Design, Synthesis and Pharmacological screening of Hybrid urea/thiourea derivatives as potential antihyperglycemics

Thesis submitted by

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   1. **Tanmoy Guria**, Puspita Roy, Tapan kumar Maity. Synthesis, Pharmacological Evaluation and Ligand-Protein Interaction Study of Hybrid Urea and Thiourea Derivatives as Antihyperglycemic Agents. *International Research Journal of Pharmacy*, 2018; 9(5): 36-46.
   2. Puspita Roy, **Tanmoy Guria**, Birendra Nath Karan, Subbiah Ramasamy, Tapan Kumar Maity. Synthesis of 2,4,6-trisubstituted Pyrimidine Analogues via Chalcone Derivatives and Their Anticancer Evaluation. *International Journal of Pharmacy and Biological Sciences,* 2018; 8(3): 996-1004.
   3. Birendra Nath Karan, Tanushree Singha, Tapan Kumar Maity, Bikash Chandra Pal, **Tanmoy Guria**, Puspita Roy, Avik Maji, Sanjib Das. Evaluation of anticancer activity of methanol extract of *Commelina benghalensis* Linn. against Ehrlich ascites carcinoma in albino mice. *International Journal of Green Pharmacy*, 2018; 12 (1): S160-S168.
   4. Birendra Nath Karan, Tanushree Singha, **Tanmoy Guria,** Puspita Roy, Tapan Kumar Maity, Bikash Chandra Pal. Evaluation of Anti-Cancer Activity of Methanol Extract of *Nyctanthes arbor- tristis* Linn. *Research Journal of Pharmaceutical, Biological and Chemical Sciences,* 2017; 8(5): 412-420.
   5. Arijit Mondal, **Tanmoy Guria**, Tapan Kumar Maity, Anupam Bishayee. A Novel Tetraenoic Fatty Acid Isolated from *Amaranthus spinosus* Inhibits Proliferation and Induces Apoptosis of Human Liver Cancer Cells. *International Journal of Molecular Sciences*, 2016; 17(10): 1604.

6. Subbiah Ramasamy, **Tanmoy Guria**, Tanushree Singha, Puspita Roy, Benu P Sahu, Jayatri Naskar, Avijit Das, Tapan Kumar Maity. Synthesis and anticancer evaluation of 5-benzyl-1,3,4-thiadiazol-2-amine derivatives on Ehrlich ascites carcinoma bearing mice. *Der Pharma Chemica*, 2016; 8(4): 446-452.
7. Arup Naskar, Tanushree Singha, **Tanmoy Guria**, Jagadish Singh, Ashok Kumar B, Tapan Kumar Maity. Synthesis, characterization and evaluation of anticancer activity of some new schiff bases of 1, 3, 4-thiadiazole derivatives. *International Journal of Pharmacy and Pharmaceutical sciences*, 2015; 7(3): 397-402.
8. Arijit Mondal, **Tanmoy Guria**, Tapan Kumar Maity. A new ester of fatty acid from methanol extract of the whole plant of *Amaranthus spinosus* and its glucosidase inhibitory activity. *Pharmaceutical Biology*, 2015; 53(4): 600-604.
9. **Tanmoy Guria**, Arijit Mondal, Tanushree Singha, Jagadish Singh, Tapan Kumar Maity. Pharmacological Actions and Phytoconstituents of *Amaranthus spinosus* Linn: A Review. *International Journal of Pharmacognosy and Phytochemical Research*, 2014; 6(2): 405-413.
10. Puspita Roy, **Tanmoy Guria**, Tanushree Singha, Benu Pani Sahu, Subbiah Ramasamy, Amalesh Samanta, Tapan Kumar Maity. Synthesis, characterization and evaluation of antimicrobial activity of 2, 4, 6-trisubstituted pyrimidine derivatives. *Pharmawave*, 2014; 1(14): 44-49.
11. Sudipta Saha, **Tanmoy Guria**, Tanushree Singha, Tapan KumarMaity. Evaluation of Analgesic and Anti-Inflammatory Activity of Chloroform and Methanol Extracts of *Centella asiatica* Linn. *ISRN Pharmacology*, Volume 2013; Article ID 789613: 6 pages, doi: 10.1155/2013/789613.

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CONFERENCES:

1. Oral presentation in 3rd International Conference (INNOPHARM 3) on **"Academic and Industrial Innovations: Transitions in Pharmaceutical, Medical and Biosciences"** organized by Innovare Academic Sciences**.** Design, synthesis and

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4. 4th International Conference on **"Science for Sustainable Development"**, organized by World Science Congress, Kolkata, held at Jadavpur University, Kolkata, December 16-18, 2014.
5. Poster presentation in "International Conference on Chemical Biology: **Disease Mechanisms and Therapeutics (ICCB-2014)**", organized by Chemical Biology Society of India (CBS) in association with CSIR-Indian institute of Chemical Technology (CSIR-IICT), held at IICT, Hyderabad, February 6-8, 2014.
6. Poster presentation in 2nd Pharm. Tech. IAPST International Conference on **"New insights into diseases and recent therapeutic approaches"**, organized by Indian Association of Pharmaceutical Scientists (IAPST), Kolkata, held at Jadavpur University, Kolkata, January 17-19; 2014.

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3. **"ACS on Campus"** held at Stadel, Salt lake Stadium, Kolkata on 11th January, 2017.

4. Poster presentation in National Seminar on **"Opportunites in Medicinal Plant research"**, organized by School of Natural Product Studies, Jadavpur University and Society for Ethnopharmacology, Kolkata, November 29-30, 2014.
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6. Seminar on **"Trends in Surface Science and Related Areas (TSSRA- 2013)"**, organized by Indian Society for Surface Science & Technology (ISSST) in association with Centre for Surface Science (CSS), Department of Pharmaceutical Technology of Jadavpur University on 3rd May, 2013.
7. Poster presentation in National Seminar on "**Research and Development in Drugs and Pharmaceutivals: an Industrial Perspective**", organized by Golden Jubilee Celebration, Department of Pharmaceutical Technology, Jadavpur University, February 23-24, 2013.
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2. National workshop on **"Revisiting Intellectual Property Rights in The Context of Recent Developments in Science & Technology"**, organized by Faculty of Engineering and Technology, Jadavpur University, Kolkata on 20th October, 2016.
3. National workshop on **"Redefining the Role of Pharmacist in Health Care System"**, organized by Indian Pharmaceutical Association, Bengal Branch on 16th November; 2014.
4. Poster presentation in International Seminar and Workshop on **"Recent Advances in Clinical Research with a Special Emphasis on BA/BE Study"**, organized by Bioequivalence Study Centre, Jadavpur University, September 11-15, 2014.
5. **"National workshop on Drug Design and Discovery"**, organized by Institute of Life Sciences (autonomous institute of DBT), held at Institute of Life Sciences, Bhubaneswar, March 3-6, 2014.

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CERTIFICATE FROM THE SUPERVISOR

This is to certify that the thesis entitled **"Design, Synthesis and Pharmacological screening of Hybrid urea/thiourea derivatives as potential antihyperglycemics"** submitted by Shri **Tanmoy Guria** who got his name registered on **10.01.2013** for the award of **Ph.D (Pharmacy)** degree of **Jadavpur University** is absolutely based upon his own work under the supervision of **Prof. Tapan Kumar Maity** and that neither his thesis nor any part of the thesis has been submitted for any degree/diploma or any other academic award anywhere before.

_____

Signature of the Supervisor

Dedicated to

my family

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Last but not the least; I dedicate the pleasure of presenting this thesis at the lotus feet of Almighty.

TANMOY GURIA

Preface

The thesis consists of synthesis, characterization, docking study, the antidiabetic and antioxidant activity of hybrid urea and thiourea derivatives **(5a-5o), (5A-5Q), (6a-6f)**.

Chapter 1 deals with Introduction which includes a brief discussion of diabetes. The Future of Diabetes, cause of diabetes, diagnosis of diabetes, risk factors, pathophysiology, complications, treatment, molecular docking study have been discussed here.

Chapter 2 comprises of Literature review. This chapter extensively highlights the different method of preparations and pharmacological activities like antidiabetic, antiviral, antibacterial, antioxidant activity of different urea and thiourea derivatives.

Chapter 3 includes Aim and Objective of this research work on the synthesis of hybrid urea and thiourea derivatives and their docking study, antidiabetic and antioxidant activity which have been elaborated in Chapter 4, Chapter 5 and Chapter 6.

Chapter 4 describes synthesis, characterization, docking study and antidiabetic activity of 4'-(phenylurenyl/thiourenyl) chalcone derivatives. This chapter elaborates synthetic procedures of fifteen compounds **(5a-5o)** and their docking study, *in vitro* α- glucosidase inhibition and *in vivo* antidiabetic study with Streptozotocin (STZ) induced diabetic rat model. The antioxidant activity of the compounds has been performed with lipid peroxidation (LPO), superoxide dismutase (SOD), catalase activity (CAT), reduced glutathione level (GSH).

Chapter 5 illustrates synthesis, characterization and pharmacological study of seventeen hybrid phenylurenyl derivatives **(5A-5Q).** The pharmacological evaluation includes *in vitro* antioxidant activity (DPPH radical scavenging activity, Hydrogen peroxide radical scavenging activity and Nitric oxide radical scavenging activity) and *in vitro* α- glucosidase inhibition assay.

Chapter 6 consists of synthesis, characterization and evaluation of *in vitro* antioxidant activity (DPPH radical scavenging activity, Hydrogen peroxide radical scavenging activity and Nitric oxide radical scavenging activity) and *in vitro* α- glucosidase inhibition assay of six chalcone based hybrid phenylurenyl/thiourenyl derivatives **(6a-6f).**

Chapter 7 contains the future scope of this entire work.

Contents

Chapter 1

Introduction

Type 2 Diabetes Mellitus is a severe global health problem. Patients suffering from Type 2 Diabetes Mellitus (T2DM) are at high risk for both microvascular complications (retinopathy, nephropathy and neuropathy) and macrovascular complications (cardiovascular diseases). Environmental factors (such as unhealthy diet and physical inactivity) and genetic factors lead to the various pathophysiological changes that are responsible for impaired glucose homeostasis in T2DM. Insulin resistance and impaired insulin secretion remain the core defects in T2DM. The antidiabetic agents are required for maintaining normoglycemia in T2DM. The treatment must not only be effective and safe but also improve the quality of life. A lot of novel medications are used to enhance insulin sensitivity and prevent or reverse the microvascular complications (Ralph *et al.,* 2015). The World Health Organization (WHO) predicts that by 2030, the prevalence will double and 366 million individuals worldwide will be affected (Wild *et al.,* 2004).

Type 2 diabetes occurs due to the effect of genetic, behavioral and environmental risk factors (Neel, 1962). Diabetes is becoming a major cause of death in people under the age of 60 (International Diabetes Federation, 2014). Investment in effective diabetes prevention and management has become necessary to battle this global epidemic. Along with urbanization and economic growth, many countries have experienced dietary changes favoring increased caloric consumption (Hu, 2011). The management of diabetes on dietary prevention has been derived from Western populations. It is critically important to conduct original investigations in other populations with different disease susceptibility and eating habits. Evidence-based nutrition therapy recommendations have been developed and implemented in many developed countries (Evert *et al.,* 2014; Mann *et al.,* 2004; Canadian Diabetes Association, 2013).

Classification of Diabetes mellitus

Type 1: Insulin-dependent diabetes mellitus (IDDM), There is beta cell destruction in pancreatic islets; the majority of cases are autoimmune (Type 1A) antibodies that destroy beta cells are detectable in blood, but some are idiopathic (Type 1B) - no beta cell antibody is found. It is also known as ‘juvenile diabetes’.

Type 2: Non-insulin-dependent diabetes mellitus (NIDDM): It begins with insulin resistance. As the disease progresses, insulin may lack. This is also called ‘adult-onset diabetes’. It is caused by a combination of excessive body weight and insufficient exercise.

Signs and symptoms

Symptoms of Type 1 Diabetes

Frequent urination, unusual thirst, Extreme hunger, unusual weight loss, Extreme fatigue and Irritability.

Symptoms of Type 2 Diabetes

Excessive Urination and Thirst, Increased Hunger, Weight Gain, Irritability and Fatigue, Blurred Vision, Decelerated Healing, Skin and Yeast Infections.

Other Symptoms

Sexual Dysfunction in Men, Numbness/Tingling in hands and feet, Vaginal Infections in Women.

Cause of type 1 diabetes

Type 1 diabetes is induced by the absence of insulin due to the destruction of beta cells in the pancreas. It is an autoimmune disease which destroys the body's immune system. Generally, the immune system protects the body from infection by killing viruses, bacteria, and other harmful foreign organisms. But in autoimmune diseases, the immune system destroys the body's own cells.

Type 1 diabetes happens in children and young adults, though it can arise at any age. Latent autoimmune diabetes (LADA) is a slowly developing type 1 diabetes in adults (Zimmet *et al.*, 1994; Humphre *et al.*, 1998).

Genetic Susceptibility

Heredity plays an essential role in determining who is to develop type 1 diabetes. Genes are moved from biological parent to child. Certain gene variants that carry instructions for making proteins called human leukocyte antigens (HLAS) on white blood cells are linked to the risk of developing type 1 diabetes (Japan and Pittsburgh 1985; Zimmet 1995).

Beta Cells Destruction

White blood cells (t cells) attack and destroy beta cells.

Environmental Causes

Some environmental factors, i.e., viruses, foods and toxins also develop type 1 diabetes.

Viruses and Infections

Viruses such as cytomegalovirus, adenovirus, rubella, and mumps possibly causes type 1 diabetes.

Infant Feeding Practices

While infants take cow's milk and cereal proteins early, it may increase the risk of type 1 diabetes (Hother Nielsen *et al.,* 1988).

Causes of Type 2 Diabetes Mellitus

MODY (Mature onset diabetes of youth)**,** Pregnancy**,** Acromegaly**,** Cushing's syndrome**,** Pheochromocytoma**,** hyperthyroidism**,** mitochondrial mutations**,** insulin gene mutations**,** insulin receptor mutations.

Other causes of diabetes

Down syndrome, Turner syndrome, Cystic fibrosis, Hemochromatosis, Cushing's syndrome, acromegaly, Lupus erythematosus and Stiff-man syndrome etc.

Diagnosis of diabetes

Diabetes Test

Following blood tests are available to diagnose prediabetes and diabetes

- Fasting plasma glucose (FPG)
- Casual plasma (blood) glucose
- Oral glucose tolerance test

Fasting Plasma Glucose Test

A normal fasting blood glucose level is 80-100 mg/dl. If the fasting blood glucose level is 126 mg/dL or higher, it indicates diabetes. Values of 100–125 mg/dl indicate prediabetes.

Casual Plasma (Blood) Glucose Test

The test is done when the blood glucose level remains 200 mg/dl or higher.

Oral Glucose Tolerance Test

The criteria of this test is a two-hour blood glucose level of 200 mg/dl or higher. If the two-hour blood glucose level is 145-199 mg/dl, prediabetes is diagnosed.

Postprandial (PP) Blood Glucose Test

It measures blood glucose levels 2-3 hours after having a meal. This test is typically done in people who have symptoms of high blood sugar or when the results of a fasting glucose test report possible diabetes but are inconclusive. The values of 200 mg/dL or more indicate diabetes.

HbA1c test

It is used to monitor the effectiveness of therapy diagnosed with diabetes. HbA1c measures the amount of glucose fixed to hemoglobin, which rises as blood glucose levels increase. Since hemoglobin circulates in the bloodstream until the RBC die. The HbA1c test is an important tool for measuring average blood glucose level over the previous 2 to 3 months (Piero *et al.,* 2006).

Diagnostics Tests For Diabetes

The oral glucose tolerance, fasting and postprandial tests are generally used to diagnose diabetes mellitus (type 1 or type 2). HbA1c is used in the management of diabetes mellitus.

- To detect hyperglycemia (high blood sugar) and hypoglycemia (low blood sugar)
- To screen for diabetes, a common disease that often does not cause early symptoms.

Chemical Tests

It involves testing the urine with Benedict's reagent. Results indicate the person having diabetes based on color formation.

- Light color = normal
- Parrot green color = >120 mg/dl
- Dark yellow color = >180mg/dl
- Reddish brown color = > 250 mg/dl
- Brown color = > 350 mg/dl

Diasticks

These are strips that used to indicate the person having diabetes mellitus or not. These strips tested with urine and based on the color change, only diagnosis the diabetes mellitus.

Glucometers

These meters are also involving in diagnosing the diabetes mellitus. Within the fraction of seconds, these will give results about blood glucose levels.

Other Laboratory Tests

In addition, to measure blood glucose and HbA1c, another tests including blood urea nitrogen (BUN), blood creatinine, and protein (albumin) in the urine are also done. The risk of heart disease is increased in people with diabetes, so also need blood tests to measure levels of total cholesterol, triglycerides, low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol.

Urine Glucose/Ketones

Patient self-monitoring is easily done with urine dipsticks for detecting and semi-quantifying glucose and ketones in the urine.

Pathophysiology of Type 2 Diabetes Mellitus

To know the molecular and cellular mechanism of T2DM, it is necessary to conceptualize the framework within which glycemia is controlled. Insulin is the vital hormone for the regulation of blood glucose and, generally, Normoglycemia is maintained by the balanced interplay between insulin action and insulin secretion. Importantly, the normal pancreatic cell can adapt to changes in insulin action, i.e., a decrease in insulin action is associated with upregulation of insulin secretion (and vice versa).

Cell dysfunction is a cause of the pathogenesis of Type 2 diabetes. This concept is based not only in cross-sectional studies but also longitudinally in Pima Indians progressing from normal to impaired glucose tolerance to T2DM. However, not only deviation from but also progression along with the hyperbola affect glycemia. When insulin action reduces (as with increasing obesity), the system compensates typically by increasing the function of the cell. However, in the meantime, concentrations of blood glucose at fasting and 2 h after glucose load will increase mildly. This increase may be small, but over time, becomes damaging because of glucose toxicity and in itself a cause for cell dysfunction. Thus, even with (theoretically) unlimited cell reserve, insulin resistance paves the way for hyperglycemia and Type 2 diabetes.

Insulin secretion from the pancreas usually decreases glucose output by the liver, raises glucose uptake by skeletal muscle, and suppresses fatty acid release from fat tissue. The various factors showed that contribute to the pathogenesis of type 2 diabetes affect both insulin secretion and insulin action. Decreased insulin secretion will reduce insulin signaling in its target tissues. Insulin resistance pathways attack the action of insulin in each of the major target tissues, leading to enhanced circulating fatty acids and the hyperglycemia of diabetes. The increased

concentrations of glucose and fatty acids in the bloodstream will feedback to worsen both insulin secretion and insulin resistance.

Risk factors

Risk Factors of Diabetes Type 1

Some known risk factors include family history, genetics, geography, viral exposure, dietary factors.

Some other possible risk factors

- A mother who has a baby below the age of 25
- A mother who has preeclampsia during pregnancy
- Born with jaundice
- A respiratory infection just after birth

Risk Factors of Diabetes Type 2

Some factors increase the risk of diabetes which are age, weight, fat distribution, inactivity, family history, gestational diabetes and prediabetes.

Complications of diabetes

Type 1 diabetes affects major organs in our body, including eyes, heart, nerves, kidneys and blood vessels. The complications of Type 1 diabetes develop gradually over the years.

Heart and blood vessel disease, Nerve damage (Neuropathy), Kidney damage (Nephropathy), Diabetic cardiomyopathy, Coronary artery disease, Stroke (Mainly the ischemic type), Diabetic myonecrosis (Muscle wasting), Diabetic encephalopathy, Eye damage, Foot damage (Diabetic foot), Skin and mouth functions, Osteoporosis, Pregnancy complications, Hearing problems.

Treatment of diabetes

A) Drug treatment

B) Non-drug treatment

A) Drug Treatment for Diabetes

Oral hypoglycemic drugs lower the glucose levels in the blood in Type 2 diabetes mellitus. Oral hypoglycemic agents are administered orally. The insulin, Exenatide, and Pramlintide are administered parenterally. There are various types of anti-diabetic drugs, and they are selected by

the nature of diabetes, age and situation of the person. Insulin must be injected or inhaled in Type I diabetes. Diabetes mellitus type 2 is an insulin resistance disease.

Insulin

Insulin is a two-chain polypeptide having 51 amino acid and molecular weight about 6000. The A-chain has 21 and B-chain has 30 amino acids. In 1921, Insulin was discovered by Banting and Best. The pure crystalline form of Insulin was obtained in 1926 and chemical structure was fully worked out in 1956 by Sanger. Insulin is usually administered subcutaneously, either by injections or by an insulin pump. Insulin is distributed only extracellularly. It is a peptide, gets degraded in the GIT if given orally. Insulin may also be given intravenously. There are several types of insulin available in the market. These are characterized by the rate of distribution in the body. Type1 diabetes is treated with Insulin. The American diabetes association showed that the diabetes control and complications trial: type1 diabetic patients were randomly allocated to intensive or conventional management.

A. Insulin secretagogues

1. Sulphonylureas (K_{ATP} Channel blockers)
   First generation: Tolbutamide
   Second generation: Glibenclamide (Glyburide), Glimepiride, Glipizide, Gliclazide
2. Non-sulphonylureas – Phenylalanine analogue/ Meglitinide
   Repaglinide, Nateglinide
3. Glucagon-like peptide-1 (GLP-1) receptor agonists
   Exenatide, Liraglutide
4. Dipeptidyl peptidase-4 (DPP-4) inhibitors
   Sitagliptin, Saxagliptin, Linagliptin, Vildagliptin
5. Amylin antagonists

B. **Insulin sensitizers**

1. Biguanides (AMP_K activator)
   Metformin
2. Peroxisome proliferator-activated receptors (PPARα) antagonist
   Fibrates
3. PPAR(γ) antagonists
   Thiazolidinediones
4. Retinoid X receptors (RXRs) agonist

Rexinoids

5. Protein tyrosine kinase inhibitors
6. Antiobesity drugs
7. β_3 receptor antagonists

C. Inhibitors of GI glucose absorption

1. Alpha-Glucosidase inhibitors
   Acarbose, Miglitol
2. Amylin analogues
   Pramlintide

D. Inhibitors of intermediary metabolism

1. Antilipolytic and antihyperlipidaemic drugs
2. Fatty acid oxidation inhibitors
   Lisophyllin

E. Insulinomimetic drugs

Vanadium salts

F. Miscellaneous antidiabetic drugs

1. Dopamine D2 receptor agonist
   Bromocriptine
2. Sodium-glucose cotransport-2 (SGLT-2) inhibitor: Dapagliflozin

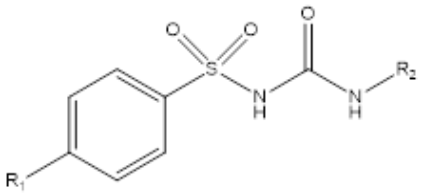
Sulfonylureas (SUs)

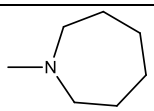
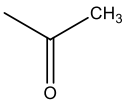
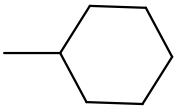
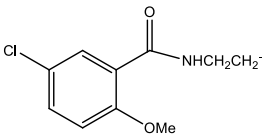
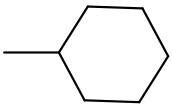
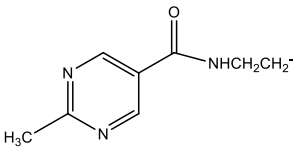
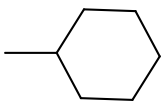
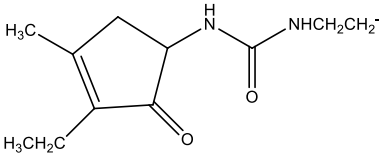
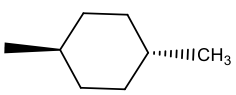
Sulfonylureas were the first widely used oral hypoglycemics. All first generation compounds have been discontinued except tolbutamide. Sulfonylureas provoke a brisk release of insulin from the pancreas. They are insulin secretagogues, activating insulin release by the action on the K_{ATP} channel of the pancreatic beta cells. The Sulfonylureas block the Sulfonylureas receptor (SUR1) which constitute a subunit of ATP sensitive K^+ channel (K_{ATP}) in the membrane of pancreatic β cells. The inward flow of K^+ ions is thereby restricted, intracellular K^+ concentration falls and the membrane is partially depolarized augmenting Ca^{2+} channel opening as well as the release of Ca^{2+} from intracellular stores. The Ca^{2+} ions promote the fusion of insulin-containing granules and exocytotic release of insulin.

First generation drugs: Tolbutamide, Chlorpropamide, Acetohexamide, Carbutamide, Glycyclamide (tolhexamide), Metahexamide and tolazamide.

Second generation drugs: Glibenclamide (glyburide), glipizide, gliclazide, glibornuride, gliquidone, glisoxepide and glyclopyramide.

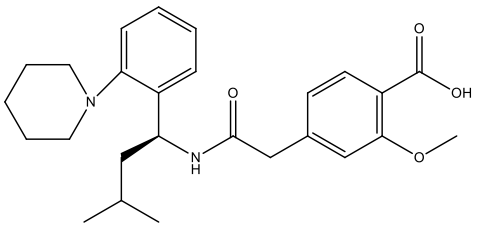
Third generation drugs: Glimepiride



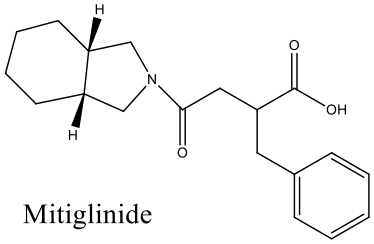
Name	R_1	R_2
Tolbutamide	$-CH_3$	$-CH_2CH_2CH_2CH_3$
Chlorpropamide	-Cl	$-CH_2CH_2CH_3$
Tolazamide	$-CH_3$	
Acetohexamide		
Glyburide		
Glipizide		
Glimepiride		

Phenylalanine analogue/Meglitinides

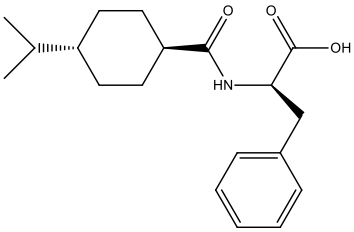
Meglitinides are also called as "short-acting secretagogues". Their mode of action is similar to the Sulfonylureas. By closing the ATP-sensitive K^+ channel (K_{ATP}) of the pancreatic beta cells, they open the calcium channels, hence enhancing insulin secretion.



Repaglinide



Mitiglinide



Nateglinide

Repaglinide is a carbamoylmethyl benzoic acid derivative. The insulinemic action of repaglinide is mediated via the inhibition of ATP-sensitive potassium ion channels (K_{ATP}) in the pancreatic beta cell. Repaglinide is quickly absorbed and rapidly metabolized. It induces fast onset short lasting insulin release. Repaglinide binds with low affinity to the classic sulphonylurea receptor. (Feinglos, 1999). Repaglinide is indicated only in selected type 2 diabetes who suffer pronounced postprandial hyperglycemia.

Glucagon-Like Peptide-1 (GLP-1) Agonist

GLP-1 is an incretin released from the gut in response to glucose. By binding with GLP-1 receptors which are cell surface $GPCR_S$ induces insulin release from pancreatic β cells. It also inhibits glucagon release from α cells. GLP-1 induces insulin release only at high glucose concentration.

Incretins such as Glucagon-like peptide 1 (GLP-1) is a potent gut hormone. It activates adenylyl cyclase and generates cAMP, which promotes exocytosis release of insulin. (Kreymann, *et al.*, 1987). Exenatide and liraglutide are GLP1 receptor agonists. The incretins GLP1 are rapidly inactivated by the enzyme Dipeptidyl peptidase-4.

Exenatide is a synthetic DPP-4 resistant analogue which activates GLP-1 receptors. Exenatide is a 39-amino-acid peptide. Being a peptide, it is inactive orally.

Liraglutide is a long-acting GLP-1 agonist. Hypoglycemia is rare with exenatide/liraglutide monotherapy, but can occur when combined with SUs/metformin.

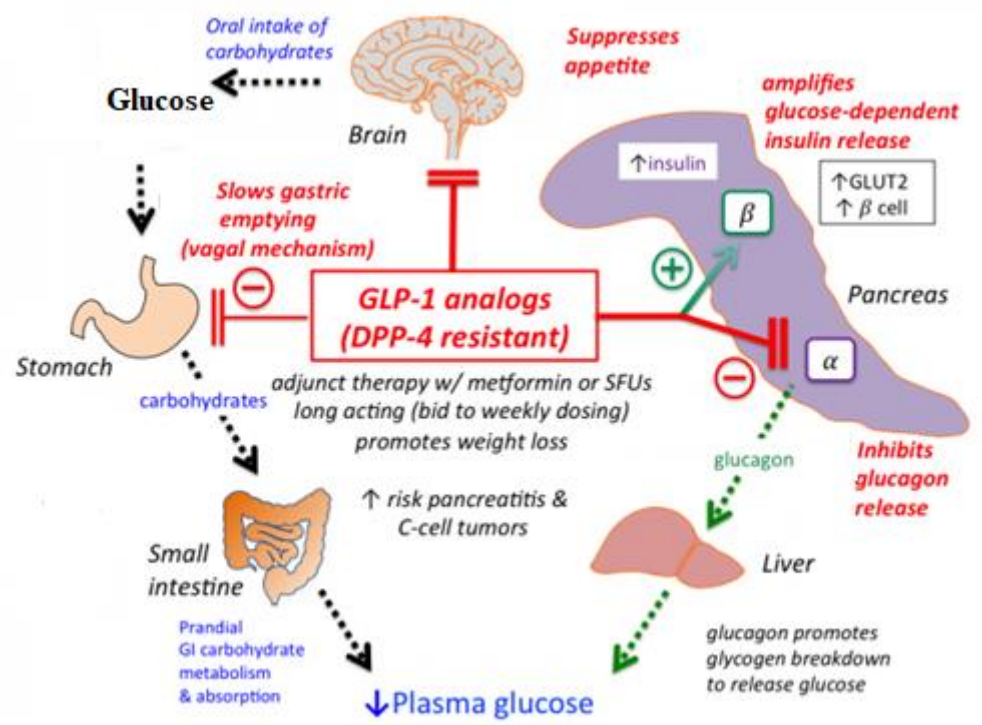


Fig 1: Mechanism of action of Glucagon-Like Peptide-1 (GLP-1) Agonist

Gastric Inhibitory Peptide (GIP) Analogs

Dipeptidyl peptidase-4 (DPP-4) Inhibitors

Dipeptidyl peptidase-4 (DPP-4) inhibitors increase the concentration of the incretin glucagon-like peptide-1 (GLP-1) in blood by inhibiting its degradation by dipeptidyl peptidase-4 (DPP-4). Sitagliptin is the first DPP-4 inhibitor. The HbA_{1c} lowering is caused by sitagliptin, which is equivalent to that metformin. Sitagliptin which is well absorbed orally is little metabolized and is largely excreted unchanged in the urine.

Vildagliptin is less selective than the sitagliptin for DPP-4. The tolerability of vildagliptin is similar to that of sitagliptin, but hepatotoxicity has been reported.

Saxagliptin binds covalently with DPP-4and acts for 24 hours despite a plasma $t_{1/2}$ of 2-4 hours.

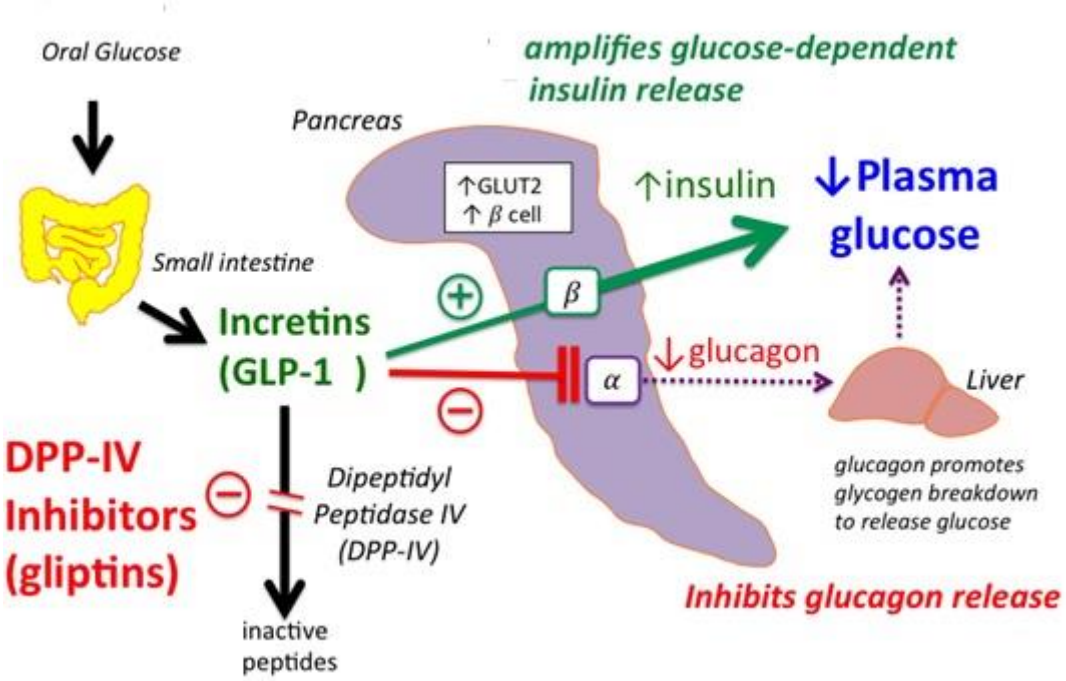


Fig 2: Mechanism of action of Dipeptidyl peptidase-4 (DPP-4) Inhibitors

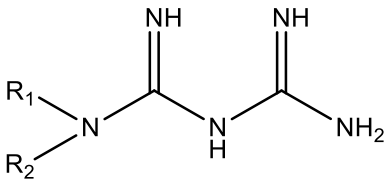
Amylin Analogues

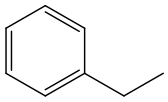
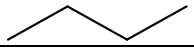
Amylin analogues are injectable drugs (subcutaneous injection) used in the treatment of both type 1 diabetes and type 2 diabetes. These compounds are administered before meals and function similarly to the hormone amylin. Amylin has several benefits in terms of weight loss and reducing blood glucose levels. Amylin, unlike GLP-1, does not have insulin secretory effects. It regulates hyperglycemia through amelioration of glucagon secretion and gastric emptying. Primitive is the only clinically available amylin analogue.

Biguanides (AMPK activator)

Biguanides reduce hepatic glucose output and increase glucose uptake. It must be used cautiously in patients suffering from liver or kidney function. Biguanides do not cause insulin release, but the presence of insulin is essential for their action.

Metformin is preferred to Phenformin as it does not inhibit mitochondrial oxidation of lactate and lactic acidosis. The activation of AMP-dependent protein kinase (AMPK) by Metformin play a crucial role in mediating the actions of metformin. Its modes of action include (a) inhibition of gluconeogenesis, (b) reduction of hepatic glucose output, (c) reduction of weight; the improvement in insulin sensitivity is a byproduct of these alterations. Metformin is widely used in the treatment of type 2 diabetes mellitus (Defronzo, *et al.*, 1995). Phenformin and Buformin are withdrawn from the market in most countries due to toxic effects.



Compounds	R_1	R_2
Metformin	CH_3	CH_3
Phenformin		H
Buformin		H

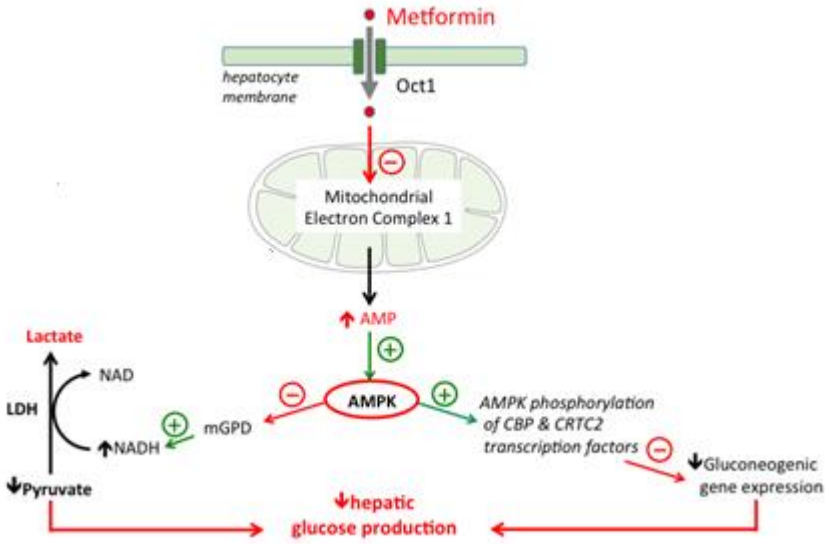


Fig 3: Mechanism of Action of Biguanides

Thiazolidinediones

Thiazolidinediones (TZDs), called as "glitazones," bind to PPARγ, a type of nuclear regulatory proteins involved in the transcription of genes regulating glucose and fat metabolism. These PPARγ act on Peroxisome Proliferator Responsive Elements (PPRE). The PPREs influence insulin-sensitive genes, which enhance the production of mRNAs of insulin-dependent enzymes. Peroxisome proliferator-activated receptor (PPAR) and their subtypes have led to the discovery of a new generation of drugs. The two major PPAR receptors are α & γ and both are expressed by obligate heterodimerization with retinoic acid x receptor (Rx Rα and Rx Rγ). The PPAR (α) is primarily responsible for lipolysis by activation of enzymes such as acyl CoA oxidase, lipoprotein lipase, malic enzyme, bifunctional enzyme, and medium chain acyl CoA

dehydrogenase. On the other hand, PPAR(γ) is primarily responsible for the adipocyte differentiation and at the metabolic level, in FFA and lipid anabolism and storage. The pronounced hypoglycemic effect seen by PPAR(γ) agonists is attributed primarily to adipocyte differentiation and/or activation (Bocl, 1999).

Pioglitazone is only one thiazolidinedione currently available in India. Pioglitazone is indicated in type 2 DM, but not in type 1 DM. It is primarily used to supplement SUs/metformin and in case of insulin resistance. France and Germany have suspended its use due to raising the risk of bladder cancer.

Rosiglitazone is banned in India since 2010 and has been withdrawn from the market in Europe due to an increased risk of cardiovascular events. Upon re-evaluation of new data in 2013, the FDA lifted the restrictions. Lobeglitazone has approved for use in Korea. Troglitazone (Rezulin) was withdrawn from the market due to an increased incidence of drug-induced hepatitis. Balaglitazone (DRF-2593) was developed by Indian pharma company Dr. Reddy's Laboratories. Non-marketed agents include: Ciglitazone, Darglitazone, Englitazone, Netoglitazone, Rivoglitazone. Rhodanine is a sulphur containing thiazolidinedione.

Fibrates have no direct effect as hypoglycemic action. This PPAR(α) agonists lower LDL, cholesterol and triglycerides but raise in HDL level, thus offering protection against increased coronary complication which is seen in type 2 diabetes (Saltiel *et al.*, 1996).

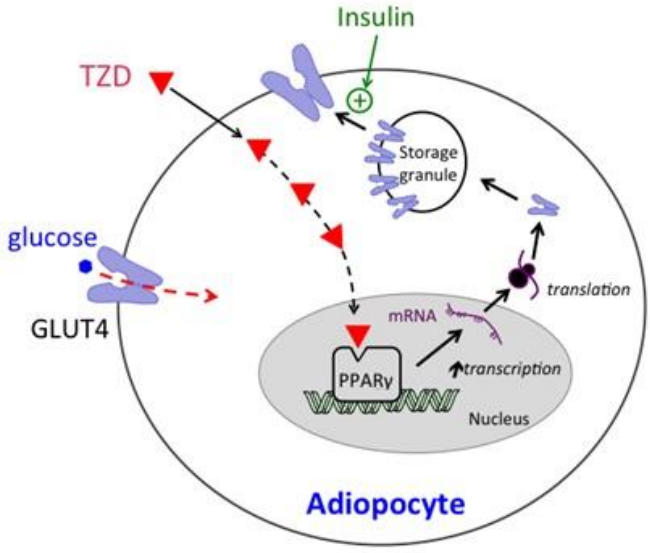
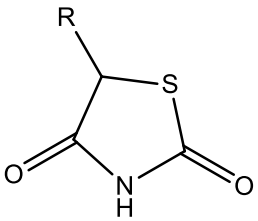
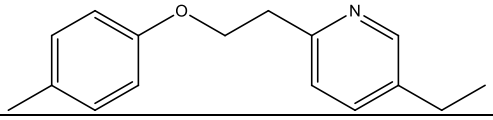
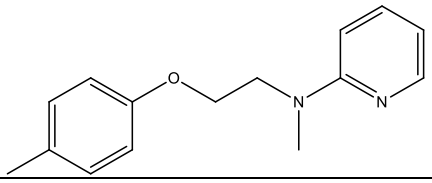
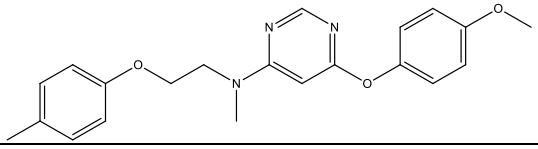
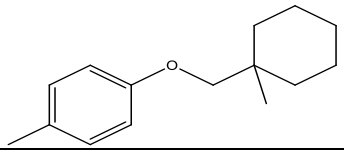
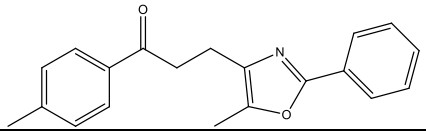
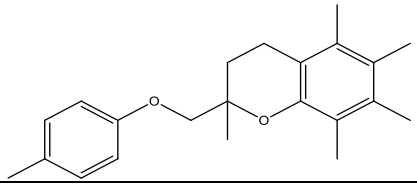
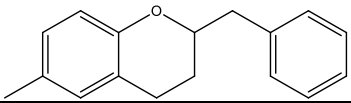
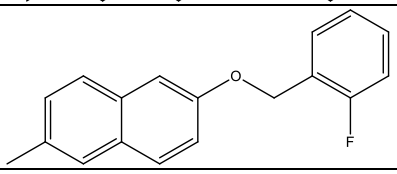
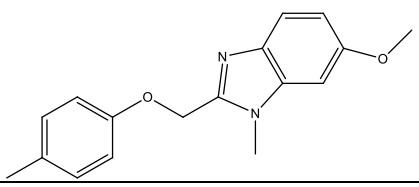


Fig 4: Mechanism of Action of Thiazolidinedione, TZD- Thiazolidinediones, PPARγ- Peroxisome proliferator-activated receptor



Compounds	R
Pioglitazone	
Rosiglitazone	
Lobeglitazone	
Ciglitazone	
Darglitazone	
Troglitazone	
Englitazone	
Netoglitazone	
Rivoglitazone	

Retinoid X receptors (RXRs) agonist

Retinoids, which activate RxR receptors are being developed to control diabetes, one such product LG 100268 has shown significant promise, in that, in addition to being an insulin sensitizer it causes weight reduction in contrast to PPAR(γ) agonists (Oglive *et al.,* 1999).

Protein tyrosine kinase inhibitor

A new class of drugs which are plant extracts and act through inhibition of protein tyrosine kinase, are being investigated. In addition to the hypoglycemic effect, it blocks the formation of proinflammatory cytokines such as TNF α. Compounds in this class include CLX 0301, CLX 0302, CLX 0900, and CLX 0901. This group of drugs also lowers cholesterol and triglycerides. Again these are sensitizers and are not effective in type 1 diabetes (Nag *et al.,* 1999).

β_3 adrenergic receptor agonists

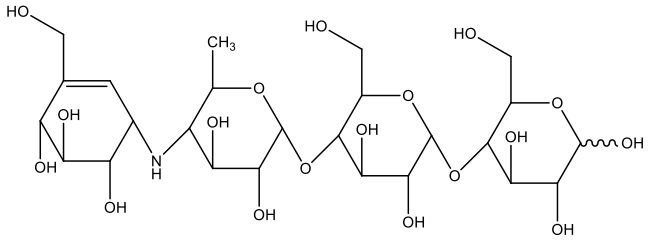
β_3 adrenergic receptor present in brown and white adipose tissues, mediate catecholamine stimulated thermogenesis and lipolysis. It has been linked to lower basal metabolic rate, greater visceral adiposity, and early onset of type 2 diabetes in these ethnic groups. This observation has stimulated the use of selective β_3 adrenoceptor agonists such as CL 316, 2443, which do not cross-react with other β- adrenoceptor, for treating obesity and improving insulin sensitivity (Dey *et al.,* 1999).

Inhibitors of GI glucose absorption

Alpha-Glucosidase Inhibitors

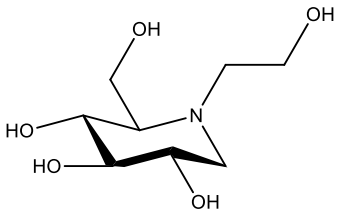
Alpha-glycosidase inhibitors are "diabetes pills" but not technically hypoglycemic agents because they do not have a direct effect on insulin secretion or sensitivity. These agents slow the starch digestion in the small intestine so that glucose from the starch of a meal slowly enters the bloodstream. These delays adsorption of carbohydrates, reducing the postprandial increase in blood glucose. E.g., Acarbose, Miglitol, Voglibose

Acarbose is an alpha-glucosidase inhibitor. Alpha-glucosidase is an intestinal enzyme that releases glucose from carbohydrates such as starch. It is composed of an acarviosin moiety and maltose.



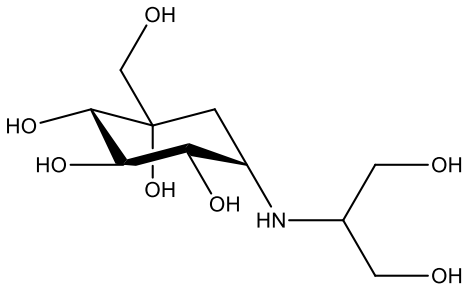
Acarbose

Miglitol is a newer-glucosidase inhibitor, derived from 1-deoxynojirimycin and is structurally similar to glucose (Wolffenbuttel *et al.,* 1996). It is absorbed from the gastrointestinal tract. It is short acting and hence is expected to have less gastrointestinal side effects than acarbose.



Miglitol

Voglibose is used for lowering postprandial blood glucose levels in people with diabetes mellitus. Voglibose delays the absorption of glucose, so that reduces the risk of macrovascular complications.



Voglibose

Amylin analogues

Amylin, also called 'islet amyloid polypeptide' (IAP) is produced by pancreatic β cells and acts in the brain to reduce glucagon secretion from α cells, delay gastric emptying, retard glucose absorption and promote satiety.

Pramlintide is a synthetic Amylin analogues which on s.c. injection before meal attenuates postprandial glycemia and exerts a centrally mediated anorectic action. It has been used as an adjuvant to mealtime insulin injection to suppress the glycemic peak in type 1 and type 2 diabetes both. The Islet amyloid polypeptide (IAPP, Amylin) suppresses insulin secretion and action, and further it has been linked with the development of insulin resistance at very high concentrations, has led to the development of amylin antagonist (IAPP) which stimulates insulin secretion (Bailey 1996).

Inhibitors of intermediary metabolism

Type 2 diabetes is invariably associated with disordered lipid metabolism. In the muscle, a glucose, fatty acid cycle has been recognized for many years. The oxidation of fatty acids decreases glucose uptake and utilization through substrate competition, causing post-receptor insulin resistance. As the rate of fatty acids oxidation determines the rate of gluconeogenesis, a good correlation exists between raised fasting NEFA levels, impaired lipid oxidation, and reesterification and hepatic glucose output (HGO) in patients with type 2 diabetes. Since the increase in HGO is responsible for fasting hyperglycemia in type 2 diabetes, drugs decreasing fatty acid levels or inhibiting fatty acid oxidation are attractive options for controlling fasting hyperglycemia (Muralidharan *et al.*, 1998).

Drugs decreasing fatty acids

These are anti-hypertriglyceridaemic drugs, e.g., acipimox and bezafibrate. Acipimox is a long-acting nicotinic acid analogue, which significantly reduces plasma NEFA and HGO. Its effects on HGO are more sustained than nicotinic acid which has a variable impact on glycemic control frequently worsening due to discrepant effects on glycogen breakdown and gluconeogenesis. In a study of obese type 2 diabetic patients, overnight suppression of plasma NEFA with acipimox significantly reduced lipid oxidation, HGO, and increased the glucose disposal rate (Fulcher *et al.*, 1992).

Fatty acid oxidation inhibitors

Suppression of fatty acid oxidation decreases hepatic gluconeogenesis and increases peripheral glucose utilization. The drugs of this class inhibit carnitine palmitoyltransferase-1 (CPT-1) which is a rate-limiting enzyme. The enzyme transfers the long chain fatty acids into mitochondria (Colher *et al.*, 1993).

Insulinomimetic drugs

Vanadium salts: Vanadium is an ultra-trace element. Its compounds such as vanadyl orthovanadate, metavanadate and peroxovanadate have been shown to have insulinomimetic effects on adipocytes, hepatocytes and the skeletal muscles as well as in hyperinsulinaemic and hypoinsulinaemic animal models of diabetes. In animal models, vanadium salts induce a decrease in body weight, attributed to its central anorectic effects. These salts act by increasing the phosphorylation of insulin receptor either by activation of the intrinsic tyrosine kinase activity or by inhibition of the phosphotyrosyl phosphatase that dephosphorylates the receptor

and may also act on post-receptor sites (mitogen-activated protein kinase and cytosolic insulin independent tyrosine kinase) (Malabar *et al.*, 1994).

Miscellaneous antidiabetic drugs

Dopamine D2 receptor agonist

Bromocriptine is used for adjunctive treatment of type 2 DM. It acts on the hypothalamic dopaminergic control of the circadian rhythm of hormone (GH, prolactin, ACTH) release and reset it to reduce insulin resistance.

Sodium-glucose cotransport-2 (SGLT-2) inhibitor

The SGLT-2 inhibitor induces glucosuria and lowers blood glucose in type 2 DM. Dapagliflozin as SGLT-2 inhibitor has been recently tested in type 2 DM patients. Glycosuria produced by dapagliflozin which can predispose to urinary and genital infections, electrolytes imbalance and increased urinary frequency.

IGF-1 receptor agonists are also being developed for selective hypoglycemic action, prolonged duration of effects, and perhaps for use by the oral route.

Additionally, new drugs are being developed to counter complications in diabetes. These include aminoguanidine, tenilsetam, OPB-9195 which are AGE receptor antagonists; protein kinase C inhibitors (LYS 333531, WAY 151003, and Cremophor EL), nerve growth factors, octreotide, hismanal, topical clonidine, picotamide, and a lot of research is focused on antioxidants such as vitamin E and α lipoic acid (Chandalia *et al.*, 2000).

B) Non-Drug Treatment for Diabetes

1. Lifestyle changes which are used to controlling diabetes

Lifestyle change is defined as the way of living which has been altered by variety manner. It has seven principles of good diabetes care: Learn as much as you can about diabetes, Learn how to control your diabetes, Get regular care for diabetes, Take care of your diabetic ABC's, Monitor your diabetic ABC's Prevent long term diabetes problems, Get checked for long term problems and treat them.

2. Exercise

It is essential in helping to prevent diabetes and is having a vital role in our treatment.

1. It helps in losing weight
2. It can reduce blood glucose levels and keep it low for several hours
3. It can lower cholesterol and blood pressure

4. It helps to reduce stress
5. It makes the tissues in your body more sensitive to the effects of insulin

3. Diet

The diet should be higher in fiber and low in fat. It also involves weight loss which is another way to increase diabetic patient's body sensitivity to the effects of insulin.

Molecular Docking:

The modern medicinal chemist has focused their interest in the study of structure-activity relationships (SAR) of newly synthesized molecules by using molecular modeling methods. (Hughes *et al.,* 2011). These methodologies have also been used in the study of pharmacodynamics (e.g., potency, affinity, efficacy, selectivity) as well as pharmacokinetic properties (ADMET: absorption, distribution, metabolism, excretion and toxicity) of synthetic molecules (Lipinski et al., 2012). The new biomolecular spectroscopic methods such as X-ray crystallography and nuclear magnetic resonance (NMR) which help in the progress of molecular and structural biology. These techniques produce vital structural information about more than 100,000 three-dimensional protein structures for targeting macromolecular drug interaction (Berman 2000).

Based on this perspective, the correlation of in silico and experimental methods has given the up-to-date understanding of the intricate aspects of intermolecular recognition (Weigelt 2010). Structure-based drug design (SBDD) methods are a component of modern medicinal chemistry (Salum *et al.,* 2008). Molecular docking, Molecular dynamics (MD) and Structure-based virtual screening (SBVS) are the frequently used SBDD strategies due to their wide range of uses in the study of molecular recognition events such as binding energetics, molecular interactions and induced conformational changes (Kalyaanamoorthy *et al.,* 2011).

A distinct approach in drug design contains the use of bioactive small-molecule libraries. The unique chemical diversity available in these libraries represents the space occupied by ligands known to interact with a specific target. This type of information is employed in Ligand-based drug design (LBDD) methods (Acharya *et al.,* 2011). Ligand-based virtual screening (LBVS), QSAR modeling, similarity searching and pharmacophore generation are some of the most useful LBDD methods (Bacilieri *et al.,* 2006). SBDD and LBDD approaches have been used as drug discovery tools in academia and industry. The integration of these approaches is

successfully employed in several investigations of structural, chemical and biological data (Trossini *et al.,* 2013; Valasani *et al.,* 2014).

Structure-Based Drug Design (SBDD) understanding the theory by which small-molecule ligands recognize and interact with macromolecules is of great importance in pharmaceutical research (Blaney 2012). SBDD refers to the systematic use of structural data (e.g., macromolecular targets, also called receptors), which are usually got experimentally or through computational homology modeling (Mandal *et al.,* 2009). The availability of three-dimensional macromolecular structures enables to know the binding site topology, including the presence of clefts, sub-pockets and cavities. Electrostatic properties, such as charge distribution, can also be carefully examined.

Current SBDD methods allow for the design of ligands consisting of the essential features for efficient modulation of the target receptor. Selective modulation of a validated drug target by high-affinity ligands interact with specific cellular processes, ultimately leading to the desired therapeutic and pharmacological effects (Urwyler 2011).
SBDD is a cyclic process comprising of stepwise knowledge acquisition. Starting from a known target structure, in silico studies can be directed to identify potential ligands. These molecular modeling procedures are taken by the synthesis of the most promising compounds (Wilson *et al.,* 2011).

Urea was the first organic compound synthesized in the laboratory, which brought a green revolution throughout the world. Later on, its similar structural compound thiourea had been discovered, which also had significant importance in agriculture. Urea is a natural chemical compound, produced in the human organism as a metabolite of proteins and other nitrogen-containing compounds as a part of the urea cycle. Urea production occurs in the liver and it is found in the urine of mammals, plants, birds, yeast and many microorganisms. It is highly soluble in water and ethanol. It was for the first time synthesized in the synthetic laboratory in 1828 by a German chemist Friedrich Wohler.

Urea contains maximum nitrogen among all the solid nitrogenous fertilizers. It is the most popular of all nitrogen-based fertilizers used worldwide. It is versatile and can be used on a wide variety of crops. Urea is also a raw material for the manufacture of various plastics and adhesives.

Urea and its derivatives constitute an important class of heterocyclic compounds which possess a wide range of therapeutic and pharmacological properties whereas, thiourea is organosulfur compound and it is similar to urea in which oxygen is replaced by sulphur. Urea/Thioureas are used as purification agents in the effluent of organic and inorganic industrial, agricultural and mining wastes. Due to the unique binding mode, they act as kinase inhibitors and novel therapeutics in cancer treatment.

References

Acharya, C., Coop, A., Polli, J.E., Mackerell, A.D., 2011. Recent advances in ligand-based drug design: Relevance and utility of the conformationally sampled pharmacophore approach. Curr. Comput. Aided Drug Des. 7, 10–22.

Bacilieri, M., Moro, S., 2006. Ligand-based drug design methodologies in drug discovery process an overview. Curr. Drug Discov. Technol. 3, 155–165.

Bailey, C.J., 1996. Novel compounds for NIDDM. Diabetes Reviews International. 5, 9-12.

Berman, H.M., 2000. The protein data bank. Nucleic Acids Res. 28, 235–242.

Bocl, E., 1999. Designing drugs for the treatment of diabetes in the next millennium. Nov Nordisk Update 1999 Proceeding. Kapur A, Joshi JK (eds). Communication Workshop (P) Ltd 221.

Blaney, J. A., 2012. Very short history of structure-based design: How did we get here and where do we need to go? J. Comput. Aided Mol. Des. 26, 13–14.

Canadian Diabetes Association Clinical Practice Guidelines Expert Committee. 2013. Clinical Practice Guidelines: Nutrition Therapy. Can J Diabetes. 37, S45–S55.

Chandalia, H.B., Lamba, P.S., 2000. Futuristic antidiabetic agents. The Indian Practioner. 53 (1), 37-44.

Colher GR, Traiaedes K, Acaulay, S.C., O'Dea, K., 1993. Effect of fatty acid oxidation on glucose metabolism in diabetic rats. Horm Metab Res. 25, 9-12.

Defronzo, R.A., Goodman, A.M., 1995. Multicentre metformin study group. Efficacy of metformin in patients with noninsulin dependent diabetes mellitus. N Engl J Med. 333, 541-6.

Dey, D., Medicherle, S.D., Neogi, P., Nag, B., 1999. CLX-0901. A new class of orally active insulin sensitizer. Diabetes. 48 (1), Abstract 0512.

Evert, A.B., Boucher, J.L., Cypress, M., 2014. Nutrition therapy recommendations for the management of adults with diabetes. Diabetes Care. 37(Supplement 1), S120–S43.

Feinglos RE. 1999. Repaglinide therapy in treatment of type 2 diabetes. Therapeutic Trends 17, 57-66.

Fulcher, G.R., Walber, M., Catalano, C., Aguist Alberti, KGMM., 1992. Metabolic effects of suppression of NEFA levels with acipomox in obese NIDDM subjects. Diabetes 41: 1400-08.

Hother Nielsen, O., Faber, O., Sorensen, N.S., Beck–Nielsen, H., 1988. Classification of newly diagnosed diabetic patients as insulin requiring or non-insulin requiring based on clinical and biochemical variables. Diabetes Care, 11, 531–537.

Hu, F.B., 2011. Globalization of diabetes: the role of diet, lifestyle, and genes. Diabetes Care 34(6): 1249–57.

Hughes, J.P., Rees, S., Kalindjian, S.B., Philpott, K.L., 2011. Principles of early drug discovery. Br. J. Pharmacol. 162, 1239–1249.

Humphrey, ARG., Mccarty, D.J., Mackay, I.R., Rowley, M.J., Dwyer, T., Zimmet, P., 1998. Autoantibodies to glutamic acid decarboxylase and phenotypic features associated with early insulin treatment in individuals with adult– onset diabetes mellitus. Diabetic Med, 15, 113–119.

International Diabetes Federation. [accessed January 30, 2014] IDF Diabetes Atlas. 62013. http:// www.idf.org/diabetesatlas.

Japan and Pittsburgh childhood diabetes research groups. 1985. Coma at onset of young insulin–dependent diabetes in Japan: the result of a nationwide survey. Diabetes, 34, 1241–1246.

Kreymann, B., Williams, G., Ghatei, M.A., Bloom, S.R., 1987. Glucagon like peptide-1 7-36: A physiological incretin in man. Lancet 2(8571), 1300-4.

Kalyaanamoorthy, S., Chen, Y.P., 2011. Structure-based drug design to augment hit discovery. Drug Discov. Today 16, 831–839.

Lipinski, C.A., Lombardo, F., Dominy, B.W., Feeney, P.J., 2012. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Deliv. Rev. 64, 4–17.

Malabar, U.H., Dryden, S., McCarthy, H.D., 1994. Effects of chronic vandate administration in the STZ-induced diabetes rat. Diabetes 43: 9-15

Mandal, S., Moudgil, M.N., Mandal, S.K., 2009. Rational drug design. Eur. J. Pharmacol. 625, 90–100.

Mann, J.I., De Leeuw, I., Hermansen, K., Karamanos, B., Karlström, B., Katsilambros, N., Riccardi, G., Rivellese, A.A., Rizkalla, S., Slama, G., Toeller, M., Uusitupa, M., Vessby, B., 2004. Evidence-based nutritional approaches to the treatment and prevention of diabetes mellitus. Nutr Metab Cardiovasc Dis. 14(6), 373–94.

Muralidharan, R., Dash, R.J., 1998. Newer modes of therapy for NIDDM. Jour Assoc Phys India 46 (8): 716-23.

Nag, B., Neogi, P., Dey, D., Sathyanarayana, M., 1999. CLX-0301. A new tryphoetin molecule for the treatment of diabetes. Diabetes 48 (1), Abstract 0485.

Neel, J.V., 1962. Diabetes mellitus: a “thrifty” genotype rendered detrimental by “progress“?. Am J Hum Genet. 14, 353-362.

Oglive, K.M., Saladin, R., Nagy, T.R., 1999. Reduced obesity in zucker fatty rates treated with a retinal x receptor agonist. Diabetes 48 (1), Abstract 0024.

Piero, NM., Joan, MN., Cromwell, KM., Joseph, NJ., Wilson, NM., Daniel M., Gathumbi K Peter, GM., Eliud, NN., 2011. Hypoglycemic Activity of Some Kenyan Plants Traditionally used to Manage Diabetes Mellitus in Eastern Province. J Diabetes Metab 2:155.

Ralph, A., DeFronzo, Ele F., Leif G., Robert, R., Henry, William H., Herman, Jens Juul H., Frank, B., Hu, C., Ronald, K., Itamar Raz, Gerald I., Shulman, Donald C., Simonson, Marcia A., 2015. Testa & Ram Weiss, Type 2 diabetes mellitus, Nature Reviews Disease Primers 23, 1, 15019.

Saltiel, A.R., Olefsky, J.M., 1996. Thiazolidinediones in the treatment of insulin resistance any type II diabetes. Diabetes 45, 1661-9.

Salum, L., Polikarpov, I., Andricopulo, A.D., 2008. Structure-based approach for the study of estrogen receptor binding affinity and subtype selectivity. J. Chem. Inf. Model. 48, 2243–2253.

Trossini, G.H.G., Guido, R.V.C., Oliva, G., Ferreira, E.I., Andricopulo, A.D., 2013. Quantitative structure-activity relationships for a series of inhibitors of cruzain from Trypanosoma cruzi: Molecular modeling, CoMFA and CoMSIA studies. J. Mol. Graph. Model. 28, 3–11.

Urwyler, S., 2011. Allosteric modulation of family C G-protein-coupled receptors: From molecular insights to therapeutic perspectives. Pharmacol. Rev. 63, 59–126.

Valasani, K.R., Vangavaragu, J.R., Day, V.W., Yan, S.S., 2014. Structure based design, synthesis, pharmacophore modeling, virtual screening, and molecular docking studies for identification of novel cyclophilin D inhibitors. J. Chem. Inf. Mod. 54, 902–912.

Wild, S., Roglic, G., Green, A., Sicree, R., King, H., 2004. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 27, 1047–1053.

Wilson, G.L., Lill, M.A., 2011. Integrating structure-based and ligand-based approaches for computational drug design. Future Med. Chem. 3, 735–750.

Weigelt, J., 2010. Structural genomics-Impact on biomedicine and drug discovery. Exp. Cell Res. 316, 1332–1338.

Wolffenbuttel, BHR., Graal, M.B., 1996. New treatments for patients with type 2 diabetes mellitus. Postgrad. Med. J. 72: 657-62.

Zimmet, P.Z., Tuomi, T., Mackay, I.R., Rowley, M.J., Knowles, W., Cohen, M., Lang, D.A., 1994. Latent autoimmune diabetes mellitus in adults (LADA): The role of antibodies to glutamic acid decarboxylase in diagnosis and prediction of insulin dependency. Diabetic Med, 11, 299–303.

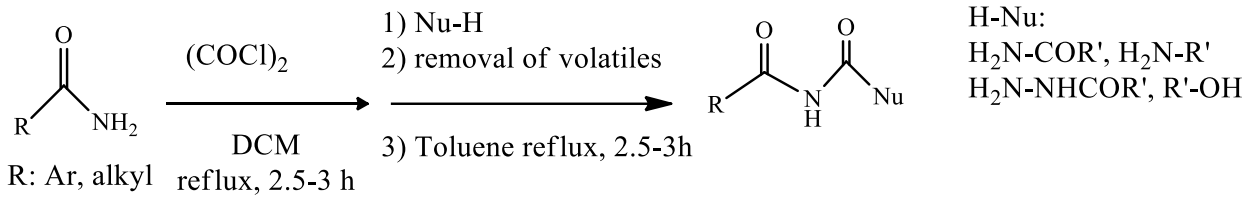
Zimmet, PZ., 1995. The pathogenesis and prevention of diabetes in adults. Diabetes Care, 18, 1050–64.

Chapter 2

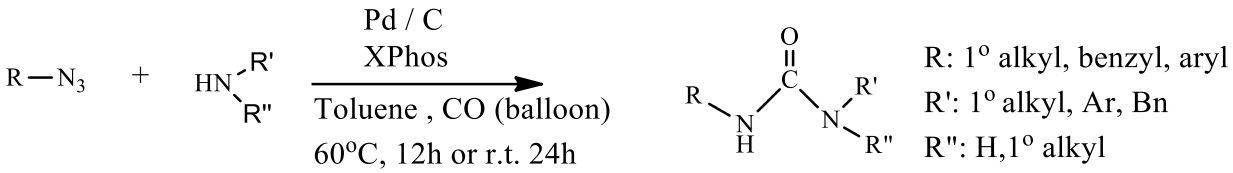
Review of Literature

Methods of Preparation of Urea derivatives

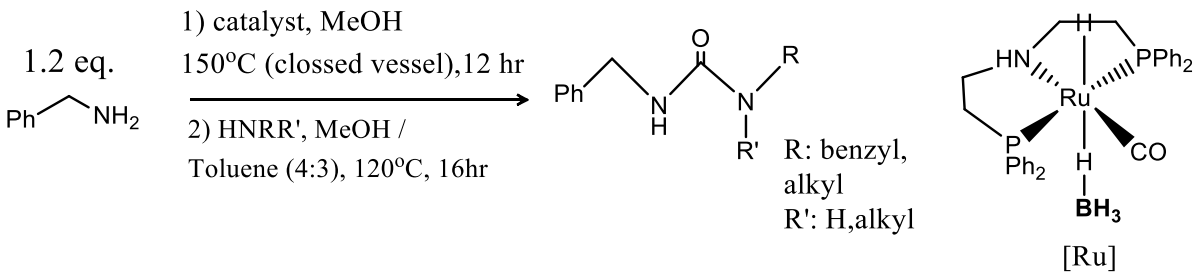
Symmetrical, unsymmetrical diacyl urea derivatives and acyl ureas/carbamates/thiocarbamates were synthesized by reacting between amides, hydrazides, amines, alcohols, carbazate, and sulfonate with Acyl isocyanates which was generated by the reaction of primary amides with oxalyl chloride (Hernandez *et al.*, 2017).



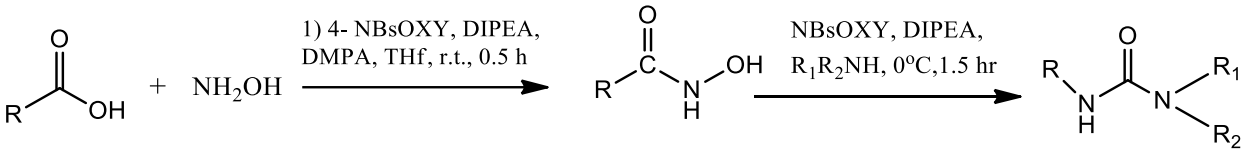
Unsymmetrical ureas were synthesized by Pd/C-catalyzed carbonylation of both aliphatic and aromatic azides in the presence of amines under CO atmosphere where N_2 as the only byproduct (Zhao *et al.*, 2016).



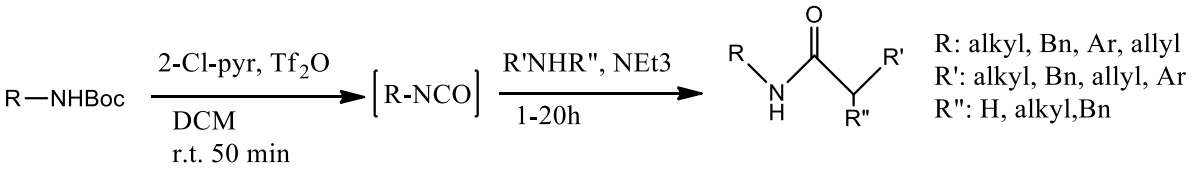
Ruthenium pincer complexes as catalysts enabled a urea synthesis directly from methanol and amine without additives, such as base, oxidant, or hydrogen acceptor. The reaction produced hydrogen as the byproduct. Unsymmetrical urea derivatives were obtained via a one-pot, two-step reaction (Kim *et al.*, 2016).



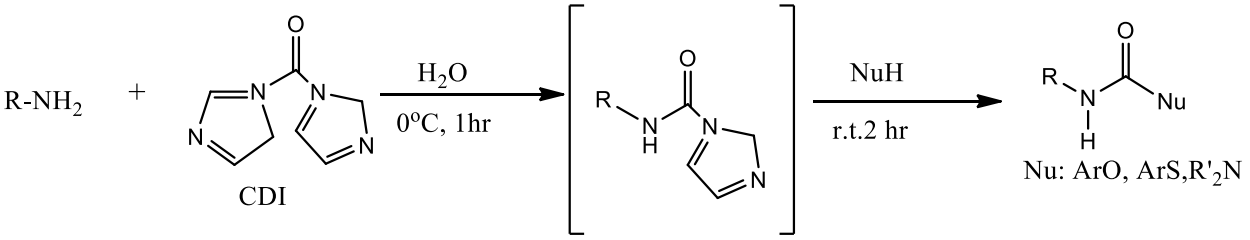
Carboxylic acids and hydroxylamine hydrochloride produced Hydroxamic acids in the presence of ethyl 2-cyano-2-(4-nitrophenyl sulfonyl oxyimino) acetate (4-NBsOXY). 4-NBsOXY also stimulated the Lossen rearrangement of hydroxamic acids in the presence of amines to yield ureas. The reactions are compatible with common *N*- and *O*-protecting groups (e.g-Boc, Fmoc, Cbz) and prevent racemization (Thalluri *et al.,* 2014).



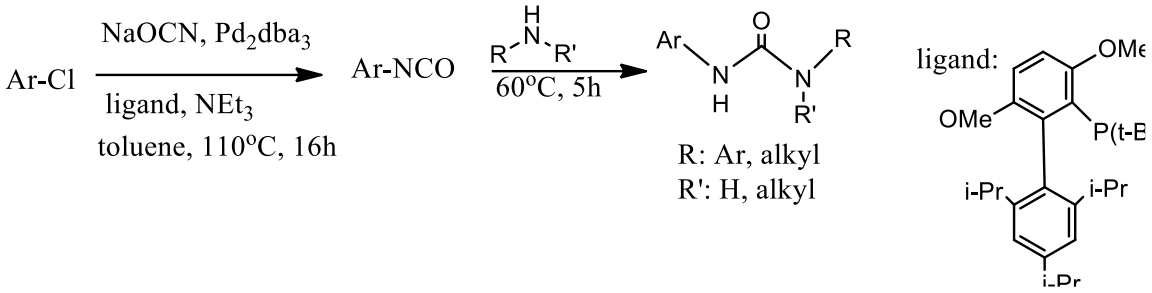
Boc-protected amines were transformed into nonsymmetrical and symmetrical disubstituted and trisubstituted ureas via in situ generations of isocyanates using 2-chloropyridine and trifluoromethanesulfonyl anhydride. A variety of amines were employed successfully, leading to high yields of isolated ureas (Spyropoulos *et al.,* 2014).



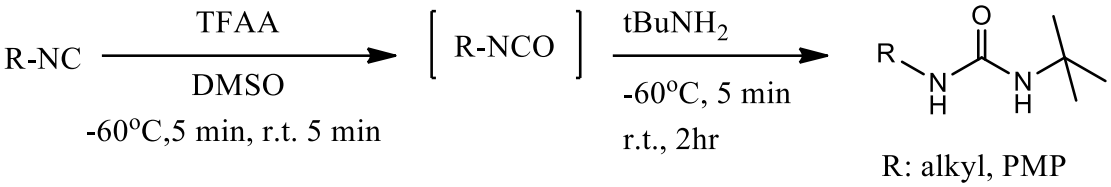
A one pot reaction of carbonylimidazolide with a nucleophile in water provided an efficient urea, carbamates and thiocarbamate preparation without an inert atmosphere (Padiya *et al.,* 2012).



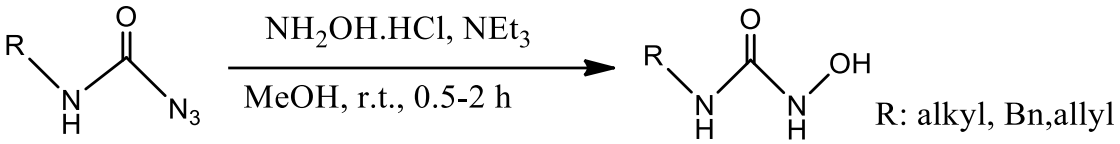
The unsymmetrical *N, N′*-di- and *N, N, N′*-trisubstituted ureas were synthesized by aryl chlorides and triflates with sodium cyanate in one pot. (Vinogradova *et al.,* 2012).



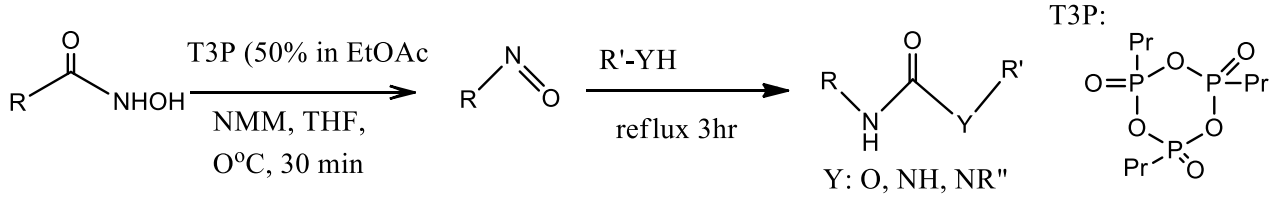
The oxidation of isonitriles produced isocyanates by DMSO with the catalyzing effect of trifluoroacetic anhydride. The process was complete in a few minutes, forming dimethyl sulfide (Le *et al.,* 2011).



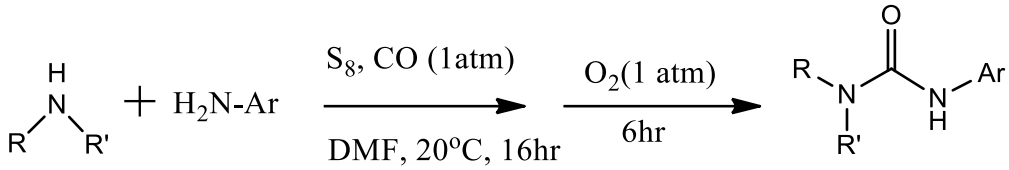
N-hydroxyureas were produced by the reaction of carbamoyl azides with hydroxylamine, whereas the reaction of carbamoyl cyanides with hydroxylamine gives carbamoyl amidoxime derivatives. (Paz *et al.,* 2010).



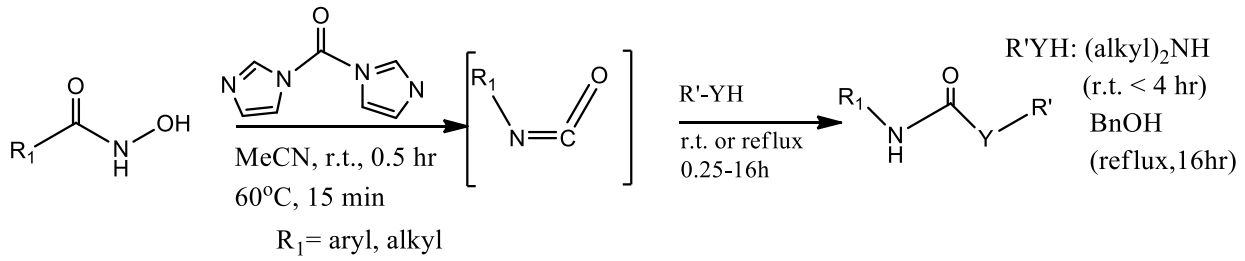
1-Propanephosphonic acid cyclic anhydride (T3P) promoted the synthesis of hydroxamic acids. Further, T3P was also used to synthesize isocyanates by activating the hydroxamates via Lossen rearrangement. Trapping with suitable nucleophiles afforded the corresponding ureas and carbamates (Vasantha *et al.,* 2010).



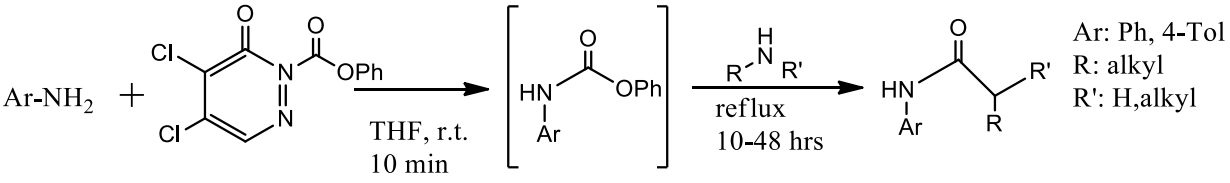
At room temperature, with ambient pressure of carbon monoxide and oxygen, *N, N*-dialkyl-*N′*-aryl ureas were synthesized from secondary amines, aromatic amines, and sulfur in very good yields (Mizuno *et al.,* 2009).



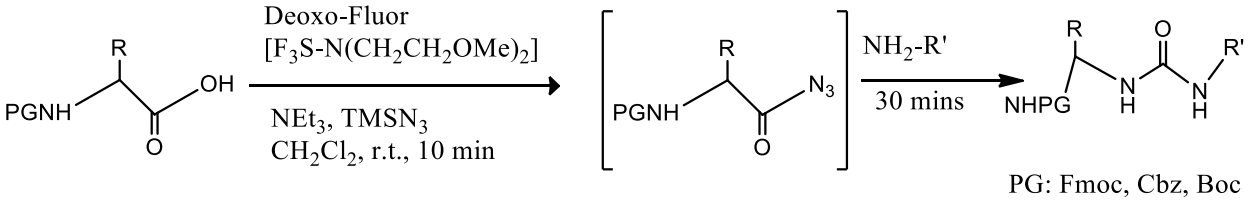
Carbonyldiimidazole leads the Lossen rearrangement of various hydroxamic acids to isocyanates. The method avoided the use of hazardous reagents and thus represented a green alternative for the Curtius and Hofmann rearrangements (Dubé *et al.,* 2009).



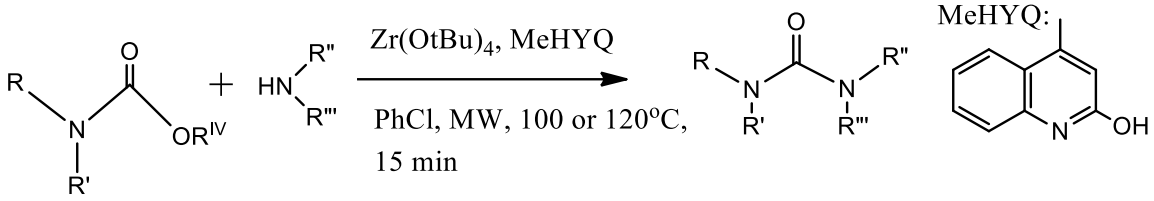
Selective and convenient synthesis of carbamates, symmetric ureas, and unsymmetrical ureas were synthesized by the reaction of amines and phenyl 4, 5-dichloro-6-oxopyridazine-1(6*H*)-carboxylate under mild conditions (Lee *et al.,* 2009).



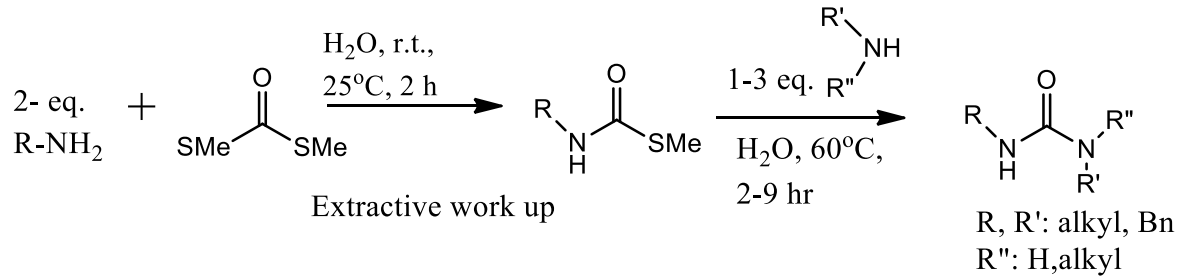
Deoxo-Fluor and $TMSN_3$ produced urea-linked peptidomimetics and neoglycopeptides under Curtius rearrangement conditions. It leads to the isolation of acyl azide and isocyanate intermediates. The reaction was carried out under ultrasonication (Hemantha *et al.,* 2009).



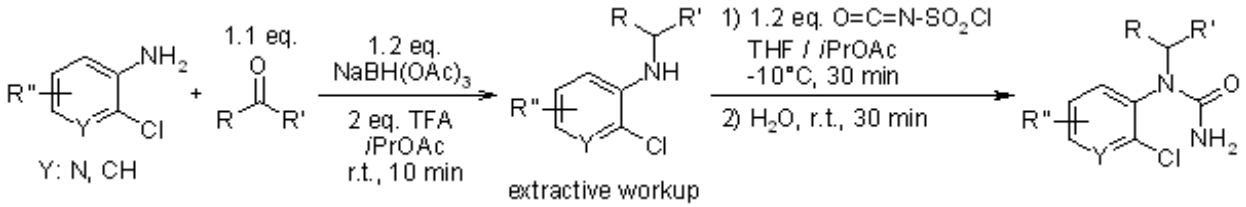
Dialkyl carbonates and carbamates, as well as 2-hydroxypyridine (HYP) and 4-methyl-2-hydroxyquinoline (MeHYQ), respectively produced carbamates and ureas in the presence of amines. A microwave acceleration effect was noticed in Zr(IV)-catalyzed carbamate-urea exchange (Han *et al.,* 2007).



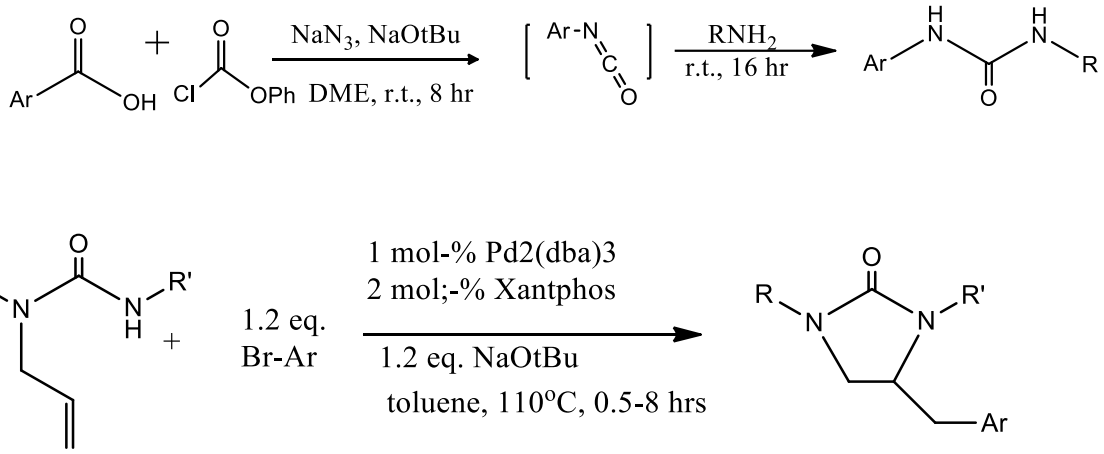
S, S-dimethyl dithiocarbonate (DMDTC) yielded *N*-alkylureas, *N, N′*-dialkyl ureas (both symmetrical and unsymmetrical), and *N, N, N′*-trialkyl ureas by carbonylation of aliphatic amines in water (Artuso *et al.,* 2007).



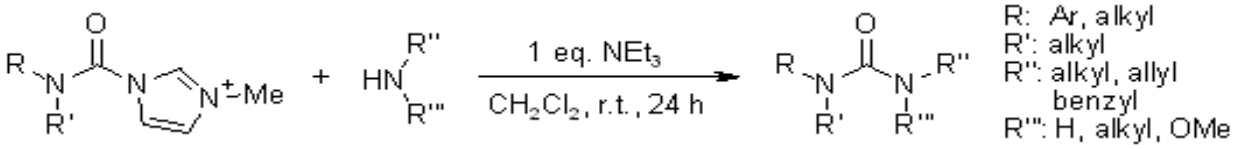
The reaction of di-*tert*-butyl decarbonate/chloroformate and sodium azide with an aromatic carboxylic acid produced the corresponding acyl azide. The acyl azide possesses a Curtius rearrangement to produce an isocyanate derivative which is either by an alkoxide or by an amine to produce the aromatic carbamate or urea (Lebel *et al.,* 2006).



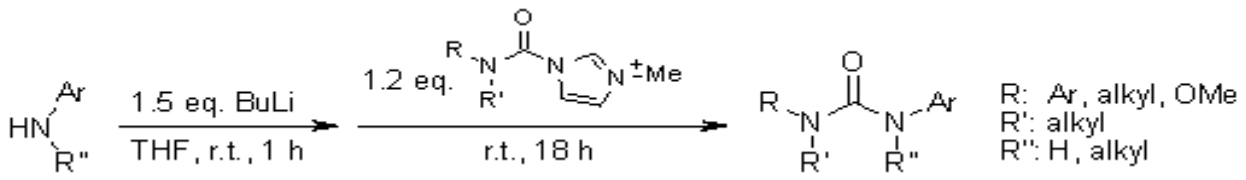
Alkylation of electron-deficient *o*-haloarylamines afforded primary ureas by the treatment with *N*-chlorosulfonyl isocyanate. A Pd-catalyzed urea cyclization reaction formed imidazopyridinones and benzoimidazolones in excellent yields (McLaughlin *et al.*, 2006).



Imidazolidin-2-ones were prepared in two steps from readily available *N*-allylamines. Addition of the amine starting materials to isocyanates afforded *N*-allylureas, which were converted to imidazolidin-2-ones with the generation of two bonds and up to two stereocenters in the presence of aryl bromides, a catalytic amount of $Pd_2(dba)_3$/Xantphos and NaO*t*Bu (Fritz *et al.*, 2006).

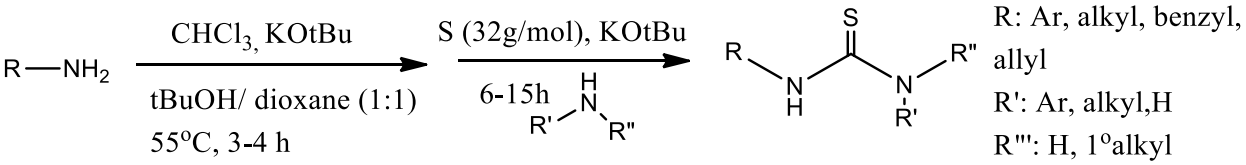


Readily prepared carbamoyl imidazolium salts acted as efficient *N, N*-disubstituted carbamoylating reagents, as a result of the 'imidazolium' effect. The salts reacted with amines, thiols, phenols/alcohols and carboxylic acids in high yields and produced ureas, thiocarbamates, carbamates, and amides, respectively (Bertrand *et al.,* 2005).

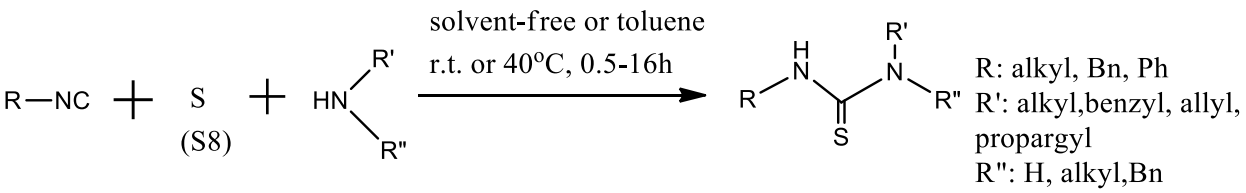


Methods of Preparation of Thiourea Derivatives

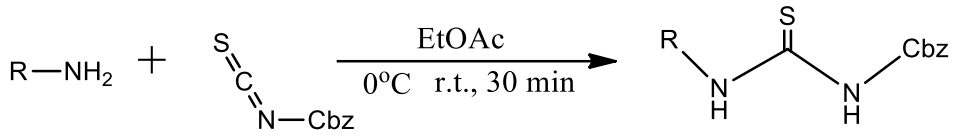
The combination of sulfur and chloroform enabled an efficient and practical synthesis of a thiocarbonyl surrogate. Various thiocarbamides and oxazolidinethiones were synthesized, including chiral thiourea catalysts and chiral oxazolidinethione auxiliaries with high selectivity (Tan *et al.,* 2017).



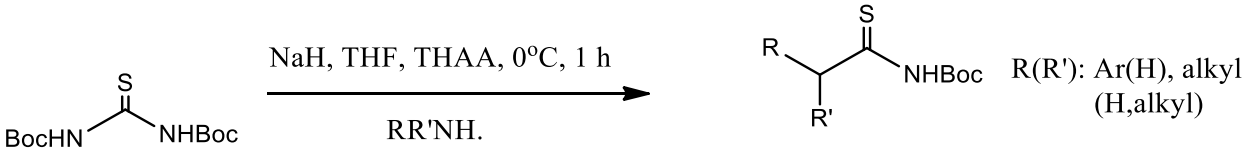
A completely atom-economic reaction of isocyanides with aliphatic amines in the presence of elemental sulfur proceeded efficiently at ambient temperature to produce thioureas in excellent yields (Nguyen *et al.,* 2014).



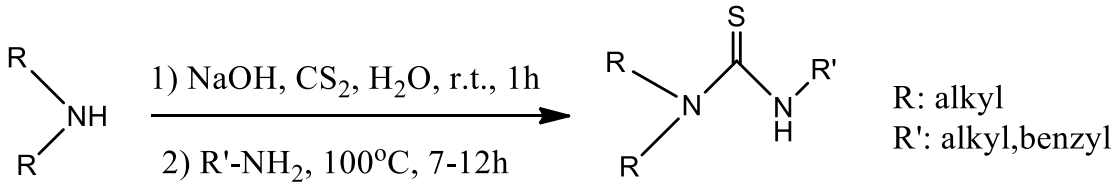
Various thioureas were derived from primary amines and carbamoyl-protected isothiocyanates. They react with the Burgess reagent to give the corresponding guanidines via either a stepwise or one-pot procedure (Maki *et al.,* 2014).



Stable and readily available *N, N'*-di-Boc-substituted thiourea was used as a mild thioacylating agent when activated with trifluoroacetic acid anhydride. Through thioacylation of nucleophiles, such as amines, alcohols, thiols, sodium benzenethiolate, and sodium malonates, a series of thiocarbonyl compounds were prepared with good chemical selectivity and functional group tolerance (Yin *et al.,* 2010).



A simple condensation between amines and carbon disulfide in aqueous medium allowed an efficient synthesis of symmetrical and unsymmetrical substituted thiourea derivatives. This protocol worked smoothly with aliphatic primary amines to afford various di- and trisubstituted thiourea derivatives (Maddani *et al.,* 2010).

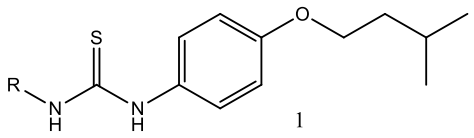


Pharmacological activity:

Urea and thiourea are important functional groups in numerous natural products and drug intermediates and building blocks for various heterocycles.

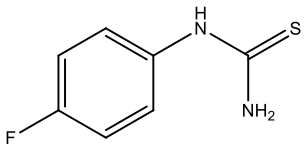
Urea and thiourea derivatives possess many promising biological activities, such as herbicidal, antimicrobial, antioxidant, antiviral, anti-HIV, antitumor, antimalarial and antidiabetic activity.

Thiocarlide (**1**) was a pharmacologically important thiourea drug used as a therapeutic agent in the treatment of tuberculosis (Liav *et al.,* 2008).



1

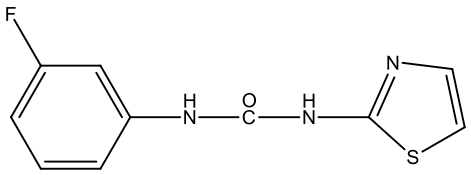
Fluorinated thioureas (**2**) constituted a novel class of potent influenza virus neuraminidase inhibitors (Saeed *et al.,* 2009).



2

Thioureas have versatile application in the field of agriculture. These are used to control the growth of insects, effect on plant growth and seed germination and also used as fungicide and herbicide.

N-substituted-N'-(2-thiazolyl and furfuryl) ureas and thioureas derivatives showed herbicidal activity and selectivity on seedlings of wheat and cucumber. N-(3-fluorophenyl)-N'-(2-thiazolyl)urea (**3)** possessed the greatest activity at 10 µM while the corresponding compound with 3-chlorophenyl (**4**) substitution showed active cytokinin like substance (Yonova *et al.,* 2004).



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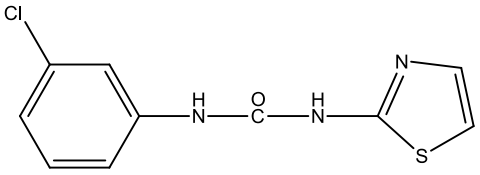
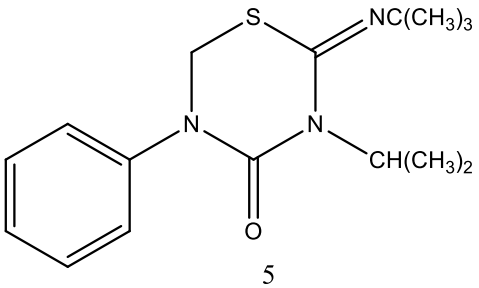


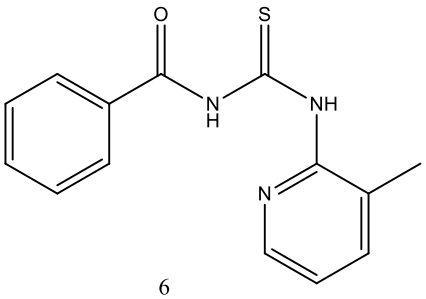
4

Thiourea derivative (**5**) controlled the growth of insects by destroying nymph at a concentration of less than 1 ppm. This is environment-friendly because they didn't destroy beneficial insects (Tunaz *et al.,* 2004).



5

Many organic compounds affect the germination and growth of the seed. The thiourea compound (**6**) elongated the roots of linseed (Brown e*t al.,* 1973). Most of the compounds belonging to the series of (7) showed plant growth regulating properties (Yonova *et al.,* 1997).



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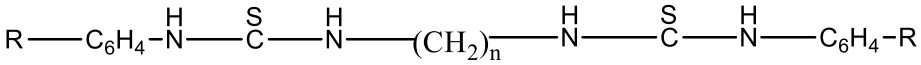
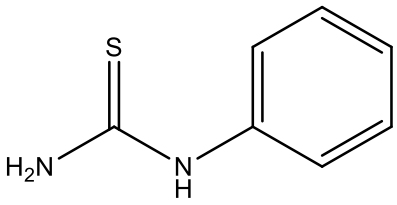


7

The chemicals or biological compounds that are used to kill fungus and fungal spores are called fungicide. Fungicides are very important in agriculture. Phenylthiourea (**8**), trichloro ethyl thiourea (**9**) and pyridyl thiourea (**10**) were active fungicides. Phenylthiourea (8) and its p-chloro and p- nitro derivatives were the most active ones. N-Butylmethylamine (**11**), N-ethyl isopropylamine (**12**) thiourea showed significant antifungal activity against the fungus yeast *Saccharomyces cerevisiae* and *Penicillium digitatum* (Rodriguez-Fernandez *et al.,* 2005).

Thiourea Derivatives of (**13**) showed the significant antifungal and antiviral activity of curative rates (Wu *et al.*, 2012).



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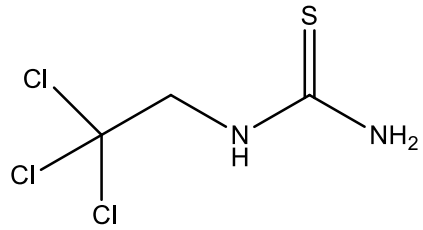

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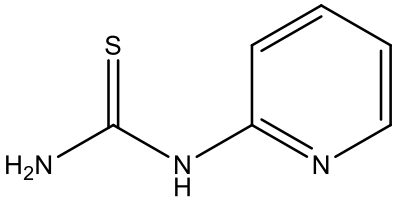

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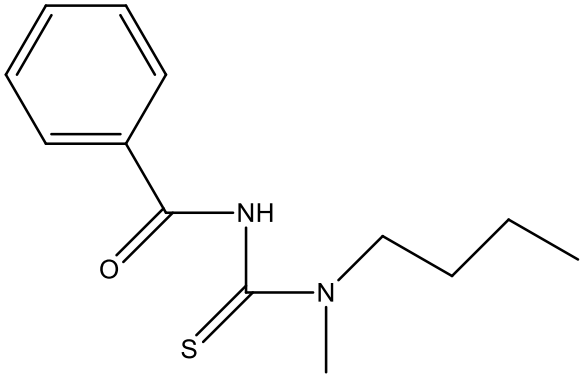

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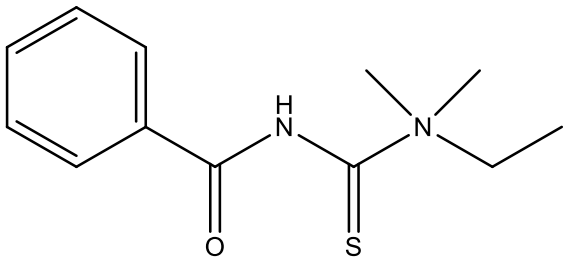

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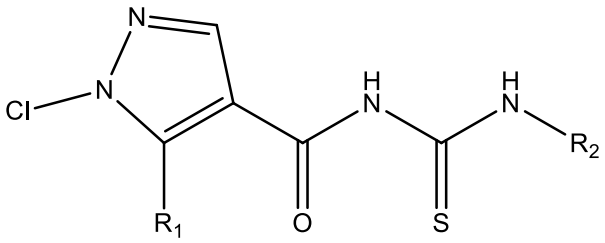
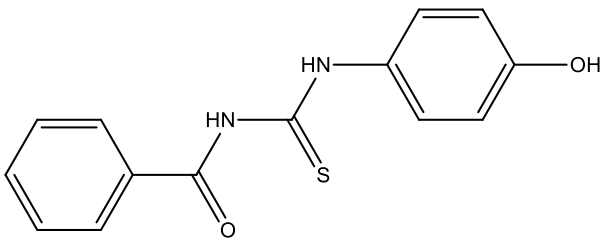


13

An antioxidant is an agent which prevent the oxidation of other substances. Antioxidants have the capability of functioning chemically as reducing agents. (1-benzoyl-3-(p-hydroxyphenyl) thiourea) (**14**), (1-benzoyl-3-(p-methoxyphenyl) thiourea (**15**), (1-morpholino-3-phenethylthiourea (**16**) and (1-phenethyl-3-(piperidine-1-yl) (17) thiourea were excellent antioxidant (Venkatesh *et al.*, 2009).



