An-Najah National University

Faculty of Graduate Studies

Biological Activity of Newly Synthesized Aromatic Thio and Amino Acid Ester Derivatives

By
Eman Assaf

Supervisors

Dr. Nidal Jaradat

Dr. Ahmad Khasati

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This thesis was defended successfully on 24/11/2021 and approved by:

Defense Committee Members	**Signature**
– **Dr. Nidal Jaradat / Supervisor**	.....
– **Dr. Ahmad Khasati /Supervisor**	Khasati.....
– **Dr. Orwa Houshia External Examiner**	.....
– **Dr. Morad Abo AL-Hssan / Internal Examiner**	.....

Dedication

إلى روحي أمي

إلى من كانت وطني ,ولم هناك يكن لي يوما ولهنا سواك

, إلى من زرع النجاح في دربي وغادر قبل أن يحصد ثماري

إلى أبي

إلى من احتضني وأعطاني عينه وقلبه وأمنه وأمانه

إلى إخوتي وأخواتي

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I want to thank my supervisors **Dr. Nidal Jaradat and Dr. Ahmad Khasati**, for their support throughout the several months of working on my master thesis, keeping me going when times were tough, asking insightful questions, and offering invaluable advice.

الاقرار

انا الموقعة ادناه مقدم الرسالة التي تحمل العنوان:

Biological activity of newly synthesized aromatic thio and amino acid ester derivatives

أقر بأن ما اشتملت عليه هذه الرسالة إنما هو نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أي درجة أو بحث علمي أو بحثي لدى أي مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's work and has not been submitted elsewhere for any other degree or qualification.

اسم الطالب: إيمان عدنان ابراهيم عساف **Student's name:**

التوقيع: **Signature:**

التاريخ: ٢١/١١/٢٠٢٤ **Date:**

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List of Abbreviations

Symbol	Abbreviation
FT-IR	Fourier Transform Infrared
^{1}H-NMR	Proton Nuclear Magnetic Resonance
ROS	Reactive Oxygen Species
BHT	Butylated Hydroxyl Toluene
HPLC	High- Performance Liquid Chromatography
TLC	Thin Liquid Chromatography
UV	Ultra Violet
GC	Gas Chromatography
DPPH	2,2-diphenyl-1-picrylhydrazyl
MIC	Minimum inhibitory concentration
PNPB	para-Nitro phenyl butyrate

Biological Activity of Newly Synthesized Aromatic Thio and Amino Acid Ester Derivatives

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Abstract

Nine corresponding substituted acid esters were synthesized by reacting two different alcohols [2-(phenylthio) ethan-1-ol and 2-(phenylamino) ethan-1-ol)] with five derivatives of benzoic acid (4-bromo benzoic acid, 4-methoxy benzoic acid, 4-amino benzoic acid, 4-tertary benzoic acid, and nitro benzoic acid) by the Fisher esterification method. Structures of these esters were established by Fourier-transform infrared (FT-IR) spectroscopy, proton nuclear magnetic resonance (^{1}H-NMR), and carbon 13 nuclear magnetic resonance (^{13}C-NMR). Acid esters were tested for their anti-oxidant, anti-microbial, anti-amylase, and anti-lipase activities.

The anti-oxidant test showed that compounds 2-phenylamino-4-terary butyl benzoate and 2-phenylthio-4-tertarybutylbenzoate had IC_{50} values of 10.5 and 13.6, µg/mL respectively, while Trolox had an IC_{50} of 0.6 µg/mL. In addition, all acid esters revel activity had activities against Gram-positive and Gram-negative bacteria, with minimum inhibition concentration (MIC) values of 0.47–7.5 µg/mL.

However, most acid esters showed more than 90%inhibition against Candida albicans, with a MIC of 1.875 µg/mL, compared with the

antifungal drug fluconazole (1.62µg/mL. The acid esters showed promising results in the α-amylase assay compared with acarbose as the standard. Compounds 2-phenylthioethyl-4-nitrobenzoate and 2-phenylthioethyl-4-methoxy benzoate both revealed an IC_{50}value of 0.021 µg/mL, while for acarbose the IC_{50}value was 64.6 µg/mL at the same concentration. On the other hand, compounds 2-phenylthioethyl-4-tertarybutyl benzoate and 2-phenylaminoethyl-4-tertarybutyl benzoate showed 51.94% and 67.122% inhibition, respectively, against the lipase enzyme when at a concentration of 600 µg/mL, despite the absence of the amide functional group, which is responsible for the activity. The biological activities of the acid ester derivatives show promising results for future in vivo, pre-clinical, and clinical investigations.

Chapter one

Introduction

2.1 Chemical synthesis

The ongoing search for new chemical molecules, especially anti-microbial, anti-oxidant, and anti-cancer agents, plays an important role in the prevention and treatment of human diseases and serves humanity. The most important recent problems are the global emergence of multi-drug resistant bacteria, which spread widely around the world, and cancer, which is becoming the second-leading cause of death in the world [1,2]. Cancer is thought to be a result of free radicals from different sources in the environment, such as sun rays, reactive oxygen species (ROS), nitrogen (RNS), chlorine (RCS), smoke, and other pollutants. Antioxidants are very important organic compounds, especially in the prevention of diseases by neutralizing free radicals. Modern biochemistry and drug discovery are directed toward identifying molecules that potentially reveal unexpected insights into new biologically active targets of more immediate use [3]. In recent decades, investments in the synthesis of potential chemical compounds and the determination of their desired biological activities have become important for drug discovery and development processes [4]. In some cases, these investments natural products as an important source for such molecules, but more often they involve collections of synthetic small molecules prepared by organic chemistry strategies, which rapidly yield large collections of relatively pure compounds [5]. Recent bio-organic approaches have focused on the discovery of novel targets and new lead molecules for the treatment of human diseases.

2.1.1 Anti-oxidant

Anti-oxidant agents are substances that have the detoxifying action of free radicals and that reduce oxidative stress by different mechanisms, including radical scavengers, hydrogen donors, electron donors, peroxide decomposers, singlet oxygen quenchers, enzyme inhibitors, synergists, and metal-chelating agents, thus preventing or decreasing capacity for cellular damage [6]. Some such endogenous anti-oxidants, including guttation, ubiquinal, and uric acid, are found in the diet [6]. A free radical, and other ROS, can be defined as a very unstable reactive species because of a missing electron in its outer shell, making it a very toxic reactive compound [7]. There are two sources of free radicals: one resulting from normal biological metabolic processes and the other resulting from exposure to environmental factors, such as X-rays, cigarette smoking, air pollutants, and industrial chemicals [7,8].

Hydroxyl radicals, superoxide anion radicals, hydrogen peroxide, oxygen singlet hypochlorite, nitric oxide radicals, and peroxy-nitrite radicals are toxic free-radical compounds that react rapidly with many components inside cells, such as lipids, proteins, and DNA, causing oxidative stress [6]. Oxidative stress results from oxidative damage when free radical generation is greater than anti-oxidant defaces and is now thought to contribute significantly to many degenerative diseases, such as cardiovascular disease, inflammatory conditions, certain cancers, and the process of aging [9]. Antioxidants such as B-carotene or vitamin E play a vital role in the prevention of various cardiovascular diseases and stop induce carcinogenesis by different mechanisms specially direct scavenging property[10].

2.1.2 Anti-microbial

Infectious diseases involve the invasion and rapid growth of germs in bodily

tissues [11]. They are considered major health problems around the world, causing

premature death and killing almost 50,000 people each day.

Infections are caused by a variety of organisms, such as bacteria, fungi, and viruses, but most prominently bacteria [11]. Bacterial infections are among the important infectious diseases that cause millions of deaths, and they continue to be a significant problem. Bacteria are prokaryotic microorganisms that can be pathogenic, and they can cause diseases or even be helpful [12].

There are many microbials that can invade the body, causing illness, and one can become exposed to them in a variety of ways. The signs and symptoms of microbial infection vary depending on the infected area and the type of microbial, but common symptoms include pain and fever [13,14]. Mild infections may respond to rest and home remedies, while some life-threatening infections may require hospitalisation. Anti-microbial agents such as antibiotics and anti-fungals are substance designed to stop or inhibit growth and are used to treat infections [14]. Microbes, not humans or animals, can become antibiotic resistant [15].

Anti-microbial resistance happens when germs such as bacteria and fungi change in response to antibiotics, thus fighting back and finding new ways to survive. New resistance mechanisms are emerging and spreading globally, threatening our ability to treat common infectious diseases [15]. Each year in the U.S., at least 2.8 million people are infected with antibiotic-resistant bacteria or fungi, and more than 35,000 people die as a result [14].

Antibiotic resistance leads to dangerous problems in all parts of the world and causes many problems, including higher medical costs, prolonged hospital stays, and increased mortality. Thus, there is an urgent need to discover and develop new anti-microbial agents to be safe in the future [15,16].

2.1.3 Diabetes

Diabetes mellitus (DM) is a chronic disorder of carbohydrate metabolism. It occurs because either the pancreas lost all or part of its function, which is the secretion of insulin. This occurs in type1 DM, called "insulin-dependent diabetes mellitus" (IDDM),or body tissues cells resistance to insulin action, and this occurs in type 2 DM, called “non-insulin-dependent diabetes mellitus" (NIDDM) or "adult-onset diabetes." Both types cause a high blood sugar level over a prolonged period of time [17,18]. About 422 million people worldwide have diabetes, with the majority living in low- and middle-income countries. The number of cases and the prevalence of diabetes have been steadily increasing over the past few decades [19,20]. People with high blood sugar have symptoms such as increased thirst, increased hunger, weight loss, and blurred vision. Severe hyperglycaemia is often associated with illness conditions such as acute infective, traumatic, circulatory or other stress may be transitory[17]. Living with diabetes for an extended period of time and uncontrolled blood sugar levels will lead to very dangerous complications that may be disabling or even life threatening The complications can be divided into two types: (i) acute complications, including diabetic ketoacidosis and nonketotic hyperosmolar coma, and (ii) chronic complications, including heart disease, stroke, kidney failure, foot ulcers, and the dysfunction of various organs such as the eyes [17,18].

Type 1 diabetes must be treated and controlled using insulin injections. There are different types of insulin, and the differences depend on different factors, such as the onset of action and the duration of action [21]. Type 2 diabetes may be treated with oral anti-diabetic agents with or without insulin. Oral anti-diabetic agents for treating type 2 diabetes include the following groups:metformin (the first medication prescribed for type 2 diabetes), sulfonylureas (second line therapy), glinides, thiazolidinediones, DPP-4 inhibitors, GLP-1 receptor agonists, SGLT2 inhibitors, and α-amylase inhibitors. Every group acts through a different mode of action and in different locations in the body, but all have the same aim decrease blood glucose level [22,23].

2.1.3.1 α -Amylase Activity:

Amylase is an enzyme that helps digest carbohydrates. It is made in the pancreas and the glands that make saliva. Amylase was the first enzyme to be discovered and isolated (by Anselme Payen in 1833). Amylase can be classified into 3 types: 1)α-amylase, 2) β-amylase, and 3) γ-amylase.

All amylases are glycoside hydrolases and act on α-1,4-glycosidic bonds [21]. A-amylase or 1,4-α-D-glucan glucanohydrolase; glycogenase is a catabolic enzyme that acts through the breakdown of starch molecules and convert into sugar molecules to yield products such as glucose and maltose to supply the body with energy. They act during the initial step of the digestion of starch, which occurs in the mouth. α-Amylase is also produced by plants, animals, and microorganisms. Inhibition of the α-amylase inhibitor is helpful in the prevention and medical treatment of type 2 diabetes and obesity.

2.1.4Obesity

Obesity is a complex health issue that threatens global wellbeing. It is caused by genetic susceptibility and the consumption of more calories than you burn off through physical activity [23,24]. In some cases, the reason for obesity is medical problems, such as endocrine disorders and mental disorders, or medications [24]. Medications associated with weight gain include certain antidepressants and anticonvulsants such as carbamazepine [25], some diabetes medications, such as insulin, sulfonylureas, and thiazolidinediones and certain hormones, such as oral contraceptives[25]. There is a link between social issues and obesity. A lack of money to purchase healthy foods or a lack of safe places to walk or exercise can increase the risk of obesity People suffering from obesity are defined according to body mass index (BMI), with a BMI over 25 considered overweight and a BMI over 30 considered obese BMI depends on the weight and height of the person and is expressed in units of kg/m^2[25]. From1975 to 2016, a large increase in the prevalence of obesity in both children and adults occurred. There has been a marked increase in obesity in countries viewed to have urban lifestyles However, the number of people considered overweight and obese has increased in low- and middle-income countries and it is more common in women than men. In 2013, the American Medical Association classified obesity as a disease [26].

Obesity is serious and potentially life-threatening because it leads to diabetes, high blood pressure, heart attack, and coronary artery disease. Therefore, it is very important to tackle obesity [27,28]. The best way to overcome obesity requires changes in eating habits and increases in regular exercise. Change in personal eating habits' alone is not enough to lose weight, so some medication with different mechanism of action is needed to decrease fat accumulation by using such :Beta-methyl-phenyl

ethylamine (Fasten). This acts by stimulating fat metabolism [29].Orlistat (Xenical) works by blocking about 30% of dietary fat from being absorbed [29].Sibutramine (Meridia) is an appetite suppressant approved for long-term use [29,30].The goal of obesity treatment is to reach and stay at a healthy weight. This improves overall health and lowers the risk of developing complications related to obesity.

2.1.4 Lipase Activity:

Lipase is a very important enzyme for fat digestion and lipid transport [31]. It is responsible for breaking down triglyceride and converting to free fatty acids and glycerol to become available for absorption in the intestines[31]. Gastrointestinal lipase inhibitor drug that inhibit action of lipase enzyme and prevent fat break down and absorber then tend to be excreted rather than being absorbed to be used as a source of caloric energy, and this can result in weight loss in individuals [32].

Orlistat is a potent, specific, irreversible inhibitor of pancreatic and gastric lipases [33,34]. The pharmacologic activity of Orlistat occurs when forming covalent bonds with the active serine site of gastric and pancreatic lipases in the lumen of the gastrointestinal tract and has been shown to block the absorption of around 30% of dietary fat at a therapeutic oral dose of 120 mg three times a day [33,34].

2.2 Ester compounds and synthesis

Esters are a common class of organic chemical compounds and biological materials. The functional group –COO is known as the ester link [35]. Esters are secondary organic metabolites that occur widely in nature and are often responsible for the fruity odour of many plants, such as methyl salicylate, which has the odour and flavour of oil of wintergreen, while propyl ethanoate has that of a pea [36]. Esters compounds can be derived

from the chemical reaction called esterification. The main use of esters is for flavourings and perfumes; however, they can also be used in the chemical industry as solvents, which can then be analysed using gas chromatography, gas–liquid chromatography, or mass spectrometry. Fischer esterification is atypical procedure to synthesize ester compounds. It is a reversible condensation reaction in which carboxylic acid is treated with an alcohol in the presence of a mineral inorganic acid catalyst to facilitate the nucleophilic attack of the alcohol at the carbonyl carbon of the carboxylic acid [37]. In a condensation reaction, two molecules join and produce a larger molecule with simultaneous loss of water [37].

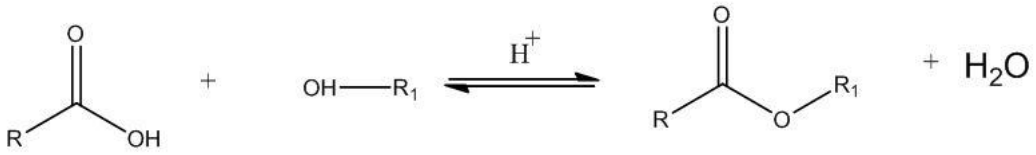
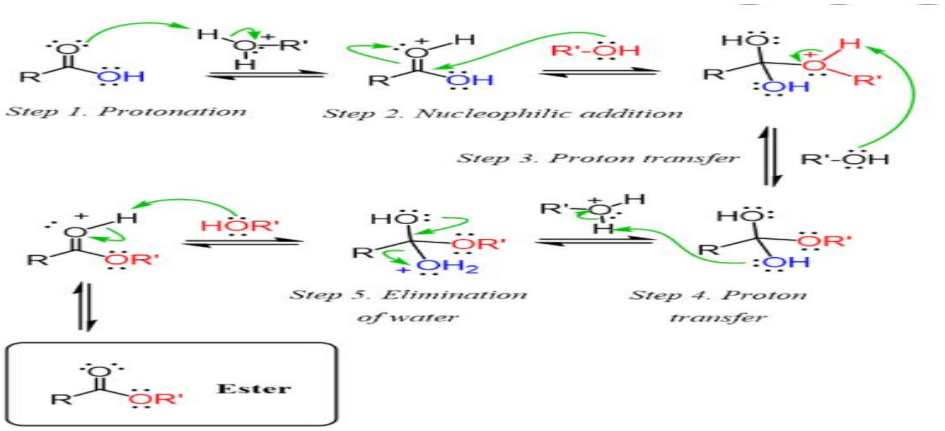


Figure 1.1: general equation of Fischer esterification.



Scheme 1.2: general mechanism of Fischer esterification.

2.3 Carboxylic acid compounds

A carboxylic acid is an organic acid consisting of a carbonyl (C=O) with a hydroxyl group (O–H) attached to the same carbon atom and is usually written as –COOH or CO_2H. Carboxylic acids occur widely. The molecular weight of organic acids varies widely from relatively small compounds,

such as formic acid, to much larger compounds (fatty acids) with higher numbers of carboxylic and phenolic functional groups [35].Carboxylic acid derivatives have varied applications in different fields, such as medicine, agriculture, pharmaceuticals, and the food industry, because of their organoleptic properties(e.g., taste, aroma, and colour) and the stability of food items. Carboxylic acids and their derivatives are used in the production of polymers, biopolymers, coatings, adhesives, and pharmaceutical drugs. They can also be used as solvents, food additives, anti-microbials, and flavourings[35].They occur widely in nature, such as the acids present in fruits and vegetables(citric acid in citric fruits, malic acid in grapes and apples, oxalic acid, broccoli), or added artificially, as acidulates (citric acid), preservatives(lactic acid), emulsifiers (tartaric acid), antioxidants (ascorbic acid), or flavours (propionic acid) in a wide variety of products for human consumption (foods and beverages). Organic acids are well-known as effective preservatives, and their anti-microbial action is due to the ability to change from un-dissociated to dissociated form, depending on the environmental pH, making them effective anti-microbial agents. For example, some organic salts (calcium and sodium propionate) are used as preservatives in dairy and bakery food products. However, there are carboxylic acids that have a beneficial effect on microorganisms, helping them growth by acting as vitamins for microbial nutrition (e.g., folic acid, nicotinic acid, or p-amino benzoic acid). They also play many important roles in the pharmaceutical industry, such as acting as solubilisers. Prodrug and/or bio-precursor acting as compounds not biologically active but converted into active ones in specific conditions and Pharmacophore providing specific interactions with an enzyme, triggering, or blocking its biological response (e.g., blood-cholesterol-reducing drugs, non-steroidal anti-inflammatory drugs.

2.4 Chromatography

Chromatography is an important biophysical analytical technique for the separation, identification, and purification of the components of a mixture into their individual components by passing it in a stationary phase through a mobile phase in which the components move at different rates Different factors will be at play during separation, including gmolecular characteristics related to adsorption (liquid–solid), partition (liquid–solid), and affinity or differences among their molecular weights [38].

2.4.1 Thin layer chromatography

Thin-layer chromatography is a type of "solid–liquid adsorption" chromatography.

It is used for non-volatile mixtures [39]. The stationary phase is a solid made of silica gel, aluminium oxide, or cellulose, and the mobile phase is a solvent that has different polarity according to mixture [40]. A fluorescent powder is mixed into the stationary phase to simplify the visualisation later on (e.g., bright green when you expose it to 254 nm UV light) [41]

2.4.2 Column chromatography

Column chromatography (CC) is widely used for the isolation, purification, and separation of large amounts of a sample from a mixture, and the process of separation is based on the differential adsorption of compounds to the adsorbent, i.e., solute distribution between the mobile phase and the stationary phase [42].The main advantage of CC is the relatively low cost and disposability of the stationary phase used in the process [42].

2.5 Physical measurements

2.5.1 Infrared spectroscopy (IR)

Infrared (IR) spectroscopy is used to study the functional groups in solid, liquid, or gaseous forms. IR spectroscopy transacts with the electromagnetic spectrum infrared (IR) region interaction with the sample's substance. The measurement of the interaction of IR radiation with matter by absorption, emission, or reflection. A common laboratory instrument that uses this technique is a Fourier-transform infrared (FT-IR) spectrometer [43].

2.5.2 Nuclear Magnetic Resonance (NMR)

Nuclear magnetic resonance (NMR) spectroscopy involves the use of NMR phenomena to study the physical, chemical, and biological properties of matter. Chemists use it to determine molecular identity and structure [44].

2.8 Aim of the thesis

The main objectives of this thesis are the following:

- ➢ To synthesize a series of the substituted phenolic acid ester by reaction with 2-(phenyl amino) ethan-1-ol and 2-(phenylthio) ethan-1-ol.
- ➢ To explore some biological activities of synthesize esters compounds.
- ➢ To enrich the literature with the physical data of these esters.

Chapter Two

Materials and Methods

3.1 Chemicals and Reagents

Materials that used in the synthesis of compounds were purchased from Aldrich-sigma chemical Co. including benzoic acid derivatives (4-Bromo benzoic acid, 4-Nitro benzoic acid, 4-Amino benzoic acid, 4-Methoxy benzoic acid and 4-tertbutyl benzoic acid), 2-(phenylthio)ethan-1-ol and 2-(phenylamino (ethan-1-oland many other materials were used during biological analysis such as DPPH, Pancreatic lipase enzyme. While chloroform, ethanol, Tween-40, sodi

3.2 Microbiology

Microorganisms used in this experiment were from An-Najah University lab. /Nablus .Types of bacteria were Klebesiella pneumonia (ATCC13883), Staphylococcus aureus (ATCC25923), Pseudomonas aeruginnosa (ATCC9027), proteus volgaris (ATCC 8427), Echerichia coli (ATCC 25922), Enterococcus faecium (ATCC 700221), MRSA (Clinical strain) and candida albicans (ATCC90028).

