An-Najah National University

Faculty of Graduate Studies

NOVEL 3,4-METHYLENEDIOXYBENZENE DERIVATIVES: SYNTHESIS, CHARACTERIZATION AND EVALUATION FOR THERAPEUTIC ACTIVITY

By

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Supervisor

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This Thesis is Submitted in Partial Fulfillment of the Requirements for the Degree of Master in Pharmaceutical Sciences, Faculty of Graduate Studies, An-Najah National University, Nablus, and Palestine.

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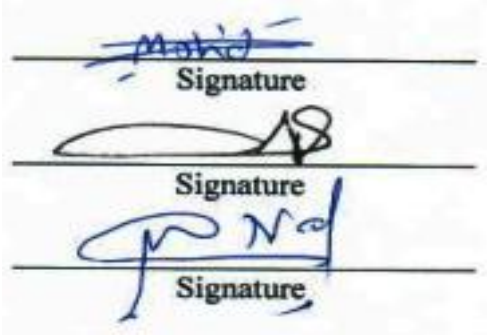

Dr. Hani Ishtaye
External Examiner

Dr. Murad Abu-Alhasan
Internal Examiner

Dedication

I am dedicating this thesis to every person who never lost hope in me.

To my parents who have forever been supporting and a true inspiration for happiness

To my colleagues at work who stood by me and supported me

To my mentor thank you from all my heart for not giving up on me

To all my friends

I dedicate this work

Acknowledgement

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Special thanks to the staff of the laboratories of the College of Pharmacy, Science and Medicine for their help without any doubts especially Tahreer Ishtayeh and Ahmad Mousa.

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Finally, my family deserves endless gratitude for supporting me and providing me with endless encouragement throughout my study years. This dream would not have been possible without them.

Declaration

I, the undersigned, declare that I submitted the thesis entitled:

NOVEL 3,4-METHYLENEDIOXYBENZENE DERIVATIVES: SYNTHESIS, CHARACTERIZATION AND EVALUATION FOR THERAPEUTIC ACTIVITY

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

Student's Name: ضياء محمود عبد الجليل حميدي

Signature:

Date: 19/1/2022

List of Contents

List of Tables

List of Figures

List of Scheme

List of Appendices

NOVEL 3,4-METHYLENEDIOXYBENZENE DERIVATIVES: SYNTHESIS, CHARACTERIZATION AND EVALUATION FOR THERAPEUTIC ACTIVITY

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ABSTRACT

Non-steroidal anti-inflammatory drugs (NSAIDs) are within the most used treatment worldwide; they inhibit the cyclooxygenase enzymes (COX) that mediates the biotransformation of arachidonic acid to inflammatory prostaglandins. In this master thesis, new benzodioxol derivatives with aryl acetate and aryl acetic acid groups were synthesized, identified and evaluated for their potency and selectivity toward COX-1 and COX-2 using an *in vitro* COX inhibition assay kit. The cytotoxicity for the synthesized compounds were evaluated by using MTS assay against cervical carcinoma cells line (HeLa). All synthesized novel compounds were identify by using FTIR, HRMS, ^{1}H-NMR, and ^{13}C-NMR techniques. The results revealed that the most potent compound against the COX-1 enzyme was 4f with IC_{50} =0.725 µM. Compound 3b exhibited potent activity against both COX-1 and COX-2 with IC_{50}=1.12 and 1.3 µM, respectively, and its selectivity ratio (0.862) was determined to be better than Ketoprofen (0.196). However, compound 4d was the most selective with a COX-1/COX-2 ratio value of 1.809 in correlation with the Ketoprofen ratio. All compounds exhibited cytotoxic activity against the HeLa Cervical cancer cell line at a high concentration range (0.219– 1.94 mM), and the most cytotoxic compound was 3e with a CC_{50} value of 219 µM. This was ten fold more than its IC_{50} values of 2.36 and 2.73 µM against COX-1 and COX-2, respectively. In conclusion, the synthesized library has significant activity against both COX-1 and COX-2 enzymes and ortho halogenated compounds were more potent than the Meta ones.

Keywords: NSAIDs, COX, Benzodioxol, HeLa, Ketoprofen

Chapter One

Introduction

1.1 Introduction

Compounds such as safrole, iso-safrol, and myristicin are also known as 1,2-methylenedioxybenzene (Benzodioxole) compounds, the 1,2-methylenedioxybenzene compounds can be found in various plants, including parsnip, carrot, parsley, and nutmeg. Benzodioxole-containing compounds exhibit various biological activities, including anticancer, antidepressant, anti-infective agents, analgesic, and anti-inflammatory [1].

The structure and functional chemistry of aliphatic and aromatic compounds make sense in the same way that the chemistry of heterocyclic compounds makes sense [2]. The term "heterocyclic compound" refers to a compound that is cyclic but contains at least one atom of a different type than carbon in its structure. However, depending on the manufacturer, the compound can be made from a variety of other compounds as well, such as phosphorus and silicon. The most common type contains high concentrations of heteroatomic nitrogen, oxygen, and Sulphur [3].

Heterocyclic compounds, which contain atoms not commonly found in nature, have been fascinating in recent years. Many heterocyclic compounds are known, but they have complex chemistry and are synthesized with a high degree of knowledge. When compared to many naturally identified heterocycles, those containing oxygen or sulfur are the most abundant and numerous due to their extensive distribution in nucleic acid instants and involvement in practically all physiological processes in both plants and animals [4].

Several organic molecules, including many organic chemicals with vital applications in industry and daily life, contain inorganic heterocyclic complexes. Heterocyclic systems are found in a variety of natural and manufactured organic substances. The main structure of most sugars and their derivatives, such as vitamin C, is a five-membered ring (Furanosied str.) or a six-membered ring (Pyranoized str.) with only one oxygen atom as its central ring structure. To illustrate this point, consider the vitamin pyridoxine (vitamin B6), a pyridine derivative required for the metabolism of amino

acids. Most vitamin B members contain heterocyclic rings that contain nitrogen; Vitamin B12 is one such example (Thiamine) [5]. There are numerous other examples of the importance of heterocyclic compounds in biological systems that can be provided. From the beginning of human civilization, natural products containing heterocyclic compounds, such as alkaloids and glycosides, have been used as remedies to treat various infections [6]. An example of heterocyclic compounds is all the alkaloids found in Chang Shan, ancient Chinese medicine, and reserpine found in Indian alkaloids found in poison arrow and drugs Codenin and J Tropin and Strychnine, respectively. The antimicrobial agent heterocyclic ring systems, which are found in many antibiotics such as penicillin and cephalosporin and norfloxacin and streptomycin, are included as an additional component and their other components [7].

The American Chemical Society has estimated that heterocyclic compounds account for a disproportionately large proportion of the many medications introduced into pharmacopoeia in recent years. Veterinary medications, such as Pyrantel and Morantel, are recommended to treat worm infestations on a large scale. Pyrantel and Morantel are two examples of such medications. Pyrantel and Morantel are two examples of such medications, to name a few [8]. Heterocyclic agrochemicals (herbicides such as atrazine and simazine, for example) and various other compounds are commonly found in agricultural products, including agrochemicals. Plant pigments like Indigo, hemoglobin, anthocyanins, and chlorophyll have contributed significantly to color chemistry and have been in use from ancient times in a variety of different heterocyclic colors. Tetraselena fulvalene, the heterocyclic ionic molecular crystal, was the first to show superconductivity in an ionic molecular crystal [9].

The word drug is derived from the French word drough, which is literally "dry herb." According to the World Health Organization definition, a medication is any substance or product used for modification or exploration of the physiological system or pathological status in the beneficiary's interest. The field of medicinal chemistry consists of two main fields. The first chemical treatment is the treatment of infections, parasites, or malignant diseases with chemical agents. These agents are often exclusively poisonous to the pathogen being treated. The other division also covers body-type disorders, and the medicines utilized are primarily chemicals that interfere with enzyme activity, pulse transmission, or hormone receptors action [10].

Heterocyclic compounds are used in all of these applications due to the high degree of chemical reactivity that they exhibit. Sulfa pharmaceuticals contain heterocyclic groups that can be incorporated into medications and impact their physical properties, such as the dissociation constant of the sulfa pharmaceuticals. They may affect their absorption patterns, metabolism, and toxicities as well. A new medication was desperately needed during the years 1930-1950 to treat diseases with a high mortality rate that were prevalent at the time, and new medications were developed in response to this need [11].

However, the potential hazards of new medications were not fully understood, and toxicology tests were undertaken before clinical trials were rudimentary. Showing the proverb due to the need as the mother of invention, many medications were introduced in the 1930s and 1940s. As a result, this period is called the "Golden Period" to discover new drugs. The following steps must be followed to obtain heterocyclic compounds [12].

Alkaloids, amino acids, indigo hues, and other active compounds from natural sources are extracted using a process known as enzymatic extraction. Environmental degradation can occur in the presence of naturally occurring products such as acridine, furfural, indol, pyridine, quinoline, and thiophene, among others. When it comes to the synthesis of heterocyclic compounds, three different types of methods can be used [11]. The first type of reaction is a tighter ring reaction, the second type is known as additional reactions, and the third type is known as substitution reactions (or substitution reactions). Removing a few small molecules, such as water or ammonia, from a long enough chain to achieve cycling is a typical approach [13]. Because of their unique chemical reactivity, histidine-containing heterocyclic compounds have great potential in the pharmaceutical industry. They appear to be essential metabolism and can act in the biosynthetic reaction as false synthesis. Organic chemists and members of the pharmacological community have been deeply interested in the building, by many, of vast searchable libraries of organic compounds. Work in several labs targeted at enhancing chemical variety has recently been evaluated. The efficiency of this technique has been shown by pharmacologically important molecules for libraries with a large number of chemical compositions [14].

The most crucial goals for pharmaceutical research are to produce new medications, improved exist medications, and successfully introduce them into clinical practice. Therefore, the pharmaceutical industry's efforts to remain competitive include developing novel and inventive pharmaceutical substances and formulations. Additional to mentioned goals, the search for medications that provide a distinct advantage over an existing medication should be prioritized for further investigation. Among the numerous advantages are the enhancement of qualitative or quantitative activity, the absence of unpleasant side effects, lower toxicities levels, increased stability, and cost savings, to name a few examples. Another advantage is the increased stability and cost savings that come with it. [15]. Before proceeding any further, it is critical to emphasize that the term "drug discovery" in pharmaceutical research and development does not have a precise definition because it is ambiguous. The approaches can be divided into several categories, each of which has several different options available. This is true whether the approach is programmatic or organizational (or both). Understanding and explicitly defining the topic's variability is critical to effectively deal with this problem [16].

Pyrazoline is an analgesic and antipyretic agent. Antipyrine 1 was first commercially available in 1984 as a combination antipyretic and analgesic, and it has been in use ever since. The first acyclic and carbocyclic chemicals were synthesized in the nineteenth century, respectively. Baritone, the first barbiturate, was introduced in 1903, making it the first invention. Additionally, during this century, the first acyclic chemical was discovered, a process that had begun in the nineteenth century and continued into this century. Guttmann discovered the acriflavine trypanocidal in 1912 while treating malaria patients with methylene blue, which he had discovered in 1891. Guttmann had previously discovered methylene blue. It has been demonstrated that this compound is more effective than an antiseptic in treating malaria parasite infection. In clinical trials, the acriflavine trypanocidal, which Guttmann discovered earlier in his career, was more effective in treating parasitic infections than an antiseptic in the treatment of parasitic infections. It was developed in 1926 for the same purpose as phenazopyridine (Pyridium) 3 [16]; however, even though it was generally ineffective, it was still used due to its analgesic properties. It was eventually phased out in the 1970s. When it came to being phased out completely, it happened in the 1970s. When it came to being phased out completely, it was in the 1970s that this occurred [16].

Nonsteroidal anti-inflammatory medications (NSAIDs) are a structurally heterogeneous class of similar-acting chemicals that are efficient at reducing pain, inflammation, and fever [17]. They contribute to the inflammatory process in a variety of ways biologically. They limit prostaglandin and inflammatory mediator synthesis, modulate the activity of inflammatory proteases, and stabilise the liposomal membrane [18]. They are a frequently prescribed and non-administered medication [19]. NSAIDs are used therapeutically to treat musculoskeletal, rheumatoid, osteoarthritis, and other inflammatory diseases [20]. Although traditional NSAIDS derivatives are still frequently used today, their gastrointestinal adverse effects have significantly curtailed their use. NSAIDs reduce pain and contribute to the inflammatory process by inhibiting the cyclooxygenase enzyme, which inhibits prostaglandin synthesis. COX-1 suppression had a detrimental effect on the gastrointestinal system [21, 22].

The present treatment of osteoarthritis with NSAIDs has been associated with an increased risk of gastrointestinal issues [23]. Additionally, NSAIDs have been associated with an increased risk of cardiovascular events, especially in individuals with heart failure [24]. Classification of NSAIDs is accomplished in a variety of methods, one of which is based on their chemical structure [25]. NSAIDs are classified into several subgroups, including the following:

1. Diflunisal, aspirin, and other derivatives of salicylic acid
2. Ibuprofen, naproxen, and ketoprofen are all derivatives of propionic acid.
3. Acetic acid derivatives include indomethacin, etodolac, and sulindac.
4. Oxicam (enolic acid) derivatives such as piroxicam and meloxicam.
5. Fenamates or anthranalic acid derivatives include mefenamic acid and meclofenamate.

“1,3-benzodioxole (1,2-methylenedioxybenzene)” is a natural product with the chemical formula “$C_6H_4O_2CH_2$”. It is also known as “1,2-methylenedioxybenzene [26]. According to the classification system, the compound is classified as a benzene derivative and a heterocyclic compound due to the presence of the methylenedioxy functional group in the structure of the compound. No discernible smell can be detected

in this colourless and odourless liquid. Although Benzodioxole is not a particularly significant compound, it is included because many of the group's closely related compounds are bioactive and can be found in pesticides and pharmaceutical formulations [27]. There are several derivatives of these compounds, and extensive research has been carried out on this, briefly explained below.

1.2 Biological effects of The Benzodioxole

A few examples of natural products that contain the benzodioxole ring system include safrole and piperonal. This ring system can be found in a wide variety of alkaloids, as well as a plethora of other botanicals and natural products, as well as in a variety of other alkaloids. 1,2-dioxolanes are a class of compounds that act as a cofactor in the reaction of the arachidonic acid conversion into the biologically active metabolites. This natural pathway links vital fatty acids into prostaglandins and other hormones. 1,2-dioxolanes are naturally occurring compounds found in many plant and animal species, including humans [28].

According to some researchers, the endoperoxide PGH2 (121) is formed during arachidonic acid's initial oxidation. It has a half-life of 4-5 minutes when produced by enzymes in the body and can be converted into prostaglandins, prostacyclins, thromboxanes, and other inflammatory mediators in humans. Numerous physiological processes, including platelet aggregation of the platelets, dilation of the vessels, and smooth muscle contraction, are all regulated by these compounds, which act as connective tissue between the body's various systems [29].

1,3-dioxolanes are used in various synthesis processes, with one of the most important being the protection of functional groups, which allows transformations to take place in other parts of the molecule. 1,3-dioxolanes are used in various synthesis processes, with one of the most important being the protection of functional groups. According to the type of particle understudy, the 1,3-dioxolane ring in several molecules can be replaced by “1,2-glycol” or “carbonyl functions” [30]. This is particularly beneficial in the field of chemistry. Metals belong to the transition groups with optically active ligands are, as shown in some studies, extremely effective catalysts for homogeneous asymmetric hydrogenation. Particularly effective are the "rhodium complexes of chiral phosphines" and one of the most widely used is "2,3-O-isopropylidene-2,3-dihydroxide-1,4-bis

(diphenylphosphine) butanes", also known as "DIOPs or 2,3-O-Isopropylidene-2,3-dihydroxy-1,4-bis(diphenylphosphine)". According to the manufacturer, Diop can be used in addition to cancer and other diseases to treat many conditions. This symbol is available in (+) and (-) forms, depending on the context, on the commercial marketplace [31].

In terms of polymers and copolymers of 1,3-dioxolane, there are many patents covering the compound that can be found online. Whether any polymers have found widespread commercial application is not known at this time [32]. Materials with a molecular weight of 104 are rigid and solids with polyethene oxide-like properties. They endow copolymers with a hydrophilic nature that would not otherwise exist due to their solubility in water. Rather than using monomers that are not surface active in the water, it has been proposed that copolymers containing active "hydrogens", in the form of "alcohols", "amines", and "carboxylic acids", be used in their place [33].