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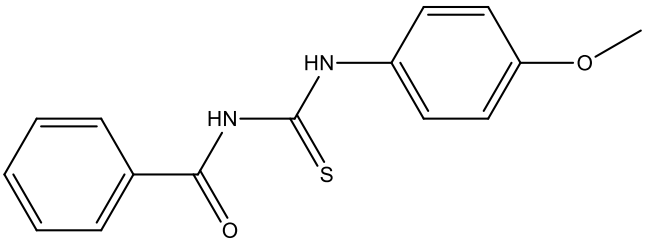

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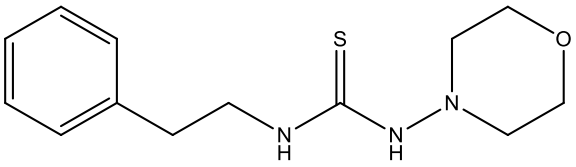

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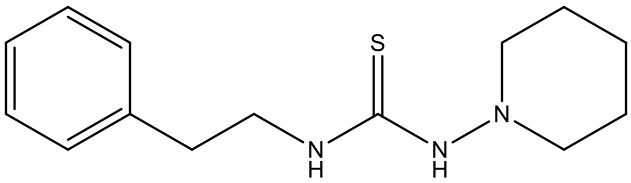
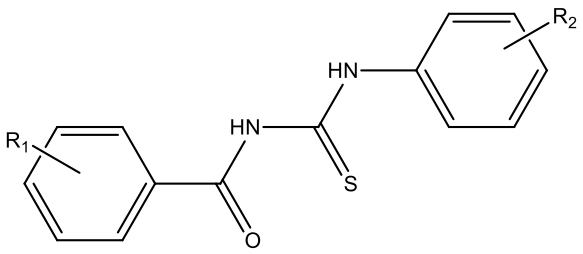


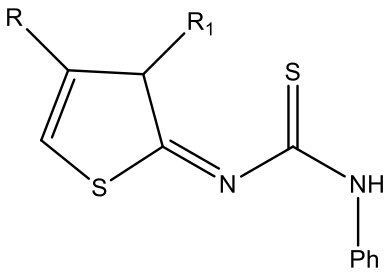
17

Thiourea derivatives were potent to act as antibacterial substances. 1-aroyl-3-aryl thioureas (**18**) had activity against *Staphylococcus aureus*, *Bacillus subtilis* and *E coli* (Saeed *et al.,* 2009)



18

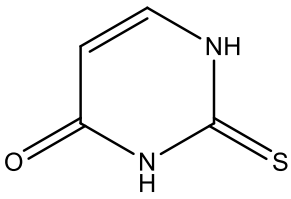
Iminothiazolines on reaction with phenyl isothiocyanate yielded thioureas (**19**) having anti-inflammatory activity (Sondhi *et al.,* 2000).



19

Antithyroid drugs were used for the treatment of goiter. Some of the synthetic thiourea derivatives were potent for treatment of hyperthyroidism which were 2-thiouracil (**20**), 6-n-propylthiouracil (**21**), 6-methylthiouracil (**22**), carbimazole (**23**), methimazole (**24**), 1, 3-

diethylthiourea (**25**), and 2-thiobarbituric acid (**26**) (Rosove 1977).



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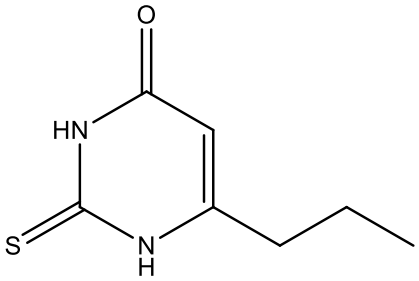

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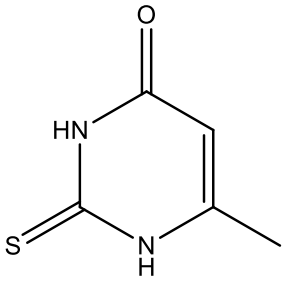

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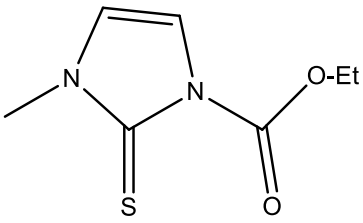

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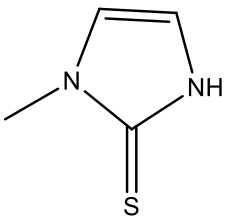

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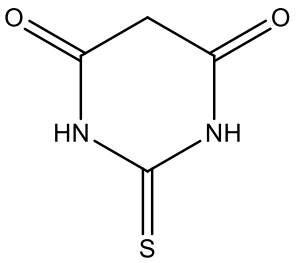

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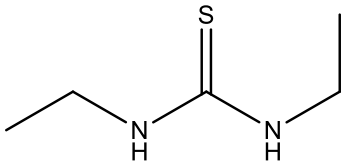
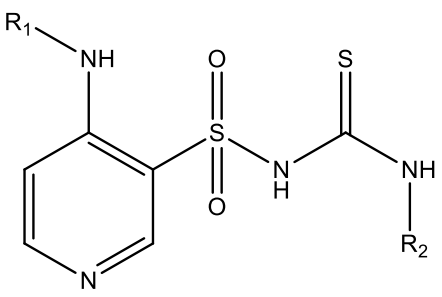


26

Thiourea (**27**) and urea (**28**) derivatives were active anti-convulsant (Masereel *et al.*, 1997).



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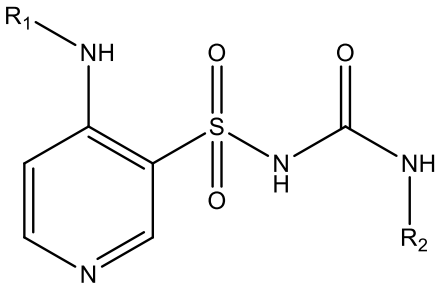
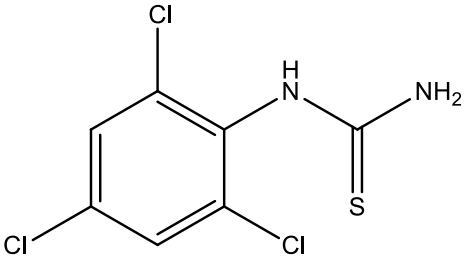


28

Mono and Disubstituted phenylthiourea acted as an anti-hypertensive compound. Thiourea derivatives (**29**), (**30**), (**31**), (**32**) and (**33**) showed anti-hypertensive activity. Among these compound, compound 32 was most active (Love *et al.*, 1972).



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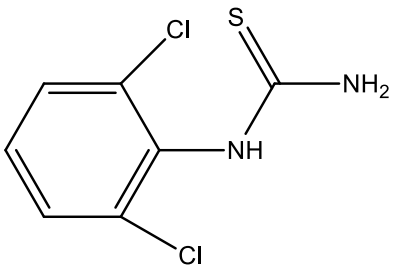

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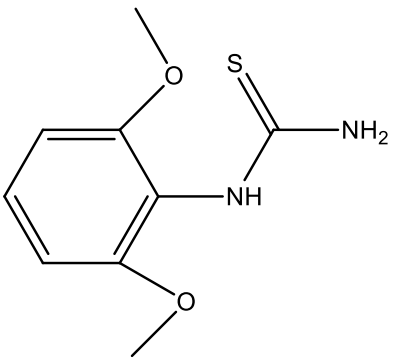

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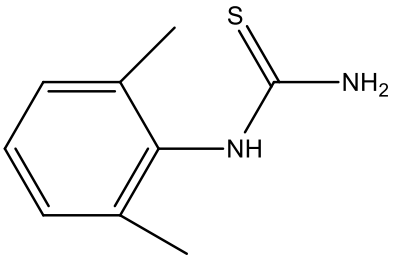

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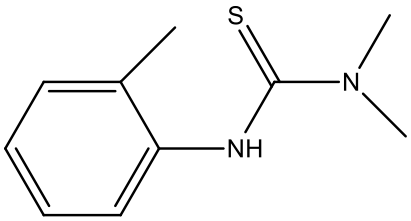
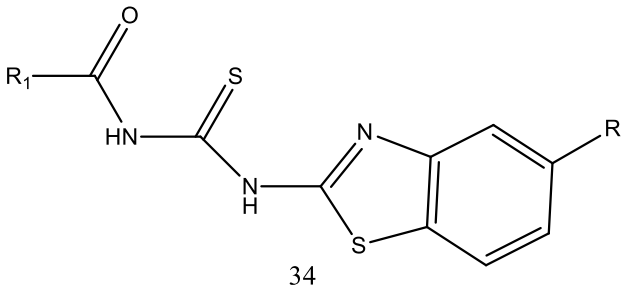


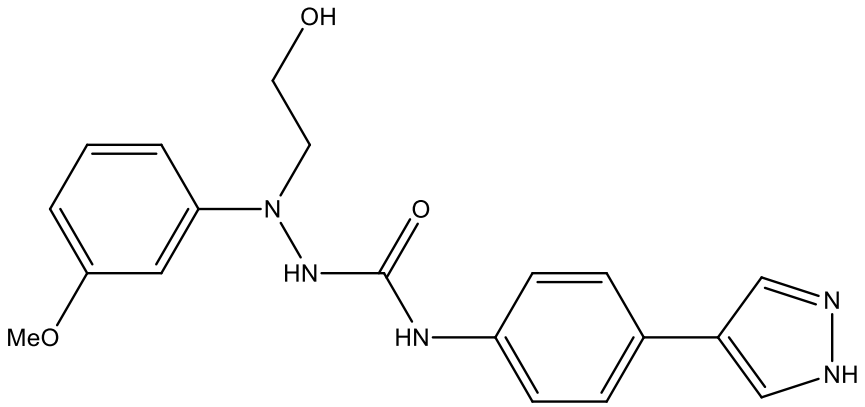
33

Novel thiourea derivatives having the general formula (**34**) were efficient in cytotoxicity activity. Their anti-cancer activity was screened by Hela cells and MCF-7 (Saeed *et al.*, 2010).



34

Urea Derivatives (35) and (36) was highly potent and selective Rho Kinase Inhibitors (Yin *et al.,* 2013).



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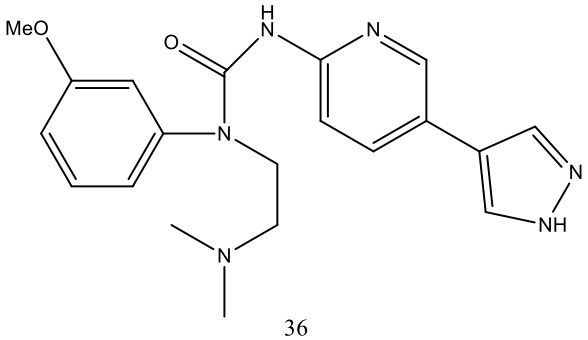
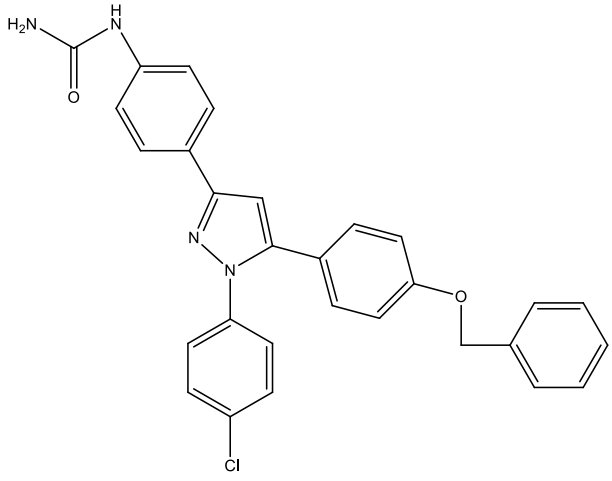


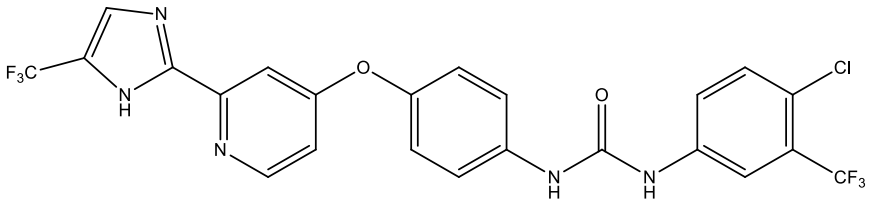
36

Pyrazolyl urea derivatives have antioxidant and anti-inflammatory activity. Among the synthesized compounds, Compound (**37**) was found to have superior antioxidant and anti-inflammatory activity (Somakala *et al.*, 2016).



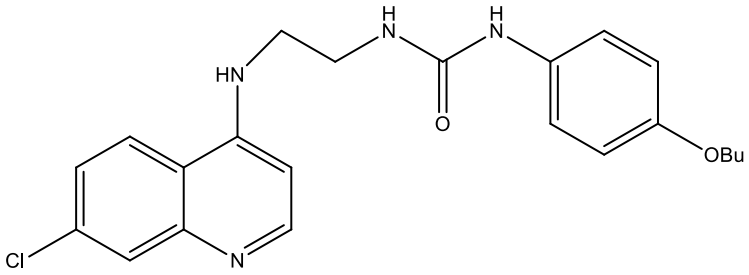
37

Bis-aryl urea derivatives exhibited moderate to excellent antiproliferative activities against three human cancer cell lines (MDA-MB-231, BGC-823 and SMMC-7721) and inhibitory activities against C-Raf kinase *in vitro*. The compound (**38**) showed maximum antiproliferative activity (Zhan *et al.,* 2012).



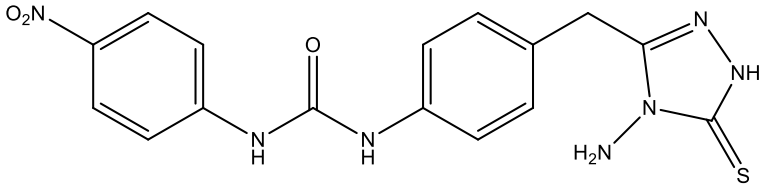
38

Quinoline hybrids from chloroquine have antiprotozoal, antimycobacterial activities. N-(4-Butoxyphenyl)-N-{2-[(7-chloroquinolin-4-yl)amino]ethyl}urea (**39**) was the most active compound against all parasites tested. Compound (**39**) was 670 times more active than metronidazole, against *G. intestinalis* (Nava-Zuazo *et al.,* 2010).



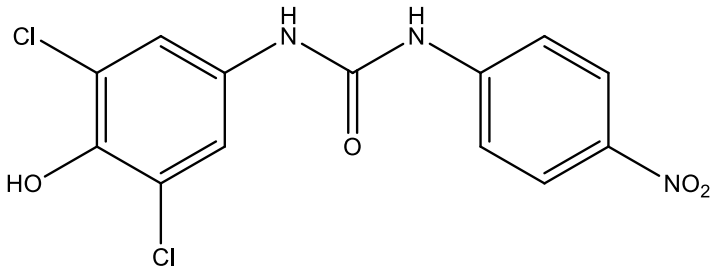
39

Urea derivatives containing 1, 2, 4-triazole moieties showed antifungal and larvicidal activity. Urea compound (**40**) showed sufficient potential to antifungal and insecticidal activity (Kaymakcioglu *et al.*, 2013).



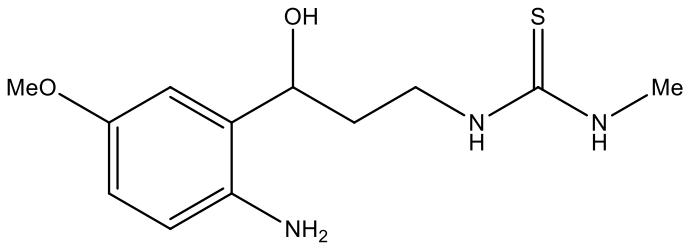
40

Urea and thiourea derivatives of 3, 5-dichloro-4-hydroxy aniline exhibited potent antioxidant and antimicrobial activities. Compound (**41**) showed good inhibitory activity.



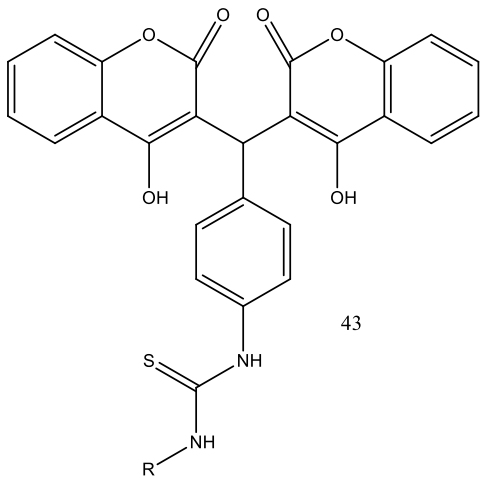
41

N, N′-disubstituted thiourea and urea derivatives have both neuronal nitric oxide synthase (nNOS) and inducible nitric oxide synthase (iNOS) inhibitors activity. N-[3-(2-Amino-5-methoxyphenyl)-3-hydroxypropyl]-N′-methylthiourea (**42**) showed significant nNOS (80.6%) and iNOS (76.6%) inhibition activity.



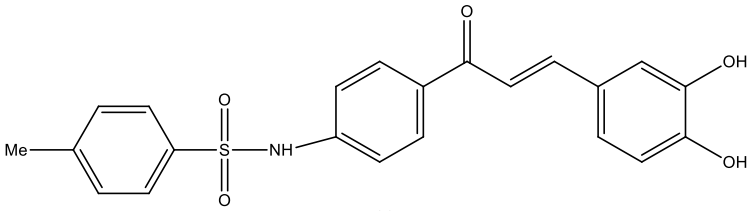
42

Abdullah Zawawi *et al.*, in 2015, studied the biscoumarin thiourea (**43**) as a new inhibitor of α glucosidases (Zawawi *et al.*, 2015).



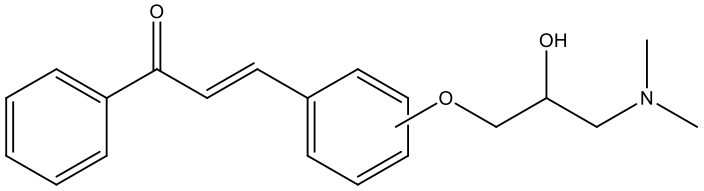
43

Seo W.D. *et al.,* had designed and synthesized a hybrid moiety of sulfonamide chalcones in 2005 and found that aminated chalcones (**44**) were a new class of highly specific α–glucosidase inhibitors showing promising results as compared to non-aminated chalcones (Seo *et al.,* 2005).



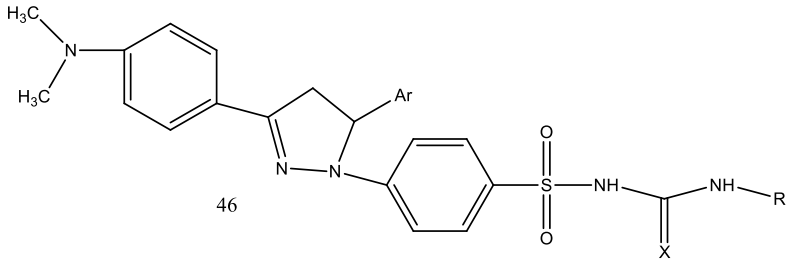
44

Shukla *et al.,* have demonstrated the synthesis and antihyperglycemic action of chalcone based aryloxypropanolamines (**45**) which claimed to elicit antioxidant action as well (Shukla *et al.,* 2007).



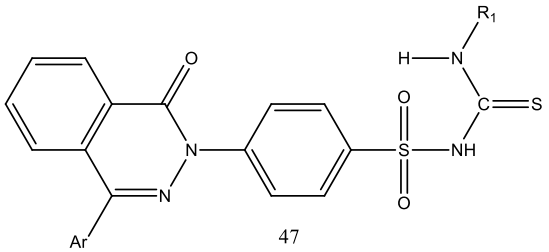
45

Ovais *et al.,* (2014) showed the anti-hyperglycaemic and aldose reductase inhibitory activity of some new pyrazoline substituted benzenesulfonylurea/thiourea (**46**) derivatives (Ovais et al., 2014).



46

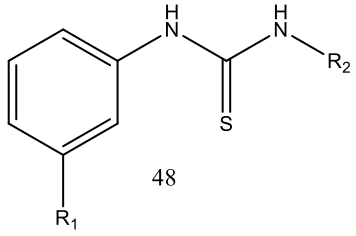
Yaseen, Shafiya *et al.,* synthesized some novel benzenesulfonylthiourea derivatives substituted with 4-aryl-1-oxophthalazin-2(1H)yl-ones (**47**) and showed their blood glucose lowering activity (Yaseen *et al.,* 2014).



47

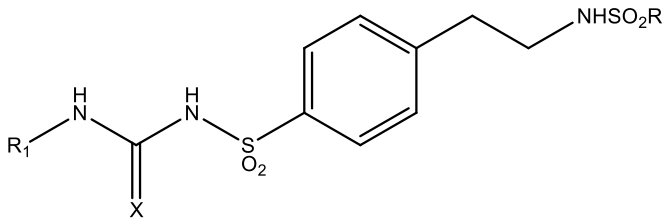
Goncharenko ON *et al.,* evaluated the efficiency and safety of early combination therapy with sulfonylurea derivatives (SUD) and insulin sensitizers in patients with type 2 diabetes mellitus (T2DM) (Goncharenko *et al.,* 2012).

In vitro urease inhibitory activity of N, N-disubstituted thioureas (**48**) derivatives were studied by Khan *et al.,* in 2014 (Khan *et al.,* 2014).



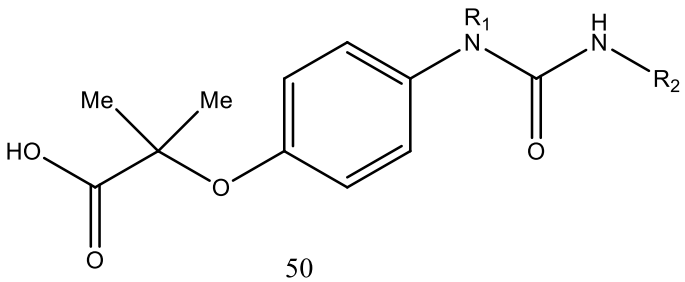
48

Alessandra Mascarello *et al.,* studied novel sulfonyl(thio)urea derivatives (**49**) which acted efficiently both as insulin secretagogues and as insulinomimetic compounds (Mascarello *et al.,* 2014).



49

The novel urea derivative (50) had a potent dual-target ligands of both Glucokinase and PPARγ. (Li *et al.,* 2014).



50

References

Artuso, E., Degani, I., Fochi, R., Magistris, C., 2007. Preparation of Mono-, Di-, and Trisubstituted Ureas by Carbonylation of Aliphatic Amines with S, S-Dimethyl Dithiocarbonate. Synthesis. 3497-3506.

Bertrand, MB., Wolfe, JP., 2005. Stereoselective synthesis of N-protected pyrrolidines via Pd-catalyzed reactions of γ-(N-acylamino) alkenes and γ-(N-Boc-amino) alkenes with aryl bromides. Tetrahedron. 61, 6447-6459.

Brown, B., Harris, R., 1973. Pesticide Science 4, 215-25.

Dubé, P., Nathel, NFF., Vetelino, M., Couturier, M., Abossafy, CL., Pichette, S., Jorgensen, ML., Hardink, M., 2009. Carbonyldiimidazole-Mediated Lossen Rearrangement. Org. Lett.11, 5622-5625.

Fritz, JA., Nakhla, JS., Wolfe, JP., 2006. A New Synthesis of Imidazolidin-2-ones via Pd-Catalyzed Carboamination of N-Allylureas. Org. Lett. 8(12), 2531-2534.

Goncharenko, ON., Ametov, AS., Isakova, MR., 2012. Evaluation of the efficiency and safety of therapy with a combination of sulfonylurea derivatives and insulin sensitizers for type 2 diabetes mellitus. TerArkh. 84(8), 67-71.

Han, C., Porco, J. A., 2007. Synthesis of Carbamates and Ureas Using Zr (IV)-Catalyzed Exchange Processes. Org. Lett., 9, 1517-1520.

Hemantha, HP., Chennakrishnareddy, G., Vishwanatha, T. MV., Sureshbabu, V., 2009. One-Pot Synthesis of Ureido Peptides and Urea-Tethered Glycosylated Amino Acids Employing Deoxo-Fluor and TMSN3. Synlett. 407-410.

Hernandez, AG., Grooms, GM., El-Alfy, AT., J. Stec., 2017. Convenient One-Pot Two-Step Synthesis of Symmetrical and Unsymmetrical Diacyl Ureas, Acyl Urea/Carbamate/Thiocarbamate Derivatives, and Related Compounds. Synthesis. 49, 2163-2176.

Khan, MK., Naz, F., Taha, M., Khan, A., Perveen, S., Choudhary, M.I., Voelter, W., 2014. Synthesis and in vitro urease inhibitory activity of N, N -disubstituted thioureas. European Journal of Medicinal Chemistry. 74, 314-323.

Kim, SH., Hong, SH., 2016. Ruthenium-Catalyzed Urea Synthesis Using Methanol as the C1 Source. Org. Lett. 18, 212-215.

Kaymakcioglu B K., Ahmet, OC., Nurhayat, T., Ali, A., Shabana, IK., Ikhlas AK., David, EW., 2013. Synthesis and Biological Activity of Substituted Urea and Thiourea Derivatives Containing 1, 2, 4-Triazole Moieties. Molecules. 18, 3562-3576.

Liav, A., Angala, S.K., Brennan, P. J., Jackson, M., 2008. N-D-aldopentofuranosyl-N'-[p-(isoamyloxy) phenyl]-thiourea derivatives: potential anti-TB therapeutic agents. Bioorg Med ChemLett. 8, 2649-51.

Lebel, H., Leogane, O., 2006. Curtius Rearrangement of Aromatic Carboxylic Acids to Access Protected Anilines and Aromatic Ureas. Org. Lett. 25, 8, 5717-5720.

Le, HV., Ganem, B., 2011. Trifluoroacetic Anhydride-Catalyzed Oxidation of Isonitriles by DMSO: A Rapid, Convenient Synthesis of Isocyanates. Org. Lett. 13(10), 2584-2585.

Lee, H.G. Kim. M.J., Park, S.E., Kim, J.J., Lee, S.G., Yoon, Y.J., 2009. Phenyl 4, 5-Dichloro-6-Oxopyridazine-1(6H)-Carboxylate as Carbonyl Source: Facile and Selective Synthesis of Carbamates and Ureas under Mild Conditions. Synlett. 2809-2814.

Li, Y., Tian, K., Qin, A., Zhang, L., Huo, L., Lei, L., Shen, Z., Song, H., Feng, Z., 2014. Discovery of novel urea derivatives as dual-target hypoglycemic agents that activate glucokinase and PPARγ. European Journal of Medicinal Chemistry, 76, 182-192.

Loev, B., Bender, PE., Bowman, H., Helt, A., McLean, R., Jen, T., 1972. Thioureas Possessing Antihypertensive Activity. Journal of medicinal chemistry. 15, 1024-27.

Maddani, MR., Prabhu, KR., 2010. A Concise Synthesis of Substituted Thiourea Derivatives in Aqueous Medium. J. Org. Chem. 75, 2327-2332.

Mascarello, A., Frederico, MJS., Gomes Castro, AJ., Mendes, CP., Dutra, MF., Woehl VM., Yunes, RA., Barreto Silva, FRM., Nunes, RJ., 2014. Novel sulfonyl (thio)urea derivatives act efficiently both as insulin secretagogues and as insulinomimetic compounds. European Journal of Medicinal Chemistry 86, 491-501.

Masereel, B., Lambert, D., Dogné, J., Poupaert, J., Delarge, J., 1997. Anticonvulsant activity of pyrid-3-yl-sulfonyl ureas and thioureas. Epilepsia. 38, 334-37.

Maki, T., Tsuritani, T., Yasukata, T., 2014. A Mild Method for the Synthesis of Carbamate-Protected Guanidines Using the Burgess Reagent. Org. Lett. 16, 1868-1871.

McLaughlin, M., Palucki, M., Davies, I. W., 2006. Efficient Access to Cyclic Ureas via Pd-Catalyzed Cyclization. Org. Lett. 8, 3311-3314.

Mizuno, T., Nakai, T., Mihara, M., 2009. Synthesis of Unsymmetrical Ureas by Sulfur-Assisted Carbonylation with Carbon Monoxide and Oxidation with Molecular Oxygen under Mild Conditions. Synthesis. 2492-2496.

Nava-Zuazo, C., Estrada-Soto, S., Guerrero-Álvarez, J., León-Rivera, I., Molina-Salinas, GM., Said-Fernández, S., Chan-Bacab, JM., Cedillo-Rivera, R., Moo-Puc, R., Mirón-López f, G., Navarrete-Vazquez, G., 2010. Design, synthesis, and in vitro antiprotozoal, antimycobacterial activities of N-{2-[(7-chloroquinolin-4-yl)amino]ethyl}ureas. Bioorganic& Medicinal Chemistry. 18, 6398–6403.

Nguyen, TB., Ermolenko, L., Al-Mourabit, A., 2014. Three-Component Reaction between Isocyanides, Aliphatic Amines and Elemental Sulfur: Preparation of Thioureas under Mild Conditions with Complete Atom Economy. Synthesis. 46, 3172-3179.

Padiya, KJ., Gavade, S., Kardile, B., Tiwari, M., Bajare, S., Mane, M., Gaware, V., Varghese, S., Harel, D., Kurhade, S., 2012. Unprecedented "In Water" Imidazole Carbonylation: Paradigm Shift for Preparation of Urea and Carbamate. Org. Lett. 14, 2814-2817.

Paz, J., Pérez-Balado, C., Iglesias, B., Muñoz, L., 2010. Different Reactivity of Hydroxylamine with Carbamoyl Azides and Carbamoyl Cyanides: Synthesis of Hydroxyureas and Carbamoyl Amidoximes. J. Org. Chem. 75, 8039-8047.

Rosove, MH., 1977. Agranulocytosis and antithyroid drugs. Western Journal of Medicine. 126, 339-343.

Rodriguez-Fernandez, E., Manzano, JL., Benito, JJ., Hermosa, R., Monte, E., Criado, JJ., 2005. Thiourea, triazole and thiadiazine compounds and their metal complexes as antifungal agents. Journal of inorganic biochemistry. 99, 1558-72.

Saeed, A., Shaheen, U., Hameed, A., Naqvi, SZH., 2009. Synthesis, characterization and antimicrobial activity of some new 1-(fluorobenzoyl)-3-(fluorophenyl) thioureas. J. Fluor. Chem. 130, 1028–1034.

Saeed, A., Abbas, N., Rafique, H., Rashid, S., Hameed, A., 2009. Chemistry. 18, 152-8.

Saeed, S., Rashid, N., Jones, PG., Ali, M., Hussain, R., 2010. Synthesis, characterization and biological evaluation of some thiourea derivatives bearing benzothiazole moiety as potential antimicrobial and anticancer agents. European journal of medicinal chemistry. 45, 1323-31.

Seo, WD., Kim, JH., Kang, JE., Ryu, HW., Curtis-Long, MJ., Lee, HS., Yanga, MS., Parka, KH., 2005. Sulfonamide chalcone as a new class of alpha-glucosidase inhibitors. Bioorg. Med. Chem. Lett. 15(24), 5514-5516.

Somakala, K., Amir, M., 2017. Synthesis, characterization and pharmacological evaluation of pyrazolyl urea derivatives as potential anti-inflammatory agents. Acta. Pharmaceutica. SinicaB. 7(2), 230–240.

Sondhi, S., Sharma, VK., Singhal, N., Verma, R., Shukla, R., Raghubir, R., Dubey, M., 2000. Synthesis and anti-inflammatory activity evaluation of some acridinyl amino antipyrine, acridinyl amino anthraquinone, acridino thiourea and thiazolino thiourea derivatives. Phosphorus, Sulfur, and Silicon and the Related Elements. 156, 21-33.

Spyropoulos, C., Kokotos, CG., 2014. One-Pot Synthesis of Ureas from Boc-Protected Amines. J. Org. Chem. 79, 4477-4483.

Shukla, P., Singh, AB., Srivastava, AK., Pratap R., 2007. Chalcone based aryloxypropanolamines as potential antihyperglycemic agents. Bioorg. Med. Chem. Lett. 17(3), 799-802.

Ovais, S., Pushpalatha, H., Reddy, BG., Rathore, P., Bashir, R., Yaseen, S., Dheyaa, A., Yaseen, R., Tanwar, O., Akthar, M., Samim, M., Javed, K., 2014. Synthesis and biological evaluation of some new pyrazoline substituted benzenesulfonylurea/thiourea derivatives as anti-hyperglycaemic agents and aldose reductase inhibitors. Euro J Med Chem. 80, 209-217.

Tan, W., Wei, J., Jiang, X., 2017. Thiocarbonyl Surrogate via Combination of Sulfur and Chloroform for Thiocarbamide and Oxazolidinethione Construction. Org. Lett. 19, 2166-2169.

Thalluri, K., Manne, SR., Dev, D., Mandal, B., 2014. Ethyl 2-Cyano-2-(4-nitrophenylsulfonyloxyimino) acetate-Mediated Lossen Rearrangement: Single-Pot Racemization-Free Synthesis of Hydroxamic Acids and Ureas from Carboxylic Acids J. Org. Chem. 79, 3765-3775.

Tunaz, H., Uygun, N., 2004. Insect growth regulators for insect pest control. Turkish Journal of Agriculture and Forestry 28, 377-87.

Vinogradova, EV., Fors, BP., Buchwald, SL., 2012. Palladium-Catalyzed Cross-Coupling of Aryl Chlorides and Triflates with Sodium Cyanate: A Practical Synthesis of Unsymmetrical Ureas. J. Am. Chem. Soc.134, 11132-11135.

Vasantha, B., Hemantha, HP., Sureshbabu, VV., 2010. 1-Propanephosphonic Acid Cyclic Anhydride (T3P) as an Efficient Promoter for the Lossen Rearrangement: Application to the Synthesis of Urea and Carbamate Derivatives. Synthesis. 2990-2996.

Venkatesh, P., Pandeya, S., 2009. Synthesis, characterisation and anti-inflammatory activity of some 2-amino benzothiazole derivatives. International Journal of ChemTech Research 1, 733-41.

Wu, J., Shi, Q., Chen, Z., He, M., Jin, L., Hu, D., 2012. Synthesis and bioactivity of pyrazole acyl thiourea derivatives. Molecules. 17, 5139-50.

Yaseen, S., Bashir, R., Ovais, S., Rathore, P., Samim, M & Javed, K., 2014. Synthesis and blood glucose lowering activity of some novel benzenesulfonylthiourea derivatives substituted with 4-aryl-1-oxophthalazin-2(1H)yl-ones. Journal of Enzyme Inhibition and Medicinal Chemistry. 29(3), 362-366.

Yonova, P.A., Stoilkova, G.M., 2004. Synthesis and biological activity of urea and thiourea derivatives from 2-amino heterocyclic compounds. J Plant Growth Regul. 23, 280-291.

Yonova, P., Guleva, E., 1997. Bulgarian Journal of Plant Physiology 23, 72-9.

Yin, B.L., Liu, Z.G., Zhang, J.C.,. Li, Z.R., 2010. N,N'-Di-Boc-Substituted Thiourea as a Novel and Mild Thioacylating Agent Applicable for the Synthesis of Thiocarbonyl Compounds Synthesis. 991-999.

Yin, Y., Lin, L., Ruiz, C., Khan, S., Cameron, DM., Grant, W., Pocas, J., Eid, N., Park, JH., Schröter, T., LoGrasso, VP and Feng, Y., 2013. Synthesis and Biological Evaluation of Urea Derivatives as Highly Potent and Selective Rho Kinase Inhibitors. J. Med. Chem. 56(9), 3568-3581.

Zawawi, AKN., Taha, M., Ahmat, N., Ismail, HN., Wadood, A., Rahim, F., Rehman, UA., 2015. Synthesis, in vitro evaluation and molecular docking studies of biscoumarin thiourea as a new inhibitor of a-glucosidases. Bioorganic Chemistry. 63, 36–44.

Zhan, W., Li, Y., Huang, W., Zhao, Y., Yao, Z., Yu, S., Yuan, S., Jiang, F., Yao, S., Li, S., 2012. Design, synthesis and antitumor activities of novel bis-aryl ureas derivatives as Raf kinase inhibitors. Bioorganic & Medicinal Chemistry. 20, 4323–4329.

Zhao, J., Li, Z., Yan, S., Xu, S., Wang, M.-A., Fu, B., Zhang, Z., 2016. Pd/C Catalyzed Carbonylation of Azides in the Presence of Amines. Org. Lett. 18(8), 1736-1739.

Chapter 3

Aim & objective

Diabetes exists as one of the common cause of mortality in the world. Prevention of diabetes by synthetic molecules is well known. A lot of synthetic drugs (Sulfonyl Urea, Biguanides) are available in the market. Though many synthetic drugs are used in the different mechanism for the treatment of diabetes, still those are very costly and beyond the financial capacity of the common people. Our effort is to design & synthesis of newer drugs with the least cost & less toxic for prolonged treatment, as diabetes treatment is lifelong so far.

In this context, urea and thiourea derivatives are important functional groups in numerous natural products and drug intermediates and building blocks for various heterocycles. Urea and thiourea derivatives possess many promising pharmacological activities, such as herbicidal, antimicrobial, antioxidant, antiviral, anti-HIV, antitumor, antimalarial and antidiabetic activity.

Based on the literature, it was found that urea and thiourea derivatives possess different biological activities. So, our motto is as follows:

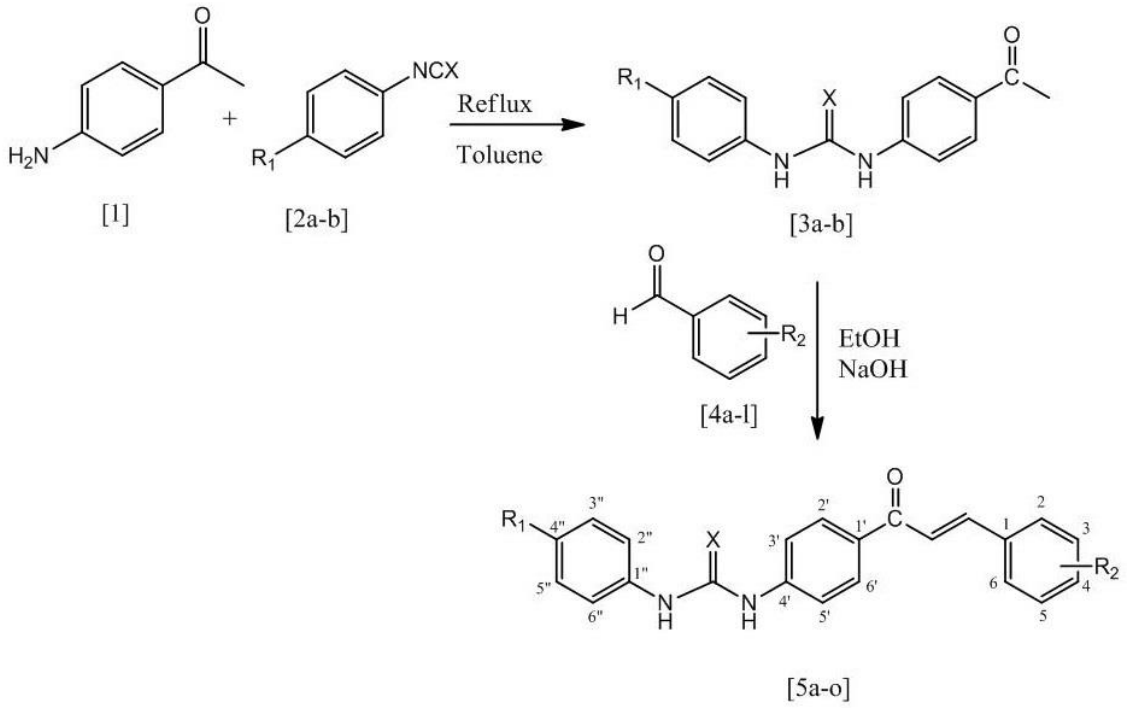
- Synthesis of hybrid molecules comprising of chalcone moiety with urea/thiourea derivatives using standard Claisen Schmidt condensation reaction procedure.
- Characterization of the synthesized compounds based on ^{1}H NMR, ^{13}C NMR, FT-IR and Mass Spectrometry.
- Docking study
- Evaluation of the synthesized compounds on standard diabetic models
    - *In vitro* assay of α- glucosidase inhibition
    - *In vivo* assay of synthesized compounds: Streptozotocin (STZ) model
- Evaluation of the *in-vitro* antioxidant activity of synthesized compounds
    - DPPH radical scavenging activity
    - Hydrogen peroxide scavenging assay
    - Nitric oxide scavenging activity

Chapter 4

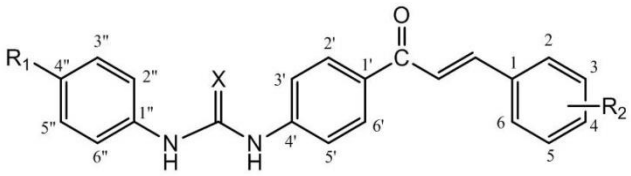
Synthesis, docking study and antidiabetic activity of 4'-(phenylureny/lthiourenyl) chalcone

Synthesis, docking study and antidiabetic activity of 4'-(phenylurenyl/thiourenyl) chalcone

General methods and experimental work



Scheme 1- General synthetic pathway for the preparation of 4'-(phenylurenyl/thiourenyl) chalcone **(5a-5o)**



Compounds	X	R_1	R_2
5a	O	Cl	4-Cl
5b	O	Cl	4-N, N- $(CH_3)_2$
5c	O	Cl	H
5d	O	Cl	2-Cl
5e	O	Cl	3-Br
5f	O	Cl	4-Br
5g	O	Cl	3-Cl
5h	O	Cl	2,6-di-Cl
5i	O	Cl	2-OCH_3
5j	O	Cl	3-OCH_3
5k	O	Cl	2,4-di-Cl
5l	O	Cl	4-OCH_3
5m	S	H	H
5n	S	H	3-OCH_3
5o	S	H	2-Cl

General procedures for the synthesis of 4-(phenylurenyl/thiourenyl) acetophenone

A mixture of the p-aminoacetophenone **(1)** and phenyl isocyanate/thiocyanate **(2a-b)** derivatives was dissolved in toluene. The mixture was refluxed; yellow solid was filtered out and dried. Recrystallization afforded the desired 4-(phenylurenyl/thiourenyl) acetophenone **(3a-b)** derivatives (Sonmez *et al.*, 2011).

General Procedure for the synthesis of 4'-(phenylurenyl/thiourenyl) chalcone Derivatives:

4'-(phenylurenyl/thiourenyl)chalcone **(5a-5o)** derivatives were synthesized by reacting equimolecular quantities of 4-(phenylurenyl/thiourenyl) acetophenone **(3a-b)** derivatives and the corresponding benzaldehyde **(4a-l)** in the presence of excess sodium hydroxide in methanol. At room temperature, the mixture was stirred; the resulting precipitate was filtered and dried in air. The precipitate was recrystallized from ethanol (Domínguez *et al.*, 2005; Dos Santos *et al.*, 2008; Sonmez *et al.*, 2011).

4-N-(N′-p-chlorophenylurenyl) acetophenone (3a)

Yield: 96%; mp: 220-223°C; IR ν_{max} 3374 (NH), 1714 (COMe), 1648 (CO) cm^{-1}; ^{1}HNMR (300MHz, DMSO-$d_{6,}$ ppm): δ 2.49 (s, 3H, COMe), 7.33 (d, 2H, J=8.7Hz), 7.48 (d, 2H, J=8.7Hz), 7.56 (d, 2H, J=8.7Hz), 7.89 (d, 2H, J=8.7Hz), 8.93 (br s, 1H, NH), 9.12 (br s, 1H, NH); ^{13}C NMR (400 MHz, DMSO-$d_{6,}$ ppm): δ 26.30, 117.24, 119.97, 125.76, 128.65, 129.61, 130.56, 138.29, 144.15, 152.07, 196.25; HRMS-ESI^{+}: $C_{15}H_{13}N_2O_2Cl$ calcd $[M+H]^{+}$: 289.738, found 289.08.

4-N-(N′-phenylthiourenyl) acetophenone (3b)

Yield: 74%;mp: 157-160°C; IR ν_{max} 3287 (NH), 1710 (COMe), 1642 (CO) cm^{-1}; ^{1}HNMR (400MHz, DMSO-$d_{6,}$ ppm): δ 2.51 (s, 3H, COMe), 7.16 (t, 1H, J=8 Hz), 7.36 (t, 2H), 7.49 (d, 2H, J=8 Hz), 7.70 (d, 2H, J=8 Hz), 7.93 (d, 2H, J=8 Hz), 10.12 (br s, 1H, NH), 10.06 (br s, 1H, NH); ^{13}C NMR (400 MHz, DMSO-$d_{6,}$ ppm): δ 26.98, 122.17, 124.14, 125.21, 129.01, 129.38, 132.60, 139.67, 144.64, 179.84, 197.07; HRMS-ESI^{+}: $C_{15}H_{14}N_2OS$ calcd $[M]^{+}$: 270.35, found 270.9.

1-[4′-N-(N′-p-chlorophenylurenyl)phenyl]-3-(4-chlorophenyl)-2-propen-1-one (5a)

Yield: 98%; mp: 258-261ºC; IR ν_{max} 3283 (NH), 1657 (CO-Urea), 1639 (CO, α, β unsaturated) cm-1; ^{1}HNMR (300MHz, DMSO-$d_{6,}$ ppm): δ 7.33 (d, 2H, J=8.7Hz), 7.49-7.52 (m, 4H), 7.63 (d, 2H, J=8.7Hz), 7.70 (d, 1H, Hα, J=15.6Hz), 7.92 (d, 2H, J=8.7Hz), 7.97 (d, 1H, J=16.5Hz), 8.13

(d, 2H, J=8.7Hz), 8.99 (br s, 1H, NH), 9.22 (br s, 1H, NH); ^{13}C NMR (400 MHz DMSO-d_6, ppm): δ 117.87, 120.49, 123.25, 126.27, 129.15, 129.40, 130.63, 130.95, 131.48, 134.30, 135.35, 138.76, 142.09, 144.88, 152.56, 187.66; HRMS-ESI$^+$: $C_{22}H_{16}N_2O_2Cl_2$ calcd $[M+H]^+$: 412.28, found 412.10.

1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(4-N, N-dimethylphenyl)-2-propen-1-one (5b)

Yield: 77%; mp: 228-230°C; IR ν_{max} 3334 (NH), 1716 (CO-Urea), 1637 (CO, α, β-Unsaturated) cm-1; ^{1}HNMR (300MHz, DMSO-d_6, ppm): δ 2.99 (s, 6H, $(CH_3)_2$), 6.73 (d, 2H, J=8.7Hz), 7.33 (d, 2H, J=8.7Hz), 7.50 (d, 2H, J=8.7Hz), 7.58-7.69 (m, 6H), 8.08(d, 2H, J=8.4Hz), 8.94 (br s, 1H, NH), 9.12 (br s, 1H, NH); ^{13}C NMR(400MHz, DMSO-d_6, ppm): δ 40.13, 111.71, 116.06, 117.31, 119.92, 122.14, 125.68, 128.61, 129.61, 130.51, 131.85, 138.31, 143.67, 144.16, 151.81, 152.07, 186.91; HRMS-ESI$^+$: $C_{24}H_{22}N_3O_2Cl$ calcd $[M+H]^+$: 420.92, found 420.17.

1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(phenyl)-2-propen-1-one (5c)

Yield: 96%; mp: 207-209°C; IR ν_{max} 3293 (NH), 1713 (CO-Urea), 1641 (CO, α, β-Unsaturated) cm-1; 1HNMR (300MHz, DMSO-d_6, ppm): δ 7.34 (d, 2H, J=8.7Hz), 7.44-7.51 (m, 5H), 7.64 (d, 2H, J=8.7Hz), 7.72 (d, 1H, J=15.6Hz), 7.86-7.90 (m, 2H), 7.96 (d, 1H, J=15.6Hz), 8.13 (d, 2H, 8.7Hz), 8.98 (brs, 1H, NH), 9.20 (br s, 1H, NH); 13C NMR(400MHz, DMSO-d_6, ppm): δ 117.36, 119.97, 122.01, 125.75, 128.62, 128.69, 128.83, 130.03, 130.35, 131.09, 134.79, 138.25, 143.02, 144.26, 152.04, 187.28; HRMS-ESI+: C22H17N2O2Cl calcd [M+H]+: 377.84, found 377.11.

1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(2-chlorophenyl)-2-propen-1-one (5d)

Yield: 98%; mp: 250-254ºC; IR ν_{max} 3326 (NH), 1716 (CO-Urea), 1636 (CO, α, β-Unsaturated) cm^{-1}; ^{1}HNMR (300MHz, DMSO-d_6, ppm): δ 7.34 (d, 2H, J=8.4Hz), 7.44-7.51 (m, 4H), 7.56 (d, 1H, J=8.7Hz), 7.63 (d, 2H, J=8.4Hz), 7.99 (2H), 8.15 (d, 2H, J=8.4Hz), 8.21(d, 1H, J=8.7Hz), 8.97 (br s, 1H, NH), 9.20 (br s, 1H, NH); ^{13}C NMR (400MHz, DMSO-d_6, ppm): δ 117.37, 119.97, 124.76, 125.77, 127.59, 128.46, 128.61, 129.94, 130.18, 130.77, 131.71, 132.42, 134.19, 137.60, 138.21, 144.50, 152.01, 187.02; HRMS-ESI$^+$: $C_{22}H_{16}N_2O_2Cl_2$ calcd $[M]^+$: 411.28, found 411.09.

1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(3-bromophenyl)-2-propen-1-one (5e)

Yield: 99%; mp: 227-230°C; IR ν_{max}3493 (NH), 1711 (CO-Urea), 1641 (CO, α, β-Unsaturated) cm^{-1}; ^{1}HNMR (300MHz, DMSO-d_6, ppm): δ 7.33 (d, 2H, J=8.82Hz), 7.40 (t, 1H, J=7.90Hz), 7.49 (d, 2H, J= 8.84Hz), 7.61-7.68 (m, 4H), 7.83 (d, 1H, J=7.52Hz), 8.01 (d, 1H, J=15.60Hz),

8.16 (m, 3H), 8.95 (br s, 1H, NH), 9.18 (br s, 1H, NH); ^{13}C NMR (400MHz, DMSO-d_6, ppm): δ 117.33, 119.97, 122.33, 123.52, 125.76, 128.11, 128.61, 130.18, 130.63, 130.85, 130.90, 132.77, 137.33, 138.23, 141.24, 144.41, 152.02, 187.09; HRMS-ESI$^+$: $C_{22}H_{16}N_2O_2ClBr$, calcd $[M+2H]^+$: 457.75, found 457.02.

1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(4-bromophenyl)-2-propen-1-one (5f)

Yield: 96%; mp: 244-249°C; IR ν_{max} 3270 (NH), 1711 (CO-Urea), 1639 (CO, α, β-Unsaturated) cm^{-1}; ^{1}HNMR (300MHz, DMSO-d_6, ppm): δ 7.34 (d, 2H, J=8.78Hz), 7.50 (d, 2H, J=8.80Hz), 7.61-7.69 (m, 5H), 7.84 (d, 2H, J=8.11Hz), 7.97 (d, 1H, J=15.66), 8.13 (d, 2H, J=8.58Hz), 8.97 (br s, 1H, NH), 9.19 (br s, 1H, NH); ^{13}C NMR (400MHz, DMSO-d_6, ppm): δ 117.35, 119.96, 122.81, 123.66, 125.75, 128.61, 130.08, 130.61, 130.97, 131.79, 134.11, 138.23, 141.62, 144.35, 152.02, 187.15; HRMS-ESI$^+$: $C_{22}H_{16}N_2O_2ClBr$ calcd $[M+2H]^+$: 457.75, found 457.18.

1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(3-chlorophenyl)-2-propen-1-one (5g)

Yield: 97%; mp: 218-220°C; IR ν_{max} 3287 (NH), 1711 (CO-Urea), 1641 (CO, α, β-Unsaturated) cm^{-1}; ^{1}HNMR (300MHz, DMSO-d_6, ppm): δ 7.30-7.35 (m, 3H), 7.46-7.51 (m, 4H), 7.56 (d, 1H, J=8.70Hz), 7.62 (d, 2H, J= 8.87Hz), 7.69 (s, 1H), 7.89 (d, 1H, J=8.73Hz), 8.02 (d, 1H, J=15.65Hz), 8.15 (d, 1H, J=8.78Hz), 8.96 (br s, 1H, NH), 9.16 (br s, 1H, NH); ^{13}C NMR (400MHz, DMSO-d_6, ppm): δ 117.33, 119.97, 123.55, 125.76, 127.71, 127.78, 128.61, 129.87, 130.17, 130.58, 130.90, 133.74, 137.08, 138.23, 141.28, 144.41, 152.02, 187.11; HRMS-ESI$^+$: $C_{22}H_{16}N_2O_2Cl$ calcd $[M]^+$: 411.28, found 411.06.

1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(2, 6-dichlorophenyl)-2-propen-1-one (5h)

Yield: 95%; mp: 246-248°C; IR ν_{max} 3332 (NH), 1711 (CO-Urea), 1632 (CO, α, β-Unsaturated) cm^{-1}; ^{1}HNMR (300MHz, DMSO-d_6, ppm): δ 7.33 (d, 2H, J= 8.87Hz), 7.42 (t, 1H, J= 8.07Hz), 7.49 (d, 2H, J=8.90Hz), 7.57-7.68 (m, 5H), 7.79 (d, 1H, J= 15.97Hz), 8.03 (d, 2H, J=8.77Hz), 8.96 (br s, 1H, NH), 9.21 (br s, 1H, NH); ^{13}C NMR (400MHz, DMSO-d_6, ppm): δ 117.50, 119.96, 125.78, 128.61, 129.04, 130.15, 130.37, 130.47, 130.82, 132.22, 134.04, 135.91, 138.19, 144.70, 151.97, 186.98; HRMS-ESI$^+$: $C_{22}H_{15}N_2O_2Cl_3$ calcd $[M]^+$: 445.72, found 445.03.

1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(2-methoxyphenyl)-2-propen-1-one (5i)

Yield: 98%; mp: 200-203°C; IR ν_{max} 3334 (NH), 1713 (CO-Urea), 1639 (CO, α, β-Unsaturated) cm^{-1}; ^{1}HNMR (300MHz, DMSO-d_6, ppm): δ 7.02 (t, 1H, J= 7.56Hz), 7.10 (d, 1H, J= 8.22Hz), 7.33 (d, 2H, J=8.86Hz), 7.43 (t, 1H, J=7.18Hz), 7.50 (d, 2H, J=8.91Hz), 7.62 (d, 2H, J= 8.78Hz), 7.86 (d, 1H, J= 15.73Hz), 7.95 (d, 1H, J=7.72Hz), 8.01 (d, 1H, J= 15.76Hz), 8.09 (d, 2H,

J=8.76Hz), 8.94 (br s, 1H, NH), 9.16 (br s, 1H, NH), 3.89 (s, 3H, OCH_3); ^{13}C NMR (400MHz, DMSO-d_6, ppm): δ 55.65, 111.72, 117.37, 119.95, 120.62, 121.80, 123.08, 125.74, 128.36, 128.61, 129.91, 131.24, 131.99, 137.59, 138.26, 144.15, 152.04, 158.13, 187.43; HRMS-ESI$^+$: $C_{23}H_{19}N_2O_3Cl$ calcd $[M+H]^+$: 407.85, found 407.26.

1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(3-methoxyphenyl)-2-propen-1-one (5j)

Yield: 98%; mp: 203-206°C; IR ν_{max} 3292 (NH), 1716 (CO-Urea), 1640 (CO, α, β-Unsaturated) cm^{-1}; ^{1}HNMR (300MHz, DMSO-d_6, ppm): δ 3.82 (s, 3H, OCH_3), 7.01 (d, 1H, J= 7.86Hz), 7.34 (d, 2H, J=8.98Hz), 7.40 (d, 2H, J=7.35Hz), 7.46 - 7.51 (m, 3H), 7.62 (d, 2H, J= 8.90Hz), 7.69 (s, 1H), 7.93 (d, 1H, J=15.59Hz), 8.14 (d, 2H, J= 8.72Hz), 8.95 (br s, 1H, NH), 9.17 (br s, 1H, NH); ^{13}C NMR (400MHz, DMSO-d_6, ppm): δ 55.26, 113.22, 116.46, 117.35, 119.97, 121.51, 122.26, 125.75, 128.62, 129.84, 130.068, 131.06, 136.20, 138.25, 143.03, 144.28, 152.04, 159.61, 187.29; HRMS-ESI$^+$: $C_{23}H_{19}N_2O_3Cl$ calcd $[M+H]^+$: 407.85, found 407.30.

1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(2, 4-dichlorophenyl)-2-propen-1-one (5k)

Yield: 96%; mp: 244-247ºC; IR ν_{max} 3325 (NH), 1710 (CO-Urea), 1654 (CO, α, β-Unsaturated) cm^{-1}; ^{1}HNMR (300MHz, DMSO-d_6, ppm): δ 7.34 (d, 2H, J= 8.83Hz), 7.48-7.56 (m, 3H), 7.62 (d, 2H, J= 8.75Hz), 7.74 (s, 1H), 7.97 (d, 2H, J= 13.77Hz), 8.14 (d, 2H, J=8.70Hz), 8.24 (d, 1H, J=8.53Hz), 8.96 (br s, 1H, NH), 9.19 (br s, 1H, NH); ^{13}C NMR (400MHz, DMSO-d_6, ppm): δ 117.36, 119.98, 125.33, 125.77, 127.86, 128.61, 129.41, 129.72, 130.23, 130.67, 131.50, 134.96, 135.34, 136.34, 138.21, 144.58, 152.01, 186.86; HRMS-ESI$^+$: $C_{22}H_{15}N_2O_2Cl_3$ calcd $[M]^+$: 445.72, found 445.19.

1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(4-methoxyphenyl)-2-propen-1-one (5l)

Yield: 93%; mp: 219-222°C; IR ν_{max} 3339 (NH), 1708 (CO-Urea), 1638 (CO, α, β-Unsaturated) cm^{-1}; ^{1}HNMR (300MHz, DMSO-d_6, ppm): δ 3.83 (s, 3H, OCH_3), 7.03 (d, 2H, J= 8.65Hz), 7.36 (d, 2H, J=8.82Hz), 7.52 (d, 2H, J=8.84Hz), 7.63 (d, 2H, J= 8.69Hz), 7.69 (d, 1H, J= 15.90Hz), 7.75 (d, 1H, J=21.90Hz), 7.85 (d, 2H, J= 8.50Hz), 8.13 (d, 2H, J= 8.67Hz), 8.98 (br s, 1H, NH), 9.18 (br s, 1H, NH). ^{13}C NMR (400MHz, DMSO-d_6, ppm): δ 55.31, 114.34, 117.33, 119.48, 119.95, 125.72, 127.43, 129.87, 130.56, 131.36, 138.27, 142.99, 144.04, 152.05, 161.16, 187.17; HRMS-ESI$^+$: $C_{23}H_{19}N_2O_3Cl$ calcd $[M+H]^+$: 407.86, found 407.10.

1-[4′-N-(N′-phenylthiourenyl) phenyl]-3-phenyl-2-propen-1-one (5m)

Yield: 86%; mp: 162-165°C; IR ν_{max} 3316 (NH), 1643 (CO, α, β-Unsaturated) cm^{-1}; ^{1}HNMR (400MHz, DMSO-d_6, ppm): δ 7.16 (t, 1H),7.36 (t, 2H, J= 8 Hz), 7.46-7.51 (m, 5H), 7.72-7.77

(m, 3H), 7.88-7.98 (m, 3H), 8.16 (d, 2H, J= 12 Hz), 10.10 (br s, 1H, NH), 10.18 (br s, 1H, NH); ^{13}C NMR (400MHz, DMSO-d_6, ppm): δ 121.67, 121.99, 123.59, 124.69, 128.75, 128.85, 129.27, 130.44, 132.66, 134.73, 139.13, 143.35, 144.22, 179.26, 187.597; HRMS-ESI$^+$: $C_{22}H_{18}N_2OS$ calcd $[M+H]^+$: 359.46, found 359.0.

1-[4′-N-(N′-phenylthiourenyl) phenyl]-3-(3-methoxyphenyl)-2-propen-1-one (5n)

Yield: 97%; mp: 144-147°C; IR ν_{max} 3316 (NH), 1646 (CO, α, β-Unsaturated) cm^{-1}; ^{1}HNMR (400MHz, DMSO-d_6, ppm): δ 3.83 (s, 3H, OCH_3), 7.03 (d, 1H, J= 8 Hz), 7.16 (t, 1H), 7.34-7.39 (m, 4H), 7.43-7.51 (m, 3H), 7.68-7.77 (m, 3H), 7.96 (d, 1H, J=16 Hz), 8.16 (d, 2H, J= 8 Hz), 10.10 (br s, 1H, NH), 10.19 (br s, 1H, NH); ^{13}C NMR (400MHz, DMSO-d_6, ppm): δ 55.26, 113.20, 116.58, 121.60, 121.64, 122.23, 123.59, 124.69, 128.48, 129.31, 129.86, 132.63, 136.14, 139.13, 143.35, 144.24, 159.62, 179.26, 187.59; HRMS-ESI$^+$: $C_{23}H_{20}N_2O_2S$ calcd $[M+H]^+$: 389.48, found 389.0.

1-[4′-N-(N′-phenylthiourenyl) phenyl]-3-(2-chlorophenyl)-2-propen-1-one (5o)

Yield: 96%; mp: 152-154°C; IR ν_{max} 3316 (NH), 1643 (CO, α, β-Unsaturated) cm^{-1}; ^{1}HNMR (300MHz, DMSO-d_6, ppm): δ 7.14 (t, 1H), 7.33 (t, 2H), 7.44-7.49 (m, 4H), 7.55 (t, 1H), 7.74 (d, 2H, J= 9 Hz), 8.00 (m, 2H), 8.13-8.22 (m, 3H), 10.11 (br s, 1H, NH), 10.19 (br s, 1H, NH); ^{13}C NMR (400MHz, DMSO-d_6, ppm): δ 121.63, 123.59, 124.72, 124.74, 127.62, 128.49, 129.43, 129.97, 131.83, 132.31, 132.35, 134.24, 137.89, 139.11, 144.48, 179.25, 187.35; HRMS-ESI$^+$: $C_{22}H_{17}N_2OSCl$ calcd $[M]^+$: 392.90, found 392.90.

α-glucosidase inhibition assay

The α-glucosidase inhibition assay was performed according to the modified method described by Patil (Patil *et al.,* 2013). 40 µL of yeast α-glucosidase (0.1 unit/ml; Sigma Aldrich, USA) was mixed with 20 µL of each of the samples (5 mg/mL DMSO solution and reconstituted in 100 µL of 100 mM phosphate buffer pH 6.8) and incubated for 1 h at 37ºC in 96-well microplate. Enzyme action for α-glucosidase was initiated by addition of 50 µL substrate (5 mM, *p*-nitrophenyl- α-D-glucopyranoside prepared in 100 mM phosphate buffer of pH 6.8) and stopped by adding 100 µL of 0.1 M Na_2CO_3 after an incubation of 10 min at 37ºC. The release of *p*-nitrophenol was measured at 420 nm spectrophotometrically (Spectra Max plus 384, Molecular Devices Corporation, Sunnyvale, CA, USA) 5 min after incubation with the substrate. Individual blanks for test samples were prepared to correct background absorbance where the substrate was replaced with 50 µL of the buffer. Control sample contained 10 µL DMSO in place of test samples. Percentage of enzyme inhibition was calculated as (1 - B/A) × 100, where [A]

represents absorbance of control without test samples, and [B] represents absorbance in the presence of test samples.

Acute toxicity study

Healthy Wistar albino rats (180 ± 20 g) of either sex were starved overnight and divided into different groups (n=4). The synthesized compounds were orally fed in rising dose levels of 0.5, 1.0, 1.5 and 2.0 g/kg b.w. The animals were noticed continuously for the first 2 h for any gross change in behavioural, neurological and autonomic profiles or any other symptoms of toxicity and mortality and randomly for the next 6 h, 24 h , 48 h and 72 h for any lethality or death. For the experiment, one-tenth and one-fifth of the maximum safe dose of the tested compounds for acute toxicity were selected (Ghosh, 1984).

***In vivo* antidiabetic activity of synthesized compounds**

Animals

Wistar rats of either sex (180 - 250 g) were taken from our University Animal House. The animals were housed in standard polyvinyl cages and the temperature of the room was maintained at 22 ± 2º C with an alternating12 h light dark cycle. Water and food were provided *ad libitum*. Experiments were performed as per the Institutional Animal Ethical Committee norms.

Study in STZ induced diabetic rats

All the animals were kept for overnight fasting. A solution of streptozotocin in 100 mM citrate buffer (pH 4.5) was prepared and calculated amount of the fresh solution was administered to overnight fasted rats (65 mg/kg b.w/rat) intraperitoneally (Pillai *et al.*, 2013). Fatal hypoglycemia is induced by streptozotocin as a result of high insulin secretion. So, 10% glucose solution was provided after 6 h for the next 24 h to prevent STZ induced hypoglycemia (Fischer and Rickert, 1975). The blood sugar level was measured by glucometer (Model: Accuchek) after 24h of glucose feeding. Animals showing 200-400 mg/dL of blood sugar level were selected as a diabetic animal. The rats were divided into eighteen groups. Group I (Normal), group II (diabetic control), group III-XVII (test compounds **5a-5o**) and a group XVIII with standard drug Metformin. Six rats in each group were taken. Rats of experimental groups were administered a suspension of the desired test sample (prepared in 1% gum acacia) orally (100 mg/kg body weight/day). Normal group animals were fed with 1% gum acacia.

Estimation of fasting blood glucose (FBG) level and body weight

FBG level and body weight of each animal were measured at 0, 5th, 10th and 15th day. Blood was taken from the tip of the tail vein of each rat and FBG level was measured using one-touch glucometer (Accucheck).

Estimation of serum biochemical parameter

Blood was collected from overnight fasted rats from each group by cardiac puncture after 24 hrs of the last dose for estimation of serum biochemical parameters like serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) (Reitman and Frankel, 1957) and alkaline phosphatase (ALP) (Duncombe, 1963). Plasma protein, blood urea, serum creatinine, lipid profile (Total cholesterol, Triglycerides, HDL, LDL and VLDL) were also estimated. All the analyses were done using commercially available kits from Span Diagnostics Ltd.

Estimation of liver biochemical parameters and antioxidant status

The liver was removed and homogenized in cold 0.1 M phosphate buffer, pH 7.0, using a glass homogenizer. Lipid peroxidation (LPO) and endogenous antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and nonenzymatic antioxidant, i.e. reduced glutathione (GSH) were estimated in liver tissue.

Estimation of lipid peroxidation level

Lipid peroxidation (LPO) was assayed according to the previously reported method (Ohkawa *et al.*, 1979). To 1 ml of tissue homogenate, 1 ml of normal saline (0.9%, w/v) and 2.0 ml of 10% trichloroacetic acid were added and mixed well. The mixture was then centrifuged (3000 × g) at room temperature for 10 min. 0.5 ml 1.0% thiobarbituric acid was added to 2 ml of supernatant, followed by heating at 95ºC for 60 min to generate the pink colored malondialdehyde (MDA). The absorbance of the samples was measured at 532 nm using spectrophotometer (Spectramax 5). The level of lipid peroxides, i.e. thiobarbituric acid reactive substances (TBARS), was estimated and expressed as nM of MDA/mg wet tissue using extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}.

Superoxide dismutase (SOD) activity assay

The assay of superoxide dismutase was performed by the method of Nishikimi *et al*. 1972. The mixture contained 100 µl tissue homogenate, 100 µl of 186 µM phenazine methosulphate, 1.9 ml of 300 µM nitroblue tetrazolium, 1 ml NADPH (700 µM). After incubation at 30ºC for 5 min,

the reaction was stopped by the addition of 500µl glacial acetic acid. Colour intensity was measured at 560 nm (Nishikimi *et al.*, 1972).

Estimation of catalase (CAT) activity

Catalase activity was determined based on the ability of the enzyme to break down H_2O_2. Catalase (CAT) was assayed and expressed as moles of H_2O_2 decomposed/min/mg of tissue (Aebi, 1974). 10 µl sample was taken in a tube containing 3 ml of H_2O_2 in phosphate buffer (M/15 phosphate buffer; pH-7.0). The time required for 0.05 optical density changes was observed at 240 nm against a blank containing the enzyme source in H_2O_2 free phosphate buffer. One unit CAT activity is the amount of enzyme that liberates half the peroxide oxygen from H_2O_2 solution of any concentration in 100s at 25ºC which is determined by CAT activity expression:

$$\text{Moles of } H_2O_2 \text{ consumed/min (units/mg)} = \frac{2.3}{\Delta t} \times ln\left(\frac{E_{initial}}{E_{final}}\right) \times 1.63 \times 10^{-3}$$

Where E is the optical density at 240 nm and t is the time required for a decrease in the absorbance.

Estimation of reduced glutathione (GSH) level

Reduced glutathione (GSH) was estimated spectrophotometrically by determination of DTNB (dithiobis-2- nitrobenzoic acid) reduced by SH-groups, expressed as g/mg wet tissue. To 0.1 ml of different tissue samples, 2.4 ml of 0.02 M EDTA solution was added and kept on an ice bath for 10 min. Then 2 ml of distilled water and 0.5 ml of 50% (w/v) trichloroacetic acid were mixed. This mixture was kept on ice for 10–15 min and then centrifuged at 3000 × g for 15 min. To 1 ml of supernatant, 2.0 ml of Tris buffer (0.4 M) was added. Then 0.05 ml of DTNB solution (Ellman's reagent; 0.01 M DTNB in methanol) was added and vortexed thoroughly. OD was measured (within 2-3 min after the addition of DTNB) at 412 nm in a spectrophotometer against a reagent blank. Appropriate standards were run simultaneously (Ellman, 1959).

Statistical analysis

The data were statistically estimated by one-way analysis of variance (ANOVA) followed by post hoc Dunnett's test using GraphPad Prism. A value of $P < 0.001$, $P < 0.01$ and $P < 0.05$ was considered to indicate statistical significance. All the results were indicated as mean Standard Error Mean (SEM) for six rats in each group.

Histopathologic study

The parts of the liver, pancreas and kidney tissues were collected for histological studies. The collected tissues were washed in normal saline and transferred immediately in 10% formalin solution for a period of at least 24 h, dehydrated with alcohol and embedded in paraffin, cut into 4 to 5µm thick sections and stained with hematoxylin-eosin dye for photomicroscopic observation.

Molecular modeling study

Homology modeling

The x-ray crystal structure of a few bacterial α-glucosidase has been reported. However, the 3D structure of α-glucosidase used in biological assays from yeast has not yet been reported (Ferreira *et al.,* 2010). To find a proper structural template for homology modeling, we searched for the protein data bank (PDB) from the protein sequence data bank and server-based Bioinformatics Tool.

Alpha-glucosidase MAL 12 Reference Sequence

The sequence of alpha-glucosidase MAL 12 protein (Gene ID-853209, NP_011808.3) consists of 584 amino acids (AAs), was retrieved from the National Center for Biotechnology Information (NCBI) at http://www.ncbi.nlm.nih.-gov/protein.

Primary Template and Protein Structure Prediction Using Online Server-based Bioinformatics Tool

In addition to using NCBI's PDB to search for 3D structures of homologous proteins, various online-based structure prediction tools were applied, including (i) Phyre2 (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index) (Kelley and Sternberg, 2009) (ii) Swiss Model (http://swissmodel.expasy.org/) (Biasini *et al.*, 2014). These were used to develop a 3D alpha glucosidase MAL 12 protein structure-based on a suitable template.

Homology model for *Saccharomyces cerevisiae* α-glucosidase was built using the crystallographic structure of *Saccharomyces cerevisiae* isomaltase (PDB Code 3A47; Resolution 1.30Å) with 72% of sequence identity with α-glucosidase was selected as a template. Protein sequence for Baker's yeast α-glucosidase (MAL12) was obtained from uniport (http://www.uniprot.org/). Sequence alignment and homology modeling were done using phyre2 model, which is fully automated homology modeling (http://www.sbg.bio.ic.ac.uk/phyre2/).

Docking Study

Protein-ligand docking study on urea/thiourea derivatives was carried out against the active site of the α-glucosidase enzyme to understand the ligand-enzyme interactions using patchDock software (Duhovny *et al.*, 2002; Schneidman-Duhovny *et al.*, 2005). PatchDock determined the best starting candidate based on shape complementarity of soft molecular surfaces. The clustering RMSD (root mean square deviation) was set to 4.0 Å for larger molecules and the complex type was fixed to default. We can get the smaller the number of the results as the higher the value of clustering RMSD. From the protocol of patchDock, the recommended values are 4 Å for protein-protein docking and 1.5 Å for protein-small molecule docking (http://bioinfo3d.cs.tau.ac.il/PatchDock/help.html). Since the α-glucosidase (protein), we decided to use the value of 4.0 for the clustering of the RMSD. The PatchDock algorithm divides the Connolly dot surface representation of the molecules into concave, convex and flat patches. Then, complementary patches are matched to generate the candidate transformations of docked complex (the candidate transformations are the docked complexes of specified receptor and ligand molecule based on the patchDock theory). PatchDock created the 1000 best-docked candidate transformations, which were selected by global energy, aVdW, rVdW and atomic contact energy. All molecular representations in this study were generated using Pymol (www.pymol.org/) and UCSF Chimera (visualization system for research and analysis) (Pettersen et al., 2004).

Result and discussion

Chemistry

The 4'-(phenylurenyl/thiourenyl) chalcone **(5a-5o)** derivatives were synthesized, as shown in **scheme 1**. *P*-aminoacetophenone **(1)** was refluxed with phenyl isocyanate/thiocyanate **(2a-b)** in toluene for 24 hours. The yellow solid 4-(phenylurenyl/thiourenyl) acetophenone **(3a-b)** were obtained. Finally, stirring of **(3a-3b)** with corresponding benzaldehydes **(4a-l)** in methanol at room temperature yielded 4'-(phenylurenyl/thiourenyl) chalcone **(5a-5o)** derivatives. The structures of the synthesized compounds (**5a-5o)** were characterized by ^{1}H NMR, ^{13}C NMR, FT-IR and Mass spectra. For instance, IR ν_{max} of compound **5l** (R_2 - 4-Methoxy) is 3339 (NH), 1708 (CO-Urea), 1638 (CO, α, β-Unsaturated) cm^{-1}. The ^{1}HNMR spectrum of **5l** showed a singlet at δ 3.83 ppm due to methoxy proton of the phenyl ring. Two broad singlet signals at δ 8.98 and δ 9.18 ppm were corresponded to the protons of –NH-CO- and -CO-NH- respectively. The twelve aromatic protons appeared in the region of δ 7.03-8.13 ppm. The signals at δ7.69 and 7.81 corresponded to α and β protons of the enone system, respectively. The ^{13}C NMR spectra of 5l

showed δ187.17 (CO), 152.05 ($CO(NH)_2$), 144.04 (C_β) and 119.95 (C_α). All these data are in agreement with the structure of compound **5l**.

α-Glucosidase inhibition assay

All synthesized compounds were evaluated through inhibitory assay for their yeast α-glucosidase (*Saccharomyces cerevisiae*) inhibitory activity. It was observed that most of the urea/thiourea derivatives exhibited significant inhibitory activity against α-glucosidase (**Table 1**). Importantly, compound **5l** was found to be the most active, showing concentration-dependent inhibition of α-glucosidase activity with 97.05% inhibition at 100 µM concentration. From the dose-response curve, the IC_{50} value of **5l** was calculated as 12.88 µM (**Fig. 1**) whereas Acarbose (marketed α-glucosidase inhibitor) showed 89.69% inhibitory activity at 100 µM concentration under similar assay conditions. The IC_{50} value of Acarbose was calculated as 11.92 µM. This data indicated that molecule **5l** was more potent than the Acarbose. Among the chloro substituted urea derivatives, compound **5a** with 4-chloro substitution exhibited highest and dose-dependent activity. It inhibited the α-glucosidase by 51.64% at 10 µM, 56.04% at 25 µM, 80.40% at 50 µM and 86.42% at 100 µM. The compound **5f** with 4-bromo substitution inhibited the α-glucosidase by 41.13%, 62.95%, 78.38% and 83.42% at 10, 25, 50 and 100 µM concentrations respectively.

Table 1.α-Glucosidase inhibition assay

Compounds	**Concentration (µM)**			
	10	25	50	100
5a	51.64±0.95	56.04±2.04	80.40±1.11	86.42±1.92
5b	ND	7.48±0.51	16.35±0.55	54.39±2.12
5c	14.09±1.72	31.15±1.3	64.61±1.42	72.69±1.46
5d	ND	12.71±1.49	22.87±1.46	54.87±1.62
5e	36.49±1.91	41.37±1.43	63.31±1.17	76.65±0.66
5f	41.13±2.64	62.95±1.42	78.38±1.60	83.42±1.31
5g	57.97±1.40	61.24±1.55	76.75±1.40	86.64±1.88
5h	7.28±1.50	20.72±1.42	43.30±1.49	65.93±1.32
5i	40.19±1.71	55.91±3.81	83.38±1.55	88.42±0.91
5j	57.87±0.95	60.23±1.38	76.36±0.59	88.05±0.35
5k	9.76±0.58	26.35±2.10	33.05±1.49	55.36±1.59
5l	43.87±1.38	64.62±1.21	85.28±1.24	97.05±0.75
5m	14.95±1.46	32.46±1.19	37.75±1.18	63.47±1.03
5n	38.78±1.78	62.10±0.77	82.10±0.91	91.07±0.75
5o	12.61±1.21	24.15±1.85	21.65±0.89	53.14±1.33
Acarbose	40.28±1.12	60.61±1.00	79.10±0.94	89.69±1.29

Values are mean ± SEM; experiment performed in triplicate; ND = Not Determine.

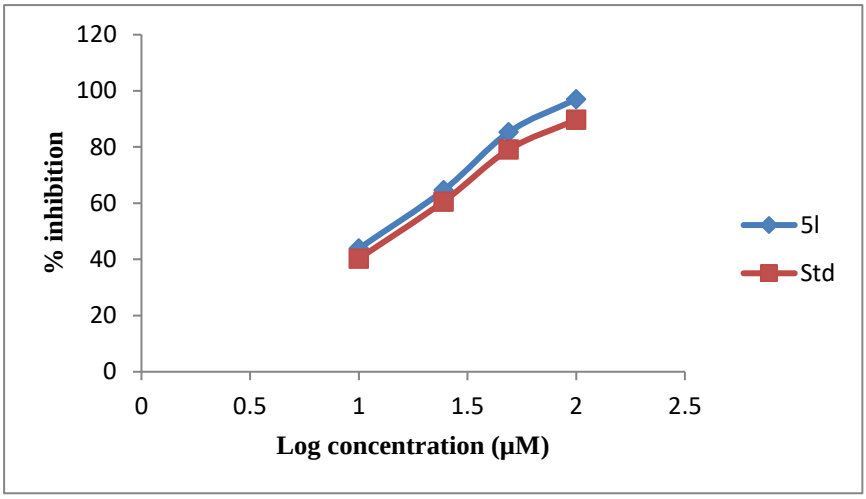


Fig. 1. Dose-response curve of α-glucosidase inhibition by compound 5l.

Among the thiourea derivatives, compound **5n** with 3-methoxy substitution inhibited the α-glucosidase by 38.78%, 62.10%, 82.10% and 91.07% at 10, 25, 50 and 100 µM concentrations respectively. Thus, methoxy and chloro substituted urea/thiourea derivatives had better activity profile than the Acarbose.

***In- vivo* antidiabetic activity**

In the acute toxicity study, synthesized compounds did not exhibit any mortality or toxic effect up to the dose of 2 g/kg b.w.; accordingly, 100 mg/kg b.w./day was taken as the dose for the *in vivo* experiment. All the synthesized compounds were further evaluated for its antihyperglycemic activity in STZ-induced diabetic rats for 14 days experiment. The increased fasting blood glucose (FBG) level in STZ induced diabetic rats was significantly (**p<0.001**) reduced after 14 days of experiment whereas compound **5b, 5c, 5d, 5h** and **5o** showed reduced blood glucose level (**p<0.01**) when compared to the diabetic control group (**Fig. 2A**). Compound **5l** showed a maximum reduction of FBG in contrast with the standard drug Metformin. The fasting blood glucose (FBG) levels of normal and experimental rats at different periods (0, 5, 10 and 15 days) upon treatment with synthesized compounds were depicted in **Table 2**.

Table 2. Level of fasting blood glucose levels in control and experimental groups of rats at different periods (0, 5, 10 and 15 days) upon treatment with synthesized compounds.

Group	Fasting blood glucose level (mg/dL)				
	Dose	Day 0	Day 5	Day 10	Day 15
I (Normal)	5 mL/kg	74.166±3.156	76.0±4.83	73.17±3.70	77.83±4.19
II (Diabetic control)	65 mg/kg	294.6±11.548	294.5±8.72	324.5±6.26	344.17±5.94
III (STZ+5a)	100 mg/kg	291.5±11.729	286.67±7.64	269.17±9.55**	242.83±3.34***
IV (STZ+5b)	100 mg/kg	296.33±19.209	271.5±11.64	284.77±3.11**	287.83±5.74**
V(STZ+5c)	100 mg/kg	276.83±8.334	277.33±5.69	263.66±7.57**	275.33±8.46**
VI (STZ+5d)	100 mg/kg	291.166±4.57	278.5±5.81	278.16±5.08**	284.83±6.33**
VII (STZ+5e)	100 mg/kg	277.5±4.95	272.0±4.57	268.16±4.29***	224.67±4.08***
VIII (STZ+5f)	100 mg/kg	285.5±4.75	286.67±3.4	260.5±4.48***	231.83±2.45***
IX (STZ+5g)	100 mg/kg	281.17±3.96	268.83±3.96*	274.5±5.9**	270.17±2.84***
X (STZ+5h)	100 mg/kg	292.00±2.71	260.17±5.19**	261.83±6.66**	289.33±5.54**
XI (STZ+5i)	100 mg/kg	304.5±8.71	284.17±4.02	279.5±3.73**	251.83±5.89***
XII (STZ+5j)	100 mg/kg	270.67±6.61	264.83±5.4*	259.17±5.64***	209.00±2.32***
XIII (STZ+5k)	100 mg/kg	298.5±6.58	283.33±3.86	269.50±5.17**	227.50±3.96***
XIV (STZ+5l)	100 mg/kg	316.0±13.27	265.0±6.46*	199.33±3.71***	166.33±3.10***
XV (STZ+5m)	100 mg/kg	276.5±2.01	269.5±5.09	265.0±4.63***	270.16±4.74***
XVI (STZ+5n)	100 mg/kg	299.83±6.01	287.33±3.08	235.83±4.08***	203.5±4.75***
XVII (STZ+5o)	100 mg/kg	286.67±3.73	273.0±2.27	263.33±6.45**	282.50±7.11**
XVIII (STZ+Metformin)	100 mg/kg	297.83±7.531	222.0±1.98***	182.66±3.69***	103.66±3.13***

Values represent the mean± S.E.M. (n =6). *p < 0.05, **P<0.01 and ***P< 0.001 compared with diabetic control group.

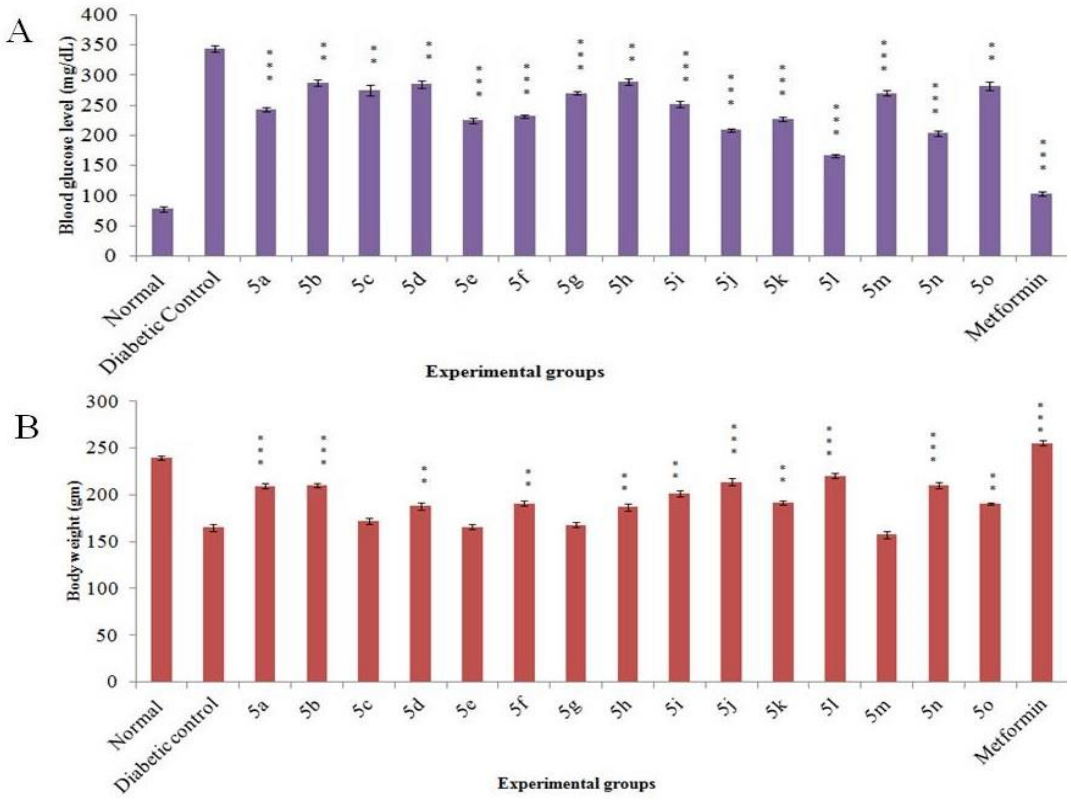


Fig. 2. Level of fasting blood glucose levels (A) and body weight (B) in diabetic control and experimental groups of rats after 14 days of treatment. Values represent the mean± S.E.M. (n =6). *p < 0.05, **P<0.01 and ***P< 0.001 compared with diabetic control group.

Diabetic group of rats displayed a notable reduction in body weight when compared with normal group of rats. Compound **5a, 5b, 5j** and **5l** showed significant (**P < 0.001**) increase in body weight after treatment of 14 days. However, there was no improvement in body weight of diabetic rats treated with **5c, 5g** and **5m** in contrast with the diabetic control group (**Fig. 2B**). The body weight of normal and experimental groups of rats are shown in **Table 3.**

Table 3. Effects of synthesized compounds on body weight in streptozotocin (STZ)- induced diabetic rats

Group	Mean body weight (g)				
	Dose	Day 0	Day 5	Day 10	Day 15
I (Normal)	5 mL/kg	210.66±2.09	215.33±2.49	227.16±3.12	239.50±2.01
II (Diabetic control)	65 mg/kg	202.83±3.61	196.66±3.96	186.5±4.53	165.16±3.98
III (STZ+5a)	100 mg/kg	201.5±3.88	204.83±1.99	206.5±3.40*	209.66±2.56***
IV (STZ+5b)	100 mg/kg	205.0±2.16	211.0±2.12*	203.33±1.97*	210.0±2.30***
V(STZ+5c)	100 mg/kg	199.16±3.88	196.16±3.00	188.16±3.01	172.0±3.30
VI (STZ+5d)	100 mg/kg	191.0±3.16	193.66±4.55	186.83±3.19	188.16±3.61**
VII (STZ+5e)	100 mg/kg	178.66±2.66	174.33±3.08**	168.5±3.62**	166.0±3.00
VIII (STZ+5f)	100 mg/kg	199.66±1.89	204.83±2.67	194.83±2.30	191.0±2.79**
IX (STZ+5g)	100 mg/kg	196.83±2.99	191.33±4.96	178.0±2.73	168.0±2.70
X (STZ+5h)	100 mg/kg	189.0±4.2	192.16±3.90	181.16±4.52	187.0±3.60**
XI (STZ+5i)	100 mg/kg	199.33±3.28	194.66±4.00	197.33±3.56	201.16±3.47**
XII (STZ+5j)	100 mg/kg	204.66±3.77	209.16±3.45	210.83±3.37**	213.83±4.15***
XIII (STZ+5k)	100 mg/kg	200.5±4.08	192.16±3.66	179.33±3.14	191.33±2.06**
XIV (STZ+5l)	100 mg/kg	204.5±4.08	212.66±3.48*	215.33±3.21**	220.33±2.96***
XV (STZ+5m)	100 mg/kg	194.5±6.41	182.66±6.10	164.83±3.87*	157.5±4.11
XVI (STZ+5n)	100 mg/kg	199.0±3.04	202.66±4.38	206.83±3.39*	210.5±3.03***
XVII (STZ+5o)	100 mg/kg	205.33±2.69	207.83±3.30	195.66±1.96	190.83±1.24**
XVIII (STZ+Metformin)	100 mg/kg	215.33±4.27	221.66±3.79***	232.33±4.8***	255.33±2.78***

Values represent the mean± S.E.M. (n =6). *p < 0.05, **P<0.01 and ***P< 0.001 compared with Diabetic control group.

Under normal physiological conditions, the activities of enzymes SGOT, SGPT and ALP are low in serum and their activities are elevated during tissue damage. A rise in SGOT and SGPT activity indicates the hepatocellular damage followed by cardiac tissue damage. Further, ALP is a marker of biliary function and cholestasis. The observed increase in activities of these enzymes in the serum of diabetic rats may be due to the leakage of these enzymes from the liver cytosol into the bloodstream as a consequence of the hepatic tissue damage. The elevated activities of SGOT, SGPT and ALP were significantly ($P < 0.001$) altered in diabetic rats administered with synthesized compounds (**5a-5o**). Reduction in the levels of activities of SGOT ($P < 0.001$), SGPT ($P < 0.001$) and ALP ($P < 0.001$) was noted in the diabetic rats treated with **5d, 5i, 5j, 5l** and **5n (Fig. 3)**. But there was no alteration in the levels of SGOT in rats treated with **5g** and **5k**

when compared to diabetic control rats. The significant reversal in SGOT, SGPT and ALP activities in diabetic rats indicate the tissue protective nature of the compounds. The levels of activities of SGOT, SGPT and ALP in the serum of the diabetic control and experimental groups of rats have been shown in **Table 4.**

Table 4. The activities of SGOT, SGPT and ALP in the serum of diabetic control and experimental groups of rats after 14 days experimental period.

Group	SGOT (U/L)	SGPT (U/L)	ALP (U/L)
I(Normal)	83.16±1.74	71.5±2.95	127.5±2.74
II (Diabetic control)	176.0±4.50	148.0±2.53	297.0±3.32
III (STZ+5a)	146.0±4.85**	104.66±3.12***	239.66±3.81***
IV (STZ+5b)	131.66±3.85***	106.66±9.36**	266.33±5.39**
V(STZ+5c)	150.16±4.51**	87.0±4.7***	246.66±5.45***
VI (STZ+5d)	126.66±2.51***	103.16±3.4***	254.33±4.36***
VII (STZ+5e)	139.0±2.75***	89.16±9.58**	281.5±3.37*
VIII (STZ+5f)	151.33±3.83**	127.83±3.86**	279.83±3.13*
IX (STZ+5g)	163.16±3.24	105.16±8.38**	260.0±4.89**
X (STZ+5h)	158.67±1.6*	107.5±8.94**	243.5±5.13***
XI (STZ+5i)	135.33±2.45***	99.5±3.51***	218.5±5.08***
XII (STZ+5j)	130.83±3.61***	111.0±4.21***	203.66±2.80***
XIII (STZ+5k)	160.33±6.34	97.83±7.06**	190.0±4.09***
XIV (STZ+5l)	108.33±2.21***	74.0±3.41***	145.16±3.14***
XV (STZ+5m)	155.0±3.6*	111.33±10.22*	269.0±4.94**
XVI (STZ+5n)	138.33±2.56***	90.0±2.43***	200.16±2.67***
XVII (STZ+5o)	153.66±3.89*	109.33±2.34***	276.0±3.86**
XVIII (STZ+Metformin)	87.16±1.57***	82.33±2.33***	142.5±3.37***

Values represent the mean± S.E.M. (n =6). *p < 0.05, **P<0.01 and ***P< 0.001 compared with STZ control group.

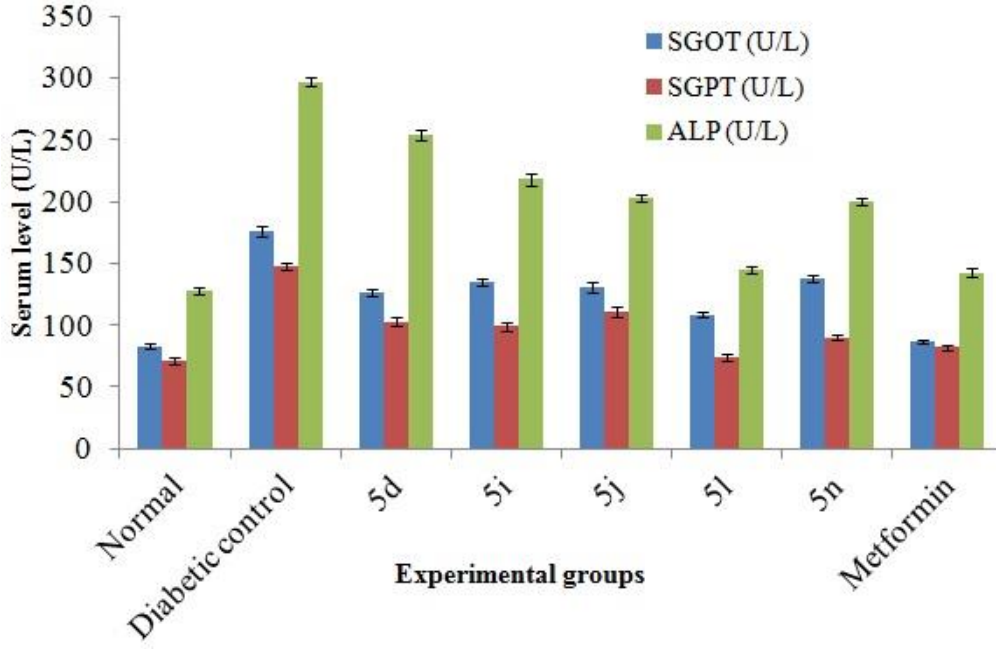


Fig. 3. The activities of SGOT, SGPT and ALP in the serum of diabetic control and experimental groups (5d, 5i, 5j, 5l, and 5n with significance value p<0.001 when compared with the diabetic control group) of rats after 14 days experimental period.

The increased levels of urea and creatinine and reduced levels of plasma protein in diabetic rats were significantly ($P < 0.001$) improved in **5a, 5i, 5j** and **5l** treated diabetic rats (**Fig. 4**). Whereas, the diabetic rats treated with **5g** and **5m** showed significant improvement in urea ($P < 0.05$) and creatinine ($P < 0.01$) levels but not in protein levels. The plasma protein, blood urea and serum creatinine levels in diabetic control and experimental groups of rats are shown in **Table 5**.

Table 5. The levels of plasma protein, blood urea and serum creatinine in diabetic control and experimental groups of rats after 14 days experimental period.

Group	Plasma protein (g/dL)	Urea (mg/dL)	Creatinine (mg/dL)
I(Normal)	8.095±0.073	35.66±3.71	0.3±0.017
II (Diabetic control)	4.85±0.179	78.83±4.11	1.075±0.046
III (STZ+5a)	6.65±0.12***	41.5±3.35***	0.55±0.04***
IV (STZ+5b)	6.48±0.31**	54.0±3.74**	0.53±0.03***
V(STZ+5c)	5.93±0.25**	61.5±3.54*	0.66±0.048**
VI (STZ+5d)	6.05±0.25*	58.66±3.31*	0.49±0.02***
VII (STZ+5e)	6.38±0.39**	57.33±4.21*	0.68±0.04**
VIII (STZ+5f)	5.95±0.15**	46.5±3.45**	0.57±0.036***
IX (STZ+5g)	5.49±0.23	59.83±2.65*	0.74±0.021**
X (STZ+5h)	6.75±0.21**	46.0±3.07**	0.56±0.03***
XI (STZ+5i)	6.86±0.20***	44.83±2.65***	0.52±0.04***
XII (STZ+5j)	6.8±0.20***	42.50±2.63***	0.51±0.04***
XIII (STZ+5k)	6.33±0.14**	54.33±3.31**	0.66±0.04**
XIV (STZ+5l)	7.17±0.09***	38.83±3.38***	0.37±0.04***
XV (STZ+5m)	5.33±0.23	62.83±3.05*	0.72±0.04**
XVI (STZ+5n)	6.30±0.29**	44.50±3.58**	0.465±0.03***
XVII (STZ+5o)	6.0±0.11**	54.83±3.89**	0.83±0.043*
XVIII (STZ+Metformin)	7.59±0.057***	38.66±2.02***	0.315±0.018***

Values represent the mean± S.E.M. (n =6). *p < 0.05, **P<0.01 and ***P< 0.001 compared with STZ control group.

