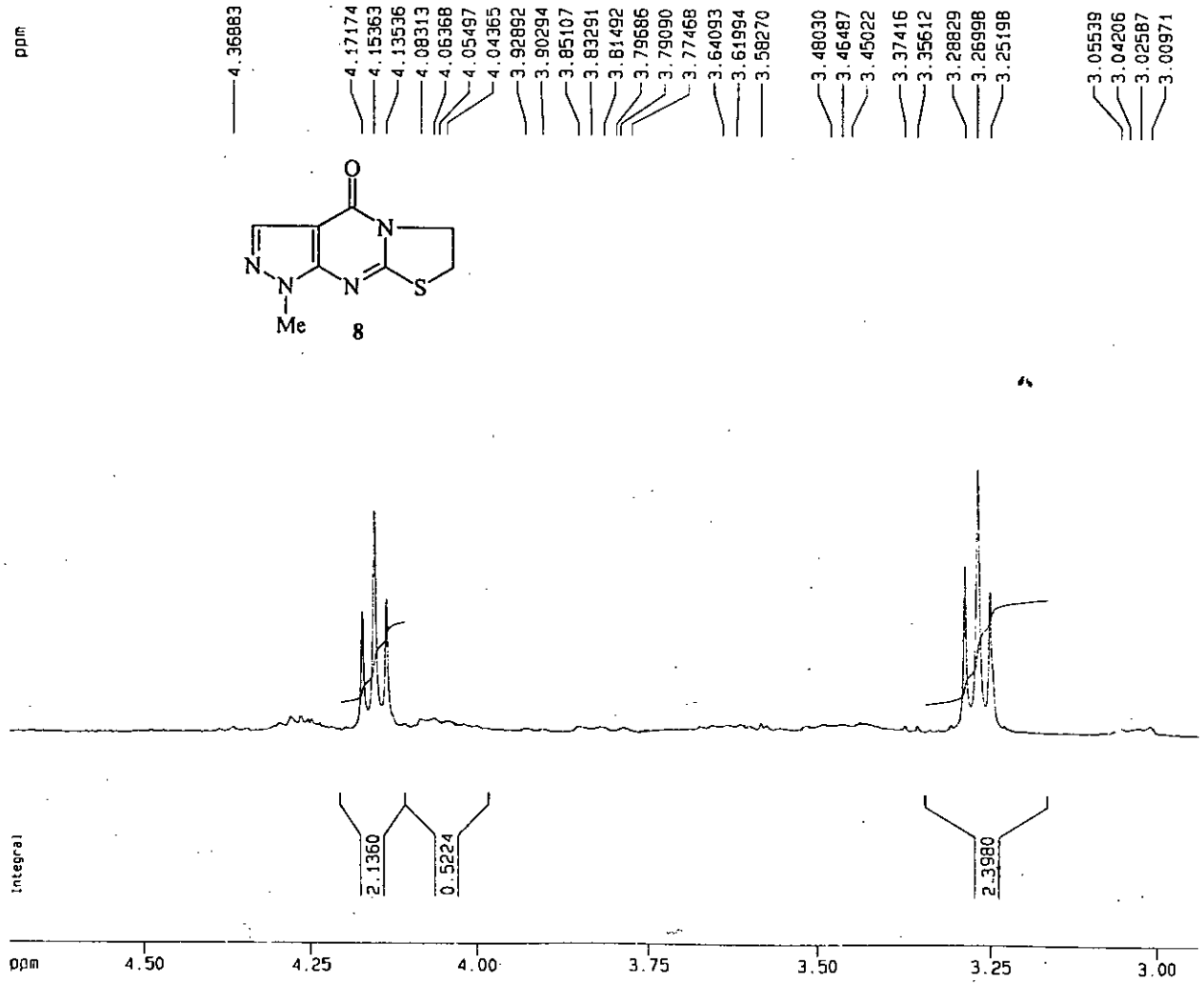


Fig. 25: ^{1}H NMR Spectrum of Compound **8** (Expansion)

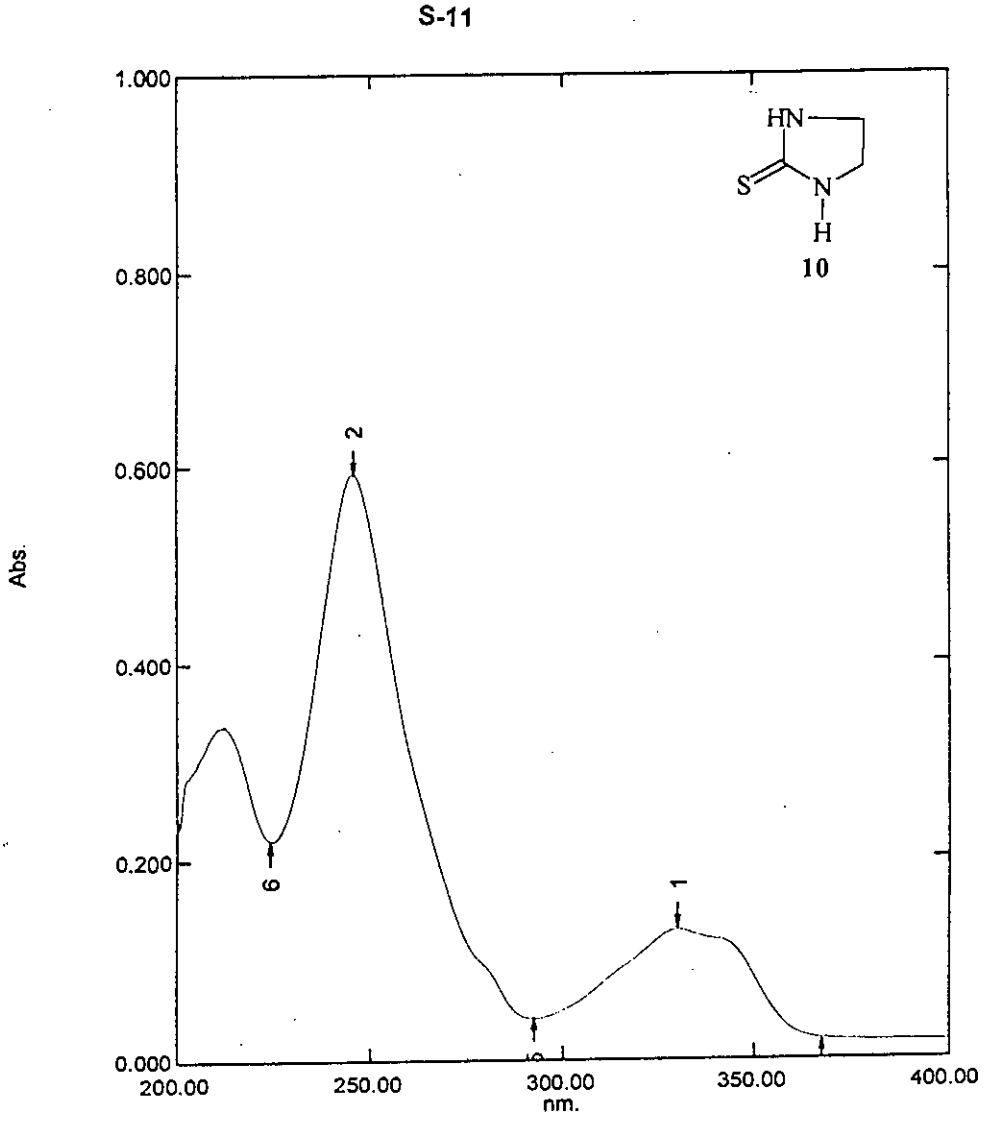
```
Current Data Parameters
NAME                  A1813
EXPNO                     1
PROCNO                    1

F2 - Acquisition Parameters
Date_              20050517
Time                  13.33
INSTRUM             dpx400
PROBHD     5 mm Multinuc
PULPROG               zg30
TD                   32768
SOLVENT              CDCl3
NS                     128
DS                       2
SWH              6410.256 Hz
FIDRES           0.195625 Hz
AQ             2.5559540 sec
RG                     362
DW                78.000 usec
DE                 6.00 usec
TE                310.0 K
D1             1.00000000 sec

============ CHANNEL f1 ============
NUC1                   1H
P1                  8.30 usec
PL1                -6.00 dB
SFO1         400.1428010 MHz

F2 - Processing parameters
SI                   32768
SF           400.1400128 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.40

1D NMR plot parameters
CX                  20.00 cm
F1P                 4.704 ppm
F1                1882.39 Hz
F2P                 2.938 ppm
F2                1175.48 Hz
PPMCM            0.08833 ppm/cm
HZCM            35.34563 Hz/cm
```



Wavelength	Abs.
245.60	0.593
212.00	0.335

Fig. 26: UV Spectrum of Compound **10**

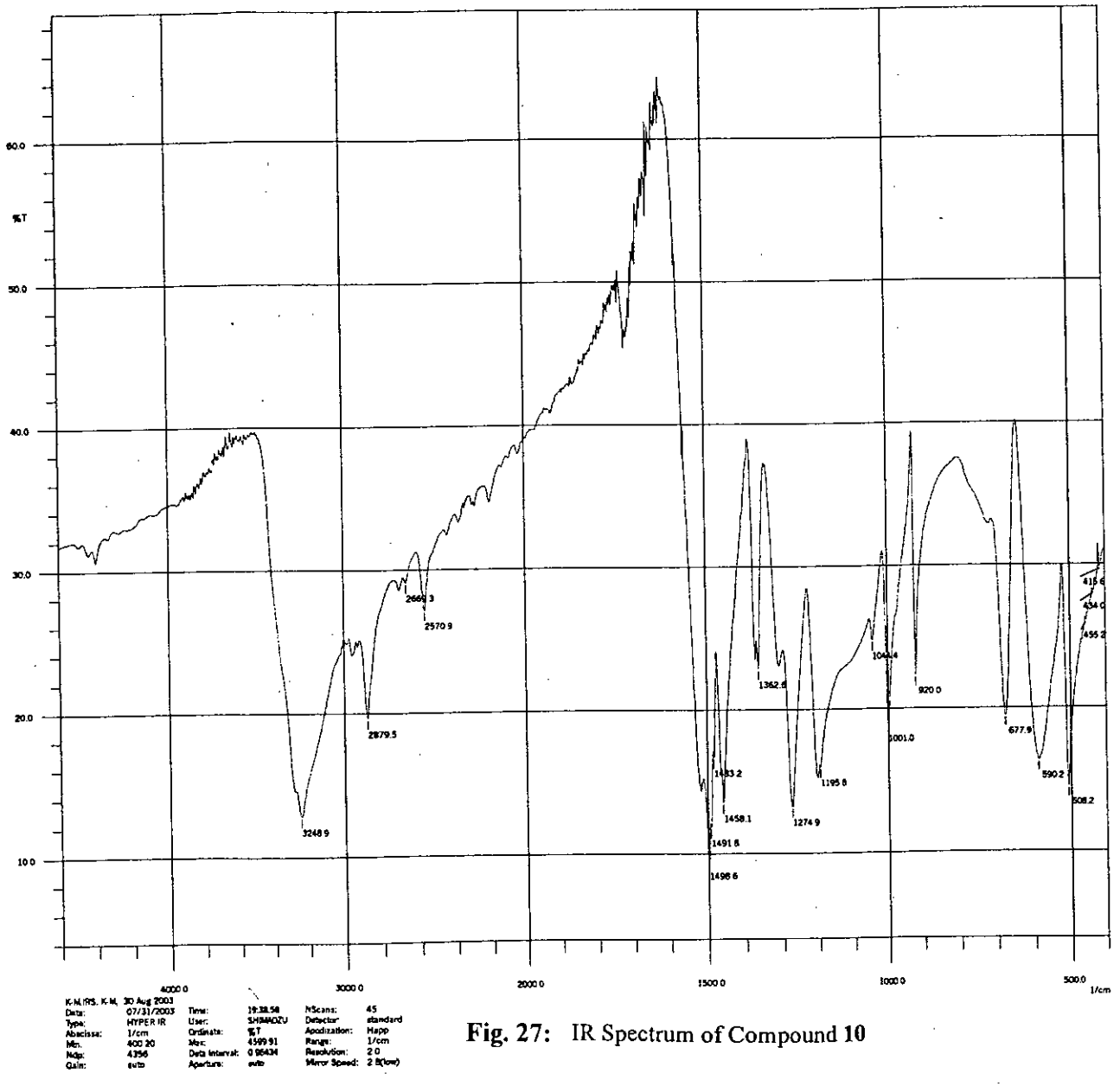
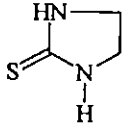


Fig. 27: IR Spectrum of Compound **10**

Peaktable of K-M.IRS, 20 Peaks
Threshhold: 30, Noise: 2, No Range Selection

No.	Pos. (1/cm)	Inten. (%T)
1	415.6	29.784
2	434.0	28.111
3	455.2	26.075
4	508.2	14.483
5	590.2	16.313
6	677.9	19.460
7	920.0	22.307
8	1001.0	18.987
9	1044.4	24.766
10	1195.8	15.976
11	1274.9	13.234
12	1362.6	22.815
13	1458.1	13.523
14	1483.2	16.660
15	1491.8	11.834
16	1498.6	9.542
17	2570.9	27.149
18	2669.3	29.085
19	2879.5	19.629
20	3248.9	12.819



10

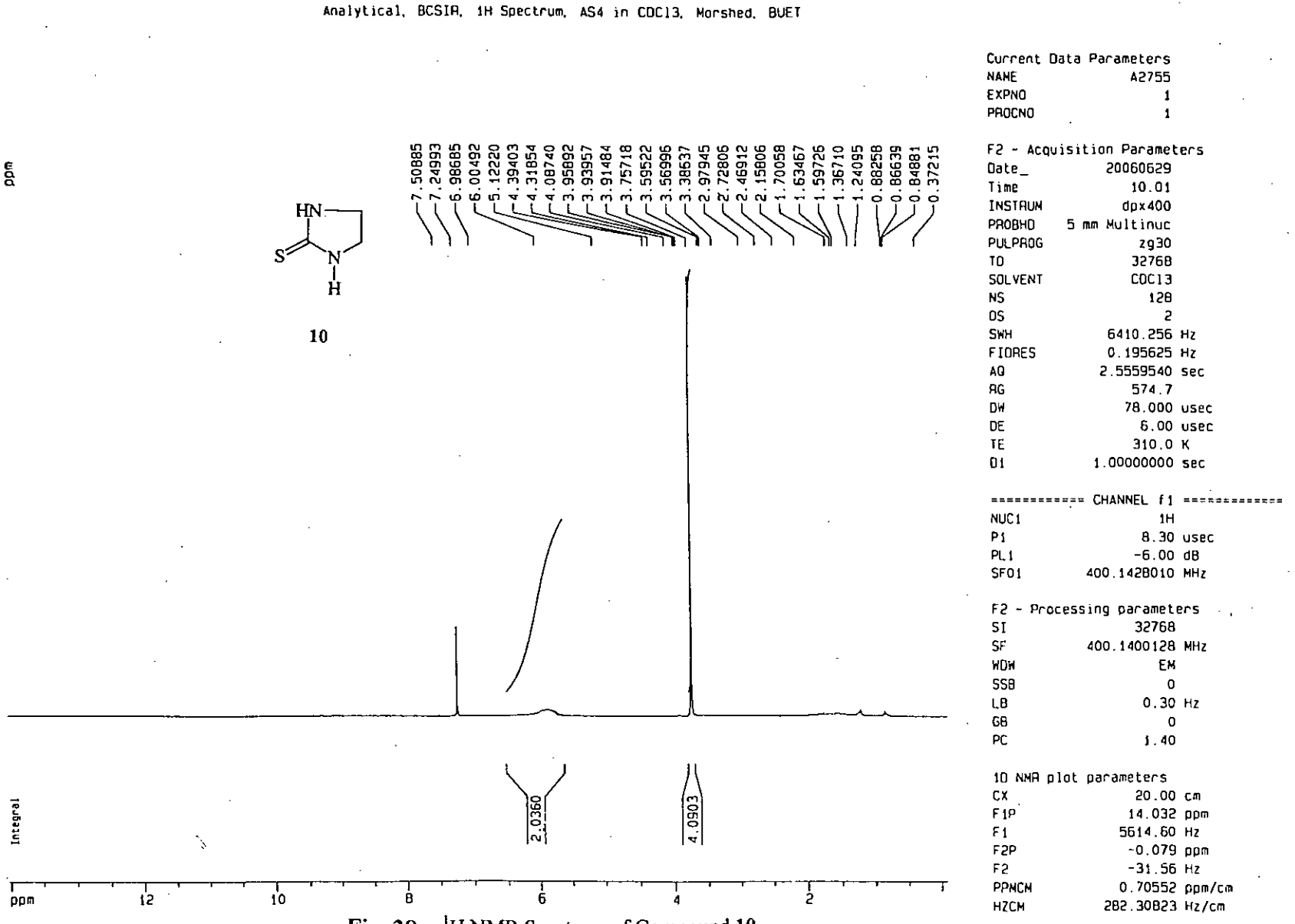
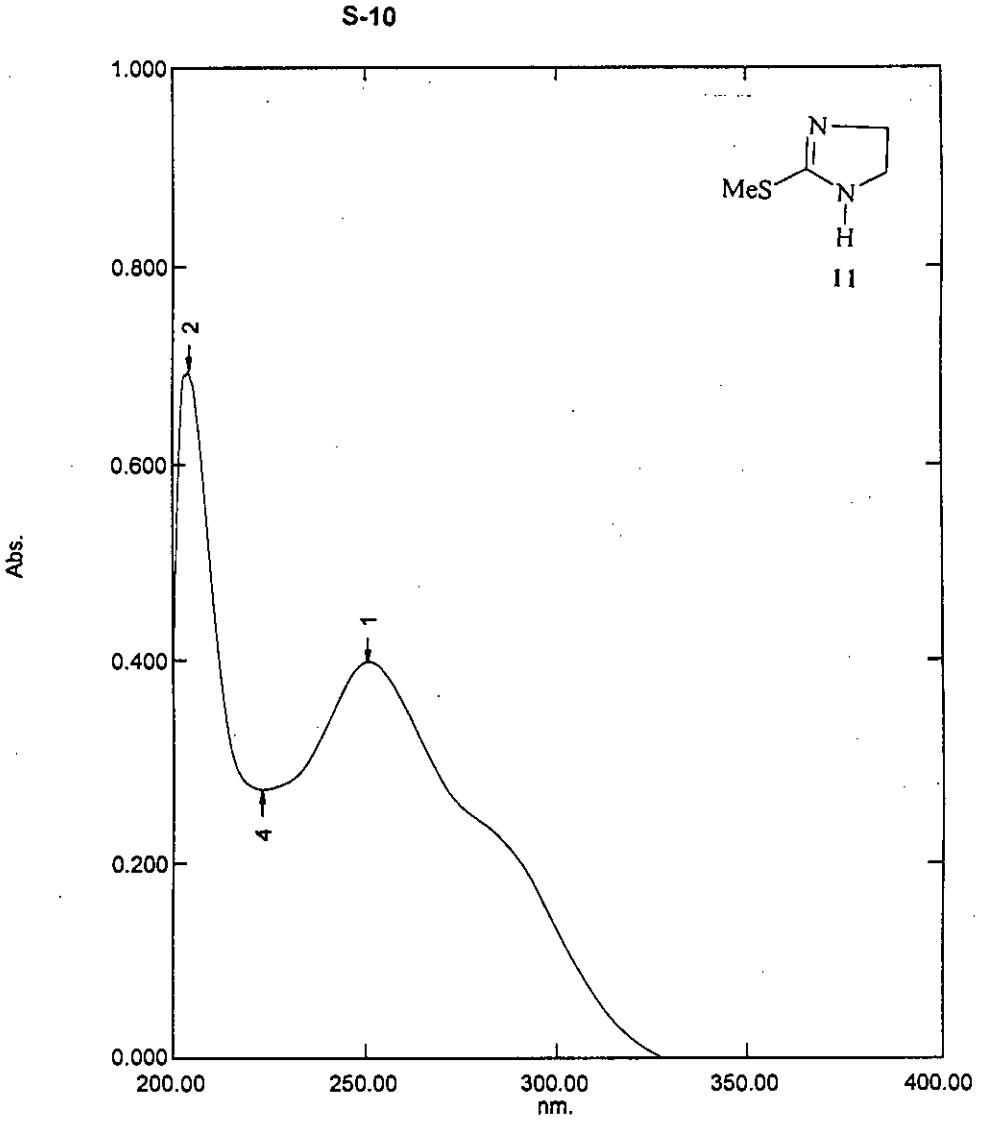


Fig. 28: ^{1}H NMR Spectrum of Compound **10**



Wavelength	Abs.
251	0.398
204	0.695

Fig. 29: UV Spectrum of Compound **11**

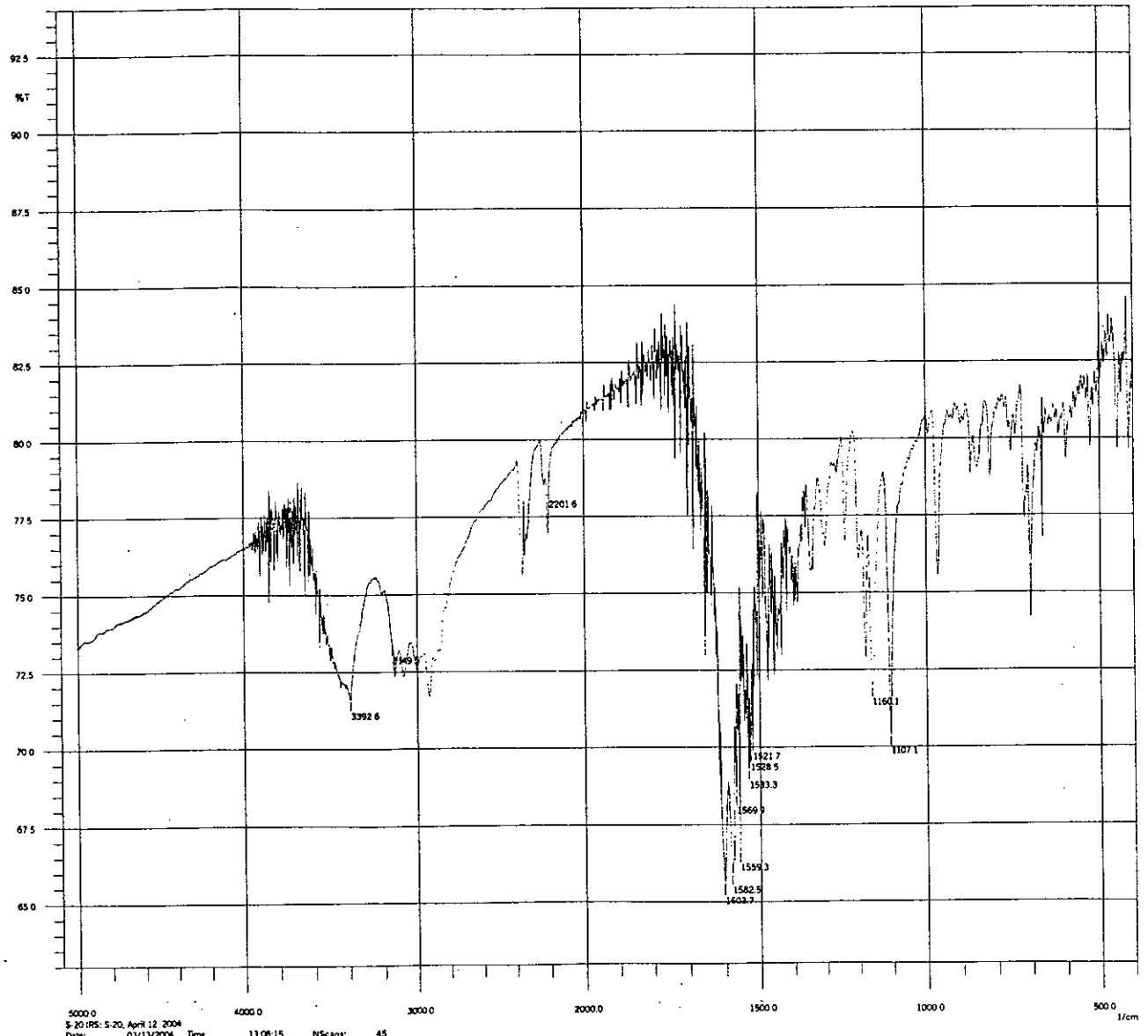
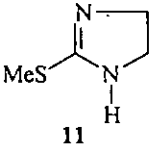


