SYNTHESES AND ANTIMICROBIAL STUDY OF HETEROCYCLIC COMPOUNDS CONTAINING QUINOXALINE MOIETY

M.Sc. THESIS

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF

MASTER OF SCIENCE (M.Sc.) IN CHEMISTRY

SUBMITTED BY

MD. DIDARUL KARIM

STUDENT ID: 1017032602 F

REG. NO.: 1017032602

SESSION: OCTOBER-2017

OCTOBER- 2022

SYNTHETIC ORGANIC CHEMISTRY LABORATORY

DEPARTMENT OF CHEMISTRY

BANGLADESH UNIVERSITY OF ENGINEERING AND TECHNOLOGY (BUET), DHAKA-1000

DEPARTMENT OF CHEMISTRY

BANGLADESH UNIVERSITY OF ENGINEERING AND TECHNOLOGY (BUET) DHAKA-1000

The thesis titled **"SYNTHESES AND ANTIMICROBIAL STUDY OF HETEROCYCLIC COMPOUNDS CONTAINING QUINOXALINE MOIETY"** submitted by **MD. DIDARUL KARIM**, Roll No.: 1017032602 F, Registration No.: 1017032602, Session: **October-2017** has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Master of Science (M.Sc.) in Chemistry on **October -22, 2022**.

Board of Examiners

1. **Dr. Md. Abdur Rashid**
   Professor — Chairman
   Department of Chemistry, BUET, Dhaka
   (Supervisor)

2. **Dr. Al-Nakib Chowdhury**
   Professor and Head — Member (Ex-Offico)
   Department of Chemistry, BUET, Dhaka

3. **Dr. Shakila Rahman**
   Professor
   Department of Chemistry, BUET, Dhaka — Member

4. **Dr. Md. Aminul Haque**
   Professor of Chemistry — Member(External)
   Jagannath University, Dhaka.

DEPARTMENT OF CHEMISTRY

BANGLADESH UNIVERSITY OF ENGINEERING AND TECHNOLOGY (BUET), DHAKA-1000

STUDENT'S DECLARATION

I hereby certify that this material, which I now submit for assessment on the program of study leading to the award of M.Sc. is entirely my own work, and I also declare this thesis or any part of it has not been submitted elsewhere for the award of any degree or diploma.

Signature of the Student

Date:……..

……...……

MD. DIDARUL KARIM

(Candidate)

St. ID: 1017032602 F

Dedicated

to

My Beloved Parents

&

Honorable Supervisor

Acknowledgement

I humbly acknowledge my deepest gratitude to the Almighty, the most gracious, benevolent and merciful creator for His infinite mercy bestowed on me in carrying out the research work presented in the dissertation.

I am extremely delighted to express my deepest gratitude and sincerely thank to my supervisor, **Prof. Dr. Md. Abdur Rashid**, for his helpful advice, keen interest, thoughtful suggestions and encouragement offered throughout the progress of my research work. It is obvious that his attributive contribution and efforts have greatly shaped me into what I am today. In fact, I am quite lucky to be a part of his ambitious research team.

I am thankful to all other respected teachers of the Department of Chemistry, BUET, for their continuous support. I would also like to thank all of the officers and staffs of the Department of Chemistry, BUET for their relentless help during my study period. My special thanks to Md. Moniruzzaman, Department of Chemistry, BUET, Dhaka for his cooperation in taking FT-IR spectrums.

I am highly thankful to Md. Emdad Hossain, Scientist, WMSRC, Jahangirnagar University for spectroscopic data of my samples.

I am highly grateful to all members of the board of examiners for their valuable suggestions and appreciated comments.

I am thankful to my all lab-mates Sabikun Nahar Marufa, Zannatul Ferdous Sonia,Sanjay Belowar, Sanjoy biswas for their friendly cooperation and lovely encouragement throughout my research period.

Finally, I would like to express my heartfelt indebtedness and profound gratitude to my beloved parents for their continuous inspiration and immeasurable sacrifices throughout the period of my study.

Author
(Md. Didarul Karim)

Contents

List of Abbreviations of Technical Symbols and Terms

Elaborations	Abbreviations
Broad singlet	bd, s
Singlet	s
Doublet	d
Double doublet	dd
Triplet	t
Multiplet	m
Hertz	Hz
Mega Hertz	MHz
Infrared spectroscopy	IR
Nuclear Magnetic Resonance	NMR
Proton NMR	$^{1}H - NMR$
Carbon-13 NMR	$^{13}C - NMR$
Coupling Constant	J

ABSTRACT

Heterocyclic compounds containing quinoxline moiety have received considerable attention during the last two decades as they possesses different pharmacological and biological activities. This study aims to synthesize and characterize heterocyclic compounds containing quinoxaline moiety. The starting compounds 3-methylquinoxalin- 2(1H)-one was prepared from *o*-phenylenediamine and pyruvic acid upon refluxing in ethanol for 3 hours in single step. Then the different hydrazonomethyl Quinoxaline compounds were synthesized from the coupling reaction between different diazonium salts and 3-methylquinoxalin-2(1H)-one as the active methylated compound. The structures of all the synthesized compounds were elucidated by the spectroscopic methods such as IR, ^{1}H NMR and ^{13}C NMR. Then the synthesized compounds were evaluated for their in vitro antibacterial activity against one Gram-positive (G+ve) bacteria *Staphylococcus aureus(+)*, five Gram-negative (G-ve) bacteria *Bacillus subtilis(-), Escherichia coli (-), Pseudomonas aeruginosa (-), Salmonella typhimurium (-) ,Citrobacter freundii (-)* and antifungal activity against two fungi *Aspergillus niger* and *Tricodarma harzianum*. All the tested compounds show moderate activity against one Gram-positive, five Gram-negative (G-ve) bacteria and two fungi.

Keywords: Heterocyclic; quinoxline; diazonium salt, coupling reaction;

CHAPTER-1

Introduction

1.1 Introduction

Quinoxalines have played a crucial role in the history of heterocyclic chemistry and also been used extensively as important synthons in organic synthesis. The chemistry of quinoxalines have attracted the focus of the scientific community in the past ten years **[1]** due to their potential applications as antiviral **[2],** antibacterial **[3],** antiprotozoal **[4],** anticancer **[5],** antidepressant **[6]** agents and as kinase inhibitors **[7].** They are also used in the agricultural fields as fungicides, herbicides and insecticides **[8].** Quinoxaline derivatives are highly potent NMDA receptor antagonist **[9].** In addition, quinoxaline moieties are present in the structure of various antibiotics such as echinomycin, levomycin, and actinoleutin, which are known as inhibitor of the gram-positive bacteria and they are active against various transplantable tumors **[10].** Moreover, quinoxaline containing condensed pyrimidine rings have been used as effective antitumor agents **[11],**herbicide antidotes **[12],** antibacterials **[13],** antileishmanial **[14],** and anticancer **[15].** Several dihydrobenzo and imidazo quinoxaline derivatives have been tested and presented antimicrobial activity as antifungal **[16]** and antibacterial agents **[17].** The antibacterial activity observed covers the Gram-positive and Gram-negative bacteria.

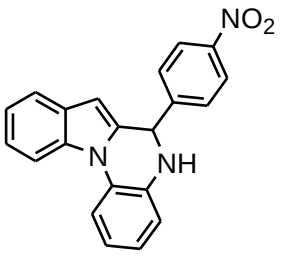
1.2 Application in Agriculture

Quinoxaline have versatile application in the field of agriculture. They are used in the agricultural fields as fungicides, herbicides and insecticides.

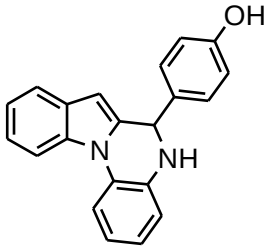
a) Antifungal Activity

The chemicals or biological organisams that are used to kill fungus and fungal spores are called fungicide. Fungicides are very important in agriculture because the fungus causes serious damage to crop . A series of new 5,6-dihydro-indolo[1,2-*a*]quinoxaline derivatives exhibited promising antifungal activities in vitro against the phytopathogenic fungi **[18].** The antifungal activity of 5,6-dihydro-indolo[1,2-*a*]quinoxaline derivatives **1-17** against five phytopathogenic fungi (i.e., Fusarium graminearum, Pyricularia oryzae, Fusarium oxysporum f. sp. vasinfectum, Alternaria alternata, and Alternaria brassicae) were investigated at the concentration of 50mg/mL in vitroby poisoned food technique**[19].** Hymexazol, a commercially available agricul-tural fungicide, was used as a positive control at 50mg/mL. among all the derivatives, compounds **2, 5, 7 ,14** and **15** exhibited the good and broad spectrum of antifungalactivities against the five phytopathogenic fungi. Meanwhile, some interesting results through a comparative study on the SAR of **1-17** were found as follows: (1) Generally, when the hydroxy group was introduced at the 4-position on the E-ring, the

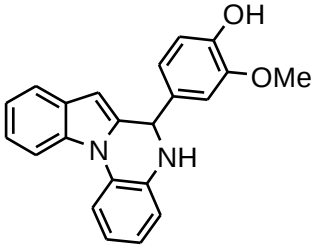
corresponding derivatives showed the potent activities. Interestingly, if the methoxy group was simultaneously introduced at the 3-position on the E-ring of **2** or **7** to give **3** or **11** the corresponding antifungal activities was decreased sharply **(2** vs. **3**; **7** vs. **11**). Moreover, introduction of hydroxyl group at the 2-position on the E-ring of 8 gave 9, the antifungal activities of which were decreased as compared with **7** bearing hydroxy group at the 4-position on the E-ring. (2) Introduction of the electron-withdrawing group (e.g.,cyano) on the A-ring of **1** usually led to the more potent compound than those possessing the electron-donating group (e.g., methyl) on the A-ring (**15** vs. **4**; **6** and **13**). (3) When the methyl group was introduced at the 6-position on the C-ring of **8** to give **16**, or the E-ring of **5** was substituted by the furanyl ring to afford **17**, the corresponding activities of **16** and **17** were not increased at all.



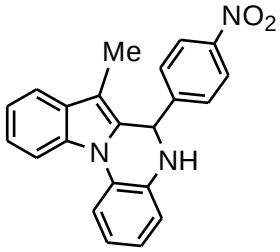
1



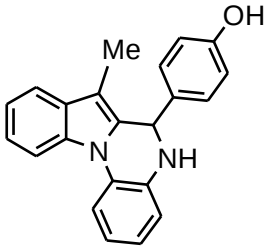
2



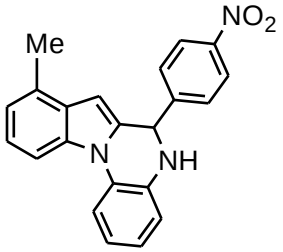
3



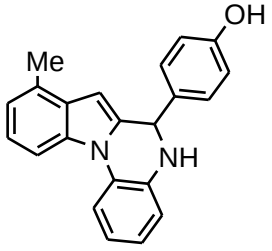
4



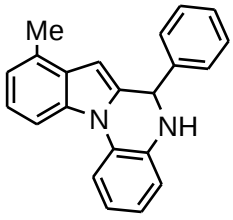
5



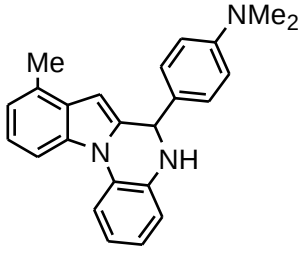
6



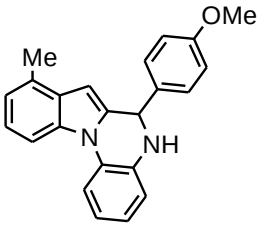
7



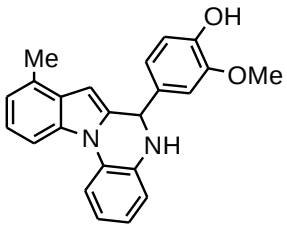
8



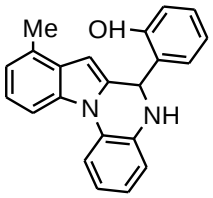
9



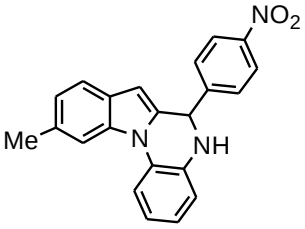
10



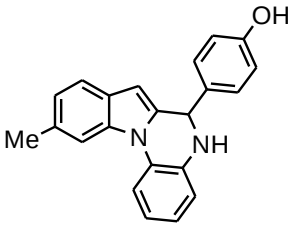
11



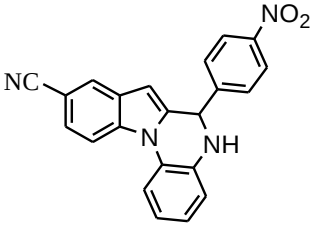
12



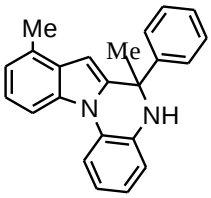
13



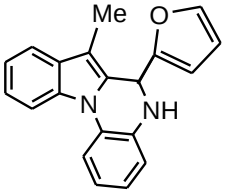
14



15



16



17

b) Growth promoting effect on plants

Many organic compounds show growth promoting effect on plants. 2-hydroxy substituted quinoxaline**18-21** showed significant on crop plants growth **[20]**. The bed of black cotton soil with fairly good drainage was prepared on an open field. The seed of three species like soybean, groundnut and chickpea under examination were so wed in these beds separately by conventional method. The plant bends were irrigated as and when required with tap water. The plants from each bed were divided into two groups A and B. The group A plants were keptunsprayed and termed as control group whereas treated group B plants were sprayed with the compound being tested. The seeds of group B were also treated with test compounds before sowing to screen growth promoting effects. The spraying solution of synthesized quinoxaline was prepared in l,4- dioxane (0.0l M) separately and sprayed at fortnightly intervals (l5, 30, 45, 60 and 75 days). All the field experiments were conductedto compare the treated plants of group B with the plants from control group A. The samples were taken at l5, 30, 45, 60 and 75 days after sowing. The plants were

carefully examined and height, number of functional leaves per plant, number of pods per plant and seed yields per hectare was recorded.

When the comparison of morphological character was made between those of treated and controlled group plants, it was interesting to note that all treated plants exhibited remarkable growth and considerable increase in height, number of leaves, number of pods per plant and yield as compared to the untreated ones .

Soybean

In soybean crop, L_2 ligand shows the maximum height, highest number of leaves, maximum number of pods per plant and maximum yield. But control plant shows minimum value of results than treated plant. The trend of ligands as follows,

$L_2 > L_3 > L_l > L_4 >$ Control

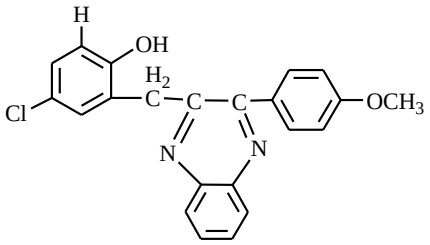
Groundnut

In groundnut crop, L_4 ligand shows the maximum height as compared to other ligands. Maximum number of leaves found in ligand L_l. L_3 ligand shows maximum number of pods per plant and yield than other ligands. But the control plants show minimum values as compared to other all treated plants.

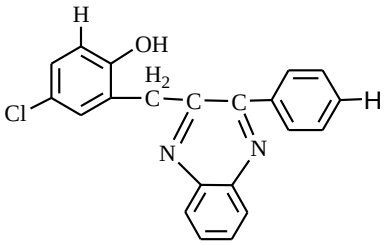
Chickpea

In the crop chickpea, L_4 ligand shows the maximum number of branches, number of pods per plant and yield at 30, 60 and 75 days as compared to other ligands. The activity trend of ligands as follows,

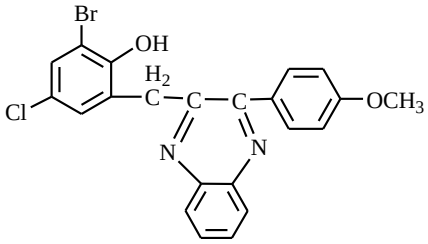
$L_4 > L_2 > L_3 > L_l >$ Control



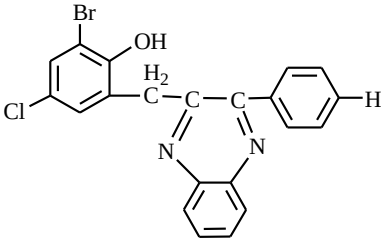
18(L1)



19(L2)



20(L3)



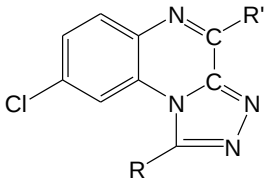
21(L4)

1.3 **Medicinal Applications Of Quinoxaline Derivatives**

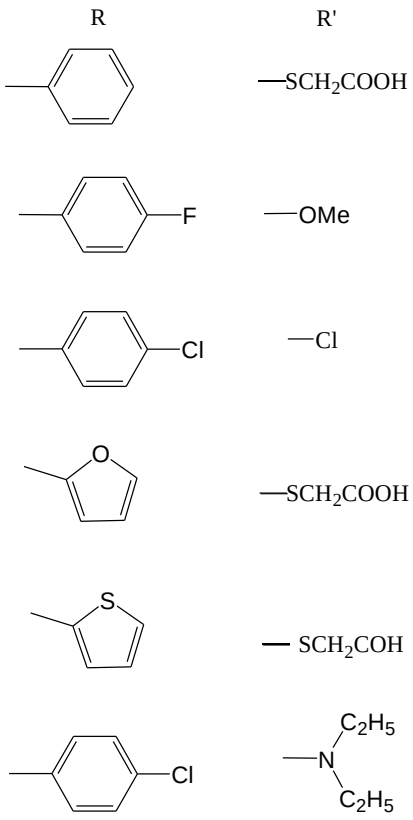
Applications of Quinoxaline derivatives in the field of medicine can't be neglected. These are being used in all aspects of medicine.

a) Antibacterial activity of Quinoxaline derivatives

Quinoxaline derivatives are potentially active as antibacterial agents.A new series of 8-chloro-1,4-substituted-[1,2,4]triazolo[4,3-a]quinoxaline**22** derivatives was synthesized and screened for antibacterial activity**[21].** The Antibacterial activity was screened against *Staphylococcus aureus*, *Bacillus subtilis* (Gram-positive bacteria) and *Proteus vulgaris, Klebsiella pneumoniae* (Gram-negative bacteria) using chloramphenicol as reference drug.



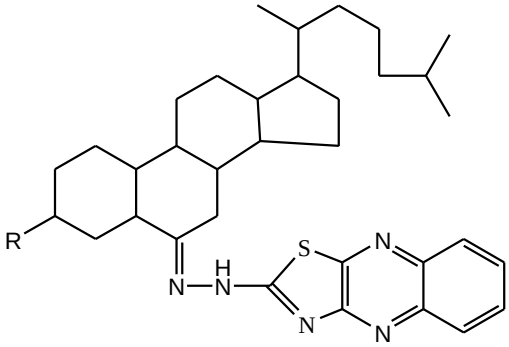
22



Salman et al reported the synthesis of different steroidal thiazolo quinoxaline derivatives as antibacterial agents against *Escherichia coli***[22**]. Steroidal ketone thiosemicarbazones were obtained from corresponding ketones by refluxing with thiosemicarbazide. The thiosemicarbazones on reaction with 2,3-dichloroquinoxalines at 80^{o}C gives 3b-acetoxy-5a-cholestan-6-[thiazolo(4,5-*b*)quinoxaline-2-yl-hydrazone] **23a,**

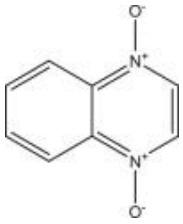
3b-chloro-cholestan-6-[thiazolo(4,5-*b*)quinoxaline-2-yl-hydrazone] **23b**, and 5a-cholestan-6-[thiazolo(4,5-*b*)quinoxaline-2-yl-hydrazone]**23c.** The structures of the compounds were evident by elemental, IR, ^{1}H NMR and FAB mass spectral analyses. The antibacterial activities of these compounds were evaluated by disk diffusion method against the culture of *E.coli* and the results were compared with the standard drug amoxicillin. The results reveal that the compounds are better

antibacterial agents as compared to amoxicillin. Among all the three compounds, compound **23b** showed better zone of inhibition.

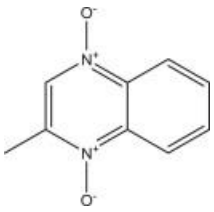


23a: R= CH_3C00
23b : R=Cl
23C : R= H

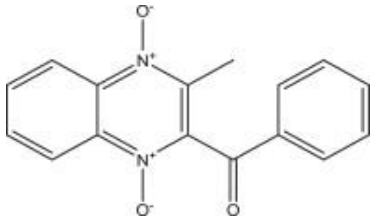
Quinoxaline is a chemical compound that presents a structure that is similar to quinolone antibiotics. The present work reports the study of the antimicrobial activity of quinoxaline N,N-dioxide and some derivatives against bacterial and yeast strains**[23**]. The compounds studied were quinoxaline-1,4-dioxide **24,** 2-methylquinoxaline-1,4-dioxide **25,** 2-methyl-3-benzoylquinoxaline-1,4-dioxide **26,** 2-methyl-3-benzylquinoxaline-1,4-dioxide **27,** 2-amino-3-cyanoquinoxaline-1,4-dioxide **28,** 3-methyl-2-quinoxalinecarboxamide-1,4-dioxide **29**, 2-hydroxyphenazine-N,N-dioxide **30** and 3-methyl-N-(2-methylphenyl)quinoxalinecarboxamide-1,4-dioxide **31**. The prokaryotic strains used were Staphylococcus aureus ATCC 6538, S. aureus ATCC 6538P, S. aureus ATCC 29213, Escherichia coli ATCC 25922, E. coli S3R9, E. coli S3R22, E. coli TEM-1 CTX-M9, E. coli TEM-1, E. coli AmpC Mox-2, E. coli CTX-M2 e E. coli CTX-M9. The Candida albicans ATCC 10231 and Saccharomyces cerevisiae PYCC 4072 were used as eukaryotic strains. For the compounds that presented activity using the disk diffusion method, the minimum inhibitory concentration (MIC) was determined. The alterations of cellular viability were evaluated in a time-course assay. Death curves for bacteria and growth curves for S. cerevisiae PYCC 4072 were also accessed. The results obtained suggest potential new drugs for antimicrobial activity chemotherapy since the MIC's determined present low values and cellular viability tests show the complete elimination of the bacterial strain.



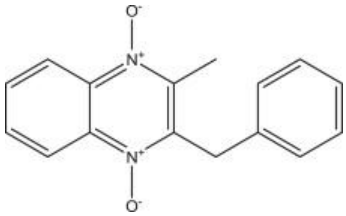
24



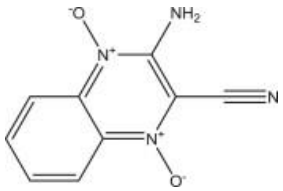
25



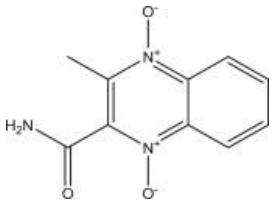
26



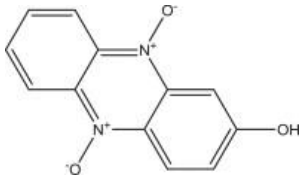
27



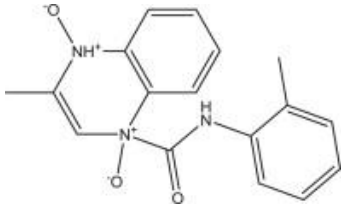
28



29



30

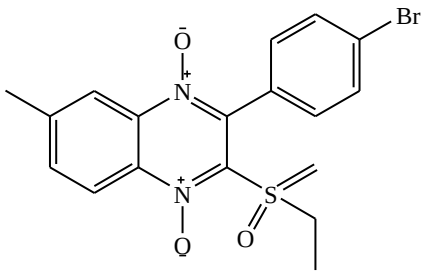


31

b) Anticancer activity of Quinoxaline derivatives

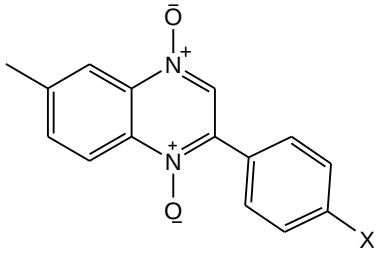
Quinoxaline nucleuses are known to exhibit potential anticancer activity. The search for anticancer drugs led to the discovery of several quninoxaline derivatives. 3-(4-Bromophenyl)-2-(ethyl sulfonyl)-6-methyl quinoxaline-1,4-di-*N*-oxide**32** or **Q39** was synthesized from quinoxaline-1,4-di-*N*-oxide and evaluated for in vitro anticancer activity in hypoxia **[24].** Cytotoxic assay demonstrated that **Q39** is a potential and highlyefficient anti-cancer compound in all the tested cell line with IC50 values of 0.18±0.03- 8.88 ±1.12µM in

hypoxia and 0.33 ±0.04-8.74 ±1.28 µM in normoxia. The mechanism of **Q39** in hypoxia confirmed that this compound could cause the opposite of K562 cell ina time dependent manner.



32

A new series of quninoxaline-1,4-di-*N*-oxides and fused quninoxaline-di-*N*-oxides were synthesized **33** and evaluated for hypoxic-cytotoxic activity on EAC cellline [**25**], compound **33a** was the most potent cytotoxine with IC_{50} 0.9 µg/mL, potency 75 µg/mL and was approximately 15 times more selective cytotoxine (HCR>111) than 3- aminoquinoxaline-2-cabonitrile which had been used as a standard (HCR>7.5).



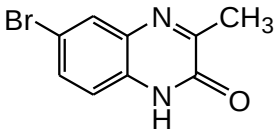
33

a : X = Cl

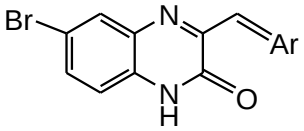
b : X = CH_3

In an effort to develop potent anti-cancer agents, some substituted quinoxaline derivatives have synthesized **[26**]. Reaction of 6-bromo-3-methylquinoxalin-2(1*H*)-one **34** with aromatic aldehydes furnished the styryl derivatives **35a–e**. Alkylation of **34** with ethyl chloro acetate produced the *N*-alkyl derivatives **36**. Hydrazinolysis of the ester derivative **36**with hydrazine hydrate afforded the hydrazide derivative **37**. In addition, chlorination of **34**with phosphorus oxychloride afforded the 2-chloro derivative **38** which was used as a key intermediate for the

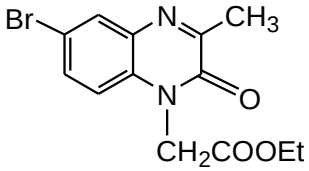
synthesis of substituted quinoxaline derivatives **39–41**,*N*-pyrazole derivative **42**, tetrazolo[1,5-*a*]quinoxaline derivative **43**,thiophenol derivatives **44**,aminophenols such as o-amino or/and p-aminopheno derivatives **45a,b**, 4-hydroxybenzaldehyde derivative **47** and Schiff base derivatives **46, 48** via reaction with several nucleophiles reagents. Docking methodologies were used to predict their binding conformation to explain thedifferences of theirtested biological activities. All the tested compoundswere screened in vitro for their cytotoxic effect on three tumor cell lines. Some new quinoxaline derivatives were stud- ied as inhibitors of c-Met kinase, a receptor associated with high tumor grade and poor prognosis in a number of human cancers. Compounds **35e, 37, 40a, 45a, 45b** and **46** showed the highest binding affinity with CDOCKER energy score, while showed the lowest IC_{50} values against three types of cancer cell lines. It is worth to mention that, compounds **35e, 40a** and**46**showed comparable inhibition activity to the reference drug, while compound**45a** showed a more potent inhibition activity than Doxorubicin.



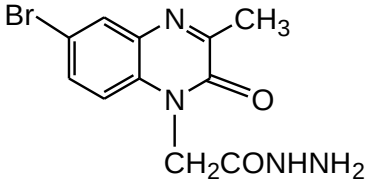
34



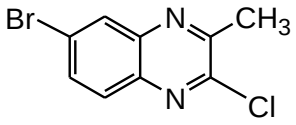
35a-e
Ar ; a= 4-C6H4Cl
b=4-C6H4OH
c= 4-C6H4CH3
d= 4-C6H4CH3
e = 4-C6H4OCH3



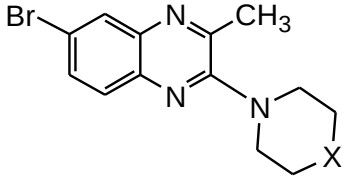
36



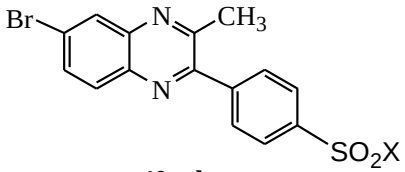
37



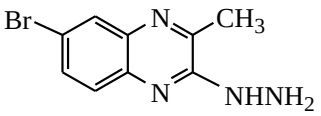
38



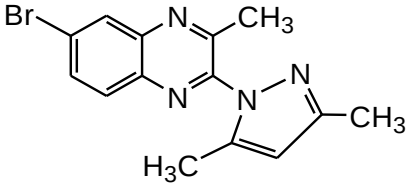
39,a,b
a,X=CH2
b, X= O



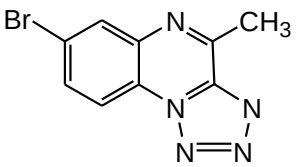
40,a,b
a,X= N
b, X= NH(CH2)3CH3



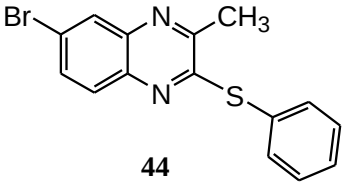
41



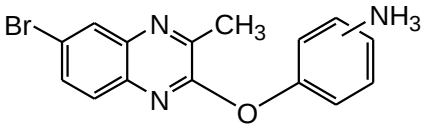
42



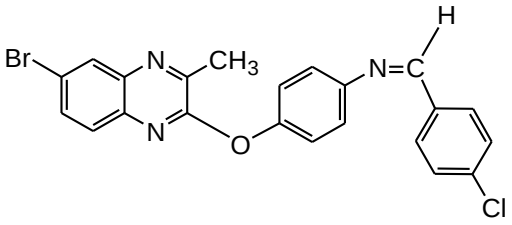
43



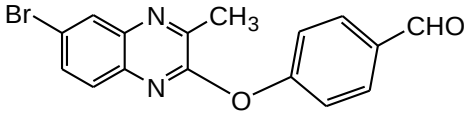
44



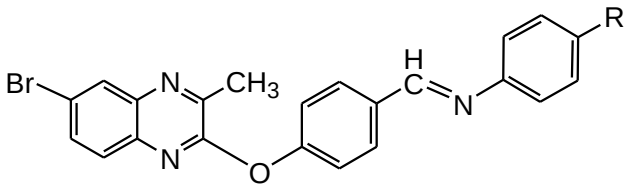
45a, O^-
45b, P^-



46



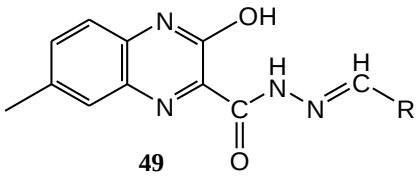
47



48a,R= H
48b, R=Cl

c) Quinoxaline derivatives as Anti-inflammatory

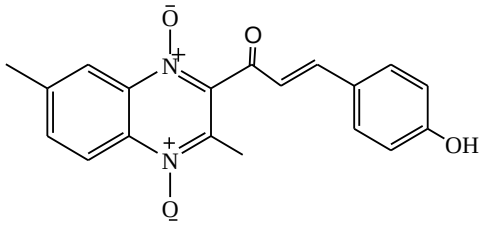
Ten substituted quinoxaline derivatives **49a-j**were synthesized and some of these com- pounds **49a, 49e, 49f, 49g, 49i & 49j**evaluated for their anti-inflammatory activity against the carrageenan-induced rat paw edema in albino Wister rats; out of which compound **49f, 49g& 49j**showed significant anti-inflammatory activities [**27].**



49

49f, R = 4-F-C_6H_4
49g, R = 2-Cl-C_6H_4
49j, R = 3-OH-C_6H_4

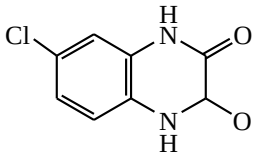
Burguete et al reported the synthesis and anti-inflammatory activities of novel ring rat substituted 3-phenyl-1-(1,4-di-*N*-oxide quinoxalin-2-yl)-2-propen-1-ones and their 4,5- dihydro-(1H)-pyrazole analogues**.[28**]. The tested compounds inhibited carrageenan-induced paw edema method (4.5–56.1%). Compound **50a** emerged as promising candidate with56.1% edema inhibition and soybean lipoxygenase inhibition (IC50 < 1M).



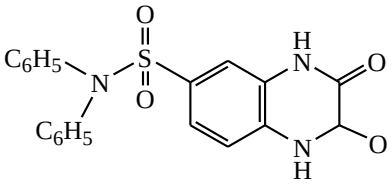
50a-c, R = H, F, CH_3O

d) Anticonvulsant activity of Quinoxaline derivatives

For anticonvulsant activity a large number of quinoxaline derivatives were evaluated and found to possess significant activity against various types of seizures. In the search of new anticonvulsant agents having quinoxaline nucleus Olayiwola et al **[29]** synthesized eight quinoxalinones and investigated for neuropharmacological effects (analgesia, sedation, convulsion, anxiety, memory and psychosis) in mice and rats. From the eight compounds 6-Chloro-1,4-dihydro-quinoxaline-2,3-dione **51** and N,N-dibenzyl-2,3-dioxo-1,2,3,4-tetrahydroquinoxaline-6-sulfonamide **52** showed significant anticonvulsant action.

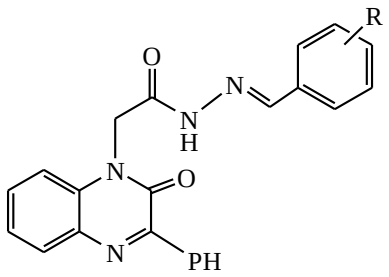


51



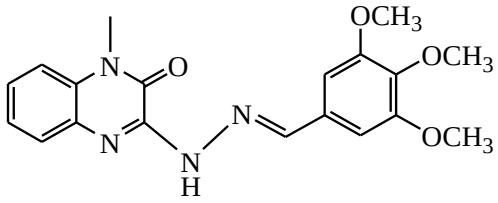
52

In view of expected anticonvulsant activity, some new derivatives of quinonxaline **53$_{a-g}$**were designed and synthesized by condensation of different aromatic aldehydes with 2-(2-oxo-3-phenyl-quinoxalin-1(2H)-yl)acetohydrazide [**30**]. All synthesized compounds were isolated and confirmed by IR, 1H-NMR, MS, elemental analysis and then tested as anticonvulsant agents.Compound **53$_c$**and **53$_a$**showed the highest anticonvulsant effect with anticonvulsant potency relative to Phenobarbital sodium of 0.8 and 0.75 whereas compound **53$_e$** exhibited the lowest relative potency of 0.09.The other compounds showed variable activity between these values as follows: **53$_b$**=0.19, **53$_d$**=0.41,**53$_f$**=0.1 and **53$_g$**=0.15. All compounds showed less activity than the reference compound phenobarbital. But the compounds provided a basis for further optimization.



53,a-g
53a, R= H
53b, R= 4-CH_3
53c, R= 4-OCH_3
53d, R= 4-Cl
53e, R= 4-OH
53f, R= 4NO_2
53g, R= 3,4,5-$(OCH_3)_3$

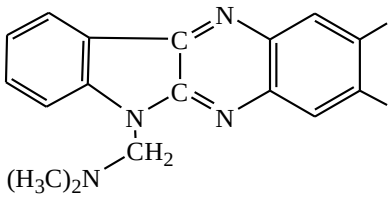
A new series of quinoxaline-2-one derivatives was synthesized and evaluated for their anticonvulsant activities **[31].** The anticonvulsant evaluation was carried out using pentylenetetrazole (PTZ) induced-convulsions mice model and phenobarbitone sodium as a standard. Docking studies were preformed to rationalize the anticonvulsant activity of the prepared compounds. There is a notable correlation between the calculated binding free energy and the in vivo anticonvulsant activity. The highest calculated binding free energy value was noticed for compound 3-[2-(3,4,5-Trimethoxybenzylidene)hydrazinyl]-1-methylquinoxaline-2(1H)-one**54**which revealed the highest anticonvulsant activity.