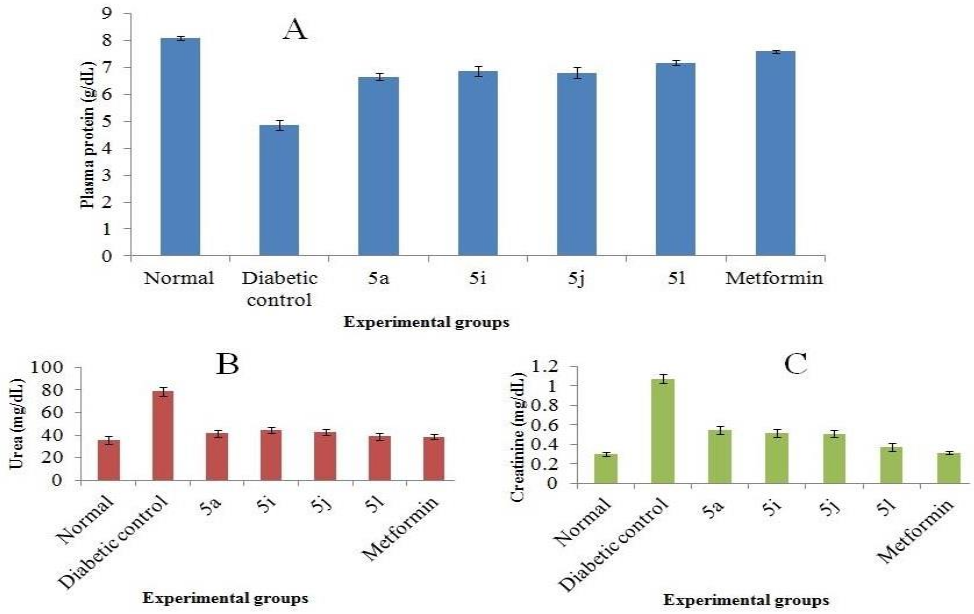


Fig. 4. The levels of plasma protein (A), blood urea (B) and serum creatinine (C) in diabetic control and experimental groups (5a, 5i, 5j and 5l with significance value p<0.001 when compared with the diabetic control group) of rats after 14 days experimental period.

It was observed that there was an increase in the triglyceride (TG), total cholesterol (TC), LDL and VLDL level and decrease in the HDL levels in the diabetic rats when compared with normal groups (**Table 6**). There was significant (P< 0.001) reduction in triglyceride levels in all groups when compared to the diabetic control group while **5b, 5c** (p< 0.01) and **5e, 5k, 5m** (p<0.05) exhibited the said significance. The total cholesterol (**5j, 5l** (p<0.001)) and LDL (**5b, 5f, 5i, 5j, 5l, 5n** (p<0.001)) level were also significantly reduced to the normal level. The VLDL level was significantly reduced to the normal level except **5e** and **5k**. There was significant (p<0.001) increase in HDL level with compounds **5j, 5l** and **5n** in the treated animal when compared with the diabetic control group.

Table 6. Level of Total cholesterol, Triglycerides, HDL, LDL, and VLDL in diabetic control and experimental groups of rats after 14 days experimental period.

Groups	Total cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
I(Normal)	127.00±2.59	104±2.65	51.00±2.52	55.20±2.62	20.80±1.47
II (Diabetic Control)	157.00±3.34	212±5.68	22.00±1.59	92.60±1.32	42.40±1.90
III (STZ+5a)	141.00±4.14*	140±3.15***	30.00±2.95	83.00±2.12*	28.00±2.07**
IV (STZ+5b)	137.00±5.80*	158±5.75**	40.00±2.18**	65.40±3.25***	31.60±2.54*
V(STZ+5c)	150.00±3.89	172±2.89**	30.00±2.94	85.60±3.23	34.40±1.13*
VI (STZ+5d)	136.00±3.80**	150±6.12***	39.00±2.03**	67.00±3.57**	30.00±2.37**
VII (STZ+5e)	143.00±3.83*	177±6.80*	40.00±3.04**	67.60±4.50**	35.40±2.54
VIII (STZ+5f)	134.00±1.75**	128±2.97***	42.00±3.97**	66.40±3.52***	25.60±1.72**
IX (STZ+5g)	138.00±3.89*	126±3.18***	37.00±3.39*	75.80±2.52**	25.20±1.98**
X (STZ+5h)	139.00±3.56*	129±3.80***	41.00±3.27**	72.20±3.00**	25.80±2.28**
XI (STZ+5i)	135.00±2.19**	132±4.45***	43.00±4.33**	65.60±1.61***	26.40±2.14**
XII (STZ+5j)	132.00±1.37***	129±2.93***	46.00±2.86***	60.20±2.19***	25.80±2.51**
XIII (STZ+5k)	147.00±2.65	185±4.34*	29.00±2.52	81.00±2.11**	37.00±3.45
XIV (STZ+5l)	133.00±0.97***	127±1.59***	47.00±2.24***	60.60±2.81***	25.40±1.73**
XV (STZ+5m)	135.00±4.12**	132±3.90***	33.00±2.27*	75.60±4.19*	26.40±3.28**
XVI (STZ+5n)	134.00±2.58**	128±3.77***	40.00±1.79***	68.40±2.77***	25.60±1.63**
XVII (STZ+5o)	139.00±3.83*	186±3.06*	32.00±3.56	69.80±3.14**	37.20±3.57
XVIII (STZ+Metformin)	128.00±2.58***	112±2.82***	49.00±3.23***	56.60±2.23***	22.40±1.72***

Values represent the mean± S.E.M. (n =6). *p < 0.05, **P<0.01 and ***P< 0.001 compared with diabetic control group.

Hyperglycemia increases the generation of free radicals by glucose auto-oxidation and the increment in free radicals thereby depletes the activity of antioxidant defense system and thus promotes de novo free radicals generation that may lead to liver cell damage (Baynes and Thrope, 1996). The LPO level was significantly (P<0.001) increased whereas reduced GSH, SOD and Catalase level were significantly (P<0.001) depleted in diabetic control animals as compared to normal group (**Table 7**). Treatment with **5b** and **5l** significantly (P<0.001) reduced LPO level when compared with diabetic control animals. Reduced GSH level was significant (P<0.001) elevated towards normal level in all groups whereas **5g** (p<0.01) and **5c, 5k, 5o**

($p<0.05$) showed less significant when compared with the diabetic control group. The level of SOD was significantly ($P<0.01$) improved with the synthesized compounds **5i, 5j, 5l** and **5n**. The administration of **5a, 5i, 5j** and **5l** recovered CAT activity significantly ($P<0.001$) towards normal level.

Table 7. Tissue lipid peroxide (LPO), reduced glutathione (GSH) Superoxide dismutase (SOD) and catalase (CAT) levels in diabetic control and experimental groups of rats after 14 days experimental period.

Groups	LPO level (nM/mg wet tissue)	GSH level (µg/mg wet tissue)	SOD level (unit/min/gm tissue)	CAT level (µM of H_2O_2decomposed/min/mg wet tissue)
I (Normal)	2.67±0.30	59.08±0.47	6.06±0.08	69.95±0.78
II (Diabetic control)	5.33±0.22	27.60±0.44	4.25±0.185	40.31±1.00
III (STZ+5a)	3.77±0.27**	51.54±0.79***	4.47±0.33	50.64±0.83***
IV (STZ+5b)	3.27±0.18***	44.33±1.30***	5.05±0.07*	45.67±0.92*
V(STZ+5c)	4.03±0.23*	34.58±1.72*	4.92±0.12*	45.57±1.20*
VI (STZ+5d)	4.005±0.21**	44.34±1.08***	5.02±0.14*	44.47±0.60*
VII (STZ+5e)	3.54±0.26**	37.46±1.09***	5.15±0.14*	47.28±0.63**
VIII (STZ+5f)	3.49±0.151**	47.51±1.27***	5.11±0.14*	48.04±0.52**
IX (STZ+5g)	3.91±0.20**	33.84±1.47**	4.85±0.08*	43.33±0.73
X (STZ+5h)	4.30±0.17*	40.13±1.09***	4.81±0.13	47.19±0.92**
XI (STZ+5i)	3.60±0.40*	41.97±1.47***	5.19±0.09**	52.39±0.73***
XII (STZ+5j)	3.13±0.28**	43.32±0.93***	5.17±0.10**	52.34±1.24***
XIII (STZ+5k)	3.83±0.25**	33.63±1.61*	4.83±0.14	44.27±1.32
XIV (STZ+5l)	3.02±0.24***	49.11±0.60***	5.48±0.11**	57.44±1.32***
XV (STZ+5m)	3.91±0.21**	40.04±1.41***	4.88±0.08*	43.34±0.86
XVI (STZ+5n)	3.22±0.35**	46.21±1.69***	5.26±0.13**	47.97±1.29**
XVII (STZ+5o)	4.19±0.09**	33.92±1.59*	4.71±0.13	45.53±1.13*
XVIII (STZ+Metformin)	3.66±0.20**	48.14±0.69***	5.75±0.06***	59.59±2.44***

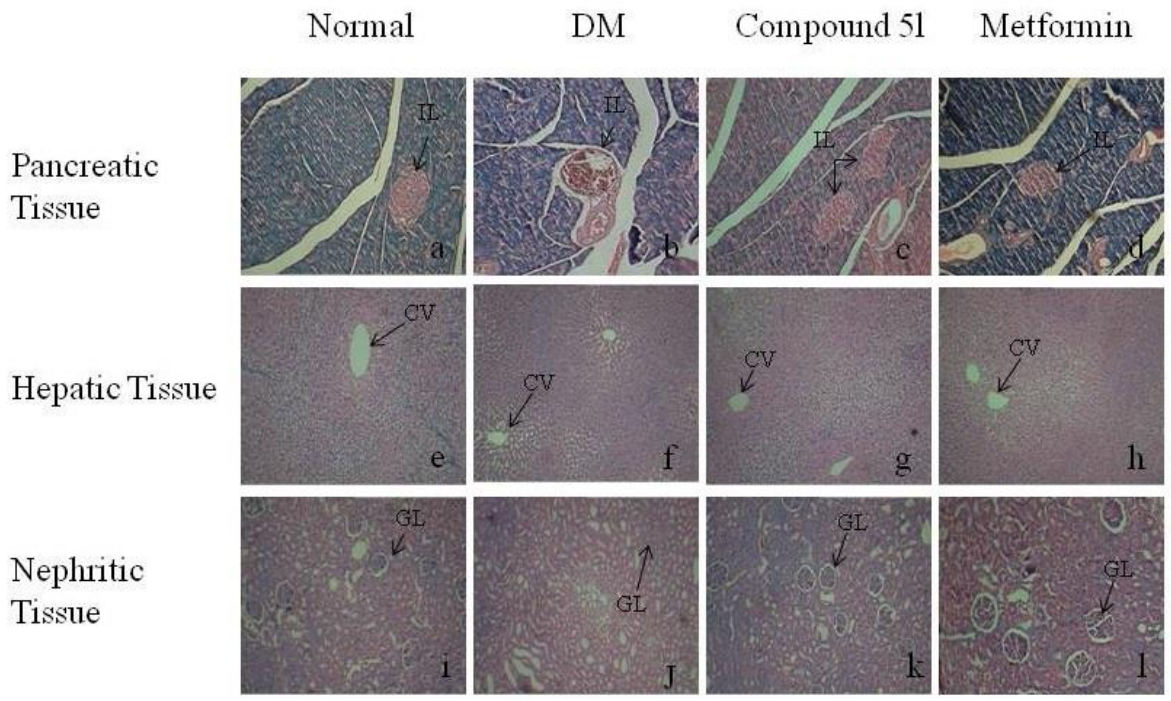
Values represent the mean± S.E.M. (n =6). *p < 0.05, **P<0.01 and ***P< 0.001 compared with diabetic control group.

Histologic examination showed degeneration and necrosis of pancreatic islets in the diabetic control group. The cytoplasm of peri-acinar hepatocytes showed either a single large or multiple small round empty vacuoles that distended the cell cytoplasm and displaced the nucleus to the periphery in histologic liver sections stained with hematoxylin and eosin. Parenchymatous degeneration was observed in peripheral regions.

Dissociation of hepatocytes and sinusoidal dilatation occurred due to these changes. Degenerated cortex and medulla, as well as necrosis of tubules, were observed in nephrons of diabetic groups. The glomerulus was emptied and distal tubules were also damaged in diabetic nephrons. These histopathological changes were restored to normal condition with compound **5l** treated animal.

Histological changes (**Fig. 5**) of pancreatic tissues of rat: a) the histological structure of pancreas of normal rat shows normal islet b) diabetic control rat shows necrosis of cells c) the rat treated

with compound **5l** shows slight regeneration of β-cells when compared with diabetic control d) the pancreas of Metformin treated rat shows mild protection from STZ-induced changes in the pancreatic islets. Photomicrographs of rat livers are showing: e) central vein with normal hepatocytes and sinusoidal spaces f) diabetic rat shows loss of the normal architecture due to distended central veins in different areas g) diabetic animals treated with compound **5l** display overall remarkable recovery changes toward normal histology h) the degenerated hepatocytes of Metformin treated animals are recovered to normal structure. Nephritic tissue of i) normal animal shows normal morphology of nephritic tissues j) diabetic rat shows degenerated cortex and medulla, necrosis of tubules in nephrons k) compound **5l** treated rat restores normal tissue



architecture of nephron l) standard drug Metformin repaired the damage of cortex and medulla.

Fig. 5. Histological analysis of rat pancreatic tissues, hepatic tissues and nephritic tissues. DM-Diabetes Mellitus, IL-Islets of Langerhans, CV-Central Vein, GL-Glomerulus.

3D Protein structure modeling of α-Glucosidase and molecular docking

We made a preliminary biocomputational analysis and we observed that experimental 3D protein α-glucosidase is not determined. Therefore, in the present study, we used *insilico* methods including Phyre2 and Swiss model to resolve α-glucosidase's 3D protein structure.

Structure prediction using Phyre2

Phyre2 suggested the 3A47_A (589 AAs) template as one of the best homologous templates for a possible 3D α-glucosidase MAL12 protein structure (Fig. 6-7). Phyre2 predicted the 3D α-glucosidase MAL12 (584 AAs) protein structure with 581 AAs based on the 3A47_A template

(589 AAs). Data obtained were prechecked by various means such as Ramachandran plot and others (Fig. 8-10).

Fig. 6. The sequence of the alpha-glucosidase enzyme (Gene ID-853209, NP_011808.3) consists of 584 amino acids and has been retrieved from the National Center for Biotechnology Information (NCBI) at http://www.ncbi.nlm.nih.gov/protein in FASTA format.

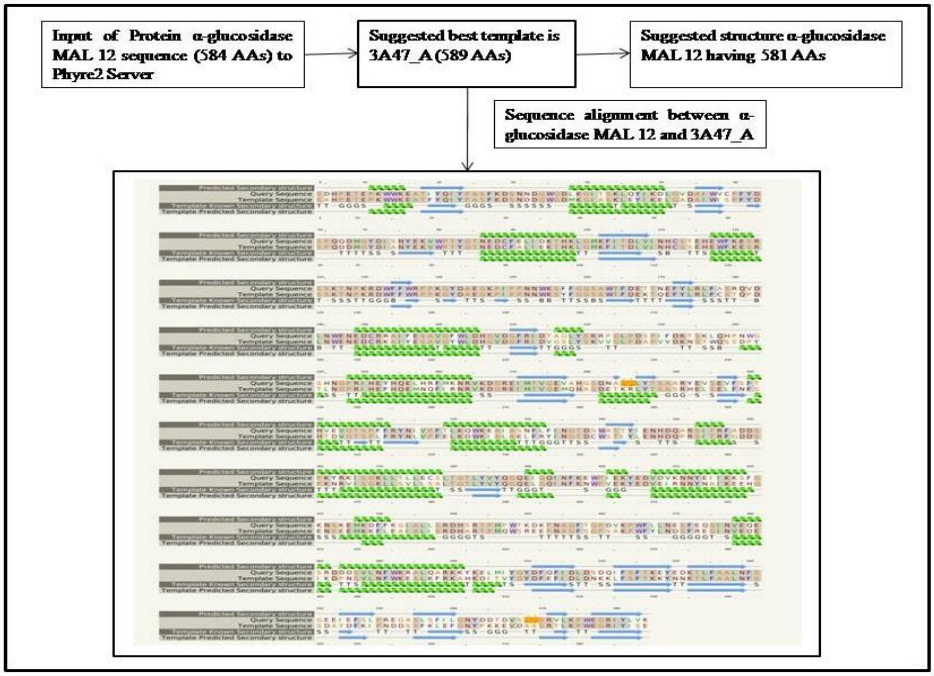


Fig. 7. The Phyre2 server suggested the 3A47_A (589 AAs) protein structure as the best homologous template for a possible 3D α-Glucosidase MAL12 (584 AAs) protein structure upon submission of the α-GlucosidaseMAL12protein sequence and the sequence alignment between α-Glucosidase MAL12 and 3A47_A is shown.

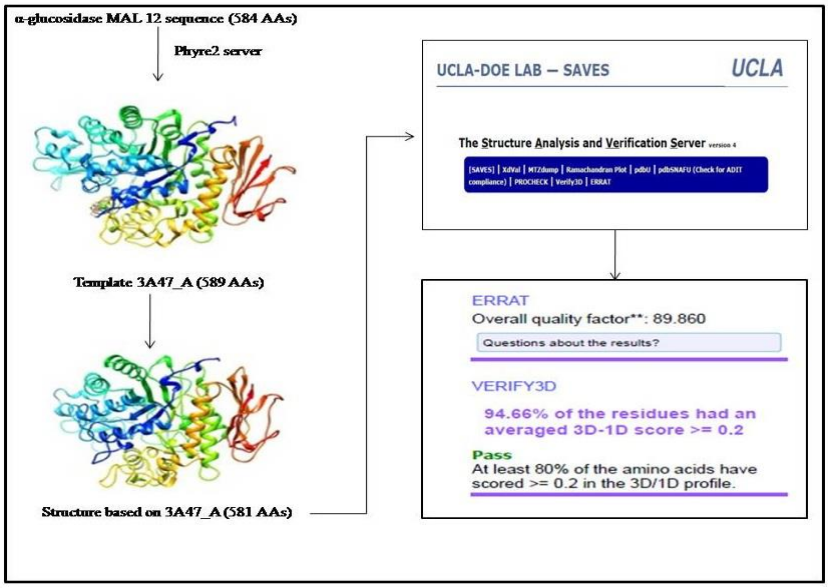


Fig. 8. The flow chart shows the 3D α-Glucosidase MAL12 protein structure prediction using the Phyre2 server and the subsequent structure evaluation steps using SAVEs (http://services.mbi.ucla.edu/SAVES/). Phyre2 server suggested the 3A47_A protein (589 AAs) as the best homologoues for a possible 3D α-Glucosidase MAL12 protein structure and the subsequently generated 3D α-Glucosidase MAL12 protein structure has 581 amino acids.

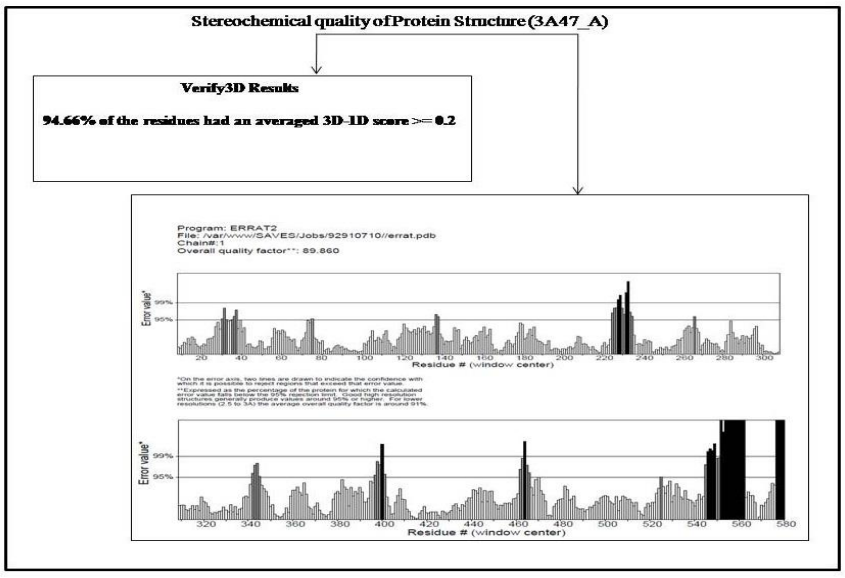


Fig. 9. An overview of the stereochemical quality-check results of the 3D α-Glucosidase MAL12 protein structure obtained from the Phyre2 server, based on the 3A47_A (581 AAs) as the best homologous template, is shown. PROCHECK(http://services.mbi.ucla.edu/SAVES/) analysis of the predicted 3D α-Glucosidase MAL12 protein structure evaluation: Ramachandran plot, main chain and side chain parameters are given. An additional overview of the stereochemical quality-check of the predicted 3D α-Glucosidase MAL12 protein structure, by VERIFY3D and ERRAT (http://services.mbi.ucla.edu/SAVES/), is also given.

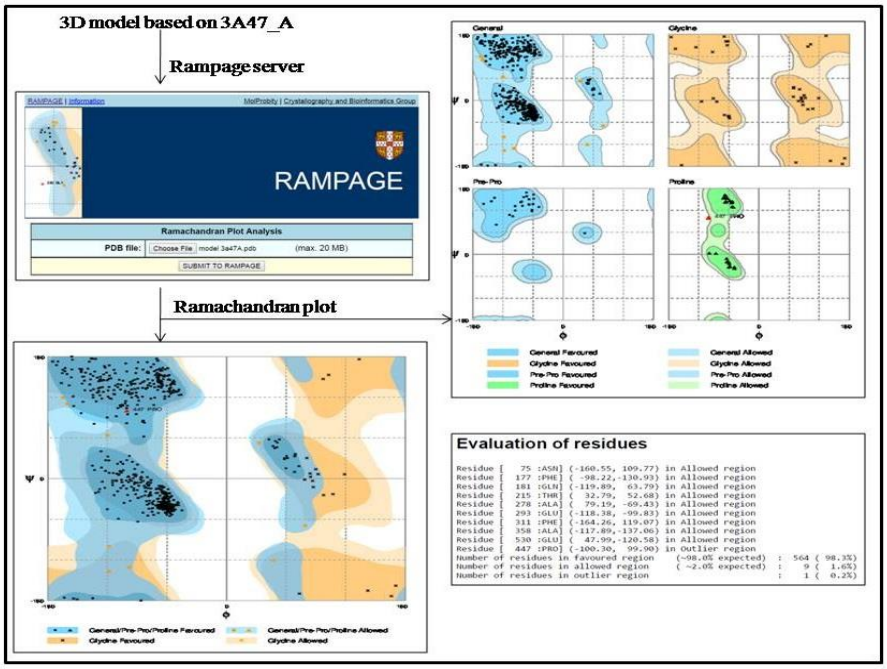


Fig. 10. Rampage (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php) assessment of Ramachandran plot of the 3D α-Glucosidase MAL12 protein structure obtained from the Phyre2 server, based on the 3A47_A (589 AAs) as the best homologous template, is shown.

Structure Prediction Using SWISS-MODEL

Submission of α-glucosidase MAL12 (584 AAs) to the SWISS-MODEL server generated two 3D α-glucosidaseMAL12 protein structure model using two different templates (3AJ7_A (589AAs) and 3AXH_A (589AAs)). The proposed 3D α-glucosidase MAL12 protein structures were pre-checked by Ramachandran plot and other methods (Fig. 11–19). Based on stereochemical properties, we observed that the model obtained from the Phyre2 method is most suitable for further ligand-protein interaction analysis.

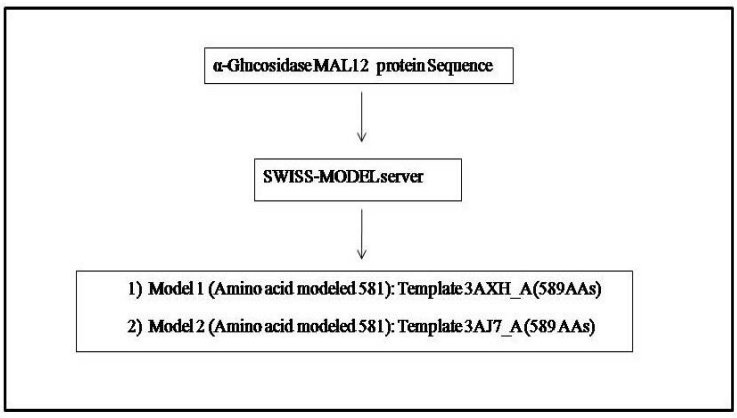


Fig. 11. Basic workflow of the α-Glucosidase MAL12 protein structure prediction, using the online SWISS-MODEL server (http://swissmodel.expasy.org/), is shown. The SWISS-MODEL server provided three (2) (best) potential 3D protein template structures (3AXH_A and 3AJ7_A) and three respective 3D α-Glucosidase MAL12 protein structure.

Fig. 12. SWISS-Model suggested Model 1 (3axh_A (589 AAs)) as one of the two best 3D protein structure templates for α-GlucosidaseMAL12. The preliminary 3D α-Glucosidase MAL12 protein structure and the protein sequence alignment between 3axh_A and α-Glucosidase MAL12 are shown.

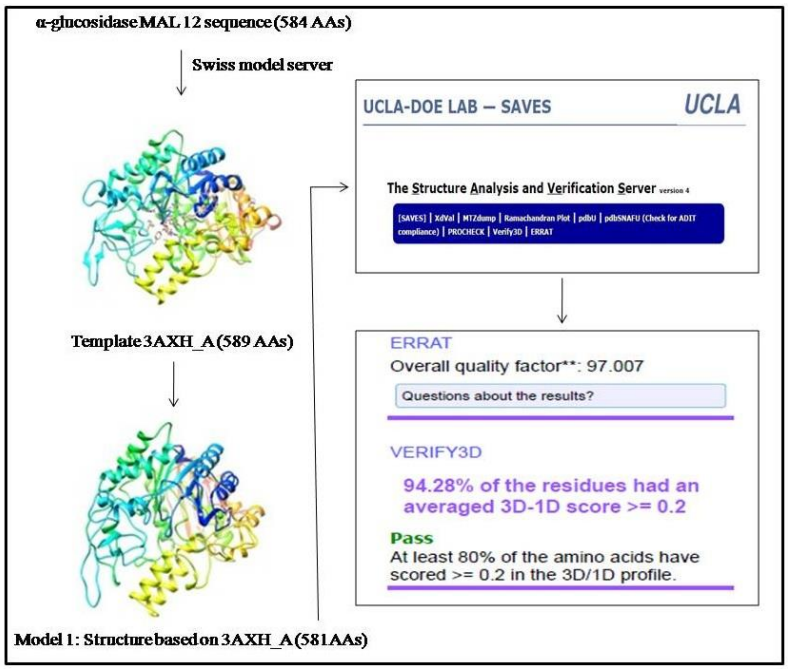


Fig. 13. The flowchart shows the 3D α-Glucosidase MAL12 protein structure prediction using the SWISS-MODEL server and the subsequent structure evaluation steps using SAVES (http://services.mbi.ucla.edu/SAVES/). The SWISS-MODEL server suggested the Model 1(3AXH_A (589 AAs)) as one of the two best 3D protein structures for α-Glucosidase MAL12 as the best homologous template for a possible 3D α-Glucosidase MAL12 protein structure and the subsequently generated 3D α-Glucosidase MAL12 protein structure has 581 amino acids.

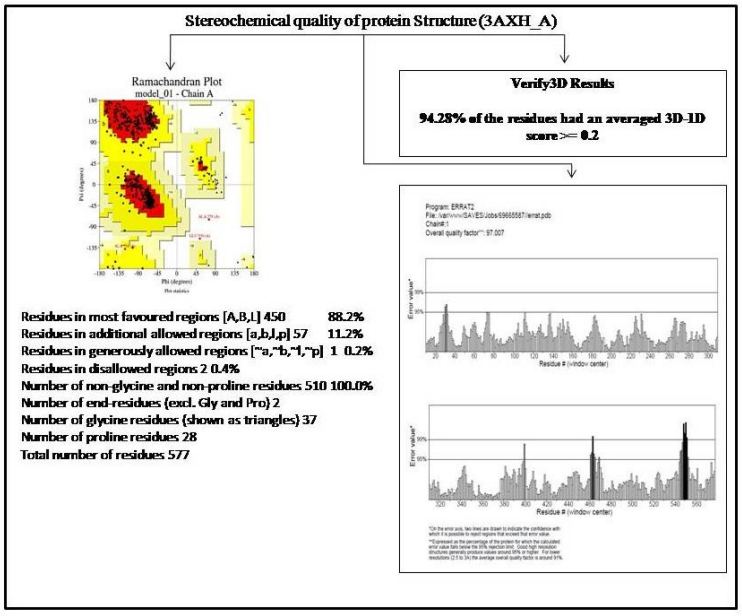


Fig. 14. An overview of the stereochemical quality-check results of the 3D α-Glucosidase MAL12 protein structure obtained from the SWISS-MODEL server, based on the 3AXH_A protein (589 AAs) as one of the three best homologous templates, is shown. PROCHECK (http://services.mbi.ucla.edu/SAVES/) analysis of the predicted 3D α-Glucosidase MAL12 protein structure evaluation: Ramachandran plot, main chain and side chain parameters are given. An additional overview of the stereochemical quality-check of the predicted 3D α-Glucosidase MAL12 protein structure, by VERIFY3D and ERRAT (http://nihserver.mbi.ucla.edu/SAVES/), is also given.

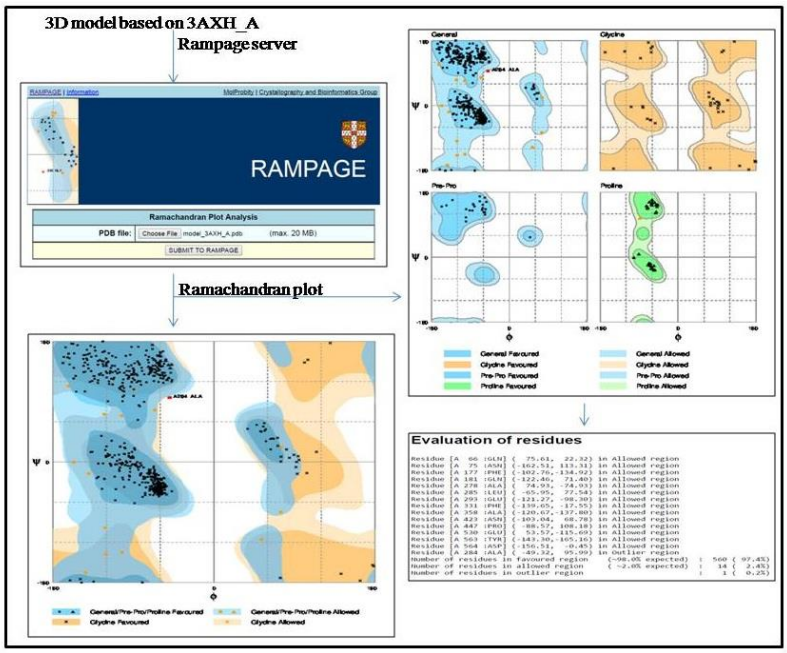


Fig. 15. Rampage (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php) assessment of Ramachandran plot of the 3D α-Glucosidase MAL12 protein structure obtained from the Swiss model server, based on the 3AXH_A (589 AAs) as the best homologous template, is shown.

Fig. 16. The SWISS-Model suggested Model 2 (3AJ7_A (589 AAs)) as one of the two best 3D protein structure templates for α-Glucosidase MAL12. The preliminary 3D α-Glucosidase MAL12 protein structure and the protein sequence alignment between 3AJ7_A and α-Glucosidase MAL12 are shown.

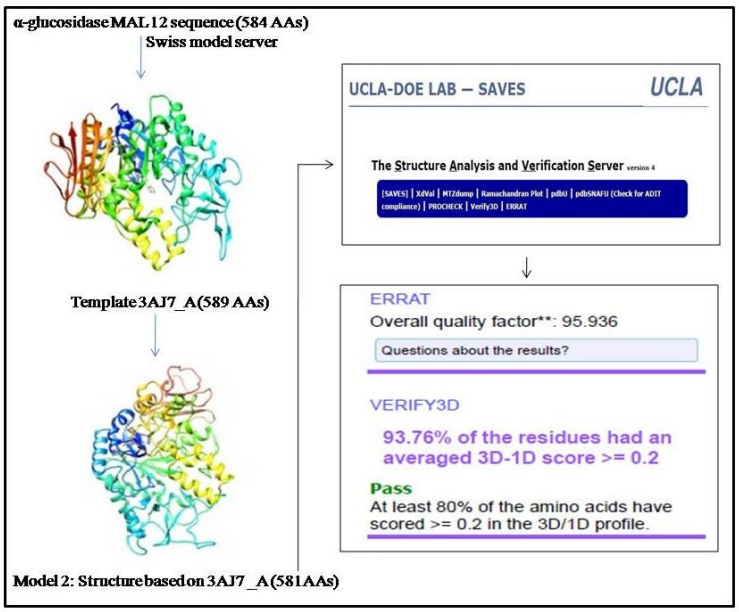


Fig. 17. The flowchart shows the 3D α-Glucosidase MAL12 protein structure prediction using the SWISS-MODEL server and the subsequent structure evaluation steps using SAVES (http://services.mbi.ucla.edu/SAVES/). The SWISS-MODEL server suggested the Model 2 (3AJ7_A (589 AAs)) as one of the three best 3D protein structures for α-Glucosidase MAL12 as the best homologous template for a possible 3D α-Glucosidase MAL12 protein structure and the subsequently generated 3D α-Glucosidase MAL12 protein structure has 581 amino acids.

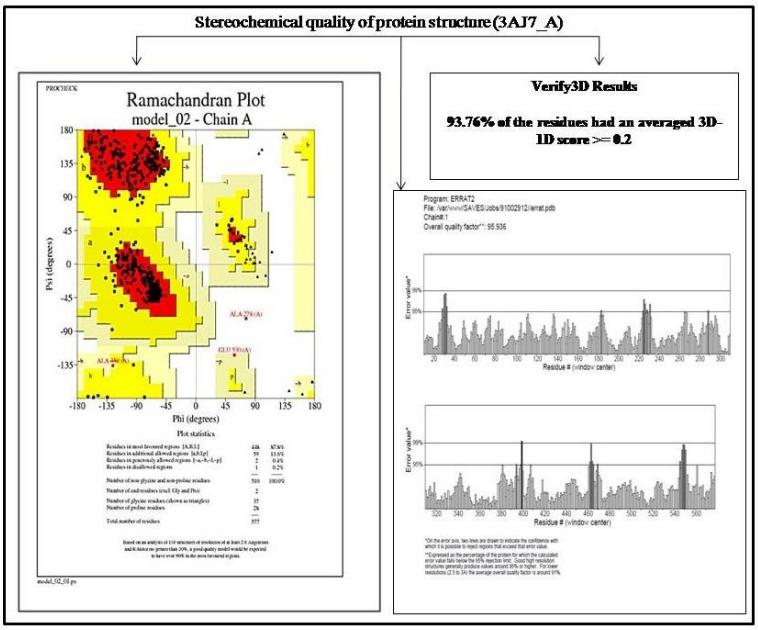


Fig. 18. An overview of the stereochemical quality-check results of the 3D α-Glucosidase MAL12 protein structure obtained from the SWISS-MODEL server, based on the 3AJ7_A protein (589 AAs) as one of the two best homologous templates, is shown. PROCHECK (http://services.mbi.ucla.edu/SAVES/) analysis of the predicted 3D α-Glucosidase MAL12 protein structure evaluation: Ramachandran plot. An additional overview of the stereochemical quality-check of the predicted 3D α-Glucosidase MAL12 protein structure, by VERIFY3D and ERRAT (http://services.mbi.ucla.edu/SAVES/), is also given.

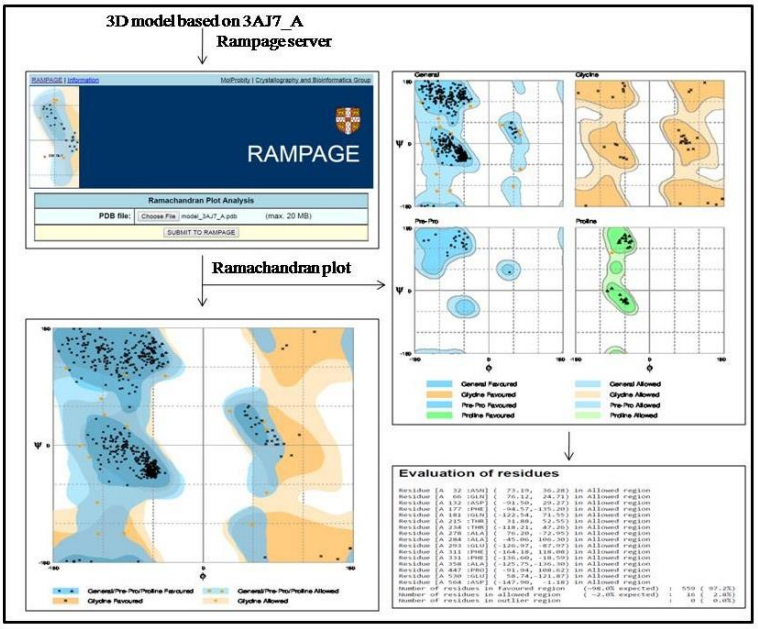


Fig. 19. Rampage (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php) assessment of the Ramachandran plot of the 3D α-Glucosidase MAL12 protein structure obtained from the Swiss model server, based on the 3AJ7_A (589 AAs) as the best homologous template, is shown.

For the binding ligand-protein interaction analysis, we performed a geometry-based molecular docking algorithm PatchDock for all synthesized molecules **5a-5o (Table 8).** The applied PatchDock docking program accurately defines specific binding sites of **5a-5o** molecules.

Table 8. Ligand-protein docking study of synthesized molecules (**5a-5o**) with the α-Glucosidase model based on 3a47_A template using PatchDock method.

Compounds	Score	Area	ACE*	Transformation
5a	5656	677.90	-124.35	-0.73 -0.56 2.30 32.55 -18.38 13.73
5b	5536	730.50	-239.60	-2.41 0.01 -2.65 35.48 -12.03 11.77
5c	5556	656.90	-118.30	-1.11 -0.68 -0.29 26.81 -9.49 9.54
5d	5644	690.30	-169.27	-0.65 -0.66 2.41 32.86 -19.65 15.52
5e	5476	669.30	-155.37	2.62 -0.69 2.32 32.55 -19.50 15.45
5f	5794	656.60	-82.06	2.83 0.76 -1.69 27.39 -8.59 10.48
5g	5458	703.50	-234.42	0.31 0.31 -2.28 32.23 -15.71 12.97
5h	5620	692.50	-73.41	2.20 1.19 -1.47 26.74 -8.06 8.93
5i	5330	672.80	-198.29	1.97 -0.25 1.02 33.37 -22.96 16.31
5j	5962	702.60	-178.37	-2.46 0.17 -2.21 34.08 -19.85 17.94
5k	5822	716.00	-116.58	-0.52 -0.49 2.49 32.84 -18.46 15.33
5l	5980	700.10	-65.50	1.15 0.27 0.06 25.76 -6.68 6.97
5m	5658	623.90	-117.64	-0.94 -0.52 -0.00 27.64 -8.28 10.12
5n	5640	696.50	-260.64	-0.61 -1.20 1.94 35.52 -13.79 13.44
5o	5614	665.70	-147.17	-0.60 -0.69 2.49 33.11 -19.19 15.24

*ACE-Atomic contact energy

According to the docking score, the highest docking score was selected for further studies. The docked structure showed atomic contact energy in the range of -65.50 to -260.64 Kcal/mol. Among the synthesized compounds, **5l** showed highest docking score and lowest atomic contact energy. The ligand-protein interaction has been shown in **Table 9**.

Based on overall results, the most effective molecule, **5l** interacts with Tyr 71, Asp 68, Val 108, His 111, Lys 155, Phe 157, Phe 177, Asp 214, His 239, Asn 241, His 279, Tyr 313, His 348, Asp 349, Asp 408, Asn 412, Arg 439 of α-glucosidase (**Fig. 20**).

Table 9. The interactive Amino acid of receptor molecule with compound (5a-5l).

Compound	Protein residue name
5a	Lys 155, Phe 157, Asp 232, His 239, Asn 241, Glu 304, Phe 311, Arg 312, Ile 415
5b	Lys155, Phe 157, Asp 232, Thr 234, Ser 235, His 239, Asn 241, His 245, Phe 311, Asn 314, Asn 412, Ile 415, Ile 416, Ser 419, Phe 420
5c	Lys 155, Phe 157, Phe 158, Phe 177, Asp 214, His 239, Asn 241, Phe 311, Tyr 313, Asp 349, Arg 439, Asp 408, Asn 412.
5d	Lys 155, His 239, Glu 304, Phe 311, Arg 312, Asp 408, Ile 415, Phe 420
5e	Lys 155, Phe 157, Asp 232, His 239, Asn 241, Glu 304, Arg 312, Asp 408, Asn 412, Ile 415, Phe 420
5f	Lys 155, Phe 157, Asn 241, Thr 215, Asp 214, His 239, Glu 276, His 279, Glu 304, Arg 312, Tyr 313, His 348, Asp 349, Asp 408, Arg 439
5g	Lys 155, Phe 157, Asp 232, Thr 234, Ser 235, His 239, Phe 311, Asn 314, Asn 412, Ile 415, Ile 416, Ser 419, Phe 420
5h	Tyr 71, His 111, Phe 157, Phe 177, Asp 214, Thr 215, Glu 276, His 279, Phe 311, Arg 312, Tyr 313, His 348, Asp 349, Asp 408, Asn 412, Arg 439
5i	Lys 155, Phe 157, Ser 235, His 239, Glu 304, Phe 311, Arg 312, Asn 314, Asp 408, Ile 415, Phe 420, Glu 426
5j	Lys 155, Phe 157, Asp 232, Thr 234, His 239, Glu 304, Phe 311, Arg 312, Asn 314, Asn 412, Ile 415, Phe 420
5k	Lys 155, Phe 157, Asp 232, Asn 241, Glu 304, Phe 311, Arg 312
5l	Tyr 71, Asp 68, Val 108, His 111, Lys 155, Phe 157, Phe 177, Asp 214, His 239, Asn 241, His 279, Tyr 313, His 348, Asp 349, Asp 408, Asn 412, Arg 439
5m	Phe 177, Asp 214, Thr 215, His 239, Asn 241, Glu 276, Glu 304, Phe 311, Arg 312, Tyr 313, Asp 349, Asp 408, Arg 439,
5n	Lys 155, Phe 157, Asp 232, Thr 234, Ser 235, His 239, Phe 311, Asn 314, Ile 416, Asn 412, Phe 420
5o	Lys 155, Phe 157, Asp 232, Asn 241, Glu 304, Phe 311, Arg 312, Asn 412, Ile 415, Phe 420

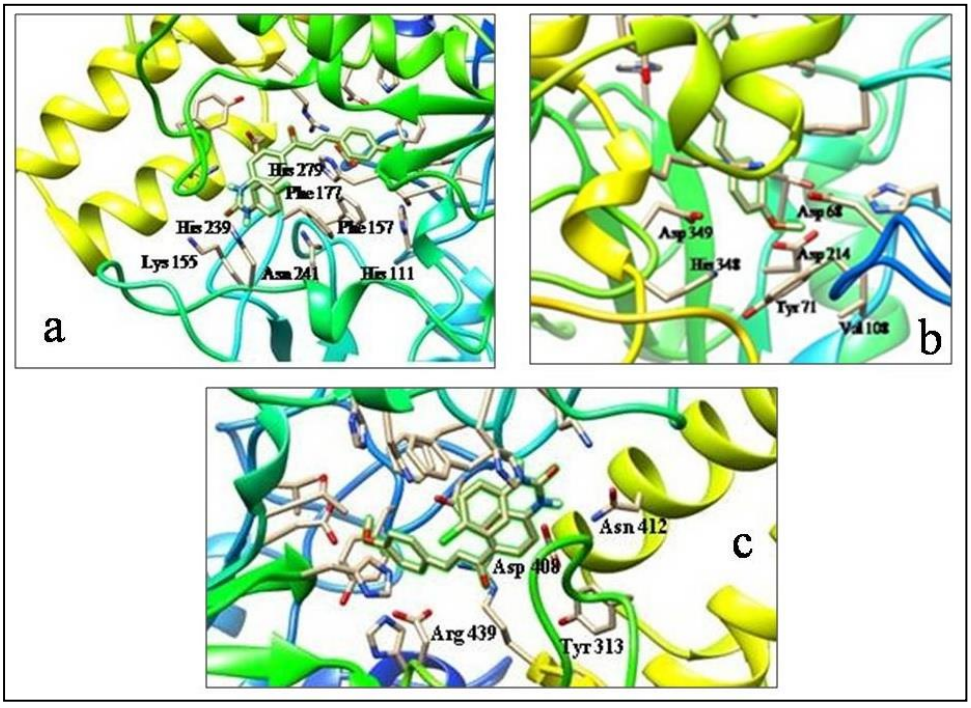


Fig. 20. Molecular docking analysis of the ligand-binding pocket of α-Glucosidase MAL12 model structure obtained from the phyre2 server, based on the 3A47_A template. The docked complexes were analyzed using Chimera 3d viewer. Compound **5l** and the interacting amino acids (**a**-His 111, Lys 155, Phe 157, Phe 177, His 239, Asn 241, His 279; **b**- Tyr 71, Asp 68, Val 108, Asp 214, His 348, Asp 349; **c**- Tyr 313, Asp 408, Asn 412, Arg 439) are depicted. Molecular Docking was done by using online PatchDock Server (https://bioinfo3d.cs.tau.ac.il/PatchDock/).

Conclusion

A series of urea/thiourea derivatives have been synthesized (**5a- 5o**). All the synthesized compounds were tested for their α-glucosidase inhibitory activity. Among them, compound **5l** (IC_{50} -12.88 µM) having 4-Methoxy substitution at phenyl ring was found to be the most active compound when compared to the standard drug Acarbose (IC_{50} - 11.92 µM). In *in vivo* study, compound **5l** showed significant improvement of the body weight, blood glucose level, serum enzyme parameter, lipid profile as well as tissue antioxidant parameters. The Molecular docking studies showed that these urea/thiourea derivatives were binding to the active site of α-glucosidase enzyme with the hydrophobic interactions, noncovalent and hydrogen bond interactions. The docking studies are in good agreement with the *in vitro* and *in vivo* studies. Hence, this study identified a new structural type of α-glucosidase inhibitors which may be used as lead molecules for further research and development of potent α-glucosidase inhibitors for the treatment of diabetes.

References

Abdel-Rahman, H., Morsy, M.A., 2007. Novel benzothiazolyl urea and thiourea derivatives with potential cytotoxic and antimicrobial activities. J. Enzyme Inhib. Med. Chem. 22, 57–64.

Adeoye, O., Ayandele, A.A., Odunola, O.A., 2007. Synthesis and bactericidal studies of some copper (ii) carboxylates with urea.J. AgricBiol Sci. 2, 4-5.

Aebi, H.,1974. Catalase: In methods in Enzymatic Analysis, vol. II. Academic Press, New York, pp. 673–684.

AG, H.B.B., 1994. Pharmacology of α-glucosidase inhibition. Eur. J. Clin. Invest. 24, 3–10. 1994

Ahmad, S., Javed, K., Rathish, I.G., Bano, S., Alam, M.S., 2009. Synthesis and Blood Glucose Lowering Activity of Novel Benzenesulfonyl-Urea Derivatives.Phosphorus Sulfur Silicon Relat Elem. 184, 2516-2524.

Baynes,J.W., Thorpe, S.R.,1996. The role of oxidative stress in diabetic complications. CurrOpinEndocrinol Diabetes Obes. 3, 277–284.

Biasini, M., Bienert, S., Waterhouse, A., Arnold, K., Studer, G., Schmidt, T., Keifer, F., Gallo, C., Bertoni, M., Bordoli, L., Torsten, S., 2014. SWISS-MODEL: modeling protein tertiary and quaternary structure using evolutionary information. - PubMed - NCBI. Nucleic Acids Res. 42, W252–W258.

Bloom, J.D., Dushin, R.G., Curran, K.J., Donahue, F., Norton, E. B., Terefenko, E., Jones, T.R., Ross, A.A., Feld, B., Lang, S.A., DiGrandi, M.J., 2004. Thiourea inhibitors of herpes viruses. Part 2: N-Benzyl-N′- arylthiourea inhibitors of CMV. Bioorganic Med. Chem. Lett. 14, 3401–3406.

Davis, S.N., Granner, D.K.,1996. Goodman & Gilman'sThe Pharmacological Basis of Therapeutics. 9th ed. McGraw-Hill, New York, pp. 1487-1517.

Derosa, G., Maffioli, P., 2012. α-Glucosidase inhibitors and their use in clinical practice. Arch. Med. Sci.

Deshpande, A.D., Harris-hayes, M., 2008. Diabetes Special Issue Diabetes-Related Complications. Am. Phys. Ther. Assoc. 88, 1254-1264.

Domínguez, J.N., León, C., Rodrigues, J., De Domínguez, N.G., Gut, J., Rosenthal, P.J., 2005. Synthesis and evaluation of new antimalarial phenylurenyl chalcone derivatives. J. Med. Chem. 48, 3654–3658.

Dos Santos, L., Lima, L.A., Cechinel-Filho, V., Corrêa, R., de Campos Buzzi, F., Nunes, R.J., 2008. Synthesis of new 1-phenyl-3-{4-[(2E)-3-phenylprop-2-enoyl]phenyl}-thiourea and urea derivatives with anti-nociceptive activity. Bioorganic Med. Chem. 16, 8526–8534.

Duhovny, D., Nussinov, R., Wolfson, H.J., 2002. Efficient Unbound Docking of Rigid Molecules. In Gusfield et al., Ed. Proceedings of the 2'nd Workshop on Algorithms in Bioinformatics(WABI) Rome, Italy, Lecture Notes in Computer Science, Springer Verlag, 2452, pp. 185-200.

Duncombe, W.G.,1963. The colorimetric micro-determination of long-chain fatty acids. *Biochem J.* 88, 7-10.

Ellman, G.L., 1959. Tissue sulfhydryl groups. Arch. Biochem. Biophys. 82, 70–77.

Faidallah, H.M., Al-Saadi, M.S., Rostom, S.A.F., Fahmy, H.T.Y., 2007. Synthesis of some sulfonamides, disubstituted sulfonylureas or thioureas and some structurally related variants. A class of promising antitumor agents. Med. Chem. Res. 16, 300–318.

Faidallah, H.M., Khan, K.A., Asiri, A.M., 2011. Synthesis and biological evaluation of new 3-trifluoromethylpyrazolesulfonyl-urea and thiourea derivatives as antidiabetic and antimicrobial agents. J. Fluor. Chem. 132, 131–137.

Faidallah, H.M., Rostom, S.A.F., Basaif, S.A., Makki, M.S.T., Khan, K.A., 2013. Synthesis and biological evaluation of some novel urea and thiourea derivatives of isoxazolo[4,5-d]pyridazine and structurally related thiazolo[4,5-d]pyridazine as antimicrobial agents. Arch. Pharm. Res. 36, 1354–1368.

Ferreira, S., Sodero, A.C.R., Cardoso, M.F.C., Lima, E.S., Kaiser, C.R., Silva, F.P., Ferreira,

V.F., 2010. Synthesis, Biological Activity, and Molecular Modeling Studies of 1H-1,2,3-Triazole Derivatives of Carbohydrates as a-Glucosidases Inhibitors. J. Med. Chem. 53, 2364–2375.

Fischer,L.J., Rickert, D.E., 1975. Pancreatic islet-cell toxicity.CRC Crit. Rev. Toxicol., 3, 231-263

Ghosh, M.N.,1984. Fundamentals of experimental pharmacology, 2nd ed. Calcutta: Scientific Book Agency; 192-194.

He, Z.-X., Zhou, Z.-W., Yang, Y., Yang, T., Pan, S.-Y., Qiu, J.-X., Zhou, S.-F., 2015. Overview of clinically approved oral antidiabetic agents for the treatment of type 2 diabetes mellitus. Clin. Exp. Pharmacol. Physiol. 42, 125–38.

Hirsh, A., Yao, S., Young, J., Cheeseman, C., 1997. Inhibition of glucose absorption in the rat jejunum: A novel action of alpha-D-glucosidase inhibitors. Gastroenterology 113, 205–211.

Huizinga, M.M., Rothman, R.L., 2006. Addressing the diabetes pandemic: A comprehensive approach. Indian J. Med. Res. 124, 481–484.

Joshi, S.R., Standl, E., Tong, N., Shah, P., Kalra, S., Rathod, R., 2015. Therapeutic potential of α-glucosidase inhibitors in type 2 diabetes mellitus: An evidence-based review. Expert Opin. Pharmacother. 16, 1959–1981.

Kelley, L.A., Sternberg, M.J.E., 2009. Protein structure prediction on the web: A case study using the phyre server. Nat. Protoc. 4, 363–373.

Lopez-Candales, A., 2001. Metabolic syndrome X: a comprehensive review of the pathophysiology and recommended therapy. J Med 32, 283–300.

Meneilly, G.S., Ryan, E.A., Radziuk, J., Lau, D.C.W., Yale, J.F., Morais, J., Chiasson, J.L., Rabasa-Lhoret, R., Maheux, P., Tessier, D., Wolever, T., Josse, R.G., Elahi, D., 2000. Effect of acarbose on insulin sensitivity in elderly patients with diabetes. Diabetes Care 23, 1162–1167.

Nishikimi, M., Appaji Rao, N., Yagi, K., 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. Biophys. Res. Commun. 46, 849–854.

Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351–358.

Patil, V.S., Nandre, K.P., Ghosh, S., Rao, V.J., Chopade, B.A., Sridhar, B., Bhosale, S. V., Bhosale, S. V., 2013. Synthesis, crystal structure and antidiabetic activity of substituted (E)-3-(Benzo [d]thiazol-2-ylamino) phenylprop-2-en-1-one. Eur. J. Med. Chem. 59, 304–309.

Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., Ferrin,

T.E., 2004. UCSF Chimera - A visualization system for exploratory research and analysis. J. Comput. Chem. 25, 1605–1612.

Pillai, S.I., Subramanian, S.P., Kandaswamy, M., 2013. A novel insulin mimetic vanadium-flavonol complex: Synthesis, characterization and *in vivo* evaluation in STZ-induced rats. Eur. J. Med. Chem. 63, 109–117.

Reitman, S., Frankel, S., 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol. 28, 56–63.

Ross, S.A., Gulve, E.A., Wang, M., 2004. Chemistry and biochemistry of type 2 diabetes. Chem. Rev. 104, 1255–1282.

Schneidman-Duhovny, D., Inbar, Y., Nussinov, R., Wolfson, H.J., 2005. PatchDock and SymmDock: Servers for rigid and symmetric docking. Nucleic Acids Res. 33, W363-367.

Scott, L.J., Spencer, C.M., 2000. Miglitol: a review of its therapeutic potential in type 2 diabetes mellitus. Drugs 59, 521–549.

Sonmez, F., Sevmezler, S., Atahan, A., Ceylan, M., Demir, D., Gencer, N., Arslan, O., Kucukislamoglu, M., 2011. Evaluation of new chalcone derivatives as polyphenol oxidase inhibitors. Bioorg. Med. Chem. Lett. 21, 7479–7482.

Suyoga Vardhan, D.M., Shantharam, C.S., Suhas, R., Gowda, D.C., 2017. Synthesis and evaluation of novel ureido/thioureido derivatives of amino acid conjugated 2,3-dichlorophenyl piperazine as highly potent antiglycating agents. J. Saudi Chem. Soc. 21, S248–S257.

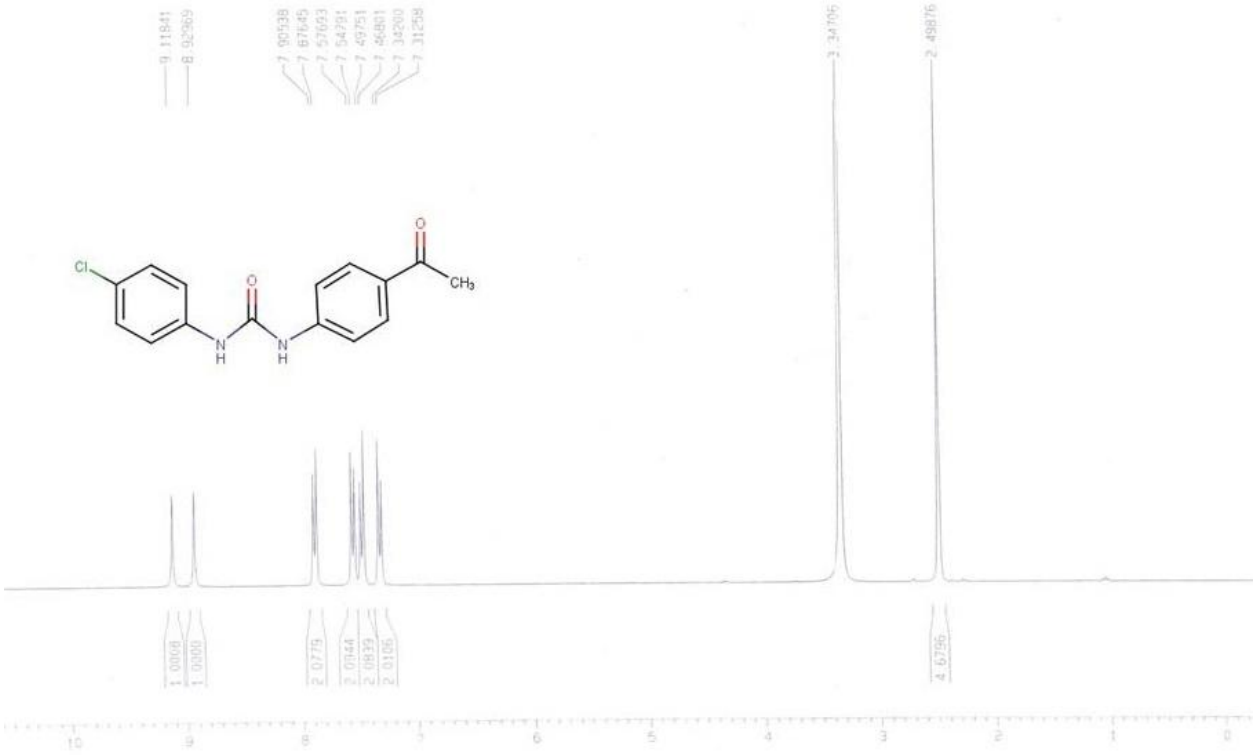
Tobe, Y., Sasaki, S.I., Mizuno, M., Hirose, K., Neamura, K.,1998. Novel Self-Assembly of m-Xylylene Type Dithioureas by Head-to-Tail Hydrogen Bonding. J. Org Chem. 63, 7481-7489.

Venkatachalam, T.K., Sudbeck, E.A., Mao, C., Uckun, F.M., 2001. Anti-HIV activity of aromatic and heterocyclic thiazolyl thiourea compounds. Bioorganic Med. Chem. Lett. 11, 523–528.

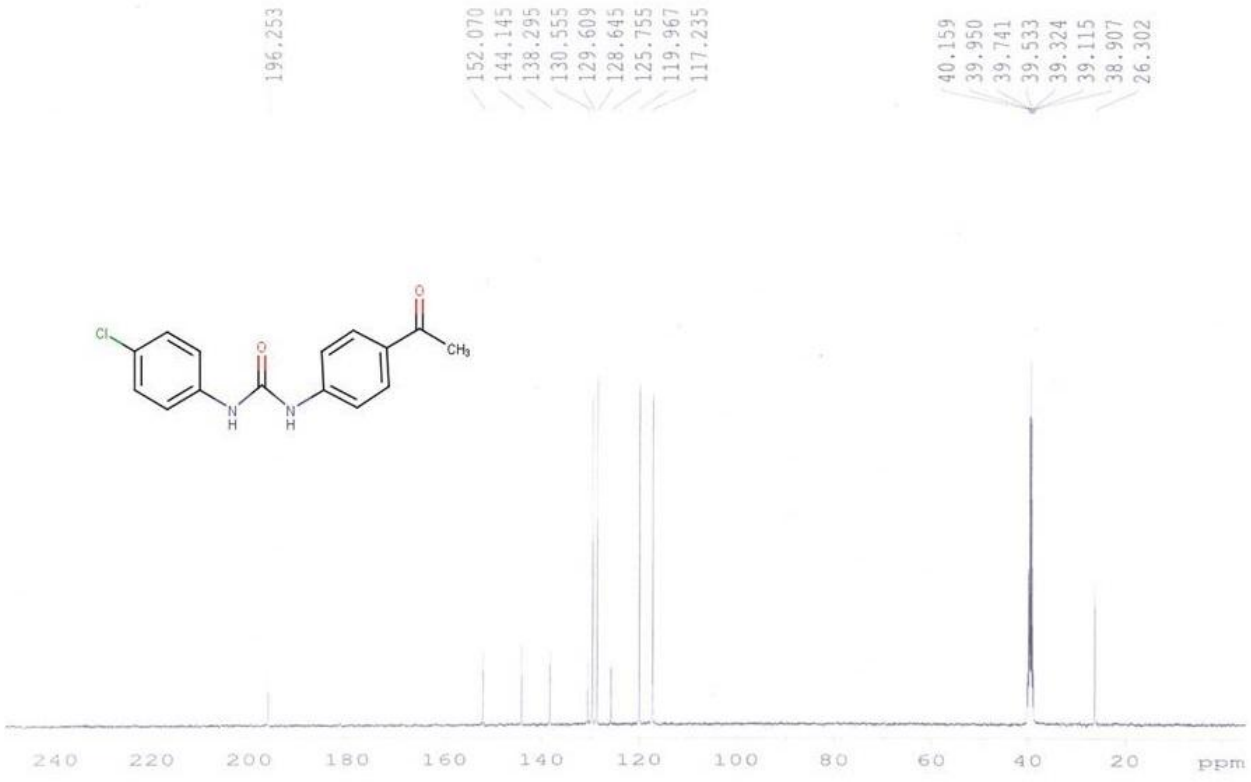
Yonova, P.A., Stoilkova, G.M., 2004. Synthesis and biological activity of urea and thiourea derivatives from 2-aminoheterocyclic compounds. J. Plant Growth Regul. 23, 280–291.

Zimmet, P., Alberti, K.G.M.M., Shaw, J., 2001. Global and societal implications of the diabetes epidemic. Nature.

Zhang, S., Zhan, T., Cheng, K., Xia, Y., Yang, B., 2008. Simple and efficient synthesis of novel glycosyl thiourea derivatives as potential antitumor agents. European J. Med. Chem. 43, 2778–2783.



^{1}H NMR of 4-N-(N′-p-chlorophenylurenyl) acetophenone (3a)



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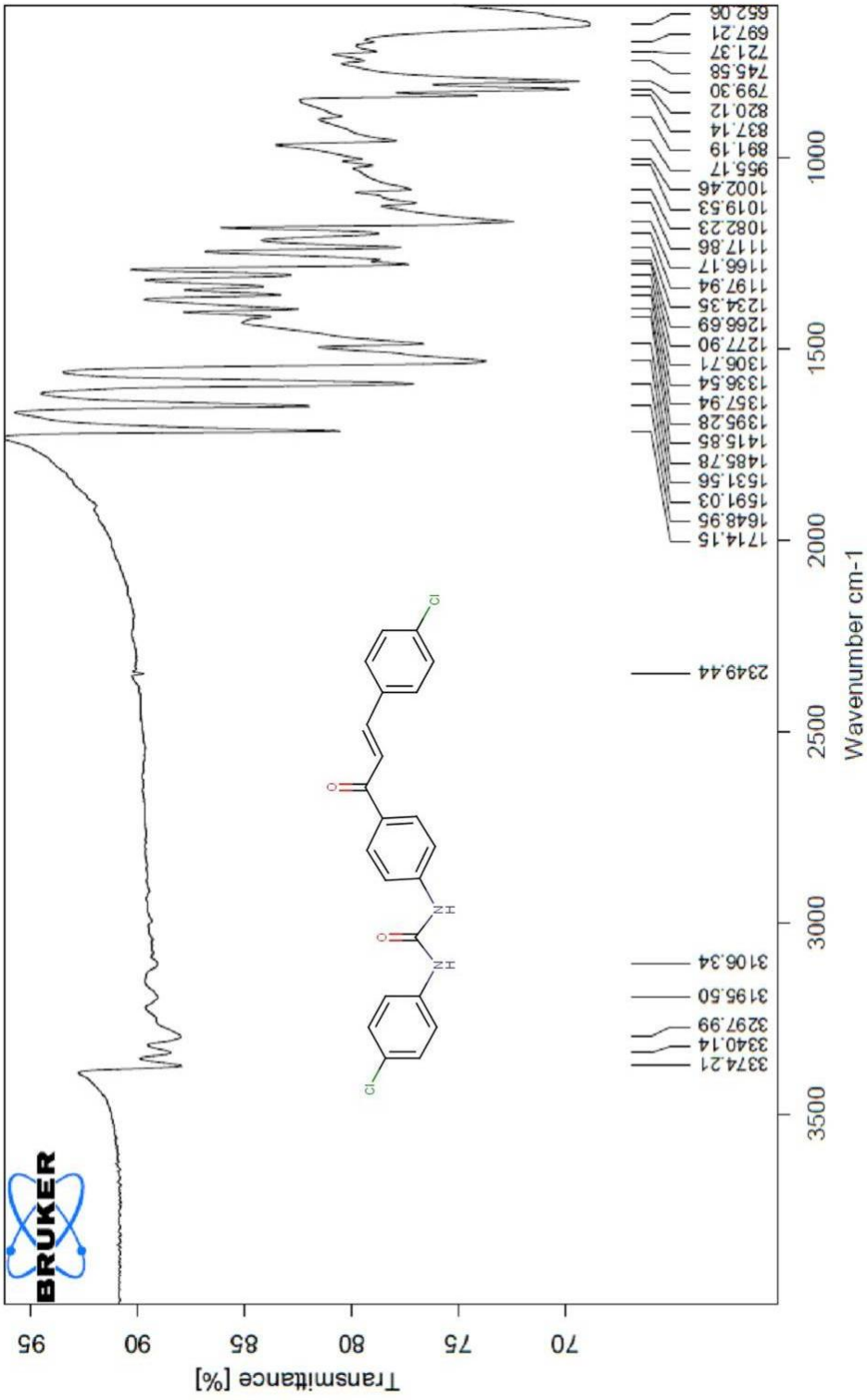

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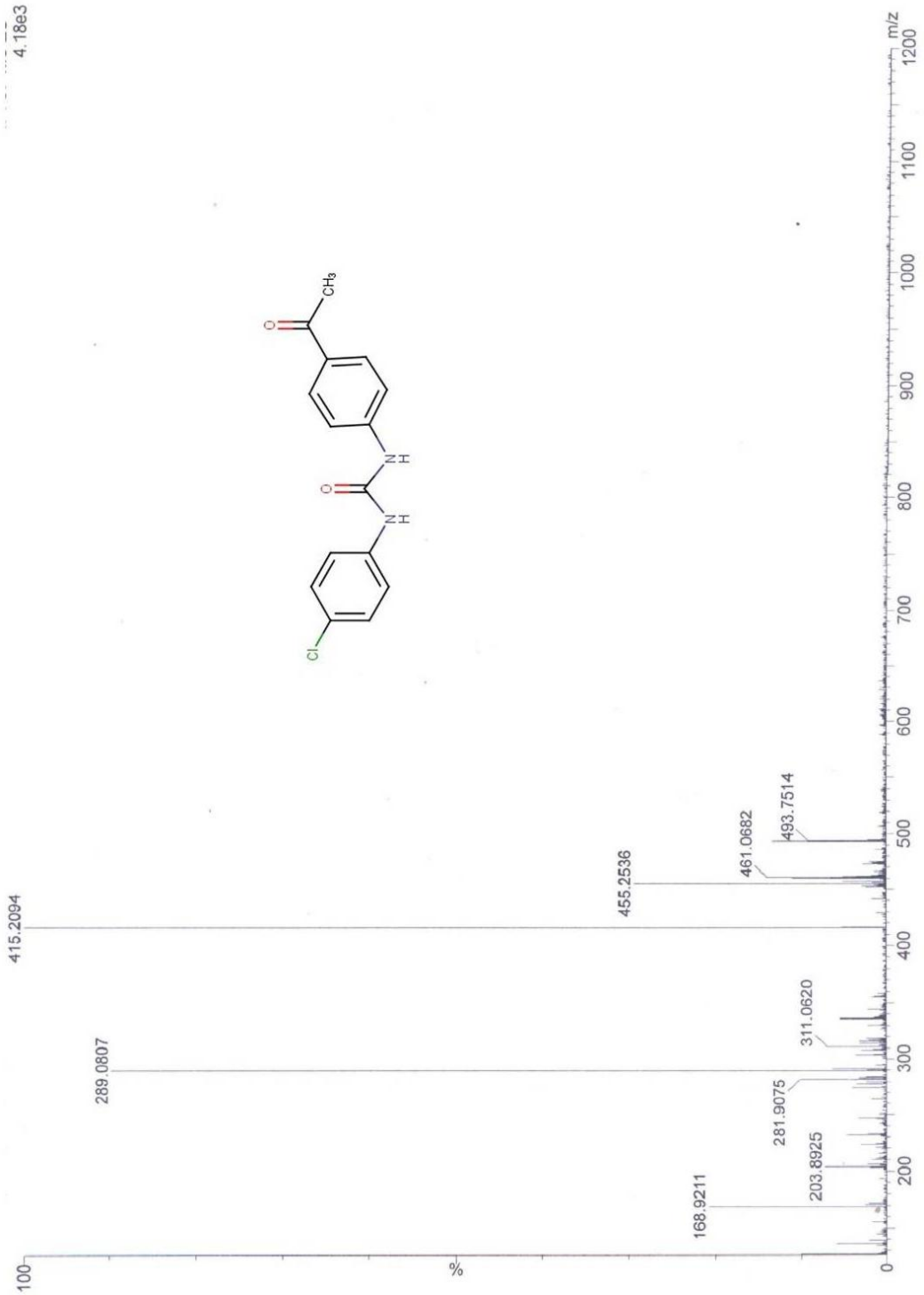

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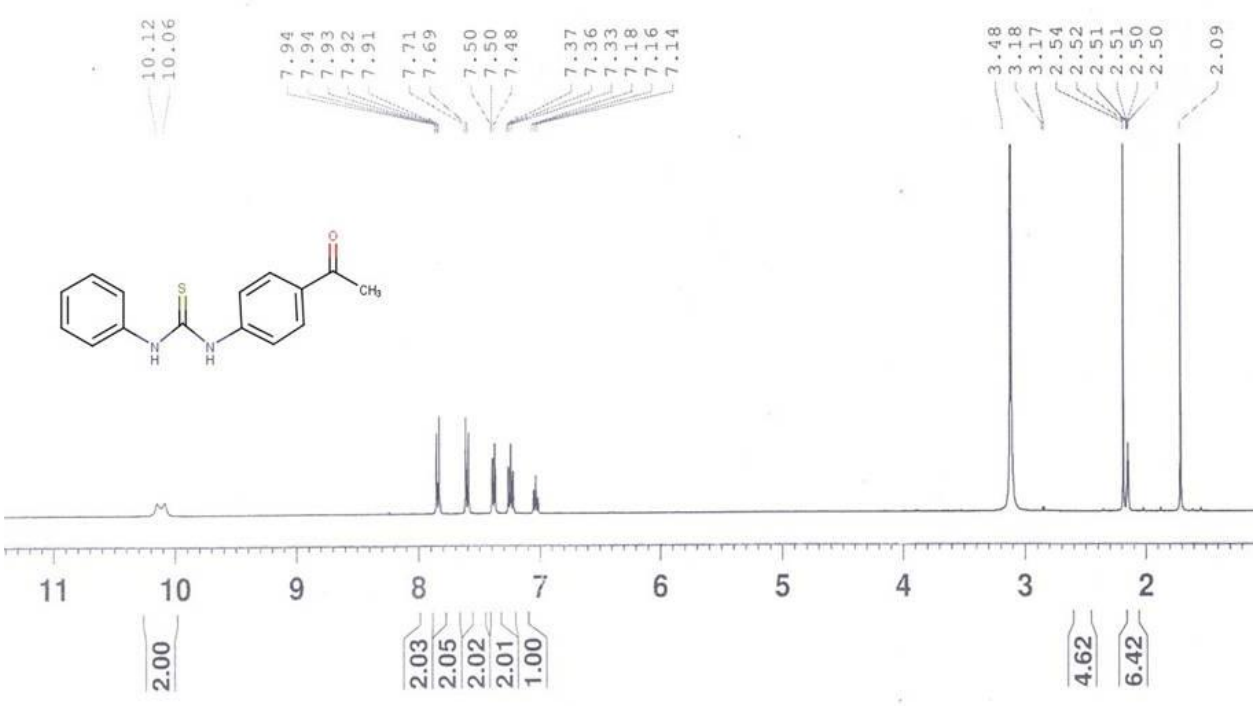
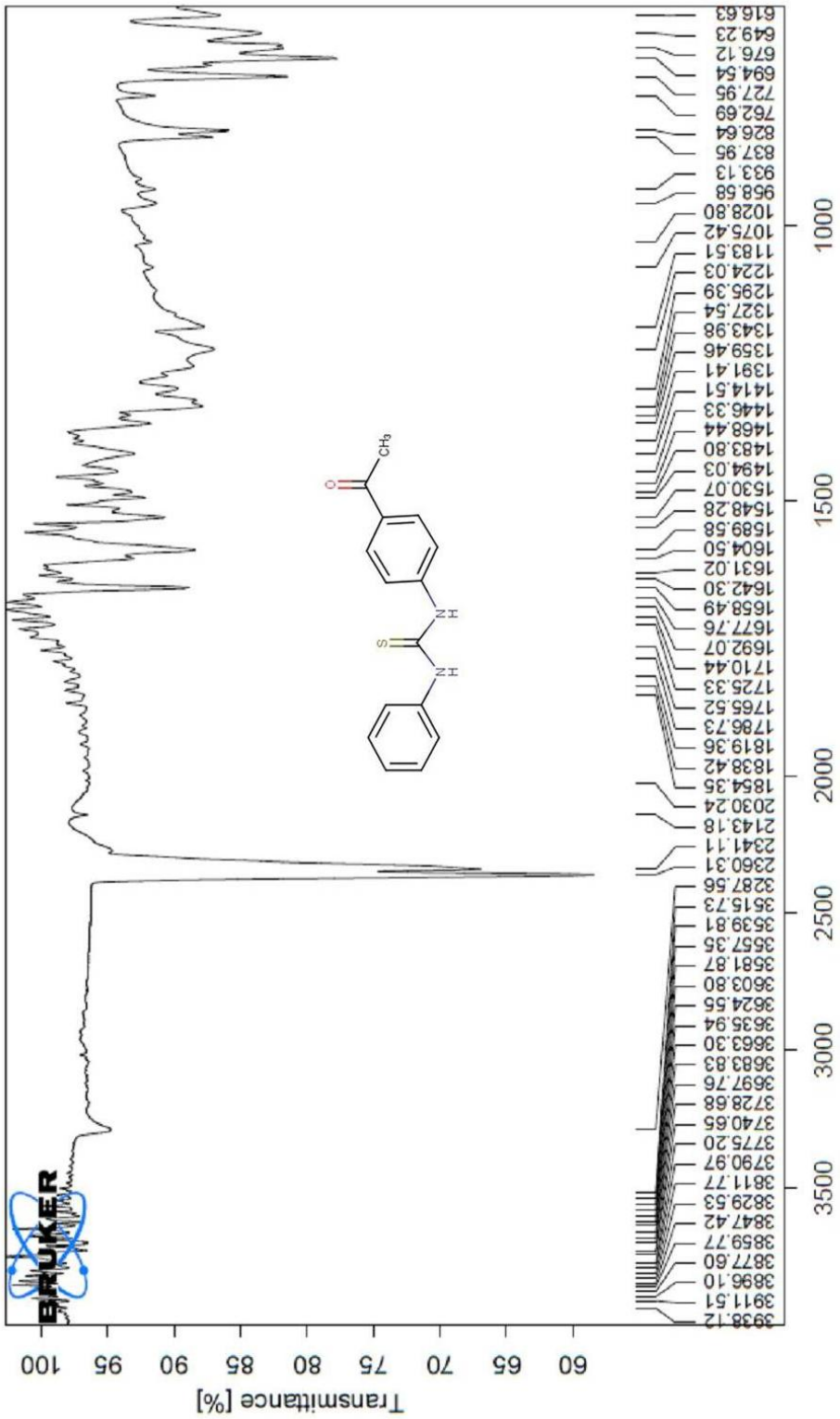


^{1}H NMR of 4-N-(N′-phenylthiourenyl) acetophenone (3b)

^{13}C NMR 4-N-(N′-phenylthiourenyl) acetophenone (3b)



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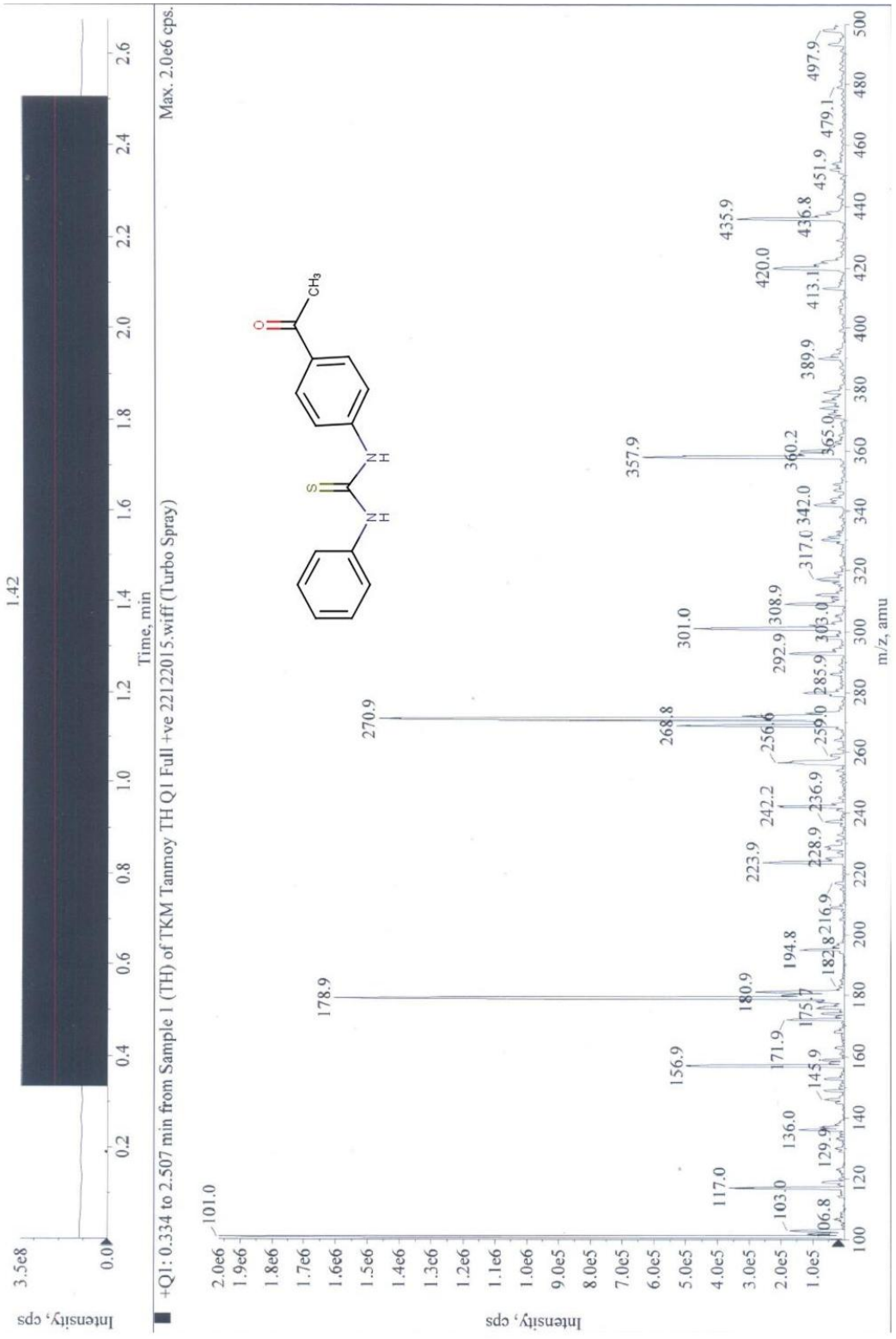
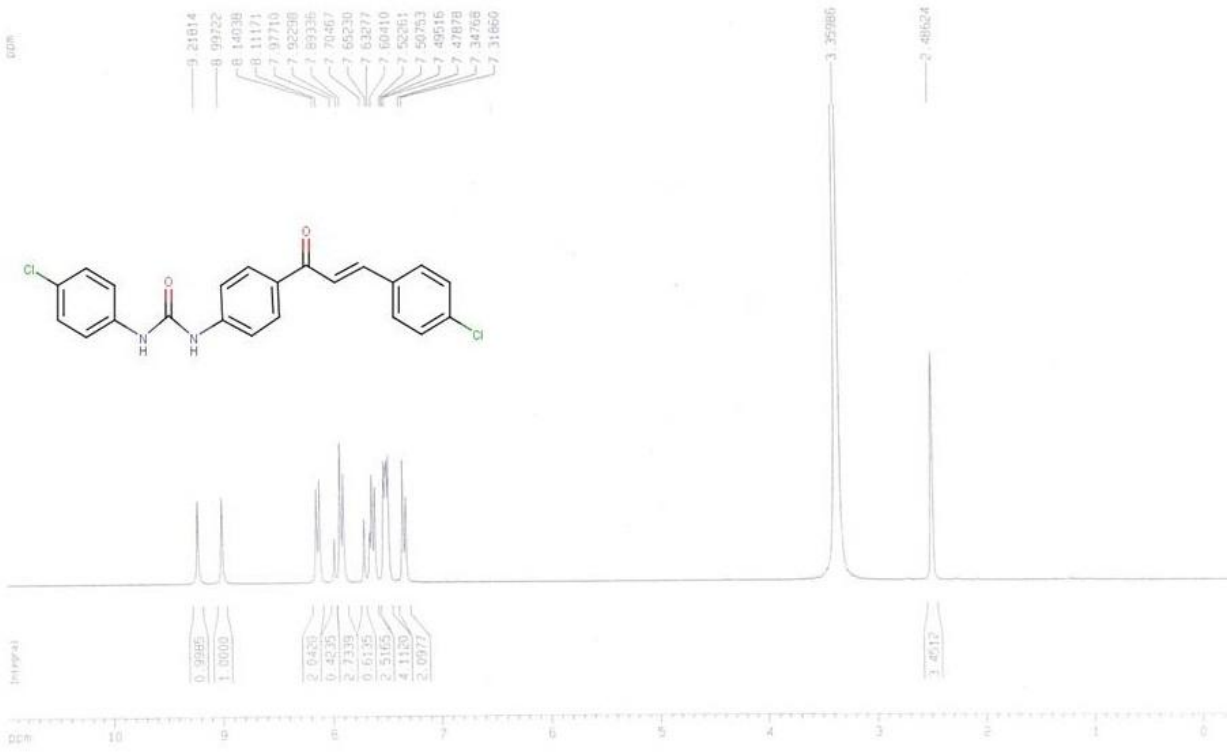


Mass spectra of 4-N-(N'-phenylthiourenyl) acetophenone (3b)



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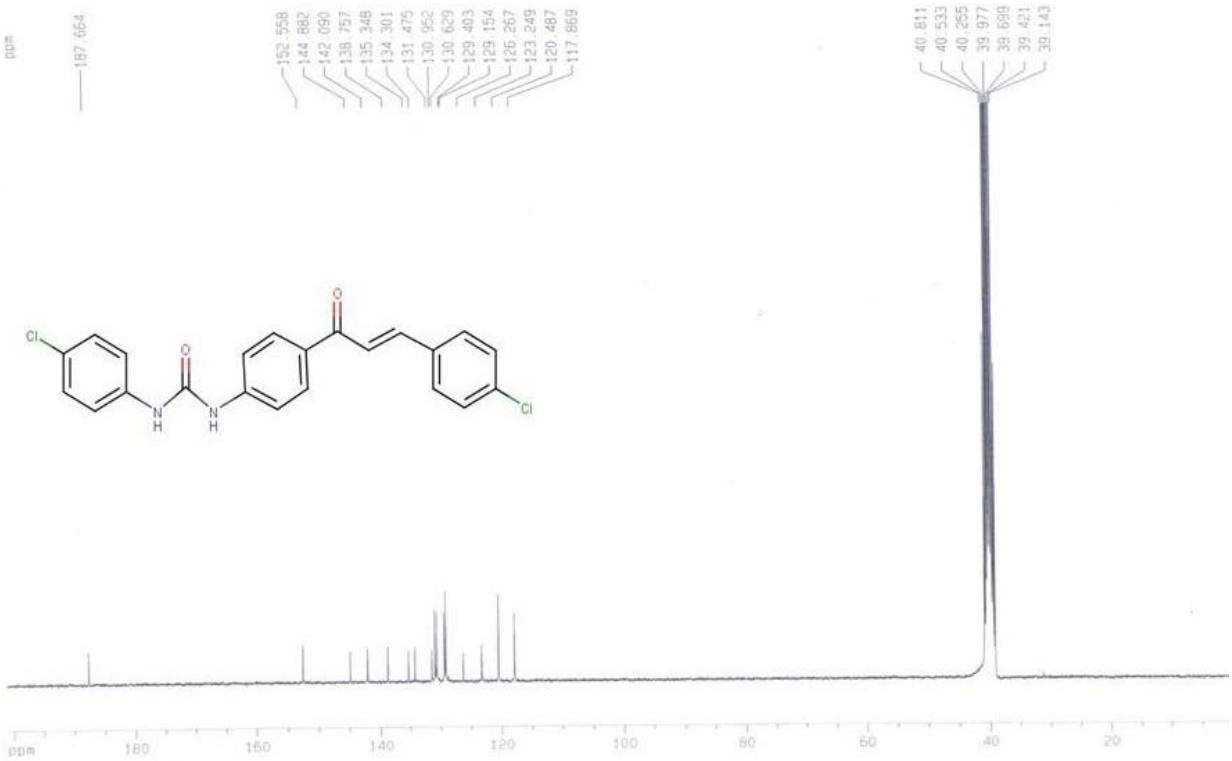

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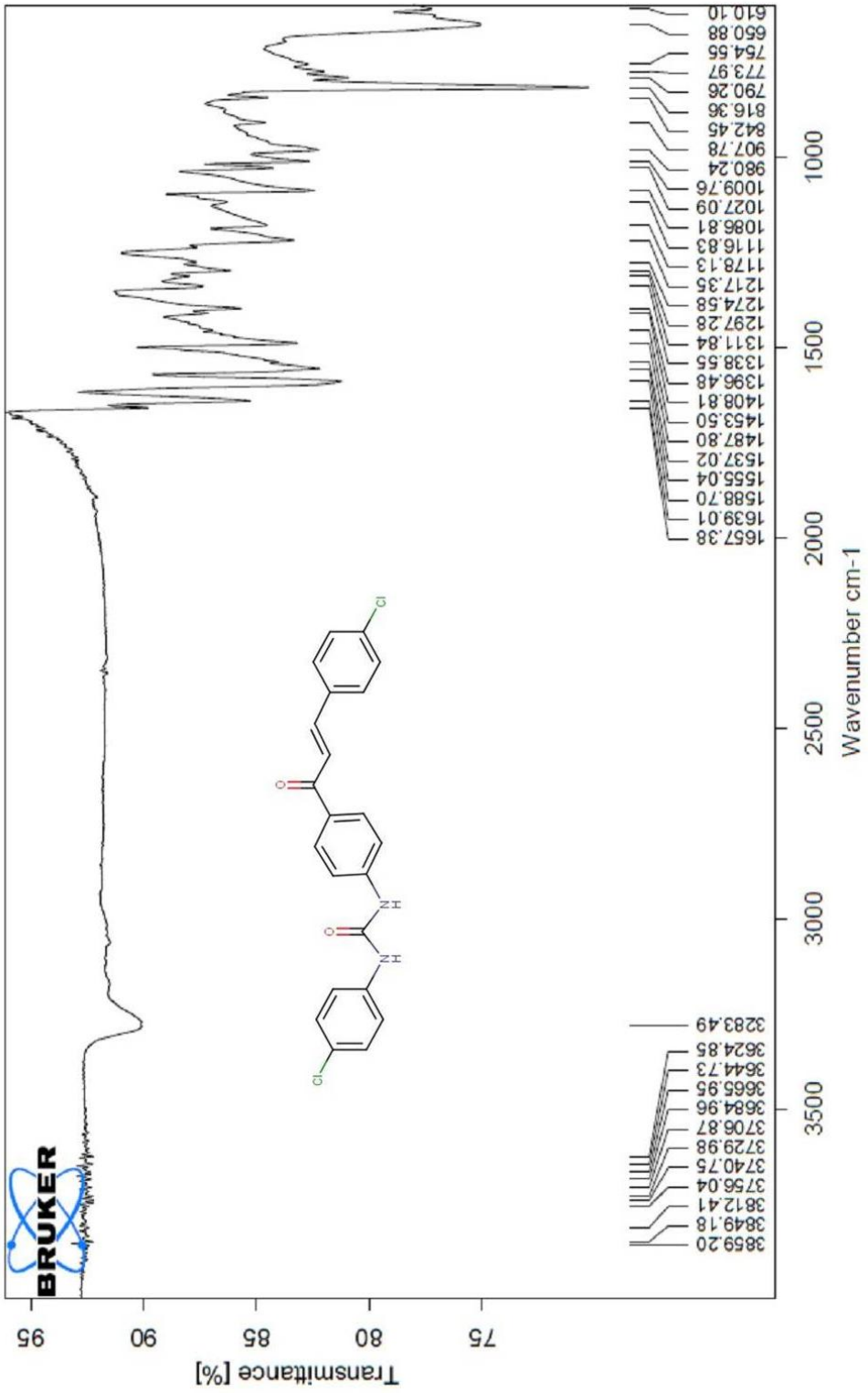

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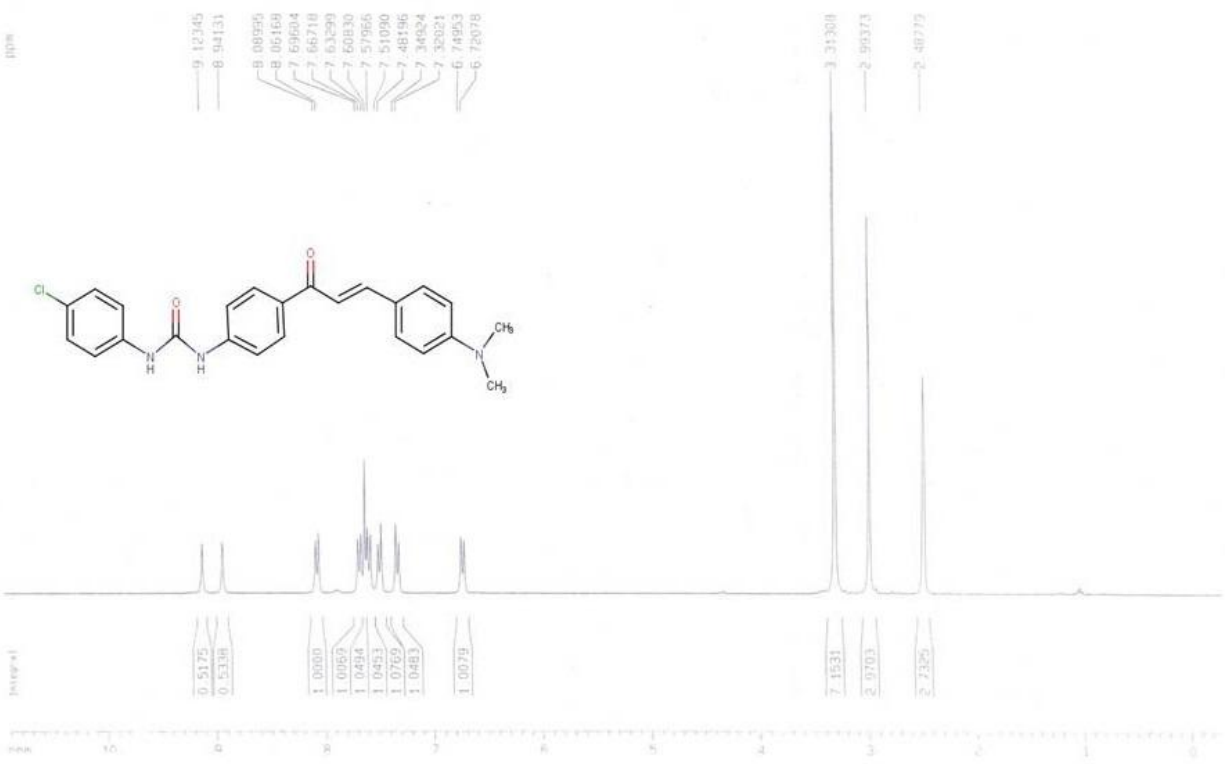

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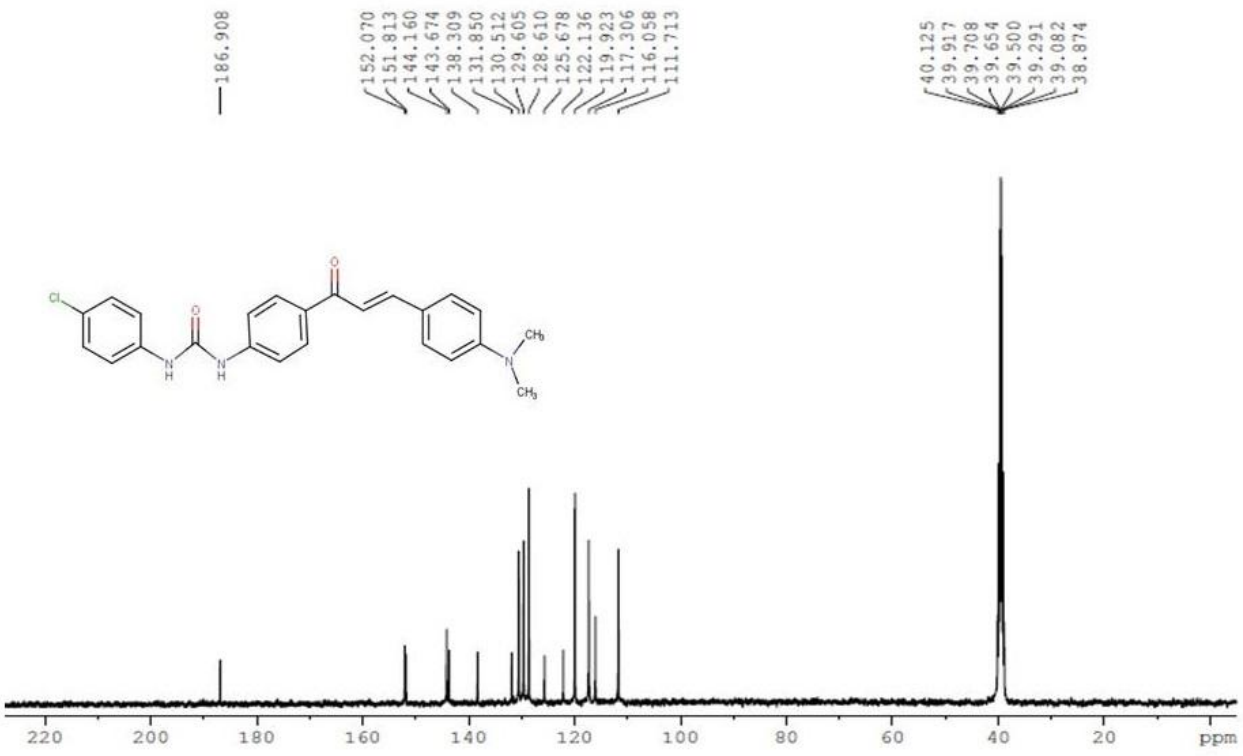

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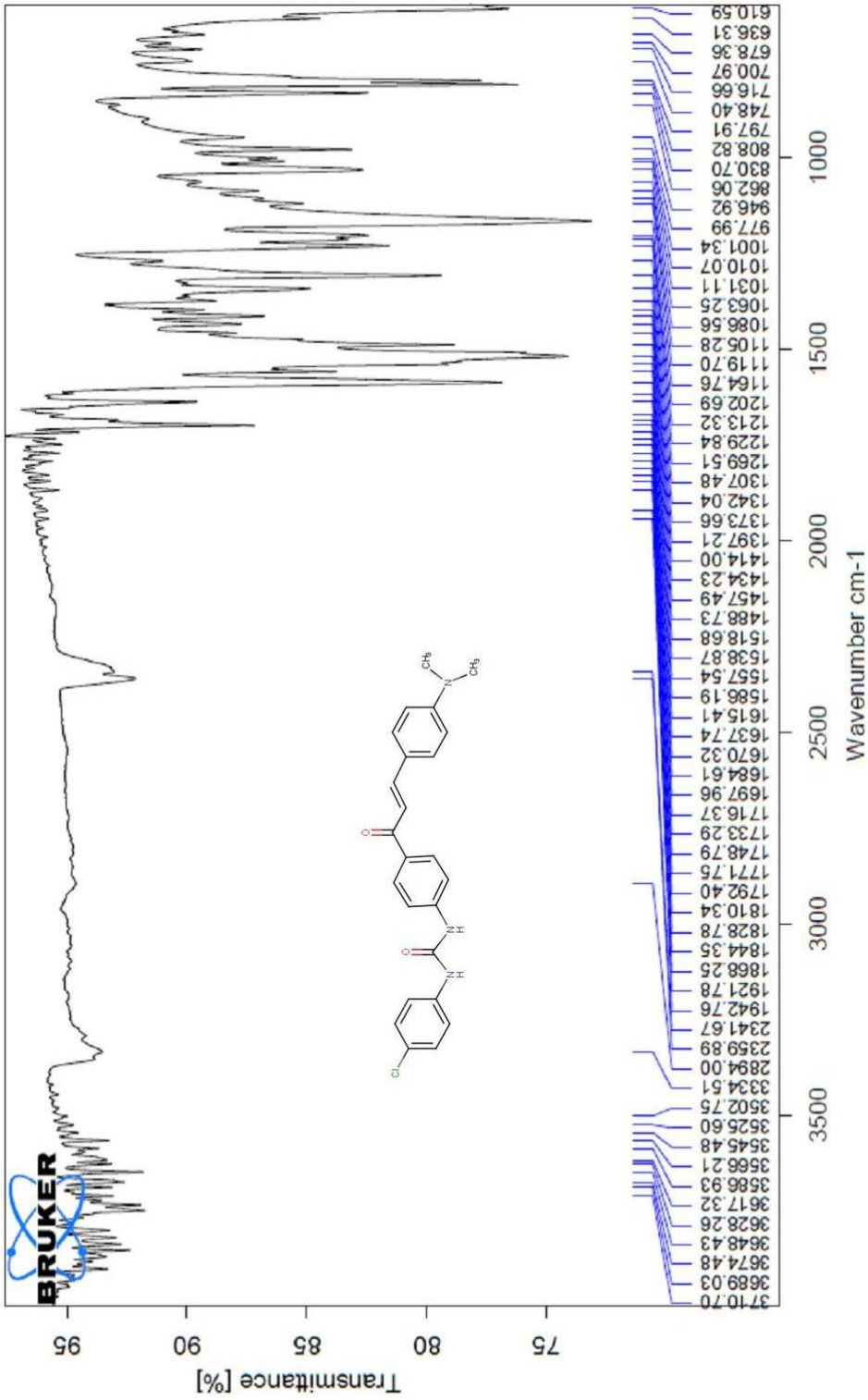