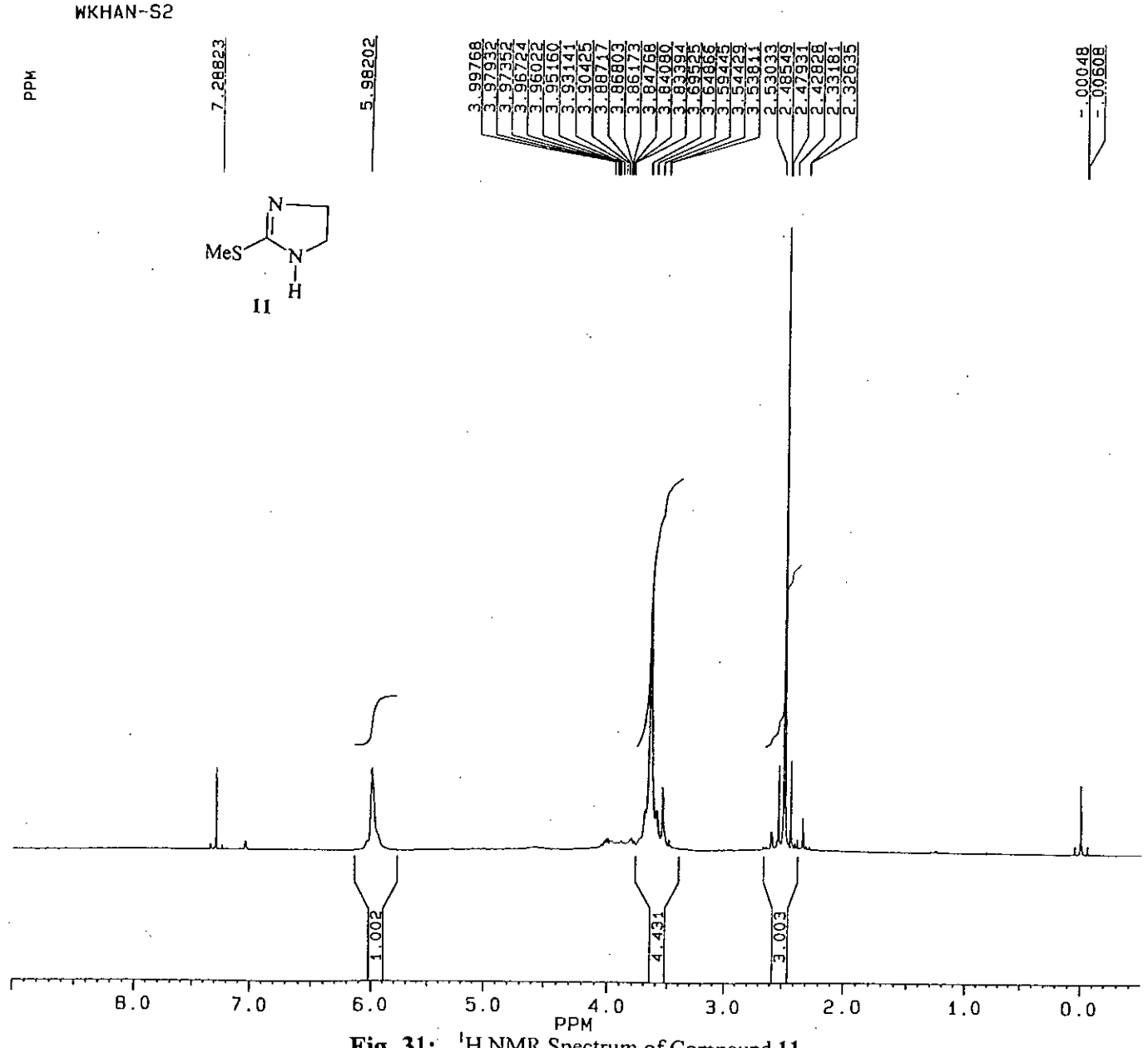
Fig. 30: IR Spectrum of Compound 11

Peaktable of S-20.IRS, 12 Peaks
Threshhold: 72, Noise: 2, No Range Selection

No.	Pos. (1/cm)	Inten. (%T)
1	1107.1	70.393
2	1160.1	71.969
3	1521.7	70.236
4	1528.5	70.297
5	1533.3	69.310
6	1559.3	66.648
7	1569.9	68.477
8	1582.5	65.918
9	1603.7	65.661
10	2201.6	78.432
11	3149.5	73.382
12	3392.6	71.599



11

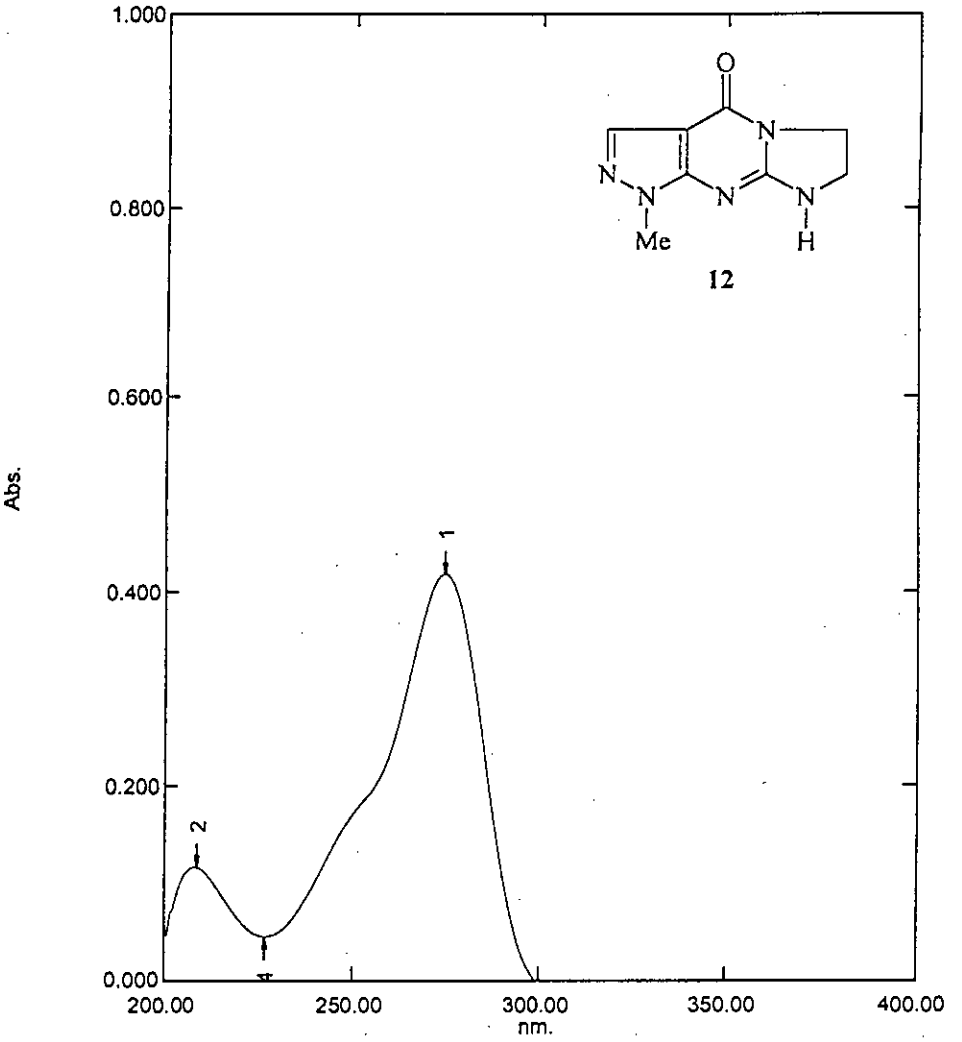


KSMDKHAN.001
DATE 13-1-4

SF	400.134
SY	230.0
O1	6395.963
SI	32768
TD	32768
SW	8064.516
HZ/PT	.492
PW	6.2
RD	2.000
AQ	2.032
RG	10
NS	8
TE	297
FW	10100
O2	20.000
DP	63L PO
LB	.200
GB	0.0
CX	22.00
CY	12.00
F1	9.001P
F2	-.498P
HZ/CM	172.769
PPM/CM	.432
SR	4383.77

Fig. 31: ^{1}H NMR Spectrum of Compound **11**

S-12



Wavelength	Abs.
275	0.419
208	0.118

Fig. 32: UV Spectrum of Compound **12**

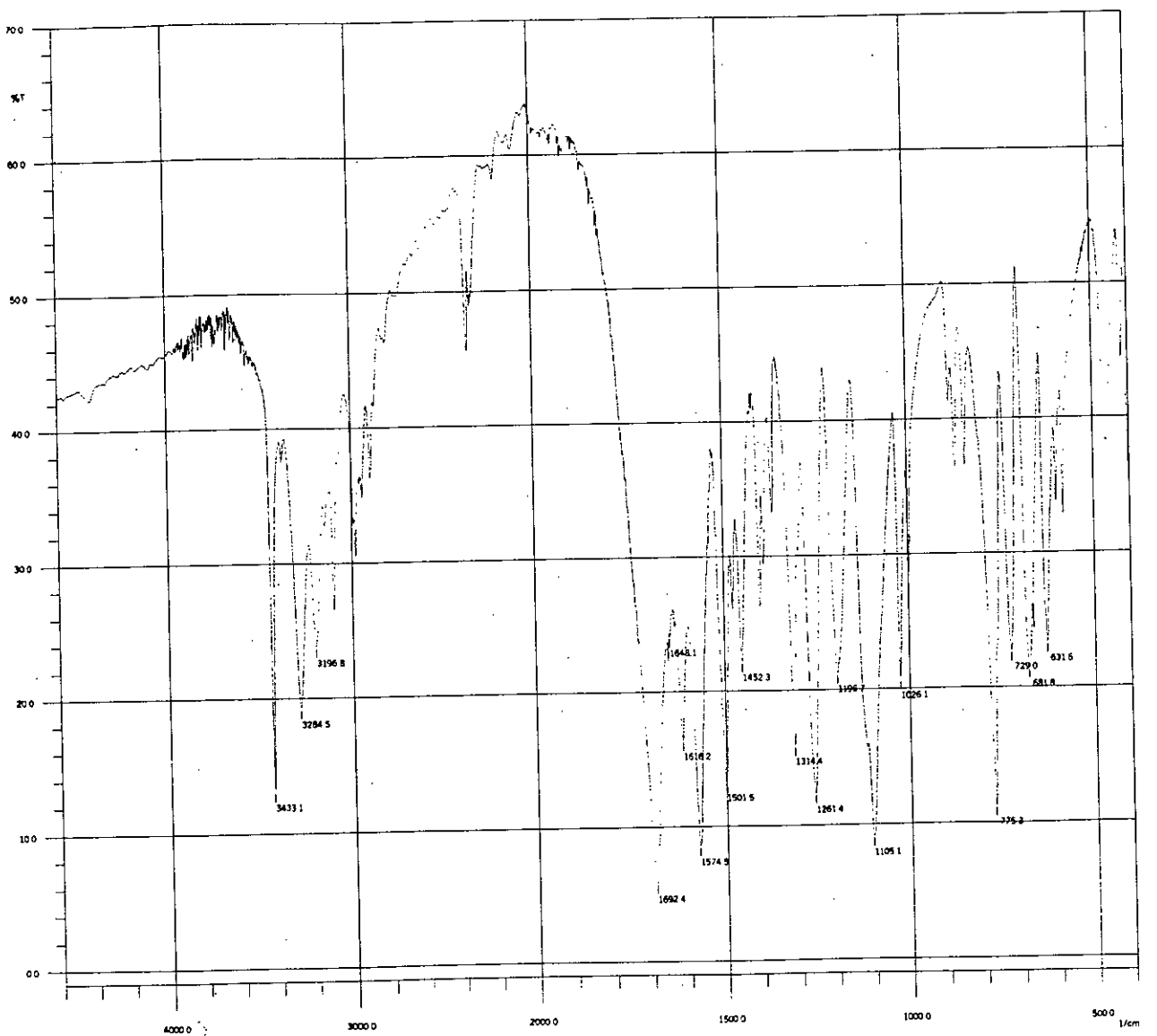
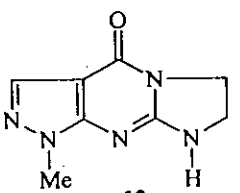


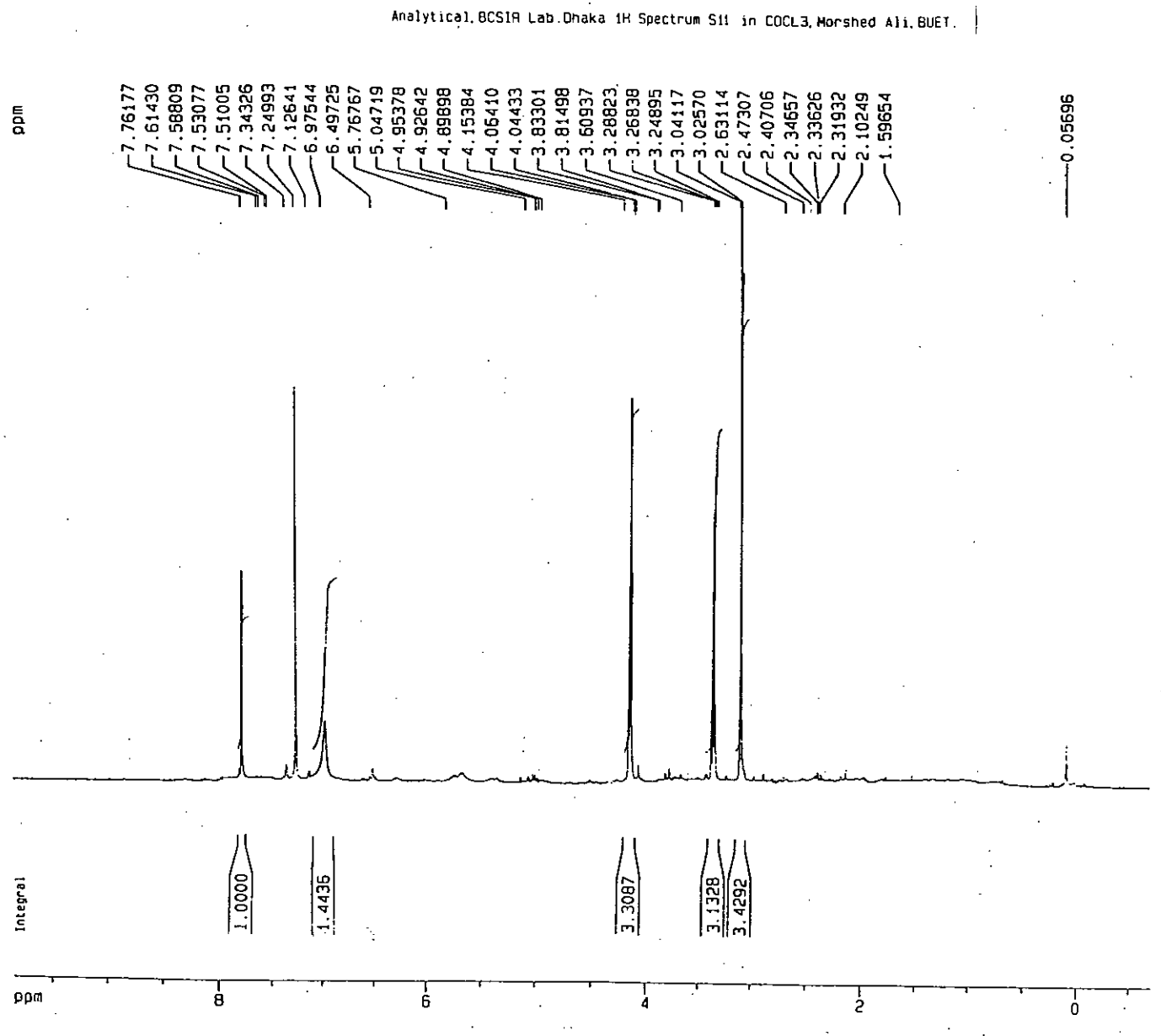
Fig. 33: IR Spectrum of Compound **12**

Peaktable of DEFAULT.IRS, 18 Peaks
Threshhold: 25, Noise: 2, No Range Selection

No.	Pos. (1/cm)	Inten. (%T)
1	631.6	23.332
2	681.8	21.427
3	729.0	22.739
4	775.3	11.247
5	1026.1	20.694
6	1105.1	9.134
7	1196.7	21.260
8	1261.4	12.371
9	1314.4	15.944
10	1452.3	22.190
11	1501.5	13.284
12	1574.8	8.576
13	1616.2	16.387
14	1648.1	24.081
15	1692.4	5.843
16	3196.8	23.794
17	3284.5	19.298
18	3433.1	13.132



12



Current Data Parameters

NAME	A1939
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20050704
Time	10.14
INSTRUM	dpx400
PROBHD	5 mm Multinuc
PULPROG	zg30
TD	32768
SOLVENT	CDCl3
NS	128
DS	2
SWH	6410.256 Hz
FIDRES	0.195625 Hz
AQ	2.5559540 sec
RG	322.5
DW	78.000 usec
DE	6.00 usec
TE	310.0 K
D1	1.00000000 sec

========== CHANNEL f1 ==========

NUC1	1H
P1	8.30 usec
PL1	-6.00 dB
SFO1	400.1428010 MHz

F2 - Processing parameters

SI	32768
SF	400.1400125 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.40

1D NMR plot parameters

CX	20.00 cm
F1P	9.863 ppm
F1	3946.61 Hz
F2P	-0.713 ppm
F2	-285.41 Hz
PPMCM	0.52882 ppm/cm
HZCM	211.60071 Hz/cm

Fig. 34: ^{1}H NMR Spectrum of Compound **12**

Part-I

Section-3

SYNTHESIS OF 5-ALKYNYL PYRAZOL DERIVATIVES BY PALLADIUM CATALYZED REACTIONS.

1.3.0. INTRODUCTION:

There are relatively few basic type reactions that generate a new carbon-carbon bond, although this is one of the most critical operations in the synthesis of organic molecules. Acetylenes and vinylic derivatives are versatile compounds in the synthetic organic chemistry and hence various methods for their synthesis have been explored.

A conventional method for the preparation of arylacetylene derivatives is the coupling reaction of arylhalides with copper (I) acetylides, known as Castro reaction[82]. The cross coupling of organotin reagents with variety of organic electrophiles, catalyzed by palladium, provides a novel method for generating a carbon-carbon bond[83] known as Stille coupling. The palladium-catalyzed coupling of haloarenes and alkenes with alkenes known as the Heek reaction is well-established[84]. The Sonogashira coupling reaction of terminal alkynes with aryl halides provides an efficient route to arylalkynes. T. Jeffery has established palladium catalyzed vinylation of vinylic halides[85] and vinylation of acetylenic iodides under solid-liquid phase-transfer conditions[86].

1.3.1. PRESENT WORK:

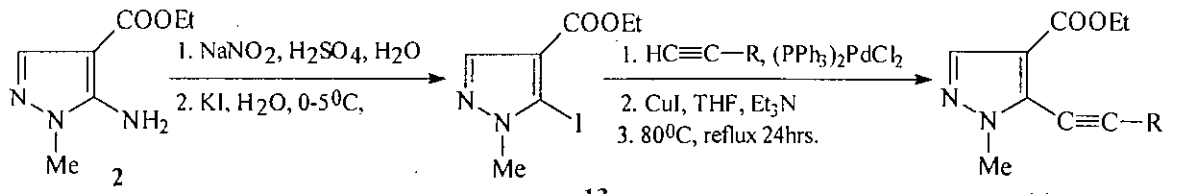
Synthesis of 5-substituted alkynyl pyrazol derivatives by palladium catalyzed reactions.

Rationale:

Pyrazoles (ethyl-5-amino-1-methylpyrazol-4-carboxylate) are a class fused heterocycles, has arose great interest in recent years due to their wide variety of biological activities[86] and pharmacological studies[87] and use as a common building block of a wide variety of alkaloids (as described in section-1). Although various methods have been developed previously for the synthesis of pyrazoles but only a few of them were mediated through palladium catalysis.

Palladium catalyzed[88] reactions have been extensively utilized for carboannulation[89] and hetero annulation[90] process. Many research groups have reported the synthesis of various aromatic heterocycles via. palladium catalyzed annulation of internal alkynes[91]. Other have shown the palladium catalyzed cyclizations to be valuable synthetic tools for the synthesis of a wide variety of heterocycles[92] using vinylic compounds, terminal alkynes, allenes and other substrates.

A new strategy for the synthesis of 5-substituted alkynyl pyrazol derivatives (**14-17**) by palladium catalyzed reactions of ethyl-5-iodo-1-methylpyrazol-4-carboxylate (**13**) with terminal alkynes followed by coupling reactions in a highly regio- and stereo-selective manner has been demonstrated. In continuation of our preceding work and in view of the natural occurrence and biological importance of the pyrazol derivatives and lack of convenient general procedures for their synthesis, we were interested in developing a general and facile method for the synthesis of 5-substituted alkynyl pyrazole derivatives.



Scheme-4

14). R = 
15). R = -Si(CH3)3
16). R = -CH2-OH
17). R = -(CH2)3-CH3

1.3.2. RESULTS AND DISCUSSION:

At first we attempted for the synthesis of ethyl-5-iodo-1-methylpyrazol-4-carboxylate (**13**) (**Scheme-4**) from ethyl-5-amino-1-methylpyrazol-4-carboxylate (**2**) through diazotization reaction followed by sandmeyer reaction. A new strategy for the synthesis of 5-substituted alkynyl pyrazol derivatives (**14-17**) by palladium catalyzed reactions of ethyl-5-iodo-1-methylpyrazol-4-carboxylate (**13**) with terminal alkynes followed by substitution reactions in a highly regio- and stereo-selective manner has been demonstrated. The reactions were usually carried out by heating a mixture of ethyl-5-iodo-1-methylpyrazol-4-carboxylate (**13**) with terminal alkynes (**18-21**) (1.2 eqv.) in DMF/THF (6 ml) at 80-85^{0}C for 16/24 hrs. in the presence of bis (triphenylphosphine) palladium (II) Chloride (3 mol%), copper (I) iodide (8 mol%) and triethylamine (4 equiv.).

After usual work-up the condensed products of the pyrazole derivatives (**14-17**) were obtained usually in excellent yields. The crude product was purified by column chromatography using silica gel (70-270 mesh). It was observed that the terminal alkynes (i.e. phenylacetylene) underwent considerable dimerization[87] during the heteroannulation reaction which was easily separable by column chromatography. It is also investigated that Cu (I) and base are necessary for the palladium-catalyzed reaction to afford the desired products.

1.3.2.A. Mechanism of palladium-catalyzed reactions of ethyl-5-iodo-1-methylpyrazol-4-carboxylate with terminal alkynes:

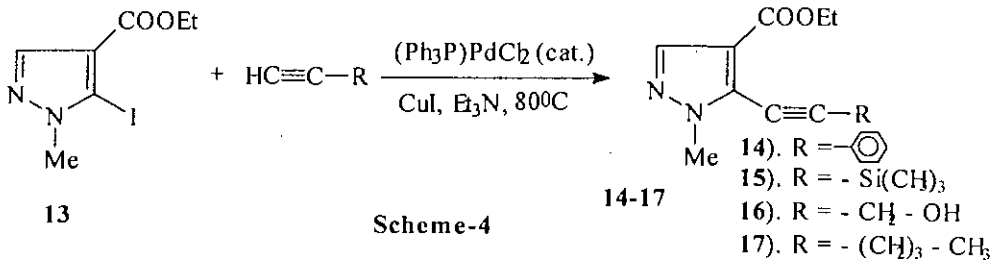
Although the detailed mechanism of the reaction is yet to be clarified, it can be perceived that the reactions proceed according to **scheme-5**. From our observations it was clear that the presence of palladium catalyst and base was very essential for the success of the heteroannulation. The key steps of the plausible mechanism were based on the following observations.

Sonogashira et al. in Japan demonstrated that terminal alkynes react smoothly with bromoalkynes, iodoarenes and bromopyridines in the presence of catalytic amounts of bis(triphenylphosphine)palladium dichloride and cuprous iodide in dimethylamine at room temperature in **scheme-5**. This mild process has significantly extended the utility of the Stephens-Castro type reaction and has performed very well in a variety of contexts in organic synthesis. In recognition of the valuable contribution of Sonogashira et al; the Pd^0/Cu^I-catalyzed coupling of *sp*-, sp^2- hybridized carbon atoms is often referred to as the Sonogashira coupling reaction.

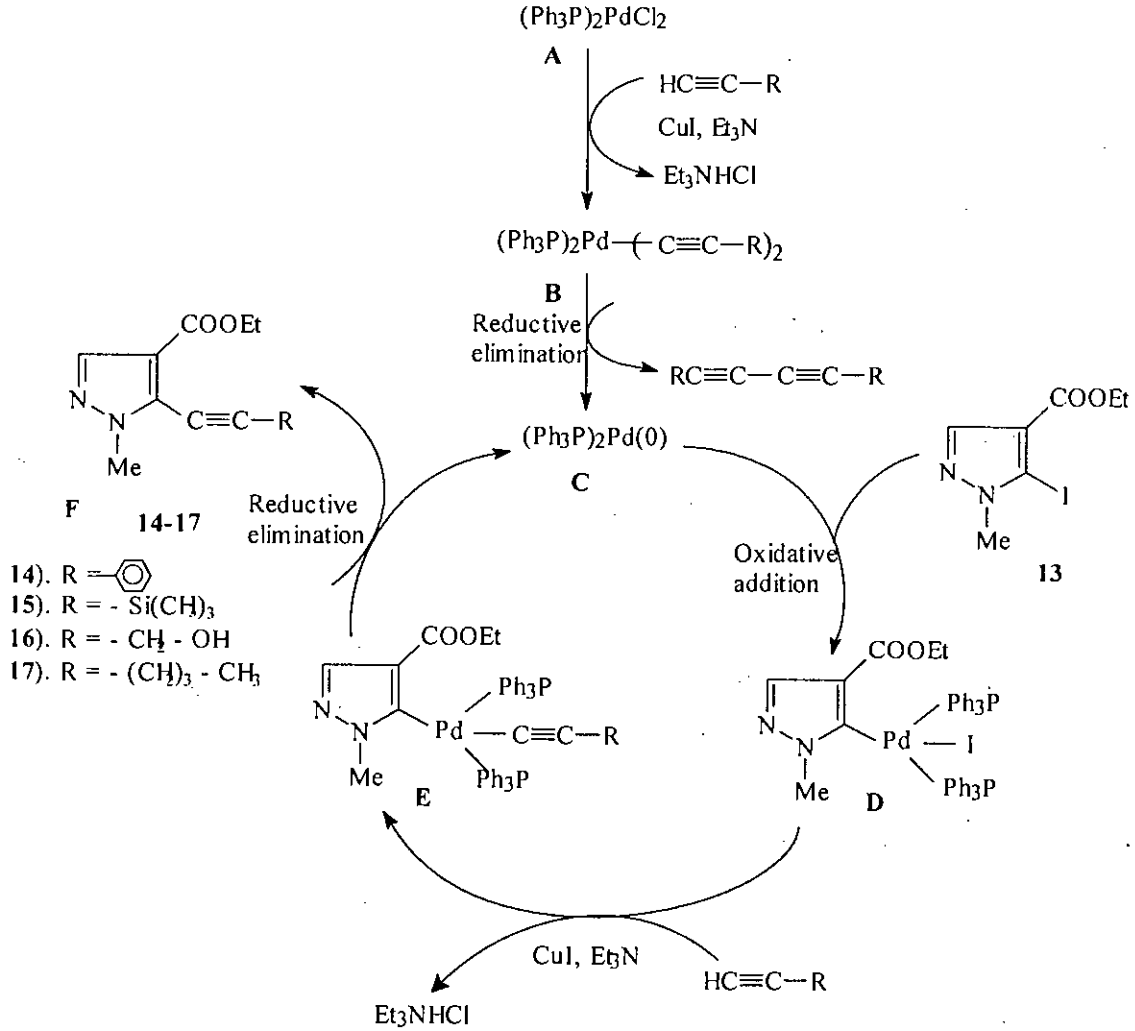
The presumed catalytic cycle for the Sonogashira coupling is shown in sheme-5. Bis(triphenylphosphine)palladium(0) **C**, the putative active catalyst, could conceivably be formed in situ through sequential copper(I)iodide-catalyzed bis-alkynylation and reductive elimination reactions (**A→B→C, Scheme-5**). Once formed, the highly co-ordinatively unsaturated 14-electron palladium(0) complex **C** participates in an oxidative addition reaction with the ethyl-5-iodo-1-methylpyrazol-4-carboxylate (**13**) to give the 5-pyrazole palladium (II) complex **D**. Then the terminal alkynes could be co-ordinates with palladium (II) complex **D,** then furnishes to co-ordinated complex **E**.