54

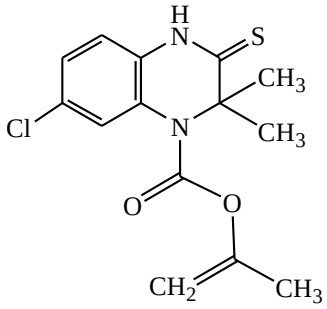
e) Antiviral activity of Quinoxaline derivaties

Great efforts have been directed to get potential antiviral agents having quinoxalinenucleus **.**Harmenberg et al **[32**] synthesized a series of al 6*H*-indolo-(2,3-b)quinoxalines and evaluated for antiherpes virus activity. The most active compound was 2,3-dimethyl(dimethylaminoethyl)5*H*-indolo-(2,3- b)quinoxaline**55**, named **B-220.**This compound was furthertested for antiviral effect and mechanism of action. Indoloquinoxalines in high concentrations (around 300, uM) inactivate virions, while at lower concentrations (around 3FM), synthesis of viral DNA and protein appears to bereduced. B-220 was tested against herpes simplex virus type-1 (HSV-1), cytomegalovirus (CMV), and varicella-zostervirus (VZV). A comparison was made with three previouslyknown active substances: acyclovir (ACV; 3) for HSV-1,phosphonoformate (PFA; 15) for CMV, and the guanosineanalog (RS)-9-[4-hydroxy-2-(hydroxymethyl)butyl]guanine(1) for VZV.



55

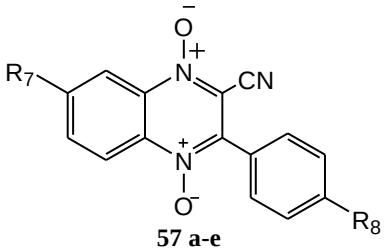
6-Chloro-3,3-dimethyl-4(isopropenyloxycarbonyl)-3,4-dihydroquinoxalin-2(1*H*)-thione (S-2720)**56**was synthesized by reacting 2,4-dichloronitrobenzene with 2-aminoisobutyric acid in the presence of KOH **[33].** The compound S-2720 was evaluated for enzyme activity,and was found to be a very potent inhibitor of both human immunodeficiency virus type1 reverse transcriptase (HIV-1 RT) activity and HIV-1 replication in tissue cultures. Likeother nonnucleoside RT inhibitors, S-2720 did not affect the HIV-2 RT.



56

f) Antimalarial Activity of Quinoxaline derivatives

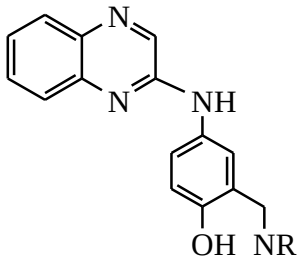
Plasmodium falciparum and *Plasmodium vivax* are the two major human malaria para- sites. *P. falciparum* is responsible for most deaths, and it has developed resistance to almost all available drugs. It is not surprising that the antimalarial activity of quinoxalines has generated a lot of interest. Many quinoxaline derivatives have been described for their potential antimalarial activity. A number of mono and di-*N*-oxide derivatives of quinoxaline heterocyclic system have been prepared and their biological activities against *Plasmo- dium falciparum* have been reported. Vicente et al [**34**] synthesized a new series of 3-phenylquinoxaline 1,4-di-*N*-oxide derivatives(**57)** and evaluated their in vitro antiplasmodial activity against *P. falciparum*. Cytotoxicity was also tested in KB cells by Alamar Blue assay. 21 Compounds out of 60 compounds were assayed against 3D7 C(Q-sensitive) showed enough activity to be also evaluated against K1 (CQ-resistant) strain. Ten of them were shown to be more active than chloroquine in the resistant strain. The most interesting compounds were 7-(methyl or methoxy)-3-(4-fluoro or chloro)phenylquinoxaline-2-carbonitrile-1,4-di-*N*-oxides **(57b, 57c, 57d & 57e)** because of their low IC50 and their high SI shown for the K1 strain, making them valid new leads.



57 a-e

	R_7	R_8
a	H	Cl
b	CH_3	Cl
c	OCH_3	Cl
d	CH_3	F
e	OCH_3	F

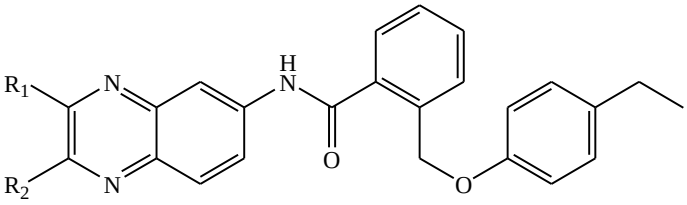
2-Arylaminoquinoxalines were prepared by the condensation of 2-chloroquinoxaline with the appropriate Mannich bases in the presence of HCl [**35**]. To synthesize the Mannich bases, 4-acetamidophenol was reacted with formaldehyde and dialkylamine to yield 3-[(dialkylamino)methyl]-4-hydroxyacetanilide, followed by hydrolysis. Antimalarial activities of the new aryl aminoquinoxalines were evaluated against the rodent malaria parasite Plasmodium yoeliiata dose of 75 mg kg−1. Three compounds synthesized (2-[3-[(diethylamino) methyl]-4-hydroxy-anilino]-quinoxaline dihydrochloride **58b**, 2-[3-[(pyrrolidinyl) methyl]-4-hydroxyanilino]-quinoxaline dihydrochloride **58f**, and 2-[3-[(piperidinyl) methyl]-4-hydroxyanilino]-quinoxaline dihydrochloride **58g** showed moderate antimalarial activity.



58b, Diethylamino
58f, Pyrrolidinyl
58g, piperidinyl

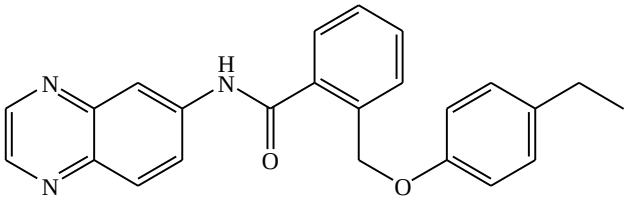
g) Antileishmanial Activity of Quinoxaline derivatives

Leishmaniasis is a widespread parasitic disease that is caused by protozoan parasites of thegenus Leishmania in tropical and subtropical areas of the World. It occurs in two major forms; cutaneous/muco-cutaneous (CL) and visceral leishmaniasis (VL, or Kala-azar), depending uponparasite species. *L. donovani* and *L. infantum* are major causative agents of VL, while *L.major, L. tropica, L. aethiopica, L. braziliensis, L. panamensis, L. amazonensis* and *L.mexicana* cause CL [**36**].A series of 29 new quinoxaline derivatives were synthesized andevaluated for their in vitro antiparasite activities against several parasites (*L. donovani,Trypanosoma brucei brucei*, and *Trichomonas vaginalis*) [**37].** Several of these compoundsdisplayed interesting activities, and particularly four quinoxaline amides showedsignificant antileishmanial properties (IC50 less than 20 μM). Compounds **59a**, **59b**, **59c**,and **60**were the most active ones (with IC50: 12.5, 8.2, 18.5, and 18.4 μM, respectively)being slightly less potent than the reference drug, miltefosine (IC50: 7.3 μM) against thepromastigote forms of *L. donovani*.



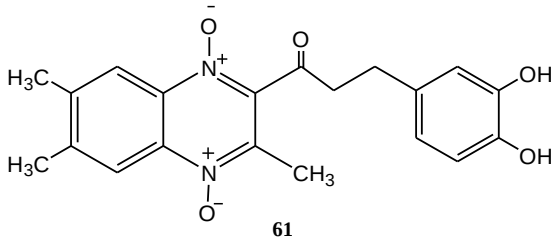
59a-c

59a: R_1=H, R_2= CH_3
59b : R_1=R_2= CH_3
59C : R_1=R_2= H



60

Burguete et al **[38]** synthesized a series of ring substituted 3-phenyl-1-(1,4-di-*N*-oxide quinoxalin-2-yl)-2-propen-1-one derivatives and tested for in vitro leishmanicidal activity against amastigotes of *L. amazonensis* in axenical cultures and murine infected macrophages. Structure-activity relationships demonstrated the importance of a radical methoxy at position R3‘, R4‘and R5‘. (2*E*)-3-(3,4,5-Trimethoxyphenyl) -1- (3,6,7- trimethyl-1,4-dioxy-quinoxalin-2-yl)-propenone **61** was the most active compound. Cytotoxicity on macrophages revealed that this product was almost six times more activethan toxic.

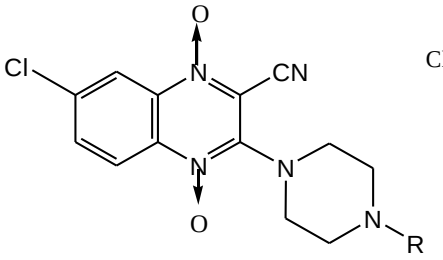


61

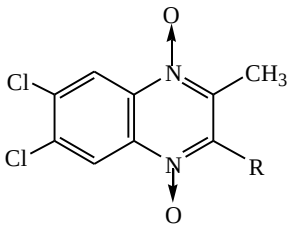
h) Trypanocidal Activity of Quinoxaline derivatives

The causative agent of Chagas’ disease or American trypanosomiasis is the haemo flagellate protozoan *Trypanosoma cruzi* (*T. cruzi*), which is transmitted in rural areas to humans and other mammals by reduviid bugs such as *Rhodnius prolixus* and Triatoma infestans [**39].** Aguirre et al

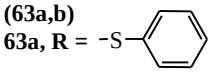
[**40**] reported the biological activity of 33 quinoxaline-N,N'-dioxide derivatives and related compounds against epimastigote form of two strain of *T. cruzi*. Compounds **62a**, **62b**, **63a** and **63b** emerged as the most active compounds against the Tulahuen strain. So, these were selected to study on Brener strain. All of selected derivatives showed similar bio-activities in both strain of *T. cruzi* as that of the reference drug, Nifurtimox. Compounds **64a** and **64b**, which displayed good ID50 in both the strains, presented an excellent electrophilic centre on the heterocycle's carbon-3 that could unselectively react with biological nucleophiles (amines and phosphates from DNA, thiols, alcohols and amines from proteins).



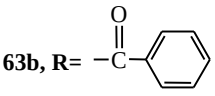
62a, R= -Ph-o-Me
62b, R= -Ph-m-CF_3

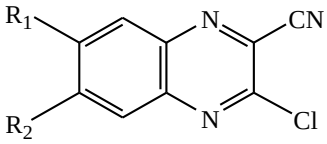


(63a,b)
63a, R =



63b, R=





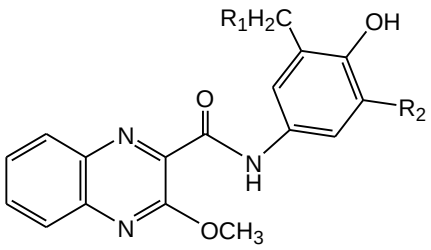
(64a,b)
64a, R_1= Cl ,R_2 = H

64b, R_1= R_2= CH_3

i) 5-HT_3 Receptor Antagonist Activity of Quinoxaline derivatives

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter involved in various pharmacologicaleffects in peripheral and central nervous systems. Fifteen 5-HT receptor subtypesbelonging to 7 major classes (5-HT1–5-HT7) have been reported so far. Recently, 5-HT3 receptor subtype has gained much attention because of the clinical use of 5-HT3 receptorantagonists

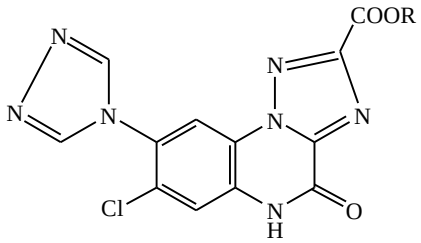
(RAs) in the treatment of cancer chemotherapy-induced nausea and vomiting,and also in postoperative nausea and vomiting. A new series of 3-substituted quinoxalin-2-carboxamides were synthesized as per the pharmacophoric requirement for 5-HT3 receptorantagonists [**41**]. The compounds were evaluated for 5-HT3 antagonisms in longitudinalmuscle-myenteric plexus preparation from guinea pig ileum against 5-HT3 agonist, 2-methyl-5-HT. Among the tested compounds, N-{3-[(4-methylpiperazin-1-yl)methyl]-4-hydroxyphenyl}-3-methoxyquinoxalin -2-carboxamide **65e** showed most favorable 5-HT3receptor antagonism.



65e, R_1= N-Methyl-Piperazinyl, R_2= H

j) AMPA Antagonist Activity of Quinoxaline derivatives

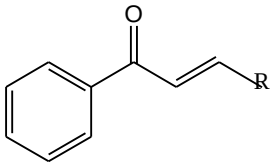
Quinoxaline derivatives are an interesting class of specific and potent competitive non- NMDA glutamate receptor antagonists. Two new compounds, 7-chloro-4,5-dihydro-8-(1,2,4-triazol-4-yl)-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylates, **66a** and **66b**, were synthesized and tested at iGluRs and analyzed for the influence of the 8-(1,2,4- triazol-4-yl) substituent at this crucial position in AMPA receptor recognition [**42].** Compound 7-chloro-4,5-dihydro-8-(1,2,4-triazol-4-yl)-4-oxo-1,2,4-triazolo[1,5- *a*]quinoxaline-2-carboxylic acid (**66b**, TQX-173) was found to be the most potent and selective AMPA antagonist from the electrophysiological data. The inhibitory action of**66b** on depolarization induced by 5 μM AMPA (IC_{50}=2.3□□ 0.4 μM) was much higher thanthat on NMDA-evoked responses (IC_{50} =46± 4 μM).



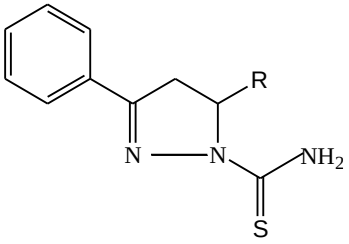
66a&b,
66a, R= Et
66b, R= H

k) Antiamoebic Activity of Quinoxaline derivatives

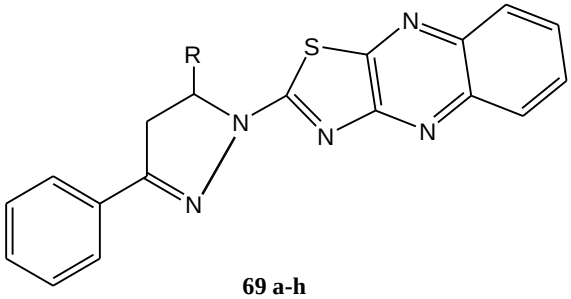
In vitro antiamoebic activities against HM1:IMSS strain of *E. histolytica* of several chalcones **67a-h**, amino-5-substituted-(3-phenyl(2-pyrazolinyl))methane-1-thiones **68a-h**and 2-(5-substituted-3-phenyl-2-pyrazolinyl)-1,3-thiazolino[5,4-b]quinoxalines **69a-h**have been reported**[43**]. The activity data showed that modification of the compounds from chalcones, topyrazolines, and further to quinoxalines, resulted in an increase in antiamoebic activity.



67 a-h



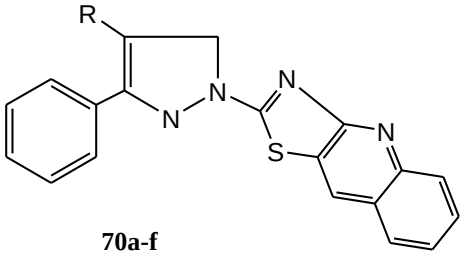
68 a-h



69 a-h

A new series of 1-N-thiocarboxamide-3-phenyl-2-pyrazolines1–6was synthesized by cyclization of different Mannichbases with unsubstituted thiosemicarbazide**[44**].The reaction of cyclized pyrazoline derivatives with 2,3-dichloroquinoxaline afforded the title compounds**70a-f**. The structures of the new compounds were confirmed by elemental analyses as well as ^{1}H, ^{13}C NMR,IR and electronic spectral data. TheHM1: IMSSstrain ofEntamoeba histolyticaparasite was cultured in vitro and the sensitivity ofthe parasite to the synthesized compounds was evaluated using the

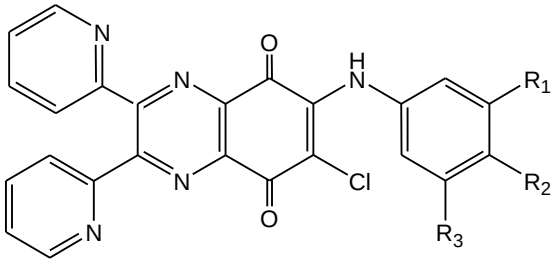
microdilution method.. The quinoxaline derivatives 70d, 70eand70f were found to be potent inhibitors ofE. histolytica.



70a-f
70a, X= H, R=H
70b, X= Br, R= H
70c, X= Cl, R= H
70d, X= H, R= CH3
70e, X= Br, R= CH3
70f, X= Cl, R= CXH3

l) Vascular smooth muscle cell proliferation inhibitor Activity of Quinoxaline derivatives

A series of 6-arylamino-2,3-bis(pyridin-2-yl)-7-chloro-quinoxaline-5,8-diones **71a-i**was synthesized and evaluated for their potential inhibitory activity on rat aortic smooth muscle cell (RAoSMC) proliferation [**45**]. Inhibition of proliferation of these cells was determined by WST colorimetric assay. The IC50 values were determined and compared to the positive control mycophenolic acid (MPA). Most of the test compounds exhibited good activity. In particular, quinoxaline-5,8-diones **71c, 71e**, and **71h**revealed potent inhibitory activities and were also comparable to that of MPA. In contrast, compounds **71b, 71f**, and **71g**exhibited relatively lower activity. These results suggested that 6-arylamino-2,3-bis(pyridin-2-yl)-7-chloro-quinoxaline-5,8-diones might be a promising lead for the development of potential inhibitors of SMC proliferations.

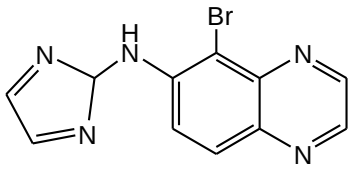


71a-i

	R_1	R_2	R_3
71a	H	Cl	H
71b	H	OH	H
71c	H	F	H
71d	H	CF_3	H
71e	H	OCF_3	H
71f	H	OCH_3	H
71g	H	H	H
71h	Cl	Cl	H
71i	F	F	F

m) Antiglaucoma Activity of Quinoxaine derivatives

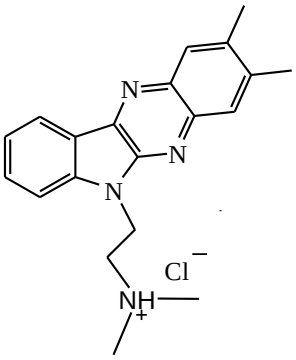
The importance of quinoxalines as pharmaceutical agents was manifested by themarketing of Brimonidine (5-bromo-*N*-(4,5-dihydro-1H-imidazol-2-y1)-6-quinoxaline**72**as an antiglucoma agent [**46]**. The drug acts through reducing the intraocular pressure, thus alleviating the symptoms of glaucoma.



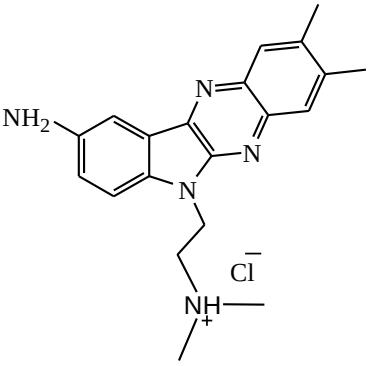
72

n) **Quinoxaline derivatives as DNA binder**

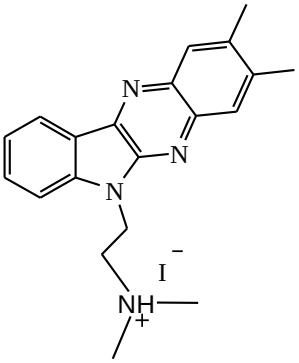
Wilhelmsson et al **[47]** synthesized five novel indoloquinoxaline**73-77** derivatives and investigate the DNA binding properties of these monomeric as well as dimeric compounds using absorption, fluorescence, and linear dichroism. Several of the mono- and dicationic derivatives presented have previously demonstrated an excellent antiviral effect that is higher than already acknowledged agents against human cytomegalovirus(CMV), herpes simplex virus type 1 (HSV-1), and varicella-zoster virus (VZV). We find that the DNA binding constants of the monomeric and dimeric derivatives are high ($\sim 10^6$) and very high ($\sim 10^9$), respectively. Results from the spectroscopic measurements show that the planar aromatic indoloquinoxaline moieties upon interaction with DNA intercalate between the nucleobases. Furthermore, we use poly(dAdT) $_2$ and calf thymus DNA in a competitive binding experiment to show that all our derivatives have an AT-region preference. The findings are important in the understanding of the antiviral effect of these derivatives and give invaluable information for the future optimization of the DNA binding properties of this kind of drugs.



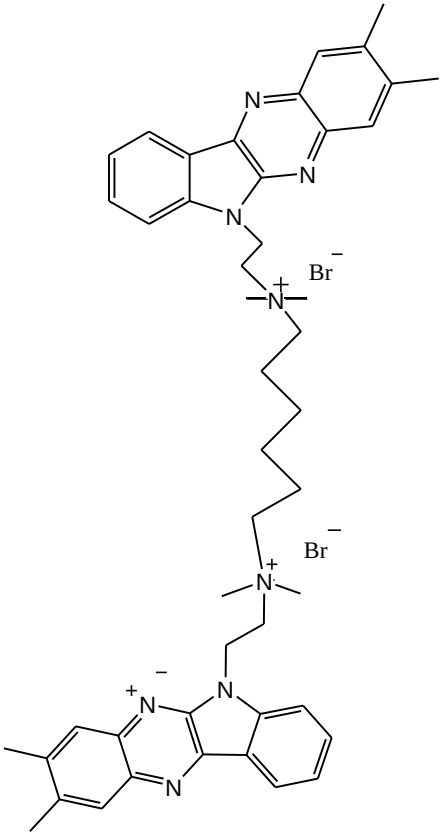
73



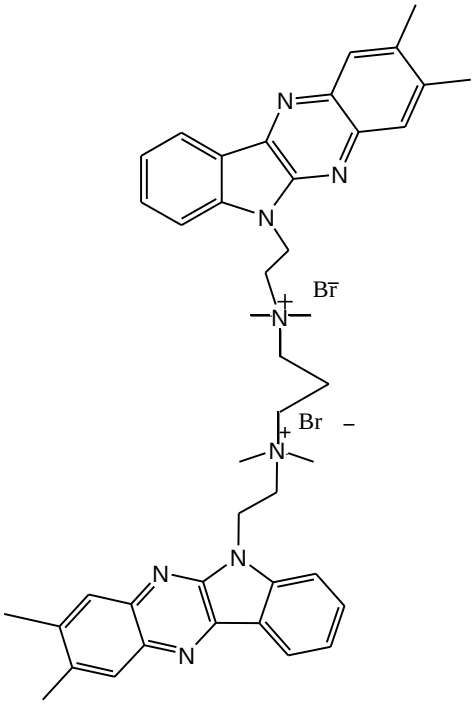
74



75



76

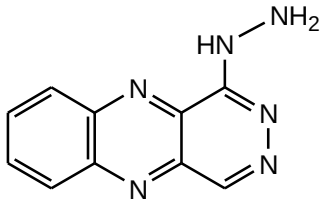


77

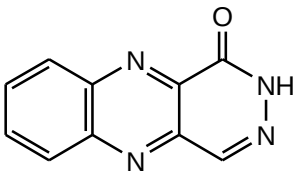
o) Quinoxaline as anti-hypertensive

1-hydrazinopyridazino[4,5-*b*]quinoxaline **78** and 1,2-dihydro-1-oxopyridazino[4,5-*b*]quinoxaline **79** showed antihypertensive activity in rats **[48]** after oral or intraperitoneal administration at doses from 5mg/kg to 25mg/kg. Both the compounds are characterized elemental analysis and 1H NMR spectra.

.



78

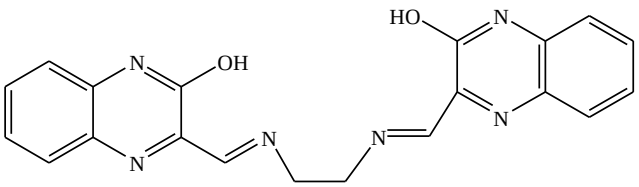


79

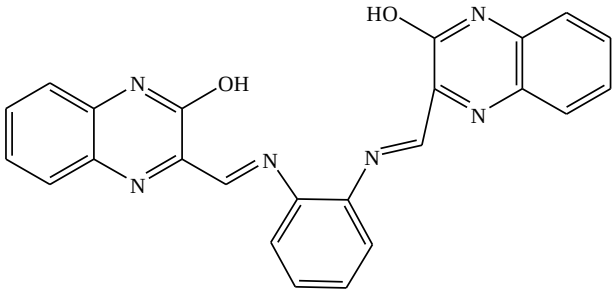
1.4 Application of Quinoxaline-based Schiff base transition metal complexes

Studies on synthesis of quinoxaline-based Schiff base complexes have considerable importance because of their interesting chemical and biological properties. Among complexing ligands, Schiff bases have special interest. Schiff bases, named after Hugo Schiff **[49],** are formed by condensation of primary amine with an active carbonyl compound. They have an azomethine group –RC=NR,' where R and R' are alkyl, cycloalkyl, aryl or heterocyclic group. Schiff bases with an additional donor close to the imino nitrogen form stable chelates with metal ions. Quinoxaline-based Schiff base complexes were synthesized and characterized by several researchers.

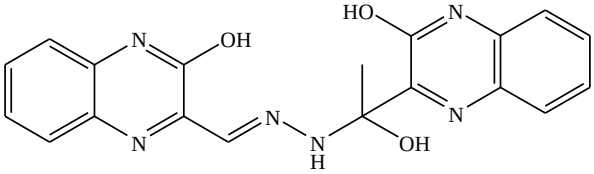
synthesis, characterization and catalytic activity studies on manganese(II), iron(III),cobalt(II), nickel(II) and copper(II) complexes of the Schiffbases derived from, N,N'-bis(1-hydroxyquinoxaline-2-carboxalidene)ethylenediamine**80,**N,N'-bis(3-hydroxyquinoxaline-2-carboxalidene)-ophenylenediamine **81**, and N,N′-bis-(3-hydroxyquinoxaline-2-carboxilidene)diethylenetriamine **82,** were reported. The complexes of **80**-**82** exhibit anomalous magnetic behavior [**50].**



80

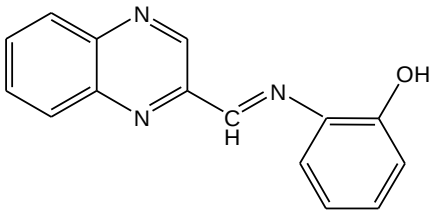


81

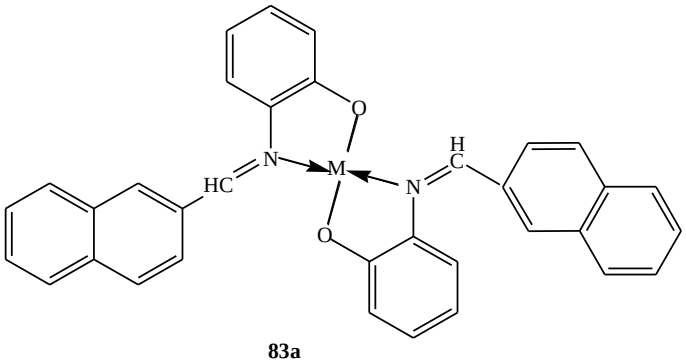


82

Some new transition metal complexes of the Schiff base quinoxaline-2-carboxalidene-2-aminophenol(QAP) **83** were synthesized and characterized by elemental analysis, molar conductance, magnetic measurements, IR, and UV–Vis studies. The complexes of Mn(II), Co(II), Ni(II), and Cu(II) have empirical formula $[M(QAP)_2]$ and Fe(III) complex has the empirical formula $[Fe(QAP)_2Cl]$. The very low molar conductance values indicate non-electrolytes. A tetrahedral geometry was assigned for Mn(II), Co(II), Ni(II), and Cu(II) complexes **83**a except Fe(III) which has an octahedral geometry [**51**].



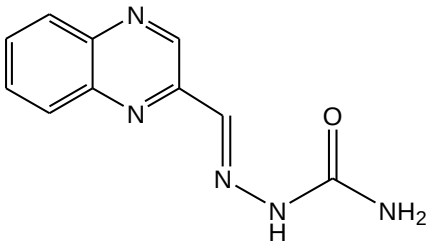
83



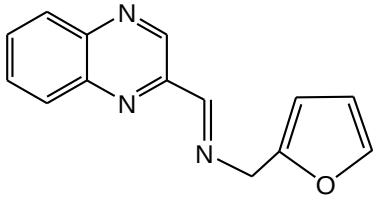
83a

[M = Mn(II), Co(II), Ni(II) or Cu(II)]

Two series of transition metal complexes of Schiff bases derived fromquinoxaline-2-carboxaldehyde with semicarbazide (QSC) **84** and furfurylamine(QFA) **85** were synthesised and characterised by elemental analyses,molar conductance and magnetic susceptibility measurements, IR, electronicand EPR spectral studies [**52].** The QSC complexes have the generalformula $[M(QSC)Cl_2]$. A tetrahedral structure has been assigned for theMn(II), Co(II) and Ni(II) complexes and a square-planar structure for theCu(II) complex. The QFA complexes have the formula $[M(QFA)_2Cl_2]$.An octahedral structure has been assigned for these complexes. All ofthe complexes exhibit catalytic activity towards the oxidation of 3,5-di-tert-butylcatechol (DTBC) to 3,5-di-tert-butylquinone (DTBQ) usingatmospheric oxygen. The cobalt (II) complex of the ligand QFA wasfound to be the most active catalyst.



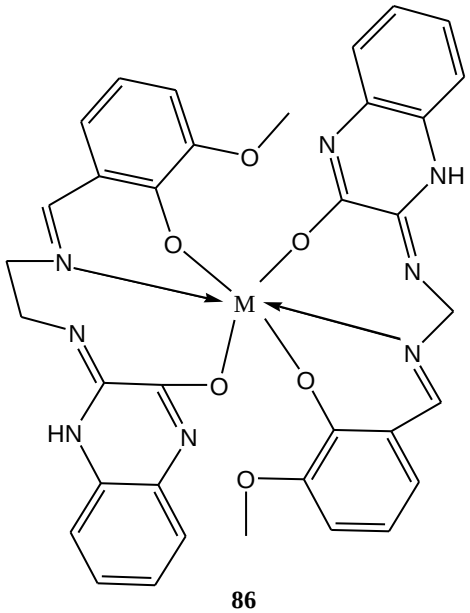
84



85

Co(II), Ni(II), Cu(II) and Zn(II) complexes of a tridentate ONO donor Schiff base ligand derived from 3-(2-aminoethylamino)quinoxalin-2(1H)-one **86** were synthesized **[53].** The ligand and its metal complexes werecharacterized using elemental analysis, molar conductance, IR,1H NMR, mass, magnetic susceptibility,electronic spectra and ESR spectral studies. Electrochemical behavior

of the synthesized compounds was studied using cyclic voltammeter. The grain size of the synthesized compounds was determined by powder XRD. The Schiff base and its complexes have been screened for their antimicrobial activitiesagainst the bacterial speciesE. coli,K. pneumoniae,P. aeruginosaandS. aureus; fungal species include,A. niger, and C. albicans by disc diffusion method. The results show that the complexes have higher activ-ity than the free ligand. The interaction of the complexes with calf thymus DNA (CT DNA) has been inves-tigated by electronic absorption method. Furthermore, the DNA cleavage activity of the complexes wasstudied using agarose gel electrophoresis.In vitroanticancer studies of the ligand and its complexes usingMTT assay was also done.

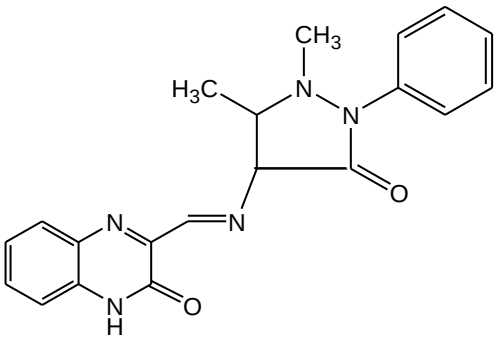


86

Where M = Co(II), Ni(II), Cu(II) and Zn(II))

Schiff base, 3-hydroxyquinoxaline-2-carboxalidine-4-aminoantipyrine **87** was synthesized by the condensation of 3-hydroxyquinoxaline-2-carboxaldehyde with 4-aminoantipyrine **[54].** HPLC, FT-IR and NMR spectral data revealed that the compound exists predominantly in the amide tautomeric form and exhibits both absorption and fluorescence solvatochromism, large stokes shift, two electron quasi reversible redox behaviour and good thermal stability, with a glass transition temperature of 104 _C. The third-order non-linear optical character was studied using open aperture Z-scan methodology employing 7 ns pulses at 532 nm. The third-order non-linear absorption coefficient, b, was 1.48 _ 10_6 cm W_1 and the imaginary part of the third-order non-linear optical susceptibility,

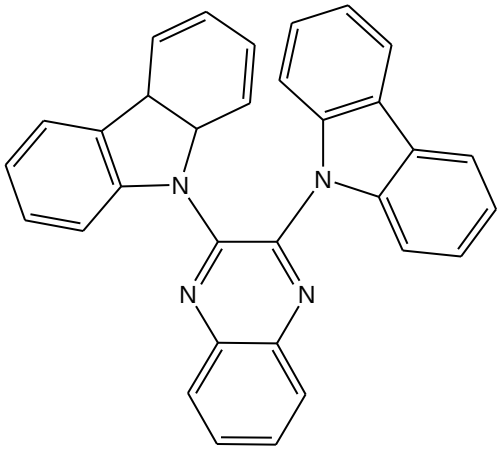
Im c(3), was 3.36 _ 10_10 esu. The optical limiting threshold for the compound was found to be 340 MW cm^{-2}.



87

1.5 Quinoxaline derivatives as organic light-emitting diodes:

Organic light-emitting diodes (OLEDs) are paying an enormous attention owing to their excellent performance in lightening and high-quality display applications and unique characteristics, such as flexibility, high efficiency, lifespan, and manufacturing cost. While the researchers have been focusing on red, green, and blue emissions, the investigations on yellow emission, which is an essential part in high quality RGBY-TV, signal light, lithography, and hybrid white OLEDs are still required. A novel bipolar donor-acceptor-donor (D-A-D) type quinoxaline derivative, 2,3-di(9*H*-carbazol-9-yl)quinoxaline (DCQ) **88** was designed and synthesized **[55]**.The spectroscopic, thermal, photophysical and electrochemical properties of DCQ were systematically investigated. DCQ was employed as a yellow host material for phosphorescent organic light emitting diodes (PHOLEDs) having both a good electron and hole transport properties. Importantly, DCQ as a host material exhibits excellent device performance having triplet energy of 2.46 eV The maximum quantum efficiency of 24.6% at 3% doping concentration and power efficiency of 49.6 lm/W at 5% doping concentration in yellow phosphorescent device.



88

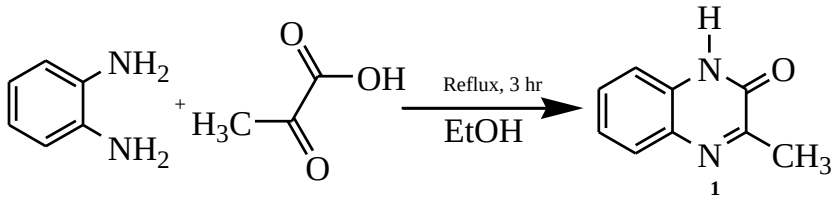
Quinoxaline, also called a benzopyrazine, is a heterocyclic compound containing a benzenering and a pyrazine ring. Quinoxalines are isomeric with cinnolines, phthalazines, andquinazolines. Studies on synthesis of quinoxaline derivatives have considerableimportance because of their interesting chemical and biological properties. Quinoxaline Derivatives are widely distributed in nature and have a variety of biological applications.

In view of the above mentioned facts and importance of biological activities it is considered worthwhile to synthesize some novel compounds containing quinoxaline moiety such as hydrazono-hydrazinyl quinoxalines compounds which might have potential biological activity.

