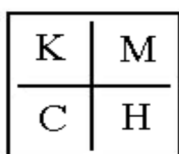**Design and Synthesis of some Novel Michael Addition derivatives of 2,4-disubstituted Oxazol-5(4H)-one as possible Anti-diabetic, Anti-oxidant and Anti-fungal agents**

Dissertation Submitted to

The Tamil Nadu Dr. M.G.R Medical University, Chennai

In partial fulfillment for the requirement of the Degree of

MASTER OF PHARMACY

(Pharmaceutical Chemistry)

April - 2012

Department of Pharmaceutical chemistry

KMCH COLLEGE OF PHARMACY

KOVAI ESTATE, KALAPATTI ROAD,

COIMBATORE 641-048.

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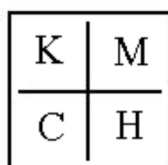**(Pharmaceutical Chemistry)**

Submitted by

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Under the guidance of

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CERTIFICATE

       This is to certify that the dissertation work entitled *"Design and Synthesis of some Novel Michael Addition derivatives of 2,4-disubstituted Oxazol-5(4H)-one as possible Anti-diabetic, Anti-oxidant and Anti-fungal agents"* submitted by **Mr. S M GUPTHA JULURI** is a bonafide work carried out by the candidate under the guidance of **Mrs. S.HURMATH UNNISSA**, **M.Pharm.** Asst Professor, to The Tamilnadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmaceutical Chemistry** at the Department of Pharmaceutical Chemistry, KMCH College of Pharmacy, Coimbatore, during the academic year 2011-2012.

                                        Dr. A. Rajasekaran, M.Pharm., Ph.D.

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</div>

DECLARATION

I do hereby declare that the dissertation work entitled *“Design and Synthesis of some Novel Michael Addition derivatives of 2,4-disubstituted Oxazol-5(4H)-one as possible Anti-diabetic, Anti-oxidant and Anti-fungal agents”* submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmaceutical Chemistry** at the Department of Pharmaceutical Chemistry was done by me under the guidance of Mrs . S. HURMATH UNNISSA, M.Pharm, Asst.Professor at the Department of Pharmaceutical Chemistry, KMCH College of Pharmacy, Coimbatore, during the academic year 2011-2012.

S. M. GUPTH JULURI

EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled *"Design and Synthesis of some Novel Michael Addition derivatives of 2,4-disubstituted Oxazol-5(4H)-one as possible Anti-diabetic, Anti-oxidant and Anti-fungal agents"* submitted by **Mr. S M GUPTHA JULURI, (Reg.No. 26107133)** to The Tamilnadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmaceutical Chemistry** is a bonafide work carried out by the candidate at the Department of Pharmaceutical Chemistry, KMCH College of Pharmacy, Coimbatore and was evaluated by us during the academic year 2011-2012.

Internal Examiner   **External Examiner**

Convener of Examinations

Examination Center : KMCH College of Pharmacy,

                                             Coimbatore.

Date   :

ACKNOWLEDGEMENT

This dissertation entitled**" *"Design and synthesis of some novel Michael Addition derivatives of 2-4 disubstituted oxazol-5(4H)one as possible Anti-diabetic, Anti-oxidant and Anti-microbial agents***"** would not have been a feasible one without the grace of god almighty who gave me moral till the completion of my project.

First and foremost I am extremely beholden to my esteemed guide, **Mrs. S. HURMATH UNNISSA, M.Pharm.,** Asst. professor, Dept. of Pharmaceutical Chemistry, for her constant insight, personal advice, countless serenity and pain taking effort in all stages of study.

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I submit my sincere thanks and respectful regard to our beloved Chairman, **Dr. Nalla G. Palanisami** and Managing Trustee, **Dr. Thavamani D. Palanisami** for all the facilities that were provided to me at the institution enabling me to do the work of this magnitude

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Above all I dedicate myself before the unfailing presence of **GOD** and constant love and encouragement given to me by my beloved **Parents, Sister, and Brother in law** who deserves the credit of success in whatever work I did.

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S M GUPTHA JULURI

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ABBREVIATIONS

Ar	Aromatic
DM	Diabetes millitus
AR	Aldose reductase
ROS	Reactive Oxidative Stress
NADPH	Nicotinamide adenine di nucleotide phosphate
e.g.	Example
%	Percentage
^{1}HNMR	Nuclear Magnetic Resonance
Mg	Milligram
Ml	Milliliter
μg	Microgram
mm	Millimeter
w/w	Weight by weight
v/v	Volume by volume
μg/ml	Microgram per liter
Hrs	Hours
^{0}C	Degree centigrade
Fig.	Figure
Tab.	Table
UV-VIS	Ultraviolet and visible spectroscopy
min.	Minutes

IR	Infrared spectroscopy
Std	Standard
TLC	Thin layer chromatography
KBr	Potassium bromide
FTIR	Fourier transform infrared spectrometer
IC	Inhibitory concentration
EC	Effective concentration
Cont	Control
DMSO	Dimethyl sulfoxide
DPPH	Diphenylpicrylhydrazyl
FRAP	Ferric reducing antioxidant power assay

Compound code	IUPAC name
A1	4-(1-(diethylamino)ethyl)-2-methyloxazol-5(4H)-onE
A2	4-((diethylamino)(4-methoxyphenyl)methyl)-2-methyloxazol-5(4H)-one
A3	4-((diethylamino)(phenyl)methyl)-2-methyloxazol-5(4H)-one
A4	4-(1-(diethylamino)-3-phenylallyl)-2-methyloxazol-5(4H)-one
A5	4-((diethylamino)(2-chlorophenyl)methyl)-2-methyloxazol-5(4H)-one
A6	4-((diethylamino)(4-(dimethylamino)phenyl)methyl)-2-methyloxazol-5(4H)-one
B1	2-benzyl-4-(1-(diethylamino)ethyl)oxazol-5(4H)-one
B2	4-((diethylamino)(4-methoxyphenyl)methyl)-2-benzyloxazol-5(4H)-one
B3	4-((diethylamino)(phenyl)methyl)-2-benzyloxazol-5(4H)-one
B4	2-benzyl-4-(1-(diethylamino)-3-phenylallyl)oxazol-5(4H)-one
B5	4-((diethylamino)(2-chlorophenyl)methyl)-2-benzyloxazol-5(4H)-one
B6	4-((diethylamino)(3-chlorophenyl)methyl)-2-benzyloxazol-5(4H)-one
B7	4-((diethylamino)(4-(dimethylamino)phenyl)methyl)-2-benzyloxazol-5(4H)-one
B8	4-((diethylamino)(4-hydroxyphenyl)methyl)-2-benzyloxazol-5(4H)-one

Abstract

Diabetes mellitus (DM) is a major degenerative disease in the world today. Several epidemiological and clinical studies indicate a direct relationship between hyperglycemia and long-term complications such as retinopathy, nephropathy, neuropathy and various fungal infections etc. This study was undertaken to evaluate the activity of some Novel Michael Addition derivatives of 2,4-disubstituted Oxazol-5(4H)-one derivatives against Diabetes and its complications by using *in vitro* enzyme inhibition technique. These derivatives showed considerable biological efficacy when compared to pioglitazone, a potent and well known anti diabetic agent as a reference drug. All the compounds were effective, amongst them di methyl amino benzaldehyde (A6 and B7) and acetaldehyde derivatives (A1 and B1) shown more prominent activity.

Key words: Oxazolone scaffold; retinopathy; neuropathy and nephropathy .

INTRODUCTION

Diabetes mellitus [1,2] (DM) is a major degenerative disease in the world today. Several epidemiological and clinical studies indicate a direct relationship between hyperglycemia and long-term complications such as retinopathy, nephropathy, neuropathy and angiopathy etc. India has today become the diabetic capital of the world with over 20 million diabetics and this number is set to increase to 57 million by 2025.DM is a multifactorial disease which is characterized by hyperglycemia ,lipoprotein abnormalities, raised basal metabolic rate, defect in reactive oxygen species scavenging enzymes and high oxidative stress-induced damage to pancreatic beta cells. It is ranked seventh among the leading causes of death and is considered third when its fatal complications are taken into account.

The term DM describes a metabolic disorder of   aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long–term damage, dysfunction and failure of various organs. Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, ketoacidosis or a non– ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death. Often symptoms are not severe, or may be absent, and consequently hyperglycaemia sufficient to cause pathological and functional changes may be present for a long time before the diagnosis is made. The long–term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation, Charcot joints, and features of autonomic dysfunction, including sexual dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease.

Based on the aetiology DM is classified into different types they are,

1. Type 1
2. Type 2 and
3. Gestational diabetes

TYPE 1 [3]**:**

Type 1 diabetes occurs in about 10-15% of all cases of diabetes. It usually occurs in people under the age of 30, but can happen at any age.

Type 1 indicates the processes of beta–cell destruction that may ultimately lead to diabetes mellitus in which "insulin is required for survival" to prevent the development of ketoacidosis, coma and death. An individual with a Type 1 process may be metabolically normal before the disease is clinically manifest, but the process of beta–cell destruction can be detected. Type 1 is usually characterized by the presence of anti–GAD, islet cell or insulin antibodies which identify the autoimmune processes that lead to beta–cell destruction. In some subjects with this clinical form of diabetes, particularly non–Caucasians, no evidence of an autoimmune disorder is demonstrable and these are classified as "Type 1 idiopathic". Aetiological classification may be possible in some circumstances and not in others. Thus, the aetiological Type 1 process can be identified and sub–categorized if appropriate antibody determinations are performed. It is recognized that such measurements may be available only in certain centres at the present time. If these measurements are performed, then the classification of individual patients should reflect this.

TYPE 2 [3,4]:

Type 2 diabetes has emerged recently as a problem among adolescents and young adults, particularly in high-prevalence populations. Although type 1 diabetes remains the most prevalent form of the disease in children worldwide, it is likely that type 2 diabetes will be the predominant form within 10 years in many populations. Type 2 diabetes has already been reported in children from Japan, the Pacific Islands, Hong Kong, Singapore, China, Malaysia, Korea and Australia. Among children in Japan, it is already more common than type 1 diabetes, accounting for 80% of childhood diabetes; the incidence has almost doubled between 1976–80 and 1991–95.

Initially insulin is still produced by the pancreas, but is less effective than normal. This is called insulin resistance and is an inherited characteristic made worse by carrying extra body fat. Because insulin is necessary for glucose to move from the blood stream into the body cells and the liver, excess glucose remains in the blood stream resulting in higher than normal blood glucose levels (BGLs). After several years of diabetes, the pancreas may become "exhausted" and produce less insulin.

GESTATIONAL DIABETES [5,6]:

Gestational diabetes is carbohydrate intolerance resulting in hyperglycaemia of variable severity with onset or first recognition during pregnancy. It does not exclude the possibility that the glucose intolerance may antedate pregnancy but has been previously unrecognized. The definition applies irrespective of whether or not insulin is used for treatment or the condition persists after pregnancy. Women who become pregnant and who are known to have diabetes mellitus which antedates pregnancy do not have gestational diabetes but have "diabetes mellitus and pregnancy" and should be treated accordingly before, during, and after the pregnancy.

In the early part of pregnancy (e.g. first trimester and first half of second trimester) fasting and postprandial glucose concentrations are normally lower than in normal, non–pregnant women. Elevated fasting or postprandial plasma glucose levels at this time in pregnancy may well reflect the presence of diabetes which has antedated pregnancy, but

Criteria for designating abnormally high glucose concentrations at this time have not yet been established. The occurrence of higher than usual plasma glucose levels at this time in pregnancy mandates careful management and may be an indication for carrying out an OGTT (Annex 1).

Nevertheless, normal glucose tolerance in the early part of pregnancy does not itself establish that gestational diabetes may not develop later.

OTHER SPECIFIC TYPES OF DIABETES [6]:

1. Genetic defects of beta-cell function
2. Genetic defects in insulin action
3. Diseases of the exocrine pancreas
4. Endocrinopathies
5. Drug- or chemical-induced
6. Infections
7. Uncommon forms of immune-mediated diabetes
8. Other genetic syndromes Sometimes Associated with Diabetes

Diagnostic Procedure:

- Random blood glucose test — for a random blood glucose test, blood can be drawn at any time throughout the day, regardless of when the person last ate. A random blood

glucose level of 200 mg/dL (11.1 mmol/L) or higher in persons who have symptoms of high blood glucose suggests a diagnosis of diabetes.

- Fasting blood glucose test — fasting blood glucose testing involves measuring blood glucose after not eating or drinking for 8 to 12 hours (usually overnight). A normal fasting blood glucose level is less than 100 mg/dL. A fasting blood glucose of 126 mg/dL (7.0 mmol/L) or higher indicates diabetes. The test is done by taking a small sample of blood from a vein or fingertip. It must be repeated on another day to confirm that it remains abnormally high.

- Hemoglobin A1C test (A1C) — The A1C blood test measures the average blood glucose level during the past two to three months. It is used to monitor blood glucose control in people with known diabetes, but is not normally used to diagnose diabetes. Normal values for A1C are 4 to 6 percent. The test is done by taking a small sample of blood from a vein or fingertip.

- Oral glucose tolerance test — Oral glucose tolerance testing (OGTT) is the most sensitive test for diagnosing diabetes and pre-diabetes. However, the OGTT is not routinely recommended because it is inconvenient compared to a fasting blood glucose test.

Tab1:Diagnostic criteria for diabetes and pre-diabetes (non-pregnant adults)

Normal
1. Fasting plasma glucose <100 mg/dl.
or
2. Oral glucose tolerance test (OGTT) 2-hr plasma glucose <140 mg/dl.
Pre-diabetes
1. A1C range of 5.7–6.4%.
or
2. Impaired fasting glucose (IFG) = fasting plasma glucose of 100–125 mg/dl.
3. Impaired glucose tolerance (IGT) = OGTT 2-hr plasma glucose of 140–199 mg/dl.
Diabetes
1. A1C >6.5%. The test should be performed in a laboratory using a method that is certified by the National Glycohemoglobin Standardization Program (NGSP) and standardized to the Diabetes Control and Complications trial (DCCT) assay.
or

2. Fasting plasma glucose >126 mg/dl. Fasting is defined as no caloric intake for at least eight hours.
3. 2-h plasma glucose >200 mg/dl during an oral glucose tolerance test. The test should be performed using a glucose load containing the equivalent of 75-g anhydrous glucose dissolved in water.
4. Symptoms of diabetes and a casual plasma glucose >200 mg/dl. "Casual" is defined as any time of day, without regard to the time since the last meal. The classic symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss.

Treatment:

Tab 2: Summary of Antidiabetic interventions as monotherapy for type 2 diabetes:

Interventions	Expected total decrease in A1C (%)	Advantages	Disadvantages
Step 1: Lifestyle intervention and metformin			
Lifestyle to: ↓ weight & ↑ activity	1–2	Low cost, many benefits	Fails for most in the first year
Metformin	1.5	Weight-neutral, no hypoglycemia, inexpensive	GI side effects, rare lactic acidosis
Step 2: Add a sulfonylurea or insulin			
Insulin	1.5–2.5	No dose limit, inexpensive, improved lipid profile	Injections, requires frequent blood glucose self-monitoring, hypoglycemia, weight gain
Sulfonylureas	1.5	Inexpensive	Weight gain, hypoglycemia
Alternative medications			
Glitazones (TZDs– thiazolidinediones)	0.5–1.4	Improved lipid profile	Restricted use. Fluid retention, weight gain, expensive, increased risk of congestive heart failure
Alpha-glucosidase inhibitors	0.5–0.8	Weight-neutral	Frequent GI side effects, 3x/day dosing, expensive
Exenatide	0.5–1.0	Weight loss	Infections, 3x/day dosing, frequent GI side effects, expensive, little experience
Glinides (meglitinides)	1–1.5	Short duration	three times/day dosing, expensive
Pramlintide	0.5–1.0	Weight loss	Infections, 3x/day dosing, frequent GI side effects, expensive, little experience

Adapted from: Nathan DM, Buse JB, Davidson MB, et al. Management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy. *Diabetes Care* 2006;29(8):1964.

Tab 3:Newer derivatives for Diabetes treatment:

Anti- Diabetic Agents	Biological effects
Thiazolidinone substituted biphenyl scaffold derivatives	• Increases IRb tyrosine Phosphorylation • Hypoglycemic
Benzylidene-2,4-thiazolidinedione derivatives substituted at ortho position of the phenyl group	• Suppresses weight gain • Hypoglycemic

	• Reduces circulating triglycerides, • total cholesterol, and NEFA
5-(3-((Z)-((Z)-2-(4-chlorophenylimio)-4-oxothiazolidin-5-ylidene) methyl)-2,5 dimethyl-1H-pyrrol-1-yl) isophthalic acid	• Competitive PTP1B inhibitor • Increases IRb tyrosine • phosphorylation
Methylenedisalicylic acid derivatives	• Improves glucose tolerance • Reduces body weight, adipose
Isothiazolidinone inhibitors (imidazole and imidazoline analogs)	• hypoglycemic
4-[(5-Arylidene-4-oxo-2 phenyliminothiazolidin-3-yl)methyl]-benzoic acids	• Lipophilic • Improved cellular permeability
Monosodium({[5-(1,1-dimethylethyl)thiazol-2-yl]methyl}{[(4-{4-[4-(1-propylbutyl)phenoxy]methyl}phenyl)thiazol-2-yl]methyl}amino)acetate	• Increases insulin-stimulated • glucose uptake
1-Biphenyl-4-yl-2-(4-nitro-phenoxy)-ethanone	• Acute and chronic hypoglycemic activity • Reduces circulating HbA1c,Triglycerides and LDL
Bidentate a-ketoacid–based inhibitors	• Potential as therapeutics for diabetes and obesity.
Triazole-linked glycosylated a-ketocarboxylic acid derivatives	• Competitive inhibitor
Aryl diketoacid derivatives	• Noncompetitive inhibitors
Difluoro-methylenephosphonate bioisosteres on a sulfonamide scaffold	• PTP1B activity inhibitors
b-C-glycosiduronic acids quinones and b-C-glycosyl compounds	• PTP1B activity inhibitor
2-(Oxalylamino) benzoic acid	• Predictive high biological activities
Benzofuran and benzothiophene biphenyls (BBB)	• hypoglycemic
Substituted phenoxy-3-piperazin-1-yl-propan-2-ols	• Hypoglycemic
Thiophene derivatives	• Permeable to cell membranes • Increases Akt phosphorylation
2-(4-Methoxyphenyl) ethyl] acetamide derivatives	• Hypoglycemic
Mixed-ligand oxovanadium(IV) complexes Chemical synthesis	• Potential insulin-enhancing agent
INTA	• Highly selective PTP1B inhibitor
INTB	• Highly selective PTP1B inhibitor

Diabetes can affect almost all body parts including eyes, kidneys, feet, nerves, heart and skin. Chronic diabetes can give rise to several life-threatening complications such as increased

risk of developing bone and joint disease, stroke, nervous system disorder, infection of the respiratory system, kidney disease, hypertension, heart disease and visual impairment and loss of digits or limbs.

Diabetes may remain unnoticed for many years until these complications manifest themselves. That is why diabetes is known as the 'Silent Killer'.

Diabetes can give rise to several skin conditions, including bacterial infections such as sties, boils and carbuncles; fungal infections such as Candidiasis; and dry, itchy skin. High glucose levels in the blood can enhance the growth of fungi and skin is the flourishing site for it. Tinea pedis occurring between the toes and sometimes the fingers is most frequently caused by Trichophyton mentagrophytes and Trichophyton rubrum. Candida albicans is another common fungus that causes skin infections and it most commonly affects the vaginal and groin areas.

Three other common fungal infections are athlete's foot (affecting the skin between the toes), jock itch (red, itchy area on the genitals as well as inside of the thighs) and ringworm (ring-shaped, itchy, scaly patches or blisters that can appear on groin, feet, abdomen, chest and scalp or nails). Itching, blistering, swelling and dry flaky skin or severe scaling are the common symptoms of fungal infections.