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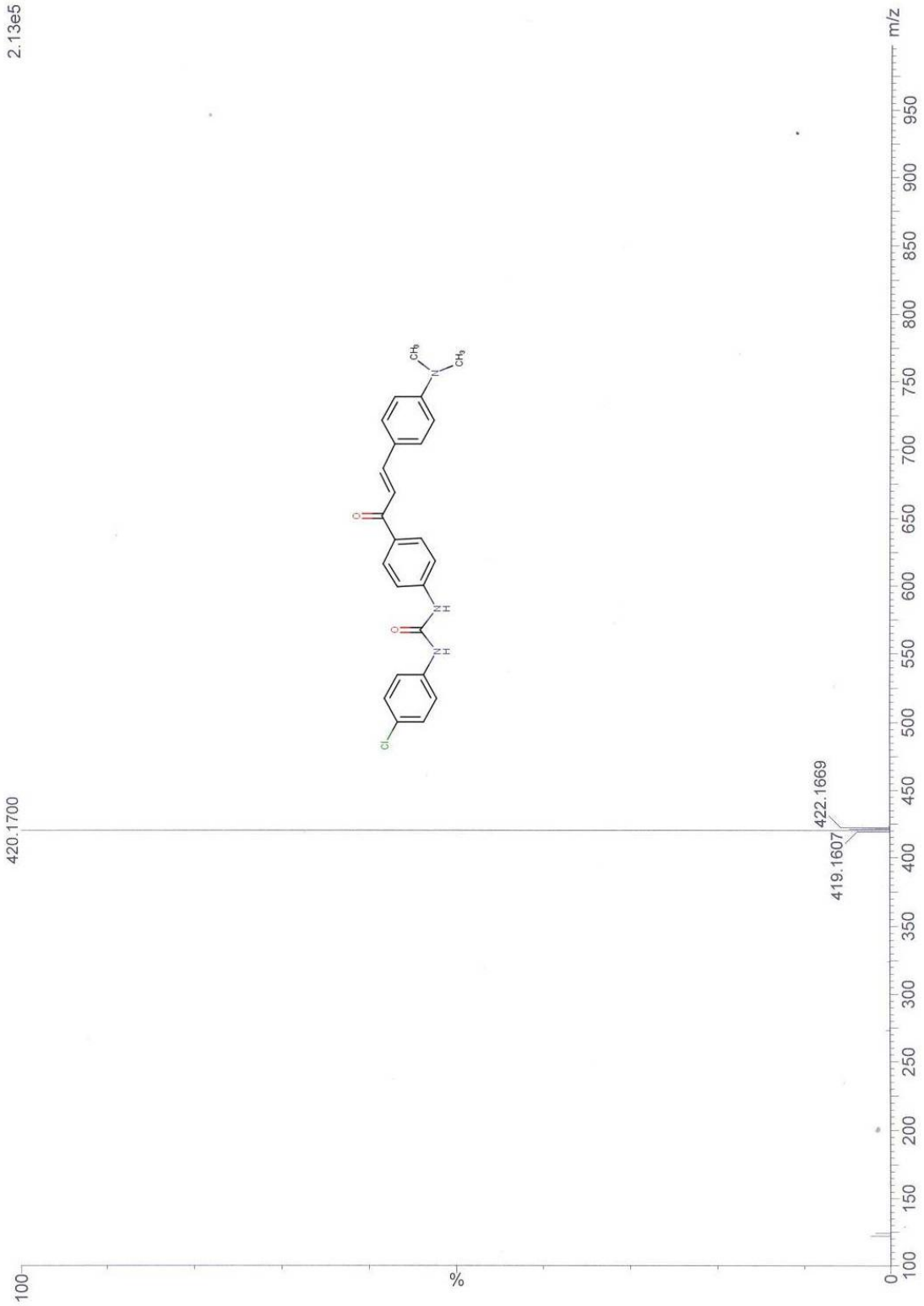
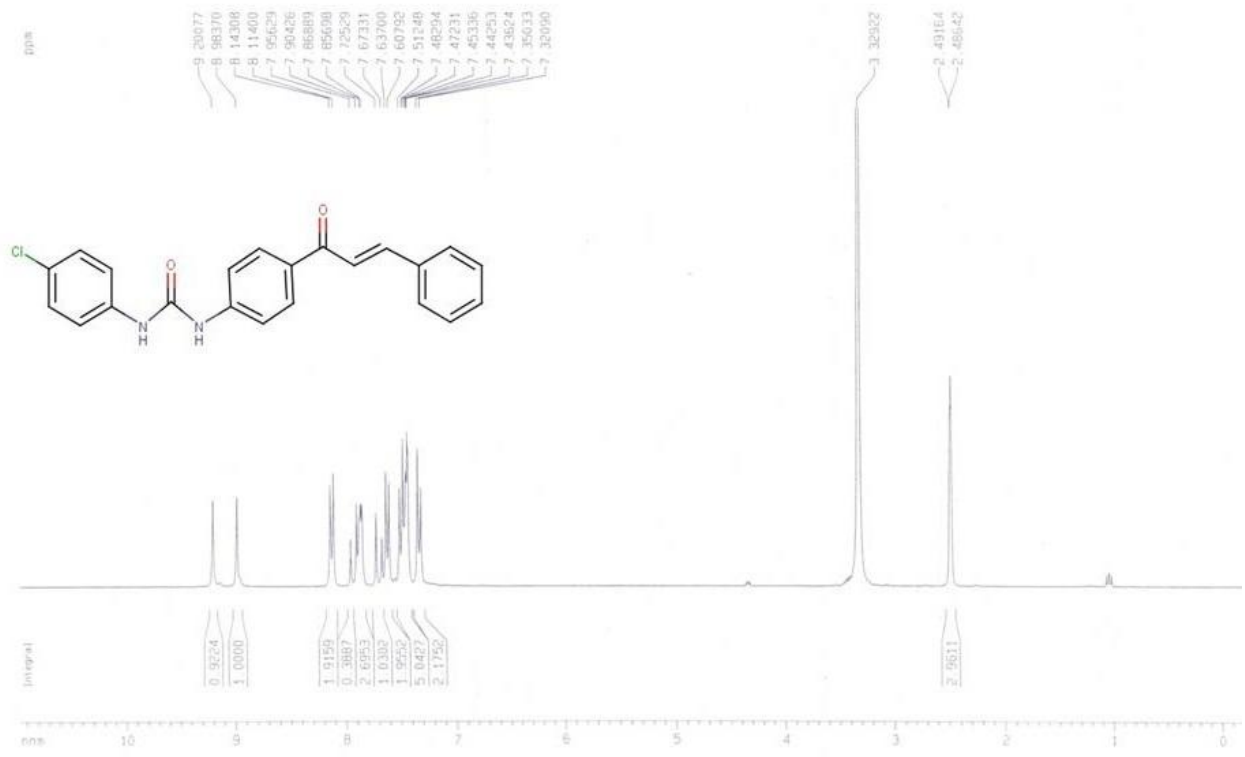


Mass spectra of 1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(4-N, N- dimethylphenyl)-2-propen-1-one (5b)



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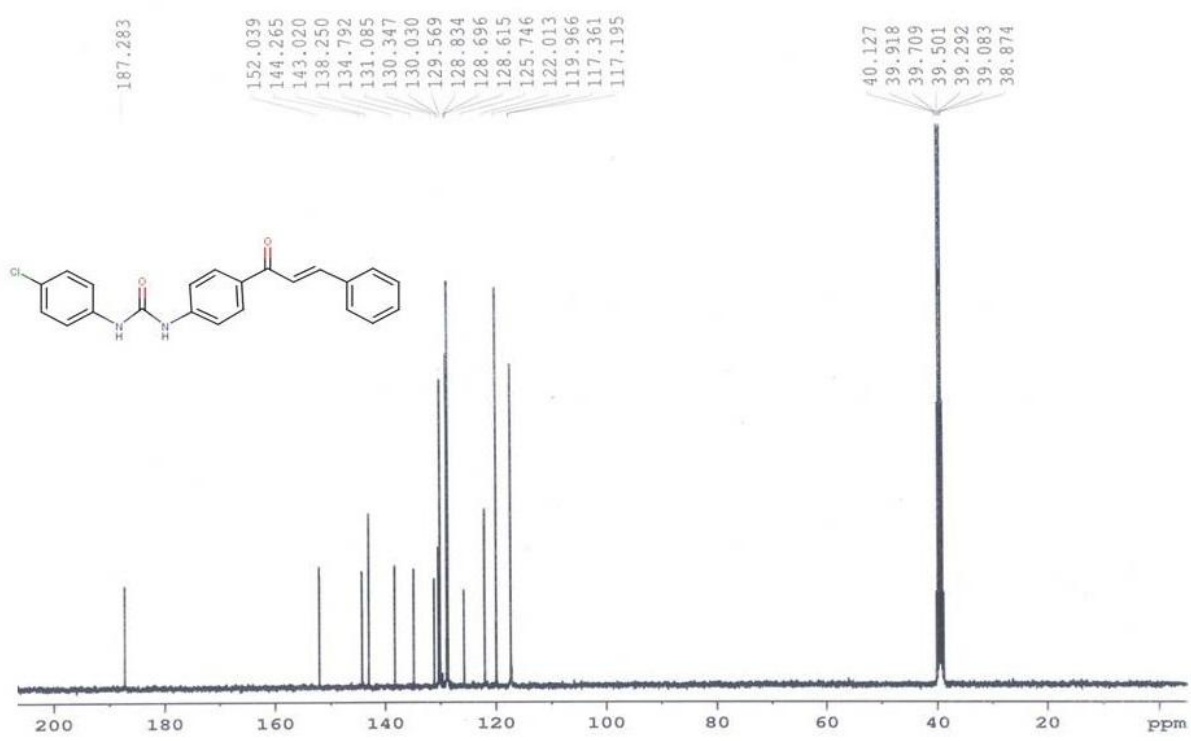

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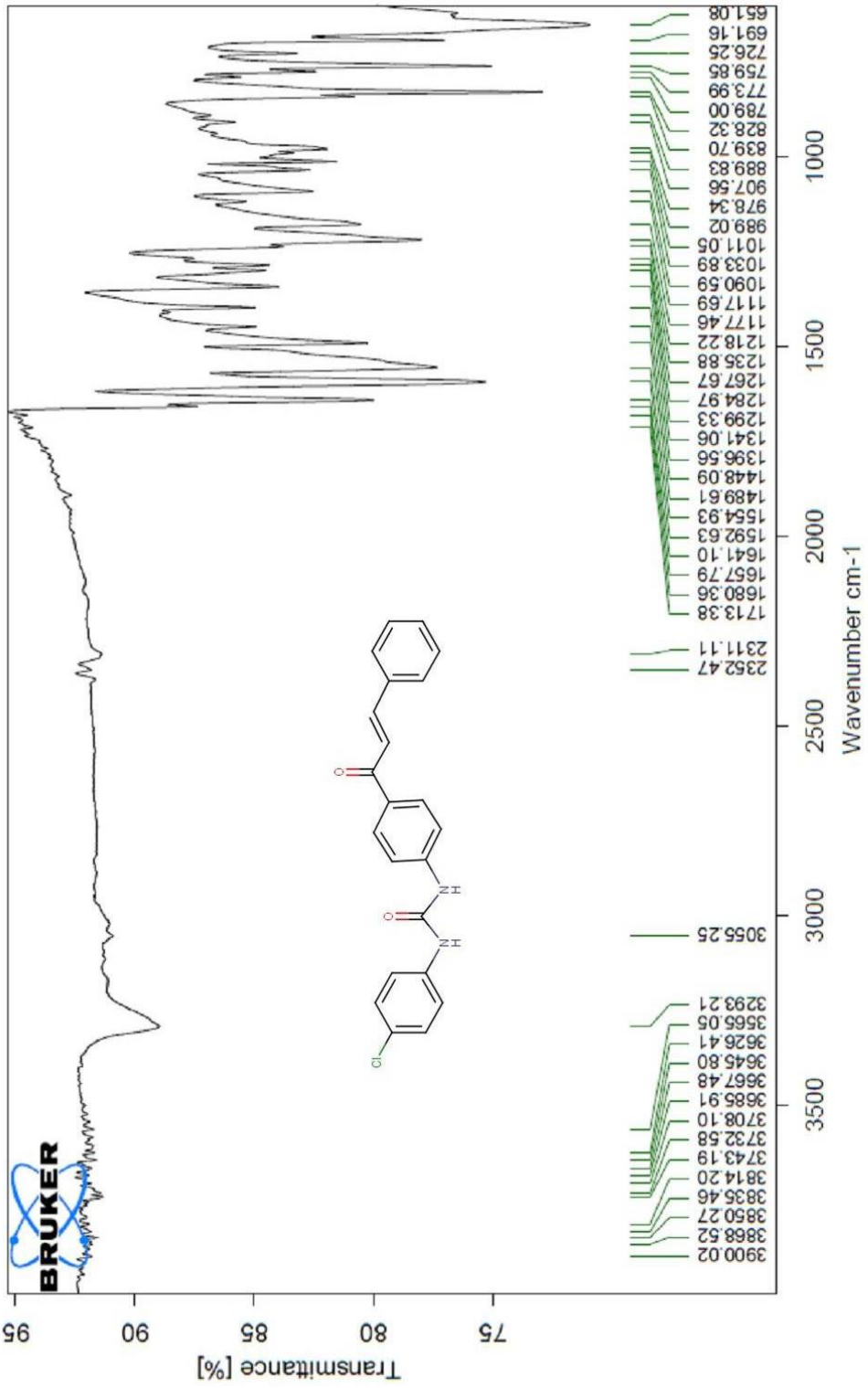

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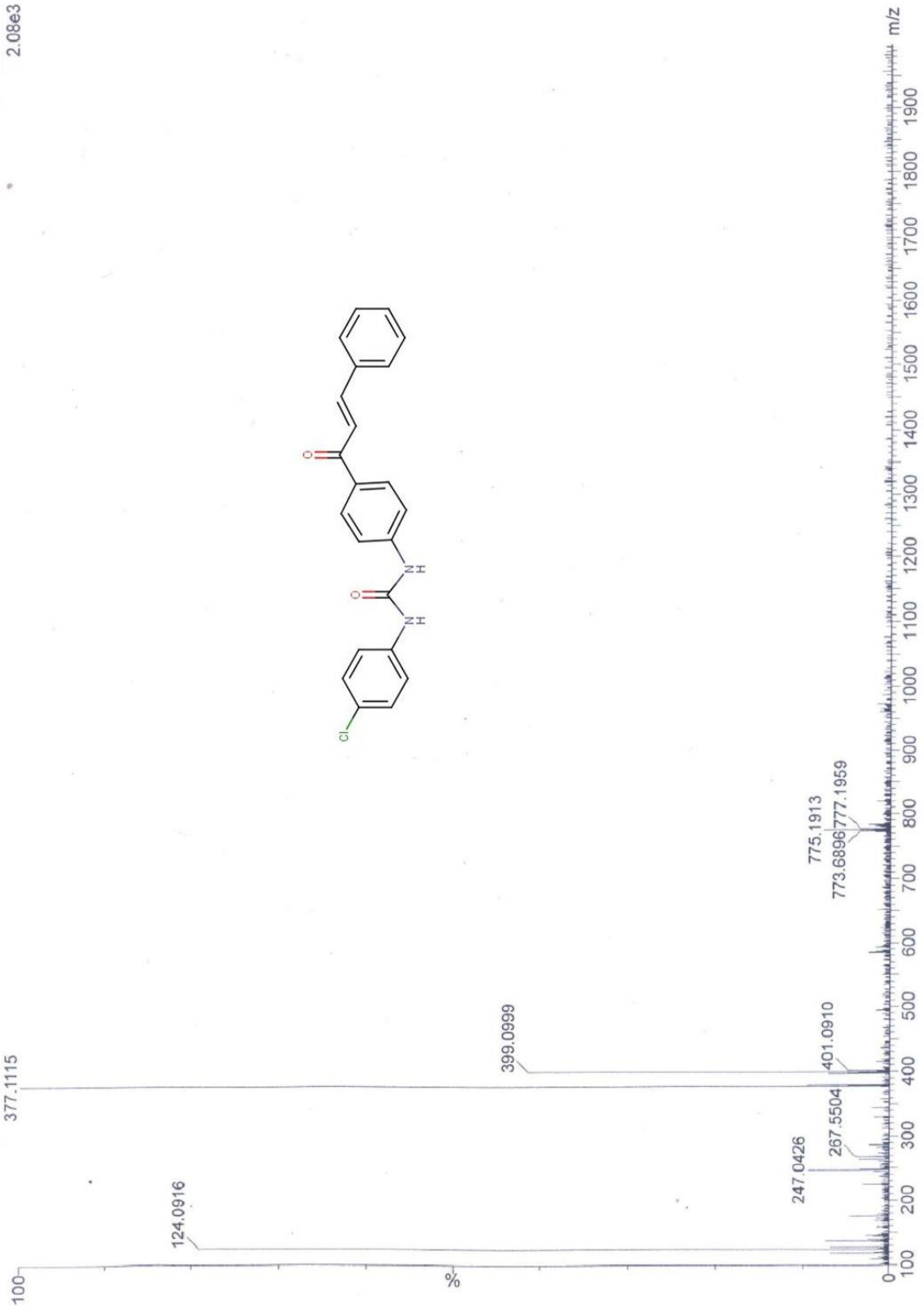

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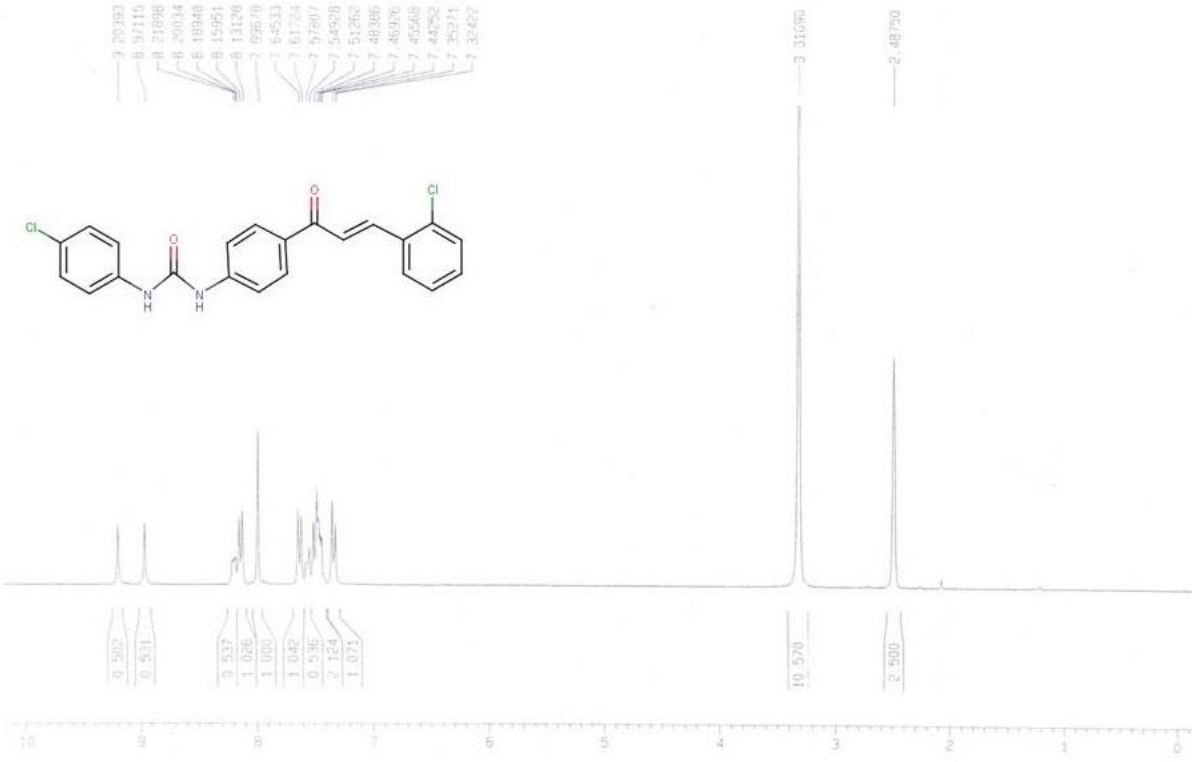

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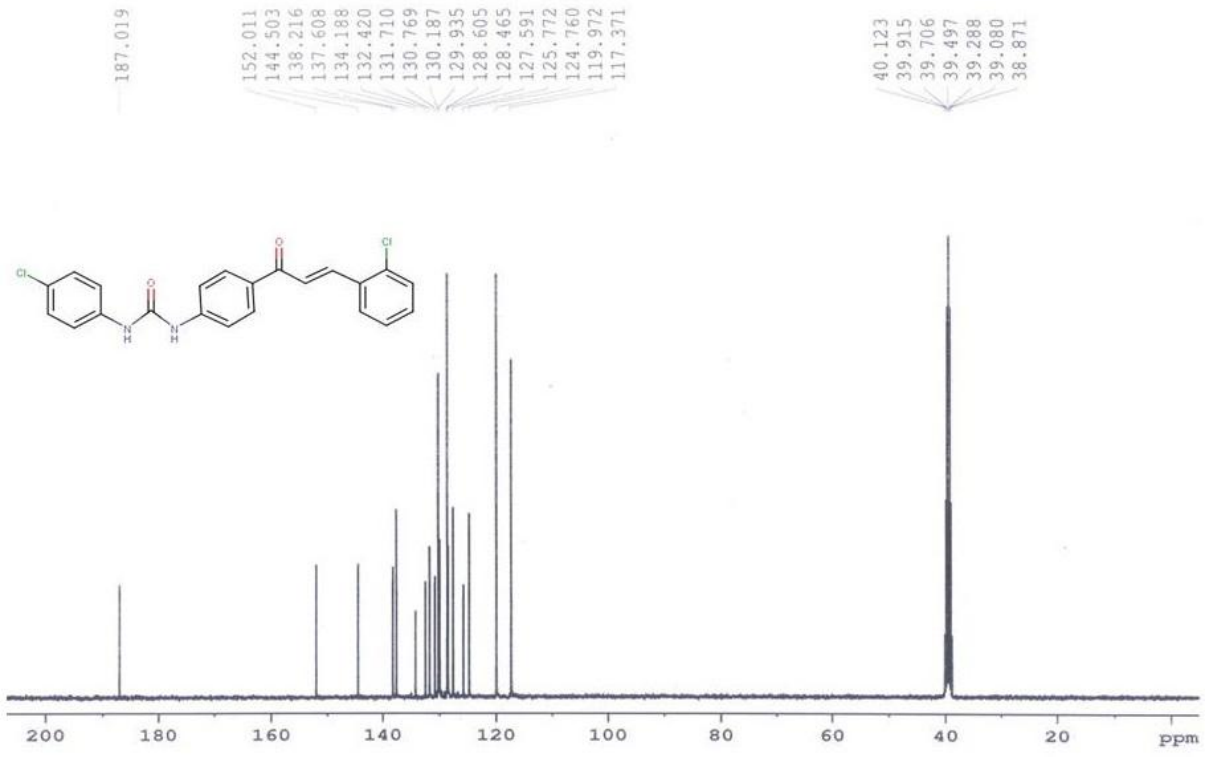

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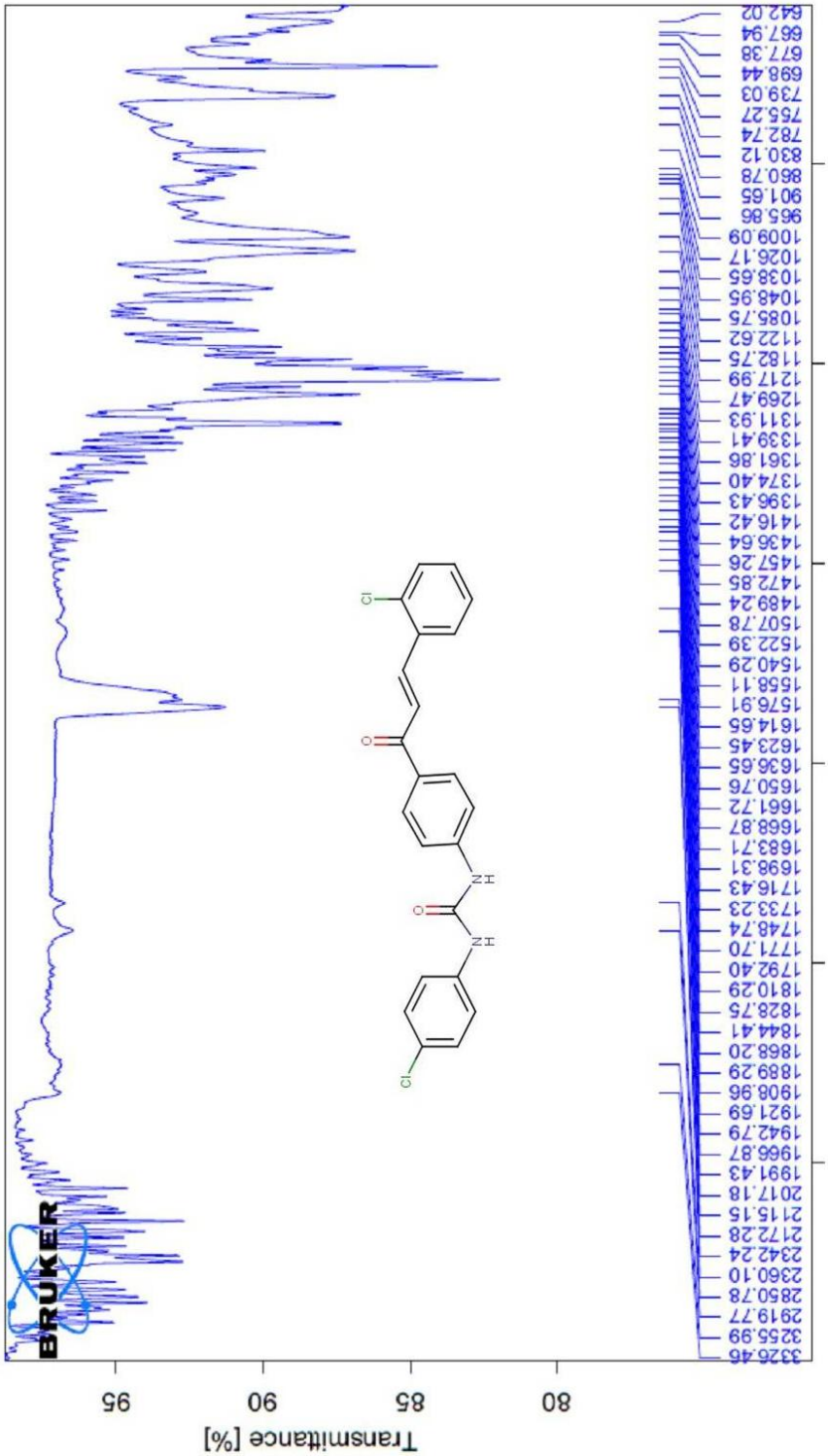

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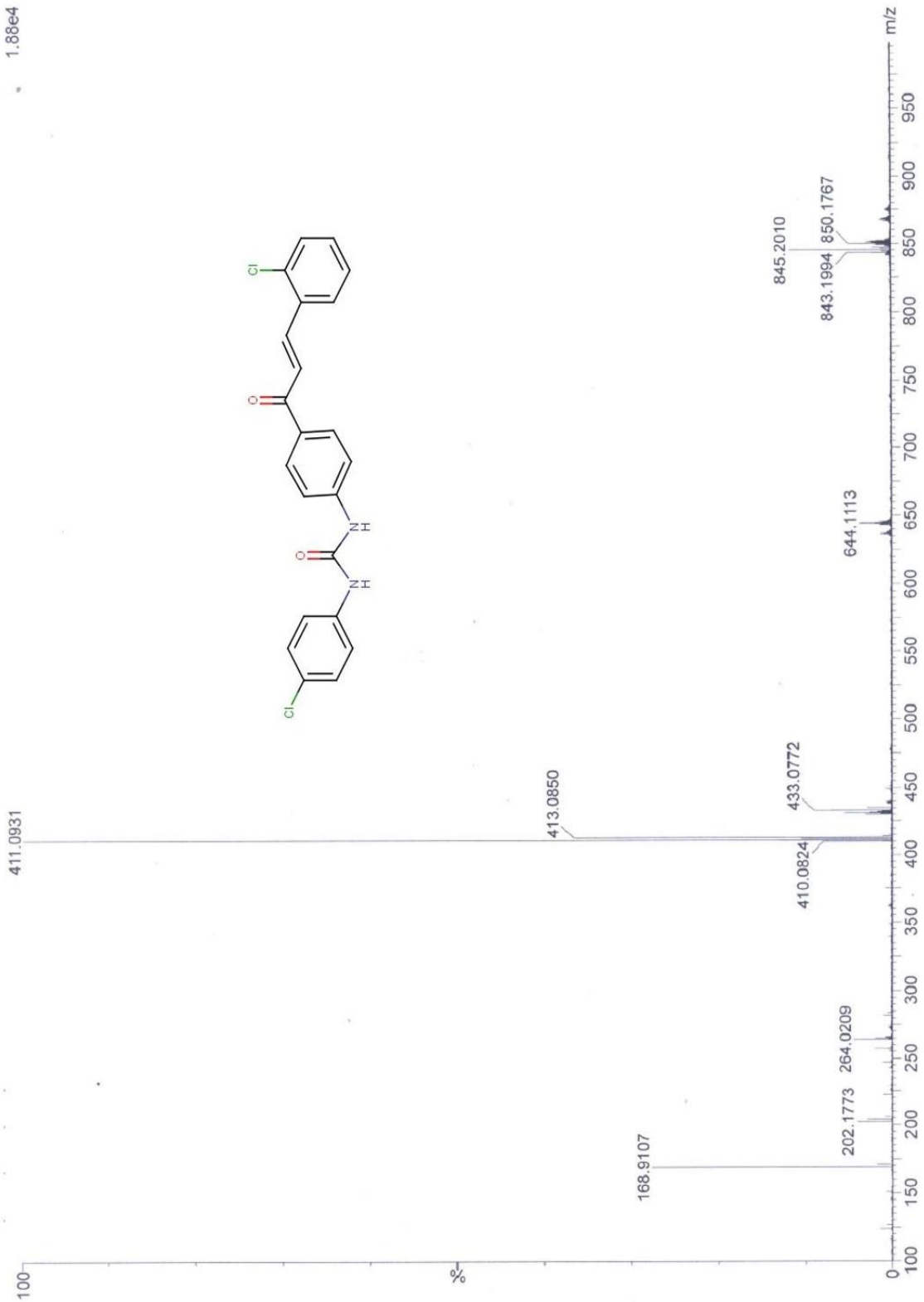

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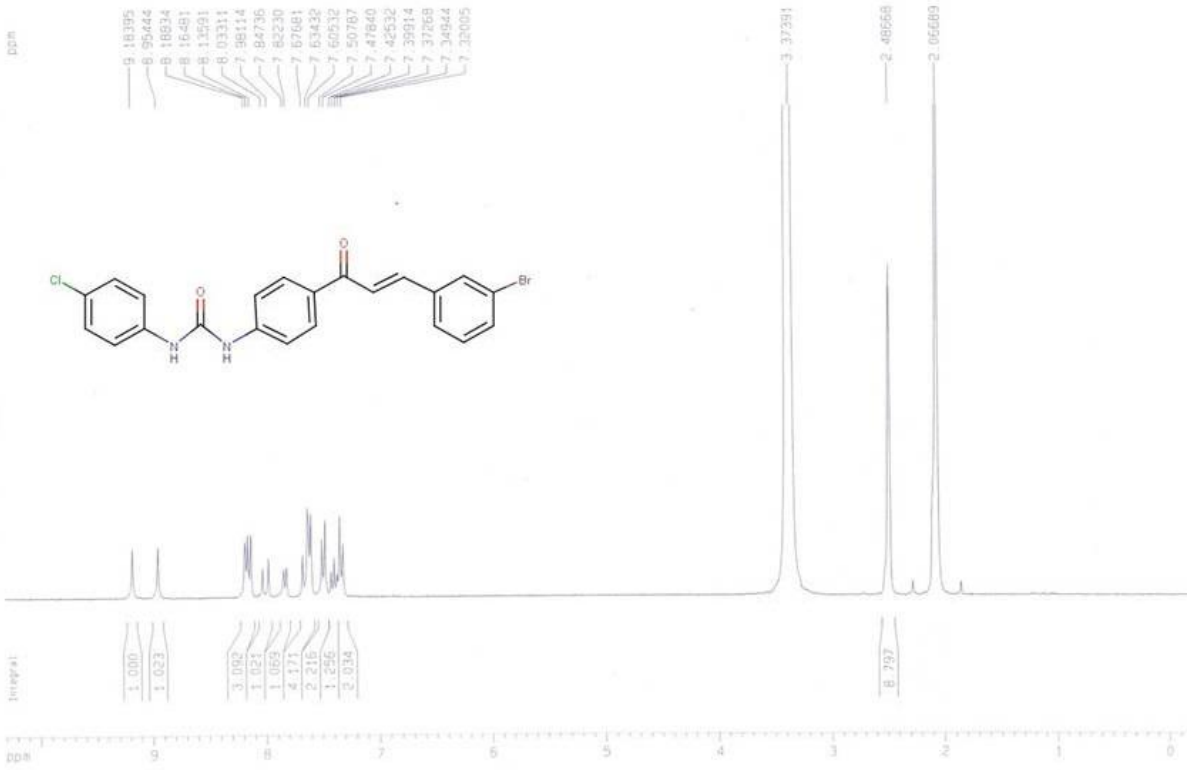
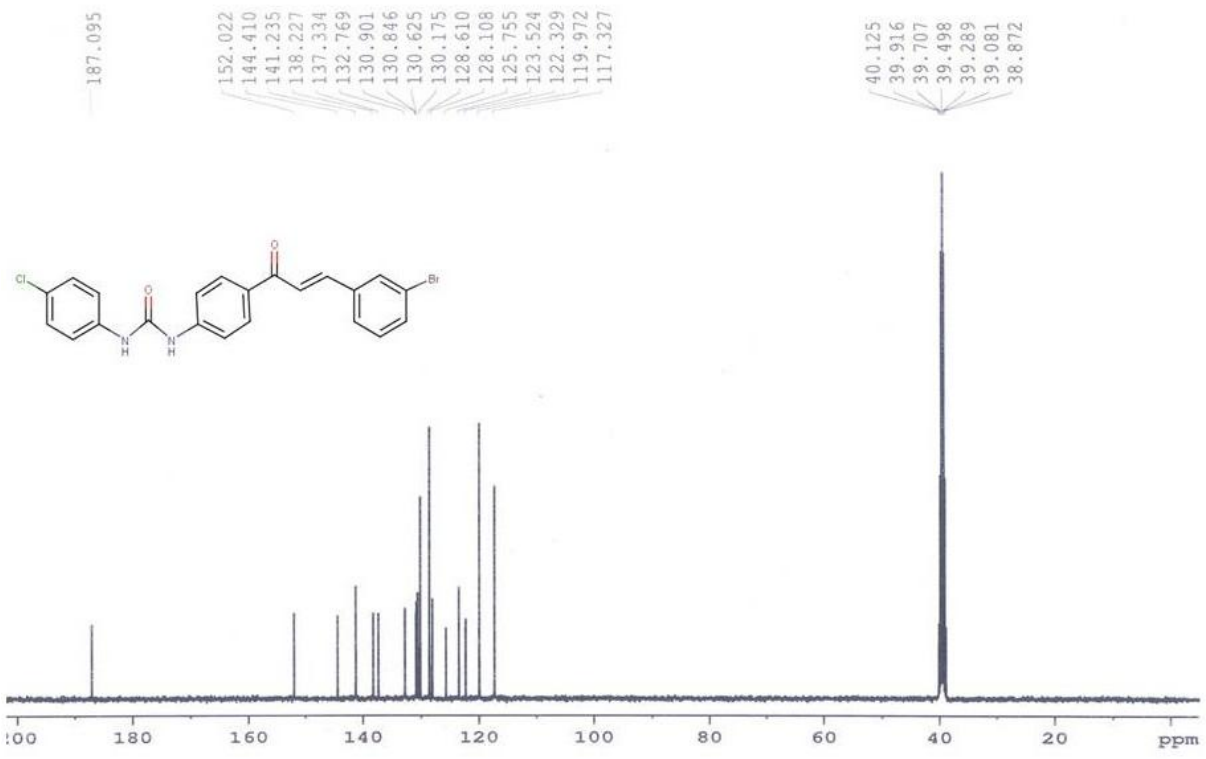


^{1}H NMR of 1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(3-bromophenyl)-2-propen-1-one (5e)



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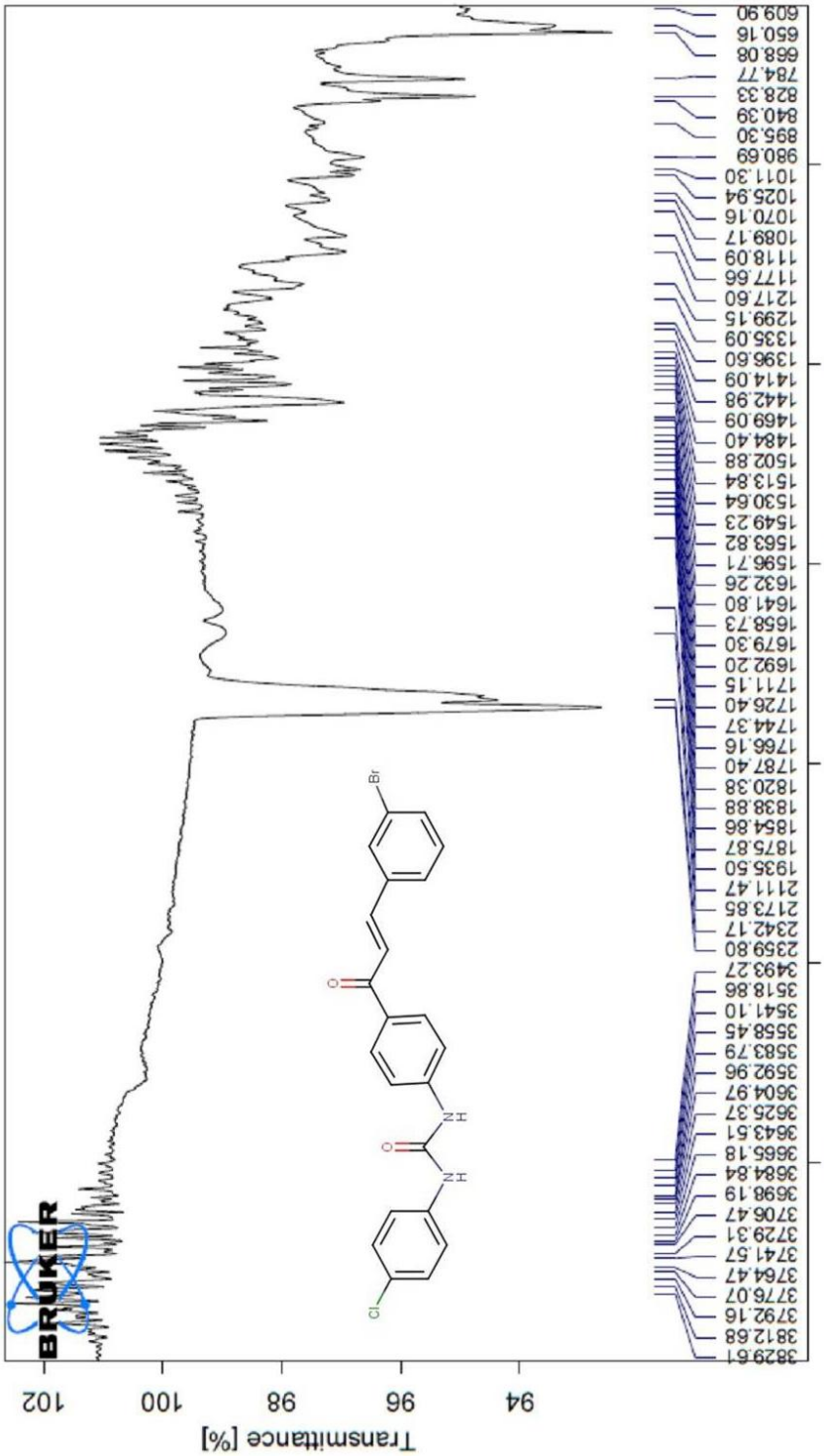
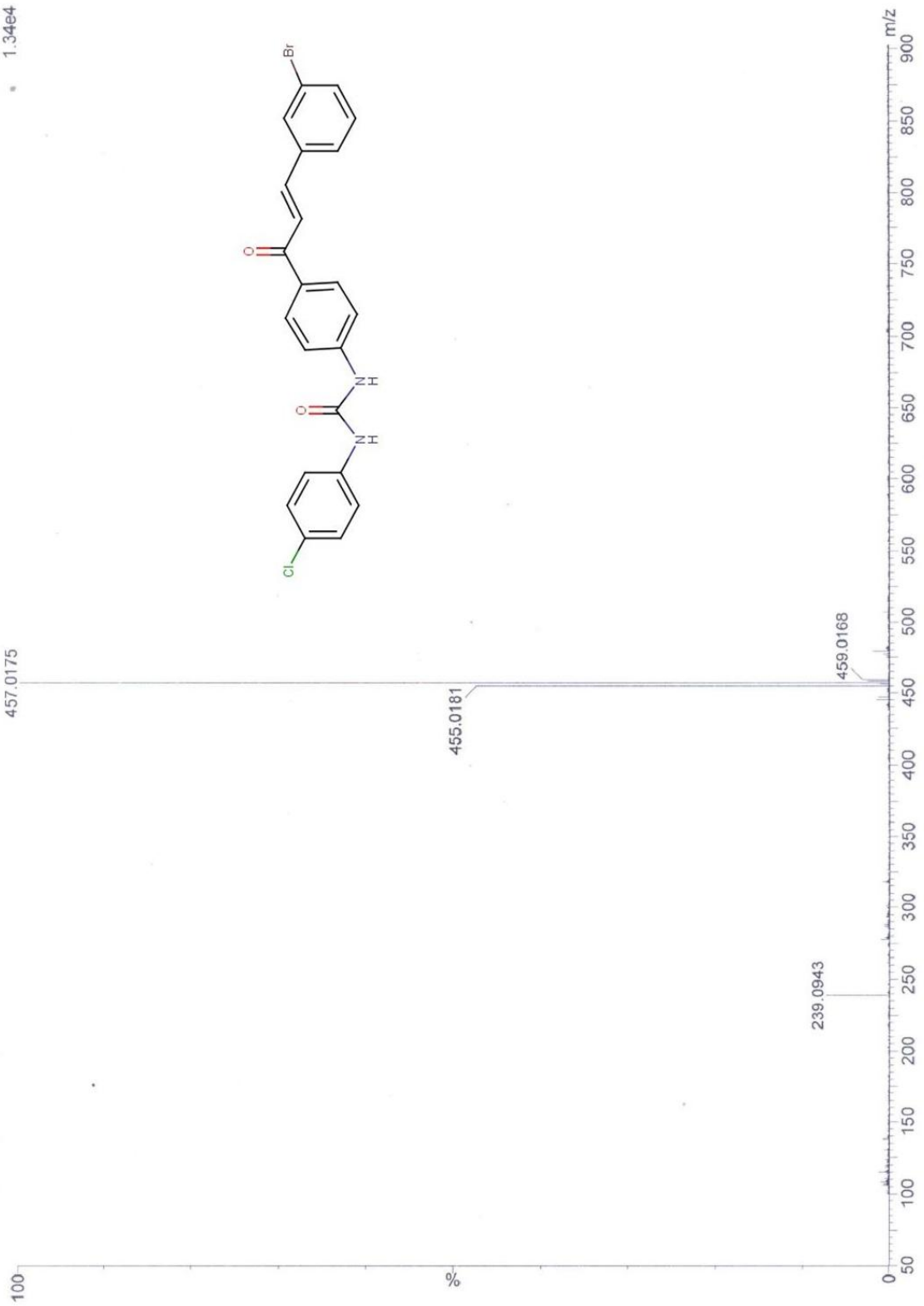


FT-IR of 1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(3-bromophenyl)-2-propen-1-one (5e)



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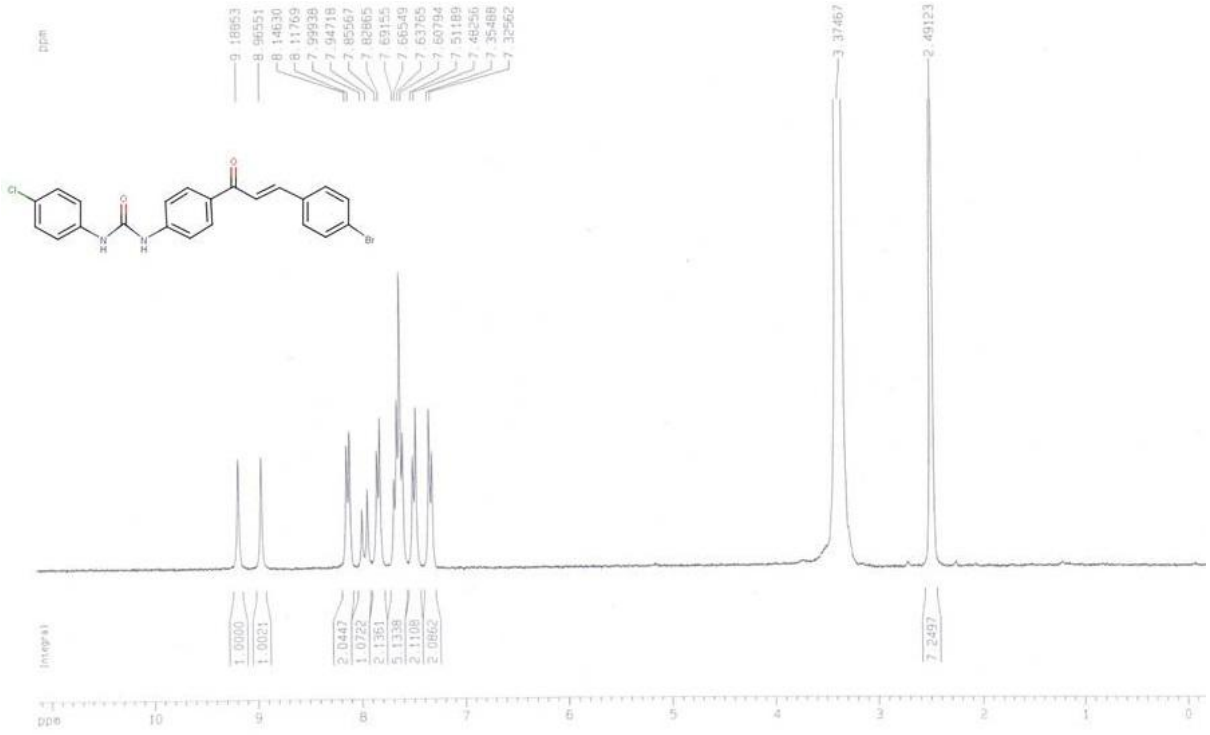

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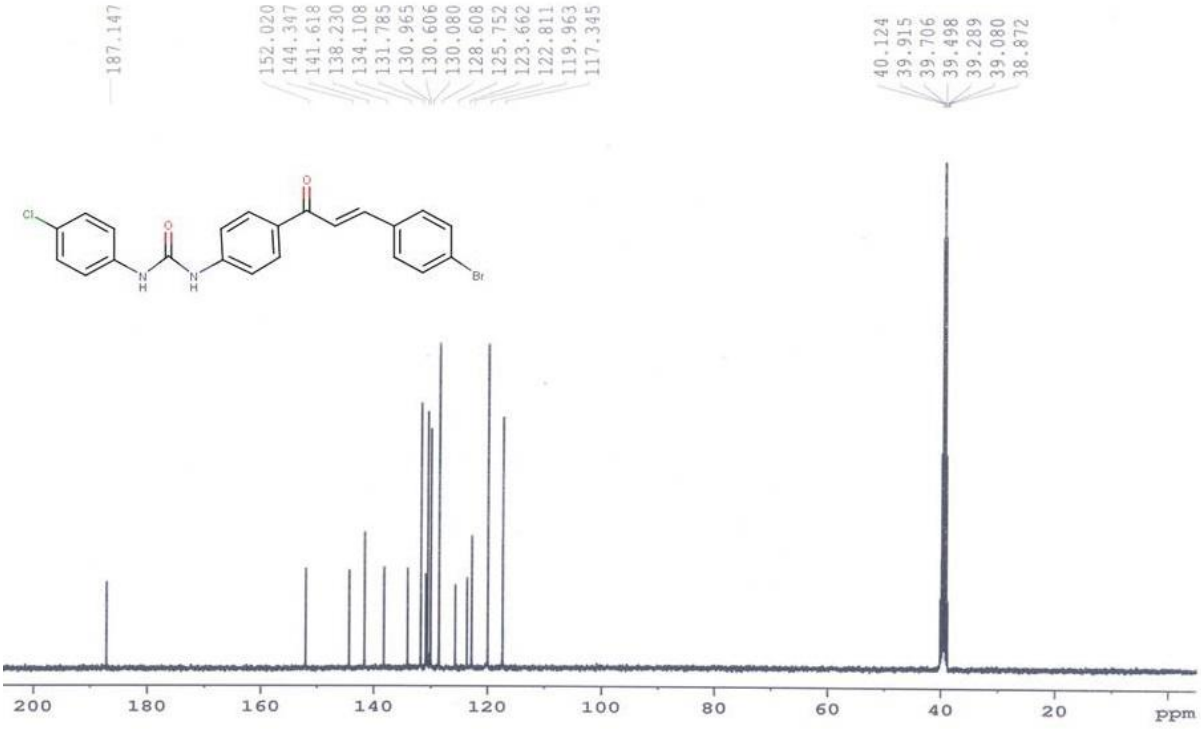

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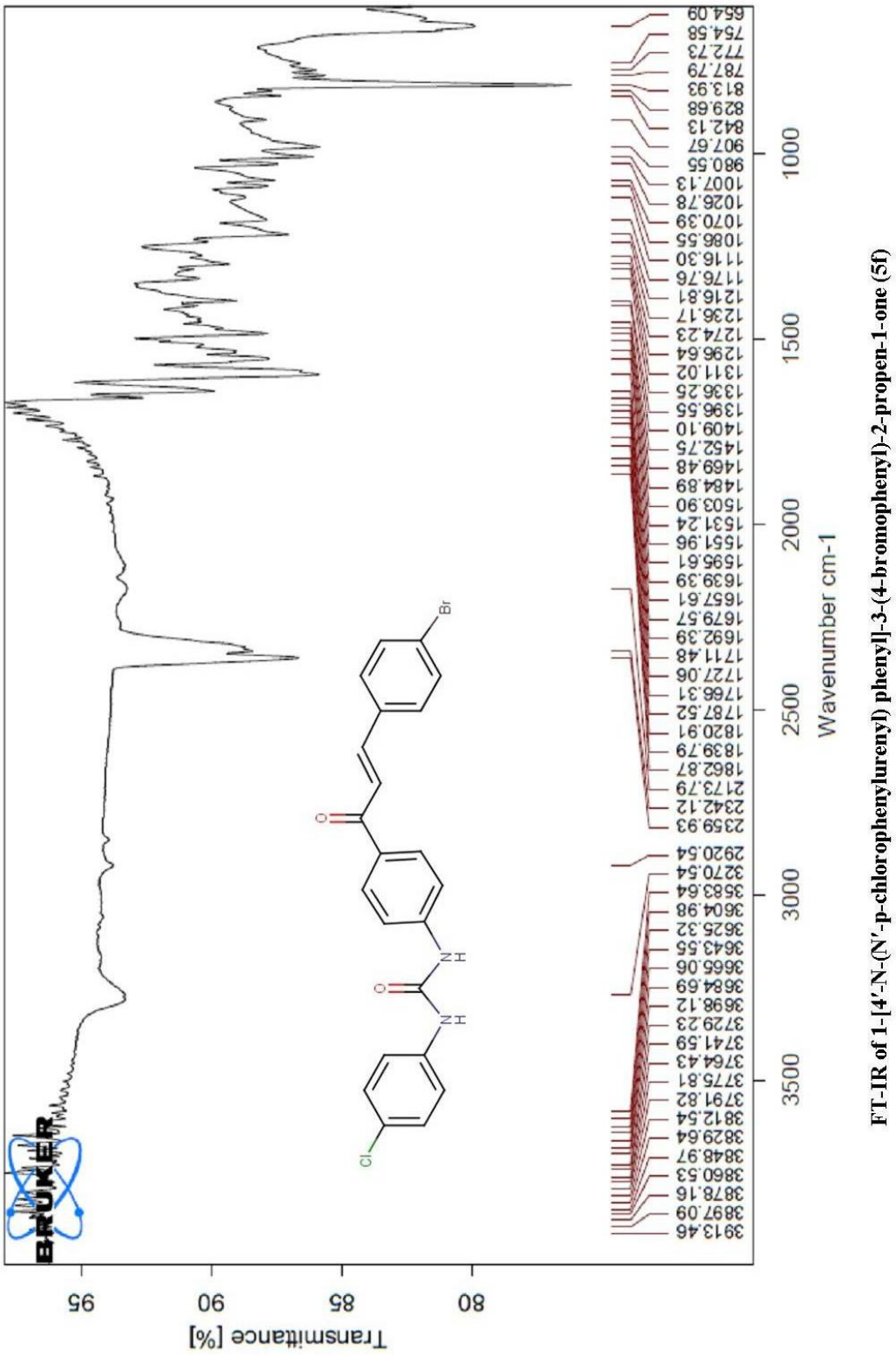

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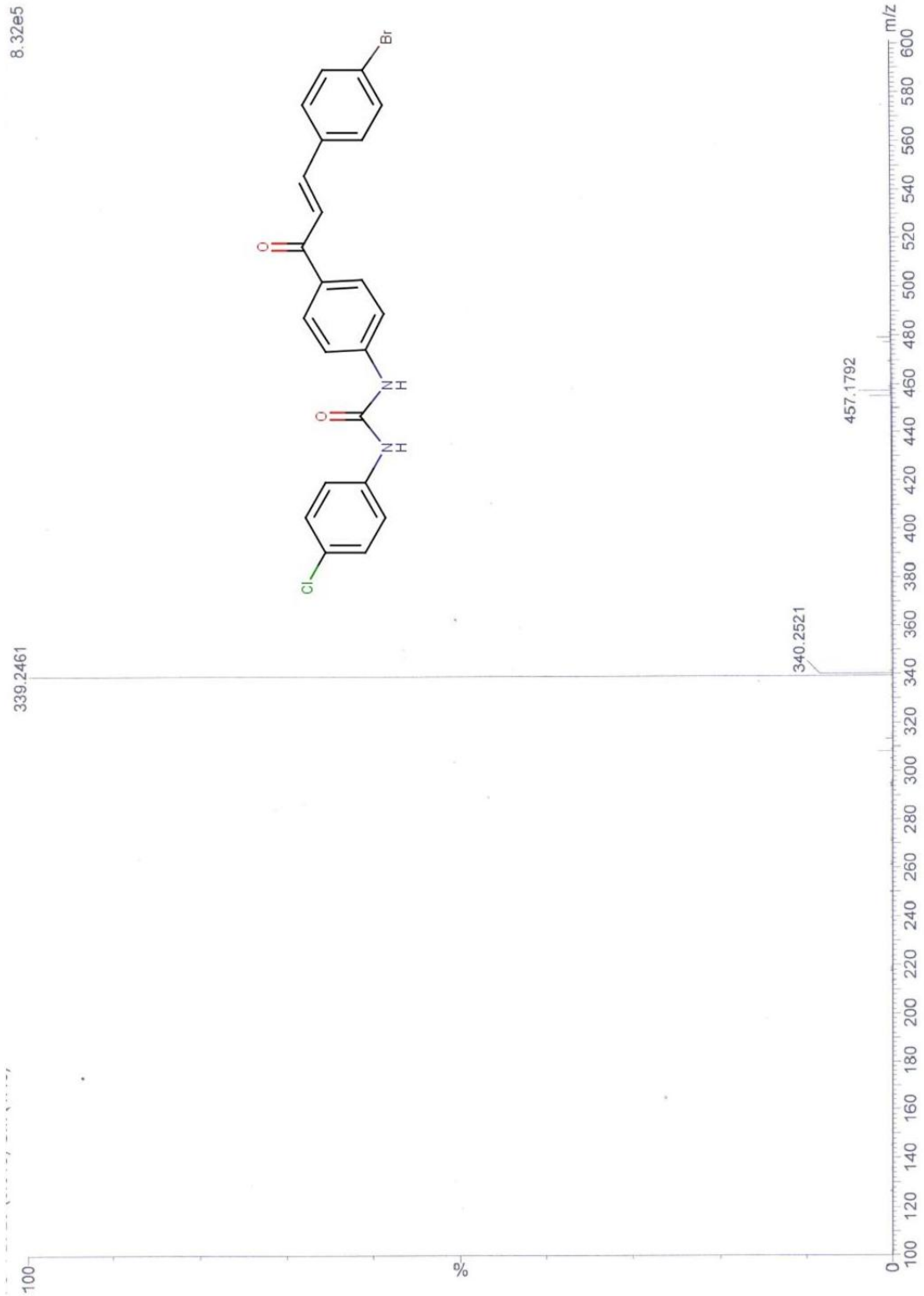
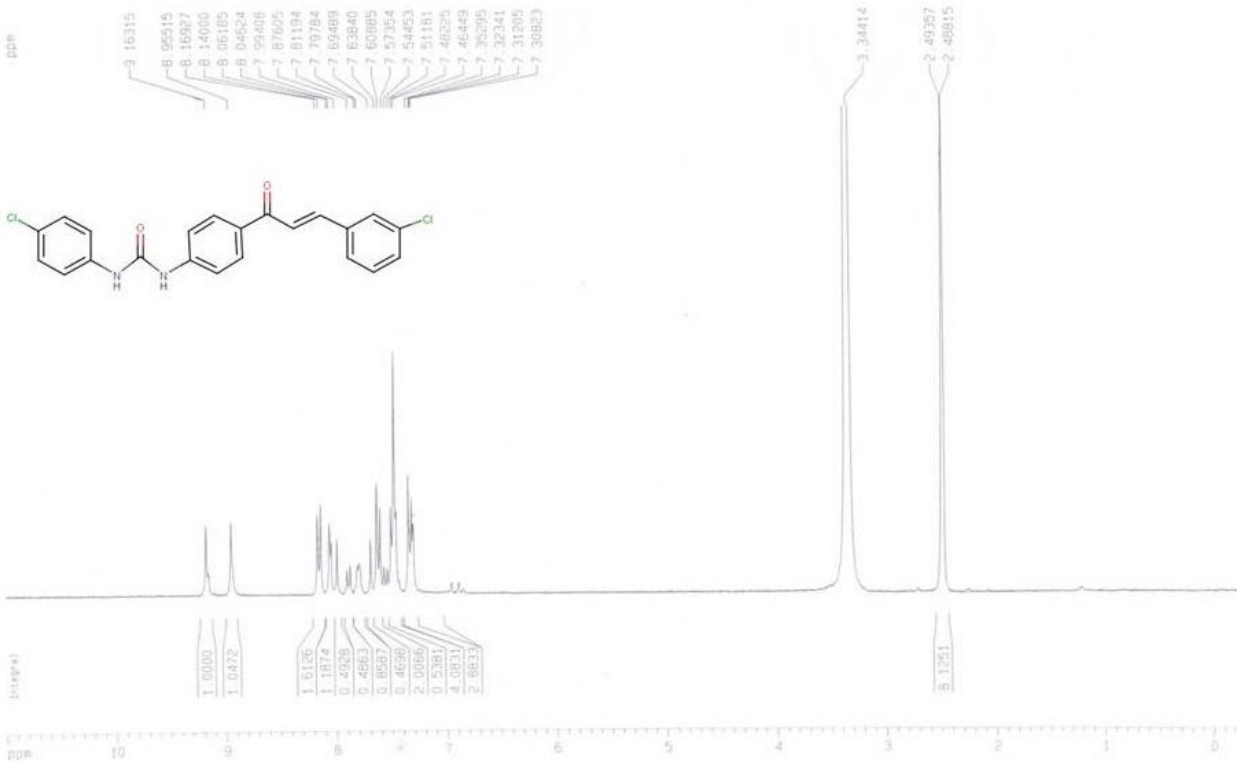
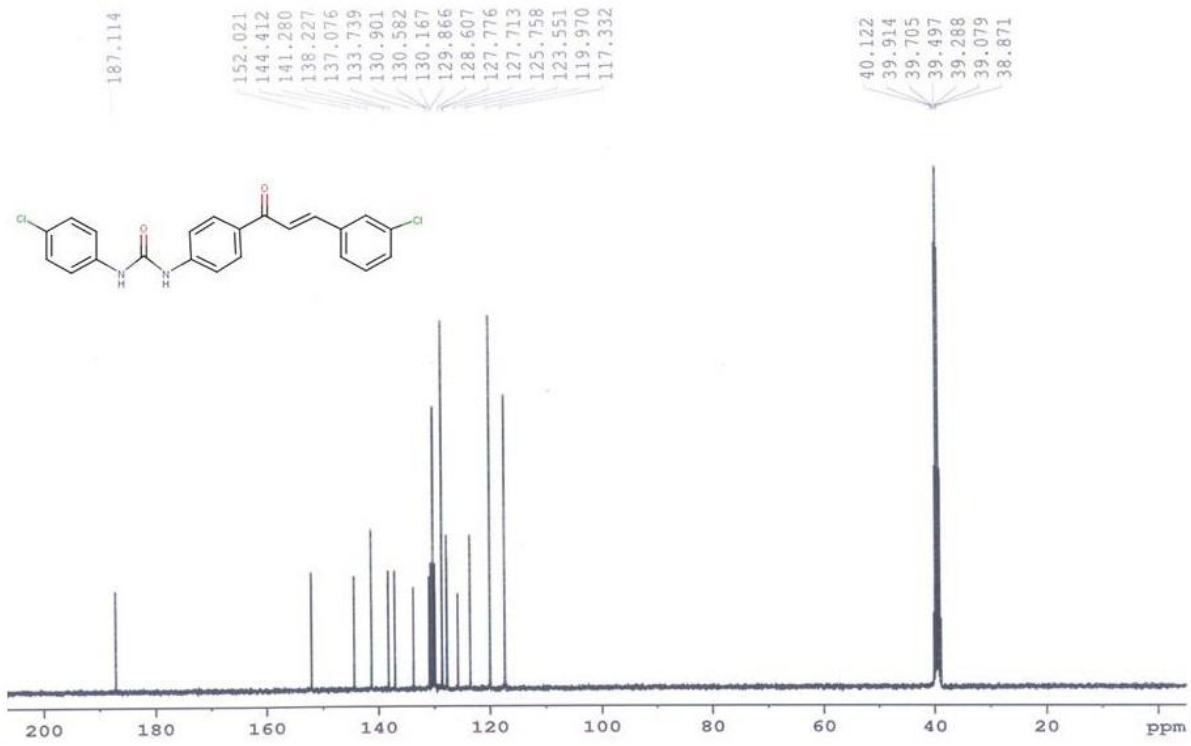


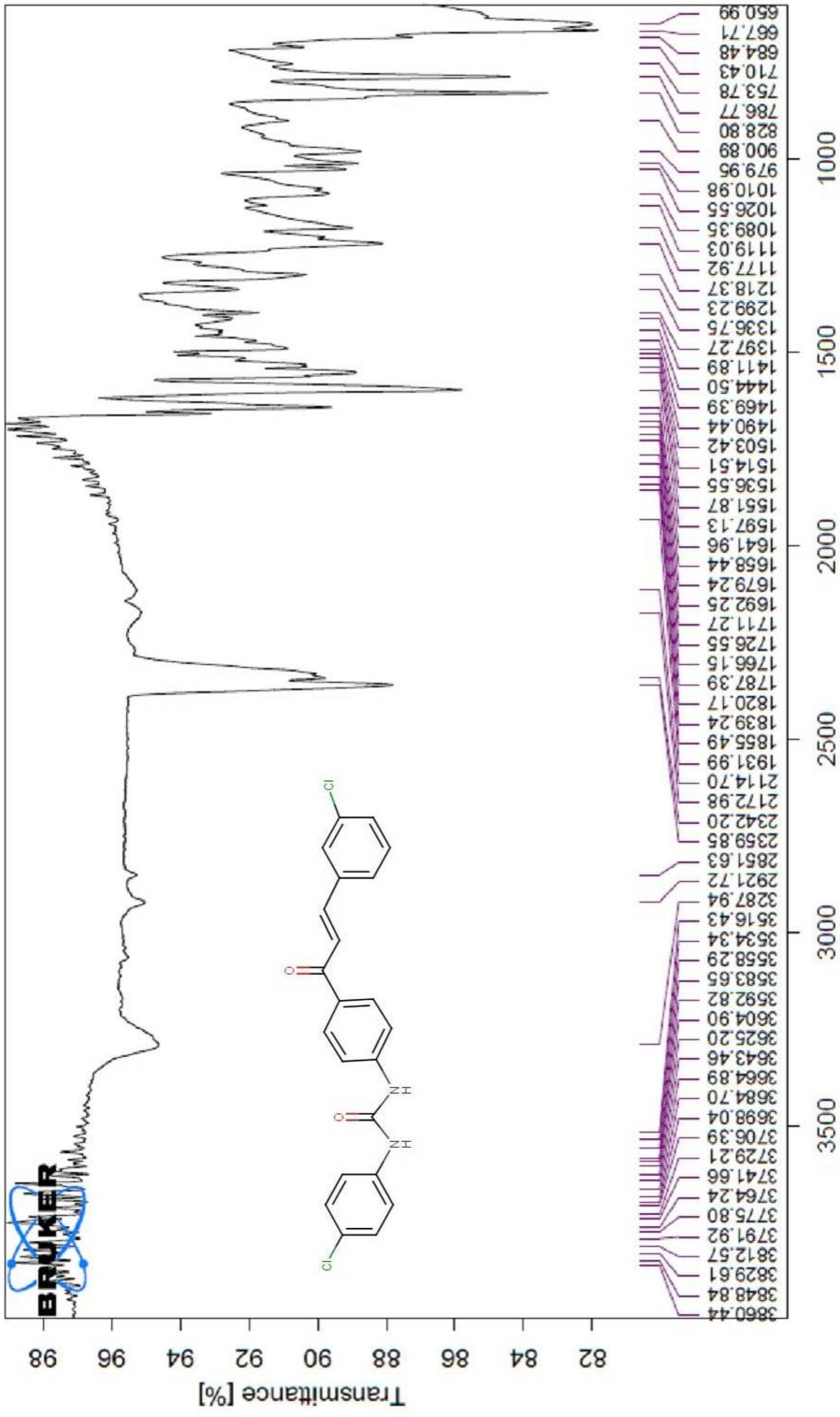
Mass spectra of 1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(4-bromophenyl)-2-propen-1-one (5f)



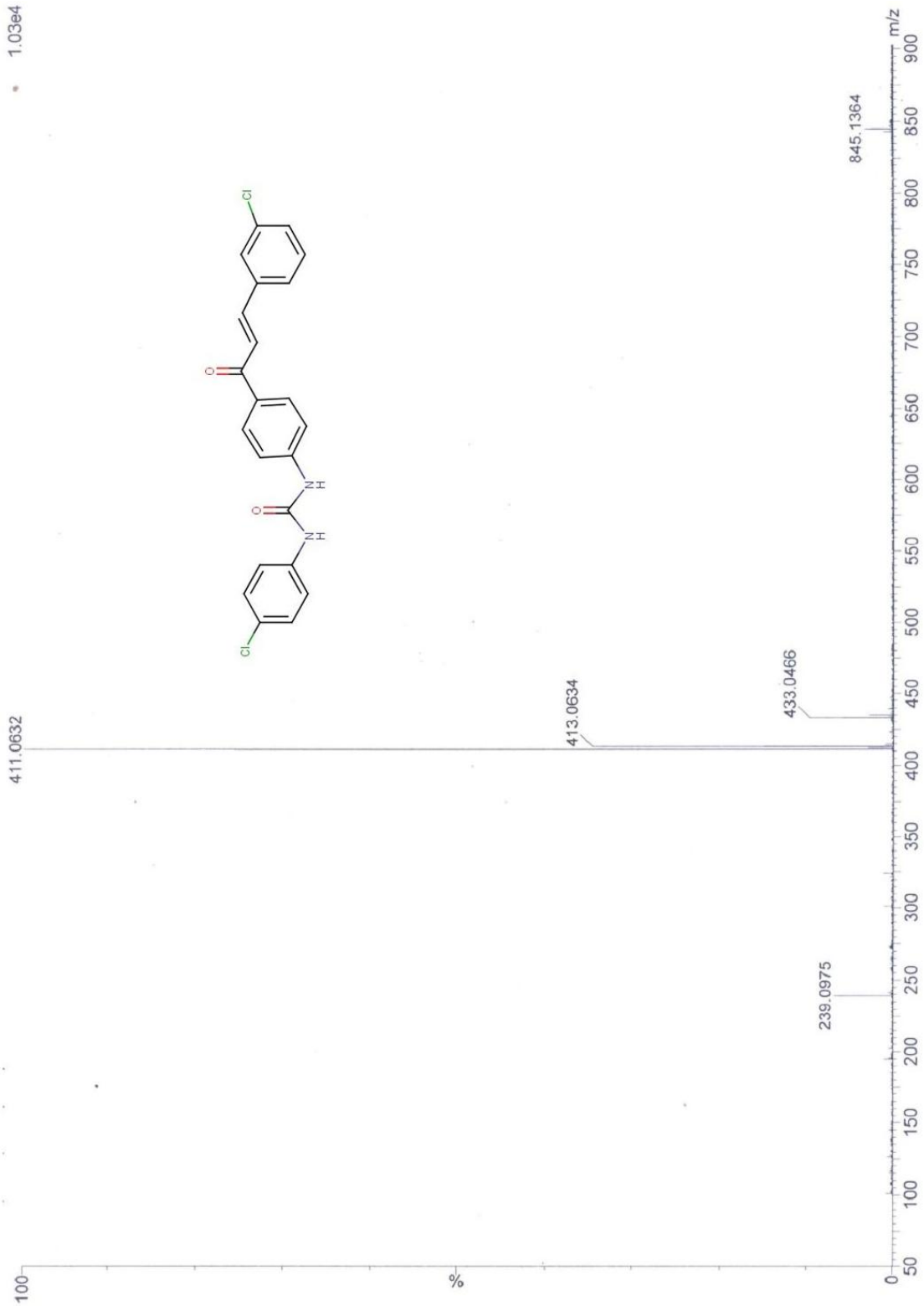
^{1}H NMR of 1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(3-chlorophenyl)-2-propen-1-one (5g)



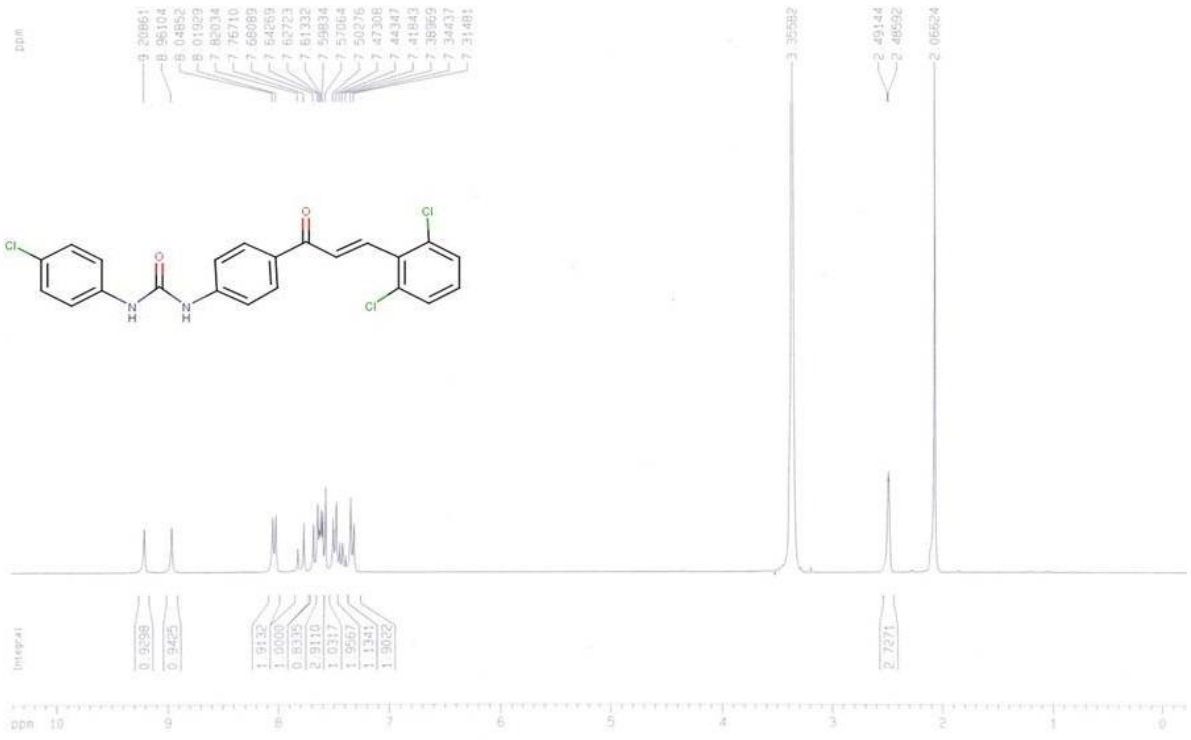
^{13}C NMR of 1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(3-chlorophenyl)-2-propen-1-one (5g)



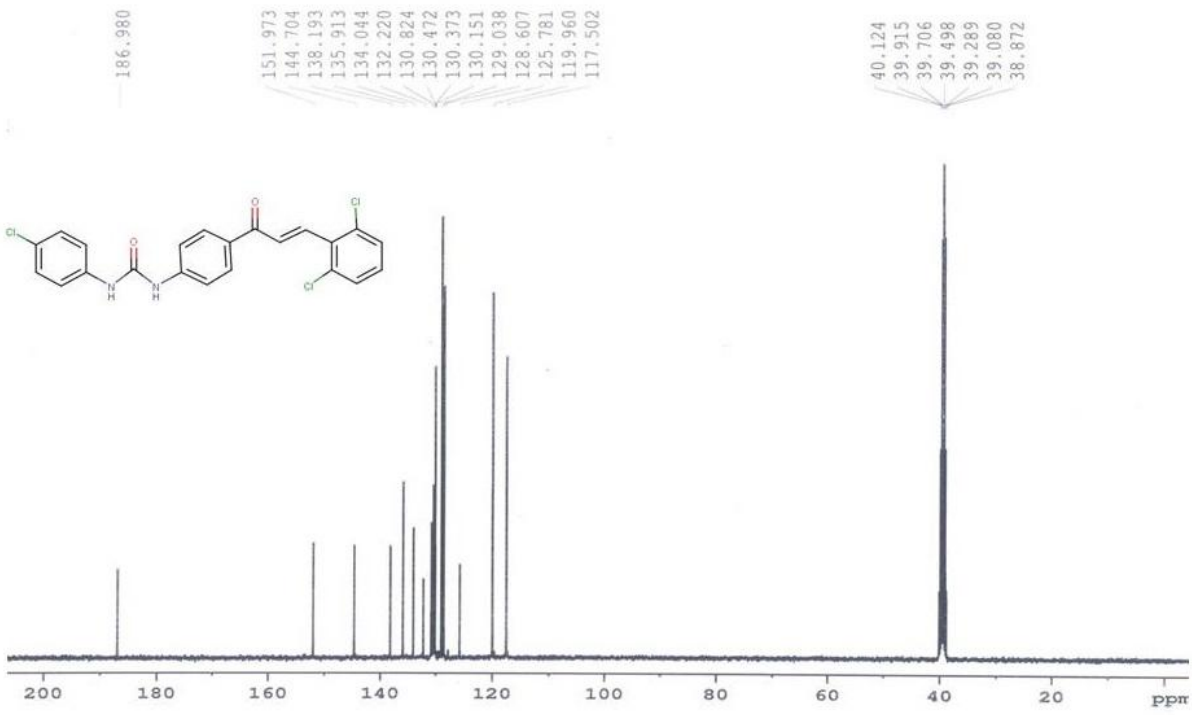
FT-IR of 1-[4'-N-(N'-p-chlorophenylurenyl) phenyl]-3-(3-chlorophenyl)-2-propen-1-one (5g)



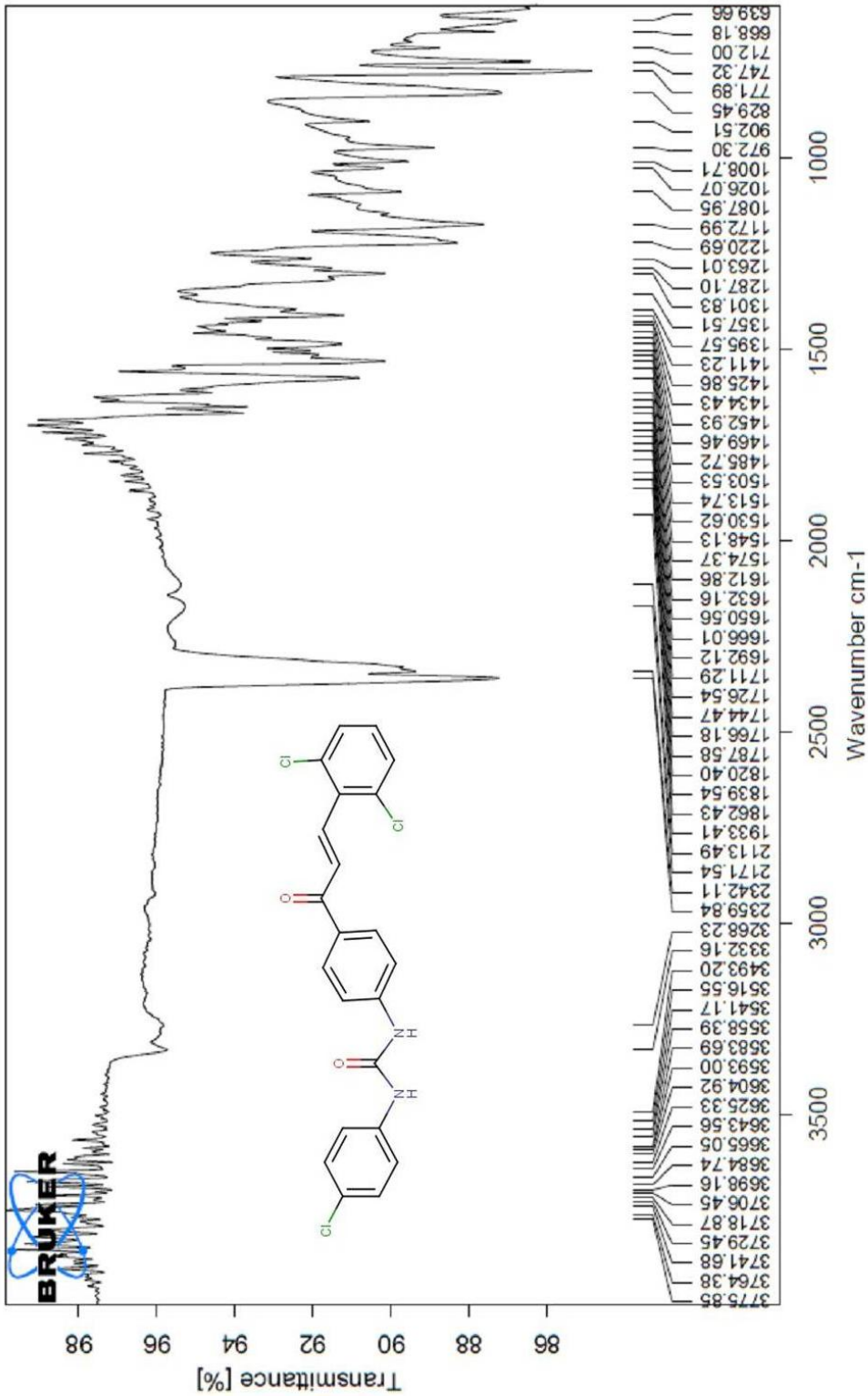
Mass spectra of 1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(3-chlorophenyl)-2-propen-1-one (5g)



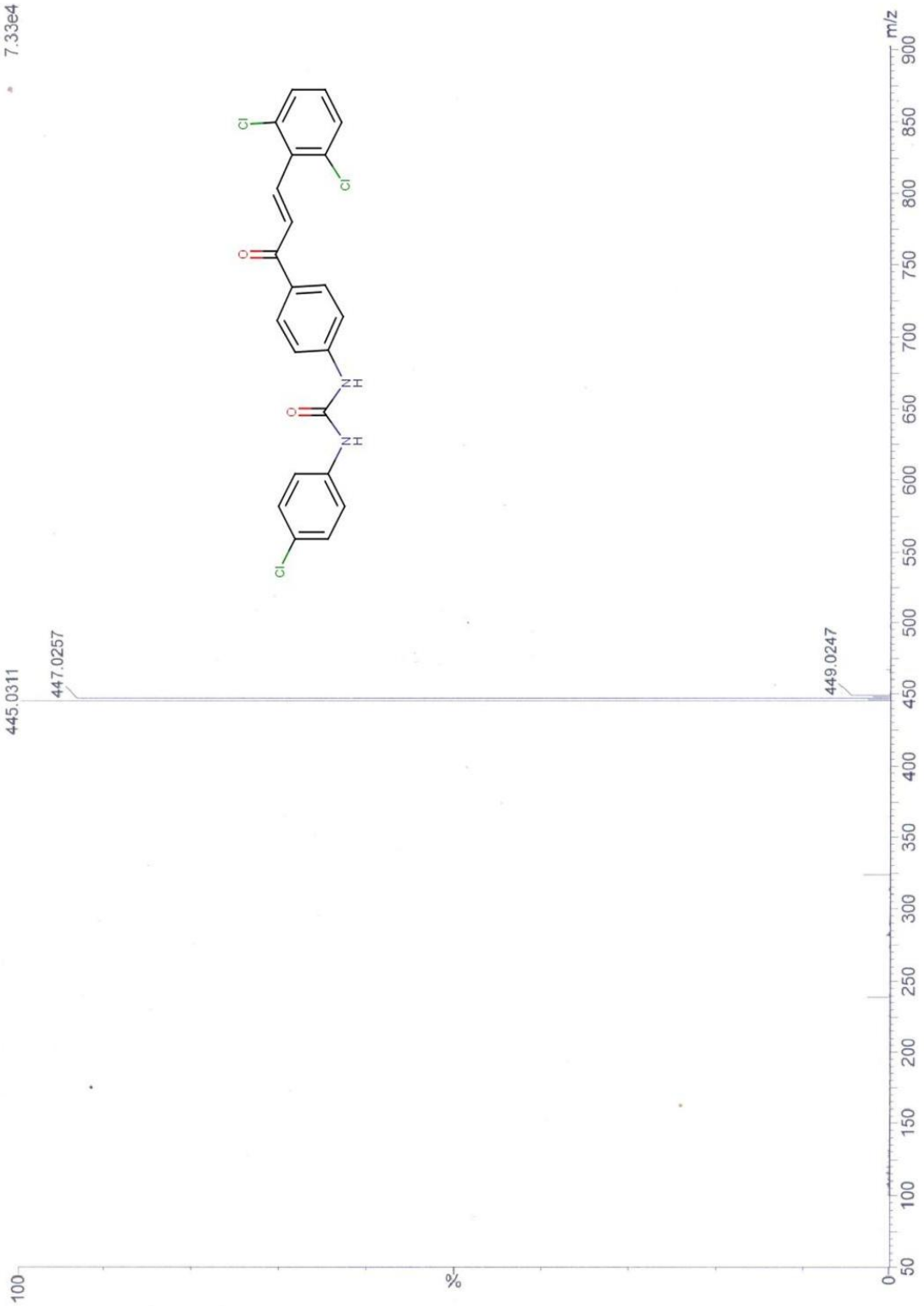
^{1}H NMR of 1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(2, 6-dichlorophenyl)-2-propen-1-one (5h)



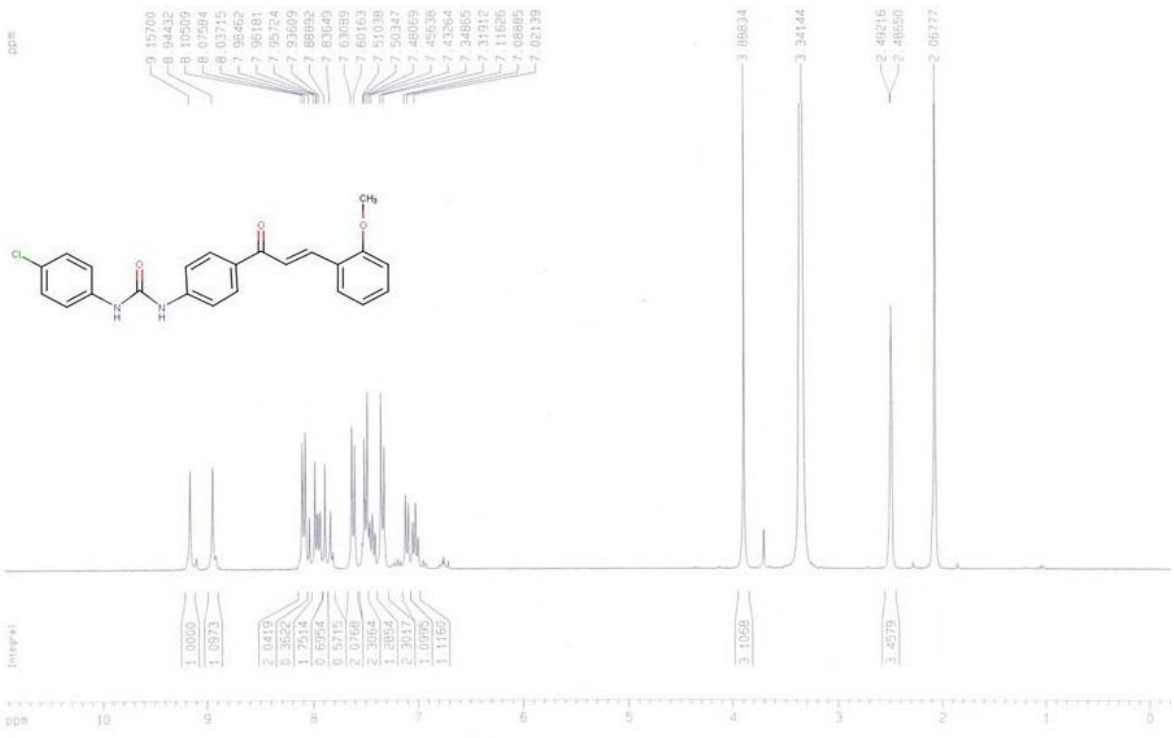
^{13}C NMR of 1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(2, 6-dichlorophenyl)-2-propen-1-one (5h)



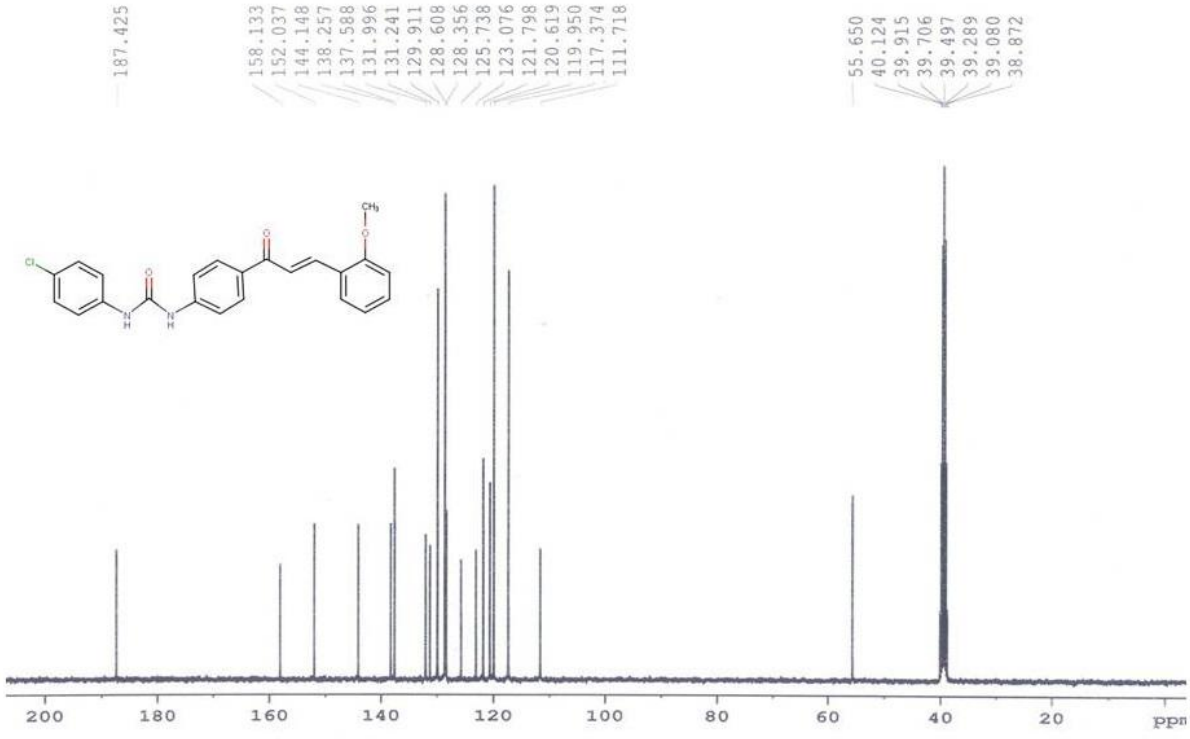
FT-IR of 1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(2, 6-dichlorophenyl)-2-propen-1-one (5h)



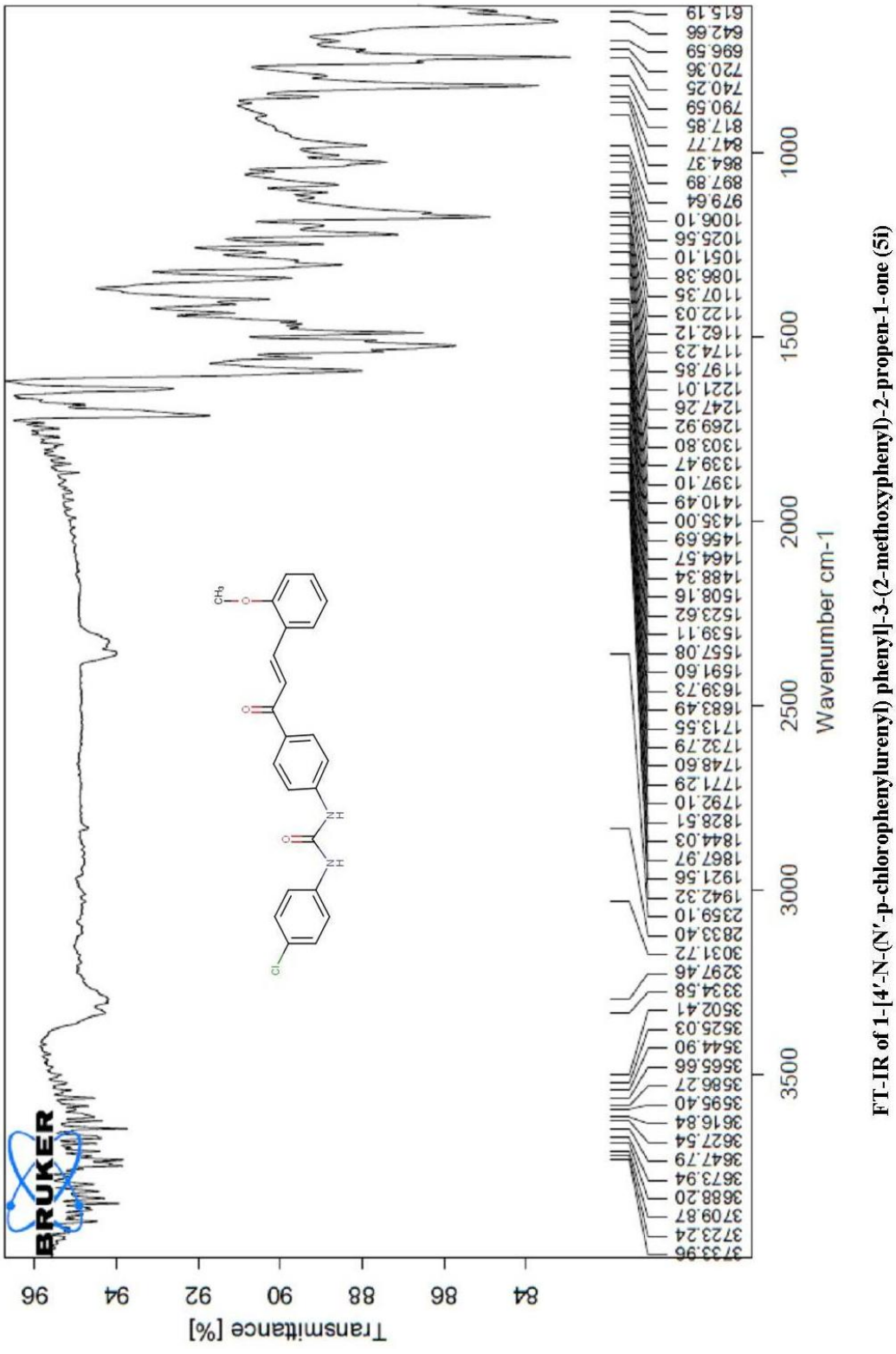
Mass spectra of 1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(2, 6-dichlorophenyl)-2-propen-1-one (5h)



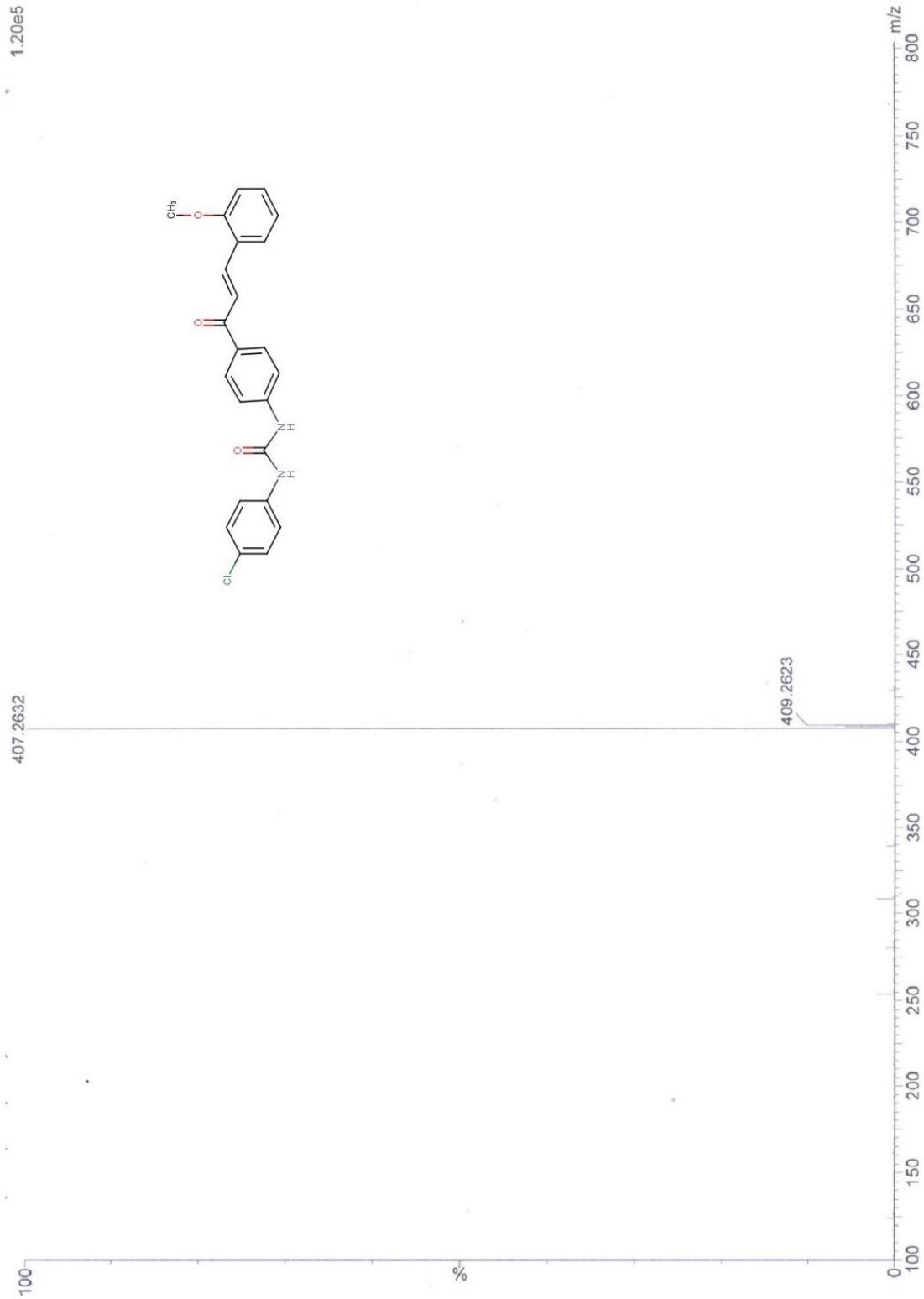
^{1}H NMR of 1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(2-methoxyphenyl)-2-propen-1-one (5i)