CHAPTER-2

Experimental

2.1 Synthesis of 3-methylquinoxalin-2(1H)-one



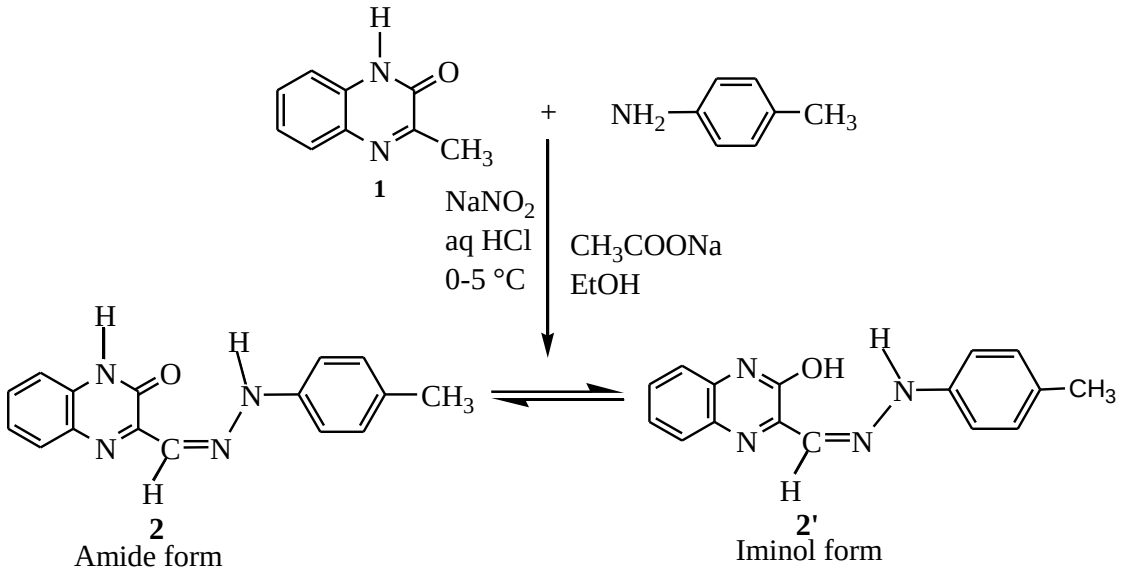
1

Equimolar amounts of pyruvic acid (0.88g, 0.01 mol) and *o*-phenylenediamine (1.084g, 0.01mol) were refluxed in ethanol for 3 hours [56]. The progress of the reaction was monitored by TLC. After completion, the reaction mixture was thencooled to room temperatureand theresultant crude solid precipitate was filtered, dried and purified by recrystallization from ethanol. A yellowish solid compound **1** with 80% yield was obtained and the melting point was recorded as 215-217 °C.

IR (KBr, cm^{-1},Fig.1): 3550-3360 (w,N-H), 3010 (C-H, Ar), 2902(C-H, aliphatic), 1668(w, NH-C=O), 1607 (C = N), 1569,1500,1487 (C = C, Ar).

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm, Fig.2): 2.66 (S, 3H, -CH_3), 7.37 (d, 2H, Ar-H, J= 8.00 Hz), 7.51 (bd, s,1H,Ar-H), 7.83 (d,1H, Ar-H, J = 8.00 Hz), 12.05 (s,1H, N-H).

2.2 Synthesis of Amide-Iminol tautomer of 3-(*P*-Tolyl-hydrazonomethyl)-1H- 3-quinoxalin-2-one



P-Toluidine (0.321g, 0.003 mol) was dissolved in a mixture of concentrated HCl (8ml) and water (6ml) and cooled to 0°Con an ice bath. To this, a cold aqueous solution of sodium nitrite (0.414g, 0.006 mol) was addeddrop wisecarefully for 30 minutes. Then thisice cold diazonium salt solution was added drop wiseinto a cold ethanolic solution of 3-methylquinoxalin-2(1H)-one (0.24g, 0.0015mol)**1** and sodium acetate (0.0075 mol, 0.615g).The resultant reaction mixture was stirred for further 2 hours. The progress of the reaction was monitored by TLC. Then the crudesolid reaction mixture was filtered, dried and purified by recrystallization from ethanol. A dark brown solid product**2** and **2'**were obtained with 70% yield and the melting point was recorded as 260-264 °C.

IR (KBr, cm^{-1}, Fig. 3): 3500-3350 (N-H, O-H), 3010 (C-H,Ar-H), 2977,(C-H, aliphatic), 1675(NH-C=O), 1605(C=N), 1545,1520,1470 (C = C, Ar).

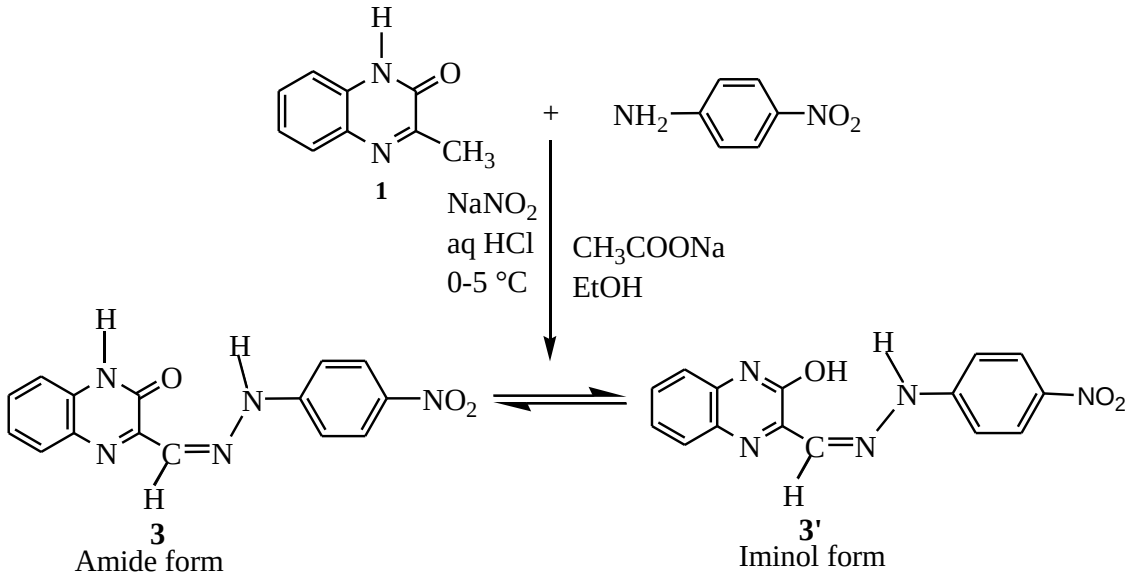
^{1}H NMR (400 MHz,DMSO-d$_6$, δ ppm, Fig.4): Compound **2** (amide form): 2.287 (S, 3H,-CH_3), 7.16 (d, 4H, J = 8.0 Hz, Ar-H), 7.321 (t, 2H,J = 8.0 Hz,Ar-H), 7.51 (t, 1H,J = 8.00 Hz , J = 4.0 Hz, Ar-H),8.01(d, 1H, J = 8.0 Hz, Ar-H), 8.324 (s,1H, CH=N), 12.535 (s, 1H, N-H), 14.538 (s, 1H, N-H).

^{1}H NMR (400 MHz,DMSO-d$_6$, δ ppm, Fig.4): Compound **2'** (Iminol form); 2.250(S, 3H, CH_3), 7.07 (d, 4H, J = 4.0 Hz, Ar-H), 7.301 (t, 2H,J = 8.0 Hz,Ar-H), 7.45 (t, 1H,J = 8.00 Hz , Ar-H), 7.68 (s,1H, CH=N), 7.76 (d, 1H, J = 8.0 Hz, Ar-H), 11.16 (s, 1H, O-H), 12.269 (s, 1H, N-H).

^{13}C NMR(100MHz,DMSO-d$_6$, δ ppm, Fig. 5): Compound **2** (amide form) ; 155.26 (1C, N-C=O), 151.43 (1C, C=N), 142.289 (1C, N=C-C=O), 133.226 (1C, Ar), 131.863 (3C, Ar), 130.188 (2C, Ar), 129.817(1C, Ar), 128.864(1C, Ar) , 124.122 (1C, Ar), 121.874 (1C, Ar), 115.867 (1C, Ar), 113.506 (1C, Ar), 20.802 (1C, CH_3).

The ^{13}C NMR(100MHz,DMSO-d$_6$, δ ppm,Fig. 5): Compound **2'** (Iminol form): 155.03 (1C, C=O), 149.939 (1C, C=N), 141.433 (1C, N=C-C=O), 131.963 (3C, Ar), 130.617 (1C, Ar) , 129.949 (1C, Ar), 129.653 (1C, Ar) , 128.697 (1C, Ar), 123.36 (1C, Ar), 115.836 (2C, Ar), 114.472 (2C, Ar), 20.874 (1C, CH_3).

2.3 Synthesis of Amide-Iminol tautomer of 3-[(4-Nitro-phenyl)-hydrazonomethyl]-1H- 3-quinoxalin-2-one



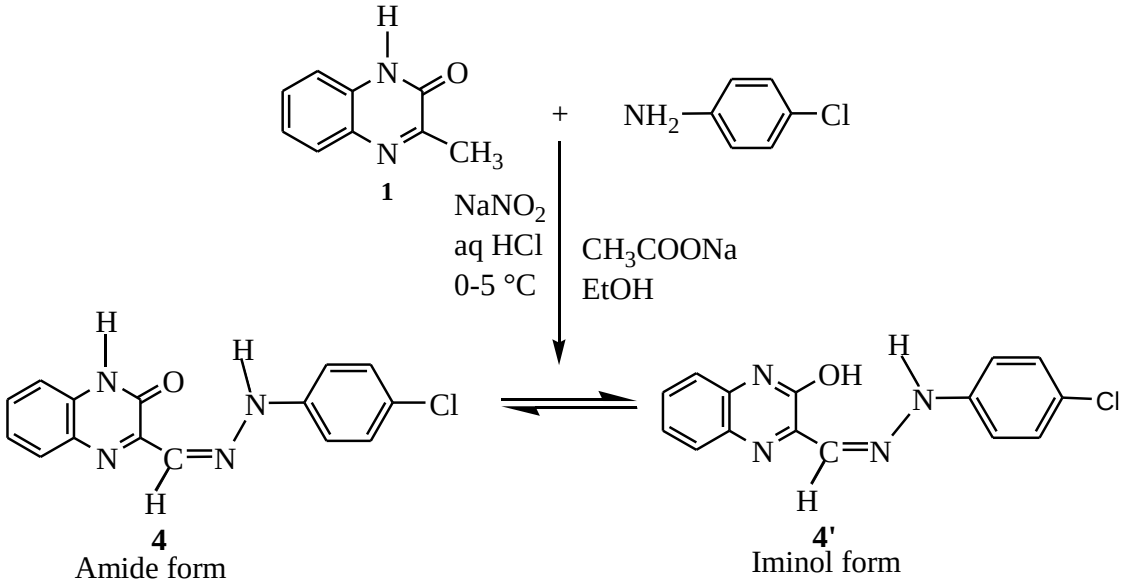
p-Nitroaniline (0.003 mol, 0.414g) was dissolved in a mixture of concentrated HCl (8ml) and water (6ml) and cooled to 0 °Con an ice bath. To this, a cold aqueous solution of sodium nitrite (0.414g, 0.006 mol) was added drop wise carefully for 30 minutes. Then this ice cold diazonium salt solution was added drop wise into a cold ethanolic solution of 3-methylquinoxalin-2(1H)-one (0.24g, 0.0015mol) **1**and sodium acetate (0.0075 mol, 0.615g).The resultant reaction mixture was stirred for further 2 hours. The progress of the reaction was monitored by TLC. Then the crude solid reaction mixture was filtered, dried and purified by recrystallization from ethanol. An reddish brown solid products **3** and **3'**were obtained with 80% yield and the melting point was recorded as 282-285°C .

IR (KBr, cm^{-1}, Fig. 6):3350-3550 (N-H, O-H), 3050 (C-H,Ar-H), 1667 (NH-C=O), 1600(C = N), 1560,1515,1473 (C = C, Ar).

^{1}H NMR (400 MHz,DMSO-d_6, δ ppm, Fig.7): Compound **3** (amide form): 7.27 (d, 2H, J = 8.0 Hz, Ar-H), 7.52 (t, 2H,J = 8.0 Hz,Ar-H), 7.83 (d, 2H,J = 8.0 Hz , Ar-H),7.88 (s,1H, CH=N), 8.20 (d, 1H, J = 4.0 Hz, Ar-H), 12.71 (s, 1H, N-H), 14.54 (s, 1H, N-H).

^{1}H NMR (400 MHz,DMSO-d_6, δ ppm, Fig.7): Compound **3'** (Iminol form); 7.34 (m, 2H, Ar-H), 7.57 (d, 2H,J = 8.0 Hz,Ar-H), 8.12 (d, 2H,J = 8.00 Hz , Ar-H),8.21 (d, 2H, J = 8.0 Hz, Ar-H),8.50 (s,1H, CH=N), 11.880 (s, 1H, O-H), 12.517 (s, 1H, N-H).

2.4 Synthesis of Amide-Iminol tautomer of 3-[(4-Chloro-phenyl)-hydrazonomethyl]-1H- 3-quinoxalin-2-one



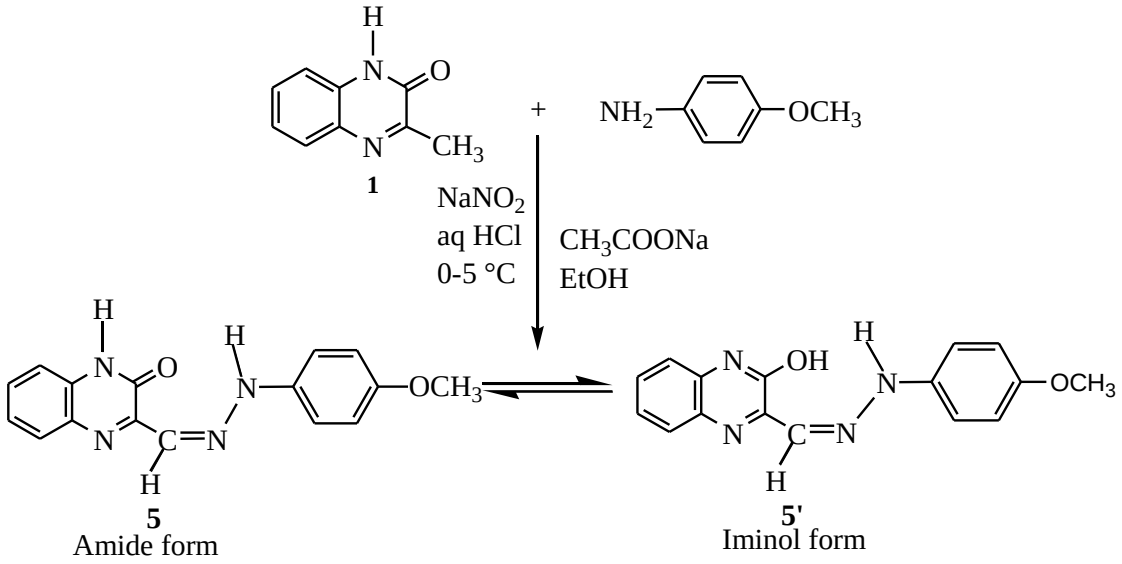
4-Chloroaniline (0.003 mol, 0.383g) was dissolved in a mixture of concentrated HCl (8ml) and water (6ml) and cooled to 0°Con an ice bath. To this, a cold aqueous solution of sodium nitrite (0.414g, 0.006 mol) was added drop wise carefully for 30 minutes. Then this ice cold diazonium salt solution was added drop wise into a cold ethanolic solution of 3-methylquinoxalin-2(1H)-one (0.24g, 0.0015mol)**1** and sodium acetate (0.0075 mol, 0.615g).The resultant reaction mixture was stirred for further 2 hours. The progress of the reaction was monitored by TLC. Then the crude solid reaction mixture was filtered, dried and purified by recrystallization from ethanol. An orange solid products **4** and **4'**were obtained with 70% yield and the melting point was recorded as 277-280°C .

IR (KBr, cm^{-1}, Fig. 8):3370-3500 (N-H, O-H), 3010 (C-H,Ar-H), 1674 (NH-C=O), 1597 (C = N), 1548,1500,1475 (C = C, Ar).

^{1}H NMR (400 MHz,DMSO-d$_6$, δ ppm, Fig.9): Compound **4** (amide form);7.16 (d, 2H, J = 8.0 Hz, Ar-H), 7.38 (d, 1H,J = 8.00 Hz , Ar-H),7.45 (d, 4H,J = 8.00 Hz , Ar-H),7.78 (d, 1H,J = 8.00 Hz , Ar-H),7.74 (s,1H, CH=N), 12.602 (s, 1H, N-H), 14.740 (s, 1H, N-H).

^{1}H NMR (400 MHz,DMSO-d$_6$, δ ppm, Fig.9): Compound **4'** (Iminol form);7.32(m, 6H, Ar-H), 7.54 (t, 1H,J = 8.0 Hz,Ar-H), 8.08 (d, 1H,J = 8.00 Hz , Ar-H),8.358(s,1H, CH=N), 11.30 (s, 1H, O-H), 12.40 (s, 1H, N-H).

2.5 Synthesis of Amide–Iminol tautomer of 3-(4-Methoxy-phenyl -hydrazonomethyl)-1H-3-quinoxalin-2-one



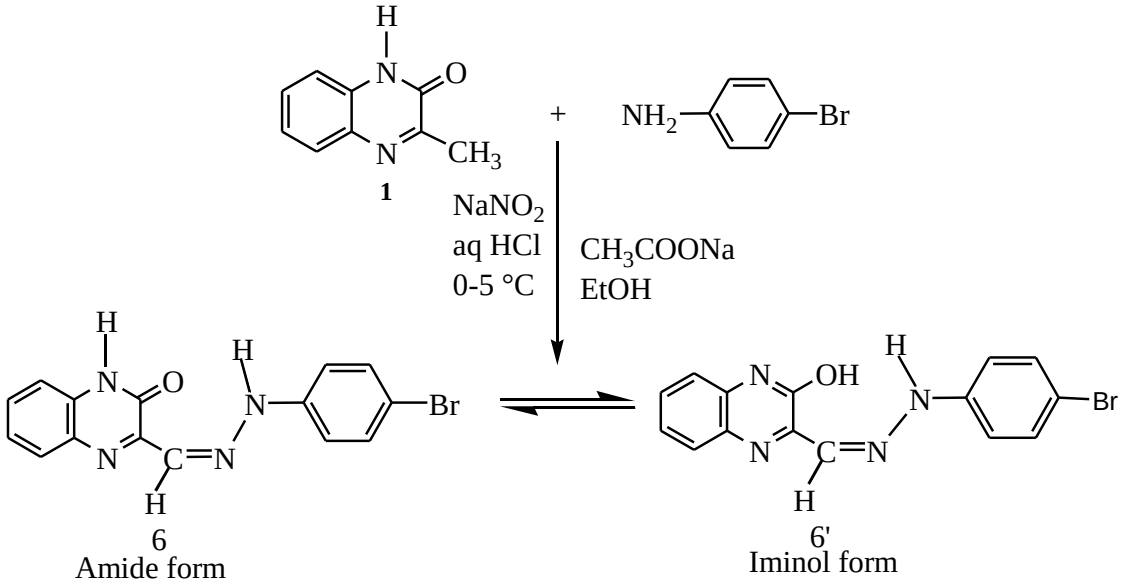
4-Methoxyaniline (0.369 g, 0.003 mol) was dissolved in a mixture of concentrated HCl (8ml) and water (6ml) and cooled to 0°Con an ice bath. To this, a cold aqueous solution of sodium nitrite (0.414g, 0.006 mol) was added drop wise carefully for 30 minutes. Then this ice cold diazonium salt solution was added drop wise into a cold ethanolic solution of 3-methylquinoxalin-2(1H)-one (0.24g, 0.0015mol)**1** and sodium acetate (0.0075 mol, 0.615g).The resultant reaction mixture was stirred for further 2 hours. The progress of the reaction was monitored by TLC. Then the crude solid reaction mixture was filtered, dried and purified by recrystallization from ethanol. A dark brown solid products**5** and **5'** were obtained with 70% yield and the melting point was recorded as 290-293°C.

IR (KBr, cm^{-1}, Fig. 10):3360 -3520 (N-H, O-H), 3091 (C-H,Ar-H), 2972,2893,2835(C-H, aliphatic), 1672(NH-C=O), 1607(C = N), 1551,1513,1469 (C = C, Ar).

^{1}H NMR (400 MHz,DMSO-d_6, δ ppm, Fig.11): Compound **5** (amide form); 3.75 (S, 3H,-OCH_3), 6.95 (d, 2H, J = 12.0 Hz, Ar-H), 7.28 (m, 2H ,Ar-H), 7.38 (d, 2H,J = 8.00 Hz ,Ar-H),7.49 (t, 1H, J = 8.0 Hz, Ar-H), 7.64 (s,1H, CH=N),7.99 (d, 2H,J = 10.0 Hz , J = 4.0 Hz, Ar-H) 13.51 (s, 1H, N-H), 14.57 (s, 1H, N-H).

^{1}H NMR (400 MHz,DMSO-d_6, δ ppm, Fig.11): Compound **5'**(Iminol form): 3.726(S,3H, -OCH_3), 6.92 (d, 2H, J = 12.0 Hz, Ar-H),7.12 (d, 1H, J = 12.0 Hz, Ar-H), 7.33 (t, 2H,J = 8.0 Hz ,Ar-H), 7.44 (d, 1H, J=12.0 Hz, Ar-H),7.76(d, 1H, J=8.0 Hz, Ar-H), 8.29 (s,1H, CH=N), 11.13 (s, 1H, O-H), 12.40 (s, 1H, N-H).

2.6 Synthesis of Amide–Iminol tautomer of 3-[(4-Bromo-phenyl)-hydrazonomethyl]-1H- 3-quinoxalin-2-one



4-Bromoaniline (0.003 mol, 0.516g) was dissolved in a mixture of concentrated HCl (8ml) and water (6ml) and cooled to 0°Con an ice bath. To this, a cold aqueous solution of sodium nitrite (0.414g, 0.006 mol) was added drop wise carefully for 30 minutes. Then this ice cold diazonium salt solution was added drop wise into a cold ethanolic solution of 3-methylquinoxalin-2(1H)-one (0.24g, 0.0015mol)**1** and sodium acetate (0.0075 mol, 0.615g).The resultant reaction mixture was stirred for further 2 hours. The progress of the reaction was monitored by TLC. Then the crude solid reaction mixture was filtered, dried and purified by recrystallization from ethanol. An orange solid products**6** and **6'**were obtained with 75% yield and the melting point was recorded as 272-275°C .

IR (KBr, cm^{-1}, Fig. 12):3350-3510 (N-H, O-H), 2968 (C-H, aliphatic), 1677(NH-C=O), 1590 (C = N), 1548,1520,1476 (C = C, Ar).

^{1}H NMR (400 MHz,DMSO-d$_6$, δ ppm, Fig.13): Compound **6**(amide form); 7.10 (d, 2H, J = 8.0 Hz, Ar-H), 7.35 (m, 2H,Ar-H),7.47 (d, 3H,J = 8.00 Hz , Ar-H),7.78 (d, 1H,J = 8.00 Hz , Ar-H),7.74 (s,1H, CH=N), 12.611 (s, 1H, N-H), 14.56 (s, 1H, N-H).

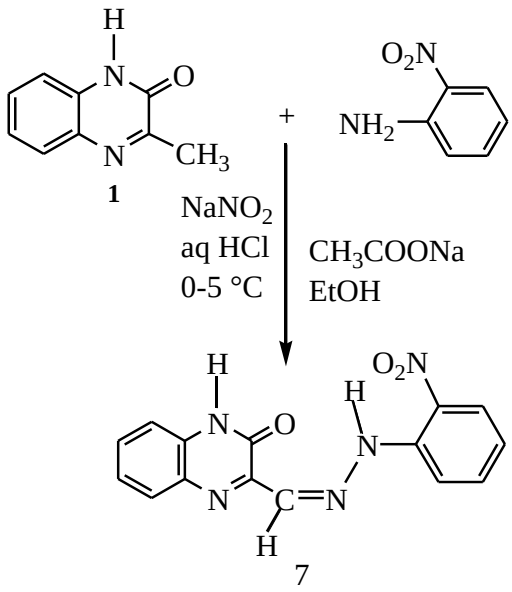
^{1}H NMR (400 MHz,DMSO-d$_6$, δ ppm, Fig.13): Compound **6'** (Iminol form); 7.32 (d, 2H, J = 8.0 Hz Ar-H), 7.42 (d, 2H, J = 8.0 Hz Ar-H), 7.51 (m, 3H,J = 8.0 Hz ,Ar-H), 8.07 (d, 1H,J = 8.00 Hz , Ar-H),8.36 (s,1H, CH=N), 11.31 (s, 1H, O-H), 12.41 (s, 1H, N-H).

^{13}C NMR(100MHz,DMSO-d$_6$, δ ppm, Fig. 14): Compound **6** (amide form);155.20 (1C, N-C=O), 151.26 (1C, C=N), 143.96 (1C, N=C-C=O), 133.12(1C, Ar-C), 132.45(1C, Ar-C), 132.05 (1C, Ar-

C) , 131.36(3C, Ar-C),130.6(1C, Ar-C) , 129.01 (1C, Ar-C), 124.05 (1C, Ar-C), 116.463 (1C, Ar-C), 115.73(1C, Ar-C) ,115.35(1C, Ar-C).

^{13}C NMR(100MHz,DMSO-d$_6$, δ ppm at Fig. 14): Compound**6'**(Iminol form); 154.95 (1C, N-C=O), 149.97 (1C, C=N), 143.146(1C, N=C-C=O), 132.56(1C, Ar-C), 132.13(1C, Ar-C), 131.75 (1C, Ar-C), 131.03(3C, Ar-C),129.05(1C, Ar-C) , 124.39 (1C, Ar-C), 123.40 (1C, Ar-C), 115.87 (1C, Ar-C), 114.14(1C, Ar-C),112.06 (1C, Ar-C).

2.7 Synthesis of 3-[(2-Nitro-phenyl)-hydrazonomethyl]-1H-3-quinoxalin-2-one:



2-Nitroaniline (0.003 mol, 0.414g) was dissolved in a mixture of concentrated HCl (8ml) and water (6ml) and cooled to 0°Con an ice bath. To this, a cold aqueous solution of sodium nitrite (0.414g, 0.006 mol) was added drop wise carefully for 30 minutes. Then this ice cold diazonium salt solution was added drop wise into a cold ethanolic solution of 3-methylquinoxalin-2(1H)-one (0.24g, 0.0015mol)**1** and sodium acetate (0.0075 mol, 0.615g).The resultant reaction mixture was stirred for further 2 hours. The progress of the reaction was monitored by TLC. Then the crude solid reaction mixture was filtered, dried and purified by recrystallization from ethanol. An reddish Brown solid products **7** were obtained with 60% yield and the melting point was recorded as 268-270°C .

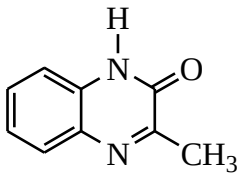
IR (KBr, cm^{-1}, Fig. 15):3360-3520 (N-H), 3099 (C-H,Ar-H), 2972(C-H, imino), 1676 (NH-C=O), 1612 (C = N), 1580,1556,1520 (C = C, Ar).

^{1}H NMR (400 MHz,DMSO-d_6, δ ppm, Fig.16): Compound **7** : 7.16 (t, 1H, J = 8.0 Hz, Ar-H), 7.35 (d, 1HJ = 8.00 Hz,Ar-H),7.45 (t, 1H,J = 8.00 Hz , Ar-H),7.61 (t, 1H,J = 8.00 Hz , Ar-H), 7.79(t, 1H,J = 8.00 Hz , Ar-H), 8.05 (s,1H, CH=N), 8.15 (d, 1H,J = 8.00 Hz , Ar-H),8.18 (d, 1H,J = 8.00 Hz , Ar-H),8.25 (d, 1H,J = 8.00 Hz , Ar-H), 12.76 (s, 1H, N-H).

CHAPTER-3

Results and Discussion

3.1 Characterization of the compound 3-methylquinoxalin- 2(1H)-one

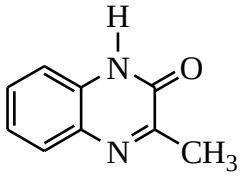


1

The IR Spectrum **Fig.1** of the compound **1** showed a wide band at 3500-3360 cm^{-1} for N-H moiety. The peak at 3010 cm^{-1} was detected for aromatic C-H stretching. The aliphatic C-H stretching was identified at 2902 cm^{-1}. A broad peak detected at 1668 cm^{-1} was assigned for C=O of NH-C=O moiety. The peak at 1607 cm^{-1} was assigned for C=N moiety.The characteristic peaks at 1569, 1500, 1487 cm^{-1} were distinguished for aromatic C=C bond.

The ^{1}HNMR spectrum **Fig.2** of the compound **1** showed a sharp singlet at 2.66 for three protons of CH_3. The doublet with the coupling constant J=8.0Hz was attributable for two aromatic protons at 7.37. The broad singlet at 7.51 was assigned for one aromatic proton. The doublet with the coupling constant J=8.0 Hz was designated for one aromatic proton at 7.83. The downfielded sharp singlet at 12.05 was attributed for one N-H proton.

All the spectral evidences expressed harmony with the structure of the **1** as



1

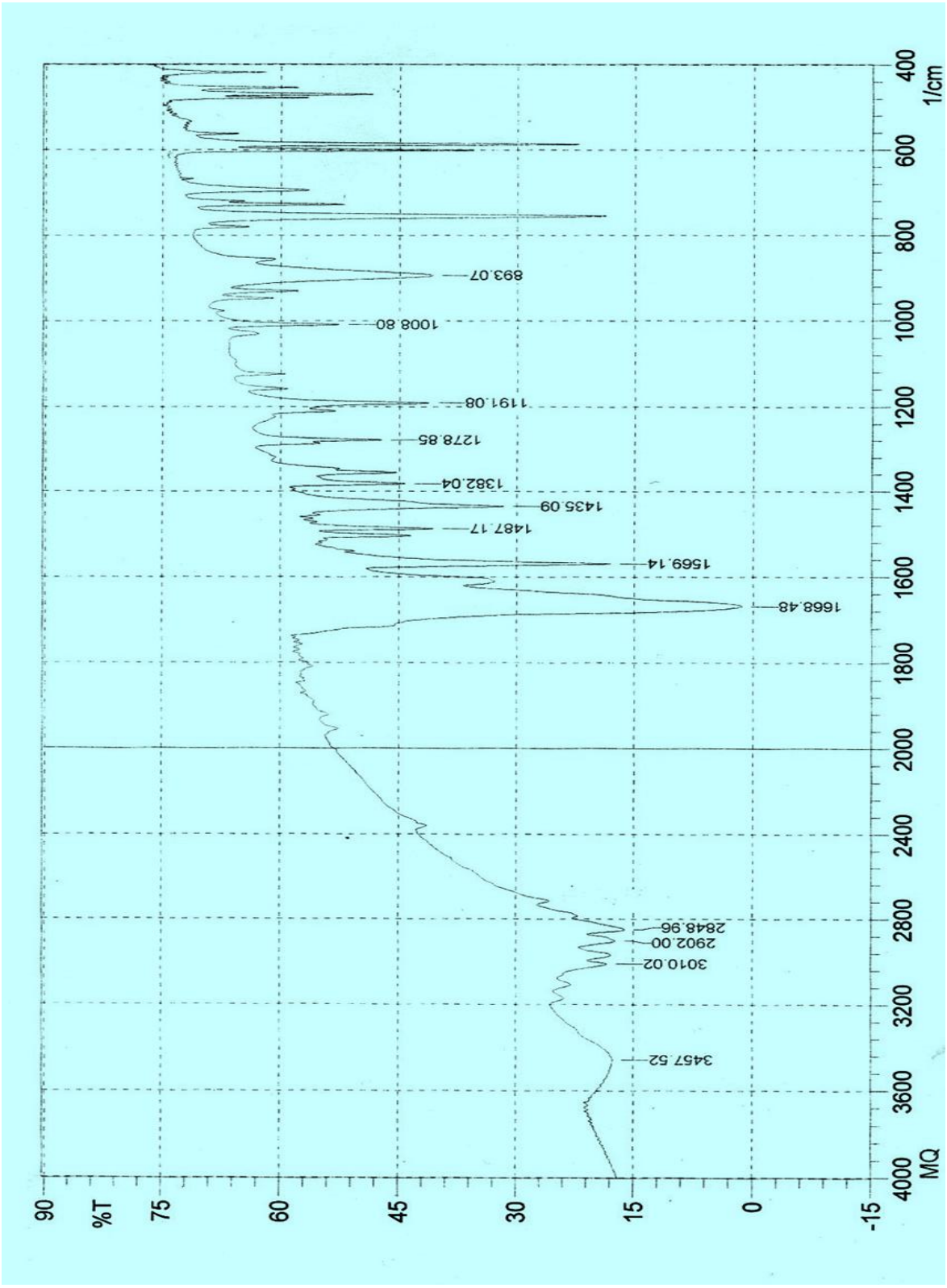


Figure 1: The IR Spectrum of the compound **1**

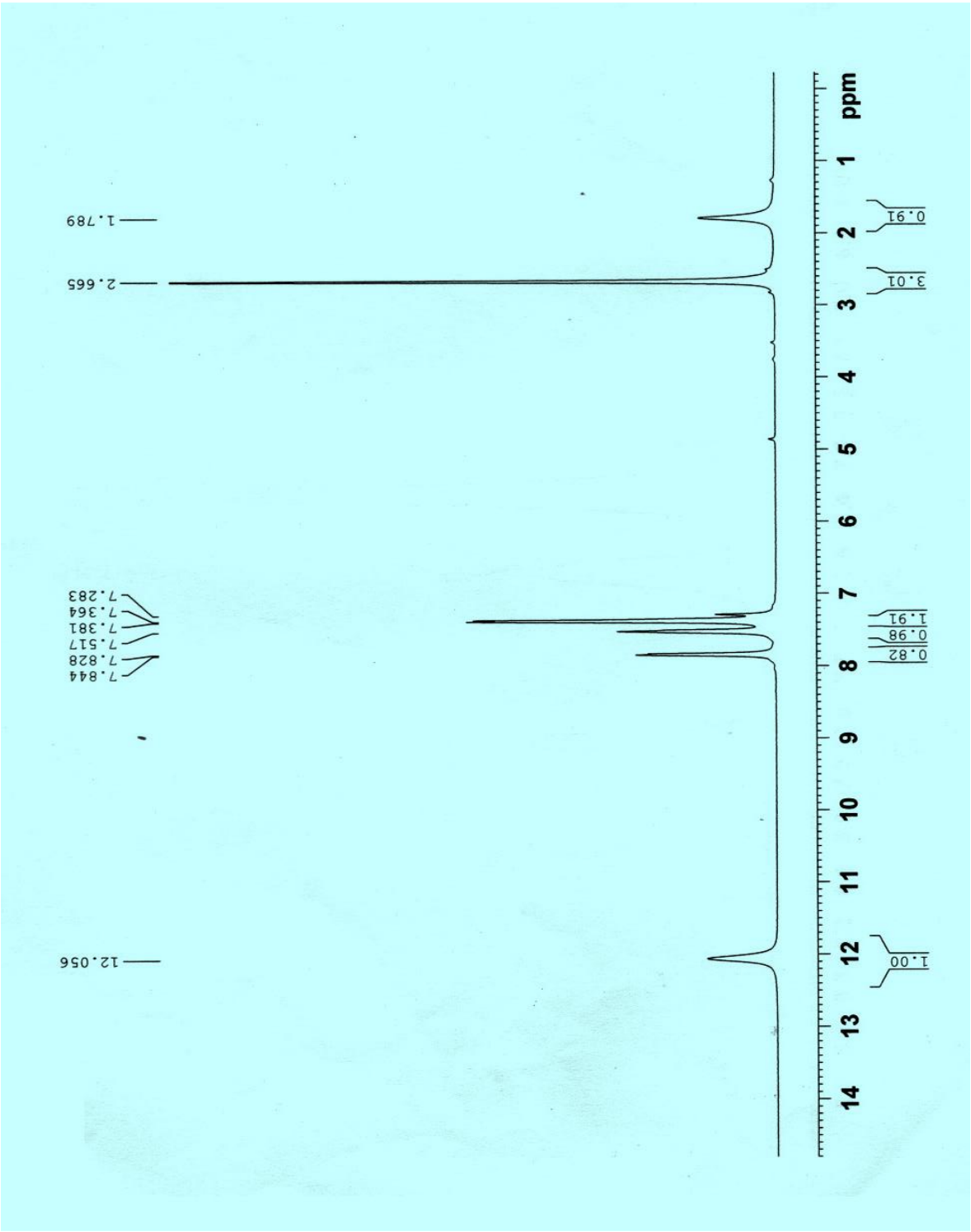


Figure 2: The [1]HNMR spectrum of the compound **1**

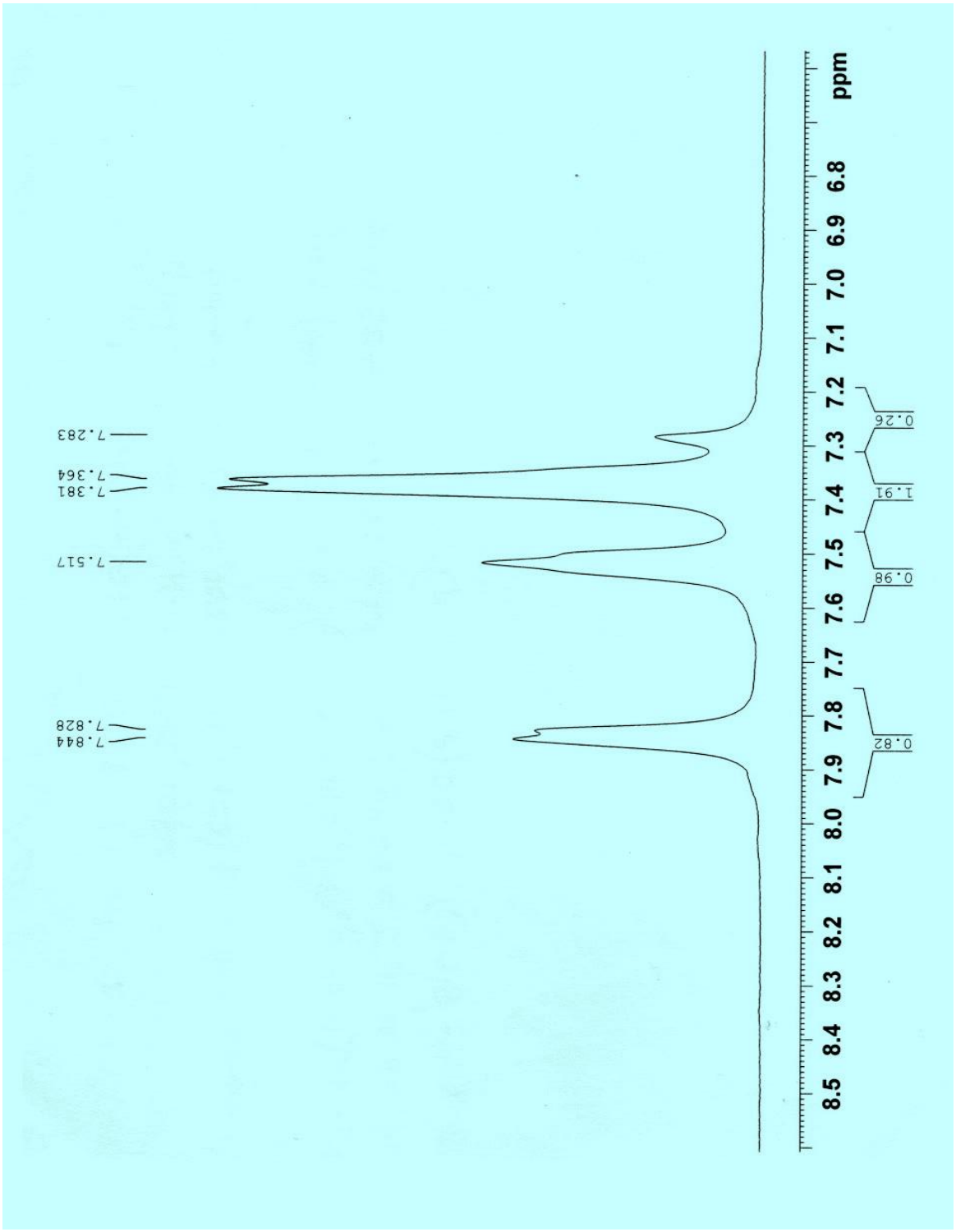
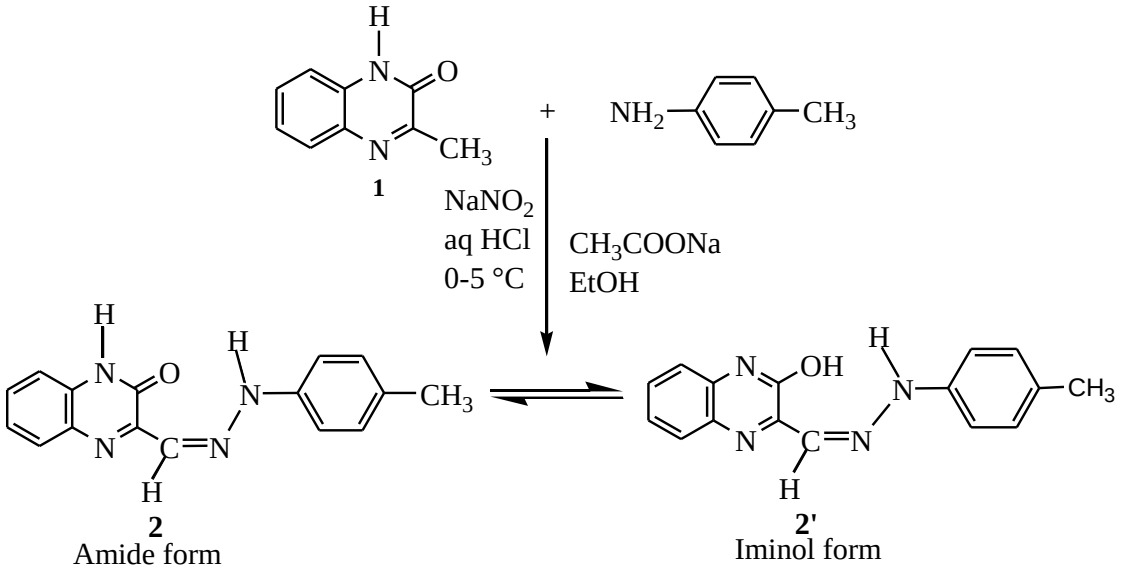


Figure 2: The ^{1}HNMR spectrum of the compound **1**

3.2 Characterization of the Amide-Iminol tautomer of 3-(*P*-Tolyl-hydrazonomethyl)-1H-3-quinoxalin-2-one

The amide-iminol tautomeric compounds **2** and **2'**were synthesized from the coupling reaction between the *p*-methyl benzenediazonium salt and **1,**the active methylated 3-methylquinoxalin-2(1H)-one. A dark brown solid products were obtained with 70% yield and the melting point was recorded as 260-264 °C.



