An-Najah National University

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Synthesis of aromatic thio-acid esters of 2-thiophenylethanol and exploring some of their biological activities

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Dedication

To my husband Salah Alden for his support, love, and encouragement.

To my son Eyad for their patience for being far from me, as I was busy all the time.

To my parents for helping, taking care and praying for me.

To my sisters Manar and Sireen who supported me and shared my worries.

To my all family who supported me.

To my brothers Tariq and Salem for their love, sincere feelings and their moral support.

To my friends for their continuous support.

To all who prayed for me.

To all whom I loved and knew.

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Finally, my family, siblings, son and close friends deserve my deep gratitude. Thank you for being in my life, which made this achievement and success possible. Lastly and exclusively, my warmest thanks must be to my husband, Salah, for his continuous and unfailing love. With his support and understanding, he underpinned my persistence in my Master study and made the completion of my thesis possible

الاقرار

انا الموقعة ادناه مقدم الرسالة التي تحمل العنوان:

Synthesis of aromatic thio-acid esters of 2-thiophenylethanol and exploring some of their biological activities

أقر بأن ما اشتملت عليه هذه الرسالة إنما هو نتاج جهدي الخاص، باستثناء ما تمت الاشارة 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 اخرى.

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.

Student's name: **اسم الطالب:**

Signature: **التوقيع:**

Date: **التاريخ:**

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List of Abbreviations

Symbol	Abbreviation
BERC	Biodiversity & Environmental Research Center
NARC	National Agriculture Research *Center*
DPPH	1,1-Diphenyl-2-picryl-hydrazyl
PABA	*p*-amino benzoic acid
DCFC	Dry column flash chromatography
RNS	Reactive nitrogen species
ROS	reactive oxygen species

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Abstract

Four compounds of thio-acid esters were prepared from the reaction of benzoic acid derivatives including 2-hydroxy, 3-hydroxy, 4-hydroxy benzoic acid with 2-thiophenylethanol. The structures of these thioesters were established by Fourier Transform Infrared (FT-IR), Proton Nuclear Magnetic Resonance (^{1}H-NMR) and C13. The aromatic thioesters were tested for their anti-oxidant, anti-fungal, anti-bacterial, anticancer anti-diabetic and anti-obesity activities. The compounds activity as antioxidants in DPPH was about (IC_{50}=30µg/ml), the same value for Gallic acid. The compounds were tested also for their antibacterial activity against: (*Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Proteus Vulgaris, Enterococcus Faecium, Pseudomonas Aeruginosa, MRSA,* and *Candida Albicans)* and showed MIC value average (3.125-6.25mg\ml), the MIC average for ampicillin antibiotic (0.001-3.125 mg\ml). While the cytotoxic property will be assessed on MCF-7, human carcinoma cells and showed the average (G2-M phase: 21.83, 8.13, 10.66, 14, 3.66). Moreover, for amylase (IC_{50} = 10) which is the same as Acarbose.

Chapter One
Introduction

1.1 Benzoic acid

Benzoic acid ($C_7H_6O_2$) is a simple aromatic carboxylic acid, a crystalline solid, colorless and odorless, with a sweetish and astringent taste. Benzoic acid is found in many plants and is synthesized by secondary metabolites. It is also found in several foods like grains, milk, eggs, and meat. Also, it is prepared by different methods, such as neutralization with the corresponding hydroxides or by heating with the corresponding concentrated carbonates. Benzoic acid and its derivatives have many industrial applications, such as food preservatives, plasticizers, and pediculicide agents, and they are used as hydroxyl-radical scavengers for antioxidant activity or as drugs for fibrotic skin disorders, such as Peroni's disease (1).

In many countries, the interest in phenols, polyphenols, and acid esters has increased, since several types of these compounds exert a wide variety of biological effects, in particular inflammation control, anti-atherogenic, inhibition of bacterial strains, and antiviral and anti-cancer activities. Also, they are used as food additives and preservatives. These effects may be due to their antioxidant role, though different mechanisms may be included (2).

Salicylic acid, which is a derivative of hydroxyl benzoic acids, and phenyl esters were proven to be some of the most important active compounds present in many plant sources (3, 4). It has been observed that the anti-

oxidative effect of acid esters and phenolic derivatives can be identified by their molecular structures and the hydroxyl group position (4, 5). For instance, the unpaired electrons can be delocalized to fix the formed radical after the observed reaction with the initiator radical(5). Some previous research carried out by scientific groups reported that the catechol observed moiety, with the 3, 4-dihydroxyl configuration, is a basic factor in the scavenging effect of this free radical for these phenolic and acid ester types of organic compounds (4). On the other hand, some studies have shown that this structure is not required for this activity (5). In addition, there are two other series of acid esters, thio-acid esters, and amino acid esters, but these have not yet been studied. Phenolic compounds and acid esters usually absorb wavelengths in the UV region, and so they are usually used in detectors in an HPLC or UV Vis detectors (6, 7). There is no single wavelength that is ideal for controlling all types of phenolic compounds because they show maximum absorbance values at various reported wavelengths (7). Most of the benzoic acid derivatives record their maxima wavelength at 246_262 nm, except gallic acid and syringic acid which exerted absorption maxima at 271 and 275 nm, respectively (8, 9). In addition, hydroxycinnamic acids absorb light in two UV regions, in the first one the maximum ranges between 225 and 235 nm and for the other the maximum ranges between 290 and 330 nm (10). At 320 nm, cinnamic acid derivative compounds may be recorded without any interference from benzoic acid derivatives that have an absorption value that is higher than

254 nm. Despite this, recording at 280 nm is the perfect alternative for the identification of both types of phenolic compounds (10).

1.2 Esterification and thio-acid esters

Esters are organic compounds derived from the reaction of an acid (organic or inorganic) and alcohol, catalyzed by a small amount of concentrated sulfuric acid or hydrochloric acid through a condensation reaction known as Fischer esterification, as illustrated in Figure 1.1 (11).

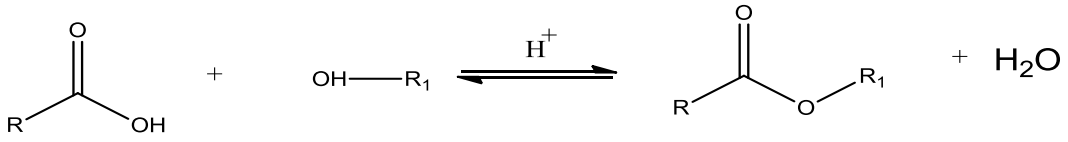


Figure 1.1: General equation of Fischer esterification**.**

Fischer esterification can be reversible with an equilibrium shifted forwards in simple alcohols and simple carboxylic acids, but also backwards when the alcohol and/or carboxylic acid are bulky. The process of hydrolysis is also preferred in aqueous solutions (11-13).

A none reversible scientific method for the preparation of none hindered esters is the reaction of alcohols with the known acid chloride (11, 14) or acid anhydrides, but the obtained yield for these bulky esters is still low (11).

Thioesters are organic compounds that have ‘RSCOR' as the functional group. The preparation of these compounds is achieved by esterification

Among thiol and a carboxylic acid. In biochemistry, the most well-known thioesters are derivatives of coenzyme A, e.g., acetyl-CoA.

The process of the esterification of thiols (RSH) is one of the preferred chemical reactions in the organic lab, which concerns the synthesis of thioester derivatives (15-17). The S acylation of thiols is also an effective and inexpensive way to safely produce sulfhydryl groups (R-S-H) with multiple steps in the preparation of the synthetic organic compound (18, 19). Thioesters are important intermediate substances in food, medical applications, and also in cosmetic chemical synthesis, as well as for the production of new materials (20, 21). Furthermore, thioesters are organic structures, which are also widely used in the preparation of heterocyclic molecules, and structures that have carbonyl functional groups that are needed for chemical processes (22, 23). The structures of thioesters also form the skeleton of many antibiotics and natural products (24). The preeminent effect of thioesters in pharmaceutical, biological, and as well as industrial applications is the most valuable reason for developing the preference of these structures adopted by scientific researchers with the following tiny review (25, 26). Acylation of thiols has been reported in many protocols. The sources for the acyl group in these transformational syntheses are traditionally carboxylic acid as well as acid chlorides or acid anhydride, which are used with strong bases, such as triethylamine, pyridine or DMAP under reflux set up for a few hours in order to start the process. During the last decade, these traditional methods have been developed with some new protocols that use catalysts like triflates, CsF_3,

titanocenebis (perfluorooctanesulfonate), and dodecylbenzene sulfonic acid for thiol acylation assay (27-29).

1.3 Testing the biological effect of the various modified compounds

Producing or influencing a change in the living tested tissue or the ability to make an alteration in a biological process is the definition of biological activity of substances. The related effects among the molecular terminal part and the biological action can be assessed by obtaining the structure and its biochemical mechanism of action. The value of observing biological processes is that it provides a description of the functional relationships among the biological influences and the chemical species that are involved (30).

1.3.1 Compounds with antioxidant activity

Free radical scavenging species, also known as antioxidants, are expected materials that may prevent or delay several forms of cell damage by reacting with the cells and the inhibition effects of these free radicals can prevent cell damage and therefore prevent or delay various diseases. Also, these free radicals are well-known as highly reactive species that carry an odd number of negatively charged electrons in their structures, and this makes them highly reactive and they can damage cells which are called cellular pathologies. Some of these negative effects can result in cancer.

A biological system which involves oxygen and nitrogen will give rise to many free radicals and different reactive species, which are all known as ‘reactive oxygen species’ (ROS) and ‘reactive nitrogen species’ (RNS), respectively. They have dual roles as they are both beneficial and also deleterious species (31, 32). Antioxidants are valuable organic substances especially when designing new drugs. There are two types of these antioxidant compounds. The first type is created by natural pathways in our bodies; the second type is supplied to our bodies from external sources like smoking, sun exposure, and other dangerous pollution sources. However, the body also needs exogenous sources of antioxidants or dietary antioxidants from fruits and vegetables, which are named as external sources of antioxidants (33, 34).

The high reactivity of free radicals makes them a highly destructive species that can rapidly harm body cells. These radicals are produced when an atom or a molecule either gains or loses an electron (a tiny negatively charged particle present in atoms) (35).

‘As the concentration of free radicals increases so too does the hazard that they pose to the body. They can damage important components of body cells, such as proteins, DNA, and cell membranes. Various forms of these mutagenic substances and carcinogens may act via the ejection of the oxygen radicals, due to the destruction of DNA. These conditions are the preferred environments for establishing and progressing cancer (36, 37).

1.3.2 Compounds with antimicrobial effects

Extremely small organisms that are identified under a microscope are referred to as microbes which are found in rocks, plants, air, soil, bodies and in water. Microbes can replicate and spread rapidly. These organisms are split into the following classes: bacteria, fungi, viruses, and protozoal types. Some microbes are responsible for disease and are called parasites. Furthermore, a variety of these organisms are present in the body as normal flora and are actually beneficial and not harmful to body cells (38).

Drugs, which have antimicrobial effects, are prepared in such a way so that they inhibit these microbes without having any bad effects on the human body (39). Antibiotics are valuable weapons for fighting bacterial strains, and the preparation of antibiotics has a strong relationship with the nature of life concerned with patients' healthy state. Nowadays, these health values have involved limitations due to natural selection; bacterial strains' resistance to these drugs is currently a critical issue. So, developing medicinal preparations derived from naturally occurring sources play a critical role in preventing and curing disorders in people (40). An antifungal medication is a medical preparation that selectively acts to get rid of fungal pathogens from the infected tissues with minimal side effects for the target (32). Compared with bacterial diseases, fungal diseases are more difficult to treat. Topical and oral treatments are long term and partially effective in managing these fungal infections. Several of these infections can be chronic and these treatments can stop the infection in

body tissues, but there is evidence that in some cases the disease can reoccur (33).

The most available and widespread classes of all mycoses are usually related to fungal infections of the skin. They affect more than 20–25 percent of the world's population, and skin mycoses are the most common type of infection…(41).

1.3.3. Anti-cancer

Nowadays, cancer is the most important health issue in the world. In many countries, cancer is the second leading cause of death, after heart disease. The incidence of different carcinomas, worldwide, is estimated to be about 10 million, and half of these incidences are in developed countries (42, 43). Almost five decades of systemic drug discovery and developments have led to a respectable accumulation of useful and important chemotherapeutic agents (44, 45), and several important successes in the curing and management of human cancer (46). In the scientific literature, several citations reference epidemiological research that supports and shows significant differences in the occurrence of carcinoma between oriental and occidental populations (47, 48).

Since it was first used almost half a century ago, chemotherapy as a cancer treatment has faced dramatic problems. Also, the lack of selectivity by conventional anti-cancer agents, leading them to damage not only malignant but also normal body and blood cells, has confused scientists and

made them aware of the need for more specifically selective medications (49). Another reported drawback that arose just after cancer chemotherapy was established, was the appearance of drug resistant cancer cells (50). This, in turn, increased the interest in searching for possible anti-cancer agents from the flora present in several countries, which are available in the market as "natural products" (51). There are no perfect and effective synthetic drugs present in the pharmacopeias. Therefore, scientists and chemists do their best to synthesize chemical compounds that may have anti-cancer activity. The compound Oenothein B, which is separated from the *Epilobium parvijlorum* plant, is used for the treatment of prostate disorders. It inhibits 5-of reductase and the 5-α reductase, which converts testosterone to the more potent androgen, dihydrotestosterone (DHT), in the prostate (52).

1.3.4 Diabetes

Diabetes mellitus (DM) is a chronic disease that presents as both postprandial or fasting hyperglycemia, with disturbances in metabolizing carbohydrates and proteins. Hyperglycemia in diabetes is caused by an absolute deficiency in either insulin secretion (type 1 DM) or insulin action (type 2 DM) or sometimes by both. The worldwide incidence of diabetes has increased in recent years. The estimated number of people with diabetes reached 30 million in 1985, 150 million in 2000, and then 246 million in 2007, based on information from the International Diabetes Federation which expects this number to hit 380 million by 2025 (53).

1.3.5 α-Amylase activity

Alpha amylase is defined as an enzyme, which catalyzes the biochemical pathway for the hydrolysis of starch (Latin asylum) into simple sugars. This enzyme is present in the saliva in the human body and in some other mammals' body saliva, where the enzyme starts the chemical process of digestion (54, 55). Foods such as bread, rice, and potatoes that contain a high percentage of starch, but low levels of simple sugars, may have a slightly sweet taste as they are chewed because the amylase degrades some of the starch into a simple sugar. In addition, the pancreas and salivary glands make amylase (alpha amylase) to digest dietary starch into disaccharides and trisaccharides which are then finally transformed by other enzymes into glucose in order to provide the body with energy (56). Plants and some bacterial strains can also produce amylase. As *diastase,* amylase was the first enzyme to be discovered and isolated (by Anselme Payen in the year of 1833). Specific amylase proteins are designated by different Greek letters. All types of amylase are glycoside hydrolases and their action depends on the α-1, 4-glycosidic bonds. The α-amylases (alternative names: 1, 4-α-D-glucan glucanohydrolase; glycogenase) are calcium metalloenzymes. As they act at random locations along the starch chain, α-amylase as a result breaks down long-chain saccharides, finally producing either malt triose and maltose from amylose, or maltose, glucose, and "limit dextrin" from amylopectin.

Since this enzyme can act anywhere on its substrate, α-amylase seems to be faster acting than β-amylase. In animals, it is a major digestive enzyme, and its optimum pH is 6.7–7.0.

Both the salivary and pancreatic amylases in human physiology are α-amylases.

The α-amylases forms are also present in plants, fungi (ascomycetes and basidiomycetes), and bacteria (Bacillus).

The activity of α-amylase was usually determined by the method of McCue and Shetty, which involved starch as a substrate in a colorimetric chemical reaction using 3, 5-dinitrosalicylic acid. A standard calibration curve was established for the hydrolyzed products (reducing groups) using D-(+)-maltose monohydrate. Then the activity was calculated as units per milligram of protein, as each one unit was defined as the amount of enzyme required to produce one mmol of maltose under these protocol conditions. Also, the protein content was estimated using the Bio-Rad protein assay kit. Data were reported as amylase inhibition (AI) index values, defined herein as the ratio of α-amylase activity of the control (just the enzyme) to that of the enzyme/clonal extract mixture and values greater than 1 indicate AI (57).

1.3.6 Glucosides

One of the non-reducing organic compounds the glycosides that by hydrolysis with acids, alkalis or enzymes will produce: A sugar part (or glycone, formed of one or more simpler sugar units). A non-sugar part (or aglycone, also called genin) (58).

An α-glucosidase, such as *maltase*, *glucoinvertase*, *glucosidosucrase*, *maltase-glucoamylase*, *alpha-glucopyranosidase*, *glucosidoinvertase*, *alpha-D-glucosidase*, *alpha-glucoside hydrolase*, *alpha-1,4-glucosidase*, or *alpha-D-glucosideglucohydrolase*, is a glucosidase found on the border of the small intestine which acts upon α(1_4) bonds. This is a contrast to beta-glucosidase. Alpha-glucosidase hydrolyzes starch and disaccharides to glucose. Maltase seems to be a similar enzyme that cleaves maltose, which is almost functionally equivalent (59, 60).

Alpha-glucosidase breaks down terminal non-reducing (1→4)-linked alpha-glucose residues to produce a single alpha-glucose residue(61). Alpha-glucosidase is a carbohydrate-hydrolase that produces alpha-glucose as opposed to beta-glucose. Beta-glucose residues can be produced by glucoamylase, a functionally similar enzyme. In addition, the substrate selectivity of alpha-glucosidase is related to the subsite affinities of the enzyme´s active site. Two suggested mechanisms involve a nucleophilic displacement and an intermediate, which is an oxocarbenium ion (62).

1.3.7 Overweight and obesity

One of the recent widespread health problems is obesity, which can be defined as a medical case in which a high amount of body fat has increased to such a level that it may have serious effects on human health. In general, a person is characterized as obese when their measured body mass index (BMI), a measurement obtained by dividing the individual's weight by the square of the individual height, is more than 30 kg/m^2, and a BMI between 25 and 30 kg/m^2 is classed as overweight(63). Other countries in East Asia use lower values. Obesity leads to an increase in the occurrence of different conditions and diseases, like cardiovascular disorders, type two diabetes, sleep apnea (characterized by obstruction in the respiratory tract), specific forms of cancer, osteoarthritis conditions, and depression.

In general, obesity is due to several factors starting with a high food intake, then low physical training, and it may depend on genetic susceptibility (63, 64). A few cases have appeared to be primarily due to genes, endocrine disturbances, some ingested medications, or mentally related problems(65). The view that obese people eat little yet gain weight because of a slow metabolism has not been medically confirmed. On average obese individuals have higher energy expenditure than their normal counterparts, since more energy is needed to stabilize an increased measured body mass.

Obesity can mostly be restricted by various social changes and personal choices. Some changes in diet and exercising are the main treatments. The quality of diet can also be altered through a reduction in the consumption of

food rich in energy supply, like those high in fats or sugars, and by increasing the consumption of fibers. Drugs can be used, along with a suitable diet, to reduce appetite or interfere with fat absorption. In some cases, if diet, exercise, and also drugs are not effective, a gastric balloon or surgery may be the solution to reduce stomach volume or length of the intestines, resulting in feeling full earlier or decreasing the capacity to absorb nutrients from the obtained diet.

Obesity seems to be a preventable cause of death worldwide, with increasing rates in both adults and children. As reported in 2015, 600 million adults (12%) and 100 million children were recorded as obese in 195 countries. Obesity seems to be more common in women than in men. Authorities view it as one of the most serious public health issues of the 21st century. Obesity is stigmatized in much of the modern world (especially in the Western world), though it was seen as a symbol of wealth and fertility at certain times in history and still is in some parts of the world. Moreover, in 2013 the American Medical Association listed the condition of obesity as one of the present diseases (66, 67).