ANTIOXIDANT
FREE RADICAL FORMATION

Atoms are most stable in the ground state. An atom is considered to be "ground" when every electron in the outermost shell has a complimentary electron that spins in the opposite direction. By definition a free radical is any atom (e.g. oxygen, nitrogen) with at least one unpaired electron in the outermost shell, and is capable of independent existence . A free radical is easily formed when a covalent bond between entities is broken and one electron remains with each newly formed atom. Free radicals are highly reactive due to the presence of unpaired electron(s). The following literature review addresses only radicals with an oxygen center. Any free radical involving oxygen can be referred to as reactive oxygen species (ROS). Oxygen centered free radicals contain two unpaired electrons in the outer shell. When free radicals steal an electron from a surrounding compound or molecule a new free radical is formed in its place. In turn the newly formed radical then looks to return to its ground state by stealing electrons with antiparallel spins from cellular structures or molecules. Thus the chain reaction continues and can be "thousand of events long." The electron transport chain (ETC), which is found in the inner mitochondrial membrane, utilizes oxygen to generate energy in the form of adenosine triphosphate (ATP). Oxygen acts as the terminal electron acceptor within the ETC. The literature

suggests that anywhere from 2 to 5% of the total oxygen intake during both rest and exercise have the ability to form the highly damaging superoxide radical via electron escape. During exercise oxygen consumption increases 10 to 20 fold to 35-70 ml/kg/min. In turn, electron escape from the ETC is further enhanced. Thus, when calculated, .6 to 3.5 ml/kg/min of the total oxygen intake during exercise has the ability to form free radicals. Electrons appear to escape from the ETS at the ubiqunone-cytochrome c level.
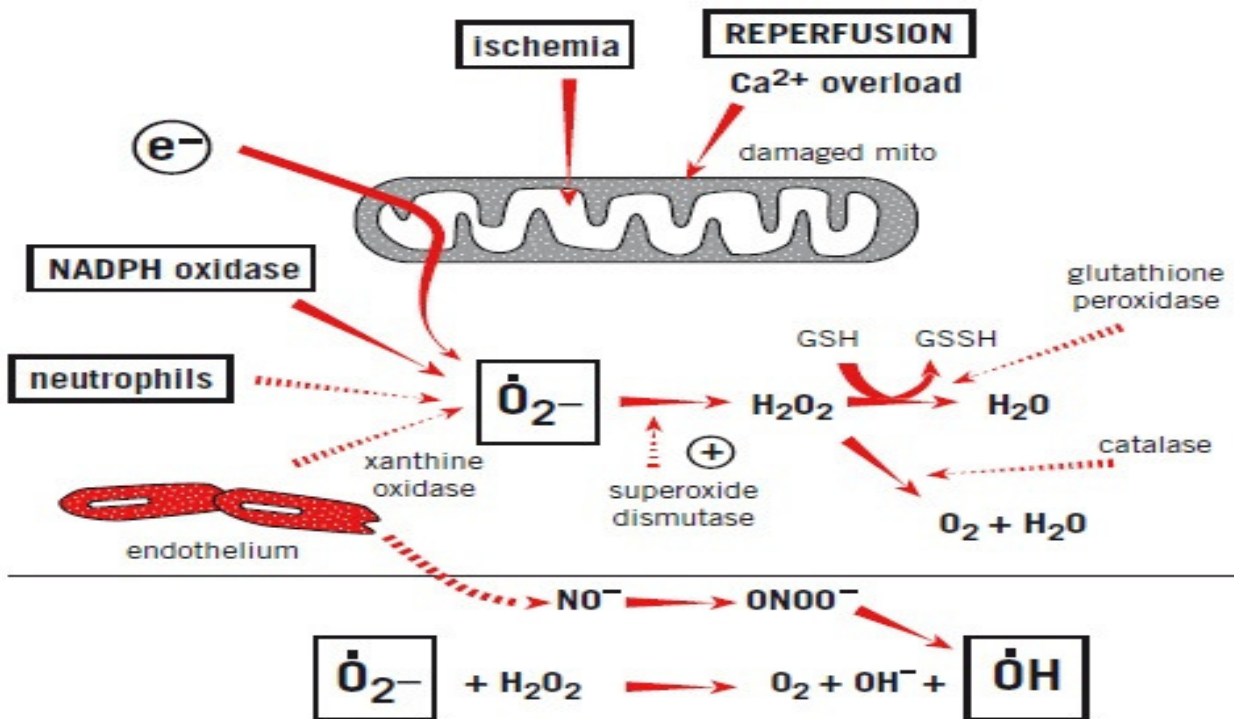
PEROXIDATION

Polyunsaturated fatty acids (PUFAs) are abundant in cellular membranes and in low-density lipoproteins (LDL). The PUFAs allow for fluidity of cellular membranes. A free radical prefers to steal electrons from the lipid membrane of a cell, initiating a free radical attack on the cell known as lipid peroxidation. Reactive oxygen species target the carbon-carbon double bond of polyunsaturated fatty acids. The double bond on the carbon weakens the carbon-hydrogen bond allowing for easy dissociation of the hydrogen by a free radical. A free radical will steal the single electron from the hydrogen associated with the carbon at the double bond. In turn this leaves the carbon with an unpaired electron and hence becomes a free radical. In an effort to stabilize the carbon-centered free radical molecular rearrangement occurs. The newly arranged molecule is called a conjugated diene (CD). The CD then very easily reacts with oxygen to form

a proxy radical. The proxy radical steals an electron from another lipid molecule in a process called propagation.

TYPES OF FREE RADICALS

There are numerous types of free radicals that can be formed within the body. This web site is only concerned with the oxygen centered free radicals or ROS. The most common ROS include: the superoxide anion (O_2^-), the hydroxyl radical (OH^-), singlet oxygen (O_2), and hydrogen peroxide (H_2O_2) Superoxide anions are formed when oxygen (O_2) acquires an additional electron, leaving the molecule with only one unpaired electron. Within the mitochondria $O_2^- \cdot$ is continuously being formed. The rate of formation depends on the amount of oxygen flowing through the mitochondria at any given time. Hydroxyl radicals are short-lived, but the most damaging radicals within the body. This type of free radical can be formed from O_2^- and H_2O_2 via the Harber-Weiss reaction. The interaction of copper or iron and H_2O_2 also produce $OH \cdot$ as first observed by Fenton. These reactions are significant as the substrates are found within the body and could easily interact. Hydrogen peroxide is produced in vivo by many reactions. Hydrogen peroxide is unique in that it can be converted to the highly damaging hydroxyl radical or be catalyzed and excreted harmlessly as water. Glutathione peroxidase is essential for the conversion of glutathione to oxidized glutathione, during which H_2O_2 is converted to water. If H_2O_2 is not converted into water O_2^- is formed. Singlet oxygen is not a free radical, but can be formed during radical reactions and also cause further reactions. Singlet oxygen violates Hund's rule of electron filling in that it has eight outer electrons existing in pairs leaving one orbital of the same energy level empty. When oxygen is energetically excited one of the electrons can jump to empty orbital creating unpaired electrons . Singlet oxygen can then transfer the energy to a new molecule and act as a catalyst for free radical formation. The molecule can also interact with other molecules leading to the formation of a new free radical.

An **antioxidant** is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols [17].

Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione,

vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells. As oxidative stress might be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases. However, it is unknown whether oxidative stress is the cause or the consequence of disease.

Reactive oxygen species (ROS), capable of causing damage to DNA, has been associated with carcinogenesis, coronary heart disease, and many other health problems related to advancing age [18]. In low concentrations, synthetic antioxidants are also in use for many industrial processes e.g. inhibition of radical formation for preventing premature polymerization during processing, storage and transportation of unsaturated monomers. They exert their effects by scavenging or preventing the generation of ROS [19] which can protect the formation of free radicals and retard the progress of many chronic diseases[20] including cancer, neurodegenerative, inflammation and cardiovascular diseases [21].

Fungus [24] is a member of a large group of eukaryotic organisms that includes microorganisms such as yeasts and mushrooms. These organisms are classified as a kingdom, Fungi. Abundant worldwide; most fungi are inconspicuous because of the small size of their structures, and their cryptic lifestyles in soil, on dead matter, and as symbionts of plants, animals, or other fungi. Fungi can break down manufactured materials and buildings, and become significant pathogens of humans and other animals. Losses of crops due to fungal diseases (e.g. rice blast disease) or food spoilage can have a large impact on human food supplies and local economies.

Pathogens and parasites [25, 26]:

Many fungi are parasites on plants, animals (including humans), and other fungi. Serious pathogens of many cultivated plants causing extensive damage and losses to agriculture and forestry include the rice blast fungus Magnaporthe oryzae, tree pathogens such as Ophiostoma ulmi and Ophiostoma novo-ulmi causing Dutch elm disease, and Cryphonectria parasitica responsible for chestnut blight, and plant pathogens in the genera Fusarium, Ustilago, Alternaria, and Cochliobolus. Some carnivorous fungi, like Paecilomyces lilacinus, are predators of nematodes, which they capture using an array of specialized structures such as constricting rings

or adhesive nets. Some fungi can cause serious diseases in humans, several of which may be fatal if untreated. These include aspergilloses, candidoses, coccidioidomycosis, cryptococcosis, histoplasmosis, mycetomas, and paracoccidioidomycosis. Furthermore, persons with immuno-deficiencies are particularly susceptible to disease by genera such as Aspergillus, Candida, Cryptoccocus, Histoplasma, and Pneumocystis. Other fungi can attack eyes, nails, hair, and especially skin, the so-called dermatophytic and keratinophilic fungi, and cause local infections such as ringworm and athlete's foot. Fungal spores are also a cause of allergies, and fungi from different taxonomic groups can evoke allergic reactions.

Antibiotic resistance [27]:

Antibiotic resistance is a type of drug resistance where a microorganism is able to survive exposure to an antibiotic. Many antibiotic resistance genes reside on plasmids, facilitating their transfer. If a bacterium carries several resistance genes, it is called multiresistant or, informally, a superbug or super bacterium. The primary cause of antibiotic resistance is antibiotic use both within medicine and veterinary medicine. The greater the duration of exposure the greater the risk of the development of resistance irrespective of the severity of the need for antibiotics. As resistance becomes more common there becomes a greater need for alternative treatments. However despite a push for new antibiotic therapies there has been a continued decline in the number of newly approved drugs. Antibiotic resistance therefore poses a significant problem.

Causes for antibiotic resistance [27]:

- ❖ Antibiotic misuse
- ❖ Resistant transmitted through animals.

Oxazole [28] is a five membered heterocyclic compound. These are azoles with oxygen and nitrogen separated by a carbon atom. The Oxazole ring system is not abundant in nature although there are number of macrocyclic antibiotics and antifungal agents which contain oxazole nucleus.
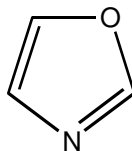
Eg., Griseovirdin and Helichondramide.
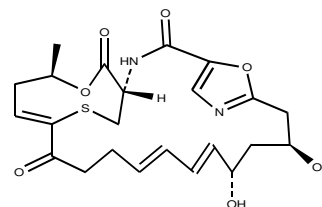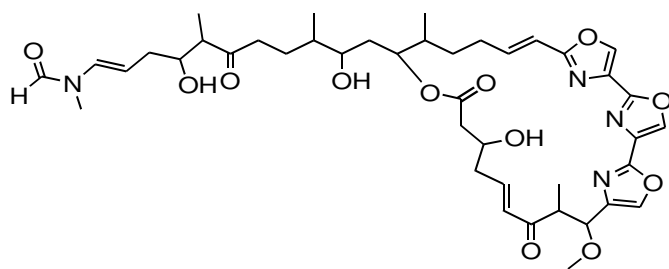
Oxazolone [3] is a class of small heterocycles, which are important intermediates in the synthesis of several small molecules, including amino acids, peptides, antimicrobial or antitumor compounds ,heterocyclic precursors as well as biosensors coupling and or photosensitive composition devices for proteins. Some Oxazolones have shown a wide range of pharmaceutical properties.
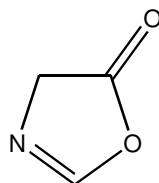

In continuation of our drug discovery program, we synthesized a variety of oxazolones and screened their effects on different aspects of diabetic response.

CHEMISTRY OF OXAZOLES

Methods of synthesis of Ox azoles:

Robinson – Gabriel synthesis [27]:

The Robinson – Gabriel synthesis is a named reaction used to synthesize oxazoles by dehydrating acylamino-ketones. The dehydrating agents used are sulphuric acid or phosphorus oxychloride.
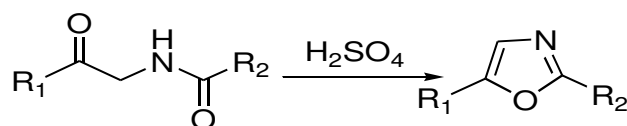
Fischer Oxazole synthesis[28]:

The Fischer oxazole synthesis is a chemical synthesis of the aromatic heterocyclic oxazole from cyanohydrins and aldehydes in the presents of An. HCl. This method was discovered by Hermen emil in 1896.
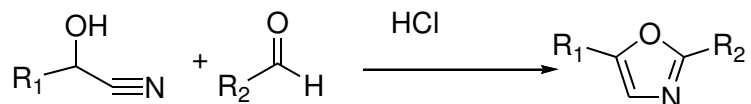
Van leusen oxazole synthesis[29]:

In this reaction the oxazoles are prepared from aldehydes by reaction with tosyl methyl isocyanide.
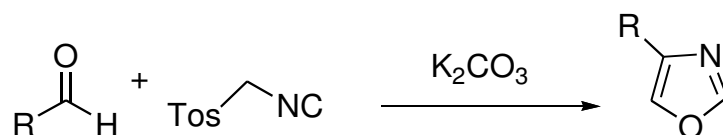
Oxazoles[30] are formed via butanol or iodine mediated doimino oxidative cyclization from readily available starting materials under mild conditions.
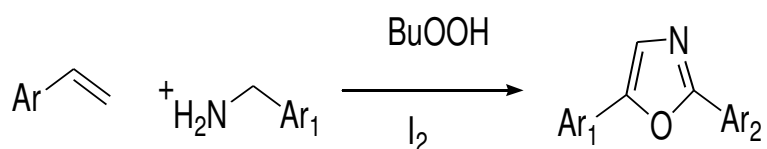
Di substituted oxazoles [31] were formulated via Iodine catalyzed tanden oxidative cyclization method at 110 ^{0}C by using DMSO as a solvent.
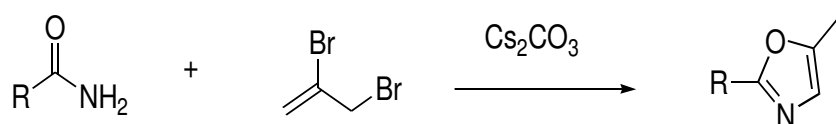
Oxazoles [32] were prepared by Cs_2CO_3 mediated reaction of aromatic and unsubstituted primary amides with 2, 3 di bromo propene. This leads to the synthesis of 2- aryl – 5- alkyl substituted oxazoles in a single step.
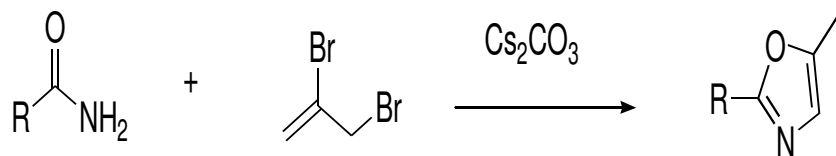

Oxazoles [33] were formulated by cyclization of propyl alcohols with anilines in the presents of $Zn(OTf)_2$ catalyst, at 100 ^{0}C without additives.
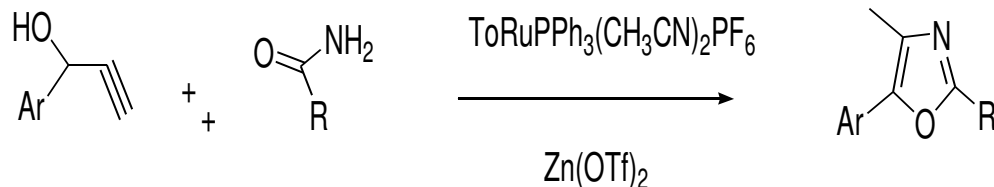

Tri substituted Oxazoles [34] were prepared by copper catalysed tandem oxidative cyclization by using DMSO as a solvent.
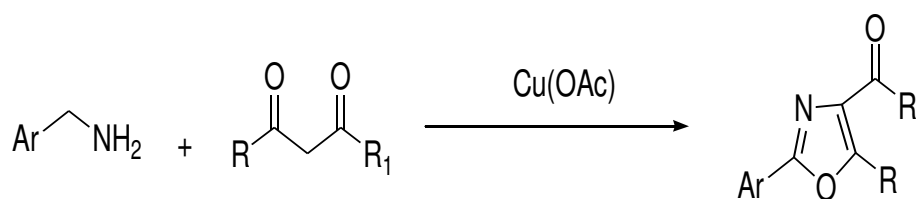
Substituted cyanoamides produces tri substituted oxazoles[35] in the presence of lithium bromide, by using toluene as a solvent.
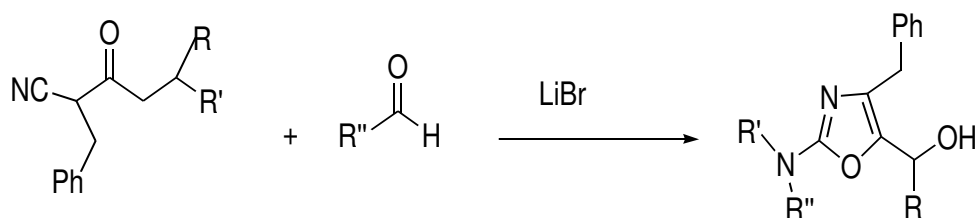
Double acylation of a protected glycine affords intermediate amino keto ester, which inturn can be dehydrated to form 1,3 oxazoles [36].
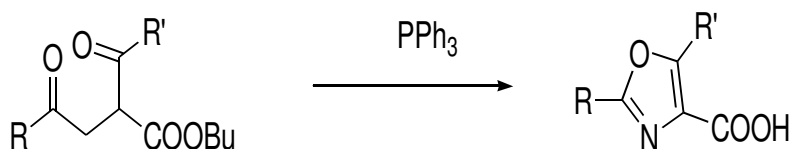
CHEMICAL PROPERTIES [3, 28]:

Oxazole is a very weak base and its salts are unstable . N-alkyloxazolium salts have been formed from several substituted oxazoles. These salts easily undergo hydrogen deuterium exchange at C2 position.

Neutral oxazoles are deprotonated preferentially at C2 by strong bases. 2 methyl substituents in oxazoles were activated by C=N bonds of the ring systems and these groups can be deprotonated by alkyl-lithium reagents and strong bases. Oxazolythinium derivatives are thermally unstable and undergo reversible ring opening.
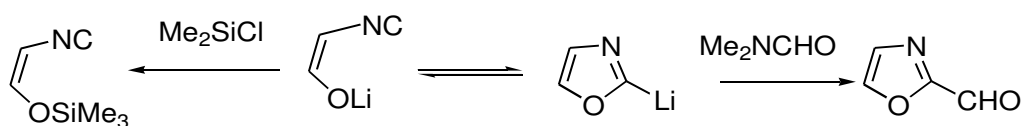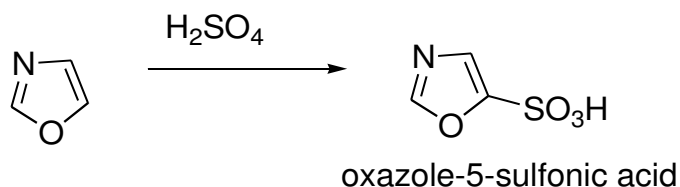

Electrophilic aromatic substitution:

Nitration:

Oxazole undergoes Electrophilic aromatic substitution at C5 position. The nitrating mixture used is nitric acid and sulphuric acid.
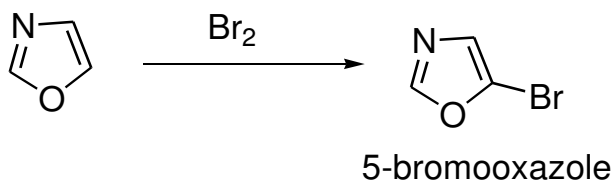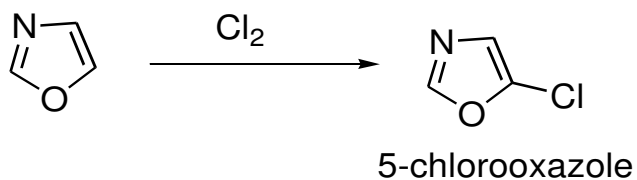
5-nitrooxazole

Sulphonation:

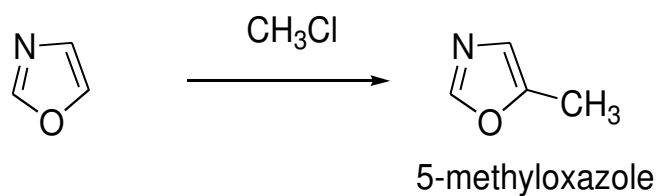Oxazole undergoes sulphonation at C2 positon. The sulphonating agent used is sulphuric acid.

oxazole-5-sulfonic acid

Halogenaton:

Oxazole undergoes halogenaton at C2 position. The reagents used are chlorine, bromine etc.