When 1,3-dioxolane reacts with vinyl compounds like styrene or isobutene, it forms a Liquid with a low molecular weight with a hydroxyl group at the surface. These polymers can be used as pre-polymers in producing polyurethane rubber elastomers and other products, for example. Dioxolane rings dangle from the surface of a polymer when 2-vinyl-1,3-dioxolane is added. Dioxolans are well-suited for use in a wide range of air-cured coating systems, adhesives, and films due to their rapid autoxidation to form hydroperoxides [34].

It is one of only a few 1,2-disubstituted ethylenes known to be capable of undergoing straightforward radical-induced homo-polymerization. Vinylene carbonate is one of the very few radical-initiated homo-polymers that have been discovered to date. Oxygen, peroxides can trigger the initiation process, or cobalt-60 rays, among other things [35]. Co-polymerisation occurs if high pressure is applied to vinylene carbonate, and the resultant material contains approximately 10% vinylene carbonate by volume. According to the manufacturer, this polymer, when combined with polyvinyl chloride, is suitable for use in injection moulding applications [36].

Majority of the antifungal compounds contained 1,3-dioxolane ring such as ketoconazole. This antifungal drug is one of the most broad spectrum orally active compounds and has been used against different type of fungal infections including superficial and deep infections [37].

Such as study conducted by Song et al [38] shows a series of γ-lactam analogues containing the 1,3-benzodioxole moiety were designed, and these derivatives were synthesized via a structural diversity-oriented synthesis from lactam. Their structures were confirmed using 1HNMR, 13CNMR, and ESI-MS spectroscopy. Their antifungal activity was assessed against four serious and typically crop-threatening agricultural fungi: *Rhizoctonia solani, Alternaria tenuis Nees, Gloeosporium theae-sinensis,* and *Fusarium graminearum*. Certain derivatives, such as compounds 7a, 7b, and 7i, demonstrated activity against *Alternaria tenuis Nees* that was superior to that of the commercial fungicide carbendazim, when compared to *eosporium theae-sinensis* and *Fusarium graminearum*. Several of these derivatives, such as compounds 7a, 7b, and 7i, demonstrated activity against *Alternaria tenuis Nees* that was greater than that of the commercial fungicide carbendazim. When compared to the blank control, several of these derivatives demonstrated good antifungal activity against *Gloeosporium theae-sinensis* and *Fusarium graminearum*. Besides this, many researchers across the world, particularly in Russia, suggest that simple oxathiolanes can be used as radio-protectant [38].

A large number of compounds have been discovered that are sulfonic acid analogues of phenolphthalein, and these compounds are referred to as phenolphthalein analogues. Although they are more commonly known by their dye or indicator designations such as phenol red and bromophenol blue are examples of such dyes [39].

1.3 Similar Structures

1.3.1 Benzo [B] Thiophene Derivatives

There is a various biological and pharmacological activity in the histidine-containing heterocyclic compounds and their derivatives with the benzo[b]thiophen ring system [40]. According to the literature review, heterocyclic sulfur compounds in remedial science have received substantial interest due, for example, to their biological and

pharmacological activity, including anti-HIV, antituberculosis, antimicrobials, anticonvulsants, anticancer, and antivirals [41].

Arylaminomethyl derivatives are a modest class of biologically active compounds that are intensively examined in the hunt for new prospective medications, and they are one of the most promising options. These have, among other things, been claimed to have antibacterial, anti-tumour, and insecticide activities [42]. Given these critical findings, various new Arylaminomethyl compounds with higher biological activity have been proposed to be synthesized as follows: (e)-N-Arylmethine-5-bromo3-chlorobenzene production and biological evaluation[b] Thiophene-2-Carbohydrazides (E)-N'-Arylmethine-5-Carbohydrazides (T)[b] thiophene-2-carbohydrazides Azomethins of type -(I) have been synthesized by treating 5-bromo-3-chlorobenzo[b]thiophen-2-carbohydrazide to form azomethines with various structures with a range of aromatic aldehydes [43].

N'-arrylmethyl-5-bromo-3-chlorobenzo[b]thiophen-2-carbohydrazide was synthesized and biologically examined [44].

Figure 1.1

N'-arrylmethyl-5-bromo-3-chlorobenzo[b]thiophen-2-carbohydrazide

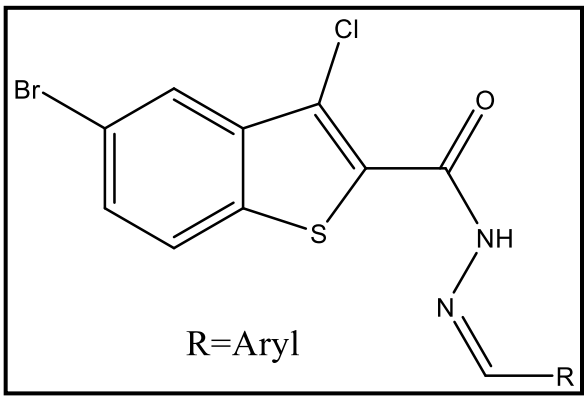
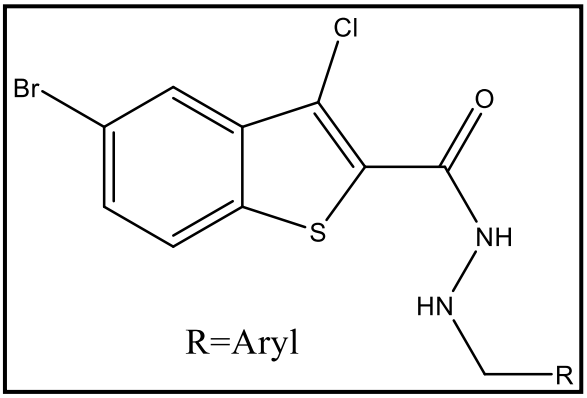


Figure 1.2

N'-arrylmethyl-5-bromo-3-chlorobenzo[b]thiophen-2-carbohydrazide

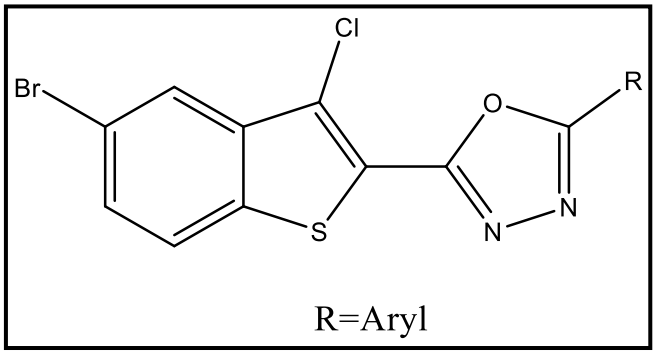


The compounds Type - (II) are generated in the form of type - (I) with anhydrous NaBH4 by reacting arylamines [45].

1,3,4-Oxadiazoles are associated with a wide range of pharmacological activities, including anaesthetic, antifungal, hypnotic, and antibacterial action, amongst other things. We were able to develop a superior therapeutic value for the 1,3,4-oxadiazole derivatives detailed below due to these valid observations [46].The synthesis of 2-(5-Bromo-3-Clorobenzo[b]thiophen-2-yl)-5-aryl-1,3,4-oxadiazoles and their biological evaluation are described.

Figure 1.3

2-(5-Bromo-3-Clorobenzo[b]thiophen-2-yl)-5-aryl-1,3,4-oxadiazoles



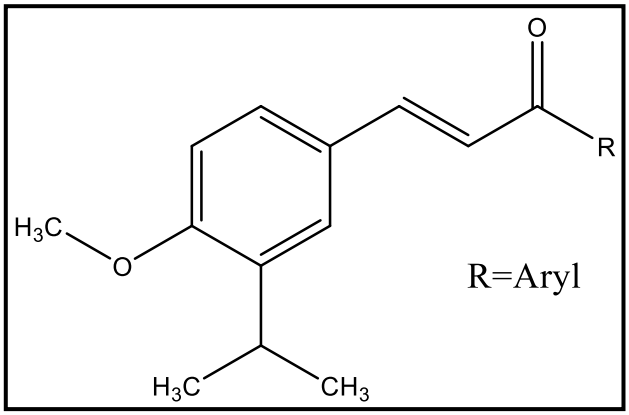
It is possible to make type-III oxadiazole derivatives by reacting 5-bromo-3-chlorobenzo[b]thiophen-2-carbohydrazide with various aromatic acids in the presence of POCl3 and then oxidizing the resulting product [47].

1.3.2 Chalcones, Ester and Ketone Derivatives

Chalcones are Phenyl acrylate ketones containing an ethylenic reactive group (-COCH=CH-) and a reactive ethylenic group. Chalcone derivatives are biologically diversified, among other things analgesic, anthelmintic, anti-inflammatory, antifungal, antibacterial. Chalcones are effective synths for a range of chemical molecules in addition to their other features. This valid observation motivated us in the following part to synthesize several new chalcones products, acetylpyrazoline and cyclohexenone derivatives with a nucleus of 3-isopropyl-4-methoxy benzaldehyde [48]. Biological screening and synthesis (E) (E) -3- 3- (3-Isopropyl-4- methoxyphenyl) -1-aryl-prop -2-en-1-ones.

Figure 1.4

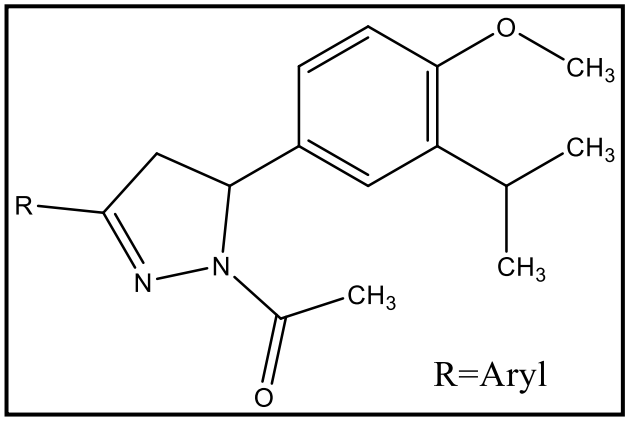
(E) (E) -3-3-(3-Isopropyl-4- methoxyphenyl) -1-aryl-prop -2-en-1-ones



Different aryl ketones have been employed to manufacture the kind of chalcone derivatives for condensing 3-isopropyl-4-methoxy benzaldehyde with different aryl ketones in the presence of 40 percent NaOH (IV). The pyrazoles 1-Acetyl-3-aryl-5-(3-isopropyl-4-methoxyphenyl) were synthesized and biologically screened.

Figure 1.5

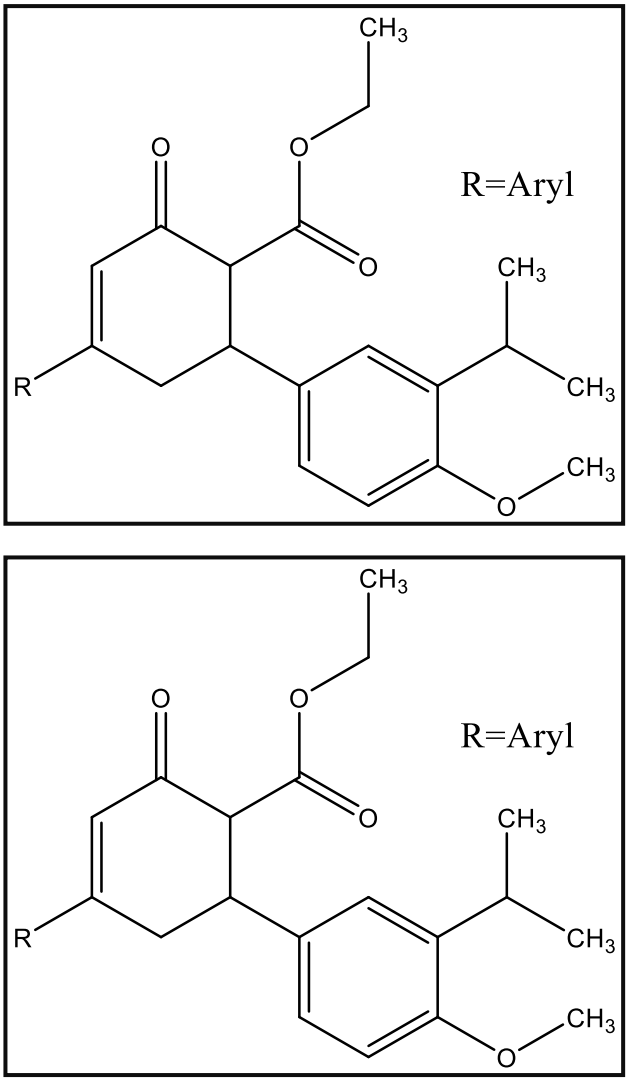
1-Acetyl-3-aryl-5-(3-isopropyl-4-methoxyphenyl)



It has been discovered that Type -(V) pyrazolines can be synthesized with condensation of type - (IV) chalcones using hydrazine hydrate in glacial acetic acid [48]. According to the researchers, ethyl 4-aryl-6-(3-isopropyl4-methoxyphenyl)-2-Oxocyclohex-3-ene-1-carboxylates have been synthesized and biologically screened [49]. A new family of cyclohexenone type (VI) derivatives was developed in the presence of an essential catalyst (K2 CO3) through cyclo-condensation of type (IV) chalcones with ethyl acetoacetate, as reported earlier [50].

Figure 1.6

ethyl 4-aryl-6-(3-isopropyl4-methoxyphenyl)- 2-Oxocyclohex-3-ene-1-carboxylates.



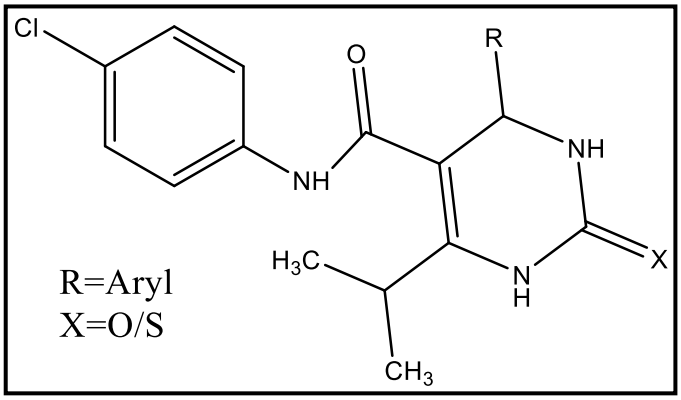
1.3.3 Tetrahydro Pyrimidine Derivatives

Pyrimidine nuclei have substantial pharmacological and biological action, and some natural derivatives such as nucleic acids and vitamin B may be discovered in the wild. There are numerous pharmacological and biological activities in the pyrimidine nuclei. Many pyrimidine derivatives have shown various pharmacological activities such as cancer, calcium channel blocker, and other bodily impacts. Due to our continuous interest in the synthesis of substituted pyrimidines, several novel potentially bioactive pyrimidine derivatives [51], detailed as follows, have been synthesized: Synthesis and biological screening of N-(4-Chlorophenyl)-1,2,3,4- tetrahydro-6-isopropyl-4-aryl-2-

oxo/thienopyridine 5-carboxamides using conventional method and molecular iodine as a catalyst [52].

Figure 1.7

N-(4-Chlorophenyl)-1,2,3,4- tetrahydro-6-isopropyl-4-aryl-2 oxo/ thienopyridine 5-carboxamides

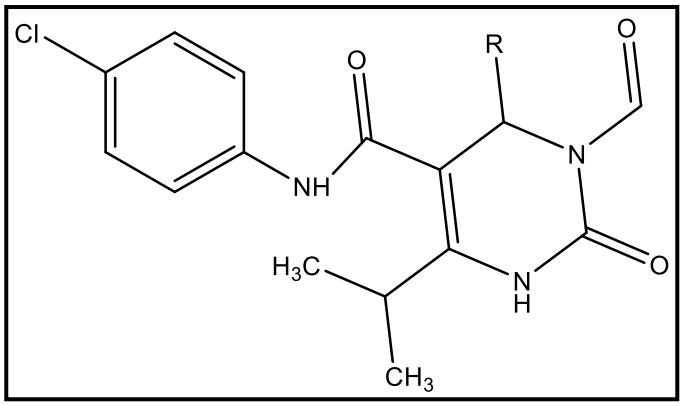


Pyrimidine derivatives of Type (VII) have been prepared by the multicomponent cyclization reaction of N-(4-Chlorophenyl)-4-methyl-3-Oxopentanamide with urea/ thiourea and different aromatic aldehydes in the presence of molecular iodine [53]. Synthesis and biological screening of N-(4-Chlorophenyl)-3- formyl6-isopropyl -2-oxo-4-aryl -1,2,3,4-tetrahydropyrimidine-5-carboxamides.