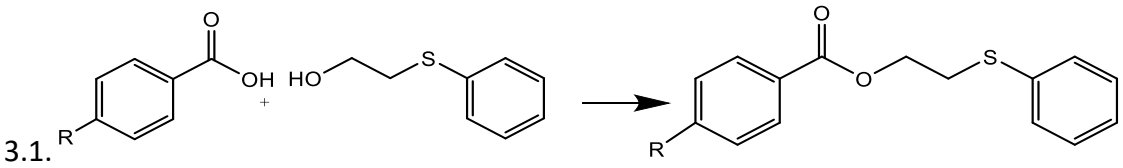
3.3 Physical measurements

Thin layer chromatography (TLC) was used to check the purity of synthesized compounds. Melting points were measured by Stuart melting Point apparatus R00102618.^{1}H –NMR and carbon 13 nuclear magnetic resonance (^{13}C-NMR) were determined by (Bruker 300 MHz-Avance III) at the University of Jordan/ Jordan. IR spectra were recorded on Fourier transform Infrared spectrophotometer (Necolet Is5 - Id3) at An-Najah

University. The purity of the compounds was confirmed by HPLC Breeze 2 HPLC system.

3.4 General synthetic procedure for aromatic thio acid esters

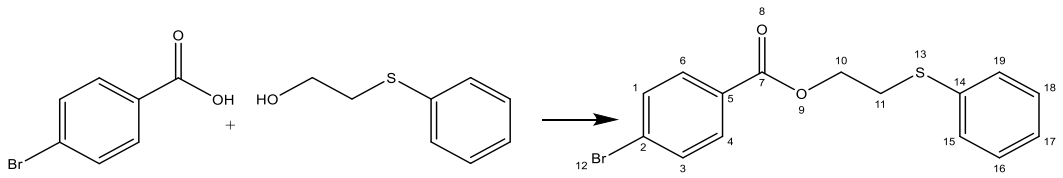
Acid esters were synthesized from the reflux of 2-(phenylthio) ethan-1-ol and benzoic acid derivatives for 2 h to produce 2-phenylthioethyl benzoates by the Fisher esterification method and were catalysed by 2 drops of diluted HCl. After cooling the system at room temperature and standing over night, the crude was checked by thin layer chromatography and purified by CC with a mobile phase of n-hexane/acetone (3:2) as the eluent. Yields were between 25 and 79% . The products are summarised in Ttable 3.1.



3.1.

Scheme3.1General synthetic procedure for aromatic thio acid esters.

3.4.1 Synthesis of 2-phenylthioethyl 4-bromobenzoate



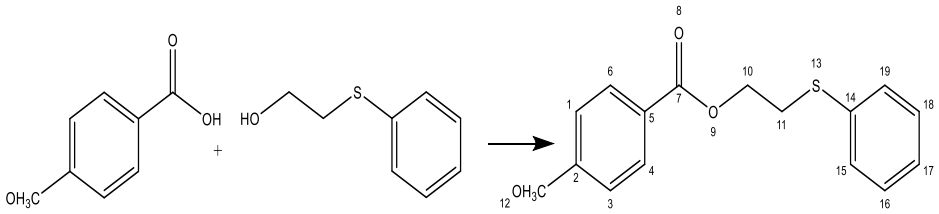
The Esterification of 2-(phenylthio) ethan-1-ol (1.8ml, 2.0574g, 0.01333mol) and 4-bromobenzoic acid (2g,0.0099mol)produced 2-phenylthioethyl 4-bromobenzoate. (57%) (m.p.= 182-184˚C)

IR;3103.96; 2977.31; 2834.83; 2676.52; 2559.30; 1678.53; 1586.19; 1482.48; 1425.15; 1399.46; 1318.85; 1301.93; 1278.01; 1177.99; 1127.31; 1109.40;1068.63; 1012.19; 929.21; 850.75;807.90; 739.73; 691.77; 627.69; 570.04; 548.80; 511.05 cm^{-1}

^{1}HNMR (δ): (7.1-7.2) 5H at C15-C19) (7.6- 7.8) 4H at C1, C3,C4, C5; (4.36)2H at C10; (3.35) 2H at C11 ppm.

^{13}C-NMR (δ): (167.1) C7; (129-136) C1-C6; (125-129) C14-C19; (64) C10; (39.6) C11 ppm.

3.4.2 Synthesis 2-phenylthioethyl 4-methoxybenzoate



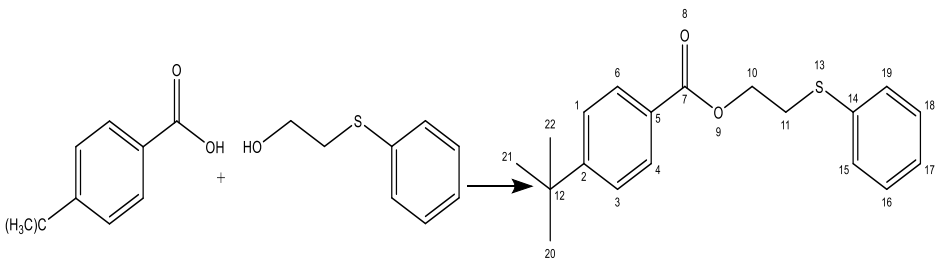
2-(phenylthio) ethan-1-ol (2.7432g, 0.0177mol) and 4-methoxybenzoic acid (2g,0.0131mol) were refluxed to produced 2-phenylthioethyl 4-methoxybenzoate. Yield 54.16% and m.p.=181-183˚C.

IR; 2841; 2555.32; 1681; 16202.95; 1578.09; 1515.63; 1427.11; 1299.03; 1258.03; 1166.78; 1106.48 cm^{-1}

^{1}HNMR (δ):(6.9) 2H at C1, C3; (7.86) 2H at C4, C6;(3.9) 3H at C12;(4.3) 2H at C10; (3.65) 2H at C11; (7.24-7.28) 5H at C15, C16, C17, C18, C19 ppm.

^{13}C-NMR(δ): (114.2) C1, C3; (131.8) C4,C6; (122.4) C5; (165.7) C(63.4) C10; (39.5) C11; (126.4-129.5) C15-C19;(131.8) C14 ppm.

3.4.3 Synthesis 2-phenylthioethyl4-t-butylbenzoate



2-(phenylthio) ethan-1-ol (2.286g, 0.0148mol) and 4-t-butylbenzoic acid (2g,0.0112mol) were refluxed to produced 2-phenylthioethyl4-t-

butylbenzoate. Yield was 34.8% and (m.p.=198-200˚C).

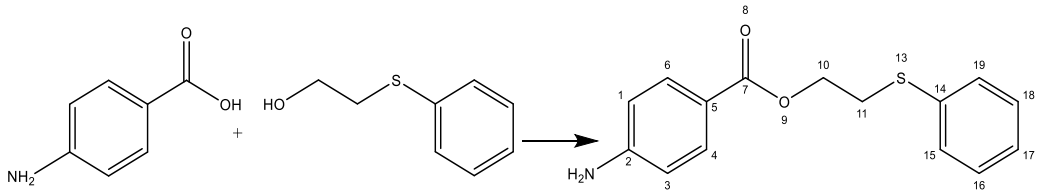
IR;3024.64; 2955.43; 1681.14; 1610.21; 1422.90; 1317.40; 1288.03; 1261.89; 1188.04; 1113.28; 1069.10; 1015.51; 941.51; 856.54; 797.33; 708.18; 543.66 cm^{-1}

^{1}HNMR (δ): (1.24) t-butyl 12H atached to C20,C21,C22; (2.5,3.5)4H attached to

C10,C11; (7.4) 5H attached to C15-C19; 7.8 4H atached to C1,C3,C4,C6 ppm.

^{13}C-NMR(δ): (39.9)for C12, C20, C21,C22; (125-129) for C14-C19, C1, C3, C4, C5, C6; (167.7) for C7; (156.3) for C2 ppm.

3.4.4Synthesis 2-phenylthioethyl 4-aminobenzoate



2-(phenylthio) ethan-1-ol (2.857g, 0.01852mol) and4-aminobenzoic acid (2g,0.01458mol)produced 2-phenylthioethyl4-aminobenzoate.Yield was 25.27% and m.p.=184-186˚C.

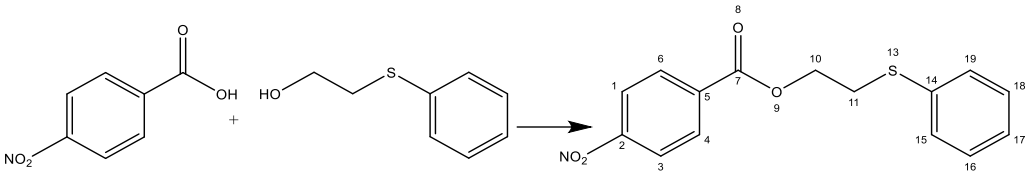
IR; 2882.48; 1786.26; 1713.70; 1657.38; 1611.12; 1584.82; 1538.99; 1511.45; 1436.34; 1316.96; 1268.11; 1187.33; 1142.87; 1055.79; 1021.11; 854.68; 799.77; 743.84; 720.99; 690; 637.05;602.53; 556.13; 527.50; 512.65 cm^{-1}

^{1}HNMR (δ):(6.47) 2H at C1,C3; (7.63) 2H at C4, C6;(4.5) 2H at C10; (3.54) 2H at

C11; (7.23-7.29) 5H at C15-C19 ppm.

^{13}C-NMR (δ):(114.5) C1,C3; (151.3) C2; (131.6) C4, C6, C14-C19;(119.3) C5;(39.5) C11 ppm.

3.4.5 Synthesis 2-phenylthioethyl 4-nitrobenzoate



2-(phenylthio) ethan-1-ol (2.7432g, 0.0177mol) and 4-nitrobenzoic acid (2g,0.0119 mol) werererefluxed to produced 2-phenylthioethyl4-nitro benzoate. Yield was 78.8% and m.p.=175-177 ˚C.

IR;3337.22; 1478; 1088.43; 797.64; 735.93;690.40;671.57; 598.14; 570.55;

555.79;533.70 cm^{-1}

^{1}HNMR (δ): (7.32-7.46) 4H at C1,C3,C4, C6; (7.19-7.31) 5H at C15-C19); (3.55) 2H at C10; (3.6) 2H at C11 ppm.

^{13}C-NMR(δ):(129-135) C1,C3,C4, C5,C6; (146.9)C7, (136) C2;(50.4) C10; (39.9) C11; (116-129) C14-C19 ppm.

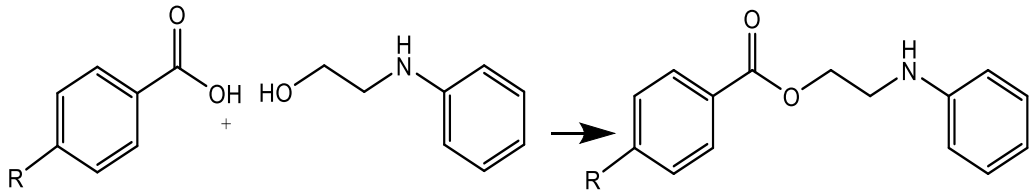
Tab.3.1-phenylthioethyl benzoates compounds melting point and % yield.

R	Name of compound	Melting point	%yield
Br2	2-phenylthioethyl 4-bromobenzoate	182-184˚C	57%
OCH3	2-phenylthioethyl 4-methoxybenzoate	181-183˚C	54.16%
t-butyl	2-phenylthioethyl4-t-butylbenzoate	198-200˚C	34.8%
NH2	2-phenylthioethyl 4-aminobenzoate	184-186˚C	25.27%

NO2	2-phenylthioethyl 4-nitrobenzoate	175-177˚C	78.8%

3.5 General synthetic procedure for aromatic amino acid esters

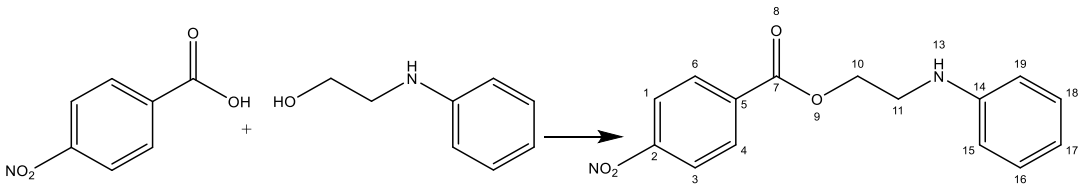
Amino acid esters were synthesized from the reflux of 2-(phenylamino) ethan-1-ol and benzoic acid derivatives to produce 2-phenylaminoethyl benzoates by the Fisher esterification method and were catalyzed by 2 drops of diluted HCL. The reactants were refluxed for 2 h in and cooling the system at room temperature over night. The products Were checked thin layer chromatography and purified by Colum chromatography with a mobile phase(n-hexane/acetone) (3:2) as eluent. Yields were between 4 and 44%. The products are summarised in table 3.2 and the reaction is shown in scheme 2.2



Scheme 3.2: General synthetic procedure for aromatic amino acid esters

3.5.1 Synthesis 2-phenylaminoethyl 4-bromobenzoate

2-(phenylamino) ethan-1-ol (1.9692g, 0.01435mol) and 4-bromobenzoic acid (2g, 0.0099mol) were refluxed to produced 2-phenylaminoethyl 4-bromobenzoate. Yield was 36.9% and m.p.=179-181˚C as shown in scheme 3.3



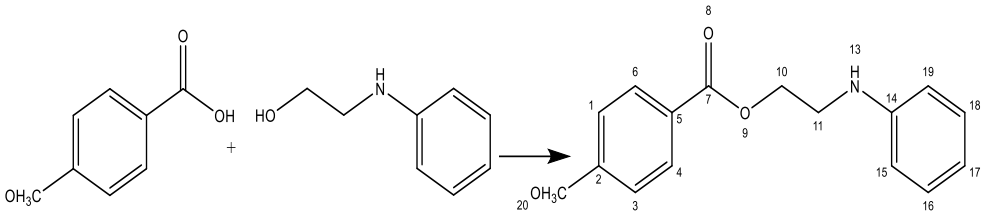
The structure of product was established by Infra-red and Proton NMR spectral, and^{13}C-NMR

IR;3393.89; 2831.64; 1719.61; 1597.53; 1503.77; 1397.49; 1328.79; 1270.02; 1228.63; 1103.26;1031.15; 1011.91;993.57;940.83; 842.35;752.01; 694.56; 636.31;613.93; 566.73;550.01;533.37 cm^{-1}.

^{1}HNMR(δ): (8.27) N-H; (8.14) 4H at C1,C3, C4, C6; ;(6.5-7.19) 5H at C15-C19 (4.11) 2H at C10; (3,51) 2H at C11 ppm

^{13}C-NMR (δ): (148.5) C7, (131.6) C2, C13; (129 C1, C3,C4,C5,C6; (113-117) C14-C19 ppm

3.5.2 Synthesis 2-phenylaminoethyl 4-methoxybenzoate



2-(phenylamino) ethan-1-ol (2.6256g, 0.01913mol) and 4-methoxybenzoic acid (2g,0.0131mol) were refluxed to produced 2-phenylaminoethyl 4-ethoxybenzoateYield 43.17% and m.p.=178-188 ˚C.

The structure of products was established by Infra-red and Proton NMR spectral, and^{13}C-NMR

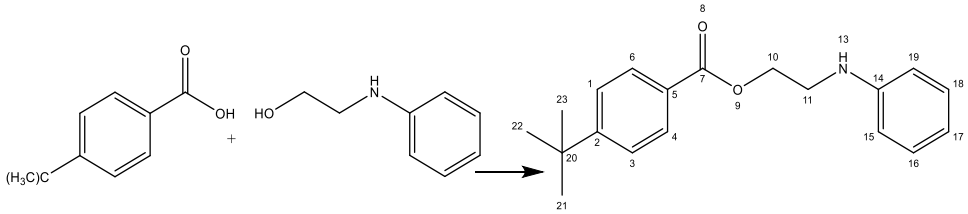
IR; 3902.22; 3801.52; 3525.61; 2841.53; 2555.98; 2364.31; 1920; 1868; 1844; 1793; 1771; 1747; 1733; 1716; 1683; 1651; 1636; 1602; 1558; 1541; 1508; 1473;1427; 1396; 1300; 1257; 1167; 1106; 1026; 927; 844; 824; 772; 696;634;614;573; 551; 538; 510 cm^{-1}.

^{1}HNMR(δ):(7.99) N-H; (6.58-8.58) 4H at C1,C3, C4,C6; (4.36) 2H at C10; (3.75) 2H at C11; (6.58-7.09) 5H at C15-C19 ppm

^{13}C-NMR(δ): (114.2) C1, C3; (131.8) C4, C6; (122.4) C5; (165.7) C7; (63.4)

C10; (39.5) C11; (126.4-129.5) C15-C19;(131.8) C14 ppm.

3.5.3 Synthesis 2-phenylaminoethyl4-t-butylbenzoate



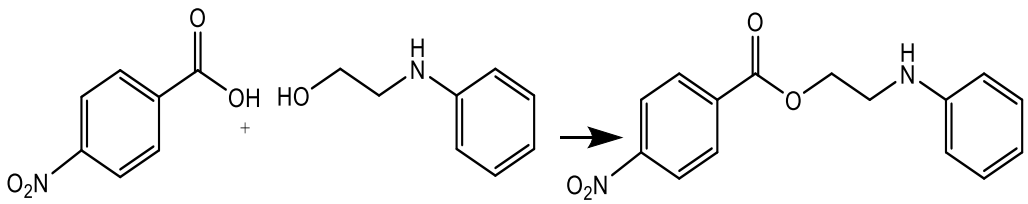
2-(phenylamino) ethan-1-ol (2.4068g, 0.0175mol) and 4-t-butylbenzoic acid (2g,0.0112mol) were refluxed to produced 2-phenylaminoethyl4-t-butylbenzoate. Yield was 3.9% and m.p.=160-162˚C.

IR; 3749;3392; 2962; 2871; 2673; 2543; 1926; 1818; 1681; 1604; 1569; 1505; 1461; 1422; 1365; 1317; 1287; 1187; 1133; 1056; 1017; 938; 856; 799; 779; 749; 707; 693; 636; 578; 543; 522 cm^{-1} .

^{1}HNMR (δ): (1.24) t-butyl 12H atached to C20, C21, C22; (2.5,3.5)4H attached to C10, C11; (7.4) 5H attached to C15-C19; 7.8 4H atached to C1,C3,C4,C6 ppm.

^{13}C-NMR(δ): (39.9)for C12, C20, C21, C22; (125-129) for C14-C19, C1, C3, C4, C5, C6; (167.7) for C7; (156.3) for C2 ppm.

3.5.4 Synthesis 2-phenylaminoethyl4-nitrobenzoate



2-(phenylamino) ethan-1-ol (1.640 g, 0.01196 mol) and 4-nitrobenzoic acid (2g,0.01196 mol) were refluxed to produced 2-phenylaminoethyl4-nitrobenzoate. Yield was 28.07% and m.p.=172-174 ˚C.

IR; IR; 3750.76; 3345.74; 1603.01; 1507.38; 1079.06; 750.24; 693.55; 658.49; 599.81; 574.90; 537.29; 522.95 cm^{-1} .

^{1}HNMR (δ):(7.46) N-H; (7.32) 4H at C1,C3,C4, C6; (7.19-7.31) 5H at C15-C19); (3.55) 2H at C10; (3.6) 2H at C11ppm.
^{13}C-NMR(δ):(129-135) C1,C3,C4, C5,C6; (146.9)C7, (136) C2;(50.4) C10; (39.9) C11; (116-129) C14-C19ppm.

Tab.3.2-phenylaminoethyl benzoates compounds melting point and % yield.

R	Name of compound	Melting point˚C	%yield
Br2	2-phenylaminoethyl 4-bromobenzoate	179-181˚C	36.9%
OCH3	2-phenylaminoethyl 4-methoxybenzoate	178-188˚C	43.17%
t-butyl	2-phenylaminoethyl4-t-butylbenzoate	160-162 ˚C	3.9%
NO2	2-phenylaminoethyl 4-nitrobenzoate	172-174˚C	28.07%

3.6 Checking for purification

The purity of the samples was confirmed by HPLC. The purity of all compounds ranged from 71–99.6%. A qualitative analysis was conducted using HPLC (Breeze 2, HPLC system) with a C18 column (5µm, 4.6×250 mm cartridge).The mobile phase was methanol (A)and acetonitrile (B) (60:40).The HPLC separation was achieved using binary-solvent gradient elution, which began with 100% of solvent A and 0% of solvent B and increased to 0% of A and 100% of B. The injection volume was 20 µL with a flow rate of 0.7 mL/min.

3.7 General procedure of anti-oxidant test for ester compounds

The free-radical scavenging capacity of acid ester compounds was determined by using the free-radical method DPPH [45]. The principle of the DPPH test depends on the decrease in absorbance at different concentrations. DPPH (2,2-diphenyl-1picryl-hydrazyl-hydrate) is free-radical stable at room temperature. It is reduced in the presence of anti-oxidant molecules. A fresh stock solution of 1 mg/mLof DPPH was prepared in methanol The concentration of ester compoundsin stock solution was 10mg/mL (100µg/mL). One millilitre (100µg/mL) of stock solution was placed in a test tube and diluted with methanol up to 10 mL. Different concentrations of stock solution were prepared (80, 50, 20, 10, and 7 µg/mL DPPH solution), and 1mg/mL was added in each test tube containing ester compounds and the test tubes were incubated for 30 min. The absorbance was determined at 517nm using a spectrometer. The IC_{50} $_0$for each compound was calculated from the percent inhibition. The reference for the test was Trolox. A control sample was prepared containing the same volume without any ester compounds. Trolox was used as the blank. The scavenging percentage was calculated according to the following equation:

% Inhibition=[$A_{control} - A_{sample}$]/$A_{control}$]*100

where $A_{control}$ is the absorbance of DPPH alone and A_{sample} is the absorbance of DPPH along with the different concentrations of ester compounds.

Table 3.3: Absorbance for samples at different concentration for DPPH test

	Concentration µg/mL	0	7	10	20	50	80	100
No.	Compounds name	Absorbance at different compounds concentration						
I.	2-phenylthioethyl 4-aminobenzoate	0	0.235	0.23	0.215	0.21	0.203	0.2
II.	2-phenylthioethyl 4-bromobenzoate	0	0.237	0.229	0.217	0.205	0.204	0.203
III.	2-phenylthioethyl 4-methoxybenzoate	0	0.232	0.223	0.215	0.203	0.194	0.18

IV.	2-phenylthioethyl 4-nitrobenzoate	0	0.235	0.225	0.217	0.206	0.203	0.2
V.	2-phenylthioethyl4-t-butylbenzoate	0	0.166	0.115	0.102	0.077	0.065	0.04
VI.	2-phenylaminoethyl 4-bromobenzoate	0	0.263	0.233	0.22	0.21	0.208	0.21
VII.	2-phenylaminoethyl 4-methoxybenzoate	0	0.228	0.215	0.205	0.179	0.165	0.15
VIII.	2-phenylaminoethyl 4-nitrobenzoate	0	0.26	0.235	0.23	0.217	0.2	0.18
IX.	2-phenylaminoethyl4-t-butylbenzoate	0	0.175	0.124	0.112	0.09	0.07	0.04
	Trolox	0	0.030	0.023	0.013	0.007	0.004	0.003

3.8 General procedure of antimicrobial test for ester compounds

The anti-microbial properties of the synthesized compounds were determined using eh broth micro-dilution method in Mueller Hinton Agar (MHA) against both Gram-positive and Gram-negative bacteria [46]. This method is considered a semi-quantitative method used to determine minimum inhibition concentration(MIC) [46]. The MIC is considered the lowest concentration of each compound that completely inhibits microbial growth. Compounds were tested against the following types of bacteria: *Pseudomonas aeruginosa* (ATCC27853), *Escherichia coli* (ATCC25922), *Staphylococcus aureus* (ATCC 25923), methicillin-resistant *Staphylococcus aureus* (MRSA), *Proteus*, *Klebsiella*, and *Candida albicans*as. Twenty milligrams per millilitre of each compound was dissolved in 500µg of dimethyl sulfoxide (DMSO) (Riedeldehan,Germany) and 500µL of media and then sterilised by UV light.