1.3.8 Anti lipase activity

Substances that are classified as lipase inhibitors are used due to their ability to reduce the action of lipases, which are present in the small intestine. In addition, lipases are ejected into the intestine from the pancreas as fat reaches this location. The function of lipase blockers is usually to reduce the absorption of these fatty materials by the GI. As a result, these

fats will be flushed away in feces, without being absorbed in order to be used as a source of caloric energy, and this can result in weight loss in individuals. These inhibitory agents could be used for the treatment of some obesity conditions, that can lead to type II diabetes and cardiovascular disorders if not well controlled. A commonly used material for lipase inhibiting agents is Orlistat (68, 69).

The effect of lipase inhibitors on the amount of absorbed fat can be observed, as the lipase inhibiting agents are not absorbed until they reach the blood, so they cannot inhibit the absorption of fat until then. The binding of lipase inhibitors to lipase enzymes occurs in the intestine, and this blocks the breaking down of dietary triglycerides that are then transformed into monoglycerides and other fatty acids. This reduces the absorption of fatty substances that are present in the diet. Furthermore, lipase inhibitors are bonded to the serine active point on lipases by such strong covalent bonds that are relatively irreversible, meaning that the lipase inhibitor will stay attached to this digestive enzyme. Researchers have reported that lipase inhibitors perform best when 40% of the daily caloric intake by each individual is taken from fatty substances. Orlistat tends to stop the absorption of 30% of the total amount of ingested fatty food present in a meal, as orlistat is usually flushed away from the body digestive tract faster than the fatty materials can act (69, 70).

Aims of our study

Main objectives of this scientific study are the following:

1- To prepare a series of substituted benzoates of 2-phenoxyethanethiol

2- To explore some of the biological activity of these esters

3- To enrich the literature with the physical data of these esters

Chapter Two
Materials and Methods

2.1 Chemicals and reagents

The following chemicals were needed for our experiment: 2-thiophenyl ethanol, ortho-hydroxybenzoic acid, benzoic acid, 3-hydroxy benzoic acid, para-hydroxyl benzoic acid, all of which were purchased from Aldrich. The following chemicals were purchased from Frutarom: ethyl acetate, $NaHCO_3$, brine, Na_2SO_4, cyclohexane, ethyl acetate, β-carotene, DPPH, Na_2HPO_4\NaH_2PO_4 (Disodium phosphate\Monosodium phosphate), NaCl, Porcine pancreatic amylase enzyme solution, starch, dinitrosalicylic acid (DNSA), methanol, Trolox, Gallic acid, pancreatic lipase enzyme, tri-HCl buffer, PNPB p-nitro phenylbutyrate acetonitrile, Orlistat, tocopherol, alpha glucosides, Na_2CO_3, acarbose. All of the chemicals were of analytical grade.

2.2 Microorganisms

The microorganisms used in this experiment were from An-Najah University lab. / Nablus. The types of bacteria were: *Klebsiella pneumonia* (ATCC13883), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 9027), *Proteus vulgaris* (ATCC 8427), *Escherichia coli* (ATCC 25922), *Enterococcus faecium* (ATCC 700221), MRSA (Clinical strain), and *Candida albicans* (ATCC90028).

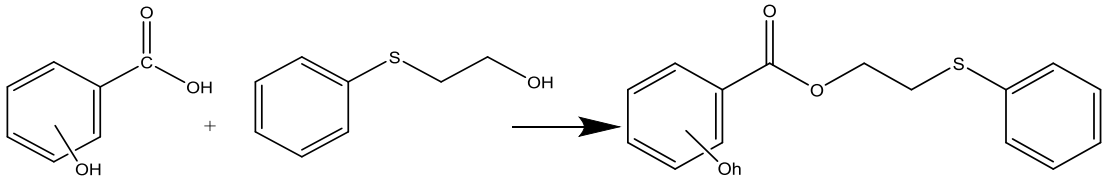
2.3 Physical measurements

The melting range of each compound was measured by start melting point apparatus, R00102618, while IR was detected by an infrared spectrophotometer (Nicolet Is5 - Id3) at An-Najah University. ^{1}H –NMR and Carbon13 were determined by a Bruker 500 MHz-Avance III at the University of Jordan/ Jordan.

2.4 General method for the preparation of thio-acid esters

Thio-acid esters were synthesized by refluxing benzoic acid and its derivatives with 2-thiophenyl ethanol for two hours. The reaction was left overnight at ambient room temperature. The prepared compound was fully dissolved in 20 ml ethyl acetate and the residue was separated using saturated $NaHCO_3$ in a separator funnel. The organic layer was thoroughly washed with a saturated solution of NaCl, dried over Na_2SO_4, and then the solvent was evaporated off. The residues were purified by flash chromatography using the mobile phase of n-hexane-ethyl acetate and a silica gel as the stationary phase.

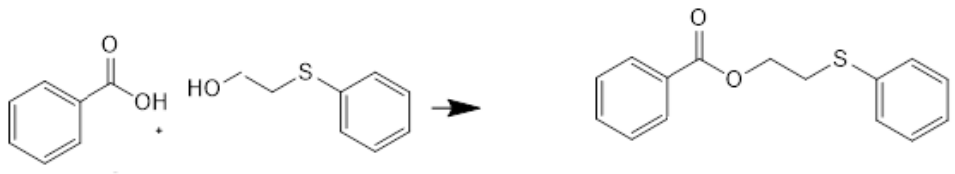
The following equation shows the expected general reactions for acid esters.



Where OH is a substituent in the places of Orth, Meta, or Para.

2.4.1 Preparation of 2-(phenylthio) ethyl benzoate (I)

Benzoic acid (3.062 g, 0.025 mol) and 2-thiophenylethanol (3.856 g, 0.025 mol) were refluxed for two hours, then left for one night. The reaction was catalyzed by adding 2 ml sulfuric acid. The product was dissolved in 20 ml ethyl acetate, then extracted with saturated sodium bicarbonate. The phases were washed with saturated sodium chloride, dried with sodium sulfate, and the solvent was evaporated. The final product was purified using flash chromatography using the mobile phase of 40% n-hexane 60% ethyl acetate and a silica gel as the stationary phase.



Scheme 2: Reaction of benzoic acid with thiophenyl ethanol.

Percentage yield is 68% and M.p. = 113–115ºC. UV λ_{max} 240–270

IR ν_{max} (1697 C=O, 1596 aromatic, 1242 ester, 1064 carboxylic acid cm^{-1}).

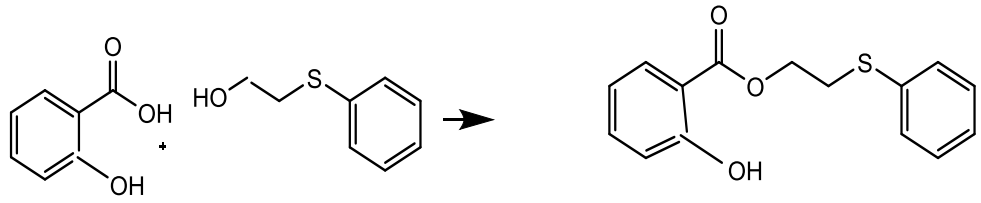
^{1}HNMR (DMSO):7.39 (2H, m; H2 and H6); 7.21 (1H, m; H4); 7.35 (2H, m; H3 and H5); 7.66 (1H, m; H15); 8.05 (2H, m; H13 and H17); 7.56 (2H,

m; H14 and H16);) 4.17 (2H, t, *J*= 6Hz, -OCH_2); 3.54 (2H, t, *J*=6 Hz, CH_2- S).

13CNMR (DMSO) δ: (171, C-ester), (158, C1'); (131.9, C4), (130.1, C-3' and C-5'), (129.0, C2- C6), (128.0, C3), (121.0, C4), (114.0, C2'-C6'), (69.4, CH_2), (66.9, CH_2) ppm.

2.4.2 Preparation of 2-(phenylthio) ethyl 2-hydroxybenzoate (II)

Firstly, 2-hydroxybenzoic acid (3.425 g, 0.025 mol) was added to 2–thiophenylethanol (3.850 g, 0.025 mol) and 2 ml sulfuric acid and then they were refluxed for half an hour, then the reaction was left for one night. The product was dissolved in 20 ml ethyl acetate then extracted with saturated sodium bicarbonate. The organic layer with 10% sodium chloride was washed, the solvent was dried with sodium sulfate, then evaporated. The product was purified by flash chromatography using the mobile phase of 40% n-hexane-60% ethyl acetate and silica gel as a stationary phase. The following scheme shows the chemical reaction of 2-(phenylthiol) ethyl 2-hydroxybenzoate.



Scheme 3: Reaction of 2-thiophenyl ethanol with 2-hydroxy benzoic acid.

Percentage yield is (76%) and M.p. = 234–237ºC. UV λ_{max} 240–270.

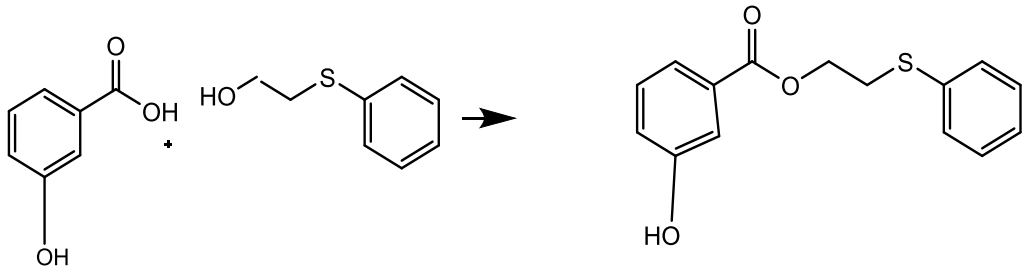
IR (3309 O-H, 1712 C=O, 1596 Aromatic ring, 1242 ester, 1069 carboxylic acid) cm^{-1}.

^{1}HNMR (DMSO): 7.39 (2H, H2, H6); 7.35 (2H , H3 , H5), 7.21(1H, m, H-4); 7.72 (1H, H14); 7.37 (1H, m, H15); 7.54 (1H,m H16'); 7.17 (1H, H17) 4.17 (2H, t, *J*= 6Hz, -OCH_2); 3.54 (2H, t, *J*=6 Hz, CH_2- S), 10.9 (O-H) ppm.

^{13}C-NMR (DMSO δ: 172, C- ester; 166, C1'; 156, C2; 135, C4; 131, C6'; 130 (C3' and C-5'); 121.0, C5'; 120, C4'; 119.0, C2'and C6'; 117.0, C1; 114.0, C3'; 69.2, O-CH_2; 62.1, CH_2-S) ppm.

2.4.3 Preparation of 2-(phenylthio) ethyl 3-hydroxybenzoate (III)

Firstly, 3-hydroxybenzoic acid (3.45 g, 0.025 mol), was mixed with 2-thiophenylethanol (3.850 g, 0.025 mol) and catalyzed with 2 ml sulfuric acid and then refluxed for an hour, then the reaction was left for one night. The product was then dissolved in 20 ml ethyl acetate and extracted with saturated sodium bicarbonate. The layer with 10% sodium chloride was washed, the solvent was dried with sodium sulfate and then evaporated. The product was purified by flash chromatography using the mobile phase 60% ethyl acetate -40% n-hexane and silica gel as a stationary phase. The following scheme shows the chemical reaction of 2-(phenylthio) ethyl 3-hydroxybenzoate.



Scheme 4: Reaction of 2-thiophenyl ethanol with 3-hydroxy benzoic acid**.**

Percentage yield is 56% and M.p. = 165–167ºC. UV λ_{max} 240–270.

IR ν_{max} (3309 O-H, 1689 C=O, 1596 Aromatic, 1496, 1242ester , 1087 carboxylic acid alkene , 673, 692 CH2) cm^{-1}.

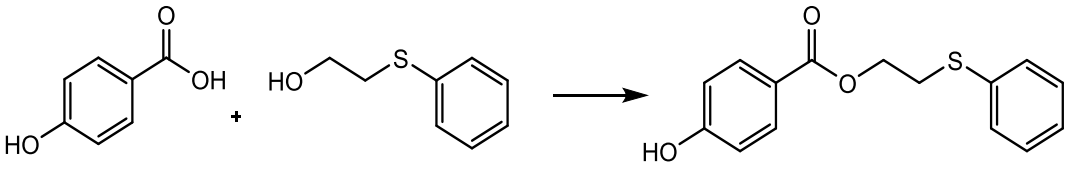
^{1}HNMR (DMSO):δ 7.39(2H,m,H2 and H6),7.21 (1H,H4);7.35 (2H, m,H3, H5'); 7.61 (1H, H13) 7.691H ,H14), 7.16 (1H ,H15)7.56 (1H, H17), 8.5(O-H), 4.17 (2H,t, *J*=7.0 Hz ,-OCH_2), 3.54 (2H, t, *J*=7 Hz, CH_2-S) ppm.

^{13}CNMR (DMSO):165.8, C-ester; 157.8, C1'; 156.5, C3; 132.0, C1; 129.4, C'3 and C5'; 125.9, C5; 124.9, C6; 120.8, C4'; 117.4, C2 and C4; 66.3, O-CH_2; 64.5, CH_2-S) ppm.

2.4.4 Preparation of 2-(phenylthio) ethyl 4-hydroxybenzoate (IV)

The 4-hydroxybenzoic acid (0.025 mol, 3.45 g), 2-thiophenylethanol (0.025 mol, 3.850 g) and 1 ml sulfuric acid were refluxed for an hour, then the reaction was left for one night. The product was then dissolved in 20 ml ethyl acetate and extracted with saturated sodium bicarbonate. The layer was washed with 10% sodium chloride, the solvent was dried with Na_2SO_4 and then removed. The product residue was purified by flash

chromatography using the mobile phase 40% n-hexane- 60% ethyl acetate and silica gel as a stationary phase. The following scheme shows the chemical reaction of 2-(phenylthio) ethyl 4-hydroxybenzoate.



Scheme 5: Reaction of 2-thiophenyl ethanol with4-hydroxybenzoic acid**.**

Percentage yield is 63% and the melting point is in the range 224–228°C, while UV λ_{max} 240–270.

IR v_{max} (3308O-H, 1689 C=O, 1596 Aromatic, 1495, 1241 ester, 1087 carboxylic acid, 694 alkenes, 673, 481 –CH2) cm^{-1}.

^{1}HNMR (DMSO) δ: 7.39 (2H, m, H2 and, H6), 7.35 (2H, m, H3 and, H5), 7.21(H-4) 6.88 (2H, m, H14', H16'),7.88 (2H, H13, H17) 4.43 (2H, t, *J*=7.0 Hz,-OCH_2); 3.31 (2H, t, *J*=7 Hz, CH_2-S),8.3 (O-H) ppm.

^{13}C-NMR (DMSO) δ: 167.1(C-ester), 160.4(C4), 156.8(C1') 132(C2 and C6); 129.9(C3 and C5'), 122. (C3' and C5), 121.0 (C4), 115.3 (C1'), 114.3(C2 and C6'), 69.2(O-$CH_{2)}$, 62.2(CH_2-S) ppm.

2.5 Biological activity assays

2.5.1 Antioxidant activity

2.5.1.1 DPPH (2, 2-diphenyl-1-picryl-hydroxyl hydrate) assay

Four synthetic compounds were tested for the efficiency of scavenging free radicals matched with Trolox and Gallic acid as a basic. One mg/ml concentration solutions in methanol were prepared from the compounds and the solutions that were produced were used to prepare concentrations of 5, 10, 20, 30, 40, and 50 μg/ml. The DPPH reagent (0.002% w/v) was dissolved in methanol before being mixed with working concentrations in ratios of 1:1:1 (compound: DPPH: methanol). The methanol solution was adopted as a blank. All the solutions were incubated for 30 min at room temperature in the dark. When the antioxidant compound reacts with DPPH, which can donate hydrogen, it is reduced. The color changes from deep violet to light. Absorbance values were estimated by using a UV–vis spectrophotometer, at a wavelength of 517 nm. The antioxidant potential percentage of each compound and Trolox and gallic acid was estimated according to the formula:

$$\text{Inhibition Percentage} = (A_b - A_s)/A_b \times 100\%,$$

where A_b is blank absorbance and A_S is sample absorbance. The IC_{50} values for each compound and Trolox and gallic acid were determined from the curves (71).

2.5.1.2 β-carotene methods

The effectivity of the synthesized compounds was estimated using a modified method of Gazzani and Miller (72). The method was based on the coupled oxidation of β-carotene and linoleic acid emulsion. The bleaching mechanism of β-Carotene is produced from the hydro-peroxides that are created from linoleic acid (73). During the oxidation process, the characteristic orange color of β-carotene and the chromophore will be lost. The presence of antioxidants can hinder the extent of the β-carotene bleaching. Briefly, 1 mg β-carotene was dissolved in 2 mL chloroform and 20 mg linoleic acid, and 200 mg of Tween 20 was added. Chloroform was completely vaporized by a rotary evaporator at a temperature of less than 30°C, under reduced pressure. Then 200 mL of distilled water saturated with oxygen was added to the flask which was shaken strongly for 30 min. A sample (5 ml) of the prepared emulsion was transferred to a series of tubes each containing 0.1 ml of the synthesized compounds or tocopherol (2 mg/ml).

The control sample was prepared exactly the same way but without adding antioxidants. Each sample type was prepared in triplicate. The samples were placed in water bath at 50°C for 2 h. The absorbance of the sample was read spectrophotometrically at a wavelength of 470 nm, immediately after sample preparation and at 15-min intervals until the end (t = 120 min) of the experiment.

2.5.2 Testing the antimicrobial activity

Four bacterial strains were selected for antibacterial examination and they were supplied by the American Type Culture Collection (ATCC) and they were; *Escherichia coli* (ATCC 25,922); *Pseudomonas aeruginosa* (ATCC 27,853); *Staphylococcus aureus* (ATCC 25,923); and some clinical isolates of MRSA (Methicillin-Resistant *Staphylococcus aureus*) were also examined. The *C. siliquastrum* samples were examined against the growth of *Candida albicans* to assess the fungus inhibition ability of our compounds. Furthermore, the antimicrobial effect of *C. siliquastrum* in our study was reported using the broth micro dilution process (74, 75). Fifty grams of every compound were fully dispersed in 50 ml dimethyl sulfoxide to establish a final concentration of 1 mg/ml. Filter-sterilization was carried out on the resulting solution in order to serially micro-dilute it by twofold, six times under a sterilized nutrient broth. The dilution processes were carried out using aseptic conditions in the available 96 well dishes. Inside the micro-wells which were selected to examine the antibacterial ability of the tested compounds, the concentration ranged from 1.53 to 25 mg/ml. The same conditions existed inside the micro-wells of the compounds assigned to examine the antifungal effect where the concentration also ranged from 1.53 to25 mg/ml. Also there was a micro-well plate, holding number 11, which included compounds that were free of nutrient broth, and which was adopted as a positive control for the growth of microbes. In addition, micro-well holding number 12 included compounds free of nutrient broth that was kept away from the others and was not inoculated

with any of the examined microbes. Each of the bacterial pathogen and Candida Albicans samples was examined in duplicate in this assay. All the inoculated microplates were incubated at 35°C, and plates inoculated with the examined bacterial strains were incubated for 18 h and plates inoculated with examined *Candida albicans* were incubated for 48 h. The lowest value of the assessed concentration of *C. siliquastrum* at which there was no visible microbial growth in the micro-well was also recorded, and was defined as the minimal inhibitory concentration (MIC) of the tested compound (75). This micro-well was considered as a negative reported control of bacterial growth. The micro-wells with numbers ranging between 1–11 were also inoculated aseptically with the examined microbial strains. At the time of inoculation, the final concentrations of microbial cells were about 5×10^5 and $0.5–2.5\times10^3$ colony-forming unit (CFU)/ml for the examined strains of bacteria and *Candida albicans*, respectively. Each of the involved microbes in our study were assessed in duplicate.