Various DMF and POCl3 dihydropyridines were formulated to supply the tetrahydro pyrimidines (VIII) synthesized through formylation [54].

Figure 1.8

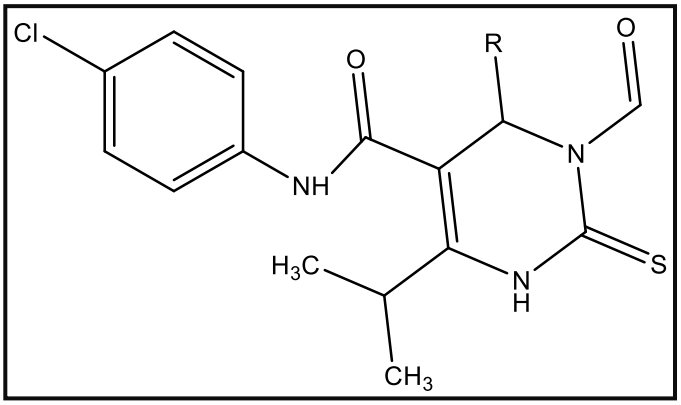
N-(4-Chlorophenyl)-3-formyl6-isopropyl-2-oxo-4-aryl-1,2,3,4-tetrahydropyrimidine-5-carboxamides



SECTION-III: Synthesis and biological screening of N-(4-Chlorophenyl)-3- formyl-6-isopropyl-2-thioxo-4-aryl-1,2,3,4-tetrahydropyrimidine-5-carboxamides.

Figure 1.9

N-(4-Chlorophenyl)-3-formyl-6-isopropyl-2-thioxo-4-aryl-1,2,3,4-etrahydropyrimidine-5-carboxamides..



Formylation of several dihydropyridines with DMF and POCl3 has been demonstrated to produce tetrahydro pyrimidines of type - (IX) [55].

Single crystal X-ray diffraction, the most widely used experimental technique for creating a detailed image of a small molecule, can resolve individual atoms in a crystal. Using a slow evaporation process in methanol as the solvent, a single crystal of 1-Phenyl-3-methyl pyrazole-2-en-5-one was synthesized at room temperature [56]. Single crystals of exceptional quality were recovered from the ground in 45 days, despite the challenging conditions. For the compositions of all synthesized compounds, elemental analysis, FT-IR, 1H NMR spectroscopy, and mass spectroscopy were used, with the results of mass spectroscopy being used to validate the results of the results of the elemental analysis. The purity of each substance was determined through the use of thin-layer chromatography. Every compound tested showed antibacterial activity against Gram +ve and Gram -ve bacteria when tested at a concentration of 40 g/ml and antifungal activity against the mould Aspergillus niger. The newly synthesized compounds outperform conventional medications by a wide margin [57].

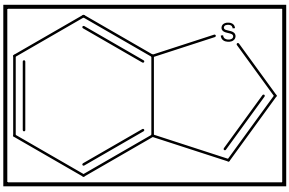
1.4 Therapeutic Importance of Benzodioxole and similar structures

Due to the biological importance of Benzodioxole and its similar structures, interest in the chemistry of this molecule has increased in recent years [57]. It has been reported that the compound Benzo[b]Thiophen and its derivatives have been synthesized and tested for their anticonvulsant efficacy in a variety of experimental settings [57].

In line with the findings of Keri et al. [58], who synthesized diamino benzo[b]thiophen and its derivatives, which act as site-directed inhibitors of thrombin activity, diamino benzo[b]thiophen and its derivatives were found to inhibit thrombin activity. As a result, according to its scientific name, Kuanuniamine A is the most fundamental member of a class of marine alkaloids that have demonstrated a potent anticancer effect in both experimental and in vivo cancer studies. Aside from that, they possess immunosuppressive and antiviral properties [59]. It was discovered by Romagnoli et al. [60], who used it to make the chemical 4,7-dioxobenzo[b]thiophene, which is an analogue of the compound and is used in its production.

Figure 1.10

structure of Benzothiophene



Liger et al. [61] developed a method for synthesizing benzo [b]thiophen derivatives, which demonstrated a significant affinity for serotonin and 5-HT1A receptor transporters, among other things. A group of researchers led by Chen et al. [62] discovered the benzo[b]thiophens, and Sakae et al. [63] discovered that they exhibited a variety of biological properties against the human T-cell leukaemia virus when combined with naphthothiophenequinones and benzo [b]thiophenequinones with ortho amino-functional on the ring (HTLV-1). Grover et al. [64] developed an organic compound known as 3-benzylbenzo[b]thiophenes that is effective in treating postmenopausal problems such as breast cancer and uterine and cervix cancer.

Researchers Wu et al. [65] have synthesized and investigated benzo[b] thiophen derivatives for their inhibitory action in NHE-1 cells and their cardioprotective efficacy in both *in vitro* and in vivo studies in the lab. Recent research by Sadig et al. [66] has resulted in the synthesis of benzo[b]thiophenes and a derivative of benzo[b]thiophenes, with the researchers currently investigating the efficacy of these compounds as anti-breast cancer agents in animal models.

According to Kupracz et al. [67] a review of four novel benzo[b]thiophene derivatives intended as serotonin N-acetyltransferase (AANAT) inhibitors has been published. It was reported in the journal Chemical Communications that the findings were made. ANAT-specific inhibitors may be beneficial in treating some physiopathological abnormalities that can occur in disorders such as seasonal affective disorder or obesity. Benzo[b]thieno[2,3-c]quinolones [68], have been the subject of research by an international team of scientists led by Dr. Kz Z Grace. They have been tested on a variety of human cell lines, including a normal cell line, to determine their antiproliferative effects.

Racané et al. [69] have developed and tested antibacterial effectiveness against gram-positive and gram-negative bacteria in the laboratory using gram-positive and gram-negative bacteria in the case of 2-Isopropylamide-3-carboxamide products.

Pieroni et al. [70] have discovered novel benzothiophene compounds that are inhibitors of protein tyrosine phosphatase1B and possess antihyperglycemic properties. Dell'Osso et al. [71] discovered the development of a new class of antidepressant medications due to their research, which resulted in the development of 5-HT1A serotonin receptors and serotonin transporters in five substituted benzo [b]thiophene compounds. It has been demonstrated that Benzothiophen-4-piperazine compounds, which Hayashi et al. [72] have successfully synthesized and demonstrated the serotonergic activity, can be used as new antagonists in the 5-HT1B vascular receptor. It is the hope that these compounds will serve as new antagonists for the 5-HT1B vascular receptor.

Meltzer-Brody et al. [73] discovered that the cytostatic/tumour-associated carbon anhydrase isozymes I, II, and IX activity were inhibited by the benzo [b]thiophene 1,1-dioxide Sulfonamides in the presence of the tumour-associated enzyme. According to Burgdorf et al. [74], novel benzo[b]thiophen-2-carboxanilide cyano and Isopropylamide

substitutes for benzo [b]thiophen-2-carboxanilide cyano and Isopropylamide were developed and demonstrated to have anti-cancer properties in animal models. Based on their research, benzo [b]thiophen 2-carboxamides are highly efficient antagonists of the human receptor H3. According to the latest news, Li et al. [75] are developing new HIV-1 reverse transcriptase inhibitors based on a tricyclic benzothiophene scaffold as their starting point. Antiproliferative benzo[b]thiophene-based histone deacetylase inhibitors, discovered and reported by Pawar et al. [76]. This same compound was discovered and reported by others as well. It was discovered by Singh et al. [77] that it is possible to synthesize 2- and 3-aminobenzo[b]thiophene derivatives that, when used in conjunction with the Thiophene Ring, can act as antimitotic agents or tubulin polymerization inhibitors. An article on the production of benzo[b]thiophene derivatives and the evaluation of their dual 5-HT1A/SSRI activity was published by Zhang et al. [78] in the Journal of the MedChemComm.

A study published in science by Gao et al. [79] discovered several 2,3 substituted 1-benzo[b]thiophen derivatives. They also discovered their pharmacological activities for synthesis as well as their cannabinoid activity. Kashadia 6-Carbethoxy-5-aryl-3-[p-(3'-chloro-2'-benzo[b] thiophenonylamino) phenyl] is a novel compound currently being researched and developed. It is effective against bacteria that cause tuberculosis—derivatives of benzo [b]thiophene that contain the nucleus of the compound. Netto et al. [80] discovered how to easily synthesize the antibacterial agents such as, azetidine and acetyl oxadiazoles. They could also easily synthesize thiosemicarbazides and 1,3,4-thiadiazoles, which were both powerful antitubercular and antibacterial agents [81]. 1,2,4-triazoles and thiosemicarbazide are heterogeneous compounds that can exist together or separately Gangu et al. [82]. 1,3,4-thiadiazoles, imidazolines, 1,2,4-triazoles, thiourea derivatives (thiophenes), 1,3,4-oxadiazoles, 1,3,4-thiadiazoles were some of the compounds developed by Khan et al. [83]. They were antibacterial substances, imidazolines, and 1,2,4-triazoles as antibacterial substances, though. See figure 1.11 in appendix B

Based on our ongoing research into benzo[b]thiophenes, which has resulted in numerous synthetic techniques and the discovery of a wide range of biological activities, we have synthesized benzo[b]thiophene for superior therapeutic agents, intending to obtain extremely potent biodynamic agents. In the ship bases, there is a wide range of pharmacological activity, including antimicrobial, antibacterial, anti-inflammatory, antiviral, and other antimicrobial characteristics, as well as other antimicrobial characteristics. Schiff's coumarin base derivatives were developed and evaluated in the laboratory by researchers [84]. The derivatives were tested for their ability to inhibit bacterial growth.

According to the researchers, spiro derivatives derived from styryl ship bases have been synthesized by Borah et al. [85], and they have the potential to exert an antibacterial and antifungal effect. Many azomethine derivatives were synthesized by Tang et al. [86] and tested in a laboratory setting. The results showed that the compounds had good antibacterial activity. According to the authors, the findings of Ahmad et al. [87] were novel. They demonstrated that the test organisms *S. aureus, E. coli, Shigella dysenteries*, and *Salmonella typhi*, among other bacteria, are naturally antibacterial. *S. aureus, E. coli, Shigella dysenteries, and Salmonella typhi*, among other bacteria, were naturally antibacterial. A team of researchers has successfully synthesized diazomethane, which has been shown to have good activity against plant hormones. Gardner et al. [88] investigated the antiviral activity of Schiff's base amino, hydroxy guanidine (SB-AHG5) against Adenovirus Type-5 and Herpes Simplex Virus Type I (HSV-1).

Cataldo et al. [89] have synthesized some of Schiff's baseline glucose derivatives with acetylenic bonds. After preparation of the compound, bactericidal activity was tested on *E. coli and Staphylococcus aureus*. A team led by Sedgwick et al. [90] has created the foundation and found that Schiff's antibiotics are effective. Researchers have summed up different azomethines and their boron complexes and then evaluated them for antibacterial and antifungal activities. It is evident that azomethines are exceedingly poisonous, yet their activity rises after compounds are complexed.

Oliver [91] found a technique of Cyclocondensation of azomethines with good antischistosomal action. Junker et al. [92] found that a new class of acetyl ferrocene derived from Schiff's foundations had an antibacterial function, which they synthesized. Certain azomethine derivatives were evaluated against diverse bacterial strains for antibacterial efficacy. In recent work, Goldberg et al. [93] synthesized an amine that was found to stabilize in phosphatidylcholine liposome, trans-Nitron-vinylidene-butylamine. According to the findings, if the cholesterol concentration in the membrane increases, the creation rate of the base ship reduces.

Volinsky et al. [94] have summed up several novel ship bases with great antibacterial activity. They have synthesized two-chlorine phenothiazines and examined for the capacity to inhibit carrageenin-induced oedema in Albino rats. According to Marsanasco et al. [95], have azomethines that have well-tolerated analgesic and antipyretic effects. In a Science study, the foundations of Lu et al. [96] synthesized and found that they showed good action with MIC ranging from 10 to 25 g/ml against the bacteria Vibrio Cholera. Some of the compounds are effective against *Salmonella typhi and Vibro Cholera*-0 (MIC 25-150 grams/ml). Fan et al. [97] synthesized the 4-hydroxy-6- carboxy hydrazine benzothiophene analog with various aldehydes and carried out pharmacology research. Ergenc and colleagues have created azomethine compounds with synthesized antifungal activity. Douadi et al. [98] have analyzed and synthesized several azomethines in their research. Swift et al. [99] have built various new ship bases, anticancer in nature and anticancer.

Mirzaei et al. [100] had synthesized and assessed the antibacterial activities of several azomethines *in vitro*. Zhang and Seidel [101] discovered azomethines that, according to their findings, exhibit antibacterial and anti-inflammatory characteristics. Prajila and Joseph [102] developed a synthesis of Schiff's bases with a benzo [b]thiophene nucleus that can be employed as antituberculosis or as antibacterial. Wałęsa-Chorab et al. [103] reported the straightforward synthesis of some new azomethines, including the physiologically potent benzo[b]thiophenic nucleus. The Mannich basis of 4-amino-3-mercapto-5-pyridine-3'-yl [1,2,4]-triazole and ship's base, both of which have a nicotinic acid nucleus with antituberculous and antibacterial assessments, have been examined and synthesized [104].

1.5 COX-1 and COX-2 overview

Through the action of phospholipase A2, cyclooxygenase enzymes (COX-1 and COX-2) catalyse the production of prostaglandin H2 and the formation of reactive oxygen species from arachidonic acid [105]. Prostaglandin H2 is a precursor for the synthesis of other prostaglandins, including prostaglandin E2, which operate as immunomodulators and inflammatory mediators. The actions of these prostaglandins, particularly prostaglandin E2, are mediated through vasodilation, pyretic effects, and the ability to proliferate [106]. Thrombboxane A2, prostaglandin E2, D2, I2 (prostacyclin), and F2 are further mediators engaged in a variety of biological responses depicts the biology of prostanoids and the manufacturing process [107]. Figure 1.12 in appendix B shows detail biochemistry of prostanoids and their pathway of formation.

COX-2 is divided into three sections: (1) an upper region containing (Phe381), (Tyr385), (Leu384), (Trp387), and (Phe518), (Leu503); (2) a lower region containing (Leu531), (Val116), (Val349), and (Leu359); and (3) a third region containing (Leu531), (Val116), (Val349), and (Leu359) (Tyr355). The amino acids (Phe518), (Leu352), (Val523), (Gln192), (Arg513), (His90), and (Ser353) [108]. According to the structure of many COX-2 inhibitors, hydrophobic interactions dominate the first region, hydrogen bonds with polar functional groups such as carboxylate and trifluromethyl dominate the second region, and additional hydrogen bonds with polar oxygen or nitrogen-containing functional groups dominate the third region [109]. Figure 1.13 in appendix B shows the domains of COX enzyme.

NSAIDs have a lower affinity for the COX-2 enzyme than selective COX-2 inhibitors. They do not form a salt bridge with the higher hydrophobic channel because they lack a carboxylic group. Tricyclics have a carbocylic or heterocyclic ring system with 1, 2-diaryl substitutions and functional groups on one aryl ring such as azido, sulfonamide, methansulfonamide, and methansulfonyl or tetrazole groups; heterocyclics have a heterocyclic ring system with 1, 2-diaryl substitutions and functional groups on one aryl ring such as azido Coxibs are a subset of this class of compounds. Acyclic compounds, on the other hand, have a cyclic centre surrounded by two or three chains. The bicyclic, tricyclic, fused, or spiro ring systems were employed to construct a variety of ring sizes and types. Rings of four, five, or six members have also been used widely as the major core of this type of molecule [110, 111].

A COX-2 inhibitor should possess a number of properties. A structure with three evenly spaced cycles is one of these characteristics. Binding with fewer steric limitations was achieved by adding a substitution on the C-3 central ring. According to previous study, the most efficient variations featured two fluorine atoms. In a study using pyrrole-based esters as a scaffold, two fluorine atoms in the same molecule were discovered to have a favourable effect on COX-2 inhibition. An electron withdrawing group, rather than an electron giving group, is favoured at the para position, and among the groups tested, CF3 performed best, followed by NO2, F CH3, H OCH3, OCH2CH3, and OH. Prior studies have mostly focused on substituted 1,4- and 1,5-diaryl and 1,2,3-triazoles with a pharmacophore comprising SO2CH3 in the para position. Furthermore, as compared to the –SCH3 group, the inclusion of –SO2CH3 significantly increased selectivity [112].