The IR spectrum **Fig.3** of the compound **2** and **2'**showed a broad band at 3350-3500 cm^{-1} for O-H and N-H moieties. The aromatic C-H stretching was identified at 3010 cm^{-1}. The aliphatic C-H stretching was detected at 2977 cm^{-1}.The C=O peak of the amide group was assigned at 1675 cm^{-1}. The peak at 1605 cm^{-1} was for C=N moiety. The characteristic peaks at 1545, 1520, 1470 cm^{-1} were distinguished for aromatic C=C bonds.

The ^{1}HNMR spectrum **Fig.4** of the compound **2** (amide form) showed a sharp singlet at 2.287 for three protons of CH_3. The doublet with the coupling constant J=8.0 Hz at 7.16 was identified for four aromatic protons. The triplet with the coupling constant J=8.0 Hz, J=4.0 Hz at 7.321 was assigned for two aromatic protons. The similar triplet with the coupling constant J=8.0 Hz, J = 4.0 Hz at 7.51 was indicative for the single aromatic proton. The doublet with the coupling constant J=8.0 Hz at 8.01was designated for one aromatic proton. The singlet of HC=N amino proton was detected at 8.324. The two singlets at 12.535 and at 14.538 were attributed for two

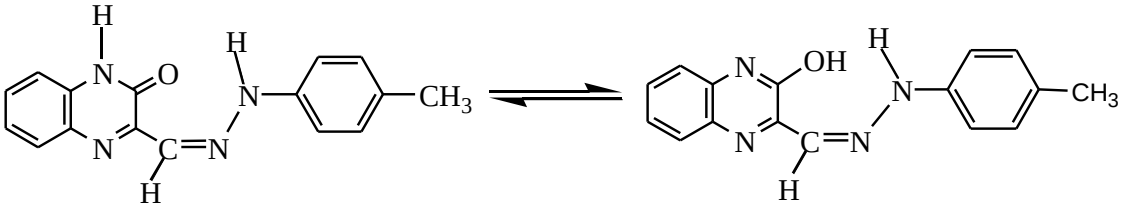
N-H moieties.

The ^{1}HNMR spectrum **Fig.4** of the compound **2'** (iminol form) showed a sharp singlet at 2.250 for three protons of CH_3. The doublet with the coupling constant J=4.0 Hz at 7.07 was detected for four aromatic protons. The triplet with the coupling constant J=8.0 Hz at 7.30 was designated for two aromatic protons. The triplet with the coupling constant J=8.0 Hz at 7.45 was attributed for one aromatic proton.The singlet of single imino proton HC=N was distinguished at 7.68. The doublet with the coupling constant J=8.0 Hz was detected at 7.76 for one aromatic proton. The singlet at 11.16 was designated for one enolic O-H proton. The other downfielded singlet at 12.369 was assigned for N-H proton.

The ^{13}C NMRspectrum **Fig.5**of the compound **2** (amide form) showed the signals at 155.26 (1C, N-C=O), 151.43 (1C, C=N), 142.289 (1C, N=C-C=O), 133.226 (1C, Ar), 131.863 (3C, Ar), 130.188 (2C, Ar), 129.817(1C, Ar),128.864(1C, Ar), 124.122 (1C, Ar), 121.874 (1C, Ar), 115.867 (1C, Ar), 113.506 (1C, Ar), 20.802 (1C, CH_3).

The ^{13}C NMR spectrum **Fig.5**of the compound **2'** (iminol form) showed the signals at 155.03 (1C, N-C=O), 149.939 (1C, C=N), 141.433 (1C,N=C-C=O), 131.963 (3C, Ar), 130.617 (2C, Ar) , 129.949 (1C, Ar),129.653 (1C, Ar) , 128.697 (1C, Ar), 123.36 (1C, Ar), 115.836 (2C, Ar), 114.472(2C, Ar),20.874 (1C, CH_3).

All the spectral evidences expressed harmony with the structure of the amide-iminol tautomeric compounds **2** and **2'** as



2 Amide form

2' Iminol form

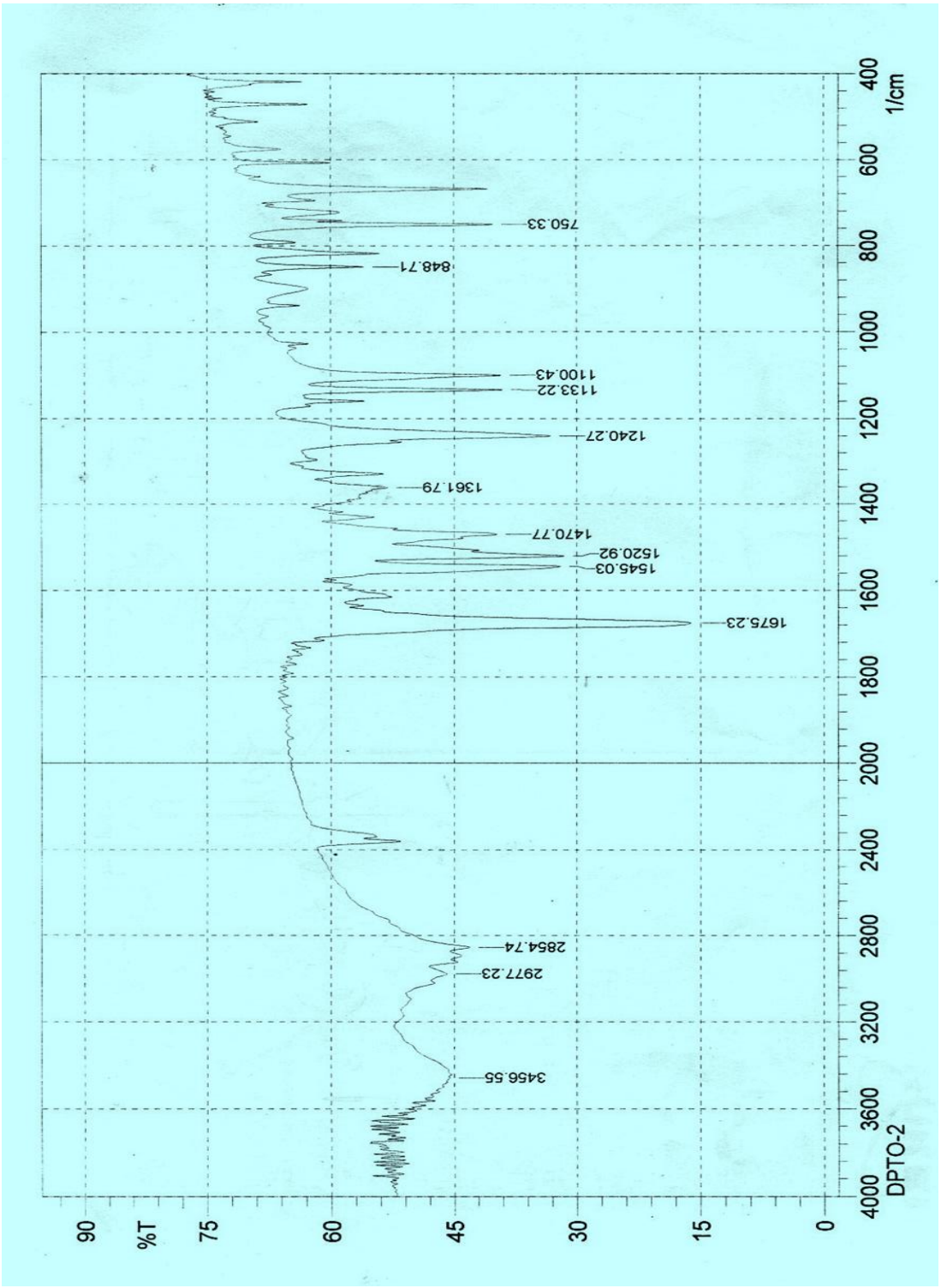


Figure 3 : The IR Spectrum of the compound **2** and **2'**

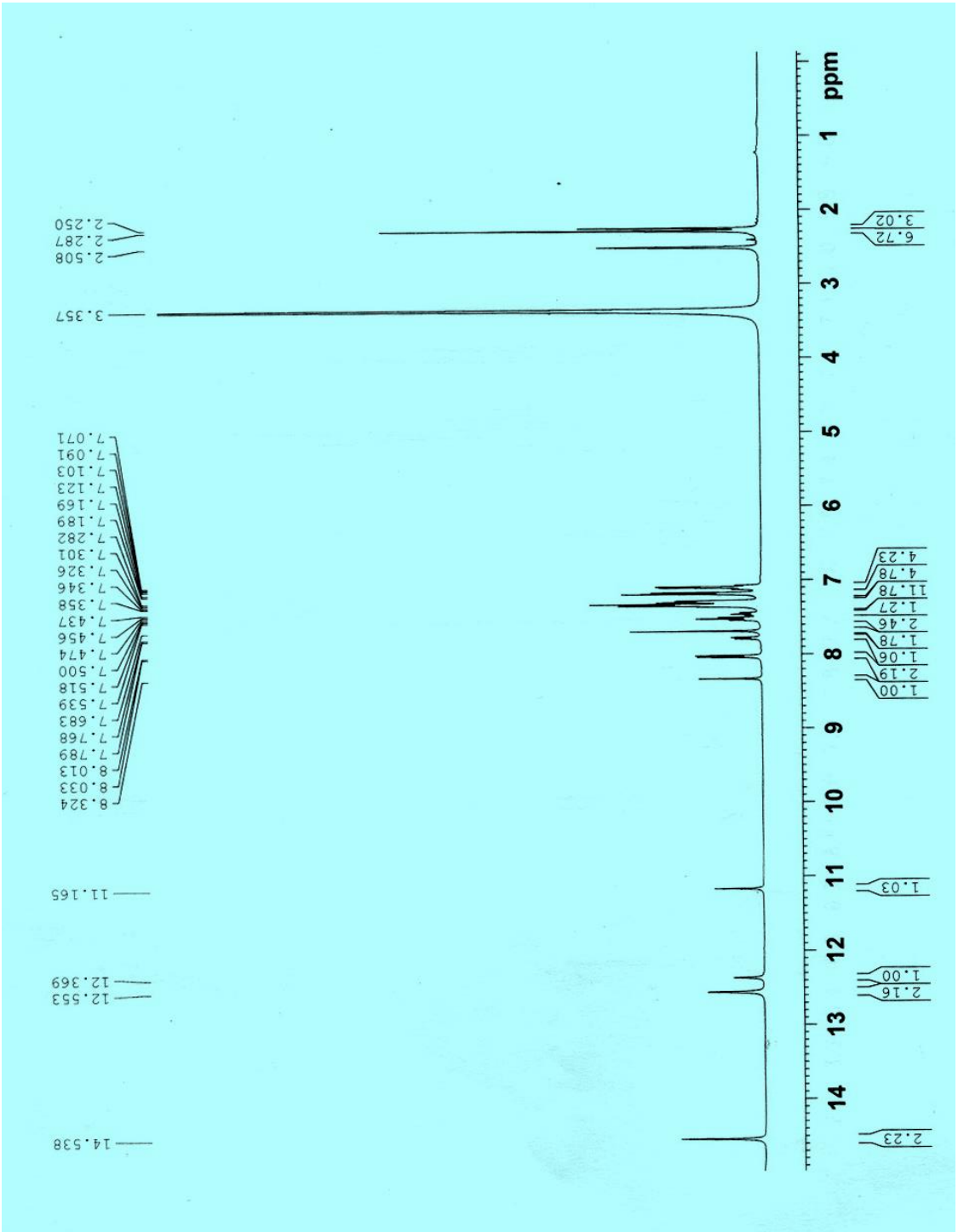


Figure 4 : The ^{1}HNMR spectrum the compound **2** and **2'**

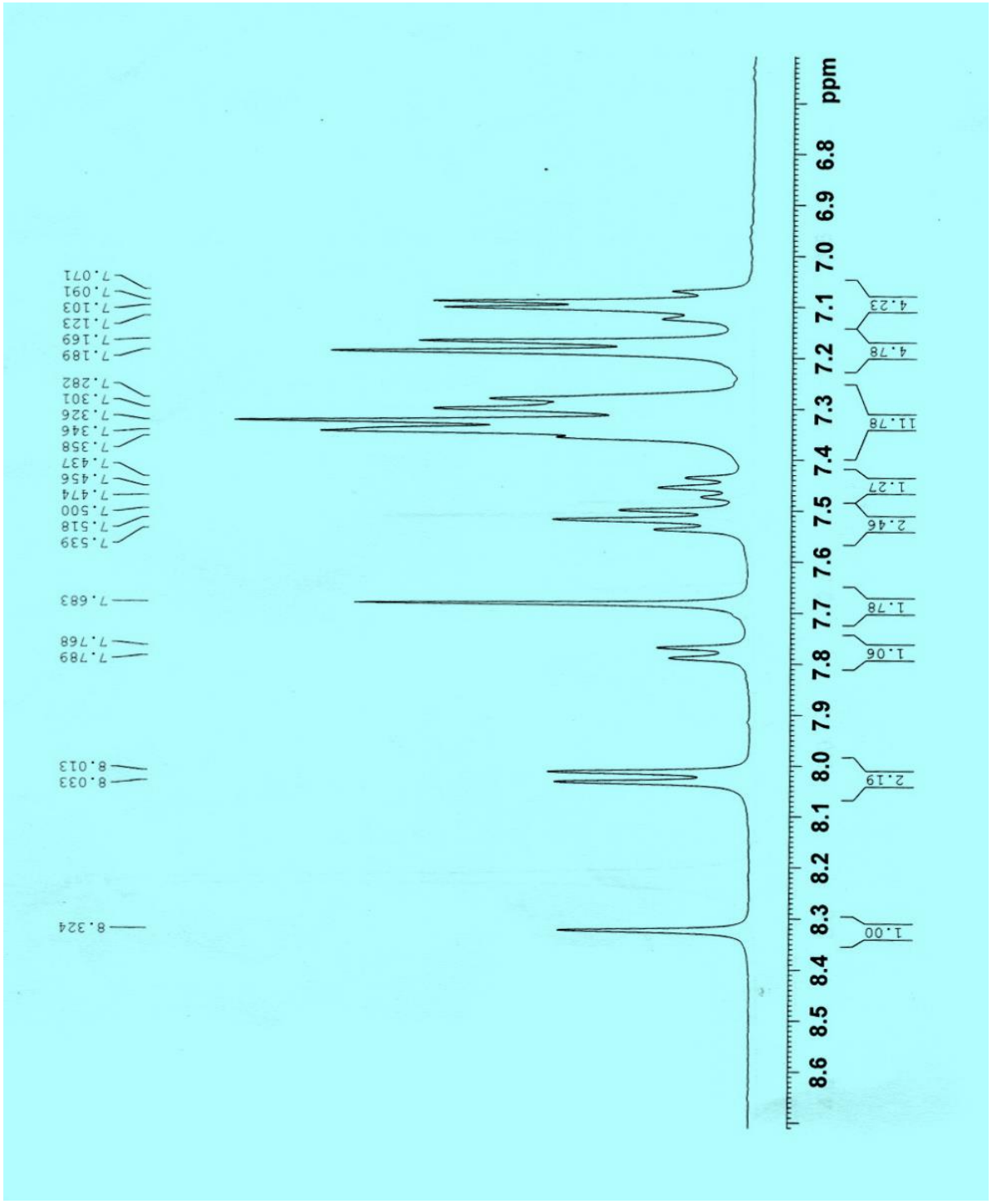


Figure 4 : The ^{1}HNMR spectrum the compound **2** and **2'**

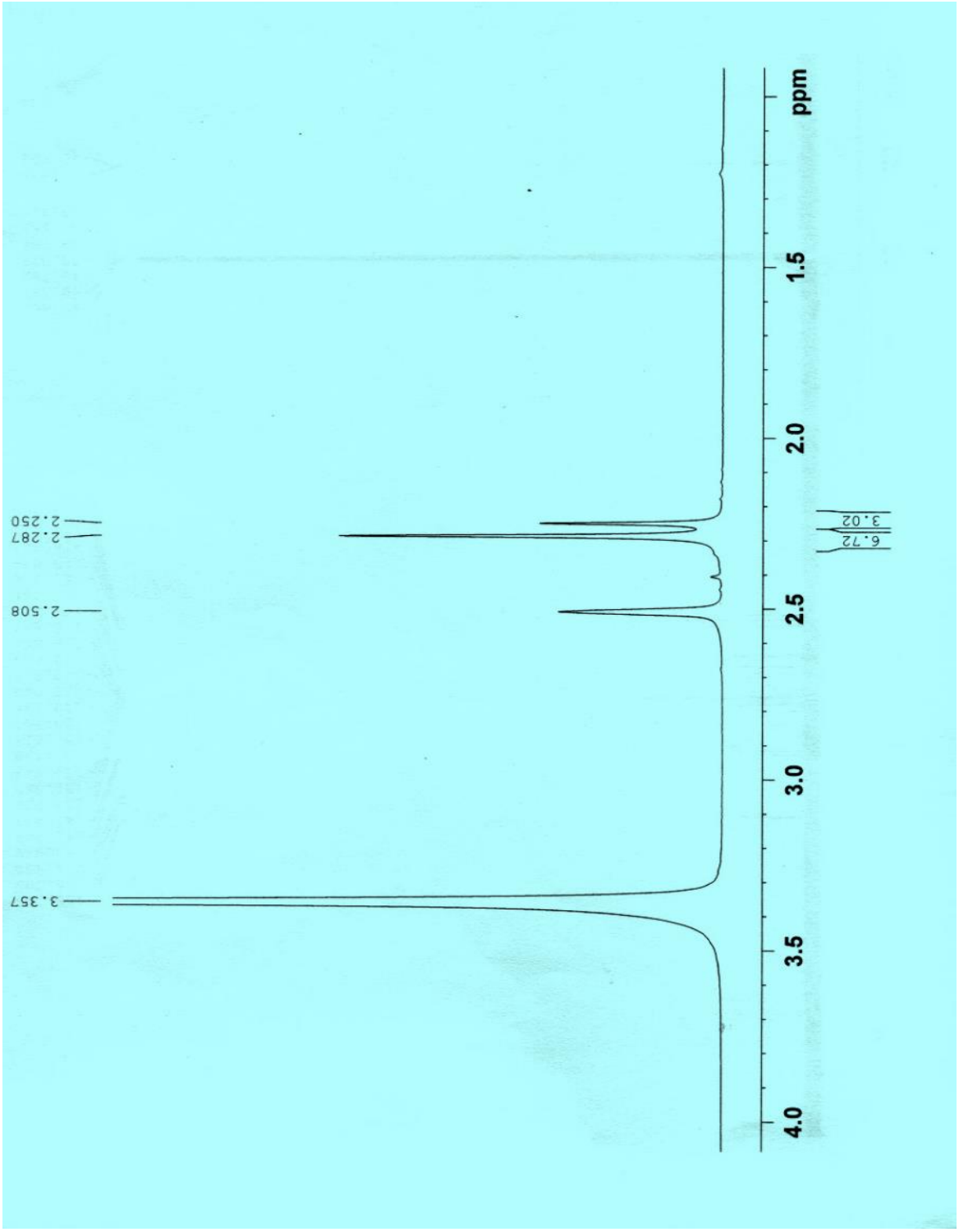


Figure 4 : The [1]HNMR spectrum the compound **2** and **2'**

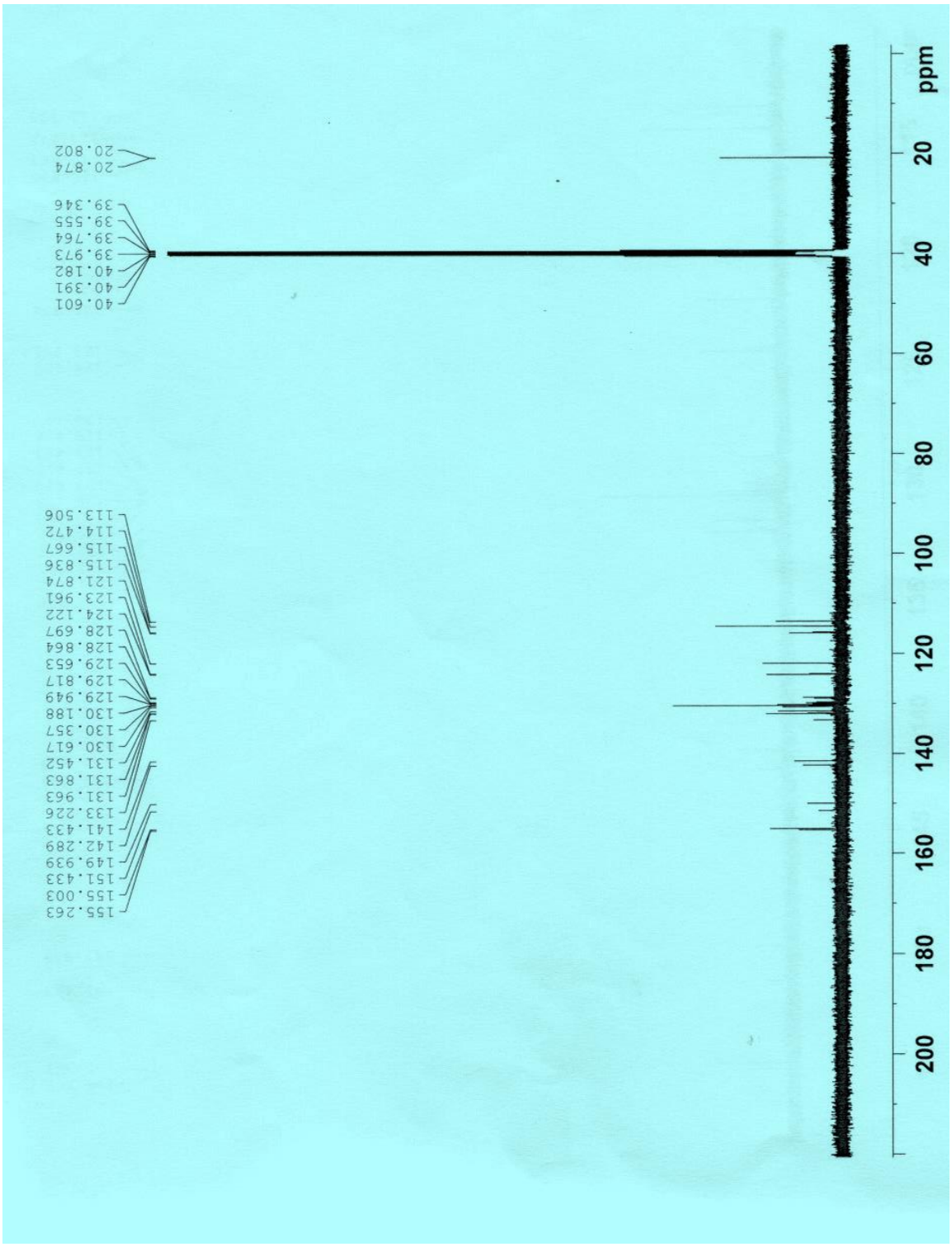


Figure 5 : The ^{13}C NMR spectrum the compound 2 and 2'

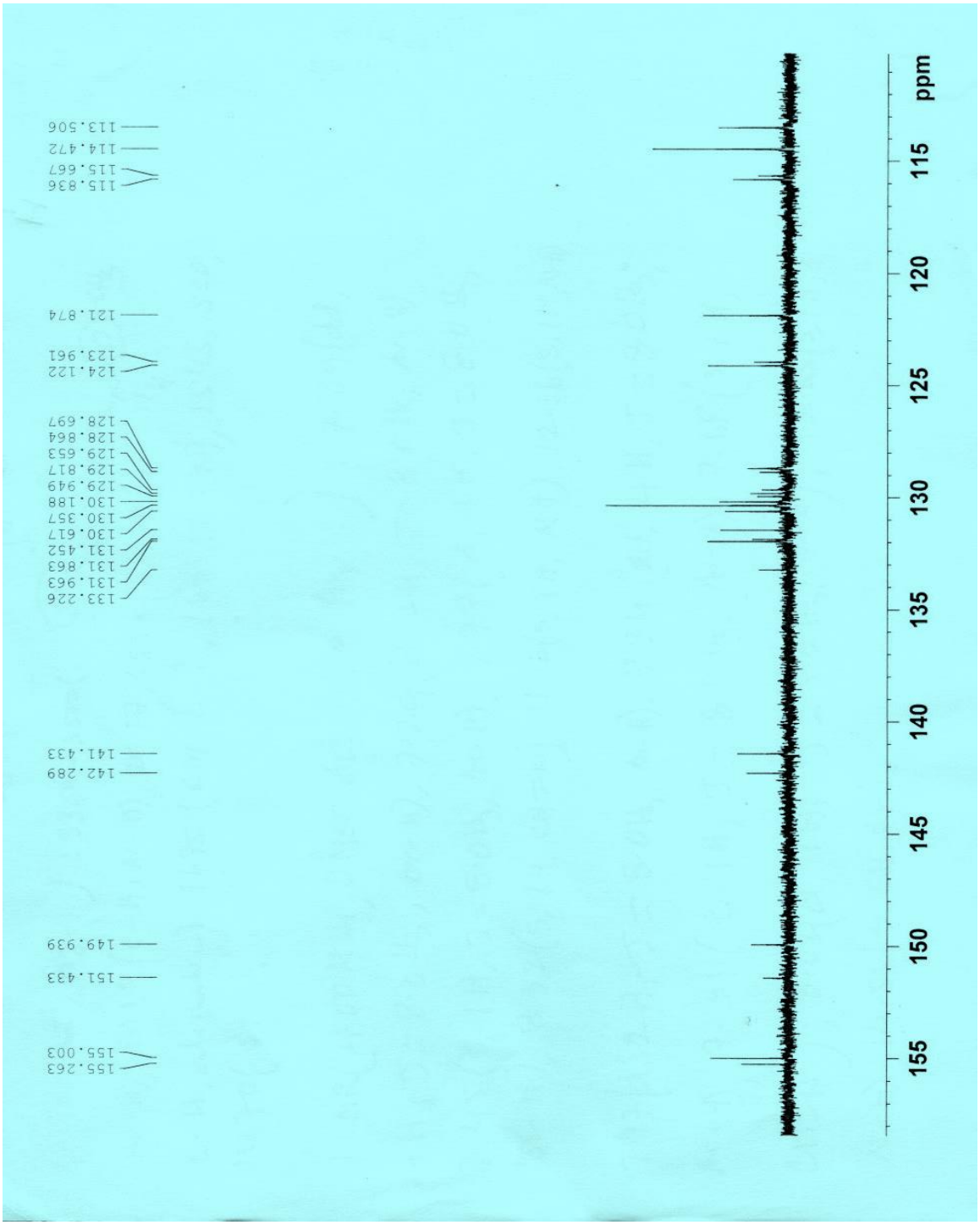
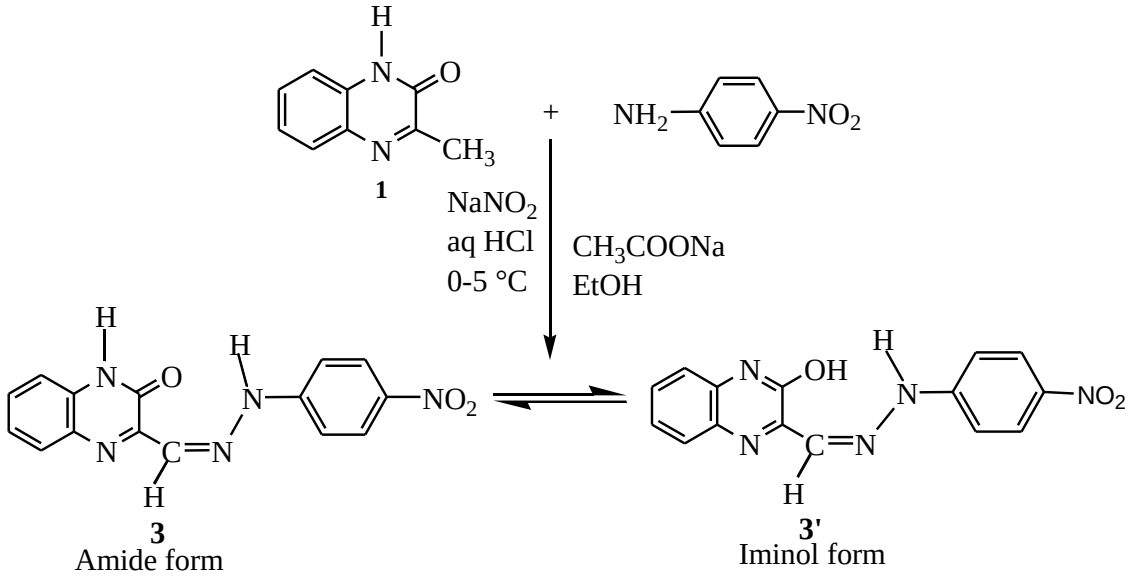


Figure 5 : The ^{13}C NMR spectrum the compound 2 and 2'

3.3 Characterization of Amide-Iminol tautomer of 3-[(4-Nitro-phenyl)-hydrazonomethyl]-1H-3-quinoxalin-2-one

The amide-iminol tautomeric compounds **3** and **3'**were synthesized from the coupling reaction between the *p*-nitrobenzenediazonium salt and **1,**the active methylated 3-methylquinoxalin-2(1H)-one. A reddish brown solid products **3** and **3'** were obtained with 80% yield and the melting point was recorded as 282-285°C .



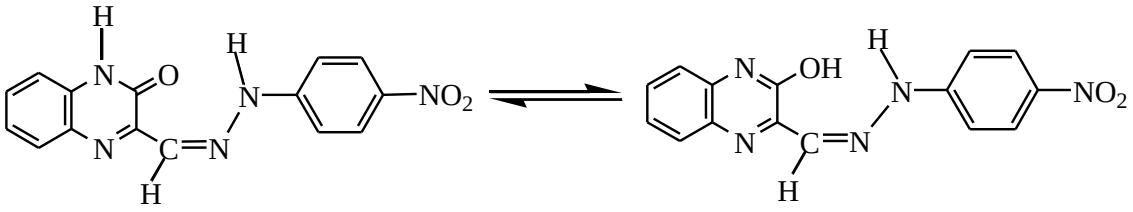
The IR spectrum **Fig.6** of the compound **3** and **3'**showed a broad band at 3350-3550 cm^{-1} for O-H and N-H moieties. The aromatic C-H stretching was identified at 3050 cm^{-1}. The aliphatic C-H stretching was detected at 2977 cm^{-1}.The peak at 1667cm^{-1} was identified for C=O of NH-CO. The peak at 1600 cm^{-1} was for C=N moiety. The characteristic peaks at 1560, 1515, 1473 cm^{-1} were distinguished for aromatic C=C bonds.

The ^{1}HNMR spectrum **Fig.7** of the compound **3** (amide form) showed a doublet with the coupling constant J=8.0 Hz at 7.27 was identified for two aromatic protons. The triplet with the coupling constant J=8.0 Hz, at 7.52 was assigned for two aromatic protons. The doublet with the coupling constant J=8.0 Hz at 7.83 was attributedfor twoaromatic protons. The iminoHC=N proton was detected at 7.88 as a singlet. The doublet with the coupling constant J=4.0 Hz at 8.20 was assigned for two aromatic protons. Thesinglet at 12.71 was distinguished for one N-H proton and the other downfielded sharp singlet at 14.54 was attributed for one N-H proton.

The ^{1}HNMR spectrum **Fig.7** of the compound **3'** (iminol form) showed a multiplet at 7.34 for two aromatic protons. The doublet with the coupling constant J=8.0 Hz at 7.57 was attributed for two aromatic protons.The doublet with the coupling constant J=8.0 Hz at 8.12 was designated for two

aromatic protons.The doublet with the coupling constant J=8.0 Hz at 8.21 was detected for two aromatic protons. The sharp singlet for imino HC=N proton was detected at 8.50. The sharp singlet at 11.88 was distinguished for one proton of enolic O-H and the other downfielded sharp singlet at 12.517 was attributed for N-H proton.