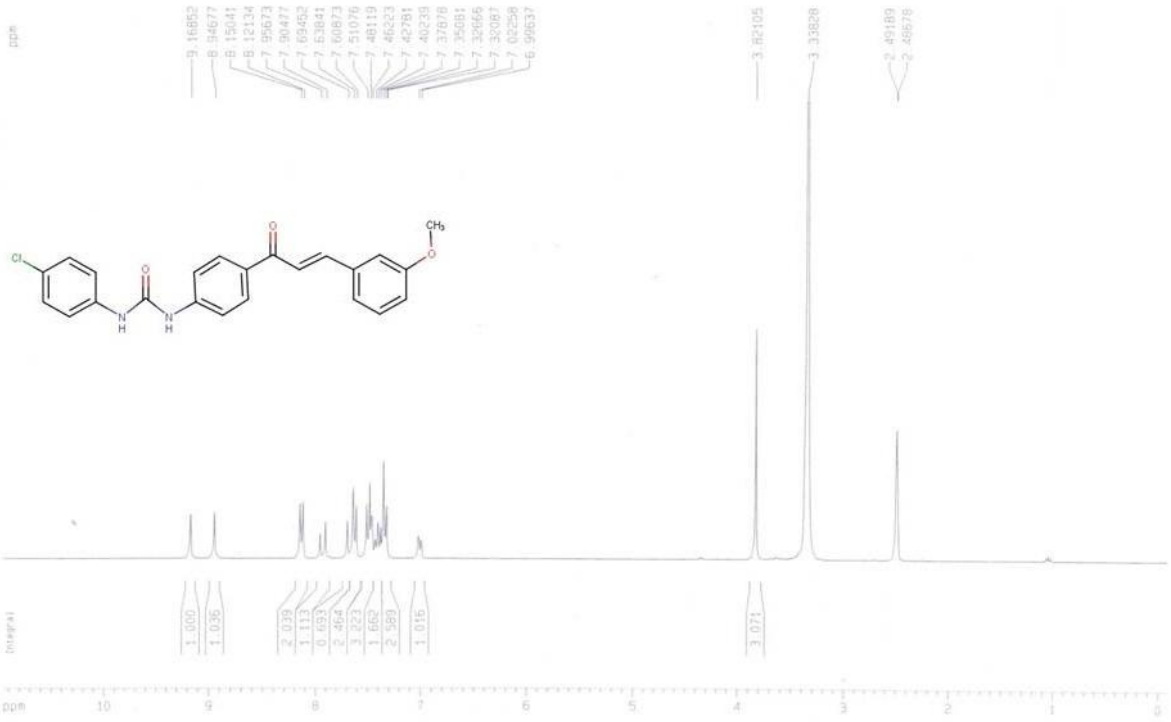
^{13}C NMR of 1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(2-methoxyphenyl)-2-propen-1-one (5i)



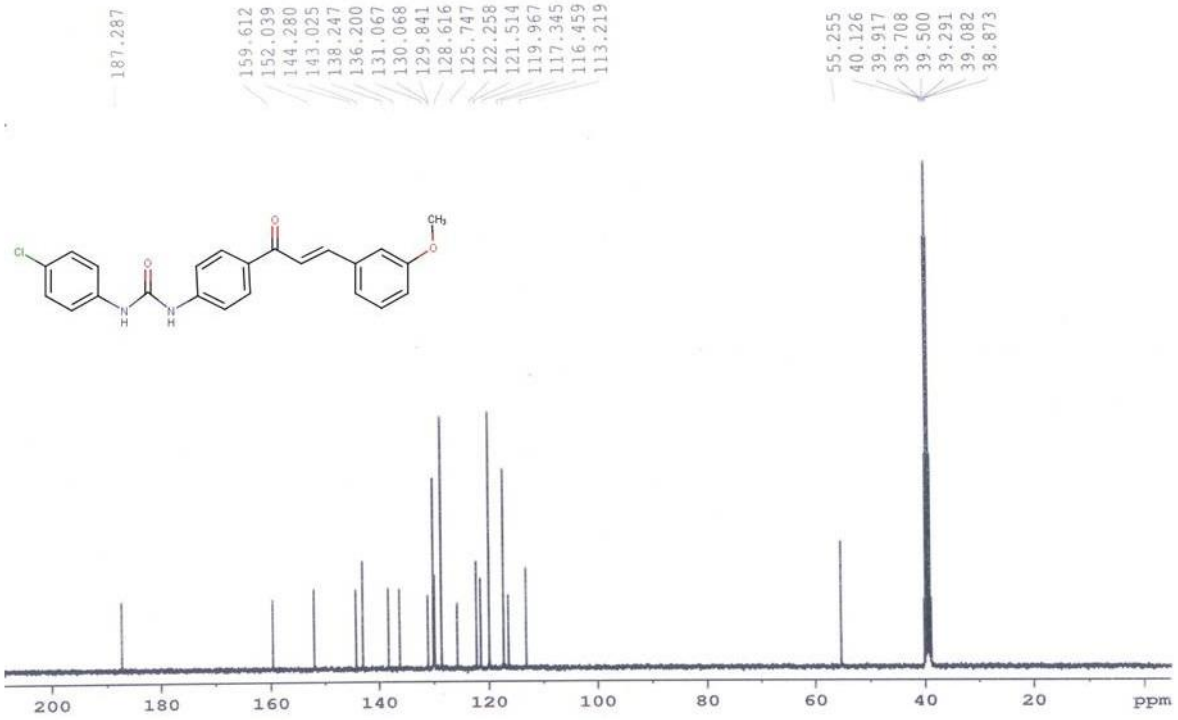
FT-IR of 1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(2-methoxyphenyl)-2-propen-1-one (5i)



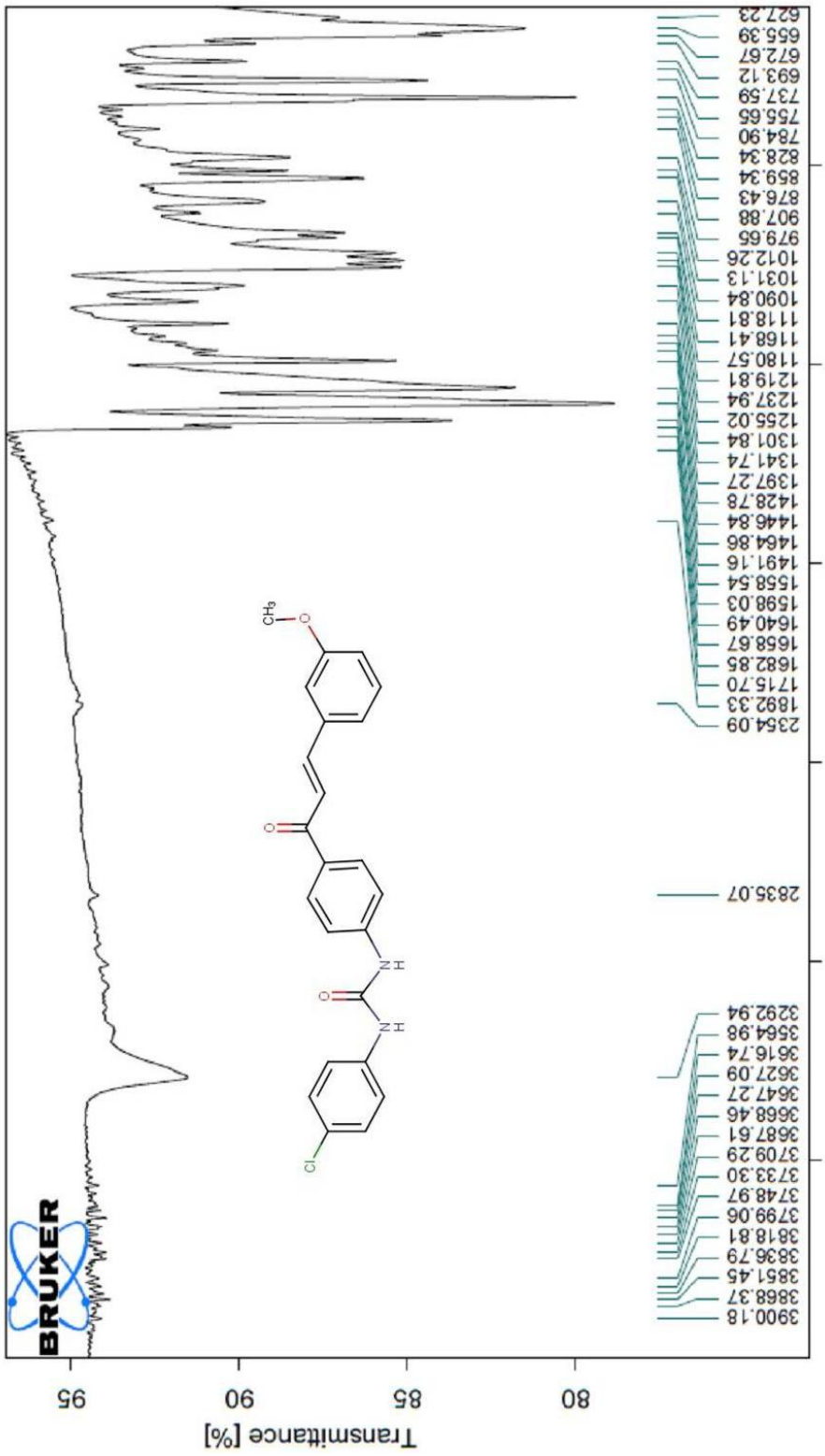
Mass spectra of 1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(2-methoxyphenyl)-2-propen-1-one (5i)



^{1} H NMR of 1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(3-methoxyphenyl)-2-propen-1-one (5j)

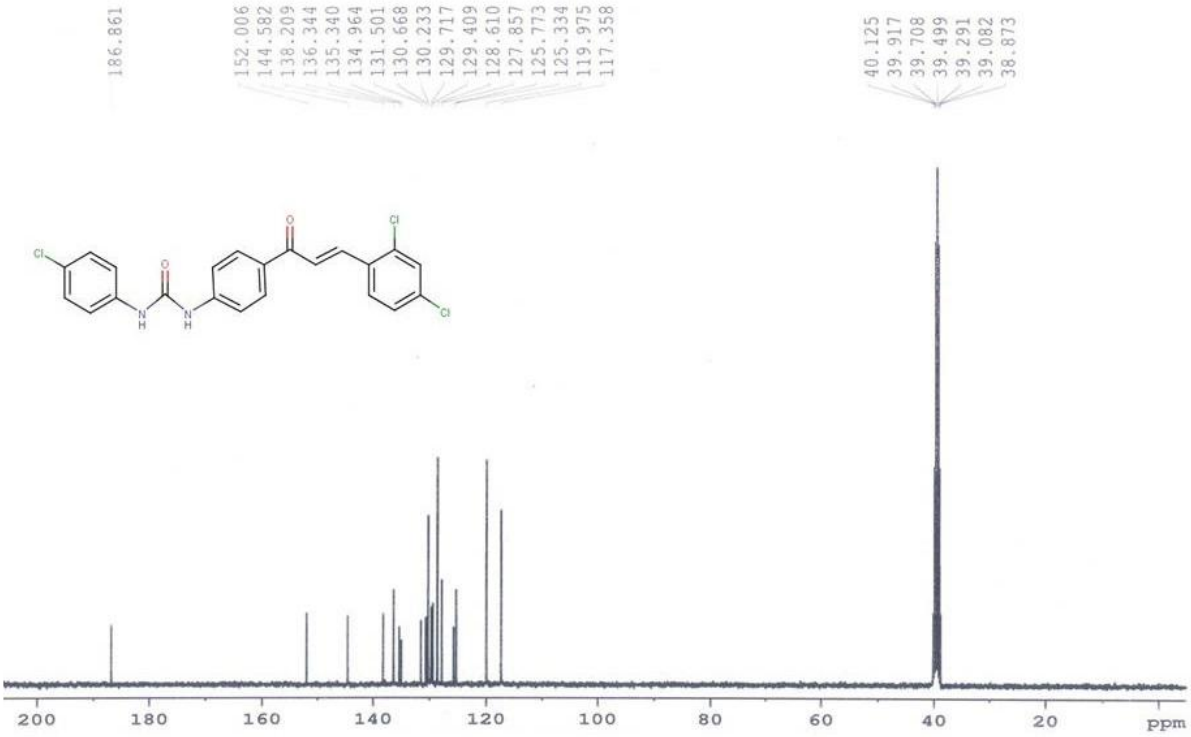


^{13}C NMR of 1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(3-methoxyphenyl)-2-propen-1-one (5j)

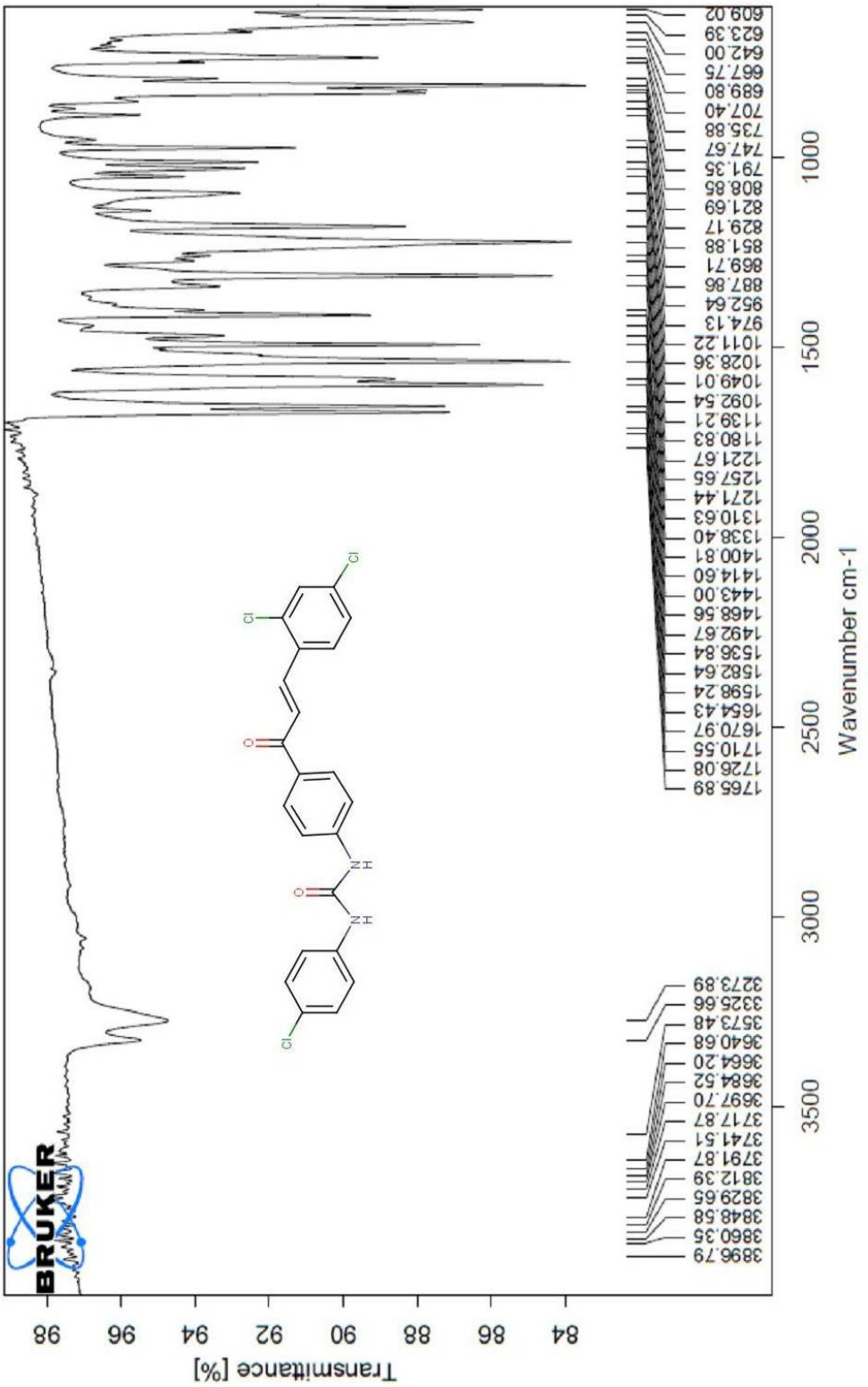


FT-IR of 1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(3-methoxyphenyl)-2-propen-1-one (5j)

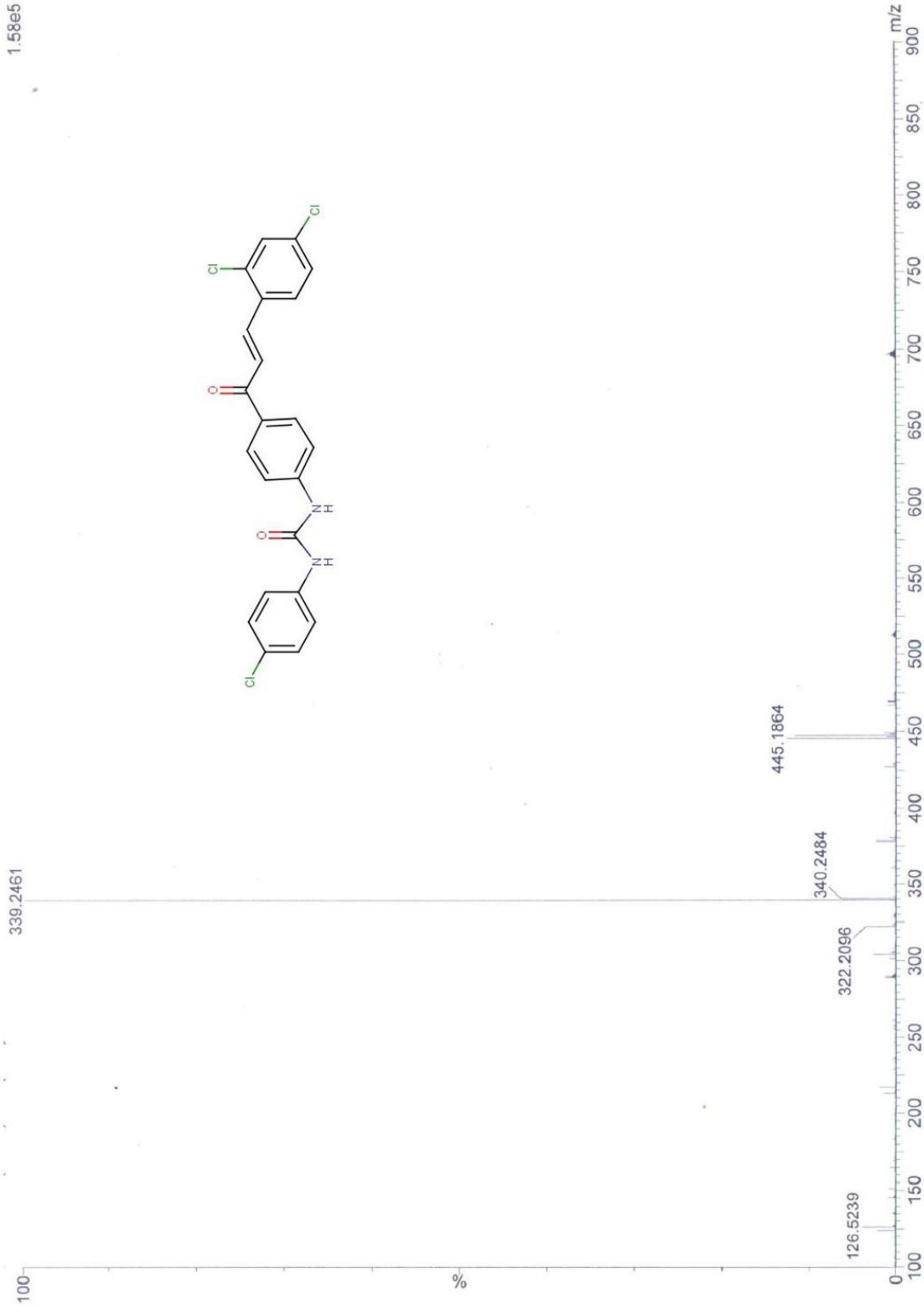
^{1}H NMR of 1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(2, 4-dichlorophenyl)-2-propen-1-one (5k)



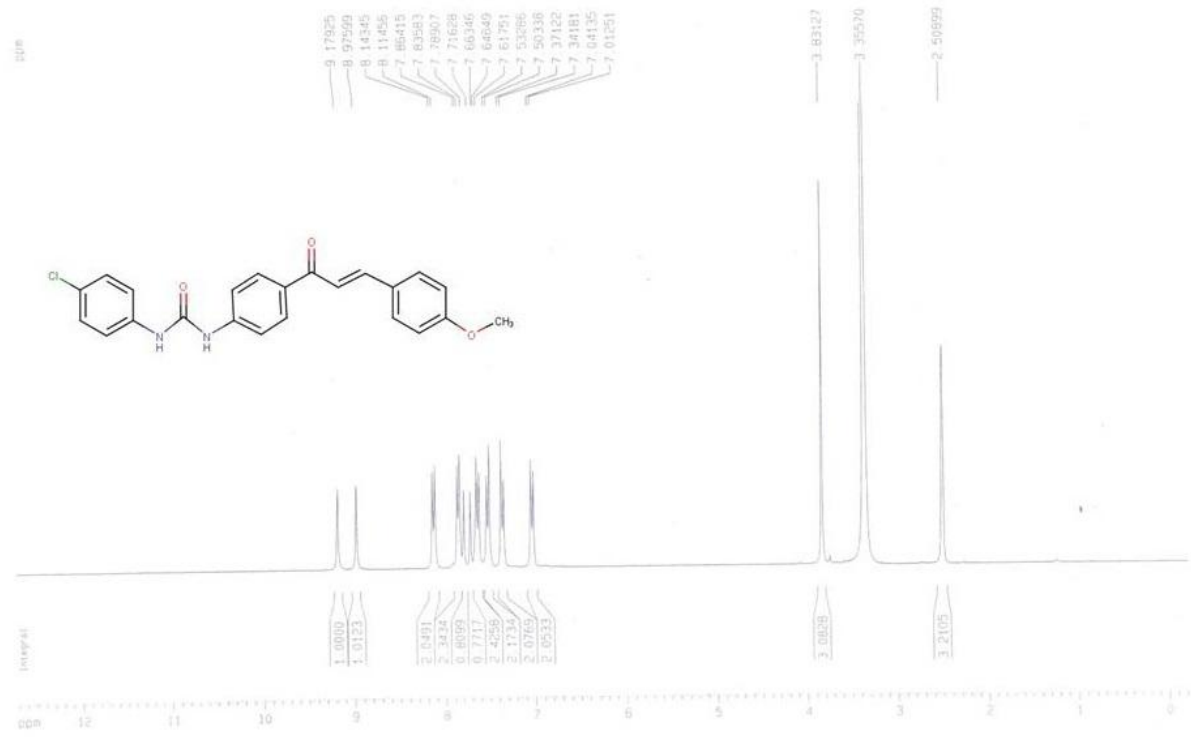
^{13}C NMR of 1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(2, 4-dichlorophenyl)-2-propen-1-one (5k)



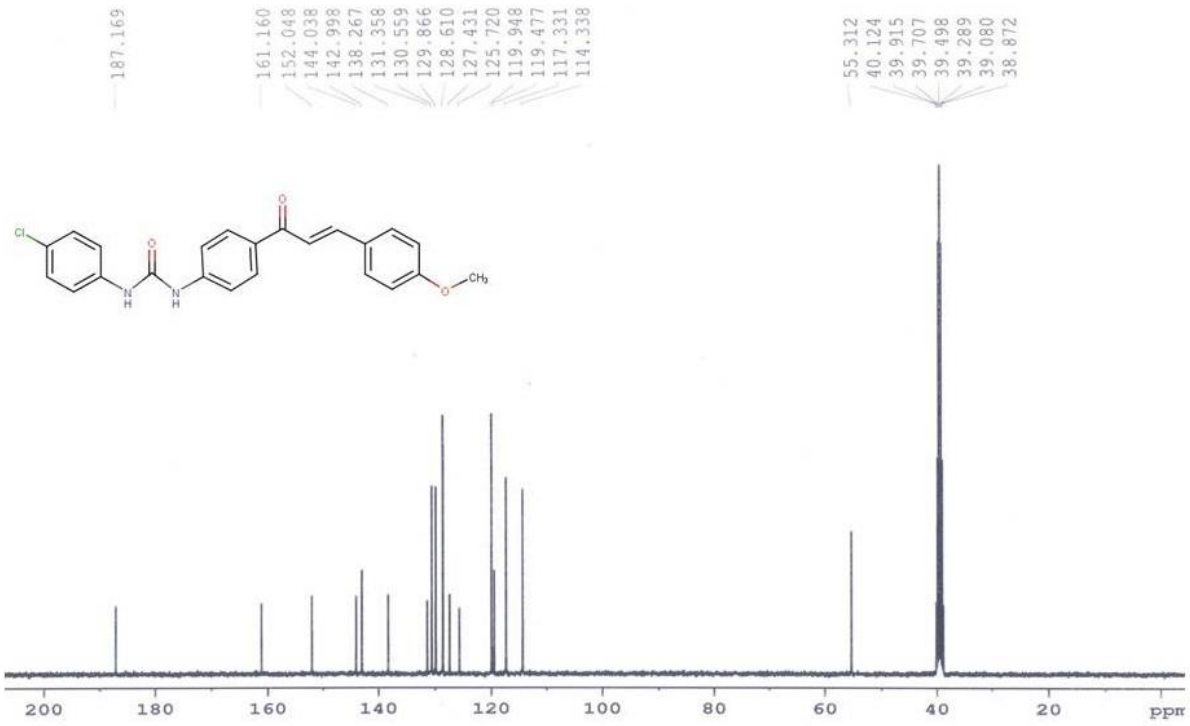
FT-IR of 1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(2, 4-dichlorophenyl)-2-propen-1-one (5k)



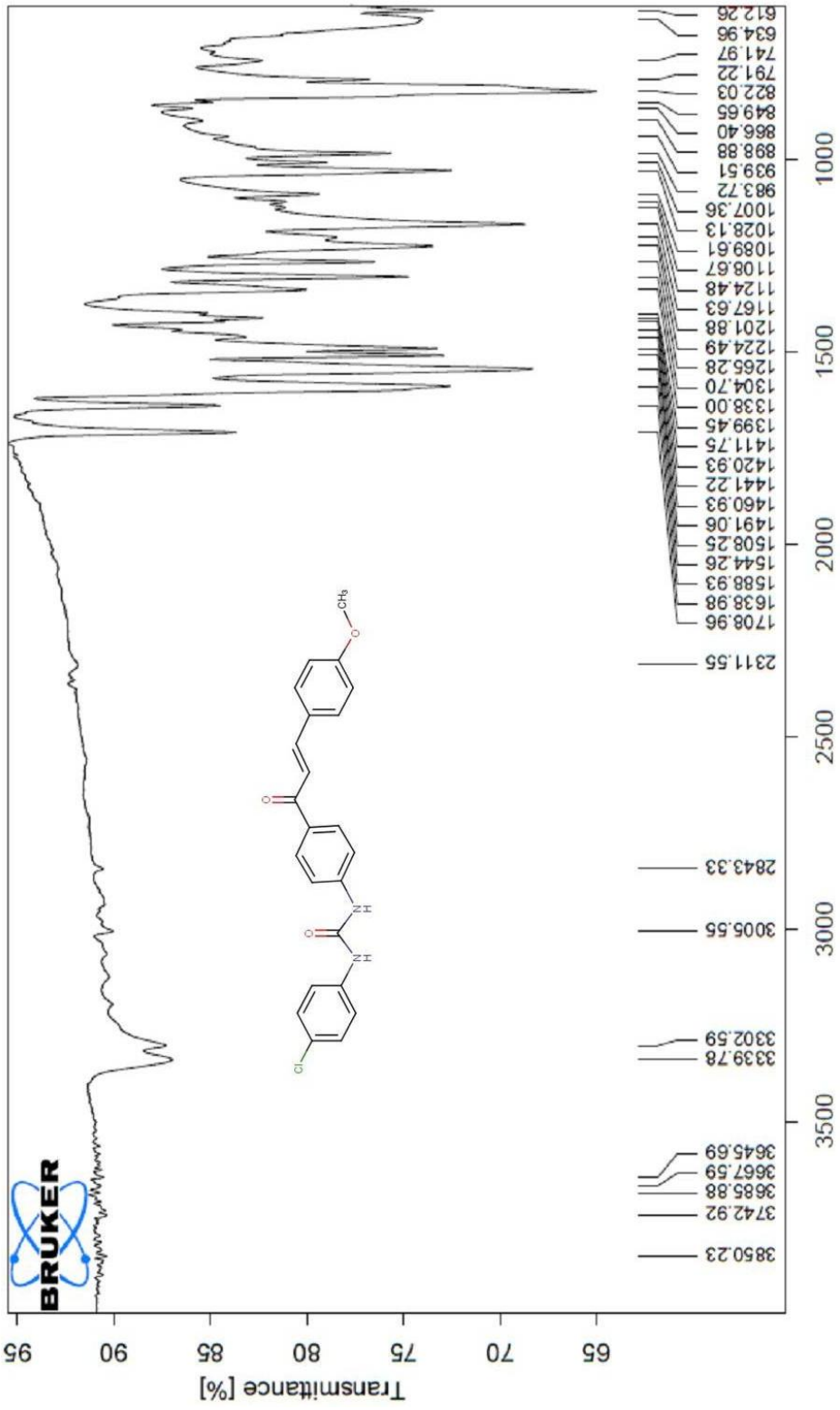
Mass spectra of 1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(2, 4-dichlorophenyl)-2-propen-1-one (5k)



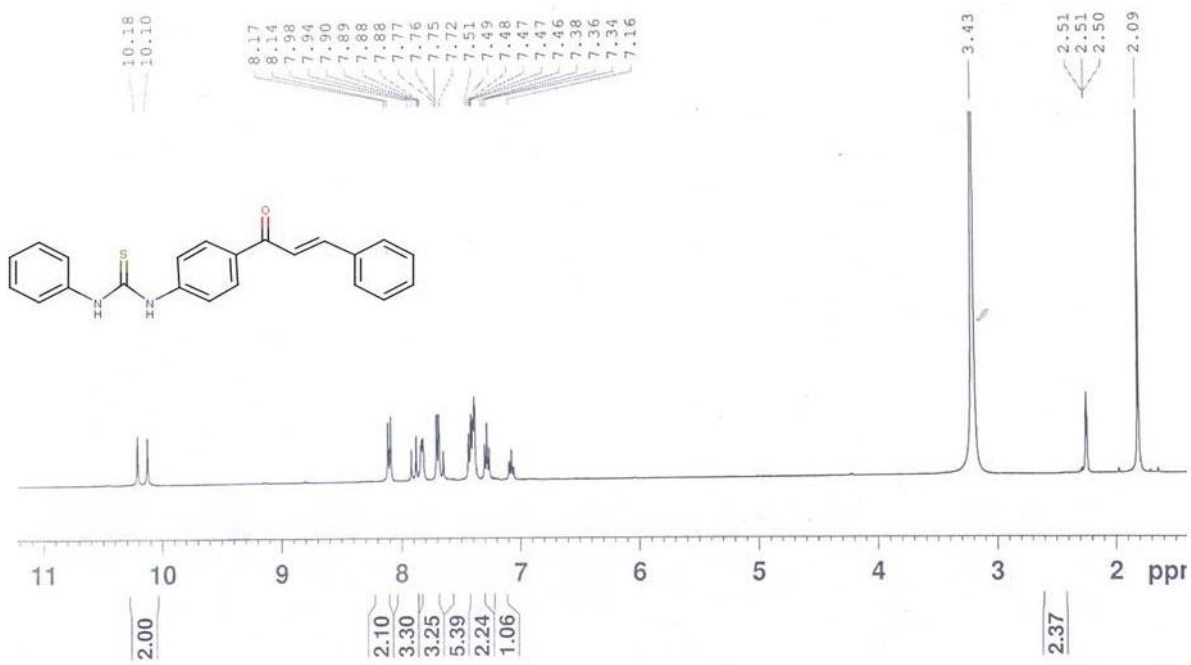
^{1}H NMR of 1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(4-methoxyphenyl)-2-propen-1-one (5l)



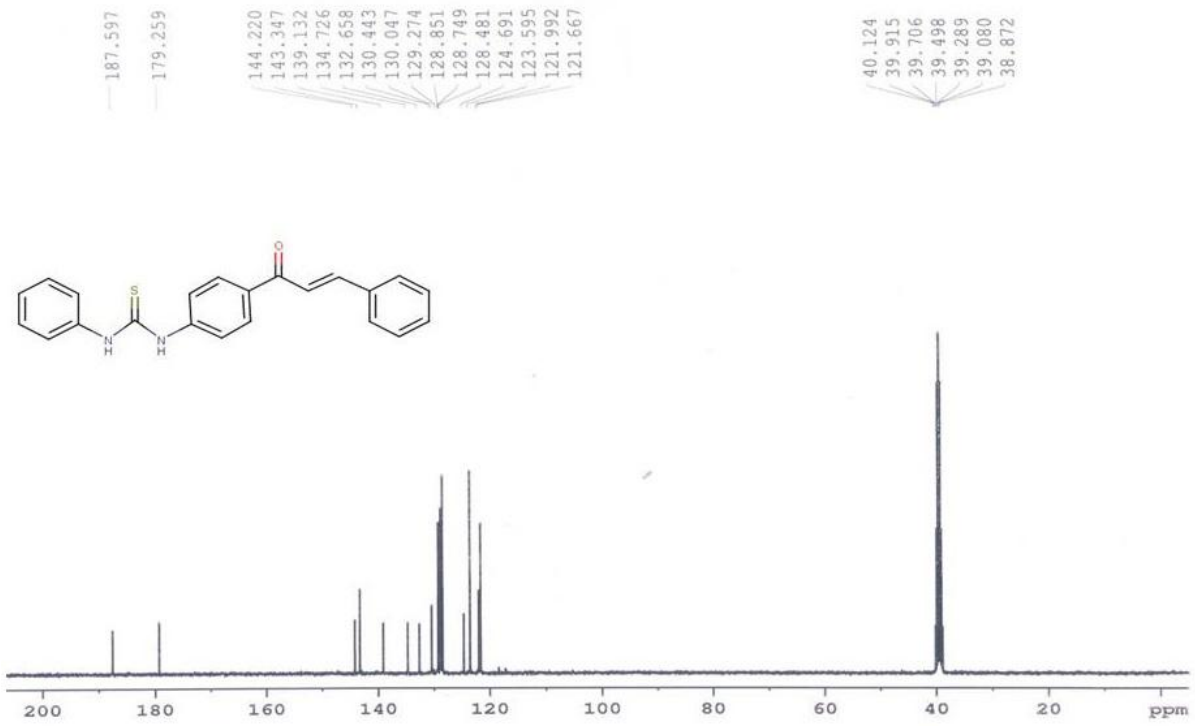
^{13}C NMR of 1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(4-methoxyphenyl)-2-propen-1-one (5l)



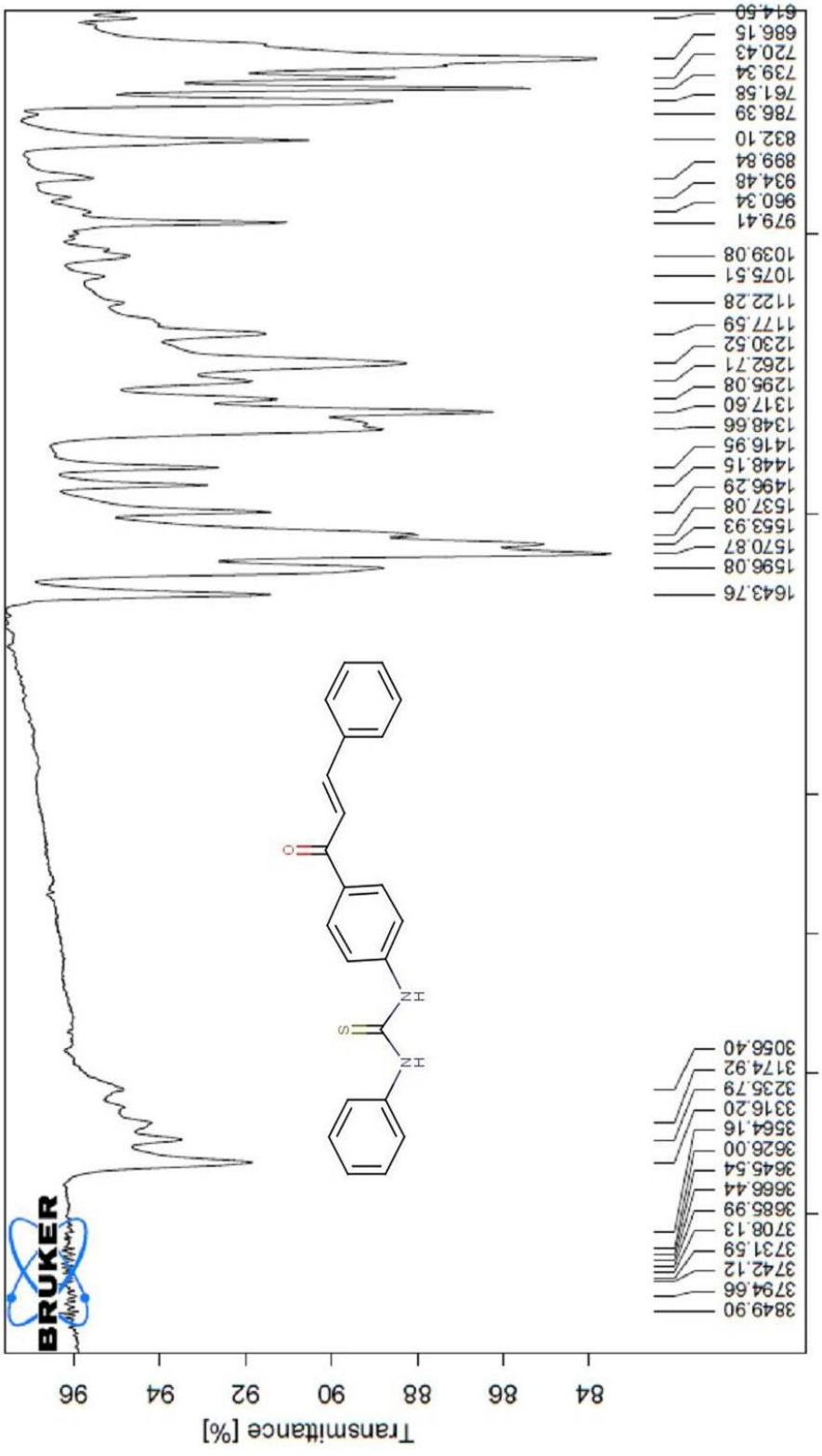
FT-IR of 1-[4'-N-(N'-p-chlorophenylurenyl) phenyl]-3-(4-methoxyphenyl)-2-propen-1-one (5l)



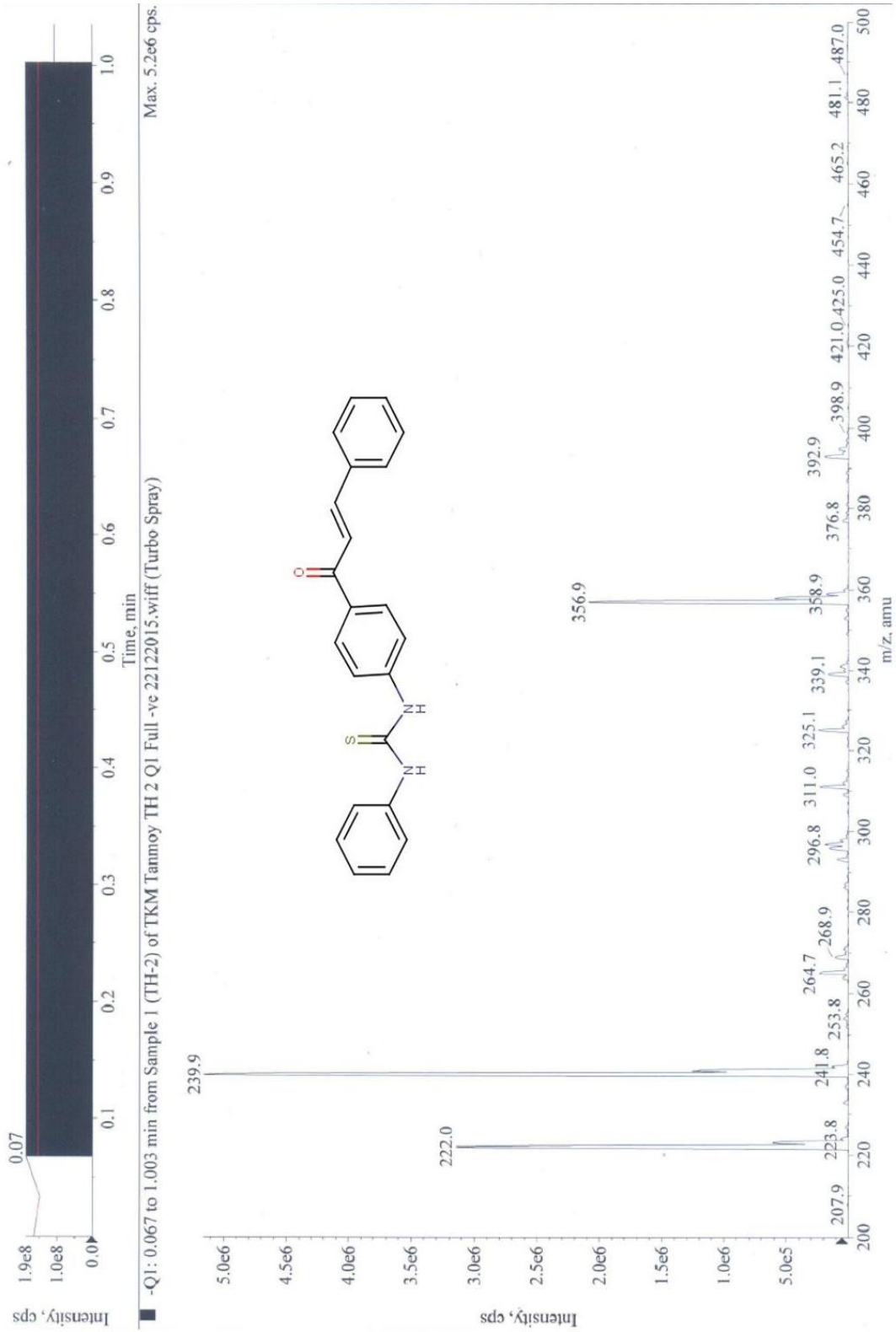
^{1}H NMR of 1-[4′-N-(N′-phenylthiourenyl) phenyl]-3-phenyl-2-propen-1-one (5m)



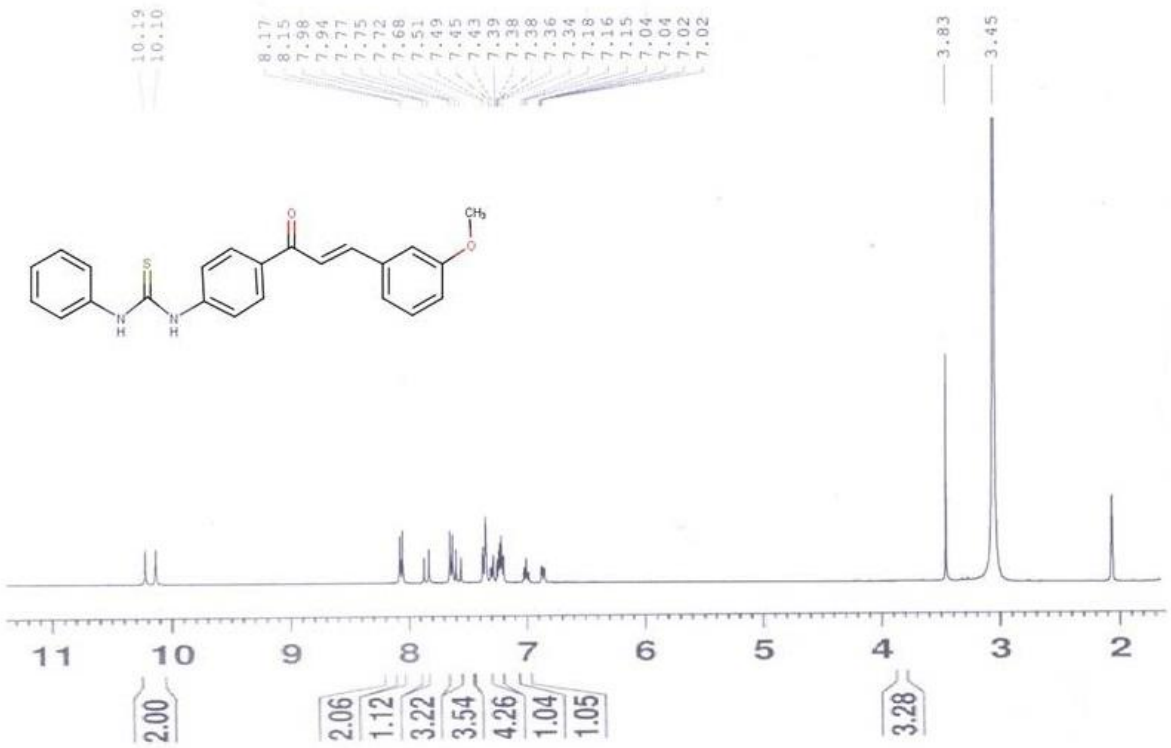
^{13}C NMR of 1-[4′-N-(N′-phenylthiourenyl) phenyl]-3-phenyl-2-propen-1-one (5m)



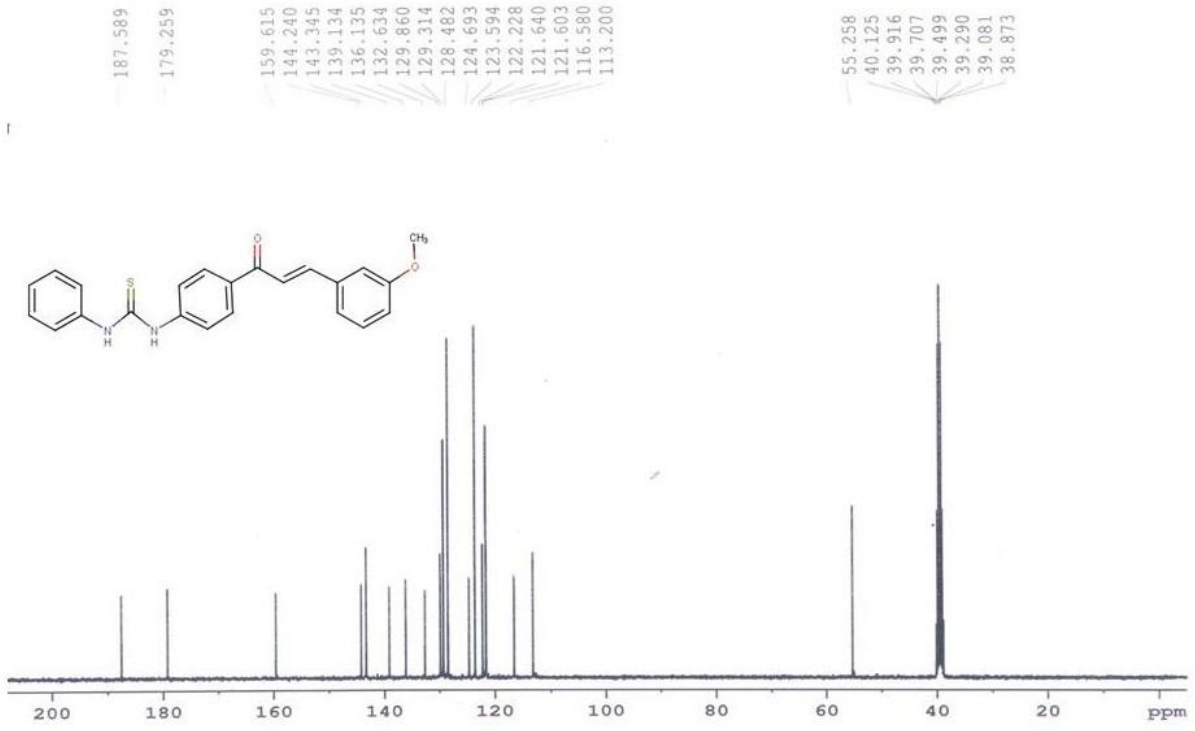
FT-IR of 1-[4′-N-(N′-phenylthiourenyl) phenyl]-3-phenyl-2-propen-1-one (5m)



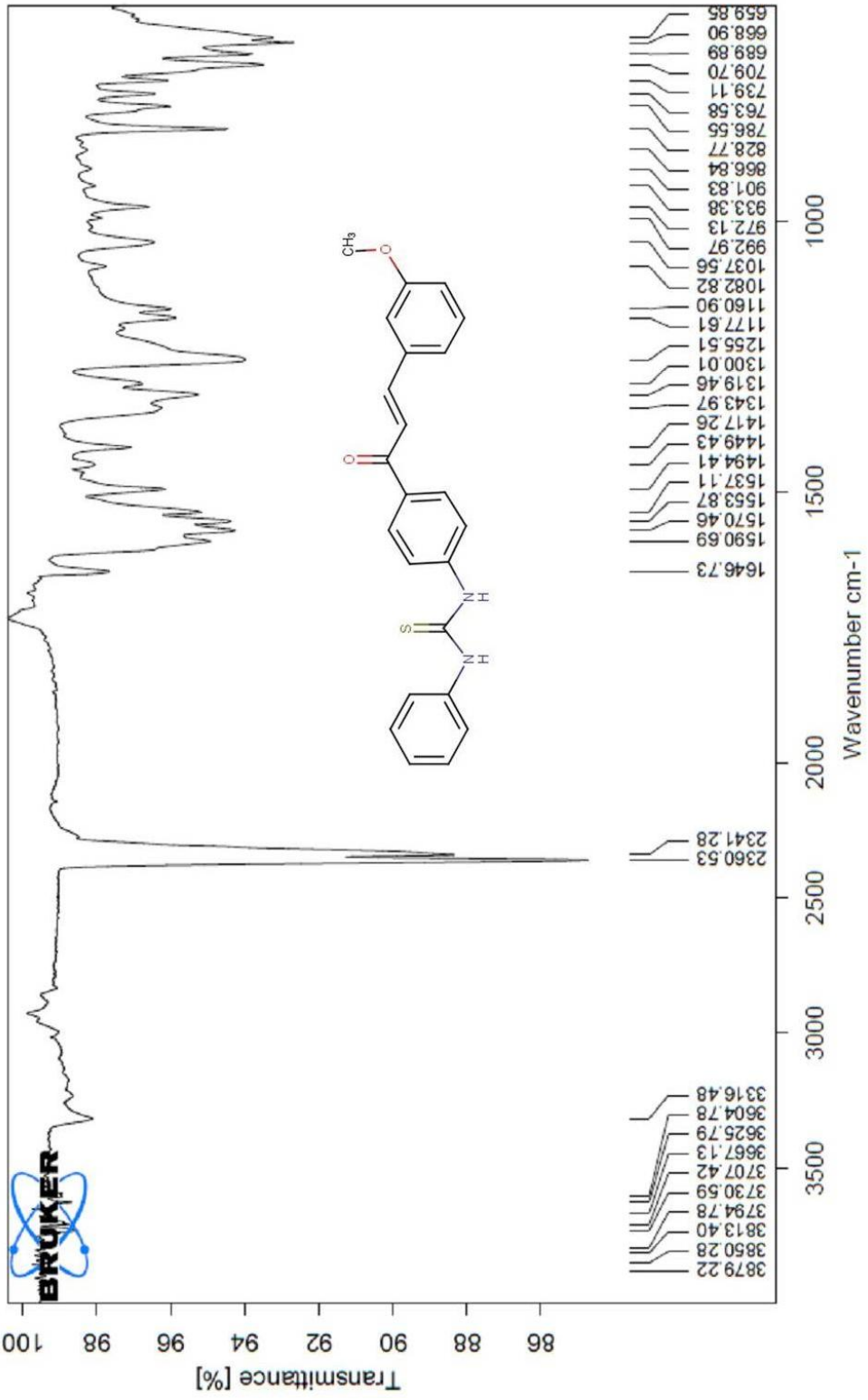
Mass spectra of 1-[4′-N-(N′-phenylthiourenyl) phenyl]-3-phenyl-2-propen-1-one (5m)



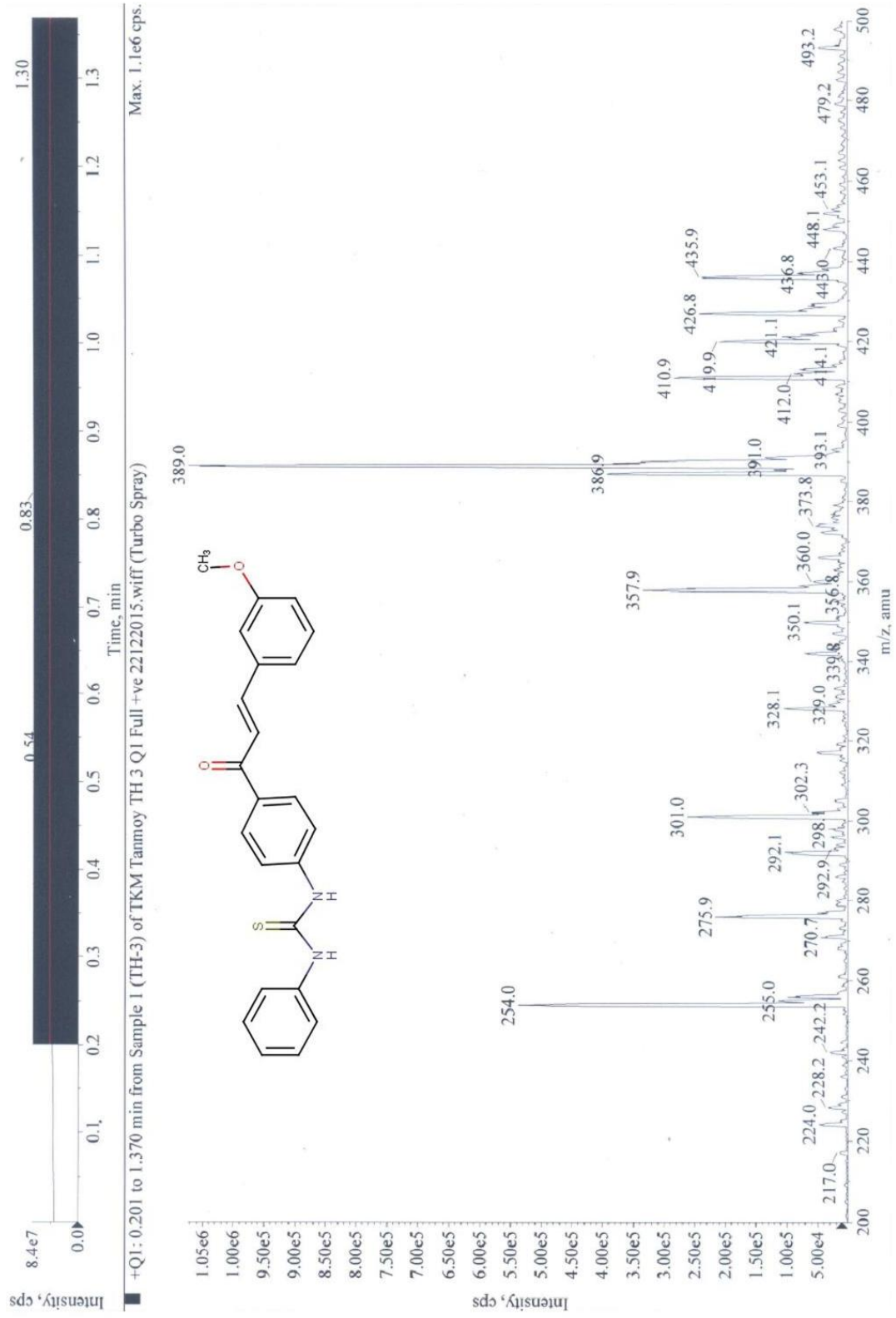
^{1}H NMR of 1-[4′-N-(N′-phenylthiourenyl) phenyl]-3-(3-methoxyphenyl)-2-propen-1-one (5n)



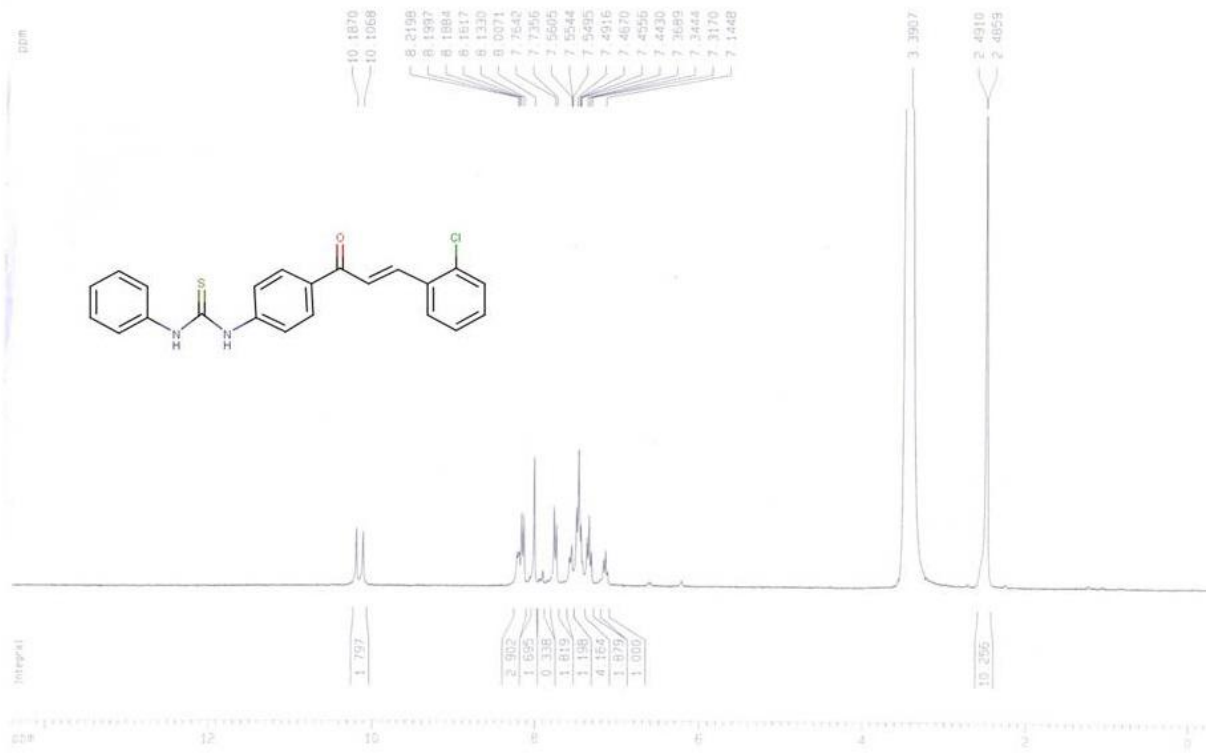
^{13}C NMR of 1-[4′-N-(N′-phenylthiourenyl) phenyl]-3-(3-methoxyphenyl)-2-propen-1-one (5n)



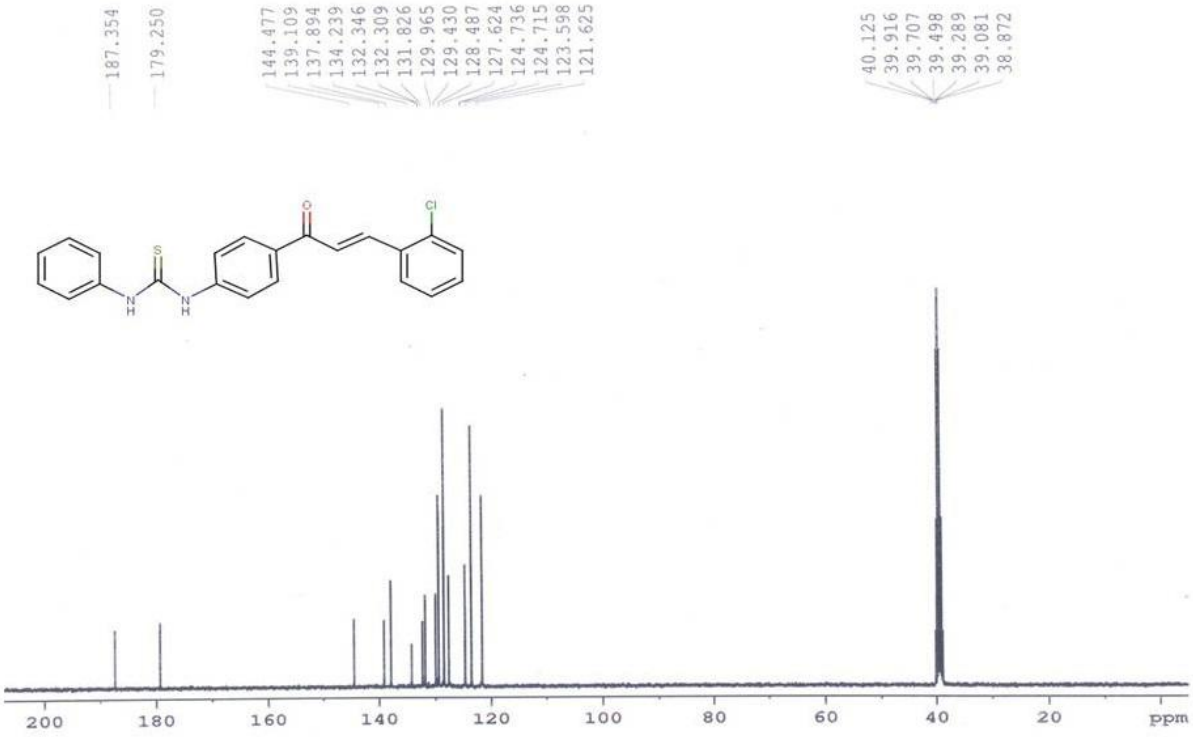
FT-IR of 1-[4′-N-(N′-phenylthiourenyl) phenyl]-3-(3-methoxyphenyl)-2-propen-1-one (5n)



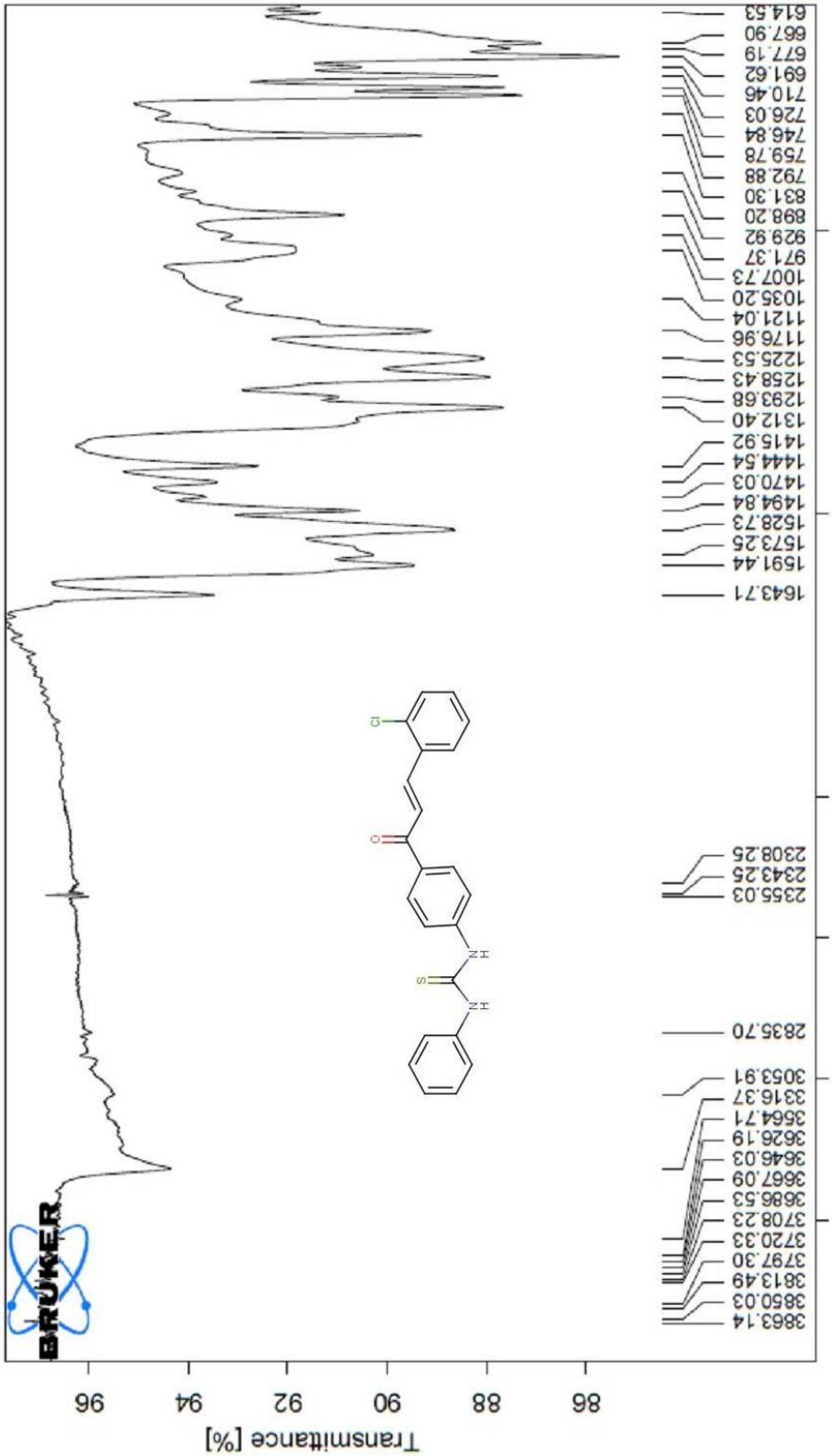
Mass spectra of 1-[4′-N-(N′-phenylthiourenyl) phenyl]-3-(3-methoxyphenyl)-2-propen-1-one (5n)



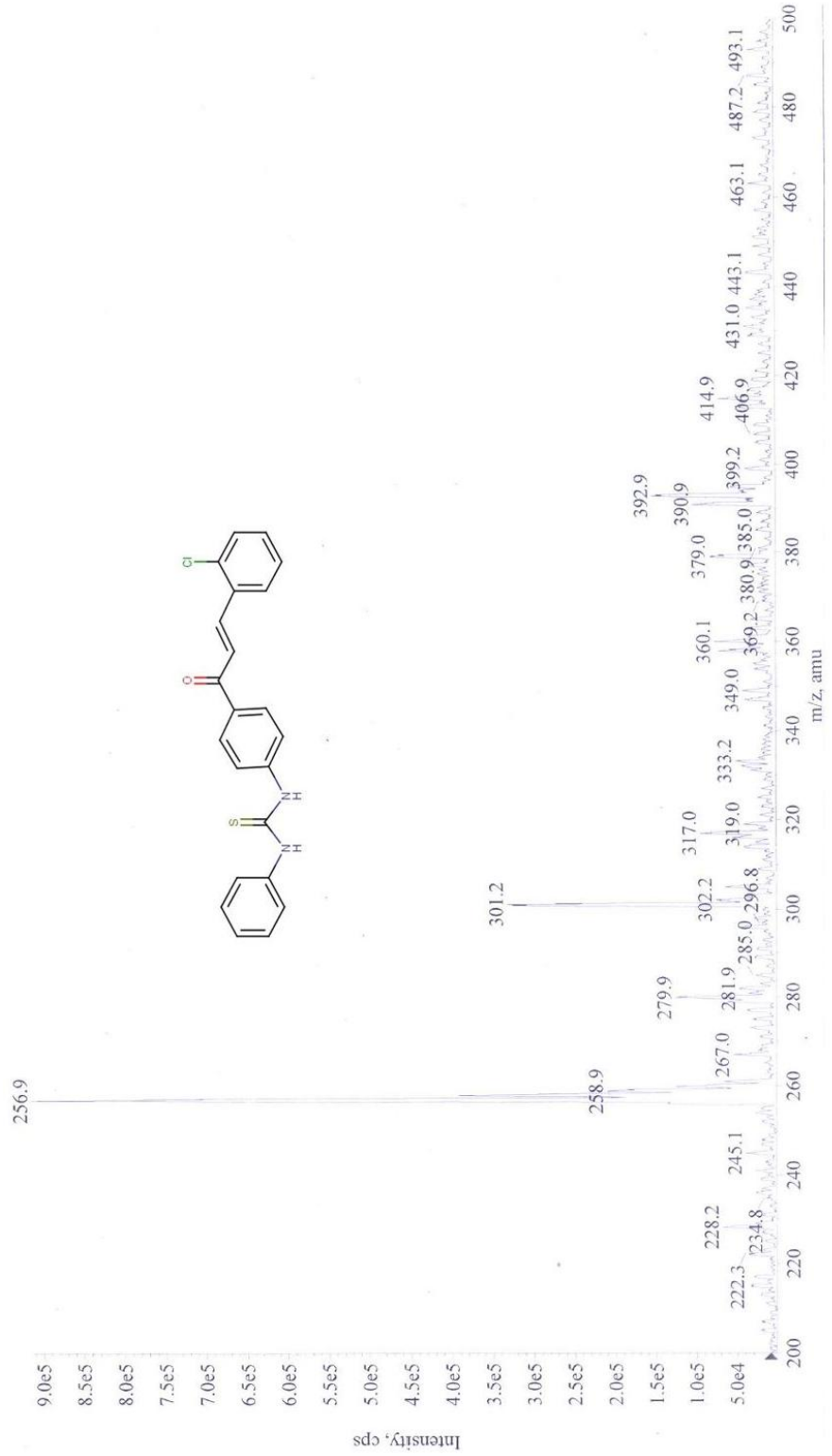
^{1}H NMR of 1-[4′-N-(N′-phenylthiourenyl) phenyl]-3-(2-chlorophenyl)-2-propen-1-one (5o)



^{13}C NMR of 1-[4′-N-(N′-phenylthiourenyl) phenyl]-3-(2-chlorophenyl)-2-propen-1-one (5o)



FT-IR of 1-[4′-*N*-(*N*′-phenylthiourenyl) phenyl]-3-(2-chlorophenyl)-2-propen-1-one (5o)



Mass spectra of 1-[4′-N-(N′-phenylthiourenyl) phenyl]-3-(2-chlorophenyl)-2-propen-1-one (5o)

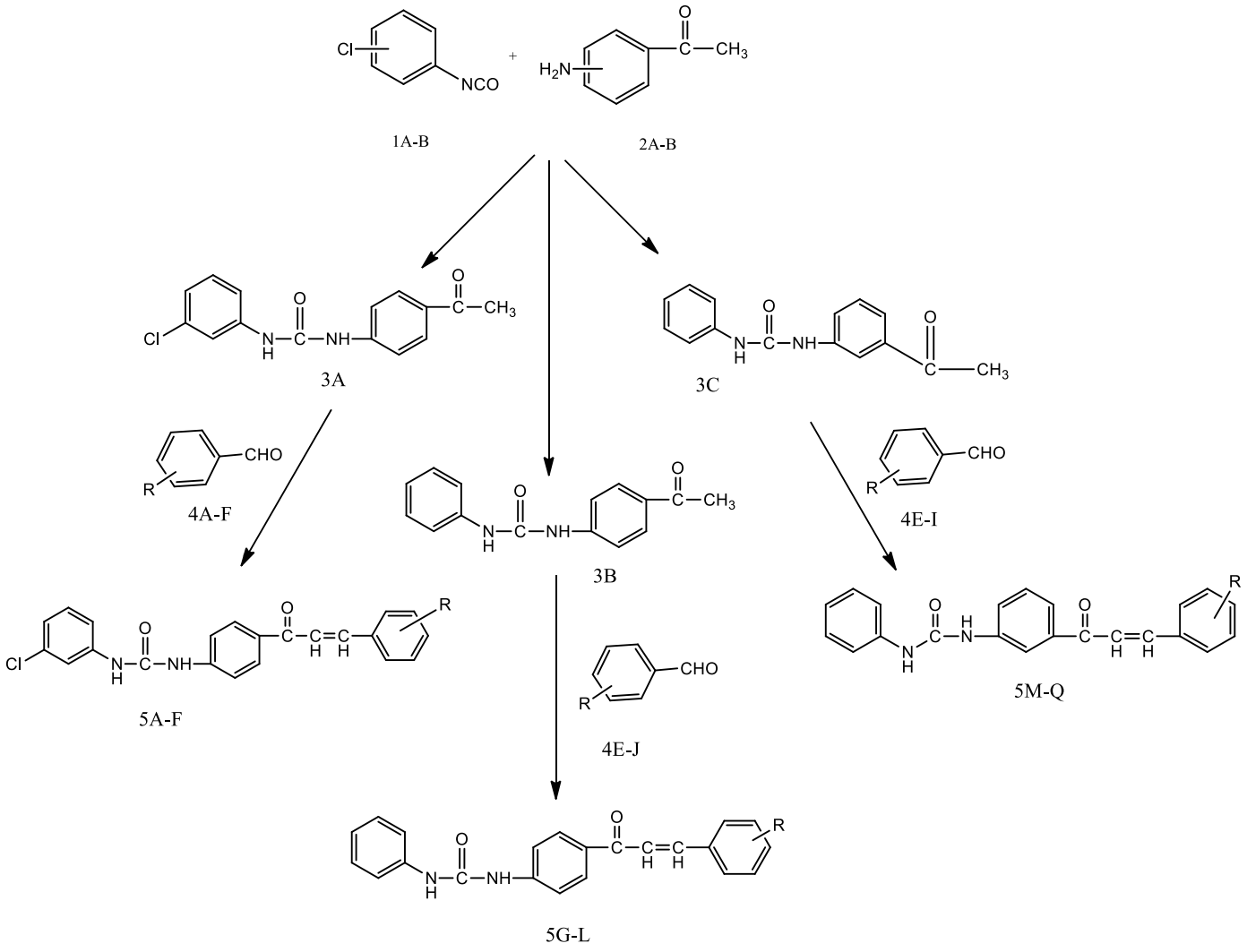
Chapter 5

Synthesis, in-vitro antioxidant and antidiabetic activity of hybrid phenylurenyl derivatives containing chalcone moiety

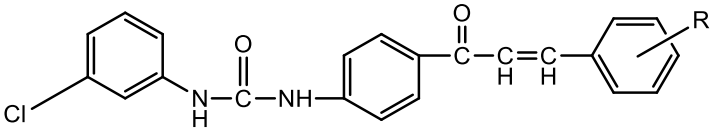
Synthesis, *in vitro* antioxidant and antidiabetic activity of hybrid phenylurenyl derivatives containing chalcone moiety

All the reagents and chemicals were purchased from Sigma Aldrich, Spectrochem, Alfa Aesar and SRL. The reaction progress was observed by Thin layer chromatography (TLC). TLC was run on commercially precoated plates (Merck, silica gel 60F254) and chloroform: methanol was used as a developing solvent system and detection of spots was made by UV light and/or by iodine vapours. Melting points were checked by Veego melting point apparatus and were uncorrected. IR spectra were obtained on a Bruker FTIR (Alpha) spectrometer. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 on a Bruker spectrometer. Chemical shifts were denoted in δ (ppm) relative to the internal reference tetramethylsilane.

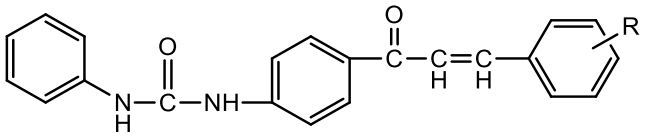
General methodology and experimental work



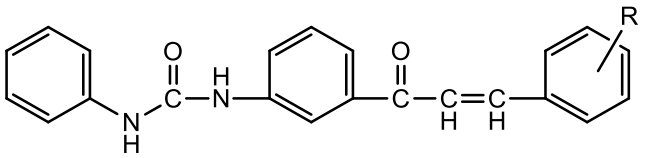
Scheme 2. Synthetic route for the preparation of hybrid phenylurenyl derivatives **(5A-Q)**



Compounds	R
5A	4-OCH_3
5B	3-OCH_3
5C	2-Cl
5D	2-OCH_3
5E	4-Br
5F	4-N, N- $(CH_3)_2$



Compounds	R
5G	3-OCH_3
5H	3-Cl
5I	3-Br
5J	2,6-di-Cl
5K	2-Cl
5L	2-OCH_3



Compounds	R
5M	3-Cl
5N	3-OCH_3
5O	2-Cl
5P	2-OCH_3
5Q	2,6-di-Cl

General procedures for the synthesis of phenylurenyl acetophenone

A mixture of the phenyl isocyanate **(1A-B)** and aminoacetophenone **(2A-B)** derivatives were dissolved in toluene. The mixture was refluxed; yellow solid was filtered out and dried. Recrystallization afforded the desired phenylurenyl acetophenone **(3A-C)** derivatives in pure form (Sonmez *et al.*, 2011).

General Procedure for the synthesis of phenylurenyl chalcone Derivatives:

Phenylurenyl chalcone **(5A-Q)** derivatives were synthesized by reacting equimolecular quantities of phenylurenyl acetophenone **(3A-C)** derivatives and the corresponding benzaldehydes **(4A-J)** in the presence of excess sodium hydroxide in methanol. At room temperature, the mixture was stirred;

the resulting precipitate was filtered and dried in air. The precipitate was recrystallized from ethanol (Domínguez *et al.,* 2005; Dos Santos *et al.,* 2008; Sonmez *et al.,* 2011).

1-(4-acetylphenyl)-3-(3-chlorophenyl)urea (3A)

Yield: 91%; mp: 171-173°C; mf: $C_{15}H_{13}N_2O_2Cl$; mw: 288.77; IR ν_{max} 3368 (NH), 1723 (CO-Urea), 1638 (CO) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6, ppm): δ 3.54 (s, 3H, OCH_3), 7.06 (d, 1H, J=6.4), 7.35-7.28 (m, 2H), 7.72-7.57 (m, 3H), 7.92 (d, 2H, J=.8), 9.03 (s, 1H, NH), 9.19 (s, 1H, NH); ^{13}C NMR (400 MHz, DMSO-d_6, ppm): δ 30.61, 113.58, 115.24, 121.88, 127.82, 128.08, 128.59, 131.09, 132.41, 138.33, 145.60, 152.02, 188.68.

1-(4-acetylphenyl)-3-phenylurea (3B)

Yield: 90%; mp: 183-186°C; mf: $C_{15}H_{14}N_2O_2$; mw: 254.28; IR ν_{max} 3341 (NH), 1655 (CO-Urea), 1584 (CO, α, β-Unsaturated) cm^{-1};^1H NMR (400 MHz, DMSO-d_6, ppm): δ 3.50 (3H, OCH_3), 7.01 (t, 1H), 7.31 (t, 2H), 7.47 (d, 2H, J=7.6 Hz), 7.59 (d, 2H, J=8.8 Hz), 7.91 (d, 2H, J=8.8 Hz), 8.82 (s, 1H, NH), 9.12 (s,1H, NH). ^{13}C NMR (400 MHz, DMSO-d_6, ppm): δ 26.25, 117.09, 118.41, 122.22, 128.79, 129.61, 130.34, 139.16, 144.28, 152.12, 196.36.

1-(3-acetylphenyl)-3-phenylurea (3C)

Yield: 86%; mp: 153-155°C; mf: $C_{15}H_{14}N_2O_2$; mw: 254.28; IR ν_{max} 3291 (NH), 1717 (CO-Urea), 1629 (CO) cm^{-1}.

1-(3-chlorophenyl)-3-(4-(3-(4-methoxyphenyl)acryloyl)phenyl)urea (5A)

Yield: 78%; mp: 192-194°C; mf: $C_{23}H_{19}N_2O_3Cl$; mw: 406.898; IR ν_{max} 3299 (NH), 1680 (CO-Urea), 1650 (CO, α, β-Unsaturated) cm-1; ^{1}HNMR (400MHz, DMSO-d_6, ppm): δ 3.81 (s, 3H, OCH3), 7.02 (t, 3H), 7.30 (d, 2H, J=6.0Hz), 7.62 (d, 2H, J=8.7Hz), 7.69-7.78 (m, 3H), 7.83 (d, 2H, J=8.7Hz), 8.12 (d, 2H, J=8.7Hz), 9.09 (br s, 1H, NH), 9.26 (br s, 1H, NH); ^{13}C NMR(400MHz, DMSO-d_6, ppm): δ 55.27, 114.31, 116.83, 117.40, 117.73, 119.32, 121.80, 127.37, 129.89, 130.39, 130.60, 131.39, 133.17, 140.79, 143.09, 143.93, 152.00, 161.14, 187.16.

1-(3-chlorophenyl)-3-(4-(3-(3-methoxyphenyl)acryloyl)phenyl)urea (5B)

Yield: 67%; mp: 184-186°C; mf: $C_{23}H_{19}N_2O_3Cl$; mw: 406.898; IR ν_{max} 3342 (NH), 1720 (CO-Urea), 1640 (CO, α, β-Unsaturated) cm-1; ^{1}HNMR (400MHz, DMSO-d_6, ppm): δ 3.82 (s, 3H, OCH3), 7.01 (d, 2H, J=7.2Hz), 7.30-7.47 (m, 5H), 7.62-7.70 (m, 4H), 7.95 (d, 1H, J=15.6Hz), 8.15 (d, 2H, J=8.7Hz), 9.12 (br s, 1H, NH), 9.31 (br s, 1H, NH); ^{13}C NMR(400MHz, DMSO-d_6, ppm): δ

55.22, 113.18, 116.47, 116.86, 117.42, 117.76, 121.53, 121.83, 122.13, 129.86, 130.09, 130.39, 131.11, 133.17, 136.15, 140.76, 143.11, 144.16, 152.60, 159.57, 187.30.

1-(3-chlorophenyl)-3-(4-(3-(4-chlorophenyl)acryloyl)phenyl)urea (5C)

Yield: 77%; mp: 249-251°C; mf: $C_{22}H_{16}N_2O_2Cl_2$; mw: 411.364; IR ν_{max} 3280 (NH), 1713 (CO-Urea), 1638 (CO, α, β-Unsaturated) cm-1; ^{1}HNMR (400MHz, DMSO-d_6, ppm): δ 7.04 (d, 1H), 7.31 (d, 2H, J=6.6Hz), 7.52 (d, 2H, J=8.1Hz), 7.62-7.71 (m, 4H), 7.91-7.99 (m, 3H), 8.14 (d, 2H, J=8.4Hz), 9.03 (br s, 1H, NH), 9.23 (br s, 1H, NH); ^{13}C NMR(400MHz, DMSO-d_6, ppm): δ 116.85, 117.42, 117.76, 121.83, 122.60, 128.85, 130.08, 130.39, 131.01, 133.17, 133.70, 134.83, 140.74, 141.60, 144.20, 151.98, 187.14.

1-(3-chlorophenyl)-3-(4-(3-(2-methoxyphenyl)acryloyl)phenyl)urea (5D)

Yield: 82%; mp: 180-182°C; mf: $C_{23}H_{19}N_2O_3Cl$; mw: 406.898; IR ν_{max} 3354 (NH), 1724 (CO-Urea), 1636 (CO, α, β-Unsaturated) cm-1; ^{1}HNMR (400MHz, DMSO-d_6, ppm): δ 3.89 (s, 3H, OCH3), 7.04 (d, 2H, J=7.5Hz), 7.11 (d, 1H, J=8.4Hz), 7.29- 7.43 (m, 3H), 7.63 (d, 2H, J=8.4Hz), 7.72-7.99 (m, 4H), 8.10 (d, 2H, J=8.4Hz), 9.03 (br s, 1H, NH), 9.22 (br s, 1H, NH); ^{13}C NMR(400MHz, DMSO-d_6, ppm): δ 55.60, 111.66, 116.84, 117.46, 117.76, 120.62, 121.65, 121.81, 122.98, 128.31, 129.58, 129.93, 130.38, 131.28, 132.06, 133.18, 137.62, 140.78, 144.03, 152.00, 158.10, 187.43.

1-(4-(3-(4-bromophenyl)acryloyl)phenyl)-3-(3-chlorophenyl)urea (5E)

Yield: 71%; mp: 282-284°C; mf: $C_{22}H_{16}N_2O_2ClBr$; mw: 455.768; IR ν_{max} 3314 (NH), 1689 (CO-Urea), 1650 (CO, α, β-Unsaturated) cm-1; ^{1}HNMR (400MHz, DMSO-d_6, ppm): δ 7.05- 7.29 (m, 3H), 7.61- 7.71 (m, 6H), 7.84 (d, 2H, J=8.4Hz), 7.97 (d, 1H, J=15.9Hz), 8.14 (d, 2H, J=8.4Hz), 9.03 (br s, 1H, NH), 9.23 (br s, 1H, NH); ^{13}C NMR(400MHz, DMSO-d_6, ppm): δ 116.85, 117.42, 117.76, 121.82, 122.76, 123.70, 130.09, 130.38, 130.61, 131.01, 131.78, 133.17, 134.04, 140.75, 141.69, 144.22, 151.98, 187.14.

1-(3-chlorophenyl)-3-(4-(3-(4-(dimethylamino)phenyl)acryloyl)phenyl)urea (5F)

Yield: 62%; mp: >220°C; mf: $C_{24}H_{22}N_3O_2Cl$; mw: 419.94; IR ν_{max} 3304 (NH), 1710 (CO-Urea), 1637 (CO, α, β-Unsaturated) cm-1; ^{1}HNMR (400MHz, DMSO-d_6, ppm): δ 3.00 (s, 6H, N(CH3)2), 6.75 (d, 2H, J=8.1Hz), 7.04 (s, 1H), 7.31- 7.71 (m, 9H), 8.09 (2H), 9.02 (br s, 1H, NH), 9.17 (br s, 1H, NH).

1-(4-(3-(3-methoxyphenyl)acryloyl)phenyl)-3-phenylurea (5G)

Yield: 74%; mp: 188-189°C; mf: $C_{23}H_{20}N_2O_3$; mw: 372.406; IR ν_{max} 3376 (NH), 1730 (CO-Urea), 1643 (CO, α, β-Unsaturated) cm-1; ^{1}HNMR (400MHz, DMSO-d_6, ppm): δ 3.83 (s, 3H, OCH3), 7.00 (t, 2H), 7.28- 7.46 (m, 7H), 7.63- 7.71 (m, 3H), 8.14 (t, 2H), 9.14 (br s, 1H, NH), 9.15 (br s, 1H, NH); ^{13}C NMR(400MHz, DMSO-d_6, ppm): δ 55.22, 113.16, 116.47, 117.24, 118.43, 121.52, 122.17, 122.24, 128.79, 129.87, 130.11, 130.88, 136.16, 139.16, 143.05, 144.46, 152.11, 159.57, 187.29.

1-(4-(3-(3-chlorophenyl)acryloyl)phenyl)-3-phenylurea (5H)

Yield: 76%; mp: 196-198°C; mf: $C_{22}H_{17}N_2O_2Cl$; mw: 376.872; IR ν_{max} 3283 (NH), 1713 (CO-Urea), 1643 (CO, α, β-Unsaturated) cm-1; ^{1}HNMR (400MHz, DMSO-d_6, ppm): δ 6.99 (d, 1H, J=6.0Hz), 7.29 (d, 2H, J=6.0Hz), 7.47-7.71 (m, 7H), 7.80 (1H), 8.00 (d, 2H, J=9.0Hz), 8.15 (d, 2H, J= 6.0Hz), 8.82 (br s, 1H, NH), 9.17 (br s, 1H, NH); ^{13}C NMR(400MHz, DMSO-d_6, ppm): δ 117.22, 118.43, 122.23, 123.46, 127.69, 127.78, 128.78, 129.87, 130.21, 130.58, 130.73, 133.74, 137.03, 139.17, 141.29, 144.60, 152.10, 187.10.

1-(4-(3-(3-bromophenyl)acryloyl)phenyl)-3-phenylurea (5I)

Yield: 81%; mp: 207-208°C; mf: $C_{22}H_{17}N_2O_2Br$; mw: 421.276; IR ν_{max} 3289 (NH), 1643 (CO, α, β-Unsaturated) cm-1; ^{1}HNMR (400MHz, DMSO-d_6, ppm): δ 6.98 (t, 1H), 7.29 (d, 2H, J=7.8Hz), 7.39-7.47 (m, 3H), 7.60-7.67 (m, 4H), 7.82 (d, 2H, J=9.0Hz), 8.16 (t, 2H, J=6.0Hz), 7.97 (2H), 8.80 (br s, 1H, NH), 9.14 (br s, 1H, NH).

1-(4-(3-(2,6-dichlorophenyl)acryloyl)phenyl)-3-phenylurea (5J)

Yield: 85%; mp: 215-217°C; mf: $C_{22}H_{16}N_2O_2Cl_2$; mw: 411.364; IR ν_{max} 3308 (NH), 1714 (CO-Urea), 1649 (CO, α, β-Unsaturated) cm-1; ^{1}HNMR (400MHz, DMSO-d_6, ppm): δ 6.98 (1H), 7.28 (t, 2H), 7.43 (t, 3H), 7.56-7.67 (m, 5H), 7.76 (1H), 8.02 (d, 2H, J=9.0Hz), 8.79 (br s, 1H, NH), 9.15 (br s, 1H, NH).

1-(4-(3-(2-chlorophenyl)acryloyl)phenyl)-3-phenylurea (5K)

Yield: 92%, mp: 213-215°C; mf: $C_{22}H_{17}N_2O_2Cl$; mw: 376.87; IR ν_{max} 3261 (NH), 1713 (CO-Urea), 1649 (CO, α, β-Unsaturated) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.021 (t, 1H), 7.32 (t, 1H), 7.67-7.45 (m, 8H), 8.24-7.98 (m, 5H), 8.86 (s, 1H, NH), 9.20 (s, 1H, NH).

1-(4-(3-(2-methoxyphenyl)acryloyl)phenyl)-3-phenylurea (5L)

Yield: 88%; mp: 212-213°C; mf: $C_{23}H_{20}N_2O_3$; mw: 372.41; IR ν_{max} 3346 (NH), 1713 (CO-Urea), 1641 (CO, α, β-Unsaturated) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 3.91 (s, 3H, OCH_3), 7.04 (q, 1H), 7.13 (d, 2H, J=8.4), 7.32 (t, 1H), 7.49-7.43 (m, 4H), 7.65 (d, 2H, J= 8.8), 8.05-7.88 (m, 3H), 8.12 (d, 2H, J=8.8), 8.85 (s, 1H, NH), 9.17 (s, 1H, NH).

1-(3-(3-(3-chlorophenyl)acryloyl)phenyl)-3-phenylurea (5M)

Yield: 93%; mp: 172-175°C; mf: $C_{22}H_{17}N_2O_2Cl$; mw: 376.86; IR ν_{max} 3292(NH), 1664 (CO-Urea), 1628 (CO, α, β-Unsaturated) cm^{-1}; ^{1}HNMR (400MHz, DMSO-d_6, ppm): δ 6.98 (t, 1H), 7.29 (t, 2H), 7.70-7.45 (m, 5H), 7.76 (d, 2H), 7.85 (d, 2H), 7.97 (d, 1H, J=4.5 Hz), 8.08 (d, 1H), 8.14 (t, 1H), 8.73 (s, 1H, NH), 8.92 (s, 1H, NH); ^{13}C NMR(400MHz, DMSO-d_6, ppm): δ 118.19, 118.82, 122.45, 122.96, 123.54, 123.77, 124.21, 128.23, 128.45, 129.24, 129.71, 130.61, 131.17, 134.27, 137.39, 138.43, 139.97, 140.59, 140.74, 142.71, 153.00, 189.53.

1-(3-(3-(3-methoxyphenyl)acryloyl)phenyl)-3-phenylurea (5N)

Yield: 87%; mp: 184-187°C; mf: $C_{23}H_{20}N_2O_3$; mw: 360.38; IR ν_{max} 2903 (NH), 1716 (CO-Urea), 1628 (CO, α, β-Unsaturated) cm^{-1}; ^{1}HNMR (400MHz, DMSO-d_6, ppm): δ 3.34 (s, 3H, OCH_3), 6.98 (t, 1H), 7.04 (d, 1H, J=7 Hz), 7.29 (t, 1H), 7.37 (t, 1H), 7.42-7.65 (m, 5H), 7.71-7.88 (m, 4H), 8.14 (d, 1H), 8.75 (s, 1H), 8.94 (s, 1H, NH), 9.04 (s, 1H,NH).

1-(3-(3-(2-chlorophenyl)acryloyl)phenyl)-3-phenylurea (5O)

Yield: 73%; mp: 169-171°C; mf: $C_{22}H_{17}N_2O_2Cl$; mw: 376.86; IR ν_{max} 3446 (NH), 1715 (CO-Urea), 1635 (CO, α, β-Unsaturated) cm^{-1}; ^{1}HNMR (400MHz, DMSO-d_6, ppm): δ 6.98 (t, 1H), 7.40-7.26 (m, 3H), 7.52-7.42 (m, 4H), 7.54 (d, 1H), 7.60 (d, 1H), 7.76 (d, 1H), 8.84 (d, 1H), 7.93 (t, 1H), 8.03 (d, 1H), 8.18 (t, 1H), 8.87 (s, 1H,NH), 8.94 (s, 1H. NH).

1-(3-(3-(2-methoxyphenyl)acryloyl)phenyl)-3-phenylurea (5P)

Yield: 74%; mp: 109-112°C; mf: $C_{23}H_{20}N_2O_3$; mw: 360.38; IR ν_{max} 3289 (NH), 1715 (CO-Urea), 1634 (CO, α, β-Unsaturated) cm^{-1}; ^{1}HNMR (400MHz, DMSO-d_6, ppm): δ 3.34 (s, 3H, OCH_3), 7.01 (dt, 2H), 7.12 (q, 1H), 7.27 (td, 2H), 7.41-7.49 (m, 4H), 7.57 (d, 1H), 7.66-7.73 (m, 3H), 7.76 (d, 1H), 7.80 (t, 1H), 8.73 (s, 1H, NH), 8.93 (s, 1H, NH).

1-(3-(3-(2, 6-dichlorophenyl)acryloyl)phenyl)-3-phenylurea (5Q)

Yield: 96%; mp: 129-132°C; mf: $C_{22}H_{16}N_2O_2Cl_2$; mw: 411.35; IR ν_{max} 3307 (NH), 1715 (CO-Urea), 1645 (CO, α, β-Unsaturated) cm^{-1}; ^{1}HNMR (400MHz, DMSO-d_6, ppm): δ 7.23 (t, 1H), 7.40 (d,

2H), 7.42-7.51 (m, 5H), 7.55 (t, 2H), 7.59-7.77 (m, 3H), 8.17 (s, 1H), 8.71 (s, 1H, NH), 8.90 (s, 1H, NH).

In vitro α-glucosidase inhibition assay

The α-glucosidase inhibition assay was performed according to the modified method described by Patil (Patil *et al.*, 2013). 40 µL of yeast α-glucosidase (0.1 unit/ml; Sigma Aldrich, USA) was mixed with 20 µL of each of the samples (5 mg/mL DMSO solution and reconstituted in 100 µL of 100 mM phosphate buffer pH 6.8) and incubated for 1 h at 37ºC in 96-well microplate. Enzyme action for α-glucosidase was initiated by addition of 50 µL substrate (5 mM, *p*-nitrophenyl- α-D-glucopyranoside prepared in 100 mM phosphate buffer of pH 6.8) and stopped by adding 100 µL of 0.1 M Na_2CO_3 after an incubation of 10 min at 37ºC. The release of *p*-nitrophenol was measured at 420 nm spectrophotometrically (Spectra Max plus 384, Molecular Devices Corporation, Sunnyvale, CA, USA) 5 min after incubation with the substrate. Individual blanks for test samples were prepared to correct background absorbance where the substrate was replaced with 50 µL of the buffer. Control sample contained 10 µL DMSO in place of test samples.

Percentage of enzyme inhibition was calculated as (1 - B/A) × 100, where [A] represents absorbance of control without test samples, and [B] represents absorbance in the presence of test samples.

In vitro antioxidant study

DPPH scavenging activity

DPPH scavenging activity is one of the most widely used methods for screening the antioxidant activity (Blois, 1958). The DPPH test gives information on the reactivity of the test compounds with a stable free radical (Mohana *et al.*, 2013). The radical scavenging activity was determined according to the method of Blois (Blois, 1958). 25µl of variable concentrations of synthesized compounds (10-100 µM in ethanol) was added to 100 µl of a DPPH solution (0.004% w/v in ethanol) in 96 well microplates and kept for 60 minutes in dark place at room temperature. The decrease in the solution absorbance was determined at 517 nm. The positive control used was Ascorbic acid. The percentage DPPH radical scavenging activity was calculated using the following formula:

DPPH radical scavenging activity (%) = $[(A_c - A_t)/A_c \times 100]$, where A_c is the absorbance of the control and A_t is the absorbance of tested compounds or the standard drug.

Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging activity of the tested compounds was determined by the method of Jayaprakasha *et al.,* (Jayaprakasha, *et al.,* 2004). Hydrogen peroxide solution (2mmol/l) was prepared in phosphate buffer (pH 7.4). 50µl of different concentrations (10-100µM) of synthesized compounds were added to 50 µl of hydrogen peroxide solution. The absorbance of hydrogen peroxide was determined at 230 nm after 10 min against a blank solution and compared with Ascorbic acid, the reference compound.

Hydrogen peroxide scavenging activity (%) = $[(A_c - A_t)/A_c \times 100]$, where A_c is the absorbance of the control and A_t is the absorbance of tested compounds or the standard drug.

Nitric oxide scavenging activity

The Nitric oxide scavenging was measured according to the modified method of Sreejayan *et al.,* (Sreejayan *et al.,* 1997). Sodium nitroprusside in phosphate buffer was mixed with different concentration of the synthesized compounds dissolved in ethanol and incubated at room temperature for 2 h. 50 µl of the incubation solution was removed and diluted with an equal volume of Griess reagent (1% sulfanilamide in 5% H_3PO_4 and 0.1% was diamine dihydrochloride). The absorbance was determined at 546 nm and compared with standard Ascorbic acid.

Nitric oxide scavenging activity (%) = $[(A_c - A_t)/A_c \times 100]$, where A_c is the absorbance of the control and A_t is the absorbance of tested compounds or the standard drug.