Several studies and experiments have been carried out using a range of approaches and procedures in order to create innovative, potent, and selective COX-2 inhibitors. The 1, 5-substituted tetrazole was used to make a variety of molecules. A tricyclic with a central tetrazole core and two substituted heterocycles were used to make two series. Sulfonamide and Methylsulfonyl substituted rings with the same third ring in both series bound to different functional groups make up the final series of compounds. With IC_{50} values of 6 and 7 M for 4h and 6h compounds, respectively, all produced inhibitors inhibited COX-2 to a lesser level than Celecoxib, all other inhibitors had an IC_{50} of 100M [113].

Another study generated tri aryl pyrazoline derivatives by keeping the pharmacophore-containing sulfonyl or/and sulfamoyl ring while changing the second ring and substituting the third ring. Compounds 13e, 13i, and 13h were moderately selective and active against COX-2. The most efficient compound was 13i, which had anti-inflammatory activity comparable to Celecoxib [114].

A library of pyrazoline derivatives was created in a previous work. A diaryl with one of the phenyl groups replaced by a methyl sulfone group and the other by a different group is connected to a pyrazoline core with a 1N substitution. Compounds comprising 4-methyl, 4-methoxy, 2-phenyl methoxy, and 4-Cl have the highest COX-2 selectivity [115].

1.6 Objective of the study

The main focus of our study is on introducing chemical diversity into the molecular framework to synthesize active molecules with a wide range of compositions. Specifically:

1. Synthesize library of COX-1 and COX-2 inhibitors.
2. Characterize the physical and chemical proprieties of the novel synthesized derivatives.
3. *In vitro* testing on COX-1 and COX-2 enzymes screening kits.
4. *In vitro* testing on HeLa cells and determining the cytotoxicity of each product.
5. Discover a novel compounds with potent inhibitory activities against COX enzymes.

Chapter Two

Methodology

2.1 Reagents and materials

All reagents were purchased from Sigma-Aldrich, C.S Chemicals Company and Alfa Aesar and use without further purification. 2-Iodobenzoic acid (catalog # STBH3016), 4-Iodobenzoic acid (catalog # BCBX0184), 2-Bromobenzoic acid (catalog # STBH3169), 3,4-(Methylenedioxy)-phenylacetic acid (catalog # 10206812), 3-Bromobenzoic acid (catalog #B17Q36), 2,4-Dichlorobenzoic acid (catalog # S34634), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (catalog # A10807), COX inhibitor screening assay kit No. 560131 (Cayman Chemical, USA).

Acetone, methanol (MeOH), ethanol (EtOH), dichloromethane (DCM), Ethyl acetate (EtOAc), and chloroform ($CHCL_3$) were purchased from C.S Chemicals Company.

2.2 Instrumentation

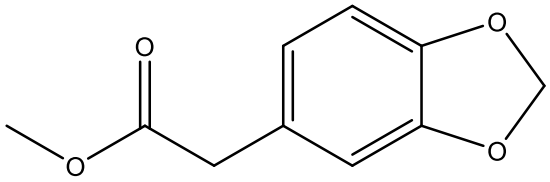
Melting points were determined with an SMP-II digital Melting Point Apparatus. Perkin Elmer spectrum 400 FTIR/FTNR spectrometer was used to obtain IR spectra. Two NMR instruments were used to perform ^{1}H-NMR and ^{13}C-NMR spectra. The first was a Burker 500MHz-Avance III High-Performance Digital FT-NMR spectrometer at the Faculty of Science, University of Jordan, Jordan. The second was Burker 300 MHz-Avance III High-Performance Digital FT-NMR spectrometer at the NMR facility at the Doping and Narcotics Analysis Laboratory of the Faculty of Pharmacy, Anadolu University, Turkey. DMSO-d6 was used as a solvent in both instruments, and Tetramethylsaline was the internal standard. All chemical shifts were recorded as d (ppm). High-resolution mass spectra data (HRMS) were collected using a water LCT premier XE Mass spectrometer using the ESI (+) method at Pharmacy Faculty Gazi University Ankara-Turkey. COX inhibitor screening assay kit NO.560131 (Cayman Chemical, USA) was used to determine the inhibitory activity of ovine COX-1 and human recombinant COX-2 enzyme. The yellow product of this enzymatic reaction is determined using a UV spectrophotometer with a Microplate reader (BioRad japan) at wavelength 415nm.

2.3 Synthesis and biological evaluation of products

All synthetic procedures and enzyme screening tests were conducted at An-Najah National University laboratories.

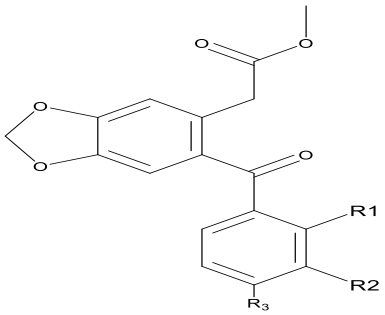
2.4 General synthetic procedures

2.4.1 Synthesis of methyl 2-(2*H*-1,3-benzodioxol-5-yl) acetate (compound 1)



In Clean round bottom flask 3-4-(methylendioxy)phenylacetic acid (8g, 44.40 mmol) was dissolved in methanol, then was cooled in an ice bath to 0 °C, then oxalyl chloride (4ml, 46.80mml) was added dropwise, and the reaction mixture was stirred for 30-45 min. The reaction mixture was then evaporated to dryness under vacuum, diluted with ethyl acetate and washed with saturated sodium bicarbonate ($NaHCO_3$) and distilled water. The organic layer was dried with sodium sulphate, filtered then evaporated again. The concentrated product was then purified by silica gel column chromatography by using hexane: ethyl acetate solvent system (50%:50%). Compound **1** was a yellow oil and the yield was 94%.

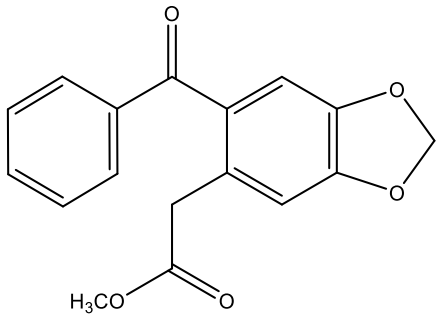
2.4.2 General procedure for ketoester (3a-3f) synthesis



In Clean round bottom flask, benzoic acid derivatives (1.46 g, 6.68 mmol) and phosphorus pentoxide (5 g) were added to a stirred solution of dichloromethane (60 mL) and compound 1 (1 g, 5.14 mmol). Then the mixture was stirred at room temperature for 18 h, before distilled water (60 mL) was cautiously added and the mixture extracted with ethyl acetate twice (60 mL) then the organic layer was separated and then treated

with 1M NaOH (60 mL), brine (60 mL) and distilled water twice (60 mL). The organic layer was dried with sodium sulphate, filtered and evaporated under vacuum pressure, and then purified by different concentration of Hexane Ethyl acetate solvent systems of silica gel column chromatography [118, 119].

2.4.2.1 Synthesis of Methyl 2-(6-benzoylbenzo[d] [1,3] dioxol-5-yl) acetate (compound 3a)



3a

Crude yellow semi-solid with **R_f:** 0.83 (Hex: EtOAc 3:2). The percentage yield is 75%.

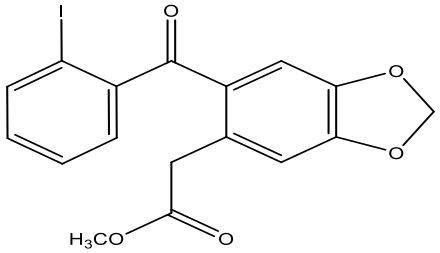
HRMS (m/z): $[M+H]^+$ calcd. For $C_{17}H_{15}O_5$, 299.0919, found. 299.0919.

IR (FTIR/FTNIR-ATR): 1737 cm-1 ester carbonyl (C=O), 1661 cm-1 keton carbonyl (C=O).

^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 7.62-7.67 (3H, m, Ar-H), 7.52 (2H, t, *J* = 7.8 Hz, Ar-H), 7.05 (1H, s, Ar-H), 6.89 (1H, s, Ar-H), 6.12 (2H, s, O-CH_2-O), 3.74 (2H, s, -CH_2-C=O), 3.47 (3H, s, O-CH_3).

^{13}C-NMR (DMSO-d_6, 300 MHz) δ ppm: 196.53, 171.61, 149.66, 146.04, 138.08, 133.39, 131.59, 130.21, 130.00, 129.73, 129.44, 128.95, 112.66, 110.34, 102.45, 51.89, and 38.36. See Appendix A for the spectrum.

2.4.2.2 Synthesis of Methyl 2-(6-(2-iodobenzoyl)benzo[d][1,3]dioxol-5-yl)acetate (compound 3b)



3b

Sticky semi-solid product with $\mathbf{R_f}$: 0.74 (Hex: EtOAc 1:1). The percentage yield is 90%.

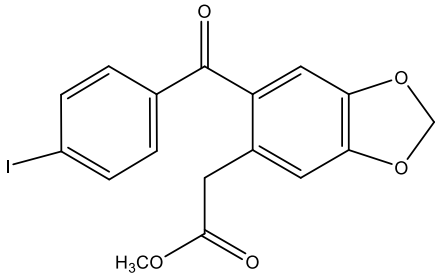
HRMS (m/z): $[M+H]^+$ calcd. For $C_{17}H_{14}IO_5$, 424.9886, found. 424.9875.

IR (FTIR/FTNIR-ATR): 1740 cm-1 ester carbonyl (C=O), 1659 cm-1 keton carbonyl (C=O).

^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 7.95 (1H, d, *J* = 7 Hz, Ar-H), 7.51 (1H, t, *J* = 7.5 Hz, Ar-H),7.23-7.30 (2H, m, Ar-H), 7.11 (1H, s, Ar-H), 6.64 (1H, s, Ar-H), 6.13 (2H, s, O-CH_2-O), 3.92 (2H, s, -CH_2-C=O), 3.59 (3H, s, O-CH_3).

^{13}C-NMR (DMSO-d_6, 300 MHz) δ ppm: 197.19, 171.49, 151.23, 146.51, 145.12, 139.78, 134.05, 133.30, 131.97, 128.95, 128.60, 113.60, 112.03, 102.93, 93.46, and 51.98. See Appendix A for the spectrum

2.4.2.3 Synthesis of Methyl 2-(6-(4-iodobenzoyl)benzo[d][1,3]dioxol-5-yl)acetate (compound 3c)



3c

Powder product with $\mathbf{R_f}$: 0.35 (Hex: EtOAc 1:1) and M.P. 119-121 ℃. The percentage yield is 87%.

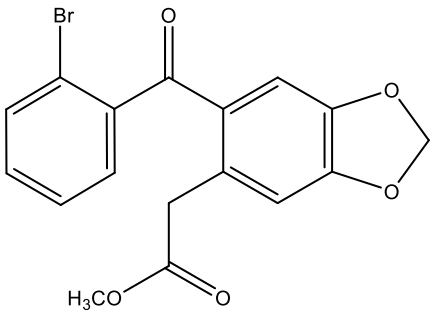
HRMS (m/z): $[M+H]^+$ calcd. For $C_{17}H_{14}IO_5$, 424.9886, found. 424.9880.

IR (FTIR/FTNIR-ATR): 1735 cm-1 ester carbonyl (C=O), 1660 cm-1 keton carbonyl (C=O).

^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 7.92 (2H, d, *J* = 8.4 Hz, Ar-H), 7.41 (2H, d, *J* = 8.8 Hz, Ar-H), 7.06 (1H, s, Ar-H), 6.92 (1H, s, Ar-H), 6.13 (2H, s, O-CH_2-O), 3.75 (2H, s, -CH_2-C=O), 3.47 (3H, s, O-CH_3).

^{13}C-NMR (DMSO-d_6, 300 MHz) δ ppm: 195.94, 171.61, 149.79, 138.52, 137.89, 137.40, 131.94, 131.20, 130.14, 112.70, 110.38, 102.49, 102.05, 52.02, 38.29. See Appendix A for the spectrum

2.4.2.4 Synthesis of Methyl 2-(6-(2-bromobenzoyl)benzo[d][1,3]dioxol-5-yl)acetate (compound 3d)



3d

Powder product with $\mathbf{R_f}$: 0.47 (Hex: EtOAc 4:1) and M.P. 85-87 ℃. The percentage yield is 85%.

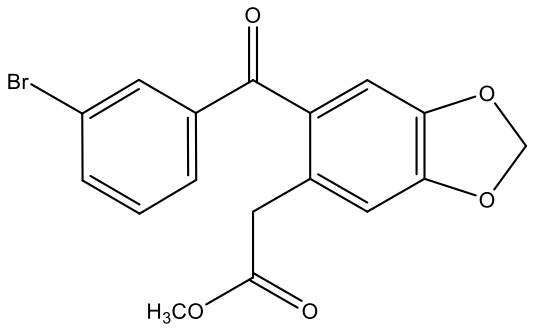
HRMS (m/z): $[M+H]^+$ calcd. For $C_{17}H_{14}BrO_5$, 377.0025, found. 377.0024.

IR (FTIR/FTNIR-ATR): 1740 cm-1 ester carbonyl (C=O), 1658 cm-1 keton carbonyl (C=O) **.**

^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 7.34-7.77 (4H, m, Ar-H), 7.11 (1H, s, Ar-H), 6.69 (1H, s, Ar-H), 6.14 (2H, s, O-CH_2-O), 3.93 (2H, s, -CH_2-C=O), 3.59 (3H, s, O-CH_3).

^{13}C-NMR (DMSO-d_6, 300 MHz) δ ppm: 196.53, 171.61, 149.66, 146.04, 138.08, 133.39, 131.59, 130.21, 130.00, 129.73, 129.44, 128.95, 112.66, 110.34, 102.45, 51.89, and 38.36. **See Appendix A for the spectrum**

2.4.2.5 Synthesis of Methyl 2-(6-(3-bromobenzoyl)benzo[d][1,3]dioxol-5-yl)acetate (compound 3e)



3e

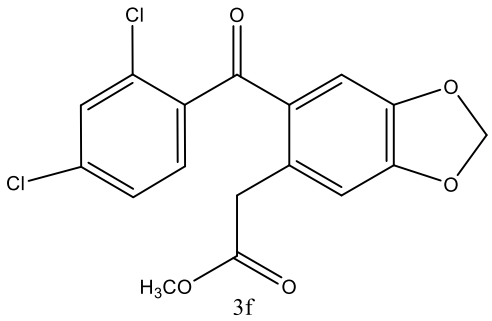
Powder product with **R_f**: 0.66 (Hex: EtOAc 3:2) and M.P. 72.5-74.5℃. The percentage yield is 79%.

HRMS (m/z): $[M+H]^+$ calcd. For $C_{17}H_{14}BrO_{5,}$ 377.0025, found. 377.0024.

IR (FTIR/FTNIR-ATR): 1742 cm-1 ester carbonyl (C=O), 1655 cm-1 keton carbonyl (C=O).

^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 7.86 (1H, d, *J* = 8 Hz, Ar-H), 7.77 (1H, s, Ar-H), 7.64 (1H, d, *J* = 8 Hz, Ar-H), 7.50 (1H, t, *J* = 8 Hz, Ar-H), 7.06 (1H, s, Ar-H), 6.95 (1H, s, Ar-H), 6.15 (2H, s, O-CH_2-O), 3.77 (2H, s, -CH_2-C=O), 3.49 (3H, s, O-CH_3). See Appendix A for the spectrum.

2.4.2.6 Synthesis of Methyl 2-(6-(2,4- dichlorobenzoyl) benzo[d] [1,3]dioxol-5-yl) acetate (compound 3f)



3f

Powder product with $\mathbf{R_f}$: 0.84 (Hex: EtOAc 1:1) and M.P. 95-97℃. The percentage yield is 83%.