2.5.3 Testing anti-cancer activity

2.5.3.1 Assessed cell line

The evaluated cytotoxicity was carried out by two cell lines, which were MCF-7 and MCF-10A. The examined MCF-7 is as known as a breast cancer cell line, characterized by the overexpression of a receptor-related to estrogen. In contrast, MCF-10A is not a tumorigenic epithelial breast cell line. The MCF-7 case was achieved by RPMI-1640 media (Germany, sigma) mixed with 10% fetal bovine serum (Germany, Sigma), 1% l-

glutamine (France, Sigma), 1% streptomycin and 1% penicillin (USA, Sigma), also a pH of 7.2 was controlled using Dulbecco's Phosphate Buffered Saline (DPBS) (USA, Sigma). The cells were grown in an atmosphere containing humidity and 95% air and 5% CO_2 at 37°C in an ESCO incubator adapted for cell-culture. Moreover, the construction of the MCF-10A case was carried out in DMEM-media (Sigma, Germany) that was nourished with 10% fetal bovine serum,1% l-glutamine, 1% streptomycin, and 1% penicillin, and the pH was kept at 7.2 using Dulbecco's Phosphate Buffered Saline (DPBS). The growth of cells was observed in an atmosphere with humidity and containing 95% air and 5% CO_2 at 37°C in an ESCO incubator adapted for cell-culture (76).

2.5.3.2 Flow cytometry analysis

After the culture applications, the MCF-7 and MCF-10A cells were collected and controlled to 106 /ml in staining buffer (in saline containing 1% bovine albumin; Israel, Biological Industries). For viability as well as for apoptosis reporting, propidium-iodide (PI) fragmented DNA was stained and phosphatidylserine was stained by Annexin V-conjugated to FITC (R&D Systems, Minneapolis, MN) were utilized depending on the manufacturer's information. Apoptosis usually defined as annexin-V (+) but propidium-iodide (-). Viable cells were defined as annexin-V (-) but propidium-iodide(-). Every experimental part involved set up, unstained controls, IgG isotype controls with FMO controls. In order to analyze the cell cycle using DNA, content quantitation was applied using the

propidium-iodide. The MCF-7 and MCF-10A cells were stabilized in cold 70% ethanol for nearly 30 min at 4°C. They were then washed by 2X in PBS and spun at 2000 rpm and the supernatant was discarded once finished. To ensure that only DNA was stained, the cells were treated with the ribonuclease enzyme (50 µl of 100 µg/ml RNase), stained with 5 µl of 50 µg propidium iodide/100ml and analysis was done using a flow cytometer (Immune fluorometry systems, Becton- Dickinson LSR II, Mountain View, CA) (77).

2.5.4 Measurement of α-glucosidase activity

The inhibition of α-glucosidase activity by the synthesized molecules was measured. The α-glucosidase inhibitory activity is expressed as percentages of inhibition (percentage): Inhibitory effect % = $(A_b - A_s)/ A_b$ *100 %, where A_b and A_s are the absorbance values of the blank (containing PBS, α-glucosidase, and PNPG a colored substrate of glucosidase) and the tested sample (containing PBS, extract, α-glycosidase and PNPG), respectively.

The α-glucosidase activity was measured using 10 and 20 mg/ml of the synthesized molecules. Each concentration was recorded by using 1, 3, 6, 9, and 12 mm PNPG. α-Glucosidase action was detected depending on the assay previously mentioned. The inhibitory pattern was assessed using a Lineweaver–Burk plot. The constant Ki of enzyme inhibitory effect was determined.

2.5.5 Anti-lipase activity

A solution of 1 mg/ml of the synthesized compounds was mixed with 10% dimethyl sulfoxide (DMSO) and then diluted with 10% DMSO to produce five dilutions (0.2, 0.4, 0.6, 0.8, and 1 mg/ml). Orlistat was considered as a reference in this inhibition protocol for pancreatic lipase and was tested following the same steps that were used previously.

A freshly utilized stock solution of pancreatic lipase enzyme was established by suspending this enzyme in 10% DMSO to form 1 mg/ml. Firstly, 25 mg of lipase was suspended in a small amount of 10% DMSO, bringing the volume up to 25 ml in V.F (25 ml), this was then put in a water bath sonicator at 37ºC for 15 min. The stock solution of PNPB was constructed depending on manufacture structures (20.9 mg was obtained from PNPB and dispersed in 2 ml of acetonitrile) by dissolving 104.5 mg of PNPB in acetonitrile brined up to the volume of 10 mL in V.F (10 ml). The pancreatic lipase (PL) inhibition assay was conducted by adopting the procedure in the references with slight modifications(78-80). From each working solution of the synthesized compounds prepared above, 200 µl of the synthesized compounds was taken and put in a separate test tube, then 100 µL of porcine pancreatic lipase (1 mg/ml) was added to it. The resulting mixture was then adjusted to 1000 µl after addition the of 700 µL of tris-HCl solution and then it was incubated in a water bath at 37°C for 15 min. After the incubation, 100 µl of PNPB (*p*-nitrophenyl butyrate) solution was added to each test tube. This mixture was then incubated again

in a water bath at 37°C for 30 min. A solution characterized as negative control was constructed without the synthesized compounds, using 100 µl of porcine pancreatic lipase (1 mg/ml) solution mixed with tris-HCl solution up to 1 ml after the addition of 900 µl. The same procedure was adopted for Orlistat, which was a positive control. A tris-HCl buffer was used to zero UV-Vis spectrophotometer at 405 nm. The effect of the pancreatic lipase was reported by measuring the hydrolysis of *p*-nitrophenolate to *p*-nitro phenol at 405 nm using a UV-Vis spectrophotometer device. The lipase inhibition activity of the synthesized compounds, or Orlistat as a reference, was identified by measuring the effect on the enzyme reaction rate after the addition of the synthesized compounds and then comparing it with the control. The % inhibition of the synthesized compounds was calculated by using the following equation:

Inhibitory lipase percentage (%) = $[(A_b - A_s)/A_b]*100$.

2.5.6 α-amylase inhibitory screening

The α-amylase inhibitory assessment was based on the Wickramaratne et al. protocol (81) with some slight alterations in some steps. The experimental part was carried out by following the 3, 5-dinitrosalicylic acid (DNSA) procedure. Solutions of 20 mm sodium phosphate mono basic and sodium phosphate dibasic buffer involving 6.7 mm sodium chloride (NaH_2PO_4 and Na_2HPO_4 both including 6.7 mm NaCl, pH 6.9) were constructed by partially filling the beaker with the NaH_2PO_4 and NaCl solution, the mixture was subjected to a magnetic stirrer, while the pH was

adjusted by inserting a calibrated pH electrode in the solution. Then, the Na_2HPO_4 and NaCl solution was gradually added until the pH reached 6.9. A weight of 5.36 g of 20 mm $Na_2HPO_4.7H_2O$ and 0.39 g of 6.7 mm NaCl were dispersed in distilled water to make 1 L and a weight of 2.76 g of NaH_2PO_4. H_2O and 0.39 g of NaCl dissolved in distilled water to make 1 L. The stock solution for the synthesized molecules had a concentration of 1 mg/ml and was put in in a minimum amount of 10% DMSO (1:100 dilution) and was then dispersed in a buffer of Na_2HPO_4/NaH_2PO_4 (0.02 M) and NaCl (0.006 M) at adjusted pH 6.9. Working solutions with concentrations of 0.01, 0.05, 0.1, 0.5, and 1 mg/ml were obtained by mixing 0.1, 0.5, 1, 5, and 10 ml of our synthesized molecules, respectively, and then diluting them with a buffer of Na_2HPO_4/NaH_2PO_4 (0.02 M) and NaCl (0.006 M) at pH 6.9 and then brined up to 10 mL using VF (10 ml). The acarbose was considered as a reference and was established following the same previous steps used for the synthesized molecules.

A solution of α-amylase was (2 unit/ml) was produced by dissolving 12.5 mg of amylase enzyme in a minimum amount of DMSO10 %, which was then brined up to 100 ml with the previous phosphates buffer in V.F (100 ml). A starch solution with a concentration of 1% (w/v) was prepared by suspending 1000 mg of starch in 100 ml of distilled water using V.F (100 mL), and then it was kept in water bath at 37ºC until use, with occasional mixing to prevent starch precipitation. DNSA was used as reactive reagent to react with reducing sugars to produce 3-animo-5- nitro salicylic acid that is highly absorbent of light at about 540 nm. It was prepared by dissolving

12 g of sodium potassium tartratetetrahydrate in 8.0 ml of 2 M NaOH (8 g in 100 ml distill. water) then further dissolved in 20 ml of 96 mm of 3.5-dinitrosalicylic acid solution.

3,5-dinitrosalicylic acid ⟹ 3-amino,5-nitrosalicylic acid

Then, 200 µl of the amylase solution (2 unit/mL) was gently shaken with 200 µl of each of the VOs established working solutions and then this was incubated at 37°C for 10 min. Then 200 µl of the starch solution was added to each test tube and there was further incubation for 3 min at 37°C. The reaction was terminated by the addition of 200 µl DNSA and then boiling for 10 min at 85–90°C. The mixture was then cooled to room temperature and diluted with 5 mL of distilled water, and the absorbance was recorded at 540 nm using a UV-Vis. spectrophotometer. Replacement of the synthesized compounds with 200 µl of buffer was established to obtain the blank sample. In this protocol, acarbose was the positive control sample. The enzyme inhibitory activity was expressed as percent inhibition and the following equation was used in order to determine IC_{50} value for the tested compounds

$$\%\,\alpha\ amylase\ inhibition = \frac{Abs100\%\ control - AbsSample}{Abs100\%\ Control} \times 100$$

Chapter Three
Results and Dissections

3.1 Identification of 2-thiophenoxyethanol benzoates:

The structures of products were established by their UV, Infrared, Proton NMR and ^{13}C spectral data.

All IR bands for all functional groups in the prepared compounds are seen obviously; even those small deviations due to the fine differences in structures can be explained. The $-CH_2-CH_2-$ stretching (symmetric and asymmetric) bands appear clearly for all products in the range 2800-3000 cm^{-1}. The aromatic proton bond stretching bands are seen just above 3000 $cm^{-1.}$ The bands for the carbonyl groups vary according to the specific structure of each compound.

The conjugated carbonyl group, with an aromatic ring, is expected to show bands in the range 1700-1710 cm^{-1}, this has been seen with exceptions for the ortho- products. The bulkiness of these groups prevents an ideal conjugation by distorting the planarity required for that and increasing the wavenumber. The C-C stretching for aromatic rings is found around 1590 and 1490cm^{-1}. The C-O single bond of the ester stretches around 1240 cm^{-1}, that of the C-O of that with the aromatic ring is about 1280cm^{-1}, while that of the alcoholic C-O stretching is found around 1040 – 1060 cm^{-1}, following those found foresters of primary alcohols. The out of plane bending of the aromatic C-H bonds for the mono-substituted ring have been

found at around 690 and 750 $cm^{-1}$and those for the di-substituted aromatic ring are found at the expected frequency.

3.2 NMR

The proton NMR spectra of the esters I – IV have been obtained and analyzed. The high resolution of the machine (500 MHz) has approximated the expected very complex spectra, such as the AA'XX' and AA'BB' for aromatic system into simple A_2X_2, AX_2 and so on. The simple coupling constants can be calculated even for the aromatic protons. The coupling constant were averaged because the resolution is not enough to show the para and Meta coupling. The alcoholic part of the ester is the same and shows very similar signals in their charts. The ethylene group shows two triplets at δ 4.2 -4.6 ppm with an average coupling constant (4.2-4.5 Hz).

3.3 Anti-oxidant activity

3.3.1 DPPH RESULT:

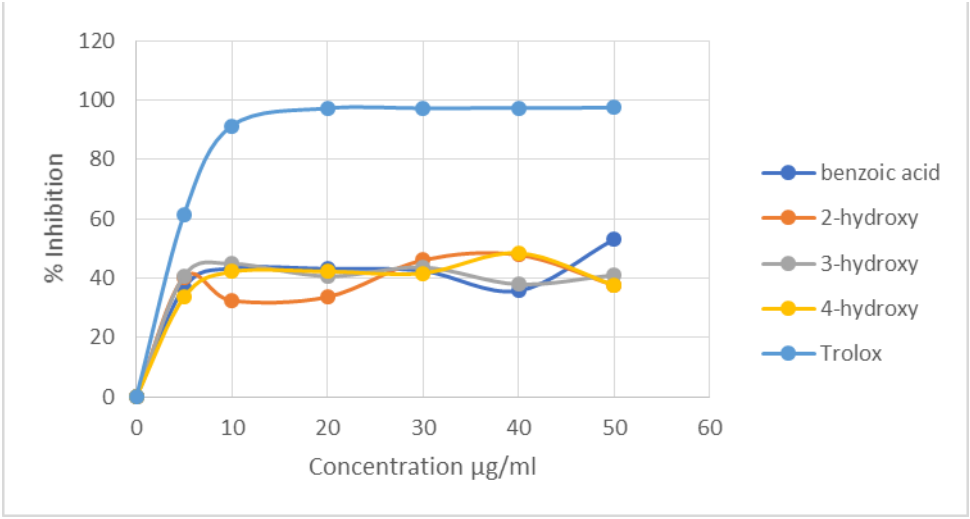
DPPH (1, 1-Diphenyl-2-picryl-hydroxyl) is one of the methods used to measure the antioxidant activity of different compounds such as phenols and phenolic acid esters. The decrease in absorbance at 517nm induced by antioxidants determines the reduction capability on the DPPH. When antioxidants donate hydrogen atoms to the radicals, they lose their purple color. This, in turn, leads to decreased absorption. The decrease in absorption is taken as a measure of the extent of radical scavenging. All the compounds showed free radical scavenging activity near to the Gallic acid

at concentrations 30 ug\ml table 3.1 and figure 3.1 the values of percent inhibition of the tested compounds. Also, the percent inhibition values of the compounds increased with increasing concentration, while the values of IC_{50} of both benzoate and 4-hydroxy is about 90% of the value of IC_{50} to the Gallic acid.

On the other hand, there is not much difference between the values of percent inhibition for the compounds. This means that the position of functional group as ortho-, para- or Meta- has a slight effect on the antioxidant activity. Our results are in good agreement with literature done on polyphenolic compounds, which showed that the structure did not affect the antioxidant activity (82).

Table 3.1 Percent inhibition of radicals by benzoate compounds at different concentrations

Conc,	% Inhibition					
comp	benzoic acid	2-hydroxy	3-hydroxy	4-hydroxy	Trolox	Gallic acid
0	0	0	0	0	0	0
5	36.8	32.5	40.8	33.6	61.5	12.5
10	35.8	33.7	44.9	34.4	91.4	26.6
20	43.2	37.7	40.7	42.2	97.3	36.1
30	42.5	40.2	43.7	42.3	97.3	45.5
40	43.4	46.1	38	41.7	97.3	61.3
50	53.1	47.9	41.1	48.5	97.4	63.7
IC50	48	>50	50	>50	4	35



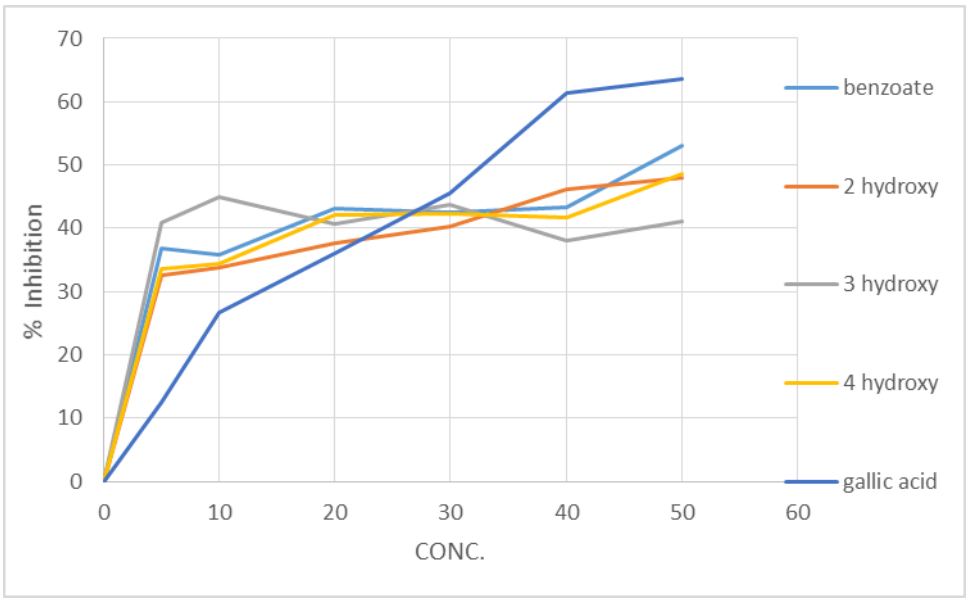


Figure3.1: % Inhibition of DPPH for the tested compounds.

3.3.2 β-carotene linoleic acid activity:

The same compounds were tested for their antioxidant activity using the emulsion system of β-carotene linoleic acid depending on the fact that β-carotene loses its color in the absence of antioxidants(83, 84). The synthetic compounds (2-(phenylthio)ethyl benzoate and 2-(phenylthio)ethyl 2-hydroxy benzoate) showed higher antioxidant efficiency in concentration 0.2 mg\ml compared with water (control) and α-tocopherol and the other

synthetic compounds antioxidant which gave the lowest antioxidant efficiency. Figure 3:2 showed the antioxidant activities of the synthetic acid esters and positive reference standard with the coupled oxidation of β-carotene in concentration 0.2 mg\ml. The antioxidant activity of all the compounds gradually decreases with the increase of time. Water showed the highest β-carotene bleaching activity followed by α-tocopherol after 1 hour. (2-thiophenylethanol benzoate revealed the best antioxidant with absorbance 1.04 after 75 min compared with the other synthetic compounds. The second one is 2-thiophenylethanol 2-hydroxy benzoate, which showed absorbance 0.84 after 75 min.

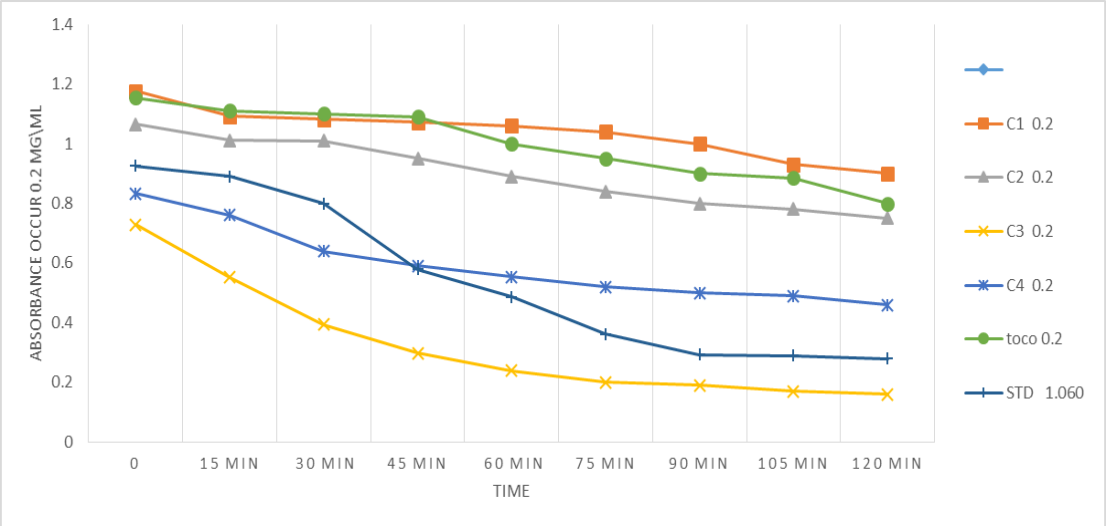


Figure 3:2. Antioxidant activities of synthesis compounds in β- Carotene-linoleic acid test.