5-chlorooxazole

5-bromooxazole

Friedel crafts alkylation:

Oxazole undergoes Friedel crafts alkylation at C2 position. Lewis acid like aluminum chloride is used as catalyst and the reagent used is alkyl halides.
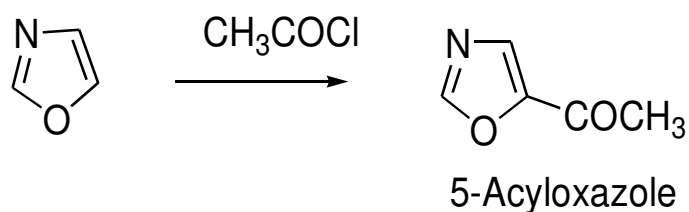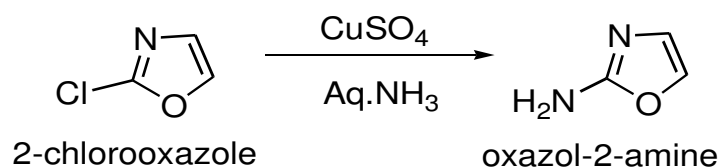
$$CH_3Cl$$

5-methyloxazole

Friedel crafts acylation:

Oxazole undergoes Friedel crafts acylation at C2 position. Lewis acid like aluminum chloride is used as catalyst and the reagent used is acyl halides.

$$CH_3COCl$$

5-Acyloxazole

Nucleophilic Aromatic substitution:

Oxazoles undergoes Nucleophilic Aromatic substitution at C2 position and form 2-nitro oxazole, as well as 2-hydroxy oxazole. The catalyst used for this is cupper sulphate.

hydroxylaton

2-chlorooxazole   oxazol-2-ol

$$CuSO_4$$
$$Aq.NH_3$$

2-chlorooxazole   oxazol-2-amine
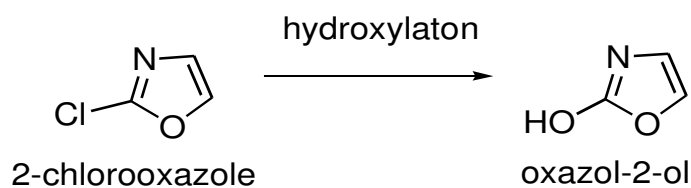
Michael addition:

As originally defined by Arthur Michael, the reaction is the addition of an enolate of a ketone or aldehyde to an α , β-unsaturated carbonyl compound at the β carbon. A newer definition, proposed by Kohler, is the 1,4-addition of a doubly stabilized carbon nucleophile to an α,β-unsaturated carbonyl compound. Some examples of nucleophiles include beta-ketoesters, malonates, and beta-cyanoesters. The resulting product contains a highly useful 1,5-dioxygenated pattern.

The Michael addition is an important atom-electronic method for diastereoselective and enantioselective C-C bond formation.

Mechanism:
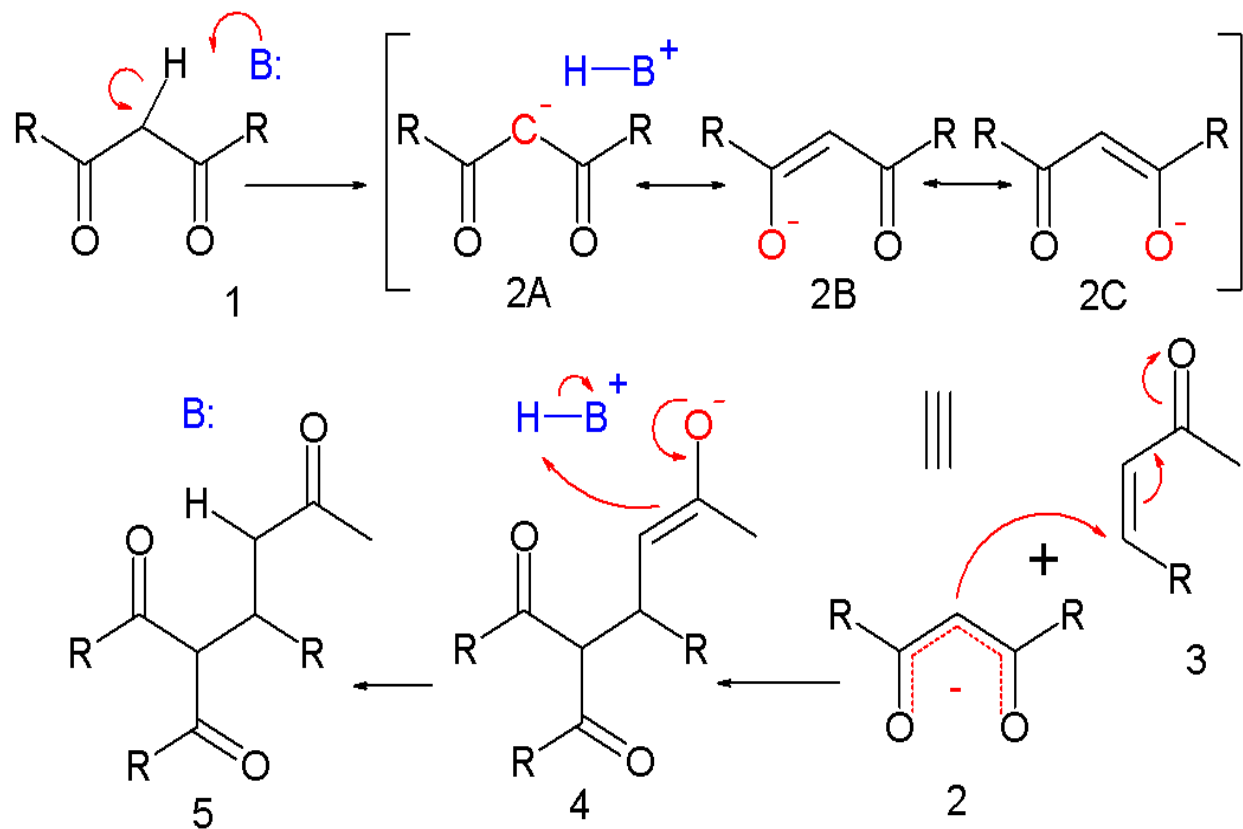
Deprotonation of 1 by base leads to carbanion 2 stabilized by its electron-withdrawing groups. Structures 2a to 2c are three resonance structures that can be drawn for this species, two of which have enolate ions. This nucleophile reacts with the electrophilic alkene 3 to form 4 in

a conjugate addition reaction. Proton abstraction from protonated base (or solvent) by the enolate 4 to 5 is the final step.

The course of the reaction is dominated by orbital, rather than electrostatic, considerations. The HOMO of stabilized enolates has a large coefficient on the central carbon atom while the LUMO of many alpha, beta unsaturated carbonyl compounds has a large coefficient on the beta carbon. Thus, both reactants can be considered soft. These polarized frontier orbitals are of similar energy, and react efficiently to form a new carbon-carbon bond.

Like the aldol addition, the Michael reaction may proceed via an enol, silyl enol ether in the Mukaiyama-Michael addition, or more usually, enolate nucleophile. In the latter case, the stabilized carbonyl compound is deprotonated with a strong base (hard enolization) or with a Lewis acid and a weak base (soft enolization). The resulting enolate attacks the activated olefin with 1,4-regioselectivity, forming a carbon-carbon bond. This also transfers the enolate to the electrophile. Since the electrophile is much less acidic than the nucleophile, rapid proton transfer usually transfers the enolate back to the nucleophile if the product is enolizable; however, one may take advantage of the new locus of nucleophilicity if a suitable electrophile is pendant. Depending on the relative acidities of the nucleophile and product, the reaction may be catalytic in base. In most cases, the reaction is irreversible at low temperature, due to least-motion arguments.

Erlenmeyer condition[76]:

Erlenmeyer condition is use full for the synthesis of α amino acids. Accroding to Erlenmeyer condition, the Oxazolones were prepared by condensation of hippuric acid or acetyl glycine with aldehydes.

LITERATURE REVIEW

ANTI-DIABETIC AGENTS

1. **G.Mariappan et al.,[1]** synthesized aryl furano oxazolone derivatives as potent anti-diabetic agents and these are having thyrosine Phosphatase inhibiting action.
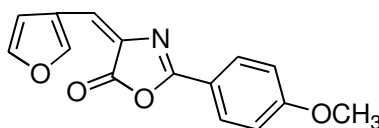
2. **Harikishore Pingali et al., [47]** synthesized oxazole containing 1,3-Dioxane-2-carboxylic acid derivatives as anti-diabetic agents by PPAR agonist action.
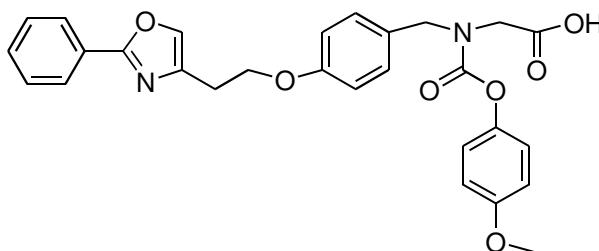
3. **Atul Kumar et al.,[48]** synthesized 2-aryl-naphtho[1,2-d]oxazole derivatives and exhibited anti-diabetic agents by its potential PTP-1B inhibitor activty.
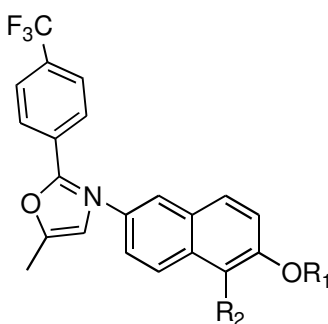
4. **Masao Shiozaki et al.,[49]** synthesized tetrahydropyrano[2,3-d]oxazole and shown potent anti-diabetic agents.

ANTI-MICROBIAL AGENTS

5. **Fumike miyake et al.,**[54] synthesized 5-(3-indolyl)oxazole derivatives, a potent anti-bacterial agents acting on both gram + and gram - microorganisms.
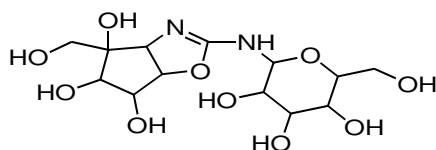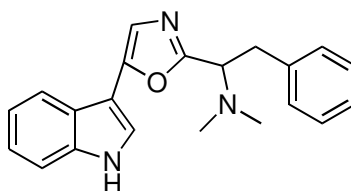

6. **Salah Belaidi et al.,**[55] **synthesized 2,4,5 tri substituted derivatives, a potent anti bacterial agents. All analogs shown moderate to good anti-bacterial activity.**
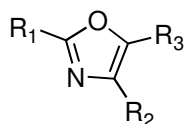
7. **Chung kyu jaa et al.,**[56] synthesized benzo[d]oxazole-4,7-diones, a potent anti-fungal agents. **All analogs shown moderate to good anti-fungal activity.**
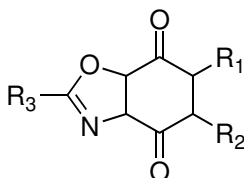
ANTI-INFLAMMATORY AND ANALGESIC

8. **Paul c. Unangst et al.,**[39] synthesized series of oxazole derivatives of 2, 6-ditert-Butylphenol shown potent anti-inflammatory activity by inhibiting lipoxygenase and cyclooxygenase enzymes.
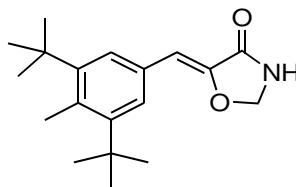
9. **Kim F. McClure et al.,**[40] synthesized series of triazolopyridine oxazole derivatives shown good analgesic activity by antagonizing action on p38 receptors.
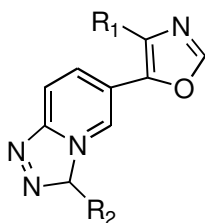
10. **Richard J. Perner et al.,**[41] synthesized series of 4,5-disubstituted-2-arylamino oxazole derivatives shown potent anti-inflammatory activity by TRPV1 antagonist action.
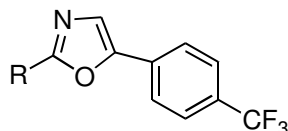
11. **M. Ravi Shashi Nayana et al.,**[42] synthesized series of triazolopyridine oxazole derivatives shown anti-inflammatory activity by p38 MAP kinase inhibitor action.
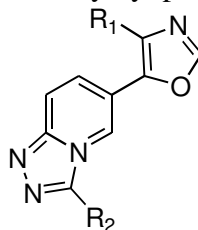
CARDIOVASCULAR AGENTS

12.   **Shankar Swaminathan et al.,**[43] synthsized 2,5-disubstituted derivatives shown potent cardiovascular activity as that of Ifetroban Sodium
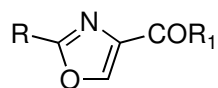
13. Jie Eun Lee et al.,[44] synthesized oxazole linked arylpiperazinylalkylamines as potent anti-hypertensive agents by calcium channel blocking action.
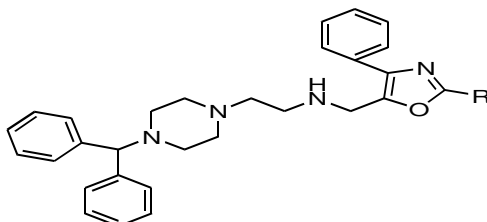
14. Kumiko Takeuchi et al. [45] synthesized Phenyl Oxazole Derivatives as potent anti-anginal agent by its Thromboxane Receptor Antagonism andThromboxane Synthase Inhibition activity.
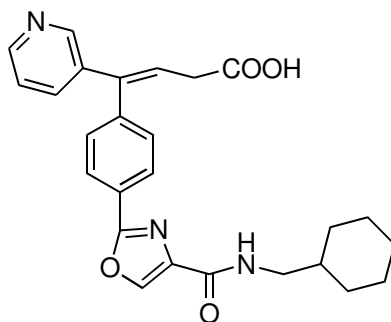
15. Francoise et al., [46]synthesized Bicyclic Oxazolone Derivatives as potent Anti-Angiogenic Agents.
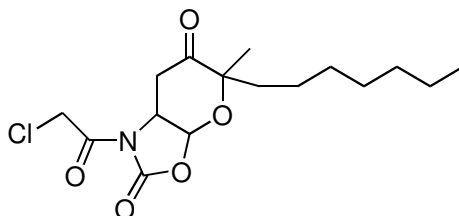
ANTIBIOTICS

16. Mikael Bergdahl et al.,[50] Synthesized   Oxazole- and Diene-Containing C9-C23 Fragment of the Type A Streptogramin as potent antibiotics.

17. **Masaki Kita et al.,**[51] synthesized tris-oxazole macrolactone derivatives and acted as macrolide antibiotics.

ANTI-TUMOR AGENTS

18. **Xin hua lee et al.,**[52] synthesized Novel 2,4,5-trisubstituted oxazole derivatives, a novel group of anticancer agents active on a multidrug resistant cell line. All analogs showed *in-vitro* cytotoxic activity against human and mureine leukemia cell line. And also showed *in-vivo* anti-leukemic activity.

19. **Vincent et al.,**[53] synthesized 2-phenyl-oxazole-4-carboxamide derivatives and shown potent apoptosis inducer activty.
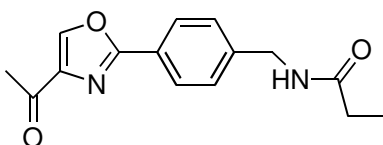
IMMUNOMODULATORY AGENTS

20. **David S millon et al.,**[57] synthesized phenyl-carboxyethyl-oxazole derivatives, a potent immunosuppressants as that of urocanic acid.
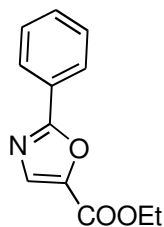
21. **Rongze Kuang et al.,**[58] synthesized quinoline linked oxazole derivatives, a potent immunostimulants which are act as phosphodiesterase 4 inhibitors.
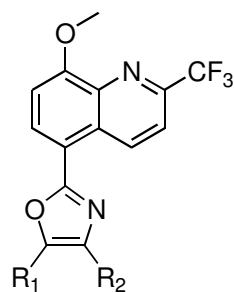
22. **Muhammad A. Mesaik et al.,**[59] synthesized aryl-oxazolone derivative, a potent immunomodulatory agents, acted by T-cell proliferation.
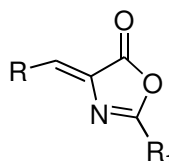
ANTI-OBESITY AGENTS

23. **H.O.Ok et al.,**[60] synthesized Substituted Oxazole Benzenesulfonamides as Potent Human Adrenergic Receptor Agonists.
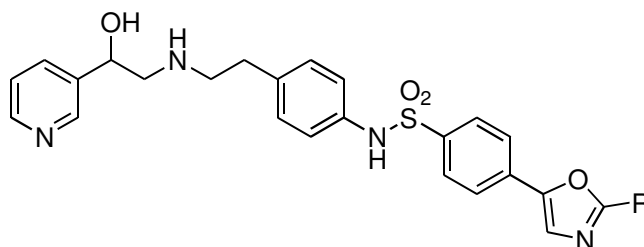
24. **Brijesh kumar et al.,**[61] synthesized dihydropyrazole of 4S-(β)-3-(4-chlorophenyl)-N-methyl-N0-   [(4-chlorophenyl)-sulfonyl]-4-phenyl-4,5-dihydro-1Hpyrazole-   1-caboxamidine oxazole as potent CB1 receptor antagonist.
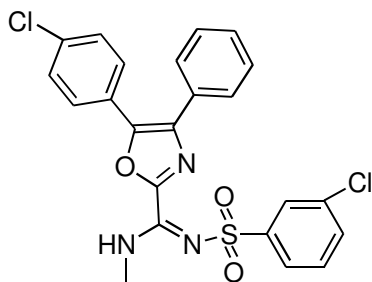
ANTI-ALZHEIMER AGENTS

25. Hossein Razavi et al.,[62] synthesized substituted aryl-carboxyl-oxazoles, a potent anti-Alzheimer agent by acting on amyloidogenesis.
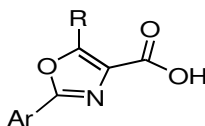
ANTI-TUBERCULAR AGENTS

26. Garrett C. Moraski et al.,[63] synthesized oxazole benzyl esters, a potent anti-tubercular agent. The compounds were tested by *in vivo* mouse infection model.
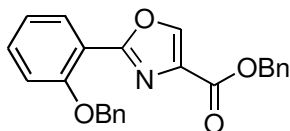
AIM AND OBJECTIVES

Type 2 diabetes comprises 90% of people with diabetes around the world and is one of the major public health challenges of the 21st century. The number of cases worldwide in 2000 is estimated to be about 171 million and is projected to rise to 366 million in 2030. The World Health Organization (WHO) projects that without urgent action, diabetes-related deaths will increase by more than 50% in the next 10 years. Especially in upper-middle income countries, diabetes deaths are projected to increase by over 80% between 2006 and 2015. This circumstance results that the demand for medical care in type 2 diabetes will continue to increase.

Over the past decade, there has been substantial interest in oxidative stress and its potential role in diabetogenesis, development of diabetic complications, atherosclerosis and associated cardiovascular disease. Over production of reactive oxygen and nitrogen species (ROS/RNS) leads to lowered antioxidant defense and alterations of enzymatic pathways. This contribute to endothelial, vascular and neurovascular dysfunction.

High glucose levels in the blood of Diabeteic Patients can enhance the growth of fungi and skin is the flourishing site for it. Fungal microorganisms cause many infections like Candidiasis, dry itchy skin, tinea pedis, athlete's foot, jock itch and ring worm.

So developing a drug moiety containing anti-diabetic activity, anti-oxidant and anti-fungal will be a relevant adjuvant pharmacotherapy.

According to literature review, Oxazolone derivatives were proven to be promising agents, showing good activity against diabetes and its complications.

Oxazolone is a versatile molecule responsible for various activities like Analgesic, Anti-angiogenic, immunomodulatory, anti-fungal and Anti-tumor etc.
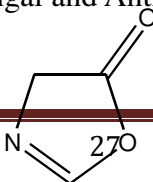
Design of work:

- ✓ 2, 4 Di substituted oxazole have attracted continuing interest because of their varied biological activities namely anti-cancer, anti-inflammatory, anti-fungal and antioxidant etc.

- ✓ Recently, growing lines of evidence have suggested that 2, 4 Di substituted oxazole derivatives are having anti diabetic activity similar to that of Thiazolidinediones.

- ✓ Thiazolidinediones were withdrawn because of increased risk of hepatotoxicity, a potent side effect which limits the use of these derivatives as safe drug candidates.

- ✓ With this background the aim of our work is to synthesize, some novel 2, 4 Disubstituted oxazolone derivatives which are having structure similarity with that of thiazolidinedione derivatives.

Rosiglitazone   pioglitazone
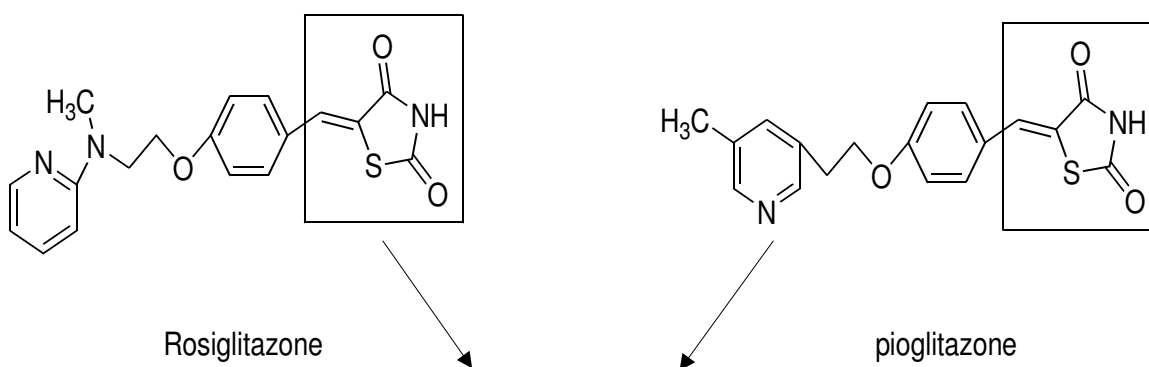
The objectives of the present work can be summarized as follows:

- ❖ Synthesis of some novel potential derivatives of Oxazolone derivatives by using experimental procedure.

Our Proto type

❖ Characterization of synthesized compounds by various analytical techniques like TLC, FT IR, NMR and Mass Spectral studies**.**

❖ Screening for anti-diabetic activity by using *in-vitro* Aldose reductase enzyme inhibition assay technique.