Two-fold serial dilution was done by transferring 2 mL of nutrient broth into mico-wells, starting with the negative control, and concentrations ranged from 10mg/mL to 0.625mg/mL. The positive control was free of the tested compounds, and the negative control was left uninoculated to test for the sterility of the media and must be clear after incubation.

Table 3.4: Micro dilution results for the samples at different concentration of ester compounds

TYPE OF BAC	I	II	III	VI	V	VI	VII	VIII	IX
MRSA	3	3	4	2	3	2	2	3.5	3.5
S.arues	3	3	3	1.5	2.5	1.5	1.5	3.5	3.5
Kleb.	2	2	2	1	3	1	1.5	5	5
E.coli	2	2	2	1	3	1	1.5	3	3
Protous	2.5	2	3	1	3	1	1.5	3	3
psedomonus	2	2	2	1	2	1	1	3	3
candida	2	2.5	3.5	2	3	2	3	3	3

3.9 α-amylase inhibitory screening

In this assay, we studied the effect of nine synthetic compounds using the standard protocol of 3,5-dinitrosalicylic acid (DNSA), with slight modifications [47]. The working solution was prepared after mixing buffer solutions of 0.02Msodium phosphate monobasic and sodium phosphate dibasic prepared using 0.006 M sodium chloride and 0.02 M of NaH_2PO_4,H_2O, and $Na_2HPO_4 .7H_2O$ at pH 6.9.Samples of each compound were prepared by dissolving 25mg of each in 25 mL of 10% DMSO to give a concentration of 1mg/mL and were further dissolved in buffer (Na_2HPO_4/NaH_2PO_4[0.02 M], NaCl [0.006 M] at pH 6.9). The following concentrations were prepared: 500, 100, 70, 50, and 10µg/mL. Fresh starch stock (1% w/v) was prepared using 1 g of starch suspended in 100 mL of phosphate buffered saline (pH 6.9). Porcine pancreatic α-amylase solution was prepared by dissolved 50mg of the amylase enzyme in a minimum amount of DMSO (10%), DNSA was used as a reactive reagent, and it

reacts with reducing sugars to form 3-animo-5-nitrosalicylic acid, which strongly absorbs light at 540 nm. It was prepared by dissolving 200 mg of DNSA in 4.0 mL of 2 M NaOH (8 g in 100 mL distilled water) and 6 g sodium potassium tartrate then completed the volume up to 20 mL. The reaction started with the addition of 250 µL of the enzyme solution (2 unit/mL) to 250µL of each synthesized compounds and incubated for 10 min at room temperature. Then, 250µL of the starch solution was added to each tube, and each tube was incubated for at least 3 min at room temperature. The reaction was stopped by the addition of 500µL of DNSA (di nitro salicylic acid) reagent and boiled for 10 min in a water bath at 100°C. The mixture was cooled to reach room temperature and diluted with 5mL of distilled water, and the absorbance was recorded at 540nm using a UV–Vis spectrophotometer. The blank with 100% enzyme activity was prepared by replacing the synthesized compounds with 250µL of buffer. All tests were run in triplicate. Acarbose was used as a standard reference following the same previous steps. The α-amylase inhibitory activity was calculated according to the following equation:

$$\% \text{ α-amylase inhibition} = (A_B - A_T)/A_B * 100\%$$

where A_B is the absorbance of the blank and A_T is the absorbance of the tested sample. The percent inhibition of α-amylase was plotted vs. the concentration of the tested compounds, and the IC_{50} values were obtained from the graph.

Table 3.5: Absorbance for samples at different concentration for anti-α-amylase test

No.	Concentration (µg/mL)	0	10	50	70	100	500
	Compounds name	Absorbance at different compounds concentration					
I	2-phenylthioethyl 4-aminobenzoate	0	0.116	0.107	0.107	0.091	0.089
II	2-phenylthioethyl 4-bromobenzoate	0	0.111	0.1	0.1	0.097	0.087

III	2-phenylthioethyl 4-methoxybenzoate	0	0.105	0.105	0.095	0.094	0.094
IV	2-phenylthioethyl 4-nitrobenzoate	0	0.235	0.229	0.222	0.179	0.121
V	2-phenylthioethyl4-t-butylbenzoate	0	0.099	0.09	0.09	0.085	0.049
VI	2-phenylaminoethyl 4-bromobenzoate	0	0.101	0.099	0.097	0.088	0.063
VII	2-phenylaminoethyl 4-methoxybenzoate	0	0.303	0.239	0.215	0.205	0.204
VIII	2-phenylaminoethyl 4-nitrobenzoate	0	0.242	0.227	0.2	0.089	0.083
IX	2-phenylaminoethyl4-t-butylbenzoate	0	0.012	0.115	0.0189	0.002	0.001
	Acrobose	0	0.141	0.1367	0.103	0.103	0,084

3.10 Anti-lipase activity test

The inhibition of digestive lipases refers to the suppression of dietary fat absorption and, to some extent, to a strategy against overweight and obesity.The assay was carried out following the standard protocol method, with slight modifications [48]. The stock solution of the synthesized molecules was prepared by dissolving 25mg of the molecules in 25mL of 10% DMSO to give a concentration of 1mg/mL and then diluted to prepare different concentrations (600, 400,300, 200, and 100µg/mL) using the dilution process.

The pancreatic lipase enzyme stock solution (1mg /mL) was prepared using 25mg of the lipase enzyme suspended in a minimum amount of DMSO (10%),then completed up to 25 mL and placed in a water bath sonicator at 37ºC for 15 min.*p*-Nitro phenyl butyrate (PNPB) was used as the substrate for the lipase enzyme [49].

The hydrolysis of 4-nitrophenyl butyrate by these enzymes releases the chromophore 4-nitrophenolate, which is spectrophotometrically active at 415 nm [50]. It was prepared by dissolving 20.9 mg of PNPB in 2.0 mL of acetonitrile by dissolving 104.5 mg of PNPB in acetonitrile “up the volume to 10 mL in V. F(10 mL).The reaction started with the addition of 200 µL

of the enzyme solution to 400µL of each diluted synthesized compound, and then 1400 µL Tris- HCl was added to each tube and each tube was incubated for 15 min at 37°C. After the incubation time, 200 µL of PNPB solution was added to each test tube, and each tube was incubated again for 30 min at 37°C.

The pancreatic lipase activity was determined by measuring the hydrolysis of *p*-nitrophenolate to *p*-nitrophenol at 405 nm using a UV–Vis spectrophotometer. The blank with 100% enzyme activity was prepared by replacing the synthesized compounds with 400µL of Tris- HCl. All tests were run in triplicate. Orlistat was used as the standard reference following the same prev

$$\% \text{ of lipase inhibition} = (A_B - A_T)/A_B * 100\%$$

where A_B is the absorbance of the blank and A_T is the absorbance of the tested sample.

The percent lipase inhibition was plotted against the synthesized molecule concentration, and the IC_{50} values were obtained from the graph. Table 3.9 shows the absorbance of the samples at different concentrations after the addition of acid esters.

Table 3.6: Absorbanc values of acid esters against lipase inhibition

	Concentrations(µg/mL)	0	100	200	300	400	600
NO.	Name of compounds	Absorbance at different compounds concentration					
I.	2-phenylthioethyl 4-aminobenzoate	0	1.495	1.426	1.388	1.388	1.35
II.	2-phenylthioethyl 4-bromobenzoate	0	1.2	1.19	1.116	1.032	0.999
III.	2-phenylthioethyl 4-methoxybenzoate	0	1.738	1.547	1.446	1.369	1.313
IV.	2-phenylthioethyl 4-nitrobenzoate	0	1.392	1.364	1.359	1.3	1.225
V.	2-phenylthioethyl4-t-butylbenzoate	0	1.099	0.999	0.956	0.882	0.703
VI.	2-phenylaminoethyl 4-	0	1.732	1.489	1.462	1.453	1.448

	bromobenzoate						
VII.	2-phenylaminoethyl 4-ethoxybenzoate	0	1.719	1.642	1.633	1.519	1.519
VIII.	2-phenylaminoethyl 4-nitrobenzoate	0	1.554	1.544	1.512	1.43	1.424
IX.	2-phenylaminoethyl4-t-butylbenzoate	0	1.334	1.233	1.008	0.774	0.481
	Orlistat	0	0.608	0.14	0.126	0.108	0.085

Chapter Three

Result and discussion

4.1 synthesis of thio and anino acid esters

Acid esters were synthesized using a typical procedure of esterification called the Fisher procedure, where five different carboxylic acids (4-bromo benzoic acid, 4-nitro benzoic acid, 4-amino benzoic acid, 4-methoxy benzoic acid and 4-tertbutyl benzoic acid) were used and refluxed with two typesof alcohol [2-(phenylthio) ethan-1-ol and 2-(phenylamino) ethan-1-ol] in the presence of a mineral inorganic acid catalyst. The products were checked by thin layer chromatography, and crudes were purified through CC with the mobile phase (3 n-hexane: 2 acetone) as the eluent. The purity of the samples was confirmed by HPLC (Breeze 2, HPLC system. The mobile phase was methanol and acetonitrile (60:40). The figures show the retention time and area under the peaks. The purity of all compounds ranged between 71 and 99.6%. Yields were between 25 and 79%. The compounds were identified and confirmed from IR, H-NMR, and C^{13} analysis.

4.2 DPPH assay result:

An anti-oxidant agent is a substance that detoxifies the action of free radicals and reduces oxidative stress by different mechanisms. The DPPH test uses a method to determine the ability of acid esters to detoxify free radical activity, depending on the decrease in absorbance at different concentrations, making a simple reduction reaction between the anti-oxidant agent and DPPH. free radical is reduced in the presence of an antioxidant molecule through the donation of a hydrogen atom, which gives rise to colour. DPPH is free-radical stable at room temperature and has a

dark violet colour. The decrease in the colour of the solution leads to a decrease in absorption, which is taken as a measure of the extent of radical scavenging. Trolox was used as a reference for the test. The percent inhibition and IC_{50} for all compounds were calculated as shown in Table 4.1 and Figure 4.1.

Nine acid ester were prepared for the first time and tested for their antioxidant activity at different concentrations ranging from 7–100µg/mL. For all of these, the activity increased with increasing concentrations. Two of the compounds (2-phenylthioethyl-4-t-butylbenzoate and 2-phenylaminoethyl-4-t-butylbenzoate) showed percent inhibition result at a concentration of 100 µg/mL 84.70% and 84.79%, respectively, and the IC_{50} values were 13.5664µg/mL and 10.50µg/mL, respectively. When comparing the results of the two compounds with Trolox, we concluded that the activity of the two compounds was about 75%compared with Trolox. The percent inhibition of Trolox was 99%, and the IC_{50} was0.58µg/mL, while the other compounds did not show any significant activity. On the other hand, a few studies held about hydroxyl acid esters and showed IC_{50} range 790–1000 µg/mL[51].

Table 4.1 percent inhibition and IC_{50} for tested compounds at different concentration for DPHH test.

	Concentration (µg/mL)	0	7	10	20	50	80	100	IC_{50}
NO.	**Name of compounds**	**%Inhibition**							
I.	2-phenylthioethyl4-aminobenzoate	0	10.64	12.5	18.25	20.15	22.8	23.9	108.5407
II.	2-phenylthioethy 4-bromobenzoate	0	9.8	12.9	17.5	22	22.4	22.8	58.8445
III.	2-phenylthioethyl4-methoxybenzoate	0	11.78	15.2	18.2	22.8	26.23	31.55	1956.2547
IV.	2-phenylthioethyl4-nitrobenzoate	0	10.64	14.4	17.4	21.67	22.8	23.95	199.5801
V.	2-phenylthioethyl4-t-butylbenzoate	0	36.8	56.27	61.21	70.7	75.2	84.7	13.5664
VI.	2-phenylaminoethyl4-bromobenzoate	0	0	11.40	16.34	20.1	20.9	21	678.0756
VII.	2-phenylaminoethyl4-methoxybenzoate	0	13.3	18.25	22.05	31.9	37.26	42.9	39.2144
VIII.	2-phenylaminoethyl4-nitrobenzoate	0	1.14	10.64	12.54	17.5	24	31.5	83.3109
IX.	2-phenylaminoethyl4-t-butylbenzoate	0	33.5	52.85	57.41	65.8	73.4	84.79	10.50
	Trolox	0	88.9	91.4	95.4	97.4	98.5	99	0.5917

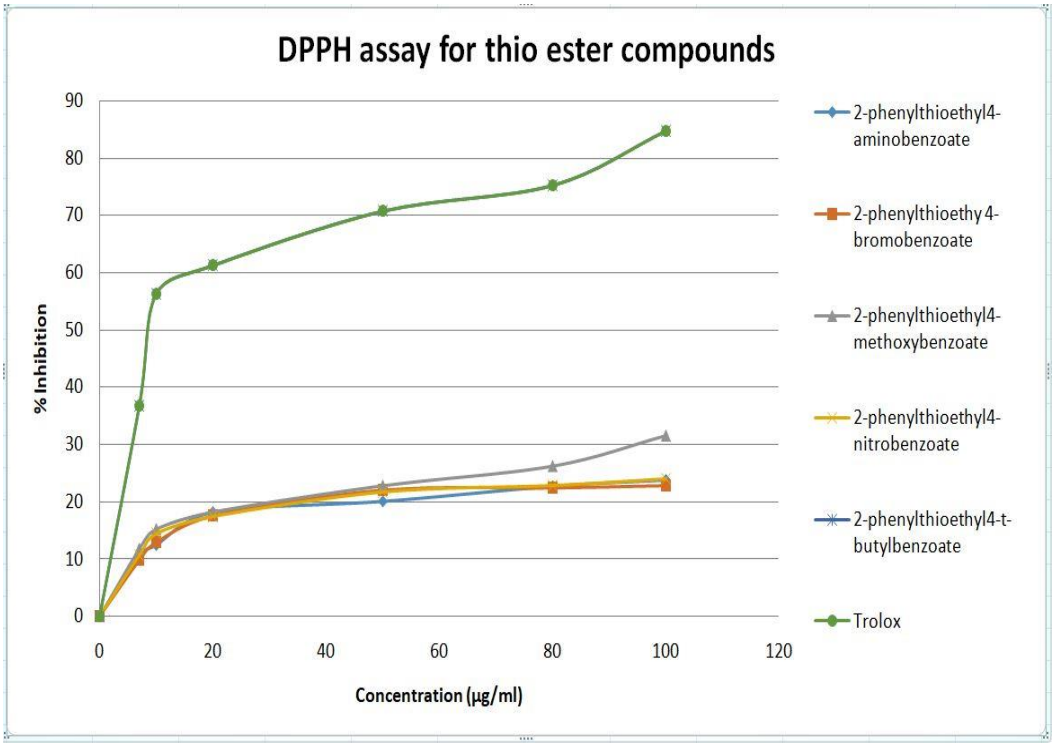


Figure (4.1) percent inhibition of the tested thio ester compounds

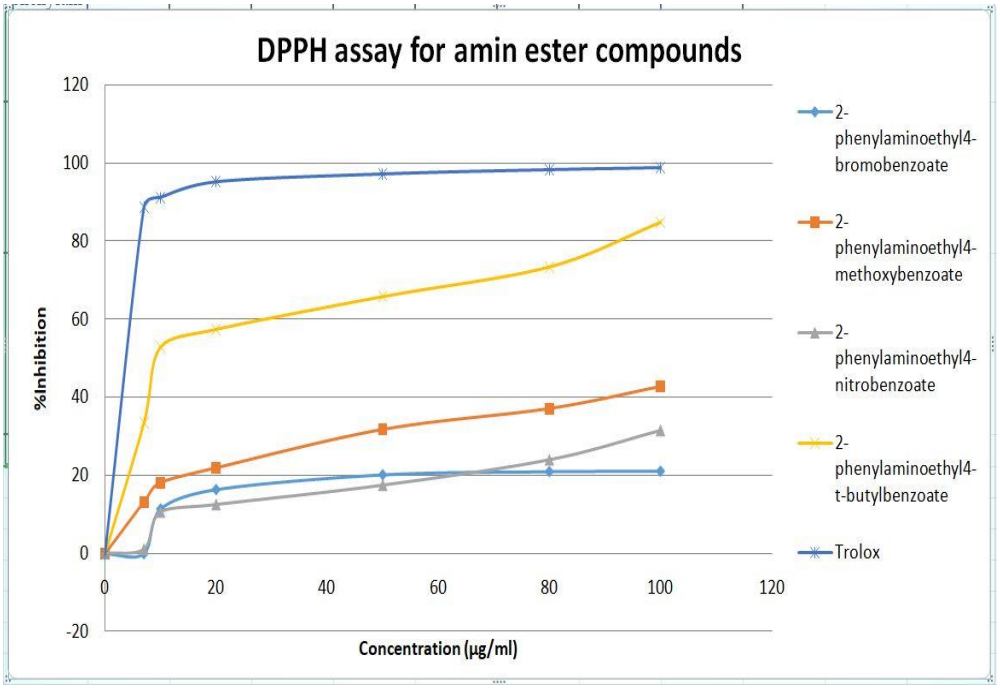


Figure (4.2): percent inhibition of DPPH for the tested amino ester compounds

4.3 Anti-microbial assay result

Synthetic ester compounds were assayed against microbial activity and MICby the broth micro dilution test [46]. Seven types of pathogenic microbes were used, including Gram-positive bacteria, Gram-negative bacteria and one type of fungi called *Candida albicans*. The references of the assay were the anti-fungal drug fluconazole and the anti-bacterial drugs ampicillin and ciprofloxacin.The MICs for the compounds are shown in Table 4.2 and Figure 4.2.

Table 4.2 (MIC) values for the compounds shown at different concentrations

TYPE OF BACTERIA	I	II	III	VI	V	VI	VII	VIII	IX	fluconazol	Ampicillin	Ciprofloxacin
MRSA	1.875	1.875	0.9375	3.75	1.875	3.75	3.75	1.875	1.875	R	R	0.15
S.arues	1.875	1.875	1.875	7.5	3.75	7.5	7.5	1.875	1.875	R	2.12	0.87
Kleb.	3.75	3.75	3.75	7.5	1.875	7.5	7.5	0.46625	0.46625	R	0.002	0.132
E.coli	3.75	3.75	3.75	7.5	1.875	7.5	7.5	1.875	1.875	R	2.12	1.45
Protous	3.75	3.75	1.875	7.5	1.875	7.5	7.5	1.875	1.875	R	R	0.87
psedomonus	3.75	3.75	3.75	7.5	3.75	7.5	7.5	1.875	1.875	R	2.01	0.016
candida	3.75	3.75	1.875	3.75	1.875	3.75	1.875	1.875	1.875	1.62	R	R

All types of microbes, including MRSA (resistance to amoxicillin), were susceptible to all ester compounds. MIC values were ranged from 7.5–0.47µg/mL. Compound III had a MIC value of 0.94µg/mL against MRSA,while for MIC of each following compounds I, II, III, VIII and IX were 1.875µg/mL against *aureus*, which is slightly lower than MIC of amoxicillin 2.12µg/mL. In addition, compounds VIII and V showed the lowest MIC values against*klebsilla* (0.47µg/mL), which is about 75% of ciprofloxacin and amoxicillin. As for the Gram-negative bacteria *protous*, despite their resistance to amoxicillin they were susceptible to all compounds, and the best result was found for compounds V, VIII, and IX, with a MIC value of 1.875 µg/mL. In addition, compounds VIII and IX showed significant activity against *E.coli* and *pseudomonas*.The MIC value 1.875 µg/mL better than MIC for amoxicillin against *E. coli* and *pseudomonas.* MIC values for compounds VIII and IX were close to the MIC value of ciprofloxacin against *E. coli* and *pseudomonas.* Distinguished results appeared with *Candida*, which was susceptible to compounds III,V,VIII, and IX. The MIC value was nearly the same as the value for the anti-fungal drug fluconazole.

4.4 α-amylase inhibitory assay result

The α-amylase inhibitory enzyme acts as a competitive, reversible inhibitor of pancreatic α-amylase and membrane-bound intestinal α-glucoside hydrolase.

Pancreatic α-amylase hydrolyses complex carbohydrates to oligosaccharides in the small intestine. Nine tested compounds were tested for their α-amylase inhibition activity,and IC_{50} values were compared with the standard compound (acarbose).

The results are shown in Table 4.3.

Table 4.3 percent inhibition and IC50value of α-amylase assay for tested compounds

	Concentration (µg/mL)	0	10	50	70	100	500	IC_{50}
NO.	Name of compounds	% Inhibition						
I.	2-phenylthioethyl 4-aminobenzoate	0	61.7	64.7	64.7	69.9	70.6	2.567
II.	2-phenylthioethyl 4-bromobenzoate	0	63.4	66.9	66.9	67.9	73.6	34.22
III.	2-phenylthioethyl 4-methoxybenzoate	0	65.32	68.6	68.6	68	68	0.0206
IV.	2-phenylthioethyl 4-nitrobenzoate	0	22.4	24.4	26.7	40.9	60	99.89
V.	2-phenylthioethyl4-t-butylbenzoate	0	67.3	67.3	67.3	72	83.82	50.3
VI.	2-phenylaminoethyl 4-bromobenzoate	0	66.6	67.3	67.9	71	79.2	66.03
VII.	2-phenylaminoethyl 4-methoxybenzoate	0	0	21.1	29	32.34	32.67	120.7
VIII.	2-phenylaminoethyl 4-nitrobenzoate	0	20.1	25.1	33.9	70.6	72.6	77.6275
IX.	2-phenylaminoethyl4-t-butylbenzoate	0	60.4	62	93.8	99.33	99.66	19.5
	Acarbose	0	53.2	54.9	66.1	66.1	72.5	52.17

Table 4.3 and figures (4.3,4.4) showed the percent inhibition against α-amylase enzyme of the synthesized acid esters. Most of them showed better results than $IC_{50}$of Acarbose. IC_{50} for Acarbose was 52.17 µg/mL and percent inhibition was 72.5 at 500 µg/mL. IC_{50} I, II, III, V and IX were showed better than result of Acarbose, the IC_{50} for mention compounds 2.567µg/mL, 34.22 µg/mL 0.0206µg/mL, 50.3 µg/mL and 19.5 µg/mL respectively.