3.4 Testing antimicrobial activity:

The benzoate compounds were tested against seven bacteria and one fungus that cause dermic and mucosal infections, besides other infections, in humans(52). For all the bacteria we tested here, we had four controls: 1)

positive control which contains media and bacteria; 2) negative control which only contains media; 3) compound control (compound+ media) to be sure that there is no contamination and turbidity and that the changes are not due to the compound itself (so compounds were serially diluted in this control); and 4) DMSO which were tested for every microbe separately to check the effect on each one, and the antimicrobial activity of DMSO was also considered.

All the compounds studied in this work showed antibacterial activity. The 2-thiophenylethanol 3-benzoate showed significant activity, especially at concentrations of 3.125–6.25 mg\ml against all the bacteria we tested, but *Candida* showed resistance. The second compound, 2-thiophenylethanol 4-benzoate, showed good activity against all the bacteria for a concentration range of 3.125–6.25 mg\ml, except the MRSA and *Candida,* were resistant. The 2-thiophenylethanol benzoate was the only compound that showed some activity against *Candida* at a concentration of 1.56mg\ml and it had antibacterial activity against *S. aureus*, *Proteus vulgaris*, *Enterococcus faecium,* and MRSA at concentrations of 3.125, 6.25, 6.25, and 3.125 mg\ml, respectively. While 2-phenylthioethyl 2-hydroxy benzoate showed the highest antibacterial activity for *S .aureus*, *Proteus vulgaris*, *Klebsiella,* and MRSA with concentrations of 3.125, 3.125, 6.25, and 6.25 mg\ml, respectively. When we compared the MIC for the four compounds with good antibiotics, we found that benzoate 2-thiophenylethanol has the same MIC as fluconazole (1.56) for the inhibition of the growth of *Candida*. Moreover, benzoate 2-thiophenylethanol, 2 hydroxyl 2-thiophenylethanol,

and 4 hydroxyl 2-thiophenylethanol have the same MIC as ampicillin (3.125) for the inhibition of the growth of *S. aureus* bacteria. It is noteworthy to take attention for those modified compounds although they possess less activity than synthetic antibiotics. It is important to prepare new therapeutics because some kinds of bacteria become resistant to certain drugs after a period of time.

Table 3.2: Microbial growth inhibition MIC values of *synthesis compounds (mg\ml)*

	ATCC 25923	ATCC 25922	ATCC13883	ATCC8427	ATCC700221	ATCC927	CLINICAL STRAIN	ATCC90028
	S. aureus	E coli	klebsiella	Proteus vulgaris	Enterococcus faeaium	Pseudomonas aeruginosa	MRSA	Candida Albian's
C1	3.125	R	R	6.25	6.25	R	3.125	1.56
C2	3.125	R	6.25	3.125	R	R	6.25	R
C3	6.25	6.25	6.25	6.25	3.125	6.25	6.25	R
C4	3.125	6.25	6.25	3.125	6.25	6.25	R	R
Fluconazole	R	R	R	R	R	R	R	1.56
Ampicillin	3.12	3.12	0.001	0.018	0.78	3.12	R	R
Ciprofloxacin	0.78	1.56	0.125	3.12	0.78	0.015	0.125	R

3.5 Anticancer activity:

Cytotoxicity: Compound inhibits the DNA cell cycle of MCF-7 cells. To investigate whether the compound could induce cell cycle perturbations in breast cancer cells, flow cytometry analyses of propidium iodide stained nuclei cells were performed. Cell cycle parameters were investigated for four compounds. The compounds were incubated with MCF-7 for 24 h at a concentration of 500 mg/ml). The figure shows a slight increase in the fraction of cells in the G1 phase following treatments with compounds. Around averages of 56% were obtained in the G1 phase following treatment of concentrations of four compounds as compared to 41% in untreated

Cells ($P<0.05$) (Fig 3.3).

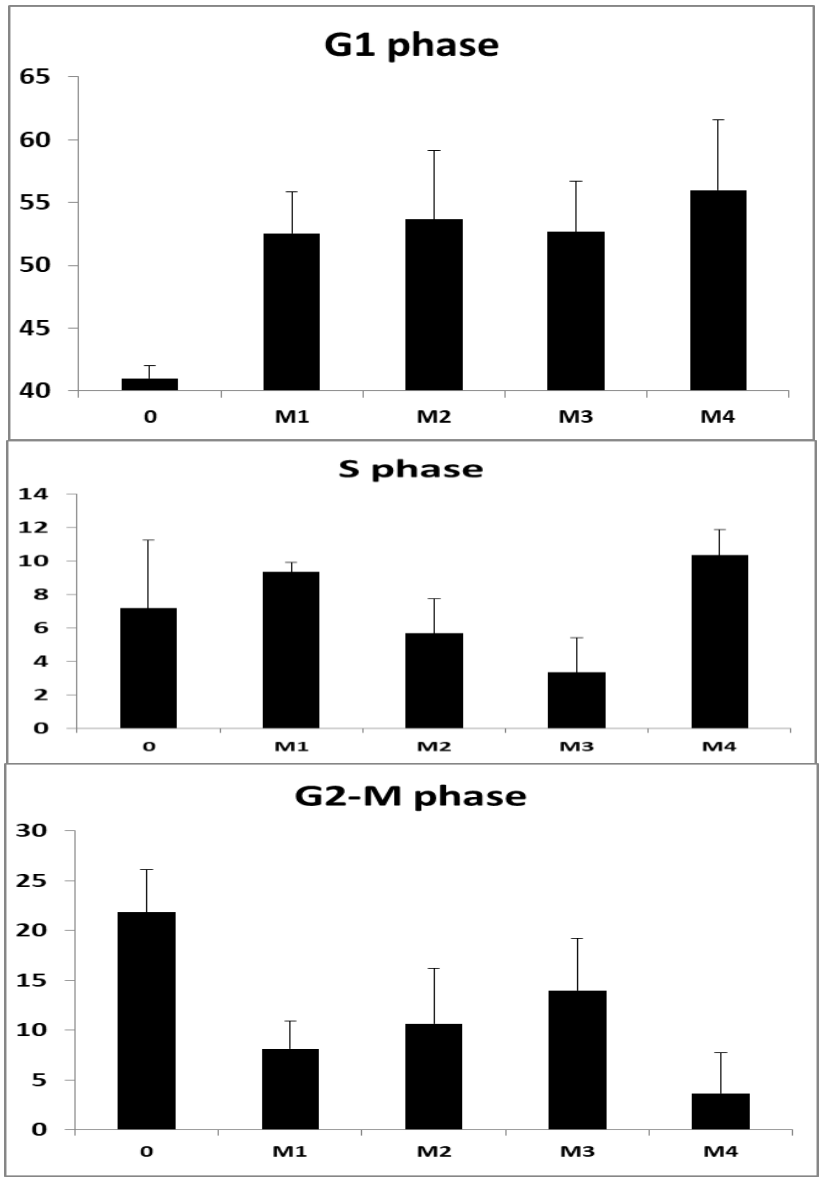


Figure 3.3: cell cycle phase treatment with four compound

Figure 3.3 shows averages of three different readings of (A) G1 phase (B) S phase and (C) G2-M phase following treatments with four compounds.

Also, significant elevations in the proportion of cells in the S phase were obtained following 500 mg/ml concentration of two compounds (C1=9.3% compared to 7.16 %, C4 10.3 %, compared to 7.16% untreated cell, respectively ;(Fig3 B) $P<0.05$).

The other compound decrease in the S phase. Cell cycle parameters were perturbed in MCF-7 cells in the G2/M phase in the 500 mg/ml FOUR COMPOUNDS leaves to 8.13%, 10.7%, 14%, and 3.67% respectively, as compared to 21.8 %in the untreated cells. P-value was significant between all groups (Fig. 3 C)

These results indicate four compounds as a potent inhibitor of cell cycle progression at the G2/M phase and might suggest anti-cancer properties.

We next determined whether the four compounds that perturb DNA content induce apoptosis (programmed cell death). Cells undergoing apoptosis have their phosphatidylserine (PS) phospholipid translocated from the inner face of the plasma membrane to the cell surface; therefore, apoptotic cells can be identified by the presence of PS on the cell surface. As mentioned in materials and methods, detection of PS was estimated by staining with a fluorescent conjugate of annexin-V, a protein that has a high affinity for PS, followed by flow cytometry analysis. Cells were also stained with propidium iodide (PI), which can enter the cell only when the plasma membrane is damaged. Early apoptosis evaluated by positive for PS, but negative for PI and was distinguished from late apoptotic and necrotic cells estimated by positive for both PS and PI.

Four compounds significantly decreased apoptosis to C1 47.3, C2 29, C3 10.6, C4 8.3%, while a large population of late apoptotic/necrotic cells. Figure 3.4 shows averages of treatment of MCF-7 cells with four compounds, respectively (P-value <0.05 in all groups).

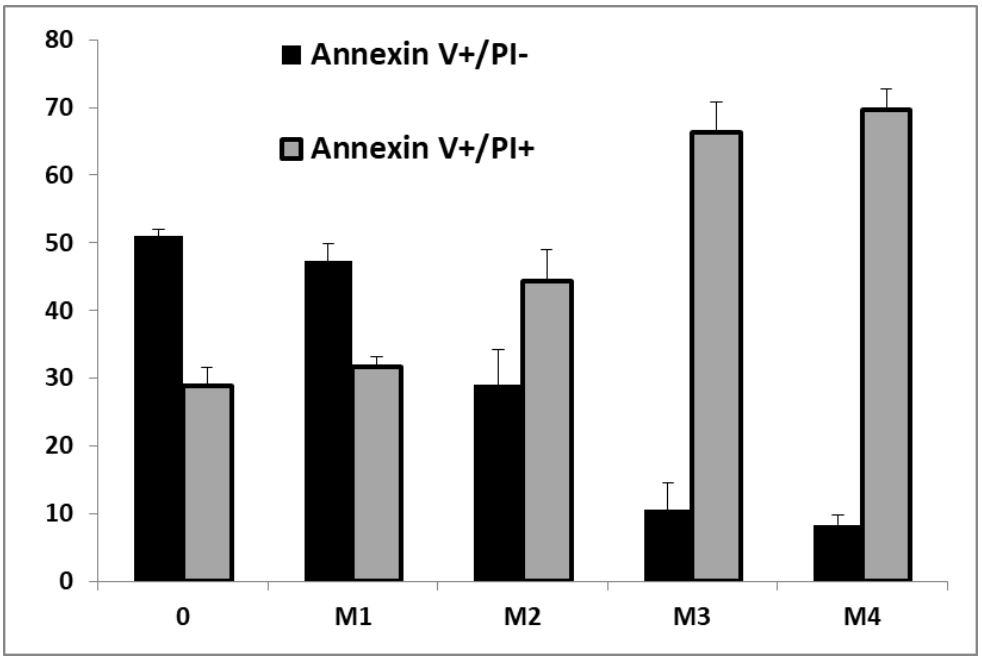


Figure 3.4: Apoptosis and necrosis averages of synthesis compounds

Taken together, the provided data suggest that four compounds could have an anti-cancer potential through the G2-M cell cycle phase arrest of MCF-7 and shifting the cells to necrosis.

3.6 α-amylase inhibitory screening

The α-amylase inhibitory activity was expressed as percent inhibition and calculated using equation 4. The percentage of α-amylase inhibition will be plotted against the concentration of the synthesized molecules and the IC_{50}values were obtained from the graph.

$$\%\,\alpha\ amylase\ inhibition = \frac{Abs100\%\ control - AbsSample}{Abs100\%\ Control} \times 100$$ Equation 4

Tables 3, 4 and figure 4 showed the percent inhibition of the synthesized compounds compared with Acarbose.

Table 3.3 Percent inhibition of the synthesized compounds compared with Acarbose.

Conc	0.1	0.5	0.7	1	5
compd.1	61.8±0.61	68±2.90	74.9±1.45	93.4±0.33	93.2±0.17
compd.2	44.3±0.89	74.8±1.68	85.9±0.93	88.0±0.17	88.2±0.44
compd.3	12±1.37	86.4±1.68	90.4±0.58	92.0±0.89	92.2±0.29
compd.4	32±0.77	90.3±1.67	93.6±0.50	93.1±0.34	92.4±0.50
Conc. µg/ml	% inhibition				
	Acarbose	Compd.1	Compd.2	Compd.3	Compd.4
0	0	0	0	0	0
10	53.22	61.8	44.3	12	32
50	54.91	68.2	74.8	86.4	90.3
70	66.1	74.9	85.9	90.4	93.6
100	66.1	93.4	88	92	93.1
500	72.54	93.2	88.2	92.2	92.4
IC_{50}	10	10	15	30	20

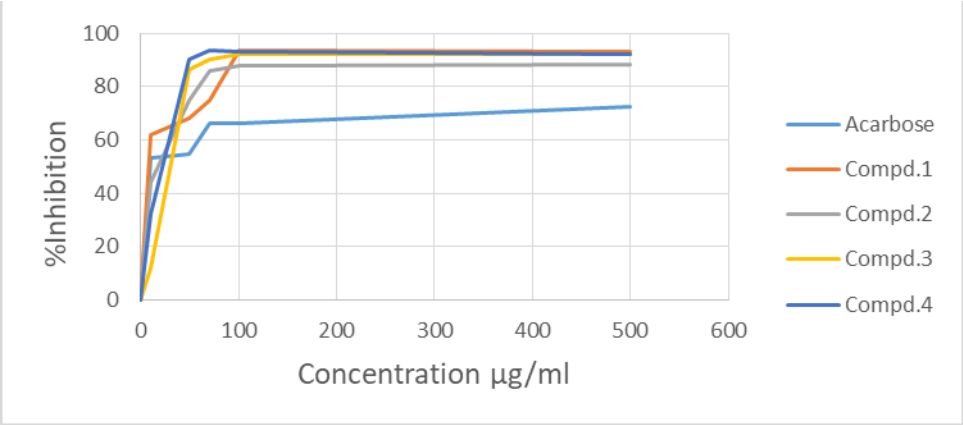


Figure 3.5: % Inhibition of α-amylase enzyme for the tested compounds

3.7 α- Glycosidase activity of thio-acid esters

Table 4 shows the percent inhibition and values of IC_{50} of the synthesized acid esters compared with Acarbose (standard compound). In general, those compounds revealed a significant effect. Compounds one and three showed

IC_{50} values 50 and 53µg/ml respectively, which is more effective against enzyme Acarbose IC_{50} is 54, while compound two showed IC_{50} 90µg/ml. The results are reasonable and justified because the synthesized compounds are modified acid esters and have the same functional groups as phenolic compounds, such as Trans - p-coumaric acid.

Table 3.4 The the percent inhibition of the synthesized compounds compared with Acarbose α- Glycosidase

	% Inhibition			
Conc.µg/ml	Acarbose	Compd.1	Compd.2	Compd.3
0	0	0	0	0
100	65.8±0.42	74.2±0.97	59±1.32	68.6±1.11
200	67.8±0.35	77.4±1.11	87.6±0.18	88.3±0.84
300	73.2±0.42	84.5±0.32	88.3±00	89.0±0.48
400	85.4±0.35	88.5±0.8	88.4±0.48	89.5±0.55
500	92.2±0.11	91.8±0.32	92.2±0.48	90.8±0.83
IC50	54	50	90	53

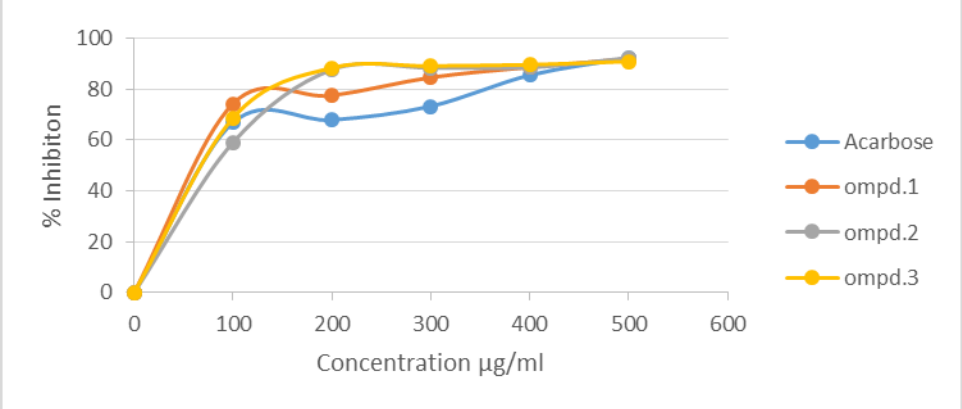


Figure 3.6: %Inhibition of α-Glycosidase enzyme for the tested compounds

3.8 Assessment in-vitro porcine pancreatic lipase enzyme activity

The hydrolysis of p- nitrophenyl butyrate to p-nitrophenyl was used to measure the influence of the synthetic acid esters on the porcine pancreatic lipase enzyme. The assay measures the percent inhibition of the compounds compared to with orlistat drug as a standard. The results of percentage inhibition and IC_{50} values for the compounds and orlistat are shown in table 3.5 and figure 3.7.

Table 3.5 the percent inhibition of the synthesized compounds compared with Orlistat porcine pancreatic lipase enzyme

Conc. µg/ml	% inhibition				
	Orlistat	Compd.1	Compd.2	Compd.3	Compd.4
0	0	0	0	0	0
50	91.1	34	35	6	40.4
100	93.1	51.5	48.3	9.2	44.4
200	94.3	57	50.9	37	56
300	97.4	65	50.4	38.9	69.7
400	97.5	69.2	54.7	58.3	74.4
IC_{50}	25	90	110	380	150

Table 3.5 and figure 3.7 show the percent inhibition and $IC_{50}$of the synthesized compounds compared with orlistat. Compound one shows the best results of percent inhibition. The value of IC_{50} of compound four was fourth fold (25µg/ml) compared with orlistat (IC_{50} =25µg/ml). The least active one against lipase enzyme was compound 3 which has (IC_{50} =380µg/ml). Those results may be justified by the lack of our compounds to the amide group which is the main one in orlistat.

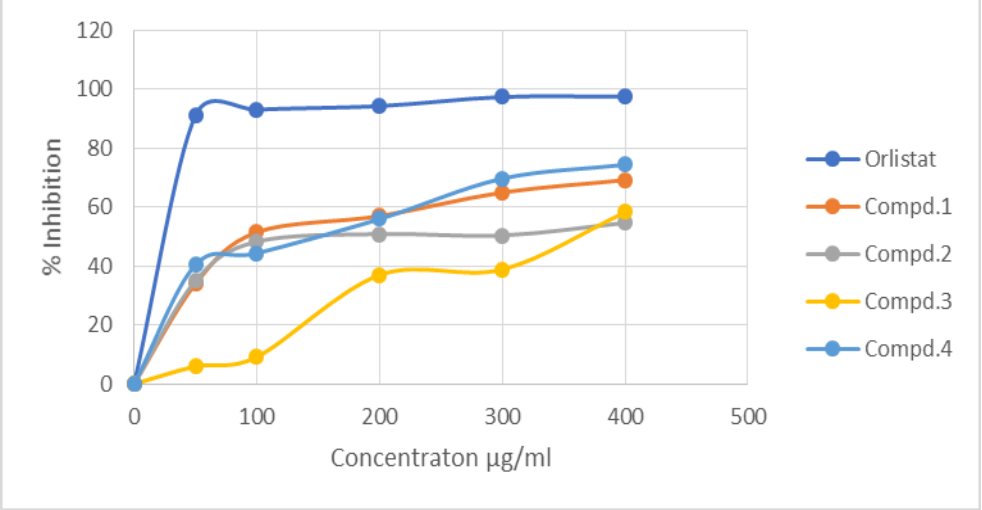


Figure 3.7: the percent inhibition and $IC_{50}$of the synthesized compounds compared with orlistat

Conclusion

The synthetic compounds were prepared easily by the reaction of acid esters and 2-thiophenyl ethanol. The compounds were identified depending on some physical properties such as melting points and thin-layer chromatography. The identity of the compound was confirmed using the spectroscopic method such as UV, IR, HNMR and carbon 13. The compounds were tested for their biological activities through many tests like antioxidant, antimicrobial, anticancer, anti-α-glucosidase, anti-α-amylase, and lipase activities. They showed significant activity in most of the tests. The best results were in antimicrobial, anticancer, anti-α-amylase and α-glycosidase. These results encourage other researchers to complete studies of those compounds and make them as best choices of human drugs.