❖ Screening for anti-oxidant activity by using in-vitro DPPH and FRAP method.

❖ Screening for anti-fungal activity against fungal organisms like *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus parasiticus* and *Candida albicans,* by Disc diffusion method and determination of Minimum inhibitory concentration (MIC) by serial dilution method.

EXPERIMENTAL PROCEDURE

SYNTHESIS OF SOME NOVEL MICHAEL ADDITION DERIVATIVES OF OXAZOLONE DERIVATIVES

STEP 1 [64]:

We synthesized various Oxazolone derivatives by using different starting materials like acetyl glycine and Hippuric acid. Hippuric acid was purchased from Sigma-aldrich chemicals and Acetyl glycine was prepared by using the below procedure.

Preparation of Acetyl glycine:

37.5 g (0.5M) of glycine was added to 150ml of water in a 500ml conical flask and stirred vigorously with mechanical stirrer until the solid was completely dissolved. 102 gm of (95 ml, 1M) of Acetic anhydride was added to above mixture for 15-20 min. the solution becomes hot and some acetyl glycine was crystallized. Then the solution was kept in refrigerator for overnight. The precipitate was separated on a Buchner funnel and washed with ice cold water and dry at 100^0C.

STEP 2 [64] :

General method for the preparation of 4-substituted-[Benzylidene]-2-(4-phenyl) oxazol-5-one:

A mixture of Hippuric acid / Acetyl glycine (0.01mol), substituted aromatic aldehyde (0.02mol), anhydrous sodium acetate (0.01 mol) and acetic anhydride (0.04mol) was refluxed for 1 h on a water bath with occasional stirring. The resulting mixture was left in refrigerator overnight. The solid thus obtained was filtered, washed with cold water, dried in hot air oven at 60∘C and recrystallized from ethanol. All the compounds were synthesized by adopting the same procedure with variation in reaction time.

STEP 3:

General method for preparation of Michael addition products[65] :

Equal moles of Oxazolone derivatives were reacted with dietylamine by using 2M of ceric ammonium nitrate as a catalyst. Heat the mixture on water bath for 5 - 60 minutes. All the title compounds were synthesized by adopting the same procedure with variation in reaction time.

The derivatives formed were recrystallized by using suitable solvents like water and DMSO.

The purity of the product was confirmed by a single spot on the TLC plate and solvent system used was methanol: chloroform (1:1).Melting point was determined and uncorrected.

SCHEME 1:

4-((diethylamino)(sub)methyl)-2-methyloxazol-5(4H)-one

A1 – A6

SCHEME 2:

hippuric acid

Aldehydes

a= acetic anhydride

b=An.sodium acetate

R= Aromatic/Aliphatic
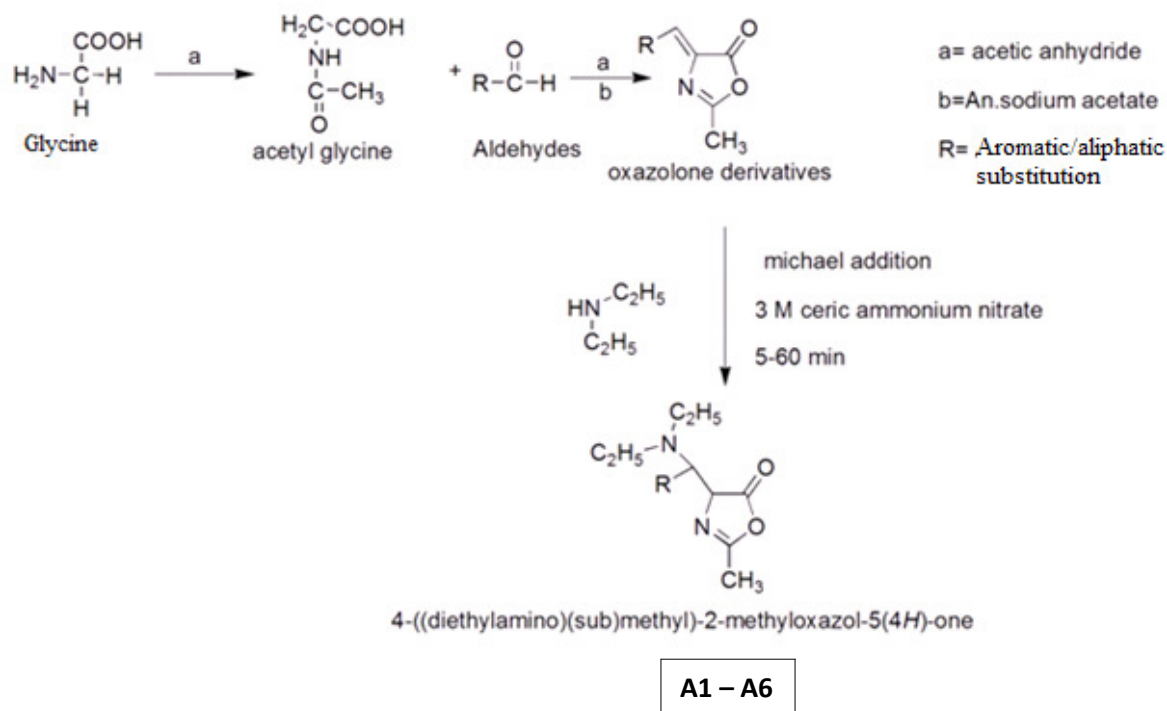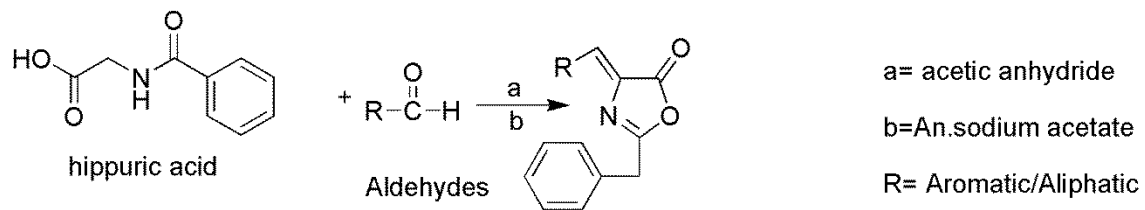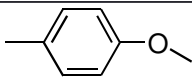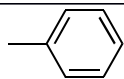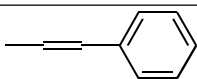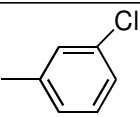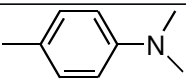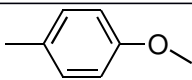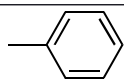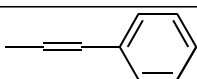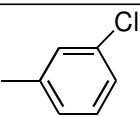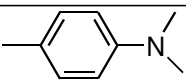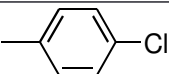
oxazolone derivatives

michael addition

3 M ceric ammonium nitrate

5-60 min
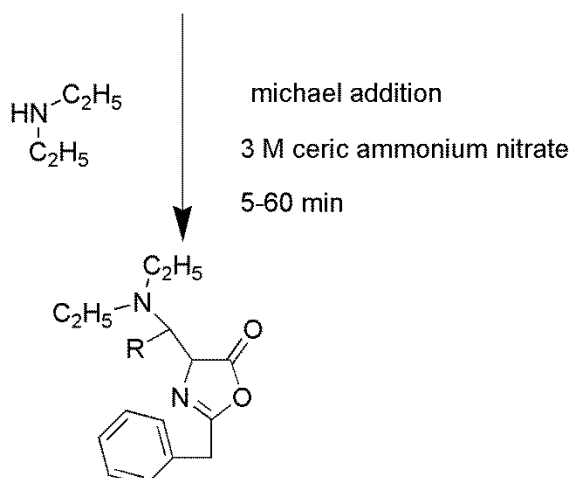
Michael addition product of Oxazol 5 one derivatives

B1-B8

B1 – B8

CODE	R
A1	— CH₃
A2	
A3	
A4	
A5	
A6	
B1	CH₃
B2	
B3	
B4	
B5	
B6	
B7	

B8	

CHARACTERIZATION OF THE SYNTHESIZED COMPOUNDS

MELTING POINT ANALYSIS

Melting points of the synthesized compounds were determined in a one end fused capillary tube method by using Thermonic Model –C-LMP- 1 CAMPVEEL melting point apparatus, and were uncorrected.

THIN LAYER CHROMATOGRAPHY ANALYSIS

Purity of the compounds was checked by TLC using silica gel G (0.5mm thickness) coated over glass plate (12 x 20 cm) as stationary phase, chloroform:methanol (1:1) as mobile phase and the spot was visualized by iodine vapor.

Log P:

The hydrophobic character of a drug can be measured experimentally by testing the drugs relative distribution in an n-octanol/water mixture.

$$P = \frac{concentration\ of\ drug \in octanol}{concentratio\ of\ drug \in aqueous\ solution}$$

 SPECTRAL STUDIES[66,67]
ULTRA VIOLET SPECTRAL ANALYSIS

$$\lambda_{max}$$

The maximum absorbance or   of synthesized compounds determined in the concentration of 0.01% w/v in DMF by using Shimadzu 2000 ultraviolet Spectrophotometer. The maximum absorbance was measured in nm.

INFRARED SPECTRAL ANALYSIS

The IR Spectra of the synthesized compounds were recorded by JASCO-FT/IR -170**0** spectrophotometer in KBr disc. The IR value was measured in cm^{-1}.

NUCLEAR MAGNETIC RESONANCE SPECTRAL ANALYSIS

The NMR Spectra of the synthesized compounds were recorded by Bruker 300 MHz FT- NMR using TMS (Tetramethylsilane) as internal standard. The PMR (Proton Magnetic Resonance) spectroscopic values are measured in δ ppm in DMSO-d$_6$.

MASS SPECTRAL ANALYSIS

The Mass Spectra of the synthesized compounds were recorded in MS (EI) JEOL GC MATE 700 EV.

BIOLOGICAL SCREENING

ANTI-DIABETIC SCREENING OF THE SYNTHESIZED COMPOUNDS BY USING *IN VITRO* ENZYME INHIBITON ASSAY TECHNIQUE

Procedure[68] :

The Aldose reductase suspension was purchased from the sigma-aldrich. The AR activity was spectrophotometrically assayed by measuring the decrease in NADPH absorption at 340 nm over a 4 min period, using DL-glyceraldehyde as a substrate. Each 1.0 mL cuvette containing equal units of enzyme, 0.1M sodium phosphate buffer (pH 6.2) and 0.3 mM NADPH either with or without 10 mM substrate and inhibitor was prepared. One set of mixtures prepared with an equivalent volume of sodium phosphate buffer instead of tested samples was used as control. The concentration of the extracts required to inhibit 50% of AR activity under the assay conditions was defined as the IC50 value. Pioglitazone was used as a standard drug.

$$IC_{50} = (1 - A/B) \times 100$$

A= Absorbance of sample
B= Absorbance of control

IN VITRO ANTI-OXIDANT SCREENING OF SYNTHESIZED COMPOUNDS

➢ In-vitro Anti-oxidant screening of synthesized compounds were done by using three methods

✓ DPPH method

✓ FRAP method

Determination of DPPH (1-1-diphenyl 2-picryl hydrazyl) radical-scavenging activity:

Procedure[69] :

The free radical-scavenging activity of the synthesized compounds were measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH. 0.1 mM solution of DPPH in ethanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in methanol at different concentrations (25-100µg/ml). Thirty minutes later, the absorbance was measured at 517 nm. Ascorbic acid was used as the reference compound. Lower absorbance of the reaction mixture indicated higher free radical-scavenging activity. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula:

$$\% \text{ inhibition} = (A_0 - A_t)/ A_0$$

where A_0 was the absorbance of the control (blank, without compounds) and A_t was the Absorbance in the presence of the compounds. All the tests were performed in triplicate and the graph was plotted with the mean values.

Ferric reducing antioxidant power (FRAP) assay[69] :

FRAP assay is based on the ability of antioxidants to reduce $Fe3+$ to $Fe2+$ in the presence of 2,4,6-tri(2-pyridyl)- s-triazine (TPTZ), forming an intense blue $Fe2+$-TPTZ complex with an absorption maximum at 593 nm. This reaction is pH-dependent (optimum pH 3.6). The

absorbance decrease is proportional to the antioxidant content (Benzie and Strain, 1996). 0.2 ml of the compound is added to 3.8 ml of FRAP reagent (10 parts of 300 mM sodium acetate buffer at pH 3.6, 1 part of 10.0 mM TPTZ solution and 1 part of 20.0 mM $FeCl_3 . 6H_2O$ solution) and the reaction mixture is incubated at 37°C for 30 min and the increase in absorbance at 593 nm is measured. $FeSO_4$ is used for calibration. The antioxidant capacity based on the ability to reduce ferric ions of sample is calculated from the linear calibration curve and expressed as mmol $FeSO_4$ equivalents per gram of sample. BHT, BHA, ascorbic acid, quercetin, catechin or trolox can be used as a positive control.

ANTI-FUNGAL SCREENING OF THE SYNTHESIZED COMPOUNDS BY DISC DIFFUSION METHOD

PROCEDURE

Preparation of Sabourands dextrose broth: [74,75]

Composition of Sabourands dextrose broth

SL.NO	INGREDIENTS	QUANTITY
1	Dextrose	40g
2	peptone	10g
3	water	1000ml

Specified amount of dextrose and peptone was taken along with 1000ml of distilled water in a conical flask and heated in a steam bath to dissolve. The pH was maintained at 7.6 ± 0.2 and sterilized in an autoclave at 15 lb pressure, 120°C for 15 minutes. The sterile medium was poured into the sterile Petri dish and allowed to solidify.

Preparation of plates:

Sabourands dextrose broth medium was prepared and transferred into sterile Petri plates aseptically (thickness of 5-6mm). The plates were allowed to dry at room temp. The plates were inverted to prevent condensate falling on the agar surface. The layers of the medium are uniform in thickness, is done by placing the plates on a leveled surface. Standardized fungal inoculums of *Aspergillus niger, Aspergillus fumigatus, Aspergillus parasiticus, Candida albicans,* were applied to the plates and spreaded uniformly over the surface of medium by using a sterile Non-absorbent cotton swab and finally the swab was passed around the edge of the medium. The inoculated plates were closed with the lid and allowed to dry at room temperature. The sample impregnated discs (10µg /disc) in dimethyl sulphoxide and standard Clotrimazole 10µg/disc were placed on the inoculated agar medium. All petriplates were incubated at 27˚C -28˚C for 24 hrs. After the incubation diameter of zone of inhibition produced by the sample were measured.

Determination of Minimum Inhibitory Concentration for Synthesized Compounds (MIC) by Serial dilution method:

❖ The serial dilutions of known concentration of compound solution are made from the stock (100 mg/ml) by using Sabourands dextrose broth using the method described below.

❖ The tubes were labeled 1 to 8 and 1 ml of Sabourands dextrose broth were added to the first 5 tubes and 8th tube, then added 0.5ml of Sabourands dextrose broth to 6th and 7th tubes.

❖ One ml of different synthesized compounds was added to the 1st tube, mixed and transfer 1ml serially up to tube 5.From the 5th tube transfer 1ml to 6th tube. Mixed and transfer 0.5 ml to the 7th tube. Each tube, 1 to 7 contains 1ml diluted extract.

❖ The 8th tube was the control.

❖ With a standardized micro pipette, add a drop of the diluted broth culture approximately 0.01ml of the test organism to all tubes, including the control, gently mixed and incubated at 26-28 ^{0} c for 18 to 24hrs.

❖ The highest dilution of particular compounds showing no turbidity was observed and recorded. This was taken as the end point, and this dilution was considered to contain the concentration of drug equivalent to MIC.

CHARECTERIZATION OF SYNTHESIZED COMPOUNDS
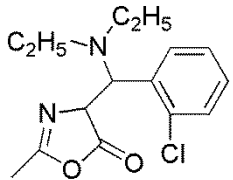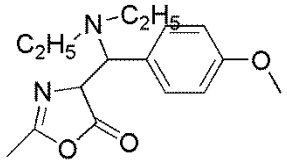
Table 4: PHYSICOCHEMICAL PARAMETERS OF SYNTHESIZED DERIVATIVES
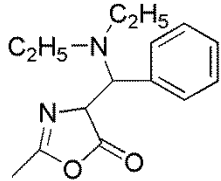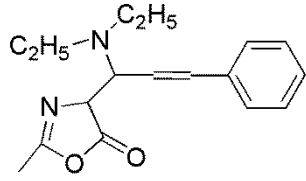
CODE	MOLECULAR FORMULA	MOLECULAR Wt (g)	MP (^{0}C)	% YIELD	Rf	log P	COLOUR	SOLUBILITY
A1	$C_{10}H_{18}N_2O_2$	198	91 – 93	45%	0.7	0.83	Pale yellow	water
A2	$C_{16}H_{22}N_2O_3$	290	220 – 222	38.4%	0.62	2.92	yellow	water
A3	$C_{15}H_{20}N_2O_2$	260	216 - 218	51%	0.81	2.24	Pale yellow	water
A4	$C_{17}H_{22}N_2O_2$	286	191 – 193	61%	0.54	2.76	white	water
A5	$C_{15}H_{19}ClN_2O_2$	294	216 – 218	48%	0.62	2.8	green	water
A6	$C_{17}H_{25}N_3O_2$	303	241 – 243	57%	0.68	2.35	black	water
B1	$C_{16}H_{22}N_2O_2$	274	185 – 187	68%	0.5	2.69	white	DMSO
B2	$C_{22}H_{26}N_2O_3$	369	231 – 234	65%	0.78	3.96	yellow	DMSO
B3	$C_{21}H_{24}N_2O_2$	336	246 – 248	48%	0.6	4.08	Pale green	DMSO
B4	$C_{23}H_{26}N_2O_2$	362	238 – 240	52.5%	0.74	4.6	orange	DMSO
B5	$C_{22}H_{26}ClN_2O_2$	385	276 – 279	74%	0.84	4.64	green	DMSO
B6	$C_{22}H_{26}ClN_2O_2$	385	269 – 272	69%	0.48	4.45	green	DMSO
B7	$C_{23}H_{29}N_3O_2$	379	274 – 276	89%	0.81	3.73	red	DMSO
B8	$C_{21}H_{24}N_2O_3$	352	283 – 285	56%	0.52	3.69	brown	DMSO
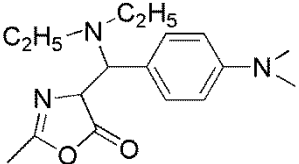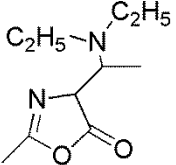
*Mobile phase – methanol: chloroform (1:1)
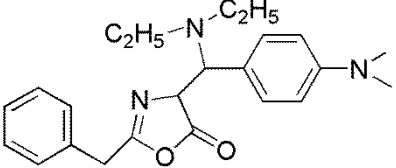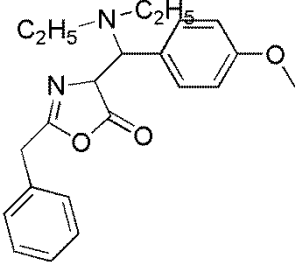
SPECTRAL DATA
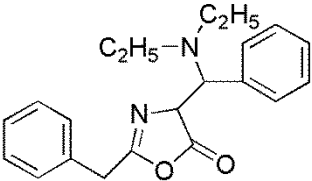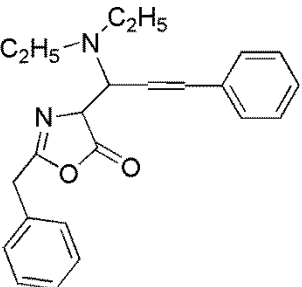
Table 5: SPECTRAL DATA OF THE SYNTHESIZED COMPOUNDS
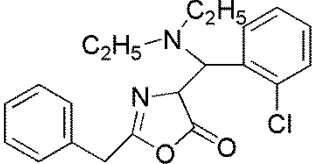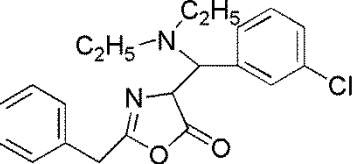
Comp. code	Molecular structure	(IR) υ_{max} (KBr/cm^{-1})	NMR (δ ppm)	MASS (m/z, amu)
A6	4-((diethylamino)(2-chlorophenyl)methyl)-2-methyloxazol-5(4H)-one	1599(C=C ar) 840(di sub ben) 1240.04(c-o-c str) 1400.04 (methyl) 1542.1(C=N)	0.9(s, 3H, CH3) 2.4-2.9(t, 2H, CH2) 2.8(d, 1H, CH) 1.0(t, 4H, CH3) 6.5-6.9(d,4H, Ar)	
A2	4-((diethylamino)(4-methoxyphenyl)methyl)-2-methyloxazol-5(4H)-one	1602(C=C ar) 1266.04(c-o-c str) 1401.04 (methyl) 1596(C=N) 1725(keto)		
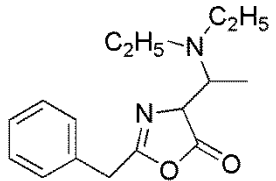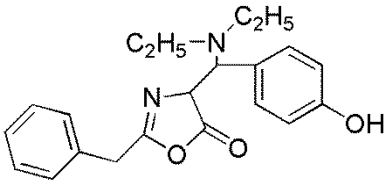
Comp. code	Molecular structure	(IR) υ_{max} (KBr/cm^{-1})	NMR (δ ppm)	MASS (m/z, amu)
A3	4-((diethylamino)(phenyl)methyl)-2-methyloxazol-5(4H)-one	1602(C=C ar) 696(mono sub ben) 1266.04(c-o-c str) 1401.04 (methyl) 1788.65(ketone) 1552.1(C=N)		
A4	4-(1-(diethylamino)-3-phenylallyl)-2-methyloxazol-5(4H)-one	1604(C=C ar) 690 (mono sub ben) 1294.97 (c-o-c str) 1400 (methyl) 1793.47(ketone) 1650.77(C=C ali) 1502.1(C=N)		