All the spectral evidences expressed harmony with the structure of amide-iminol tautomeric compounds **3** and **3'** as



3 Amide form

3' Iminol form

.

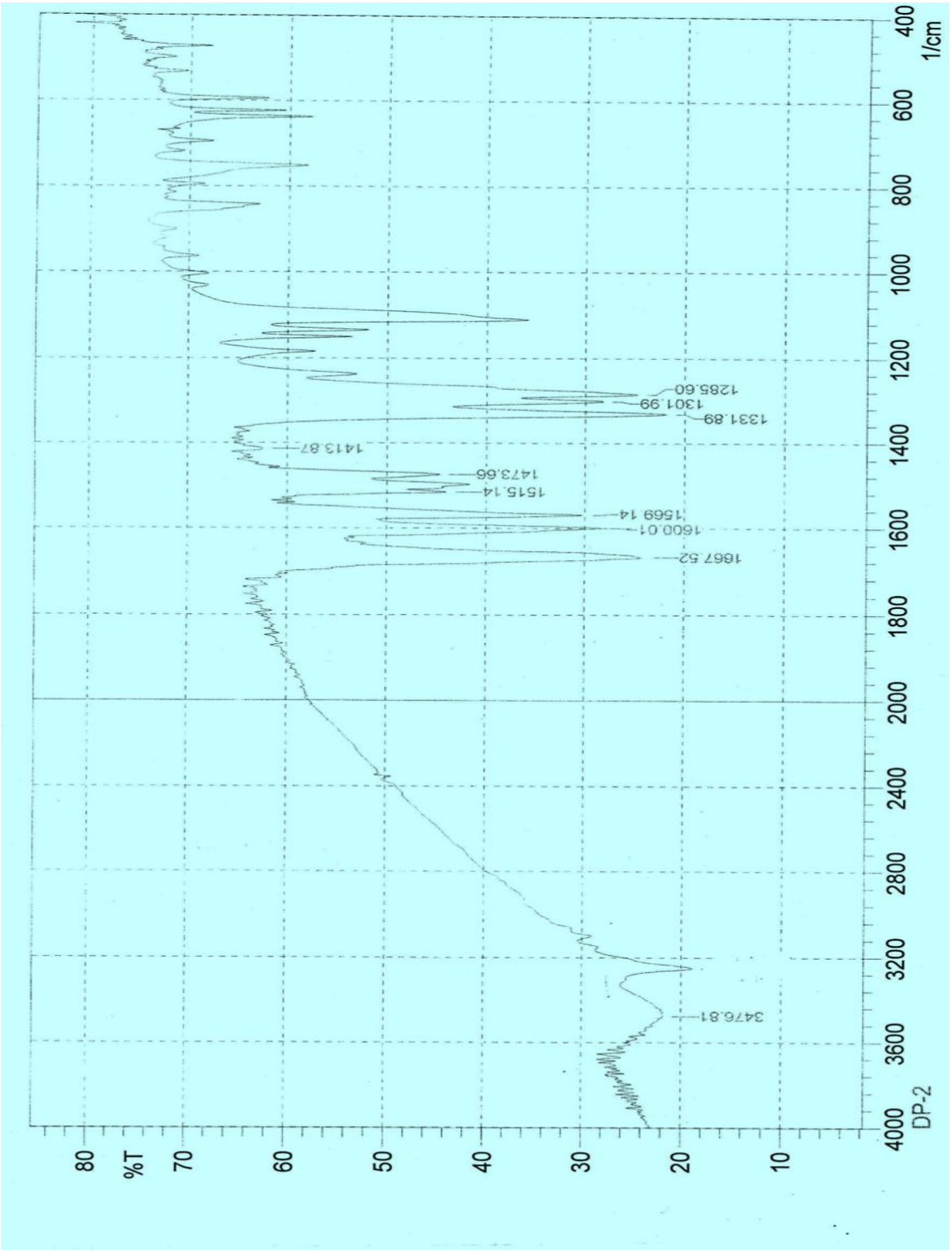


Figure 6: The IR Spectrum of the compound **3** and **3'**

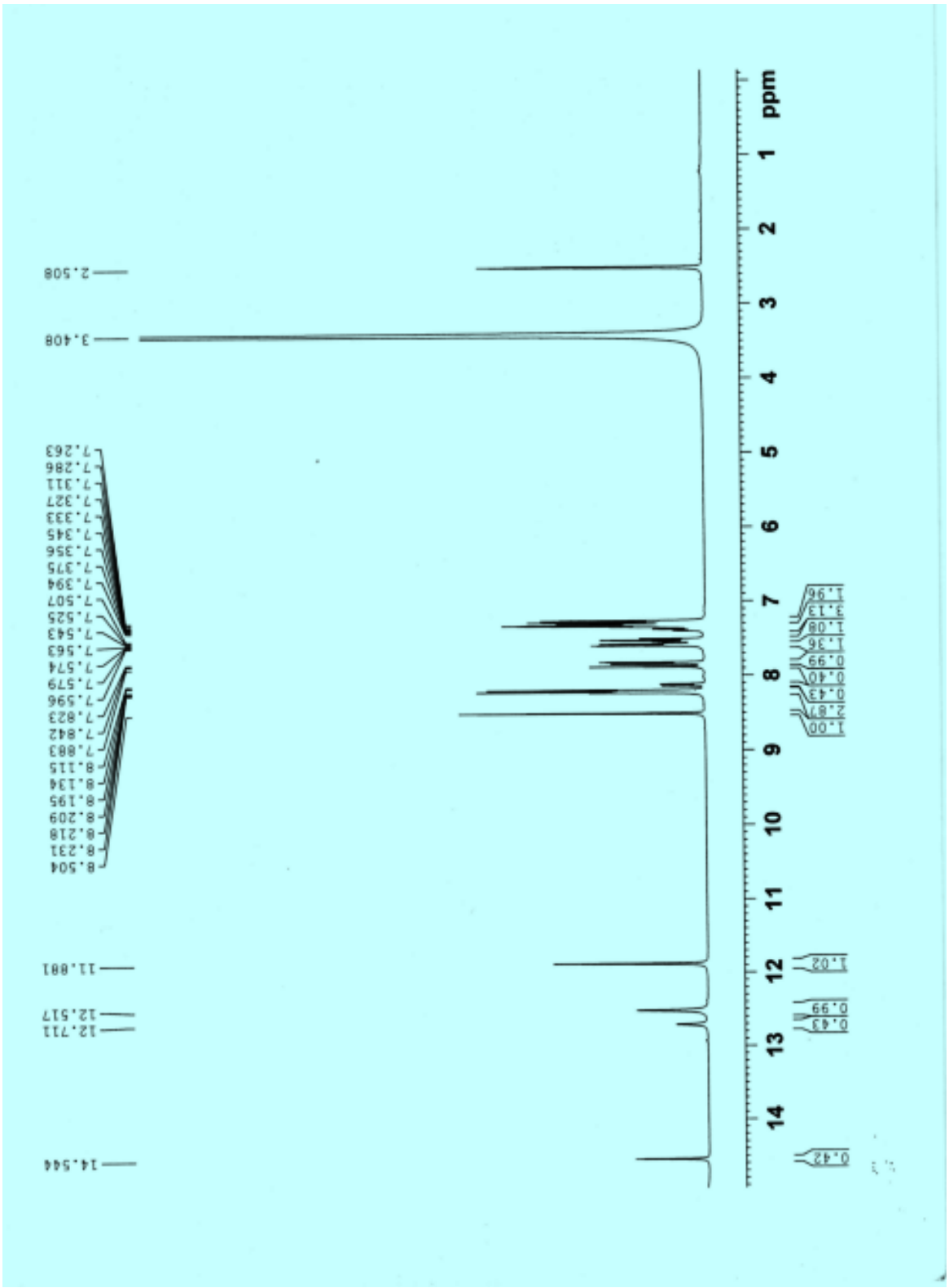


Figure 7 : The [1]HNMR spectrum the compound **3** and **3'**

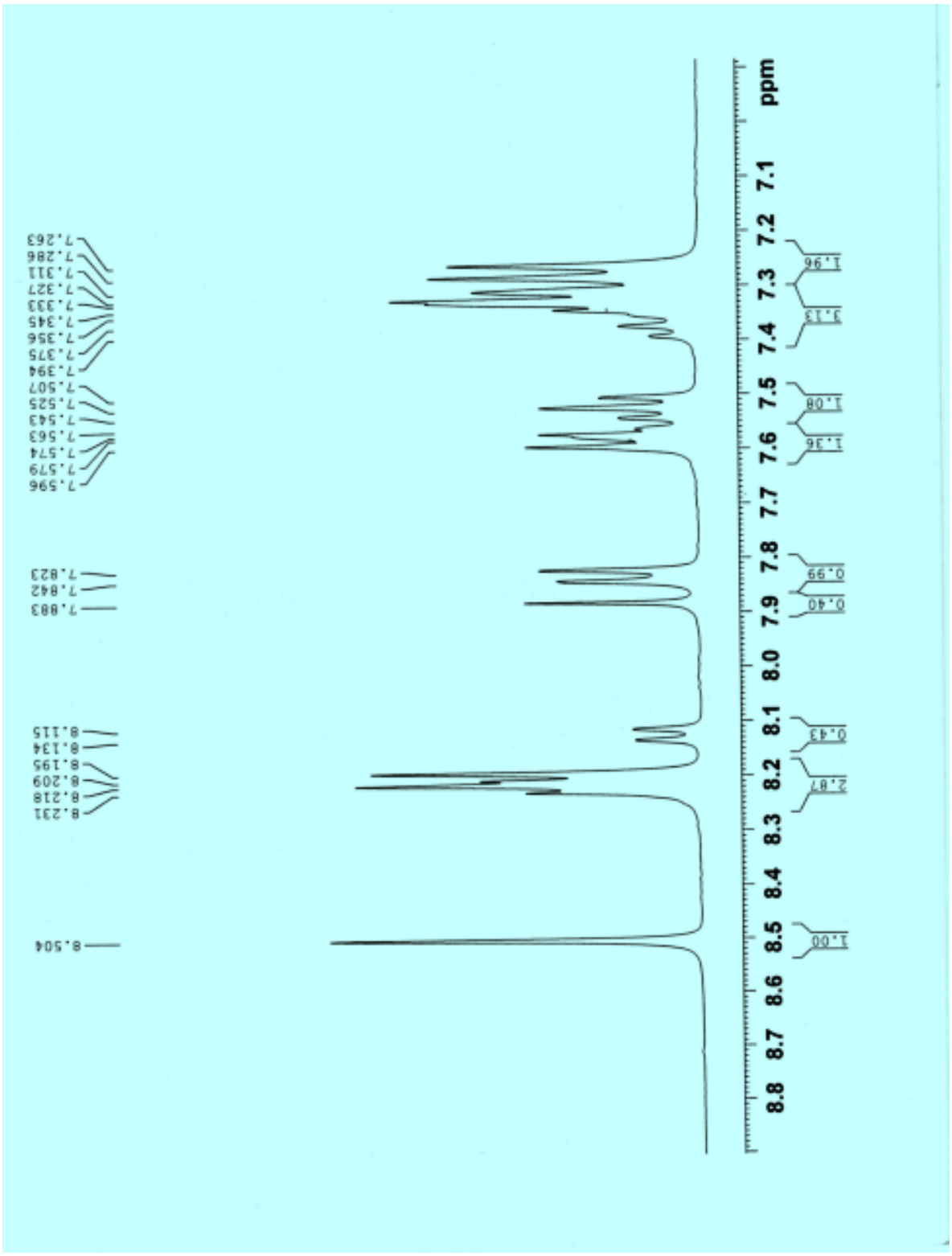


Figure 7 : The ^{1}HNMR spectrum the compound **3** and

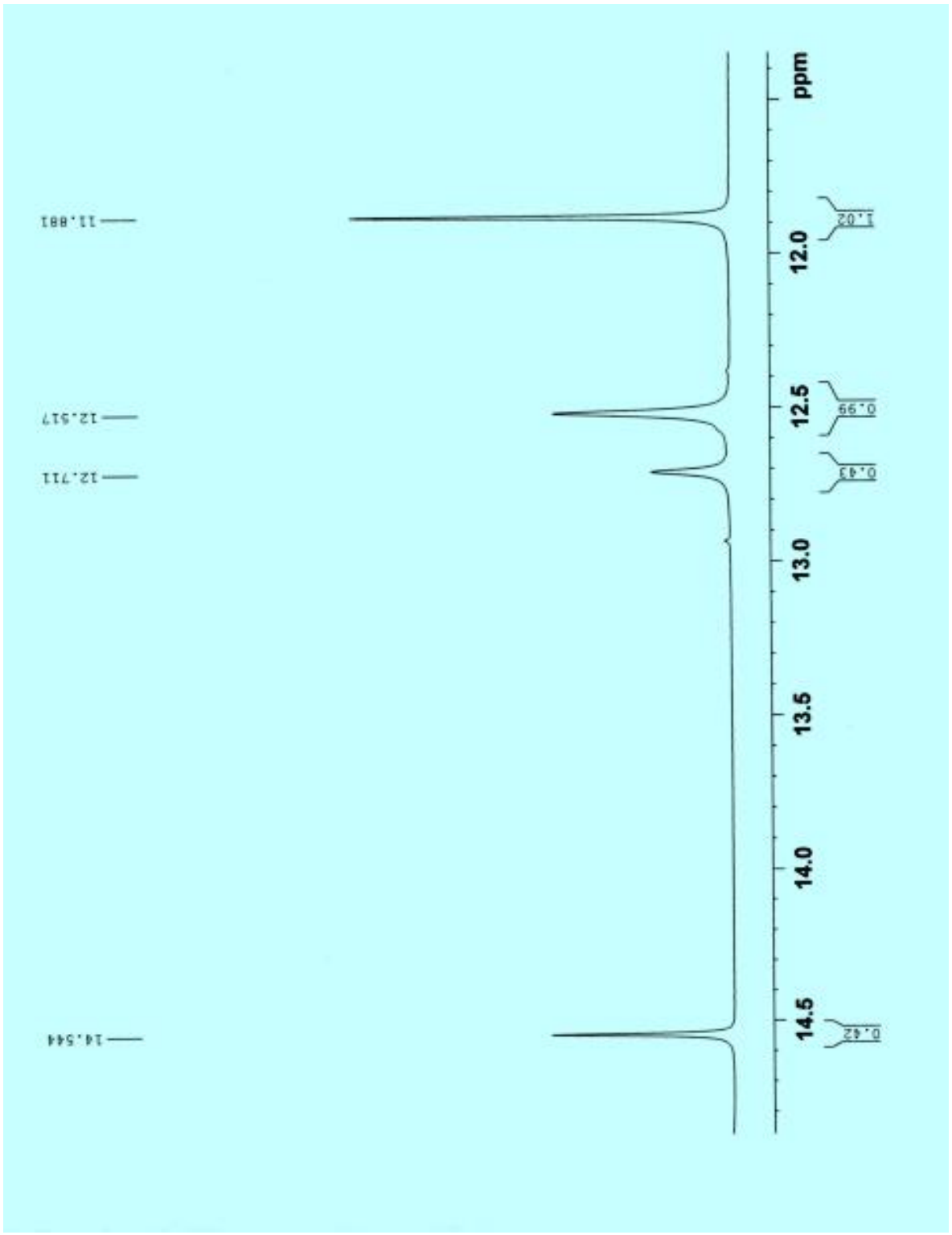
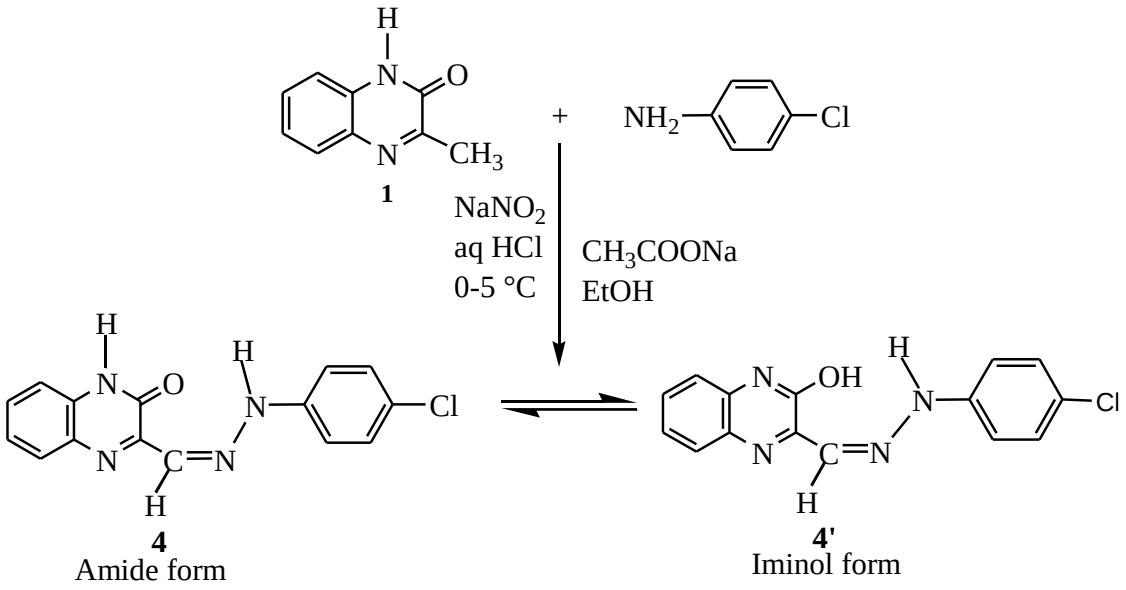


Figure 7 : The [1]HNMR spectrum the compound **3** and **3'**

3.4 Characterization of Amide–Iminol tautomer 3-[(4-Chloro-phenyl)-hydrazonomethyl]-1H-3-quinoxalin-2-one

The amide-iminol tautomeric compounds **4** and **4'**were synthesized from the coupling reaction between the *p*-chlorobenzenediazonium salt and **1**, the active methylated 3-methylquinoxalin-2(1H)-one. An orange solid products **4** and **4'**were obtained with 70% yield and the melting point was recorded as 277-280°C.



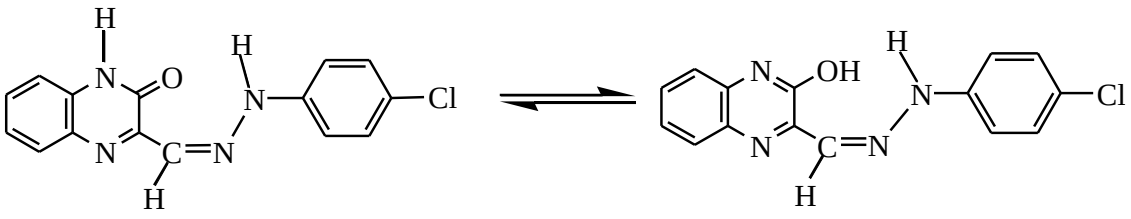
The IR spectrum **Fig.8** of the compound **4** and **4'**showed a broad band at 3370-3500 cm^{-1} for O-H and N-H moieties. The aromatic C-H stretching was identified at 3010 cm^{-1}.The peak at 1674cm^{-1} was identified for C=O of NH-CO. The peak at 1597 cm^{-1} was for C=N moiety. The characteristic peaks at 1548, 1500 and 1475 cm^{-1} were distinguished for aromatic C=C bonds.

The ^{1}HNMR spectrum **Fig.9** of the compound **4** (amide form) showed a doublet with coupling constant J=8.0 Hz at 7.16 for two aromatic protons. The doublet with the coupling constant J=8.0 Hz at 7.38 was attributed for one aromatic proton. The doublet with the coupling constant J=8.0 Hz at 7.45 was designated for four aromatic protons. The another doublet with the coupling constant J=8.0 Hz at 7.78 was assigned for one aromatic proton. The single iminoHC=N proton was detected at 7.74 as a sharp singlet. The singlet at 12.604 was distinguished for one N-H proton and the other downfielded sharp singlet at 14.470 was attributed for another N-H proton.

The ^{1}HNMR spectrum **Fig.9** of the compound **4'** (iminol form) showed a multiplet at7.32 for six aromatic protons. The triplet with the coupling constant J=8.0 Hz at 7.54 was attributed for one aromatic proton.The doublet with the coupling constant J=8.0 Hz at 8.08 was designated for one

aromatic proton. The sharp singlet for imino HC=N proton was detected at 8.358. The sharp singlet at 11.30 was distinguished for one proton of enolic O-H and the downfielded sharp singlet at 12.40 was attributed for one N-H proton.

All the spectral evidences expressed harmony with the structure of amide-iminol tautomeric compounds **4** and **4**'as



4
Amide form

4'
Iminol form

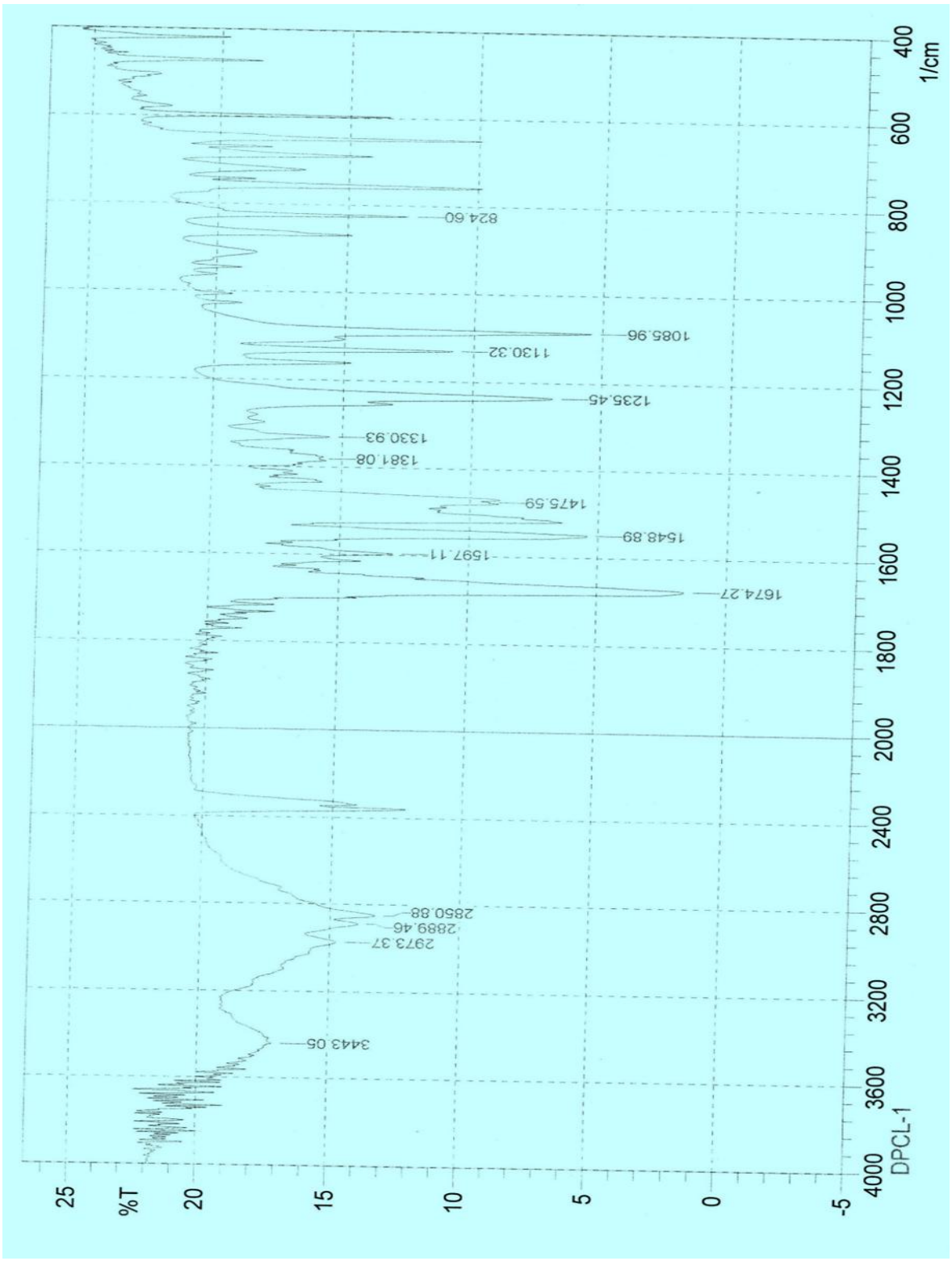


Figure 8 : The IR Spectrum of the compound **4** and **4'**

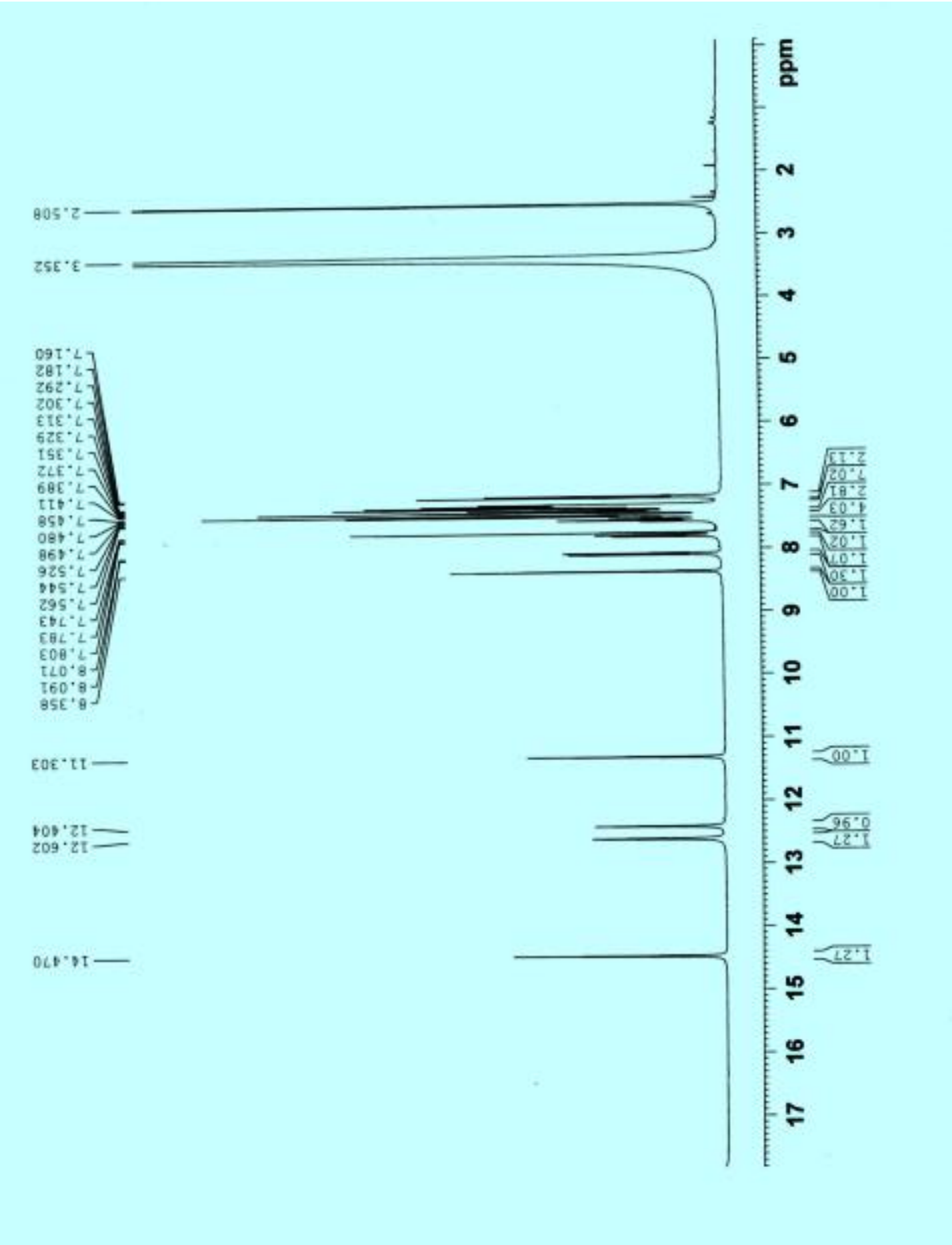


Figure 9 : The ^{1}HNMR spectrum the compound **4** and **4'**

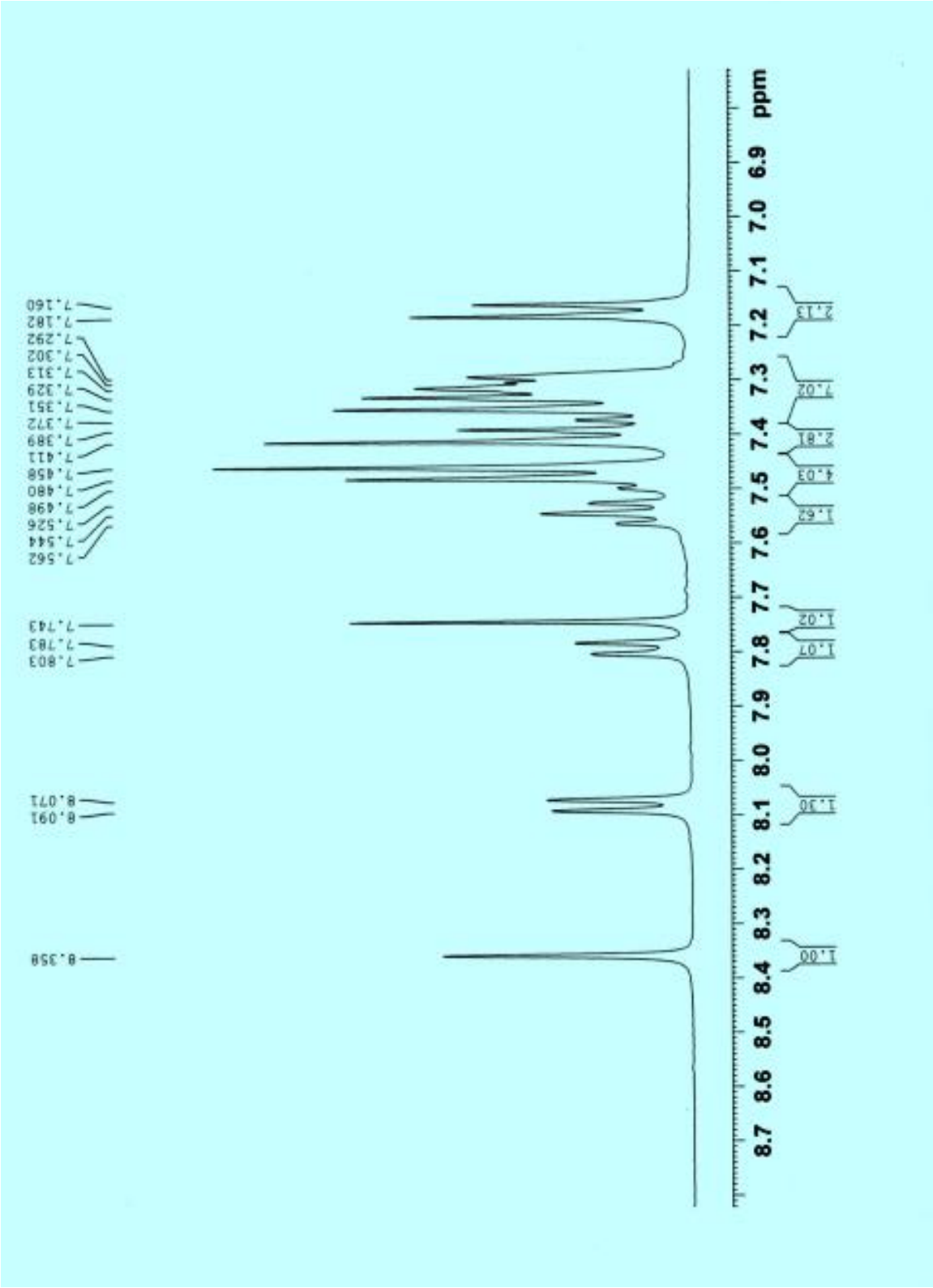
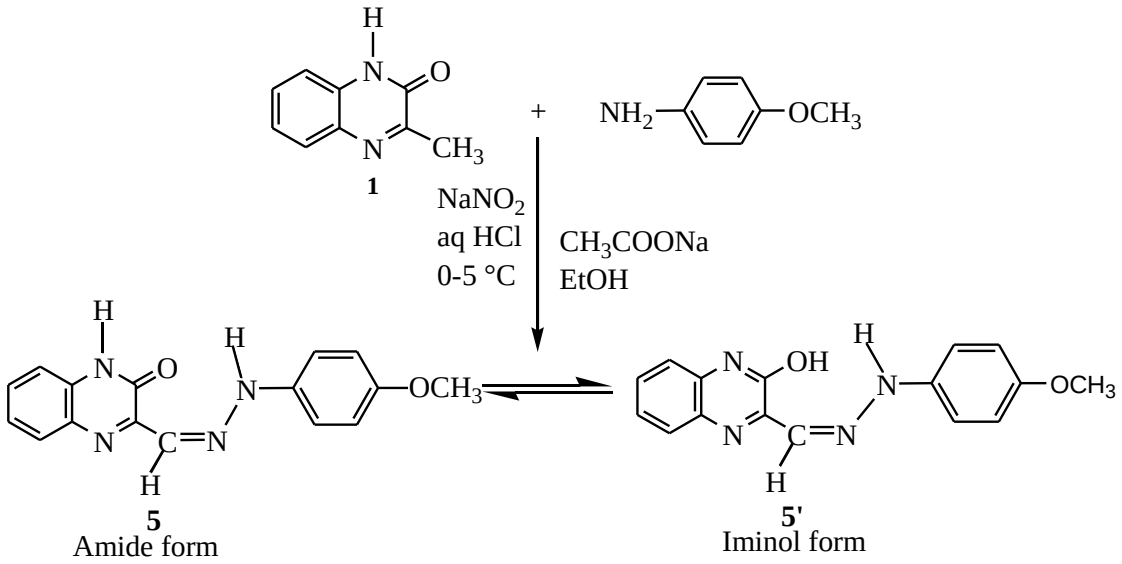


Figure 9 : The ^{1}HNMR spectrum the compound **4** and **4'**

3.5 **Characterization of Amide-iminol tautomer 3-(4-Methoxy-phenyl -hydrazonomethyl)-1H-3quinoxalin-2-one**

The amide-iminol tautomeric compounds **5** and **5'**were synthesized from the coupling reaction between the *p*-methoxybenzenediazonium salt and **1**, the active methylated 3-methylquinoxalin-2(1H)-one. A dark brown solid products**5** and **5'** were obtained with 70% yield and the melting point was recorded as 290-293°C.

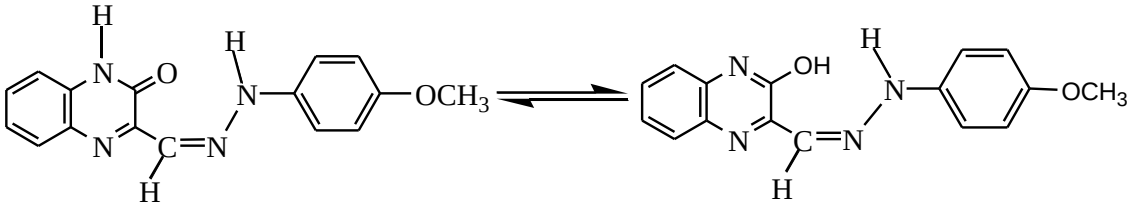


The IR spectrum **Fig.10** of the compound **5** and **5'**showed a broad band at 3360-3520 cm^{-1} for O-H and N-H moieties. The aromatic C-H stretching was identified at 3091 cm^{-1}. The peak at 2972, 2893, 2835 cm^{-1} were assigned for aliphatic C-H and imino C-H stretching. The peak 1672cm^{-1} was designated for C=O group of HN-C=O. The peak at 1607 cm^{-1} was for C=N moiety. The characteristic peaks at 1551, 1519, 1469 cm^{-1} were distinguished for aromatic C=C bonds.

The ^{1}HNMR spectrum **Fig.11** of the compound **5** (amide form) showed a sharp singlet at 3.75 for three protons of -OCH_3. The doublet with the coupling constant J=12.0 Hz at 7.16 was assigned for two aromatic protons. The multiplet for two aromatic protons was designated at 7.28.The doublet with the coupling constant J=8.0 Hz was identified at 7.38 for two aromatic protons.The triplet with the coupling constant J=8.0 Hz was detected at 7.49 for one aromatic proton. The sharp singlet for single HC=N iminoproton was detected at 7.64. The doublet with the coupling constant J=10.0 Hz at 7.99 was assigned for two aromatic protons. The sharp singlet at 13.50 was distinguished for one N-H proton and the other downfielded sharp singlet at 14.57 was attributed for another N-H proton.

The ^{1}HNMR spectrum **Fig.11** of the compound **5'** (iminol form) showed a sharp singlet at 3.726 for three protons of -OCH_3. The doublet with coupling constant J=12.0 Hz at 6.92 was detected for two aromatic protons. The doublet with the coupling constant J=12.0 Hz at 7.12 was designated for one aromatic proton.The triplet with the coupling constant J=8.0 Hz at 7.33 was identified for two aromatic protons. The doublet with the coupling constant J=12.0 Hz at 7.44 was attributed for one aromatic proton.The doublet with the coupling constant J=12.0 Hz at 7.76 was charcaterized for one aromatic proton.The singlet at 8.29 was detectable for one imino proton HC=N. The sharp singlet at 11.13 was distinguished for one proton of enolic O-H and the downfielded sharp singlet at 12.40 was attributed forone N-H proton.