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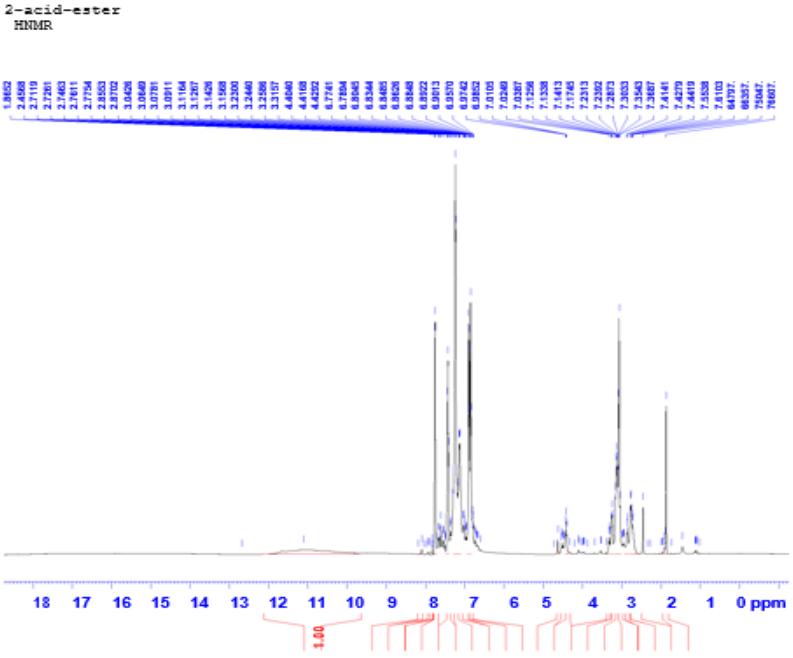
Appendices:

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

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TE                  305.2 K
D1              2.00000000 sec
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PLW1            17.39999962 W

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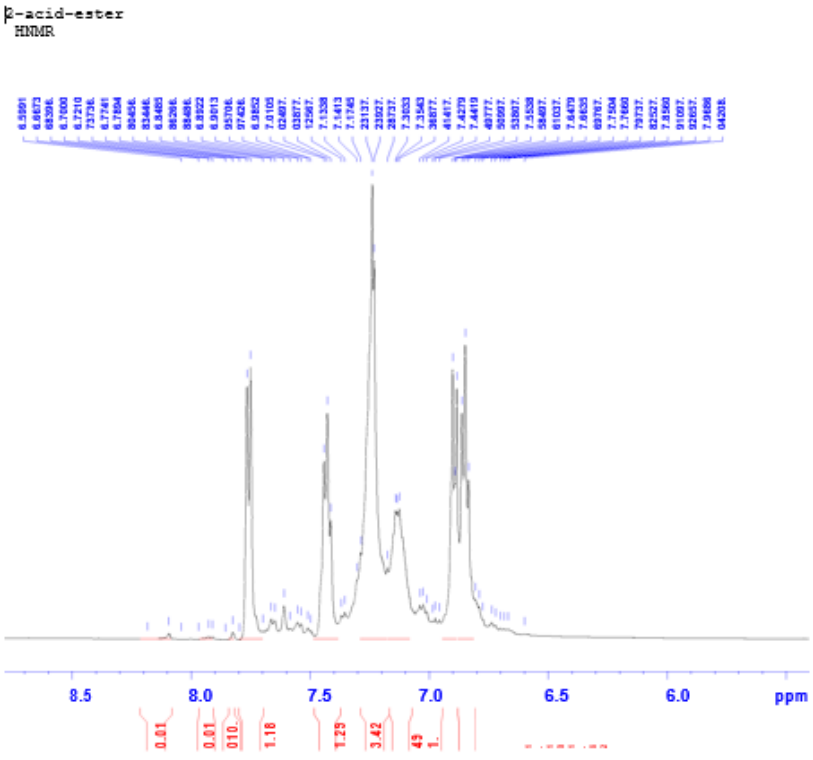

The University of Jordan
Faculty of Science
Department of Chemistry

Instrument Model:
Bruker 500 MHz-Avance III

Operator: Rola Hassouneh
nmr500@ju.edu.jo

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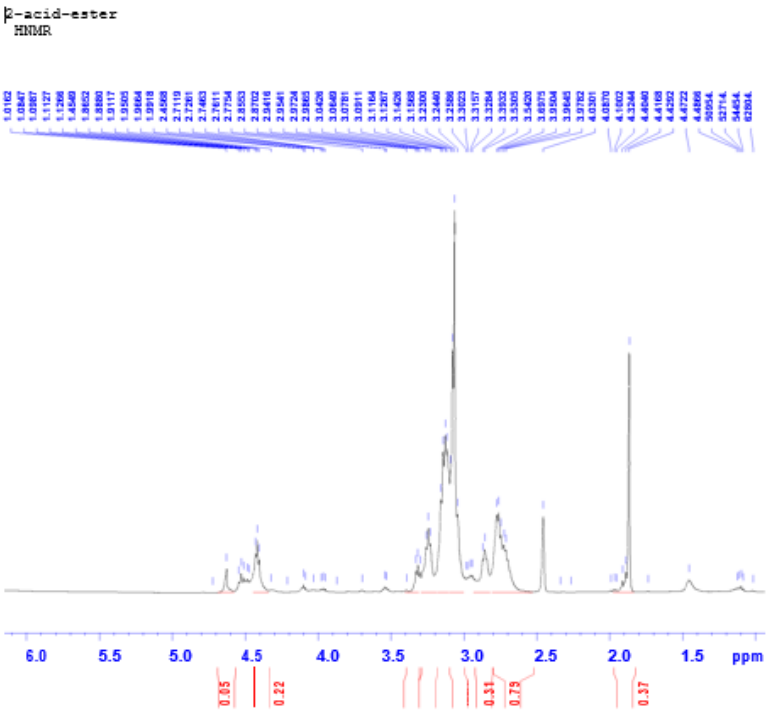
======== CHANNEL f1 ========
SFO1            500.1343971 MHz
NUC1                    1H
P1                   10.60 usec
PLW1            17.39999962 W

F2 - Processing parameters
SI                  131072
SF              500.1300270 MHz
WDW                     EM
SSB                  0
LB                    1.00 Hz
GB                   0
PC                   10.00
```



The University of Jordan
Faculty of Science
Department of Chemistry

Instrument Model:
Bruker 500 MHz-Avance III

Operator: Rola Hassouneh
nmr500@ju.edu.jo

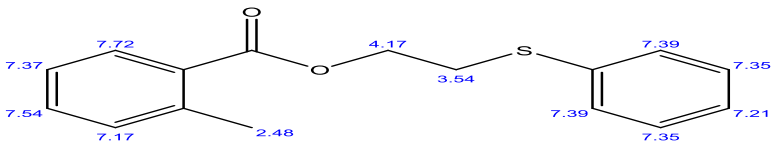
```
Current Data Parameters
NAME          15oct15jalal
EXPNO               1141
PROCNO                 1

F2 - Acquisition Parameters
Date              20151029
Time                 13.04
INSTRUM              spect
PROBHD    5 mm PABBO BB/
PULPROG                zg
TD                  65536
SOLVENT             CHCl3
NS                      8
DS                      0
SWH           10000.000 Hz
FIDRES            0.152588 Hz
AQ            3.2767999 sec
RG                   12.22
DW                 50.000 usec
DE                  6.50 usec
TE                 305.2 K
D1            2.00000000 sec
TD0                     1

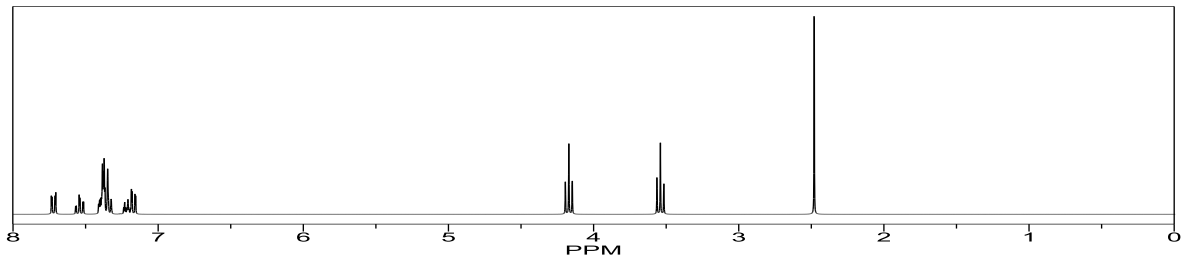
======== CHANNEL f1 ========
SFO1          500.1343971 MHz
NUC1                   1H
P1                  10.60 usec
PLW1          17.39999962 W

F2 - Processing parameters
SI                 131072
SF            500.1300270 MHz
WDW                    EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  10.00
```

ChemNMR ^{1}H Estimation



Estimation quality is indicated by color: good, medium, rough

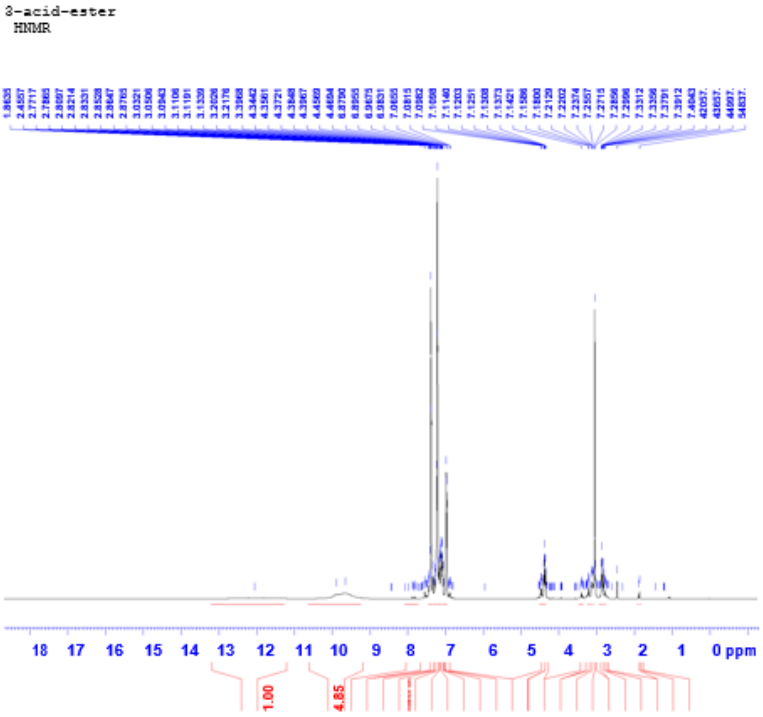


Protocol of the H-1 NMR Prediction:

Node	Shift	Base + Inc.	Comment (ppm rel. to TMS)
CH 7.39		7.26	1-benzene
		-0.08	1 -S-C
		0.21	general corrections
CH 7.72		7.26	1-benzene
		0.71	1 -C(=O)OC
		-0.12	1 -C
		-0.13	general corrections
CH 7.17		7.26	1-benzene
		0.11	1 -C(=O)OC
		-0.20	1 -C
		0.00	general corrections
CH 7.39		7.26	1-benzene
		-0.08	1 -S-C
		0.21	general corrections
CH 7.35		7.26	1-benzene
		-0.10	1 -S-C
		0.19	general corrections
CH 7.35		7.26	1-benzene
		-0.10	1 -S-C
		0.19	general corrections
CH 7.37		7.26	1-benzene
		0.11	1 -C(=O)OC
		-0.19	1 -C
		0.19	general corrections
CH 7.54		7.26	1-benzene
		0.21	1 -C(=O)OC
		-0.12	1 -C
		0.19	general corrections
CH 7.21		7.26	1-benzene
		-0.24	1 -S-C
		0.19	general corrections
CH2 3.54		1.37	methylene
		1.44	1 alpha -S-1:C*C*C*C*C*C*1
		0.42	1 beta -OC(=O)-1:C*C*C*C*C*C*1
		0.31	general corrections
CH2 4.17		1.37	methylene
		2.92	1 alpha -OC(=O)-1:C*C*C*C*C*C*1
		0.30	1 beta -S-1:C*C*C*C*C*C*1
		-0.42	general corrections
CH3 2.48		0.86	methyl
		1.49	1 alpha -1:C*C*C*C*C*C*1
		0.13	general corrections

1H NMR Coupling Constant Prediction

shift	atom index		coupling	partner, constant and vector
7.39	2			
		3	7.5	H-C*C-H
		6	1.5	H-C*C*C-H
		4	1.5	H-C*CH*C-H
7.72	14			
		15	7.5	H-C*C-H
		16	1.5	H-C*CH*C-H
7.17	17			
		16	7.5	H-C*C-H
		15	1.5	H-C*CH*C-H
7.39	6			
		5	7.5	H-C*C-H
		2	1.5	H-C*C*C-H
		4	1.5	H-C*CH*C-H
7.35	5			
		6	7.5	H-C*C-H
		4	7.5	H-C*C-H
		3	1.5	H-C*CH*C-H
7.35	3			
		2	7.5	H-C*C-H
		4	7.5	H-C*C-H
		5	1.5	H-C*CH*C-H
7.37	15			
		14	7.5	H-C*C-H
		16	7.5	H-C*C-H
		17	1.5	H-C*CH*C-H
7.54	16			
		17	7.5	H-C*C-H
		15	7.5	H-C*C-H
		14	1.5	H-C*CH*C-H
7.21	4			
		5	7.5	H-C*C-H
		3	7.5	H-C*C-H
		6	1.5	H-C*CH*C-H
		2	1.5	H-C*CH*C-H
3.54	8			
		9	7.1	H-CH-CH-H
4.17	9			
		8	7.1	H-CH-CH-H
2.48	19			



The University of Jordan
Faculty of Science
Department of Chemistry

Instrument Model:
Bruker 500 MHz–Avance III

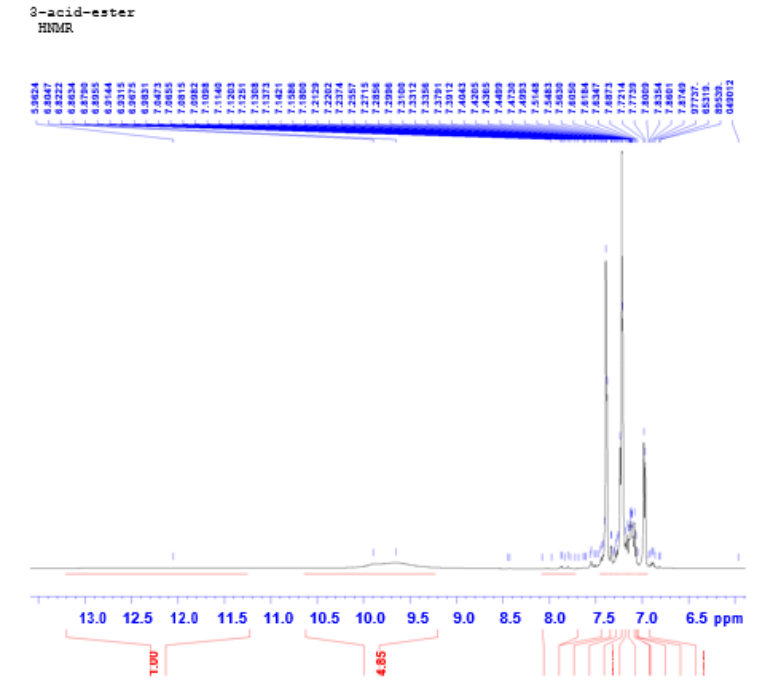
Operator: Rola Hassouneh
nmr500@ju.edu.jo

```
Current Data Parameters
NAME          15oct15jalal
EXPNO                 1161
PROCNO                   1

F2 - Acquisition Parameters
Date               20151029
Time                  12.26
INSTRUM               spect
PROBHD     5 mm PABBO BB/
PULPROG                 zg
TD                   65536
SOLVENT              DMSO
NS                       7
DS                       0
SWH              10000.000 Hz
FIDRES               0.152588 Hz
AQ                3.2767999 sec
RG                     6.52
DW                  50.000 usec
DE                   6.50 usec
TE                  305.2 K
D1               2.00000000 sec
TD0                      1

======== CHANNEL f1 ========
SFO1            500.1343971 MHz
NUC1                    1H
P1                   10.60 usec
PLW1            17.39999962 W

F2 - Processing parameters
SI                  131072
SF              500.1300270 MHz
WDW                     EM
SSB          0
LB                    1.00 Hz
GB           0
PC                    5.00
```



The University of Jordan
Faculty of Science
Department of Chemistry

Instrument Model:
Bruker 500 MHz–Avance III

Operator: Rola Hassouneh
nmr500@ju.edu.jo

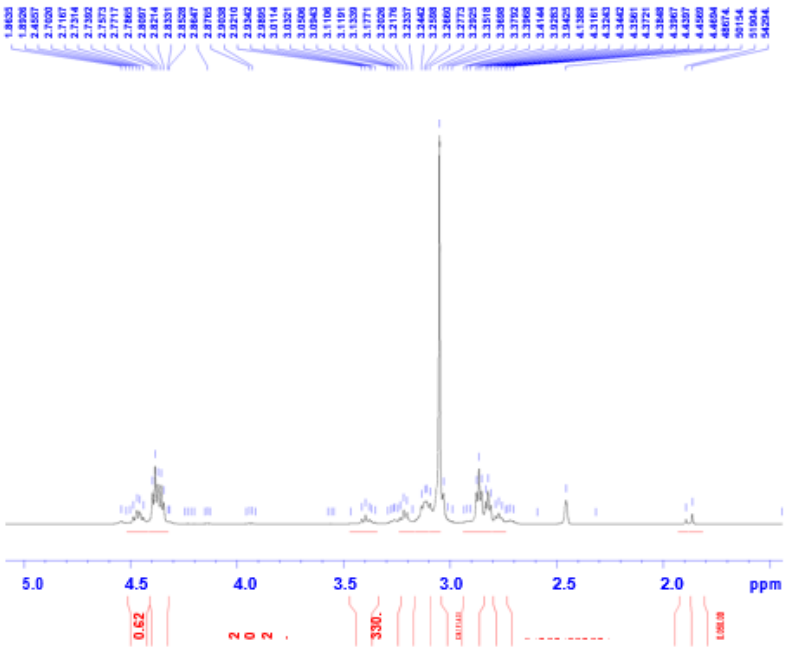
```
Current Data Parameters
NAME          15oct15jalal
EXPNO                 1161
PROCNO                   1

F2 - Acquisition Parameters
Date               20151029
Time                  12.26
INSTRUM               spect
PROBHD     5 mm PABBO BB/
PULPROG                 zg
TD                   65536
SOLVENT              DMSO
NS                       7
DS                       0
SWH              10000.000 Hz
FIDRES               0.152588 Hz
AQ                3.2767999 sec
RG                     6.52
DW                  50.000 usec
DE                   6.50 usec
TE                  305.2 K
D1               2.00000000 sec
TD0                      1

======== CHANNEL f1 ========
SFO1            500.1343971 MHz
NUC1                    1H
P1                   10.60 usec
PLW1            17.39999962 W

F2 - Processing parameters
SI                  131072
SF              500.1300270 MHz
WDW                     EM
SSB          0
LB                    1.00 Hz
GB           0
PC                    5.00
```

β-acid-ester
HNMR



The University of Jordan
Faculty of Science
Department of Chemistry

Instrument Model:
Bruker 500 MHz-Avance III

Operator: Rola Hamoudeh
nmr500@ju.edu.jo

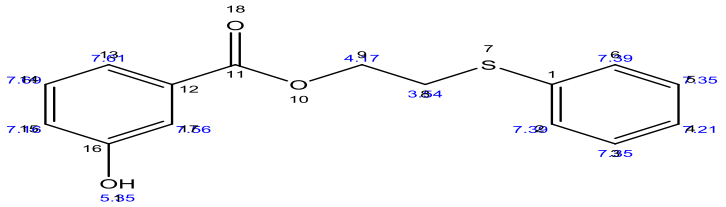
```
Current Data Parameters
NAME          18oct15jalal
EXPNO               1161
PROCNO                 1