Comp. code	Molecular structure	(IR) υ_{max} (KBr/cm^{-1})	NMR (δ ppm)	MASS (m/z, amu)
A5	4-((diethylamino)(4-(dimethylamino)phenyl)methyl)-2-methyloxazol-5(4H)-one	1602(C=C ar) 860(di sub ben) 1266.04(c-o-c str) 1401.04 (methyl) 1450.01(C-Cl) 1562.1(C=N)		
A1	4-(1-(diethylamino)ethyl)-2-methyloxazol-5(4H)-one	1597(C=C ar) 1292.04(c-o-c str) 1399(methyl) 1597.3(C=N) 1640(ketone)		

Comp. code	Molecular structure	(IR) υ_{max} (KBr/cm^{-1})	NMR (δ ppm)	MASS (m/z, amu)
B7	4-((diethylamino)(4-(dimethylamino)phenyl)methyl)-2-benzyloxazol-5(4H)-one	1638(C=C) 1262(C-O-C) 1401 (CH3) 1638(C=N) 1785(keto) 3128(Ar-H) 831(di sub ben)	2.4-2.9(t, 3H, CH2) 2.8(d, 1H, CH) 1.0(t, 4H, CH3) 6.5-6.9(d,4H, Ar)	Mol. Ion 378.2 Base peak 252
B2	4-((diethylamino)(4-methoxyphenyl)methyl)-2-benzyloxazol-5(4H)-one	1265(C-O-C) 1590.1(C=N) 1400.01(OCH3) 1664(keto) 830.31(di sub ben)		Mol. ion 369 Base peak 252

Comp. code	Molecular structure	(IR) υ_{max} (KBr/cm⁻¹)	NMR (δ ppm)	MASS (m/z, amu)
B3	4-((diethylamino)(phenyl)methyl)-2-benzyloxazol-5(4H)-one	1161.85(C-O-C) 1552.1(C=N) 1400.01(CH3) 1642(keto) 697(mono sub ben)	2.4-2.9(t, 2H, CH2) 3.4(d, 1H, CH) 0.897(t, 2H, CH3) 7.0-8.5(d,10H, Ar) 2.6(d,1H,CH2)	
B4	2-benzyl-4-(1-(diethylamino)-3-phenylallyl)oxazol-5(4H)-one	1298.85(C-O-C) 1550.1(C=N) 1650(keto) 691(mono sub ben)		

Comp. code	Molecular structure	(IR) υ_{max} (KBr/cm^{-1})	NMR (δ ppm)	MASS (m/z, amu)
B5	4-((diethylamino)(2-chlorophenyl)methyl)-2-benzyloxazol-5(4H)-one	1298(C-O) 1592.1(C=N) 1402(C-Cl) 1794(keto) 863(di sub ben) 690(mono sub ben)		
B6	4-((diethylamino)(3-chlorophenyl)methyl)-2-benzyloxazol-5(4H)-one	1282(C-O-C) 1547.1(C=N) 1794(keto) 862(di sub ben) 1436.07(C-Cl)	3.4(t, 2H, CH2) 2.8(d, 1H, CH) 1.91(t, 2H, CH3) 7.0-8.0(d,9H, Ar)	

Comp. code	Molecular structure	(IR) υ_{max} (KBr/cm^{-1})	NMR (δ ppm)	MASS (m/z, amu)
B1	2-benzyl-4-(1-(diethylamino)ethyl)oxazol-5(4H)-one	1264.85(C-O-C) 1552.1(C=N) 1400.01(CH3) 1648(keto) 693.31(mono sub ben)		
B8	4-((diethylamino)(4-hydroxyphenyl)methyl)-2-benzyloxazol-5(4H)-one	1208.85(C-O-C) 1602(C=N) 1652(keto) 831(di sub ben) 3120(OH)		

IR Spectroscopy of A1:

4-(1-(diethylamino)ethyl)-2-methyloxazol-5(4*H*)-one

Peaks labelled on spectrum:
- 3353.6 cm-1
- 3125.08 cm-1
- 1723.09 cm-1
- 1619.91 cm-1
- 1400.07 cm-1
- 601.682 cm-1

%T vs Wavenumber [cm-1]

IR Spectroscopy of IR Spectroscopy of A2:

4-((diethylamino)(4-methoxyphenyl)methyl)-2-methyloxazol-5(4*H*)-one

100

%T

80 3848.26 cm-1

60

40

30

3350.71 cm-1

3127.01 cm-1

1725.98 cm-1

1596.77 cm-1

1400.07 cm-1

1223.08 cm-1

994.125 cm-1

684.606 cm-1
625.788 cm-1

4000   3000   2000   1000   400

Wavenumber [cm-1]

IR Spectroscopy of A3

Select All

Comment...

4-((diethylamino)(phenyl)methyl)-2-methyloxazol-5(4*H*)-one

%T

3351.68 cm-1

3125.08 cm-1

1722.12 cm-1

1590.99 cm-1

276.65 cm-1

237.11 cm-1

994.125 cm-1

685.57 cm-1

624.823 cm-1

Wavenumber [cm-1]

IR Spectroscopy of A4:

4-(1-(diethylamino)-3-phenylallyl)-2-methyloxazol-5(4*H*)-one

IR Spectroscopy of A5:

4-((diethylamino)(2-chlorophenyl)methyl)-2-methyloxazol-5(4*H*)-one

IR Spectroscopy of A6:

4-((diethylamino)(4-(dimethylamino)phenyl)methyl)-2-methyloxazol-5(4H)-one

100

80

%T

60

40

30

4000   3000   2000   1400.07 cm-1   1000   400

Wavenumber [cm-1]

411.728 cr

669.178 cm-1

1599.66 cm-1

3127.01 cm-1

IR Spectroscopy of B1

4-((diethylamino)(4-methoxyphenyl)methyl)-2-benzyloxazol-5(4H)-one

%T

260

200

100

0

3836.68 cm-1
3746.05 cm-1

3445.21 cm-1

1787.69 cm-1
1400.07 cm-1
0.95 cm-1

1655.73 cm-1
1593.56 cm-1

1265.07 cm-1

1160.94 cm-1

1310.39 cm-1
1127.87 cm-1

981.69 cm-1

830.205 cm-1

693.284 cm-1
549.613 cm-1

463.656 cm-1

4000   3000   2000   1000   400

Wavenumber [cm-1]

IR Spectroscopy of B2:

4-((diethylamino)(2-chlorophenyl)methyl)-2-benzyloxazol-5(4H)-one

863.953 cm-1

1794.44 cm-1

1402 cm-1

1650.77 cm-1

487.902 cm-1

692.32 cm-1

3836.68 cm-1

3746.05 cm-1

%T

Wavenumber [cm-1]

IR Spectroscopy of B3:

4-((diethylamino)(3-chlorophenyl)methyl)-2-benzyloxazol-5(4H)-one

IR Spectroscopy of B4:

4-((diethylamino)(4-(dimethylamino)phenyl)methyl)-2-benzyloxazol-5(4H)-one

IR Spectroscopy of B5:

4-((diethylamino)(2-chlorophenyl)methyl)-2-benzyloxazol-5(4H)-one

100

80

%T

60

40

3127.01 cm-1

1788.65 cm-1

1652.7 cm-1

1602.5 cm-1

1030.77 cm-1

696.177 cm-1

1161.9 cm-1

1266.04 cm-1

1401.03 cm-1

4000   3000   2000   1000   400

Wavenumber [cm-1]

IR Spectroscopy of B6:

4-((diethylamino)(3-chlorophenyl)methyl)-2-benzyloxazol-5(4H)-one

1785 cm-1

1638 cm-1

1030 cm-1

831.169 cm-1

696.177 cm-1

1161.9 cm-1

1401.03 cm-1

3127.97 cm-1

IR Spectroscopy of B7:

4 ((diethylamino)(4 (dimethylamino)phenyl)methyl) 2 benzyloxazol 6(4H) one

3128.1 cm-1

1785 cm-1

1638 cm-1

1030 cm-1

831 169 cm-1

696.177 cm-1

1161.9 cm-1

1401.03 cm-1

IR Spectroscopy of B8:

4-((diethylamino)(4-hydroxyphenyl)methyl)-2-benzyloxazol-5(4H)-one

%T

100

80

60

40

3127.01 cm-1

1788.65 cm-1
1652.7 cm-1
1602.5 cm-1

1030.77 cm-1
696.177 cm-1
1161.9 cm-1
1266.04 cm-1

1401.03 cm-1

Wavenumber [cm-1]

4000   3000   2000   1000   400

NMR spectra of Compound A6:

A6

4-((diethylamino)(4-(dimethylamino)phenyl)methyl)-2-methyloxazol-5(4H)-one

Current Data Parameters
NAME   11_guptajulani
EXPNO   1
PROCNO   1

F2 - Acquisition Parameters
Date_   20120111
Time   11.43
INSTRUM   spect
PROBHD   5 mm PABBO BB-
PULPROG   zg30
TD   25772
SOLVENT   DMSO
NS   16
DS   2
SWH   6443.299 Hz
FIDRES   0.250012 Hz
AQ   1.9999572 sec
RG   203
DW   77.600 usec
DE   6.50 usec
TE   298.2 K
D1   1.00000000 sec
TD0   1

======== CHANNEL f1 ========
NUC1   1H
P1   14.10 usec
PL1   -3.00 dB
PL1W   13.42244530 W
SFO1   400.2330311 MHz

F2 - Processing parameters
SI   32768
SF   400.2300095 MHz
WDW   EM
SSB   0
LB   0.30 Hz
GB   0
PC   1.00

A6

9.660
8.647
7.860
7.857
7.853
7.845
7.840
7.836
7.693
7.686
7.681
7.668
7.663
7.657
7.550
7.531
7.525
7.517
7.513
7.510
7.484
7.481
7.469
7.465
7.448
7.444
6.800
6.793
6.789
6.776
6.771
6.764

3.856
3.842

3.298
3.036
2.499
2.495
2.490
2.485
2.481

0.897

11   10   9   8   7   6   5   4   3   2   1   ppm

0.94   0.45   0.97 2.00 0.48 0.98   2.12   0.99   6.35

A6

9.660

8.647

7.860
7.857
7.853
7.845
7.840
7.836
7.693
7.686
7.681
7.668
7.663
7.657
7.550
7.531
7.525
7.517
7.513
7.510
7.484
7.481
7.469
7.465
7.448
7.444

6.800
6.793
6.789
6.776

| 9.5 | 9.0 | 8.5 | 8.0 | 7.5 | 7.0 | ppm |

0.94

0.45

0.97

2.00

0.48
0.98

2.12

A6

3.856
3.842

3.298

3.036

4.0   3.9   3.8   3.7   3.6   3.5   3.4   3.3   3.2   3.1   3.0   ppm

0.99

6.35

NMR spectra of Compound B3:

B3

4-((diethylamino)(phenyl)methyl)-2-benzyloxazol-5(4H)-one

BRUKER

```
Current Data Parameters
NAME      11_guptajulani
EXPNO                  2
PROCNO                 1

F2 - Acquisition Parameters
Date_           20120111
Time               11.46
INSTRUM            spect
PROBHD     5 mm PABBO BB-
PULPROG             zg30
TD                 25772
SOLVENT             DMSO
NS                    48
DS                     2
SWH          6443.299 Hz
FIDRES      0.250012 Hz
AQ          1.9999572 sec
RG                   203
DW            77.600 usec
DE             6.50 usec
TE              298.1 K
D1        1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1            14.10 usec
PL1           -3.00 dB
PL1W      13.42244530 W
SFO1     400.2330311 MHz

F2 - Processing parameters
SI                 32768
SF       400.2300094 MHz
WDW                   EM
SSB        0
LB              0.30 Hz
GB         0
PC              1.00
```

B3

B3
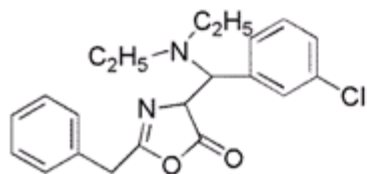
1.168
1.150
1.131
1.109

0.897

1.30   1.25   1.20   1.15   1.10   1.05   1.00   0.95   0.90   0.85   ppm

0.74   0.83   0.28   0.38

B3

8.319 8.314 8.300 8.296 8.149 8.147 8.134 8.129 8.126 7.984 7.976 7.965 7.959 7.955 7.867 7.850 7.749 7.746 7.743 7.733 7.728 7.722 7.712 7.709 7.706 7.659 7.655 7.642 7.639 7.625 7.621 7.619 7.594 7.589 7.575 7.564 7.560 7.557 7.553 7.543 7.533 7.528 7.523 7.519 7.516 7.510 7.506 7.494 7.476 7.451 7.436 7.432 7.381 7.359 7.343

8.5   8.4   8.3   8.2   8.1   8.0   7.9   7.8   7.7   7.6   7.5   7.4   7.3   7.2   7.1 ppm

1.00   1.03   0.63   0.26   0.72   1.34   3.09   1.05

NMR spectra of Compound B6:

B6

4-((diethylamino)(3-chlorophenyl)methyl)-2-benzyloxazol-5(4H)-one
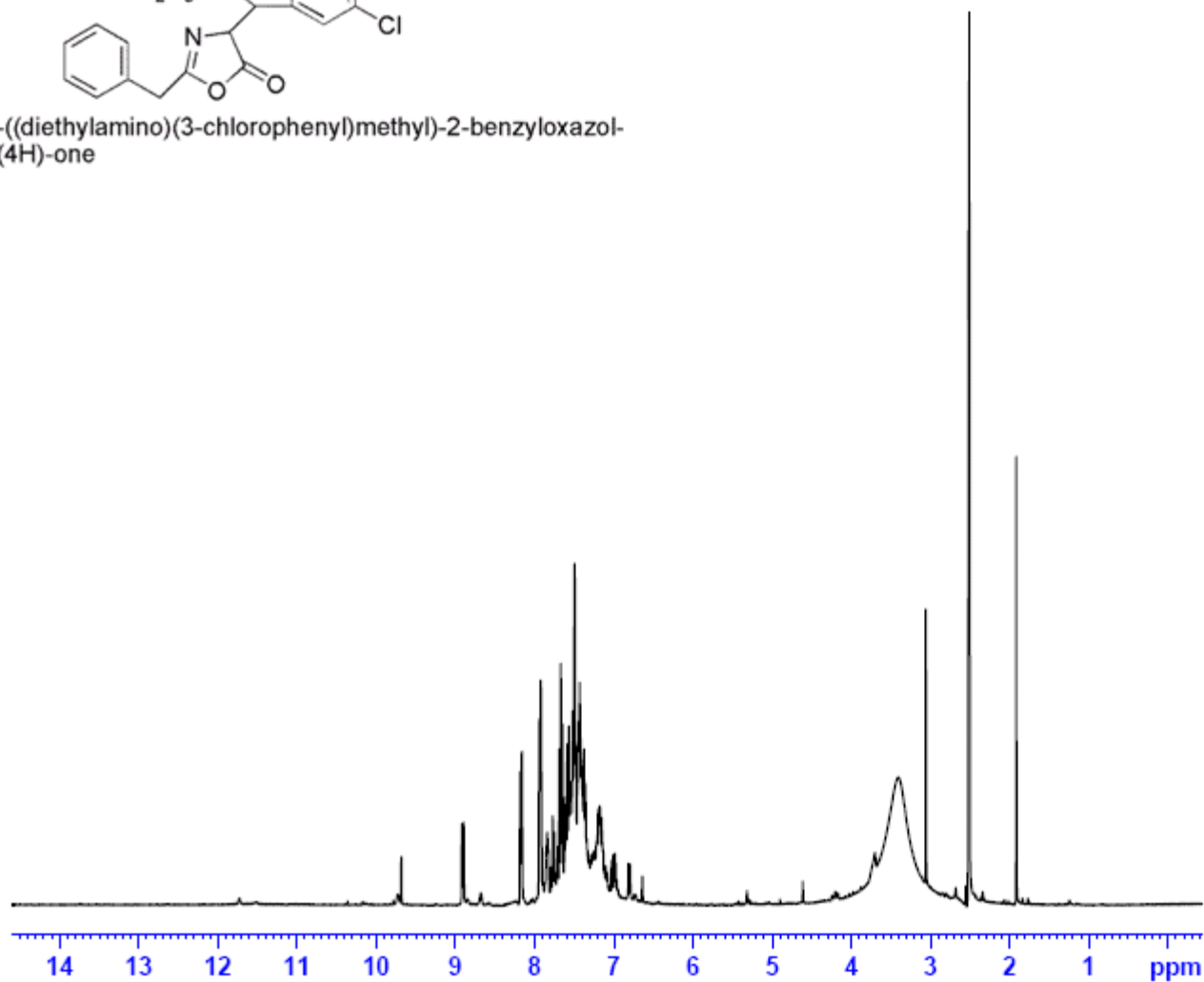
BRUKER

```
Current Data Parameters
NAME        11_guptajulani
EXPNO                     4
PROCNO                    1

F2 - Acquisition Parameters
Date_              20120111
Time                  11.53
INSTRUM               spect
PROBHD      5 mm PABBO BB-
PULPROG                zg30
TD                    25772
SOLVENT                DMSO
NS                       80
DS                        2
SWH             6443.299 Hz
FIDRES          0.250012 Hz
AQ            1.9999572 sec
RG                      203
DW               77.600 usec
DE                6.50 usec
TE                  297.8 K
D1          1.00000000 sec
TDO                       1

======== CHANNEL f1 ========
NUC1                     1H
P1               14.10 usec
PL1               -3.00 dB
PL1W         13.42244530 W
SFO1         400.2330311 MHz

F2 - Processing parameters
SI                    32768
SF          400.2300000 MHz
WDW                      EM
SSB        0
LB                  0.30 Hz
GB         0
PC                     1.00
```
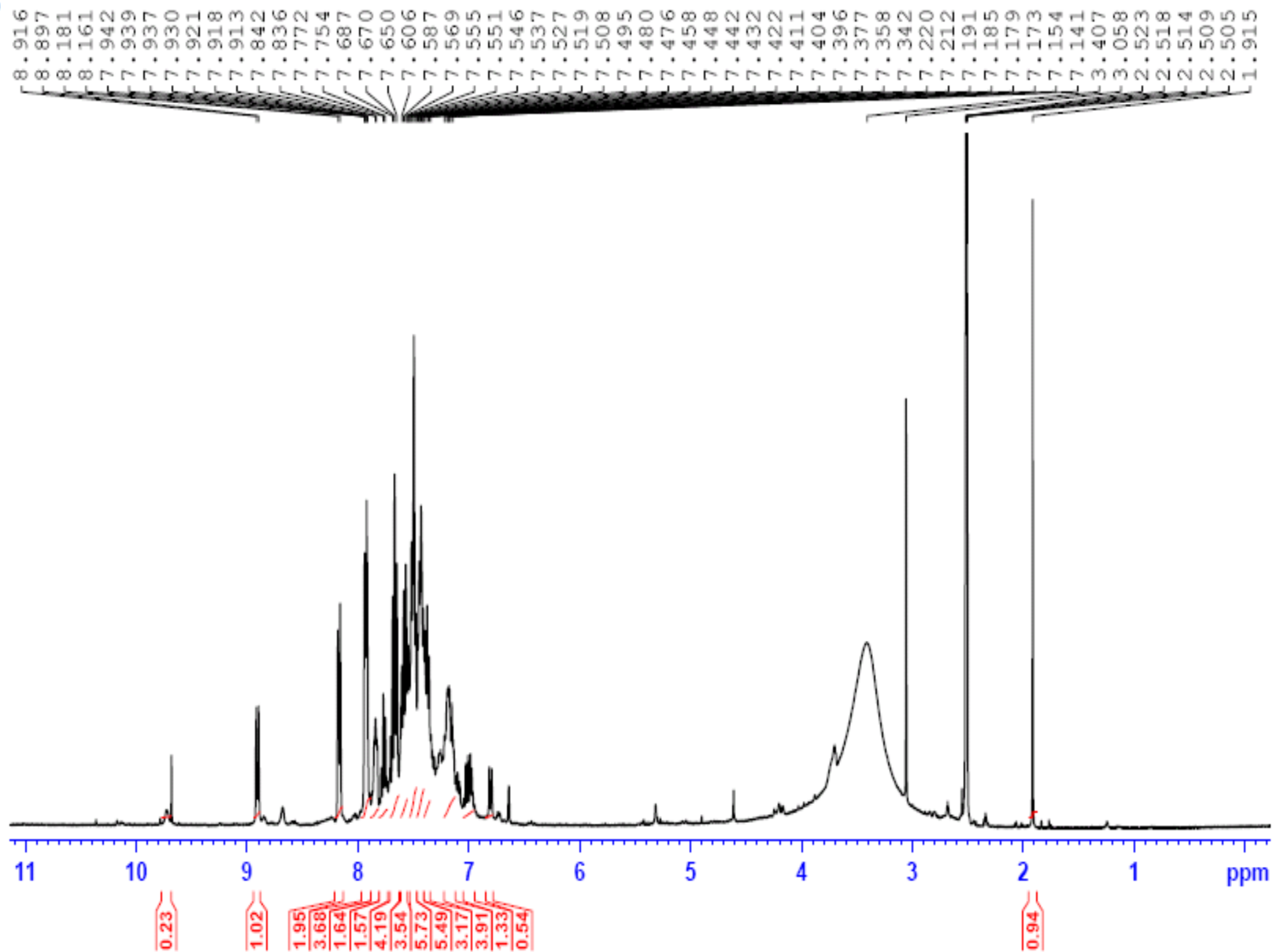
B6
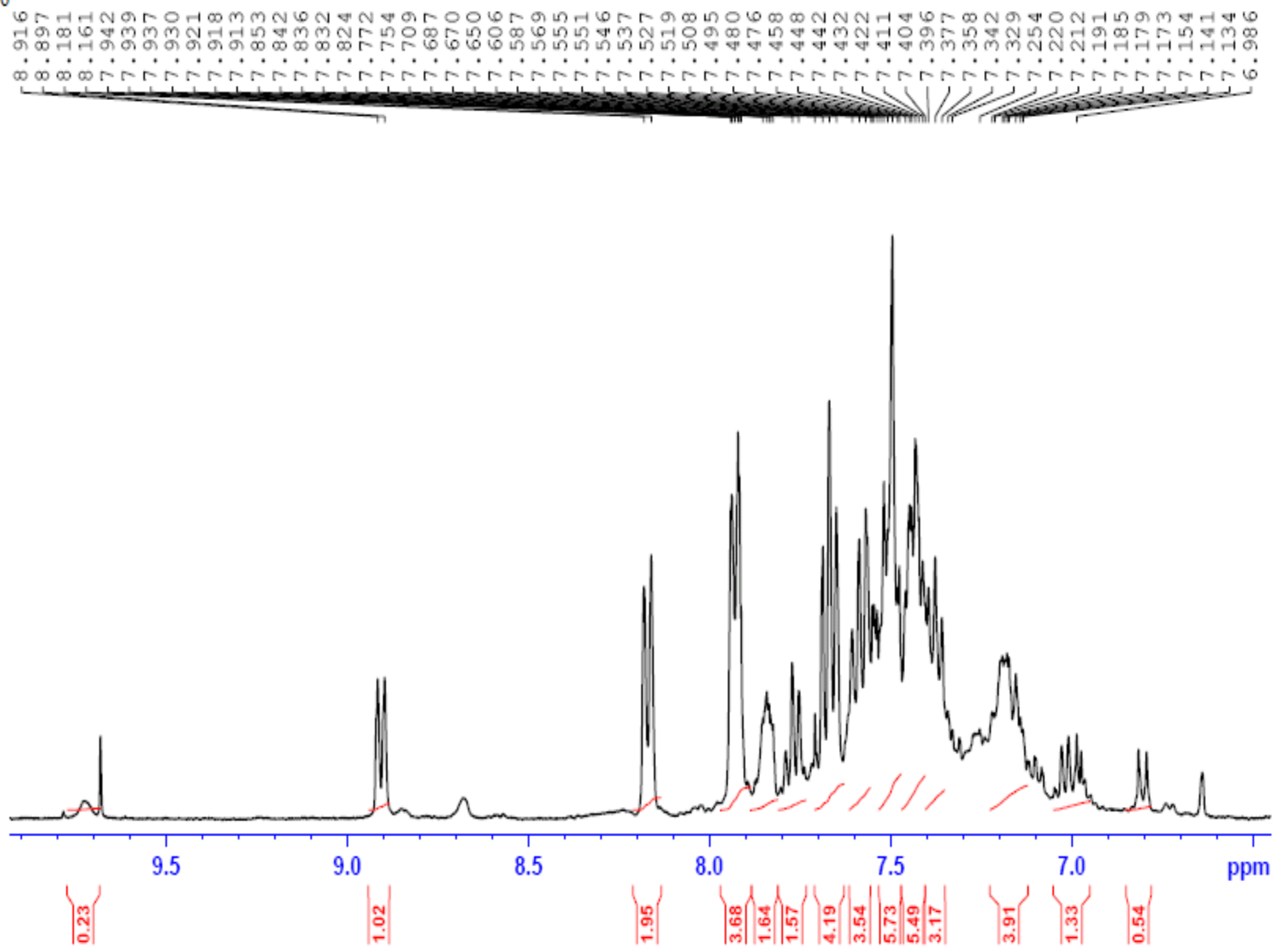
B6
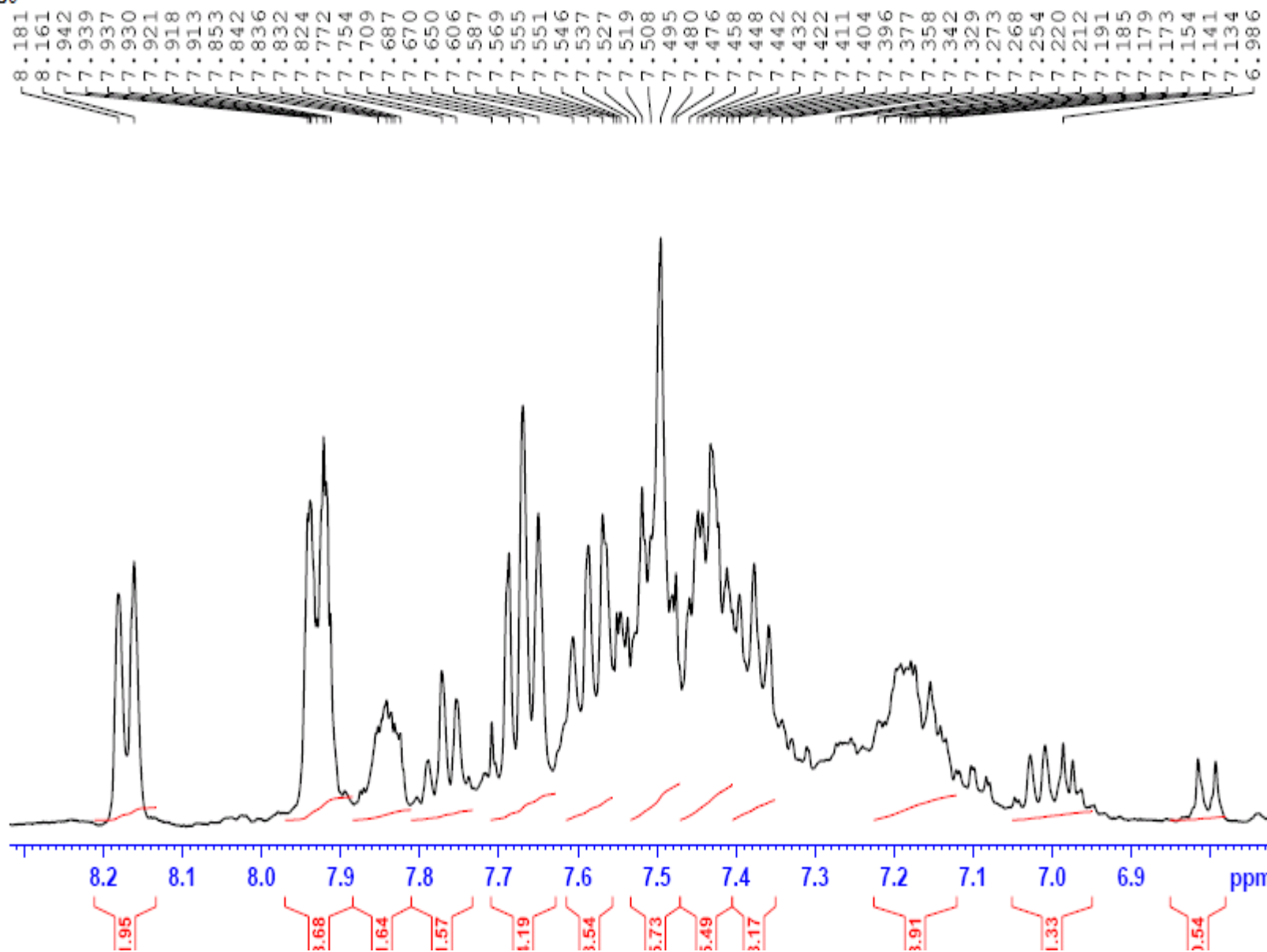
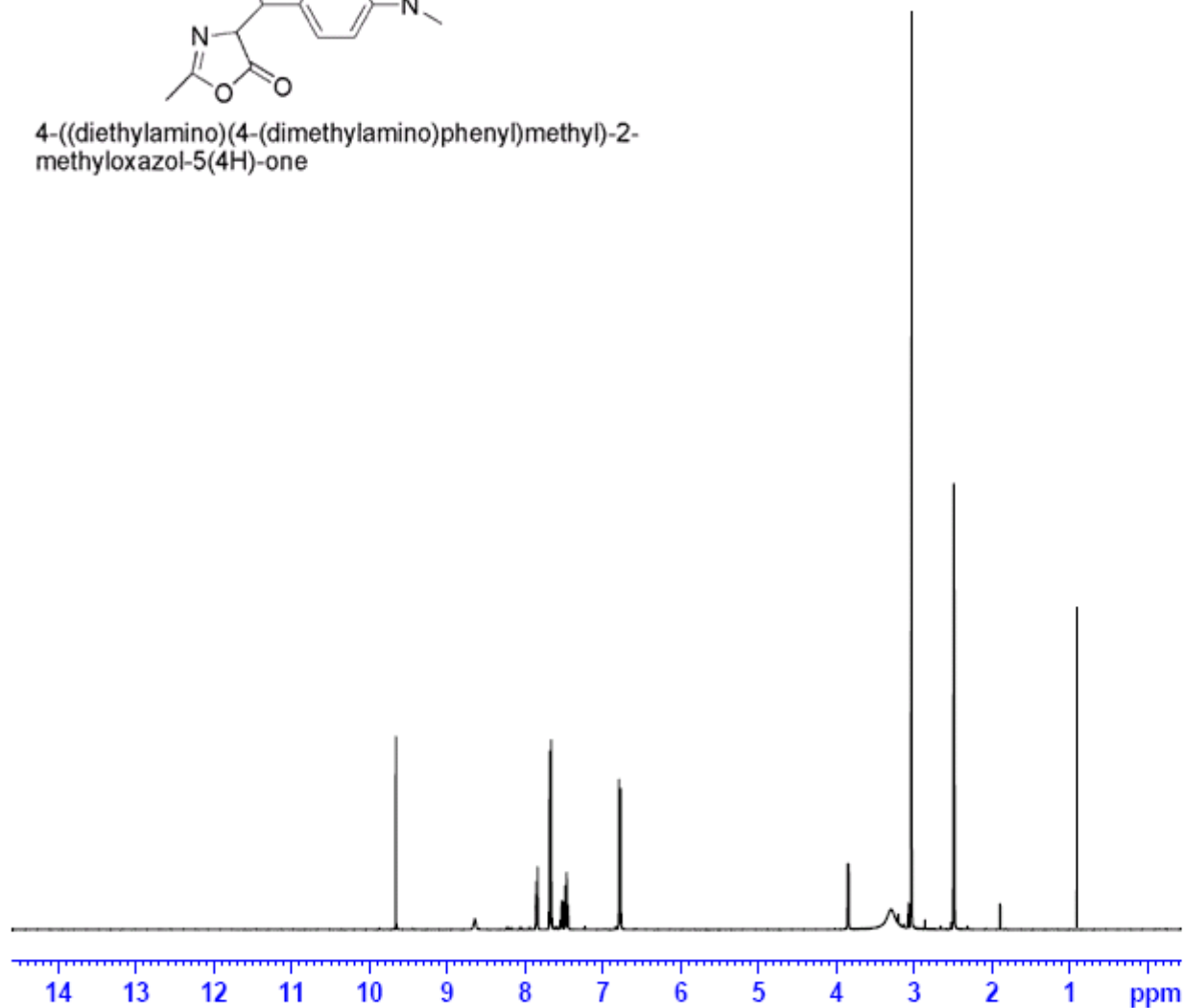## NMR spectra of Compound B7:

B7

4-((diethylamino)(4-(dimethylamino)phenyl)methyl)-2-
benzyloxazol-5(4H)-one

BRUKER

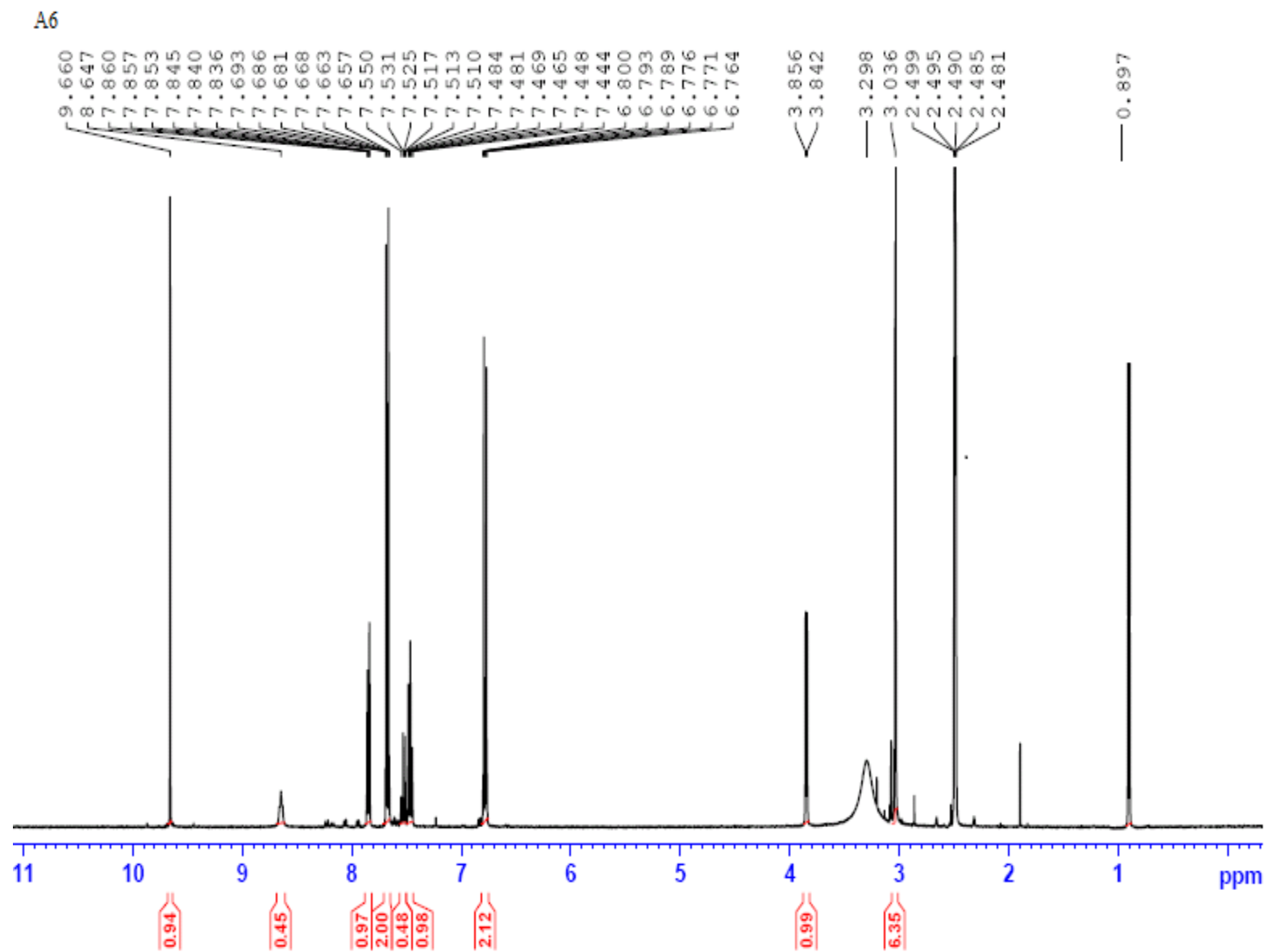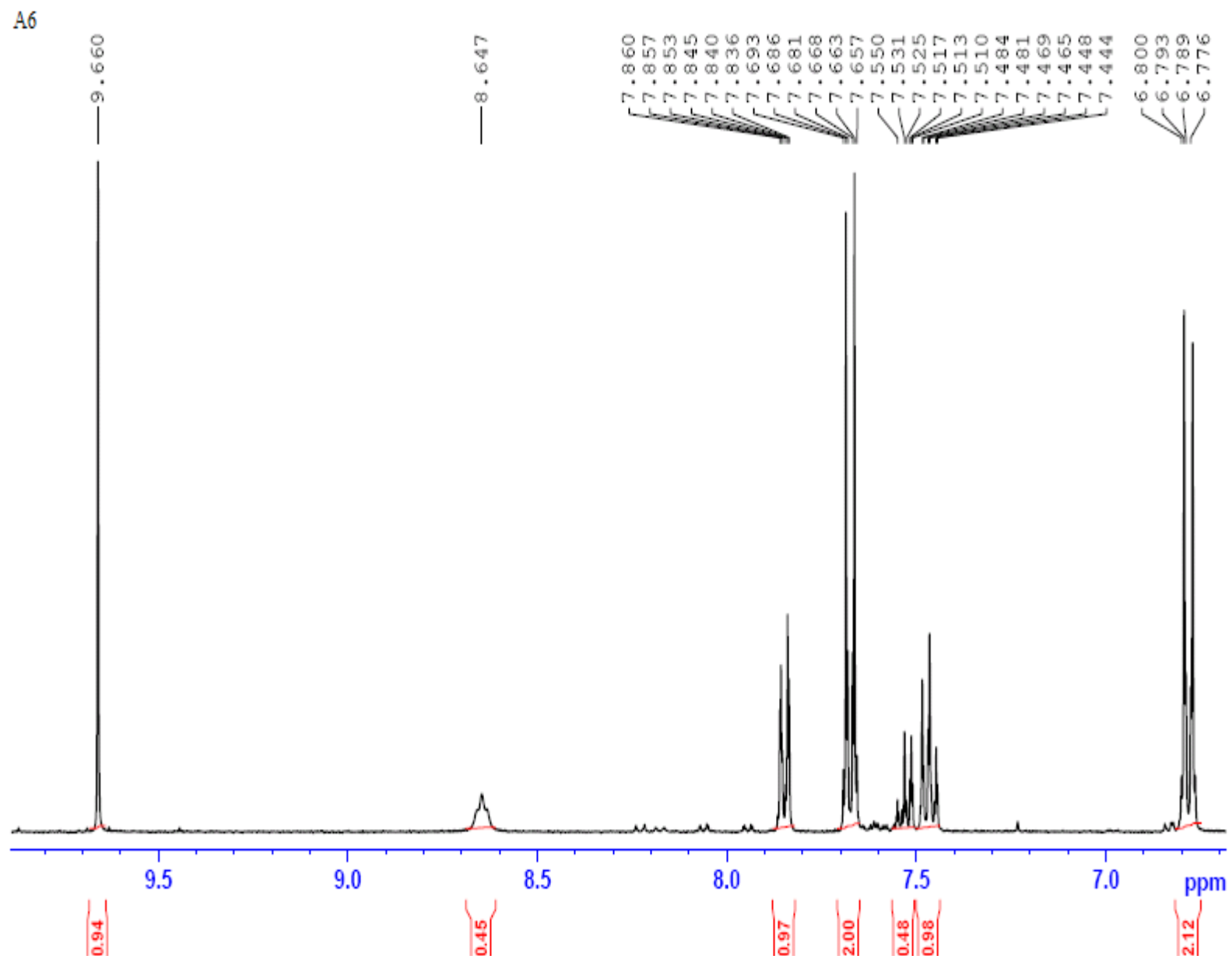Current Data Parameters
NAME   11_guptajulani
EXPNO   4
PROCNO   1