All the spectral evidences expressed harmony with the structure of amide-iminoltautomeric compounds **5** and **5'** as



5 Amide form

5' Iminol form

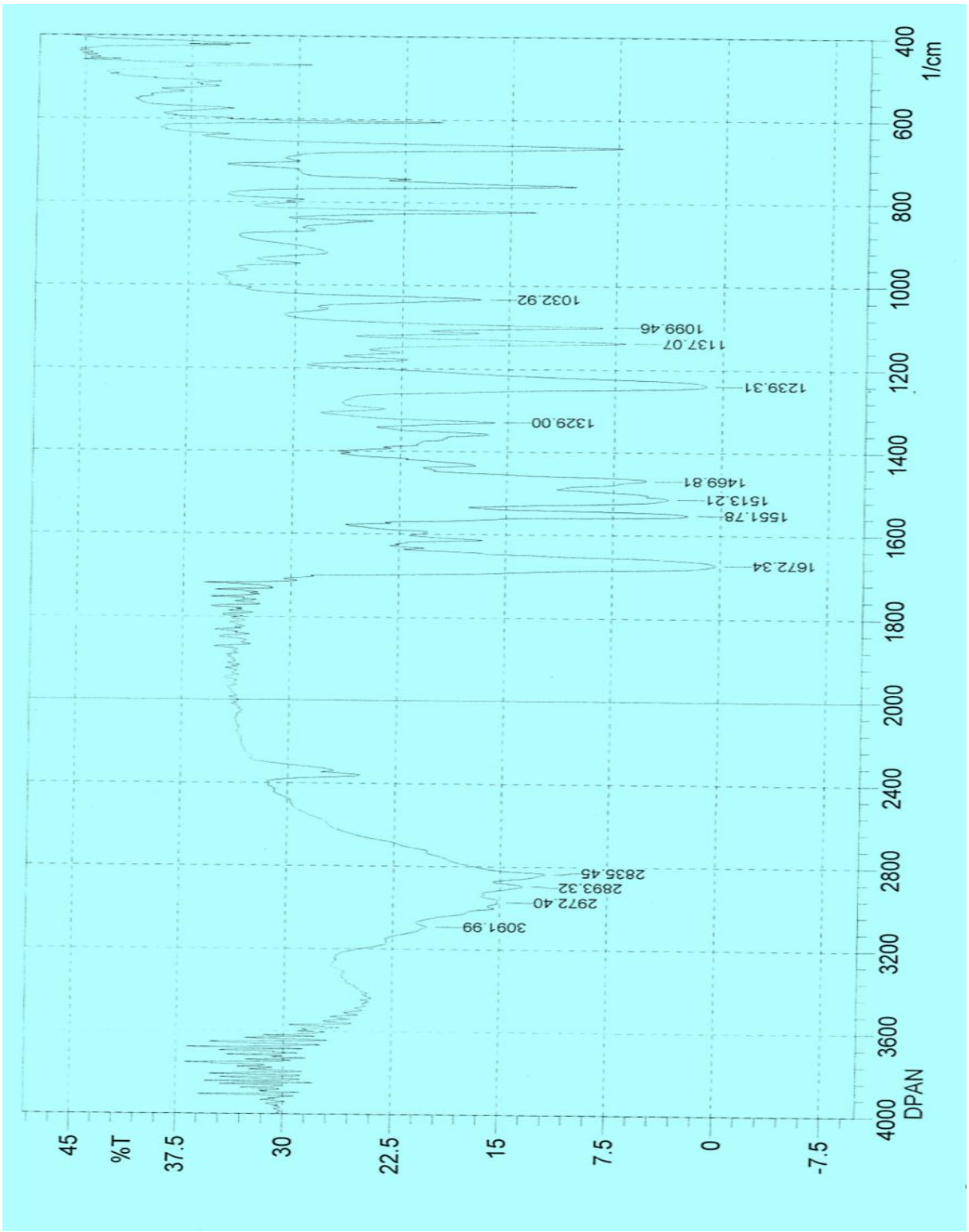


Figure 10 : The IR Spectrum of the compound **5** and **5'**

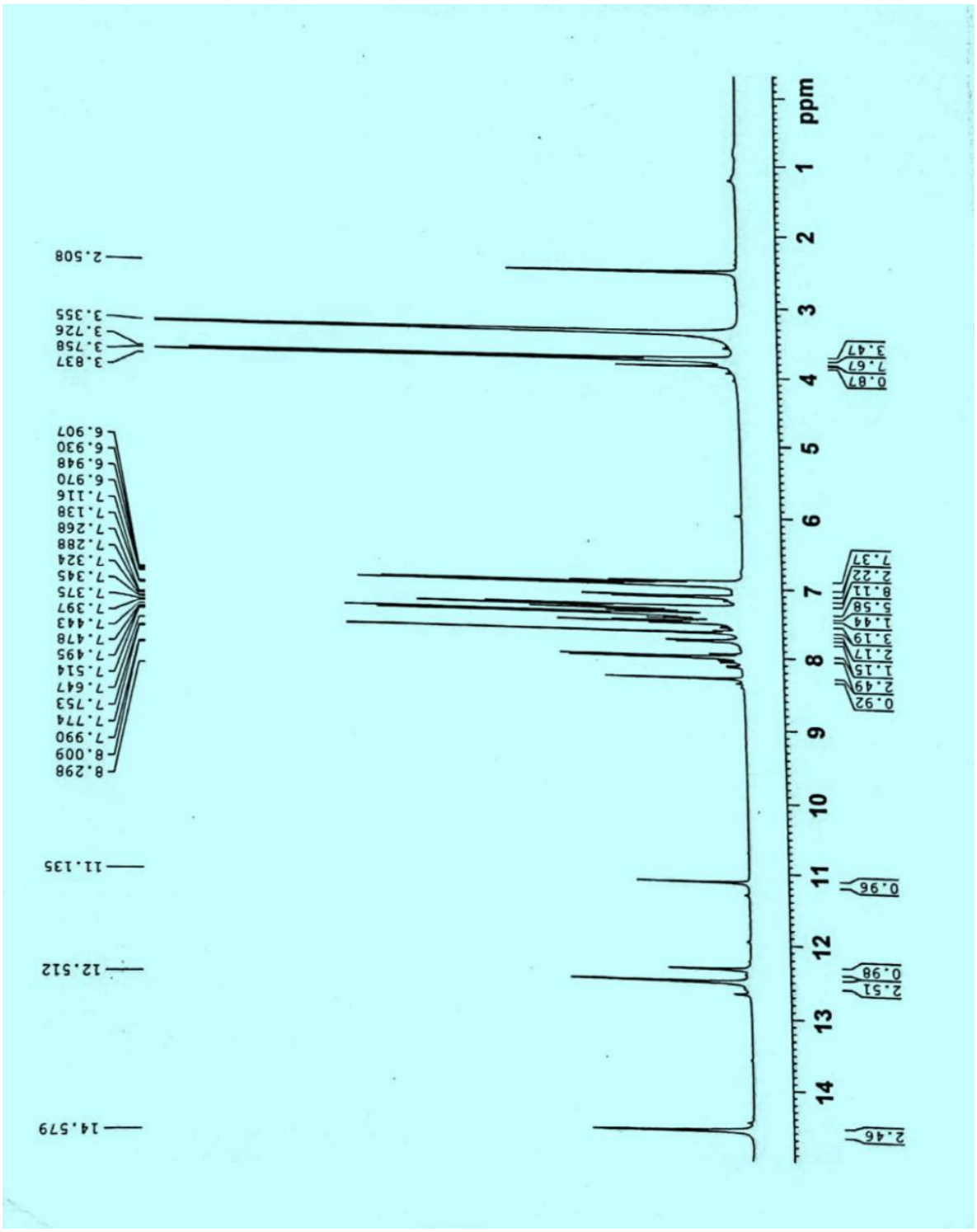


Figure 11 : The [1]HNMR spectrum the compound **5** and **5'**

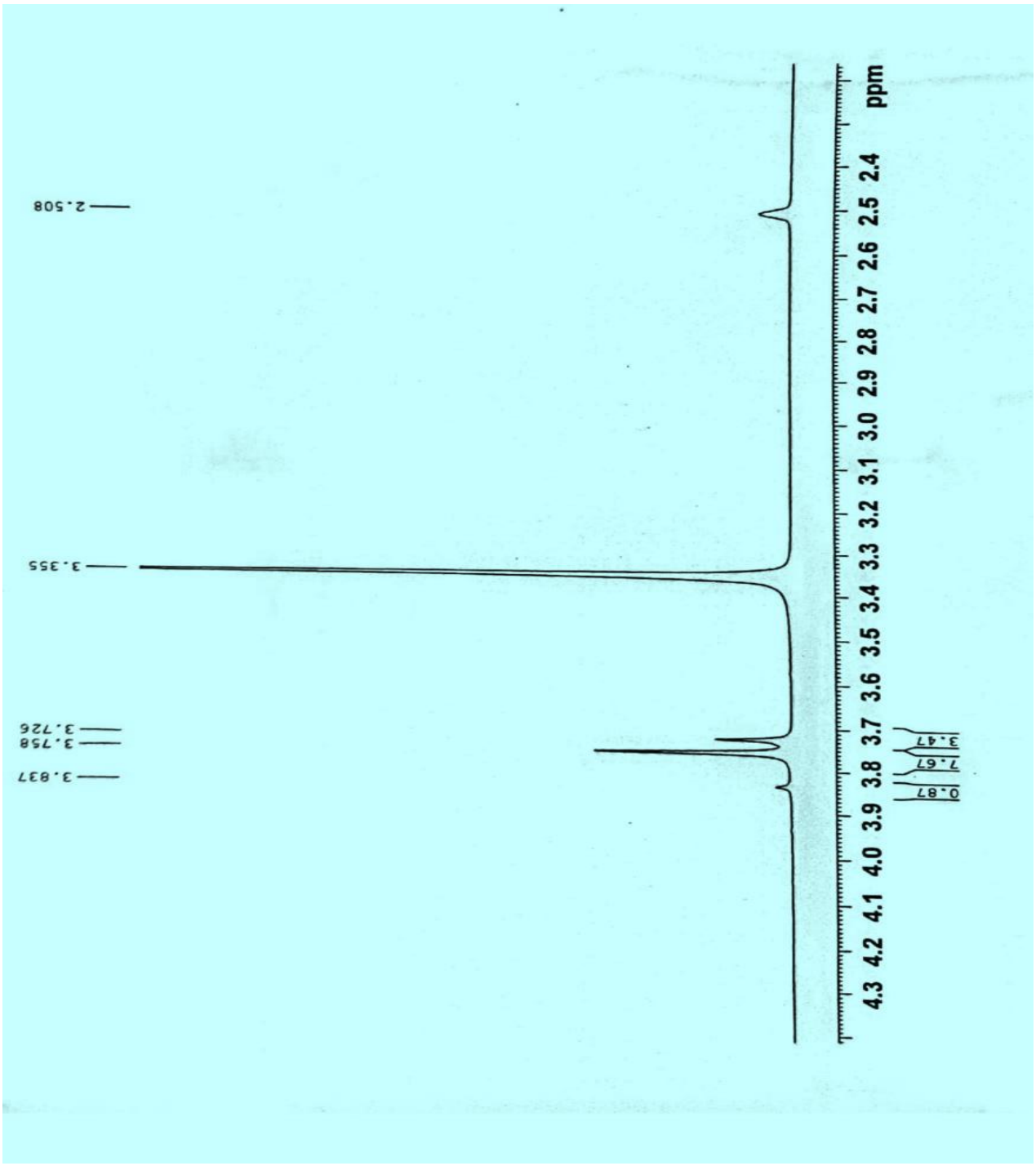


Figure 11: The [1]HNMR spectrum the compound **5** and **5'**

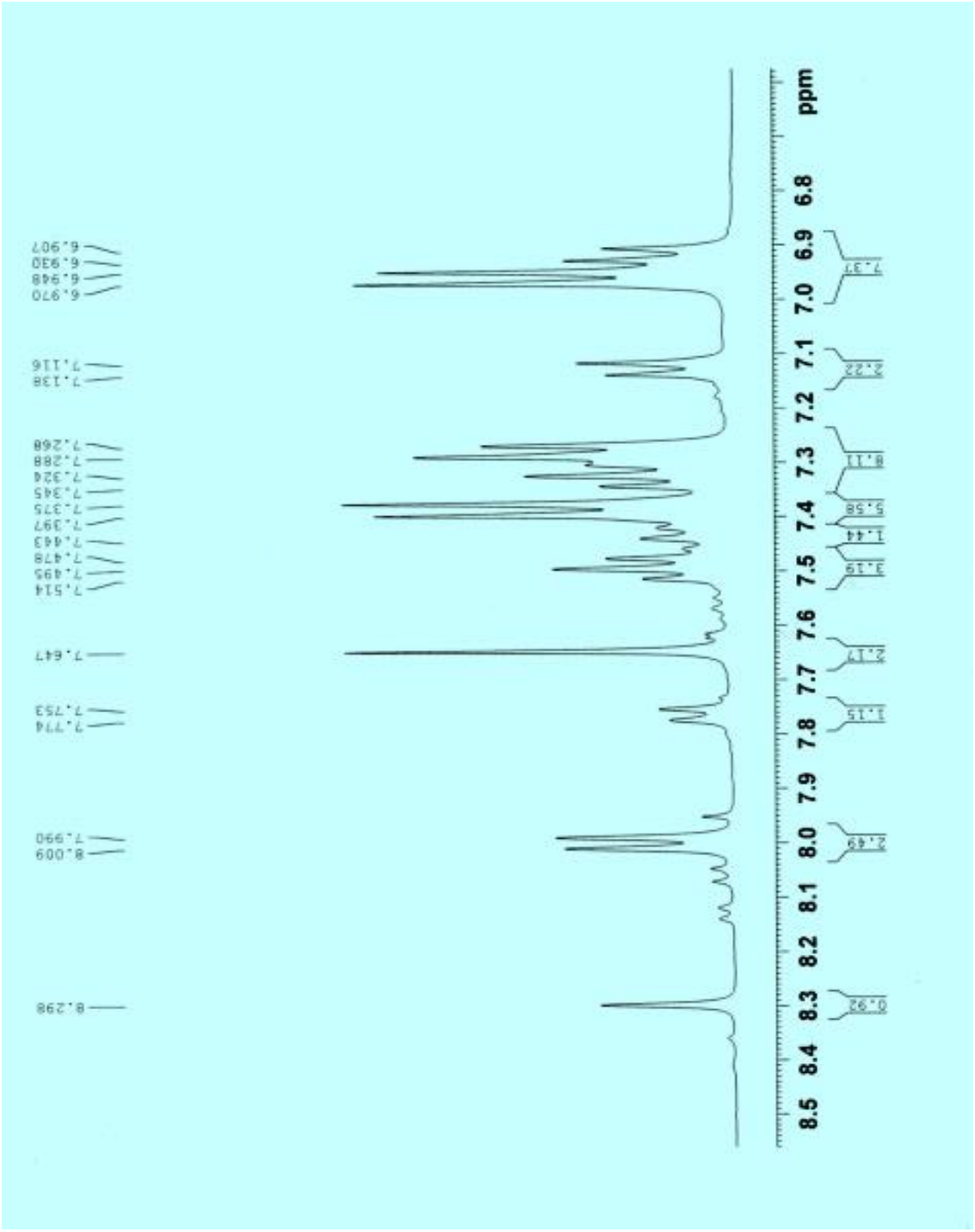
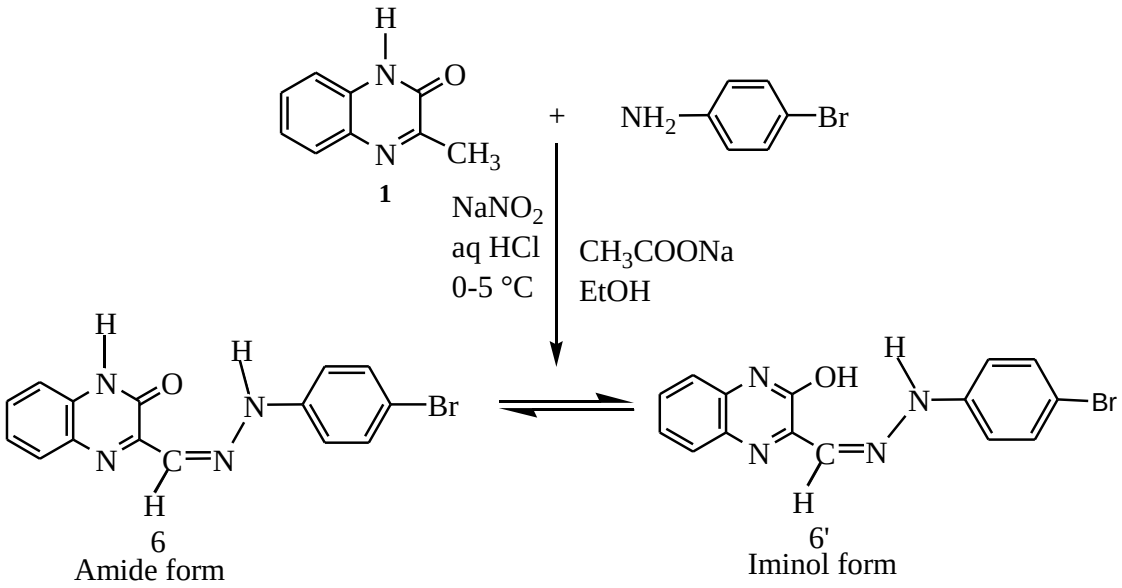


Figure 11: The ^{1}HNMR spectrum the compound **5** and **5'**

3.6 Characterization of Amide–Iminoltautomer 3-[(4-Bromo-phenyl)-hydrazonomethyl]-1H-3-quinoxalin-2-one

The amide-iminol tautomeric compounds **6** and **6'**were synthesized from the coupling reaction between the *p*-bromobenzenediazonium salt and **1**, the active methylated 3-methylquinoxalin-2(1H)-one. An orange solid products **6** and **6'**were obtained with 75% yield and the melting point was recorded as 272-275°C .



The IR spectrum **Fig.12** of the compound **6** and **6**'showed a broad band at 3510-3350 cm^{-1} for O-H and N-H moieties. The aliphatic C-H stretching was identified at 2968 cm^{-1}.The peak at 1677cm^{-1} was identified for C=O of -NH-CO. The peak at 1590 cm^{-1} was for C=N moiety. The characteristic peaks at 1548, 1520 and 1476 cm^{-1} were distinguished for aromatic C=C bonds.

The ^{1}HNMR spectrum **Fig.13** of the compound **6** (amide form) showed a doublet with coupling constant J=12.0 Hz at 7.10 fortwo aromatic protons. The multiplet at 7.35 was assignedfor two aromatic protons. The doublet with the coupling constant J=8.0 Hz at 7.47 was designated for three aromatic protons.The sharp singlet for single iminoHC=N proton was detected at 7.74. The doublet with the coupling constant J=8.0 Hz at 7.78 was designated for one aromatic proton. The sharp singlet at 12.611 was distinguished for one N-H proton and the other downfielded sharp singlet at 14.56 was attributed for another N-H proton.

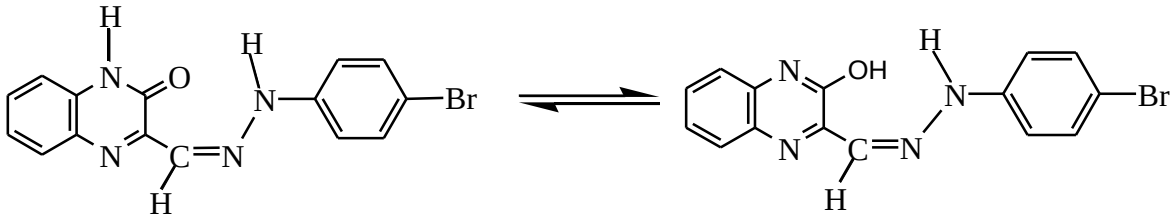
The ^{1}HNMR spectrum **Fig.13** of the compound **6'** (iminol form) showed a doublet with coupling constant J=8.0 Hz at 7.32 was detected for two aromatic protons. The doublet with the coupling constant J=8.0 Hz at 7.42 was designated for two aromatic protons. The multiplet at 7.51 was

identified for three aromatic protons. The doublet with the coupling constant J=8.0 Hz at 8.07 was attributed for one aromatic proton.The sharp singlet for single iminoHC=N proton was detected at 8.36. The sharp singlet at 11.31 was distinguished for one proton of enolic O-H and the downfielded sharp singlet at 12.41 was attributed for N-H proton.

The ^{13}C NMR **Fig.14** of the compound **6** (amide form) showed the signals at 155.20 (1C, N-C=O), 151.26 (1C, C=N), 143.96 (1C, N=C-C=O), 133.12(1C, Ar-C), 132.45(1C, Ar-C), 132.05 (1C, Ar-C), 131.36(3C, Ar-C),130.6(1C, Ar-C) , 129.01 (1C, Ar-C), 124.05 (1C, Ar-C), 116.463 (1C, Ar-C), 115.73(1C, Ar-C) ,115.35(1C, Ar-C).

The ^{13}C NMR **Fig.14** of the compound **6**' (iminol form) showed the signals at 154.95 (1C, N-C=O), 149.97 (1C, C=N), 143.146(1C, N=C-C=O), 132.56(1C, Ar-C), 132.13(1C, Ar-C), 131.75 (1C, Ar-C), 131.03(3C, Ar-C),129.05(1C, Ar-C) , 124.39 (1C, Ar-C), 123.40 (1C, Ar-C), 115.87 (1C, Ar-C), 114.14(1C, Ar-C) ,112.06 (1C, Ar-C).

All the spectral evidences expressed harmony with the structure of the amide-iminol tautomeric compounds **6** and **6**' as



6 Amide form

6' Iminol form

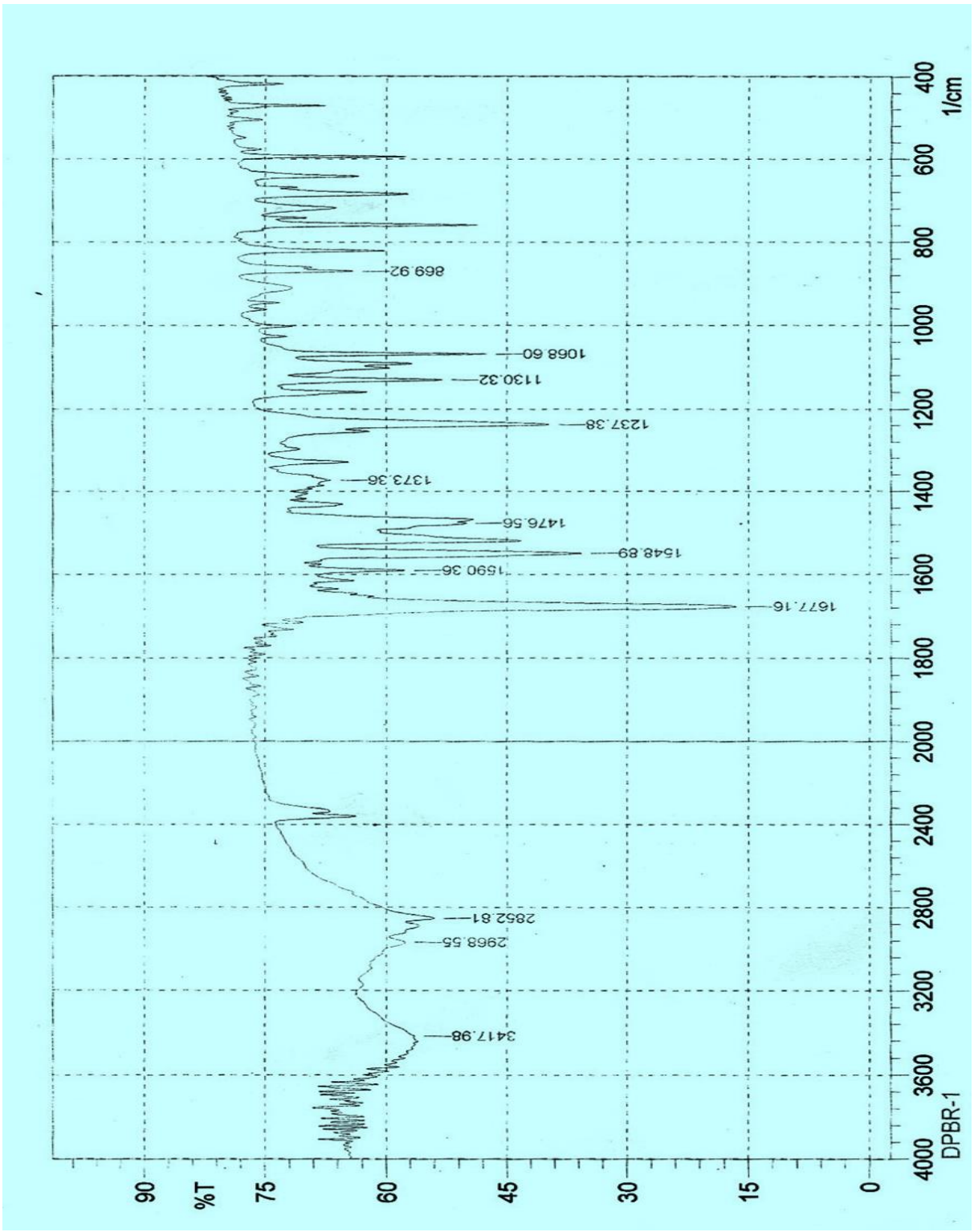


Figure 12 : The IR Spectrum of the compound **6** and **6'**

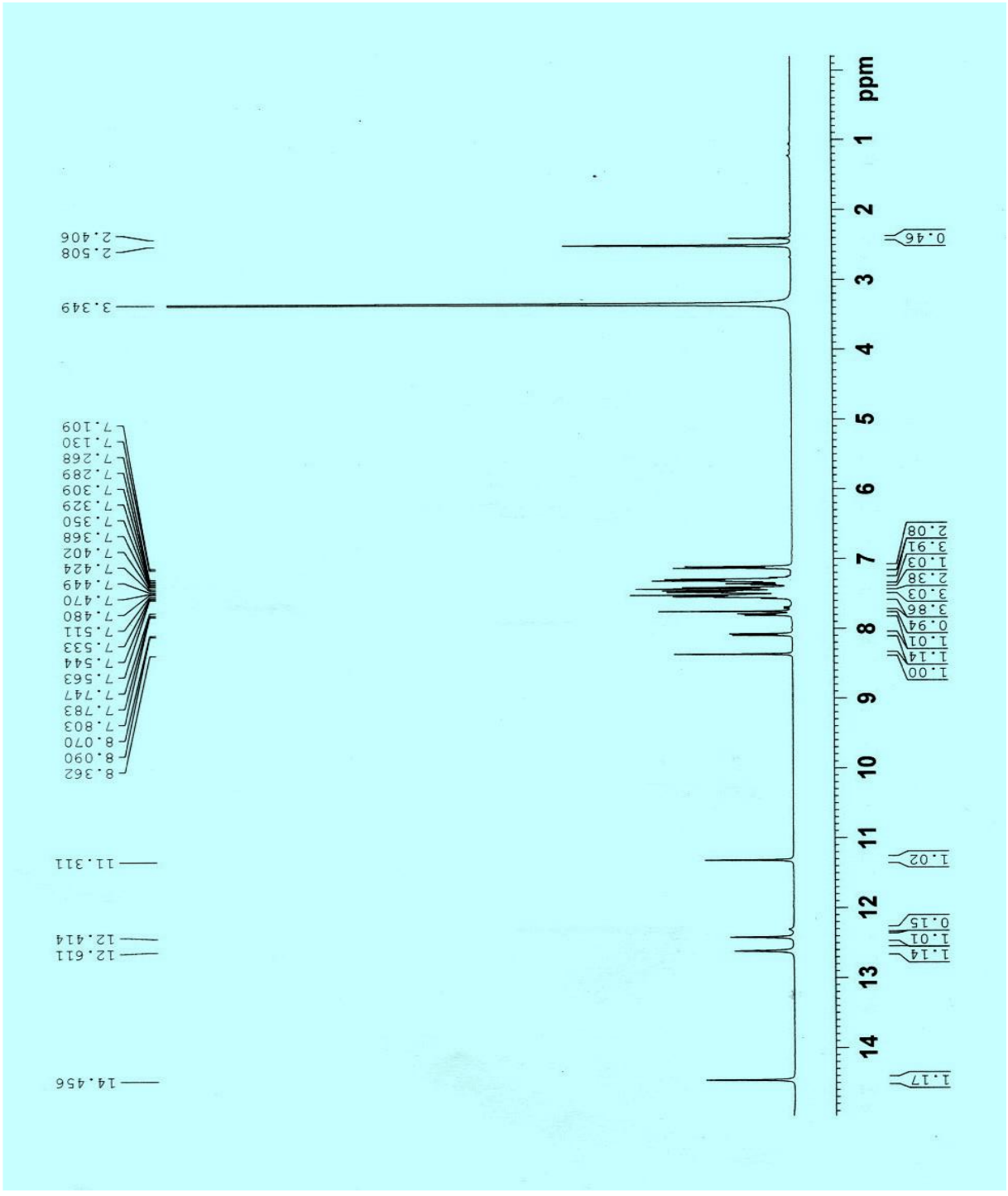


Figure 13 : The ^{1}HNMR spectrum the compound **6** and **6'**

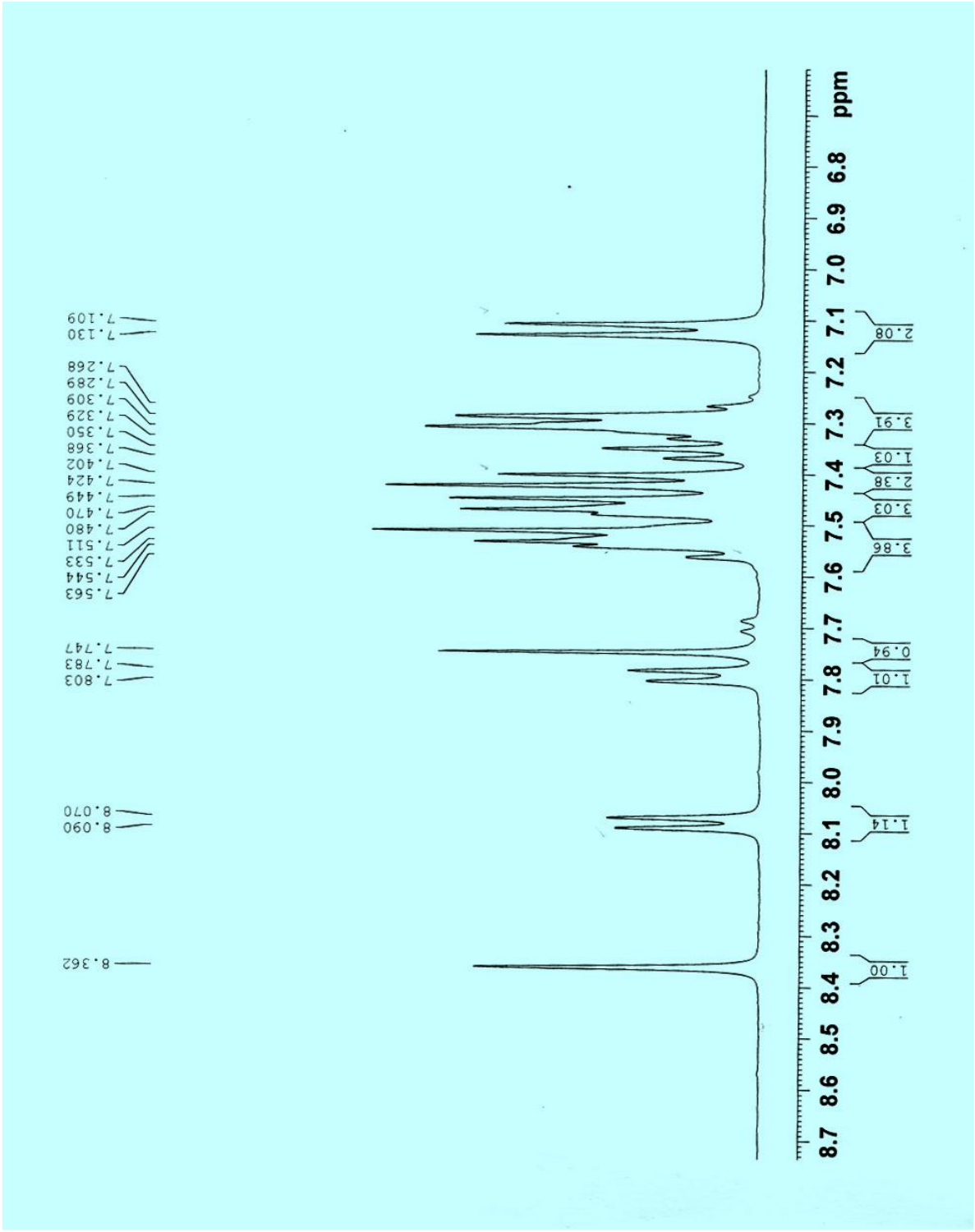


Figure 13 : The ^{1}HNMR spectrum the compound **6** and **6'**

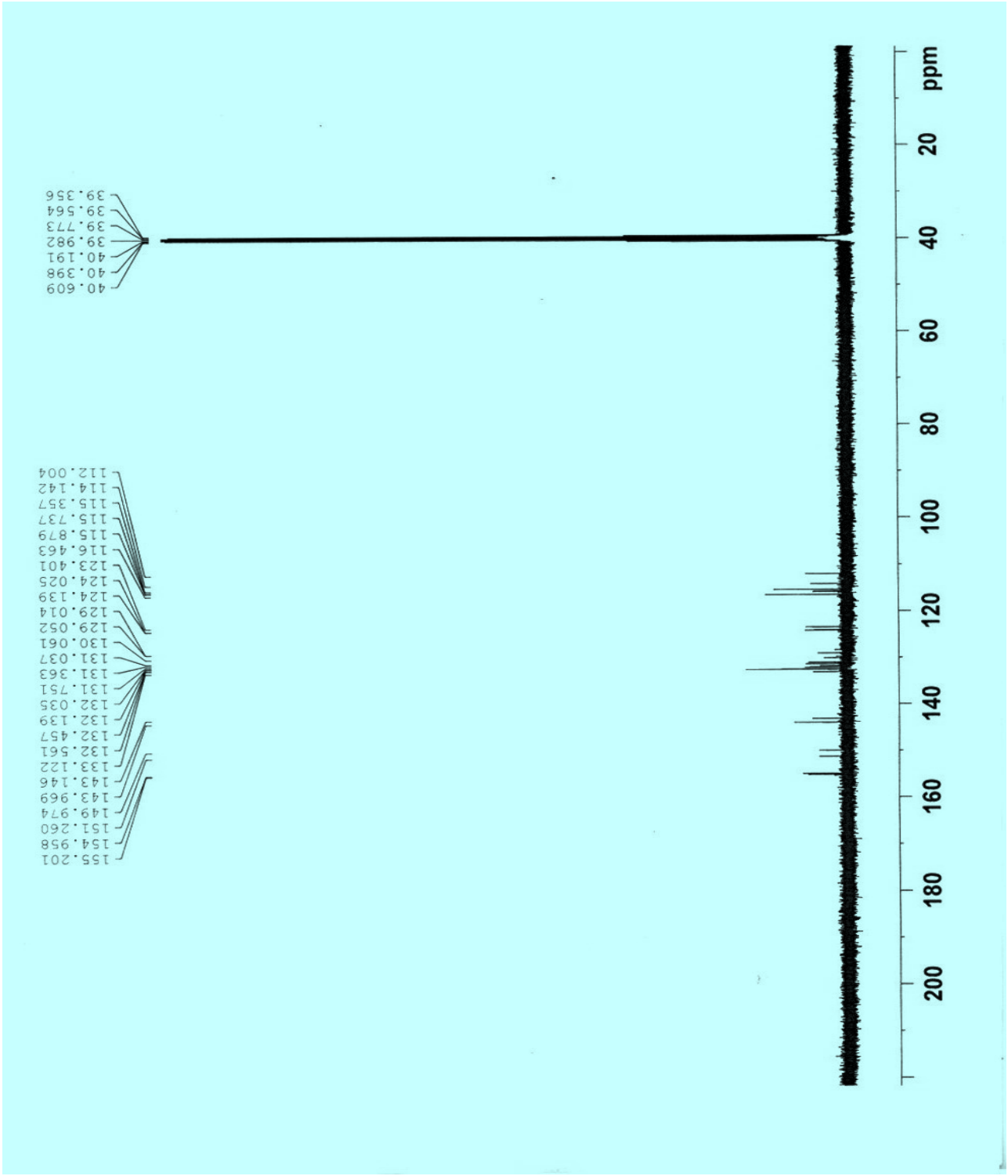


Figure 14 : The ^{13}C NMR spectrum the compound 6 and 6'