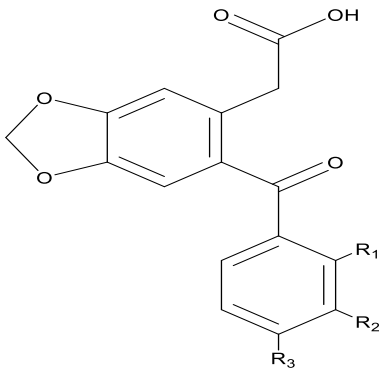
HRMS (m/z): $[M+H]^+$ calcd. For $C_{17}H_{13}Cl_2O_5$, 367.0140 , found.367.0140.

IR (FTIR/FTNIR-ATR): 1760 cm-1 ester carbonyl (C=O), 1633 cm-1 keton carbonyl (C=O).

^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 7.79-7.82 (2H, m, Ar-H), 7.59 (1H, d, *J* = 8.4 Hz, Ar-H), 7.07 (1H, s, Ar-H), 7.00 (1H, s, Ar-H), 6.14 (2H, s, O-CH_2-O), 3.78 (2H, s, -CH_2-C=O), 3.48 (3H, s, O-CH_3).

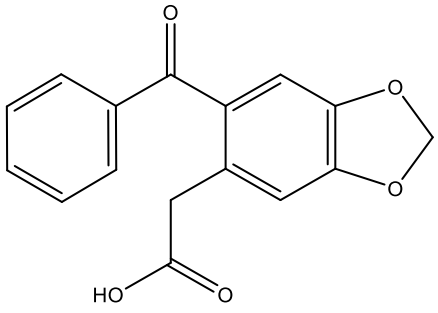
^{13}C-NMR (DMSO-d_6, 300 MHz) δ ppm: 194.29, 171.68, 150.13, 146.17, 138.56, 136.07, 131.70, 131.34, 130.62, 130.52, 130.33, 116.68, 112.82, 110.64, 102.06, 52.05, 38.33. See Appendix A for the spectrum

2.4.3 General procedure for 2-(6-benzoyl-2H-1,3-benzodioxol-5-yl)acetic acid (4a-4f) synthesis



The ketoesters 3a-3f (450 mg, 1.35 mmol) were dissolved in methanol/H_2O/THF (12/12/12 mL), then NaOH (540.9 mg, 13.5 mmol) was added. The solution was heated in an oil bath and refluxed for four h, before being cooled to room temperature. The solution was then evaporated, and the residue was made acidic by adding HCL 2 N (pH = 2). The precipitate was filtered and concentrated under vacuum to give the crude products 4a-4f.

2.4.3.1 Synthesis of 2-(6-benzoylbenzo[d][1,3]dioxol-5-yl)acetic acid (compound 4a)



4a

Powder product with **R_f**: 0.56 (Hex: EtOAc 3:2 + 500µl acetic acid) and M.P. 184.5-186.5℃. The percentage yield is 97%.

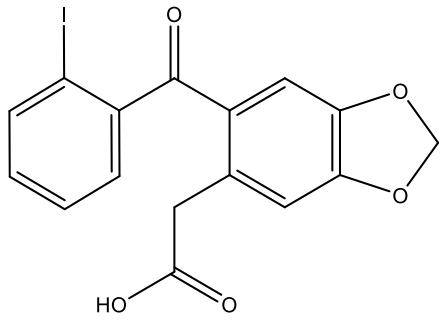
HRMS (m/z): $[M+H]^+$ calcd. For $C_{16}H_{13}O_5$, 285.0763, found. 285.0760.

IR (FTIR/FTNIR-ATR): 1770 cm-1 acetic acid carbonyl (C=O), 1655 cm-1 keton carbonyl (C=O).

^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 12.18 (1H, s, OH), 7.49-7.70 (5H, m, Ar-H), 7.03 (1H, s, Ar-H), 6.86 (1H, s, Ar-H), 6.11 (2H, s, O-CH_2-O), 3.67 (2H, s, -CH_2-C=O).

^{13}C-NMR (DMSO-d_6, 300 MHz) δ ppm: 96.64, 172.65, 149.48, 138.12, 133.68, 133.33, 131.73, 130.68, 130.26, 130.01, 129.20, 128.90, 112.62, 110.16, 102.32, 38.59. See Appendix A for the spectrum

2.4.3.2 Synthesis of 2-(6-(2-iodobenzoyl)benzo[d][1,3]dioxol-5-yl)acetic acid (compound 4b)



4b

Powder product with **R_f**: 0.60 (Hex: EtOAc 3:2 + 500µl acetic acid) and M.P 147-149 ℃. The percentage yield is 92%.

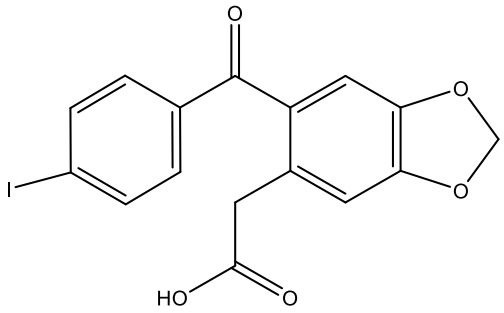
HRMS (m/z): $[M+H]^+$ calcd. For $C_{16}H_{12}IO_5$, 410.9730, found. 410.9721.

IR (FTIR/FTNIR-ATR): 1754 cm-1 acetic acid carbonyl (C=O), 1653 cm-1 keton carbonyl (C=O).

^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 7.95 (1H, d, *J* = 7.8 Hz, Ar-H), 7.50 (1H, t, *J* = 7.8 Hz, Ar-H), 7.23-7.31 (2H, m, Ar-H), 7.06 (1H, s, Ar-H), 6.61 (1H, s, Ar-H), 6.11 (2H, s, O-CH_2-O), 3.83 (2H, s, -CH_2-C=O).

^{13}C-NMR (DMSO-d_6, 300 MHz) δ ppm: 197.14, 172.60, 150.97, 146.17, 145.23, 139.81, 134.23, 131.97, 129.31, 129.14, 128.54, 113.48, 111.77, 102.75, 93.51. See Appendix A for the spectrum

2.4.3.3 Synthesis of 2-(6-(4-iodobenzoyl) benzo [d] [1,3] dioxol-5-yl) acetic acid (compound 4c)



4c

Powder product with $\mathbf{R_f}$: 0.69 (Hex: EtOAc 3:2 + 500µl acetic acid) and M.P 239.5-241.5℃. Percentage yield is 89%.

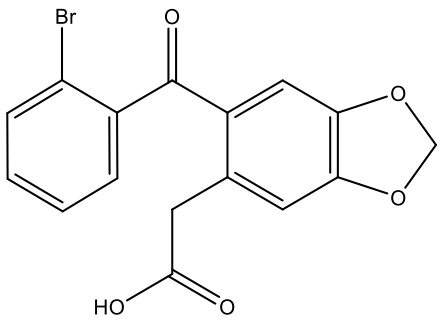
HRMS (m/z): $[M+H]^+$ calcd. For $C_{16}H_{12}IO_5$, 410.9730, found. 410.9728.

IR (FTIR/FTNIR-ATR): 1760 cm-1 acetic acid carbonyl (C=O), 1660 cm-1 keton carbonyl (C=O).

^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 12.20 (1H, s, OH), 7.91 (2H, d, *J* = 8.7 Hz, Ar-H), 7.42 (2H, d, *J* = 8.4 Hz, Ar-H), 7.03 (1H, s, Ar-H), 6.90 (1H, s, Ar-H), 6.11 (2H, s, O-CH_2-O), 3.67 (2H, s, -CH_2-C=O).

^{13}C-NMR (DMSO-d_6, 300 MHz) δ ppm: 196.04, 172.63, 149.60, 145.85, 138.14, 137.83, 137.44, 131.99, 131.30, 130.77, 112.63, 110.17, 102.37, 101.95, 38.56. See Appendix A for the spectrum.

2.4.3.4 Synthesis of 2-(6-(2-bromobenzoyl)benzo[d][1,3]dioxol-5-yl)acetic acid (compound 4d)



4d

Powder product with **R_f**: 0.54 (Hex: EtOAc 3:2 + 500µl acetic acid) and M.P 145-147℃. The percentage yield is 87%.

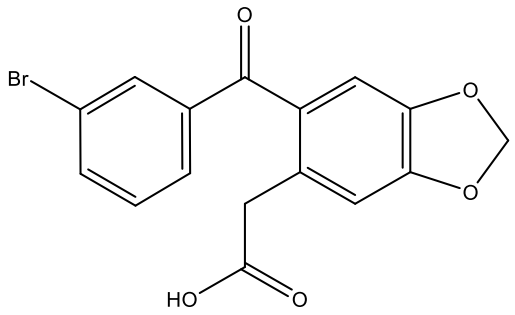
HRMS (m/z): $[M+H]^+$ calcd. For $C_{16}H_{12}BrO_5$, 362.9868, found. 362.9860.

IR (FTIR/FTNIR-ATR): 1766 cm-1 acetic acid carbonyl (C=O), 1664 cm-1 keton carbonyl (C=O).

^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 7.41-7.73 (4H, m, Ar-H), 6.96 (1H, s, Ar-H), 6.61 (1H, s, Ar-H), 6.07 (2H, s, O-CH_2-O), 3.68 (2H, s, -CH_2-C=O), 3.42 (1H, bs, O-H).

^{13}C-NMR (DMSO-d_6, 300 MHz) δ ppm: 195.59, 172.70, 150.48, 133.34, 132.50, 132.01, 130.31, 127.99, 119.14, 112.82, 112.45, 111.03, 105.10, 102.38, 101.57. See Appendix A for the spectrum

2.4.3.5 Synthesis of 2-(6-(3-bromobenzoyl)benzo[d][1,3]dioxol-5-yl)acetic acid (compound 4e)



4e

Powder product with **R_f**: 0.63 (Hex: EtOAc 3:2 + 500µl acetic acid) and M.P 154-156℃. The percentage yield is 96%.

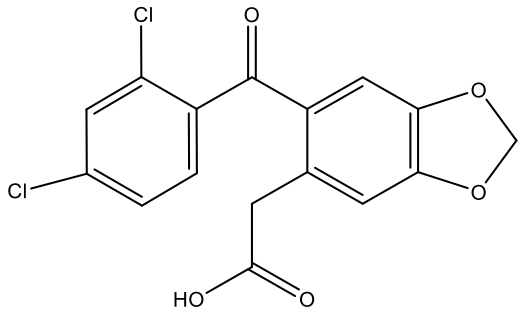
HRMS (m/z): $[M+H]^+$ calcd. For $C_{16}H_{12}BrO_5$, 362.9868, found. 362.9868.

IR (FTIR/FTNIR-ATR): 1759 cm-1 acetic acid carbonyl (C=O), 1658 cm-1 keton carbonyl (C=O).

^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 12.24 (1H, s, O-H) 7.75-7.78 (2H, m, Ar-H), 7.64 (1H, d, *J* = 8.1 Hz, Ar-H), 7.48 (1H, t, *J* = 8.1 Hz, Ar-H), 7.04 (1H, s, Ar-H), 6.92 (1H, s, Ar-H), 6.12 (2H, s, O-CH_2-O), 3.70 (2H, s, -CH_2-C=O).

^{13}C-NMR (DMSO-d_6, 300 MHz) δ ppm: :195.25, 172.72, 149.80, 145.88, 140.39, 135.85, 132.47, 131.17, 131.09, 131.04, 129.34, 122.12, 112.70, 110.31, 102.44, 38.58. See Appendix A for the spectrum

2.4.3.6 Synthesis of 2-(6-(2,4-dichlorobenzoyl)benzo[d][1,3]dioxol-5-yl)acetic acid (compound 4f)



4f

Solid product with **R_f**: 0.69 (Hex: EtOAc 1:1) and M.P 168.5-170℃. The percentage yield is 91%.

HRMS (m/z): [M+H]+ calcd. For $C_{16}H_{11}Cl_2O_5$, 352.9948, found. 352.9955.

IR (FTIR/FTNIR-ATR): 1768 cm-1 acetic acid carbonyl (C=O), 1657 cm-1 keton carbonyl (C=O).

^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 12.25 (1H, s, OH), 7.77-7.81 (2H, m, Ar-H), 7.60 (1H, dd, *J* = 8.3, 1.8 Hz, Ar-H), 7.05 (1H, s, Ar-H), 6.98 (1H, s, Ar-H), 6.13 (2H, s, O-CH_2-O), 3.71 (2H, s, -CH_2-C=O).

^{13}C-NMR (DMSO-d_6, 300 MHz) δ ppm: : 194.43, 172.73, 149.41, 145.94, 138.60, 135.99, 131.75, 131.29, 131.20, 130.74, 130.36, 129.84, 112.74, 110.40, 102.47, 38.56. See Appendix A for the spectrum

2.4.4 General procedure of performing column chromatography

A suitable column size was selected then column preparation was made by adding silica gel on the cotton capped column, followed by the sample infused in the silica gel. The gradient mobile phase optimized by TLC was used to dissolve the product. Tubes collection was finally made after elution for analysis.

2.4.5 Biological assay on COX enzyme screening kits

COX (human) Inhibitor Screening Assay Kit (supplied by Cayman chemicals (catalog # 560131) was used to assess the inhibitory effect of conversion of arachidonic acid PGH_2 by human recombinant COX-2 and bovine COX-1.

The synthesized product has a similar structure to Ketoprofen, so we used Ketoprofen as a positive control in the test.

The preparation of reagents and the testing procedures were performed as the manufacturer recommended. Three concentrations of the inhibitors and ketoprofen (50, 20, and 5 µM) were dissolved in dimethyl sulfoxide (DMSO) and incubated for 10 minutes at 37℃ with a mixture of COX-1 or COX-2 enzyme, Heme in the diluted reaction buffer. The reaction was initiated by adding 10µl of arachidonic acid and incubating for exactly thirty seconds at 37℃. Saturated stannous chloride solution (30µl) was added to stop the enzyme catalysis, followed by incubation at room temperature for 5 mins.

The produced prostaglandin was quantified by ELISA. The 96 –well plates were covered with plastic film and incubated for 18hr, at room temperature on an orbital shaker. After incubation, the plate was washed five times with wash buffer after emptying the wells, followed by adding 200µl of Ellman's reagent to each well and 5µl of tracer to the TA wells. The plate was incubated in the dark plate for 60-90 mins at room temperature until the absorbance of the B_0 well is in the range of 0.3-0.8 at 405 nm.

Unilab microplate reader 6000 was used to read the plate. The IC_{50} was calculated from the concentration inhibition response curve, and the selectivity index (SI) was calculated by dividing the IC_{50} COX-1 by the IC_{50} COX-2 [116]

2.4.6 Cell culture and cytotoxicity assay

HeLa Cervical Carcinoma was cultured in RPMI-1640 media and supplemented with 10% foetal bovine serum, 1% penicillin/streptomycin antibiotics, and 1% l-glutamine. The cells were grown in the humidified environment at 37℃ with a 5% CO_2 atmosphere, and then a 96-well plate was used to seed the cells at 2.6 x 10^4 cells/well. After 48h, the media was changed, and cells were incubated with different concentration

of tested compounds for 24h. Cell viability was assessed by CellTilter 96® aqueous one solution cell proliferation (MTS) assay according to the manufacturer's instructions (Promega Corporation, Madison, WI). Briefly, at the end of the treatment, 20µL of MTS solution per 100µL of media was added to each well and incubated at 37℃ for 2h. Absorbance was measured at 490nm [117].