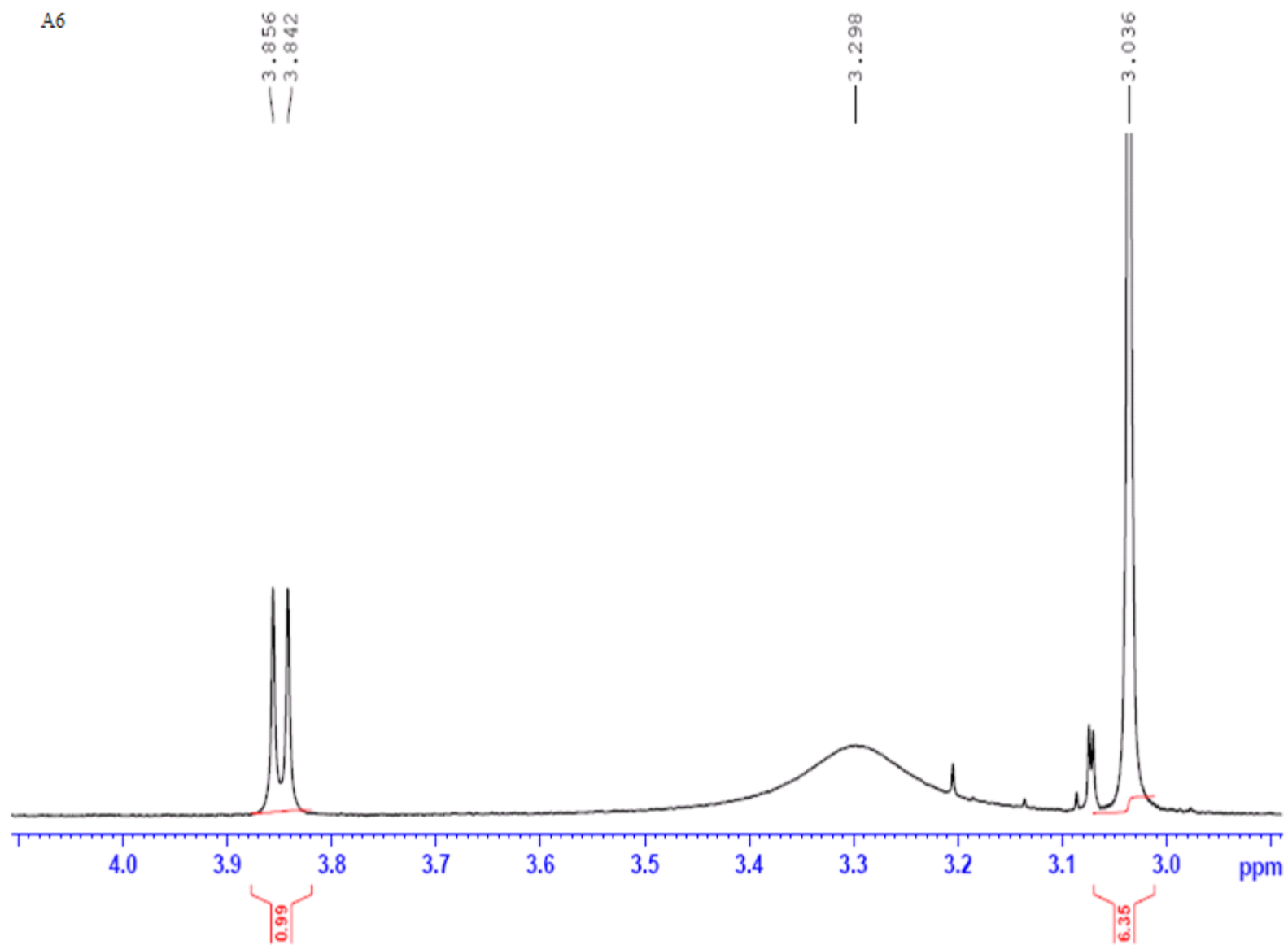F2 - Acquisition Parameters
Date_   20120111
Time   11.53
INSTRUM   spect
PROBHD   5 mm PABBO BB-
PULPROG   zg30
TD   25772
SOLVENT   DMSO
NS   80
DS   2
SWH   6443.299 Hz
FIDRES   0.250012 Hz
AQ   1.9999572 sec
RG   203
DW   77.600 usec
DE   6.50 usec
TE   297.8 K
D1   1.00000000 sec
TD0   1

======== CHANNEL f1 ========
NUC1   1H
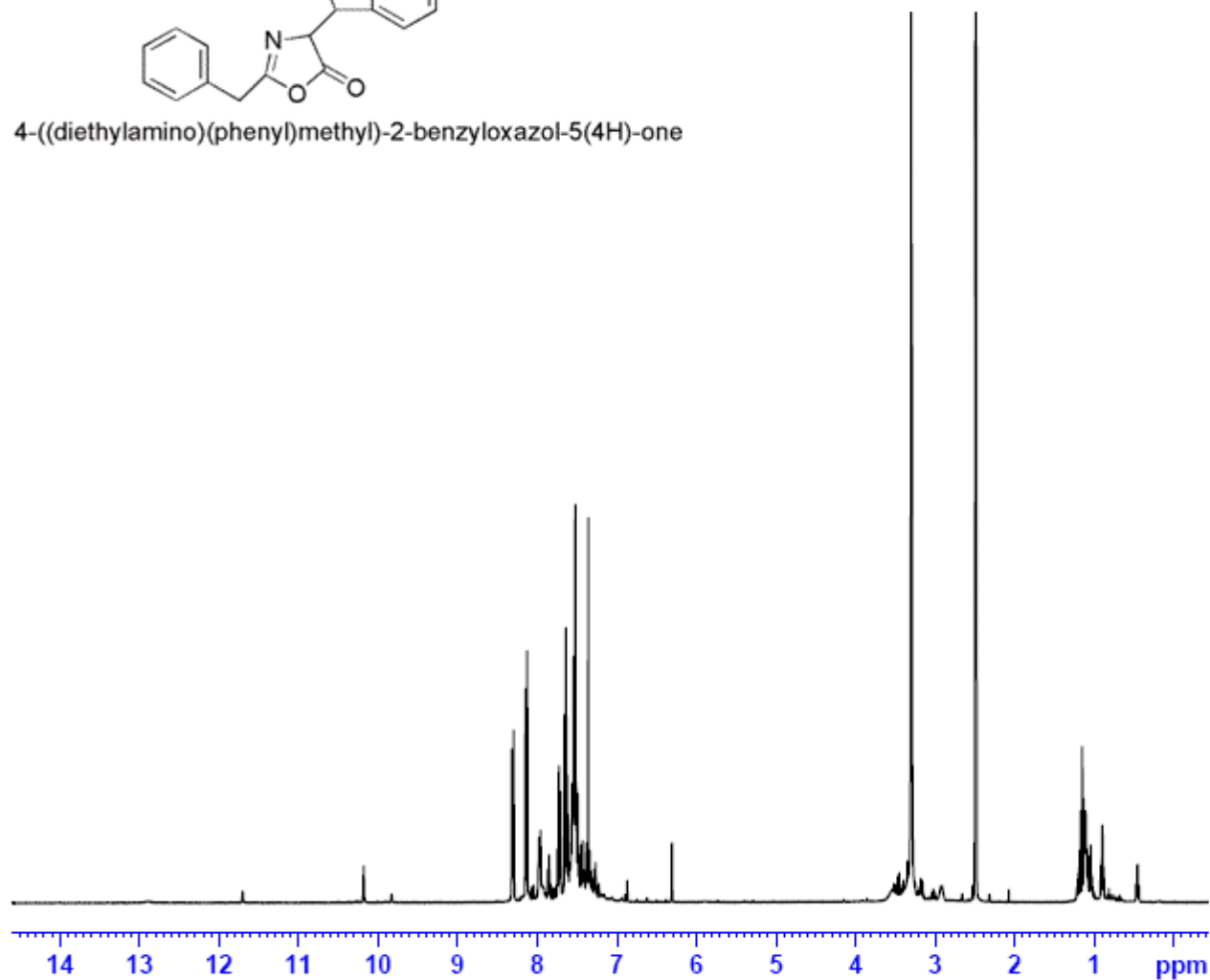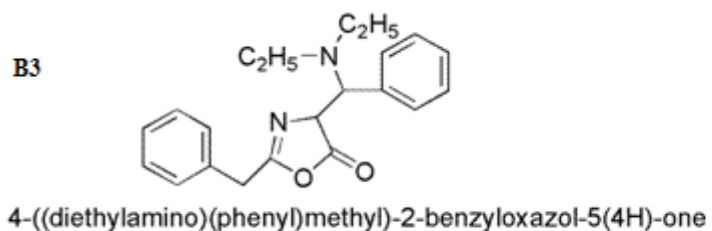P1   14.10 usec
PL1   -3.00 dB
PL1W   13.42244530 W
SFO1   400.2330311 MHz

F2 - Processing parameters
SI   32768
SF   400.2300000 MHz
WDW   EM
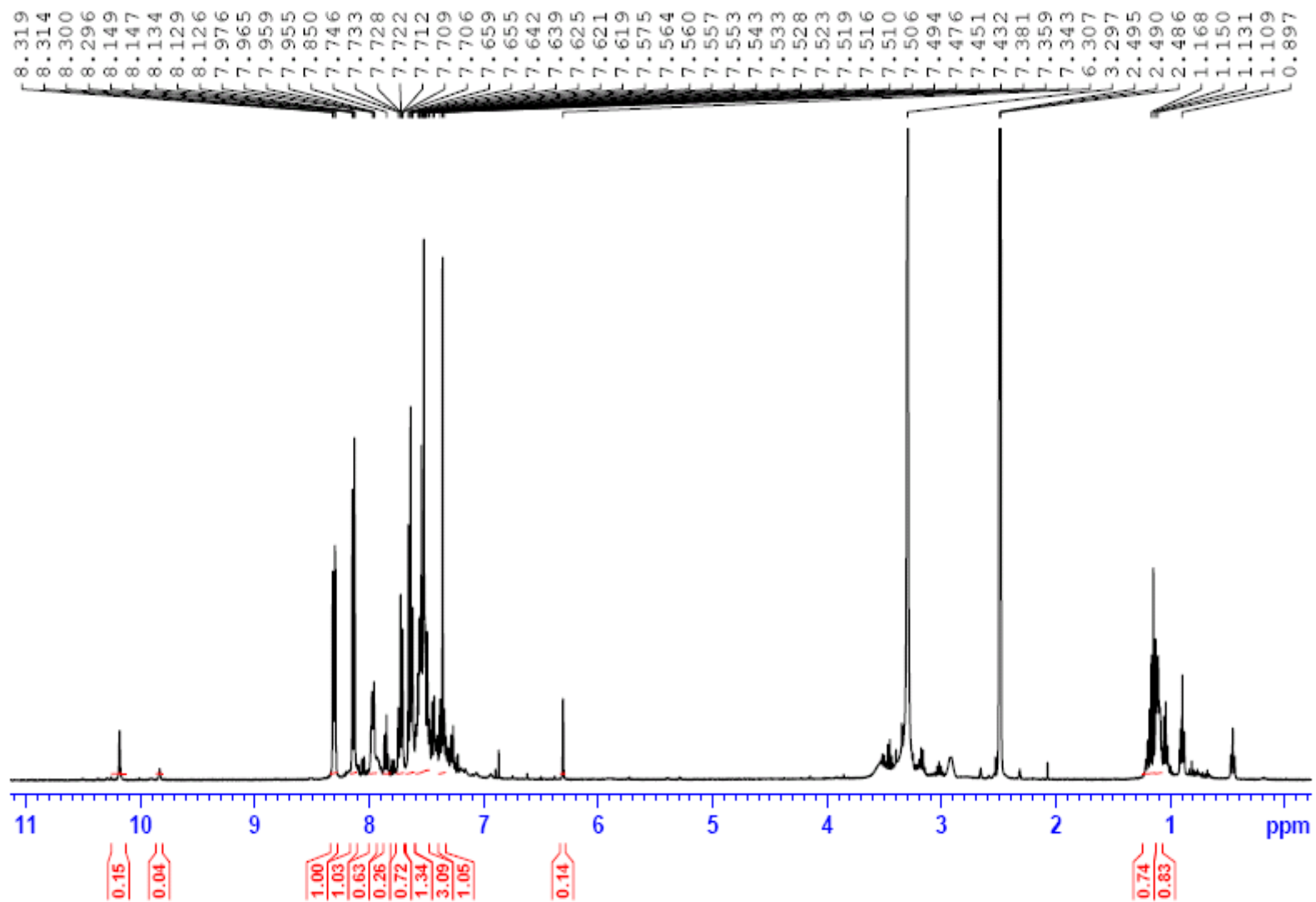SSB   0
LB   0.30 Hz
GB   0
PC   1.00

14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm

8.916
8.897
8.181
8.161
7.942
7.939
7.937
7.930
7.921
7.918
7.913
7.842
7.836
7.772
7.754
7.687
7.670
7.650
7.606
7.587
7.569
7.555
7.551
7.546
7.537
7.527
7.519
7.508
7.495
7.480
7.476
7.458
7.448
7.442
7.432
7.422
7.411
7.404
7.396
7.377
7.358
7.342
7.220
7.212
7.191
7.185
7.179
7.173
7.154
7.141
3.407
3.058
2.523
2.518
2.514
2.509
2.505
1.915

11 10 9 8 7 6 5 4 3 2 1 ppm

0.23
1.02
1.95
3.68
1.64
1.57
4.19
3.54
5.73
5.49
3.17
3.91
1.33
0.54
0.94

B7

8.916
8.897
8.181
8.161
7.942
7.939
7.937
7.930
7.921
7.918
7.913
7.853
7.842
7.836
7.832
7.824
7.772
7.754
7.709
7.687
7.670
7.650
7.606
7.587
7.569
7.555
7.551
7.546
7.537
7.527
7.519
7.508
7.495
7.480
7.476
7.458
7.448
7.442
7.432
7.422
7.411
7.404
7.396
7.377
7.358
7.342
7.329
7.254
7.220
7.212
7.191
7.185
7.179
7.173
7.154
7.141
7.134
6.986

| 9.5 | 9.0 | 8.5 | 8.0 | 7.5 | 7.0 | ppm |

0.23   1.02   1.95   3.68 1.64 1.57   4.19 3.54 5.73 5.49 3.17   3.91   1.33   0.54

B7

8.181 8.161 7.942 7.939 7.937 7.930 7.921 7.918 7.913 7.853 7.842 7.836 7.832 7.824 7.772 7.754 7.709 7.687 7.670 7.650 7.606 7.587 7.569 7.555 7.551 7.546 7.537 7.527 7.519 7.508 7.495 7.480 7.476 7.458 7.448 7.442 7.432 7.422 7.411 7.404 7.396 7.377 7.358 7.342 7.329 7.273 7.268 7.254 7.220 7.212 7.191 7.185 7.179 7.173 7.154 7.141 7.134 6.986

Mass spectroscopy of B2:

B- 2
Scan: 47 TIC=2659232 Base=20%FS #ions=1384 RT=.24

4-((diethylamino)(4-methoxyphenyl)methyl)-2-benzyloxazol-5(4*H*)-one

Calculated Mol. wt: 369
Founded Mol.wt:369.4425

51.6393

252.0278

149.0659

75.5437

207.1213

96.2065

219.0277

235.0658

113.0514

135.1173

167.1049

185.1973

261.3535

281.5739 294.2245 304.6625 320.3506

341.1975 355.2389 369.4425

m/z   100   150   200   250   300   350
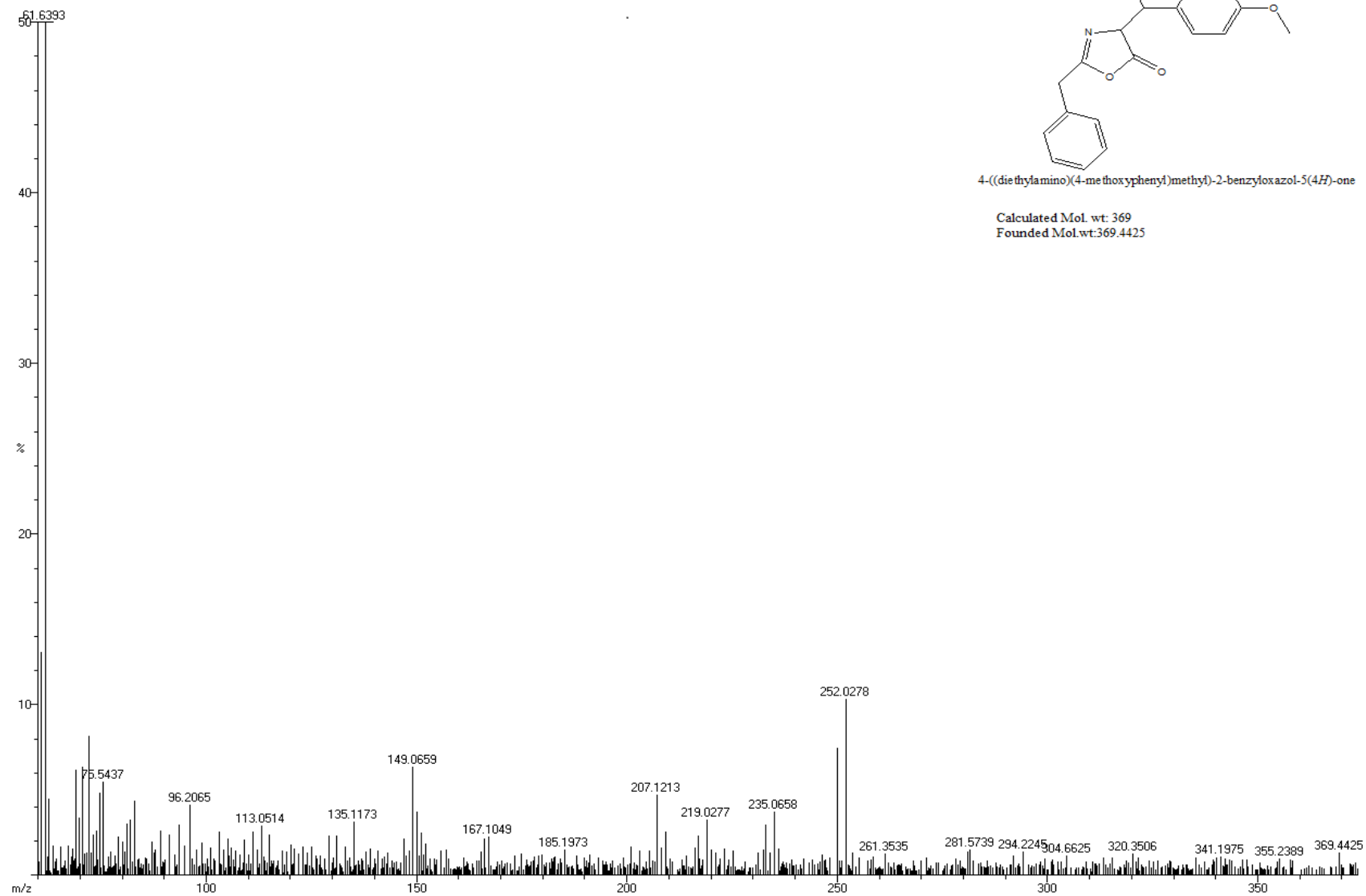
Mass spectroscopy of B7:

B7
Scan: 16 TIC=2878880 Base=21.6%FS #ions=1348 RT=.08

4-((diethylamino)(4-(dimethylamino)phenyl)methyl)-2-benzyloxazol-5(4H)-one

Calculated Mol. wt: 379
Founded Mol.wt:378.2527

61.6393
72.2178
96.2379
113.0751
129.0601
149.0296
166.0864
179.1216
207.0786
219.0167
235.0204
252.0395
260.8999
281.0166
292.2018
309.1653
320.3374
346.5739
355.1696
378.2527

m/z   100   150   200   250   300   350
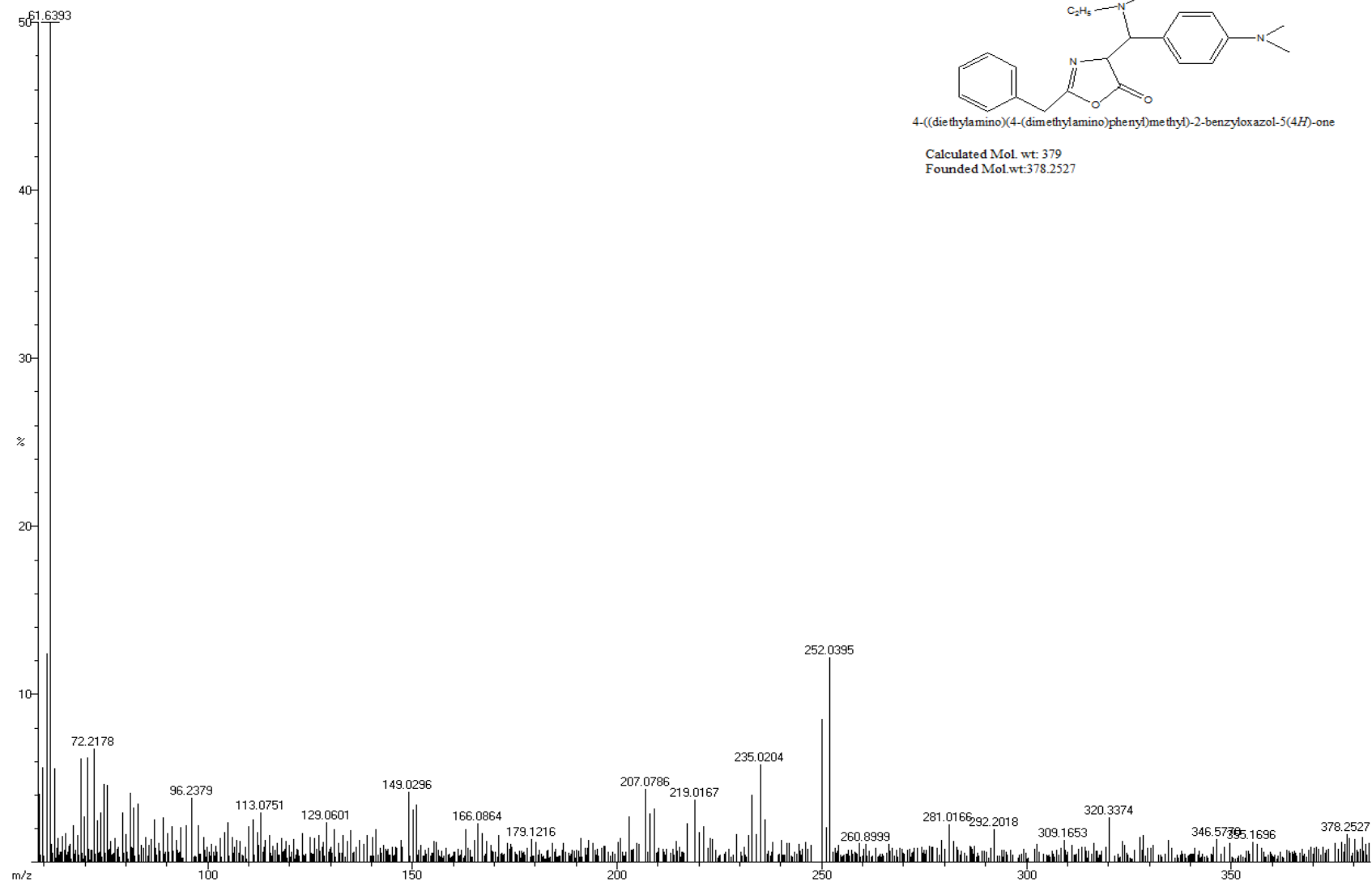
ANTI-DIABETIC SCREENING

Table 6:Anti-Diabetic activity of synthesized compounds

S.NO	CODE	IC_{50} at 10μM
1	A1	69%
2	A2	10%
3	A3	8%
4	A4	13%
5	A5	16%
6	A6	85%
7	B1	84%
8	B2	19%
9	B3	14%
10	B4	12%
11	B5	31%
12	B6	12%
13	B7	70%
14	B8	10%
15	pioglitazone	79%

ANTI OXIDANT SCREENING

Anti Oxidant activity of synthesized compounds

INVITRO ANTIOXIDANT

DPPH RADICAL SCAVENING ACTIVITY:

DPPH Radical scavenging (antioxidant) activity was determined by the method modified by *Hatano* et al., (1989).