Finally, a terminating reductive elimination step reveals the coupling products as 5-alkynyl pyrazole derivatives **F (14-17)** and regenerates the active palladium(0)

catalyst.



Scheme-4



Mechanism-3: Catalytic cycle for the Sonogashira coupling

1.3.2.B. Cherecterization of the products:

Ethyl-5-phenylacetylenethnyl-1-methylpyrazol-4-carboxylate (14):

The compound **14** was reddish solid, mp. 71-72^{0}C and R_f value 0.26 (n-hexane : chloroform, 1:1).

In the UV spectrum (**Fig. No. 41**) the λ_{max} value was found in the range of 265.60 nm. The IR spectrum (**Fig. No. 42**) of this compound showed the following characteristic peaks: 3103.3 cm^{-1} (C-H str.), 2983 cm^{-1} (C-H str.), 2150.6 cm^{-1} (C≡C str.), 1701.1 cm-1 (C=O str.), 1382.9cm-1 (-CN str.) respectively.

In its ^{1}H-NMR spectrum (**Fig. No. 43, 44 & 45**) exhibited a one-proton singlet at δ 7.89 for –CH, a two-proton doublet at 7.58 (as d, 2H, Ar-H, J = 7.8 Hz) for aromatic group Ar-H, a two-proton doublet at δ 7.39 (as d, 2H, Ar-H, J = 7.8 Hz) for Ar-H, a two-proton quartet at δ 4.32 (as t, 2H, J = 7.2 Hz) for –CH_2, a two-proton triplet at δ 1.34 and a three-proton singlet at δ 3.39 for –NCH_3 group in the molecule. This assignment is in complete agreement with the structure of the compound (**14**).

Further support for the structure of compound (**14**) was achieved by ^{13}C NMR spectrum (**Fig. No. 46**), which showed the signals at δ_c 162.36 (C=O), 142.57, 141.11, 131.76, 129.53, 128.56, 128.21, 121.66, 116.61, 100.73, 60.29, 40.39, 39.26, 29.68 and 14.37 respectively.

So, the ^{13}C NMR spectrum indicated the presence of eight carbons in the molecule corresponding to the molecular formula $C_{15}H_{14}N_2O_2$, thereby suggesting the formation of a compound (**14**).

Ethyl-5-trimethylsilylethnyl-1-methylpyrazol-4-carboxylate (15):

The compound **15** was reddish solid, mp.80-82^{0}C and R_f value 0.32 (n-hexane : chloroform, 1:1).

In the UV spectrum (**Fig. No. 47**) the λ_{max} value were found in the range of 263, 220 and 204nm. The IR spectrum (**Fig. No. 48**) of this compound showed absorption bands at 2958.6 cm^{-1} (C-H str.), 2130.5 cm^{-1} (C≡C str.) and 1710.7 cm^{-1} (C=O str.) respectively.

The ^{1}H NMR spectrum (**Fig. No. 49 & 50**) showed a one-proton singlet at δ 7.94 for –CH, a two-proton quartet at δ 4.28 (as q, 2H, CH_2, J = 7.1Hz) for –CH_2, a three-proton triplet at δ 1.33 (as t, 3H, CH_3, J = 7.1Hz) for -CH_3, a three-proton singlet at δ 3.97 for –NCH_3 group and a nine-proton singlet at δ 0.96 for –CMe_3 group, which indicated the formation of (**15**). Further the structure was confirmed by ^{13}C NMR spectrum.

Ethyl-5-propynylol-1-methylpyrazol-4-carboxylate (16):

The compound **16** was reddish solid, mp. 76-77^{0}C and R_f value 0.26 (n-hexane : chloroform, 1:1).

In its UV spectrum (**Fig. No. 51**) the λ_{max} value were found in the range of 229 nm. The IR spectrum (**Fig. No. 52**) of this compound showed the following characteristic peaks: 3301.50cm^{-1} (-OH str.), 2140.5 cm^{-1} (C≡C str.), 1708.8 cm^{-1} (C=O) and 1382 cm^{-1}(-CN str.) respectively.

Its ^{1}H NMR spectrum (**Fig. No. 53 & 54**) exhibited a one-proton singlet at δ 7.94 for –CH, a two-proton quartet at δ 4.30 (as q, 2H, CH_2, J = 7.1Hz) for methylene (-CH_2) group, a one-proton singlet at δ 3.90 for –OH group and a three-proton

triplet at δ 1.35 (t, 3H, CH_2, *J*=7.1Hz) for methyl (–CH_3) groups respectively. This assignment is in complete agreement with the structure of the compound (**16**).

Further support for the structure of compound (**16**) was achieved by ^{13}C NMR spectrum, which showed the signals at δ 165.20 (C=O), 142.57, 140.11, 131.76, 129.53, 120.35, 100.73, 65.29, 40.39, 39.26, 29.68 and 14.37 respectively. So, the ^{13}C NMR spectrum indicated the presence of ten carbons in the molecule corresponding to the molecular formula $C_{10}H_{12}N_2O_3$, thereby suggesting the formation of a compound (**16**).

Ethyl-5-hexyane-1-methylpyrazol-4-carboylate (17):

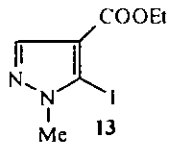
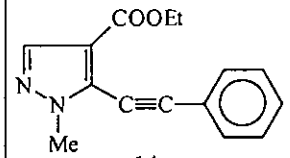
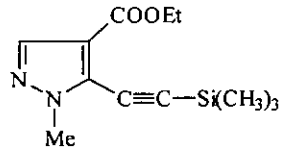
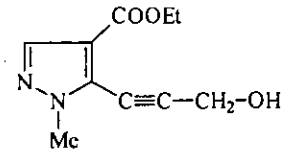
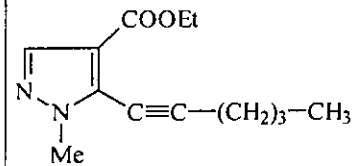
The compound **17** was afforded as a gum and R_f value 0.26 (n-hexane : chloroform, 1:1).

In the UV spectrum, the λ_{max} value were found in the range of 260, 245 and 218nm. The IR spectrum of this compound (**10**) showed absorption bands at 2948.5cm^{-1}, 2140..0, 1710.8cm^{-1} and 1382cm^{-1}corresponding to –CH, -C≡C-, –CO and –CN stretching respectively.

In its ^1H NMR spectrum showed a one-proton singlet at δ 7.80 for –CH, a two-proton quartet at δ 4.25 for methelene (-CH_2) group, a three-proton singlet at δ 3.63 for –NCH_3 group, a four-proton multiplet at δ 1.30 for (–CH_2)$_2$ group and a two-proton triplet at δ 2.05 and δ 1.64 were observed for two methelene (-CH_2) groups in the molecule.

The ^{13}C NMR spectrum also displayed the presence of thirteen number of carbon atoms corresponding to its molecular formula ($C_{13}H_{18}N_2O_3$) which was in confirm with the structure assigned for (**17**).

1.3.2.C. Comparison of some spectral data of 5-alkynyl pyrazole derivatives:

Compound	IR (cm^{-1})	^1H NMR (δ_H)	^{13}C NMR (δ_C)	UV nm
 13	3103.3 (γ_{CH}) 1701.1 (γ_{CO}) 1382.9 (γ_{CN})	δ_H 7.98 (s, 1H, CH), 4.32 (q, 2H, CH_2, *J*=7.1Hz), 3.97 (s, 3H, NCH_3), 1.35 (t, 3H, CH_3, *J*=7.1Hz).	δ_C 162.05 (C=O), 142.56, 118.51, 88.37, 60.36, 40.42, 14.31.	232.0 210.0
 14	3095.5 (γ_{CH}) 3016.5 (γ_{CH}) 1676.0 (γ_{CO}) 2150.6 ($\gamma_{C\equiv C}$)	δ_H 7.89 (s, 1H, CH), 7.58 (d, 2H, Ar-H), 7.39 (d, 2H, Ar-H), 4.32 (q, 2H, CH_2), 3.39 (s, 3H, NCH_3), 1.34 (t, 3H, CH_3).	δ_C 162.36 (C=O), 142.57, 141.11, 131.76, 129.53, 128.56, 128.21, 121.66, 116.61, 100.73, 60.29, 40.39, 39.26, 29.68, 14.37.	265.60
 15	2958.6 (γ_{CH}) 2931.6 (γ_{CH}) 1710.7 (γ_{CO}) 2130.5 ($\gamma_{C\equiv C}$)	δ_H 7.94 (s, 1H, CH), 4.28 (q, 2H, CH_2), 3.97 (s, 3H, NCH_3), 1.33 (t, 3H, CH_3), 0.96 (s, 9H, Me_3).	δ_C 165.20 (C=O), 142.57, 140.11, 131.76, 129.53, 120.35, 100.73, 65.29, 40.39, 39.26, 29.68, 14.37.	263.0 220.0 204.0
 16	3301.5 (γ_{OH}) 1708.8 (γ_{CO}) 1382.9 (γ_{CN}) 2140.0 ($\gamma_{C\equiv C}$)	7.94 (s, 1H, CH), 4.30 (q, 2H, CH_2), 3.96 (s, 3H, NCH_3), 3.90 (s, 1H, OH), 2.46 (d, 1H, C*Ha*), 2.25 (d, 1H, *CH_b*), 1.35 (t, 3H, CH_3).	163.24 (C=O), 142.57, 139.11, 130.86, 119.06, 115.21, 95.74, 62.47, 41.32, 23.28.	229 210
 17	3103.5 (γ_{CH}) 2948.5 (γ_{CH}) 1710.8 (γ_{CO}) 2140.6 ($\gamma_{C\equiv C}$)	7.80 (s, 1H, CH), 4.25 (q, 2H, CH_2), 3.63 (s, 3H, NCH_3), 2.35 (t, 2H, CH_2, *J*=7.5Hz), 2.05 (t, 3H, CH_3, *J*=7.6Hz), 1.64 (t, 2H, CH_2, *J*=7.4Hz), 1.30 (m, 4H, CH_2, *J*=7.5Hz).	164.27 (C=O), 149.52, 139.40, 128.70, 105.21, 82.09, 81.80, 79.36, 72.08, 67.04, 34.14, 24.58, 13.86.	260.0 245.5 218.0

1.3.3. Starting Material:

Synthesis of ethyl-5-iodo-1-methylpyrazol-4-carboxylate (13):

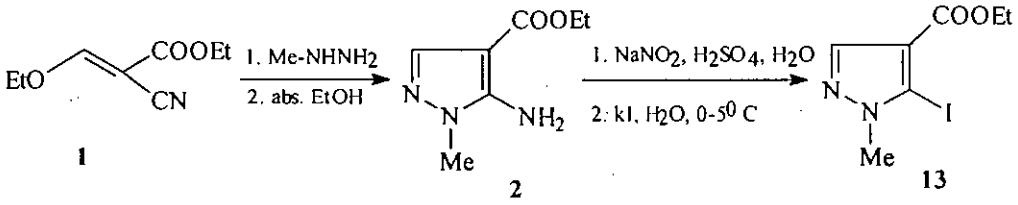
This compound ethyl-5-iodo-1-methylpyrazol-4-carboxylate (**13**) was prepared from ethyl-5-amino-1-methylpyrazol-4-carboxylate (**2**) by using diazotization of pyrazoles derivatives followed by Sandmeyer iodination procedure.

Ethyl-5-amino-1-methylpyrazol-4-carboxylate (2):

This compound (**2**) was synthesized from ethyl(ethoxymethylene)cyanoacetate (**1**) by using Gewald method[48] which was reported in previous section-2.

Ethyl-5-iodo-1-methylpyrazol-4-carboxylate (13):

Ethyl-5-iodo-1-methylpyrazol-4-carboxylate (**13**) has been used as starting materials because of their easy availability from ethyl-5-amino-1-methylpyrazol-4-carboxylate (**2**). Diazotization of pyrazole derivatives (ethyl-5-iodo-1-methyl pyrazol-4-carboxylate) followed by Sandmeyer iodination with potassium iodide furnished ethyl-5-iodo-1-methylpyrazol-4-carboxylate (**13**) as shown in **Scheme**-4.



Scheme-4

Ethyl-5-amino-1-methylpyrazol-4-carboxylate (**13**) was dissolved in distilled water containing of conc. sulphuric acid in a flask. The mixture was cooled in a freezing point (0-5^{0}C) and was stirred mechanically. A solution of sodium nitrate in water was added to mixture. The filtrate was poured into an ice cold solution of potassium iodide in water with stirring. Then the mixture was heated to boiling for 10 minutes and cooled. The precipitated product collected by filtration and

recrystallized from ethanol to give (**13**) as a yellow solid. The yield was 0.88g (86.77%); mp. 75-77^{0}C.

The products were characterized by its UV, IR, ^{1}H NMR, and ^{13}C NMR spectroscopy. In the UV spectrum (**Figure No. 35**), the peaks were found in the range of 232.00 and 210 nm.

IR spectrum (**Figure No. 36**) of this compound (**13**) showed absorption bands at 3450cm-1 and 3245cm-1, which confirmed the presence of $-NH_2$ group and at 1680cm-1 for C=O group stretching in the molecule.

In its ^{1}H NMR spectrum (**Fig. No. 37**) showed one-proton singlet at δ 7.98 indicated the presence of –CH proton, three-proton quartet at δ 4.32 for $-CH_2$, three-proton singlet at δ 3.97 for –NMe group and three-proton triplet at δ 1.35 for $-CH_3$ in the molecule.

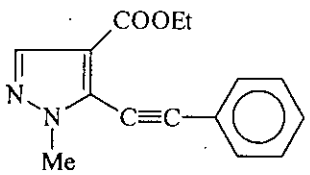
The ^{13}C NMR spectrum (**Fig. No. 40**), which showed signals at δ 162.05 (C=O), 142.56, 118.51, 88.37, 60.36, 40.42 and 14.31 respectively. The presence of seven number of carbon atoms was in good agreement with structure assigned for (**13**).

1.3.4. CONCLUSION:

We have demonstrated a convenient and facile method for the synthesis of 5-substituted alkynyl pyrazol derivatives from the reaction of ethyl-5-iodo-1-methylpyrazol-4-carboxylate (**13**) with terminyl alkynes by a $(PPh_3)_2PdCl_2$-CuI-Et_3N system. We have used THF as a solvent in the palladium catalyzed reaction. The most important features of the synthesis are that readily available, inexpensive starting materials are used relatively in mild reaction conditions and relatively good yields were obtained. Also, no toxic and hazardous compounds are produced by this procedure. This reaction is highly regio-selective in case of palladium-catalyzed reactions. A variety of functional groups could be introduced at the 5-

3. Synthesis of ethyl-5-phenylethnyl-1-methylpyrazol-4-carboxylate (14).

A mixture of ethyl-5-iodo-1-methylpyrazole-4-carboxylate (**13**) (0.1gm, 0.3573 mmol), bis(triphenylphosphine)palladium (II) chloride (0.0088gm, 3.5% mol), copper (I) iodide (0.0054, 8% mol) and triethyl amine (0.144gm, 4eqv.) was stirred in THF (4ml) under nitrogen atmosphere for 1 hrs. Then phenylacetylene (0.1094gm, 3eqv.) was added dropwise to the reaction mixture, stirring continued for 24 hrs. at 80-85^{0}C. The progress of the reaction was monitored by TLC (n-hexane : chloroform, 1:1, v/v). After completion of the reaction, the mixture was evaporated to dryness under reduced pressure and the residue was extracted with chloroform (3×50ml). The combined chloroform extract was washed with distilled water (3×50ml), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to obtain reddish solid. The latter was purified by chromatography on a column of silica gel (60-120 mesh) with n-hexane : chloroform (1:1). The n-hexane fraction afforded dimer of phenyl acetylene and n-hexane : chloroform fraction yielded the compound (**13**). The compound (**13**) was recrystallized from n-hexane : chloroform to obtain a reddish solid (0.6gm, 60.12%); mp. 71-72^{0}C.



14

M.F.: $C_{15}H_{14}N_2O_2$

UV (EtOH): 265.60 nm.

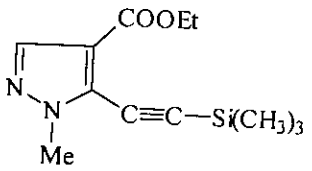
IR(KBr): ν_{max} 3095.5, 3016.5, 2896.9, 1676.0, 1598.9, 1396.4 and 927.7cm-1

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.89 (s, 1H, CH), 7.58 (d, 2H, Ar-H, J = 7.8 Hz) 7.39 (d, 2H, Ar-H, J = 7.8Hz), 4.32 (q, 2H, CH_2, J = 7.1Hz), 3.39 (s, 3H, NCH_3), 1.34 (t, 3H, CH_3, J = 7.1Hz).

^{13}C NMR (100 MHz, $CDCl_3$): δ_C 162.36 (C=O), 142.57, 141.11, 131.76, 129.53, 128.56, 128.21, 121.66, 116.61, 100.73, 60.29, 40.39, 39.26, 29.68, 14.37.

4. Synthesis of ethyl-5-trimethylsilylethnyl-1-methylpyrazol-4-carboxylate (15).

The title compound (**15**) was synthesized from ethyl-5-iodo-1-methylpyrazol-4-carboxylate (**13**) (0.1gm, 0.3573mmol), bis(triphenylphosphine)palladium(II) chloride (0.0088gm, 3.5% mol), copper (I) iodide (0.0055, 8% mol) and triethyl amine (1.44gm, 4 eqv.) and trimethylsilylacetylene (0.0778gm, 0.7126mmol) in THF (4ml) by following the procedure described above for the compound (**14**).After column chromatography, the compound was afforded as a gum. It was crystallized from n-hexane-ethyl acetate to furnish yellow solid (**14**) (0.084gm, 80.34%), mp. 80-82^{0}C.



15

M.F.: $C_{12}H_{18}N_2O_2Si$

UV (EtOH): 275.0 nm.

IR(KBr): ν_{max} 2958.6, 2931.6, 1710.7 and 1218.9 cm^{-1}.

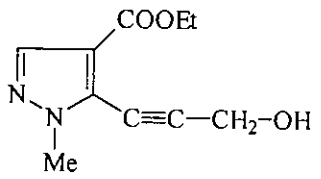
^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 7.94 (s, 1H, CH), 4.28 (q, 2H, CH_2, J = 7.1Hz), 3.97 (s, 3H, NCH_3), 1.33 (t, 3H, CH_3, J = 7.1Hz), 0.96 (s, 9H, Me_3).

^{13}C-NMR (100 MHz, $CDCl_3$): δ_C 165.20 (C=O), 142.57, 140.11, 131.76, 129.53, 120.35, 100.73, 65.29, 40.39, 39.26, 29.68, 14.37.

5. Synthesis of ethyl-5-propynylol-1-methylpyrazol-4-carboxylate (16).

Bis (triphenylphosphine) palladium (II) chloride (0.0088gm, 3.5% mol), copper (I) iodide (0.0055, 8% mol) and triethylamine (0.145gm, 4 eqv.) were added to a solution of ethyl-5-iodo-1-methylpyrazol-4-carboxylate (**13**) (0.1gm, 0.36mmol) in THF (4ml). The mixture was stirred for 1 hrs. under nitrogen atmosphere at

room temperature. Then propargyl alcohol (0.025gm, 1.2 eqv.) was added drop wise to the mixture, stirring was continued for 24 hrs. at room temperature. After usual work up and column chromatography, the compound was obtained as a yellowish gum. It was crystallized from n-hexane-chloroform to afford a yellowish solid (**16**) (0.071gm, 73.21%), mp. 76-77^{0}C.



16

M.F.: $C_{10}H_{12}N_2O_3$

UV (EtOH): 229 nm

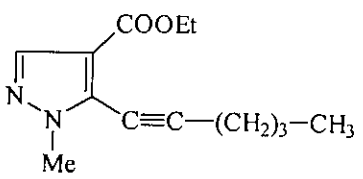
IR(KBr): ν_{max} 3301.50, 1708.8, 1514.0, 1382.9, 1220.9 and 1031.8cm^{-1}.

^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 7.94 (s, 1H, CH), 4.30 (q, 2H, CH_2, J = 7.1Hz), 3.96 (s, 3H, NCH_3), 3.90 (s, 1H, OH), 2.46 (d, 1H, CH*a*, J = 8.2Hz), 2.25 (d, 1H, CH_b, J = 8.2Hz), 1.35 (t, 3H, CH_3, J=7.1Hz).

^{13}C-NMR (100 MHz, $CDCl_3$): δ_C 163.24 (C=O), 142.57, 139.11, 130.86, 119.06, 115.21, 95.74, 62.47, 41.32, 23.28.

6. Synthesis of ethyl-5-hexyane-1-methylpyrazol-4-carboylate (17):

The title compound (**17**) was synthesized from ethyl-5-iodo-1-methylpyrazol-4-carboxylate (**13**) (0.15gm, 0.54mmol), bis(triphenylphosphine)palladium(II) chloride (0.0133gm, 3.5% mol), copper (I) iodide (0.0088, 8% mol) and triethyl amine (0.22gm, 4 eqv.) and 1-hexyne (0.023gm, 0.186mmol) in THF (4ml) by following the procedure described above for the compound (**17**). After column chromatography the compound was afforded as a gum, (0.088gm, 64.31%).



17

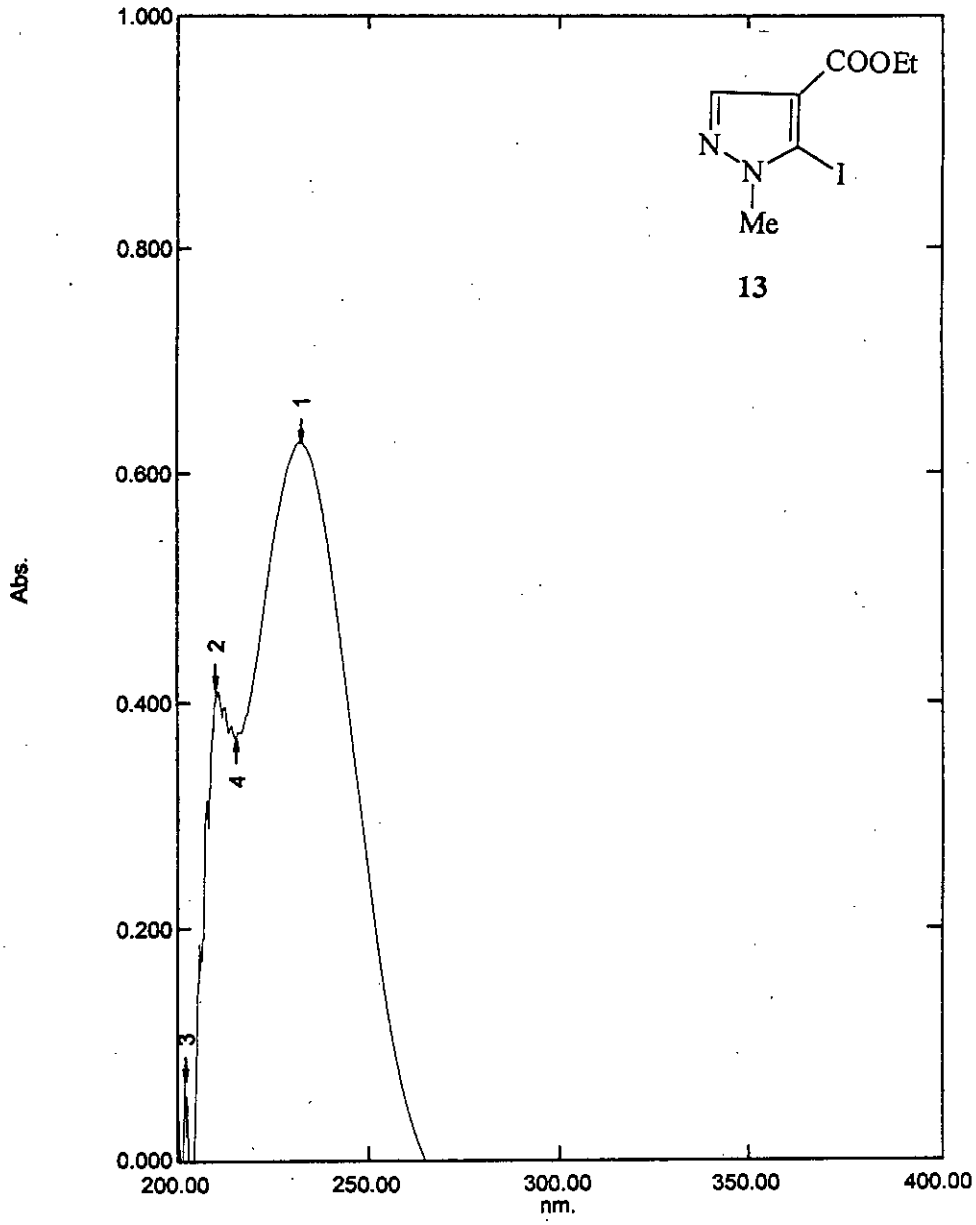
M.F.: $C_{13}H_{18}N_2O_3$

UV (EtOH): 260.0, 245.5 and 218nm

IR(KBr): ν_{max} 3103.5, 2948.5, 1710.8 and 1382cm^{-1}.

^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 7.80 (s, 1H, CH), 4.25 (q, 2H, CH_2), 3.63 (s, 3H, NCH_3), 2.35 (t, 2H, CH_2, J = 7.5Hz), 2.05 (t, 2H. CH_2, J = 7.6Hz), 1.64 (m, 4H, CH_2, J = 7.4Hz), 1.30 (t, 2H, CH_2, J = 7.5Hz).