Figure 4.3 percent inhibition of α -amylase enzyme for the tested thio ester compounds

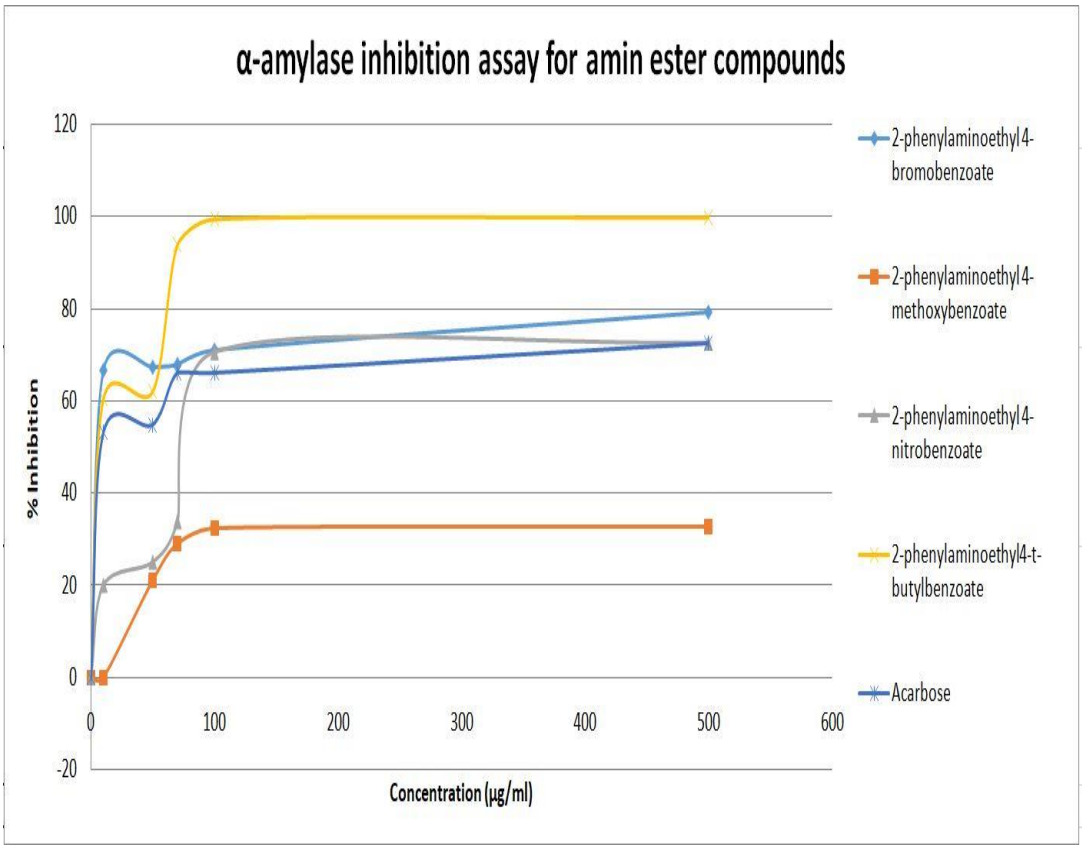


Figure 4.4 percent inhibition α –amylase assay for the tested amino ester compounds

The percent inhibition of compounds IX, V, and VI was 99.5%, 79.3%, and 73.5%, respectively, at 500 µg/mL. Percent inhibition values for compounds I, II, III , V, VI, and IX were higher than 50% at 10µg/mL, which is a very interesting result. The IC_{50} value for compound III was 0.0206 µg/mL, which is 3000 times lower than acarbose as a stander .This result deserves more attention and comprehensive medicinal studies. They may be better good alternatives in the future.

4.5 Anti-lipase activity assay result

Lipase is a very important enzyme for fat digestion and lipid transport. It is responsible for the breakdown of triglyceride and converts it to free fatty acids and glycerol to become available for absorption in the intestines [9]. The result of the lipase inhibition assay showed different percent inhibitor

of pancreatic lipase (PL)and IC_{50} and then compared with standard (orlistat) as show in Table 4.4 and Figure4.4 and Figure 4.6. **% Inhibition**

Table 4.4 percent inhibition and IC50 of lipase enzyme for tested compounds for anti-lipase test

	Concentration (µg/mL)	0	100	200	300	400	600	IC_{50}
NO.	Name of compounds							
I.	2-phenylthioethyl 4-aminobenzoate	0	13.98	17.95	17.95	20.13	23	<1000
II.	2-phenylthioethyl 4-bromobenzoate	0	30.95	31.53	31.53	40.62	42.5	<1000
III.	2-phenylthioethyl 4-methoxybenzoate	0	0	10.98	10.98	21.21	24.5	<1000
IV.	2-phenylthioethyl 4-nitrobenzoate	0	19.9	21.51	21.51	27.7	29.5	<1000
V.	2-phenylthioethyl4-t-butylbenzoate	0	36.7	42.5	42.5	49.25	59.5	600
VI.	2-phenylaminoethyl 4-bromobenzoate	0	0.34	14.3	14.3	16.6	16.39	<1000
VII.	2-phenylaminoethyl 4-methoxybenzoate	0	1.09	6.04	6.04	12.6	13.17	<1000
VII	2-phenylaminoethyl 4-nitrobenzoate	0	10.58	11.16	11.16	17.7	18.1	<1000
IX.	2-phenylaminoethyl4-t-butylbenzoate	0	23.24	29.05	29.05	55.5	72.32	400
	Orlistat	0	65	92	92.8	93.5	95.1	82.187

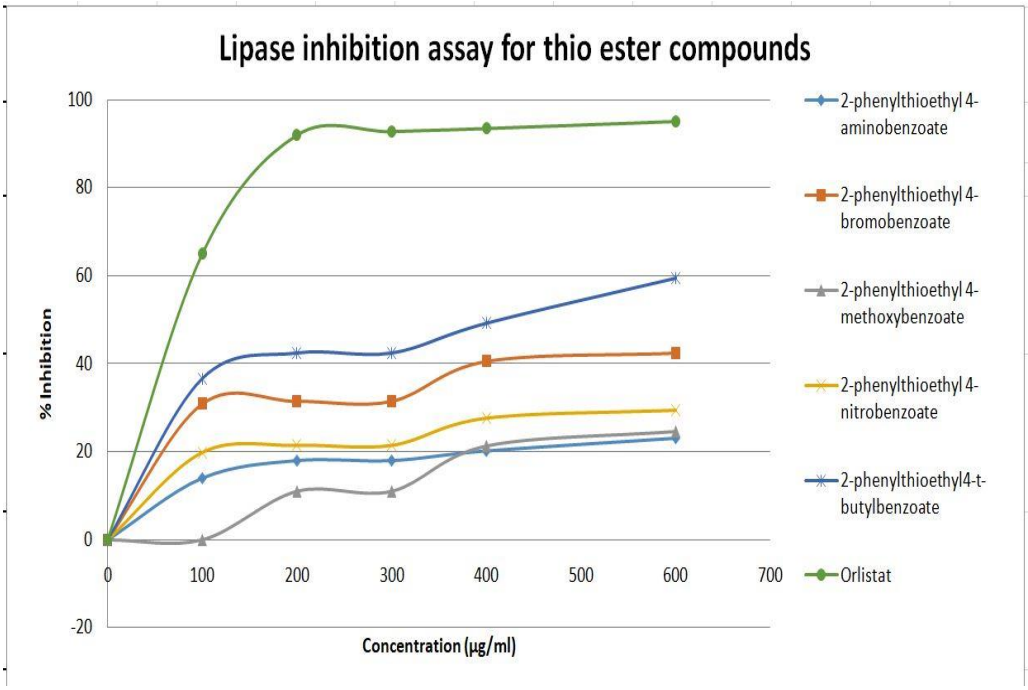


Figure 4.5 percent inhibition of lipase enzyme for the tested thioacid esters

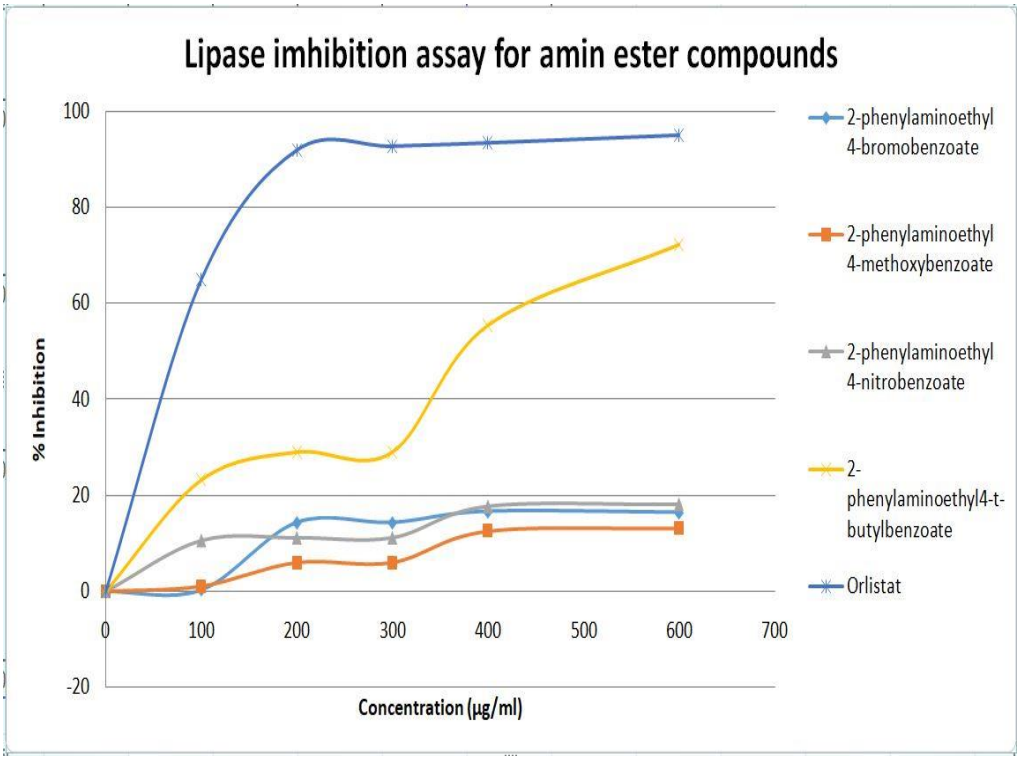


Figure 4.6 percent inhibition of lipase enzyme for tested acid esters

Most acid esters did not show significant activity against the lipase enzyme, except for two compounds (V and IX). The percent inhibitions of these were 59.5% and 72.3% at 500 µg/mL,the while IC_{50}values were 600

and 400 µg/mL, respectively. The activities of the two compounds were similar when compared with Orlistat as a reference for the test; although, they lack an amide group, which represents the main group responsible for the activity of Orlistat.

Conclusion

Nine acid esters were prepared by the Fisher esterification using five different carboxylic acid derivatives and two different alcohols [2-(phenylthio) ethan-1-ol and 2-(phenylamino) ethan-1-ol)].The compounds were identified and confirmed by necessary analyses, such as IR spectroscopy H-NMR, and ^{13}C-NMR The compounds were investigated for their anti-oxidant, anti-lipase, anti-amylase, and anti-microbial activities. The results of the investigation showed significant activity, especially against the α-amylase enzyme and fungus. Most of the compounds showed considerable activity against α-amylase inhibition, especially compounds VI, V, and IX, and they were better than standard. All compounds were found to be susceptible to all bacterial strains and the fungus Candida. Some compounds showed anti-oxidant activity that was slightly lower than Trolox as the standard. Other compounds acted as α-lipase inhibitors; although, they lack an amide group.