Chapter Three

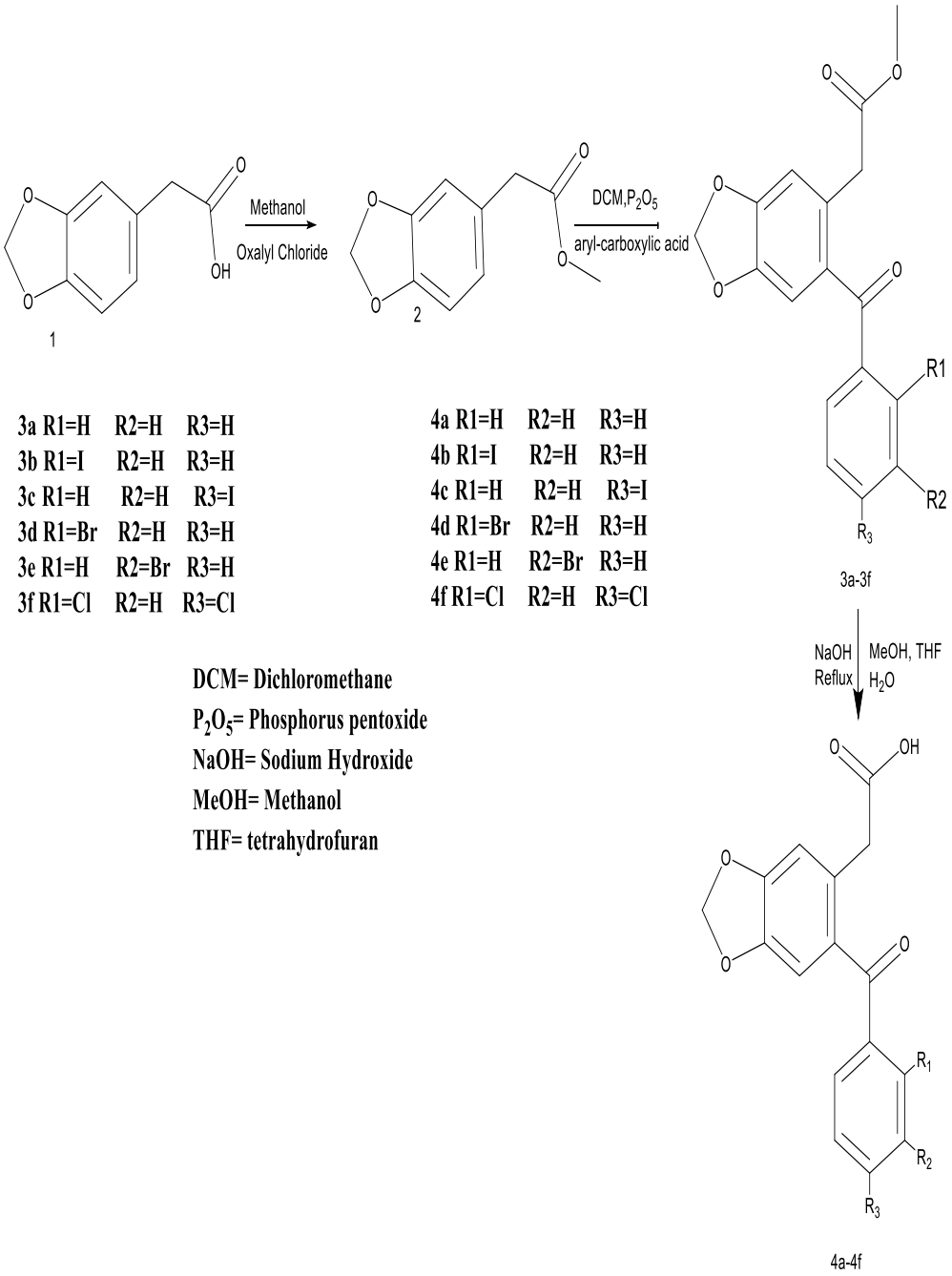
Results and Discussion

3.1 Chemistry

The benzodioxol aryl acetate derivatives (3a-3f) and acetic acid derivatives (4a-4f) were synthesized as shown in the scheme 1. The methyl 3-4(methylenedioxy) phenyl acetate compound 1 was generated by an esterification reaction of 3,4-(methylenedioxy) phenyl acetic acid. Oxalyl chloride was added dropwise to methanol and stirred for 30mins in an ice bath [118, 119]. The ketoesters 3a-3f was synthesized by dissolving the ester compound 1 in DCM with benzoic acid derivatives with an excess of phosphorous pentoxide, stirring at room temperature for approximately 18h. The Benzodioxole acetic acid derivatives 4a-4f were produced by hydrolysis reaction of the ester compounds 3a-3f using NaOH. All the synthesized products were purified by column chromatography [118].

Scheme 1

Reaction steps of the synthesis procedure.



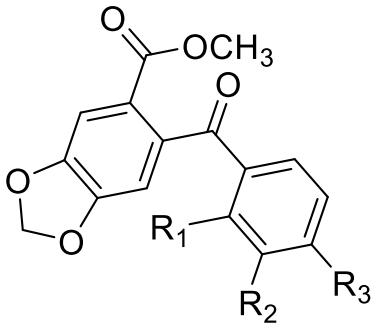
3a R1=H R2=H R3=H
3b R1=I R2=H R3=H
3c R1=H R2=H R3=I
3d R1=Br R2=H R3=H
3e R1=H R2=Br R3=H
3f R1=Cl R2=H R3=Cl

4a R1=H R2=H R3=H
4b R1=I R2=H R3=H
4c R1=H R2=H R3=I
4d R1=Br R2=H R3=H
4e R1=H R2=Br R3=H
4f R1=Cl R2=H R3=Cl

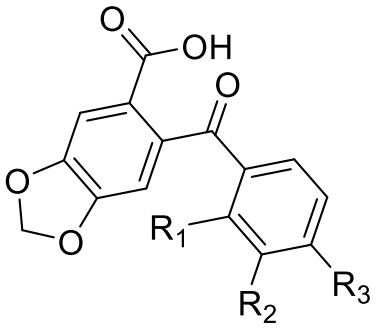
DCM= Dichloromethane
P_2O_5= Phosphorus pentoxide
NaOH= Sodium Hydroxide
MeOH= Methanol
THF= tetrahydrofuran

3.2 *In vitro* activity on COX-1 and COX-2 enzymes

Ketoprofen was used as positive control and three serial concentrations were tested (50, 20, and 5 µM) .The half-maximal inhibitory concentration (IC_{50}) and selectivity index (SI) were calculated, shown in Tables 1 and 2 for ketoesters and acetic acid, respectively.



(a)



(b)

(a) Ketoesters:

Table 3.1

Half-maximal inhibitory concentration (IC_{50}) and selectivity index (SI) for ketoesters compounds

Compound	R1	R2	R2	IC_{50} (COX-1) (µM)	IC_{50} (COX-2) (µM)	SI
3a	H	H	H	12.320	14.340	0.859
3b	I	H	H	1.120	1.300	0.862
3c	H	I	H	27.060	37.450	0.723
3d	Br	H	H	1.3	1.45	0.897
3e	H	Br	H	2.360	2.730	0.864
3f	Cl	H	Cl	5.180	4.100	1.263
Ketoprofen				0.031	0.158	0.196

P value <0.05

Standard deviation (σ) for IC_{50} COX-1 = 9.252

Standard deviation (σ) for IC_{50} COX-2 = 12.964

b) Acetic acid:

Table 3.2

half-maximal inhibitory concentration (IC_{50}) and selectivity index (SI) for acetic acid compounds

Compound	R1	R2	R2	IC_{50} (COX-1) (µM)	IC_{50} (COX-2) (µM)	SI
4a	H	H	H	1.450	3.340	0.434
4b	I	H	H	7.670	30.700	0.250
4c	H	I	H	33.700	39.140	0.861
4d	Br	H	H	4.250	2.350	1.809
4e	H	Br	H	7.110	49.300	0.144
4f	Cl	H	Cl	0.725	4.29	0.169
Ketoprofen				0.031	0.158	0.196

P value <0.05

Standard deviation (σ) for IC_{50} COX-1 = 11.279

Standard deviation (σ) for IC_{50} COX-2 = 17.808

All Benzodioxole acetate structures with halogen (Br, CL, I; 3b-3f) on the phenyl ring showed better activity against COX_1 (IC_{50} 1.12-27.06 µM) than the acetic acid benzodioxole with the same halogen (IC_{50} 4.25-33.7 µM; 4b-4e), except 4f had the most potent inhibitory activity (IC_{50} 0.725 µM) on COX_1. On the other hand, the acetic acid benzodioxole without substitutions (4a) had a stronger inhibitory effect against COX_1 and COX_2 (1.45 and 3.34 µM, respectively) than their acetate rival (3a) (12.32 and 14.34 µM, respectively). However, all halogenated benzodioxole acetate structures (3b-3f) showed better activity against COX_2 (IC_{50} 1.30-37.45 µM) than halogenated benzodioxole acetic acid (IC_{50} 2.35-39.14 µM: 4b-4f).

When the Halogen (Br or I) moved from the meta-position to the ortho-position (3b-3c, 3d-3e, 4b-4c & 4d-4e) it resulted in increase in the activity of both COX-1 and COX-2 inhibition activity .The activity inhibition for most compounds towered COX1 and COX2 were close relatively to ketoprofen but the selectivity ratio were better than Ketoprofen.

3.3 In vitro evaluation of cytotoxicity

HeLa (Cervical carcinoma cells) was used in the MTS assay to determine the cytotoxic effect of benzodioxole derivatives. Four different concentrations were used (2, 1, 0.5, and 0.1 mM) to do this test. Tables 3 and 4 show the half-maximal cytotoxic concentration (CC_{50}) for ketoesters and acetic acid, respectively.

(a) Ketoesters:

Table 3.3

half maximal cytotoxic concentration (CC_{50}) for ketoesters compounds

Compound	R1	R2	R2	HeLa Cell CC_{50} in mM
3a	H	H	H	1.49
3b	I	H	H	0.228
3c	H	I	H	1.79
3d	Br	H	H	1.61
3e	H	Br	H	0.219
3f	Cl	H	Cl	0.949

Standard deviation (σ) for CC_{50} = 0.636

(b) Acetic acid:

Table 3.4

half maximal cytotoxic concentration (CC_{50}) for acetic acid compounds

Compound	R1	R2	R2	HeLa Cell CC_{50} in mM
4a	H	H	H	1.94
4b	I	H	H	0.697
4c	H	I	H	1.049
4d	Br	H	H	0.547
4e	H	Br	H	0.437
4f	Cl	H	Cl	1.019

Standard deviation (σ) for CC_{50} = 0.497

The above results showed that all compounds have inhibition of cell growth at relatively high concentrations meaning that we need minimum 5folds of the IC_{50} concentration of COX enzyme.

By taking these results we can see that 4f is the most ideal compound in our library.

In the present research the results revealed that the library have inhibition activity against COX enzymes better than some tricyclic compounds synthesized by other researchers such as Caliskan et. al, his most active compound was one of pyrazole-3-propanoic acid derivatives with selectivity ration 0.93 and activity against COX-1 and COX-2 with IC_{50} very close to our results (1.5 and 1.6 µM, respectively). On the other hand, our synthesized product were very close or even better than their most other tested compounds [120].

Conclusion

In this thesis we can see that the library have inhibition activity against COX enzymes better than some tricyclic compounds synthesized by other researchers such as Caliskan et. al, his most active compound was one of pyrazole-3-propanoic acid derivatives with selectivity ration 0.93 and activity against COX-1 and COX-2 with IC_{50} very close to our results (1.5 and 1.6 µM, respectively). On the other hand, our synthesized product were very close or even better than their most other tested compounds [120].

Promising Benzodioxole structures were synthesized in simple reactions that showed moderate activity against COX_1 and COX_2 enzymes. Most of the compounds showed better selectivity on COX_2 than Ketoprofen. This difference may be due to the bigger moiety of 1,2-methylenedioxybenzene than the phenyl moiety in the Ketoprofen. Despise that all compounds **3a-4f** showed cytotoxic activity on HeLa cells at high doses, the effect dose towards COX enzyme was at least lesser 10 times greater than the cytotoxic concentrations.

Limitation and recommendation

Synthesizing more analogues with different substitutions is recommended in further studies and docking studies that will help us study the structure-activity relationship to achieve a better COX_2 selectivity.

List of Abbreviation

Abbreviation	Meaning
$NaNO_2$	Sodium nitrite
NaN_3	Sodium azide
Hr/s	Hour or Hours
H_2O	Water
DW	Distilled water
HCl	Hydrochloride
IC_{50}	The half maximal Inhibitory concentration
µM	Micro molar
SI	Selectivity index
DMAP	4-Dimethylaminopyridine
Hz	Hertz
MHz	Mega Hertz
MeOH	Methanol
Min	Minutes
RT	Room Temperature
MW	Molecular weight
NIR	Near infrared
NMR	Nuclear Magnetic Resonance
HRMS	High resolution mass spectroscopy
^{o}C	Celsius Degree
µl	Microliter
Mmole	Millimole
mg	Milligram
G	Gram
pH	Power of hydrogen
TLC	Thin layer chromatography
UV-Vis	Ultraviolet-Visible
λ_{max}	Lambda max
R_f	Retention factor
Rpm	Round per minute
TA	Total activity
Blk	Blank
NSB	Nonspecific binding
S	Standard
C	Concentration
millimolar	mM
CC	Cytotoxic Concentration
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium

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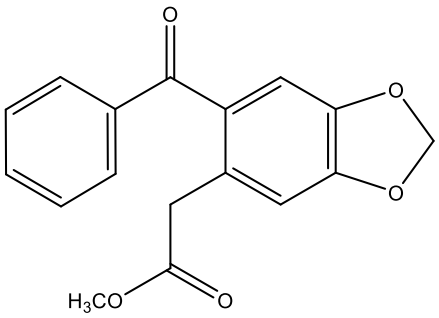
Appendices