^{13}C-NMR (100 MHz, $CDCl_3$): δ_C 164.27 (C=O), 149.52, 139.40, 128.70, 105.21, 82.09, 81.80, 79.36, 72.08, 67.04, 34.14, 24.58, 13.86.



Wavelength	Abs.
232	0.628
210	0.413

Fig. 35: UV Spectrum of Compound 13

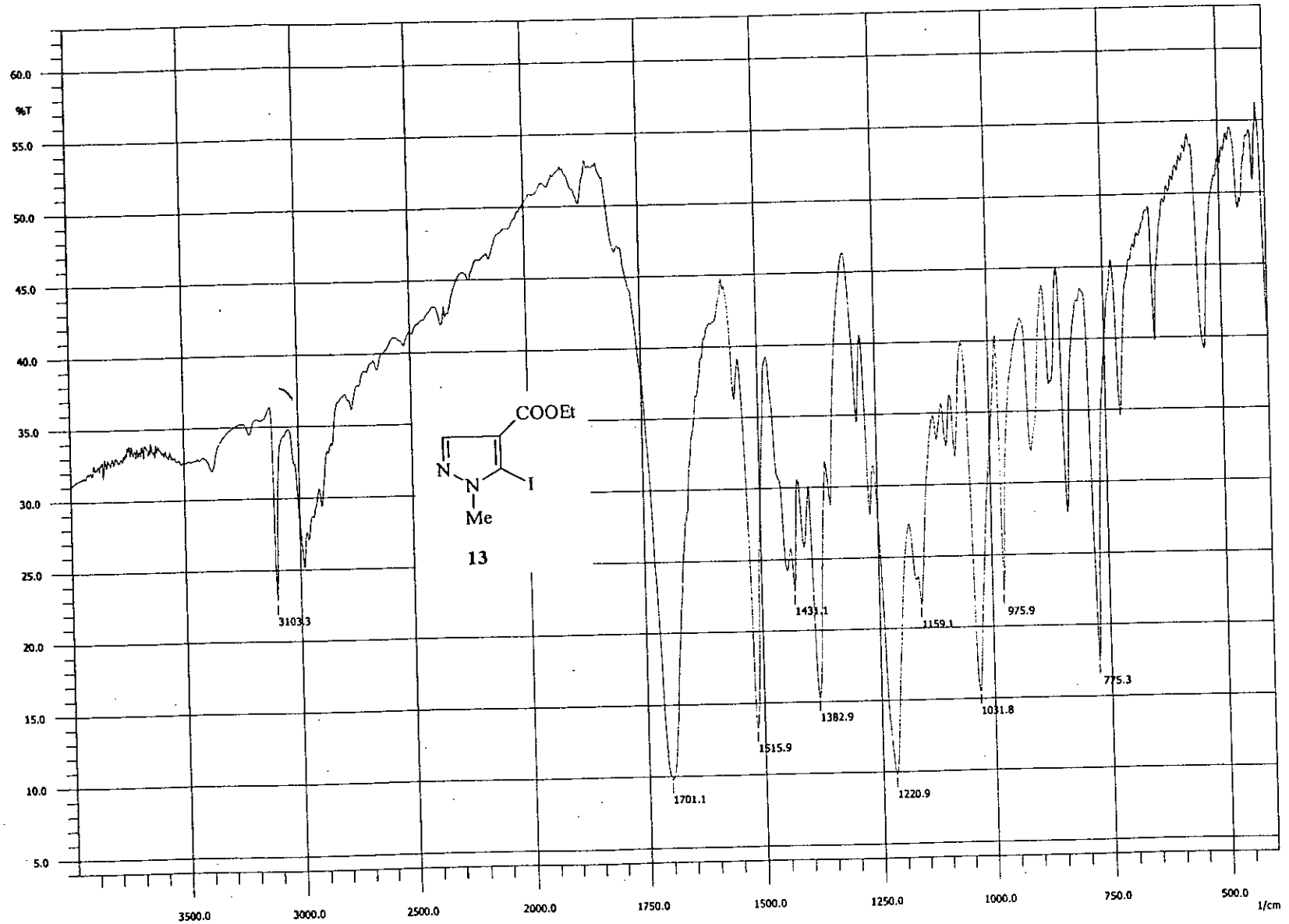
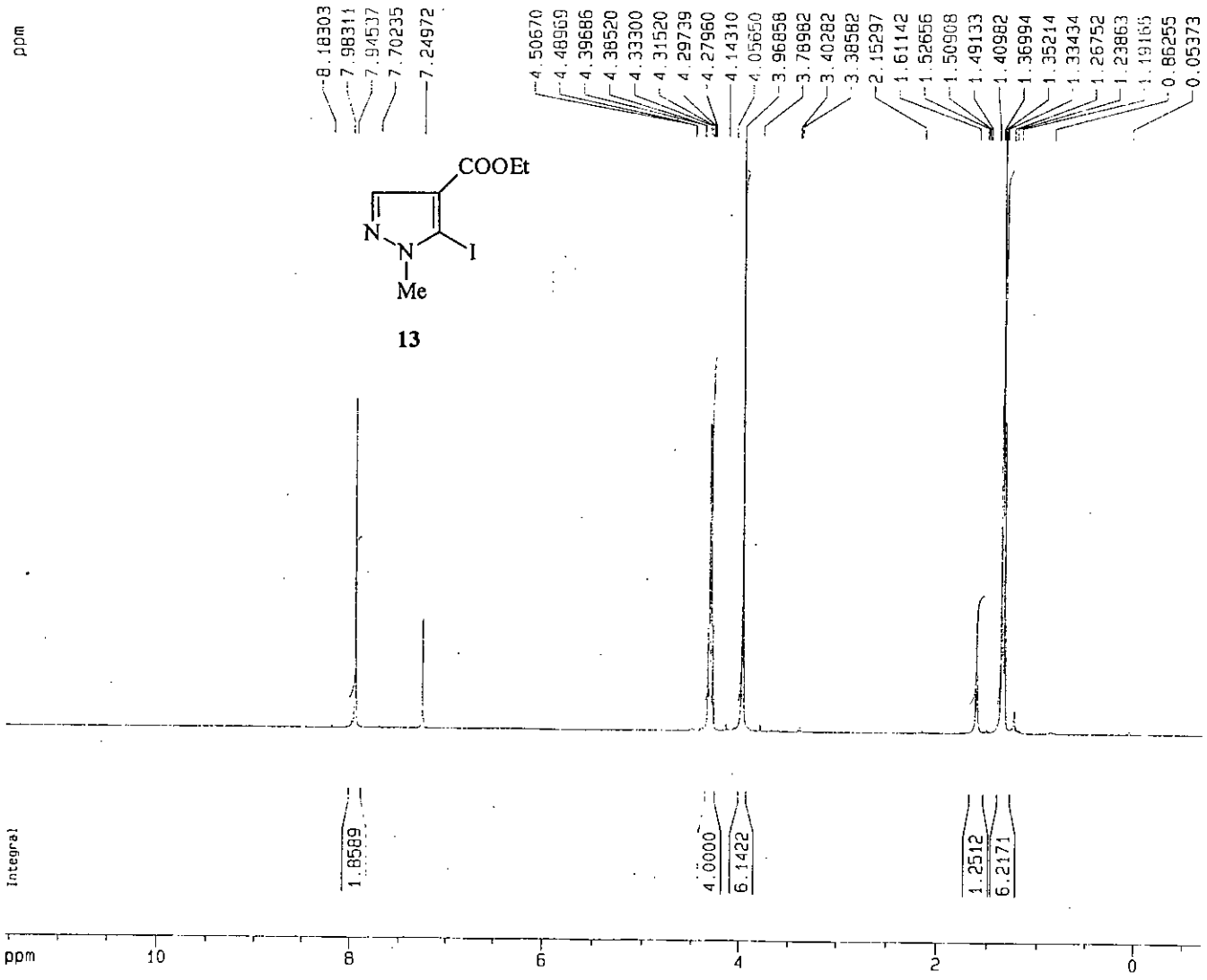


Fig. 36: IR Spectrum of Compound **13**



13

Fig. 37: ^{1}H NMR Spectrum of Compound **13**

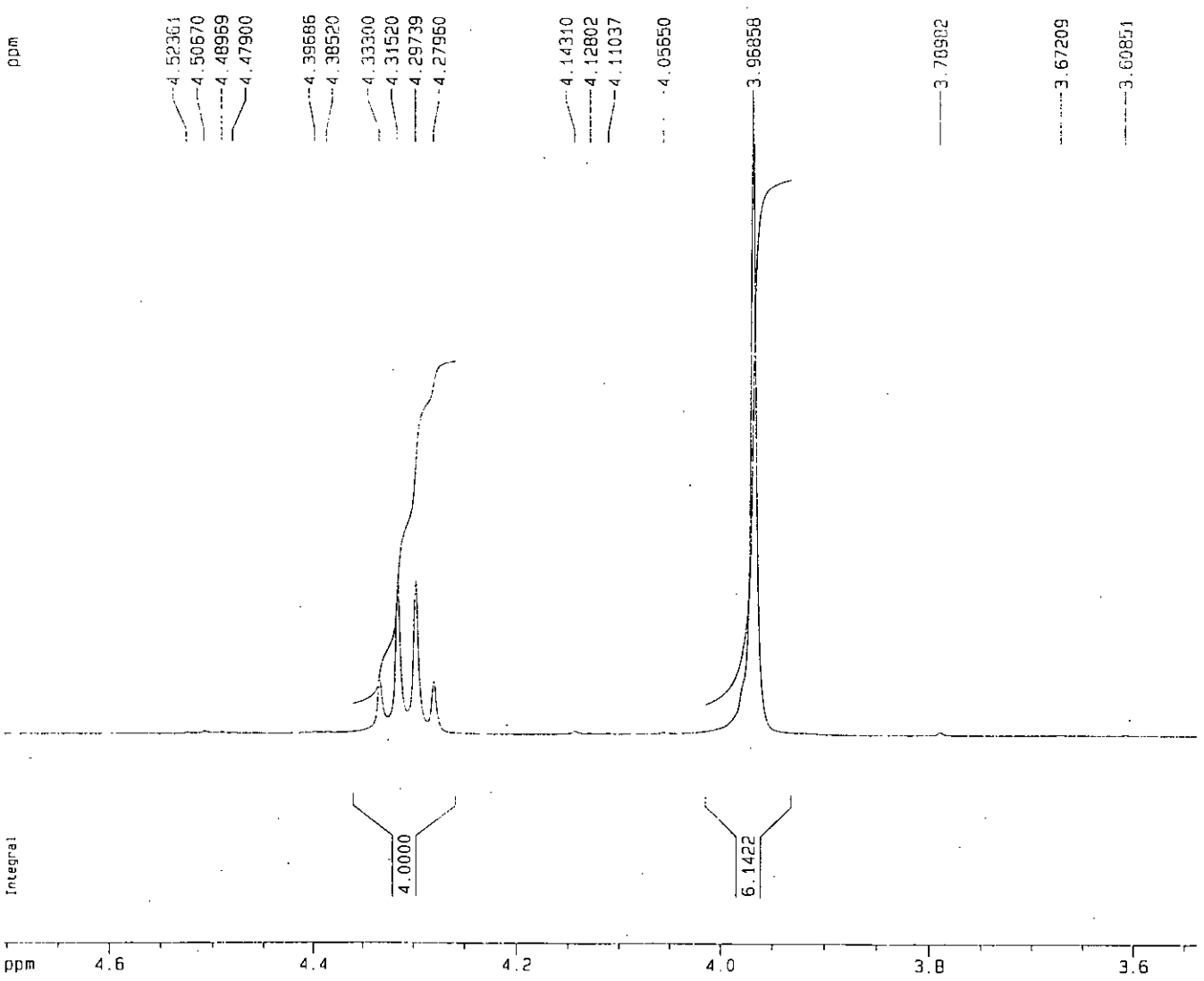
```
Current Data Parameters
NAME                  A1940
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_              20050704
Time                 12.15
INSTRUM              dpx400
PROBHD     5 mm Multinuc
PULPROG              zg30
TD                  32768
SOLVENT             CDCl3
NS                     128
DS                       2
SWH             6410.256 Hz
FIDRES          0.195625 Hz
AQ              2.5559540 sec
RG                   322.5
DW                78.000 usec
DE                 6.00 usec
TE                 310.0 K
D1              1.00000000 sec

============ CHANNEL f1 =============
NUC1                  1H
P1                 8.30 usec
PL1                -5.00 dB
SFO1          400.1428010 MHz

F2 - Processing parameters
SI                  32768
SF            400.1400126 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.40

1D NMR plot parameters
CX                 20.00 cm
F1P                11.539 ppm
F1                 4617.37 Hz
F2P                -0.696 ppm
F2                 -278.49 Hz
PPMCM           0.61177 ppm/cm
HZCM           244.79297 Hz/cm
```



Current Data Parameters

NAME	A1940
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20050704
Time	12.16
INSTRUM	dpx400
PROBHD	5 mm Multinuc
PULPROG	zg30
TD	32768
SOLVENT	CDCl3
NS	128
DS	2
SWH	6410.256 Hz
FIORES	0.195625 Hz
AQ	2.5559540 sec
RG	322.5
DW	78.000 usec
DE	5.00 usec
TE	310.0 K
D1	1.00000000 sec

========== CHANNEL f1 ==========

NUC1	1H
P1	8.30 usec
PL1	-6.00 dB
SFO1	400.1428010 MHz

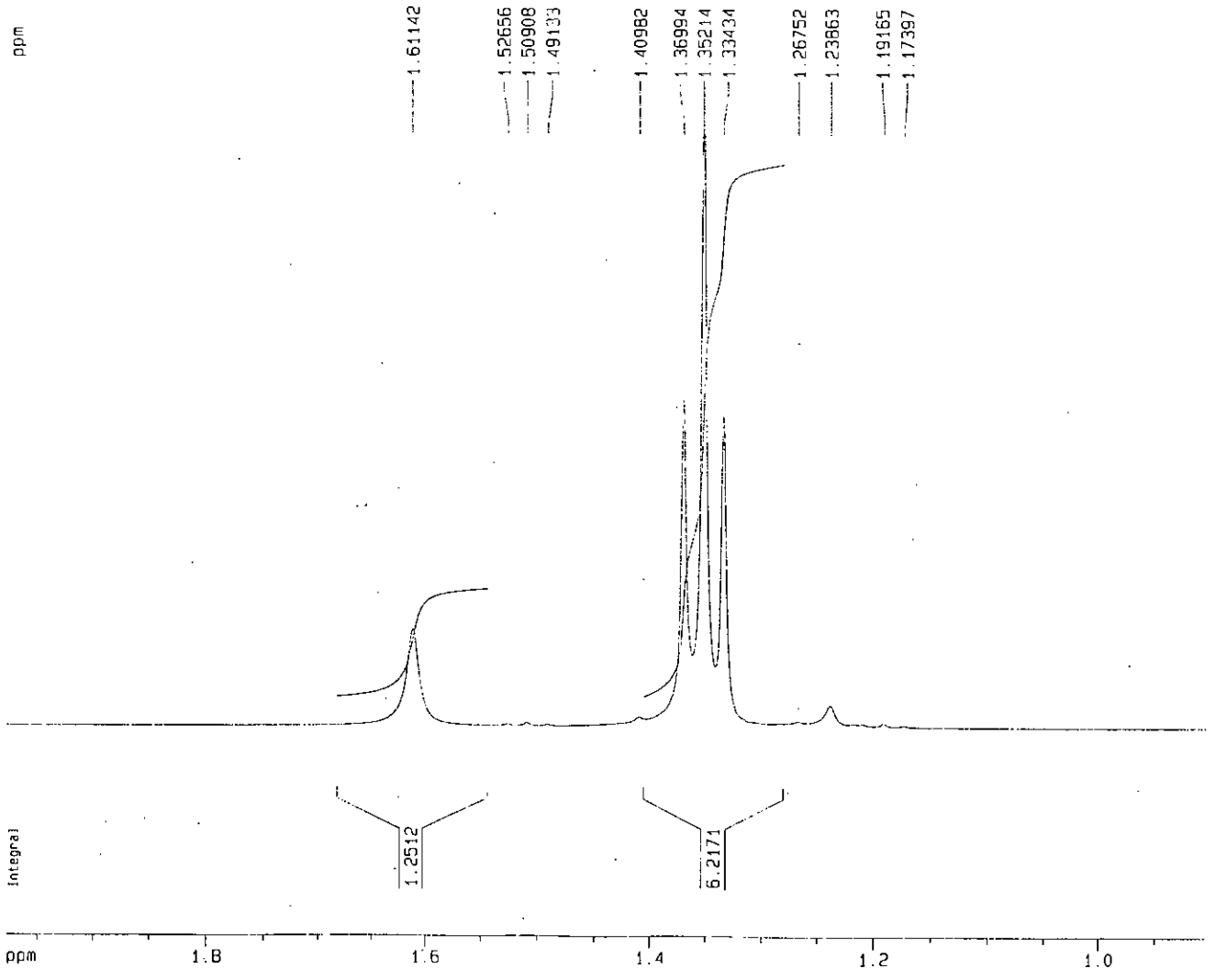
F2 - Processing parameters

SI	32768
SF	400.1400126 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.40

1D NMR plot parameters

CX	20.00 cm
F1P	4.703 ppm
F1	1881.69 Hz
F2P	3.538 ppm
F2	1415.88 Hz
PPMCM	0.05821 ppm/cm
HZCM	23.29076 Hz/cm

Fig. 38: ^{1}H NMR Spectrum of Compound **13** (Expansion)



Current Data Parameters

NAME	A1940
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20050704
Time	12.16
INSTRUM	dpx400
PROBHD	5 mm Multinuc
PULPROG	zg30
TD	32768
SOLVENT	CDCl3
NS	128
DS	2
SWH	6410.256 Hz
FIDRES	0.195625 Hz
AQ	2.5559540 sec
RG	322.5
DW	78.000 usec
DE	6.00 usec
TE	310.0 K
D1	1.00000000 sec

========== CHANNEL f1 ==========

NUC1	1H
P1	8.30 usec
PL1	-6.00 dB
SFO1	400.1428010 MHz

F2 - Processing parameters

SI	32768
SF	400.1400126 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.40

1D NMR plot parameters

CX	20.00 cm
F1P	1.978 ppm
F1	791.55 Hz
F2P	0.904 ppm
F2	361.73 Hz
PPMCM	0.05371 ppm/cm
HZCM	21.49113 Hz/cm

Fig. 39: ^{1}H NMR Spectrum of Compound **13** (Expansion)