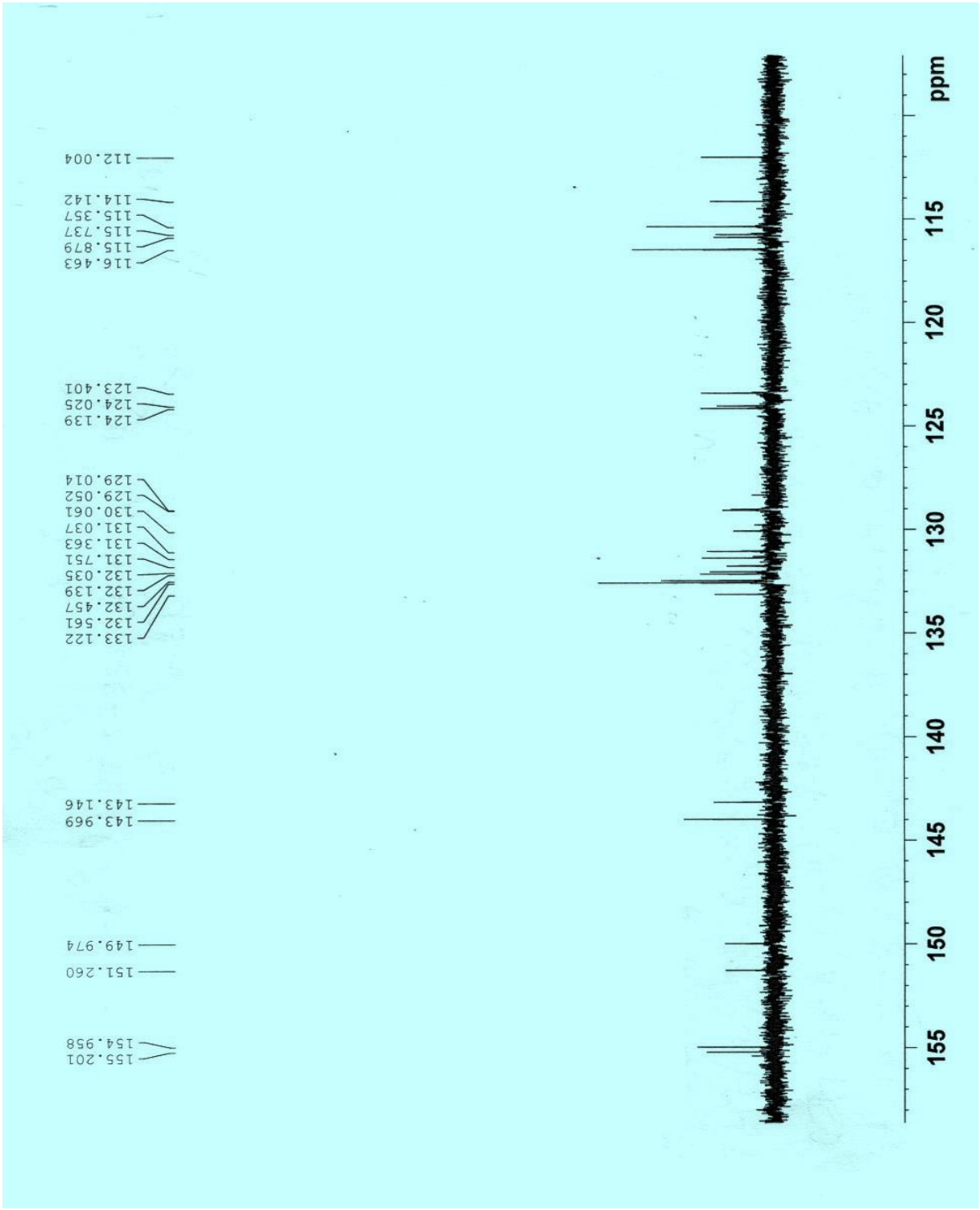
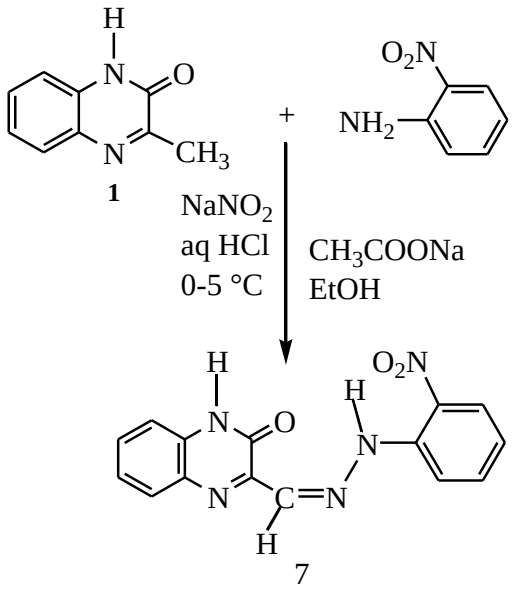


Figure 14 : The ^{13}C NMR spectrum the compound 6 and 6'

3.7 Characterization of 3-[(2-Nitro-phenyl)-hydrazonomethyl]-1H-3-quinoxalin-2-one

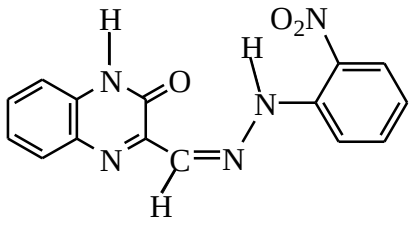
The compound **7** was synthesized from the coupling reaction between the *o*-nitrobenzene diazonium salt and **1**, the active methylated 3-methylquinoxalin-2(1H)-one. An reddish brown solid product **7** was obtained with 60% yield and the melting point was recorded as 268-270°C .



The IR spectrum **Fig.15** of the compound **7**showed a broad band at 3360-3520 cm^{-1} for free hydrogen bonded N-H. The aromatic C-H stretching was identified at 3099 cm^{-1}.The imino C-H stretching was detected at 2972cm^{-1}. The conjugated amide C=O group of -NH-C=O was assigned at 1676cm^{-1}. The peak at 1612 cm^{-1} was for C=N moiety. The characteristic peaks at 1580, 1556 and 1520 cm^{-1} were distinguished for aromatic C=C bonds.

The ^{1}HNMR spectrum **Fig.16** of the compound **7**showed a triplet with coupling constant J=8.0 Hz at 7.16 for one aromatic proton. The doublet with the coupling constant J=8.0 Hz at 7.35 was designated for one aromatic proton. The triplet with the coupling constant J=8.0 Hz at 7.45 was detected for one aromatic proton.The triplet with the coupling constant J=8.0 Hz at 7.61 was attributed for one aromatic proton. The triplet with the coupling constant J=8.0 Hz at 7.79 was characterized for one aromatic proton.The sharp singlet for one HC=N imino proton was detected at 8.05. The doublet with the coupling constant J=8.0 Hz at 8.15 was designated for one aromatic proton.The doublet with the coupling constant J=8.0 Hz at 8.18 was identified for one aromatic proton. The doublet with the coupling constant J=8.0 Hz at 8.25 was designated for one aromatic proton. The sharp singlet at 12.76 was attributable for one N-H proton.

All the spectral evidences expressed harmony with the structure of compounds **7** as



7

The compound **7** did not show any amide-iminol tautomeric form.The *o*-nitro group of the benzenediazonium salt might have formed hydrogen bonding with the N-H hydrogen and formed a stable rigid structure.

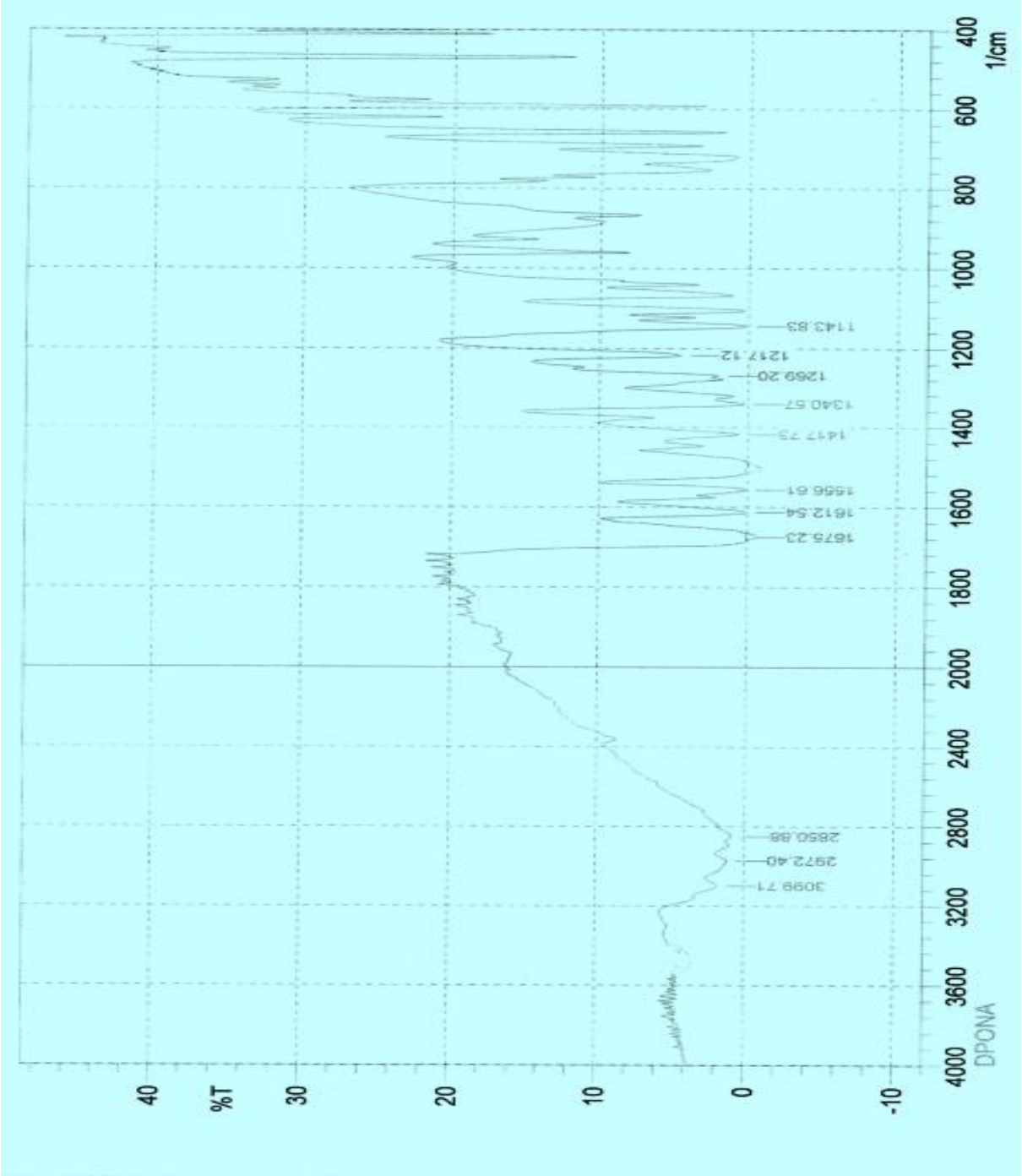


Figure 15 : The IR Spectrum of the compound **7**

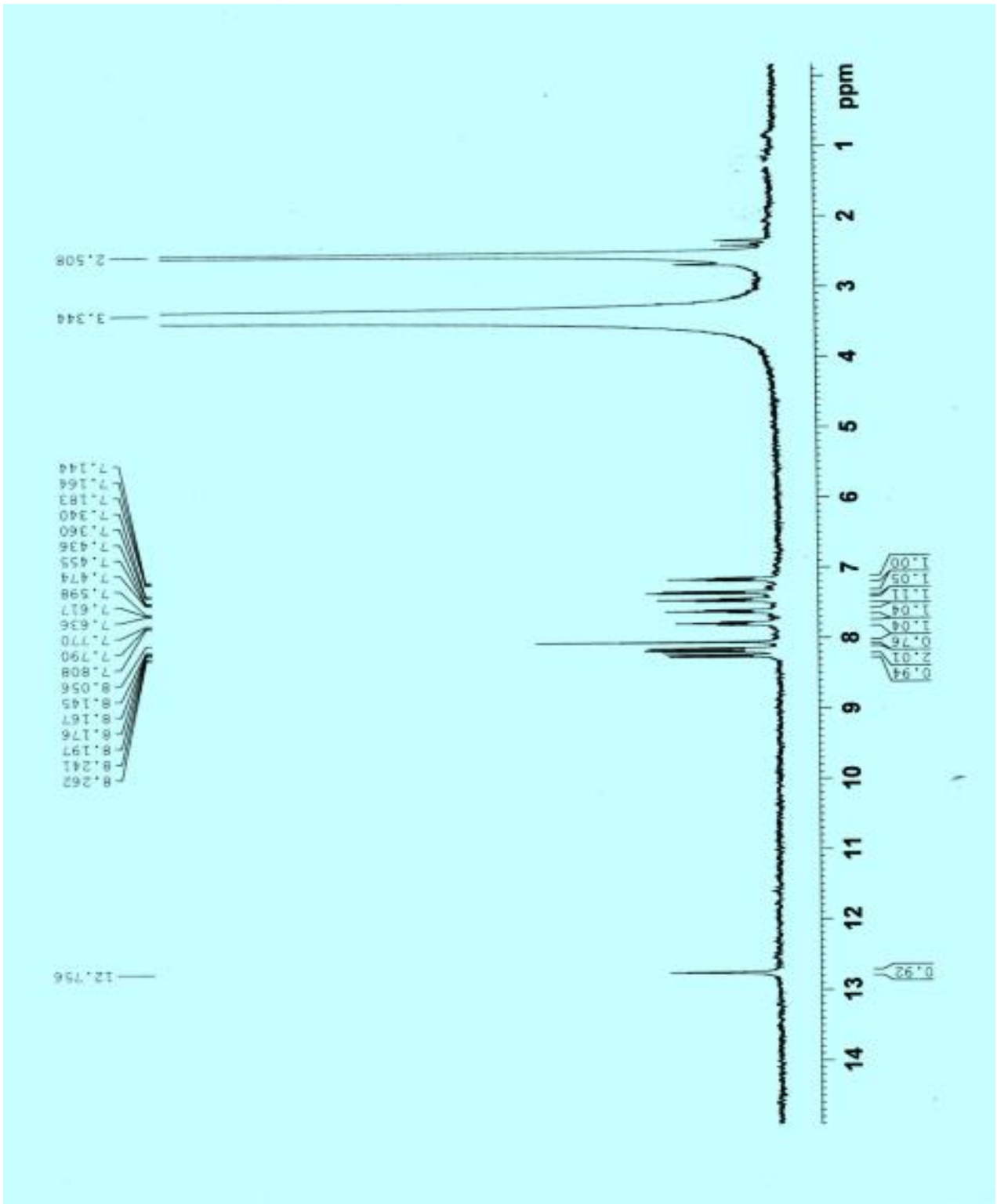


Figure 13 : The ^{1}HNMR spectrum the compound **7**

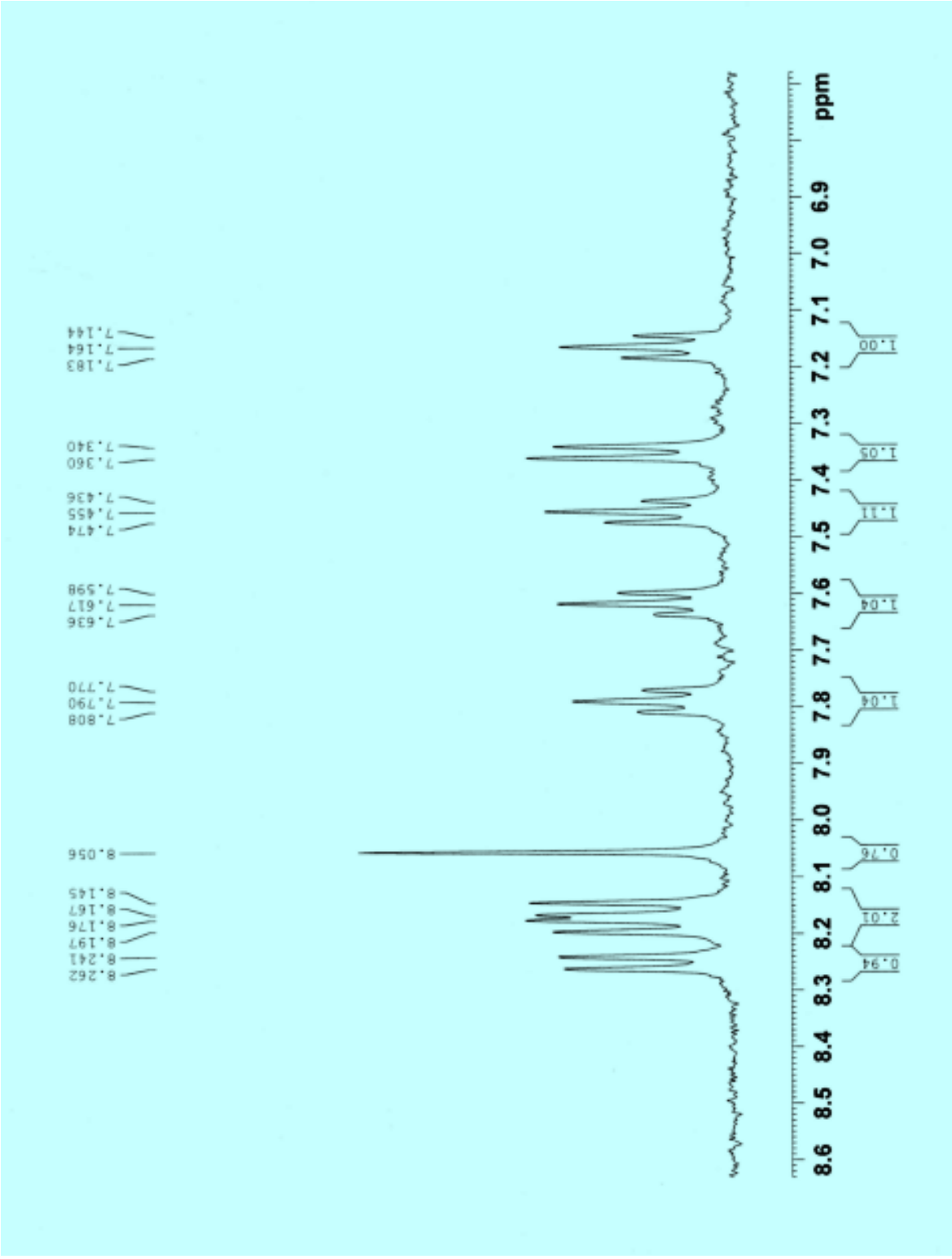


Figure 13 : The ^{1}HNMR spectrum the compound **7**

CHAPTER-4

ANTIMICROBIAL SCREENING

4.1 Introduction

Antimicrobials are one of the most successful forms of chemotherapy and have been used to save the human population from the threat of infectious diseases. The emergence of microbial resistance to conventional antibiotics is a serious threat to the effectiveness of current antimicrobial therapy. To counteract antimicrobial resistance, discovery of novel antimicrobial agents to counteract the antibiotic-resistant strains is one of the major medical concerns of the 21st century. It was found from the literature that Compounds containing the quinoxaline nucleus exhibit a broad spectrum of biological activity such as antibacterial **[57-59],** antifungal **[60-61],** antiviral **[62],** anticancer **[63],** antituberculosis **[64],** antimalarial **[65]** and anti-inflammatory properties **[66].** However, as a part of our drug discovery program, our compounds have been tested for antimicrobial activities against one Gram-positive and five Gram-negative bacteria as well as two human fungal pathogens. The use of synthesized compounds for their antimicrobial action has been the subject of research by many workers and many works have been carried out in this field recently, to discover new antimicrobial drugs. The antimicrobial screening which is the first stage of antimicrobial drug research is performed to ascertain the susceptibility of various fungi and bacteria to any agent. This test measures the ability of each test sample to inhibit the in vitro fungal and bacterial growth. This ability may be estimated by any of the following three methods.

- Disc diffusion method
- Serial dilution method
- Bioautographic method

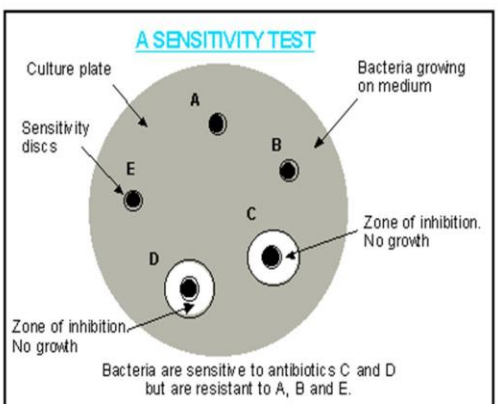
But there is no standardized method for expressing the results of antimicrobial screening. Some investigators use the diameter of zone of inhibition and/or the minimum weight of extract to inhibit the growth of microorganisms. However, a great number of factors viz., the extraction methods, inoculums volume, culture medium composition, pH, and incubation temperature can influence the results. Among the above mentioned techniques the disc diffusion is a widely accepted in vitro investigation for preliminary screening of test agents which may possess antimicrobial 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 bacteriostatic and bactericidal activity can be made by this method.

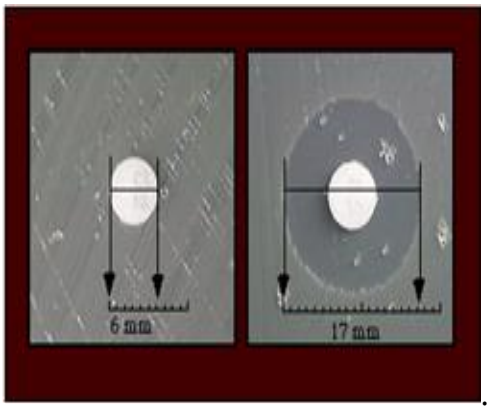
4.2 Principle of Disc Diffusion Method

In this classical method, antibiotics diffuse from a confined source through the nutrient agar gel and create a concentration gradient. Dried and sterilized filter paper discs (6 mm diameter) containing the test samples of known amounts are placed on nutrient agar medium uniformly leave seed with the test microorganisms. Standard antibiotic (Ciprofloxacin) discs and blank discs are used as positive and negative control. These plates are kept at low temperature (4° C) for 24 hours to allow maximum diffusion of the test materials to the surrounding media. The plates are then inverted and incubated at 37° C for 24 hours for optimum growth of the organisms. The test materials having antimicrobial property inhibit microbial growth in the media surrounding the discs and thereby yield a clear, distinct area defined as zone of inhibition. The antimicrobial activity of the test agent is then determined by measuring the diameter of zone of inhibition expressed in millimeter. In the present study the synthesized compounds were tested for antimicrobial activity by disc diffusion method. The experiment is carried out more than once and the mean of the readings is required.

4.3 Determination of antimicrobial activity by the zone of inhibition

The antibacterial potency of the test agents is measured by their activity to prevent the growth of the microorganisms surrounding the discs which gives clear zone of inhibition. After incubation, the antibacterial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale.





In the present study synthesized compounds were tested for antibacterial activity by disc diffusion method.

4.4 Antimicrobial Screening

4.4.1 Disc diffusion methods

The Kirby-Bauer and Stokes' methods are usually used for antibacterial susceptibility testing, with the Kirby-Bauer method being recommended by the NCCLS. The accuracy and reproducibility of this test are dependent on maintaining a standard set of procedures as described here.

4.4.2 Materials used

a) Test Organisms: The bacterial and fungal strains used for the experiment were collected as pure cultures from the department of Chemistry, Jagannath University of Bangladesh and the experiments were completed by the help of same department. Both gram positive, gram-negative and fungi organisms were taken for the test and they are listed below.

Gram-positive bacteria	**Gram negative-bacteria**
Staphylococcus aureus	Bacillus subtilis
	Escherichia coli
	Pseudomonas aeruginosa
	Salmonella typhimurium
	Citrobacter freundii

The fungal strains used for the experiment were collected as pure cultures and two organisms were taken for the test.

Aspergillus niger	*Tricodarma harzianum*

b) Growth Media: The activity was conducted on the Nutrient Agar Media produced from TSA (Tryptone Soya Agar).

Composition :

Pancreatic digest of casein	15.0 g/L
Enzymatic digest of soya bean	5.0 g/L
Sodium chloride	5.0 g/L
Agar	15.0 g/L

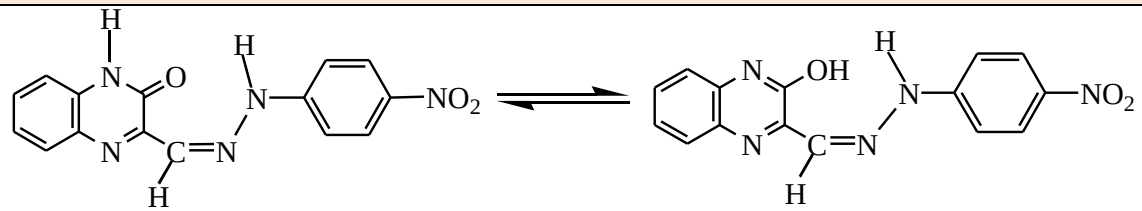
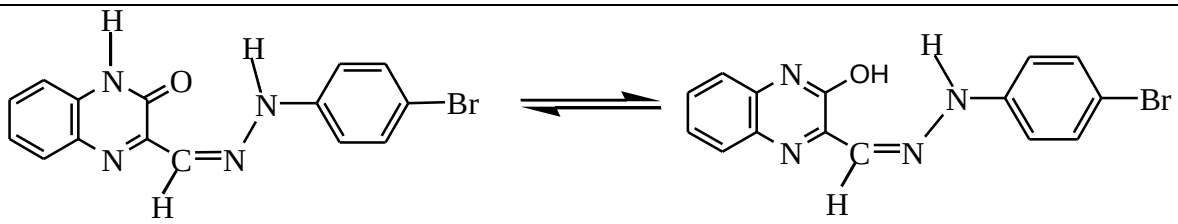
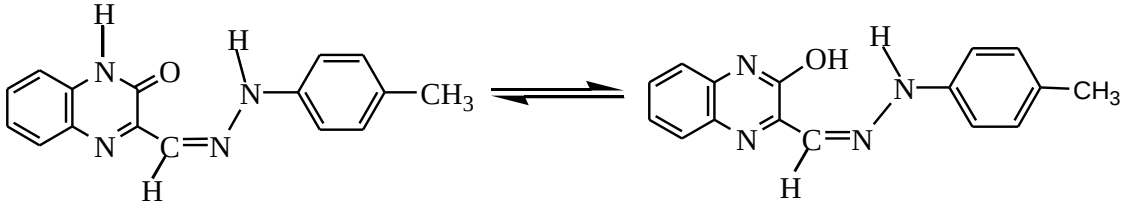
c) Apparatus Used:

- **Petri plate** : Plastic plate, which was previously sterilized.
- **Pipette :** Micropipette was used for adding the required concentration of sample to the plates.

- **Blank discs :** Susceptible blank discs were used, which was stored in 20^0C to 8^0C.
- **Glasswares :** 500 ml conical flask and test tubes were used.
- **Compounds Screened :** All the synthesized compounds**3a-s**.
- **Solvent Used :** Dimethyl sulfoxide.
- **Standard Used :** Ciprofloxacin for bacteria and Michanazole for fungi.

d) Test materials

The synthesized compounds for the antimicrobial test are numbered as 21, 22 and 23 **Table: Table of the tested compounds structure.**

Sample code	Structures of the samples/ compounds
21	 3 Amide form 3' Iminol form **Amide-Iminol tautomer of 3-[(4-Nitro-phenyl)-hydrazonomethyl]-1H- 3-quinoxalin-2-one**
22	 6 Amide form 6' Iminol form **Amide–Iminoltautomer 3-[(4-Bromo-phenyl)-hydrazonomethyl]-1H- 3-quinoxalin-2-one**
23	 2 Amide form 2' Iminol form **Amide-Iminol tautomer of 3-(*P*-Tolyl-hydrazonomethyl)-1H-3-quinoxalin-2-one**

4.5 Procedure for Performing the Disc Diffusion Test

Inoculums Preparation

4.5.1 Growth Method

The growth method is performed as follows

At least three to five well-isolated colonies of the same morphological type were selected from an agar plate culture. The top of each colony was touched with a loop.

a) growth was transferred into a tube containing 4 to 5 ml of a suitable broth medium, such as tryptic soy broth.
b) The broth culture was incubated at 37°C until it achieved or exceed the turbidity of the 0.5 McFarland standard (usually 2 to 6 hours).
c) The turbidity of the actively growing broth culture was adjusted with sterile saline or broth to obtain a turbidity optically comparable to that of the 0.5 McFarland standard.

4.5.2 Inoculation of Test Plates

a) Media was prepared by adding 40.0 gm of Nutrient agar to 1L of distilled water. Then it was sterilized by autoclaving at 15 lb/inch and at 210^{o}C temperatures for two hours.
b) Media was cooled to the temperature of approximately 40^{0}C and microorganisms were inoculated to the media. 25ml was transferred to a petriplate. Two such plates were prepared for each organism. Plates were allowed to cool for 20 minutes.
c) Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab.
d) The dried surface of a TSA plate is inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar was swabbed.
e) The lid may be left the plate for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.

4.5.3 Application of Discs to Inoculated Agar Plates

The predetermined battery of antibacterial discs was dispensed onto the surface of the inoculated agar plate. Each disc was pressed down to ensure complete

a) contact with the agar surface. The discs were placed such a way so that they were no closer than 24 mm from center to center.
b) The plates are inverted and placed in an incubator set to 37°C within 15 minutes after the discs were applied

4.5.4 Application of Samples on the discs

a) Crude fungal extract was dissolved in DMSO and diluted to get concentration of 300 µg/disc.
b) Four blank discs were placed in the petri plates. Reference standard Tetracycline was impregnated on one of the discs, and only solvent as a blank was impregnated on one of the discs, and others experimental solutions were impregnated on others discs. Each disc's was marked by a marker as a small symbol so that each of the discs could be easily identified. 25µl of solution was injected on each disc.

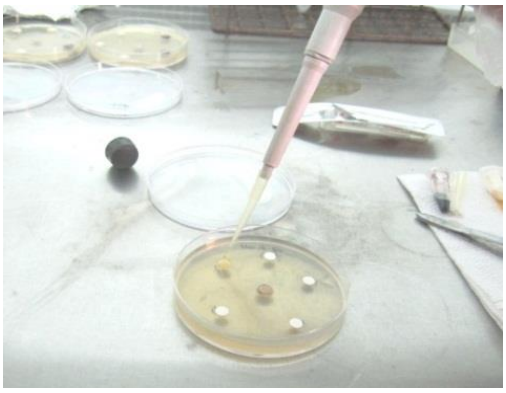


Figure : Application of Samples on the discs.

4.5.5 Reading Plates and Interpreting Results

The above culture plates were incubated at 37°C for 24 hours. The zones of inhibition produced by compounds and Ciprofloxacin for bacteria and Michanazole for fungi were recorded in mm and compared.

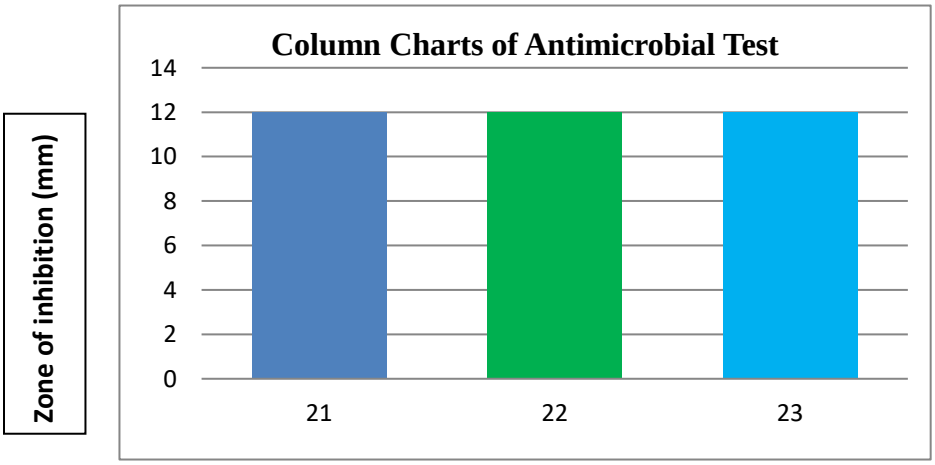
4.5.6 Determination of antimicrobial activity by measuring the Zone of Inhibition

After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with transparent scale. Synthesized compounds were tested for antibacterial activity against eight bacteria and antifungal activity against two fungi. All compounds were tested at 300µg/disc concentration.

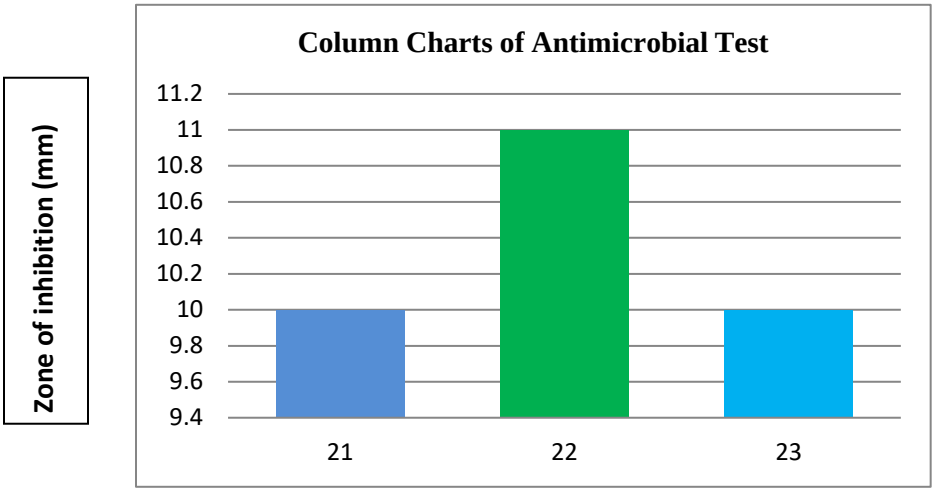
Table 2: *In Vitro* Bactericidal Profiles of **21, 22 & 23** in Terms of Zone of Inhibition

Antimicrobial Screening					
Organism	S-21	S-22	S-23	Standard	
				Miconazole	Ciprofloxacin
Gram positive					
Staphylococcus aureus(+)	10	11	10		38
Gram negative					
Bacillus subtilis (-)	13	13	12		36
Escherichia coli (-)	12	12	12		40
Pseudomonas aeruginosa (-)	10	11	10		42
Salmonella typhimurium (-)	10	11	10		50
Citrobacter freundii (-)	10	10	12		37
Fungi					
Aspergillus niger	5	5	6	28	
Tricodarma harzianum	5	5	5	25	

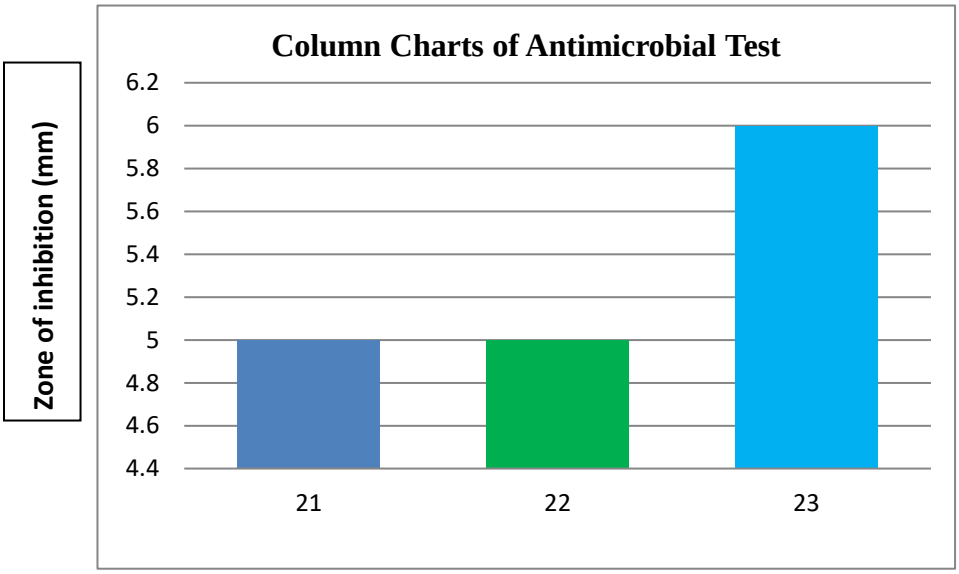
Antibacterial activity against *E.coli*



Antibacterial activity against *Staphylococcus aureus*



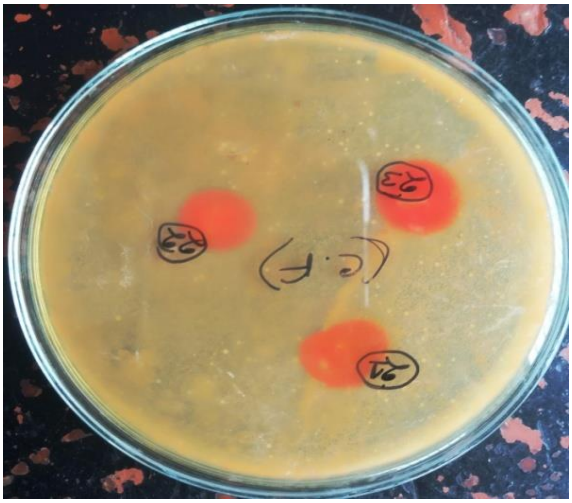
Antifungal activity against *Aspergillus niger*



Test for *E.coli*

Test for Bacillus subtilis

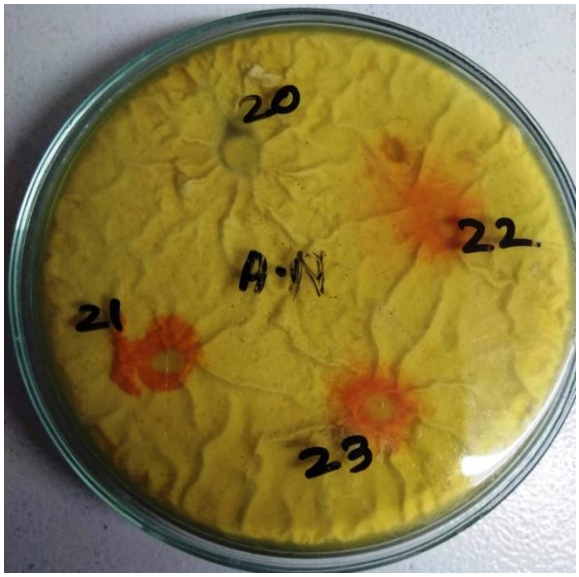
Test for Staphylococcus aureus



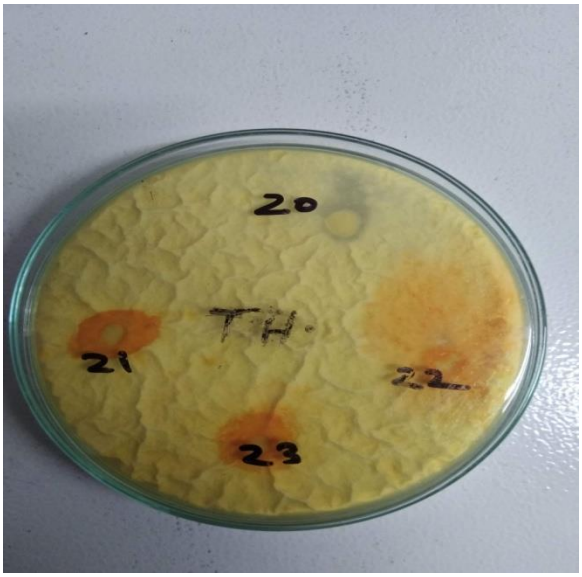
Test for Citrobacter freundii

Test for Salmonella typhimurium

Test for Pseudomonas aeruginosa



Test for Aspergillus niger



Test for Tricodarma harzianum

4.5.7 Results and discussion of in vitro Antibacterial Screening

The tested compounds showed activities at a dose of 300 µg $disc^{-1}$ comparable to Ciprofloxacin for bacteria and Michonazole for fungi (standard) at 300 µg $disc^{-1}$. The synthesized compounds Sample-21(**3** & **3')** , Sample -22(**6** & **6') and sample-**23(**2** & **2')** were evaluated for their in vitro antibacterial activity against one Gram-positive (G+ve), five Gram-negative (G-ve) bacteria and antifungal activity against two fungi. All the tested compounds show moderate activity result against one Gram-positive (G+ve), five Gram-negative (G-ve) bacteria.