Appendix A

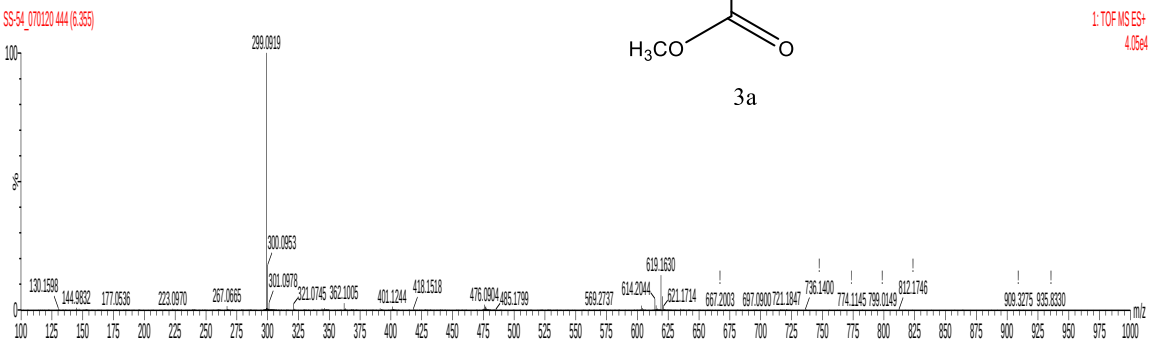
HRMS, 1 H-NMR, and 13 C-NMR spectra

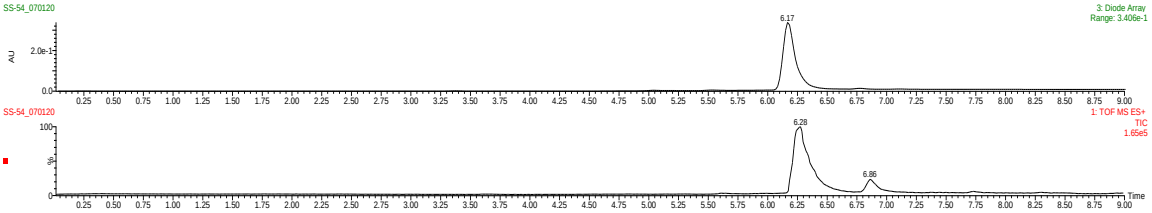
3a

HRMS

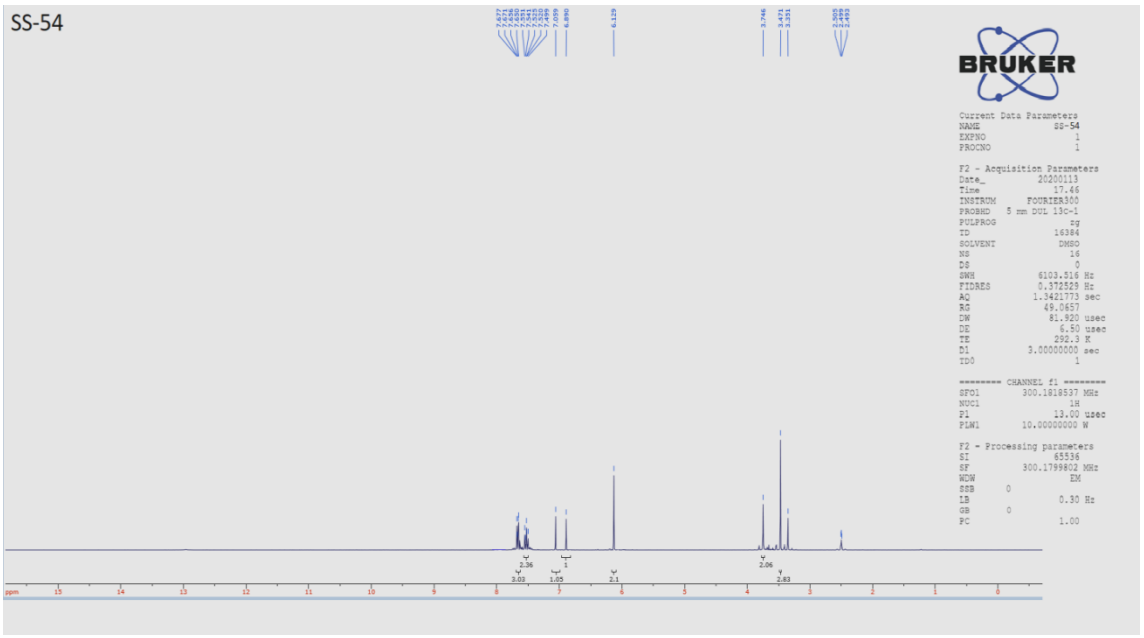


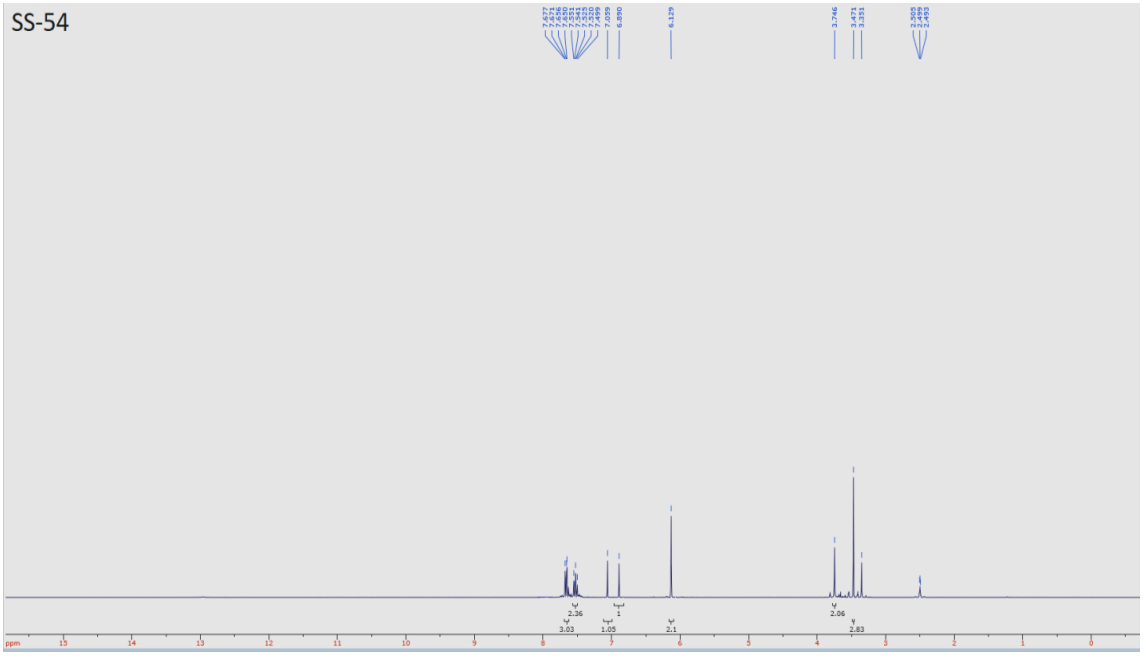
3a



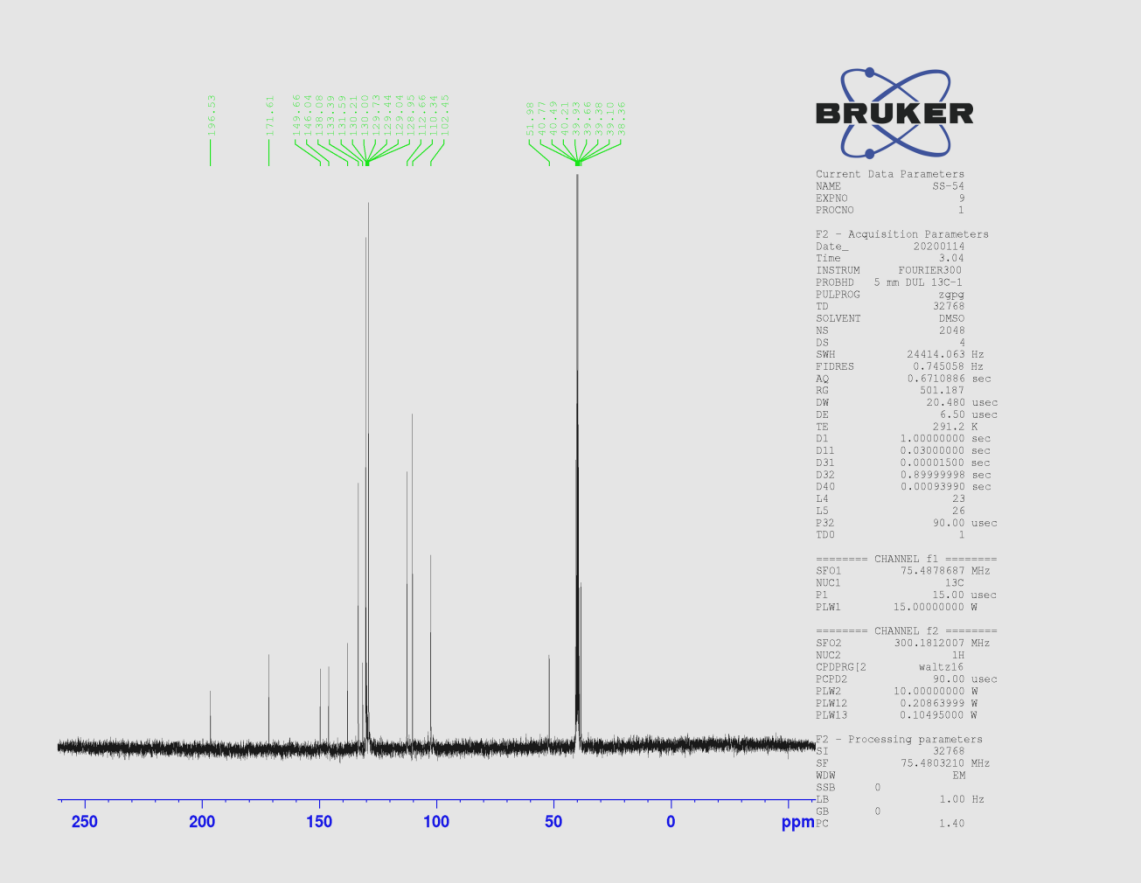


H-NMR





C-NMR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146.04
138.08
133.39
131.59
130.21
130.00
129.73
129.44
129.04
128.95
112.66
110.34
102.45
51.98
40.77
40.49
40.21
39.93
39.66
39.38
39.10
38.36
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SWH 24414.063 Hz
FIDRES 0.745058 Hz
AQ 0.6710886 sec
RG 501.187
DW 20.480 usec
DE 6.50 usec
TE 291.2 K
D1 1.00000000 sec
D11 0.03000000 sec
D31 0.00001500 sec
D32 0.89999998 sec
D40 0.00093990 sec
L4 23
L5 26
P32 90.00 usec
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NUC1 13C
P1 15.00 usec
PLW1 15.00000000 W
======== CHANNEL f2 ========
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NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 10.00000000 W
PLW12 0.20863999 W
PLW13 0.10495000 W
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LB 1.00 Hz
GB 0
PC 1.40
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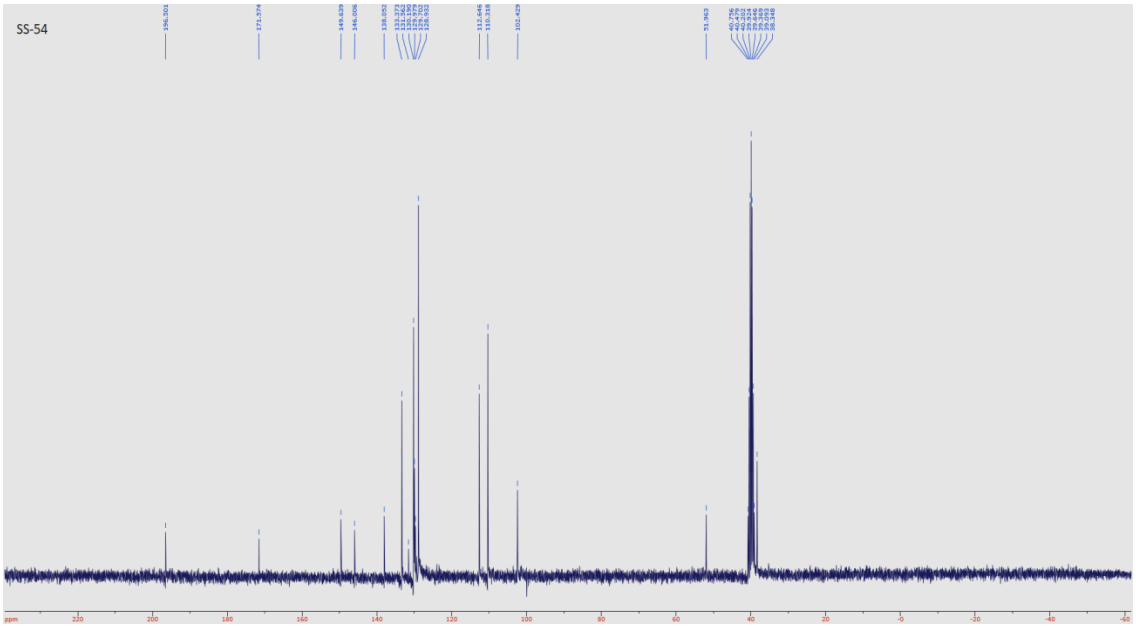
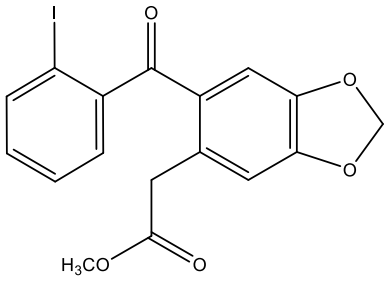
150
100
50
0
ppm

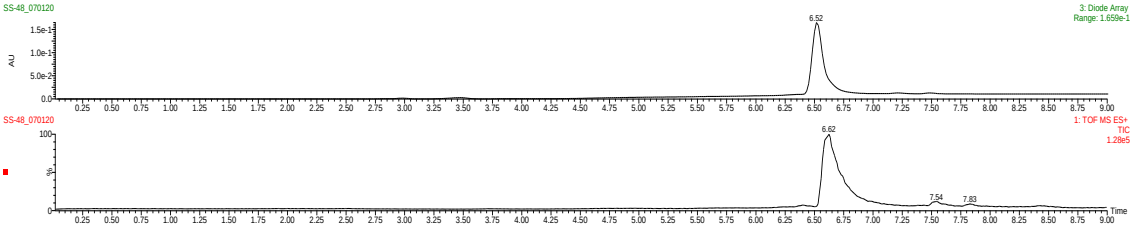

SS-54

3b


I
O
O
O
H3CO
O

3b

HRMS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AU
SS-48_070120
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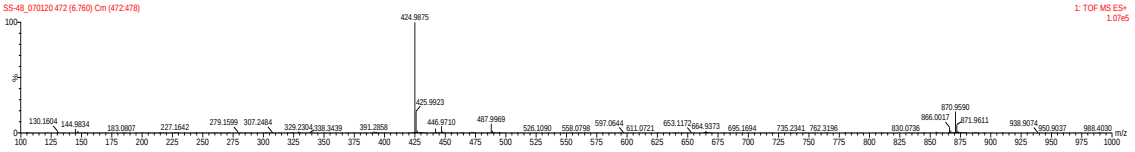
TIC
1.28e5
6.62
7.54
7.83
%
Time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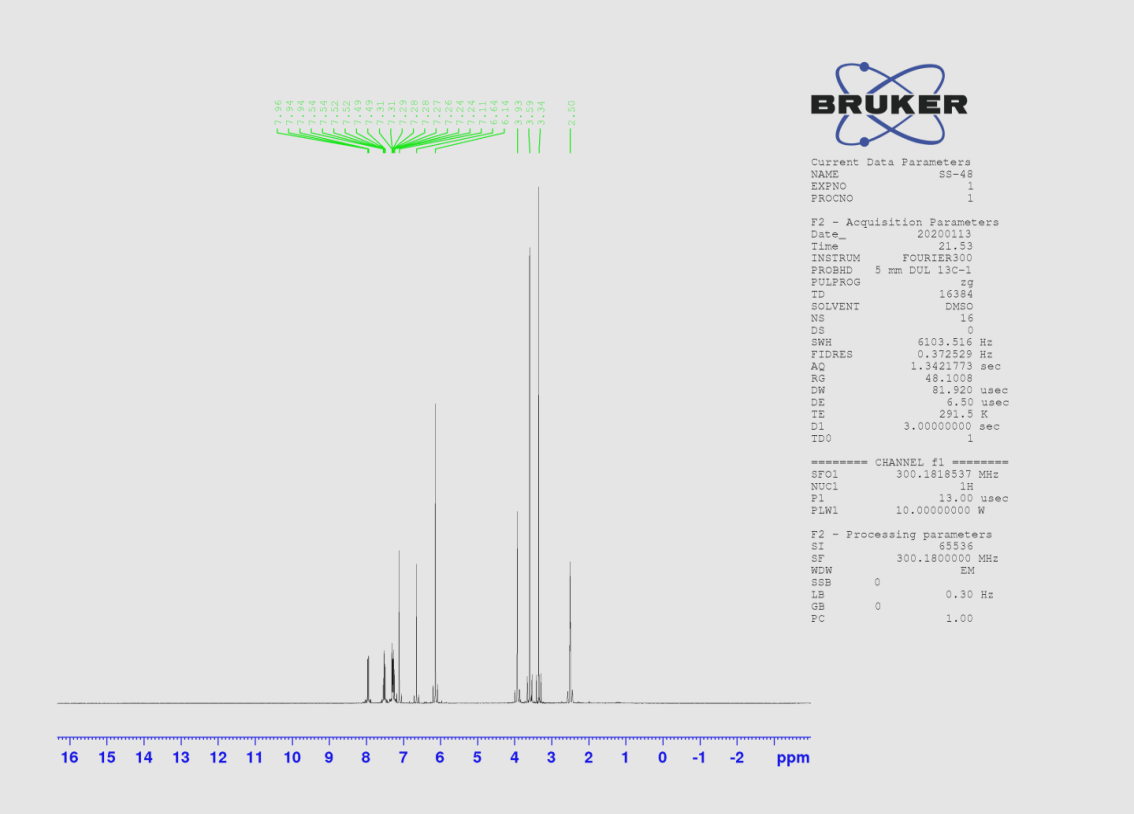
%
m/z