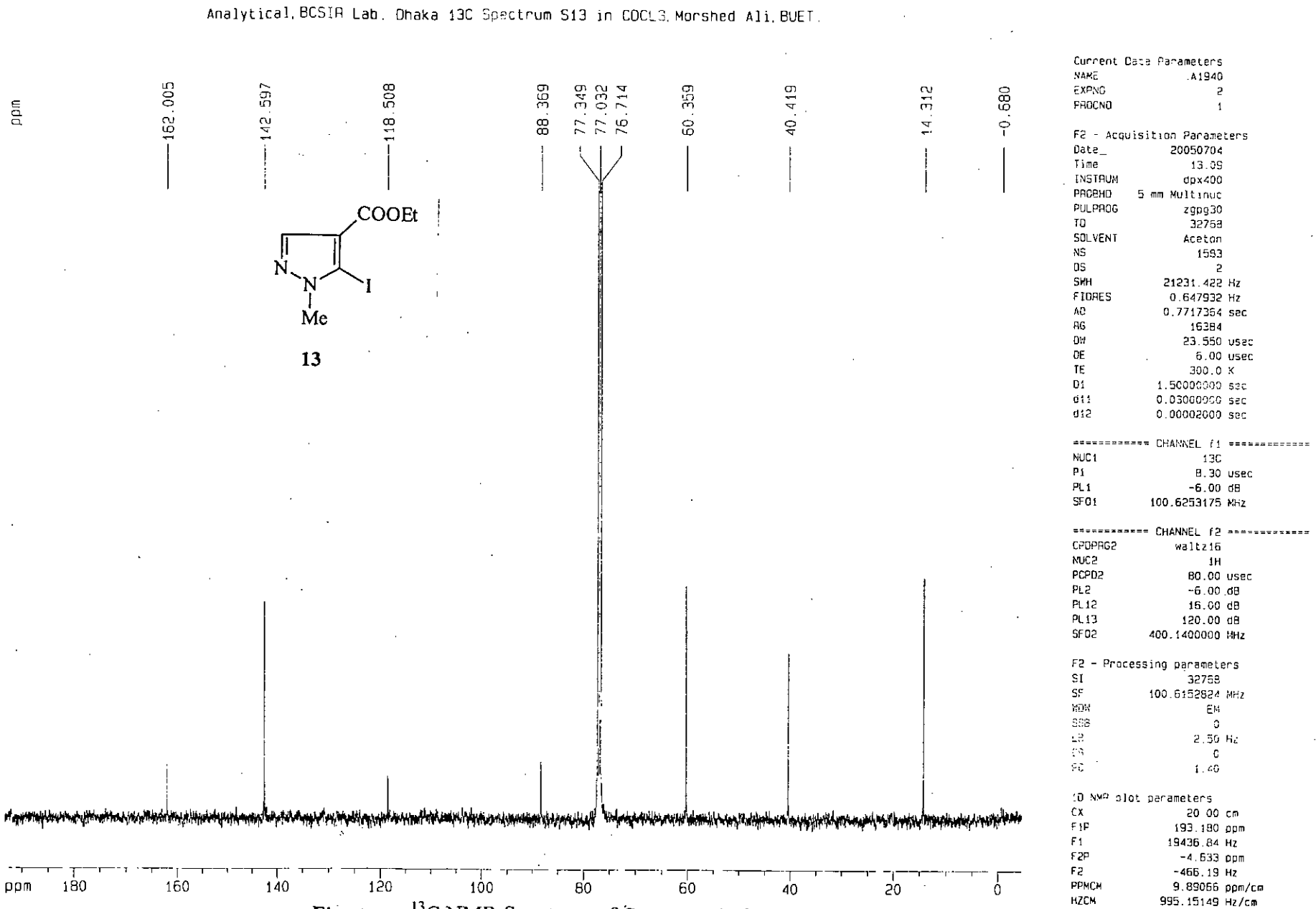


Fig. 40: ^{13}C NMR Spectrum of Compound **13**

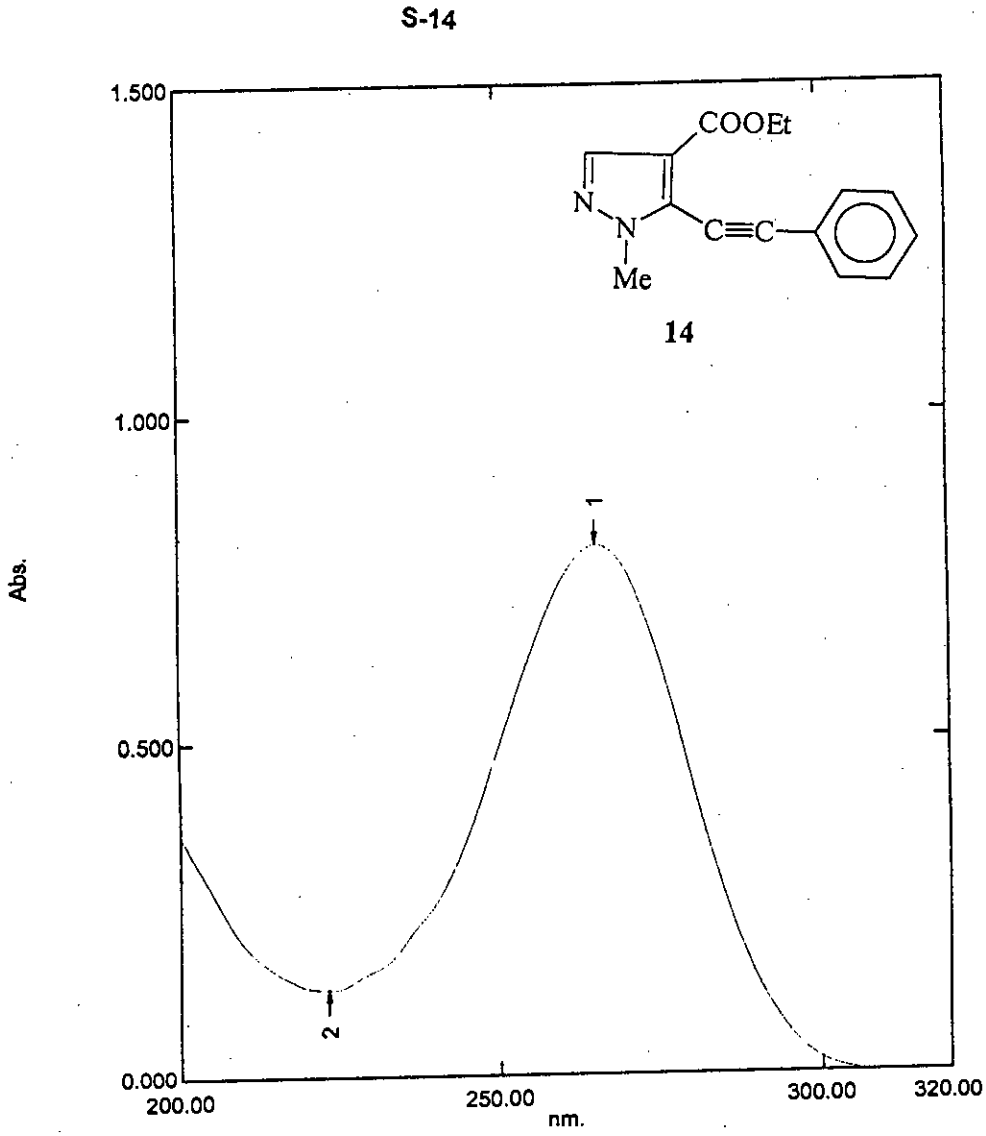


Fig. 41: UV Spectrum of Compound **14**

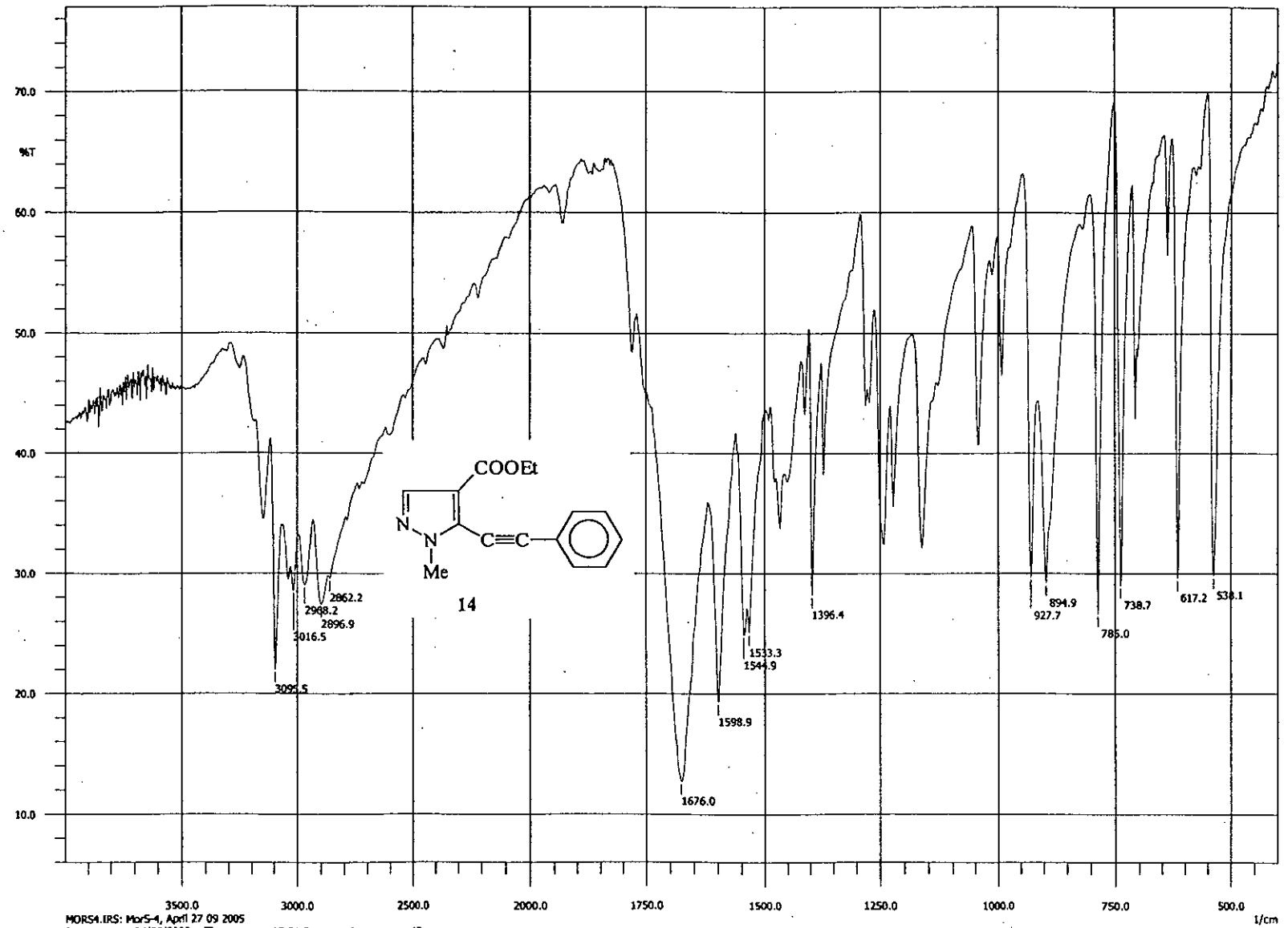


Fig. 42: IR Spectrum of Compound **14**

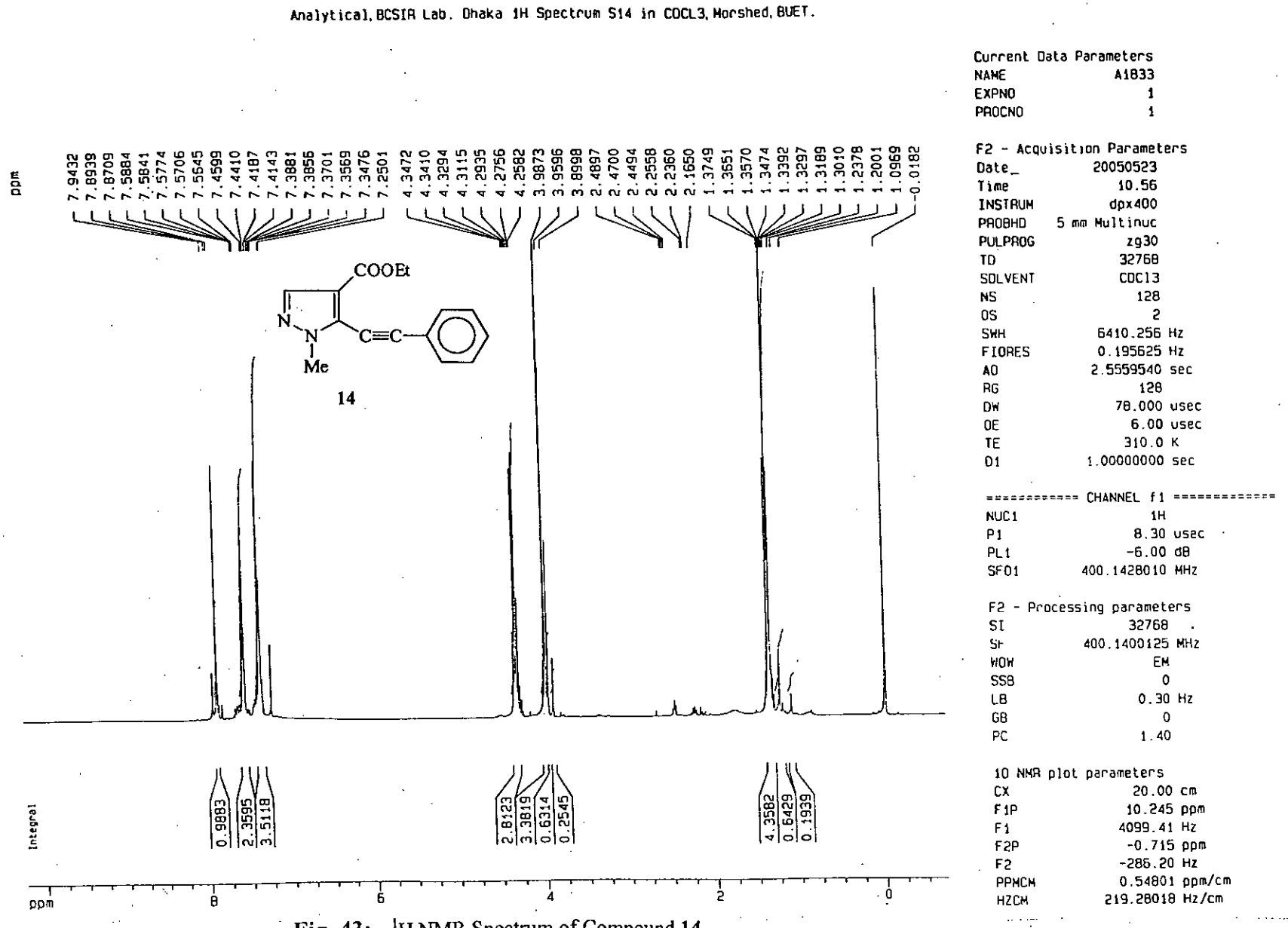


Fig. 43: ^{1}H NMR Spectrum of Compound 14

Current Data Parameters

NAME	A1833
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20050523
Time	10.56
INSTRUM	dpx400
PROBHD	5 mm Multinuc
PULPROG	zg30
TD	32768
SOLVENT	CDCl3
NS	128
DS	2
SWH	6410.256 Hz
FIDRES	0.195625 Hz
AQ	2.5559540 sec
RG	128
DW	78.000 usec
DE	6.00 usec
TE	310.0 K
D1	1.00000000 sec

========== CHANNEL f1 ==========

NUC1	1H
P1	8.30 usec
PL1	-6.00 dB
SFO1	400.1428010 MHz

F2 - Processing parameters

SI	32768
SF	400.1400125 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.40

1D NMR plot parameters

CX	20.00 cm
F1P	10.245 ppm
F1	4099.41 Hz
F2P	-0.715 ppm
F2	-286.20 Hz
PPMCM	0.54801 ppm/cm
HZCM	219.28018 Hz/cm

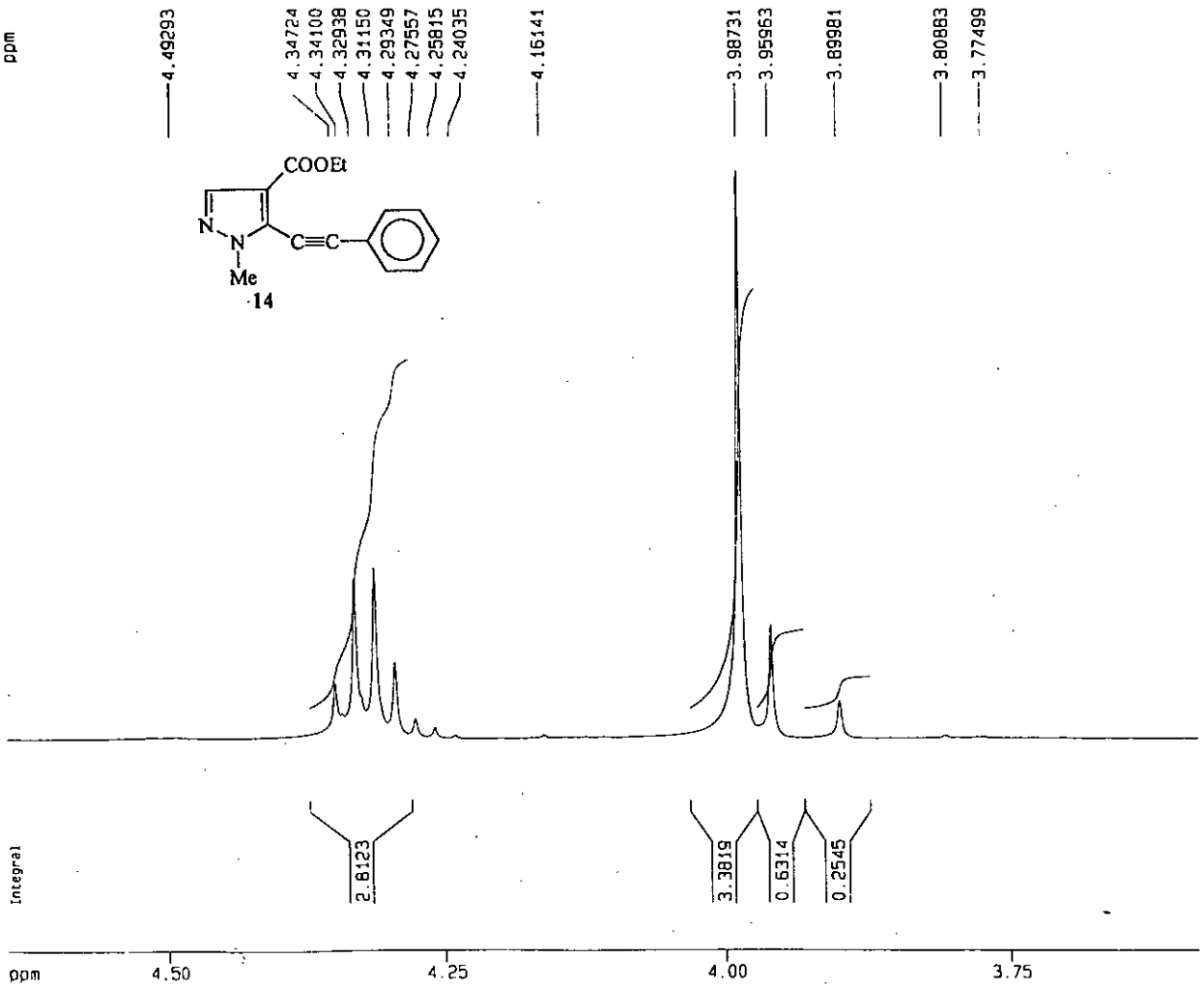


Fig. 44: ^{1}H NMR Spectrum of Compound **14** (Expansion)

```
Current Data Parameters
NAME                  A1833
EXPNO                     1
PROCNO                    1

F2 - Acquisition Parameters
Date_              20050523
Time                  10.56
INSTRUM             dpx400
PROBHD     5 mm Multinuc
PULPROG               zg30
TD                   32768
SOLVENT              CDCl3
NS                     128
DS                       2
SWH              6410.256 Hz
FIDRES           0.195625 Hz
AQ               2.5559540 sec
RG                     128
DW                78.000 usec
DE                 6.00 usec
TE                310.0 K
D1              1.00000000 sec

============ CHANNEL f1 =============
NUC1                   1H
P1                  8.30 usec
PL1                -6.00 dB
SFO1          400.1428010 MHz

F2 - Processing parameters
SI                   32768
SF            400.1400125 MHz
WDW                    EM
SSB                     0
LB                 0.30 Hz
GB                      0
PC                   1.40

1D NMR plot parameters
CX                  20.00 cm
F1P                4.643 ppm
F1                1857.66 Hz
F2P                3.582 ppm
F2                1433.46 Hz
PPMCM           0.05301 ppm/cm
HZCM           21.20988 Hz/cm
```

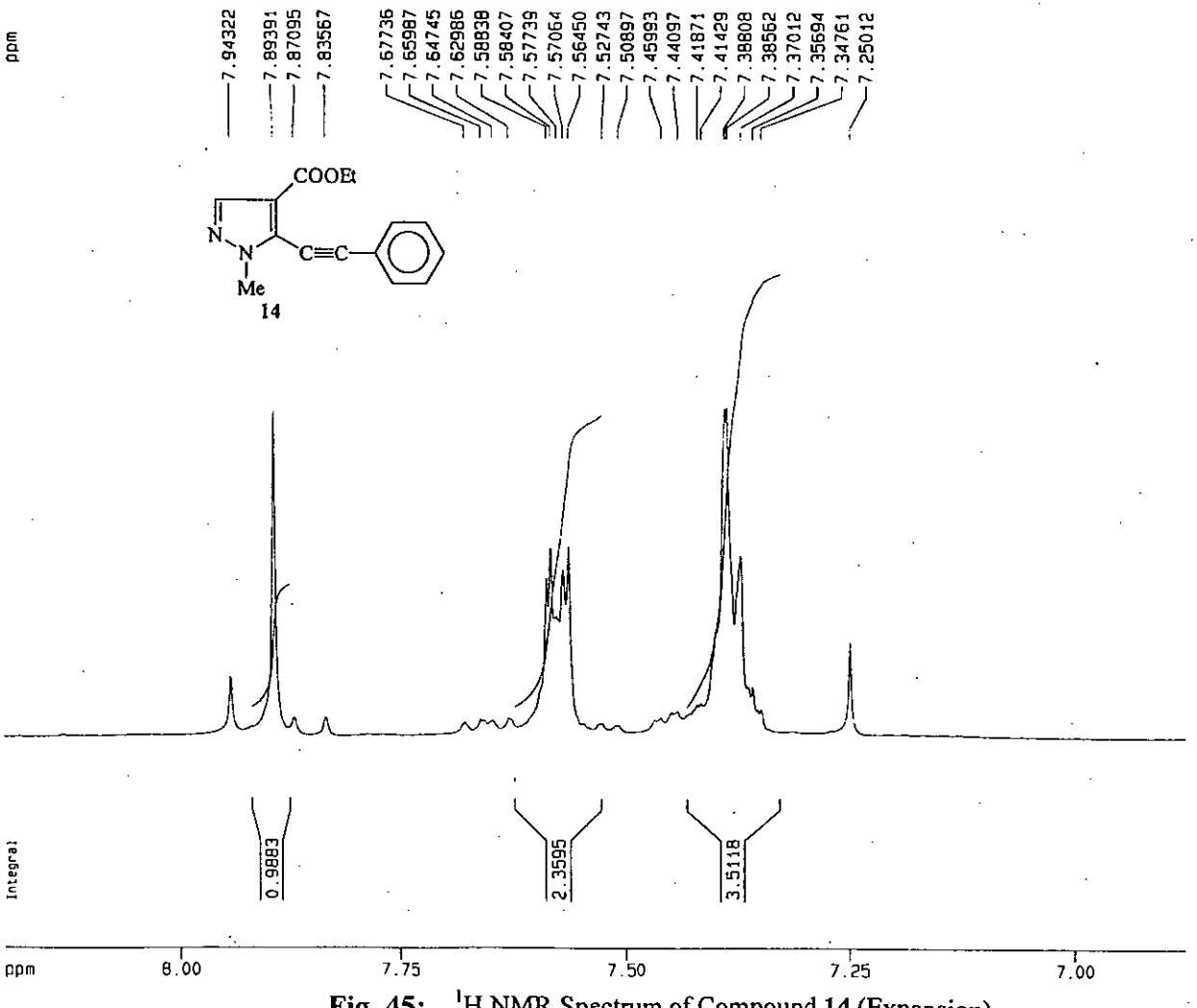
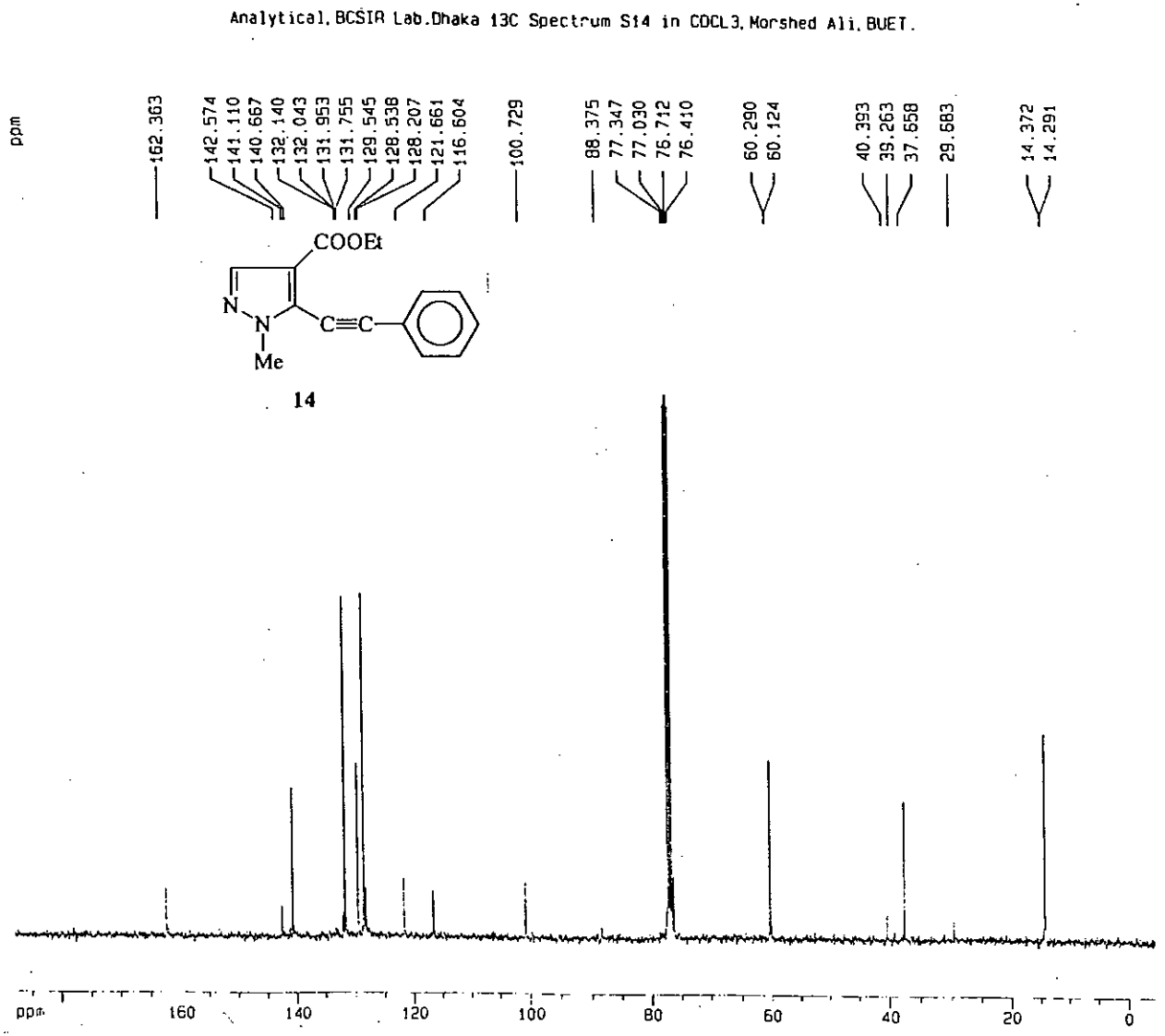


Fig. 45: ^{1}H NMR Spectrum of Compound **14** (Expansion)

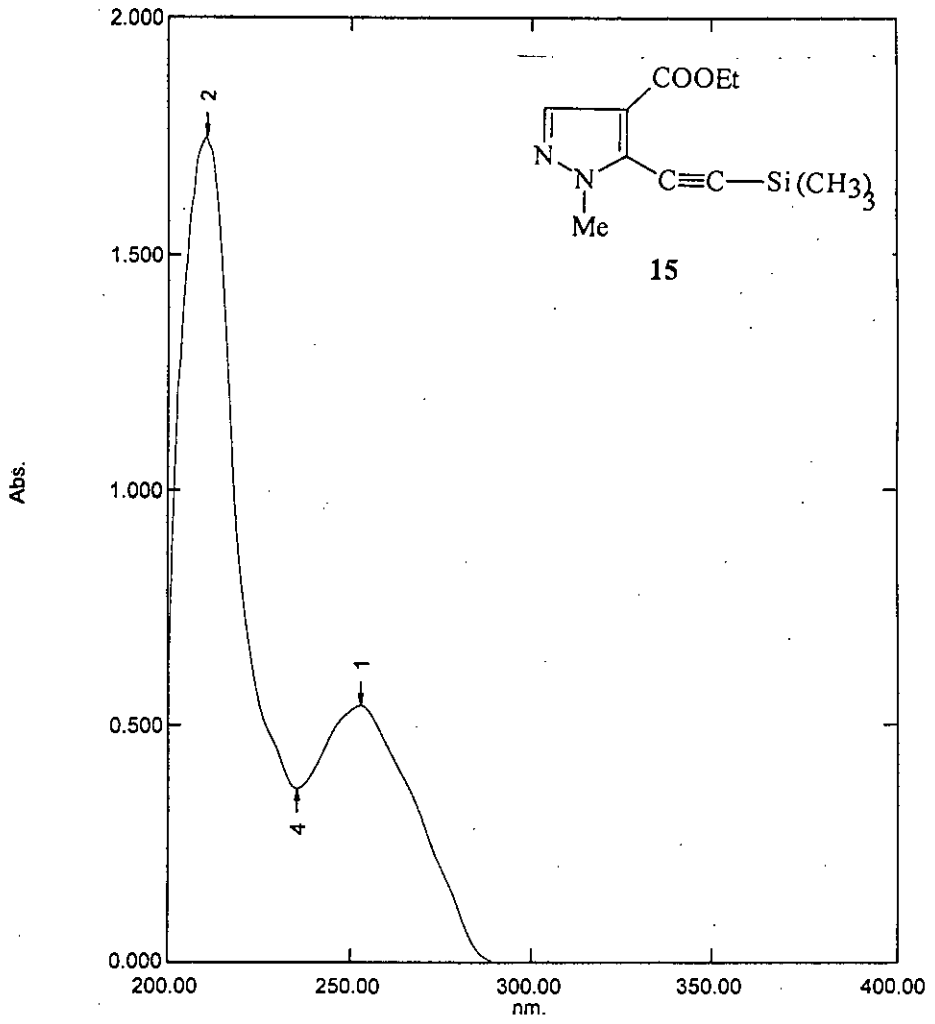
Current Data Parameters	
NAME	A1833
EXPNO	1
PROCNO	1
F2 - Acquisition Parameters	
Date_	20050523
Time	10.56
INSTRUM	dpx400
PROBHD	5 mm Multinuc
PULPROG	zg30
TD	32768
SOLVENT	CDCl3
NS	128
DS	2
SWH	6410.256 Hz
FIDRES	0.195625 Hz
AQ	2.5559540 sec
RG	128
DW	78.000 usec
DE	6.00 usec
TE	310.0 K
D1	1.00000000 sec
========== CHANNEL f1 ==========	
NUC1	1H
P1	8.30 usec
PL1	-6.00 dB
SFO1	400.1428010 MHz
F2 - Processing parameters	
SI	32768
SF	400.1400125 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.40
1D NMR plot parameters	
CX	20.00 cm
F1P	8.198 ppm
F1	3280.16 Hz
F2P	6.875 ppm
F2	2750.82 Hz
PPMCM	0.05614 ppm/cm
HZCM	26.46679 Hz/cm



14

Fig. 46: ^{13}C NMR Spectrum of Compound **14**

Current Data Parameters	
NAME	A1833
EXPNO	2
PROCNO	1
F2 - Acquisition Parameters	
Date_	20050704
Time	14.32
INSTRUM	dpx400
PROBHD	5 mm Multinuc
PULPROG	zgpg30
TD	32768
SOLVENT	Aceton
NS	1855
DS	2
SWH	21231.422 Hz
FIDRES	0.647932 Hz
AQ	0.7717354 sec
RG	16384
DW	23.550 usec
DE	6.00 usec
TE	300.0 K
D1	1.50000000 sec
d11	0.03000000 sec
d12	0.00002000 sec
========== CHANNEL f1 ==========	
NUC1	13C
P1	8.30 usec
PL1	-6.00 dB
SFO1	100.6253175 MHz
========== CHANNEL f2 ==========	
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	-6.00 dB
PL12	16.00 dB
PL13	120.00 dB
SFO2	400.1400000 MHz
F2 - Processing parameters	
SI	32758
SF	100.6152844 MHz
WDW	EM
SSB	0
LB	2.50 Hz
GB	0
PC	1.40
1D NMR plot parameters	
CX	20.00 cm
F1P	187.925 ppm
F1	18908.12 Hz
F2P	-4.425 ppm
F2	-445.23 Hz
PPMCM	9.61750 ppm/cm
HZCM	967.66742 Hz/cm