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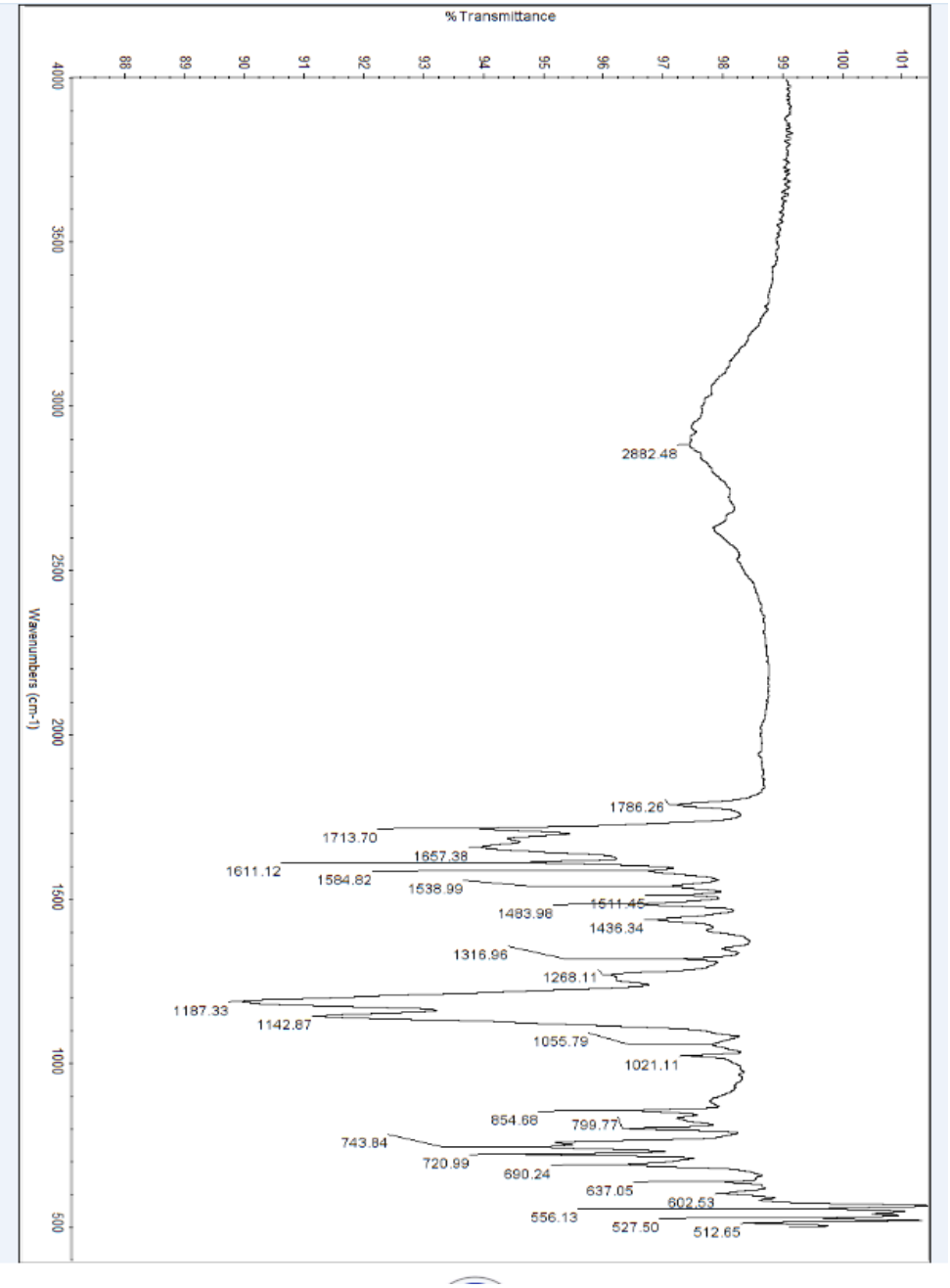
Index of IR analysis results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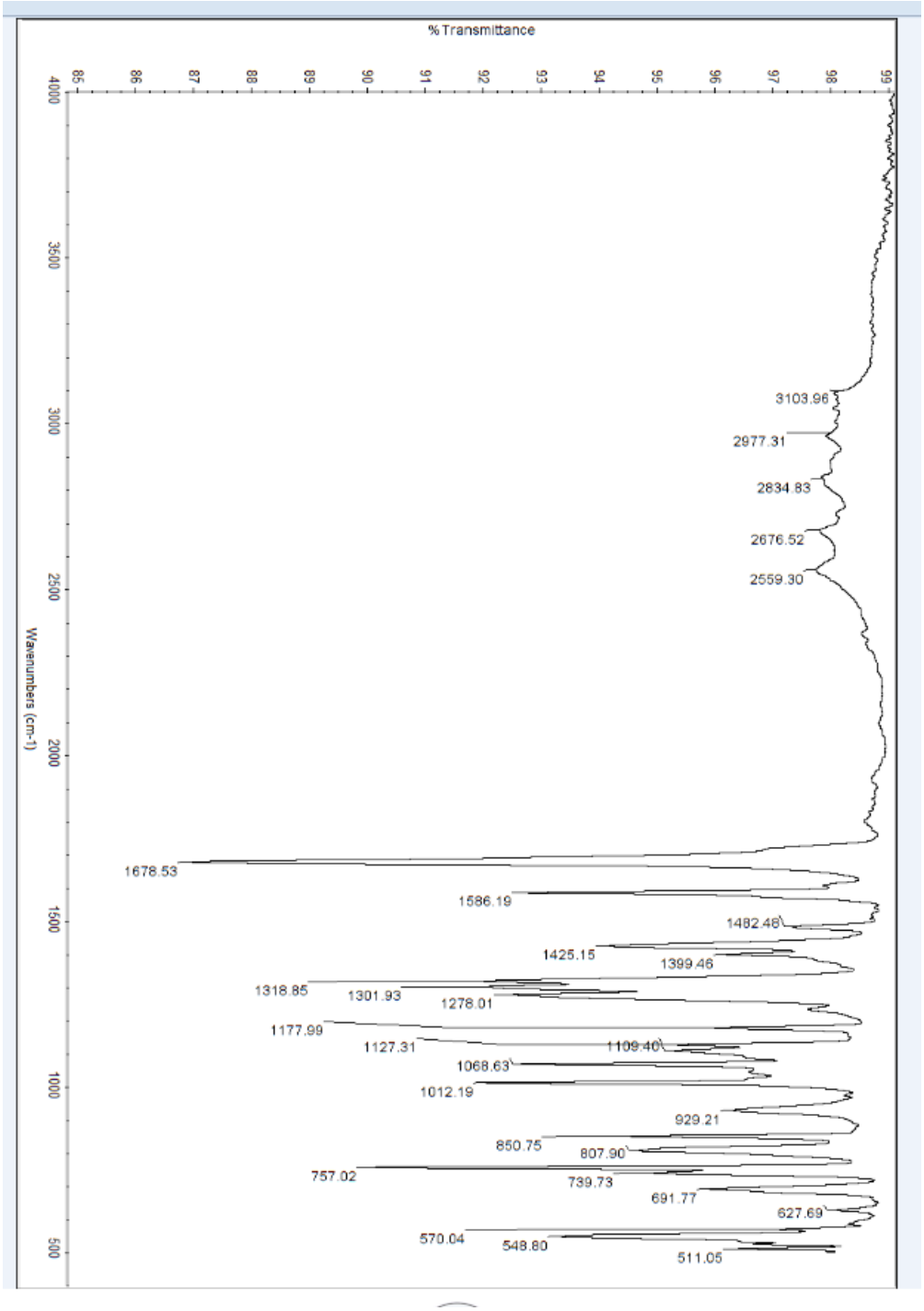
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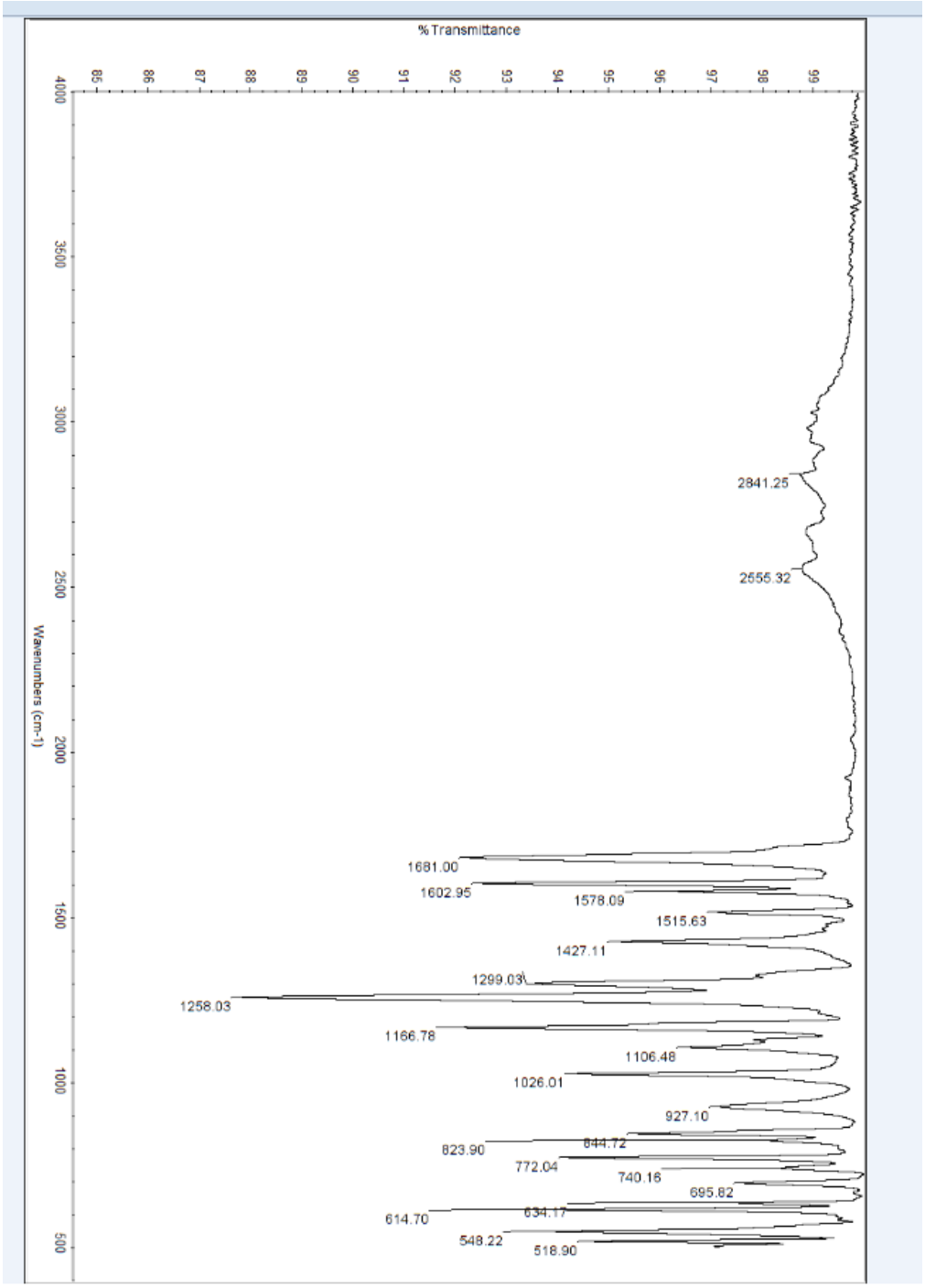
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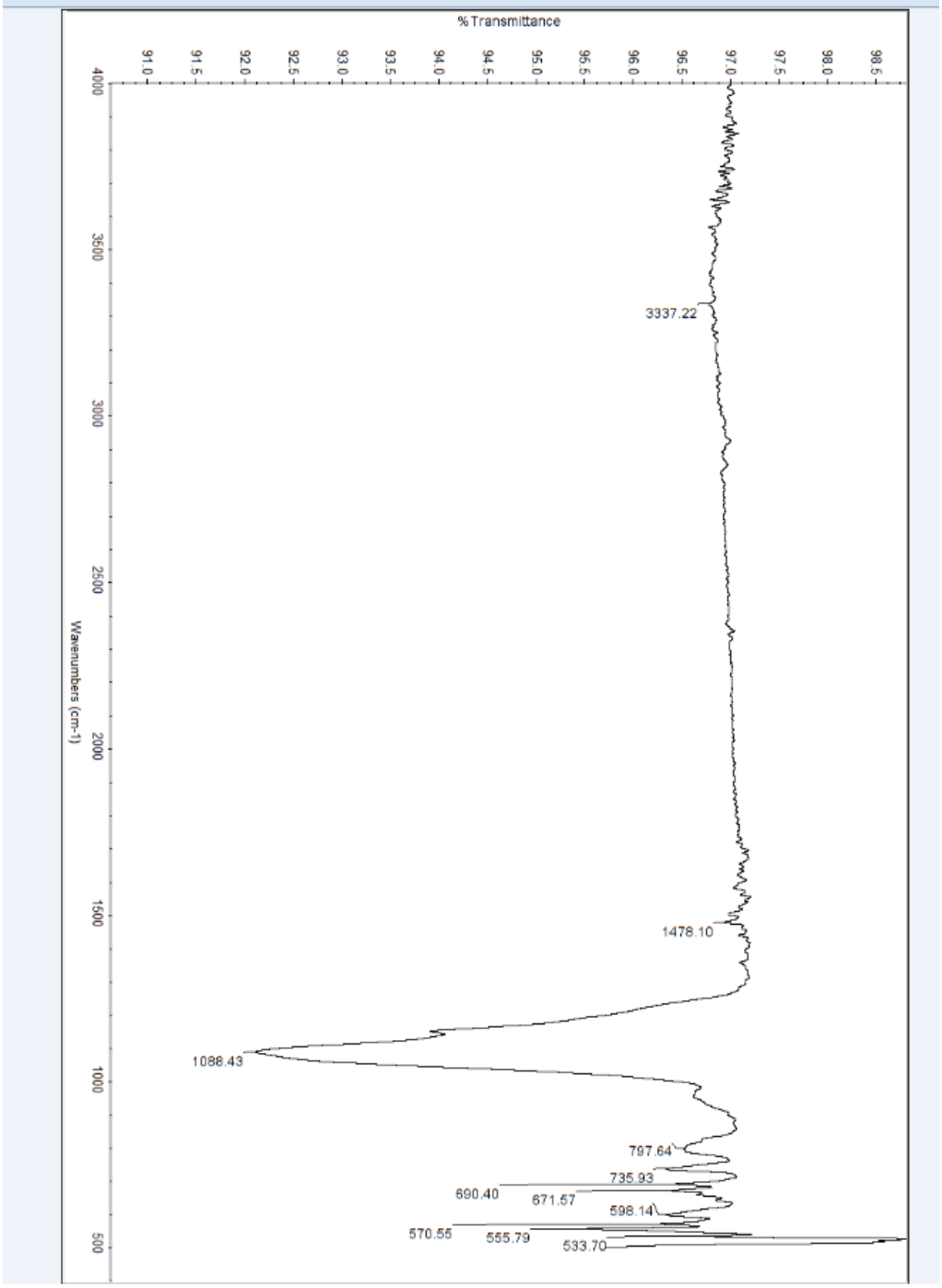
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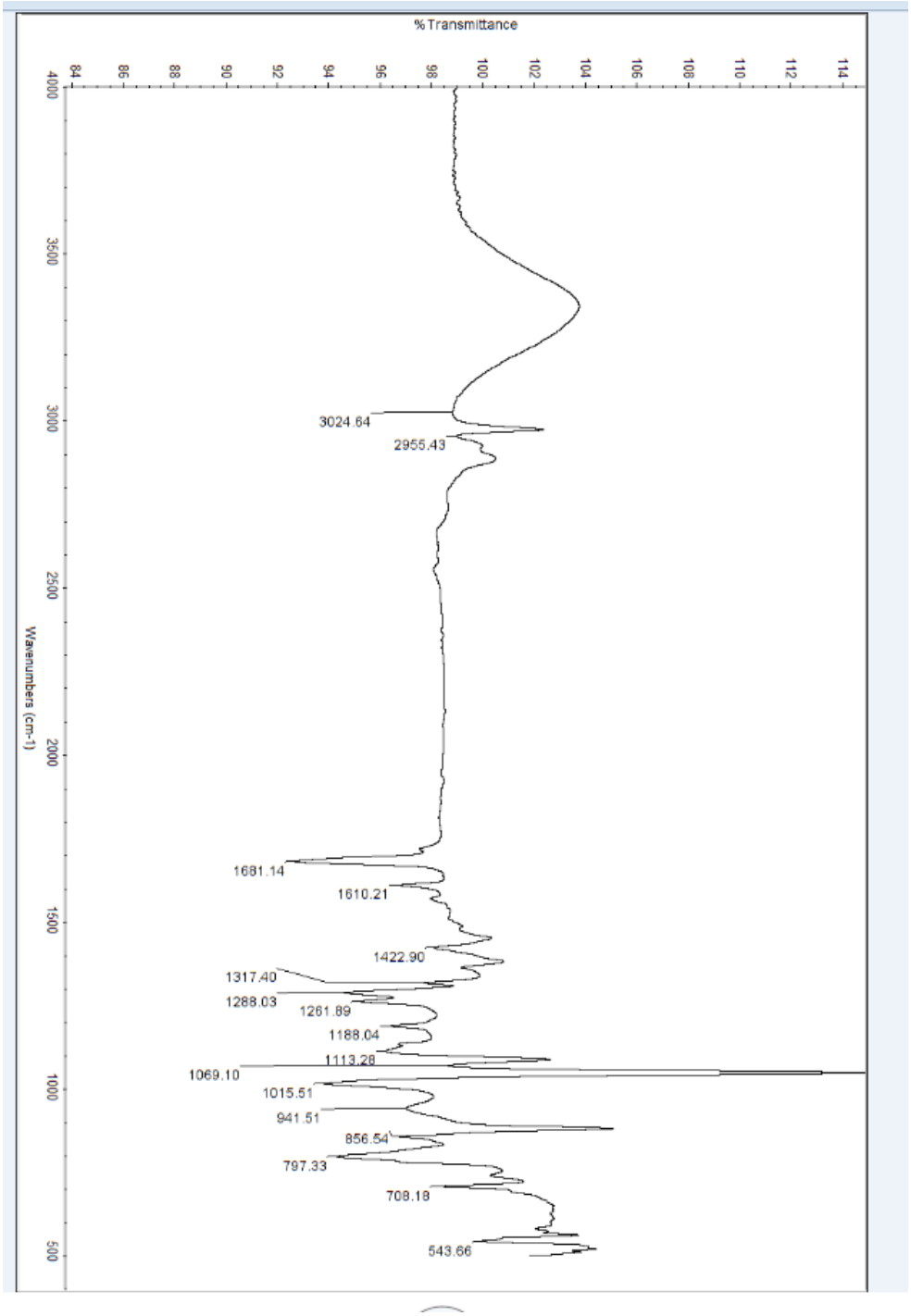
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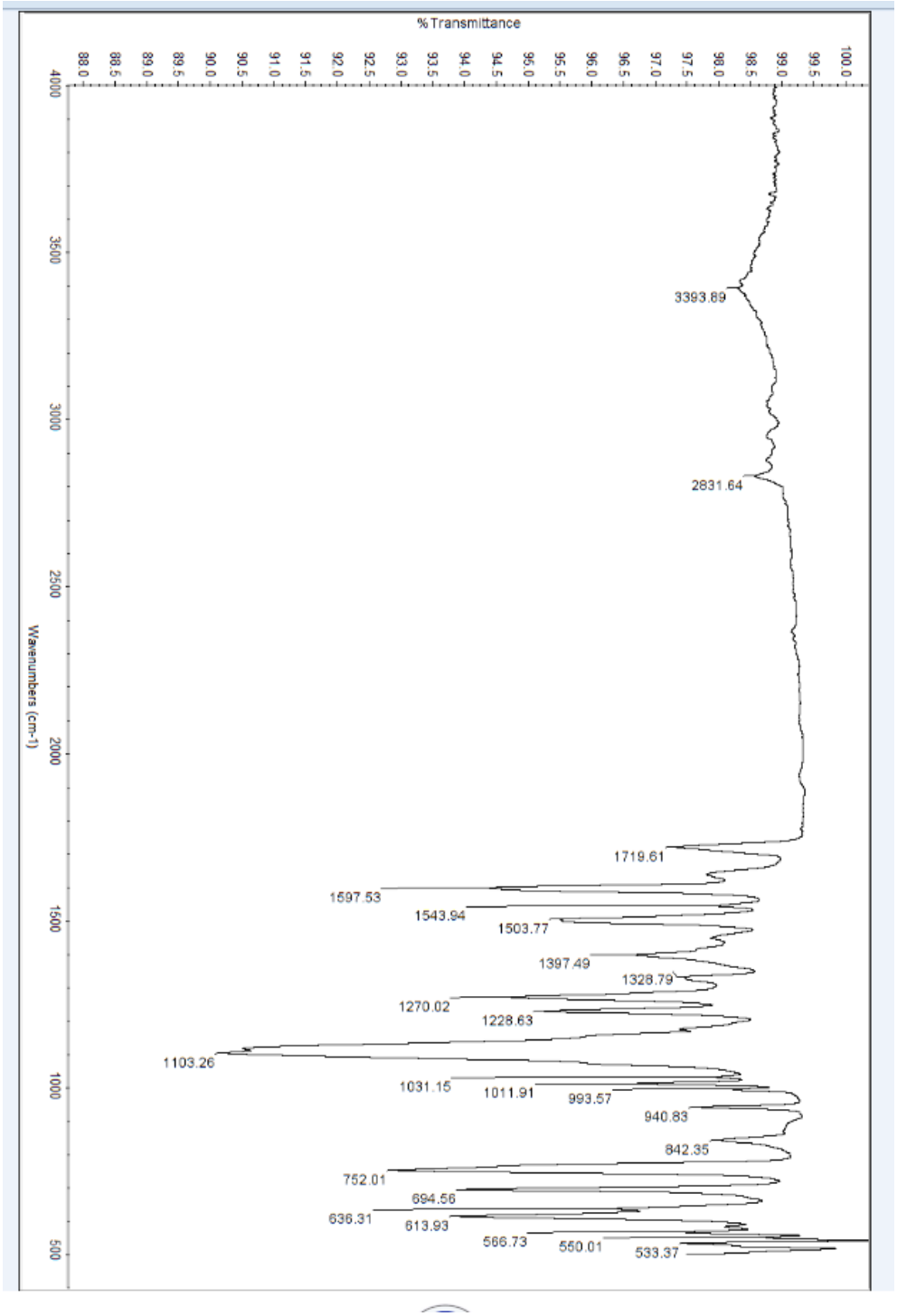
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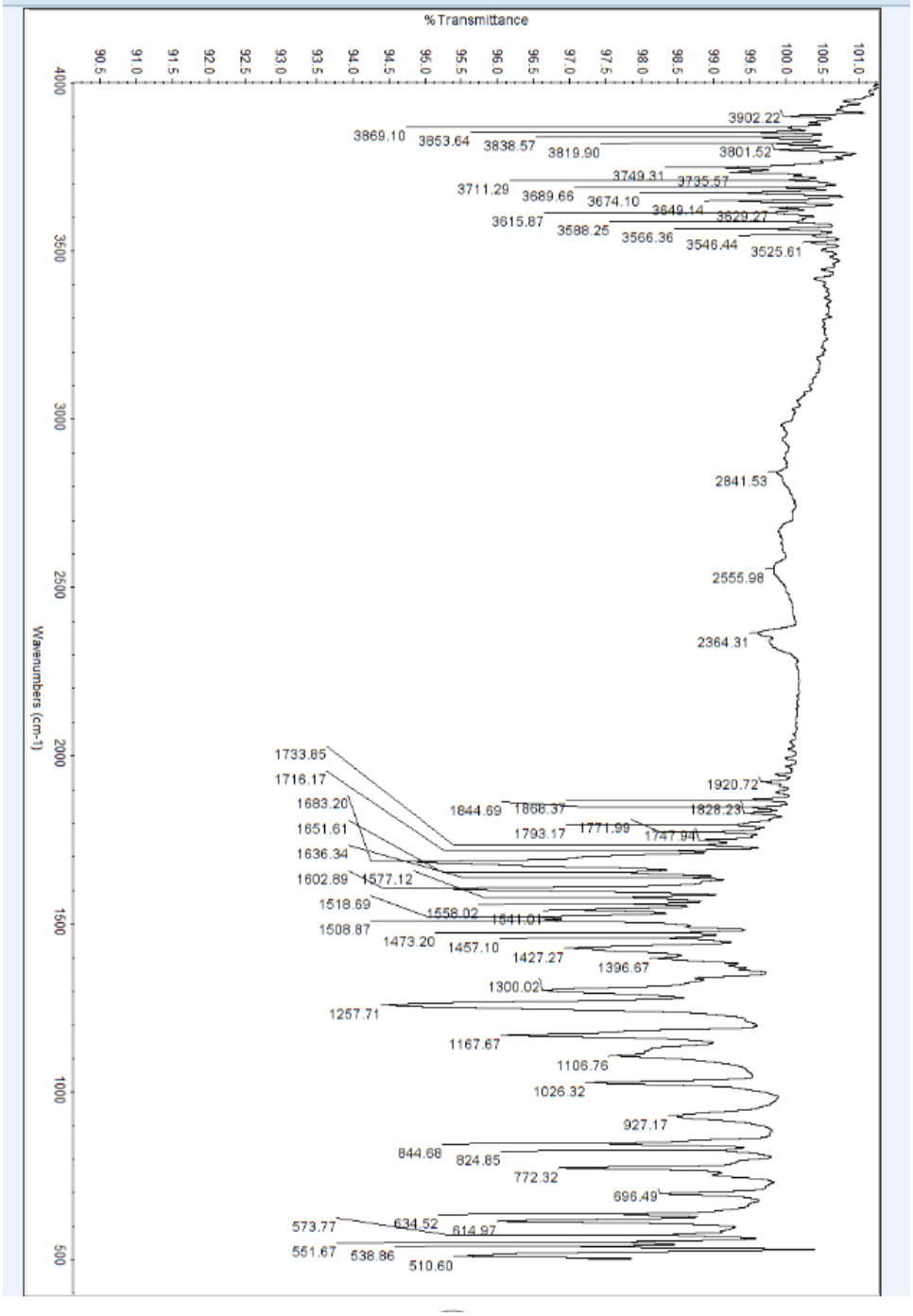
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3735.57
3711.29
3689.66
3674.10
3649.14
3629.27
3615.87
3588.25
3566.36
3546.44
3525.61
2841.53
2555.98
2364.31
1920.72
1733.85
1716.17
1683.20
1844.69
1868.37
1828.23
1793.17
1771.99
1747.94
1651.61
1636.34
1602.89
1577.12
1518.69
1558.02
1541.01
1508.87
1473.20
1457.10
1427.27
1396.67
1300.02
1257.71
1167.67
1106.76
1026.32
927.17
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824.85
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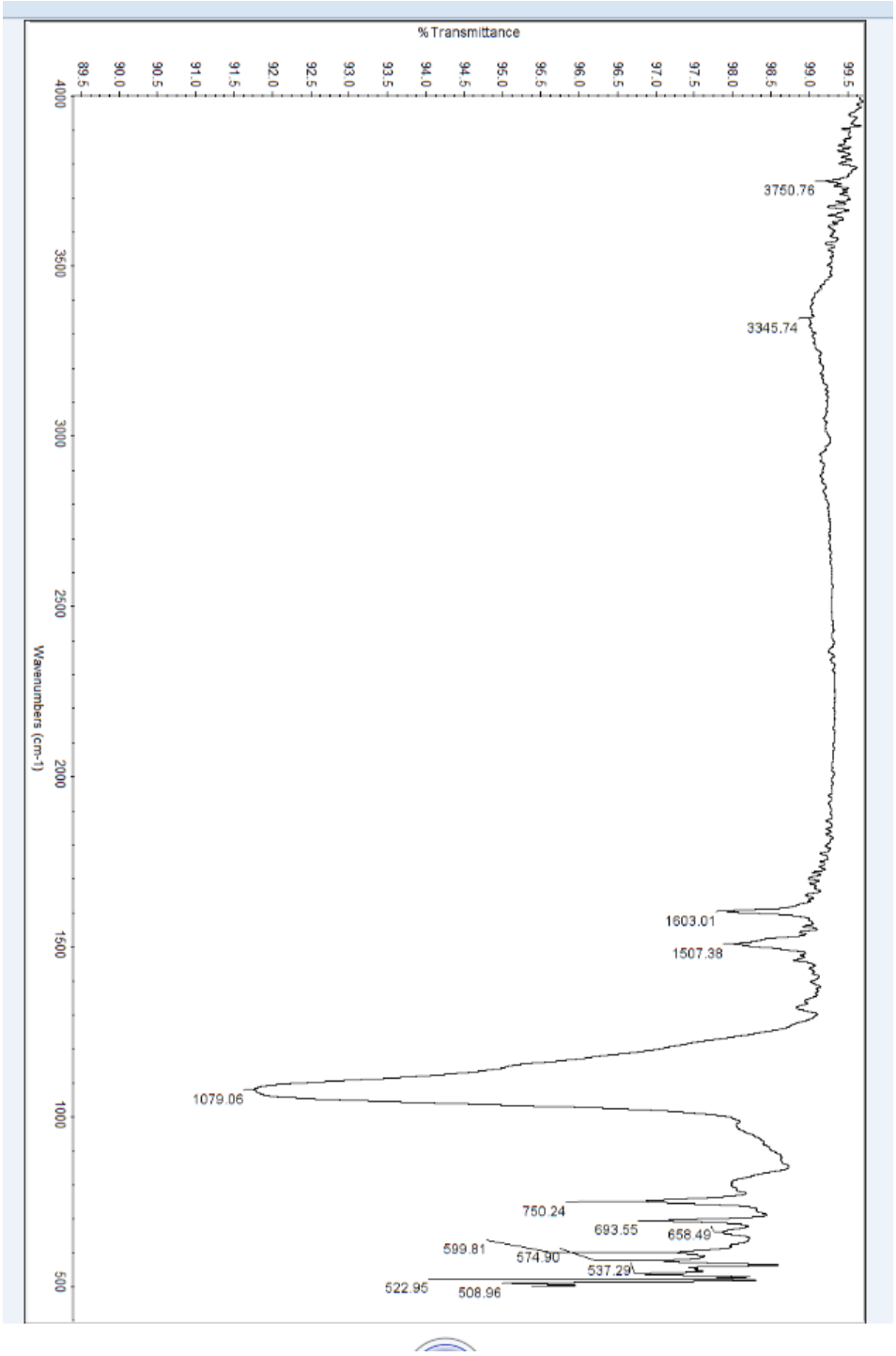
573.77
634.52
614.97
551.67
538.86
510.60