F2 - Acquisition Parameters
Date              20151029
Time                 12.26
INSTRUM              spect
PROBHD     5 mm PABBO BB/
PULPROG                zg
TD                   65536
SOLVENT              DMSO
NS                       7
DS                       0
SWH              10000.000 Hz
FIDRES               0.152588 Hz
AQ                3.2767999 sec
RG                      6.82
DW                 50.000 usec
DE                  6.50 usec
TE                  305.2 K
D1               2.00000000 sec
TD0                      1

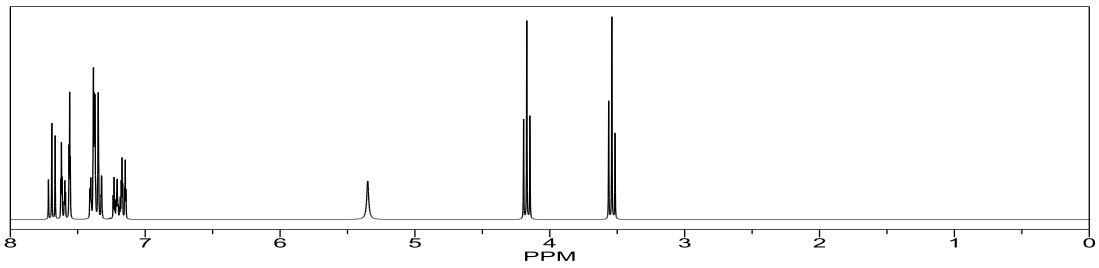
======== CHANNEL f1 ========
SFO1            500.1343971 MHz
NUC1                    1H
P1                   10.60 usec
PLW1            17.39999962 W

F2 - Processing parameters
SI                     131072
SF              500.1300270 MHz
WDW                       EM
SSB          0
LB                    1.00 Hz
GB           0
PC                    5.00
```

ChemNMR ^{1}H Estimation



Estimation quality is indicated by color: good, medium, rough



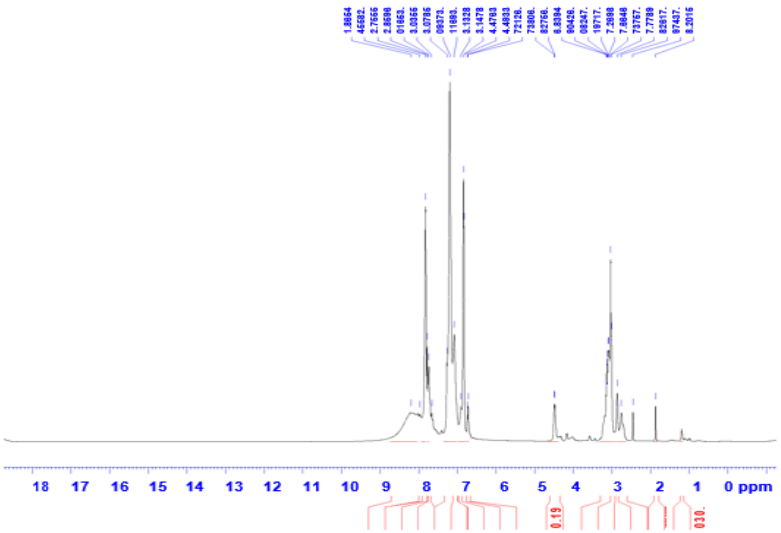
Protocol of the H-1 NMR Prediction:

Node	Shift	Base + Inc.	Comment (ppm rel. to TMS)
OH	5.35	5.00	aromatic C-OH
		0.35	general corrections
CH	7.39	7.26	1-benzene
		-0.08	1 -S-C
		0.21	general corrections
CH	7.56	7.26	1-benzene
		-0.53	1 -O
		0.71	1 -C(=O)OC
		0.12	general corrections
CH	7.61	7.26	1-benzene
		-0.44	1 -O
		0.71	1 -C(=O)OC
		0.08	general corrections
CH	7.39	7.26	1-benzene
		-0.08	1 -S-C
		0.21	general corrections
CH	7.16	7.26	1-benzene
		-0.53	1 -O
		0.21	1 -C(=O)OC
		0.22	general corrections
CH	7.35	7.26	1-benzene
		-0.10	1 -S-C
		0.19	general corrections
CH	7.69	7.26	1-benzene
		-0.17	1 -O
		0.11	1 -C(=O)OC
		0.49	general corrections
CH	7.35	7.26	1-benzene
		-0.10	1 -S-C
		0.19	general corrections
CH	7.21	7.26	1-benzene
		-0.24	1 -S-C
		0.19	general corrections
CH2	3.54	1.37	methylene
		1.44	1 alpha -S-1:C*C*C*C*C*C*1
		0.42	1 beta -OC(=O)-1:C*C*C*C*C*C*1
		0.31	general corrections
CH2	4.17	1.37	methylene
		2.92	1 alpha -OC(=O)-1:C*C*C*C*C*C*1
		0.30	1 beta -S-1:C*C*C*C*C*C*1
		-0.42	general corrections

1H NMR Coupling Constant Prediction

shift	atom index	coupling partner, constant and vector		
5.35	19			
7.39	2			
		3	7.5	H-C*C-H
		6	1.5	H-C*C*C-H
		4	1.5	H-C*CH*C-H
7.56	17			
		15	1.5	H-C*C*C-H
		13	1.5	H-C*C*C-H
7.61	13			
		14	7.5	H-C*C-H
		17	1.5	H-C*C*C-H
		15	1.5	H-C*CH*C-H
7.39	6			
		5	7.5	H-C*C-H
		2	1.5	H-C*C*C-H
		4	1.5	H-C*CH*C-H
7.16	15			
		14	7.5	H-C*C-H
		17	1.5	H-C*C*C-H
		13	1.5	H-C*CH*C-H
7.35	5			
		6	7.5	H-C*C-H
		4	7.5	H-C*C-H
		3	1.5	H-C*CH*C-H
7.69	14			
		13	7.5	H-C*C-H
		15	7.5	H-C*C-H
7.35	3			
		2	7.5	H-C*C-H
		4	7.5	H-C*C-H
		5	1.5	H-C*CH*C-H
7.21	4			
		5	7.5	H-C*C-H
		3	7.5	H-C*C-H
		6	1.5	H-C*CH*C-H
		2	1.5	H-C*CH*C-H
3.54	8			
		9	7.1	H-CH-CH-H
4.17	9			
		8	7.1	H-CH-CH-H

4-acid-ester
HNMR



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         18oct15jalal
EXPNO             1151
PROCNO               1

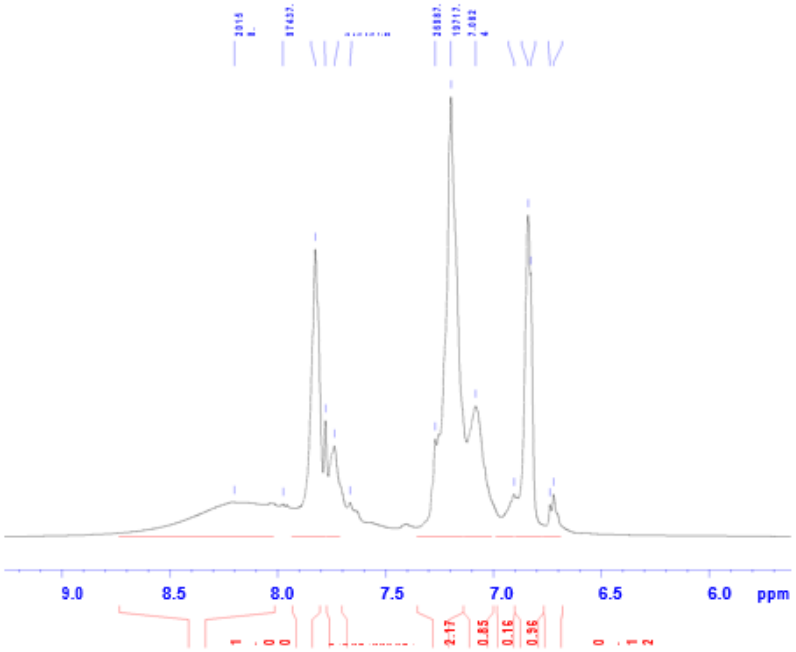
F2 - Acquisition Parameters
Date              20181029
Time                 12.19
INSTRUM              spect
PROBHD     5 mm PABBO BB/
PULPROG              zg
TD                  65536
SOLVENT              DMSO
NS                      8
DS                      0
SWH             10000.000 Hz
FIDRES              0.152588 Hz
AQ                3.2767999 sec
RG                     6.52
DW                50.000 usec
DE                 6.50 usec
TE                 308.2 K
D1              2.00000000 sec
TD0                     1

======== CHANNEL f1 ========
SFO1            500.1343971 MHz
NUC1                    1H
P1                   10.60 usec
PLW1           17.39999962 W

F2 - Processing parameters
SI                   131072
SF              500.1300270 MHz
WDW                     EM
SSB          0
LB                    1.00 Hz
GB           0
PC                    1.00
```

,

4-acid-ester
HNMR



The University of Jordan
Faculty of Science
Department of Chemistry

Instrument Model:
Bruker 500 MHz–Avance III

Operator: Rola Hassouneh
nmr500@ju.edu.jo

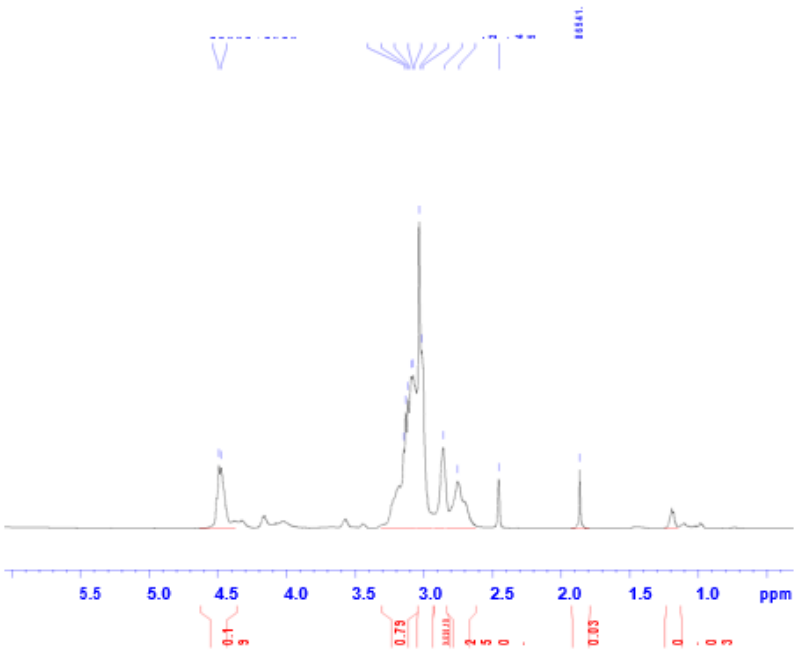
```
Current Data Parameters
NAME         18oct15jalal
EXPNO             1151
PROCNO               1

F2 - Acquisition Parameters
Date              20181029
Time                 12.19
INSTRUM              spect
PROBHD     5 mm PABBO BB/
PULPROG              zg
TD                  65536
SOLVENT              DMSO
NS                      8
DS                      0
SWH             10000.000 Hz
FIDRES              0.152588 Hz
AQ                3.2767999 sec
RG                     6.52
DW                50.000 usec
DE                 6.50 usec
TE                 308.2 K
D1              2.00000000 sec
TD0                     1

======== CHANNEL f1 ========
SFO1            500.1343971 MHz
NUC1                    1H
P1                   10.60 usec
PLW1           17.39999962 W

F2 - Processing parameters
SI                   131072
SF              500.1300270 MHz
WDW                     EM
SSB          0
LB                    1.00 Hz
GB           0
PC                    1.00
```

4-acid-ester
HNMR



The University of Jordan
Faculty of Science
Department of Chemistry

Instrument Model:
Bruker 500 MHz-Avance III

Operator: Rola Hassouneh
nmr500@ju.edu.jo

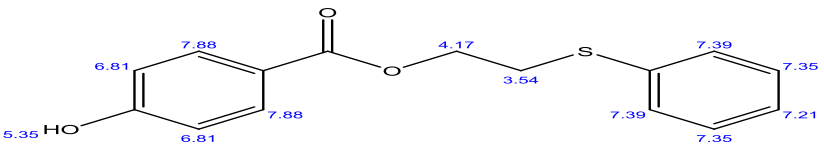
```
Current Data Parameters
NAME          15octl3jalal
EXPNO               1151
PROCNO                 1

F2 - Acquisition Parameters
Date              20151029
Time                 12.19
INSTRUM              spect
PROBHD     5 mm PABBO BB/
PULPROG                zg
TD                  65536
SOLVENT             DMSO
NS                      8
DS                      0
SWH             10000.000 Hz
FIDRES              0.152588 Hz
AQ                3.2767999 sec
RG                     6.52
DW                50.000 usec
DE                  6.50 usec
TE                 308.2 K
D1              2.00000000 sec
TD0                     1

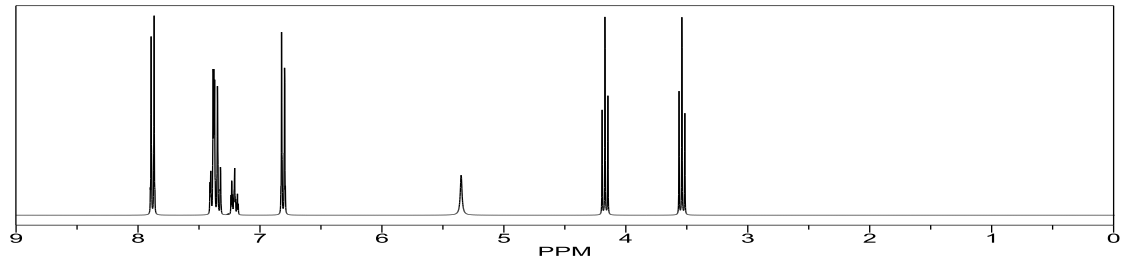
======== CHANNEL f1 ========
SFO1            500.1343971 MHz
NUC1                    1H
P1                   10.60 usec
PLW1            17.39999962 W

F2 - Processing parameters
SI                  131072
SF              500.1300270 MHz
WDW                     EM
SSB                     0
LB                   1.00 Hz
GB                      0
PC                   1.00
```

ChemNMR ^{1}H Estimation



Estimation quality is indicated by color: good, medium, rough



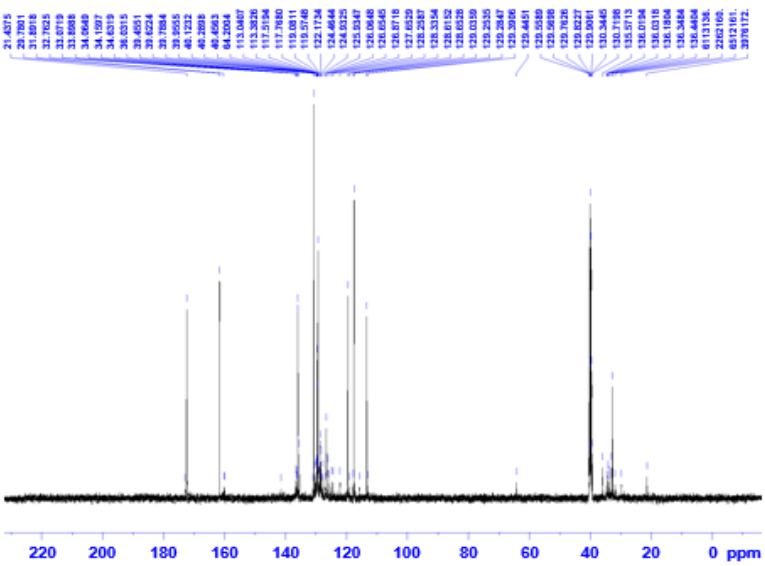
Protocol of the H-1 NMR Prediction:

Node	Shift	Base + Inc.	Comment (ppm rel. to TMS)
OH	5.35	5.00	aromatic C-OH
		0.35	general corrections
CH	7.39	7.26	1-benzene
		-0.08	1 -S-C
		0.21	general corrections
CH	6.81	7.26	1-benzene
		-0.53	1 -O
		0.11	1 -C(=O)OC
		-0.03	general corrections
CH	7.88	7.26	1-benzene
		-0.17	1 -O
		0.71	1 -C(=O)OC
		0.08	general corrections
CH	7.39	7.26	1-benzene
		-0.08	1 -S-C
		0.21	general corrections
CH	6.81	7.26	1-benzene
		-0.53	1 -O
		0.11	1 -C(=O)OC
		-0.03	general corrections
CH	7.88	7.26	1-benzene
		-0.17	1 -O
		0.71	1 -C(=O)OC
		0.08	general corrections
CH	7.35	7.26	1-benzene
		-0.10	1 -S-C
		0.19	general corrections
CH	7.35	7.26	1-benzene
		-0.10	1 -S-C
		0.19	general corrections
CH	7.21	7.26	1-benzene
		-0.24	1 -S-C
		0.19	general corrections
CH2	3.54	1.37	methylene
		1.44	1 alpha -S-1:C*C*C*C*C*C*1
		0.42	1 beta -OC(=O)-1:C*C*C*C*C*C*1
		0.31	general corrections
CH2	4.17	1.37	methylene
		2.92	1 alpha -OC(=O)-1:C*C*C*C*C*C*1
		0.30	1 beta -S-1:C*C*C*C*C*C*1
		-0.42	general corrections

1H NMR Coupling Constant Prediction

shift	atom index	coupling partner,	constant	and vector
5.35	19			
7.39	2			
		3	7.5	H-C*C-H
		6	1.5	H-C*C*C-H
		4	1.5	H-C*CH*C-H
6.81	14			
		13	7.5	H-C*C-H
		16	1.5	H-C*C*C-H
7.88	13			
		14	7.5	H-C*C-H
		17	1.5	H-C*C*C-H
7.39	6			
		5	7.5	H-C*C-H
		2	1.5	H-C*C*C-H
		4	1.5	H-C*CH*C-H
6.81	16			
		17	7.5	H-C*C-H
		14	1.5	H-C*C*C-H
7.88	17			
		16	7.5	H-C*C-H
		13	1.5	H-C*C*C-H
7.35	5			
		6	7.5	H-C*C-H
		4	7.5	H-C*C-H
		3	1.5	H-C*CH*C-H
7.35	3			
		2	7.5	H-C*C-H
		4	7.5	H-C*C-H
		5	1.5	H-C*CH*C-H
7.21	4			
		5	7.5	H-C*C-H
		3	7.5	H-C*C-H
		6	1.5	H-C*CH*C-H
		2	1.5	H-C*CH*C-H
3.54	8			
		9	7.1	H-CH-CH-H
4.17	9			
		8	7.1	H-CH-CH-H

2-acid-ester
C13



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          1HOCT1CJALAL
EXPNO                 1142
PROCNO                   1