Wavelength	Abs.
253	0.541
210	1.750

Fig. 47: UV Spectrum of Compound **15**

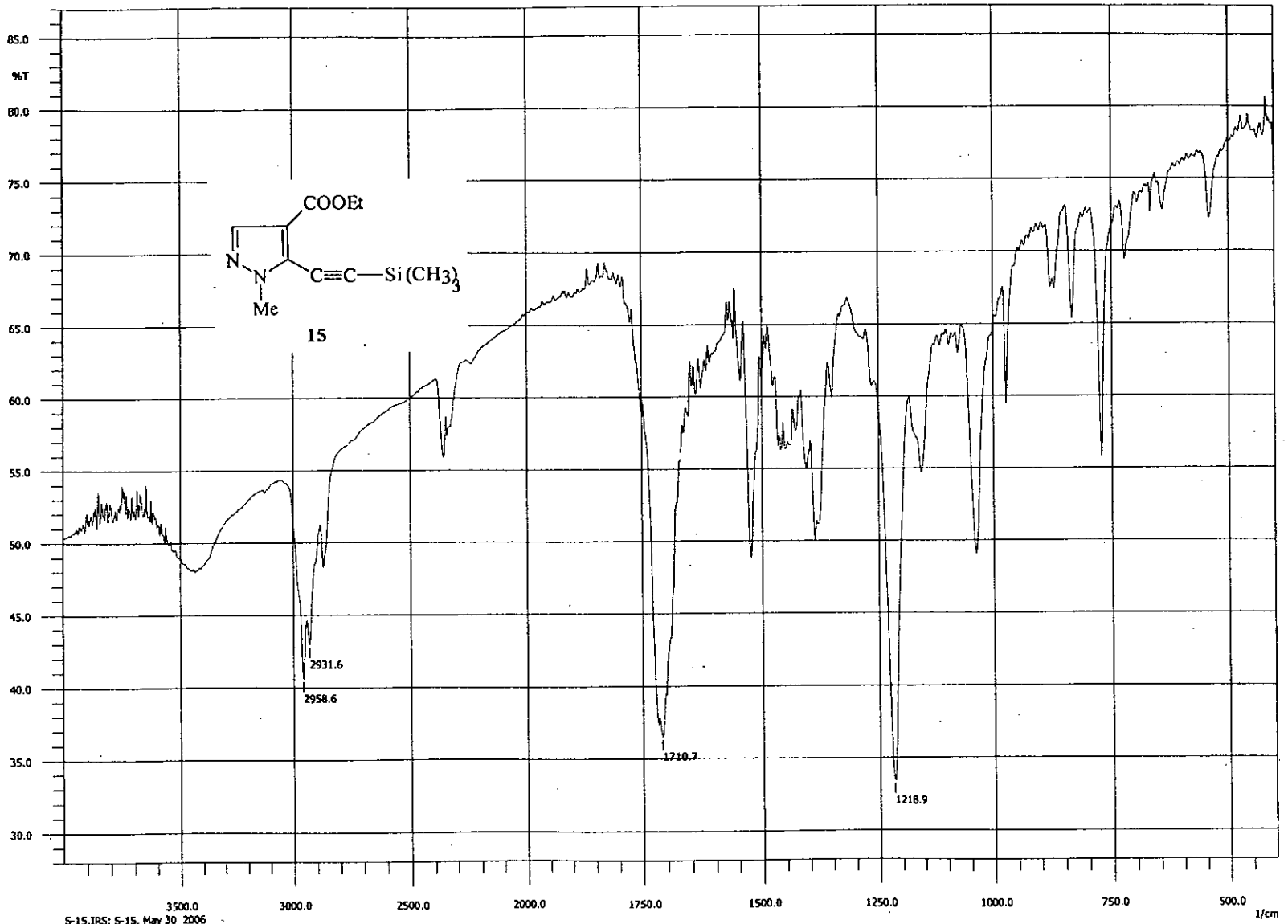
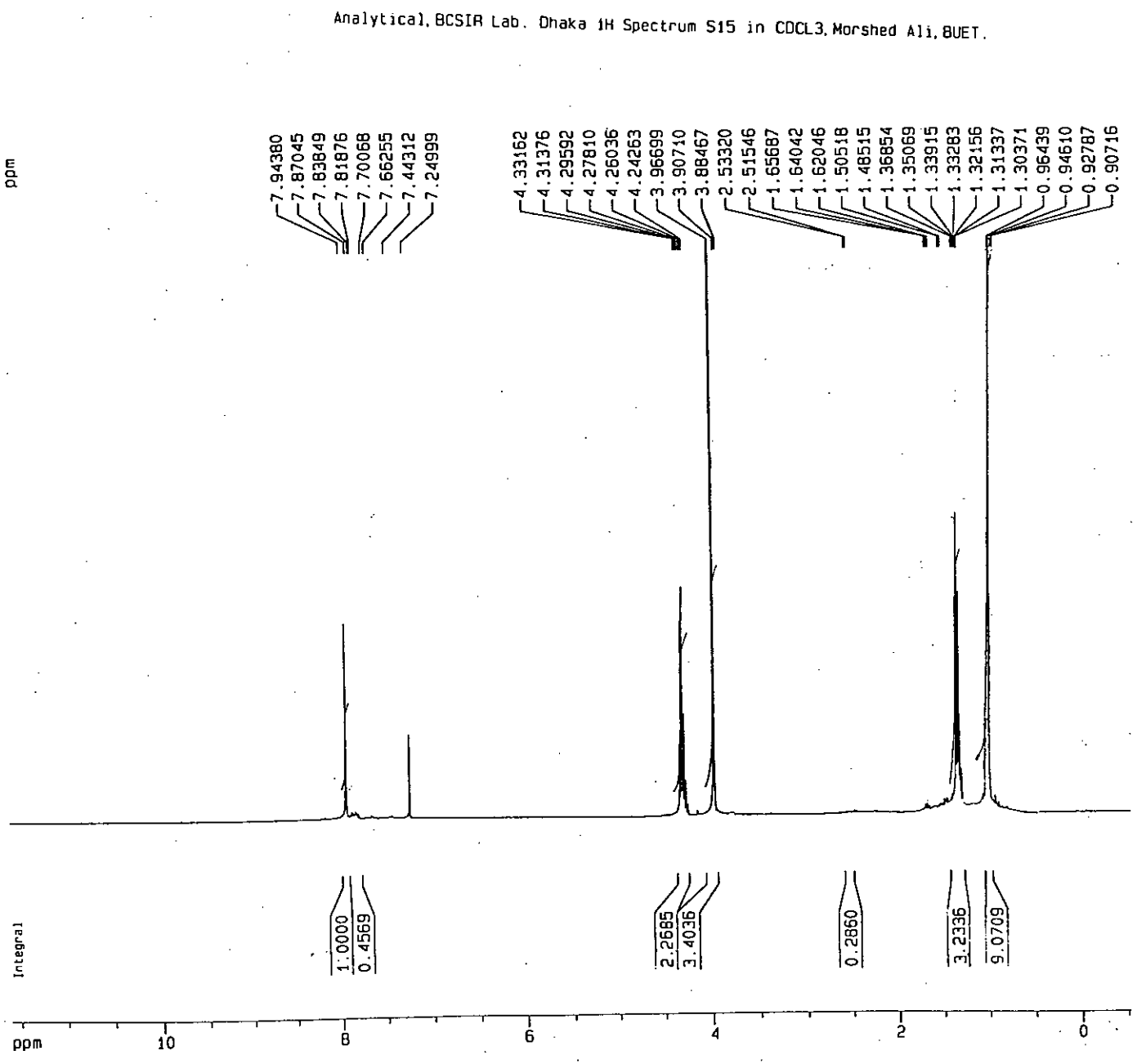


Fig. 48: IR Spectrum of Compound **15**



Current Data Parameters

NAME	A1953
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20050712
Time	10.14
INSTRUM	dpx400
PROBHD	5 mm Multinuc
PULPROG	zg30
TD	32768
SOLVENT	CDCl3
NS	128
DS	2
SWH	6410.256 Hz
FIDRES	0.195625 Hz
AQ	2.5559540 sec
RG	181
DW	78.000 usec
DE	6.00 usec
TE	310.0 K
D1	1.00000000 sec

========== CHANNEL f1 ==========

NUC1	1H
P1	8.30 usec
PL1	-6.00 dB
SFO1	400.1428010 MHz

F2 - Processing parameters

SI	32768
SF	400.1400126 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.40

1D NMR plot parameters

CX	20.00 cm
F1P	11.616 ppm
F1	4647.85 Hz
F2P	-0.518 ppm
F2	-207.18 Hz
PPMCM	0.60667 ppm/cm
HZCM	242.75127 Hz/cm

Fig. 49: ^{1}H NMR Spectrum of Compound **15**

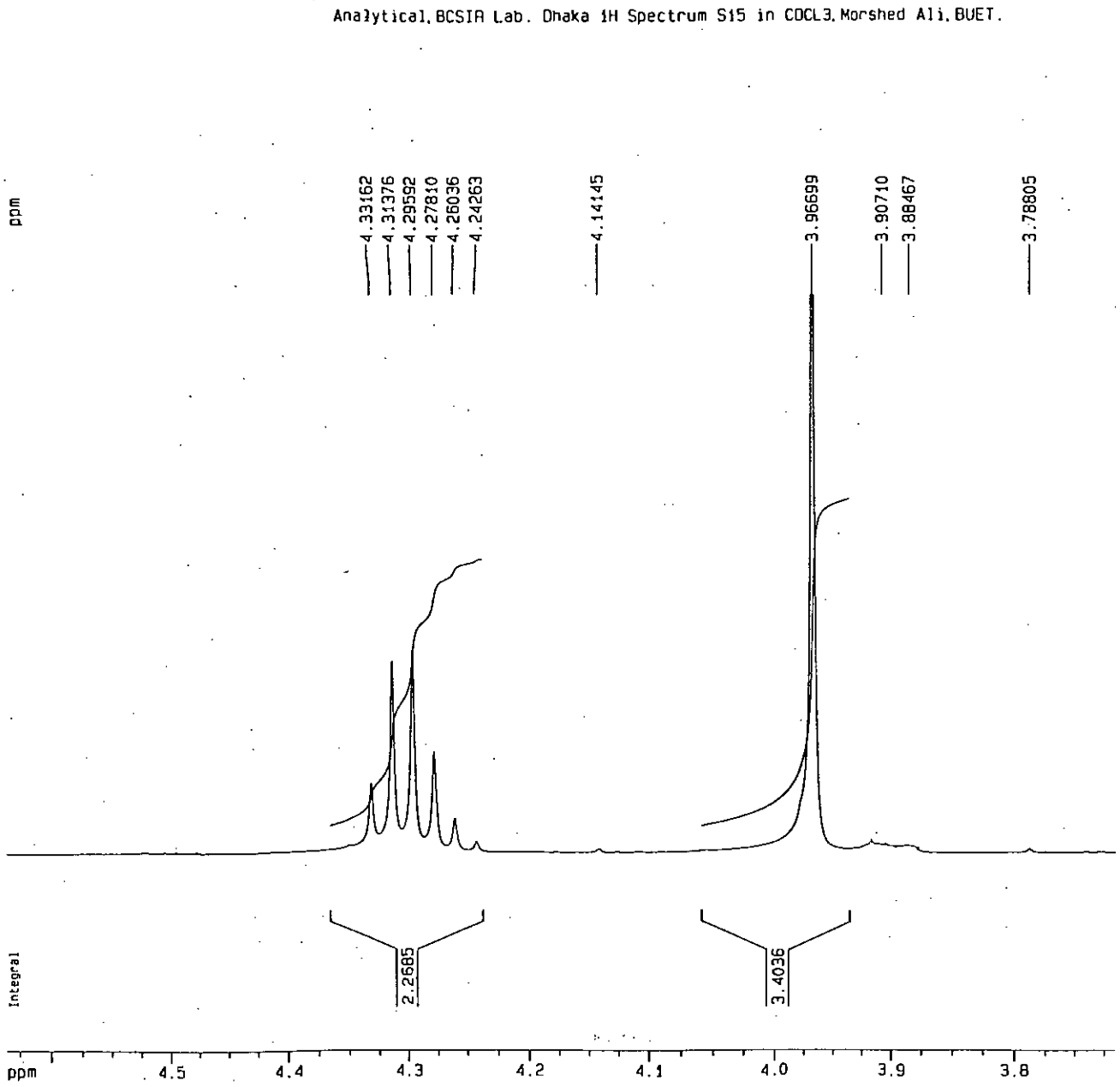
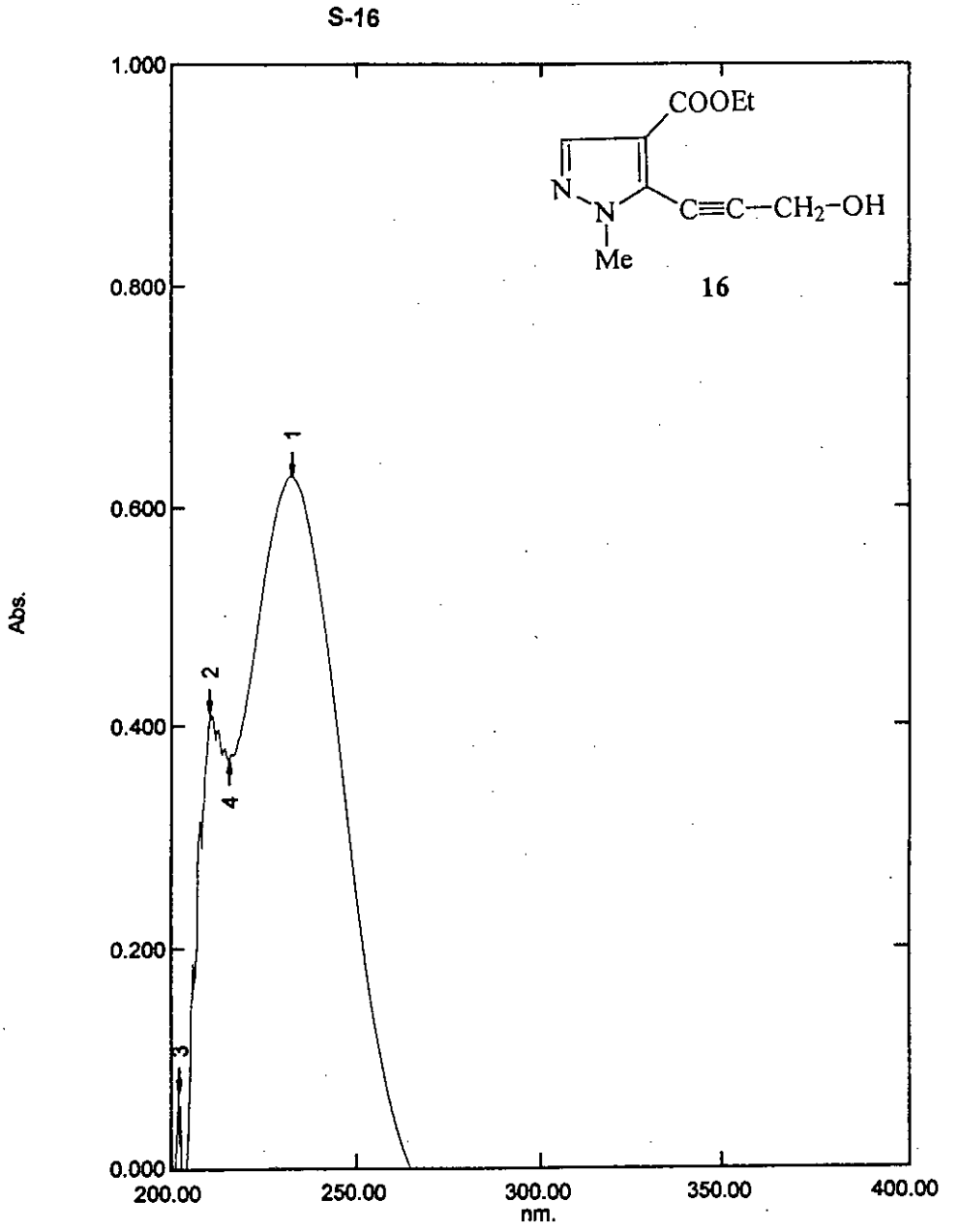


Fig. 50: ^{1}H NMR Spectrum of Compound **15** (Expansion)



Wavelength	Abs.
229	0.445
210	0.352

Fig. 51: UV Spectrum of Compound **16**

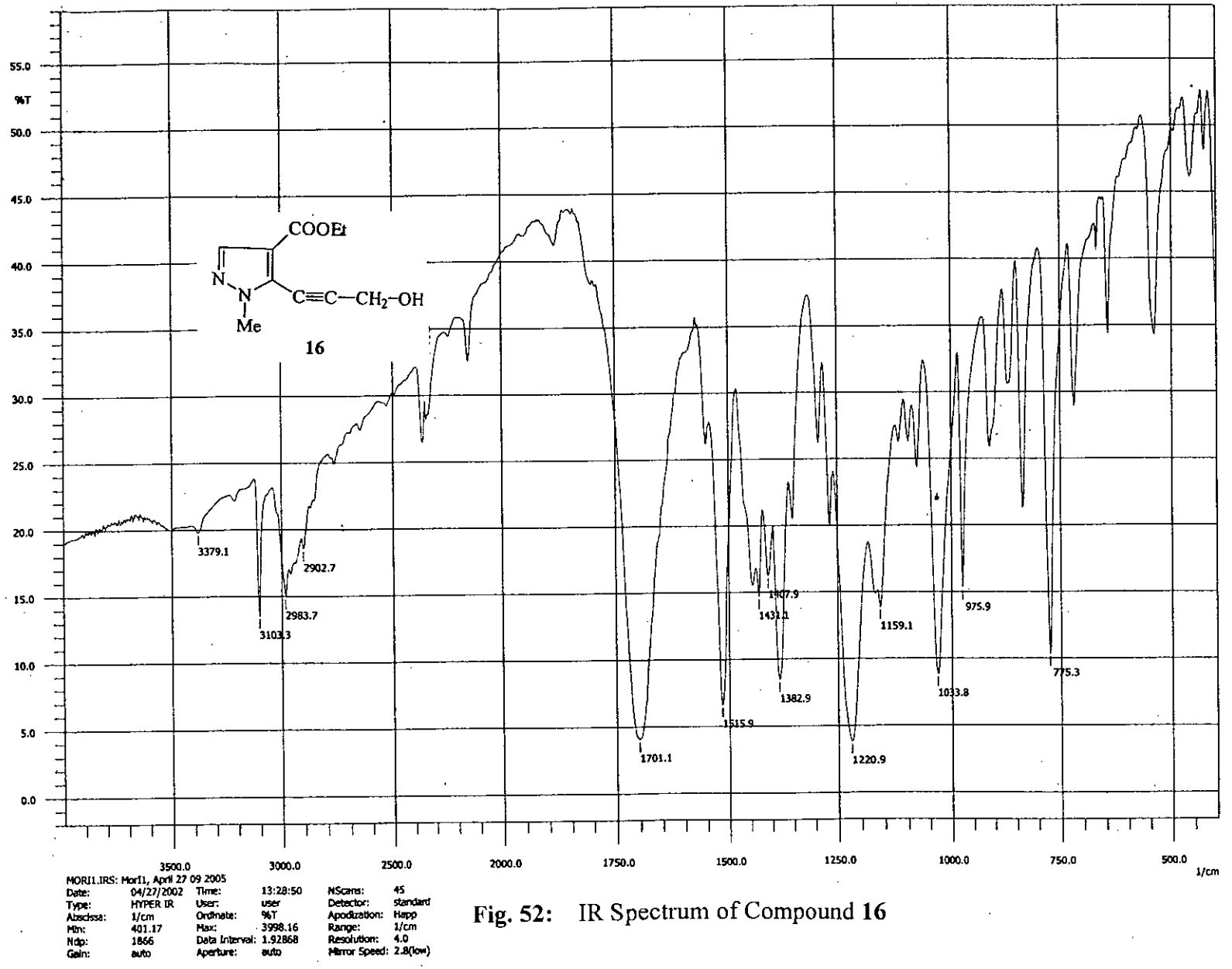


Fig. 52: IR Spectrum of Compound **16**

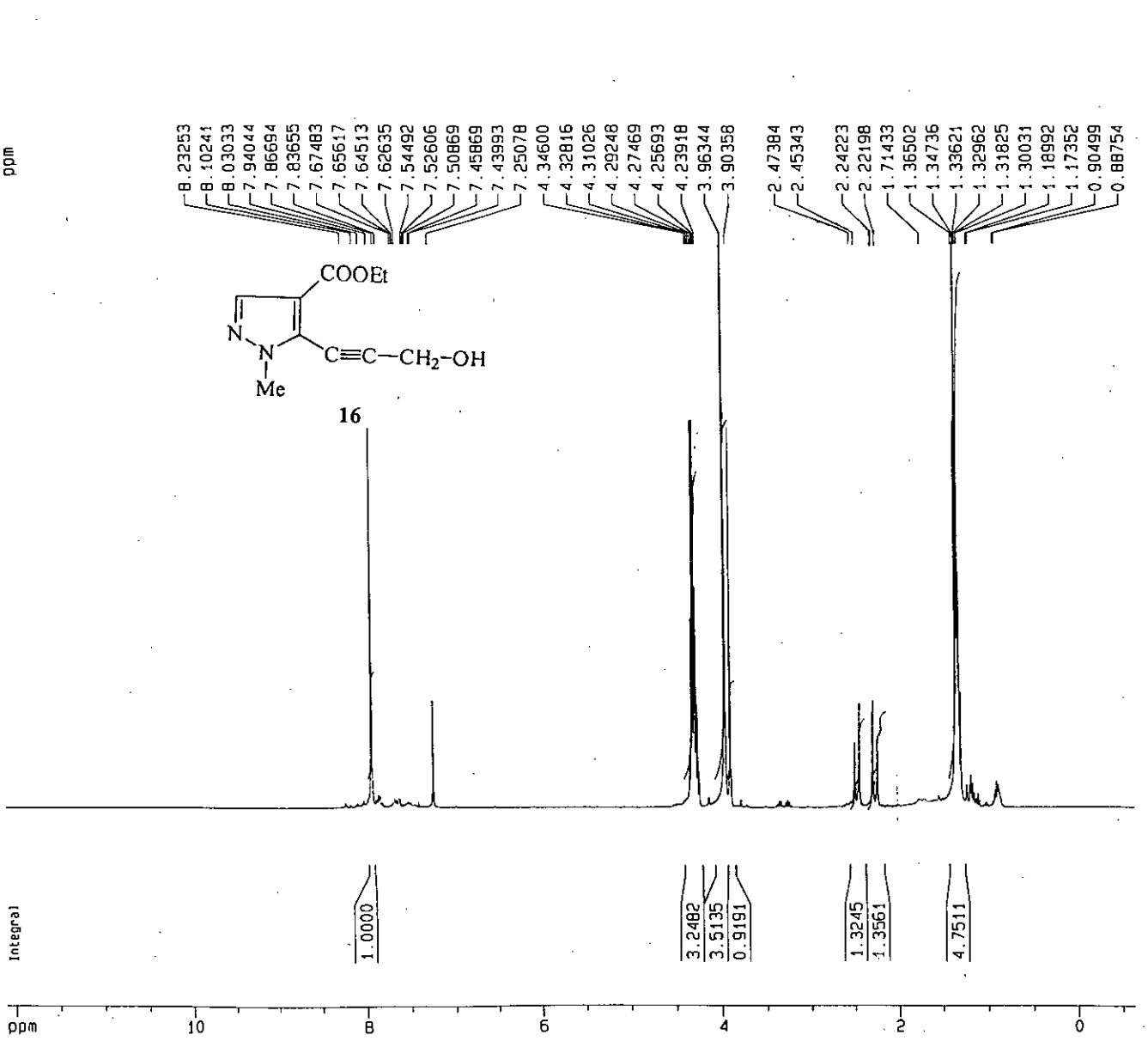


Fig. 53: ^{1}H NMR Spectrum of Compound **16**

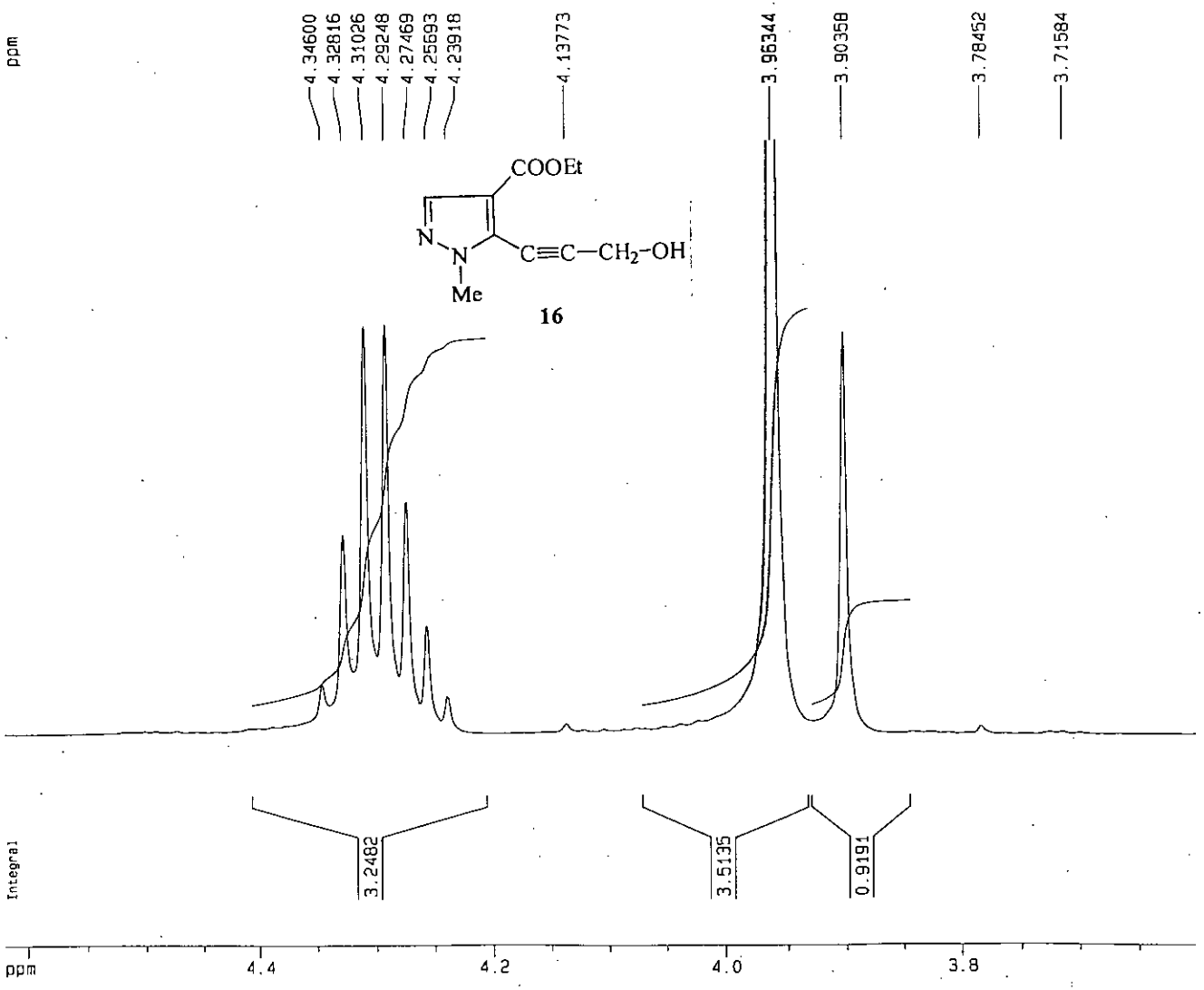
Current Data Parameters	
NAME	A1954
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters	
Date_	20050712
Time	10.25
INSTRUM	dpx400
PROBHD	5 mm Multinuc
PULPROG	zg30
TD	32768
SOLVENT	CDCl3
NS	128
DS	2
SWH	6410.256 Hz
FIDRES	0.195625 Hz
AQ	2.5559540 sec
RG	181
DW	78.000 usec
DE	6.00 usec
TE	310.0 K
D1	1.00000000 sec