%Transmittance
Wavenumbers (cm-1)
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3345.74
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1079.06
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658.49
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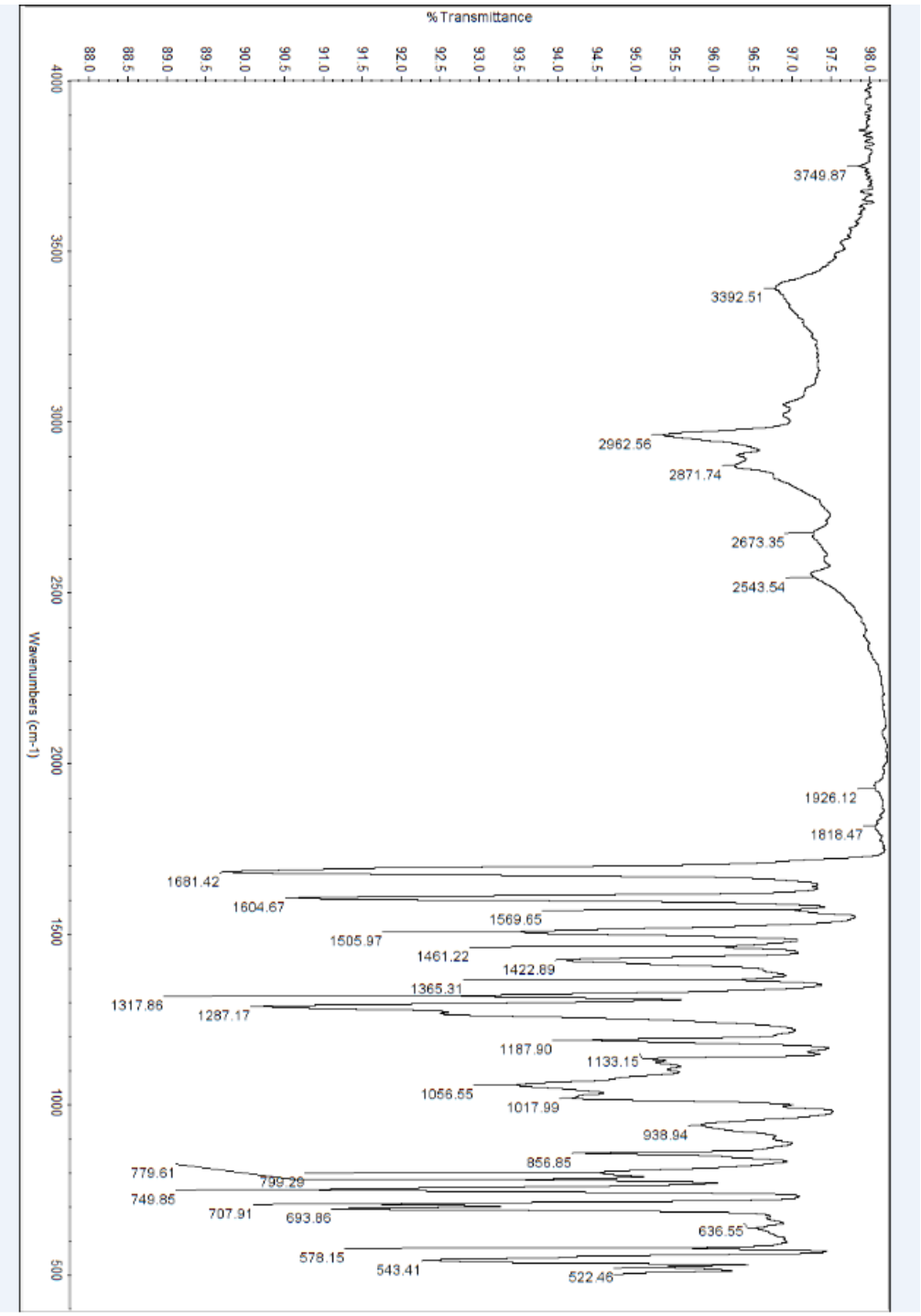
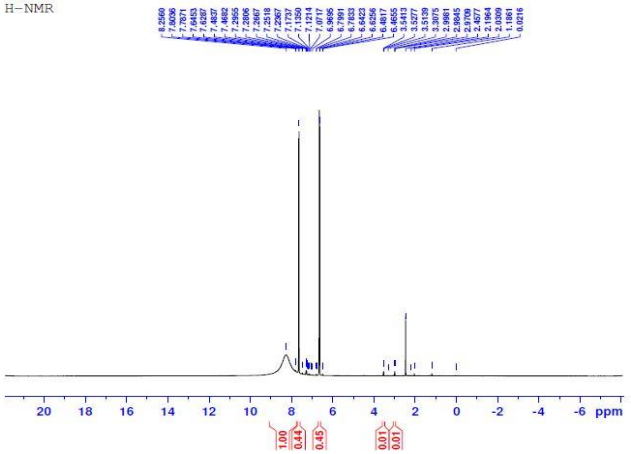
508.96


%Transmittance
Wavenumbers (cm-1)
3749.87
3392.51
2962.56
2871.74
2673.35
2543.54
1926.12
1818.47
1681.42
1604.67
1569.65
1505.97
1461.22
1422.89
1365.31
1317.86
1287.17
1187.90
1133.15
1056.55
1017.99
938.94
856.85
779.61
799.29
749.85
707.91
693.86
636.55
578.15
543.41
522.46

Index of ^{1}**HNMR (δ and** ^{13}**C−NMR(δ)** analysis results

amin-thio
H-NMR



The University of Jordan
Faculty of Science
Department of Chemistry

Instrument Model:
Bruker 500 MHz-Avance III
Operator: Rola Hassouneh
nmr500@ju.edu.jo

```
Current Data Parameters
NAME          21jun15jalal
EXPNO               351
PROCNO                1

F2 - Acquisition Parameters
Date_          20210617
Time              10.20 h
INSTRUM           spect
PROBHD   Z119470_0023 (
PULPROG             zg
TD                65536
SOLVENT            DMSO
NS                    4
DS                    0
SWH           15000.000 Hz
FIDRES            0.457764 Hz
AQ                2.1845334 sec
RG                   39.9
DW                33.333 usec
DE                 6.50 usec
TE                314.0 K
D1             2.00000000 sec
TD0                   1
SFO1         500.1334791 MHz
NUC1                 1H
P1                12.00 usec
PLW1         13.32299995 W

F2 - Processing parameters
SI                 131072
SF           500.1300270 MHz
WDW                  EM
SSB       0
LB                0.40 Hz
GB        0
PC                2.00
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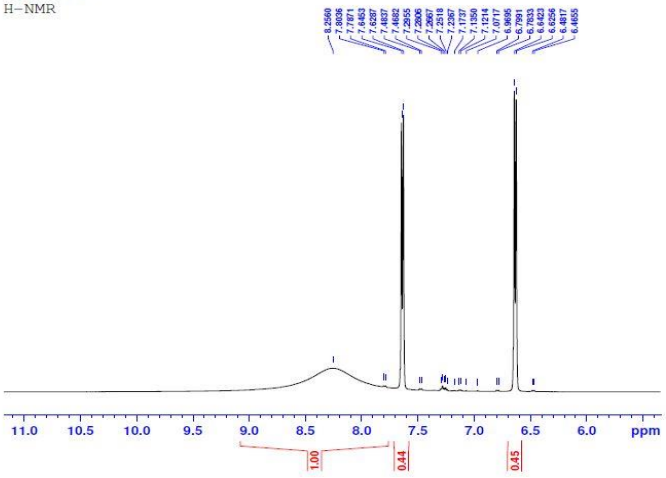
amin-thio
H-NMR



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Department of Chemistry

Instrument Model:
Bruker 500 MHz-Avance III
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```
Current Data Parameters
NAME          21jun15jalal
EXPNO               351
PROCNO                1

F2 - Acquisition Parameters
Date_          20210617
Time              10.20 h
INSTRUM           spect
PROBHD   Z119470_0023 (
PULPROG             zg
TD                65536
SOLVENT            DMSO
NS                    4
DS                    0
SWH           15000.000 Hz
FIDRES            0.457764 Hz
AQ                2.1845334 sec
RG                   39.9
DW                33.333 usec
DE                 6.50 usec
TE                314.0 K
D1             2.00000000 sec
TD0                   1
SFO1         500.1334791 MHz
NUC1                 1H
P1                12.00 usec
PLW1         13.32299995 W

F2 - Processing parameters
SI                 131072
SF           500.1300270 MHz
WDW                  EM
SSB       0
LB                0.40 Hz
GB        0
PC                2.00
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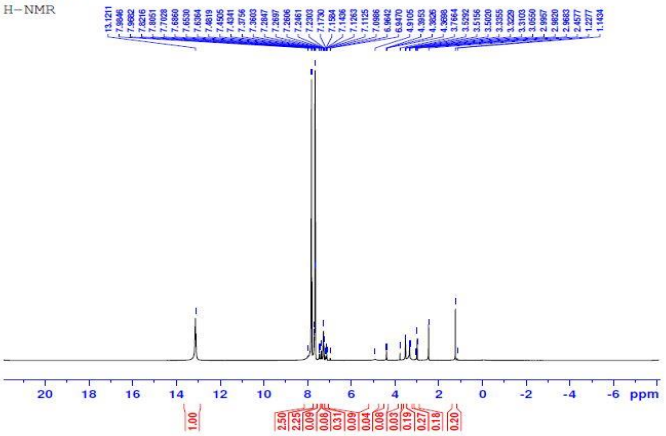

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Instrument Model:
Bruker 500 MHz-Avance III
Operator: Rola Hassouneh
nmr500@ju.edu.jo

```
Current Data Parameters
NAME                 21jun15jalal
EXPNO                         301
PROCNO                          1

F2 - Acquisition Parameters
Date_               20210616
Time                      12.06 h
INSTRUM                   spect
PROBHD     Z119470_0023 (
PULPROG                       zg
TD                         65536
SOLVENT                     DMSO
NS                             4
DS                             0
SWH                   15000.000 Hz
FIDRES                0.457764 Hz
AQ                    2.1845334 sec
RG                         39.9
DW                       33.333 usec
DE                         6.50 usec
TE                        298.0 K
D1                    2.00000000 sec
TD0                            1
SFO1                500.1334791 MHz
NUC1                        1H
P1                        12.00 usec
PLW1                13.32299995 W

F2 - Processing parameters
SI                        131072
SF                  500.1300270 MHz
WDW                           EM
SSB                 0
LB                         0.40 Hz
GB                  0
PC                         2.00
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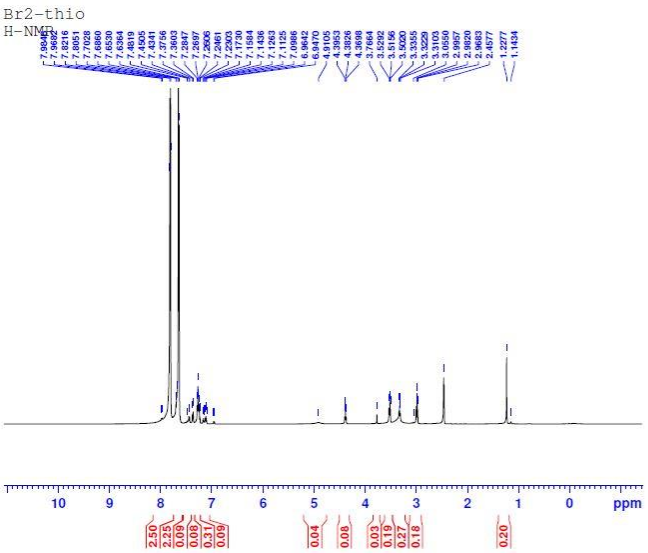

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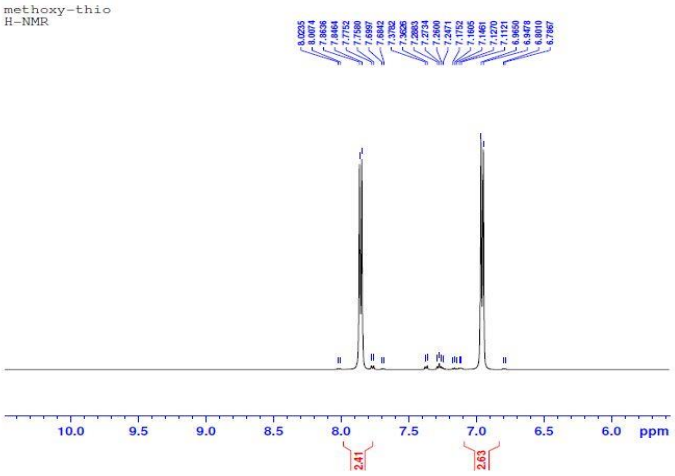
Instrument Model:
Bruker 500 MHz-Avance III
Operator: Rola Hassouneh
nmr500@ju.edu.jo

```
Current Data Parameters
NAME                 21jun15jalal
EXPNO                         301
PROCNO                          1

F2 - Acquisition Parameters
Date_               20210616
Time                      12.06 h
INSTRUM                   spect
PROBHD     Z119470_0023 (
PULPROG                       zg
TD                         65536
SOLVENT                     DMSO
NS                             4
DS                             0
SWH                   15000.000 Hz
FIDRES                0.457764 Hz
AQ                    2.1845334 sec
RG                         39.9
DW                       33.333 usec
DE                         6.50 usec
TE                        298.0 K
D1                    2.00000000 sec
TD0                            1
SFO1                500.1334791 MHz
NUC1                        1H
P1                        12.00 usec
PLW1                13.32299995 W

F2 - Processing parameters
SI                        131072
SF                  500.1300270 MHz
WDW                           EM
SSB                 0
LB                         0.40 Hz
GB                  0
PC                         2.00
```



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Instrument Model:
Bruker 500 MHz-Avance III
Operator: Rola Hassouneh
nmr500@ju.edu.jo

```
Current Data Parameters
NAME          21jun15jala1
EXPNO                  271
PROCNO                   1

F2 - Acquisition Parameters
Date_              20210616
Time                 11.47 h
INSTRUM                spect
PROBHD    Z119470_0023 (
PULPROG                  zg
TD                    65536
SOLVENT                DMSO
NS                        4
DS                        0
SWH               15000.000 Hz
FIDRES                0.457764 Hz
AQ                2.1845334 sec
RG                     17.8
DW                   33.333 usec
DE                     6.50 usec
TE                   298.0 K
D1                2.00000000 sec
TD0                       1
SFO1           500.1334791 MHz
NUC1                     1H
P1                    12.00 usec
PLW1          13.32299995 W

F2 - Processing parameters
SI                     131072
SF            500.1300270 MHz
WDW                       EM
SSB          0
LB                    0.40 Hz
GB           0
PC                    2.00
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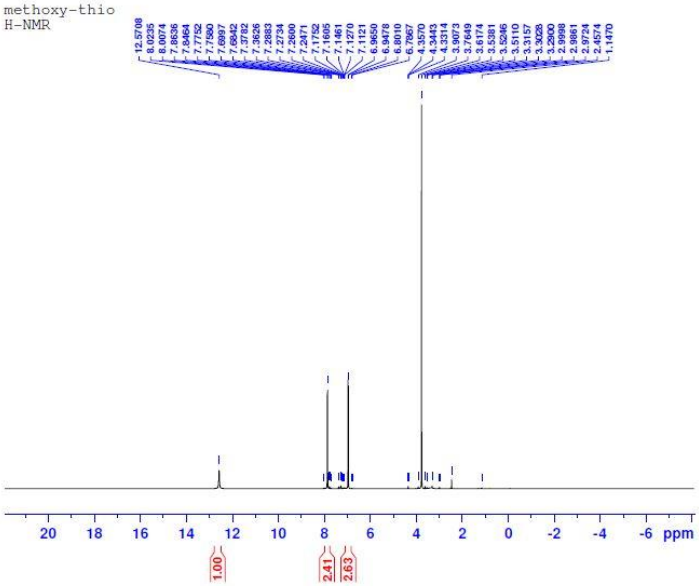

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Instrument Model:
Bruker 500 MHz-Avance III
Operator: Rola Hassouneh
nmr500@ju.edu.jo

```
Current Data Parameters
NAME          21jun15jala1
EXPNO                  271
PROCNO                   1

F2 - Acquisition Parameters
Date_              20210616
Time                 11.47 h
INSTRUM                spect
PROBHD    Z119470_0023 (
PULPROG                  zg
TD                    65536
SOLVENT                DMSO
NS                        4
DS                        0
SWH               15000.000 Hz
FIDRES                0.457764 Hz
AQ                2.1845334 sec
RG                     17.8
DW                   33.333 usec
DE                     6.50 usec
TE                   298.0 K
D1                2.00000000 sec
TD0                       1
SFO1           500.1334791 MHz
NUC1                     1H
P1                    12.00 usec
PLW1          13.32299995 W

F2 - Processing parameters
SI                     131072
SF            500.1300270 MHz
WDW                       EM
SSB          0
LB                    0.40 Hz
GB           0
PC                    2.00
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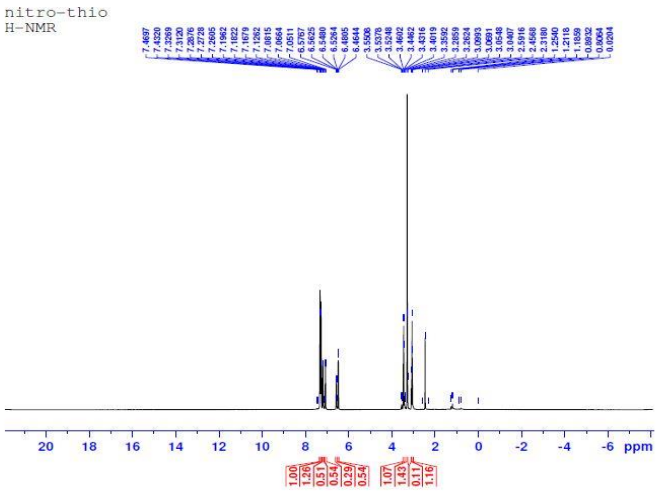

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Instrument Model:
Bruker 500 MHz-Avance III
Operator: Rola Hassouneh
nmr500@ju.edu.jo

```
Current Data Parameters
NAME          21jun15jalal
EXPNO                  451
PROCNO                   1

F2 - Acquisition Parameters
Date_              20210617
Time                 11.20 h
INSTRUM              spect
PROBHD    Z119470_0023 (
PULPROG                zg
TD                  65536
SOLVENT              DMSO
NS                       4
DS                       0
SWH            15000.000 Hz
FIDRES             0.457764 Hz
AQ              2.1845334 sec
RG                   49.66
DW                33.333 usec
DE                 6.50 usec
TE                298.0 K
D1             2.00000000 sec
TD0                      1
SFO1          500.1334791 MHz
NUC1                    1H
P1                  12.00 usec
PLW1          13.32299995 W

F2 - Processing parameters
SI                  131072
SF            500.1300270 MHz
WDW                     EM
SSB                      0
LB                  0.40 Hz
GB                       0
PC                  2.00
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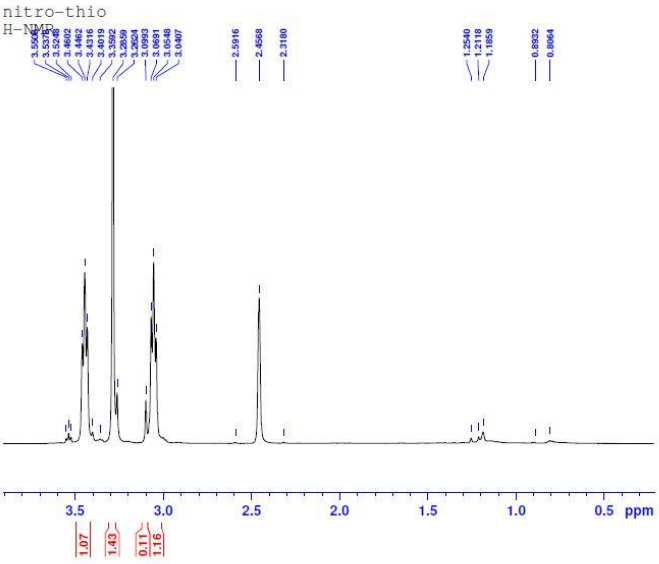

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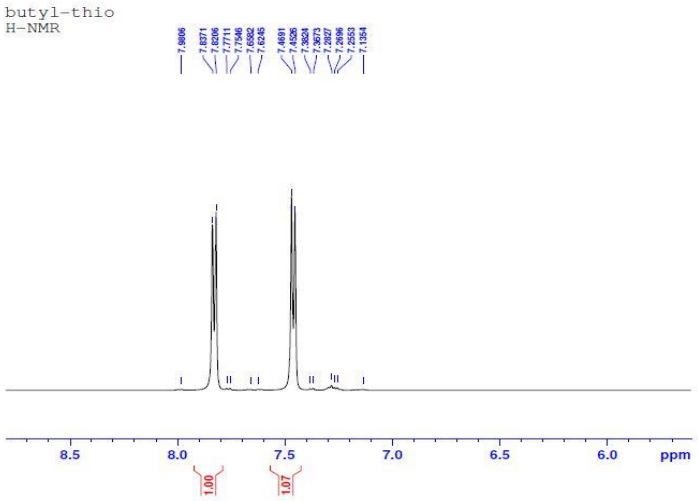
Instrument Model:
Bruker 500 MHz-Avance III
Operator: Rola Hassouneh
nmr500@ju.edu.jo

```
Current Data Parameters
NAME          21jun15jalal
EXPNO                  451
PROCNO                   1

F2 - Acquisition Parameters
Date_              20210617
Time                 11.20 h
INSTRUM              spect
PROBHD    Z119470_0023 (
PULPROG                zg
TD                  65536
SOLVENT              DMSO
NS                       4
DS                       0
SWH            15000.000 Hz
FIDRES             0.457764 Hz
AQ              2.1845334 sec
RG                   49.66
DW                33.333 usec
DE                 6.50 usec
TE                298.0 K
D1             2.00000000 sec
TD0                      1
SFO1          500.1334791 MHz
NUC1                    1H
P1                  12.00 usec
PLW1          13.32299995 W

F2 - Processing parameters
SI                  131072
SF            500.1300270 MHz
WDW                     EM
SSB                      0
LB                  0.40 Hz
GB                       0
PC                  2.00
```



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Instrument Model:
Bruker 500 MHz-Avance III
Operator: Rola Hassouneh
nmr500@ju.edu.jo

```
Current Data Parameters
NAME                  21jun15jalal
EXPNO                          461
PROCNO                           1

F2 - Acquisition Parameters
Date_                     20210617
Time                         11.31 h
INSTRUM                       spect
PROBHD     Z119470_0023 (
PULPROG                         zg
TD                           65536
SOLVENT                       DMSO
NS                               4
DS                               0
SWH                      15000.000 Hz
FIDRES                       0.457764 Hz
AQ                           2.1845334 sec
RG                              49.66
DW                           33.333 usec
DE                            6.50 usec
TE                           298.0 K
D1                        2.00000000 sec
TD0                              1
SFO1                   500.1334791 MHz
NUC1                            1H
P1                           12.00 usec
PLW1                  13.32299995 W

F2 - Processing parameters
SI                           131072
SF                    500.1300270 MHz
WDW                             EM
SSB                 0
LB                           0.40 Hz
GB                  0
PC                           2.00
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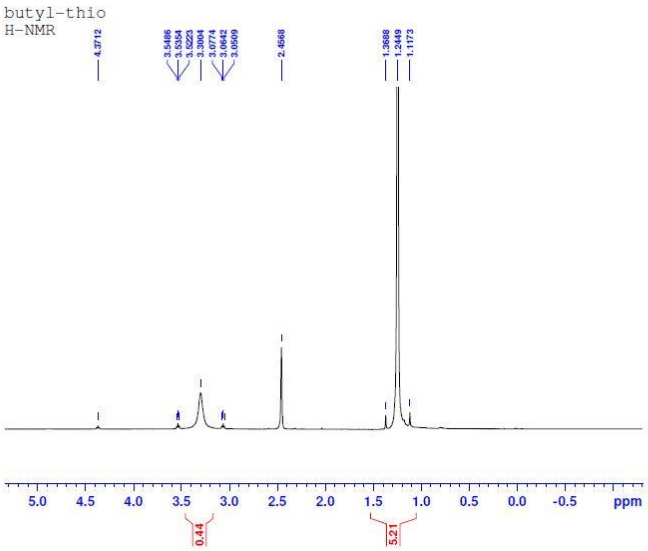