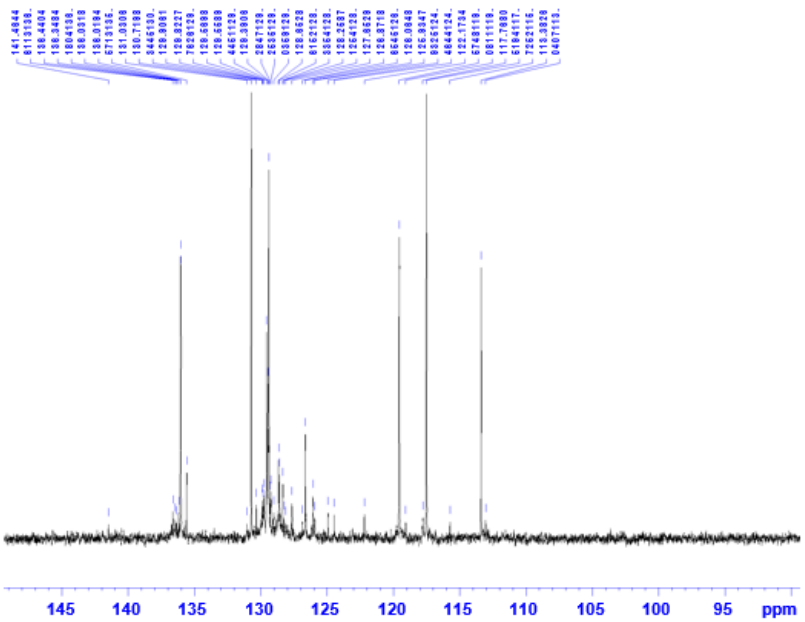
F2 - Acquisition Parameters
Date_              20181029
Time                 13.07
INSTRUM              spect
PROBHD    5 mm PABBO BB/
PULPROG          zgpg30
TD                 65536
SOLVENT             DMSO
NS                    88
DS                     4
SWH              31250.000 Hz
FIDRES               0.476837 Hz
AQ                1.0485760 sec
RG                  202.06
DW                  16.000 usec
DE                   6.50 usec
TE                  308.2 K
D1               2.00000000 sec
D11              0.03000000 sec
TD0                      1

======== CHANNEL f1 ========
SFO1            125.7713691 MHz
NUC1                    13C
P1                    9.80 usec
PL1              93.32499695 W

======== CHANNEL f2 ========
SFO2            500.1320005 MHz
NUC2                    1H
CPDPRG[2          waltz16
PCPD2                80.00 usec
PL2              17.37800026 W
PL12              0.36307999 W
PL13              0.22982000 W

F2 - Processing parameters
SI                   32768
SF            125.7577590 MHz
WDW                     EM
SSB                  0
LB                    1.00 Hz
GB                   0
PC                    1.40
```

2-acid-ester
C13



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          1Hoct1CJalal
EXPNO                 1142
PROCNO                   1

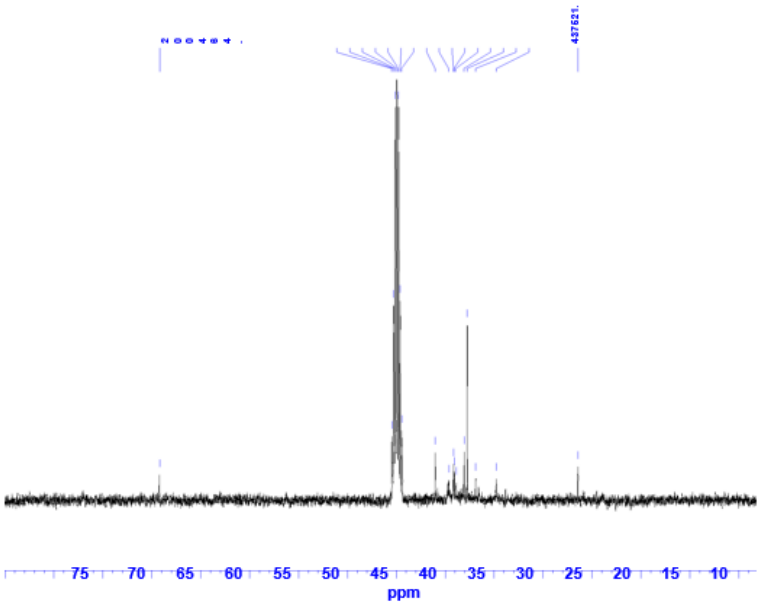
F2 - Acquisition Parameters
Date_              20181029
Time                 13.07
INSTRUM              spect
PROBHD    5 mm PABBO BB/
PULPROG          zgpg30
TD                 65536
SOLVENT             DMSO
NS                    88
DS                     4
SWH              31250.000 Hz
FIDRES               0.476837 Hz
AQ                1.0485760 sec
RG                  202.06
DW                  16.000 usec
DE                   6.50 usec
TE                  308.2 K
D1               2.00000000 sec
D11              0.03000000 sec
TD0                      1

======== CHANNEL f1 ========
SFO1            125.7713691 MHz
NUC1                    13C
P1                    9.80 usec
PL1              93.32499695 W

======== CHANNEL f2 ========
SFO2            500.1320005 MHz
NUC2                    1H
CPDPRG[2          waltz16
PCPD2                80.00 usec
PL2              17.37800026 W
PL12              0.36307999 W
PL13              0.22982000 W

F2 - Processing parameters
SI                   32768
SF            125.7577590 MHz
WDW                     EM
SSB                  0
LB                    1.00 Hz
GB                   0
PC                    1.40
```

2-acid-ester
C13



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          16oct15jalal
EXPNO             1142
PROCNO               1

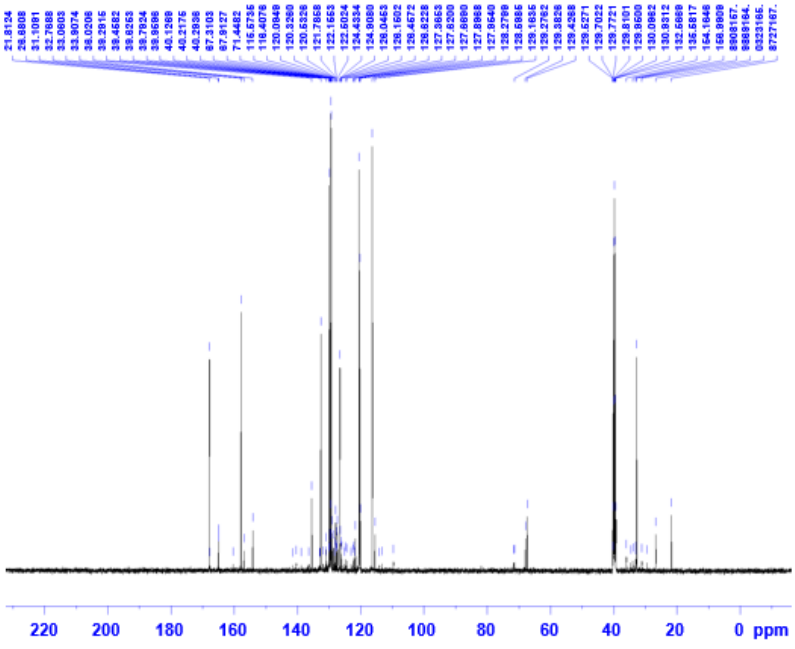
F2 - Acquisition Parameters
Date_          20151029
Time              13.07
INSTRUM           spect
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD               65536
SOLVENT            DMSO
NS                   88
DS                    4
SWH           31250.000 Hz
FIDRES        0.476837 Hz
AQ            1.0485760 sec
RG                202.06
DW               16.000 usec
DE                6.50 usec
TE                305.2 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                  1

========== CHANNEL f1 ==========
SFO1         125.7713681 MHz
NUC1               13C
P1                9.80 usec
PLW1         93.32499695 W

========== CHANNEL f2 ==========
SFO2         500.1320003 MHz
NUC2               1H
CPDPRG[2          waltz16
PCPD2            80.00 usec
PLW2         17.37800026 W
PLW12         0.36307999 W
PLW13         0.22982000 W

F2 - Processing parameters
SI                32768
SF           125.7577890 MHz
WDW                  EM
SSB                  0
LB                1.00 Hz
GB                   0
PC                1.40
```

3-acid-ester
C13



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          16oct15jalal
EXPNO             1162
PROCNO               1

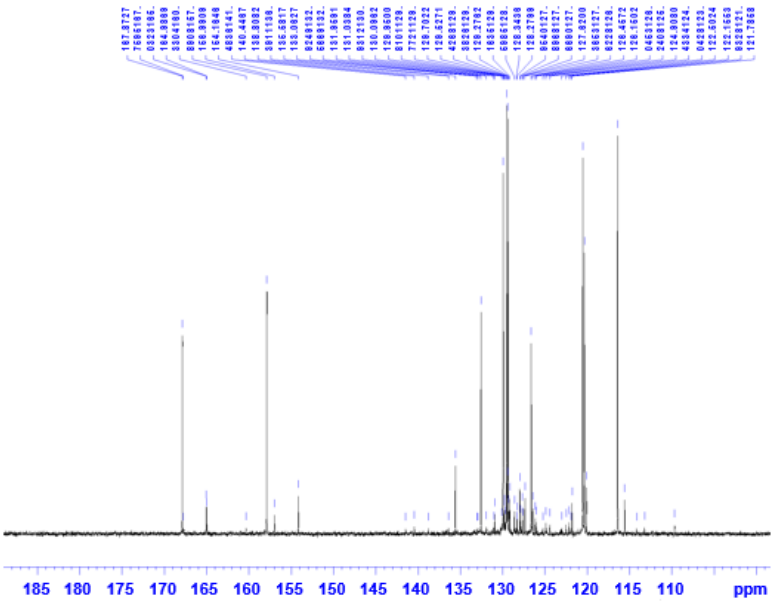
F2 - Acquisition Parameters
Date_          20151029
Time              12.25
INSTRUM           spect
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD               65536
SOLVENT            DMSO
NS                   57
DS                    4
SWH           31250.000 Hz
FIDRES        0.476837 Hz
AQ            1.0485760 sec
RG                202.06
DW               16.000 usec
DE                6.50 usec
TE                305.2 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                  1

========== CHANNEL f1 ==========
SFO1         125.7713681 MHz
NUC1               13C
P1                9.80 usec
PLW1         93.32499695 W

========== CHANNEL f2 ==========
SFO2         500.1320003 MHz
NUC2               1H
CPDPRG[2          waltz16
PCPD2            80.00 usec
PLW2         17.37800026 W
PLW12         0.36307999 W
PLW13         0.22982000 W

F2 - Processing parameters
SI                32768
SF           125.7577890 MHz
WDW                  EM
SSB                  0
LB                1.00 Hz
GB                   0
PC                1.40
```

3-acid-ester
C13



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          15oct15jalal
EXPNO             1162
PROCNO               1

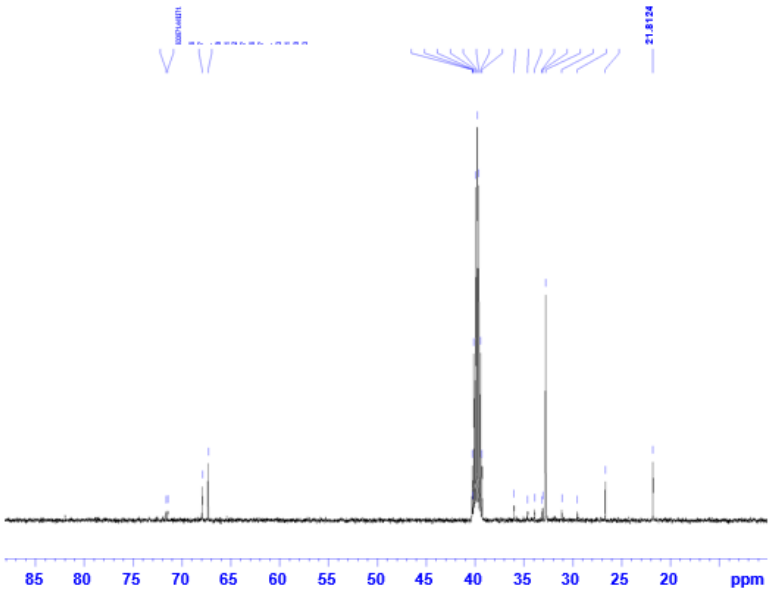
F2 - Acquisition Parameters
Date_          20151029
Time              12.28
INSTRUM           spect
PROBHD     5 mm PABBO BB/
PULPROG          zgpg30
TD               65536
SOLVENT            DMSO
NS                   57
DS                    4
SWH           31250.000 Hz
FIDRES        0.476837 Hz
AQ            1.0485760 sec
RG                203.06
DW               16.000 usec
DE                6.50 usec
TE              308.3 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                  1

======== CHANNEL f1 ========
SFO1        125.7713681 MHz
NUC1              13C
P1                9.80 usec
PLW1       93.32499695 W

======== CHANNEL f2 ========
SFO2        500.1320005 MHz
NUC2              1H
CPDPRG[2          waltz16
PCPD2            80.00 usec
PLW2       17.37500095 W
PLW12       0.38507999 W
PLW13       0.22952000 W

F2 - Processing parameters
SI               32768
SF          125.7577890 MHz
WDW                 EM
SSB          0
LB                1.00 Hz
GB           0
PC                1.40
```

3-acid-ester
C13



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          15oct15jalal
EXPNO             1162
PROCNO               1

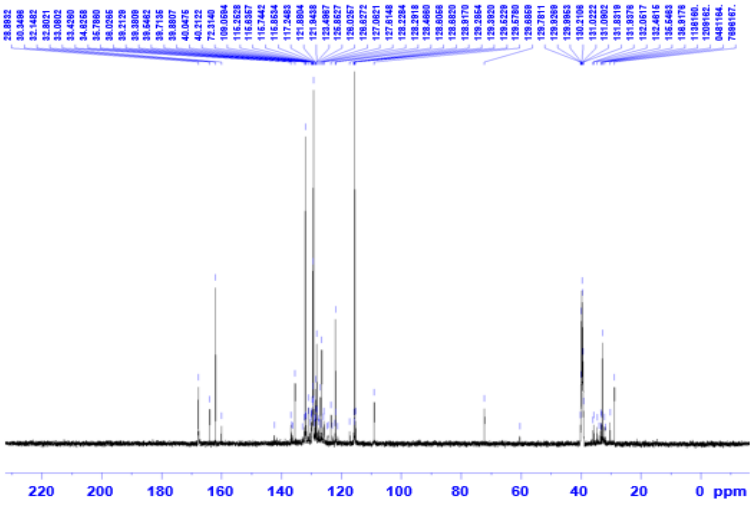
F2 - Acquisition Parameters
Date_          20151029
Time              12.28
INSTRUM           spect
PROBHD     5 mm PABBO BB/
PULPROG          zgpg30
TD               65536
SOLVENT            DMSO
NS                   57
DS                    4
SWH           31250.000 Hz
FIDRES        0.476837 Hz
AQ            1.0485760 sec
RG                203.06
DW               16.000 usec
DE                6.50 usec
TE              308.3 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                  1

======== CHANNEL f1 ========
SFO1        125.7713681 MHz
NUC1              13C
P1                9.80 usec
PLW1       93.32499695 W

======== CHANNEL f2 ========
SFO2        500.1320005 MHz
NUC2              1H
CPDPRG[2          waltz16
PCPD2            80.00 usec
PLW2       17.37500095 W
PLW12       0.38507999 W
PLW13       0.22952000 W

F2 - Processing parameters
SI               32768
SF          125.7577890 MHz
WDW                 EM
SSB          0
LB                1.00 Hz
GB           0
PC                1.40
```

4-acid-ester
C13



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          18oct19ala1
EXPNO             1102
PROCNO               1

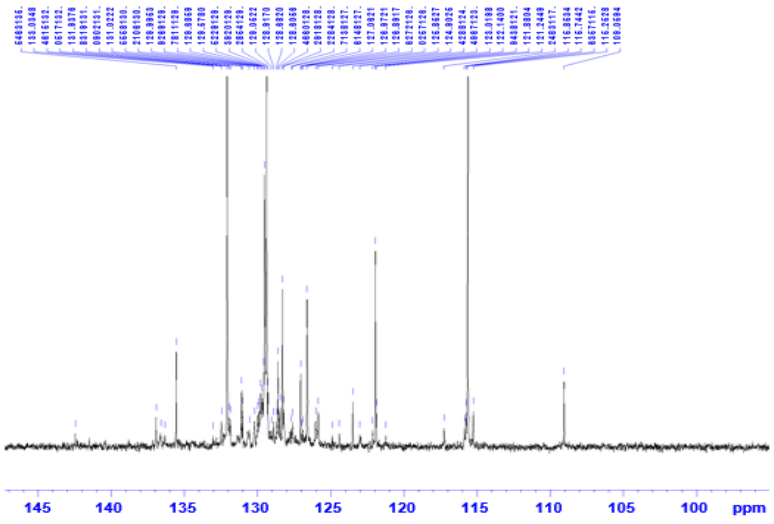
F2 - Acquisition Parameters
Date_          20191029
Time              12.20
INSTRUM           spect
PROBHD     5 mm PABBO BB/
PULPROG          zgpg30
TD               65536
SOLVENT          DMSO
NS                  64
DS                   4
SWH          31250.000 Hz
FIDRES        0.476837 Hz
AQ            1.0485760 sec
RG             202.06
DW              16.000 usec
DE               6.50 usec
TE              308.4 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                  1

========== CHANNEL f1 ==========
SFO1         125.7713681 MHz
NUC1               13C
P1                9.80 usec
PLW1         93.32499695 W

========== CHANNEL f2 ==========
SFO2         500.1320005 MHz
NUC2               1H
CPDPRG[2        waltz16
PCPD2             80.00 usec
PLW2         17.37800026 W
PLW12         0.36307999 W
PLW13         0.22982000 W

F2 - Processing parameters
SI               32768
SF          125.7577890 MHz
WDW                EM
SSB                0
LB                1.00 Hz
GB                 0
PC                1.40
```

4-acid-ester
C13



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          18oct19ala1
EXPNO             1102
PROCNO               1

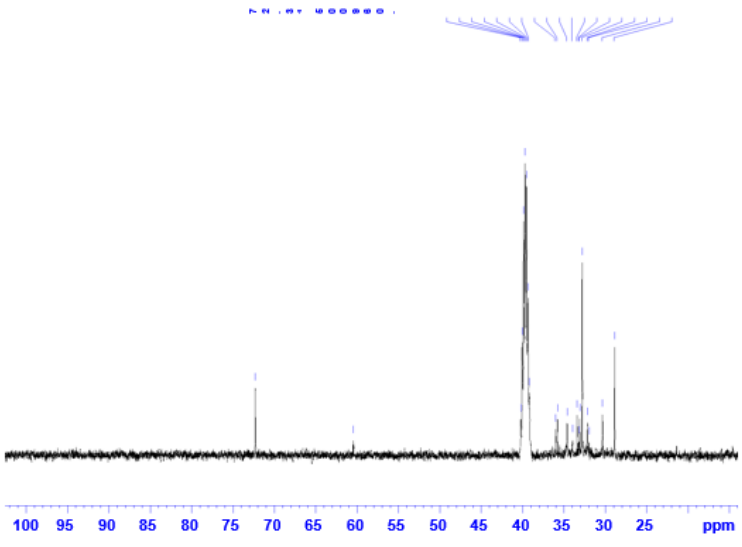
F2 - Acquisition Parameters
Date_          20191029
Time              12.20
INSTRUM           spect
PROBHD     5 mm PABBO BB/
PULPROG          zgpg30
TD               65536
SOLVENT          DMSO
NS                  64
DS                   4
SWH          31250.000 Hz
FIDRES        0.476837 Hz
AQ            1.0485760 sec
RG             202.06
DW              16.000 usec
DE               6.50 usec
TE              308.4 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                  1

========== CHANNEL f1 ==========
SFO1         125.7713681 MHz
NUC1               13C
P1                9.80 usec
PLW1         93.32499695 W

========== CHANNEL f2 ==========
SFO2         500.1320005 MHz
NUC2               1H
CPDPRG[2        waltz16
PCPD2             80.00 usec
PLW2         17.37800026 W
PLW12         0.36307999 W
PLW13         0.22982000 W

F2 - Processing parameters
SI               32768
SF          125.7577890 MHz
WDW                EM
SSB                0
LB                1.00 Hz
GB                 0
PC                1.40
```