============ CHANNEL f1 ============	
NUC1	1H
P1	8.30 usec
PL1	-6.00 dB
SFO1	400.1428010 MHz

F2 - Processing parameters	
SI	32768
SF	400.1400126 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.40

1D NMR plot parameters	
CX	20.00 cm
F1P	12.106 ppm
F1	4843.94 Hz
F2P	-0.636 ppm
F2	-254.51 Hz
PPMCM	0.63708 ppm/cm
HZCM	254.92264 Hz/cm



Current Data Parameters

NAME	A1954
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20050712
Time	10.25
INSTRUM	dpx400
PROBHD	5 mm Multinuc
PULPROG	zg30
TD	32768
SOLVENT	CDCl3
NS	128
DS	2
SWH	6410.256 Hz
FIDRES	0.195625 Hz
AQ	2.5559540 sec
RG	181
DW	78.000 usec
DE	6.00 usec
TE	310.0 K
D1	1.00000000 sec

========== CHANNEL f1 ==========

NUC1	1H
P1	8.30 usec
PL1	-6.00 dB
SFO1	400.1428010 MHz

F2 - Processing parameters

SI	32768
SF	400.1400126 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.40

1D NMR plot parameters

CX	20.00 cm
F1P	4.620 ppm
F1	1848.65 Hz
F2P	3.602 ppm
F2	1441.16 Hz
PPMCM	0.05092 ppm/cm
HZCM	20.37478 Hz/cm

Fig. 54: ^{1}H NMR Spectrum of Compound **16** (Expansion)

Part-I

Section-4

REFERENCES

REFERENCES

1. I. A. R. Katritzky and C. W. Rees, *Comprehensive Heterocyclic Chemistry* (New York), 143-221, 1984.
2. J.V. Ram, H.K. Pandey and Vlietnieck, *J. Heterocyclic Chem.*, **18**, 1277, 1981.
3. A. Albert, *Adv. Heterocyclic Chem.*, vol. **39**, Ed. A.R. Katritzky, *Academic press,* New York, 117, 1986.
4. J. Chern, Y. Liaw, C. Chen, J. Rong, C. Huang, C. Chan and A. Wang., *Heterocyclic,* **36**, 1091, 1993.
5. B. Roth and C.C. Cheng, *Progress in Medicinal Chemistry,* vol. **19**, G.P. Eillis and G.B. West, Eds., *Elsevier Biomedical press,* New York, 267, 1982.
6. a) E.F. Elslager, P. Jacob and L.M. Werbel, *J. Heterocyclic Chem.*, **9**, 775, 1972.
   b) E.F. Elslager, C. Hess, J.J. Johnson, D. Ortwins, V. Chien and L.M. Werbel, *J. Med. Chem.*, **24**, 127, 1981.
7. A. Santagati, M. Santagati and M. Modica, *Heterocycles,* **36**, 1315, 1993.
8. R.C. Petric, B.H. Cottam, A.P. Mckernan, K.R. Robins and R.G. Revankar, *J. Med. Chem.*, **28**, 1010, 1985.
9. a) T. Jen, B. Dienel, H. Bowman, J. Petta, A.Helt and B. Love, *J. Med. Chem.*, **15**, 727, 1972.
   b) V.T. Bandurco, E.M. Wong, S.D. Levine and Z.G. Hajos, *J. Med. Chem.*, **24**, 1455, 1981.
10. F. Ishikawa, J. Saegusa, K. Inamura, K. Sakuma and S. Ashida, *J. Med. Chem.*, **28**, 1387, 1985.
11. a) F. Ishikawa, J. Saegusa, K. Inamura, K. Sakuma and S. Ashida, *J. Med. Chem.*, **28**, 1387, 1985.

b) F. Ishikawa, A. Kosasyama, H. Yamaguchi, Y. Watanabe, J. Saegusa, S. Shibamura, K. Sakuma, S. Ashida and Y. Abiko, *J. Med. Chem.*, **24**, 1376, 1981.

12. E.C. Taylor, Ed. *Synthesis of fused Heterocycles* John Wiley and sons, New York, 226, 1987.

13. a) T. Jen, B. Dienel, H. Bowman, J. Petta, A.Helt and B. Love, *J. Med. Chem.*, **15**, 727, 1972.

b) V.T. Bandurco, E.M. Wong, S.D. Levine and Z.G. Hajos, *J. Med. Chem.*, **24**, 1455, 1981.

14. F. Jekeikawa, J. Saegusa, Kazue, Inamura, K. Sakumu and S. Ashida, *J. Med. Chem.*, **28**, 1387-1393, 1985.

15. a) C.J. Shishoo, M.B. Devani, G.V. Ullas, S. Ananthan and V.S. Bhadti, *J. Chem.*, **18**, 43, 1981.

b) C.J. Shishoo, M.B. Devani, G.V. Ullas, S. Ananthan and V.S. Bhadti, *J. Heterocyclic Chem.*, **22**, 825, 1985.

c) C.J. Shishoo, M.B. Devani, V.S. Bhadti, K.S. Jain and S. Ananthan, *J. Heterocyclic Chem.*, **27**, 119, 1990.

16. A.Z.M.S. Chowdhury, F. Sauter and J. Frölich, *Sci. Pharm,* **64**, 647-653, 1996.

17. Nirupama Tiwari, Bandan, Nizamuddin, *Chem. Abstracts,* **114**, 736, 1991.

18. R.C. PetricIII, B.H. Cottam, A.P. McKernan, K.R. Robins and R.G. Revenkar, Brigham Young University, Provo, *J. Med. Chem.*, **28**, 1010-1016, 1985.

19. J. Chern, P. Tao, M. Yen, G.Lu,C. Shiau, Y. Lai, S. Chen and C. Chan, *J. Med. Chem.*, **36**, 2196, 1993.

20. E.P. Papadopoulas, *J. Heterocyclic Chem.*, **18**, 515, 1981.

21. Edaward C. Taylor and M. Patel, *J. Heterocylic. Chem.*, **25**, 1857, 1991.

22. R.K. Bansal, *Heterocyclic Reactions, Synthesis, Mechanisms,* 365, 1990.

23. D.J. Brown, *Fused pyrimidine Part-I, Quinazolines,* 490, 1967.

24. O.P. Agarwal, *Chem. of Org. Natural Products,* vol. **II**, 17th Ed., 32, 1995.

25. Edward C. Taylor, Pingzhou, *Tetrahedron Lett,* **38**(25), 4339, 1997.

26. S.A. Shiba, A.A. El-Khamry, M.E. Shaban, K.S. Atia, *Chem. Abstracts,* **126**(24), 742, 1997.

27. Timermans, B.M.W.M. Van. Zwieten, *Drug future,* **9**, 41-55, 1984.

28. Nagano, *Eur Pat,* 89065, 1983, Chugai Pharmaceutical Co. Ltd. *Chem. Abstr.,* **100**, 6547, 1984.

29. Fozard, *J. Cardio. Vasc. Pharmacol.,* **131**, 257-264, 1986.

30. P. Molina, M.J. Vilaptana and J. Perez, *Tetrahedron letters,* **46**, 23, 7855-7864, 1990.

31. P. Molina, M. Alajarin and A. Vidal, *Tetrahedron,* **45**, 4263, 1989.

32. Gujral, Sexena and Tiwari, *Indian J. Med. Res.,* **43**, 637, 1955, *Chem. Abstr.* **50,** 6662, 1956.

33. J. Malecha, M.E. Le Tourneaw, *J. Heterocyclic Chem.,* **26**, 257, 1989.

34. Fumiyoshi, J. Saegusa, K. Inamura, K. Sakuma and S. Ashida, *J. Med. Chem.,* **28**, 1387-1393, 1985.

35. Schlecker, Rainer, Treiber, H. Joerg, B. Benthgold, Hofman, H. Peter, *Drug future,* **19**, 241-563, 1994.

36. A. Rosowsky, M. Chaykovsky, K.K.N. Chen, M. Lin and E.J. Mdest, *J. Med. Chem.,* vol. **16**(3), 185, 1973.

37. M.M. El-Enamy, F.S. El-Shafie, *Chem. Abstr.,* **114**, 729, 1991.

38. P. Sukumaran, K.N. Rajasekharan, *Ind. J. Chem.,* **29**B, 1074-1076, 1990.

39. R.A. Glennon, M.M. El. Kerdawy, Abd. El-Kader, M. Ismaiel and M.M. Gineinah, *J. Heterocyclic Chem.,* **27**, 497, 1990.

40. J.W. Tilley, R.A. Le Mahieu, M. Carson, R.W. Kierstead, H.W. Baruth and B. Yaremko, *J. Med. Chem.,* **23**, 92, 1980.

41. V.M. Patil, S. Sangapure and Y.S. Agasimundin, *Ind. J. Chem.*, **23**B, 135, 1984.

42. A.A. Fadda and H.M. Hassan, *Indian J. Chem.*, **29**B, 1020, 1990.

43. A. Monge, J.A. Palop, T. Goni, *J. Heterocyclic Chem.*, **23**, 647, 1986.

44. B. Venugopalan, P.D. Desai and N.J. de-Souza, *J. Heterocyclic. Chem.*, **25**, 1633, 1988.

45. H. Takashi, S. Kenji, T.Y. Nakayama, *J. Heterocyclic Chem.*, **28**(2), 263-7, 1991.

46. A.Z.M.S. Chowdhury, *J. Bang. Chem. Soc., (inpress)*.

47. M.J. Cocco, C. Congju and A. Moccioni, *J. Heterocyclic Chem.*, **27**, 1143, 1990.

48. K. Gewald, E. Shinke and H. Böttcher, *Chem. Ber.*, **99**, 94, 1966.

49. a) C. Barkenbus, V.C. Midkiff and R.M. Newman, *J. Org. Chem.*, **16**, 232, 1951.

b) M. Kazich, Diploma, Thisis, T.U. Vienna, *Austria,* 1991.

50. A. Dornow and H. Teckenburg, *Chem. Ber.*, **93**, 1103, 1960.

51. Fathy, M. Abdul Razek, *J. Prakt. Chem.*, **3324**, 479-483, 1990.

52. K. Gewald, E. Schinke and Horst Böttcher, *Jahrg,* **99**, 94-100, 1966.

53. F. Ell-Telbany and R.O. Hutchins, *J. Heterocyclic Chem.*, **22**, 401, 1985.

54. a) F.J. Trnney, W.A. Centenko, J.J. Kerbleski, D.T. Connor, R.J. Sorenson and D.J. Herzid, *J. Med. Chem.*, **24**, 878, 1981.

b) A. Rahman and A.H. Ghazala, *Synthesis.*, 372, 1980.

55. H. Wamhoff, *Chem. Ber.*, **105**, 743, 1972.

56. A. Lespagnaol, C. Lespagnol and J.L. Bernier, *L. Ann. Pharm. Fr.*, **32**, 125, 1974.

57. F. Sauter and W. Deinhammer, *Monatsh. F. Chem.*, **104**, 1593, 1973.

58. B. Stanovnik, M. Tisler, V. Golob, I. Hwala and O. Nikolic, *J. Heterocyclic Chem.*, **17**, 733, 1980.

59. A. Monge, J.A. Palop, T. Goni, *J. Heterocyclic Chem.*, **23**, 647, 1986.

60. F. Sauter, A.Z.M.S. Chowdhury, J. Fröhlich and C. Hamaterner, *Sci. Pharm.*, **65**, 83-92, 1997.

61. J. Fröhlich, A.Z.M.S. Chowdhury, F. Sauter, *Sci. Pharm.*, **64**, 647-653, 1996.

62. A. Monge, I. Aldona and J.A. Arranas, *J. Heterocyclic Chem.*, **28**, 557, 1991.

63. A.Z.M.S. Chowdhury, F. Sauter, J. Fröhlich, *Sci. Pharm.*, **64**, 647-653, 1996.

64. K. Blasl, *Diploma thesis*, Institute of organic chemistry, Technical University of Vienna, 1989.

65. F. Sautur, J. Frölich, K. Blasl and K. Gewald, *Heterocycles*, **40**, 851, 1995.

66. K.M.M. Rahman, A.Z.M.S. Chowdhury, M.M.H. Bhuiyan and M.K. Hossain, *Pakistan J. Sci. Ind. Res.*, **44**(2), 63-66, 2001.

67. K.V. Reddy, K. Mogilaiah and B. Sreenivasulu, *Ind. J. Chem.*, **23**B, 1106-1107, 1984.

68. M.P. Jain, V.N. Gupta, C.K. Atal, *Indian J. Chem.*, **24**B, 983, 1985.

69. U. Urleb, R. Neidlein and W. Kramer, *J. Heterocyclic Chem.*, **27**, 433, 1990.

70. E. Ziegler, W. Steiger and T. Koppe, *Monatsh. F. Chem.*, **99**, 1499, 1968.

71. K.O. Gelotte, D.N. Mason, H. Meckler, W.C. Sheih and C.M. Starkey, *J. Heterocyclic Chem.*, **27**, 1549, 1990.

72. H. Wamhoff, H. Wintersohl, S. Stölben, J. Paasch, N. Zhu and F. Guo, *Liebigs Ann. Chem.*, 901, 1990.

73. N.S. Girgis, A. Jorgensen and E.B. Pedersen, *Synthesis*, 101, 1985.

74. N.P. Peet, J. Malecha M.E. Le Tounea and S. Sunder, *J. Heterocyclic Chem.*, **20**, 260, 1989.

75. H.K. Gakhar, R. Gupta and J.K. Gill, *Ind. J. Chem.*, **25**A, 957-959, 1988.

76. J.E. Jansen, *U.S. Patent.*, **2**, 299, 465, 1942.

77. J.L. Fahey, B.C. Lange, J.M. Vanderveen, G.R. Young and A.K. Bose, *J. Chem. Soc. Perkin Trans.*, **1**, 117, 1977.

78. D.J. Brown and R.F. Evans, *J. Chem. Soc.*, 527, 1962.

79. a) D. Todd, *J. Am. Chem. Soc.*, **71**, 195, 1949.
b) P. Schmidt, K. Eichenberger, M. Wilhelm and J. Druey, Helv, *Chem. Acta.*, **42**, 763, 1959.

80. H. Bollinger and H. Zutter, *Swiss Patent,* **596**, 197, 1978.

81. J. Hofmann, *Chem. Ber.*, **5**, 242, 1872.

82. K. Okuro, M. Furuune, M. Enna, M. Miura and M. Nomura, *J. Org. Chem.*, **58**, 4716, 1993.

83. J.K. Stille, *Angew, Chem. Int. Ed. Engl.*, **25**, 508, 1986.

84. A. de Meijere and F.E. Meyer, *Angew, Chem. Int. Ed. Engl.*, **33**, 2379, 1994.

85. G.W. Kabalka, L. Wang and R.M. Pagni, *Tetrahedron.*, **57**, 8017, 2001.

86. T. Jeffery, *Tetrahedron letters.*, **26**, 2667, 1985.

87. R. Donvick, F. Pansy, G. Stryker and J. Bensteir, *J. Bacteriol.*, **59**,1950.

88. Yasliro Maki Nippon Yakurigaka, Zasshi, **55**, 1814, 1953 *Chem. Abstra.* **55**, 47b, 2872, 1961.

89. a) R.F. Heck, "Palladium Reagents in Organic Synthesis"; *Academic Press,* London, 1985; R.F. Heck, *Org. Reactions,* **27**, 345, 1982; R.F. Heck, *Comprehensive Organic Synthesis,* Oxford, *Pergamon Press,* 1991.
b) J.K. Stille, *Angew Chem. Int.*, Ed. Fngl., **25**, 508, 1986.
c) G.D. Cavies, Jr., and A. Hallbug, *Chem. Rev.*, **89**, 1433, 1989.
d) L.S. Hegedus, *"Transition metals in the synthesis of complex organic molecules"* University science Book, California, 1994.
e) C. Thebtaranonth and Y. Thebtaranonth, *Cyclization Reaction, CRC*

Press, London, 1994.

f) J. Tsuji, "*Palladium Reagents and Catalysts*", Wiley, Chichester, 1995.

g) J.L. Malleron, J.C. Fiaud and J.Y. Legos, *"Handbook of Palladium-catalyzed Organic Reactions", Academic Press,* London, 1997.

90. a) Y. Zhang and E. Negishi, *J. Am. Chem. Soc.,* **111**, 3454, 1989.

b) B.M. Trost and S. Shih, *J. Am. Chem. Soc.,* **115**, 12491, 1993.

c) N.C. Ithle and C.H. Heathcock, *J.Org.Chem.,* **58**, 560, 1993.

d) R.C. Larock, M.J. Doty and S. Cacchi, *J. Org. Chem.,* **58**, 5479, 1993.

e) B.M. Trost, G.J. Tanoury, M. Lautens, C. Chan and D.T. Mcpherson, *J. Am. Chem. Soc.,* **116**, 4255, 1994.

f) S. Ma and E.I. Negishi, *J. Am. Chem. Soc.,* **117**, 6345, 1995.

91. a) L.S. Hegdus, *Angew Chem. Int., Ed. Engl.,* **27**, 1113, 1988.

b) T. Sakamoto, Y. Kondo and H. Yamanaka, *Heterocycles.,* **27**, 2225, 1988.

c) A. Arcadia, S. Cacchi and F. Marinelli, *Tetrahedron lett.,* **30**, 2581, 1989.

d) J.H. Tidwell, D.R. Senn and S.L. Buchwald, *J. Am. Chem. Soc.,* **113**, 4685, 1991.

e) R.C. Larock, N.G. Berrios-Pena, C.A. Fried, E.K. Yum, and C.Tn. W. Leogn, *J. Org. Chem.,* **58**, 4509, 1993.

f) B.M. Trost and M.C. Mcintosh, *J. Am. Chem. Soc.,* **117**, 7255, 1995.

g) A. Arcadia, S. Cacchi, G. Fabrizi and F. Marinelli, *P. Paccc. Synlett.,* 568, 1996.

h) D. Bouyssi, M. Cavicchioli and G. Balme, *Synlett.,* 944, 1997.

k) M. Cavicchioli, S. Decortiat, D. Bouysis, J. Gore and Bahme, *Tetrahedron.,* **52**, 11463, 1996.

92. a) A. Arcadia, S. Cacchi and F. Marinelli, *Synthesis.,* 747, 1986.

b) J. Trsuji, *Tetrahedron.,* **42**, 4361, 1986.

c) Y. Tamaru and Z. Yoshida, *J. Organomet. Chem.,* **334**, 213, 1987.

d) Y. Sakamoto, Y. Kondo and H. Yamanaka, *Heterocycles.*, **27**, 2225, 1988.

e) S. Torii, L. H. Xu and H. Okumoto, *Synlett.*, **51**, 7965, 1992.

93. N.G. Kundu, M. Pal, J.S. Mahanty and S.K. Dasgupta, *J. Chem. Soc., Chem. Commun.*, 41,1992; J.S. Mahanty, M. De. and N.G. Kundu, *J. Chem. Soc. Perkin trans.*, **1**, 2577, 1997.

94. Sonogashira, K. Tohda and Y. Hagihara, *Tetrahedron lett.*, (1975), 4467

95. N.G. Kundu and M.W. Khan, *Tetrahedron letters;* **38**, 6937, 1997.

96. N.G. Kundu and M.W. Khan, *Tetrahedron;* **56**, 4777, 2000.

97. M.W. Khan, M.J. Alam, M.A. Rashid and R. Chowdhury., *J. Bioorg. Med. Chem.*, **13**, 4796, 2005.

98. M.W. Khan and A.F.G.M. Reza, *Tetrahedron.*, **61**, 11204, 2005.

Part-II

Section-1

STUDIES ON ANTIMICROBIAL ACTIVITIES OF SOME FUSED PYRIMIDINE AND 5-ALKYNYL PYRAZOLE DERIVATIVES.

INTRODUCTION

Human struggle against the affliction disease, decay and death in eternal. The deterioration of human population due to an enhanced prevalence of infections diseases is becoming a global problem.[1] The contemporary treatment of infection disease involves administration of a multi drug resistant strains plus a high level of patient noncompliance.[1] From the time immemorial when the men needed medicine, most probably when the men realized about the cause of disease, they have been tried to discover any preventive agent against disease, from that time. It is universal truth that disease, decay and death have always co-existed with human life. The study of disease and their treatment must also have been come together with human intellect, when the man, occupied sufficient knowledge of chemistry to able to synthesize compounds.

Bangladesh is predominantly an agricultural country, depending mainly on crop plants, agricultural and forest products for its economic development. Although crops play a vital role in economy of the country and agroecologial conditions are favourable for the production of various crops, the yield of crops is often poor. Among the various factors responsible for poor yield of crops, plant diseases caused by various micro-organisms play a significant role. Gradually men occupied sufficient knowledge of chemistry to inhibit or to kill the micro-organisms i.e. only inhibit the microbial growth are called 'statis'. But the chemicals, which have the ability to kill the micro-organisms, are called 'cidal'. But some chemicals are called "pesticides" on the basis of kinds of pathogenic microorganisms. Pesticides may be different types, e.g. fungicides, bactericides, viricides etc.

The word bactericide and fungicide have originated from latin words: bacteria, fungus and caedo. The word caedo means "to kill". Thus literally speaking a bactericide and fungicide would be any agency, which have the ability to kill a bacteria or fungus. By common usage, the word is restricted to chemicals. Hence the words bactericide and fungicide would mean a chemical capable of killing bacteria and fungus respectively.

It is not enough that a chemical has high bacterial and antifungal activity. Such as, chemicals may have no utility unless it stands out in the tests and gives proof of significant control of diseases under varied field conditions. There are several factors, which influence the performance of a bactericide and fungicide under different field conditions. They may be either physical or chemical in nature.

A good pesticide should be toxic to the parasite or inhibit the germination of its spores without causing phytotoxicity. A number of chemicals are used to control the microbial pathogen of human and other animals as medicine. The number of chemicals available for plant disease control runs into hundreds, although all are not equally safe, effective and popular. Also different types of organic, aromatic, inorganic and heterocyclic compounds are employed as antibacterial agents. Salts of toxic metals and organic acids, organic compounds of mercury and sulfur, quinones and heterocyclic nitrogen compounds are the major fungicides in used today.

The organic compounds of sulfur are highly effective and popular fungicides in used today. All these compounds are derivatives of dithiocarbamic acid, thiram, ziram ferban, nabam, zineb and maneb are well known examples of sulfur fungicides. Many aromatic compounds have significant antimicrobial activity and have been developed into fungicides. Some of these are in commercial use.

Example of these type of fungicides are Dexon (dimethylamino benzenediazo sodium sulphonate), Diconil (tetrachloro isohpthaloutrile) etc. Heterocyclic nitrogen compound used as fungicides included glyodin (2-hepto-decay-2-imidazolin acetate), oxine (8-hydroxy quinoline) etc.

It was found from the literature that nitrogen and sulfur containing heterocyclic compounds showed marked microbial activities.[2-6] When heterocyclic part like imidazoles, nitroimidazole etc. become attached to carbohydrates,[7] their efficiency to inhibit bacteria or fungus sharply increased. It was also found that a large number of biologically active compounds possess aromatic and heteroaromatic nucleus. If an active nucleus is linked to another nucleus, the resulting molecule may possess greater potential for biological activity.[8] The benzene and substituted benzene nuclei play an important role as common denominator for various biological activities. It was observed that many a time the combination of two or more nuclei enhances the biological profile many fold than its parent nuclei.

In vitro antimicrobial activities of fused pyrimidines were successfully evaluated in our laboratory.[9] it was found that the fused pyrimidine derivative showed maximum average inhibition against four Gram-positive and four Gram-negative bacteria and four phytopathogenic fungi.

M. Shehab[10] a post graduate student of our laboratory carried out *in vitro* antimicrobial activities of fused pyrimidine derivatives. He used eleven bacteria such as *Bacillus subtilis, Bacillus megaterium, Bacillus cereus, Staphylococcus aureus, Shigella dysenteriae, Shigella sonnei, Salmonella typhi, Escherichia coli, Pseudomonas (sp.), Sarcina species* and INABA-ET (*Vibrio)* and four phytopathogenic fungi such as, *Macrophomina phaseolina, Fusarium equiseti, Alternaria alternata* and *Drechslera oryzae* were also used for this screening.