Result and discussion

Chemistry

The phenylurenyl chalcone **(5A-Q)** derivatives were synthesized, as shown in **scheme 2**. Substituted phenyl isocyanates **(1A-B)** were refluxed with Substituted amino acetophenones **(2A-B)** in toluene for 18-22 hours. The yellow/white solid phenylurenyl acetophenones **(3A-C)** were obtained. Finally, stirring of **(3A-C)** with corresponding benzaldehydes **(4A-J)** in methanol at room temperature yielded phenylurenyl chalcone **(5A-Q)** derivatives.

The synthesized compounds (**5A-Q)** were characterized by ^{1}H NMR, ^{13}C NMR and FT-IR. All the ^{1}H NMR spectra of urea derivatives carried out in DMSO-d_6 exhibited a broad singlet in the range δ = 8.71–9.26 ppm to the proton on the urea nitrogen atom. The ^{13}C NMR spectra showed the signal of the urea C=O carbon atom in the range δ = 152.60–161.11 ppm and chalcone C=O carbon range δ 187.10- 189.53 ppm. The FTIR Spectra indicated the ν_{max} of 3368-3261 cm^{-1} for NH stretching, 1680-1730 cm^{-1} for CO-Urea stretching and 1628-1650 cm^{-1} for CO, α, β-Unsaturated bond.

In vitro α-Glucosidase inhibitory activity

All synthesized compounds were evaluated through inhibitory assay for their yeast α-glucosidase (*Saccharomyces cerevisiae*) inhibitory activity. It was observed that most of the urea/thiourea derivatives showed inhibitory activity against α-glucosidase (**Table 1**). Among the synthesized compounds, compound **5A** was found to be the most active, showing concentration-dependent inhibition of α-glucosidase activity with 91.04% inhibition at 100 µM concentration. From the dose-response curve, the IC_{50} value of **5A** was calculated as 17.35 µM (**Fig. 1**) in comparison with Acarbose showing 89.69% inhibitory activity at 100 µM concentration under similar assay conditions. The IC_{50} value of Acarbose was 11.92 µM. This data indicated that molecule **5A** showed good α-glucosidase inhibitory activity with 4-methoxy substitution. Other methoxy derivatives such as **5B**, **5D**, **5G**, **5L**, **5N** and **5P** showed the inhibitory activity of 87.05, 86.79, 81.27, 76.27, 78.60 and 69.61% respectively at 100 µM concentration. Among the chloro substituted urea derivatives, compound **5C** with 4-chloro substitution exhibited highest and dose-dependent activity.

Table 1. *In vitro* α-Glucosidase inhibitory activity of synthesized compounds and standard drug Acarbose

Compounds	**Concentration (µM)**			
	10	25	50	100
5A	39.89±0.82	56.08±2.07	75.89±2.35	91.04±0.42
5B	42.55±1.13	40.92±1.54	76.55±2.68	87.05±0.74
5C	22.57±1.11	36.92±1.60	65.73±1.18	79.30±1.29
5D	36.55±1.21	45.92±1.35	65.36±2.33	86.79±1.22
5E	05.89±0.71	18.01±1.09	43.84±2.38	66.00±2.71
5F	08.85±1.46	24.67±3.32	37.17±2.35	59.33±0.89
5G	33.77±1.70	54.89±1.72	71.85±1.78	81.27±1.71
5H	09.29±1.15	18.08±0.45	54.15±2.25	67.95±1.63
5I	ND	08.12±0.42	25.43±1.37	57.24±1.42
5J	ND	12.57±2.64	21.27±1.69	53.36±1.36
5K	18.42±1.17	28.74±0.73	57.16±1.09	70.29±0.69
5L	20.44±1.12	46.74±2.31	58.52±2.35	76.27±1.59
5M	ND	10.41±1.11	33.93±2.10	58.47±0.63
5N	19.77±0.65	32.74±1.93	60.16±1.44	78.60±0.58
5O	16.93±2.20	34.72±1.05	56.37±1.60	66.01±1.92
5P	14.38±2.06	24.05±2.88	51.36±0.63	69.61±1.13
5Q	05.15±0.54	12.72±1.92	19.28±0.95	47.20±2.20
Acarbose	40.28±1.12	60.61±1.00	79.10±0.94	89.69±1.29

Values are mean ± SEM; experiment performed in triplicate; ND = Not Determine

It inhibited the α-glucosidase by 22.57% at 10 µM, 36.92% at 25 µM, 65.73% at 50 µM and 79.30% at 100 µM. The compounds **5E** and **5I** with bromo substitution inhibited the α-glucosidase by 66.00%, 57.24% at 100 µM concentration. The 2, 6-dichloro substituted derivatives **(5J, 5Q)** showed poor inhibitory activity.

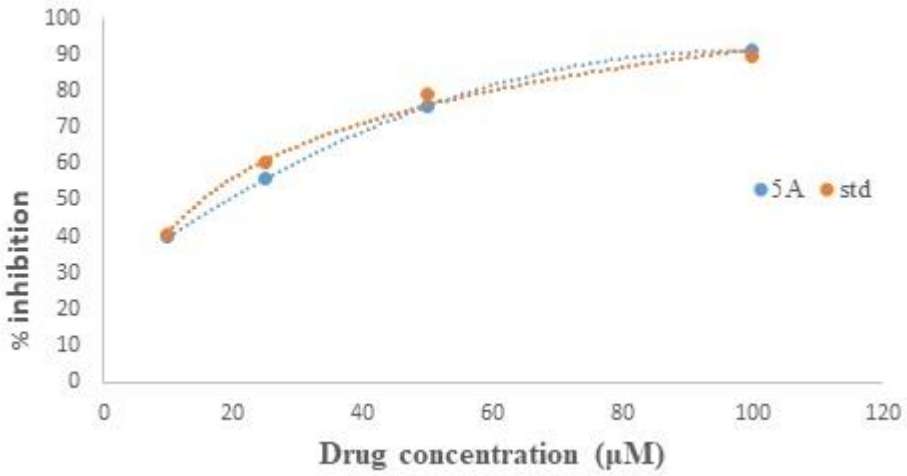


Fig. 1. Dose-response curve of α-glucosidase inhibition by compound 5A.

DPPH Scavenging activity

The antioxidant abilities of new compounds are evaluated by DPPH (2, 2- diphenyl-1-picrylhydrazyl) radical-scavenging assay which is based on to capture free radicals produced by DPPH-H (Bartolome *et al.,* 2004). DPPH is a stable nitrogen-based free radical. Its reaction rates correlate with antioxidant activity, the higher the rate, the more effective the antioxidant **(**Wright *et al.*, 2003). DPPH solution shows a deep purple color. When the purple color is changed to yellow, it leads to decreased absorbance. The absorption is maximum at 517 nm **(**Raghavendra *et al.,* 2016).

The scavenging of DPPH radicals by most of these compounds occurred in a concentration-dependent manner from 10 to 100 µM. The compounds with 4-methoxy **(5A),** 3-methoxy **(5B, 5G, 5N)** and 2-methoxy **(5D, 5L, 5P)** substitution showed maximum DPPH Scavenging activity (**Table 2**) when compared with Ascorbic acid as a standard. 4-chloro substituted derivatives **(5C)** showed maximum DPPH scavenging activity of 88.03% at 100 µM concentration among other chloro derivatives. The bromo derivatives of **5E** and **5I** showed the moderate activity of 65.36 and 65.92% at a concentration of 100 µM respectively.

Table 2. DPPH Scavenging activity

Compounds	**Concentration (µM)**			
	10	25	50	100
5A	43.63±0.58	68.81±0.32	84.58±0.75	93.43±0.27
5B	40.43±1.12	59.37±0.52	82.63±0.41	91.71±0.48
5C	25.71±1.16	56.67±1.19	75.62±0.65	88.03±0.87
5D	36.99±0.81	61.58±0.61	78.70±0.44	87.99±0.86
5E	6.99±0.80	28.50±3.85	50.88±3.20	65.36±1.51
5F	9.65±1.05	25.71±2.78	42.08±1.87	54.66±1.96
5G	33.51±0.66	65.45±1.55	72.18±0.65	84.25±0.62
5H	24.58±1.26	48.21±0.94	68.18±0.52	77.58±0.59
5I	14.58±1.26	43.55±2.90	38.03±0.41	65.92±1.29
5J	10.91±0.83	35.21±1.57	62.44±1.21	68.59±0.47
5K	16.51±0.41	37.18±0.36	56.39±0.58	76.92±0.46
5L	30.74±0.53	63.76±0.83	70.54±0.85	80.25±0.48
5M	17.07±1.54	23.76±0.83	43.57±1.69	70.25±1.38
5N	29.74±0.32	54.09±0.88	78.81±0.72	87.25±1.13
5O	17.41±2.56	34.09±0.88	67.88±0.64	78.51±0.46
5P	29.04±0.23	44.06±1.69	67.22±0.88	78.55±0.37
5Q	9.04±0.23	14.72±0.28	34.89±1.09	48.61±0.43
Ascorbic acid	46.73±0.85	65.67±0.87	88.05±0.43	98.02±0.43

Values are mean ± SEM; experiment performed in triplicate; ND = not done.

Hydrogen peroxide scavenging activity

During the inflammatory processes, several reactive species are produced in excess, the ROS, H_2O_2, peroxyl radical and HOCl. They play important roles in their pathophysiological conditions. Hydrogen peroxide (H_2O_2) is a non-radical reactive oxygen species (ROS) that can influence several cellular processes, which makes them potential targets for the therapy of inflammation (Costa *et al.*, 2005).

In vitro antioxidant activities of synthesized compounds (**5A-Q**) were evaluated against hydrogen peroxide. Compounds **5A**, **5B**, **5D**, **5G**, **5L**, **5N**, **5P** and **5C**, **5H**, **5K**, **5M**, **5O** with methoxy and chlorine as substituent showed better activity as compared with the standard Ascorbic acid (**Table 3**). Hydrogen peroxide scavenging activity of methoxy derivatives is in the order of **5A** > **5B** > **5D**> **5G**>**5L**>**5N**>**5P**. The compound **5C** with 4 -chloro substitution showed 72.33% scavenging activity at 100 µM concentration but other chloro derivatives showed less than 70% scavenging activity.

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Table 3. Hydrogen peroxide scavenging activity

Compounds	Concentration (µM)			
	10	25	50	100
5A	42.63±0.64	64.58±0.75	79.10±0.44	89.10±0.44
5B	33.77±2.41	47.71±1.27	72.63±0.41	86.71±0.61
5C	13.12±1.68	36.67±1.19	56.62±1.63	72.33±1.22
5D	31.32±0.41	41.58±0.61	66.15±0.93	83.66±0.61
5E	ND	5.17±2.42	24.88±2.24	58.69±1.51
5F	ND	14.05±2.83	25.08±2.54	48.26±0.42
5G	24.85±1.97	55.45±1.56	70.52±1.43	80.59±0.47
5H	04.58±1.26	18.21±0.94	36.11±1.69	67.58±0.59
5I	03.74±1.58	24.16±0.81	20.88±1.13	46.92±0.89
5J	07.25±1.50	12.81±1.05	24.55±1.22	51.32±0.81
5K	06.51±2.14	17.18±0.36	36.39±0.58	66.92±0.46
5L	22.74±1.46	43.76±0.83	64.21±2.34	77.59±0.97
5M	09.41±1.09	19.76±0.77	43.23±1.15	63.25±0.14
5N	20.07±0.43	44.09±0.88	71.11±0.96	77.51±0.69
5O	15.11±1.01	24.14±0.86	40.54±0.89	62.51±1.64
5P	18.04±0.94	34.99±1.52	52.57±1.70	73.55±0.66
5Q	ND	4.72±0.28	18.55±0.93	49.61±0.42
Ascorbic acid	40.77±0.53	68.33±0.57	80.55±0.41	96.92±0.38

Values are mean ± SEM; experiment performed in triplicate; ND = Not Determine.

Nitric oxide scavenging activity

Nitric oxide (NO) which is an important chemical mediator of various physiological processes, is generated by endothelial cells, macrophages, neurons, etc. (Lata *et al.,* 2003). Excess concentration of nitric oxide is associated with several diseases (Ialenti *et al.,* 1993). Nitric oxide is generated in biological tissues by specific nitric oxide synthase, which metabolizes arginine to citrulline with the formation of NO (Ross, 1993).

The principle behind the procedure is Sodium nitroprusside solution spontaneously generates the nitric oxides, that reacts with oxygen to generate nitrite ions that can be estimated using Greiss reagent. Scavengers of nitric oxides compete with oxygen leading to reduce the production of nitrite ions.

Among the synthesized derivatives with methoxy and chlorine as substituents exhibited good activity (IC_{50} value of **5A**- 43.09, **5B**- 45.53, **5D**-30.87, **5G**-54.39, **5L**-57.87, **5N**-63.79, **5P**- 67.26 and **5C**-56.03 respectively) whereas, bromo derivates (**5E**) showed poor scavenging activity.

Table 4. Nitric oxide Scavenging activity

Compounds	**Concentration (µM)**			
	10	25	50	100
5A	21.63±1.58	40.04±1.14	66.22±0.62	79.98±0.29
5B	16.83±1.63	37.83±1.39	70.95±0.98	76.20±0.56
5C	18.45±1.24	28.34±2.34	52.63±1.37	75.53±0.67
5D	29.67±1.52	61.58±0.60	57.64±1.06	77.26±1.54
5E	ND	08.84±0.97	20.80±0.39	35.36±2.54
5F	ND	07.38±1.57	19.75±0.77	44.87±2.02
5G	10.15±0.71	36.11±1.94	56.15±1.52	75.65±2.41
5H	02.25±0.23	08.43±1.08	28.69±0.55	62.17±1.04
5I	ND	09.55±0.90	32.70±2.56	67.67±1.08
5J	ND	04.48±0.71	23.88±1.39	57.99±2.70
5K	ND	12.70±1.93	26.68±0.77	60.48±0.69
5L	14.65±1.78	24.04±0.93	58.57±1.96	72.25±1.86
5M	ND	15.62±2.61	25.43±2.39	57.23±2.68
5N	14.71±2.99	25.91±0.81	46.03±1.97	70.27±0.48
5O	ND	04.45±0.72	22.52±3.53	43.85±1.30
5P	8.06±2.31	16.03±1.81	45.41±2.67	70.55±0.22
5Q	ND	ND	12.89±3.10	40.28±0.71
Ascorbic acid	37.73±0.42	65.67±0.89	82.25±0.71	93.69±0.96

Values are mean ± SEM; experiment performed in triplicate; ND = Not Determine.

Conclusion:

Diabetes mellitus, metabolic syndrome, as well as their association with oxidative stress, are co-related. Based on this relationship, seventeen hybrid urea derivatives (**5A-Q**) have been synthesized. These analogues include different substitution patterns as a key feature for antioxidant capacity as well as antidiabetic activity. It was already known that chalcone moiety is the well-known antioxidant nucleus. Synthesized hybrid urea derivatives (**5A-Q**) showed moderate to good *in vitro* antioxidant as well as *in vitro* α-glucosidase inhibitory activity. Among the synthesized compounds, compound **5A** with the 4-methoxy substitution was demonstrated to be potent antioxidant compounds by scavenging DPPH radical, hydrogen peroxide and nitric oxide radical. The α-glucosidase inhibitory activity of the compound **5A** (IC_{50}-17.35 µM) is higher than the other synthesized molecules when compared to the standard drug Acarbose (IC_{50}-16.54 µM).

The result indicates that hybrid urea derivatives are interesting antioxidant and antidiabetic entities. So, urea derivatives along with chalcone moiety will be more potent hypoglycemic compounds for the treatment of diabetes and metabolic syndrome in the near future.

References

Bartolome, B., Nunez, V., Monagas, M., Gomez Cordoves, C., 2004. *In vitro* antioxidant activity of red grape skins. Eur. Food Res. Technol. 218(2), 173.

Blois, MS., 1958. Antioxidant Determinations by the Use of a Stable Free Radical. Nature. 181(4617), 1199–1200.

Costa, A. D., Gomes, S. Reis., Lima JLFC., Fernandes, E., 2005. Hydrogen peroxide scavenging activity by non-steroidal antiinflammatory drugs. Life Sci. 76, 2841-2848.

Dos Santos, L., Lima, L.A., Cechinel-Filho, V., Corrêa, R., de Campos Buzzi, F., Nunes, R.J., 2008. Synthesis of new 1-phenyl-3-{4-[(2E)-3-phenyl prop-2-enoyl]phenyl}-thiourea and urea derivatives with anti-nociceptive activity. Bioorganic Med. Chem. 16, 8526–8534.

Domínguez, J.N., León, C., Rodrigues, J., De Domínguez, N.G., Gut, J., Rosenthal, P.J., 2005. Synthesis and evaluation of new antimalarial phenylurenylchalcone derivatives. J. Med. Chem. 48, 3654–3658.

Ialenti, S., Moncada, M., Rosa, D., 1993. Modulation of adjuvant arthritis by endogenous nitric oxide.Br. J. Pharmacol. 110, 701-705.

Jayaprakasha, GK., Jaganmohan, Rao L., Sakariah, KK., 2004.Antioxidant activities of flavidin in different *in vitro* model systems. Bioorganic & Medicinal Chemistry. 12, 5141–5146.

Lata, H., Ahuja, GK., 2003. Role of free radicals in health and disease. Ind. J. Physiol. Allied Sci. 57, 124-128.

Mohana, KN., Chikkur, B., Pradeep, K., 2013. Synthesis and Antioxidant Activity of 2-Amino-5-methylthiazol Derivatives Containing 1,3,4-Oxadiazole-2-thiol Moiety. ISRN Organic Chemistry. Article ID 620718, 8 pages.

Raghavendra, KR., Renuka, N., Kameshwar, VH., Srinivasan, B., Ajay, KK and Sheena, S., 2016. Synthesis of lignan conjugates via cyclopropanation: Antimicrobial and antioxidant studies. Bioorg. Med. Chem. Lett. 26, 3621-3625.

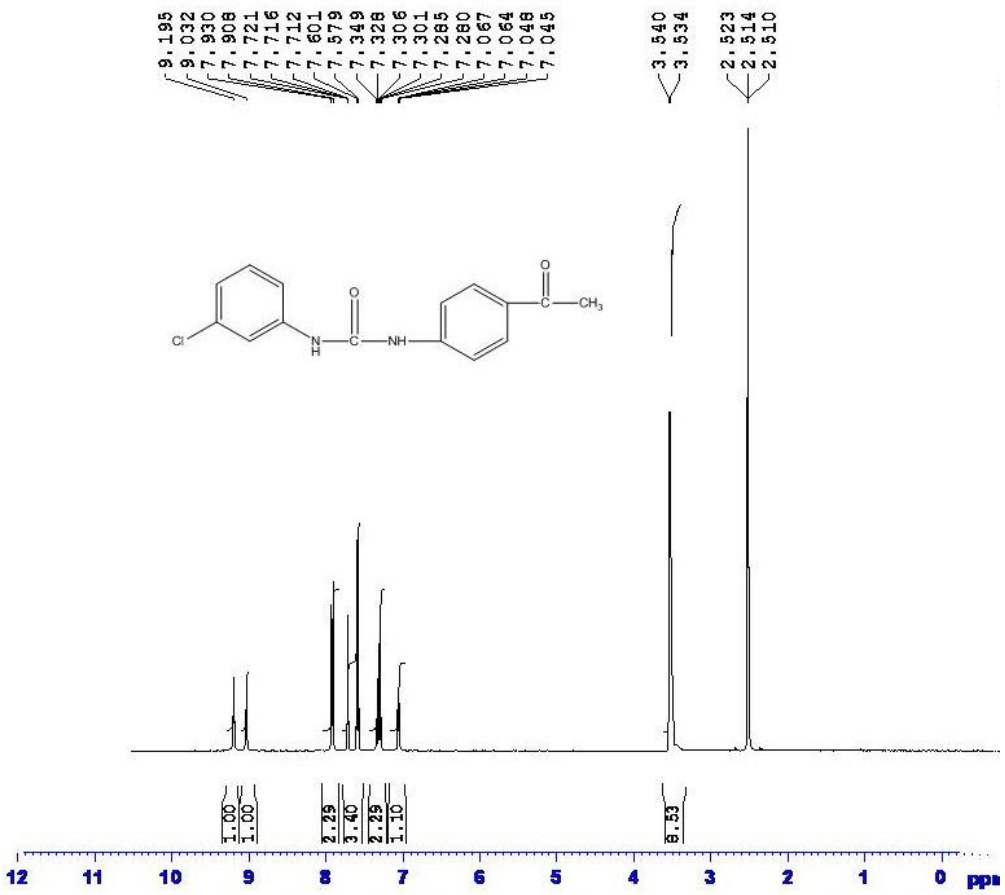
Ross, R., 1993. The pathogenesis of atherosclerosis: a perspective for the 1990's. Nature. 362, 801.

Patil, V.S., Nandre, K.P., Ghosh, S., Rao, V.J., Chopade, B.A., Sridhar, B., Bhosale, S. V., Bhosale, S.V., 2013. Synthesis, crystal structure and antidiabetic activity of substituted (E)-3-(Benzo [d]thiazol-2-ylamino) phenylprop-2-en-1-one. Eur. J. Med. Chem. 59, 304–309.

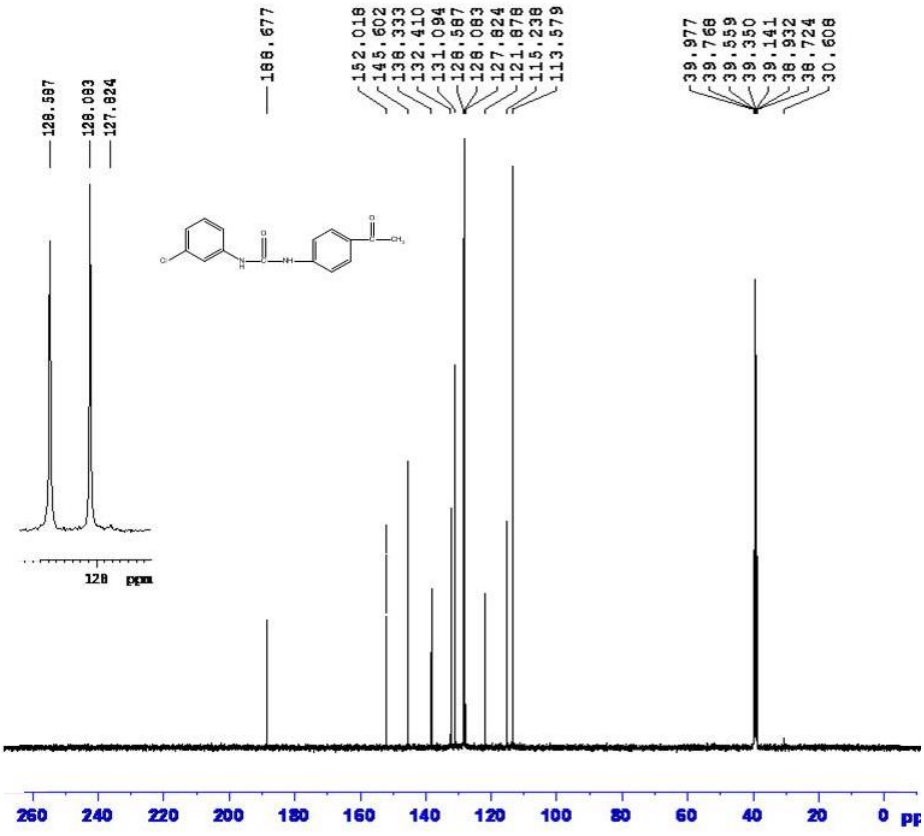
Sonmez, F., Sevmezler, S., Atahan, A., Ceylan, M., Demir, D., Gencer, N., Arslan, O., Kucukislamoglu, M., 2011. Evaluation of new chalcone derivatives as polyphenoloxidase inhibitors. Bioorg. Med. Chem. Lett. 21, 7479–7482.

Sreejayan., Rao, MN., 1997. Nitric oxide scavenging by curcuminoids. J Pharm Pharmacol. 49(1), 105-107.

Wright, J S., 2003. Searching for foundation of youth. Chem. Br., 39, 25-27.



^{1}H NMR of 1-(4-acetylphenyl)-3-(3-chlorophenyl)urea (3A)



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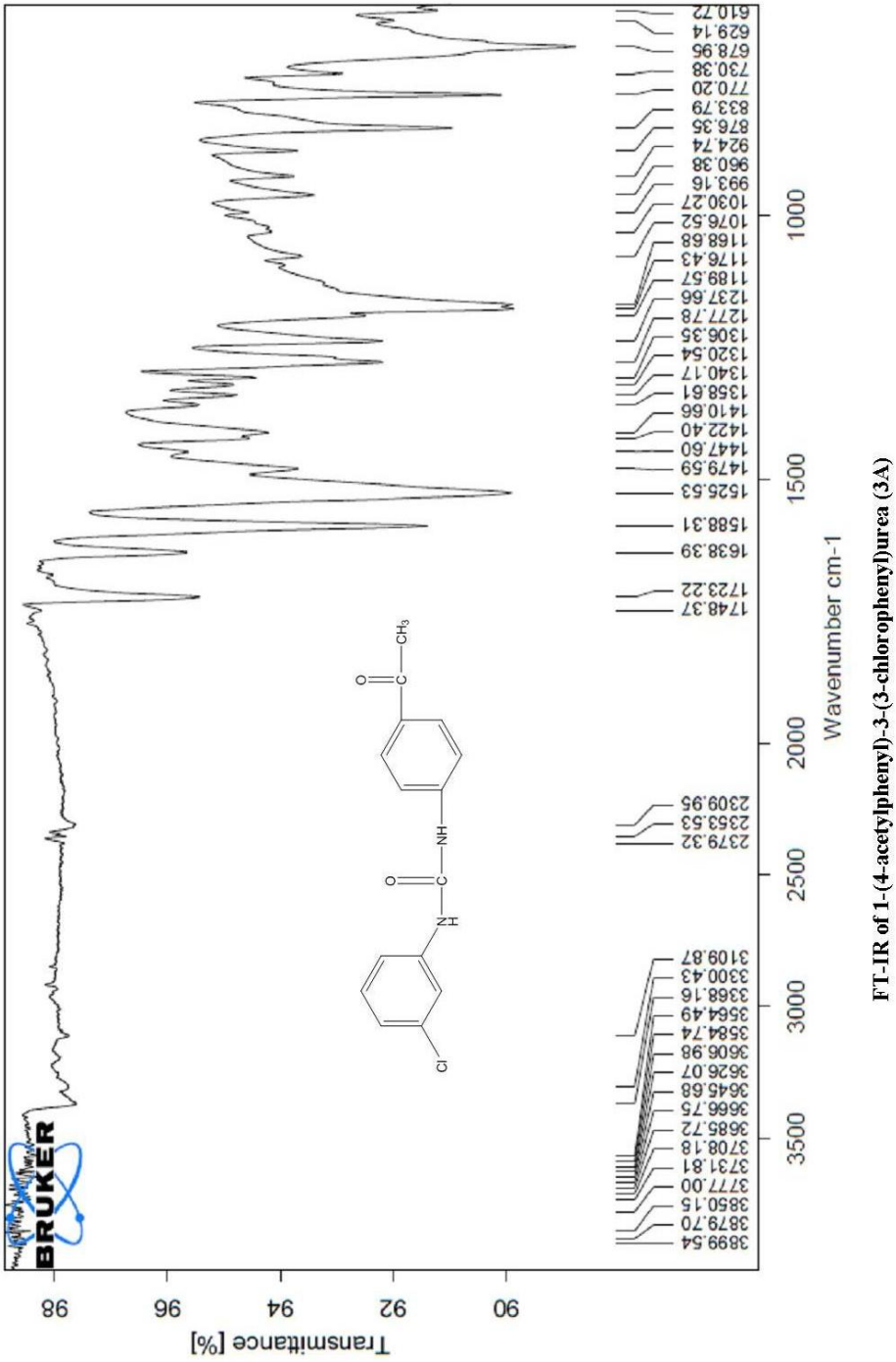

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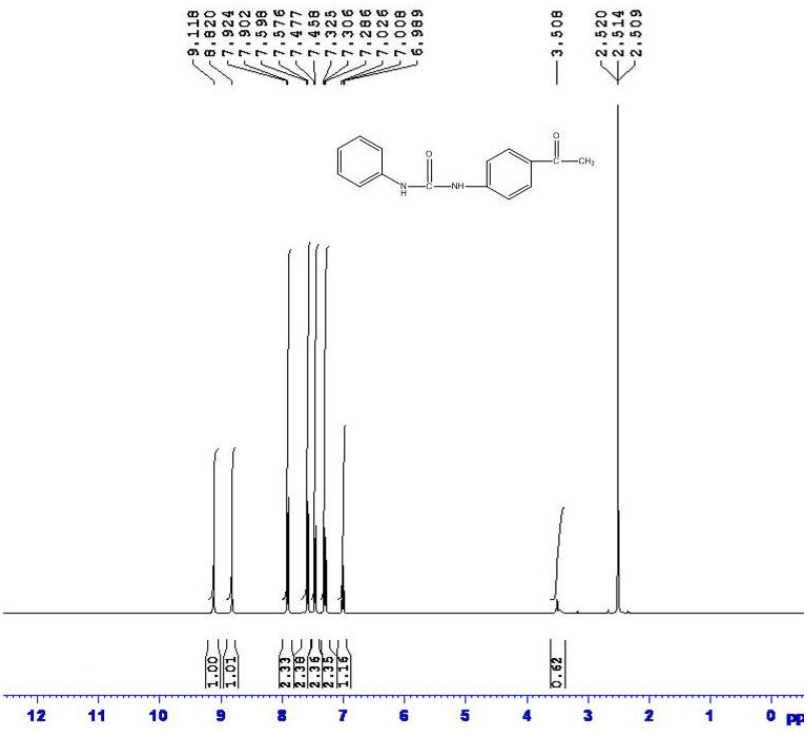

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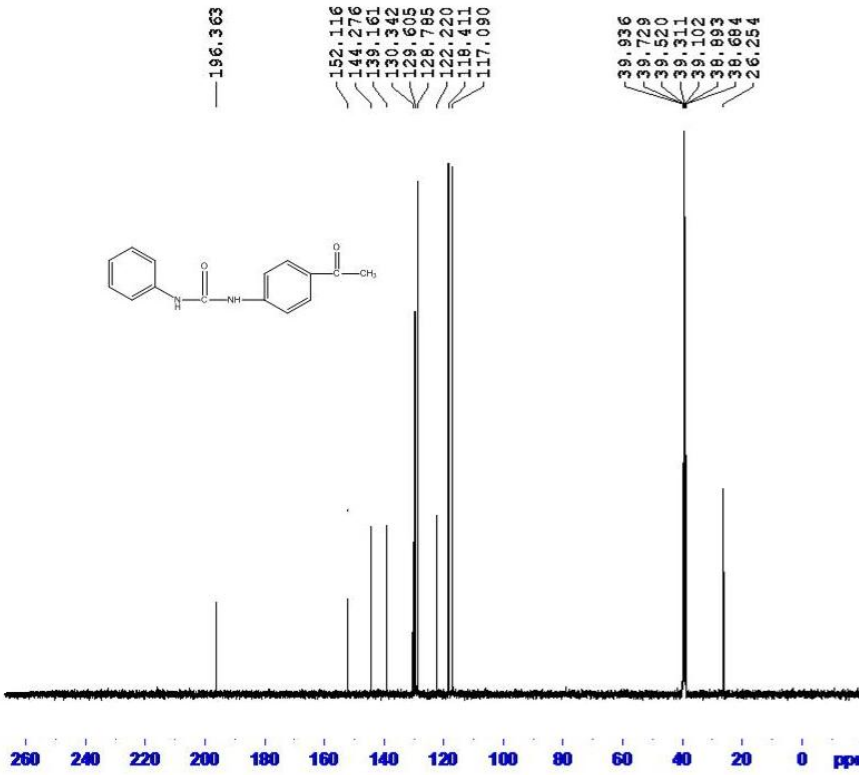

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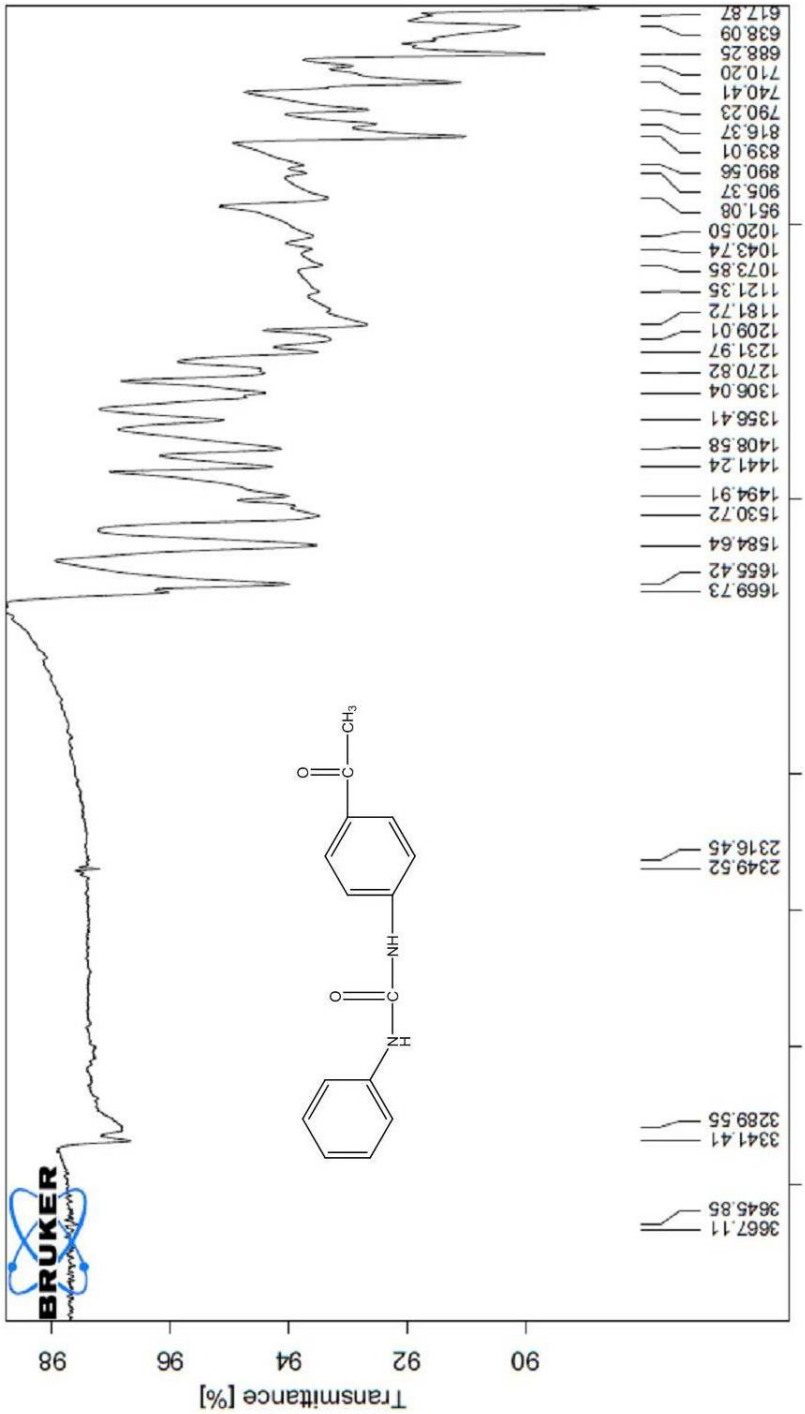

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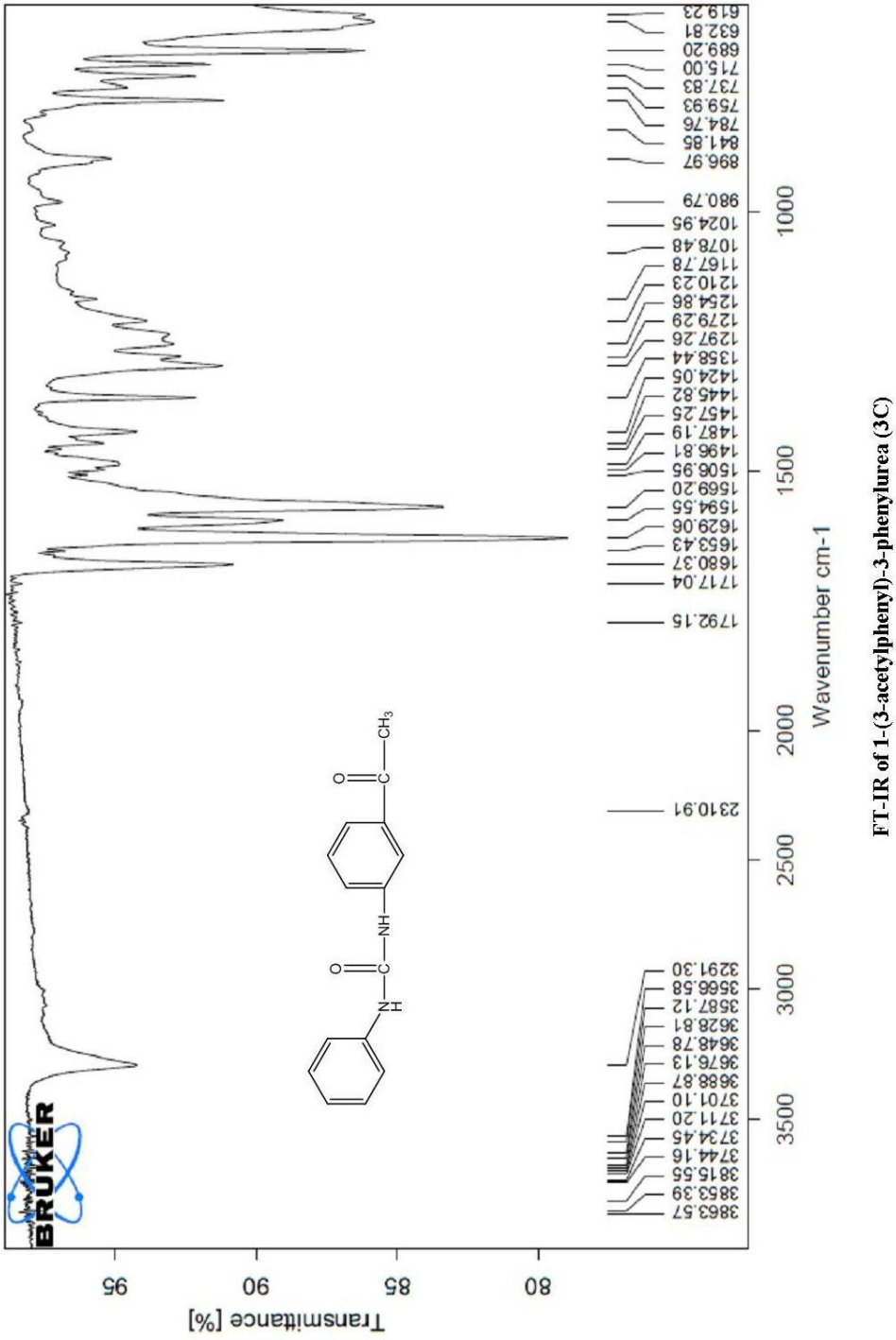

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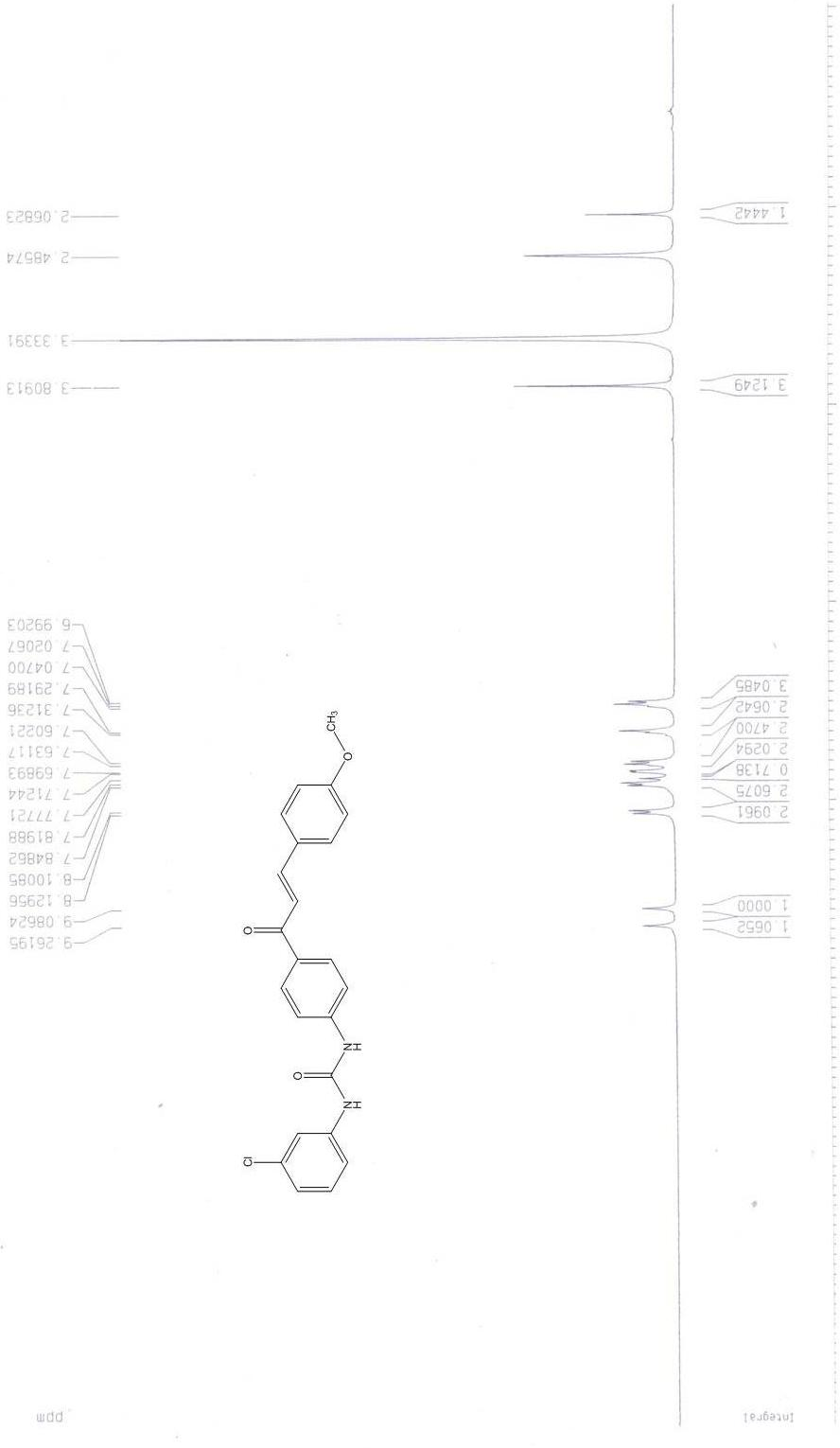

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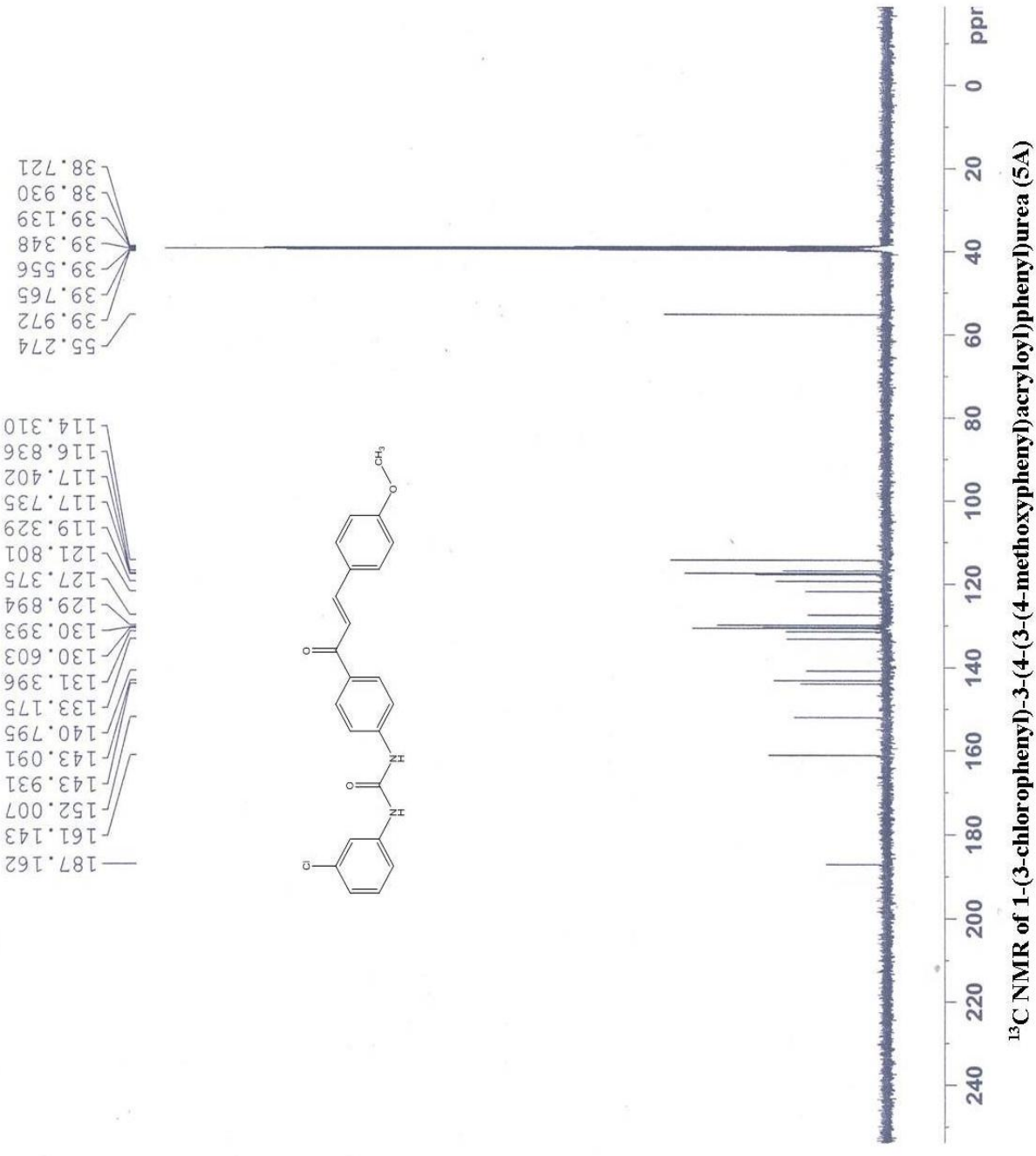

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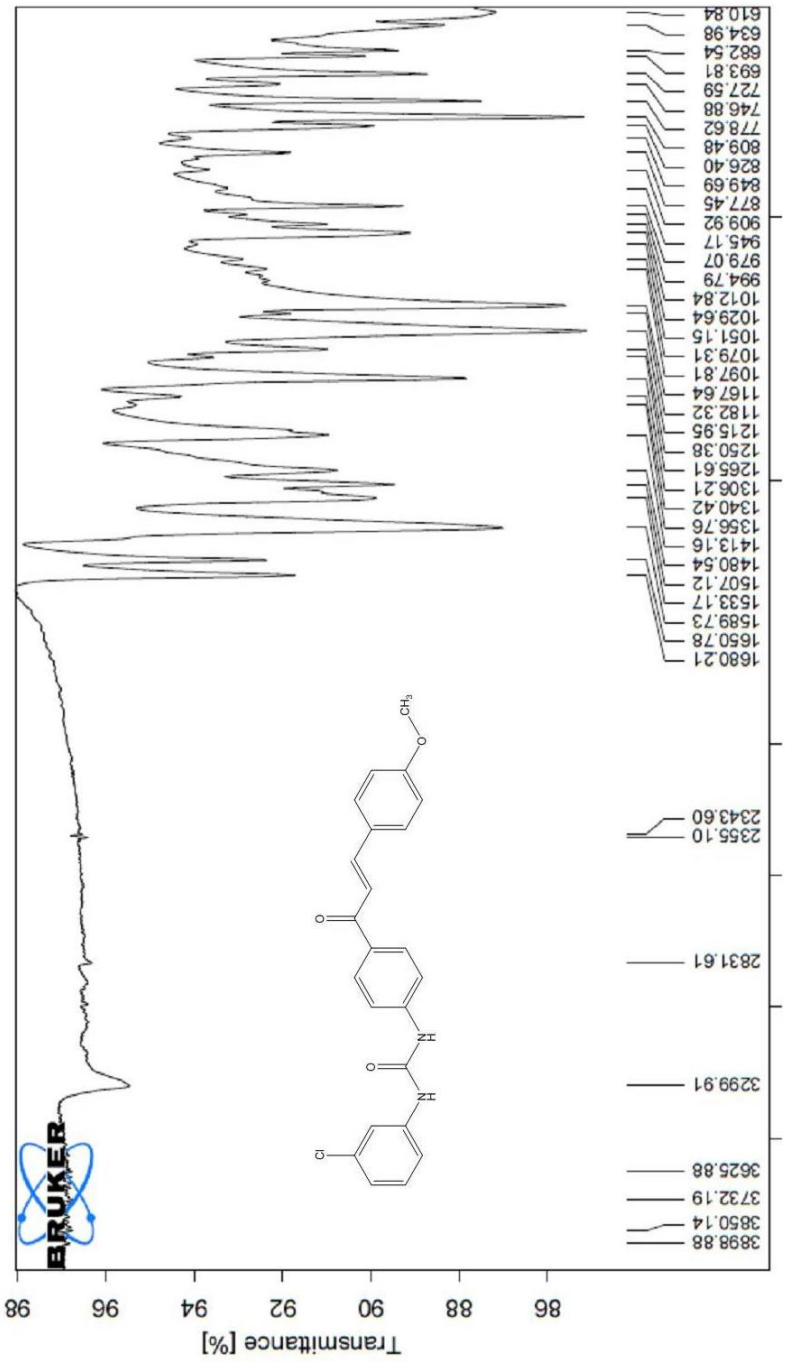

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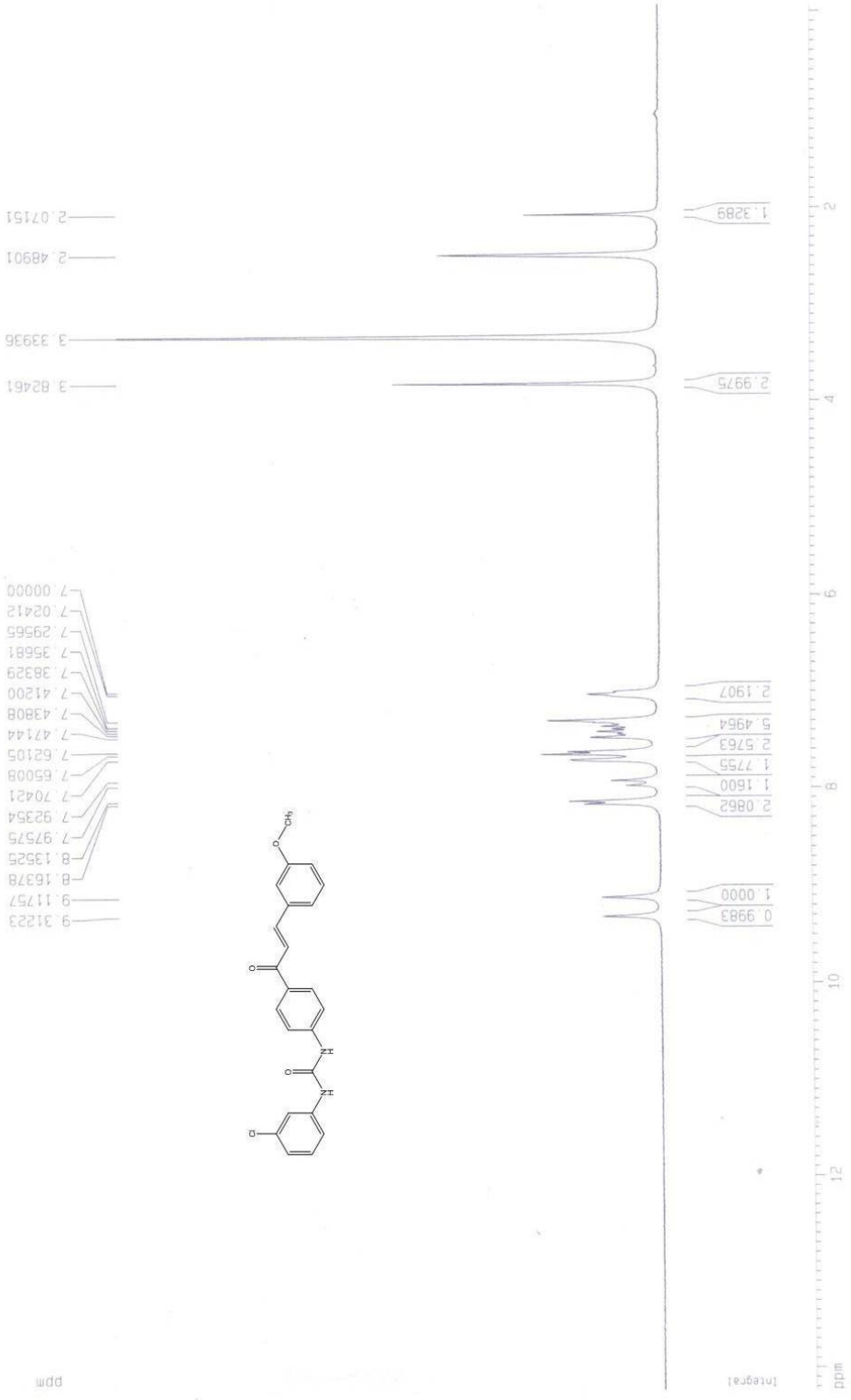

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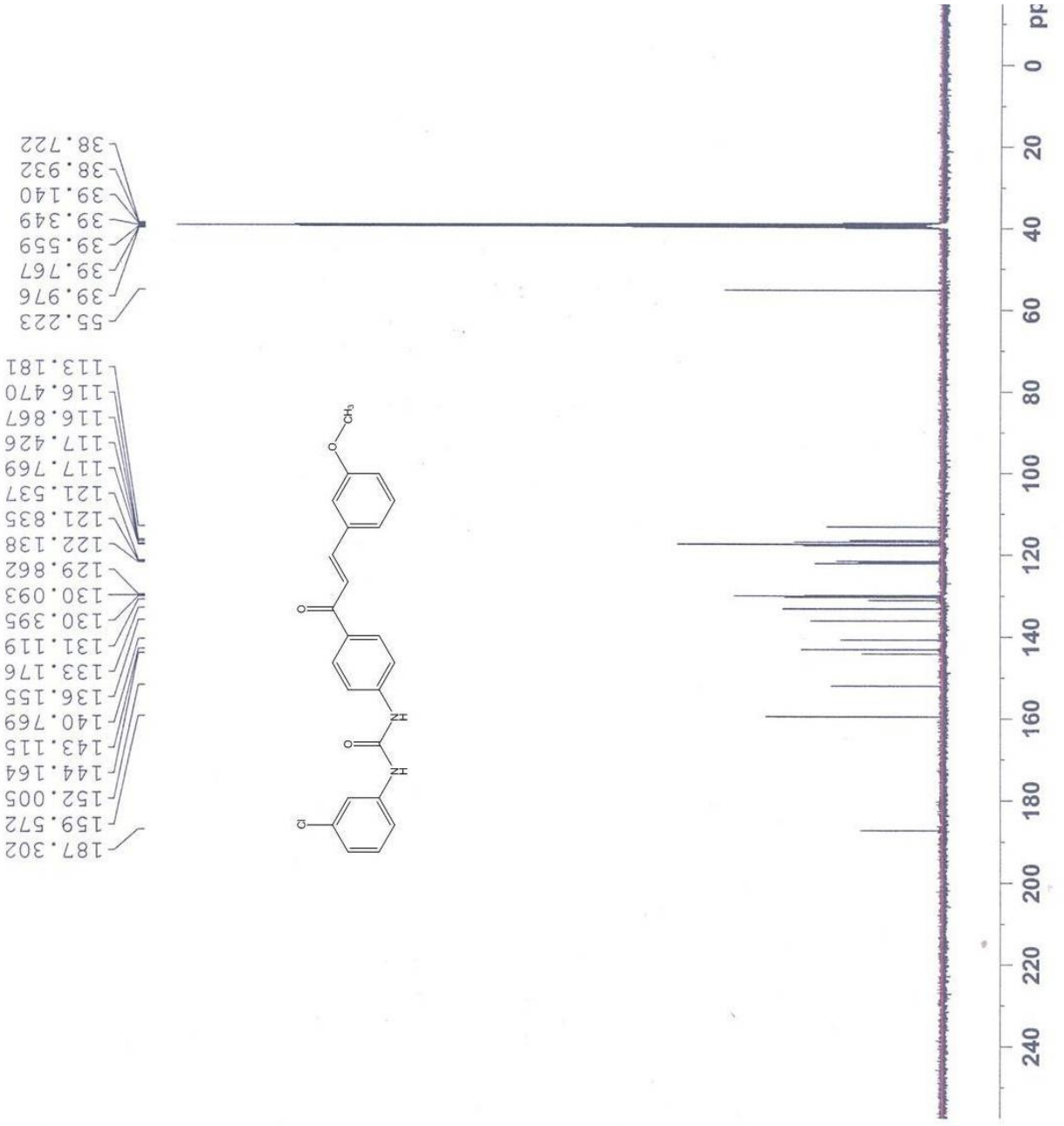

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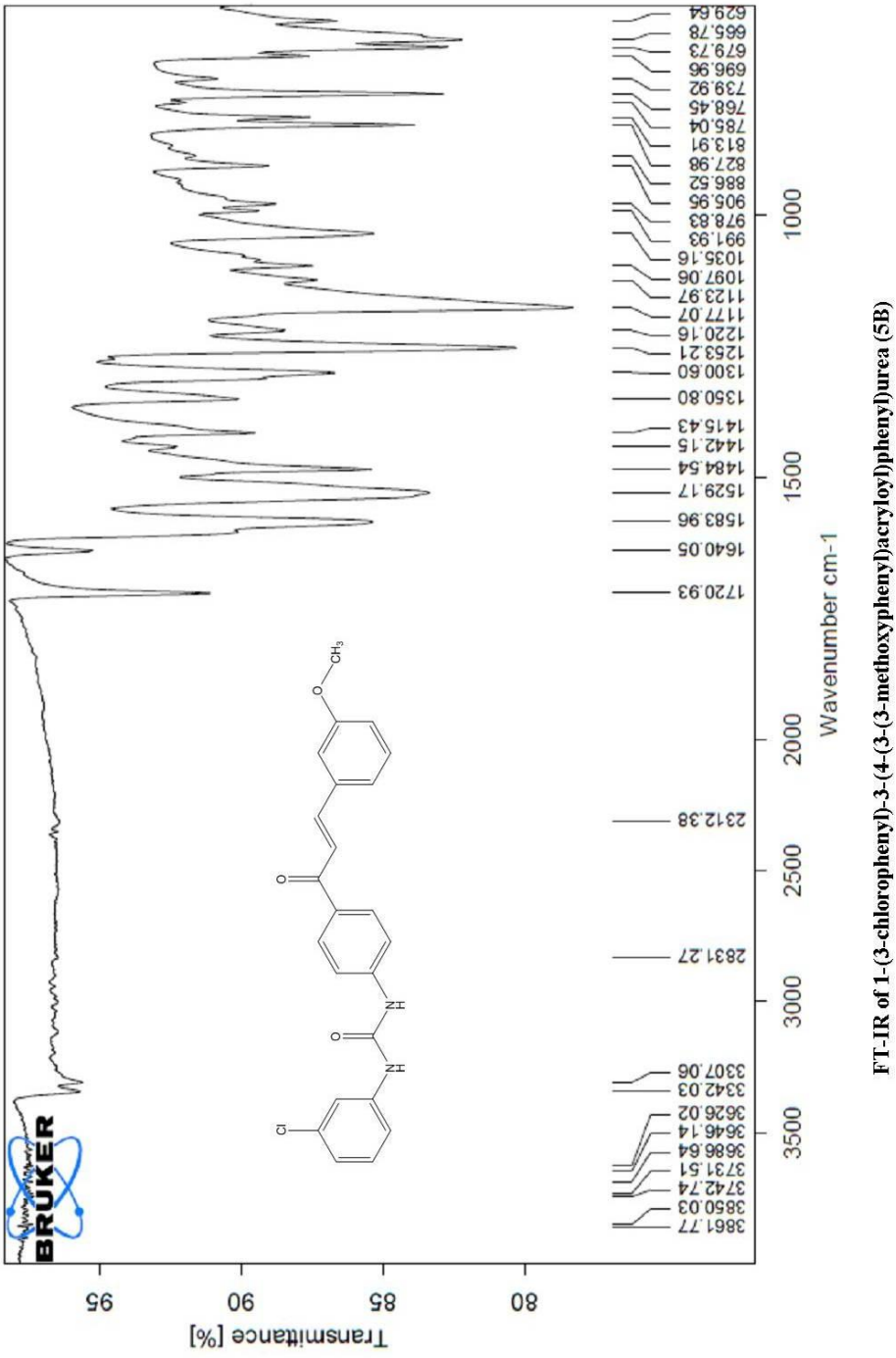

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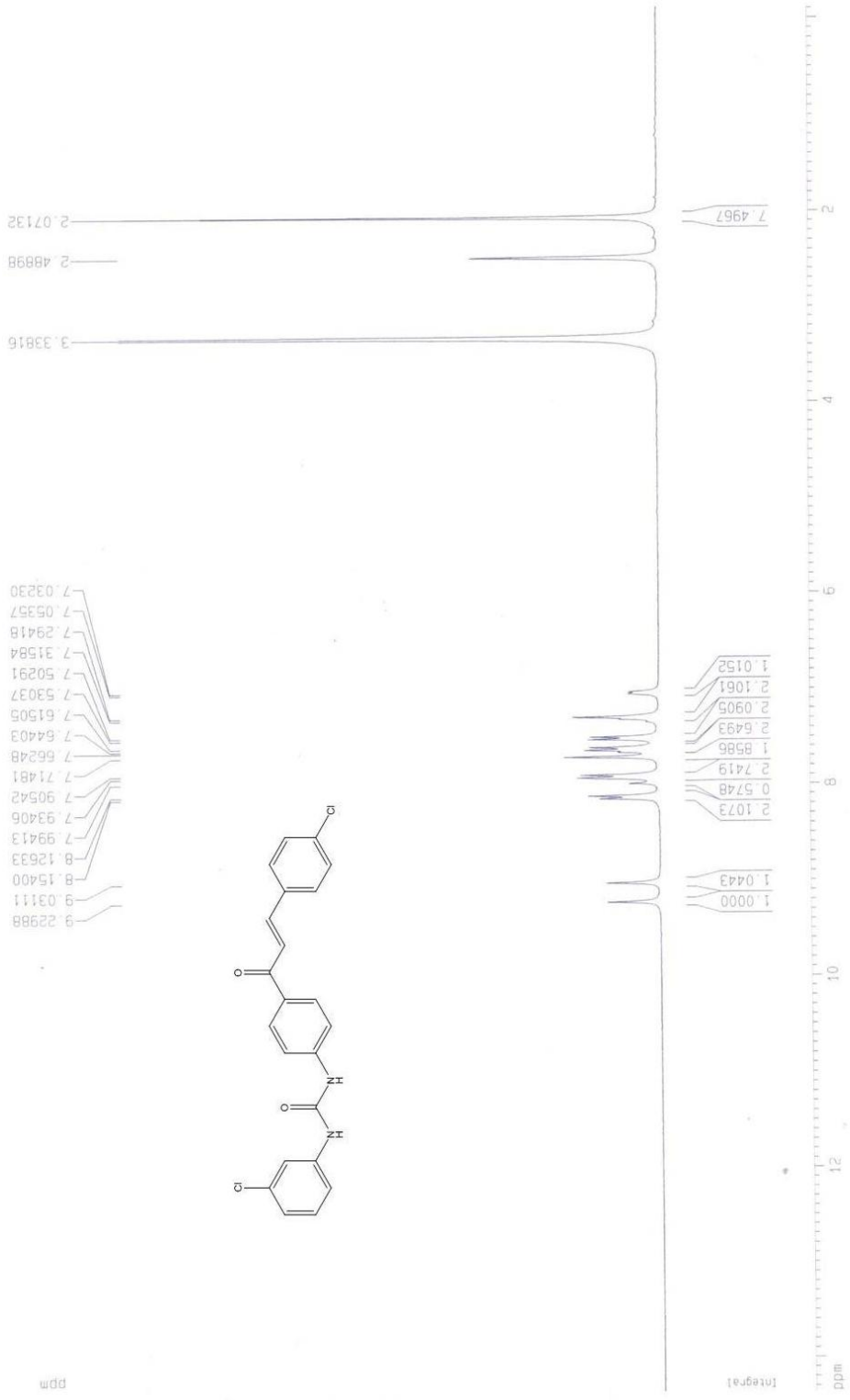

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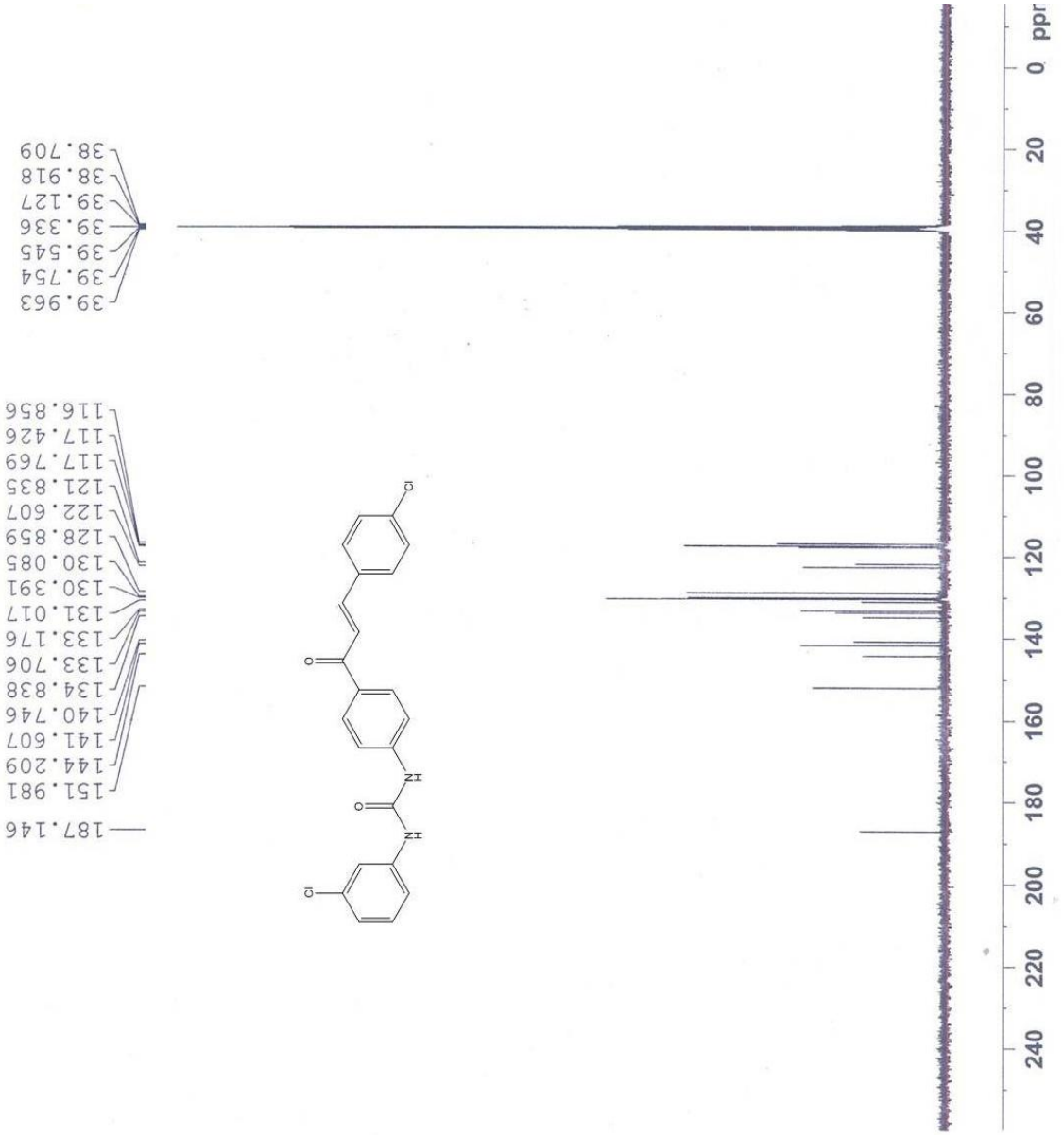

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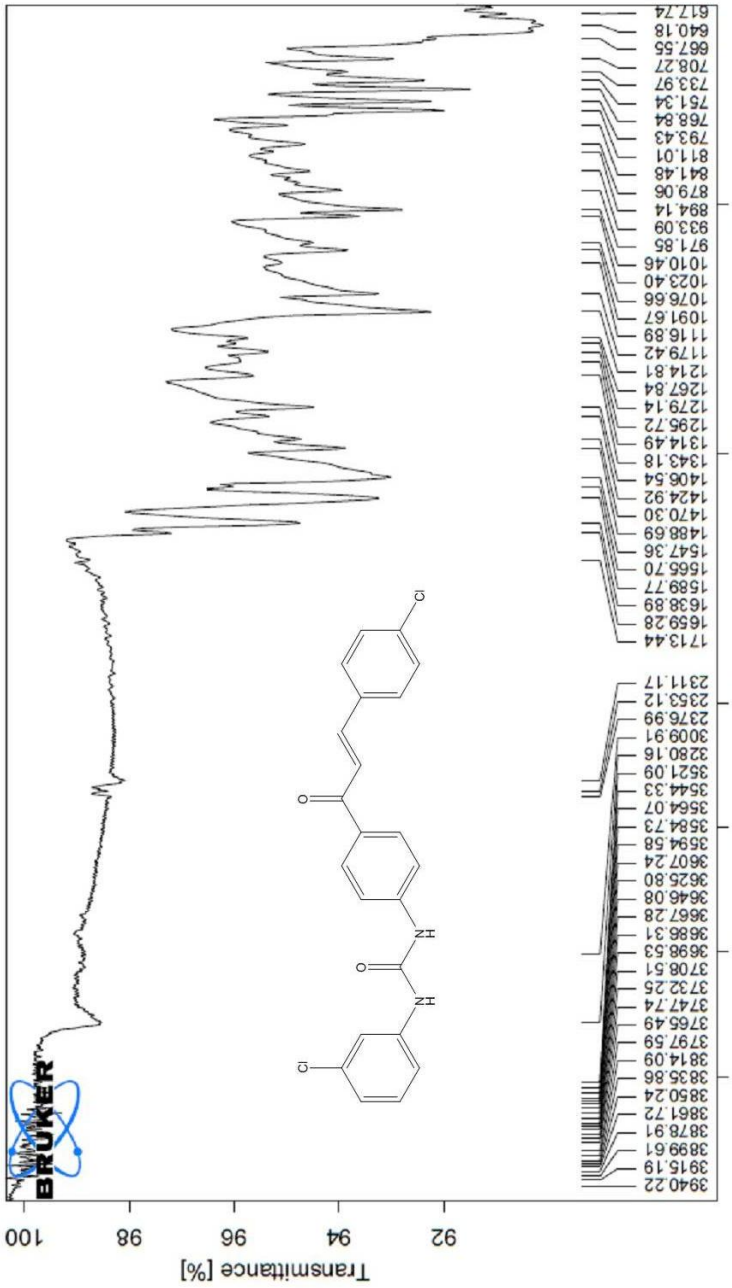

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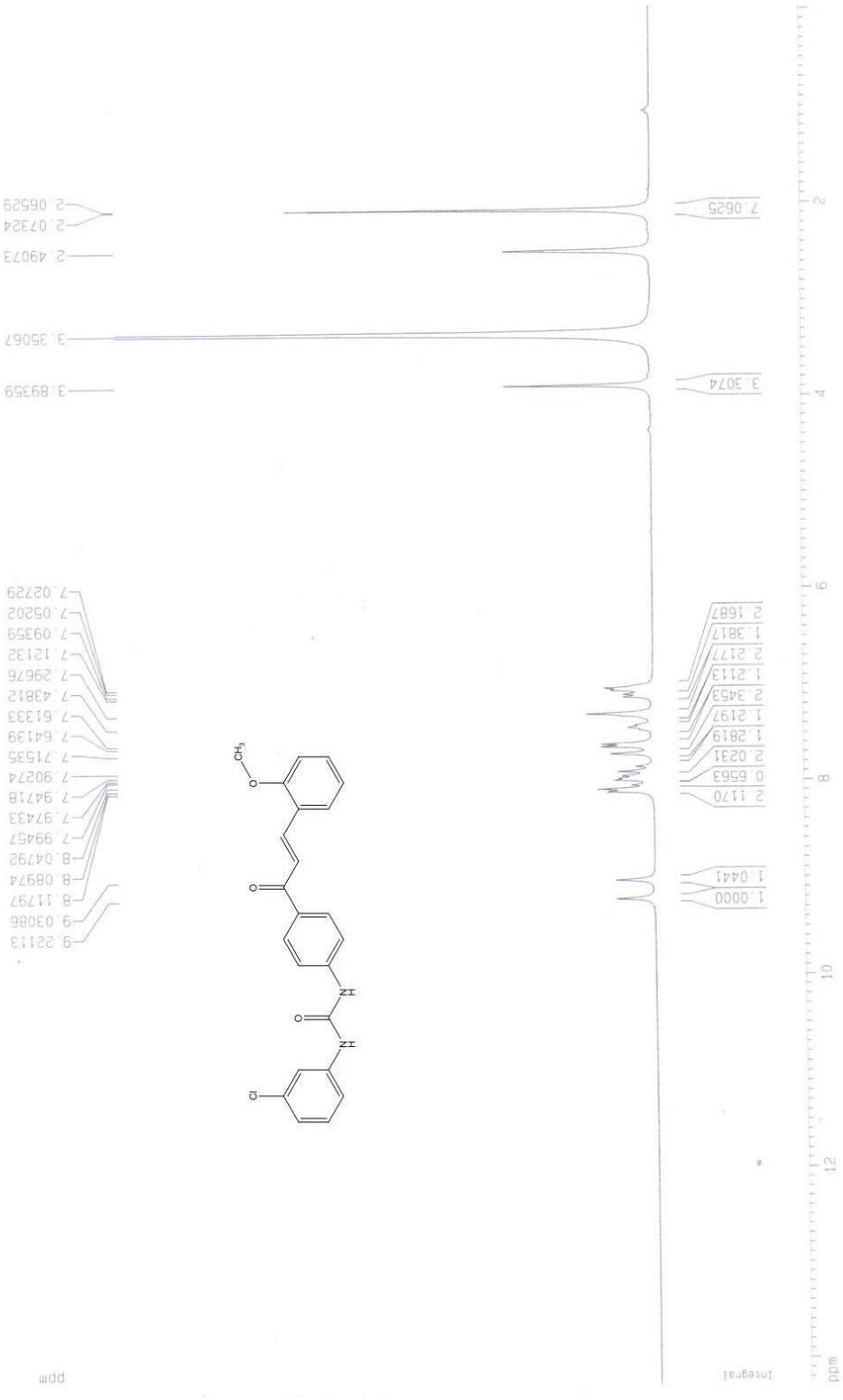

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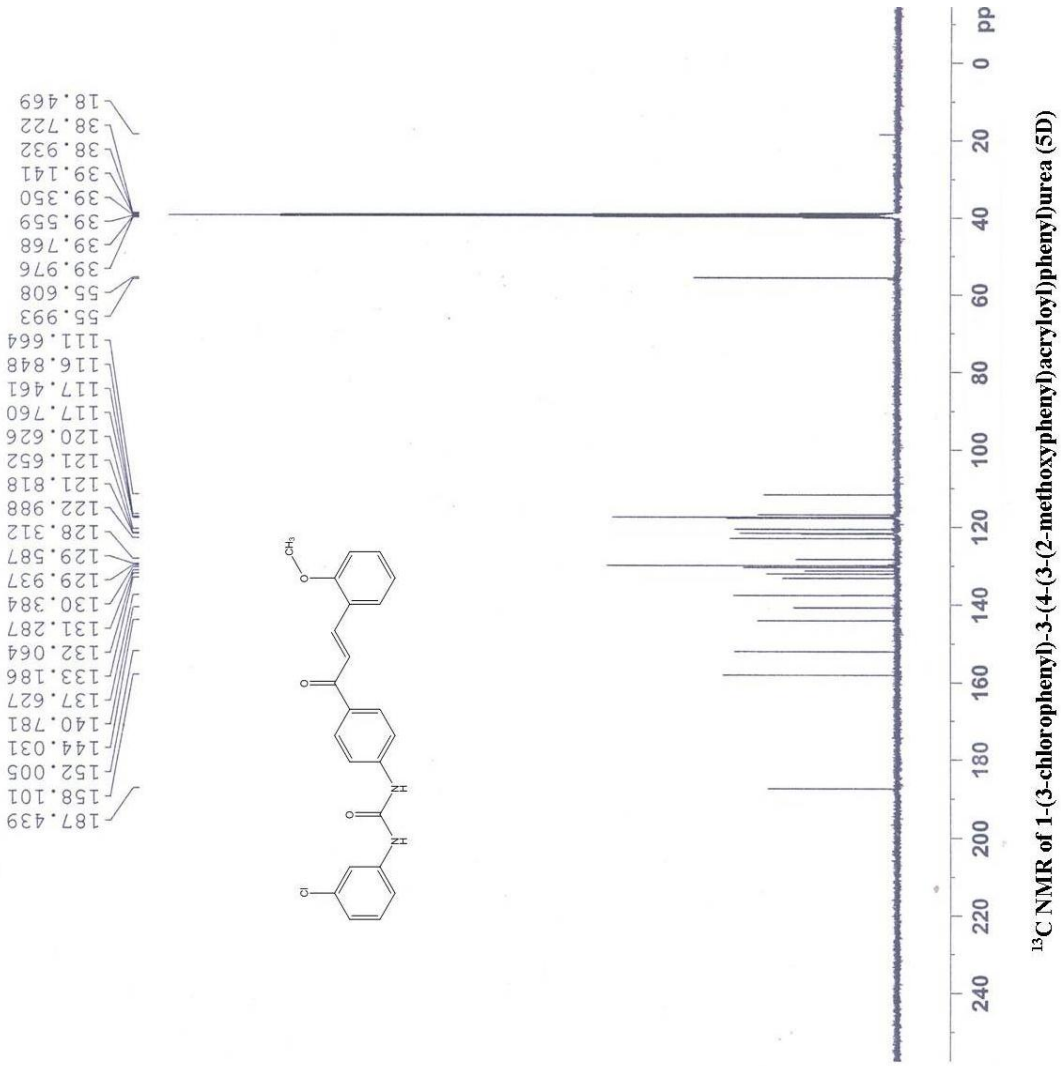

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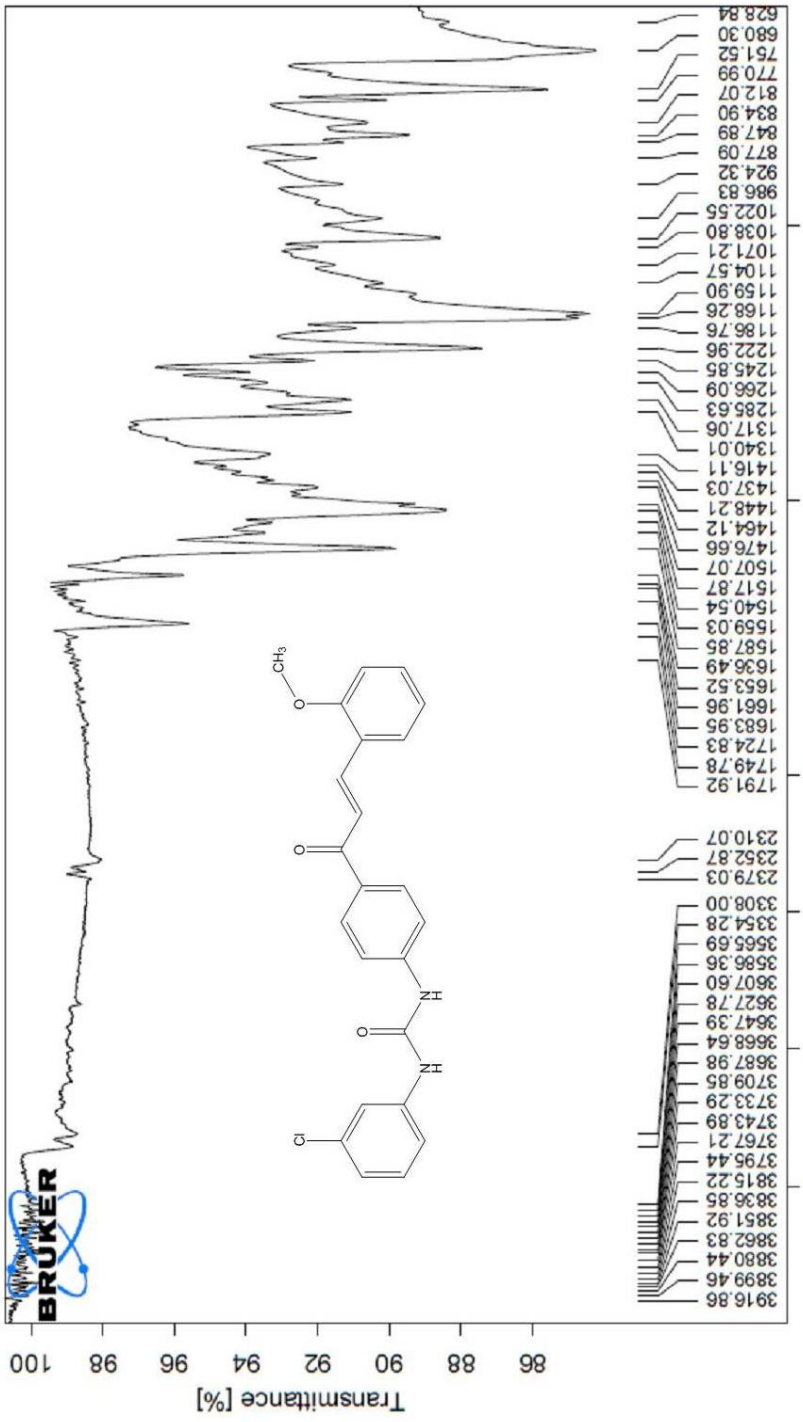

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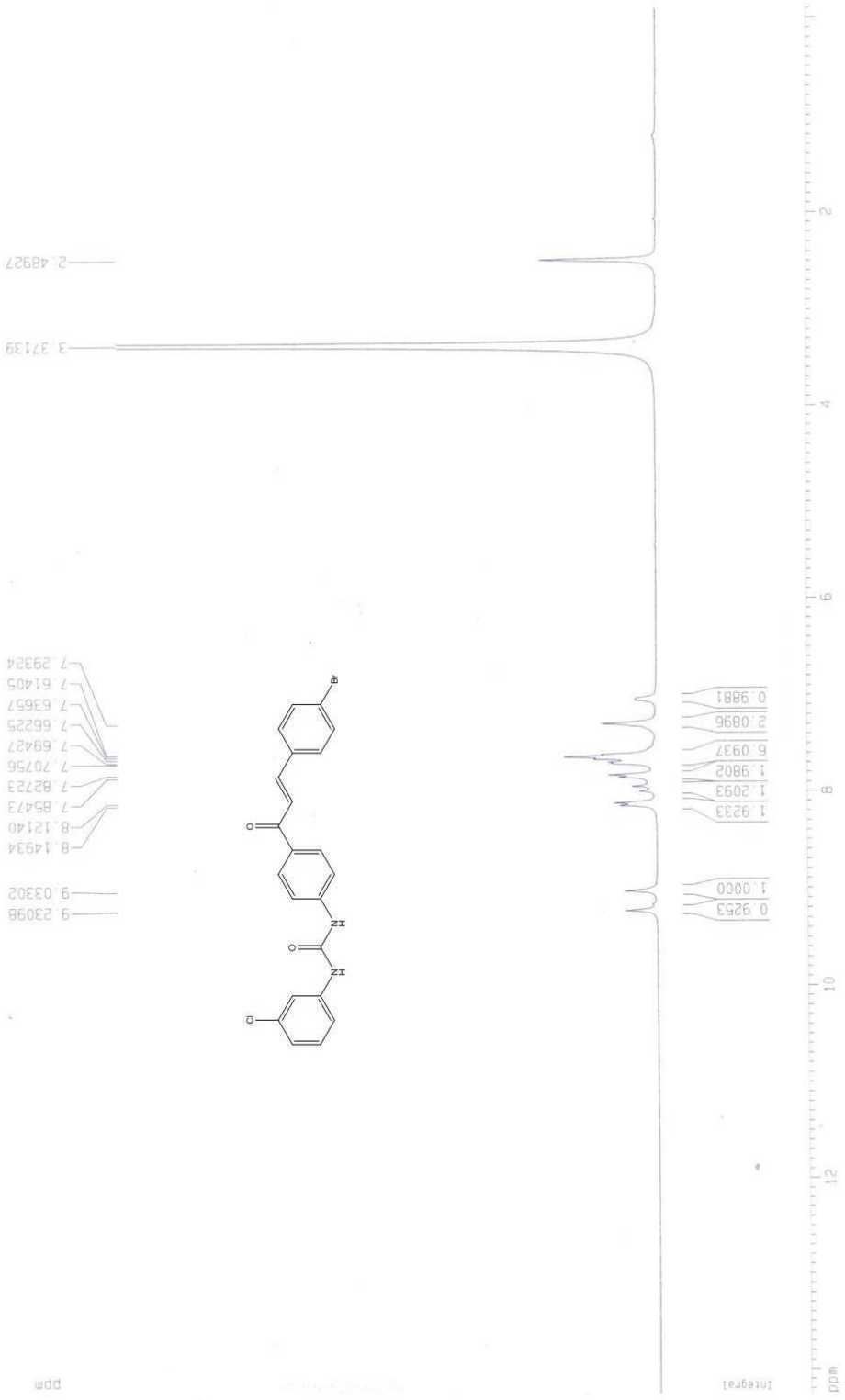

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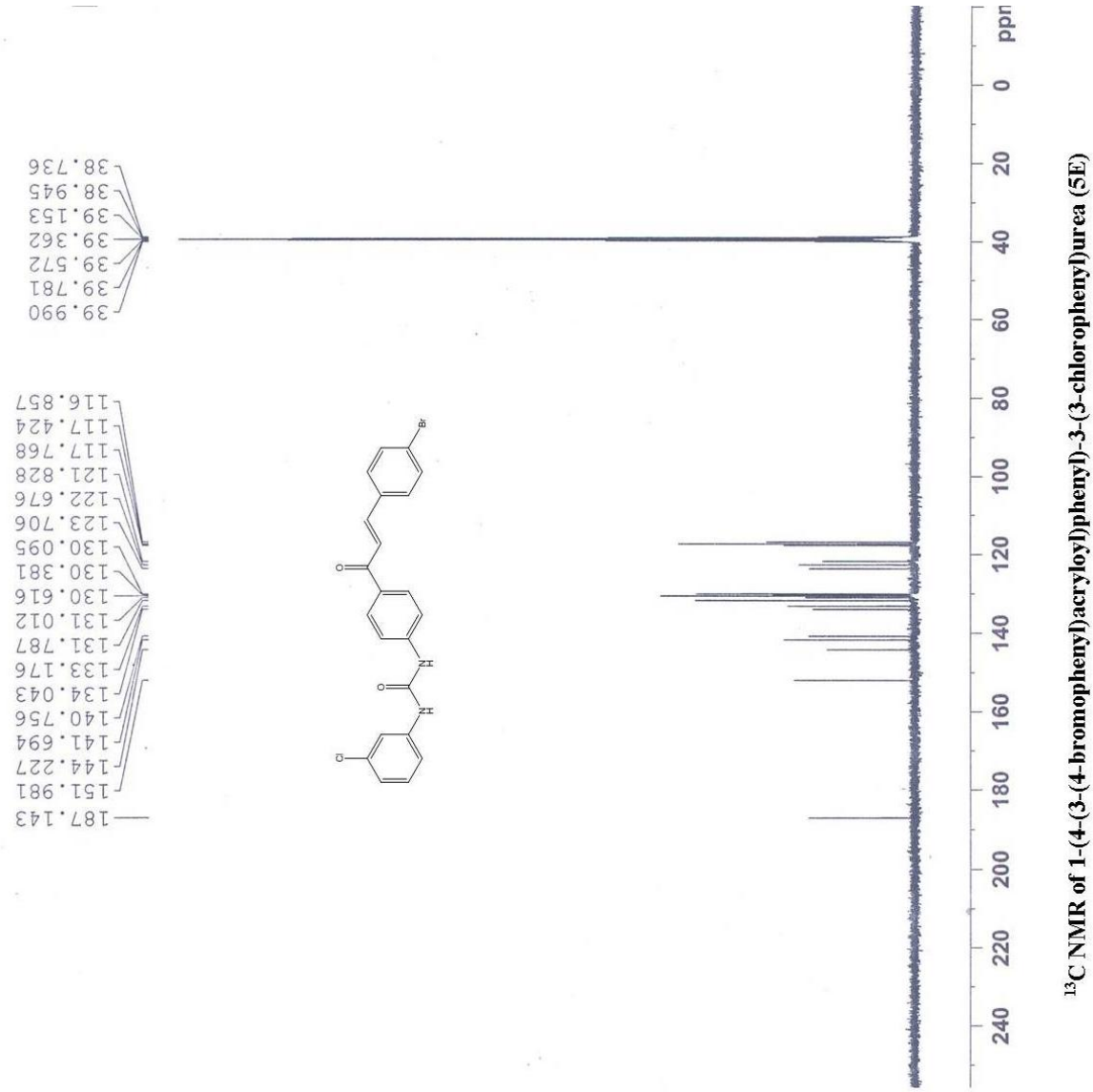

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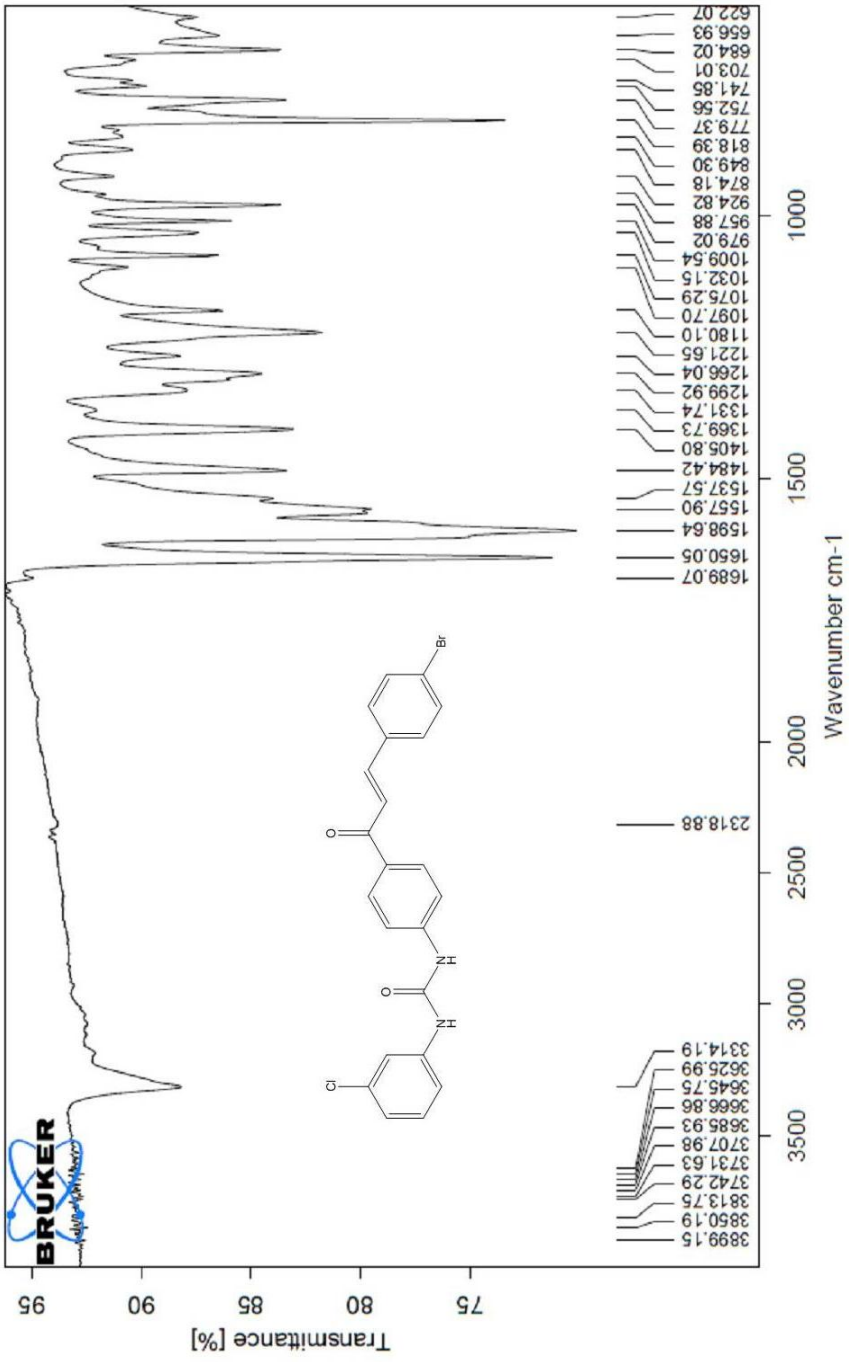
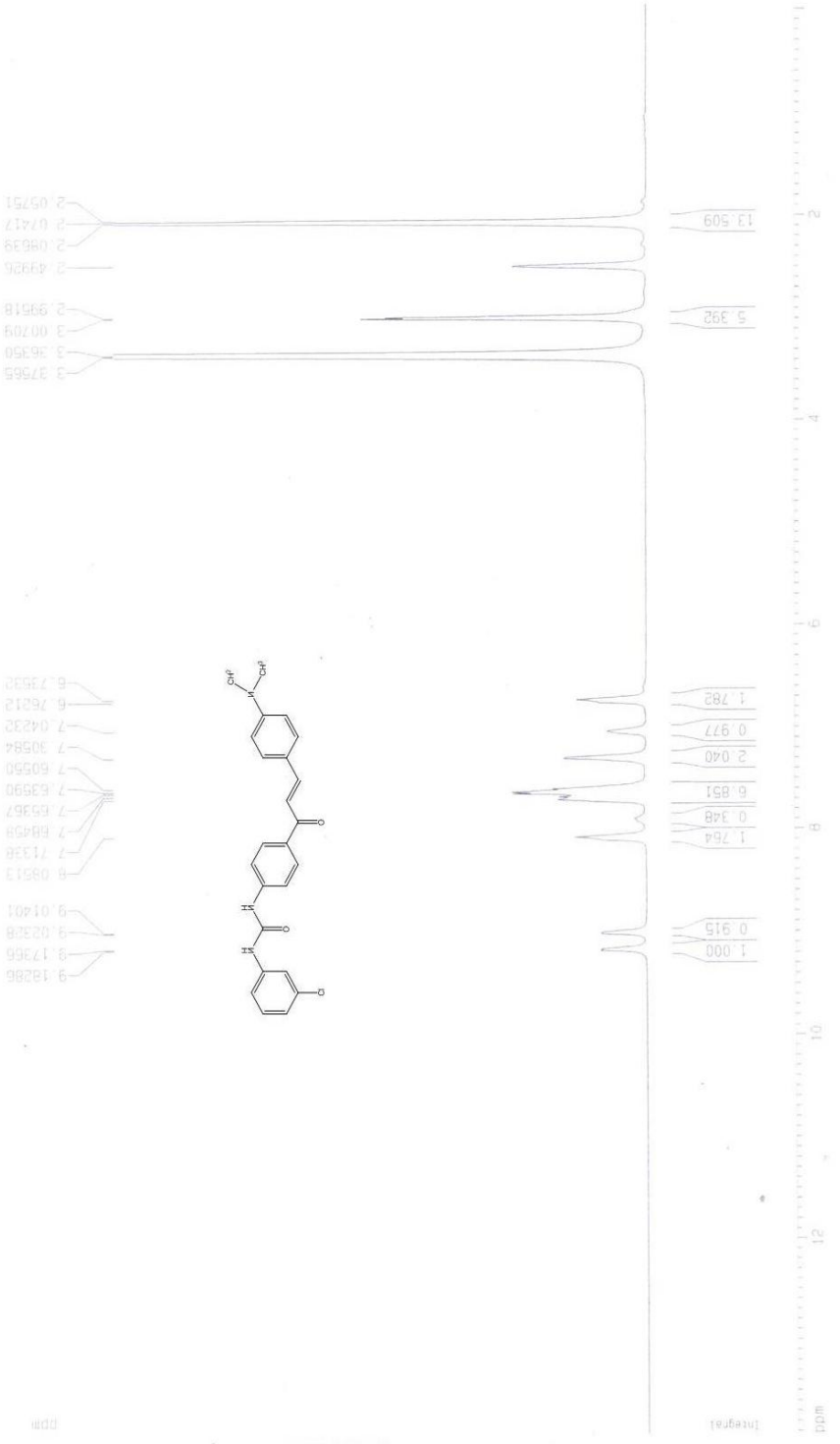


FT-IR of 1-(4-(3-(4-bromophenyl)acryloyl)phenyl)-3-(3-chlorophenyl)urea (5E)