4-acid-ester
C13



The University of Jordan
Faculty of Science
Department of Chemistry

Instrument Model:
Bruker 500 MHz–Avance III

Operator: Rola Hassouneh
nmr500@ju.edu.jo

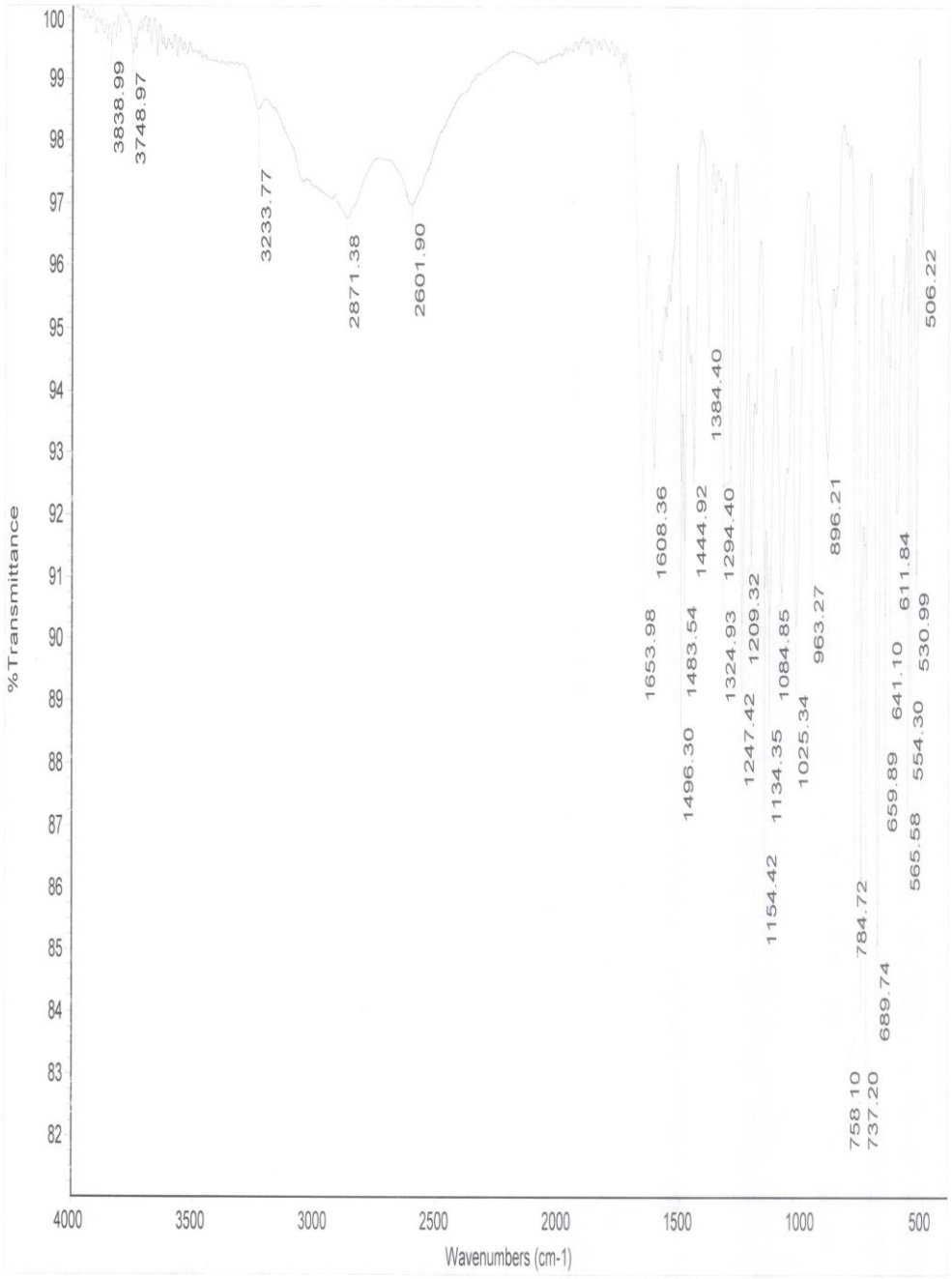
```
Current Data Parameters
NAME        15oct15jalal
EXPNO             1152
PROCNO               1

F2 - Acquisition Parameters
Date_          20151029
Time              12.20
INSTRUM           spect
PROBHD   5 mm PABBO BB/
PULPROG         zgpg30
TD              65536
SOLVENT           DMSO
NS                  66
DS                   4
SWH          31250.000 Hz
FIDRES        0.476837 Hz
AQ            1.0485760 sec
RG                202.06
DW               16.000 usec
DE                6.50 usec
TE              305.4 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                  1

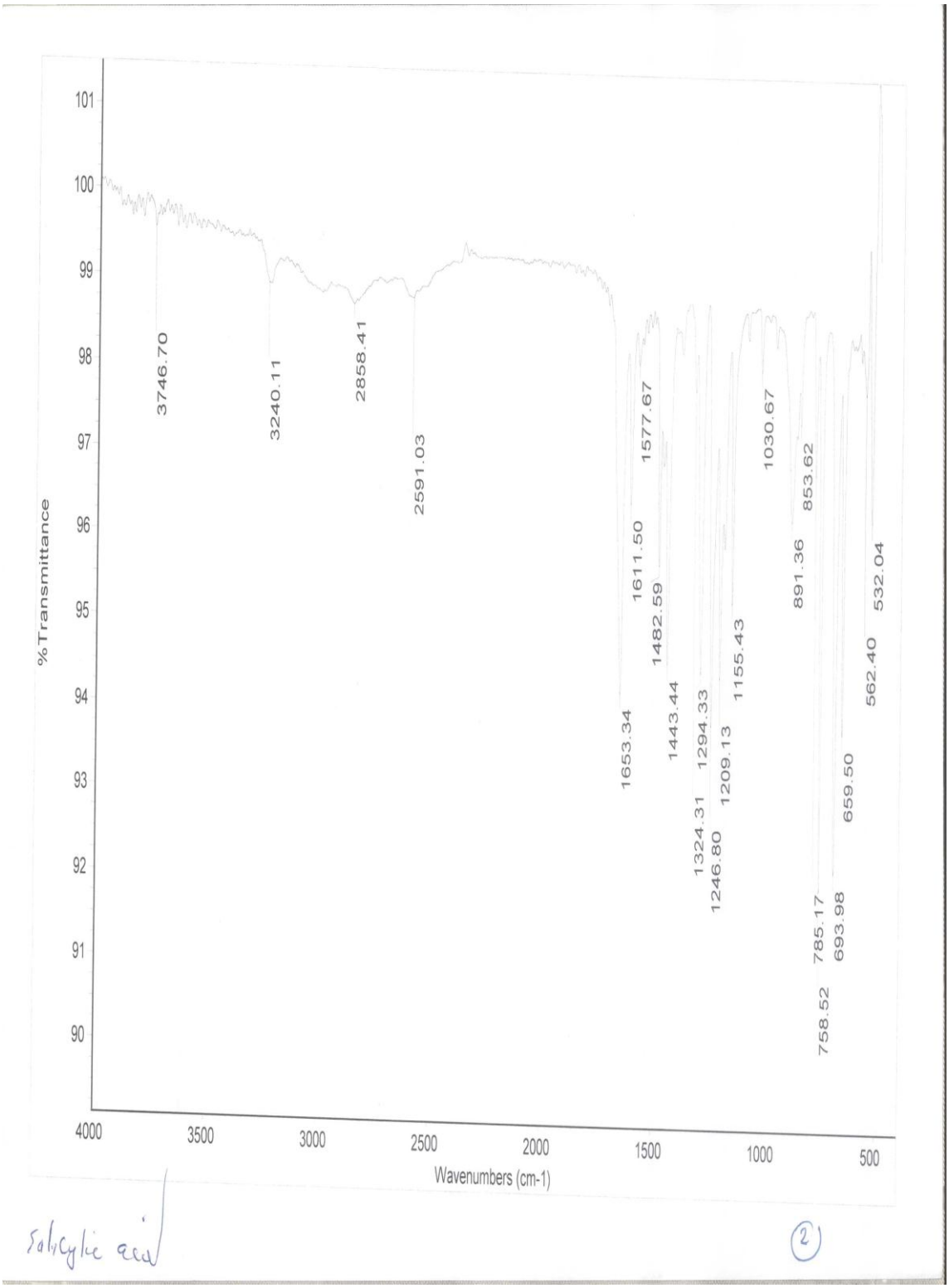
======== CHANNEL f1 ========
SFO1       125.7713681 MHz
NUC1               13C
P1                9.50 usec
PLW1       93.32499695 W

======== CHANNEL f2 ========
SFO2       500.1320005 MHz
NUC2               1H
CPDPRG[2        waltz16
PCPD2             80.00 usec
PLW2       17.37500024 W
PLW12       0.36307998 W
PLW13       0.22982000 W

F2 - Processing parameters
SI                32768
SF         125.7577592 MHz
WDW                EM
```

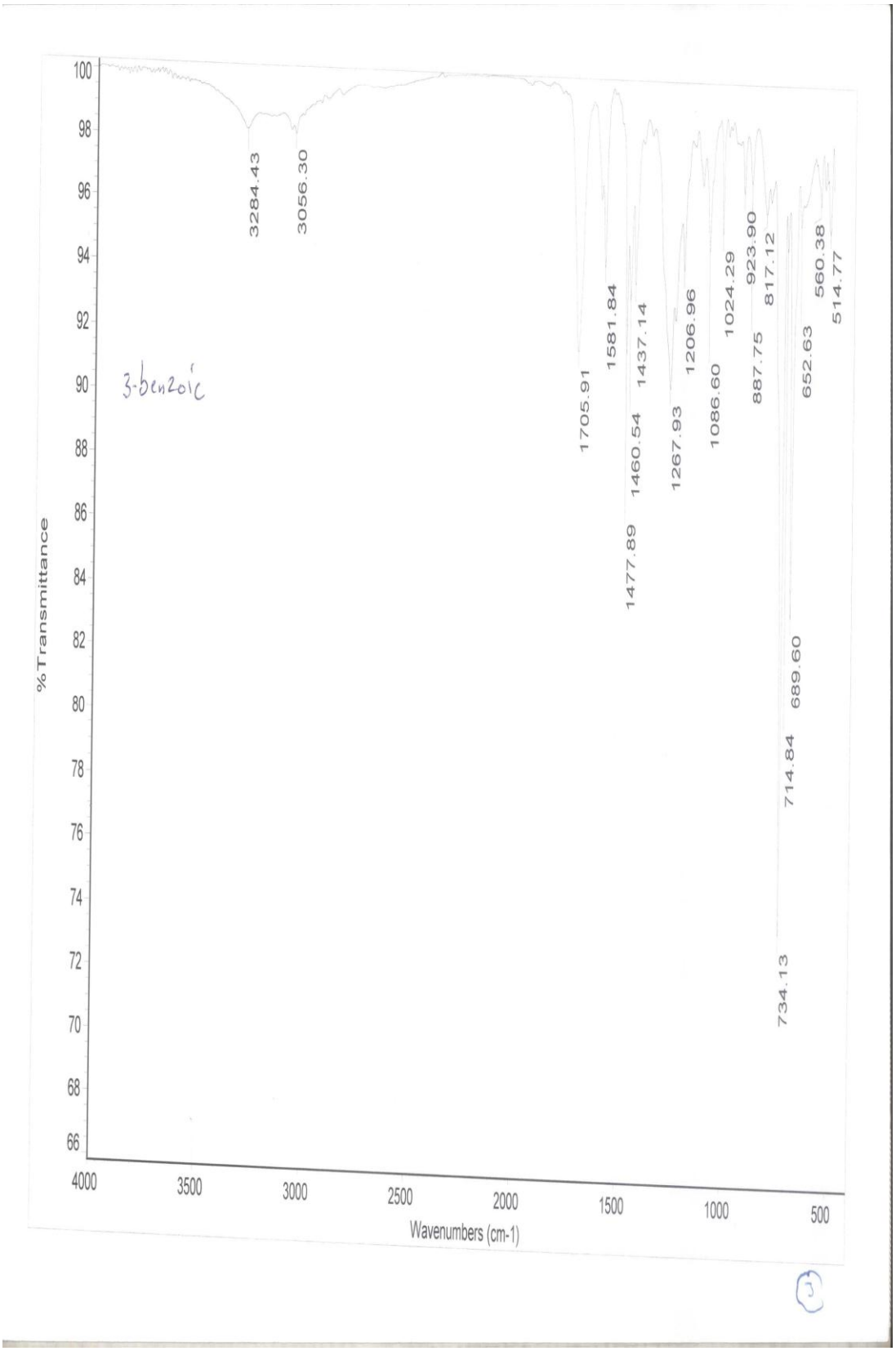


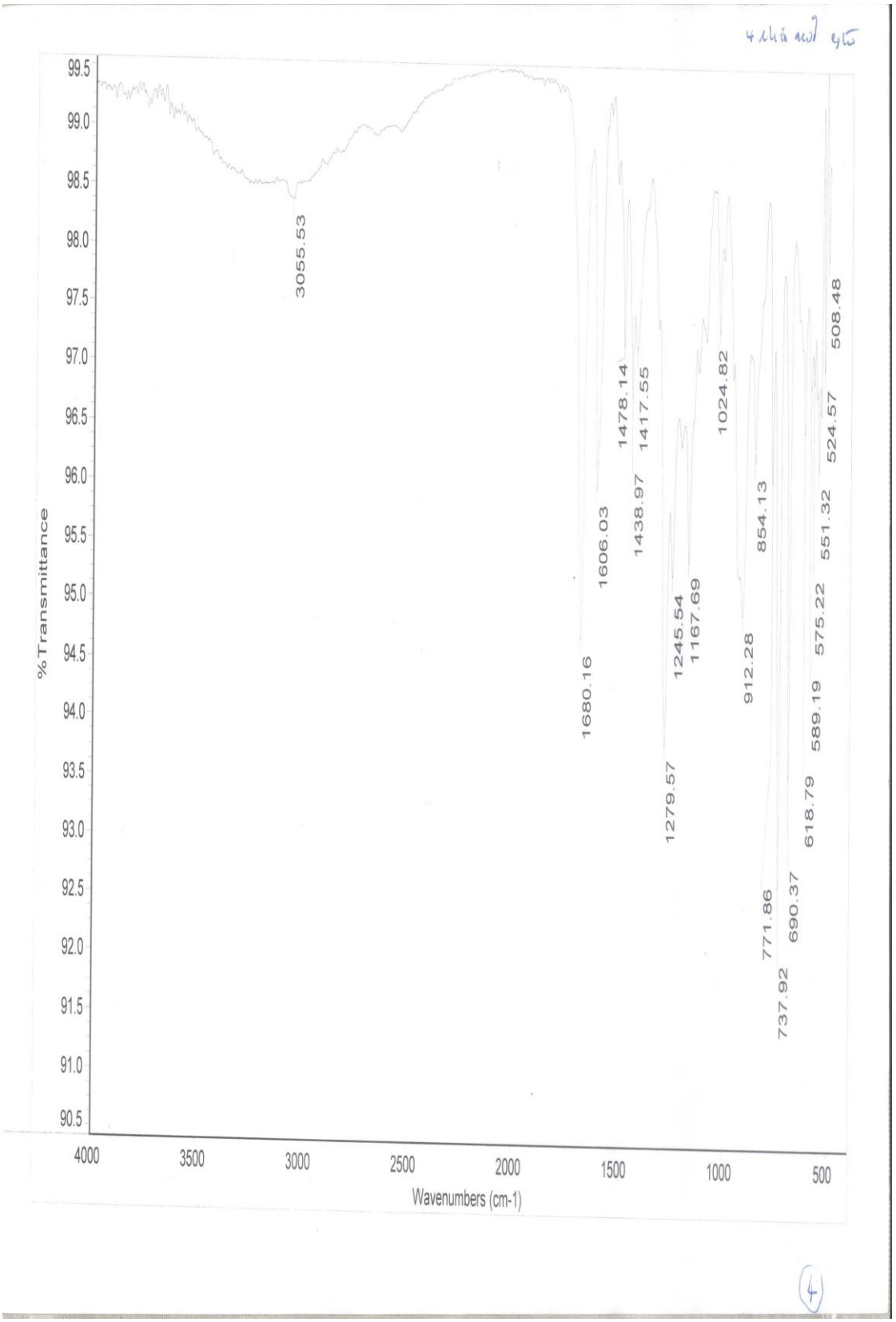
benzoic acid (1)



Salicylic acid

(2)


3-benzoic
%Transmittance
Wavenumbers (cm-1)
3284.43
3056.30
1705.91
1581.84
1477.89
1460.54
1437.14
1267.93
1206.96
1086.60
1024.29
923.90
887.75
817.12
734.13
714.84
689.60
652.63
560.38
514.77


4 chloro acid eg5
%Transmittance
Wavenumbers (cm-1)
99.5
99.0
98.5
98.0
97.5
97.0
96.5
96.0
95.5
95.0
94.5
94.0
93.5
93.0
92.5
92.0
91.5
91.0
90.5
4000
3500
3000
2500
2000
1500
1000
500
3055.53
1680.16
1606.03
1478.14
1438.97
1417.55
1279.57
1245.54
1167.69
1024.82
912.28
854.13
771.86
737.92
690.37
618.79
589.19
575.22
551.32
524.57
508.48
4

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26 February 2020

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Document title: **Synthesis of aromatic thio-acid esters of 2-thiophenylethanol and exploring some of their biological activities**

Author(s): **Maram ziad salem Hawi**

Format: **American English**

Style guide: **Not supplied**

جامعة النجاح الوطنية

كلية الدراسات العليا

تخليق ثيو – استر الحلقات الاروماتية من 2–ثيوفينيل ايثانول واستكشاف بعض أنشطتها البيولوجية

إعداد

مرام حاوي

إشراف

د. نضال جرادات

د. أحمد حسين

قدمت هذه الأطروحة استكمالاً لمتطلبات الحصول على درجة الماجستير في العلوم الصيدلانية، بكلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس– فلسطين.

2020

تخليق ثيو – استر الحلقات الاروماتية من 2–ثيوفينيل ايثانول واستكشاف بعض أنشطتها البيولوجية

إعداد

مرام حاوي

إشراف

د. نضال جرادات

د. أحمد حسين

الملخص

موضوع هذه الرسالة هو تحضير أربعة مركبات من استرات حمض الثيو من تفاعل مشتقات حمض البنزويك (2–هيدروكسي، 3–هيدروكسي، 4–هيدروكسي حمض البنزويك مع ثيوفينيل إيثانول 2.

تم إنشاء هياكل استرات ثيو بواسطة (FT-IR Fourier Transform Infrared)، والرنين المغناطيسي النووي بروتون (H-NMR1) و13C.

تم اختبار استرات ثيو العطرية لأنشطتها المضادة للأكسدة، ومضادة للفطريات، ومضادة للبكتيريا، ومضادة للسرطان، ومضادة للسكري، ومضادات السمنة.

أظهرت النتائج DPPH انه كان حوالي (IC50 = 30µg / ml)، نفس القيمة لحمض Gallic،

وتم اختبار المركبات أيضًا لنشاطها المضاد للبكتيريا ضد: (المكورات العنقودية الذهبية، الإشريكية القولونية، كليبسيلا الالتهاب الرئوي، البروتيوس الشائع، المعوية المكورات العنقودية الذهبية، الزائفة العصبية، والمبيضات البيضاء) وأظهرت معدل قيمة (MIC(3.125–6.25 mg/ml في حين نسبه MIC للمضاد الحيوي امبسلين كانت (0.001–3.125)

في حين تم تقييم الخاصية السامة للخلايا على خلايا سرطان الإنسان MCF-7 وأظهرت المتوسط (المرحلة G2-M: 21.83، 8.13، 10.66، 14، 3.66).

ايضا، تم اختبار نسبه التثبيط لأميليز (IC50 = 10) الذي اظهر انه يشابه Acarbose.