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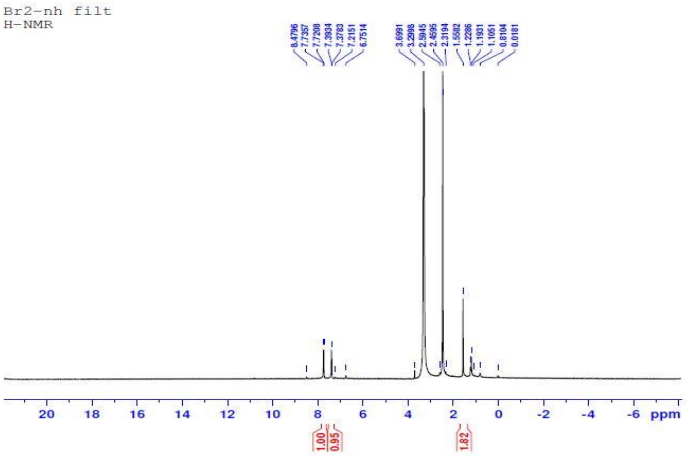
Department of Chemistry

Instrument Model:
Bruker 500 MHz-Avance III
Operator: Rola Hassouneh
nmr500@ju.edu.jo

```
Current Data Parameters
NAME                  21jun15jalal
EXPNO                          461
PROCNO                           1

F2 - Acquisition Parameters
Date_                     20210617
Time                         11.31 h
INSTRUM                       spect
PROBHD     Z119470_0023 (
PULPROG                         zg
TD                           65536
SOLVENT                       DMSO
NS                               4
DS                               0
SWH                      15000.000 Hz
FIDRES                       0.457764 Hz
AQ                           2.1845334 sec
RG                              49.66
DW                           33.333 usec
DE                            6.50 usec
TE                           298.0 K
D1                        2.00000000 sec
TD0                              1
SFO1                   500.1334791 MHz
NUC1                            1H
P1                           12.00 usec
PLW1                  13.32299995 W

F2 - Processing parameters
SI                           131072
SF                    500.1300270 MHz
WDW                             EM
SSB                 0
LB                           0.40 Hz
GB                  0
PC                           2.00
```



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Department of Chemistry

Instrument Model:
Bruker 500 MHz-Avance III
Operator: Rola Hassouneh
nmr500@ju.edu.jo

```
Current Data Parameters
NAME                  21jun15jalal
EXPNO                          291
PROCNO                           1

F2 - Acquisition Parameters
Date_               20210616
Time                    11.55 h
INSTRUM                  spect
PROBHD     Z119470_0023 (
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TD                      65536
SOLVENT                  DMSO
NS                          4
DS                          0
SWH                 15000.000 Hz
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RG                      71.89
DW                     33.333 usec
DE                      6.50 usec
TE                     298.0 K
D1                  2.00000000 sec
TD0                         1
SFO1               500.1334791 MHz
NUC1                        1H
P1                      12.00 usec
PLW1               13.32299995 W

F2 - Processing parameters
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SF                 500.1300270 MHz
WDW                        EM
SSB                 0
LB                      0.40 Hz
GB                  0
PC                      2.00
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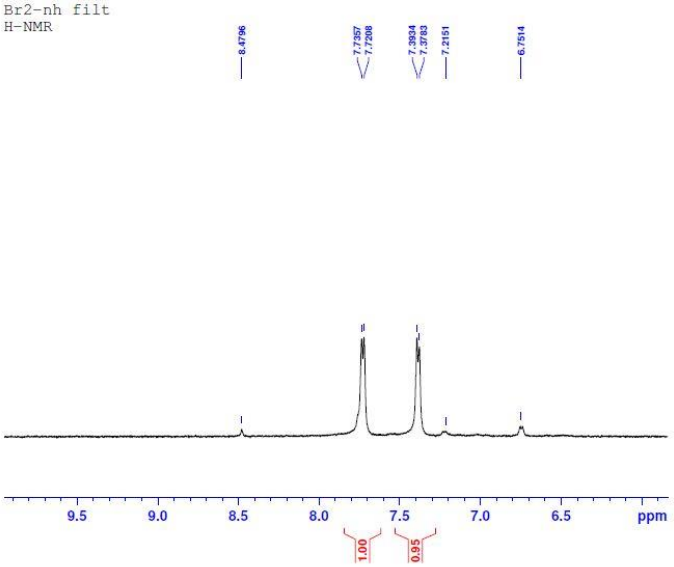

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Instrument Model:
Bruker 500 MHz-Avance III
Operator: Rola Hassouneh
nmr500@ju.edu.jo

```
Current Data Parameters
NAME                  21jun15jalal
EXPNO                          291
PROCNO                           1

F2 - Acquisition Parameters
Date_               20210616
Time                    11.55 h
INSTRUM                  spect
PROBHD     Z119470_0023 (
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TD                      65536
SOLVENT                  DMSO
NS                          4
DS                          0
SWH                 15000.000 Hz
FIDRES                0.457764 Hz
AQ                  2.1845334 sec
RG                      71.89
DW                     33.333 usec
DE                      6.50 usec
TE                     298.0 K
D1                  2.00000000 sec
TD0                         1
SFO1               500.1334791 MHz
NUC1                        1H
P1                      12.00 usec
PLW1               13.32299995 W

F2 - Processing parameters
SI                     131072
SF                 500.1300270 MHz
WDW                        EM
SSB                 0
LB                      0.40 Hz
GB                  0
PC                      2.00
```

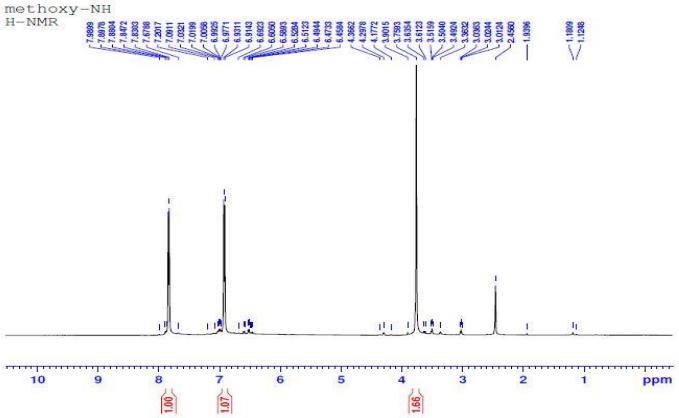

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Instrument Model:
Bruker 500 MHz-Avance III
Operator: Rola Hassouneh
nmr500@ju.edu.jo

```
Current Data Parameters
NAME                21jun15jalal
EXPNO                        441
PROCNO                         1

F2 - Acquisition Parameters
Date_               20210617
Time                    11.26 h
INSTRUM                  spect
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PULPROG                    zg
TD                      65536
SOLVENT                 DMSO
NS                             4
DS                             0
SWH                15000.000 Hz
FIDRES                 0.457764 Hz
AQ                 2.1845334 sec
RG                         28.18
DW                     33.333 usec
DE                        6.50 usec
TE                      298.0 K
D1                 2.00000000 sec
TD0                            1
SFO1               500.1334791 MHz
NUC1                        1H
P1                        12.00 usec
PLW1               13.32299995 W

F2 - Processing parameters
SI                        131072
SF                 500.1300270 MHz
WDW                          EM
SSB                0
LB                        0.40 Hz
GB                 0
PC                        2.00
```

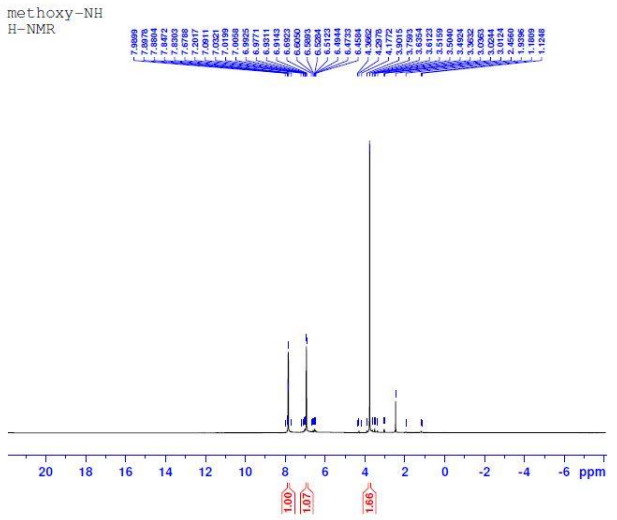

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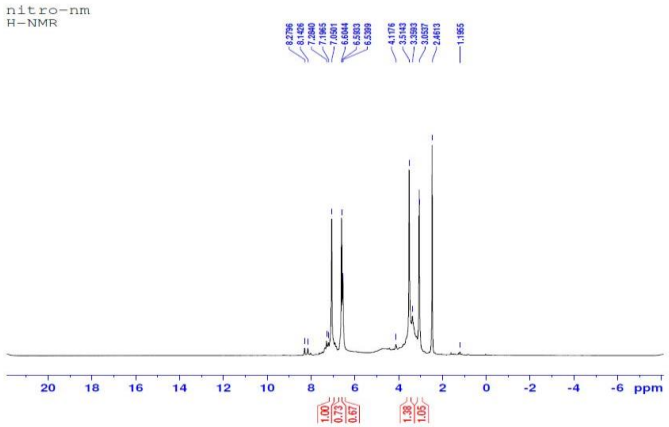
Instrument Model:
Bruker 500 MHz-Avance III
Operator: Rola Hassouneh
nmr500@ju.edu.jo

```
Current Data Parameters
NAME                21jun15jalal
EXPNO                        441
PROCNO                         1

F2 - Acquisition Parameters
Date_               20210617
Time                    11.26 h
INSTRUM                  spect
PROBHD     Z119470_0023 (
PULPROG                    zg
TD                      65536
SOLVENT                 DMSO
NS                             4
DS                             0
SWH                15000.000 Hz
FIDRES                 0.457764 Hz
AQ                 2.1845334 sec
RG                         28.18
DW                     33.333 usec
DE                        6.50 usec
TE                      298.0 K
D1                 2.00000000 sec
TD0                            1
SFO1               500.1334791 MHz
NUC1                        1H
P1                        12.00 usec
PLW1               13.32299995 W

F2 - Processing parameters
SI                        131072
SF                 500.1300270 MHz
WDW                          EM
SSB                0
LB                        0.40 Hz
GB                 0
PC                        2.00
```



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Instrument Model:
Bruker 500 MHz-Avance III
Operator: Rola Hassouneh
nmr500@ju.edu.jo

```
Current Data Parameters
NAME                  21jun15jalal
EXPNO                          341
PROCNO                           1

F2 - Acquisition Parameters
Date_                     20210617
Time                          9.45 h
INSTRUM                      spect
PROBHD     Z119470_0023 (
PULPROG                        zg
TD                          65536
SOLVENT                      DMSO
NS                               4
DS                               0
SWH                   15000.000 Hz
FIDRES                    0.457764 Hz
AQ                        2.1845334 sec
RG                            39.9
DW                          33.333 usec
DE                           6.50 usec
TE                          298.0 K
D1                    2.00000000 sec
TD0                              1
SFO1                  500.1334791 MHz
NUC1                          1H
P1                           12.00 usec
PLW1                 13.32299995 W

F2 - Processing parameters
SI                          131072
SF                    500.1300270 MHz
WDW                             EM
SSB                 0
LB                            0.40 Hz
GB                  0
PC                            2.00
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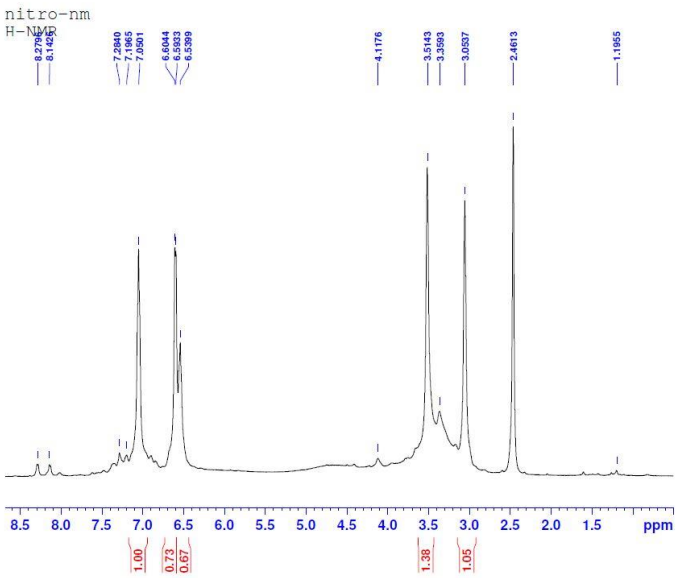

The University of Jordan
Faculty of Science
Department of Chemistry

Instrument Model:
Bruker 500 MHz-Avance III
Operator: Rola Hassouneh
nmr500@ju.edu.jo

```
Current Data Parameters
NAME                  21jun15jalal
EXPNO                          341
PROCNO                           1

F2 - Acquisition Parameters
Date_                     20210617
Time                          9.45 h
INSTRUM                      spect
PROBHD     Z119470_0023 (
PULPROG                        zg
TD                          65536
SOLVENT                      DMSO
NS                               4
DS                               0
SWH                   15000.000 Hz
FIDRES                    0.457764 Hz
AQ                        2.1845334 sec
RG                            39.9
DW                          33.333 usec
DE                           6.50 usec
TE                          298.0 K
D1                    2.00000000 sec
TD0                              1
SFO1                  500.1334791 MHz
NUC1                          1H
P1                           12.00 usec
PLW1                 13.32299995 W

F2 - Processing parameters
SI                          131072
SF                    500.1300270 MHz
WDW                             EM
SSB                 0
LB                            0.40 Hz
GB                  0
PC                            2.00
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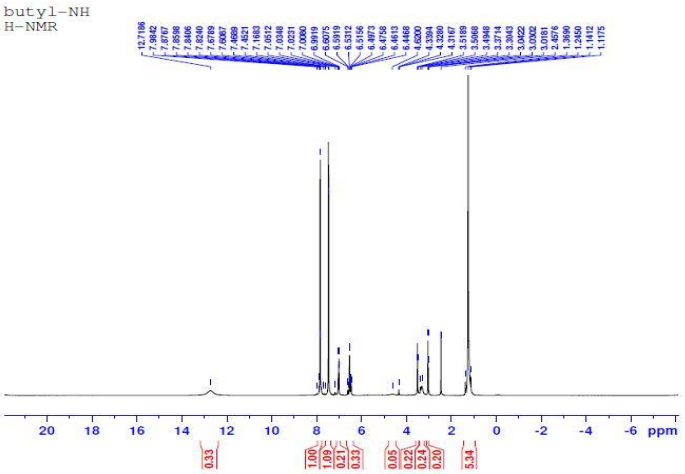

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Faculty of Science
Department of Chemistry

Instrument Model:
Bruker 500 MHz-Avance III
Operator: Rola Hassouneh
nmr500@ju.edu.jo

```
Current Data Parameters
NAME          21jun15jalal
EXPNO                  311
PROCNO                   1

F2 - Acquisition Parameters
Date_           20210616
Time              12.22 h
INSTRUM             spect
PROBHD   Z119470_0023 (
PULPROG               zg
TD                 65536
SOLVENT             DMSO
NS                     4
DS                     0
SWH           15000.000 Hz
FIDRES        0.457764 Hz
AQ            2.1845334 sec
RG                  39.9
DW               33.333 usec
DE                6.50 usec
TE               298.0 K
D1            2.00000000 sec
TD0                    1
SFO1        500.1334791 MHz
NUC1                  1H
P1                12.00 usec
PLW1         13.32299995 W

F2 - Processing parameters
SI                131072
SF          500.1300270 MHz
WDW                   EM
SSB                    0
LB                0.40 Hz
GB                     0
PC                2.00
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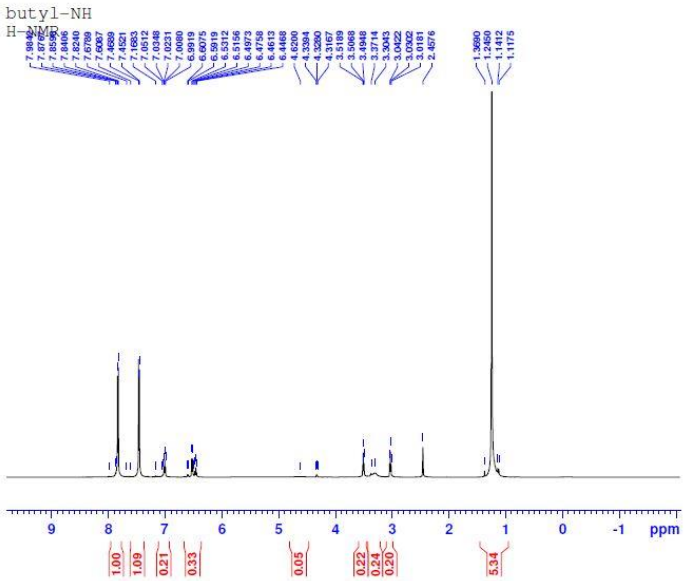

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Instrument Model:
Bruker 500 MHz-Avance III
Operator: Rola Hassouneh
nmr500@ju.edu.jo

```
Current Data Parameters
NAME          21jun15jalal
EXPNO                  311
PROCNO                   1

F2 - Acquisition Parameters
Date_           20210616
Time              12.22 h
INSTRUM             spect
PROBHD   Z119470_0023 (
PULPROG               zg
TD                 65536
SOLVENT             DMSO
NS                     4
DS                     0
SWH           15000.000 Hz
FIDRES        0.457764 Hz
AQ            2.1845334 sec
RG                  39.9
DW               33.333 usec
DE                6.50 usec
TE               298.0 K
D1            2.00000000 sec
TD0                    1
SFO1        500.1334791 MHz
NUC1                  1H
P1                12.00 usec
PLW1         13.32299995 W

F2 - Processing parameters
SI                131072
SF          500.1300270 MHz
WDW                   EM
SSB                    0
LB                0.40 Hz
GB                     0
PC                2.00
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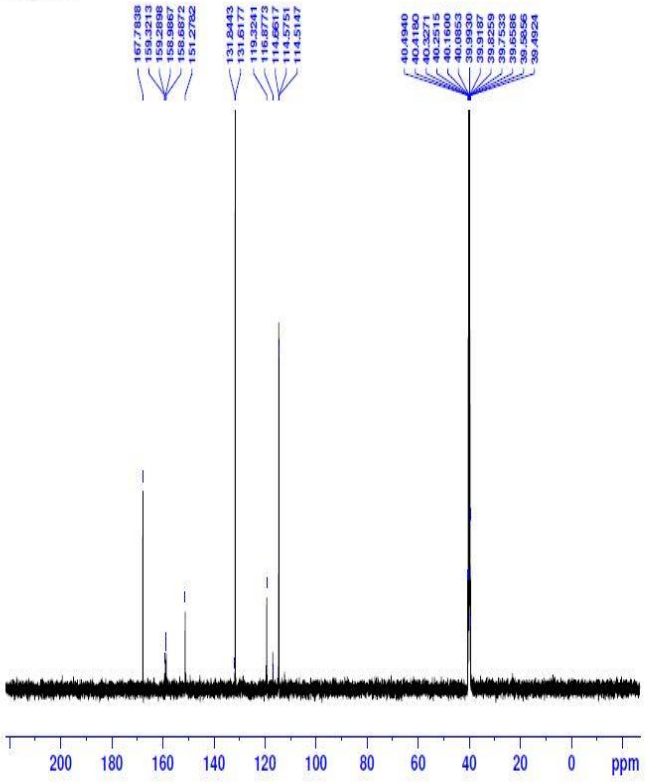
Index of ^{13}C−NMR(δ) analysis results

amin-thio
C13-NMR



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Department of Chemistry

Instrument Model:
Bruker 500 MHz-Avance III
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nmr500@ju.edu.jo

```
Current Data Parameters
NAME          21jun15jala1
EXPNO                  352
PROCNO                   1

F2 - Acquisition Parameters
Date_           20210617
Time              10.26 h
INSTRUM             spect
PROBHD   Z119470_0023 (
PULPROG           zgpg30
TD                 65536
SOLVENT             DMSO
NS                   100
DS                     4
SWH          31250.000 Hz
FIDRES        0.953674 Hz
AQ            1.0485760 sec
RG              202.06
DW               16.000 usec
DE                6.50 usec
TE              314.2 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                    1
SFO1        125.7700308 MHz
NUC1                  13c
P1                10.00 usec
PLW1        96.27500153 W
SFO2        500.1320005 MHz
NUC2                  1H
CPDPRG[2          waltz16
PCPD2             80.00 usec
PLW2        13.32299995 W
PLW12        0.29977000 W
PLW13        0.15078001 W

F2 - Processing parameters
SI                 32768
SF          125.7577890 MHz
WDW                   EM
SSB          0
LB                 1.00 Hz
GB           0
PC                 1.00
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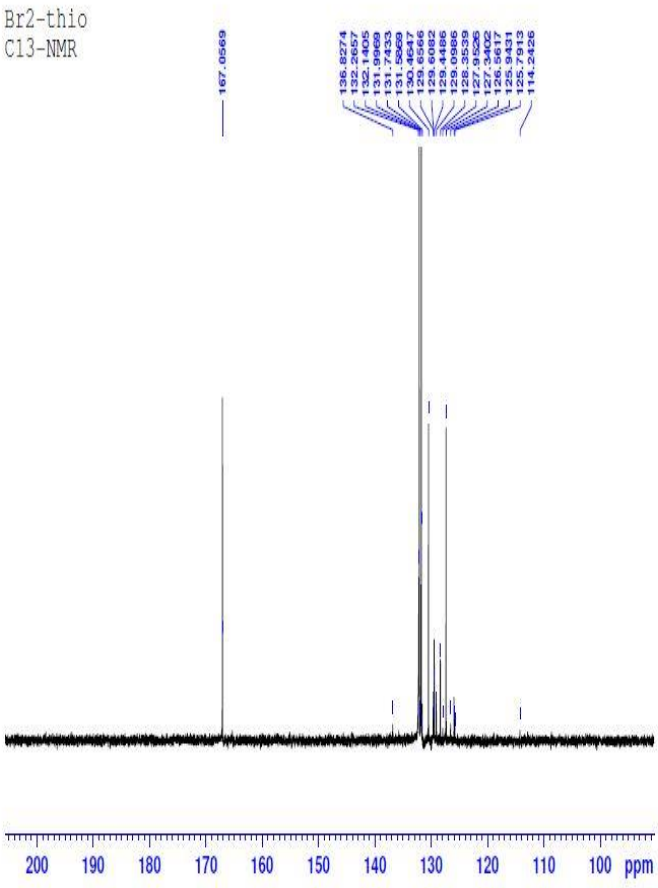