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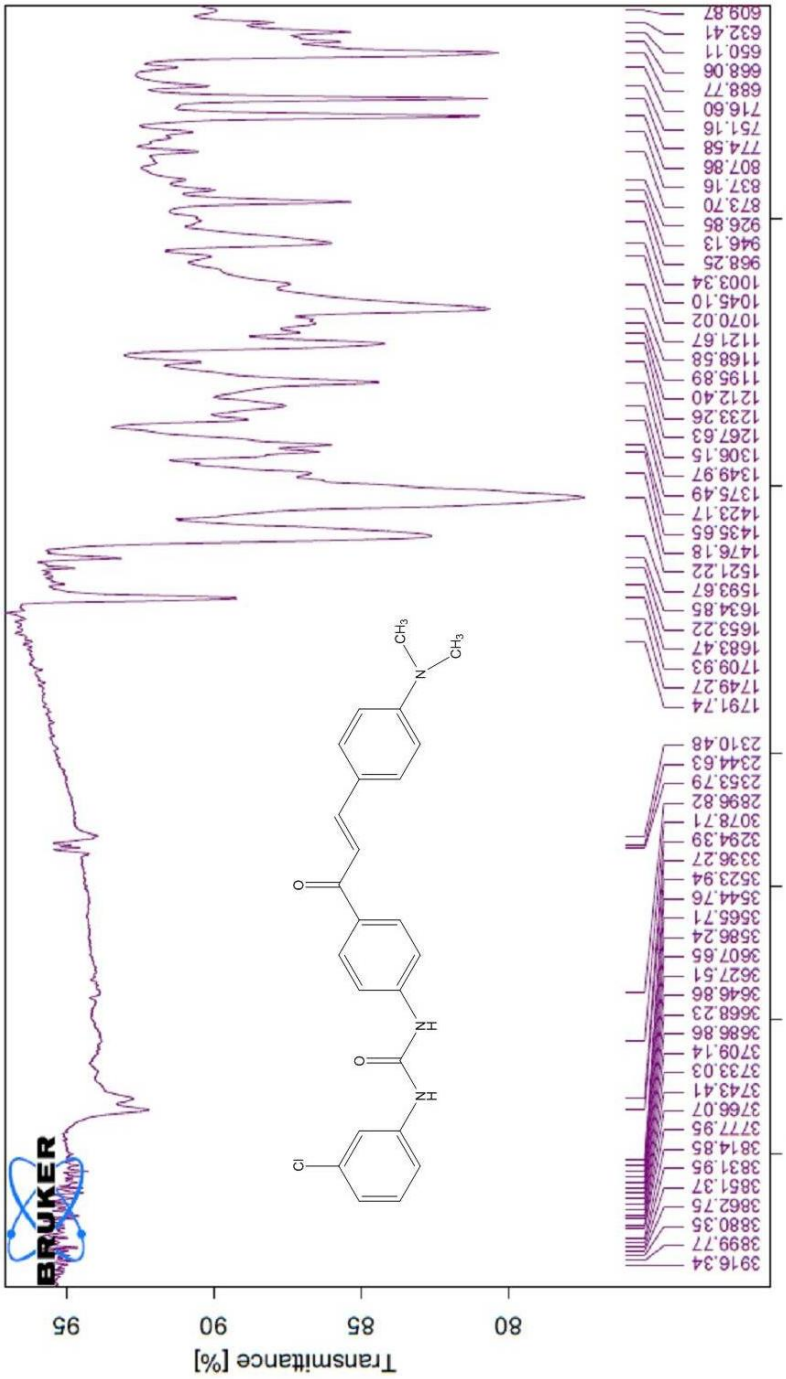
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90
85
80
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2500
2000
1500
1000
BRUKER
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3880.35
3862.75
3851.37
3831.95
3814.85
3777.95
3766.07
3743.41
3733.03
3709.14
3686.86
3668.23
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3627.51
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3566.71
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926.85
873.70
837.16
807.86
774.58
751.16
716.60
688.77
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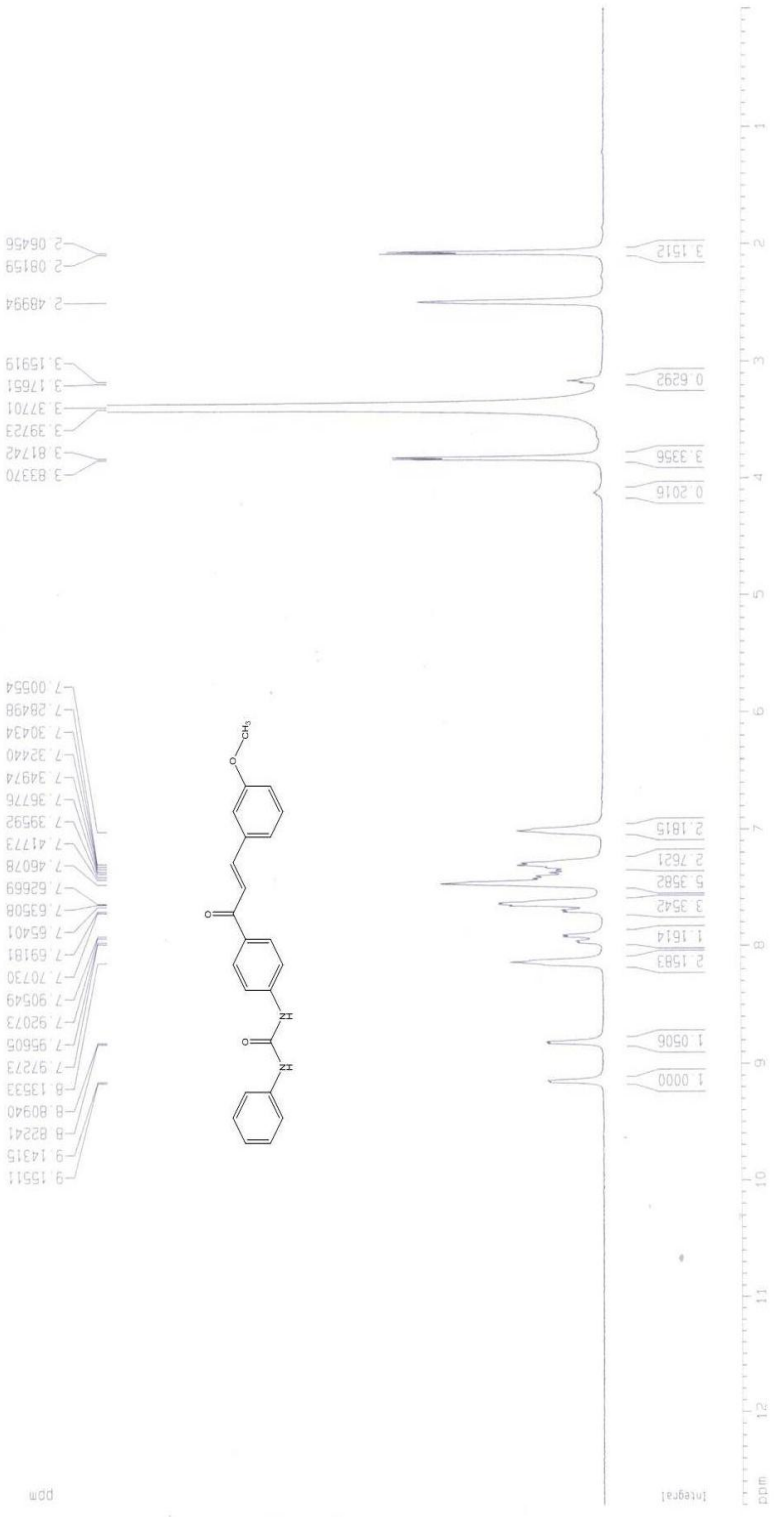
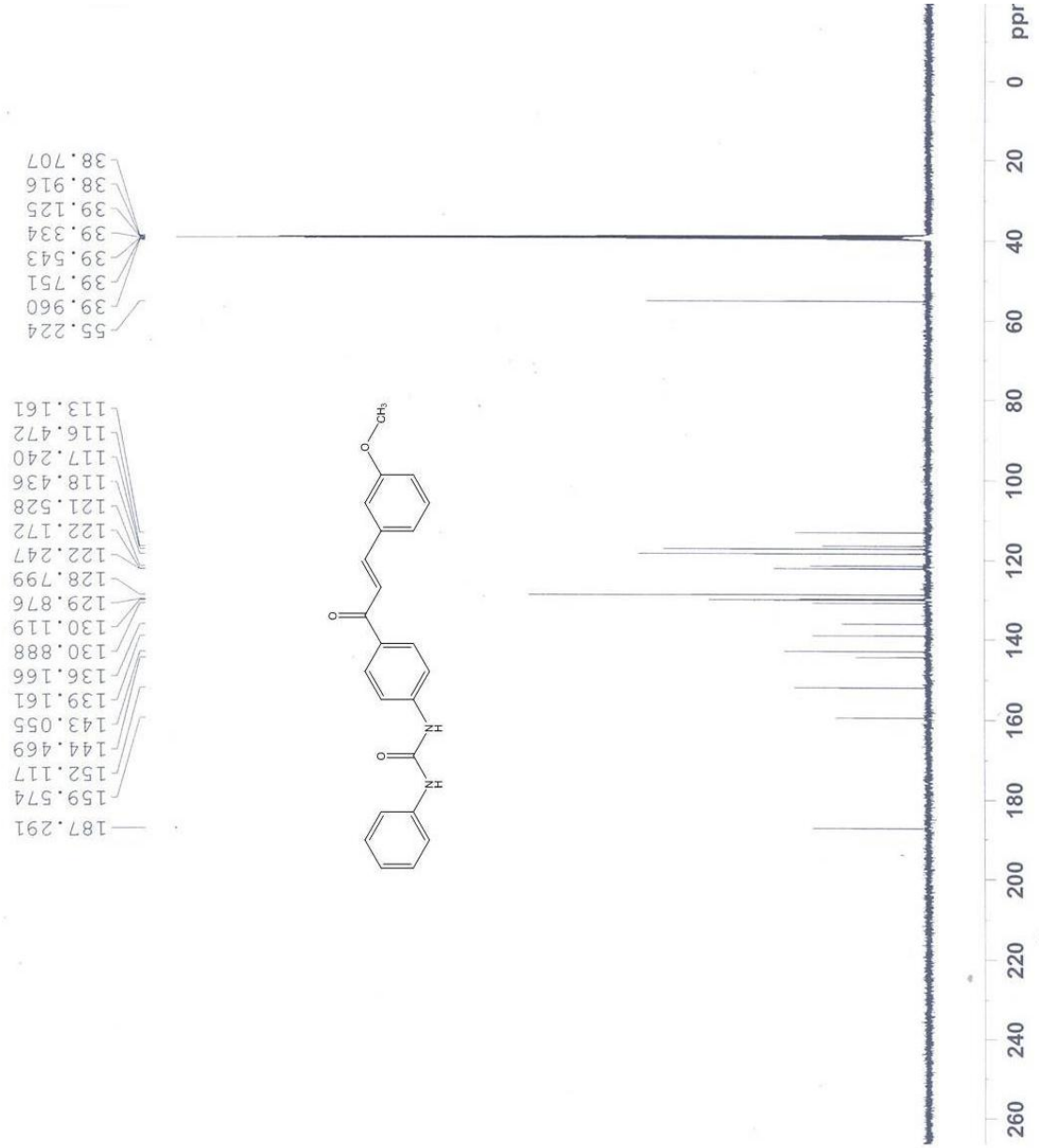
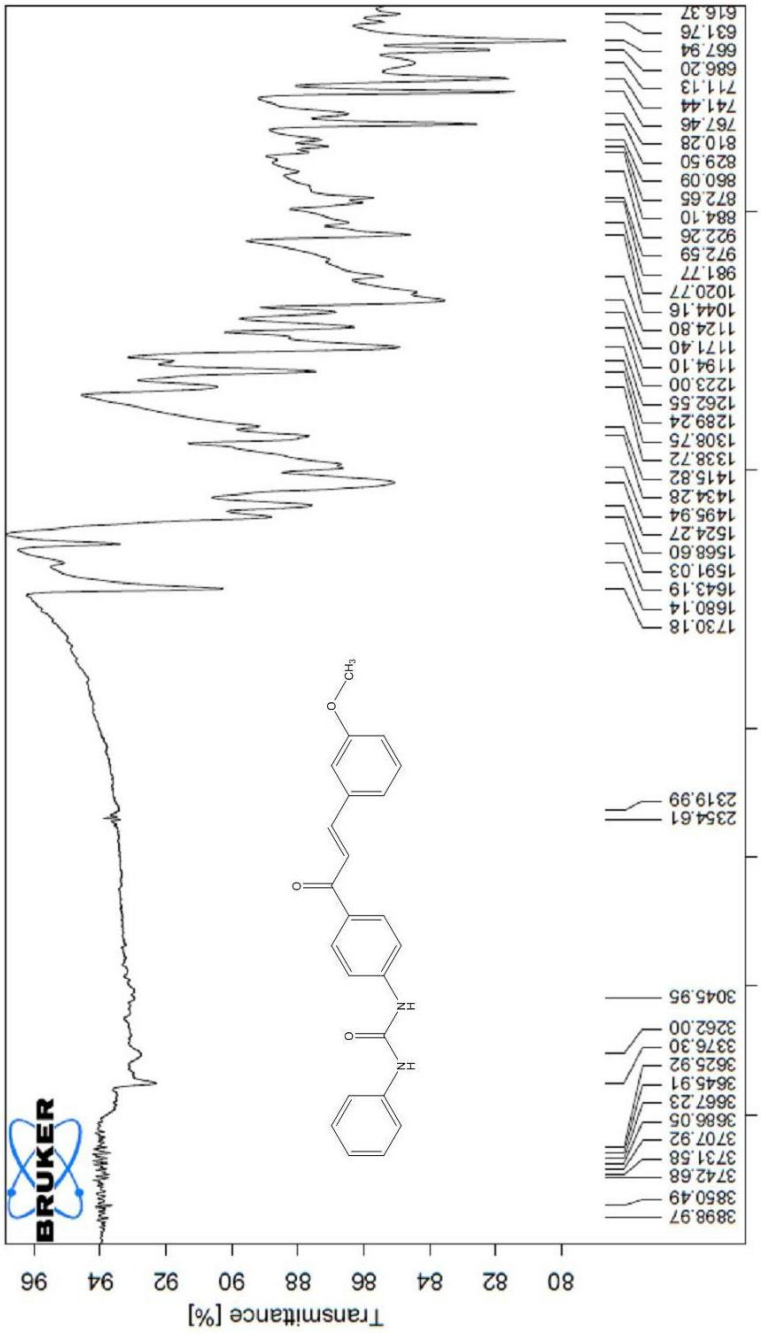


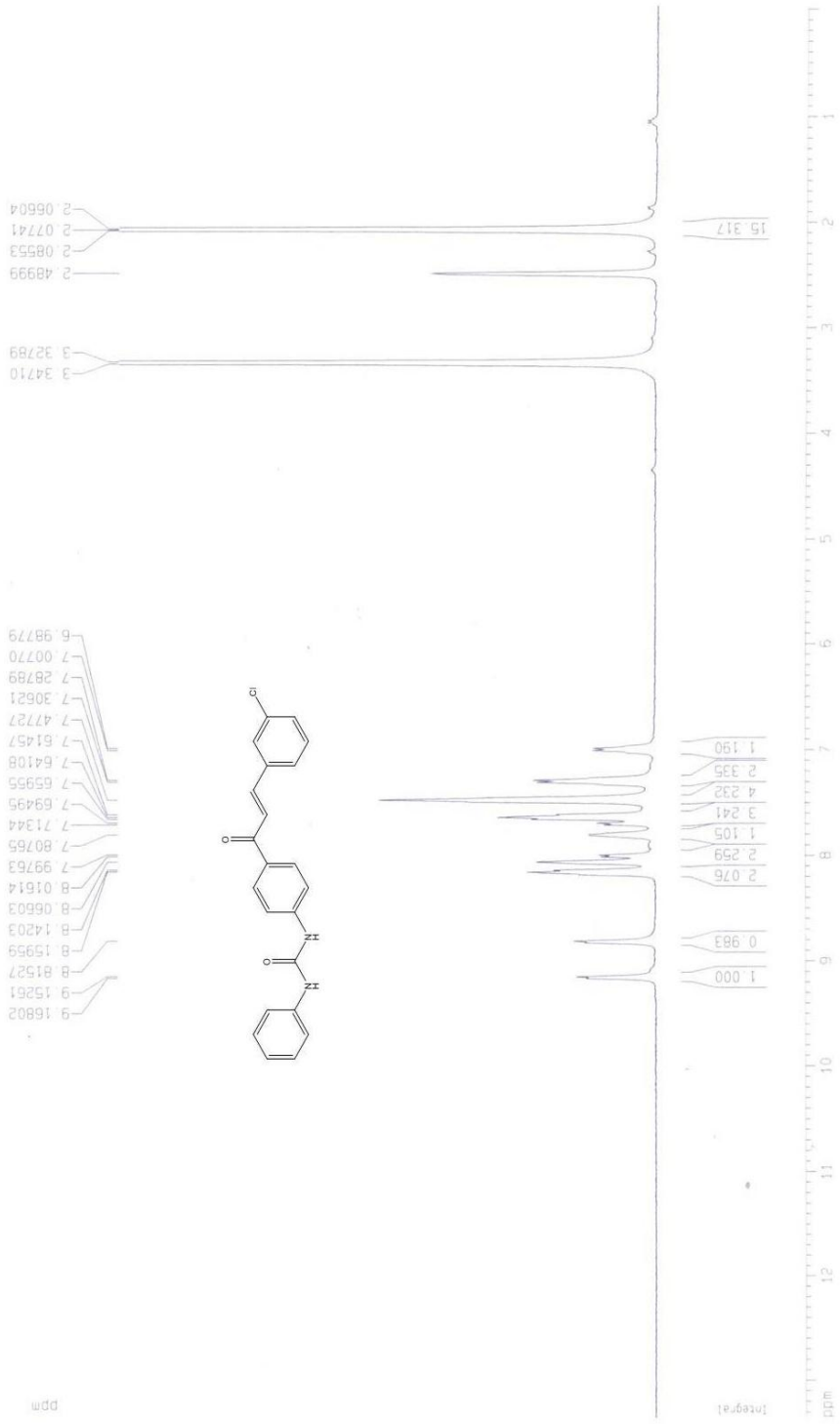
^{1}H NMR of 1-(4-(3-(3-methoxyphenyl)acryloyl)phenyl)-3-phenylurea (5G)



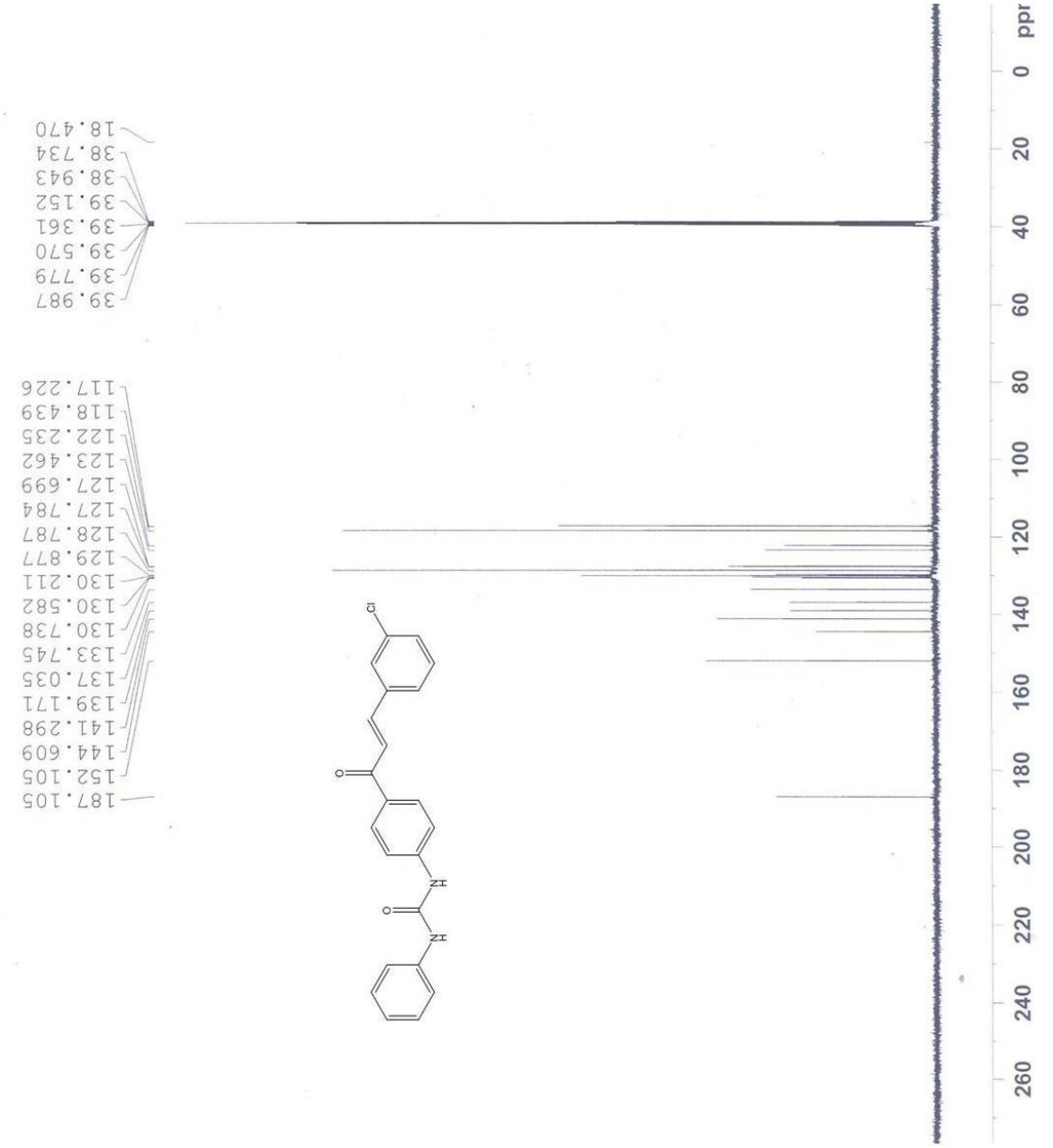
^{13}C NMR of 1-(4-(3-(3-methoxyphenyl)acryloyl)phenyl)-3-phenylurea (5G)



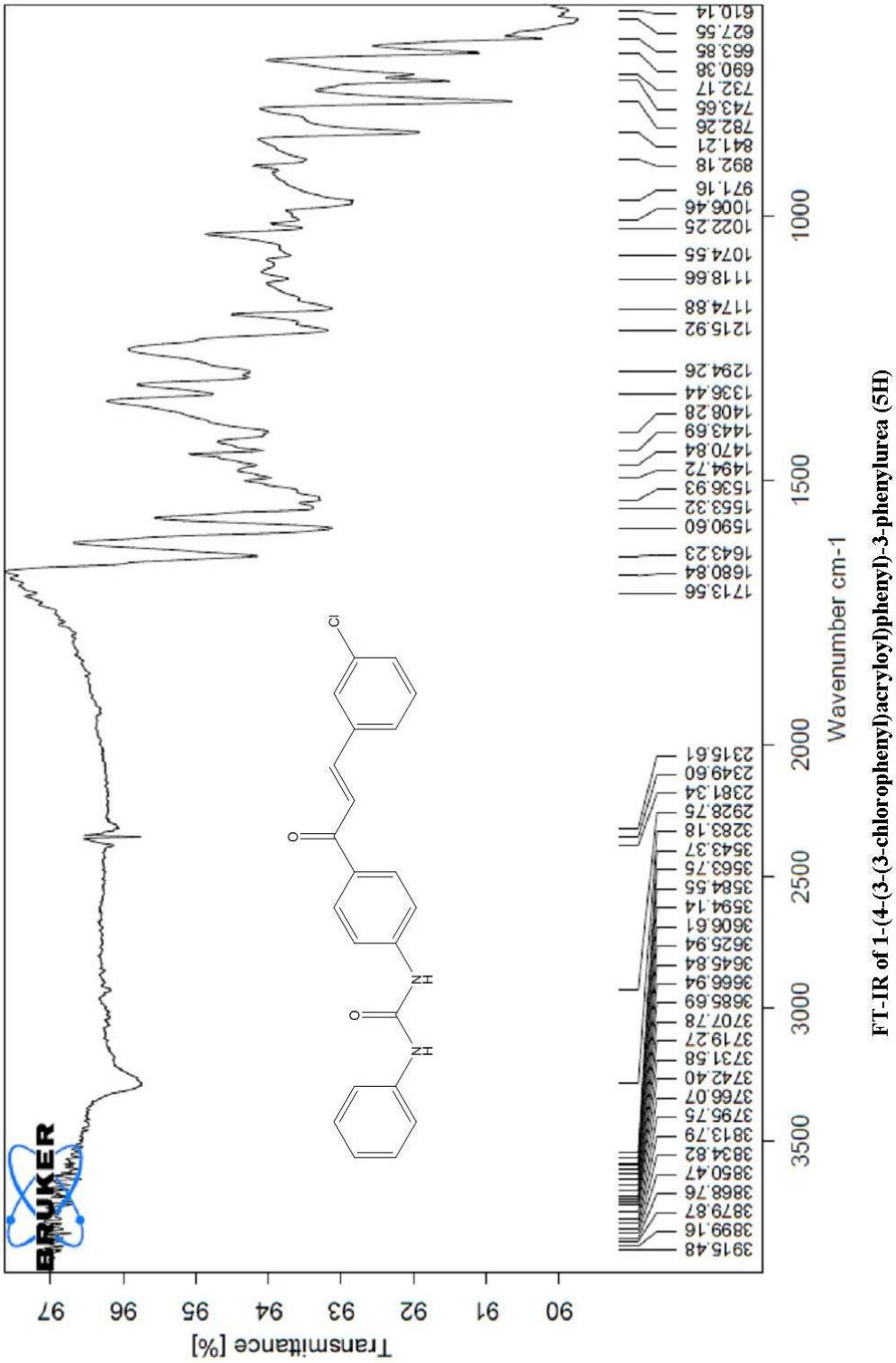
FT-IR of 1-(4-(3-(3-methoxyphenyl)acryloyl)phenyl)-3-phenylurea (5G)



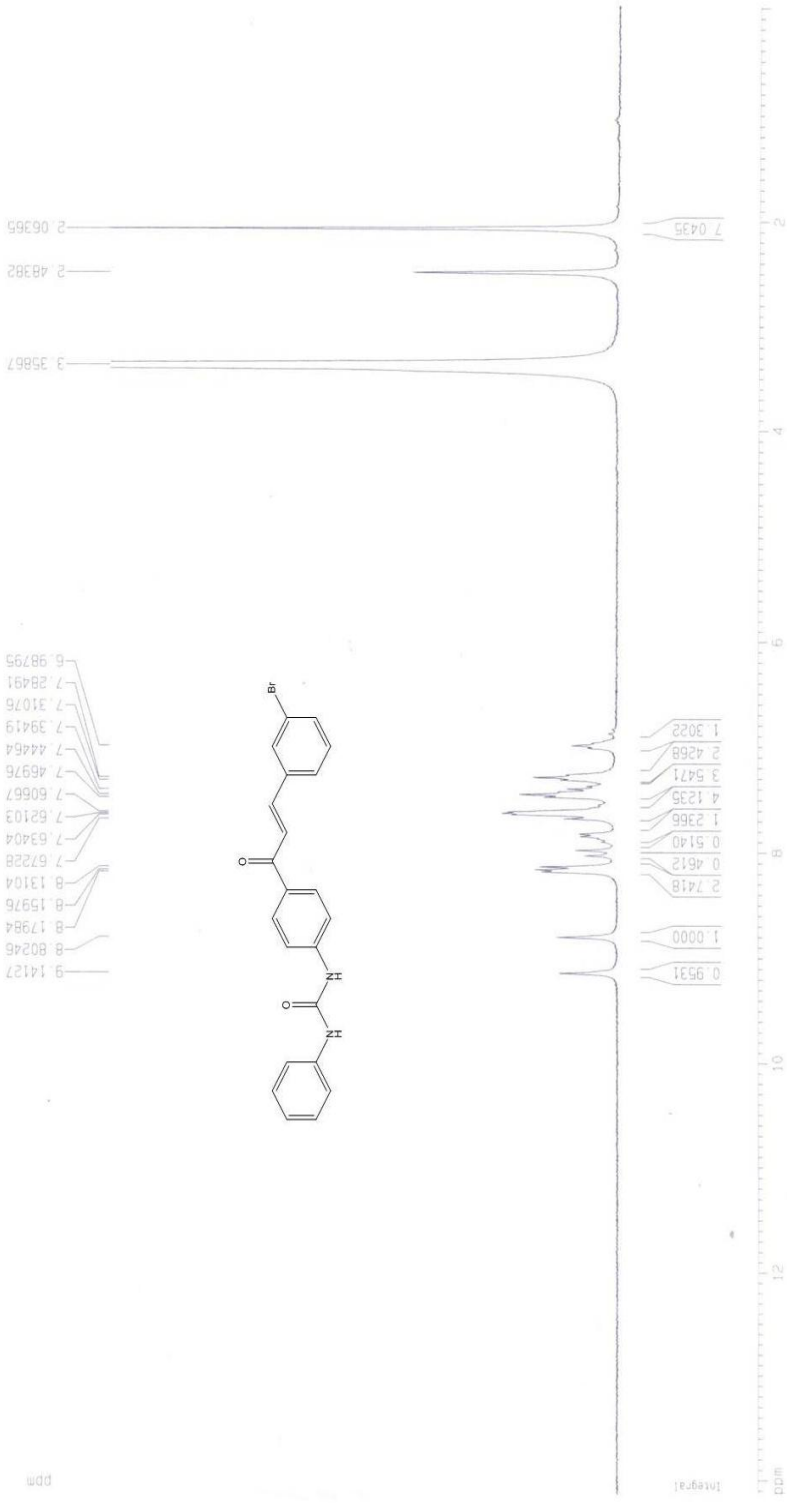
^{1}H NMR of 1-(4-(3-(3-chlorophenyl)acryloyl)phenyl)-3-phenylurea (5H)



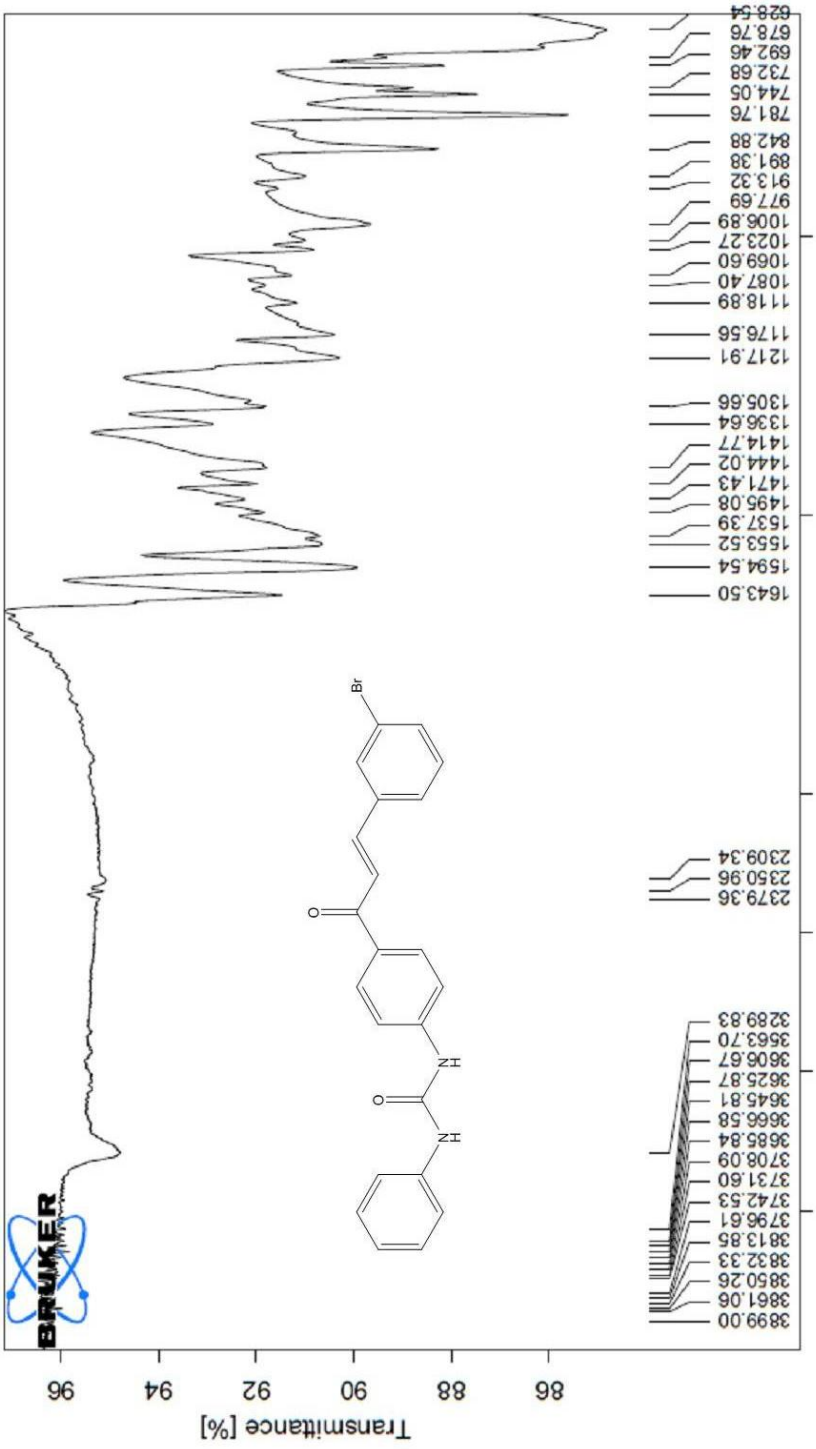
^{13}C NMR of 1-(4-(3-(3-chlorophenyl)acryloyl)phenyl)-3-phenylurea (5H)



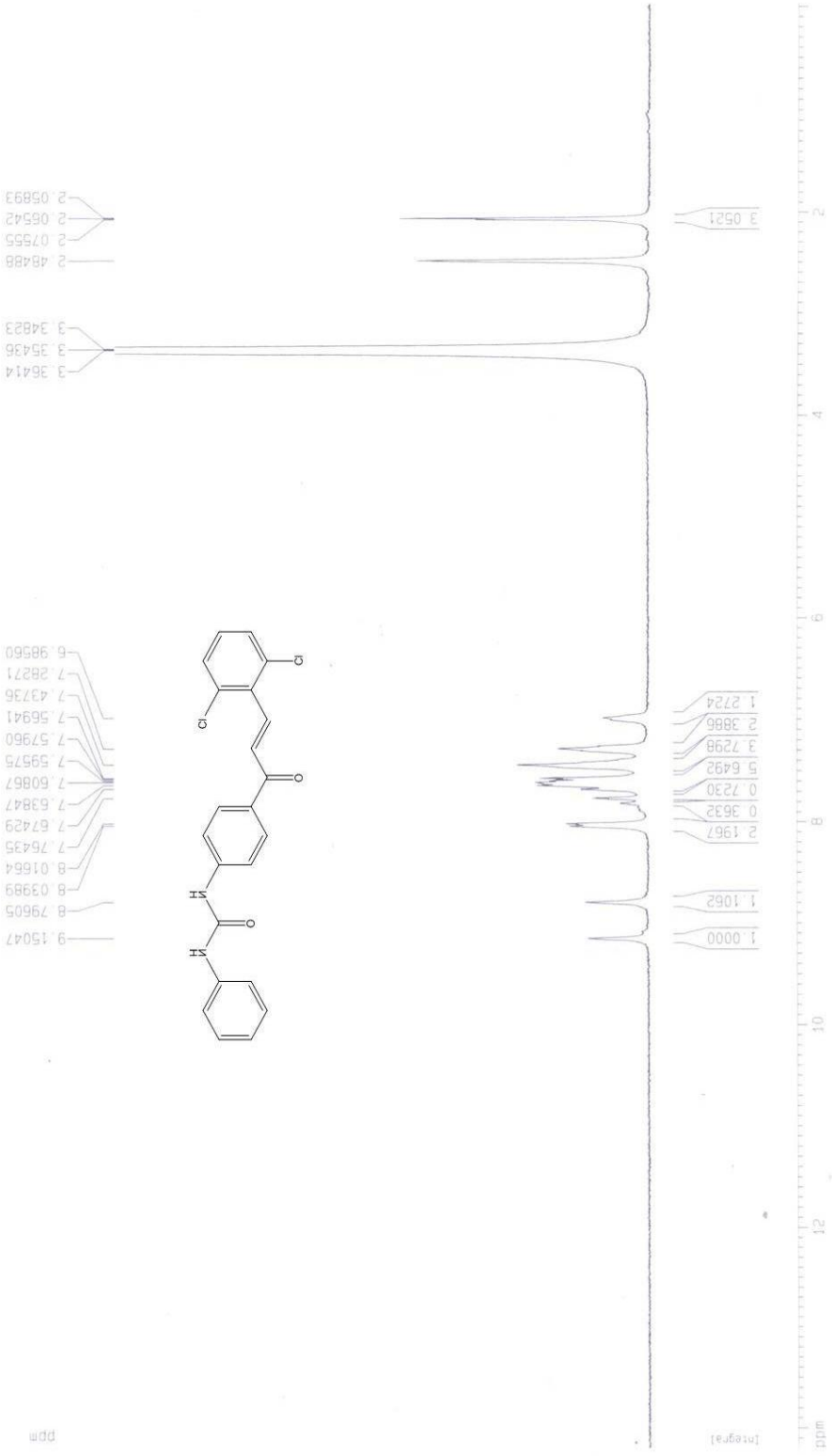
FT-IR of 1-(4-(3-(3-chlorophenyl)acryloyl)phenyl)-3-phenylurea (5H)



^{1}H NMR of 1-(4-(3-(3-bromophenyl)acryloyl)phenyl)-3-phenylurea (5I)



FT-IR of 1-(4-(3-(3-bromophenyl)acryloyl)phenyl)-3-phenylurea (5l)



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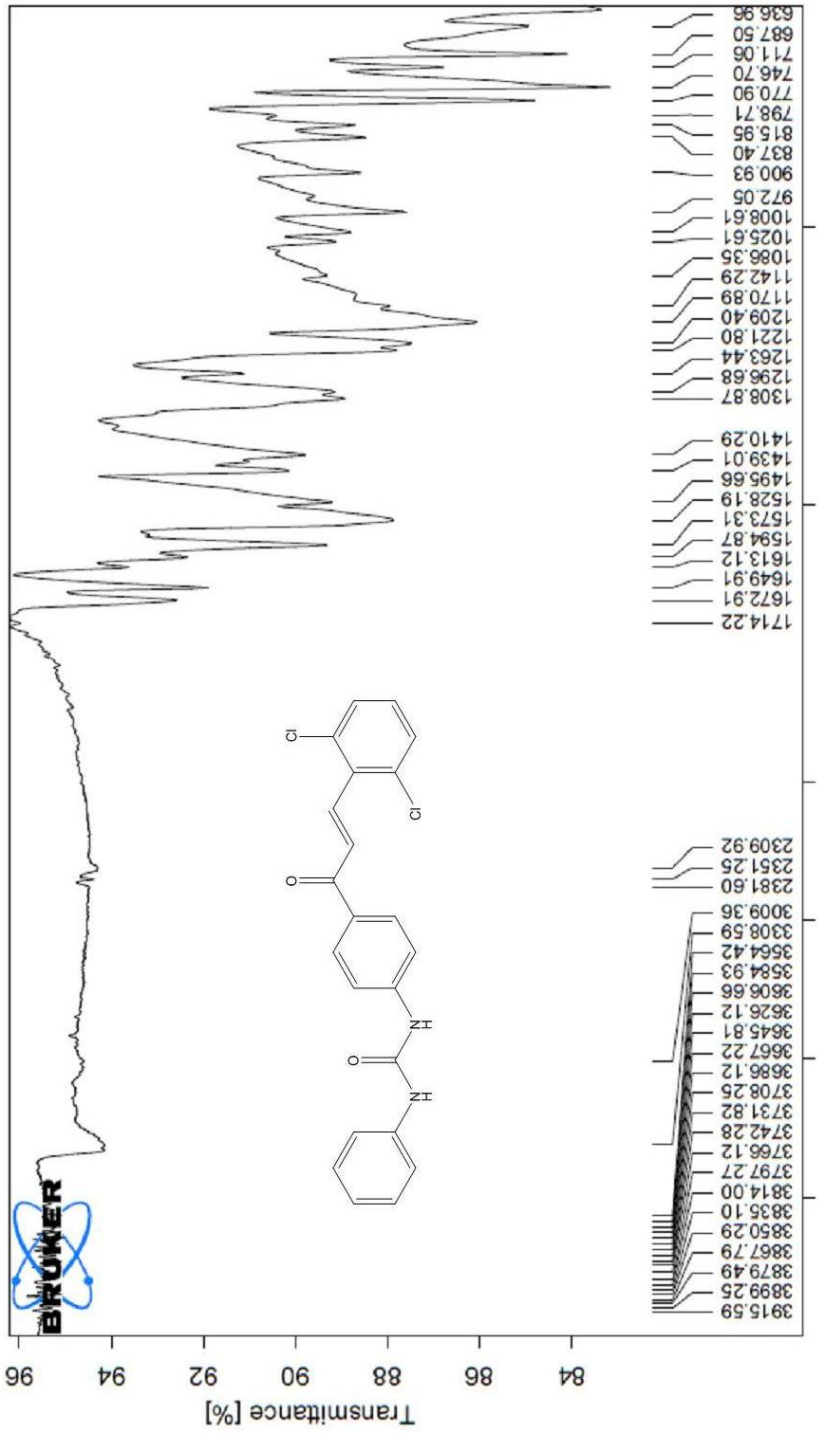
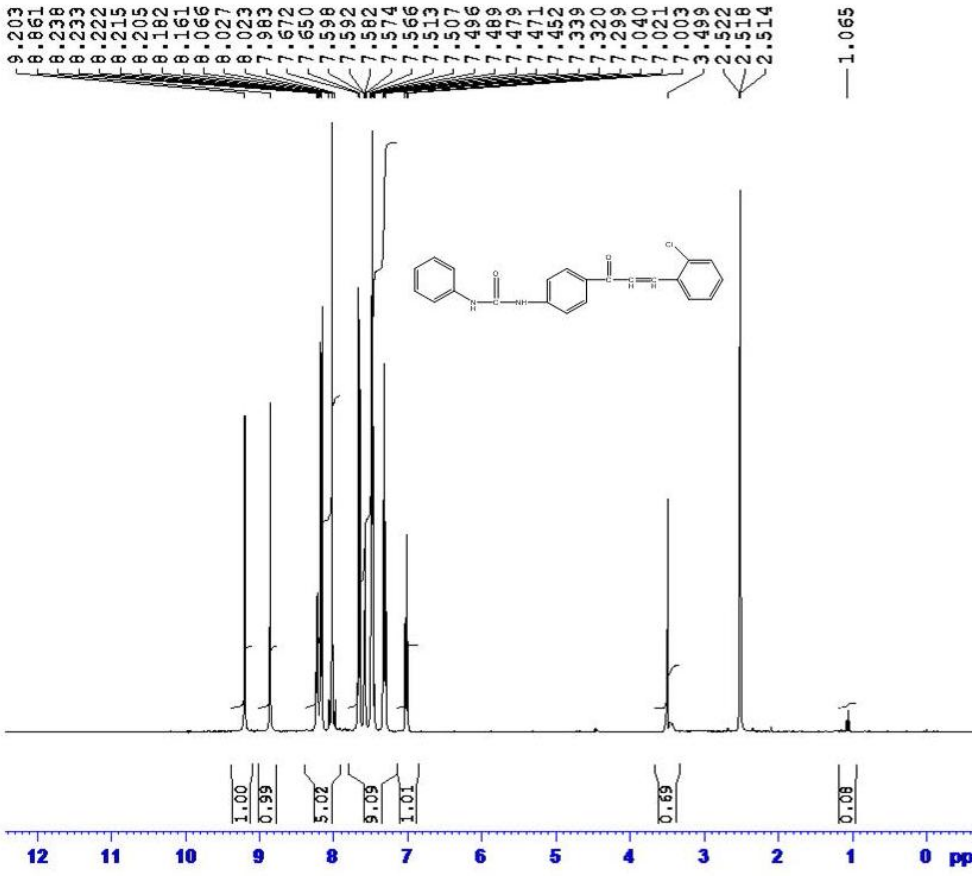


FT-IR of 1-(4-(3-(2,6-dichlorophenyl)acryloyl)phenyl)-3-phenylurea (5J)



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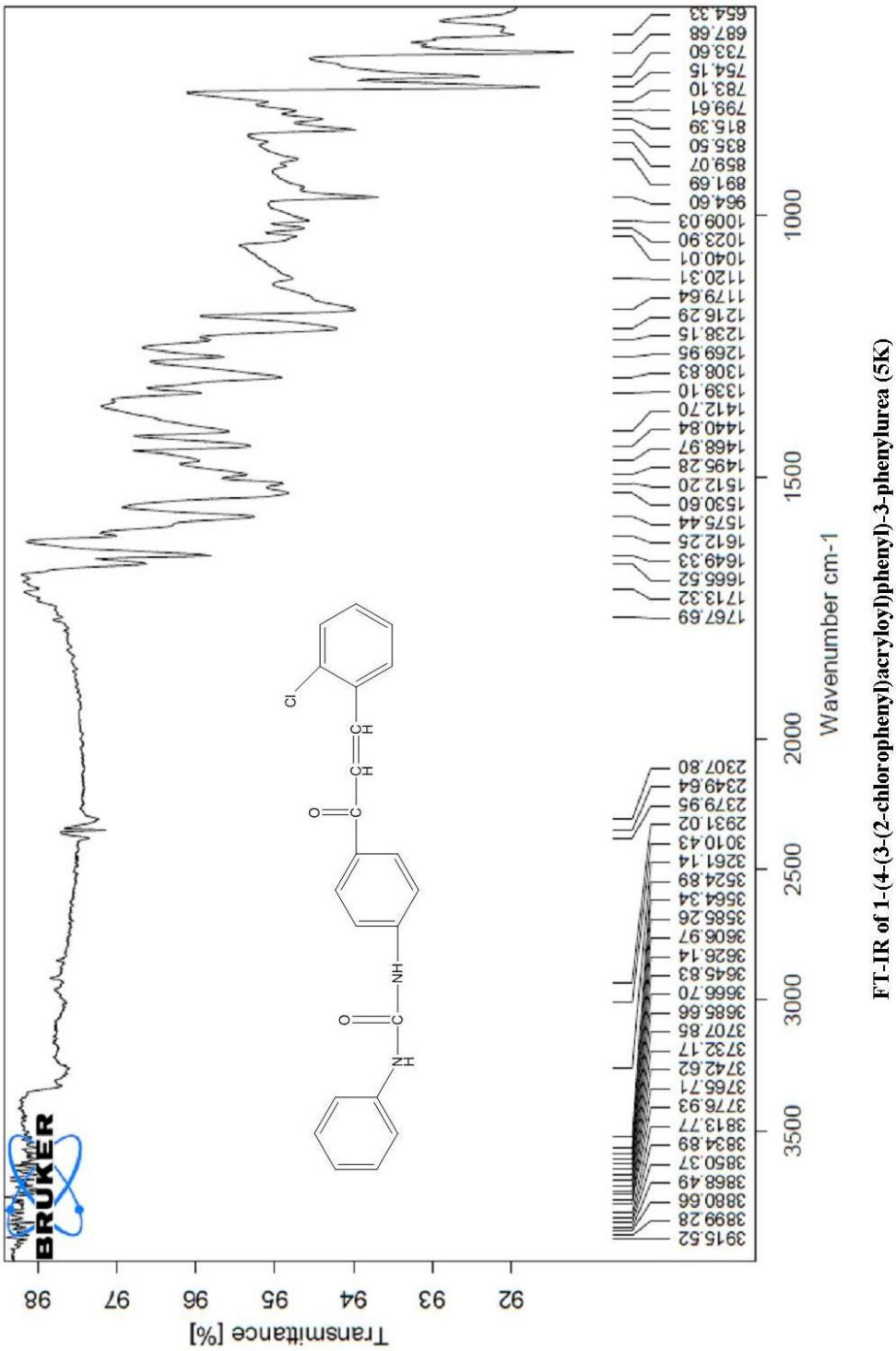
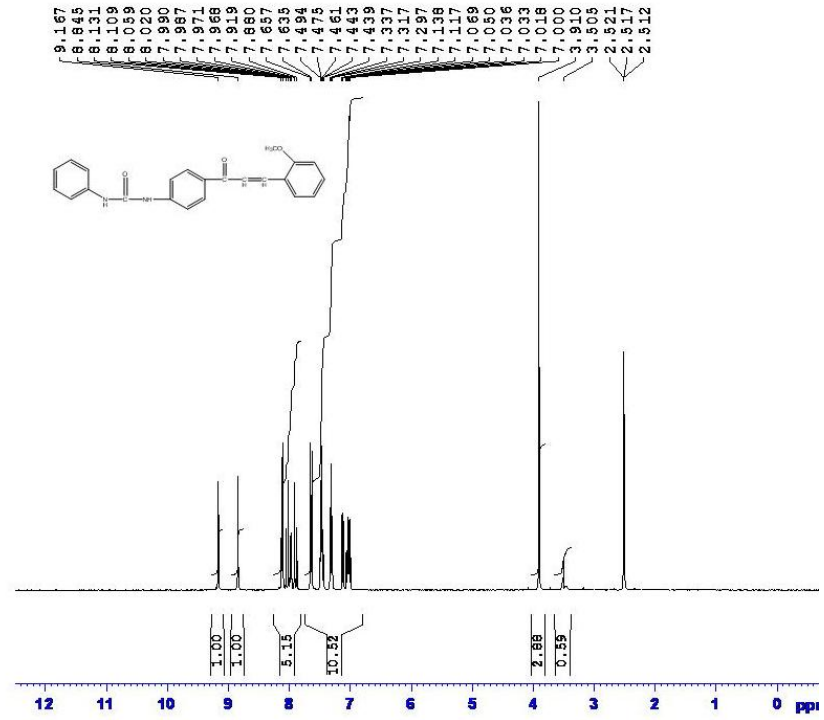


FT-IR of 1-(4-(3-(2-chlorophenyl)acryloyl)phenyl)-3-phenylurea (5K)



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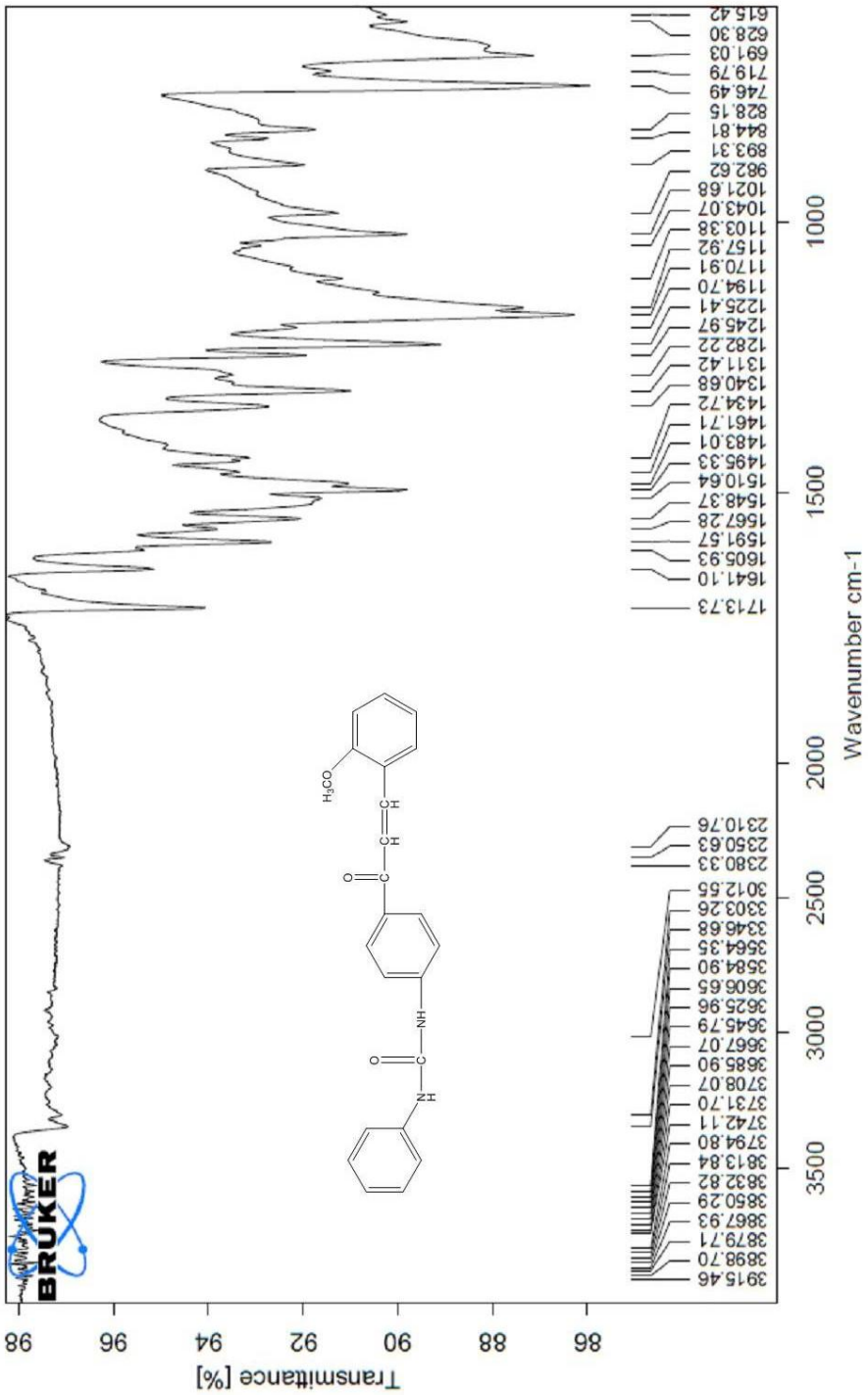
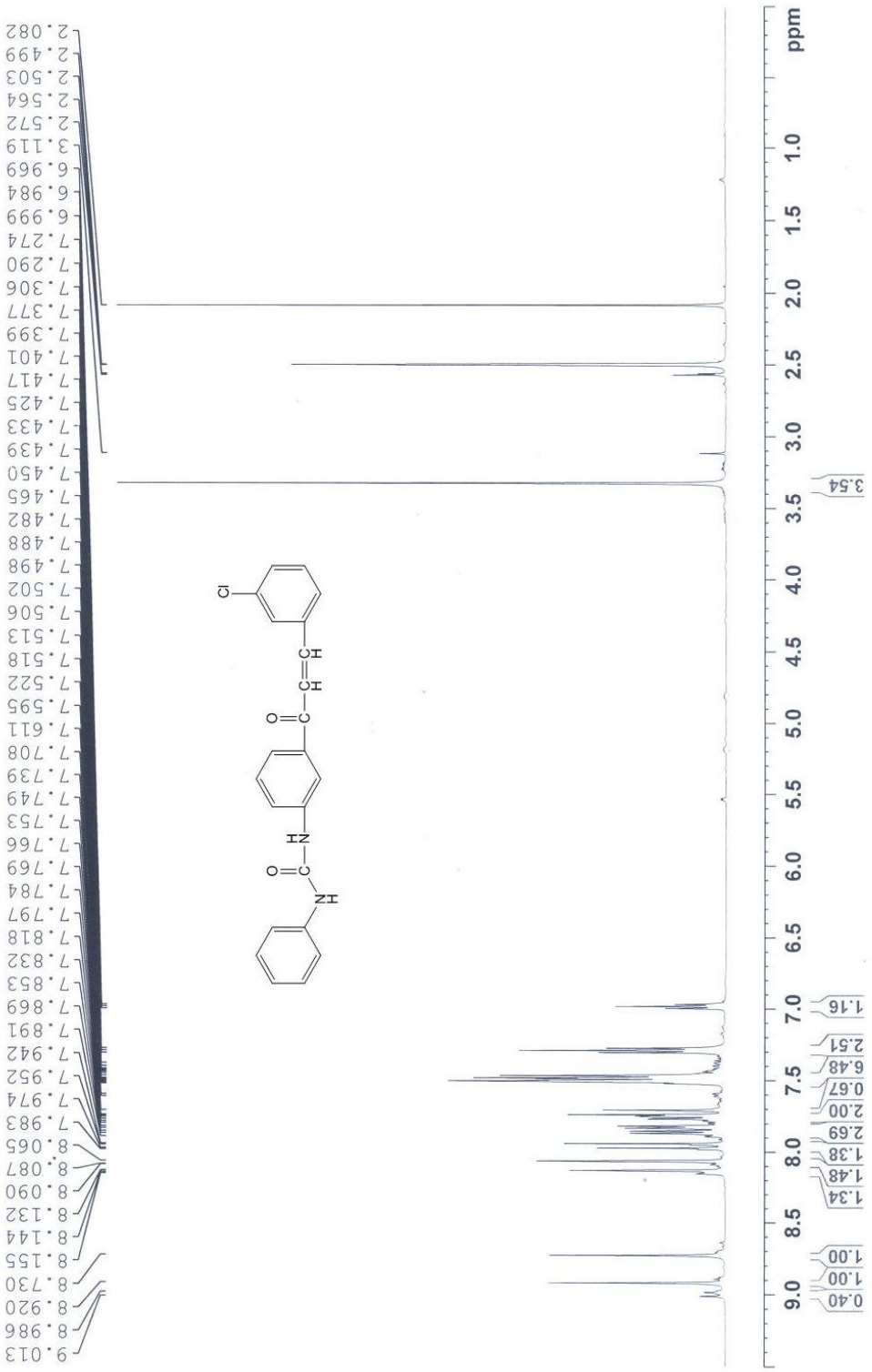


FT-IR of 1-(4-(3-(2-methoxyphenyl)acryloyl)phenyl)-3-phenylurea (5L)



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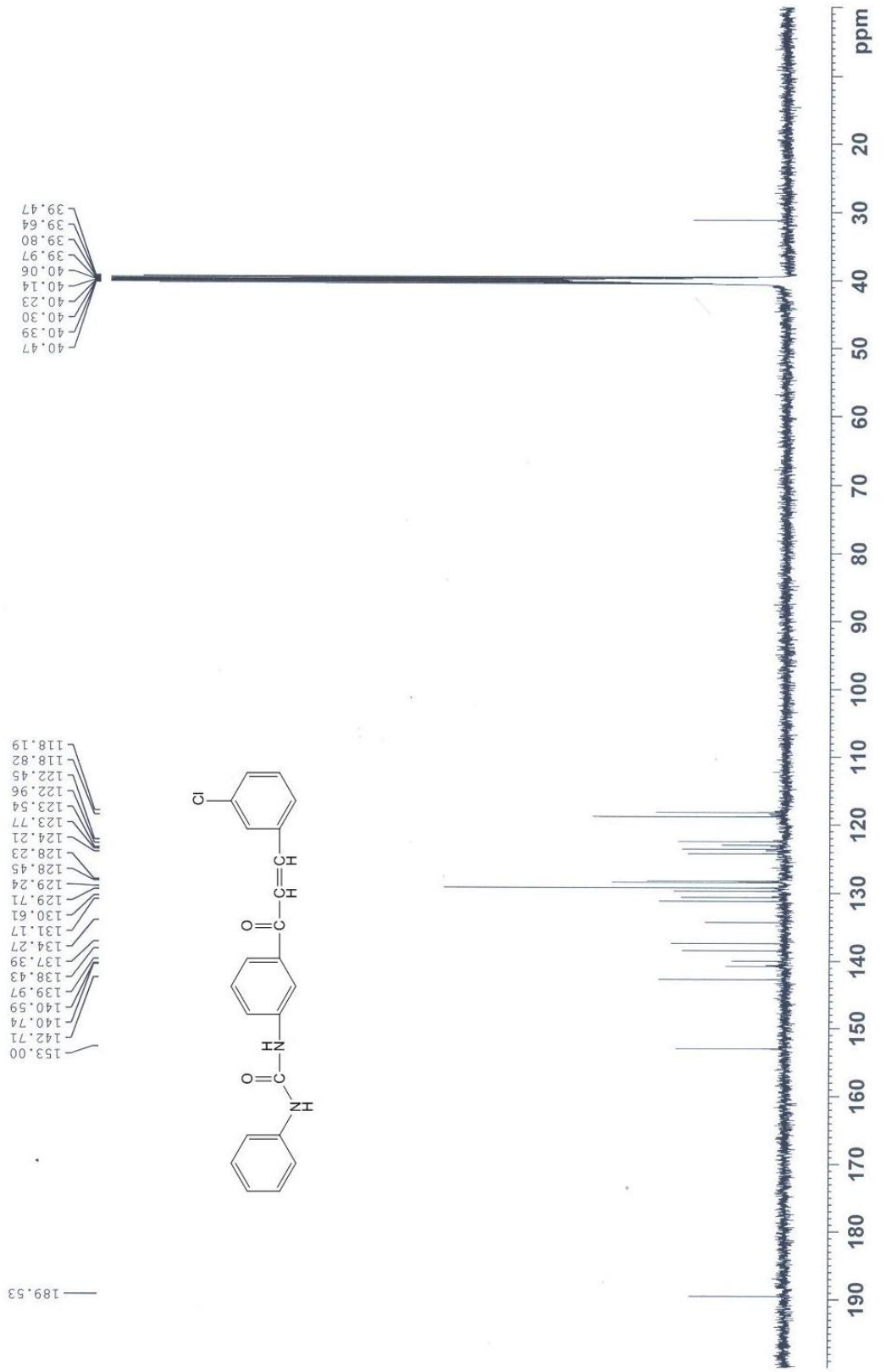
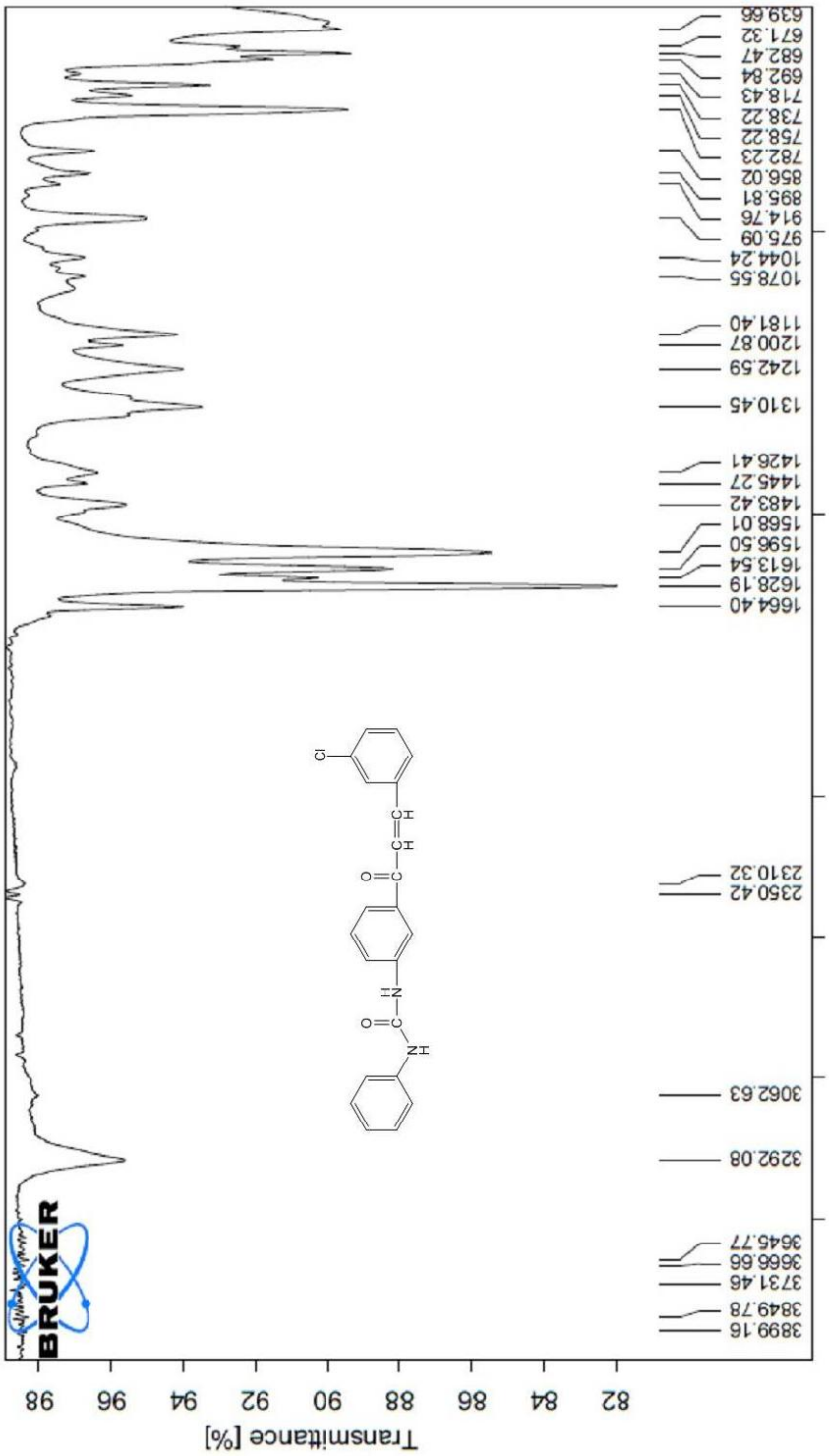
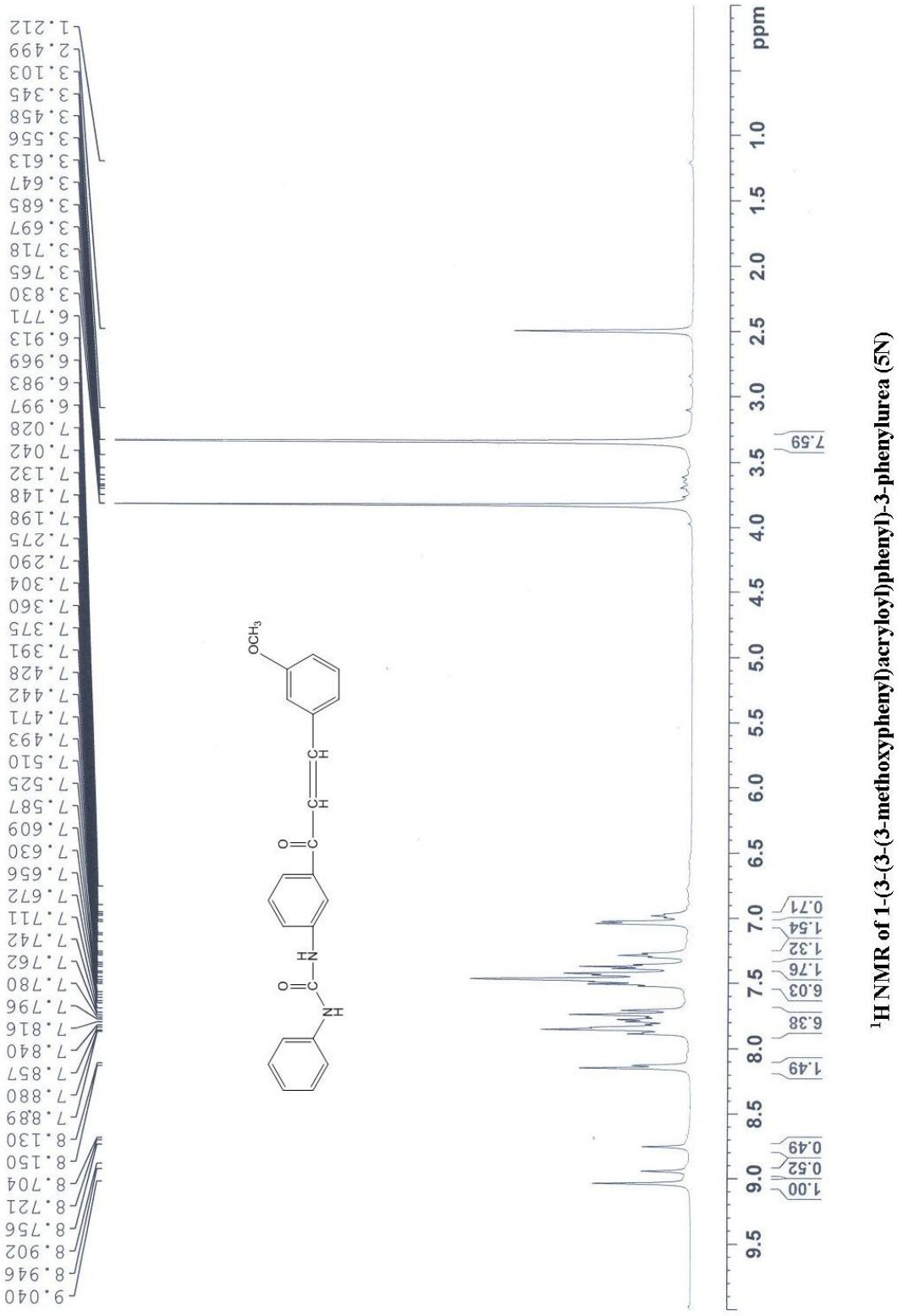


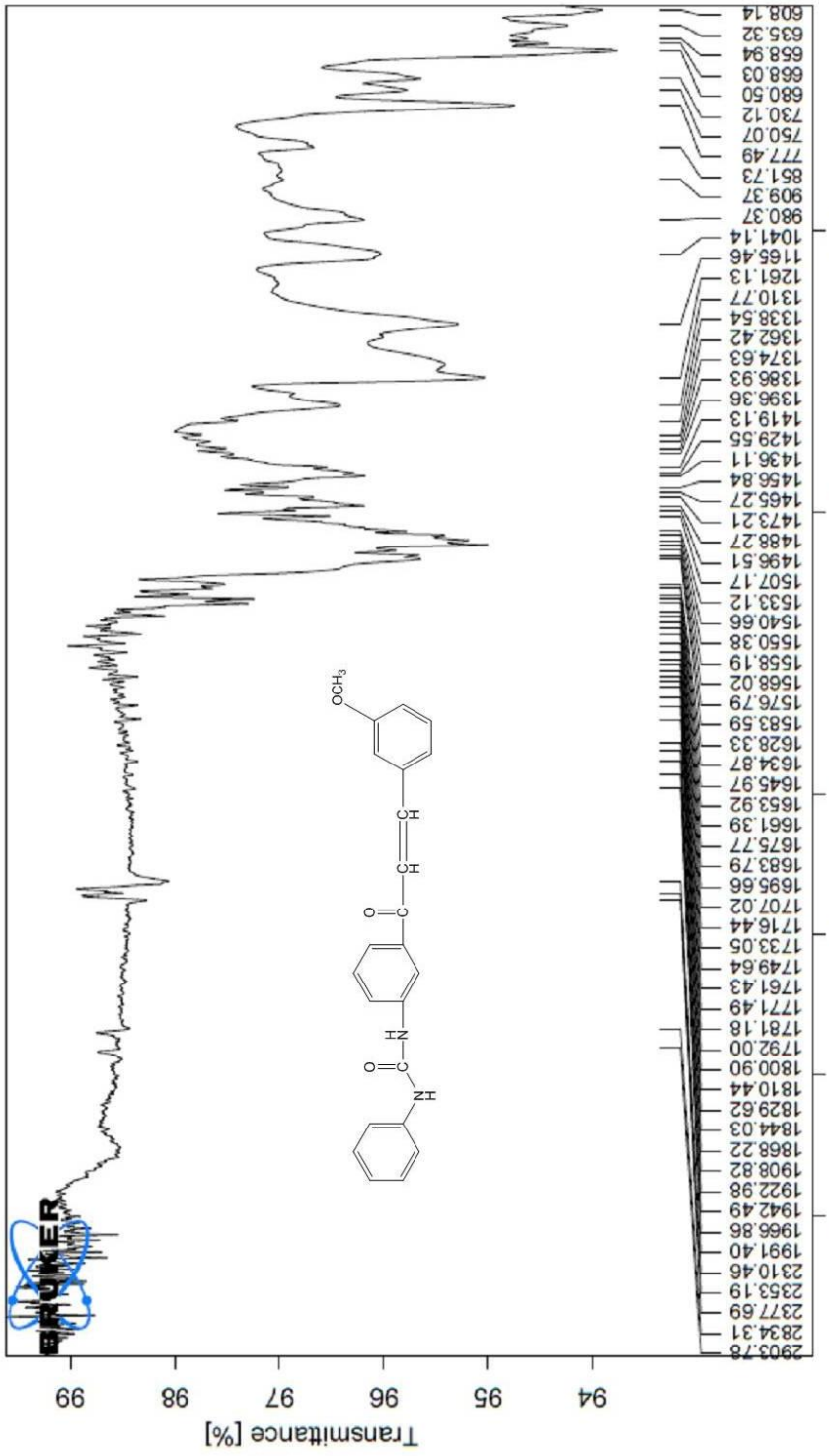
^{13}C NMR of 1-(3-(3-(3-chlorophenyl)acryloyl)phenyl)-3-phenylurea (5M)



FT-IR of 1-(3-(3-(3-chlorophenyl)acryloyl)phenyl)-3-phenylurea (5M)



^{1}H NMR of 1-(3-(3-(3-methoxyphenyl)acryloyl)phenyl)-3-phenylurea (5N)



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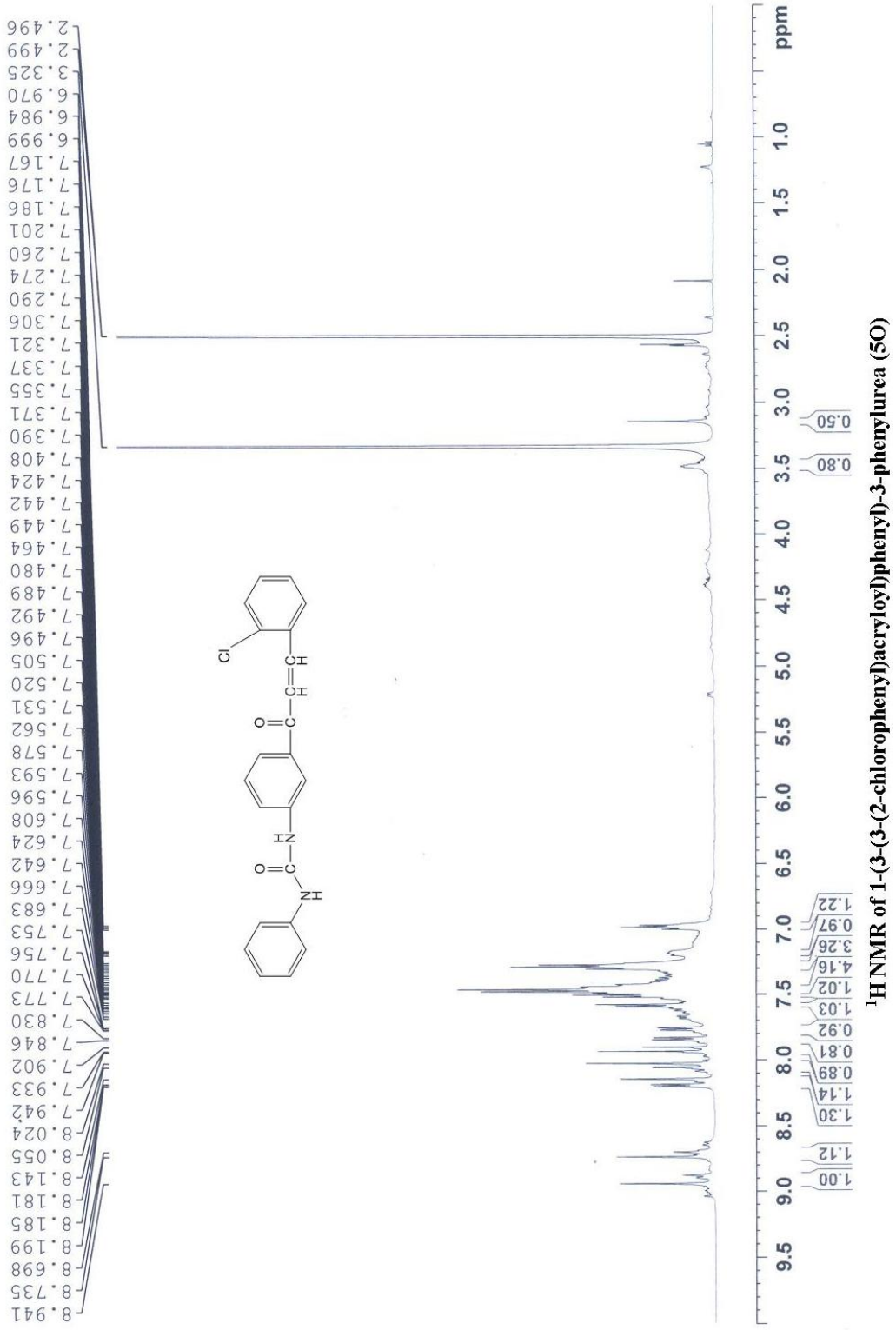
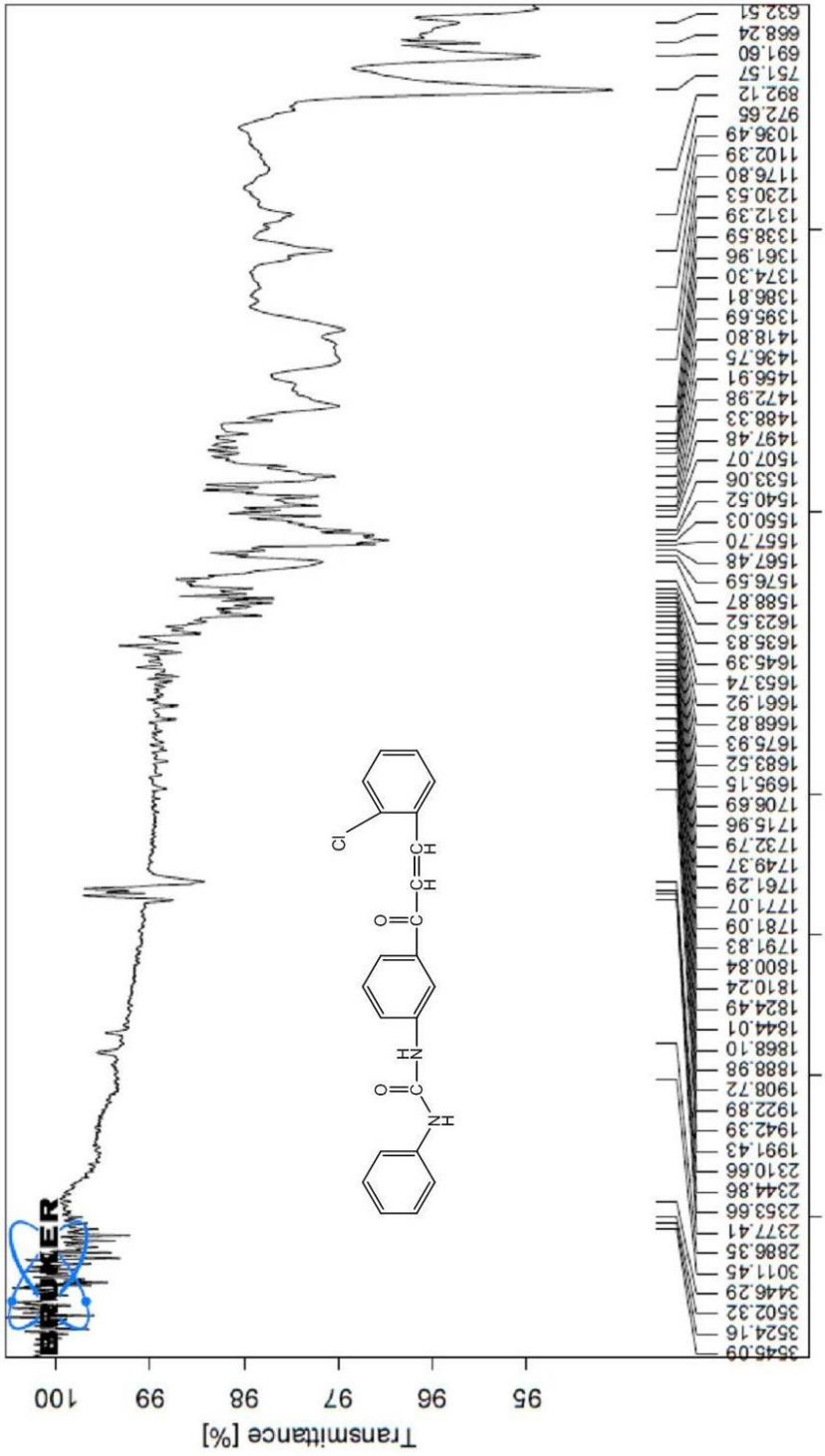
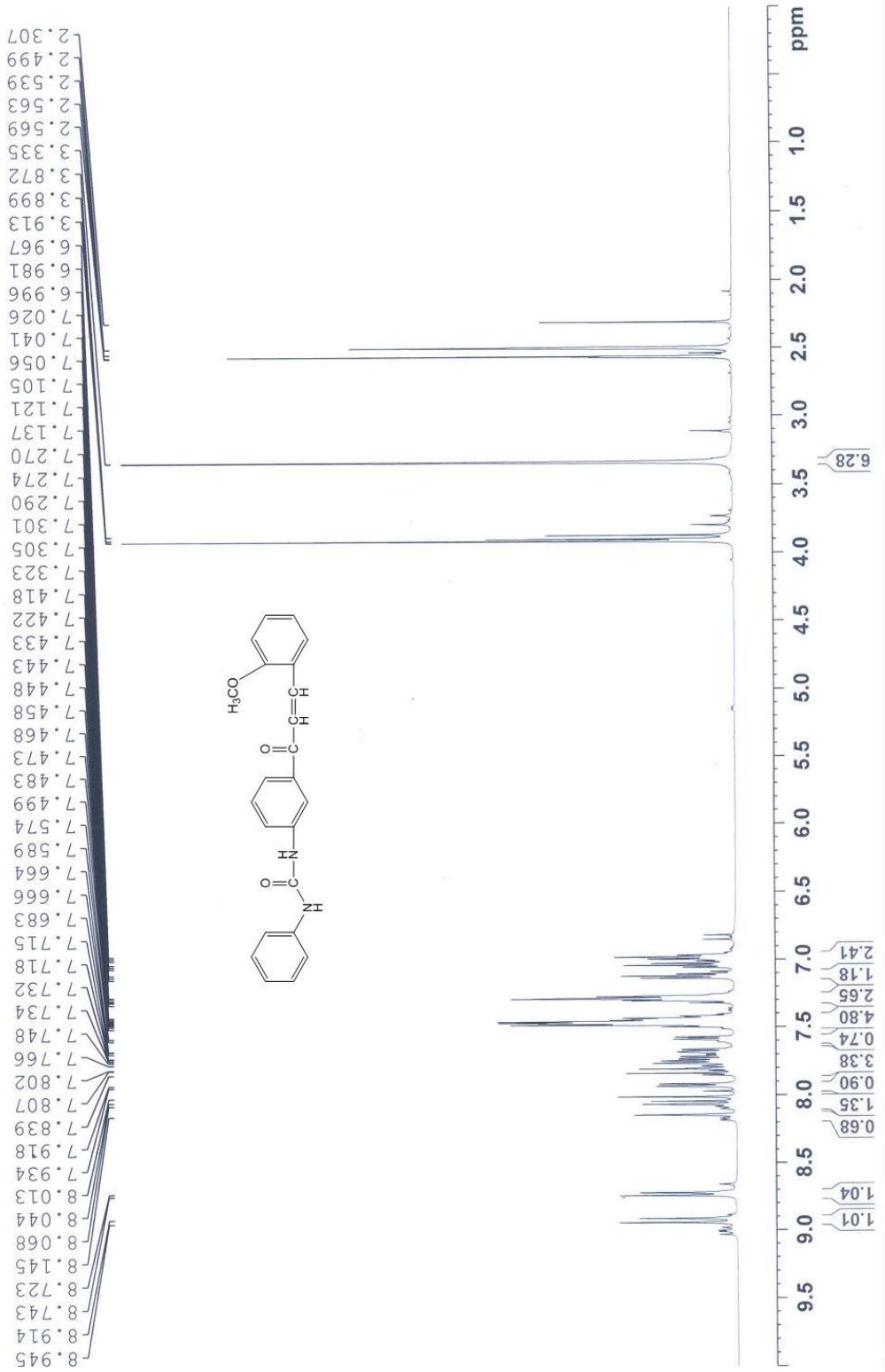


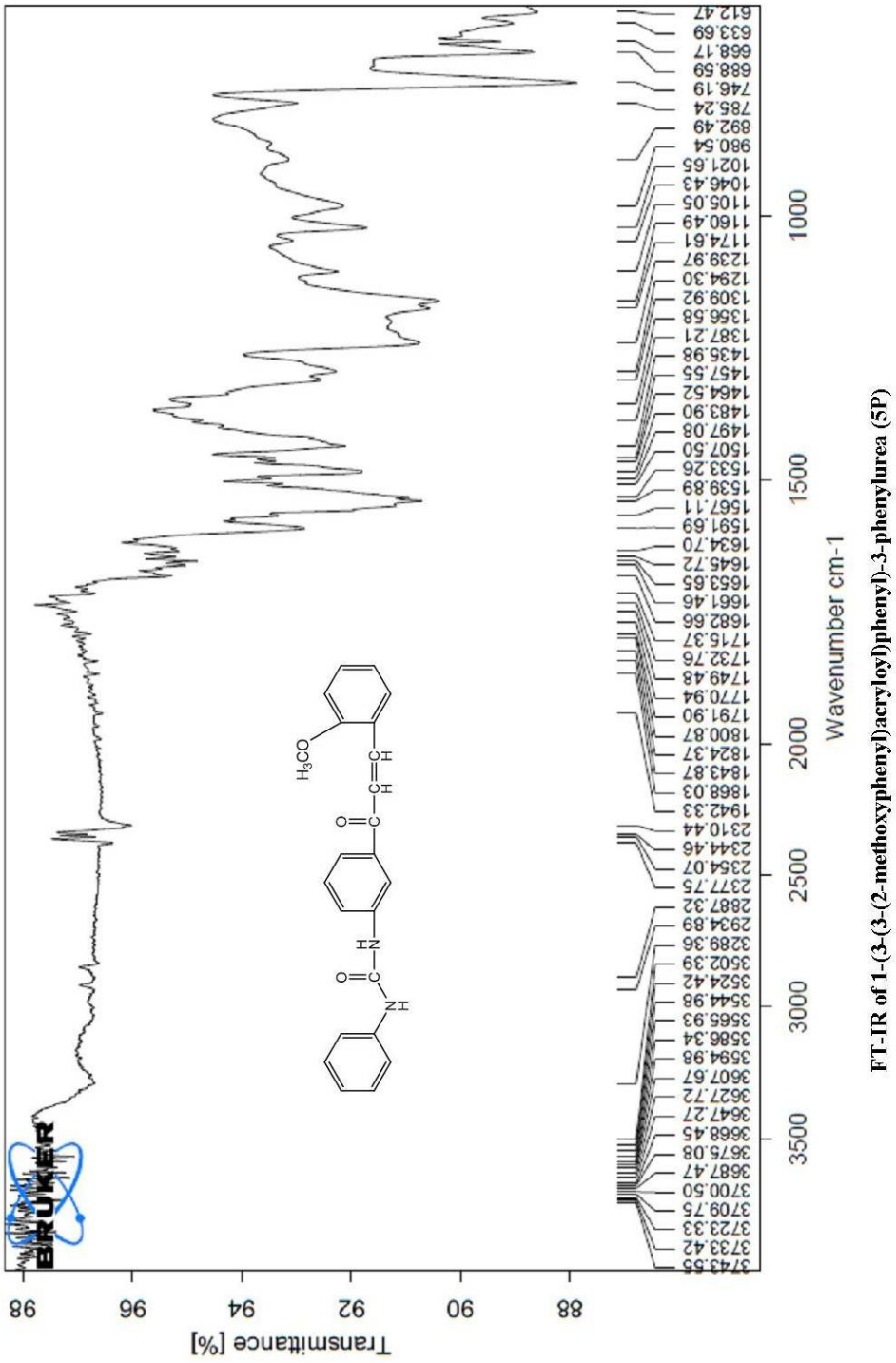
^{1}H NMR of 1-(3-(3-(2-chlorophenyl)acryloyl)phenyl)-3-phenylurea (5O)



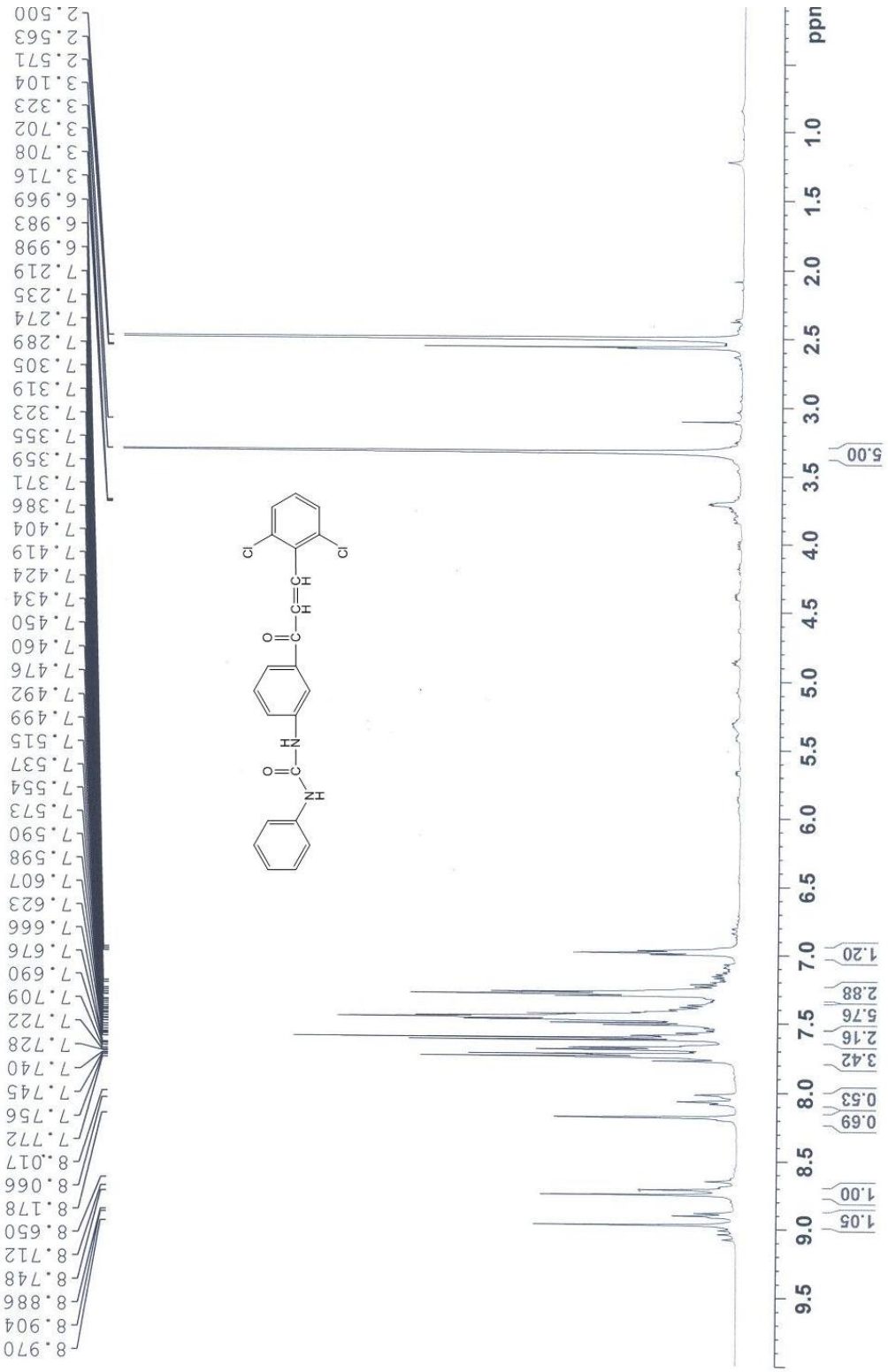
FT-IR of 1-(3-(3-(2-chlorophenyl)acryloyl)phenyl)-3-phenylurea (5O)



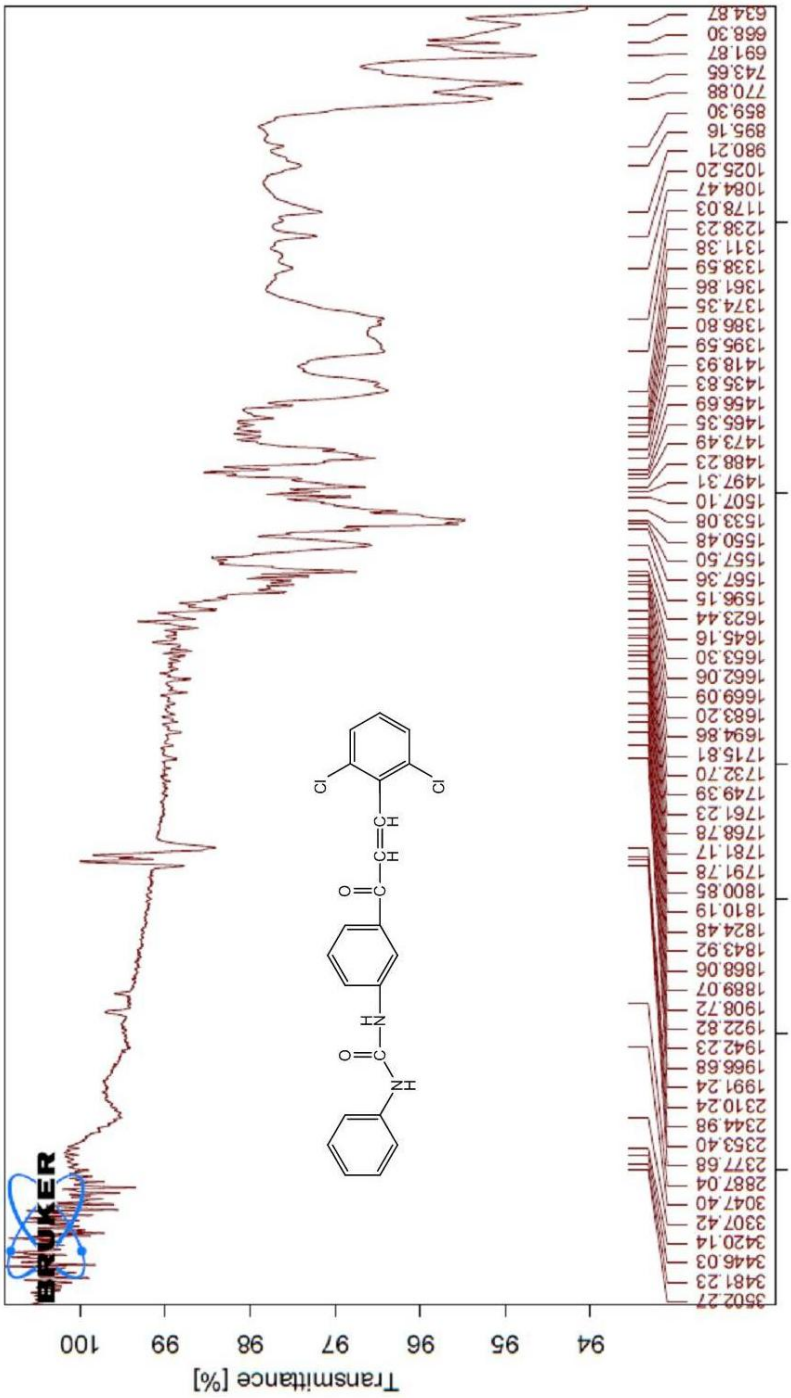
^{1}H NMR of 1-(3-(3-(2-methoxyphenyl)acryloyl)phenyl)-3-phenylurea (5P)



FT-IR of 1-(3-(3-(2-methoxyphenyl)acryloyl)phenyl)-3-phenylurea (5P)



^{1}H NMR of 1-(3-(3-(2,6-dichlorophenyl)acryloyl)phenyl)-3-phenylurea (5Q)



FT-IR of 1-(3-(3-(2,6-dichlorophenyl)acryloyl)phenyl)-3-phenylurea (5Q)

Chapter 6

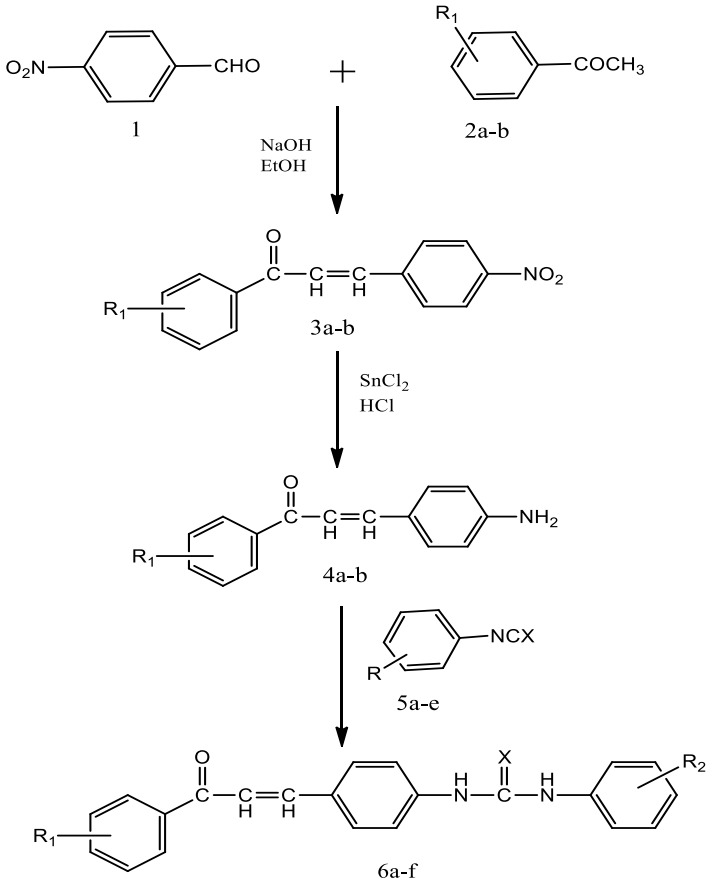
Synthesis and evaluation of chalcone based hybrid phenylurenyl/thiourenyl derivatives for their in-vitro antioxidant and antidiabetic activity

Synthesis and evaluation of chalcone based hybrid phenylurenyl/thiourenyl derivatives for their *in vitro* antioxidant and antidiabetic activity

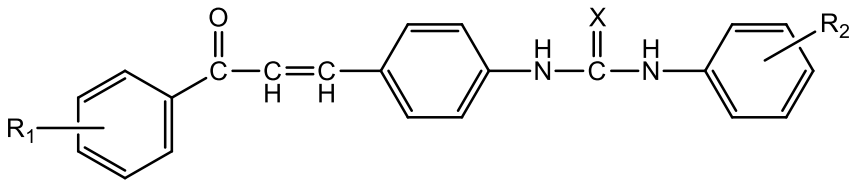
A series of hybrid urea/thiourea derivatives **(6a-f)** were synthesized by coupling isocyanate derivatives and aminochalcone derivatives (**Scheme 3**). The present investigation was carried out on the *in vitro* antioxidant and antidiabetic activity of hybrid phenylurenyl/thiourenyl derivatives.