Table 9:

Percentage of inhibition								
Conc	**A1**	**A3**	**A6**	**B1**	**B4**	**B5**	**B7**	**Ascorbicacid**
25µg	63.42	78.26	46.99	54.97	61.8	65.5	48.09	46.7
50µg	63.2	81.26	74.48	63.24	65.9	68.9	59.1	68.9
75µg	62.92	82.41	82.18	64.52	65.6	73.08	75.98	89
100µg	62.84	83.5	83.9	65.19	69.4	76.79	78.21	96
EC 50	11±0.12*	-	29±0.9*	15±0.47	18±0.61	-	24±0.94	22±0.45

ANTIOXIDANT ACTIVITY OF COMPOUNDS A1, A3, A6, B1, B4, B5, B7 AND ASCORBIC ACID:
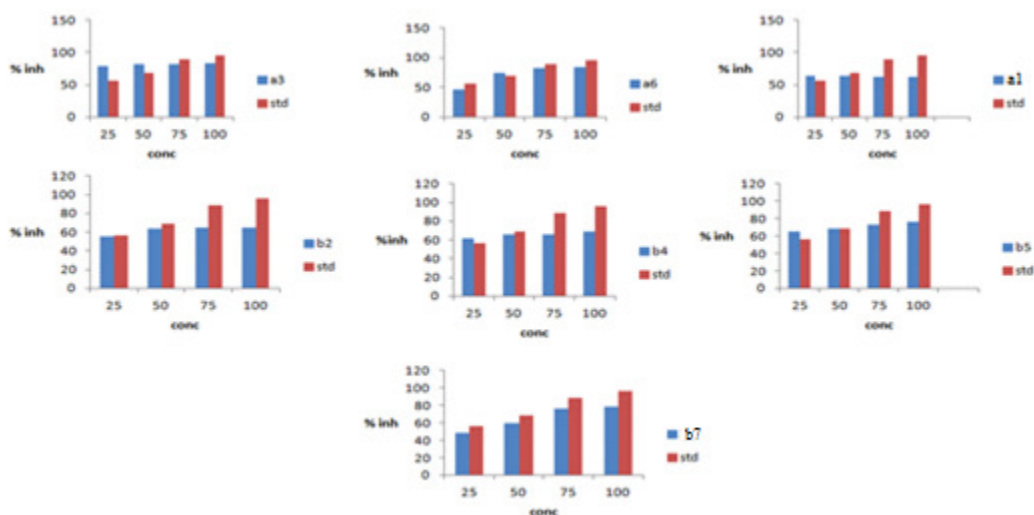
FRAP Method:

Table 10:

Absorbance								
Conc	**A1**	**A3**	**A6**	**B1**	**B4**	**B5**	**B7**	**Ascorbicacid**
25µg	1.97	3.98	3.95	3.93	3.89	3.86	3.71	3.45
50µg	1.61	3.89	3.92	3.79	3.7	3.72	3.42	2.85
75µg	1.03	3.84	3.9	3.53	3.66	3.39	3.24	2.35
100µg	0.91	3.85	3.87	3.54	3.66	3.47	3.04	1.89
r^2	0.94	0.74	0.98	0.88	0.73	0.78	0.98	0.9962
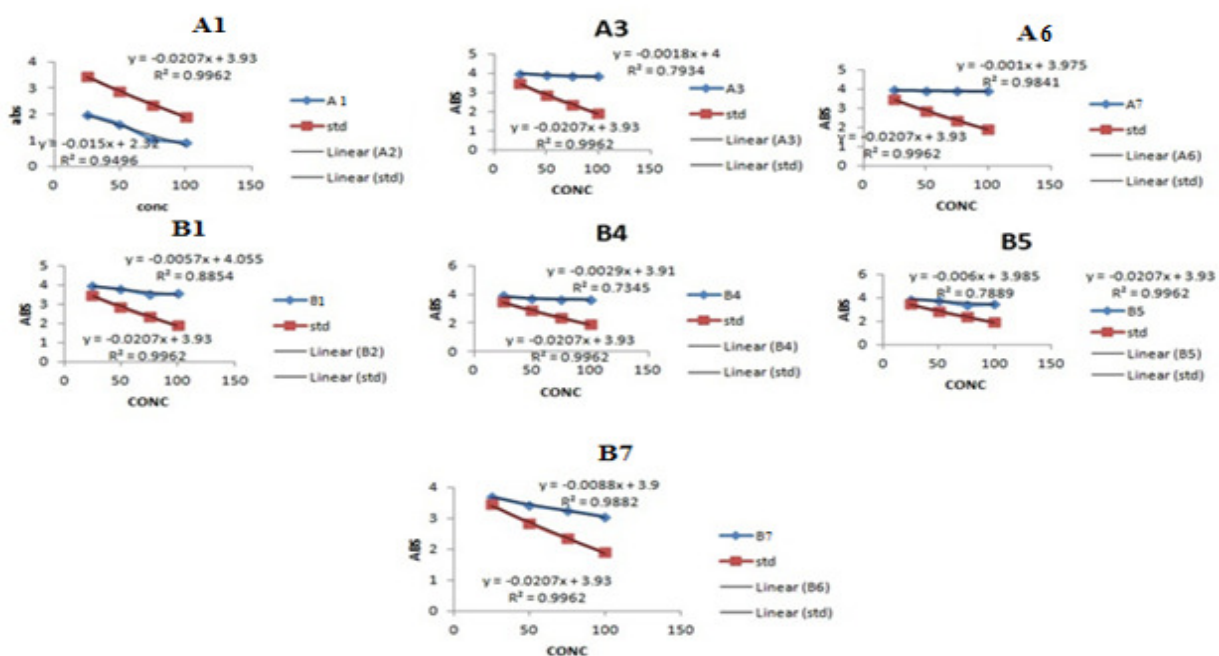
ANTI-FUNGAL SCREENING

Table 7: Anti-fungal activity of the synthesized compounds

Sl.no	Micro organisms	Zone of inhibition(in mm)														
		Compounds(10 µg/disc)														
		A-1 (1)	A-2 (2)	A-3 (3)	A-4 (4)	A-5 (5)	A-6 (6)	B-1 (7)	B-2 (8)	B-3 (9)	B-4 (10)	B-5 (11)	B-6 (12)	B-7 (13)	B-8 (14)	STD Clotrimazole 10µg/disc
1	*Aspergillus fumigalis*	3	10	3	-	6	3	-	3	-	8	7	8	-	-	15
2	*Candida albicans*	8	12	8	12	3	-	-	-	12	-	5	3	-	-	12
3	*Streptomyces griseus*	4	5	3	4	3	4	3	3	6	6	9	10	-	-	14
4	*Monascus ruber*	-	-	-	3	3	6	2	3	6	3	8	3	-	-	15

Table 8. MIC values of the synthesized compounds

Sl.no	microorganisms	MIC VALUES (µg/ml)													
		A-1 (1)	A-2 (2)	A-3 (3)	A-4 (4)	A-5 (5)	A-6 (6)	B-1 (7)	B-2 (8)	B-3 (9)	B-4 (10)	B-5 (11)	B-6 (12)	B-7 (13)	B-8 (14)
1	*Aspergillus fumigatus*	5.0	1.25	5.0	10	2.5	5.0	10	5.0	10	0.625	1.25	1.25	10	10
2	*Candida albicans*	2.5	1.25	6..25	1.25	10	-	-	-	1.25	-	2.5	2.5	-	-
3	*Streptomyces griseus*	10	5.0	10	10	10	10	10	10	5.0	2.5	2.5	1.25	-	-
4	*Monascus ruber*	-	-	-	10	10	5.0	10	10	5.0	10	2.5	10	-	-
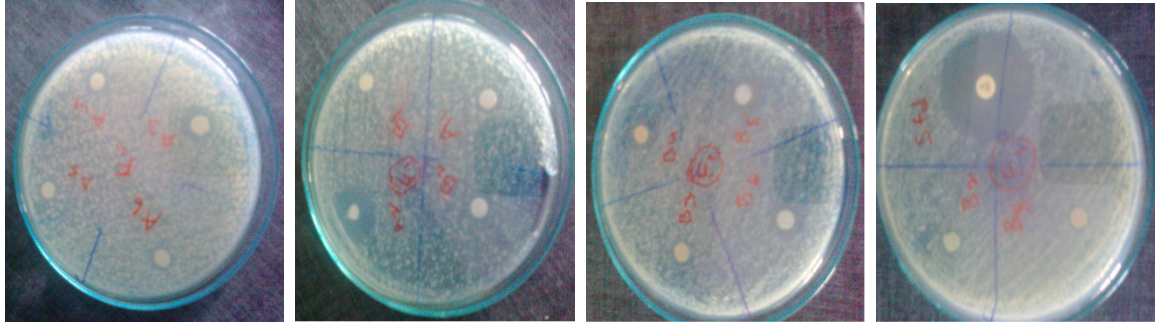
Aspergillus fumigalis
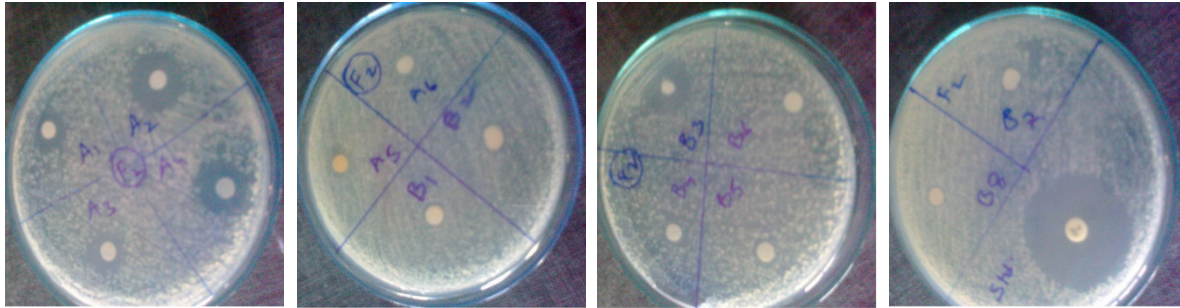
Candida albicans
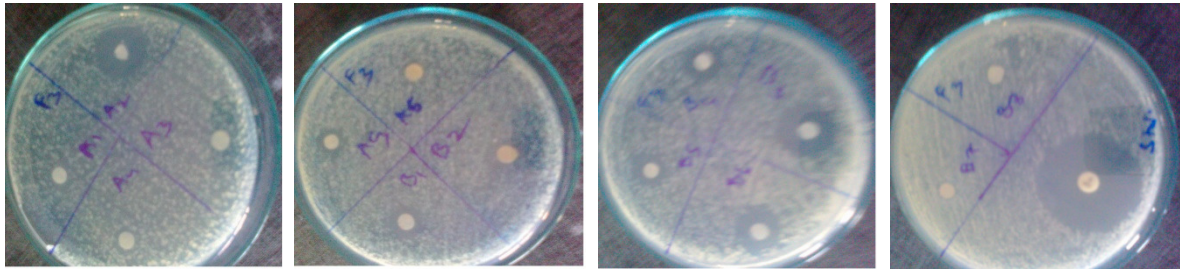
Streptomyces griseus
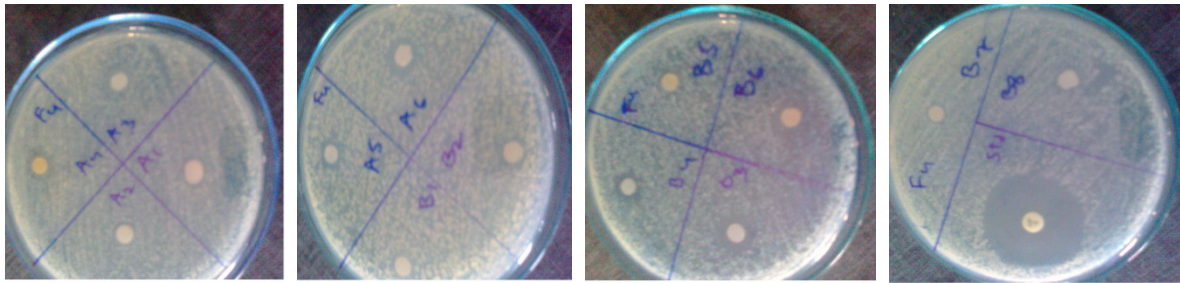
Monascus   ruber

RESULTS AND DISCUSSION

Provoked by the biological activity of the oxazolone and in view of ongoing search for the most potent anti-diabetic, anti-oxidant and anti-fungal agent, some novel 2, 3 Disubstituted derivatives of oxazolone have been synthesized and their anti-diabetic, anti-oxidant and anti-fungal activity studied**.**

Synthesis of designed oxazolone analogues:

This involves a three step procedure from a commercially available starting material

Step 1: This step involves acetylation of amino acid glycine by using acetylating agent Acetic anhydride to form acetylglycine with 75% yield. The acetic anhydride reacted with amino group which leads to the elimination of hydrogen atom, and then the acetyl group replaces the hydrogen atom of amino group.

Step 2: This step involves cyclization of acetyl glycine with acetic anhydride in the presence of sodium acetate in to 2-methyl oxazole-5-one, followed by reaction of the active methylene group with aldehydes to afford the corresponding benzylidine derivatives.

Step 3: This step involves Michael addition of oxazolone derivatives by using diethylamine as a reactant and ceric ammonium nitrate as a catalyst. The diethylamino group reacted with the active hydrogen atom in the oxazolone derivative.

All the derivatives of oxazolone (A1-A6 and B1-B8) were obtained with good yield. The percentage yield was found to be in the range of 60% - 80%.

CHARACTERIZATION OF SYNTHEZISED COMPOUNDS

The physicochemical parameters like, molecular weight, log P, solubility, melting point, and R_f of the synthesized compounds were determined.

The melting points of the synthesized compounds determined and were uncorrected, and melting range was found to be between, 280-240^{0}C

The purity of the synthesized compounds was established by single spot on the TLC Plate (mobile phase- Chloroform and methanol in 1:1 ratio) and all the compounds found to be pure.

The structures of the synthesized compounds were confirmed by IR, NMR, and mass spectral analysis.

IR Spectrum of the synthesized compounds showed the characteristic absorption band at 1480-1498cm^{-1} bands   (C=N respectively), 1620-1650 cm^{-1}due to C=O stretching vibration, 1400 cm^{-1}due to CH stretching, 1260-1280cm^{-1} due to C-O strtching confirms the chemical structure of the oxazolone derivatives.

PMR spectra of the synthesized oxazolone derivative shows 4 aromatic protons as a doublet in 6.9-7.6 ppm, 3 Protons of methyl group appeared at 0.9 ppm as a singlet, and 1 Protons of ethyl link appeared as a singlet in 4.5 ppm . Thus the proton magnetic spectrum of the compound was in full agreement with its molecular formula, with regard to proton count and the chemical shift also.

The Mass Spectral analysis of the synthesized compounds B4 and B6   were performed, and the mass spectrum of the compound was in agreement with its molecular weight.

BIOLOGICAL SCREENING

ANTI-DIABETIC ACTIVITY

The anti-diabetic studies were carried out with all synthesized oxazolone derivatives in the concentration 10µg/ml, in DMSO against Aldose reductase enzyme by using enzyme inhibition assay. Pioglitazone of same concentrations was used as standard. The anti-diabetic activities of the compounds were evaluated by estimation of IC 50 value. It could be seen that acetaldehyde(A1 and B1) and dimethylamino benzaldehyde (A6 and B7)derivatives exhibit good anti-diabetic activity. The IC 50 values of the A1, A6, B1and B7 were found to be 69%,85%,84% and 79% and the standard IC 50 was found to be 79%.

It is clear from the results that the anti-diabetic potential of compounds associated with the position of the substituent on 4th position**. Compound A6 , B7** having dimethyl amino group and **Compound A1 , B1** having methyl group, shown higher activity than the other substituents having compound.

Compound A6   4-((diethylamino)(4-(dimethylamino)phenyl)methyl)-2-methyloxazol-5(4H)-one showed excellent activity among the other compounds. When compared to standard Pioglitazine **Compound A6** shown comparable activity, where as other compounds shown slightly lesser activity than pioglitazone.

ANTI – OXIDANT ACTIVITY

The synthesized compounds were tested for anti-oxidant activity by DPPH assay method at the concentration of 25µg/ml, 50µg/ml, 75µg/ml and 100µg/ml in DMSO. Ascorbic acid was used as standard. **Compounds A6, B1 and B7** were shown good anti oxidant activity with EC-50 value of 29±0.9*, 15±0.47*,18±0.61* and 24±0.94*.

The synthesized compounds were tested for anti-oxidant activity by FRAP Assay method at the concentration of 25µg/ml, 50µg/ml, 75µg/ml and 100µg/ml in DMSO. Ascorbic acid was used as standard. **Compounds A1, A6 and B7** were shown good anti oxidant activity with linearity 0.94,0.98 and 0.98, the linearity of standard Ascorbic acid was found to be 0.996.

From the results it is clear that the compounds having dimethyl amino and methoxy groups at 4th position shown good activity when compared with standard ascorbic acid.

ANTI- FUNGAL ACTIVITY

Evaluation of the results from anti-fungal studies showed that synthesised derivatives exhibits moderate to good anti-fungal activity against *Aspergillus fumigatus, Streptomyces griseus, Aspergillus niger, Candida albicans,* With zone of inhibition range (8-15mm).

The compounds **A2, B3 and B6** are showed moderate to good activity against *Aspergillus fumigatus, Streptomyces griseus, Aspergillus niger, Candida albicans,* as compare to standard Clotrimazole With zone of inhibition *(8-15mm).*

The compound **A2, A4** shown excellent activity on *Candida albicans* with zone of inhibition (12mm).

The MIC of the synthesized compounds against *Aspergillus fumigatus, streptomyces griseus, Aspergillus niger, Aspergillus parasitus, Candida albicans* determined by serial dilution, was found to be in the range of 0.625-5.0µg/ml.

Among the synthesized compounds 4-((diethylamino)(4-methoxyphenyl)methyl)-2-methyloxazol-5(4H)-one (**A2**), 4-(1-(diethylamino)-3-phenylallyl)-2-methyloxazol-5(4H)-one (**A4**), shown excellent activity on *Candida albicans*. From this it is clear that 2-methyl oxazole compounds are more active than the 2-benzyl oxazole compounds. The activity of A2 is may be because of methoxy substituent at 4th position and A4 is may be because of the presence allyl group.

CONCLUSION

Some novel oxazolone derivatives were synthesized to get more potent drug for the treatment of Diabetes and other fungal infectious diseases.

The structures of the synthesized compounds were confirmed by spectral analysis. The synthesized oxazolone derivatives exhibited good anti-diabetic, anti-oxidant and anti-microbial activity, among those Compounds A1, A6, B1 and B7 were found to be the most potent compound with Promising activity against Aldose reductase enzyme which is responsible for Diabetes and its complications, as well as Compound A1, A6 and B7 were found to be good Anti-Oxidant agents and Compound A2 was found to be a potent anti-fungal agent. Since the compounds synthesized shows good Anti-Diabetic, Anti- Oxidant and Anti- Fungal activity. So the developed compounds will be useful for adjuvant pharmacotherapy of Diabetes and its complications along with other fungal infectious diseases in Diabetic patients.

Further studies on its possible mechanism and *in-vivo* trials on experimental animals to broaden their Pharmacological assessment may provide a potent analogue that can overcome adverse effects involved in the treatment of diabetes and will enhance the quality of life of diabetic patients due to its antioxidant and antifungal property.

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