Similarly Against fungi all Compounds showed moderate activity.

CHAPTER-5

Summary

SUMMARY

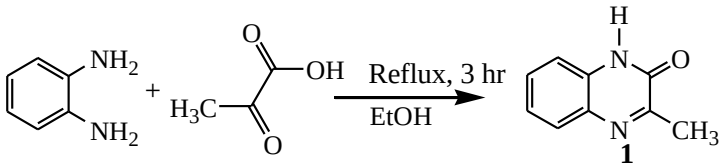
Our objective is to synthesize different quinoxaline derivatives and studies of their biological activity. We performed our aim by the following steps.

a) Preparation of methylquinoxalin as synthetic precursor.
b) Synthesized different hydrazonomethyl Quinoxaline compounds from the coupling reaction between different diazonium salts and the active methylated methylquinoxalin.
c) Characterization of the structures of the synthesized products by different physical, chemical and spectroscopic methods.
d) Evaluation of the biological activity especially antibacterial anti fungal property of the synthesized products.

1. General procedure for the synthesis Starting material of 3-methylquinoxalin-2(1H)-one

Equimolar amounts of pyruvic acid and *o*-phenylenediamine were refluxed in ethanol for 3 hours. The progress of the reaction was monitored by TLC. After completion of the reaction the product was isolated and characterized by spectral evidences such as IR, ^{1}H NMR and ^{13}C NMR.

Table-1: Synthesis of Starting material 3-methylquinoxalin 2(1H)-one



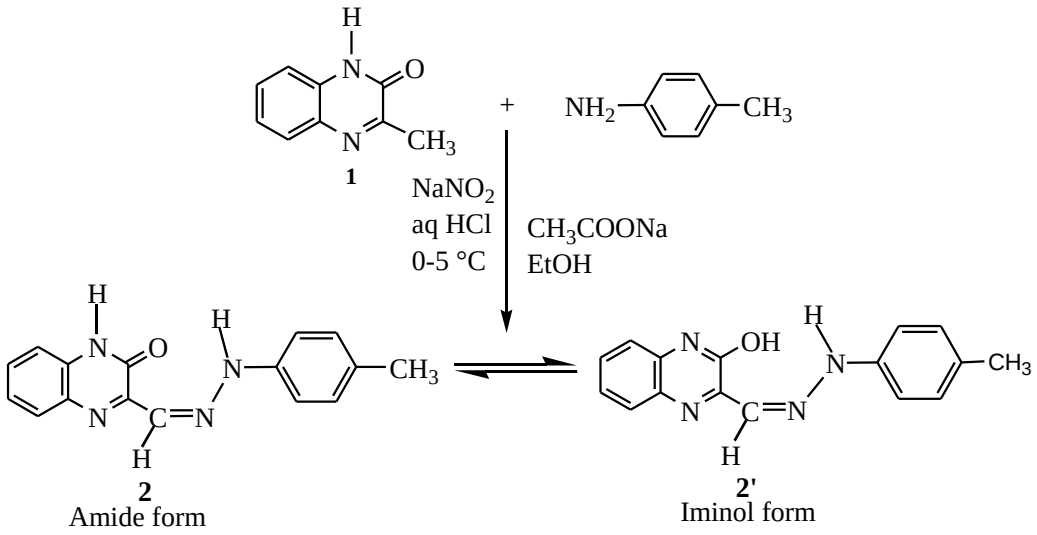
1

Yield: 80%
m.p: 215-217°C

2.General procedure for the synthesis of different hydrazonomethyl Quinoxaline compounds:

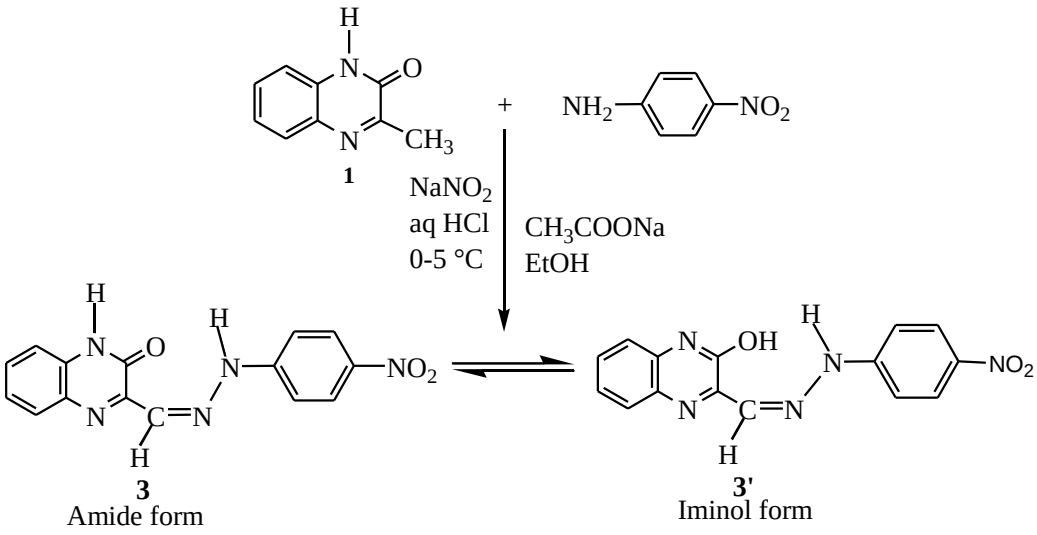
Different hydrazonomethyl Quinoxaline compounds were synthesized from the coupling reaction between different diazonium salts andthe active methylated compound 3-methylquinoxalin-2(1H)-one. The progress of the reaction was monitored by TLC. After completion of the reaction the products were isolated and characterized by spectral evidences such as IR, ^{1}H NMR and ^{13}C NMR.

a)



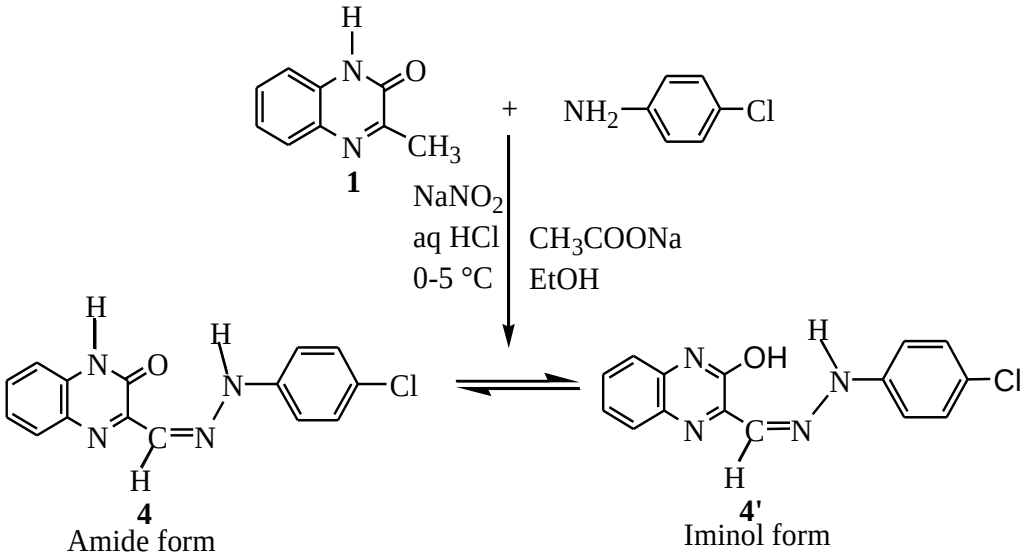
Yield: 70%
m.p: 260-264°C

b)



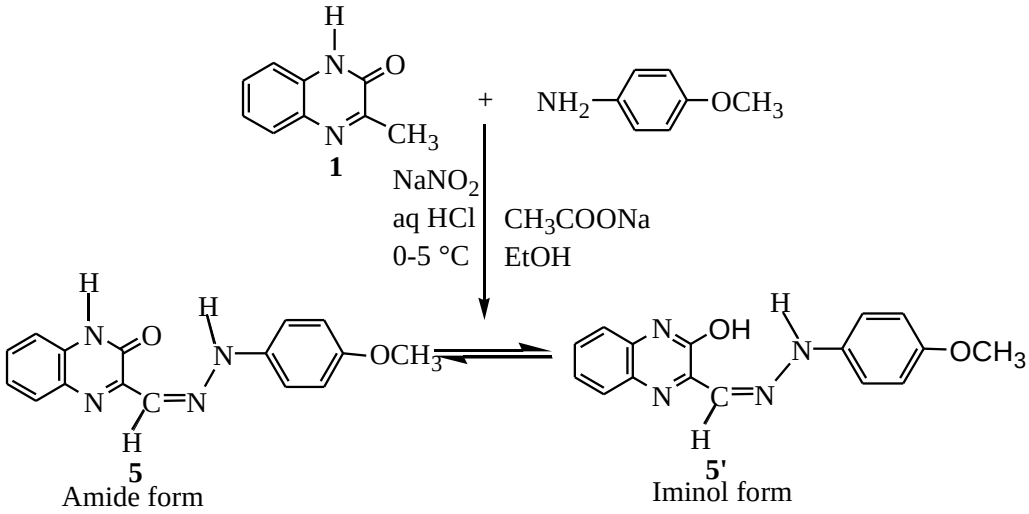
Yield: 80%
m.p: 282-285°C

c)



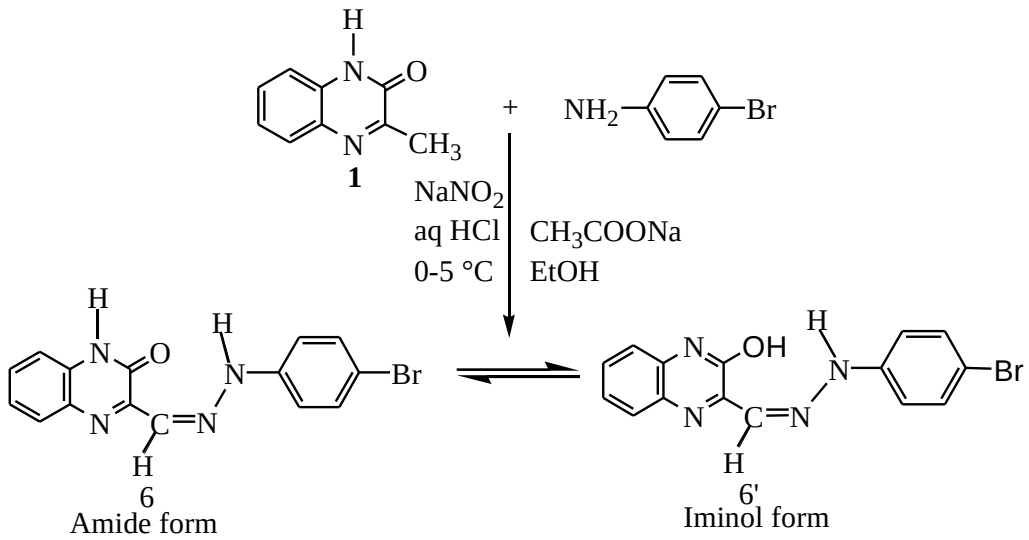
Yield: 70%
m.p: 277-280°C

d)



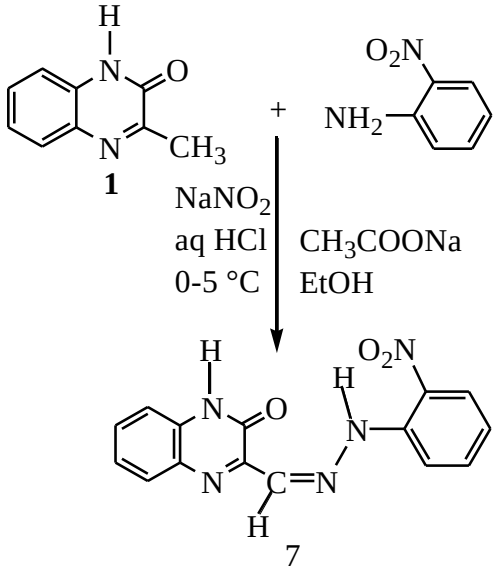
Yield: 70%
m.p: 290-293°C

e)



Yield: 75%
m.p: 272-275°C

f)



Yield: 60%
m.p: 268-270°C

3. **Antimicrobial test**:

The synthesized compounds sample-21(**3**&**3'**) ,sample-22 (**6**&**6'**) and sample-**23** (**2**&**2'**) were evaluated for their in vitro antibacterial activity against one Gram-positive (G+ve), five Gram-negative (G-ve) bacteria and antifungal activity against two fungi. All the tested compounds show moderate activity result against one Gram-positive (G+ve), five Gram-negative (G-ve) bacteria and two fungi.

Antimicrobial Screening					
Organism	S-21	S-22	S-23	Standard	
				Miconazole	Ciprofloxacin
Gram positive					
Staphylococcus aureus(+)	10	11	10		38
Gram negative					
Bacillus subtilis (-)	13	13	12		36
Escherichia coli (-)	12	12	12		40
Pseudomonas aeruginosa (-)	10	11	10		42
Salmonella typhimurium (-)	10	11	10		50
Citrobacter freundii (-)	10	10	12		37
Fungi					
Aspergillus niger	5	5	6	28	
Tricodarmaharzianum	5	5	5	25	

CHAPTER-6

References

[1] Katritzky, A. R., and Rees, C. W., *Comprehensive Heterocyclic Chemistry,* Part 2B, Vol.3, p. 157, (1984).

[2] Loriga, M., Piras, S., Sanna, P., and Paglietti, G., "Synthesis and evaluation of invitro anticancer, anti-HIV and antifungal activity," *Il Farmaco,* Vol. 52, pp. 157-166, (1997**).**

[3] Seitz, L. E., Suling, W. J., and Reynolds, R. C., "Synthesis and Antimicobacterial Activity of Pyrazine and Quinoxaline Derivatives," *J. Med. Chem,* Vol. 45, pp. 5604-5606, (2002).

[4] Hui, X., Desrivot, J., Bories, C., Loiseau, P. M., Frank, X., Hocquemiller, R., and Figadere, B., "Synthesis and antiprotozoal activity of some new synthetic substituted quinoxalines," *Bioorg. Med. Chem. Let,* Vol.16, pp. 815-820, (2006).

[5] Lindsley, C. W., Zhao, Z., Leister, W. H., Robinson, R. G., Barnett, S. F., Defeo-Jones, D., Jones, R. E., Hartman, G. D., Huff, J. R., Huber, H. E., and Duggan, M. E., "Allosteric Akt (PKB) inhibitors: discovery and SAR of isozyme selective inhibitors," *Bioorg. Med. Chem. Lett,* Vol. 15, pp. 761-764, (2005).

[6] Sarges, R., Howard, H. R., Browne, R. G., Lebel, L. A., Seymour, P. A., and Koe, B. K., " 4-Amino[1,2,4]triazolo[4,3-a]quinoxalines. A novel class of potent adenosine receptor antagonists and potential rapid-onset antidepressants," *J. Med. Chem,* Vol. 33, pp. 2240-2254, (1990).

[7] Srinivas, C., Kumar, C. N. S. S. P., Rao, V. J., and Palaniappan, S., "Efficient, convenient and reusable polyaniline-sulfate salt catalyst for the synthesis of quinoxaline derivatives," *J. Mol. Catal. A: Chem,* Vol. 265, pp. 227-230, (2007).

[8] Sakata, G., Makino, K., and Kurasawa, Y., "Recent progress in the quinoxaline chemistry. Synthesis and biological activity," *Heterocycl,* Vol. 27, pp. 2481-2485, (1988).

[9] Nagata, R., Tanno, N., Kodo, T., Ae, N., Ogita, K., Nishimura, T., Tatsuno, T., Nakamura, M., Ogita, K., and Yoneda, Y., "A facile synthesis of quinoxaline-2,3-diones as NMDA receptor antagonists," The 207th American Chemical Society National Meeting , San Diego, California, Poster, MEDI, 181, March 13-17, (1994).

[10] Dell, A., William, D. H., Morris, H. R., Smith, G. A., Freeny, J., and Roberts, G. C. K., "Synthetic approaches to 2-substituted pyrazines and 2-substituted quinoxalines and their fluorescence characteristics," *J. Am. Chem. Soc,* Vol. 97 , pp. 2497-2505, (1975).

[11]Taghreed, Z. S., Hend, M. El-Sehrawi., Reda, E. M., "Design, Synthesis, QSAR, Molecular Docking Study and Antitumor Activity of some Novel Quinazolin-4(3H)-One Derivatives," *J. Pharm. Appl. Chem*, Vol. 3 , No. 2 , pp. 161-175 , (2017).

[12] Bratz, M., Kober, R., Seele, R., Saupe, T., Meyer, N., Walker, N., Landes, A., and Walter, H., *Canadian Pat Appl* 2, 078, 4767, (1993), *Chem. Abstr,* 120, 77293b, (1994).

[13] Hurlbert, B. S., and Valenti, B. F., "Condensed pyrimidine systems. XXIV. The condensation of 2,4,6-triaminopyrimidine with malondialdehyde derivatives," *J. Med. Chem,* Vol. 11, pp. 708-71, (1968).

[14] Barea, C., Pabon, A., Castillo, D., Zimic, M., and Quiliano, M., "New salicylamide and sulfonamide derivatives of quinoxaline 1,4-di-N-oxide with antileishmanial and antimalarial activities," *Bio. Org. Med. Chem. Lett,* Vol. 21, pp. 4498-4502, (2011)**.**

[15] Ingle, Rl., Marathe, R., Magar, D., Patel, Hm., and Surana, S. J., "Sulphonamido-quinoxalines: search for anticancer agent," *Eur. J. Med. Chem,* Vol. *65*, pp. 168-186, (2013).

[16] Tandon, V. Kl., Yadav, D. B., Maurya, H. K., Chaturvedi, A. K., and Shukla, P. K., "Design, synthesis, and biological evaluation of 1,2,3-trisubstituted-1,4-dihydrobenzo[*g*]quinoxaline-5,10-diones and related compounds as antifungal and antibacterial agents," *Bioorg. Med. Chem,* Vol.14, pp. 6120-6126, (2006).

[17] Kalainin, A. Al., Voloshina, A. D., Kulik, N. V., Zobov, V. V., and Mamedov, V. A., "Antimicrobial activitry of imidazo[1,5-a]quinoxaline derivatives with pyrimidium moiety" *Eur. J. Med. Chem,* Vol. 66, pp. 345-354, (2013).

[18] Hui Xu., and Ling-ling Fan., "Synthesis and antifungal activities of novel 5,6-dihydro-indolo[1,2-a]quinoxaline derivatives," *European Journal of Medicinal Chemistry;* Vol. 46, pp. 1919-1925**,** (2011).

[19] Erwin, D.C., Sims, J.J., Borum, D.E., and Childers, J.R., *Phytopathology,* Vol. 61 , pp. 964-967, (1971).

[20] Kalambe. N. A., "Synthesis and Study Of 2–Hydroxy Substituted Quinoxaline Effects on Different Crop Plant Growth," *IJRBAT,* SpeciaI Issue (2), Vol. v, JuIy, (2017).

[21] Suresh, M., Lavanya, P., Sudhakar, D., Vashu, K., and Rao, C. V., "Synthesis and biological activity of 8-chloro-[1,2,4]triazolo[4,3-a]quinoxalines," *J.Chem.Pharm. Res.*, Vol. 2, No. 1, pp. 497-504, (2010).

[22] Salman, K. A., Kishwar, S., and Zaheer, K., "Synthesis, characterization and *in vitro* antibacterial activity of new steroidal thiazolo quinoxalines," *European Journal of Medicinal Chemistry,* Vol. 42, pp. 103-108, (2007)**.**

[23] Vieira , M., Pinheiro, C., Fernandes, R., Noronha, J. P., and Prudencio, C., "Antimicrobial activity of quinoxaline 1,4-dioxide with 2- and 3-substituted derivatives," *Microbiological Research***,** Vol. 169, No. 4, pp. 287-293, (2014).

[24] Weng, Q.,Wang, D., Guo, P., Fang, L., Hu, Y., He, Q., and Yang, B., "Q39, a novel synthetic Quinoxaline1,4-Di-N-oxide compound with anti-cancer activity in hypoxia," *Eur.J.of Pharmacol*, Vol. 581, pp. 262-269, (2008).

[25] Amin, K. M., Ismail, M. M. F., Noaman, E., Soliman, D. H., and Ammar, Y. A., "New quinoxaline 1,4-di- N-oxides. Part 1: Hypoxia-selective cytotoxins and anticancer agents derived from quinoxaline 1,4- di-N-oxides," *Bioorg. Med. Chem*, Vol. 14, pp. 6917-6923, (2006).

[26] Abbas, S. HA., Al-Marhabi, R. A., Eissa, I. S. , and Ammar, A. Y., " Molecular modeling studies and synthesis of novel quinoxaline derivatives with potential anticancer activity as inhibitors of c-met kinase," *Bioorganic & Medicinal Chemistry*, Vol. 23 pp. 6560–6572, (2015).

[**27]** Kumar, A., Verma, A., Chawla, G., and Vaishali, "Synthesis, Antiinflammatory and Antimicrobial Activities of New Hydrazone and Quinoxaline derivatives," *Int. J. Chem. Tech. Res*., Vol. 1 No. 4, pp. 1177-1181, (2009).

[28] Burguete, A., Pontiki, E., Hadjipavlou-Litina, D., Villar, R.,Vicente, E., Solano, B., Ancizu, S., Perez-Silanes, S., Aldana, I., and Monge, A., "Synthesis and anti-inflammatory/antioxidant activities of some new ring substituted 3-phenyl-1-(1,4-di-N-oxide quinoxalin-2-yl)-2-propen-1-one derivatives," *Bioorg. Med. Chem. Lett*., Vol. 17, pp. 6439-6443, (2007).

[29] Olayiwola1, G., Obafemi, C. A., and Taiwo, F. O., "Synthesis and neuropharmacological activity of some quinoxalinone derivatives," *Afr. J. of Biotechnol.,* Vol. 6, No. 6 , pp. 777-786, (2007).

[30] Elhelby, A. A., Ayyad, R. R., and Zayed, M. F., "Synthesis and biological evaluation of some novel quinoxaline derivatives as anticonvulsant agents," *Arzneimittelforschung Drug Research*, Vol. 61, No. 7, pp. 379-381, (2011).

[31] Elkaeed, E., Ghiaty, A., El-Morsy, A., El-Gamal, K., and Sakr, H.; "Synthesis and Biological Evaluation of Some quinoxaline-2-one Derivatives as Novel Anticonvulsant Agents," *ChemSci Rev Lett*, Vol. 3, No. 12, pp. 1375-1387, (2014).

[32] Harmenberg, J., Wahren, B., Bergman, J., Akerfeldt, S., and Lundblad, L., "Antiherpesvirus Activity and Mechanism of Action of Indolo-(2,3-b)Quinoxaline and Analogs*," Antimicrobial Agents and Chemo- therapy*, Vol. 32 , No. 11, pp. 1720-1724, (1988).

[33] Kleim, J., Bender, R., Billhard, U., Meichsner, C., Riess, G., Rosner, M., Winkler, I., and Paessens, A., "Activity of a Novel Quinoxaline Derivative against Human Immunodeficiency Virus Type 1 Re- verse Transcriptase and Viral Replication," *Antimicrobial Agents and Chemotherapy*, Vol. 37 , No. 8, pp. 1659-1664, (1993).

[34] Vicente, E., Lima, L. M., Bongard, E., Charnaud, S., Villar, R., Solano, B., Burguete, A., Perez-Silanes, S., Aldana, I., Vivas, L., and Monge, A., "Synthesis and structure-activity relationship of 3-phenylquinoxaline 1,4-di-N-oxide derivatives as antimalarial agents," *Eur. J. of Med. Chem*., Vol. 43,pp. 1903-1910 , (2008).

[35] Rangisetty, J. B., Gupta, C.N.V.H.B., Prasad, A. L., Srinivas, P., Sridhar, N., Parimoo, P., and Veeranjaneyulu, A., "Synthesis of new arylaminoquinoxalines and their antimalarial activity in mice," *Journal of Pharmacy and Pharmacology*, Vol. 53, pp. 1409-1413, (2001).

[36] Croft, S. L., and Coombs, G. H., "Leishmaniasis-current chemotherapy and recent advances in the search for novel drugs," *Trends Parasitol*., Vol. 19, No,11, pp. 502-508, (2003).

[37] Hui, X., Desrivot, J., Bories, C., Loiseau, P. M., Franck, X., Hocquemiller, R., and Figadere, B., "Synthesis and antiprotozoal activity of some new synthetic substituted quinoxalines," *Bioorg. Med. Chem. Lett.*, Vol. 16, pp. 815-820**,** (2006).

[38] Burguete, A.; Estevez, Y.; Castillo, D.; Gonzalez, G.; Villar, R.; Solano, B.; Vicente, E.; Silanes, S. P.; Aldana, I.; Monge, A.; Sauvain, M.; and Deharo, E., "Anti-leishmanial activity of quinoxaline derivatives," *Mem. Inst. Oswaldo Cruz, Rio de Janeiro*, Vol.103, No. 8, pp. 778-780, (2008) .

[**39]** Souza, W. D., "Basic cell biology of Trypanosoma cruzi," *Curr. Pharm. Des.*, Vol.8 No. 4, pp.269-285, (2002).

[40] Aguirre, G., Cerecetto, H., Maio, R. D., Gonzalez, M., Alfaro, M. E. M., Jaso, A., Zarranz, B., and Ortega, M. A.,"Quinoxaline N,N'-dioxide derivatives and related compounds as growth inhibitors of Trypanosoma cruzi," *Bioorg. Med. Chem. Lett*., Vol. 14, pp. 3835-3839, (2004).

[41] Perumal, R. V., and Mahesh, R., "Synthesis and biological evaluation of a novel structural type of serotonin 5-HT3 receptor antagonists," *Bioorg. Med. Chem. Lett*., Vol. 16, pp. 2769-2772, (2006).

[42] Catarzi, D., Colotta, V., Varano, F., Cecchi, L., Filacchioni, G., Galli, A., Costagli, C., and Carla, V., "7-Chloro- 4,5-dihydro-8-(1,2,4-triazol-4-yl)-4-oxo-1,2,4-triazolo[1,5-*a*] quinoxaline-2-carboxylates as Novel Highly Selective AMPA Receptor Antagonists," J. Med. Chem., Vol.43, pp. 3824-3826, (2000).

[43] Budakoti, A., Bhat, A. R., and Azam, A., "Synthesis of new 2-(5-substituted-3-phenyl-2-pyrazolinyl)-1,3- thiazolino[5,4-b]quinoxaline derivatives and evaluation of their antiamoebic activity," *Eur. J. of Med. Chem*., Vol. 44, pp. 1317-1325**,** (2009).

[44] Abid, M., and Azam, A., "Synthesis, characterization and antiamoebic activity of1-(thiazolo[4,5-b]quinoxaline-2-yl)-3-phenyl-2-pyrazoline derivatives," *Bioorganic & Medicinal Chemistry Letters*, Vol.16, pp. 2812–2816, (2006).

[**45]** Chung, H., Jung, O., Chae, M. J., Hong, S., Chung, K., Lee, S. K., and Ryu, C., "Synthesis and biological evaluation of quinoxaline-5,8-diones that inhibit vascular smooth muscle cell proliferation," *Bioorg. Med. Chem. Lett*., Vol. 15, pp. 3380-3384, (2005).

[46] Li, J. J., "Synthesis of Novel 3-Substituted Pyrrolo[2,3-*b*]quinoxalines via an Intramolecular Heck Reaction on an Aminoquinoxaline Scaffold," *J. Org. Chem*., Vol. 64, pp. 8425-8427, (1999).

[47] Wilhelmsson, M., Kingi, N., and Bergman, J., Interactions of Antiviral Indolo[2,3-*b*]quinoxaline Derivatives with DNA, *J. Med. Chem.* Vol. *51, pp.* 7744–7750, (2008).

[48] Vega, M. A., Gil, J. M., and Fernandez-alvarez, E., "Synthesis of 1-hydrazinopyridazino [4,5-*b*]quinoxaline and related compounds," *J. Heterocyclic Chem.*, Vol. 21, pp. 1271-1276, (1984).

[**49]** H. Schiff., *Ann. Suppl*., vol. 3, pp. 343, (1864).

[50] Mathew, J., "Synthesis, characterization and catalytic activity studies of some new transition metal chelates ofthe Schiff bases derived from 3-hydroxy quinoxaline-2-carboxaldehyde," PhD thesis, Cochin University of Science and Technology, (1995)**.**

[51] Mayadevi, S., and Yusuff, K.K.M., "Synthesis and Characterization of Some New Transition Metal Complexes of the Schiff Base Derived from Quinoxaline-2-Carboxaldehyde and 2-Aminophenol," *Synthesis and reactivity in inorganic and metal-organic chemistry*, Vol. 27, pp. 319-330, (1997).

[52] Mayadevi, S., Prasad, P.G., and Yusuff, K.K.M., "Studies on Some Transition Metal Complexes of Schiff Bases Derived from Quinoxaline-2-carboxaldehyde," *Synthesis and reactivity in inorganic and metal-organic chemistry*, Vol. 33, No. 3, pp. 481–496, (2003).

[53] Chellaian, D. J., and Johnson, J., "Spectral characterization, electrochemical and anticancer studies on some metal(II) complexes containing tridentate quinoxaline Schiff base," *Pectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* , Vol. 127 , pp. 396–404, (2014).

[54] Arun,V., Mathew, S., Robinson, P.P., Jose , M., Nampoori, V.P.N., and Yusuff, K.K.M., "The tautomerism, solvatochromism and non-linear optical properties of fluorescent 3-hydroxyquinoxaline-2-carboxalidine-4-aminoantipyrine," *Dyes and Pigments,* Vol.87, pp. 149-157, (2010).

[55] Reddy, M. R., Han, S. H., Lee,J. Y., and Seo, S.Y., "Synthesis and characterization of quinoxaline derivative for high performance phosphorescent organic light-emitting diodes," *Dyes and Pigments*; Vol. 153, pp. 132-136, (2018).

[56] Devendra K. S., Bhawana, T., Joshi, A., Hussain, N., Talesara, G. L., "Synthesis, Characterization and Biological Evaluation of some heterocyclic compounds ethoxyphthalimide moiety via Key intermediate 6-chloro 1,3 benzothiazole-2-amine," *Indian Journal of Chemistry*, Vol: 49B, pp. 818-825, (2010).

[57]. Badran, M.M., Abonzid, K.A., and Hussein, M.H., "Synthesis of certain substituted quinoxalines as antimicrobials agents," *Arch. Pharm. Res;* Vol. 26, pp. 107–113, (2003).

[58]. Griffith, R.K., Chittur, S.V., and Chen, Y.C., "Inhibition of glucosamine-6-Phosphate synthase from candida albicans by quinoxaline-2, 3-diones," *Med. Chem. Res;* Vol. 2, pp. 467–473, (1992).

[59]. ElGendy, A.A., ElMeligie, S., ElAnsry, A., Ahmedy, A.M., "Synthesis of some quinoxaline derivatives containing Indoline-2, 3-dione or, thiazolidine residue as potential antimicrobials agents," *Arch. Pharm. Res*. Vol.18, pp.44–47, (1995).

[60]. Sastry, R. CV., Shrinivas-Rao, K., Krishanan, V.S.H., Rastogi, K., Jain, M.L., and Narayanan, G., "Synthesis and biological activity of some new tetrazolobenzoxazines as bis-tetrazoloquinoxalines." *Indian J. Chem*. Vol. 29, pp. 396–403, (1990).

[61]. El-Hawash, S.A., Habib, N.S., and Franki, N.H., "Synthesis and antimicrobial testing of 1,2,4-triazolo[4,3-a] quinoxalines,1,2,4-triazino[4,3-a] quinoxalines and 2-pyrazolylquinoxalines," *Pharmazie,* Vol. 54, pp. 808–815, (1999).

[62]. Westphal, G., Wasiki, H., Zielinski, U., Weberr, F.G., Tonew, M., and Tonew, E., Potentielle virostatica, *Pharmazie*, Vol. 35, pp. 570–571, (1977).

[63]. Monge, A., Martinez-Crespo, F.J., Cerai, A.L., Palop, J.A., Narro, S., Senador, V., Marin, A., Sainz, Y., Gonzalez, M., Hamilton, E., Barker, A.J., "Hypoxia selective agents derived from 2-quinoxalinecarbonitrile 1, 2-di-N-oxides," *J. Med. Chem*., Vol. 38, pp. 4488–4495. (1995).

[64]. Michael, J.W., Ben-Hadda, T., Kotchevan, A.T., Ramdani, A., Touzani, R., Elkadiri, S., Hakkou, A., Boukka, M., and Elli, T., "2,3-bifunctionalized quinoxalines: Synthesis, DNA Interactions and Evaluation of Anticancer, Anti-tuberculosis and Antifungal Activity," *Molecules*; Vol.7, pp. 641–656, (2002).

[65]. Rangisetty, J.B., Gupta, C.N., Prasad, A.L., Srinavas, P., Sridhar, N., Perimoo, P., and Veeranjaneyulu, A., "Synthesis of new arylaminoquinoxalines and their antimalarial activity in mice.," *J. Pharm. Pharmacol*. Vol. 53, pp. 1409–1413, (2001).

[66]. Wagle, S., Adhikari, A.V., and Kumari, N.S., "Synthesis of some new 2-(3-methyl-7-substituted-2-oxoquinoxalinyl)-5-(aryl)-1,3,4-oxadiazoles as potential non-steroidal anti-inflammatory and analgesic agents." *Ind. J. Chem.*, Vol. 47, pp. 439–448, (2008).