The IUPAC names, chemical structures and molecular weights of the urea/thiourea derivatives used in this investigation were described in the experimental section. Reactions were routinely monitored by thin-layer chromatography (TLC) in silica gel plates and the products were visualized with iodine or ultraviolet lamp. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were determined in DMSO-d_6 solutions using a Bruker spectrophotometer. Peak positions were determined in parts per million (ppm) from tetramethylsilane as internal standard and coupling constant values (J) were given in Hz. Signal multiplicities were represented by s (singlet), d (doublet), t (triplet), q (quadruplet) and m (multiplet). Infrared (IR) spectra were established using a Bruker FTIR spectrophotometer.

General methodology and experimental work



Scheme 3. General Synthetic scheme for the preparation of chalcone based hybrid phenylurenyl/thiourenyl derivatives **(6a-f)**



Compounds	X	R_1	R_2
6a	O	H	4-Cl
6b	O	H	3-Cl
6c	O	H	4-OCH_3
6d	O	H	H
6e	O	4-OCH_3	H
6f	S	4-OCH_3	H

General Procedure for Synthesis of Nitrochalcone Derivatives

4-nitrobenzaldehyde **(1)** (50 mmol) and the substituted acetophenone **(2a-b)** (50 mmol) were dissolved in 20 mL absolute ethanol and 1N NaOH (20 mL) was added dropwise. This solution was stirred for 6-10h at room temperature and neutralized with 1N HCl. The Nitrochalcone derivatives **(3a-3b)** were filtered and washed with a minimum amount of ethanol (Domínguez, 2005; Sonmez *et al.*, 2011).

General Procedure for reduction of Nitro group to Amino group by Stannous Chloride

The nitrochalcones **(3a-b)** (10g) were dissolved in 10 mL of absolute ethanol and stirred with stannous chloride (40gm of the dihydrate) and hydrochloric acid (100ml; d 1.18). It was slowly warmed to 90°C for 8h. The solution was left overnight at 0°C and the compound was filtered out. It was mixed with an excess of aqueous ammonia and the resultant paste was drained at the pump and extracted with boiling acetone. The solvent was removed and the aminochalcones **(4a-b)** were recrystallized from alcohol.

General Procedure for Synthesis of phenylurenyl/thiourenyl Derivatives

A mixture of isocyanate/thiocyanate derivatives **(5a-e)** and aminochalcone derivatives **(4a-b)** in toluene (20 mL) were heated under reflux for 24h. The solution was evaporated and a residue was washed with toluene. The precipitated product was filtered and dried at room temperature. The final phenylurenyl/thiourenyl derivatives **(6a-f)** were recrystallized from ethanol to get the pure product (Sonmez *et al.*, 2011).

3-(4-nitrophenyl)-1-phenylprop-2-en-1-one (3a)

Yield: 92%; mp: 161-163°C; mf: $C_{15}H_{11}NO_3$; mw: 253.25, IR ν_{max} 1656 (CO, α, β-Unsaturated) cm^{-1}; ^{1}HNMR (300MHz, DMSO-d_6, ppm): δ 7.60-7.55 (m, 4H), 7.67 (d, 2H, J=9 Hz), 7.78 (s, 1H), 7.83 (s, 1H), 8.10 (t, 1H), 8.27 (d, 2H, J=9 Hz).

3-(4-aminophenyl)-1-phenylprop-2-en-1-one (4a)

Yield: 65%; mp: 146-148°C; mf: $C_{15}H_{13}NO$; mw: 223.26, IR ν_{max} 1649 (CO, α, β-Unsaturated) cm^{-1}; ^{1}HNMR (300MHz, DMSO-d_6, ppm): δ 5.90 (s, 2H, NH_2), 6.59 (d, 2H, J= 6.9 Hz), 7.54-7.65 (m, 8H), 8.07 (d, 2H, J = 6 Hz).

1-(4-methoxyphenyl)-3-(4-nitrophenyl)prop-2-en-1-one (3b)

Yield: 88%; mp: 148-150°C; mf: $C_{16}H_{13}NO_4$; mw: 283.27; IR ν_{max} 1632 (CO, α, β-Unsaturated) cm^{-1}.

3-(4-aminophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (4b)

Yield: 76%, mp: 157-160°C; mf: $C_{16}H_{15}NO_2$; mw: 253.29; IR ν_{max} 1657 (CO, α, β-Unsaturated) cm^{-1}.

1-(4-chlorophenyl)-3-(4-(3-oxo-3-phenylprop-1-en-1-yl)phenyl)urea (6a)

Yield: 96%; mp: 254-256°C; mf: $C_{22}H_{17}N_2O_2Cl$; mw: 376.87; IR ν_{max} 3288 (NH), 1715 (CO-Urea), 1631 (CO, α, β-Unsaturated) cm^{-1}; ^{1}HNMR (300MHz, DMSO-d_6, ppm): δ 7.33 (d, 2H, J=8.1 Hz), 7.47-7.63 (m, 6H), 7.65-7.72 (m, 2H), 7.79-7.83 (m, 3H), 8.12 (d, 2H, J=6.9 Hz), 8.92 (s, 1H, NH), 9.03 (s, 1H, NH).

1-(3-chlorophenyl)-3-(4-(3-oxo-3-phenylprop-1-en-1-yl)phenyl)urea (6b)

Yield: 84%; mp: 250-252°C; mf: $C_{22}H_{17}N_2O_2Cl$; mw: 376.87; IR ν_{max} 3347 (NH), 1715 (CO-Urea), 1644 (CO, α, β-Unsaturated) cm^{-1}; ^{1}HNMR (300MHz, DMSO-d6, ppm): δ 7.04 (d, 1H), 7.30 (t, 2H), 7.56 (d, 4H), 7.71-7.62 (m, 3H), 7.80-7.85 (m, 3H), 8.13 (t, 2H), 8.99 (s, 1H, NH), 9.07 (s,1H, NH); ^{13}C NMR(400MHz, DMSO-d_6, ppm): δ 55.98, 116.77, 117.66, 118.08, 119.61, 121.68, 128.29, 128.34, 128.71, 130.01, 130.38, 132.90, 133.16, 137.75, 140.91, 141.88, 143.98, 152.06, 188.98.

1-(4-methoxyphenyl)-3-(4-(3-oxo-3-phenylprop-1-en-1-yl)phenyl)urea (6c)

Yield: 79%; mp: 245-247°C; mf: $C_{23}H_{20}N_2O_3$; mw: 372.41; IR ν_{max} 3276 (NH), 1657 (CO-Urea), 1641 (CO, α, β-Unsaturated) cm^{-1}; ^{1}HNMR (300MHz, DMSO-d_6, ppm): δ 6.86 (d, 2H), 7.35 (d,

2H), 7.51-7.58 (m, 4H), 7.65-7.71 (m, 3H), 7.79 (t, 3H), 8.12 (d, 2H), 8.57 (s, 1H, NH), 8.91 (s,1H, NH).

1-(4-(3-oxo-3-phenylprop-1-en-1-yl)phenyl)-3-phenylurea (6d)

Yield: 62%; mp: 230-232°C; mf: $C_{22}H_{18}N_2O_2$; mw: 342.38; IR ν_{max} 3279 (NH), 1660 (CO-Urea), 1628 (CO, α, β-Unsaturated) cm^{-1}; ^{1}HNMR (300MHz, DMSO-d_6, ppm): δ 6.97 (t, 1H), 7.29 (t, 2H), 7.45 (d, 2H), 7.51-7.61 (m, 4H), 7.64 (q, 1H), 7.69 (s, 1H), 7.72-7.83 (m, 3H), 8.13 (d, 2H), 8.76 (s, 1H, NH), 8.99 (s, 1H, NH).

1-(4-(3-(4-methoxyphenyl)-3-oxoprop-1-en-1-yl)phenyl)-3-phenylurea (6e)

Yield: 20%; mp: 240-244°C; mf: $C_{23}H_{20}N_2O_3$; mw: 372.41; IR ν_{max} 3273 (NH), 1732 (CO-Urea), 1645 (CO, α, β-Unsaturated) cm^{-1}; ^{1}HNMR (400MHz, DMSO-d_6, ppm): δ 3.35 (s, 1H, OCH_3), 7.04 (t, 2H), 7.16-7.29 (m, 4H), 7.45 (d, 4H), 7.56 (d, 2H), 7.67 (d, 2H), 7.82 (t, 1H), 8.16 (d, 1H), 8.84 (s, 1H, NH), 9.06 (s,1H, NH).

1-(4-(3-(4-methoxyphenyl)-3-oxoprop-1-en-1-yl)phenyl)-3-phenylthiourea (6f)

Yield: 15%; mp: 278-280°C; mf: $C_{23}H_{20}N_2O_2S$; mw: 388.47; IR ν_{max} 3397 (NH), 1732 (CO-Urea), 1635 (CO, α, β-Unsaturated) cm^{-1}; ^{1}HNMR (400MHz, DMSO-d_6, ppm): δ 7.57 (d, 4H), 7.82 (d, 2H), 8.14-8.12 (m, 3H), 7.49 (d, 5H), 8.70 (d, 1H), 8.99 (s, 1H, NH), 10.10 (s, 1H, NH).

***In-vitro* α-Glucosidase inhibition assay**

The α-glucosidase inhibition assay was performed according to the modified method described by Patil *et al.*, (Patil *et al.*, 2013). 40 µL of yeast α-glucosidase (0.1 unit/ml; Sigma Aldrich, USA) was mixed with 20 µL of each of the samples (5 mg/mL DMSO solution and reconstituted in 100 µL of 100 mM phosphate buffer pH 6.8) and incubated for 1 h at 37ºC in 96-well microplate. Enzyme action for α-glucosidase was initiated by addition of 50 µL substrate (5 mM, *p*-nitrophenyl- α-D-glucopyranoside prepared in 100 mM phosphate buffer of pH 6.8) and stopped by adding 100 µL of 0.1 M Na_2CO_3 after an incubation of 10 min at 37ºC. The release of *p*-nitrophenol was measured at 420 nm spectrophotometrically (Spectra Max plus 384, Molecular Devices Corporation, Sunnyvale, CA, USA) 5 min after incubation with the substrate. Individual blanks for test samples were prepared to correct background absorbance where the substrate was replaced with 50 µL of the buffer. Control sample contained 10 µL DMSO in place of test samples.

Percentage of enzyme inhibition was calculated as (1 - B/A) × 100, where [A] represents absorbance of control without test samples, and [B] represents absorbance in the presence of test samples.

In-vitro antioxidant study

DPPH radical scavenging activity

The radical scavenging activity of the synthesized molecules was measured in terms of hydrogen donating or radical scavenging capacity using the stable radical DPPH. DPPH scavenging activity is one of the most widely used methods for screening the antioxidant activity. The DPPH test gives information on the reactivity of the test compounds with a stable free radical (Mohana *et al.,* 2013). The radical scavenging activity was determined according to the method of Blois (Blois, 1958). 25µl of variable concentrations of synthesized compounds (10-100 µM in ethanol) was added to 100 µl of a DPPH solution (0.004% w/v in ethanol) in 96 well microplates and kept for 60 minutes in dark place at room temperature. The decrease in the absorbance was reported at 517 nm. L- Ascorbic acid was used as a positive control (Ayoola *et al.*, 2008).

The percentage DPPH radical scavenging activity was calculated using the following formula:

DPPH radical scavenging activity (%) = $[(A_c - A_t)/A_c \times 100]$, where A_c is the absorbance of the control and A_t is the absorbance of tested compounds or the standard drug.

The scavenging reaction between DPPH• and an antioxidant, H-A can be written as

H_2O_2 radical scavenging assay

The hydrogen peroxide scavenging activity of the tested compounds was determined by the method of Jayaprakasha *et al.,* (Jayaprakasha *et al.,* 2004). Hydrogen peroxide solution (2mmol/l) was prepared in phosphate buffer (pH 7.4). 50µl of different concentrations (10-100µM) of synthesized compounds were added to 50 µl of hydrogen peroxide solution. The absorbance of hydrogen peroxide was determined at 230 nm after 10 min against a blank solution and compared with Ascorbic acid, the reference compound.

Hydrogen peroxide scavenging activity (%) = $[(A_c - A_t)/A_c \times 100]$, where A_c is the absorbance of the control and A_t is the absorbance of tested compounds or the standard drug.

Nitric oxide scavenging assay

The assay of nitric oxide scavenging activity was followed to the modified method of Sreejayan (Sreejayan, 1997). Sodium nitroprusside in phosphate buffer was mixed with different concentration of the synthesized compounds, dissolved in ethanol and incubated at room temperature for 2 h. 50 µl of the incubation solution was removed and diluted with an equal volume of Griess reagent (1% sulfanilamide in 5% H_3PO_4 and 0.1% naphthylethylene diamine dihydrochloride). The absorbance of the chromophore was measured at 546 nm and compared with standard Ascorbic acid.

Nitric oxide scavenging activity (%) = $[(A_c - A_t)/A_c \times 100]$, where A_c is the absorbance of the control and A_t is the absorbance of tested compounds or the standard drug.

Result and discussion

Chemistry

The structures of the synthesized compounds (**6a-f**) were characterized by ^{1}H NMR, ^{13}C NMR and FT-IR. All described products showed ^{1}H and ^{13}C NMR spectra according to the assigned structures. The infrared spectra of the compounds (**6a-f**) showed the absorptions between 3273-3397 cm^{-1} relating to NH stretch, absorptions in 1628–1645 cm^{-1} from α, β-unsaturated carbonyl moiety stretch and absorptions in 1660–1732 cm^{-1} from urea carbonyl moiety stretching. The ^{1}H NMR spectra for all the synthesized urea compounds showed signals between δ 8.57- 9.07 ppm and thiourea derivatives showed between δ 8.99-10.10 ppm relating to hydrogens attached to the nitrogen. The signals for aromatic hydrogens were between 6.86 and 8.16 ppm. The ^{13}C NMR of the compounds **6b** showed the δ 188.98 ppm relating to chalcone carbonyl and δ 152.06 ppm for urea carbonyl.

In vitro α-Glucosidase inhibition

The α-glucosidase inhibition activity of all synthesized compounds (**6a-f**) was evaluated *in vitro*. The compounds **6c** and **6a** were found better inhibition compared to other compounds with IC_{50} values of 21.86 and 28.53 µM, respectively. The compounds 6d, 6e, and 6f showed poor α-glucosidase inhibition activity compared to the standard drug Acarbose (IC_{50}- 11.92). The α-glucosidase inhibition assay was shown in **Table 1.**

Table 1. *In vitro* α-Glucosidase inhibition assay

Compounds	Concentration (µM)				IC_{50}
	10	25	50	100	
6a	30.68±0.39	54.41±1.01	68.73±0.58	85.07±1.45	28.53
6b	25.96±0.97	45.63±0.69	59.47±1.25	80.45±0.25	41.15
6c	38.25±1.41	51.38±1.46	75.38±2.03	88.57±0.58	21.86
6d	18.26±0.89	30.14±0.40	48.25±1.58	70.15±2.19	60.87
6e	04.69±1.05	12.73±0.32	24.76±2.48	52.98±1.36	95.19
6f	8.43±1.42	17.34±0.79	37.54±0.94	60.70±0.65	78.74
Acarbose	40.28±1.12	60.61±1.00	79.10±0.94	89.69±1.29	11.92

Values are mean ± SEM; experiment performed in triplicate

DPPH scavenging activity

The DPPH radical scavenging method is widely used to evaluate the free radical scavenging ability of antioxidants. DPPH is a stable nitrogen-based free radical. The violet color of the solution is altered to yellow after reduction by antioxidant. In the radical form, the DPPH molecule has an absorbance at 517 nm which disappears after acceptance of an electron or hydrogen radical from an antioxidant compound (Molyneux 2004; Sahu *et al.*, 2013).

Table 2 shows the DPPH scavenging activity of the synthesized compounds. An IC_{50} value is the concentration of the compound required to scavenge 50% of the free radicals present in the system. The drug which has lower IC_{50} value, the antioxidant activity of the drug will be higher. The compound **6c** is capable of neutralizing the DPPH free radicals by 41.63%, 57.81%, 82.41% and 90.42% at 10, 25, 50 and 100 µM concentrations respectively (**Table 2**). The IC_{50} value of **6c** is 11.55 µM. The compounds **6a** and **6b** with chloro substitution have the IC_{50} value of 19.47 and 12.17 µM respectively. DPPH scavenging was increased in a concentration-dependent manner when compared with standard antioxidant Ascorbic acid.

Table 2. DPPH Scavenging activity

Compounds	Concentration (µM)			
	10	**25**	**50**	**100**
6a	31.42±0.53	60.04±0.86	80.63±0.53	88.24±0.86
6b	34.31±0.75	65.77±0.65	77.85±0.57	81.74±0.55
6c	41.63±0.64	57.81±0.84	82.41±1.58	90.42±0.75
6d	26.94±0.82	42.89±1.51	62.92±1.51	68.70±0.96
6e	12.35±1.02	10.50±0.55	37.22±1.26	53.66±1.22
6f	3.05±0.29	22.71±0.32	31.41±1.04	55.69±1.79
Ascorbic acid	46.73±0.85	65.67±0.87	88.05±0.43	98.02±0.43

Values are mean ± SEM; experiment performed in triplicate

Hydrogen peroxide scavenging assay

Hydrogen peroxide is a poor oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly and react with Fe^{2+} and Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects (Rahul *et al.,* 2006).

As shown in **Table 3**, the hydrogen peroxide decomposition activity of the synthesized compounds occurs in a concentration-dependent manner. The methoxy substituted derivative (**6c**) has good scavenging activity with IC_{50} value of 15.98 µM when compared to the standard drug (IC_{50}-7.10 µM).

Table 3. Hydrogen peroxide scavenging activity

Compounds	Concentration (µM)			
	10	**25**	**50**	**100**
6a	28.08±0.92	57.04±1.30	73.96±0.77	86.58±0.89
6b	30.65±0.52	60.73±0.50	74.85±0.57	83.07±0.88
6c	33.66±0.52	64.73±0.29	76.41±0.75	88.08±1.01
6d	06.85±0.85	14.14±2.79	42.90±1.50	63.37±0.30
6e	ND	05.89±1.29	24.43±2.06	51.26±1.56
6f	02.59±0.58	08.37±1.49	22.41±0.52	45.33±0.76
Ascorbic acid	40.77±0.53	68.33±0.57	80.55±0.41	96.92±0.38

Values are mean ± SEM; experiment performed in triplicate; ND = Not Determine.

Nitric oxide scavenging activity

Nitric oxide is associated with inflammation, cancer, and other pathological conditions. Nitric oxide is a potentially toxic agent with a free radical character (Moncada *et al.,* 1991). An aqueous solution of Sodium nitroprusside generates nitric oxide at physiological pH which interacts with oxygen to induce nitrite ions that can be estimated using Griess reagent.

The nitric oxide scavenging activity of synthesized compounds (**6a-f**) were 80.91%, 78.40%, 82.33%, 60.88%, 50.66% and 47.67% at 100 µM concentrations respectively (**Table 4**). Incubation of a sodium nitroprusside solution with synthesized compounds at 25°C for 120 minutes resulted in linear time-dependent nitrite production, which was reduced by synthesized compounds in a concentration-dependent manner.

Table 4. Nitric oxide scavenging activity

Compounds	Concentration (µM)			
	10	**25**	**50**	**100**
6a	25.15±0.65	48.39±0.59	70.91±0.78	80.91±0.55
6b	17.65±1.22	36.73±0.50	64.18±0.28	78.40±0.32
6c	30.99±0.68	66.06±0.88	75.77±0.74	82.33±0.31
6d	24.94±0.89	20.56±0.61	34.44±2.11	60.88±0.97
6e	06.28±1.72	14.40±2.08	24.60±2.06	50.66±1.04
6f	ND	04.25±1.16	14.42±1.29	47.67±3.14
Ascorbic acid	37.73±0.42	65.67±0.89	82.25±0.71	93.69±0.96

Values are mean ± SEM; experiment performed in triplicate; ND = Not determine.

Conclusion

Here, DPPH radical scavenging, hydrogen peroxide and Nitric oxide scavenging activity of the synthesized compounds (**6a-f**) have been performed. The compounds **6a-f** showed the potent antioxidant activity in different *in vitro* models. The *in vitro* α-glucosidase inhibitory studies demonstrated that synthesized molecules showed a concentration-dependent reduction in percentage inhibition. The most active compound **6c** with 4-methoxy substitution showed maximum *in vitro* antioxidant activity at a concentration of 100µl when compared to the standard Ascorbic acid. The compound **6c** also inhibited the α-glucosidase enzyme about 88.57% at 100µM concentration with IC_{50} value of 21.86µM compared with Acarbose (IC_{50}-11.92µM). So, if any compound has both antioxidant and α-glucosidase inhibitory activity, the compound will be potent to treat the type-II diabetes mellitus.

References

Ayoola, GA., Coker, HAB., Adesegun, SA., Adepoju-Bello, AA., Obaweya, K., Ezennia. EC., Atangbayila, TO., 2008. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in southwestern Nigeria, Tropical Journal of Pharmaceutical Research. 7(3), 1019-1024.

Blois, MS., 1958. Antioxidant determinations by the use of a stable free radical. Nature. 181 (4617), 1199–1200.

Domínguez, J.N., León, C., Rodrigues, J., De Domínguez, N.G., Gut, J., Rosenthal, P.J., 2005. Synthesis and evaluation of new antimalarial phenylurenylchalcone derivatives. J. Med. Chem. 48, 3654–3658.

Jayaprakasha, GK., Jaganmohan, Rao L., Sakariah, KK., 2004. Antioxidant activities of flavidin in different *in vitro* model systems. Bioorganic & Medicinal Chemistry. 12, 5141–5146.

Molyneux, P., 2004. The use of stable free radical diphenyl picryl hydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J. Sci. Technol. 26(2), 211-219.

Moncada, S., Palmer, R. M. J., Higgs, E. A., 1991. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacological Reviews, 43(2), 109–142.

Mohana, KN., Pradeep Kumar, Chikkur B., 2013. Synthesis and antioxidant activity of 2-Amino-5-methylthiazol derivatives containing 1,3,4-Oxadiazole-2-thiol Moiety. ISRN Organic Chemistry. Article ID 620718, 8 pages.

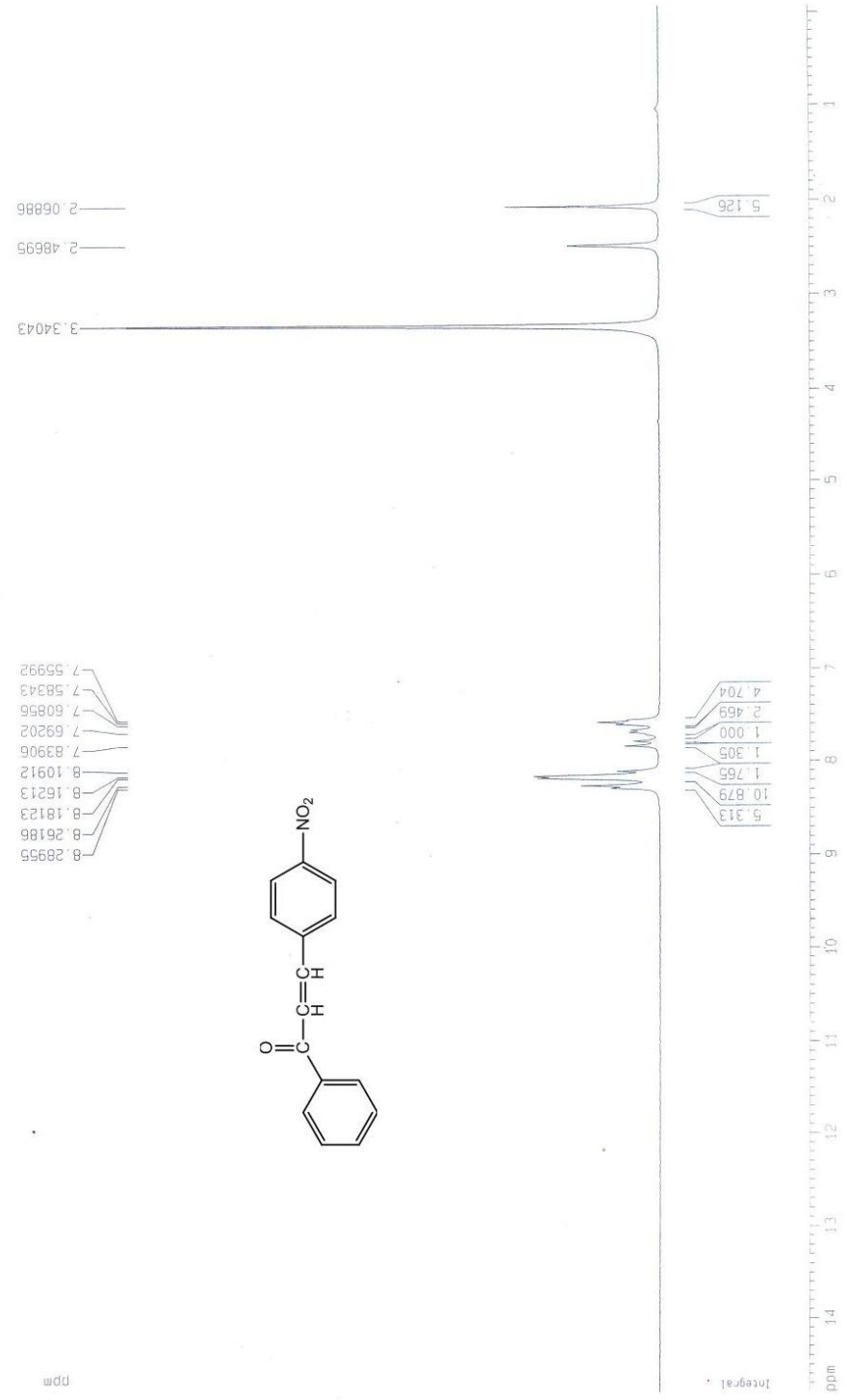
Patil, V.S., Nandre, K.P., Ghosh, S., Rao, V.J., Chopade, B.A., Sridhar, B., Bhosale, S. V., Bhosale, S. V., 2013. Synthesis, crystal structure and antidiabetic activity of substituted (E)-3-(Benzo [d]thiazol-2-ylamino) phenylprop-2-en-1-one. Eur. J. Med. Chem. 59, 304–309.

Rahul, R., Rakesh, KJ., J, Saravanan., 2016. Synthesis and *in vitro* antioxidant activity of novel 1, 3, 4- oxadiazole-2- thione. Journal of Innovations in Pharmaceuticals and Biological Sciences. 3(3), 114-122.

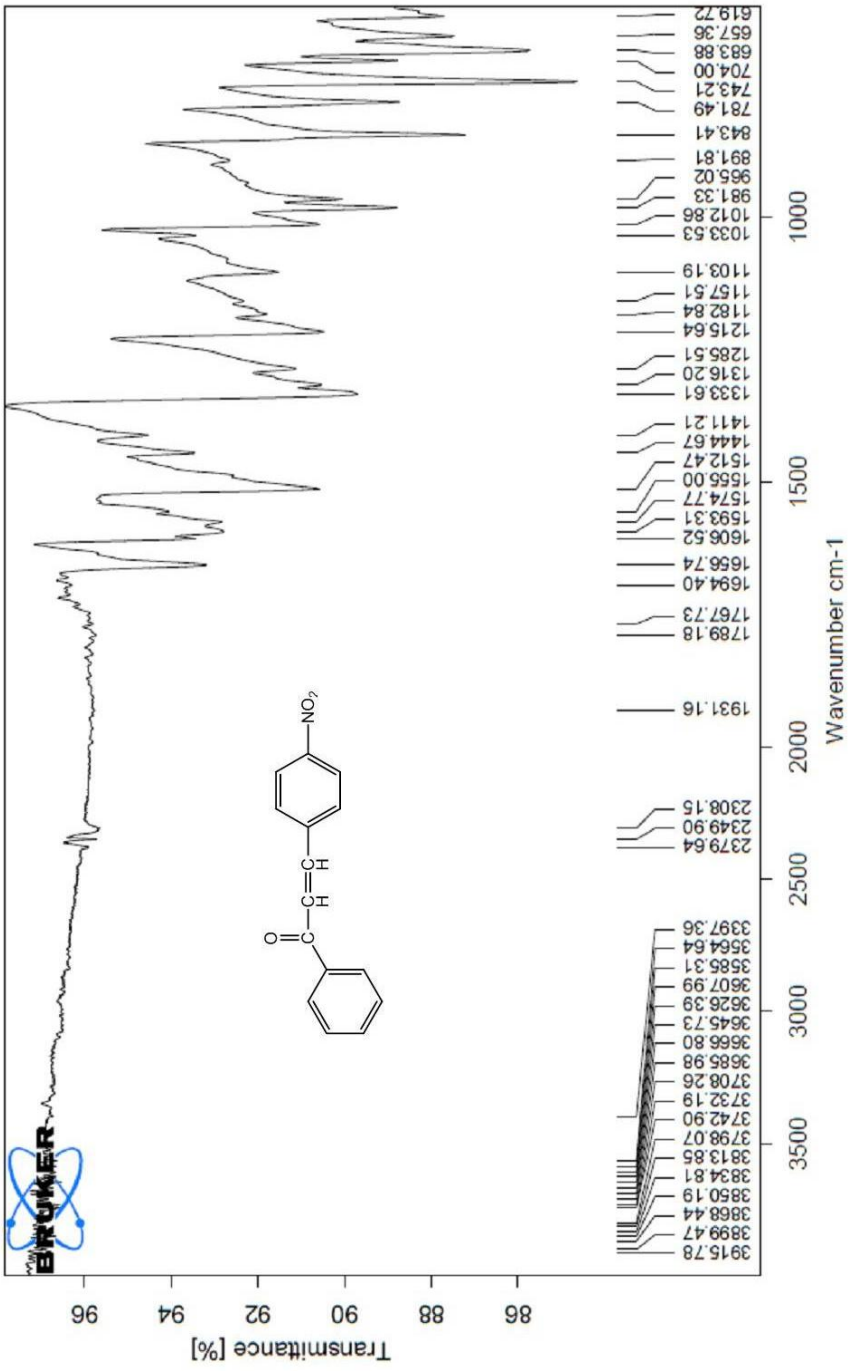
Sahu, RK., Kar, M., and Routray, R., 2013. DPPH free radical scavenging activity of some leafy vegetables used by tribals of Odisha, India. Journal of Medicinal Plants Studies. 1(4), 21-27.

Sreejayan., MN. Rao, 1997. Nitric oxide scavenging by curcuminoids. J Pharm Pharmacol. 49(1), 105-107.

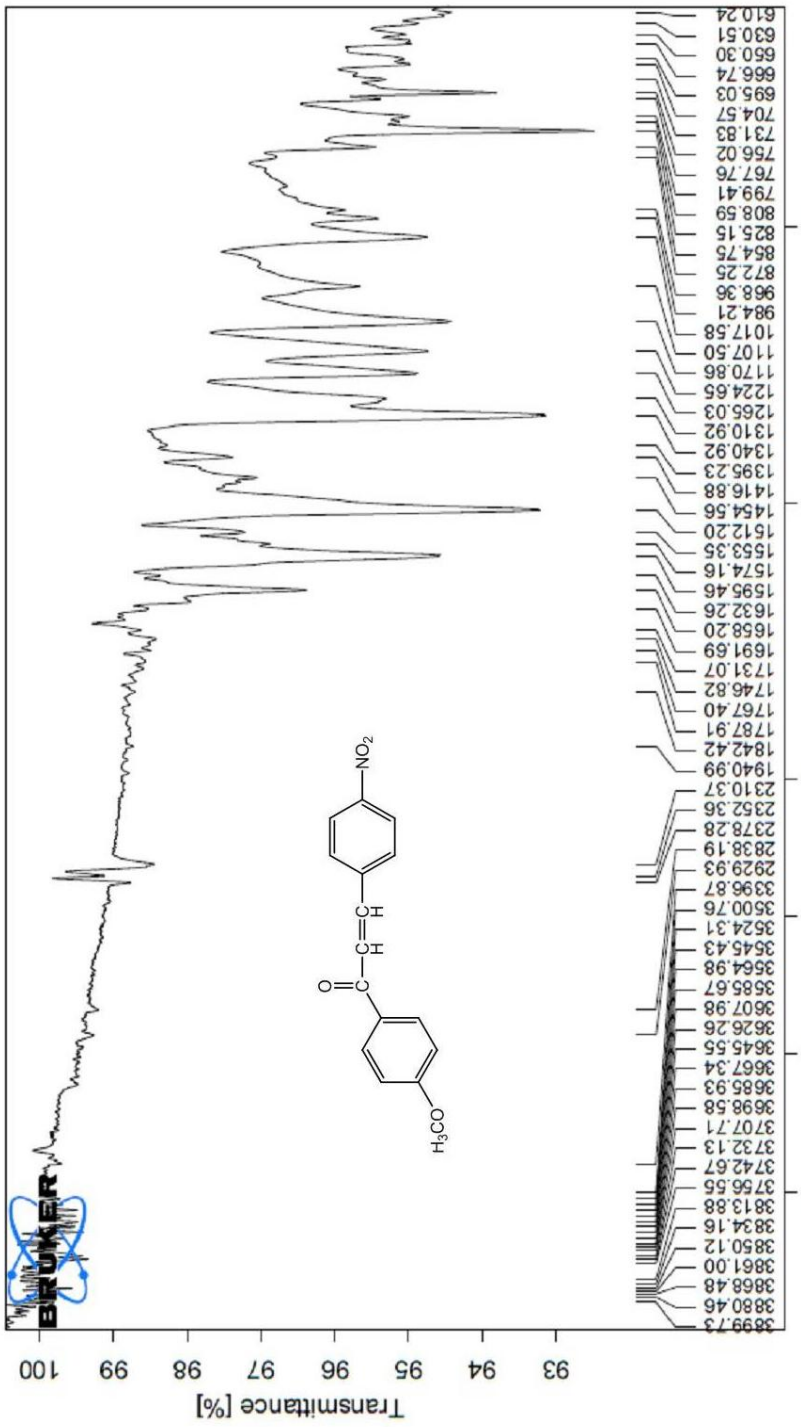
Sonmez, F., Sevmezler, S., Atahan, A., Ceylan, M., Demir, D., Gencer, N., Arslan, O., Kucukislamoglu, M., 2011. Evaluation of new chalcone derivatives as polyphenoloxidase inhibitors. Bioorg. Med. Chem. Lett. 21, 7479–7482.



^{1}H NMR of 3-(4-nitrophenyl)-1-phenylprop-2-en-1-one (3a)



FT-IR of 3-(4-nitrophenyl)-1-phenylprop-2-en-1-one (3a)



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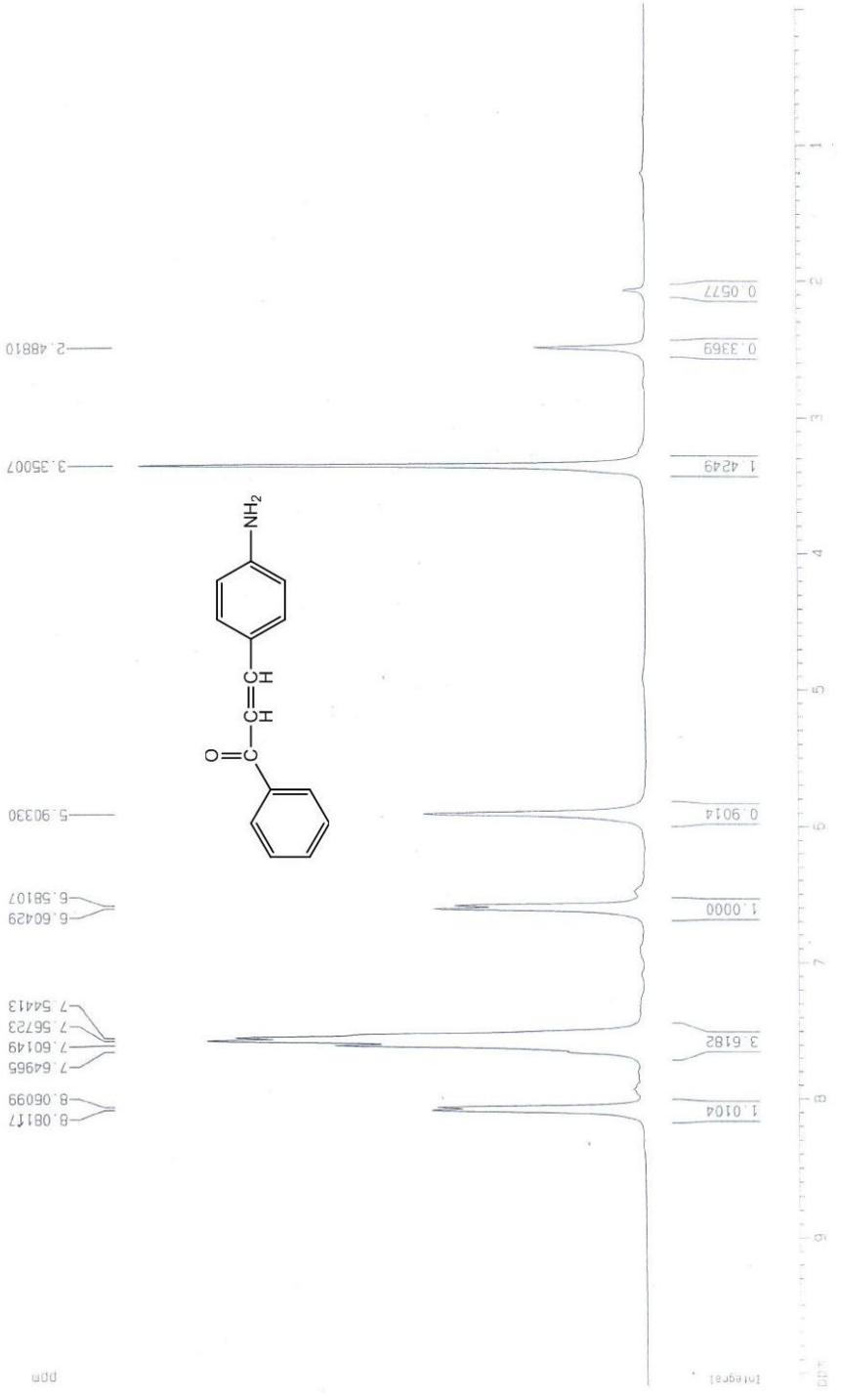

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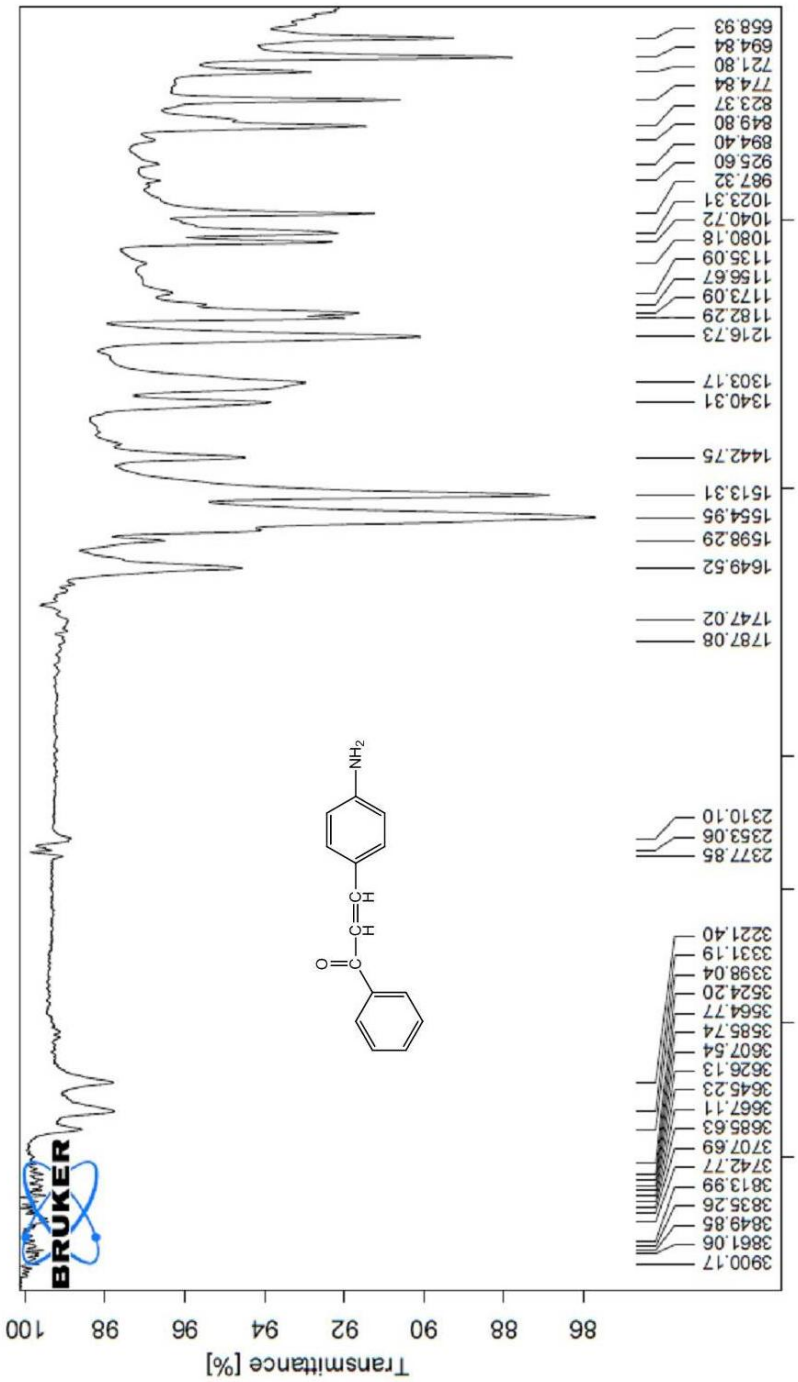

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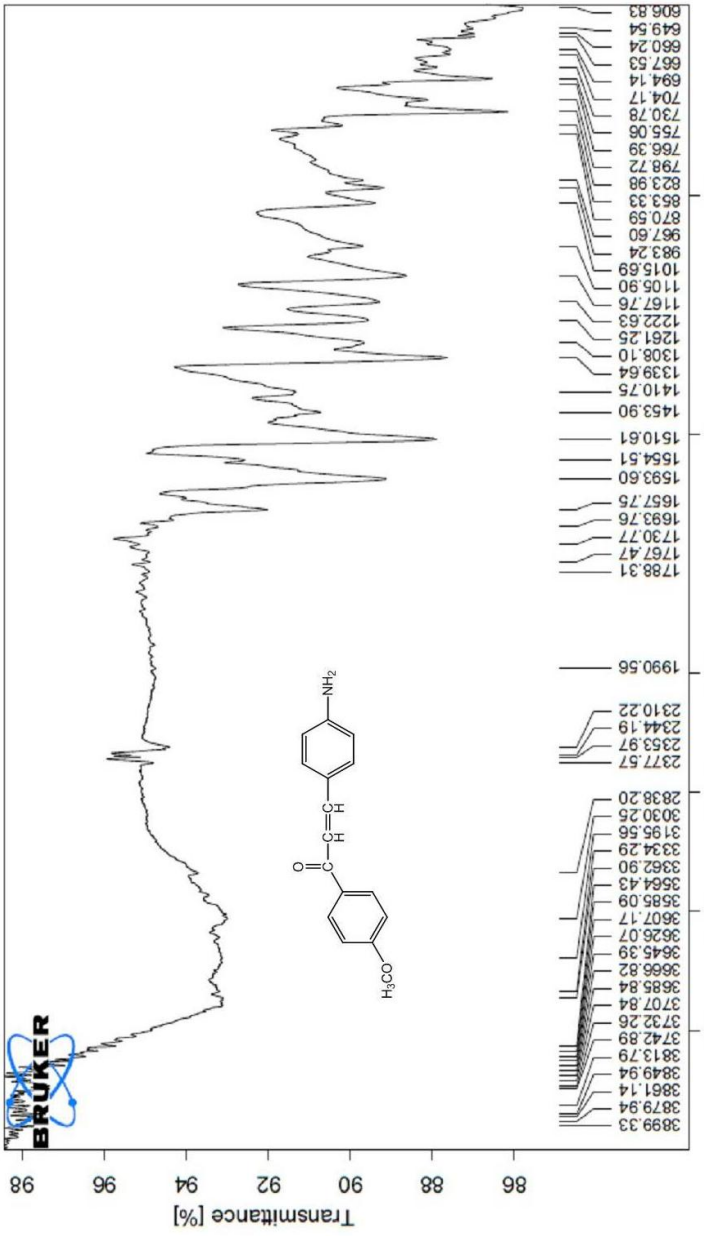

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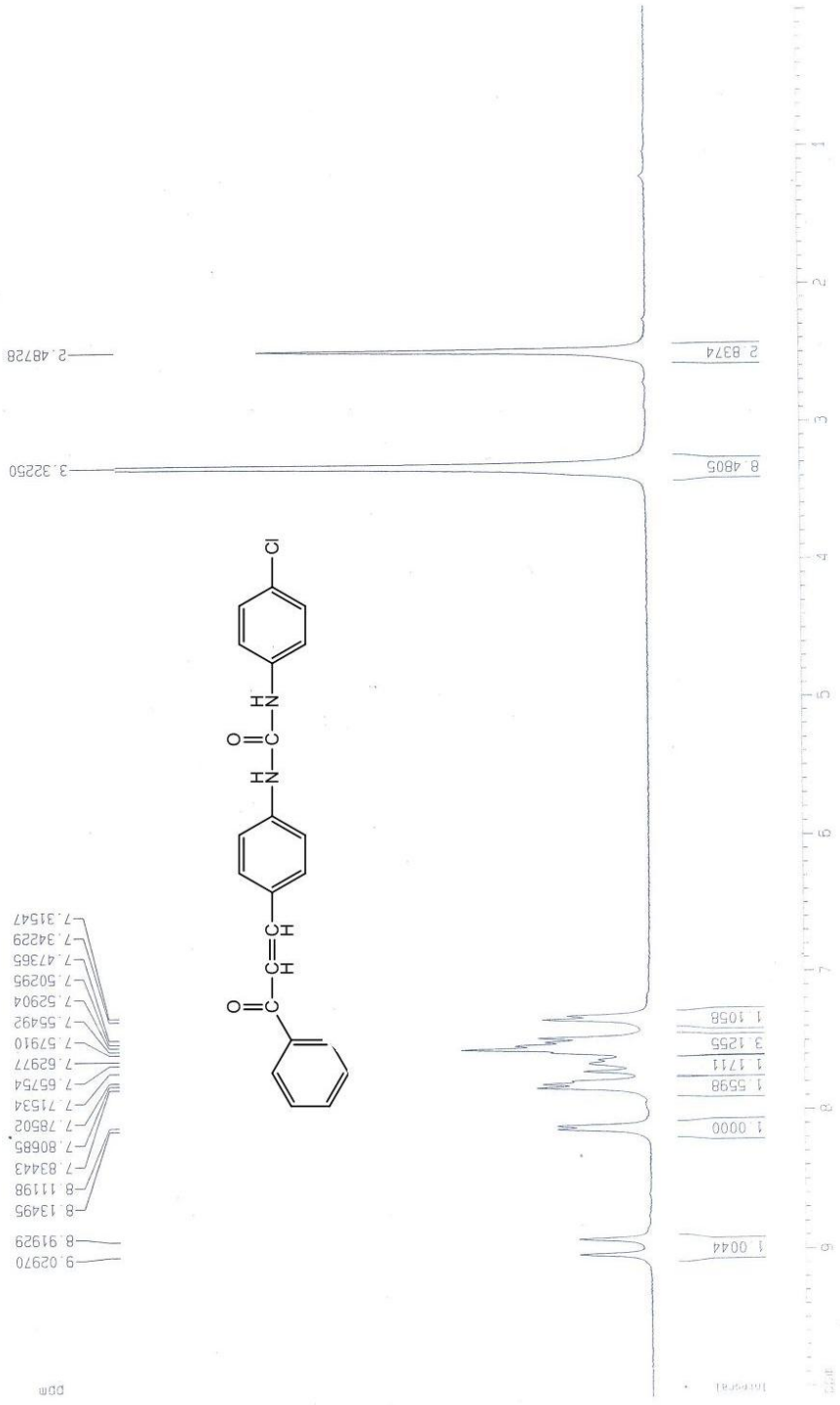
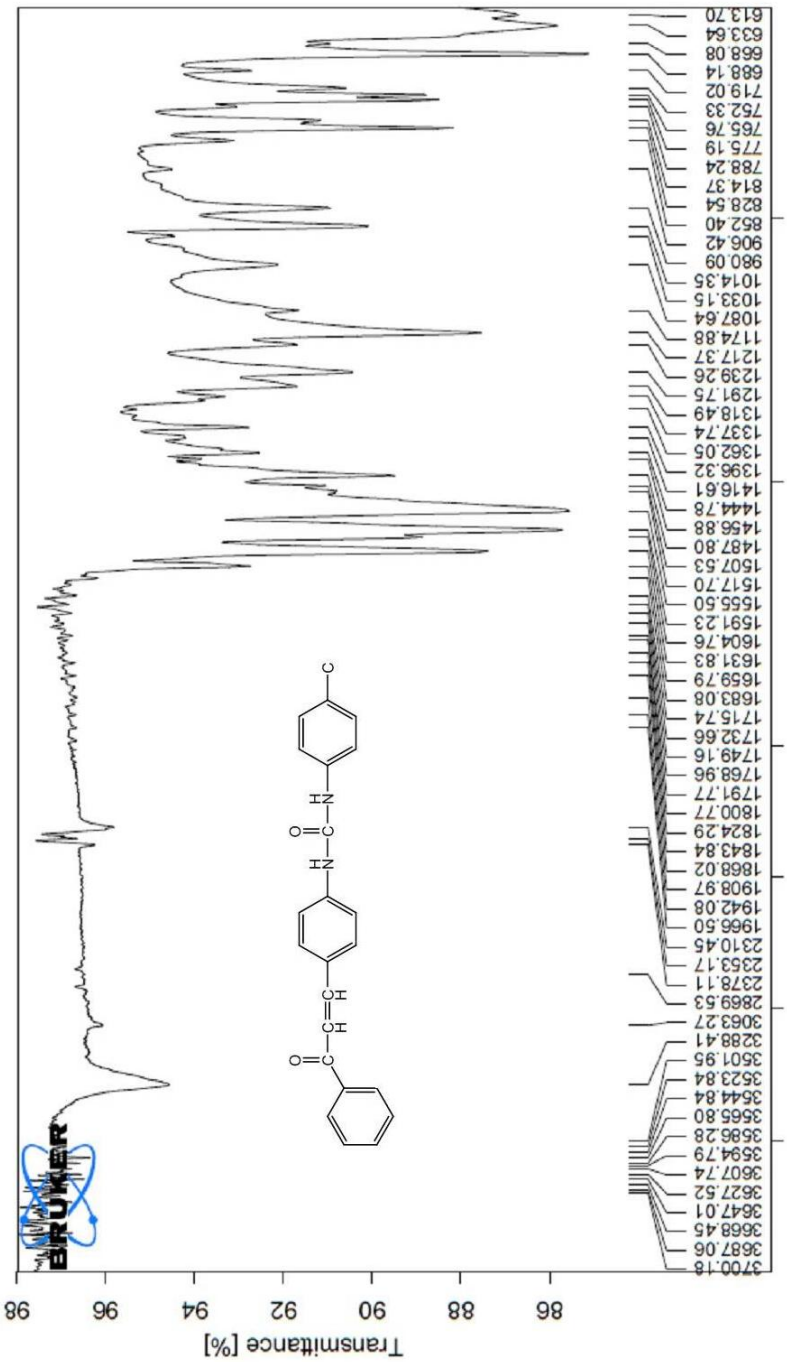


^{1}H NMR of 1-(4-chlorophenyl)-3-(4-(3-oxo-3-phenylprop-1-en-1-yl)phenyl)urea (6a)



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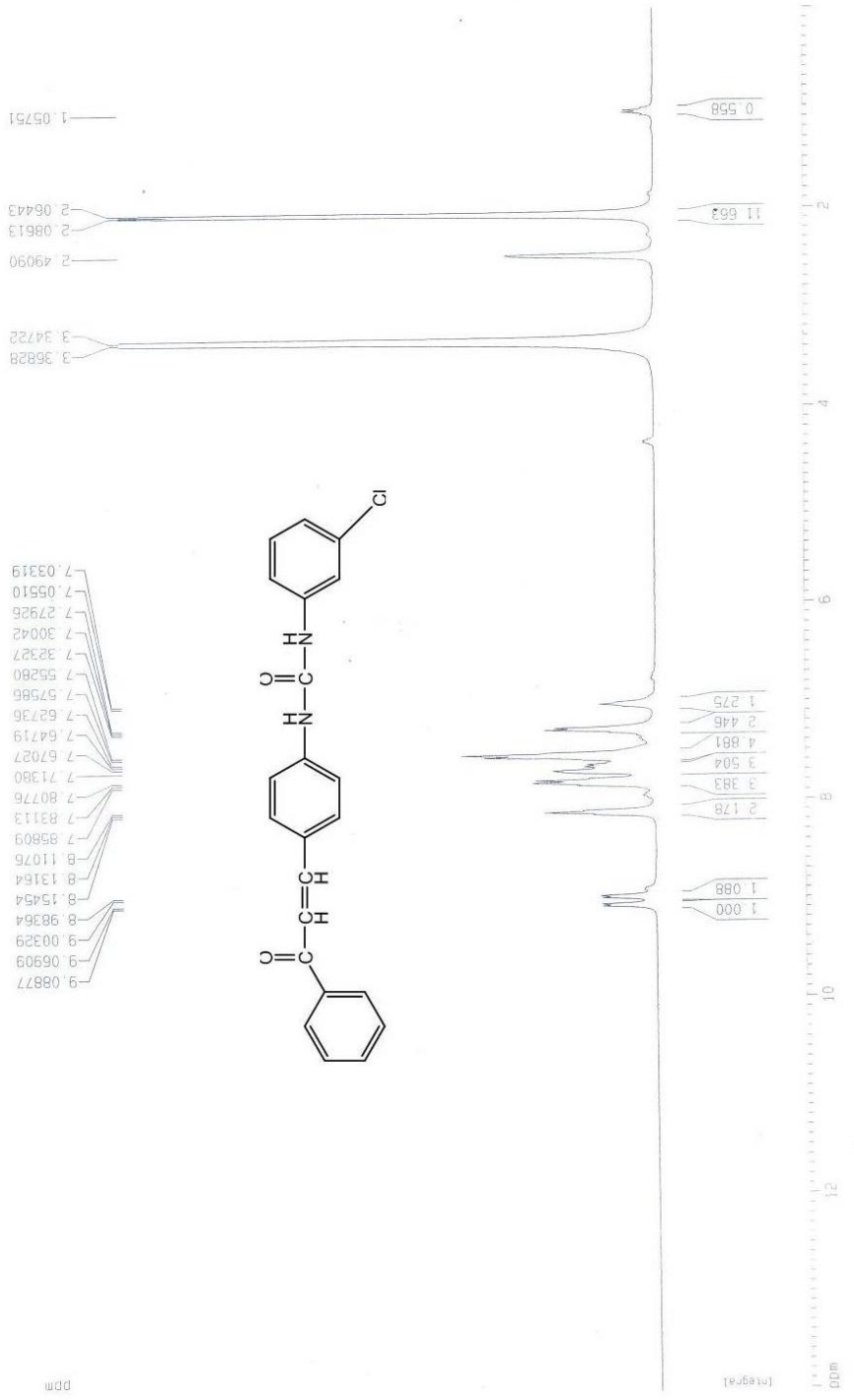

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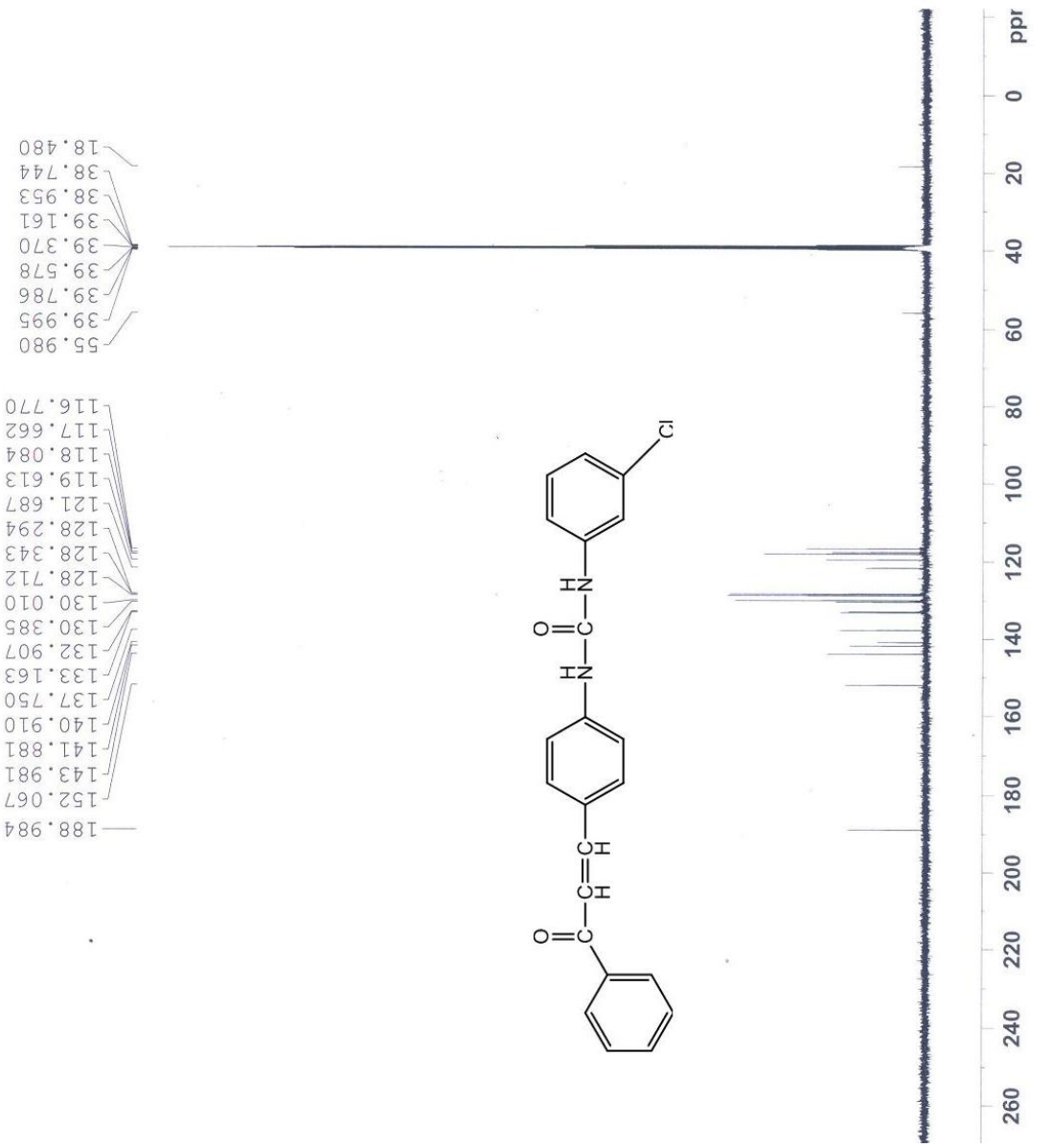
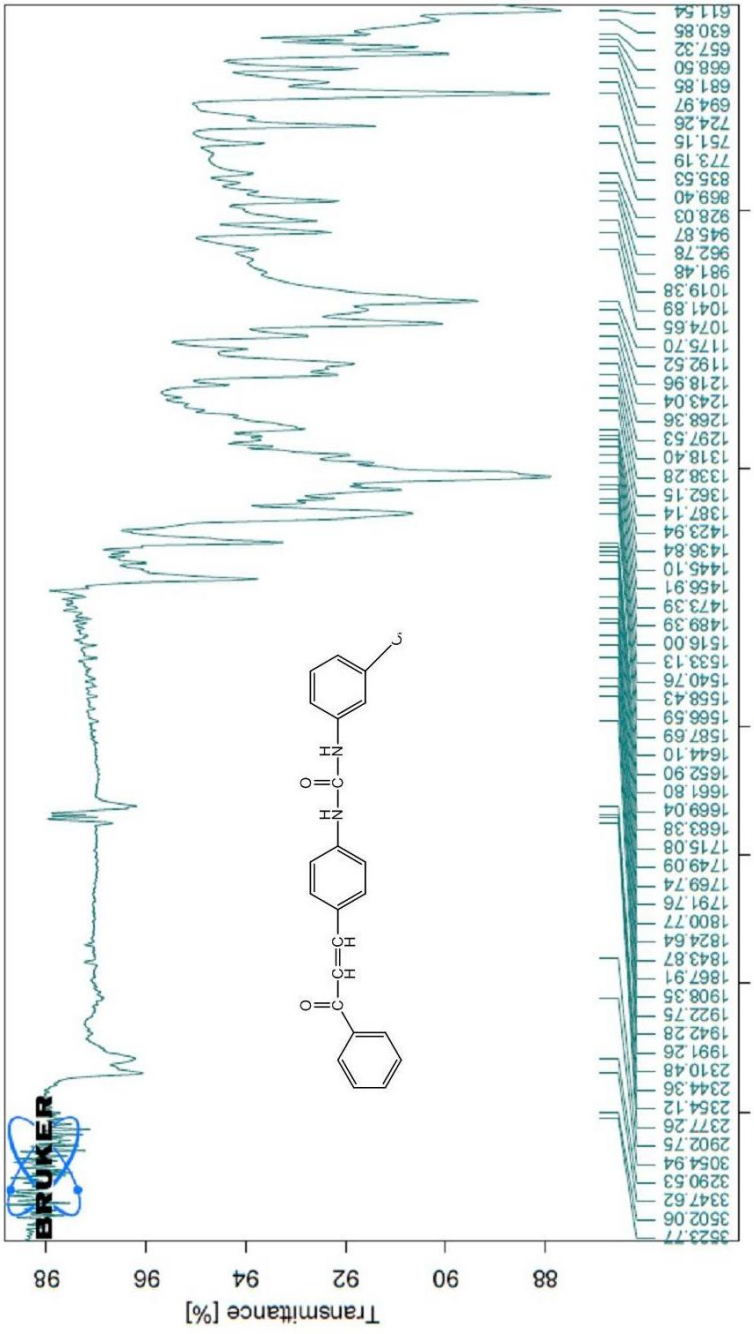
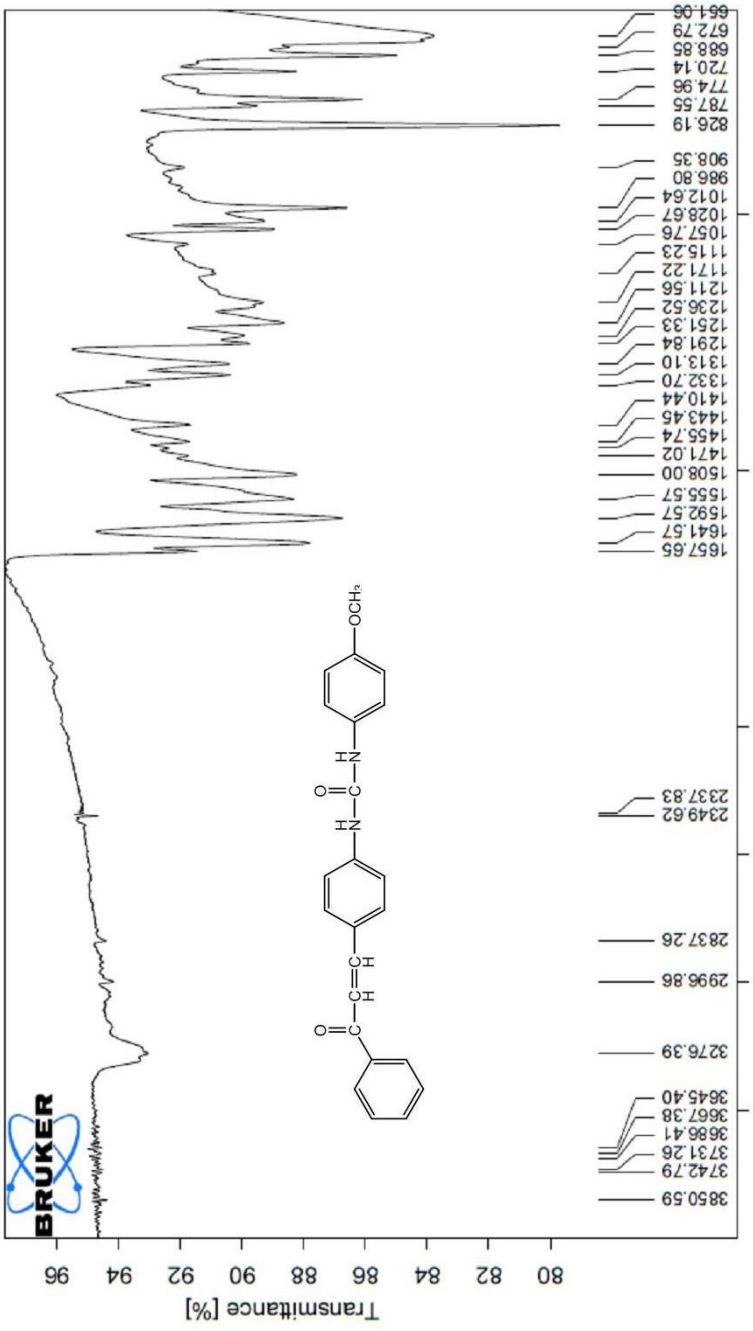


^{13}C NMR of 1-(3-chlorophenyl)-3-(4-(3-oxo-3-phenylprop-1-en-1-yl)phenyl)urea (6b)

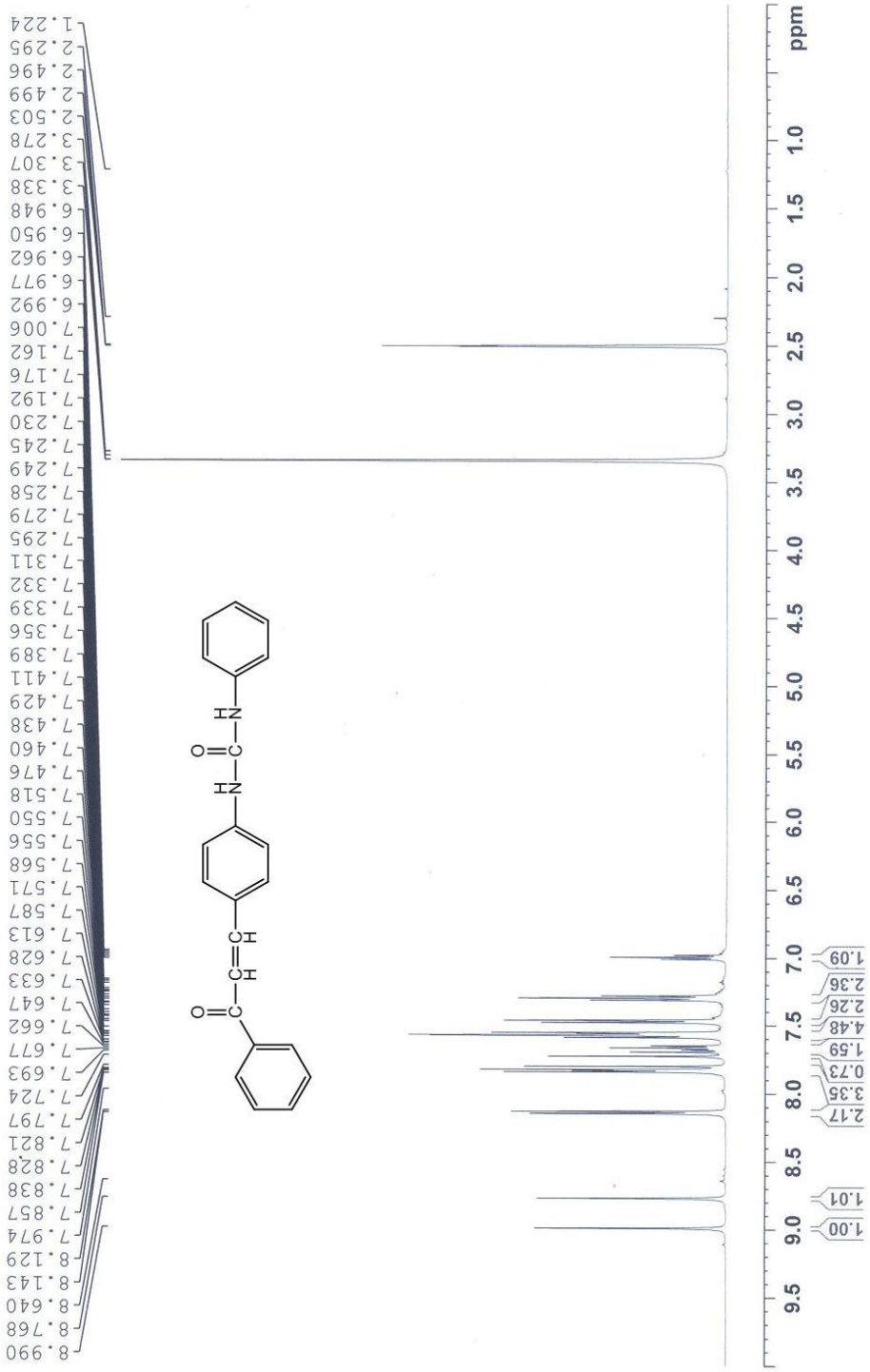


FT-IR of 1-(3-chlorophenyl)-3-(4-(3-oxo-3-phenylprop-1-en-1-yl)phenyl)urea (6b)

^{1}H NMR of 1-(4-methoxyphenyl)-3-(4-(3-oxo-3-phenylprop-1-en-1-yl)phenyl)urea (6c)



FT-IR of 1-(4-methoxyphenyl)-3-(4-(3-oxo-3-phenylprop-1-en-1-yl)phenyl)urea (6c)



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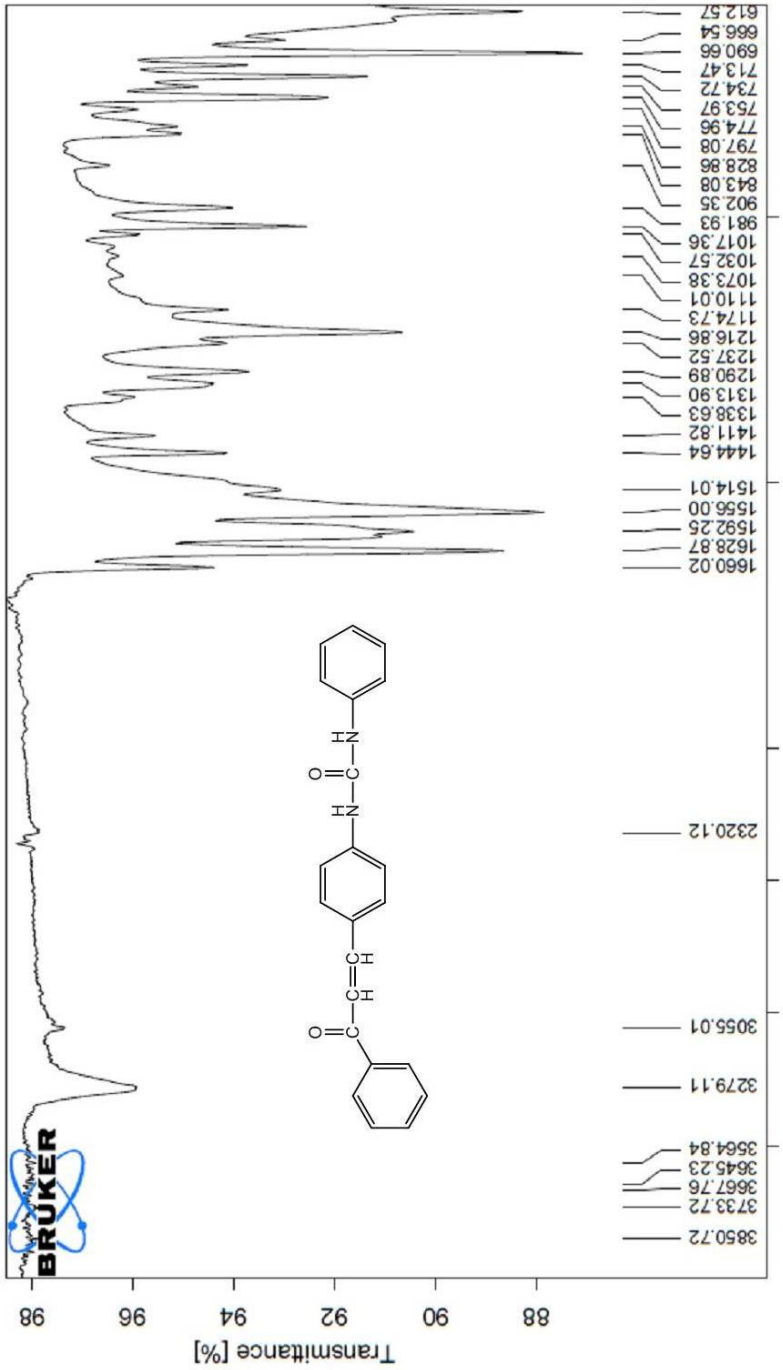
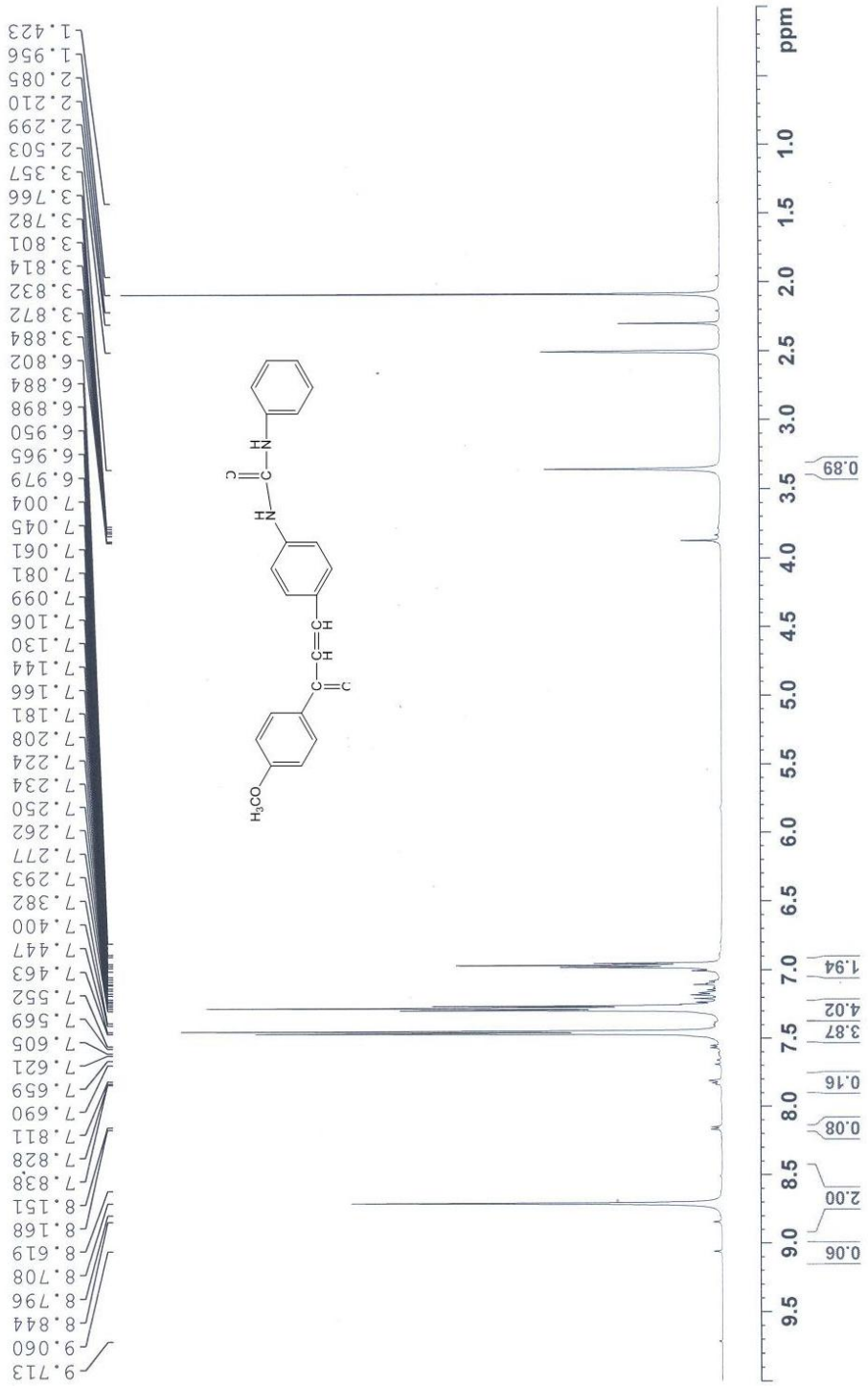
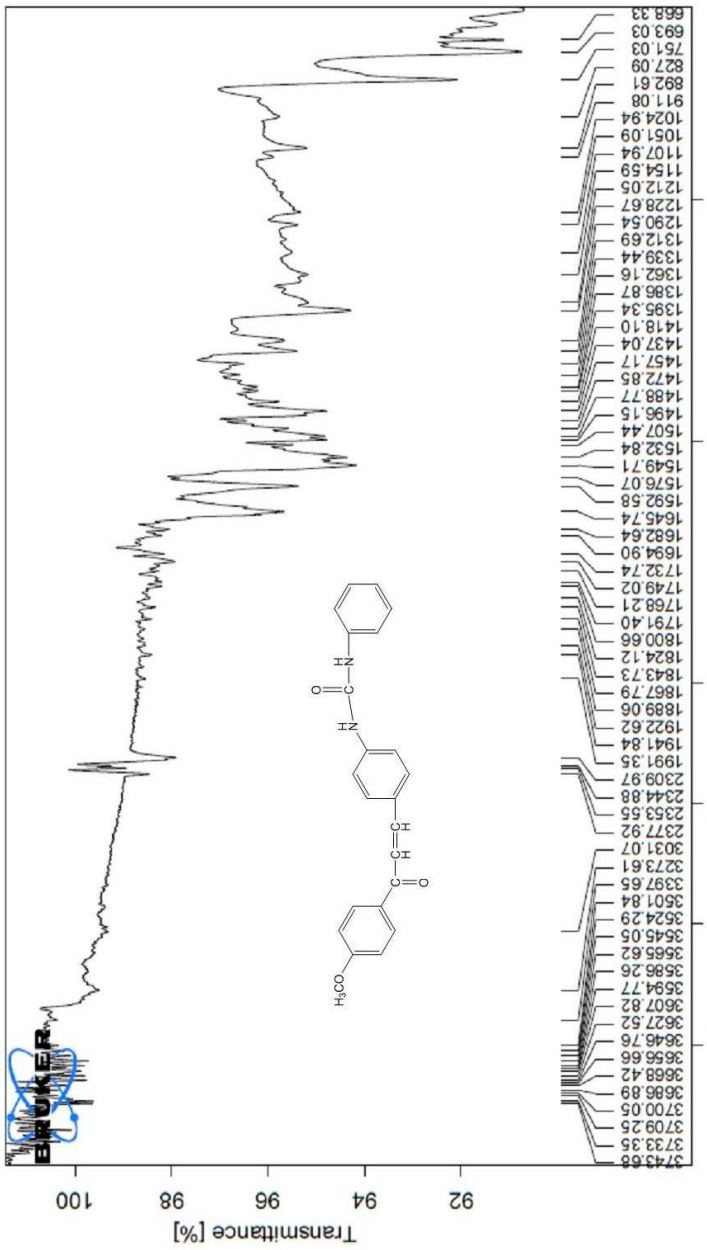


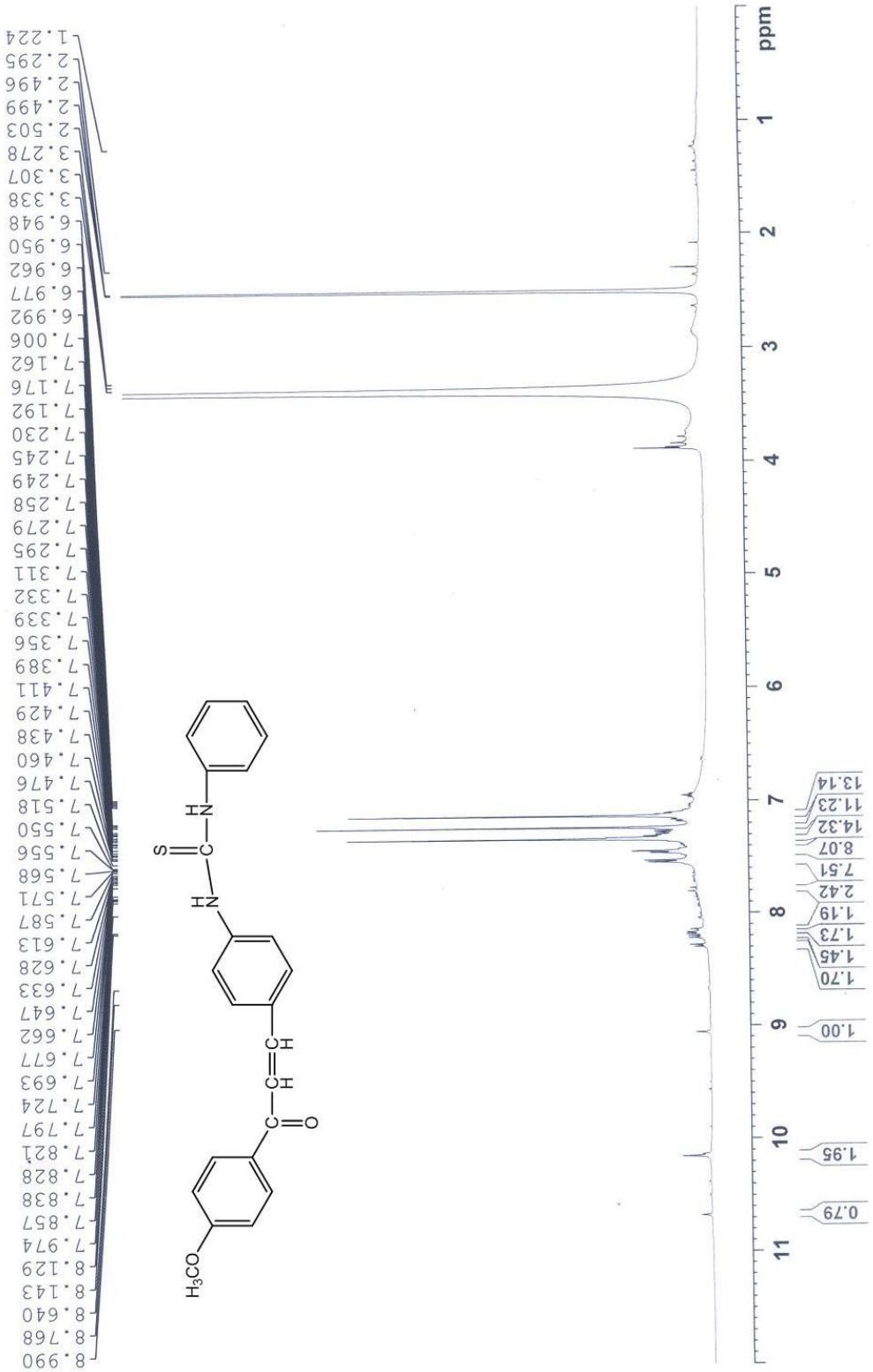
FT-IR of 1-(4-(3-oxo-3-phenylprop-1-en-1-yl)phenyl)-3-phenylurea (6d)



^{1}H NMR of 1-(4-(3-(4-methoxyphenyl)-3-oxoprop-1-en-1-yl)phenyl)-3-phenylurea (6e)



FT-IR of 1-(4-(3-(4-methoxyphenyl)-3-oxoprop-1-en-1-yl)phenyl)-3-phenylurea (6e)



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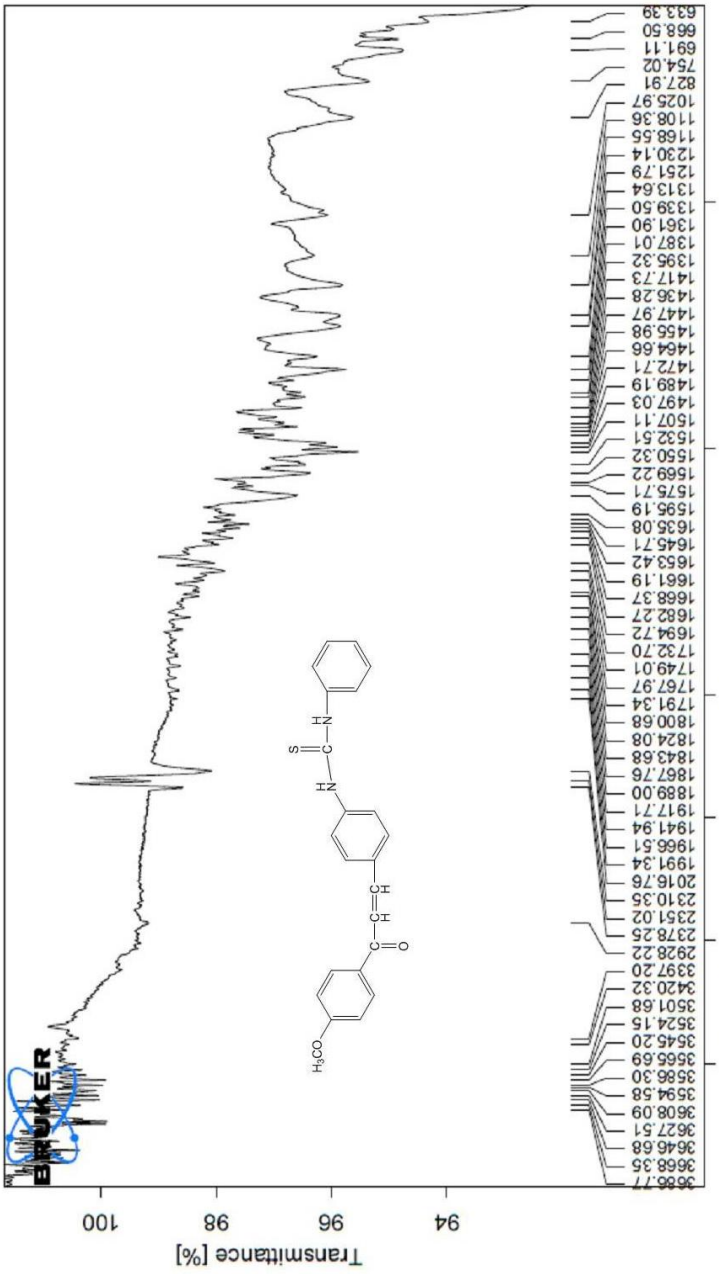

FT-IR of 1-(4-(3-(4-methoxyphenyl)-3-oxoprop-1-en-1-yl)phenyl)-3-phenylthiourea (6f)

Chapter 7

Future Scope

The purpose of the present research work is the synthesis of pharmacologically useful potent bio-active compounds. The synthetic approaches of hybrid urea and thiourea derivatives have been studied extensively. Based on the study of literature chalconyl urea and thiourea derivatives have been prepared from substituted phenyl isocyanates/thiocyanates and substituted acetophenones along with substituted benzaldehydes. All the synthesized derivatives have been screened for pharmacological activities in which some of the compounds act as a good antidiabetic and antioxidant agents. These synthesized compounds are interesting lead entities for further antidiabetic and antioxidant pharmacological evaluation. These classes of synthesized molecules possess the vital role to discover newer antidiabetic agents based on chalcones in future prospects. The further structural modification will be of interest and may result in compounds having a better therapeutic and pharmacological activity. In nearest future chalcone based urea/thiourea derivatives will exhibit a wide range of antimalarial, antioxidant, antituberculosis, antihypertensive and anticancer activity.

Annexure

Reprint of published paper and presentation of conferences

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Research Article

SYNTHESIS, PHARMACOLOGICAL EVALUATION AND LIGAND-PROTEIN INTERACTION STUDY OF HYBRID UREA AND THIOUREA DERIVATIVES AS ANTIHYPERGLYCEMIC AGENTS

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ABSTRACT

A series of hybrid urea/thiourea derivatives (5a-5f) with chalcone moiety were synthesized and pharmacological activity was evaluated using *invitro* α-glucosidase inhibition assay and *invivo* antidiabetic activity in streptozotocin (STZ) induced diabetic rat model. Among the synthesized molecules, compound 5d (1-[4′-N-(N′-p-chlorophenylurenyl) phenyl]-3-(4-methoxyphenyl)-2-propen-1-one) is more potent with IC_{50} value 12.88 µM when compared to the standard drug Acarbose (IC_{50} 16.54 µM) in *invitro* study. *Invivo* study demonstrated that compound 5d was more effective than other synthesized molecules by estimation of different biochemical parameters. For the understanding of ligand-protein interaction, molecular docking studies of the synthesized compounds were also performed. Overall, synthesized hybrid urea/thiourea derivatives might be potential a new class of compound for the treatment of type-II diabetes.

Keywords: Urea/thiourea derivatives; α-glucosidase inhibitor; antidiabetic; Molecular docking

INTRODUCTION

The prevalence of diabetes is rapidly rising all over the globe at an alarming rate in both developed and developing countries[1]. Uncontrolled hyperglycemia can lead to serious damage of vital organs including kidney damage, nerve damage and heart disease[2,3]. Type II diabetes is the majorly affective diabetes which is associated with 'diabesity' and 'metabolic syndrome'. In context of genetic susceptibility in certain ethnic groups, type II diabetes is influenced by environmental and other factors such as a sedentary lifestyle, overly rich nutrition and obesity[4]. However, treatment of diabetes mellitus principally requires the reduction of blood glucose levels and controlling cell signalling cascade. Strikingly, α-glucosidase is a membrane-bound enzyme at the epithelium of the small intestine and play an important role in carbohydrate metabolism[5]. Growing evidences suggested that inhibition of α-glucosidase might significantly decrease the postprandial hyperglycemia[6]. Thus, α-glucosidase inhibition might be a potential therapeutic target for the treatment of type-II diabetes mellitus[7]. Amongst the *α*-glucosidase inhibitors, acarbose, miglitol, and voglibose are being clinically used for the cure of type II diabetes mellitus[8-10]. However, their adverse effects are also linked to them such as abdominal discomfort, diarrhoea, and flatulence[11,12] in some cases and newly discovered additive effects (like stabling carotid plaques, and reducing inflammation)[13] encouraging the development of new therapeutic agents is an utmost interest in medicinal chemistry research.

In this context, urea and thiourea derivatives are important functional groups in numerous natural products and drug intermediates[14] and building blocks for various heterocycles. Urea and thiourea derivatives possess many promising biological activities, such as herbicidal[15], antimicrobial[16, 17], antioxidant[18], antiviral[19], anti-HIV[20], antitumor[21, 22], antimalarial[23] and antidiabetic activity[24-26].

This present study involves the synthesis of a series of urea/thiourea derivatives using previously reported methods[23, 27, 28]. Furthermore, hypoglycemic activity of urea/thiourea derivatives were investigated using well defined *in vitro* α-glucosidase assay and STZ induced diabetic rat model.

MATERIALS AND METHODS

Chemistry

^{1}H NMR (300 MHz) and ^{13}C NMR (400MHz) spectra were recorded on Bruker spectrometer in DMSO- d_6 using TMS as internal standard. IR spectra were recorded on a Bruker Alpha FTIR spectrophotometer. Mass spectra were obtained on ESI mass instrument. Melting points were determined in Veego melting point apparatus. All reactions were monitored by thin layer chromatography (TLC) on pre-coated Silica Gel 60 F_{254}; spots were visualized under UV light. All the reactions were carried out using reagent-grade solvents, and the reagents were purchased from Sigma–Aldrich, Loba, Alpha aesar and Spectrochem.

General procedures for the synthesis of 4-(phenylurenyl/thiourenyl) acetophenone

A mixture of the p-aminoacetophenone **(1)** and phenylisocyanate/thiocyanate **(2a-b)** derivatives were dissolved in toluene. The mixture was refluxed; yellow solid was filtered out and dried. Recrystallization afforded the desired 4-(phenylurenyl/thiourenyl) acetophenone **(3a-b)** derivatives in pure form[28].

National Workshop on

"BOTANICAL IDENTIFICATION
&
EVALUATION OF INDIAN MEDICINAL PLANTS"

November 20-26, 2013

Organized by

School of Natural Product Studies
Jadavpur University
Kolkata 700032, India

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2nd Pharm. Tech. IAPST International Conference on

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Theme: Interface of Chemical Biology in Drug Research

SRM University, Kattankulathur, Tamil Nadu, India; February 8-10, 2017

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This is to certify that Dr. / Mr. / Ms. **TANMOY GURIA** has presented a poster entitled **Synthesis, characterization and in-vitro antioxidant study of hybrid urea/thiourea derivatives**

in 23rd ISCB International Conference (ISCBC-2017) Theme: "Interface of Chemical Biology in Drug Research" organized by SRM College of Pharmacy in association with Indian Society of Chemists and Biologists (ISCB) held from February 8 - 10, 2017.

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P-59

Synthesis, characterization and *in-vitro* antioxidant study of hybrid urea/thiourea derivatives

Tanmoy Guria, Puspita Roy, Tapan Kumar Maity*

Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700032
Email: tguria.ju@gmail.com

Abstract: A series of urea/thiourea derivatives were synthesized and their structures were confirmed by different spectroscopic method (^{1}HNMR, Mass, FT-IR) [1]. The *in-vitro* antioxidant potential of newly synthesized hybrid urea/thiourea derivatives were carried out by different antioxidant assays such as DPPH free radical scavenging, super oxide anion radical scavenging, nitric oxide scavenging and lipid peroxidation [2]. The compounds exhibited potent DPPH free radical scavenging activity which increased with increasing the concentration of compounds when compared with standard drug ascorbic acid. In addition, all the compounds had effective lipid peroxidation, super oxide anion scavenging and nitric oxide scavenging depending on concentrations.

References:

1. L dos Santos, L A Lima, V Cechinel-Filho, R Correa, F C Buzzi, R J Nunes, Bioorganic & Medicinal Chemistry,16, 2008, 8526.
2. M Biswas, P K Haldar, A K Ghosh, Journal of Natural Science, Biology and Medicine, 1, 2010, 29.

Certificate of Poster Presentation

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Seoul, Republic of Korea
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The **International Pharmaceutical Federation (FIP)** hereby declares that

Tanmoy Guria

Puspita Roy, Tapan Kumar Maity

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Design, synthesis and antidiabetic potential of hybrid urea/thiourea derivatives

as a poster during the 77th International Congress of FIP

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Title: Design, synthesis and antidiabetic potential of hybrid urea/thiourea derivatives

In: Poster presentation on Monday, 11 September 2017, 12:00-14:30
Type: Poster
By: GURIA, Tanmoy (JADAVPUR UNIVERSITY, DEPARTMENT OF PHARMACEUTICAL TECHNOLOGY, KOLKATA, India)
Co-author(s): Tanmoy Guria: PHARMACEUTICAL TECHNOLOGY, JADAVPUR UNIVERSITY, KOLKATA, India
Puspita Roy: PHARMACEUTICAL TECHNOLOGY, JADAVPUR UNIVERSITY, KOLKATA, India
Tapan Kumar Maity: PHARMACEUTICAL TECHNOLOGY, JADAVPUR UNIVERSITY, KOLKATA, India, ()

Abstract:
BackgroundDiabetes mellitus (DM) is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period. Type 2 diabetes mellitus (T2DM) presents a major challenge to healthcare system around the world. The prevalence of diabetes is rising all over the world due to population growth, aging and urbanisation. Urea and thiourea derivatives possess many promising biological activities such as antibacterial, Antitumor, antimicrobial, anti-inflammatory etc. PurposeUrea/thiourea derivatives have been synthesized and screened for their antidiabetic activity. MethodsThis study involves the synthesis of a series of hybrid urea/thiourea derivatives (1-8) containing chalcone moiety. The synthesized compounds were characterized by FT-IR, NMR, Mass spectroscopy and evaluated for their both in vitro and in vivo antidiabetic activity. The in vitro antidiabetic activity was done by α-glucosidase inhibitory activity of synthesized compounds. The in vivo antidiabetic activity was performed on streptozotocin induced diabetic Swiss albino rats. The Blood glucose level, different enzymatic studies (SGPT, SGOT, ALT) and lipid profile (HDL, LDL, Cholesterol) of the studied animal were estimated. ResultsThe results indicated that the hybrid urea/thiourea derivatives displayed promising antidiabetic activity. Among the series, compound 3 showed potent α-glucosidase inhibitory activity when compared to the standard drug Acarbose. In in vivo study the compound 3 was found more effective when compared to the standard drug Metformin.ConclusionIt may be concluded that hybrid urea/thiourea derivatives will be a new class of antidiabetic compound in future.

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DESIGN, SYNTHESIS AND CHARACTERIZATION OF HYBRID UREA/THIOUREA DERIVATIVES AS A POTENTIANL ANTIDIABETIC

TANMOY GURIA, PUSPITA ROY, TAPAN KUMAR MAITY

DEPARTMENT OF PHARMACEUTICAL TECHNOLOGY, JADAVPUR UNIVERSITY, KOLKATA 700032

Email: tguria.ju@gmail.com

ABSTRACT

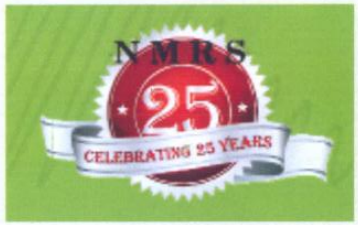
Type 2 diabetes mellitus (T2DM) presents a major challenge to healthcare system around the world. The prevalence of diabetes is rising all over the world due to population growth, aging and urbanisation. Urea and thiourea derivatives possess many promising biological activities such as antifungal, anticancer, antimicrobial, anticonvulsant etc.

Urea/thiourea derivatives have been synthesized and screened for the antidiabetic activity. The synthesized compounds (5a-5f) were characterized by FT-IR, NMR, Mass spectroscopy and evaluated for their both *in vitro* and *in vivo* antidiabetic activity. The *in vitro* antidiabetic activity was done by α-glucosidase inhibitory activity of synthesized compounds. The *in vivo* antidiabetic activity was performed on streptozotocin induced diabetic Swiss albino rats. The Blood glucose level, different enzymatic studies and lipid profile of the studied animal were estimated.

The results indicated that among the series, compound 5d showed potent α-glucosidase inhibitory activity which is supported by *in vivo* antidiabetic study.

It may be concluded that hybrid urea/thiourea derivatives will be a new class of antidiabetic compound in future.

Keywords: Diabetes mellitus, Urea/thiourea, α-glucosidase inhibitor, Antidiabetic activity



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