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Department of Chemistry

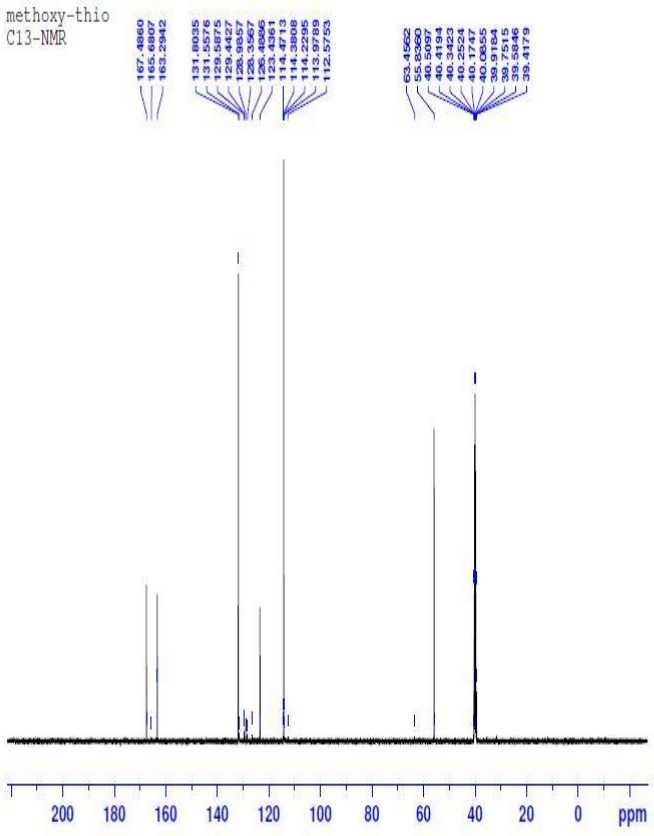
Instrument Model:
Bruker 500 MHz-Avance III

Operator: Rola Hassouneh
nmr500@ju.edu.jo

```
Current Data Parameters
NAME        21jun15jalal
EXPNO              302
PROCNO               1

F2 - Acquisition Parameters
Date_          20210616
Time              12.19 h
INSTRUM           spect
PROBHD   Z119470_0023 (
PULPROG          zgpg30
TD                65536
SOLVENT            DMSO
NS                   238
DS                     4
SWH           31250.000 Hz
FIDRES        0.953674 Hz
AQ            1.0485760 sec
RG                202.06
DW                16.000 usec
DE                 6.50 usec
TE                298.1 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                    1
SFO1        125.7700308 MHz
NUC1               13c
P1                10.00 usec
PLW1        96.27500153 W
SFO2        500.1320005 MHz
NUC2               1H
CPDPRG[2         waltz16
PCPD2             80.00 usec
PLW2        13.32299995 W
PLW12        0.29977000 W
PLW13        0.15078001 W

F2 - Processing parameters
SI                32768
SF          125.7577890 MHz
WDW                EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.00
```



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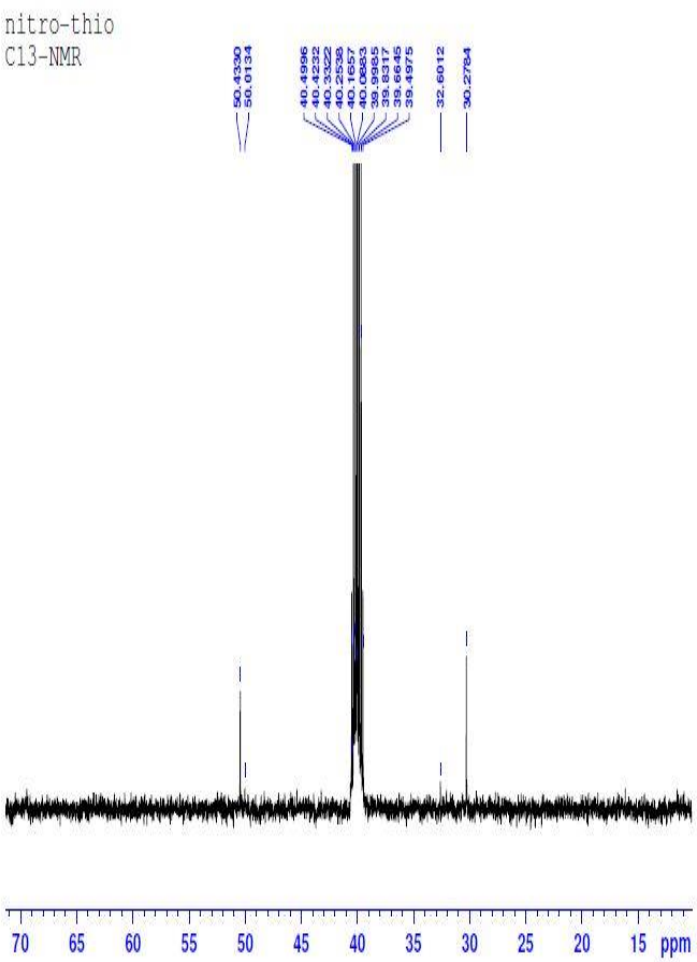
Faculty of Science
Department of Chemistry

Instrument Model:
Bruker 500 MHz-Avance III
Operator: Rola Hassouneh
nmr500@ju.edu.jo

```
Current Data Parameters
NAME        21jun15jalal
EXPNO             272
PROCNO              1

F2 - Acquisition Parameters
Date_         20210616
Time              11.51 h
INSTRUM           spect
PROBHD   Z119470_0023 (
PULPROG         zgpg30
TD               65536
SOLVENT           DMSO
NS                  67
DS                   4
SWH           31250.000 Hz
FIDRES            0.953674 Hz
AQ            1.0485760 sec
RG                202.06
DW                16.000 usec
DE                 6.50 usec
TE                298.2 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                 1
SFO1          125.7700308 MHz
NUC1               13C
P1                10.00 usec
PLW1          96.27500153 W
SFO2          500.1320005 MHz
NUC2               1H
CPDPRG[2         waltz16
PCPD2             80.00 usec
PLW2          13.32299995 W
PLW12          0.29977000 W
PLW13          0.15078001 W

F2 - Processing parameters
SI                32768
SF          125.7577890 MHz
WDW                EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.00
```



The University of Jordan
Faculty of Science
Department of Chemistry

Instrument Model:
Bruker 500 MHz-Avance III

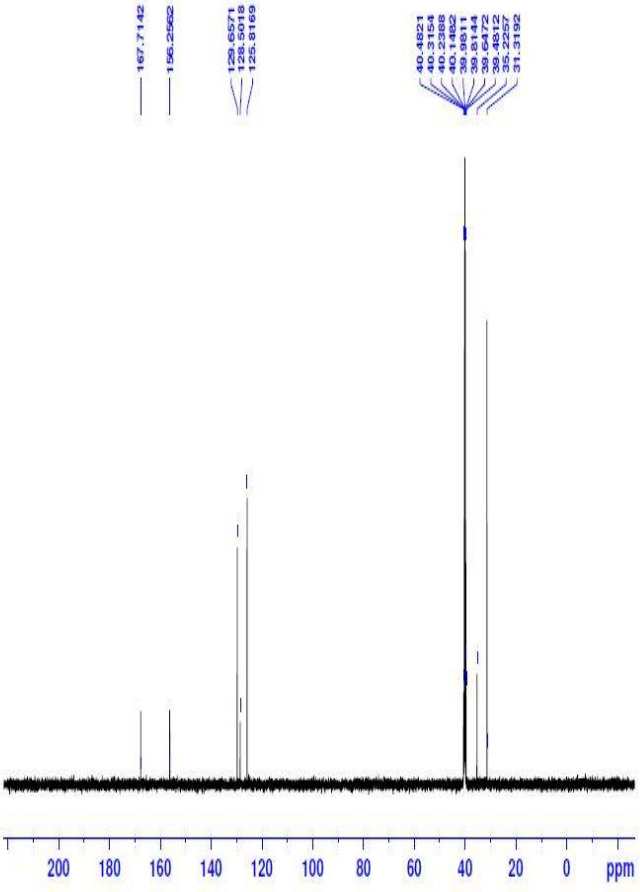
Operator: Rola Hassouneh
nmr500@ju.edu.jo

```
Current Data Parameters
NAME        21jun15jala1
EXPNO              452
PROCNO               1

F2 - Acquisition Parameters
Date_          20210617
Time              11.23 h
INSTRUM            spect
PROBHD   Z119470_0023 (
PULPROG          zgpg30
TD                65536
SOLVENT            DMSO
NS                   46
DS                    4
SWH           31250.000 Hz
FIDRES        0.953674 Hz
AQ            1.0485760 sec
RG              202.06
DW             16.000 usec
DE              6.50 usec
TE              298.2 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                  1
SFO1        125.7700308 MHz
NUC1                13C
P1               10.00 usec
PLW1        96.27500153 W
SFO2        500.1320005 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2            80.00 usec
PLW2        13.32299995 W
PLW12        0.29977000 W
PLW13        0.15078001 W

F2 - Processing parameters
SI                32768
SF          125.7577890 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.00
```

butyl-thio
C13-NMR



The University of Jordan
Faculty of Science
Department of Chemistry

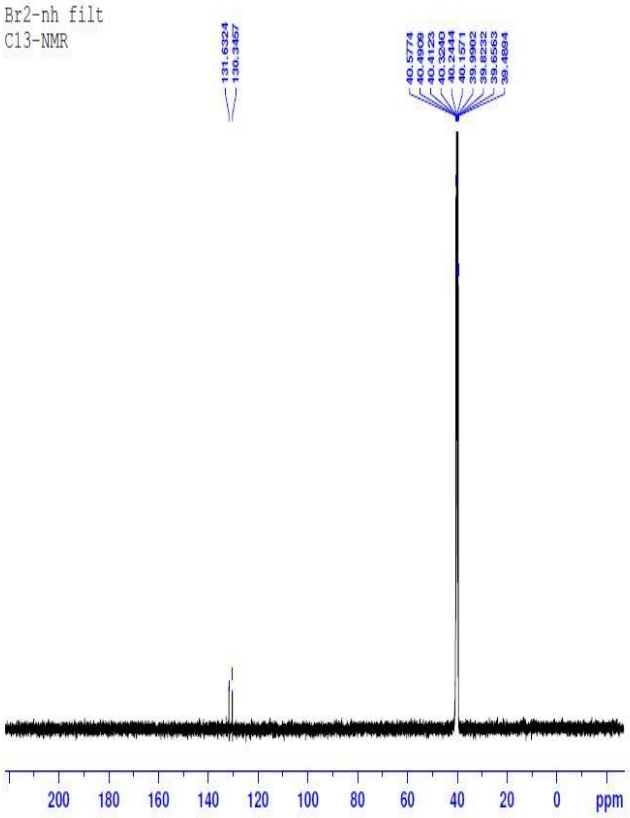
Instrument Model:
Bruker 500 MHz-Avance III

Operator: Rola Hassouneh
nmr500@ju.edu.jo

```
Current Data Parameters
NAME        21jun15jalal
EXPNO               462
PROCNO                1

F2 - Acquisition Parameters
Date_          20210617
Time              11.33 h
INSTRUM             spect
PROBHD   Z119470_0023 (
PULPROG          zgpg30
TD                65536
SOLVENT            DMSO
NS                    35
DS                     4
SWH           31250.000 Hz
FIDRES            0.953674 Hz
AQ             1.0485760 sec
RG                202.06
DW                16.000 usec
DE                 6.50 usec
TE                298.2 K
D1             2.00000000 sec
D11            0.03000000 sec
TD0                    1
SFO1         125.7700308 MHz
NUC1                  13C
P1                 10.00 usec
PLW1          96.27500153 W
SFO2         500.1320005 MHz
NUC2                   1H
CPDPRG[2         waltz16
PCPD2             80.00 usec
PLW2          13.32299995 W
PLW12          0.29977000 W
PLW13          0.15078001 W

F2 - Processing parameters
SI                 32768
SF           125.7577890 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.00
```



The University of Jordan
Faculty of Science
Department of Chemistry

Instrument Model:
Bruker 500 MHz-Avance III

Operator: Rola Hassouneh
nmr500@ju.edu.jo

Current Data Parameters

NAME	21jun15jala1
EXPNO	292
PROCNO	1

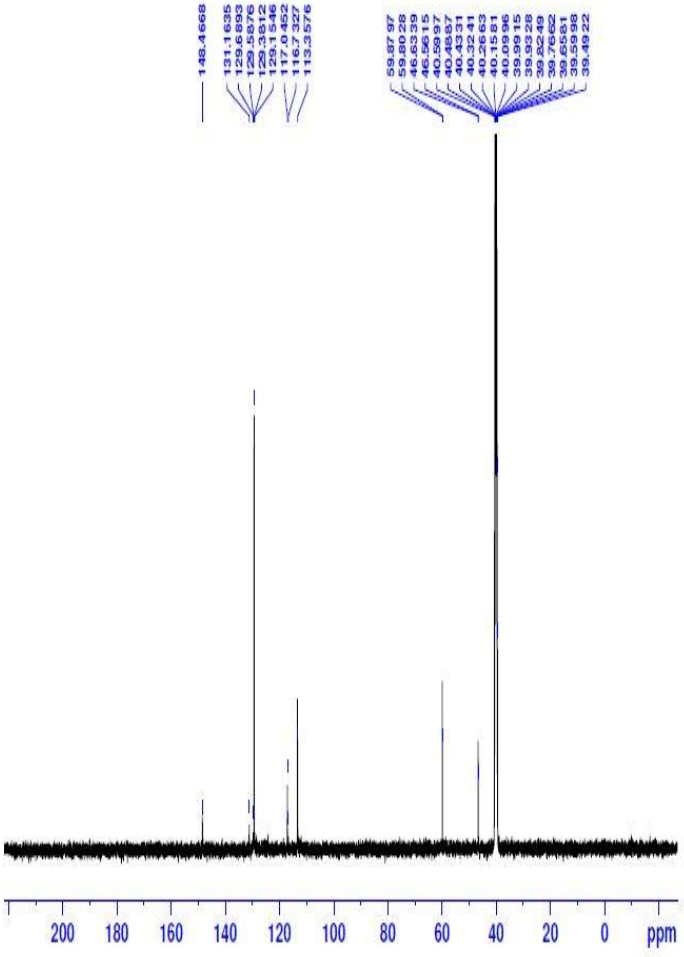
F2 - Acquisition Parameters

Date_	20210617
Time	9.42 h
INSTRUM	spect
PROBHD	Z119470_0023 (
PULPROG	zgpg30
TD	65536
SOLVENT	DMSO
NS	1409
DS	4
SWH	31250.000 Hz
FIDRES	0.953674 Hz
AQ	1.0485760 sec
RG	202.06
DW	16.000 usec
DE	6.50 usec
TE	298.0 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1
SFO1	125.7700308 MHz
NUC1	13C
P1	10.00 usec
PLW1	96.27500153 W
SFO2	500.1320005 MHz
NUC2	1H
CPDPRG[2	waltz16
PCPD2	80.00 usec
PLW2	13.32299995 W
PLW12	0.29977000 W
PLW13	0.15078001 W

F2 - Processing parameters

SI	32768
SF	125.7577890 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	2.00

nitro-nm
C13-NMR



The University of Jordan
Faculty of Science
Department of Chemistry

Instrument Model:
Bruker 500 MHz-Avance III

Operator: Rola Hassouneh
nmr500@ju.edu.jo

```
Current Data Parameters
NAME        21jun15jalal
EXPNO               342
PROCNO                1

F2 - Acquisition Parameters
Date_          20210617
Time              10.17 h
INSTRUM            spect
PROBHD   Z119470_0023 (
PULPROG          zgpg30
TD                65536
SOLVENT            DMSO
NS                   616
DS                     4
SWH           31250.000 Hz
FIDRES            0.953674 Hz
AQ             1.0485760 sec
RG                202.06
DW                16.000 usec
DE                 6.50 usec
TE                298.0 K
D1             2.00000000 sec
D11            0.03000000 sec
TD0                    1
SFO1          125.7700308 MHz
NUC1                  13C
P1                 10.00 usec
PLW1          96.27500153 W
SFO2          500.1320005 MHz
NUC2                  1H
CPDPRG[2         waltz16
PCPD2              80.00 usec
PLW2          13.32299995 W
PLW12          0.29977000 W
PLW13          0.15078001 W

F2 - Processing parameters
SI                 32768
SF            125.7577890 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.00
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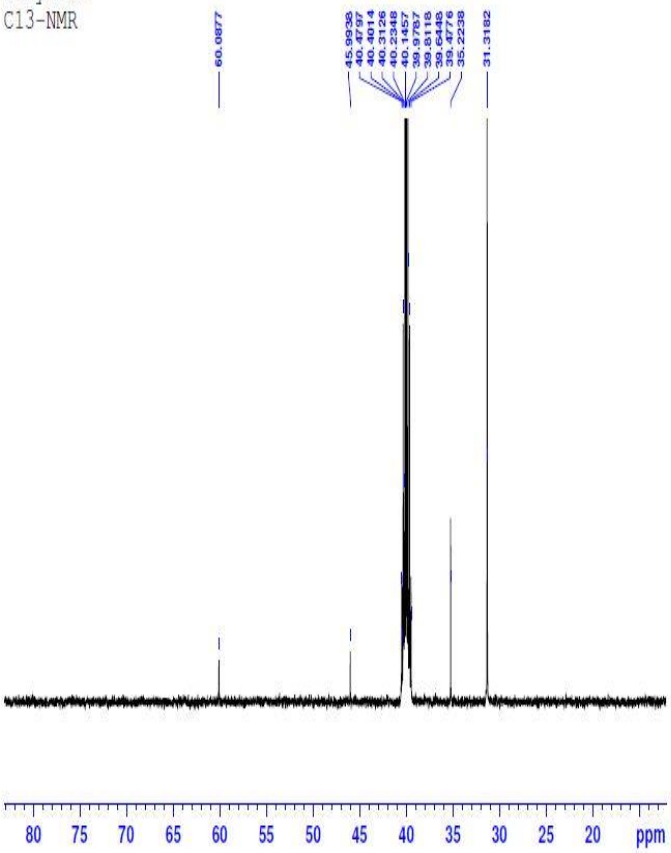
butyl-NH
C13-NMR



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Faculty of Science
Department of Chemistry

Instrument Model:
Bruker 500 MHz-Avance III
Operator: Rola Hassouneh
nmr500@ju.edu.jo

```
Current Data Parameters
NAME        21jun15jalal
EXPNO               312
PROCNO                1

F2 - Acquisition Parameters
Date_          20210616
Time              12.28 h
INSTRUM            spect
PROBHD   Z119470_0023 (
PULPROG          zgpg30
TD                65536
SOLVENT            DMSO
NS                   114
DS                     4
SWH           31250.000 Hz
FIDRES            0.953674 Hz
AQ             1.0485760 sec
RG                202.06
DW                16.000 usec
DE                 6.50 usec
TE                298.1 K
D1             2.00000000 sec
D11            0.03000000 sec
TD0                    1
SFO1         125.7700308 MHz
NUC1                 13C
P1                 10.00 usec
PLW1          96.27500153 W
SFO2         500.1320005 MHz
NUC2                 1H
CPDPRG[2         waltz16
PCPD2              80.00 usec
PLW2          13.32299995 W
PLW12          0.29977000 W
PLW13          0.15078001 W

F2 - Processing parameters
SI                 32768
SF           125.7577890 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.00
```

جامعة النجاح الوطنية

كلية الدراسات العليا

النشاط البيولوجي لمركبات ثيو إستر الأروماتيه ومركبات الامينو إستر الأروماتيه

إعداد

إيمان عدنان عساف

اشراف

د.نضال جرادات

د. أحمد خساتي

قدمت هذه الأطروحة استكمالا لمتطلبات الحصول على درجة الماجستير في الصيدليه بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس، فلسطين.

2021

النشاط البيولوجي لمركبات ثيو إستر الأروماتيه ومركبات الامينو إستر الأروماتيه

إعداد

إيمان عدنان عساف

اشراف

د.نضال جرادات

د. أحمد خساتي

الملخص

تم تخليق تسعة مركبات من السترات (*I-IX*) عن طريق تفاعل نوعين مختلفين من الكحوليات 2- (فينيل ثيو) إيثان و 2- (فينيل امينو) إيثان مع خمسة أنواع من الأحماض الكربوكسيلية المختلفة بطريقة تقليديه المعروفة باسم *(fisher reaction)*. تم فحص مركبات الإستر بواسطة *Fourier Transform Infrared (FT-IR*) ، والرنين المغناطيسي النووي البروتوني (*H-1 NMR*) ، والرنين المغناطيسي النووي للكربون 13. تم اختبار فعاليه المركبات إذ لها نشاط كمضادة للأكسدة أو مضاد للميكروبات أو إذا كان لها مقدره على تثبيط عمل كل من إنزيم الأميليز والليباز.

أظهر اختبار فحص نشاط المركبات كمضادات أكسدة أن المركبين *V* و *IX* لهما نسبة تثبيط تساوي 84.70٪ و 84.79٪ ، على التوالي ، عند تركيز 100 ميكروغرام / مل مقارنة مع ترولوكس الذي لديه قيمة تثبيط تساوي 99٪ عند نفس التركيز. بالإضافة إلى ذلك ، فإن جميع مركبات الإستر لها فعاليه لتثبيط نمو البكتيريا سواء كانت بكتيريا من النوع موجبة الجرام أو من نوع سالبة حيث تروحت قيمه التثبيط عند اقل تركيز مستخدم لتثبيط نمو البكتيريا (*MIC) (7.5 - 0.47* ميكروغرام / مل).

بينما أظهرت المركبات التاليه *III* و *V* و *VII* و *VIII* و *IX* فعاليه عاليه على تثبيط بنسبة 95٪ ضد فطريات الكانديدا حيث كانت قيمه اقل تركيز لتثبيط نشاط الفطريات يساوي (*MIC (1.875* ميكروغرام / مل) مقارنة بالعقار المضاد للفطريات فلوكونازول بقيمه تساوي (1.62 ميكروغرام / مل).

أظهرت مركبات الإستر نتائج واعده كمركبات لها المقدرة على تثبيط عمل إنزيم *α-amylase* مقارنة مع *Acarbose* كمعيار. كانت نسبة تثبيط كل من المركبات *IX , V* و *VI* *99.5 ٪* . *79.3٪* و 73.5٪ عند تركيز 500 ميكروغرام / مل على التوالي. كانت قيمه التثبيط بالنسبة المئوية ل 5 مركبات أعلى من 50٪ عند 10 ميكروغرام / مل وهي نتيجة مهمة للغاية وكانت هذه الخمس مركبات لها *IC50* أقل من *IC50* للأكاربوز. قيمة *IC50* للمركب *III* هي 0.0206 وهي أقل 3000 مرة من الأكاربوز. بالرغم من عدم وجود مجموعة أميد الوظيفية المسئولة عن نشاط إنزيم الليباز ، فإن المركبين *V* و *IX* أظهروا تثبيط 59.5٪ و 72.3٪ على التوالي عند 500 ميكروجرام / مل.