M. S. Rahman[11] showed that antimicrobial activities of the alkaloids of three plant leaves. The alkaloid fractions were screened against eight pathogenic bacteria, *viz.*, *Shigella dysenteriae, Shigella sonnei, Salmonella typhi, Bacillus subtilis, B. megaterium, B. cereus, Staphylococcus aureu, and Pseudomonas mutabilis.* The highest zone of inhibition (38 mm) was recorded with the fraction No. 4 against *Salmonella typhi.*

S.M. Shahed[12,13] a former research student of organic laboratory carried out antifungal activities of a series of acylated D-mannose derivatives. He used four phytopathogenic fungi, such as *Macrophomina phaseolina, Fusarium equiseti, Alternaria alternata and Curvularia lunata.* Most of the tested chemicals showed good inhibition (more than 50% growth against the above organism).

M. Fakruddin[14] also a former resarch student of our laboratory carried out antifungal activities of fused pyrimidine. He used five human pathogenic bacteria, *viz. Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, Salmonella typhi, Escherichia coli* and four phytopathogenic fungi, *viz. Verticillum sp., Fusarium solanae, Aspergillus sp., penicillum sp.* He found that some of the tested chemicals showed very effective antibacterial and antifungal activity.

S.M. Abe Kawsar[15,16] also a former post graduate student of the same laboratory carried out *in vitro* antibacterial activities of a series of acylated uridine derivatives. He used ten bacteria such as, *Staphylococcus aureus, Bacillus megaterium, Bacillus cereus, Bacillus subtilis, Escherichia coli, Salmonella typhi, Shigella dysenteriae, Shigella sonnei, INABA-ET* (*Vibrio*) and *Sarcina species.* It was observed that most of the acylated compounds exhibit moderate to good

antibacterial activity. Amongst the acylated compounds exhibit moderate to good antibacterial activity.

Recently, our groups synthesized 2-substituted benzofurans[17], isoindolinone and isoquinolinone[18] and tested their antibacterial and antifungal activities. The synthesized compounds demonstrated mild to significant growth inhibitors against antibiotic-susceptible standard and clinically isolated strains of gram-positive and gram-negative bacteria as well as human fungal pathogens.

In the present study, fused pyrimidine and 5-alkynyl pyrazole derivatives were used (which are shown **Table 3**) against twelve human pathogenic bacteria and five phytopathogenic fungi. Among the bacterial strains five were Gram-positive; *viz. Bacillus cereus, Bacillus megaterium, Staphylococcus aureus, Bacillus subtilies, Sarcina lutea* and seven were Gram-negative, *viz. Escherichia coli, pseudomonas aeruginosa, Salmonella paratyphi, Shigella boydii, Shigella dysenteriae, Vibrio mimicus and Vibrio parahemolyticus.* Antifungal activities of same compounds were also studied against four phytopathogenic fungi; *viz. Aspergillus niger, Candida albicans, Rhizopus oryzae and Saccharo myces cerevisiae.*

The present work was under taken to select the chemicals (fused pyrimidine and 5-alkynyl pyrazole derivatives) that have not been studied before phathogenic microorganisms of animals and plants.

Part-II

Section-2

METHODOLOGY OF THE BIOLOGICAL WORK

MATERIALS AND METHODS

The antibacterial activities of fused pyrimidine and 5-alkynyl pyrazole derivatives were studied against twelve bacteria and antifungal activities of the same compounds were also studied against four fungi. For the detection of antibacterial activities the disc diffusion method[19] was followed. The antifungal activities was assessed by poisoned food technique.[20,21]

Nutrient Agar (NA) and potato Dextrose Agar (PDA) were used as basal medium for test bacteria and fungi respectively. Methanol (MeOH)/Dimethylsulfoxide (DMSO) were used as a solvent to prepare 1% solution of the compound initially. Proper control was maintained with MeOH/DMSO. The materials and methods of the present work described detail in below:

2.2.1. Materials and Methods:

Bacteria and fungi are responsible for many infections diseases. The increasing clinical importance of drug resistant microbial pathogens has lent additional urgency to antimicrobial research. The antimicrobial screening which is the first stage of antimicrobial research is performed to ascertain the susceptibility of various microbes to any agent. This test measures the ability of each antimicrobial agent to inhibit the in *vitro* microbial growth. This ability may be estimated by any of the following three methods.

I). Disc diffusion method
II). Serial dilution method
III). Bioautographic method.

The disc diffusion technique (Bauer et al[19], 1966) is a widely accepted in *vitro* investigation for preliminary screening of agents which may possess any antibacterial activity. It is essentially a quantitative or qualitative test indicating the sensitivity or resistance of the microorganisms to the test materials. However, no distinction between baceriostatic or bactericidal activity can be made by this method. (Roland[22], R, 1982).

2.2.2. Principle of Disc Diffusion method:

Solutions of known concentration (µg/ml) of the test samples are made by dissolving measured amount of the samples in definite volume of solvents. Dried and sterilized filter paper discs (6 mm diameter) are then impregnated with known amounts of the test substances using micropipette. Discs containing the test material are placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic discs and blank discs (impregnated with solvents) are used as positive and negative control. These plates are then kept at low temperature (4°C) for 2h h to allow maximum diffusion. During this time dried discs absorb water from the surrounding media and then the test materials are dissolved and diffused out of the media. The diffusion according to the physical law that controls the diffusion of molecules through agar gel. As a result there is a gradual change of test materials concentration in the media surrounding the discs. The plates are then incubated at 37°C for 24 hrs. to allow maximum growth of the organisms. If the test materials have any antibacterial activity, it will inhibit the growth of the microorganisms giving a clear, distinct zone called "Zone of Inhibition". The antibacterial activity of the test agent is determined by measuring the diameter of zone of inhibition expressed in millimeter.

The experiment is carried out more than once and the mean of the readings is required (Bauer *et al*[19], 1966). In the present study some pure compounds were tested for antibacterial activity by disc diffusion method.

2.2.3. Experimental:

2.2.3.A. Apparatus and Reagents:

Filter Paper Discs	Screw Cap Test Tubes
Sterile Cotton	Autoclave
Micropipette	Nutrient Agar Medium
Laminar Air Flow Hood	Inoculating Loop
Refrigerator	Spirit Burner
Chloroform	Nose Mask and Hand Gloves
Petridishes	Incubator
Sterile Forceps	Ethanol

2.2.3.B. Test of Organisms:

The bacterial strains used for the experiment were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka. Both Gram-positive and Gram-negative organisms and fungi were taken for the test and they are listed in the **table-1** and **table-2.**

Table-1: List of Test Bacteria

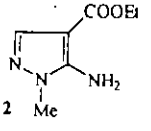
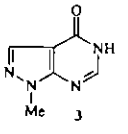
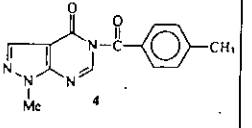
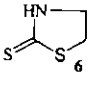
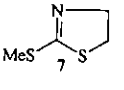
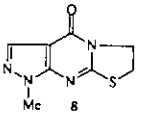
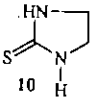
Gram positive	Gram negative
Bacillus cereus	*Esherichia coli*
Bacillus megaterium	*Pscudomonas aeruginosa*
Bacillus subtilis	*Salmonella paratyphi*
Staphylococcus aureus	*Sheigella boydii*
Sarcina lutea	*Shigella dysenteriae*
	Vibrio mimicus
	Vibrio parahemolyticus

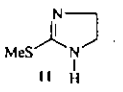
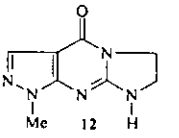
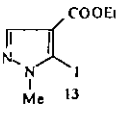
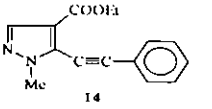
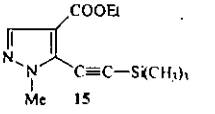
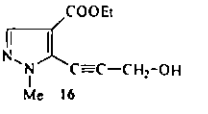
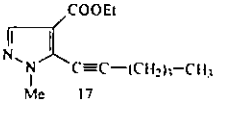
Table-2: List of test fungi

Fugi
Aspergillus niger
Candida albicans
Rhizopus oryzae
Saccharo myces cerevisiae

2.2.4. Test of Materials:

Table-3: List of Test chemicals used for antimicrobial activities.

Comd. No.	Name of the test chemicals	Molecular Formula
2	Ethyl-5-amino-1-methylpyrazol-4-carboxylate	
3	1-Methylpyrazolo[3,4-d]pyrimidin-4(5H)-one	
4	1-Methyl-4-oxo-5-(*p*-toluoyl)pyrazolo[3,4-d]pyrimidine	
6	2-Mercaptothiozoline	
7	2-Methylthio-2-thiozoline	
8	1-Methyl-6,7-dihydropyrazolo[3,4-d]thiazolo[1,2-a] pyrimidin-4-one.	
10	2-Imidazolinethione	

11	2-Methylthio-imidazoline	
12	1-Methyl-4-oxazolo-6,7-dihydropyrazolo[3,4-d]imidazo [1,2-a]pyrimidin-4(8H)-one	
13	Ethyl-5-iodo-1-methylpyrazol-4-carboxylate	
14	Ethyl-5-phenylactyleneethynyl-1-methylpyrazol-4-carboxylate	
15	Ethyl-5-trimethylsilylethynyl-1-methylpyrazol-4-carboxylate	
16	Ethyl-5-propynylol-1-methylpyrazol-4-carboxylate	
17	Ethyl-5-hexayne-1-methylpyrazol-4-carboxylate	

2.2.5. Culture Medium:

Mueller-Hinton (MH) medium and Potato Dextrose Agar (PDA) were used for making plates on which antibacterial and antifungal sensitivity tests were carried out respectively. The antibacterial activities of the materials were detected by disc diffusion method [Bauer *et al*[19], 1966] and antifungal activity of the materials were assessed by food poison technique [Miah *et al*[20], 1990 and Grover *et al*[21], 1962]. This media were also used to prepare fresh cultures.

2.2.6. Medium Used:

Nutrient Agar (NA) and potato Dextrose Agar (PDA) were used through out the work. The composition and preparation procedure of NA and PDA are described below:

Composition of Nutrient Agar Medium:

Ingredients	Amounts (gm/lit)
Peptone	5.0
Sodium chloride	5.0
Beef extract	1.5
Yeast extract	1.5
Agar	14.0
P^H (at 25°C)	7.2 – 7.6

Procedure:

To prepare required volume of this medium, calculated amount of each of the constituents was taken in a conical flask and distilled water was added to it to make the required volume. The contents were heated in a water bath to make a clear solution. The pH (at 25°C) was adjusted at 7.2–7.6 using NaOH or HCl. 10ml and 5 ml of the medium was then transferred in screw cap test tubes to prepare plates and slants respectively. The test tubes were then capped and sterilized by auto calving at 15-1bs/sq, pressure at 121°C for 20 minutes. The slants were used for making fresh culture of bacteria that were in turn used for sensitivity study.

Composition of Potato Dextrose Agar:

Ingredients	Amounts (gm/lit)
Potato	200.0
Dextrose	20.0
Agar	15.0 g

Procedure:

200g of sliced potato was boiled in 500 ml distilled water and extract was decanted after proper boiling. The extract was taken in a 1000 ml beaker and the solution was made up to the mark with distilled water. This solution was taken in suspense and 20g dextrose was added slowly in the solution. Then 15g of agar powder was added in the solution and they were mixed throughly with a glass rod. After 10 minutes of boiling the medium was transferred in 250 ml conical flash. Before autoclaving the conical flask was closed with the cotton plug and rapping with aluminium foil. The medium was autoclaved for 15 minutes at 121°C and 15–1bs/sq pressure. After autoclaving the medium was used for culture of different microorganisms.

2.2.7. Sterilization Procedures:

In order to avoid any type of contamination by the test organisms the antibacterial screening was done in Laminar Hood and all types of precautions were highly maintained. UV light was switched on an hour before working in the laminar hood. Petridishes and other glassware were sterilized by autoclaving at a temperature of 121°C and pressure of 15–1 bs/sq. inch for 20 minutes. Micropipette tips, cotton, forceps discs etc. were also sterilized.

2.2.8. Preparation of Subculture:

In an aseptic condition under laminar air cabinet, the test organisms were transferred from the pure cultures to the agar slants with help of a transfer loop to have fresh pure cultures. The inoculated strains were then incubated for 24hrs. at 37°C for their optimum growth. These fresh cultures were used for the sensitivity test.

2.2.9. Preparation of the Test Plates:

The test organisms were transferred from the subculture to the test tubes containing about 10 ml of sterilized agar medium with the help of a sterilized transfer loop in an aseptic area. The test tubes were shaken by rotation to get a uniform suspension of the organisms. The bacterial suspension was immediately transferred to the sterilized petridishes. The petridishes were rotated several times clockwise and anticlockwise to assure homogenous distribution of the best organisms in the media.

2.2.10. Preparation of Discs:

Three types of discs were used for antibacterial screening. They were:

(a) Standard Discs

(b) Blank Discs and

(c) Sample Discs

The descriptions of these discs were given below:

(a). Standard Discs:

These were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known antibacterial agent with that of produced by the test sample. In this investigation, kanamycin (30 μg / disc) standard disc was used as the reference.

(b). Blank Discs:

These were used as negative control which ensures that the residual solvents (left over the discs even after air-drying) and the filter paper were not active themselves.

(c). Preparation of Sample Discs with Test Sample:

Measured amount of each test sample was dissolved in specific volume of solvent to obtain the desired concentrations in an aseptic condition. Then discs were soaked with solutions of test samples and dried.

2.2.12. Diffusion and Incubation:

The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in the agar plates pre-inoculated with test bacteria. The plates were then kept in a refrigerator at 4°C for about 24h to allow sufficient diffusion of the materials from the discs to the surrounding agar medium. The plates were then inverted and kept in an incubator at 37°C for 24hrs.

2.2.13. Determination of Antibacterial Activity by Measuring the Zone of Inhibition:

After incubation, the antibacterial actives of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with transparent scale.

Part-II

Section-3

2.3.1. RESULTS AND DISCUSSION:

In our present study, fused pyrimidine and 5-alkynyl pyrazole derivatives (Table 3) were selected and screened for their antibacterial activity against twelve human pathogenic bacteria, *viz. B. cereus, B. megaterium, S. aureus, B. subtilies, S. lutea, E. coli, p. aeruginosa, S. paratyphi, S. boydii, S. dysenteriae, V. mimicus, V. parahemolyticus, S. flexneri and S. sonnei.*

For antifungal activities of same chemicals were also studied against four phytopathogenic fungi, *viz. A. niger, C. albicans, R. oryzae and S. myces cerevisiae.*

The results of the diameter of inhibition zone and % inhibition of mycelial growth due to the effect of chemicals are presented in **Table-4** and **Table-5.**

A total of fourteen compounds (four starting materials, four fused pyrimidine and 5-alkynyl pyrazol derivatives) have been tested for *in vitro* antimicrobial activity against five Gram-positive and seven Gram-negative bacteria as well as four human fungal pathogens. The selected microbes were collected as fresh cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Dhaka-1000. No clinically isolated resistant strains were used for the present study.

The antimicrobial activities were measured in terms of diameters of zone of inhibition (mm). All the experiments were performed thrice to minimize the experimental plus individual errors. The mean values of the diameters of zone of inhibition (M.DIZ) were taken as in disc for determining antimicrobial spectra. Sensitivity test results are interpreted in (**Table-4** and **Table-5**) and were compared with a standard antibiotic kanamycin (30 μg/dise).

The Gram positive as well as Gram-negative bacteria used in the present investigation, were found to be completely resistant against six synthesized compounds (**2, 4, 7** and **11**), at a dose level of 200 μg/disc (**Table-4 and Table-5**), compounds **3, 8** and **12** showed mild *in vitro* antimicrobial activity, especially against the fungi, *Candida albicans* **2** (M.DIZ 10), **3** (M.DIZ 11.5), **11** (M.DIZ 9.4), **12** (M.DIZ 9.8) and *Saccharomyces cerevaceae* **3** (M.DIZ 8.6), **8** (M.DIZ 8.8), **11** (M.DIZ 7.8), **12** (M.DIZ 8.6). *Aspergillus niger* and *Rhizobus oryzae* were, however, resistant to the compound (**Table-4 and Table-5**).

The compounds **3, 8** and **12** showed mild *in vitro* antimicrobial activity especially against the Gram positive *Bacillus cereus* **3** (M.DIZ 7.2), **8** (M.DIZ 7.4), **12** (M.DIZ 7.2), *Bacillus megaterium* **3** (M.DIZ 8.5), **8** (M.DIZ 9.4), **12** (M.DIZ 8.6). And also compounds **3, 8** and **12** exhibited in *vitro* antimicrobial activity, especially against the Gram negative *Escherichia coli* **3** (M.DIZ 8.2), **8** (M.DIZ 7.2), **12** (M.DIZ 7.1) and *Shigella dysenteriae* **3** (M.DIZ 7.0), **8** (M.DIZ 7.4), **12** (M.Diz 7.4). This study can therefore, confer that formation of fused pyrimidine increases antimicrobial activity.

In the arsenal of 5-alkynyl pyrazole derivatives, there appears to be no effective warhead to combat the selected organisms. The screening of five 5-alkynyl pyrazole derivatives (**14, 15, 16,** and **17**) demonstrated only mild inhibitory activity with zones of inhibitions ranging from **6.1** to **12.0** mm. (**Table-5**). Therefore, it is not possible to determine the essential structural features for antimicrobial action of this series of compounds.

2.3.2. CONCLUSION:

Twelve new synthesized heterocyclic compounds have been tested for in antimicrobial activity against five Gram-positive and seven Gram-negative bacteria as well as four human fungal pathogens. Most of this compound demonstrated mild to moderate antimicrobial activity against most of the test organism. From these structures we found that the fused pyrimidine ring causes relatively better microbial growth inhibition.

Among tested compounds 5-alkynyl pyrazole derivatives (**14, 15, 16** and **17**) exhibited relatively greater inhibition of growth of the microorganism. The higher activity of the compounds (**14-17**) could probably be due to their greater solubility in aqueous medium, which subsequently facilitated the diffusion of the chemical entities through the microbial call wall.

Substitution of iodine of the ring carbon, with bulkier terminal alkyne group increase in the antimicrobial activity of the compounds **14, 15** and **16** while hexyne substitution at the same place produce weakly active compound **17**.

Table-4: *In Vitro* Antimicrobial activity of new synthesized compound 2-11.

Diameter of Zone of Inhibition (mm)							
Done	200µg /disc	200µg /disc	200µg /disc	200µg /disc	200µg /disc	200µg /disc	**Std. 30**
Name of the Microorganism	**2**	**3**	**4**	**7**	**8**	**11**	Kan
Gram (+) bacteria							
Bacillus cereus	-	7.2	-	-	7.4	-	31.9
Bacillus megaterium	-	8.5	-	-	9.4	-	34.2
Staphylococcus aureus	-	8.1	-	-	-	-	31.6
Bacillus subtilies	-	-	-	-	-	-	30.1
Sarcina lutea	-	-	-	-	-	-	26.9
Gram (-) bacteria							
Escherichia coli	-	8.2	-	-	7.2	-	34.2
Pseudomonas aepuginosa	-	8.9	-	-	-	-	34.8
Salmonella paratyphi	-	6.5	-	-	-	-	26.9
Shigella boydii	-	-	-	-	-	-	30.2
Shigella dysenteriae	-	7.0	-	-	7.4	-	36.9
Vibrio mimicus	-	-	-	-	-	-	29.8
Vibrio parahemolyticus	-	-	-	-	-	-	31.9
Shigella flexneri	NT	NT	NT	NT	NT	NT	33.2
Shigella sonnei	NT	NT	NT	NT	NT	NT	31.5
Fungi							
Aspergillus niger	-	-	-	-	-	-	35.0
Candida albicans	10.0	11.5	-	12	-	9.4	32.5
Rhizopus oryzae	-	-	-	-	-	-	27.6
Saccharo myces cerevaceae	-	8.6	-	-	8.8	7.8	32.1

Interpretation of sensitivity test results:

Gram (+) bacteria:
> 18 mm (M.DIZ) = sensitive
14-18 mm (M.DIZ) = intermediate
< 14 mm (M.DIZ) = resistant

Gram (-) bacteria:
> 16 mm (M.DIZ) = sensitive
13 – 16 mm (M.DIZ) = intermediate
> 13 mm (M.DIZ) = resistant.

"-" indicates no sensitivity or zone of inhibition lower than 6 mm and NT refers to "Not Tested"

Table-5: *In Vitro* Antimicrobial activity of new synthesized compound 12-17.

Diameter of Zone of Inhibition (mm)							
Done	200µg /disc	200µg /disc	200µg /disc	200µg /disc	200µg /disc	200µg /disc	**Std. 30**
Name of the Microorganism	**12**	**13**	**14**	**15**	**16**	**17**	Kan
Gram (+) bacteria							
Bacillus cereus	7.2	-	8.8	7.6	8.4	-	31.9
Bacillus megaterium	8.6	-	9.2	8.1	9.8	-	34.2
Staphylococcus aureus	-	-	7.6	7.0	7.4	6.6	31.6
Bacillus subtilies	-	-	7.2	8.0	7.8	-	30.1
Sarcina lutea	-	-	7.5	6.9	8.2	7.2	26.9
Gram (-) bacteria							
Escherichia coli	7.1	-	7.9	7.6	8.6	6.6	34.2
Pseudomonas aepuginosa	-	-	6.1	6.4	7.3	-	34.8
Salmonella paratyphi	-	-	-	-	7.0	-	26.9
Shigella boydii	-	-	6.6	7.1	7.2	7.1	30.2
Shigella dysenteriae	7.4	-	7.3	8.6	8.8	-	36.9
Vibrio mimicus	-	-	7.8	8.3	8.6	-	29.8
Vibrio parahemolyticus	-	-	-	-	8.4	6.6	31.9
Shigella flexneri	NT	NT	NT	NT	NT	NT	33.2
Shigella sonnei	NT	NT	NT	NT	NT	NT	31.5
Fungi							
Aspergillus niger	-	-	9.6	10.5	12	-	28.9
Candida albicans	9.8	-	8.5	10	8.7	7.6	30.7
Rhizopus oryzae	-	-	-	-	-	-	32.6
Saccharo myces cerevaceae	8.6	-	10.0	-	8	6.4	34.5

Interpretation of sensitivity test results:

Gram (+) bacteria:
> 18 mm (M.DIZ) = sensitive
14-18 mm (M.DIZ) = intermediate
< 14 mm (M.DIZ) = resistant

Gram (-) bacteria:
> 16 mm (M.DIZ) = sensitive
13 – 16 mm (M.DIZ) = intermediate
> 13mm (M.DIZ) = resistant.

"-" indicates no sensitivity or zone of inhibition lower than 6 mm and NT refers to "Not Tested"

Part-I

Section-4

REFERENCES

REFERENCES

1. Chem Bhare, R.V. Khadse, B.G. Bobde, A.S. Bahekar and R.H. Eur *J. Med. Chem.,* 38, 89, 2003 and references therein.
2. H. Singh, K.N. Shukia, R.Dwivedi and Y.L.D. Singh, *J. Agric. Food Chem.,* **38**(7), 1483, 1990.
3. N.G. Gawande and M.S. Shingare, *Indian J. Chem.,* **26**(B), 387, 1987.
4. M.R. Chaurisia, A.K.M. Sharma and K.R. Shukia, *Indian Phys. Nat. Sci.,* 7(A), 18, 1987.
5. I. Mitsuhiro, K.Nakayama and Y. Hayase, *Heterocycles* (Tokyo)., **27**(11), 2635, 1988.
6. A.Z.M.S. Chowdhury, M.S. Rahman and M.N. Anwar, *Bangladesh J. Microbiol.,* **16**(2), 101-105, 1999.
7. Krzysztof walczak, *5th Blue Danube Symphosium of Heterocyclic Chemistry* (Proceedings)., 154s, 1994.
8. R. Gupta, S. Paul, A.K. Gupta, P.K. Kachroo and S. Bani, *Indian J. Chem.,* **36**(B), 707, 1997.
9. A.Z.M.S. Chowdhury, M.M. Matin and N.M. Anwar, *Chittagong University Studies,* Part-II (Science)., **21**(2), 79, 1997.
10. M. Shehab, *M.Sc. Thesis,* Department of Chemistry, University of Chittagong, Bangladesh, 1998.
11. M.S. Rahman, M.N. Anwar, J. Begum and Chowdhury, *Bang. J. Bot.,* **26**(1), 79-81, J.U., 1997.
12. S.M. Shahed, *M.Sc. Thesis,* Department of Chemistry, Chittagong Univ., Bangladesh, 1997.
13. A.K.M.S. Kabir, M.M. Matin, M.A.U. Mridha and S.M. Shahed *Chittagong University J. Sci.,* **22**(1), 41, 1998.

14. M. Fakruddin, *M.Sc. Thesis,* Department of Chemistry, University of Chittagong, Bangladesh, 2000.

15. A.K.M.S. Kabir, M.M. Matin and S.M. Abe Kawsar, *Chittagong University J. Sci.,* **22**(1), 39, 1998.

16. A.K.M.S. Kabir, M.M. Matin and S.M. Abe Kawsar, *Chittagong University J. Sci.,* **22**(2), 41, 1998.

17. M.W. Khan, M.J. Alam, M.A. Rashid and *J. Biorg. Med. Chem.,* **13**, 4796, 2005.

18. M. W. Khan and A.F.G.M. Reza, *Tetrahedron.,* **61**, 11204, 2005.

19. A.W. Bauer, W.M.M. kirby and M. Turk, *American J. Clinic. Pathol.,* **46**, 439, 1966.

20. M.A.T. Miah, H.U. Ahmed N.R. Sharma, A. Ali and S.A. Miah, Antifungal activity of some plant extracts, *Bangladesh J. Bot.,* **19**(1), 5, 1990.

21. R.K. Grover and J.D. Moore, *Phytopathology.*, **52,** 876,1962.

22. R. Roland, Antibiotics, An Introduction, F. Hoffman La Roche and Co., Basle, Switzerland, 1982, P 70 – 71.