H-NMR


7.96
7.94
7.94
7.54
7.52
7.49
7.31
7.31
7.28
7.28
7.27
7.26
7.24
7.24
7.11
6.64
6.14
3.93
3.53
3.34
2.50
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DE 6.50 usec
TE 291.5 K
D1 3.00000000 sec
TD0 1
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NUC1 1H
P1 13.00 usec
PLW1 10.00000000 W
F2 - Processing parameters
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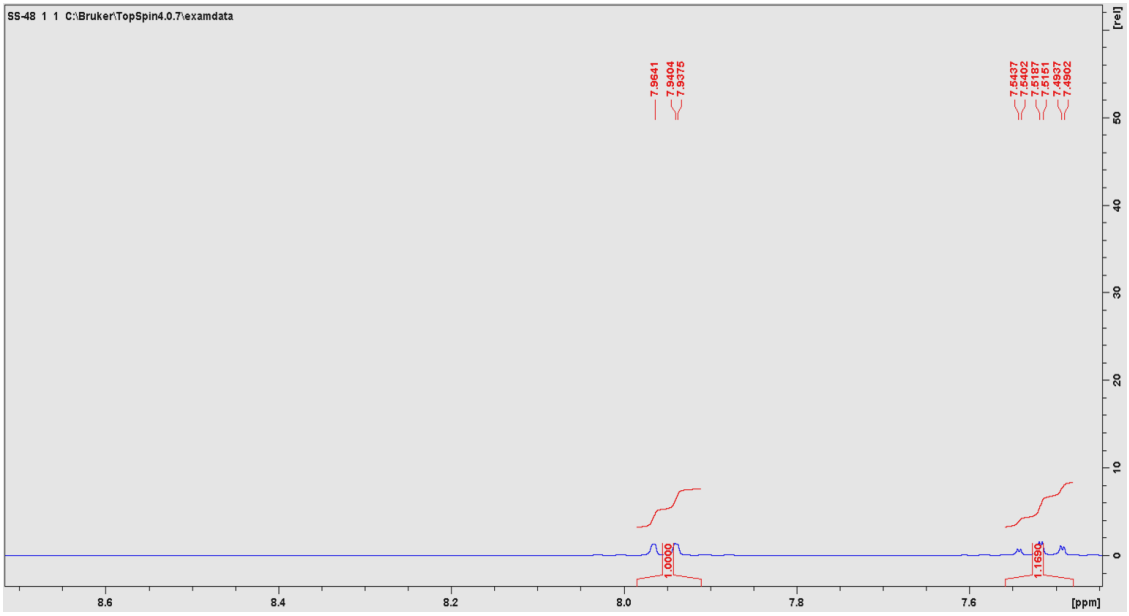
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7.5151
7.4937
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8.6
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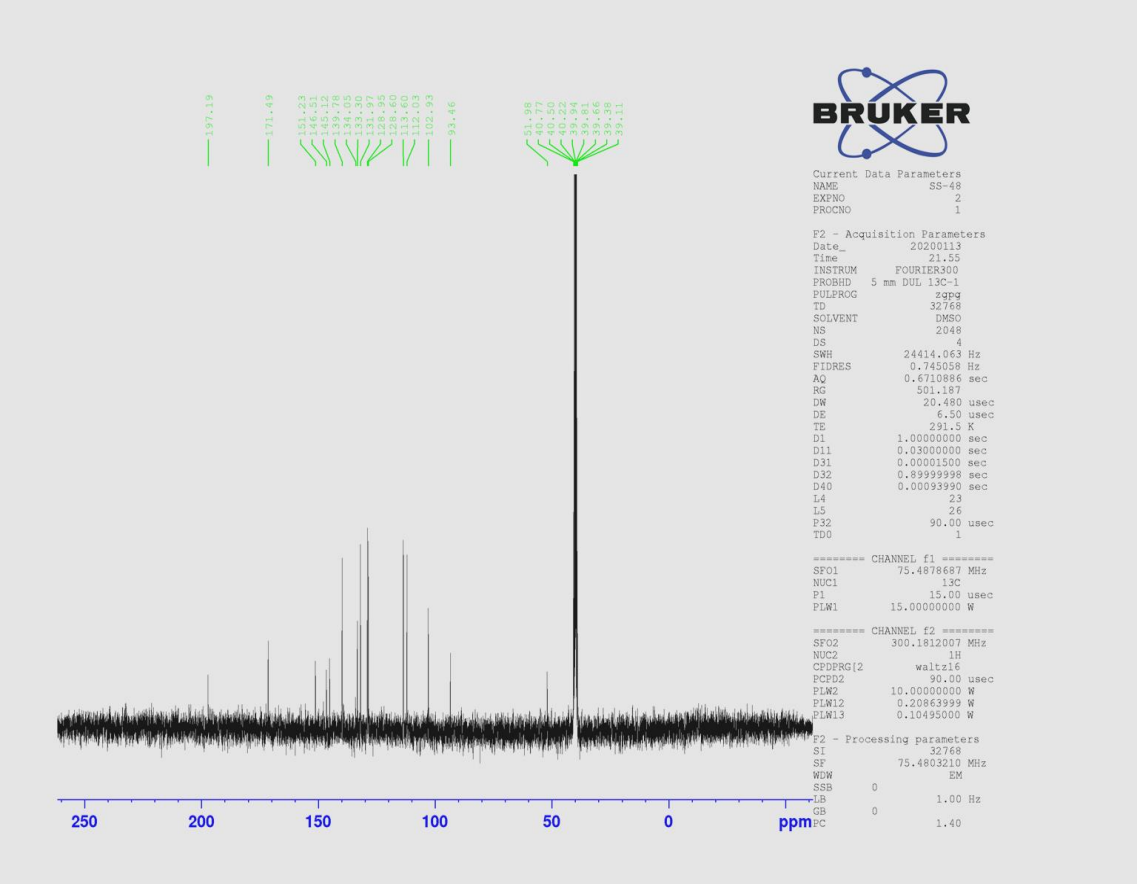
8.2
8.0
7.8
7.6
[ppm]
[rel]

C-NMR



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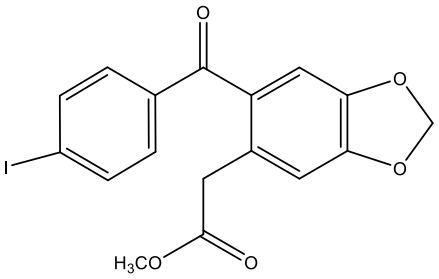
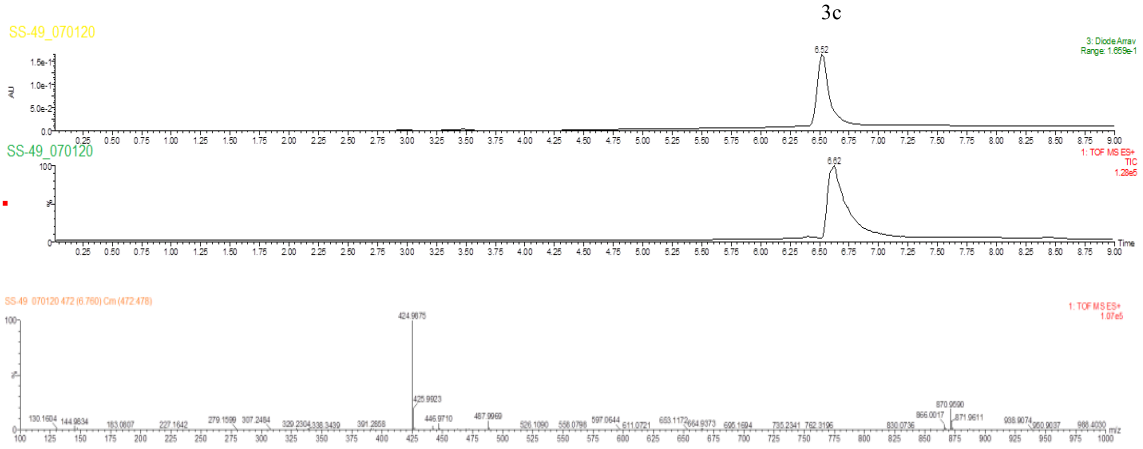


HRMS

3c



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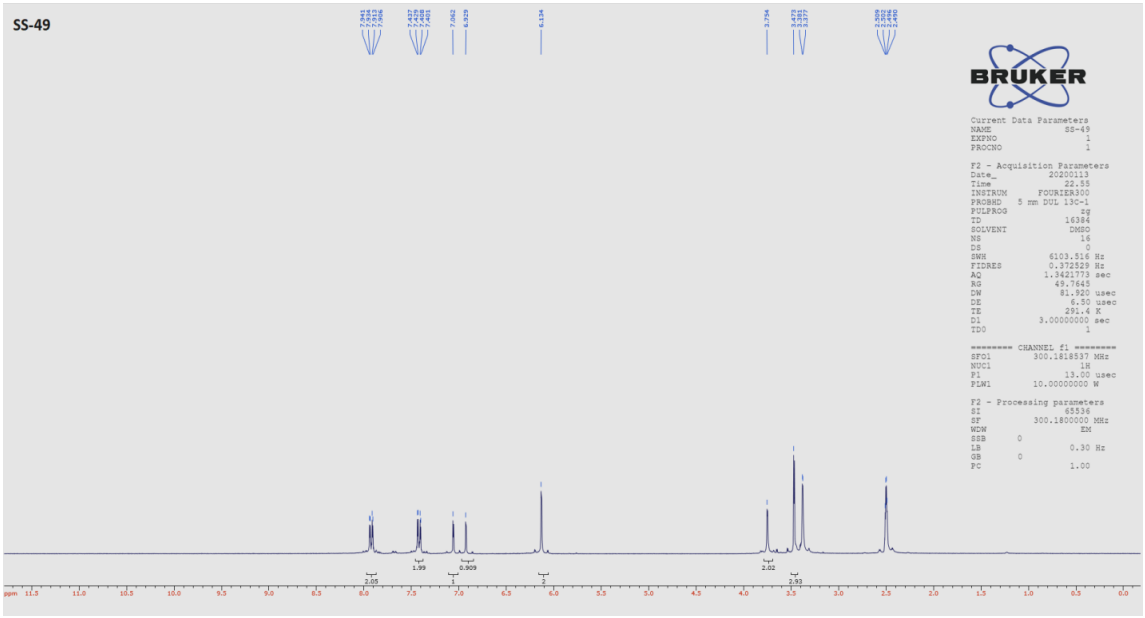
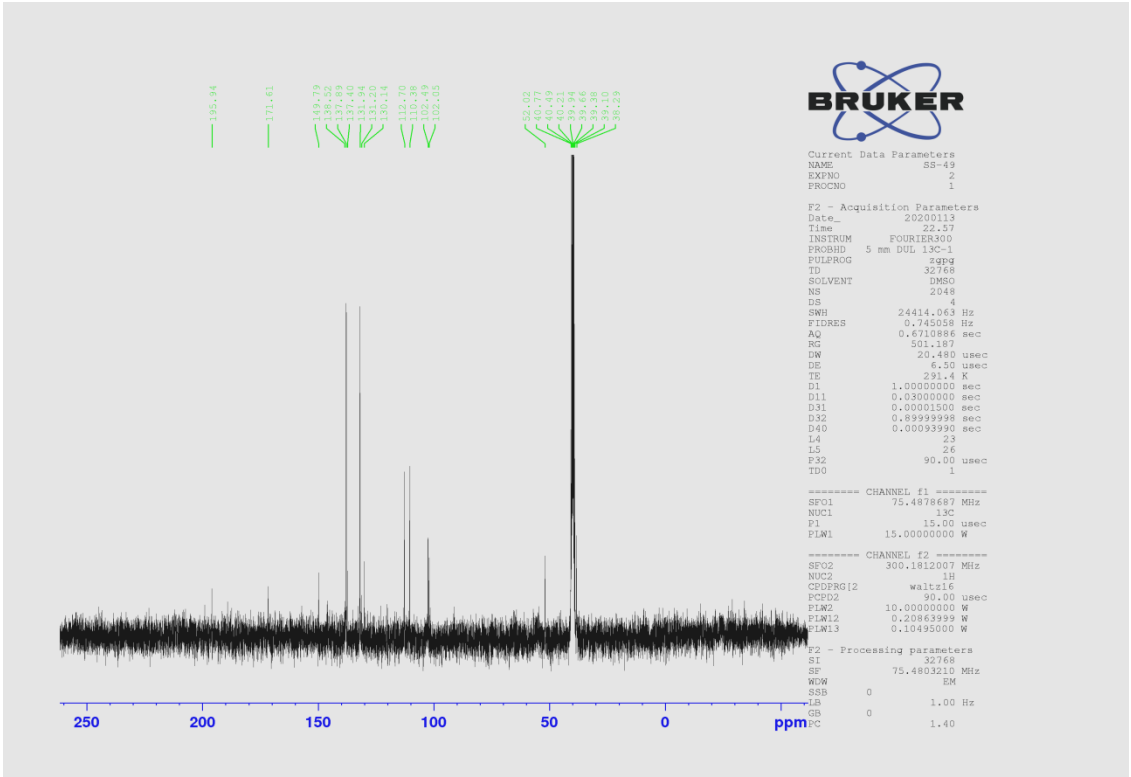


C-NMR



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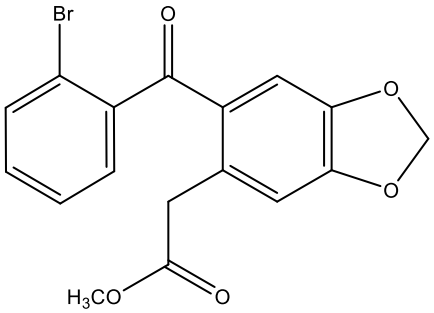

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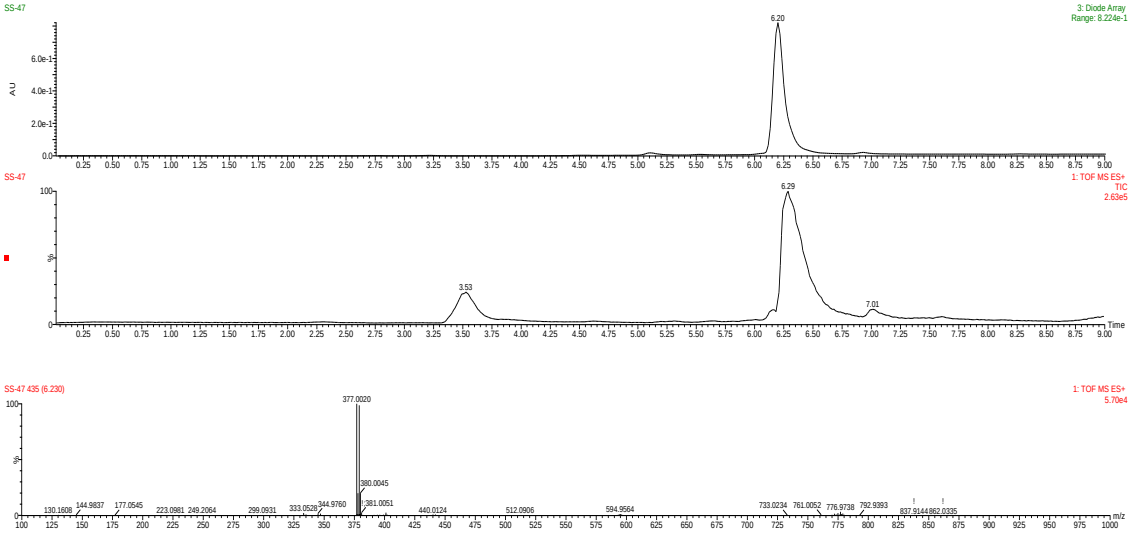

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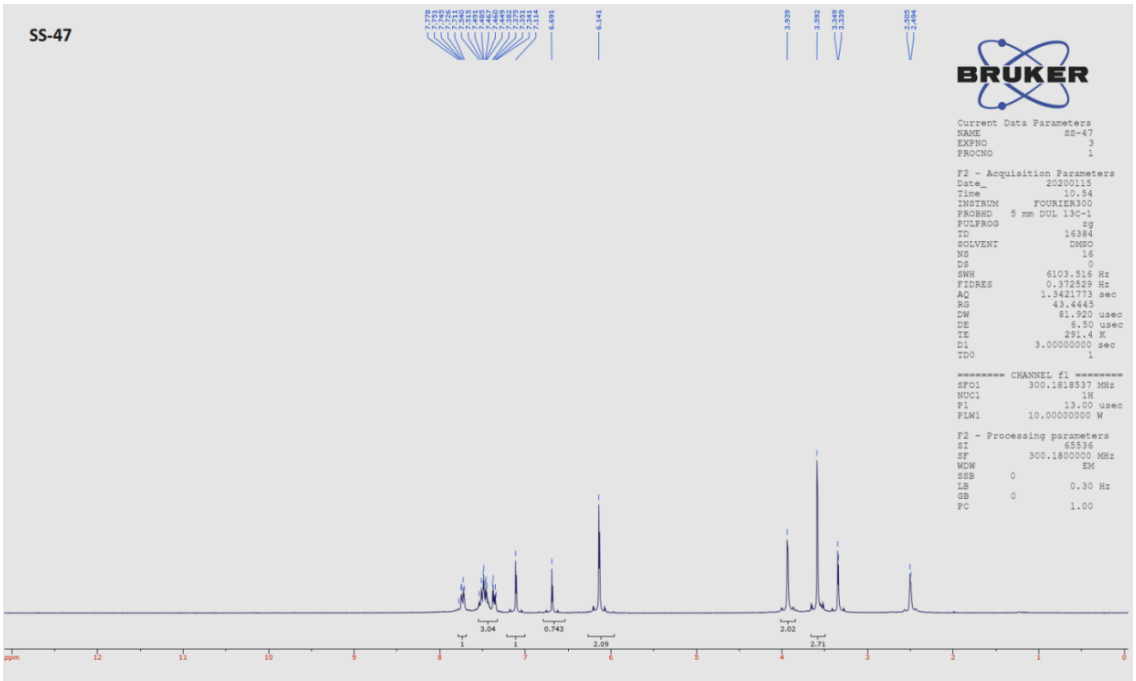

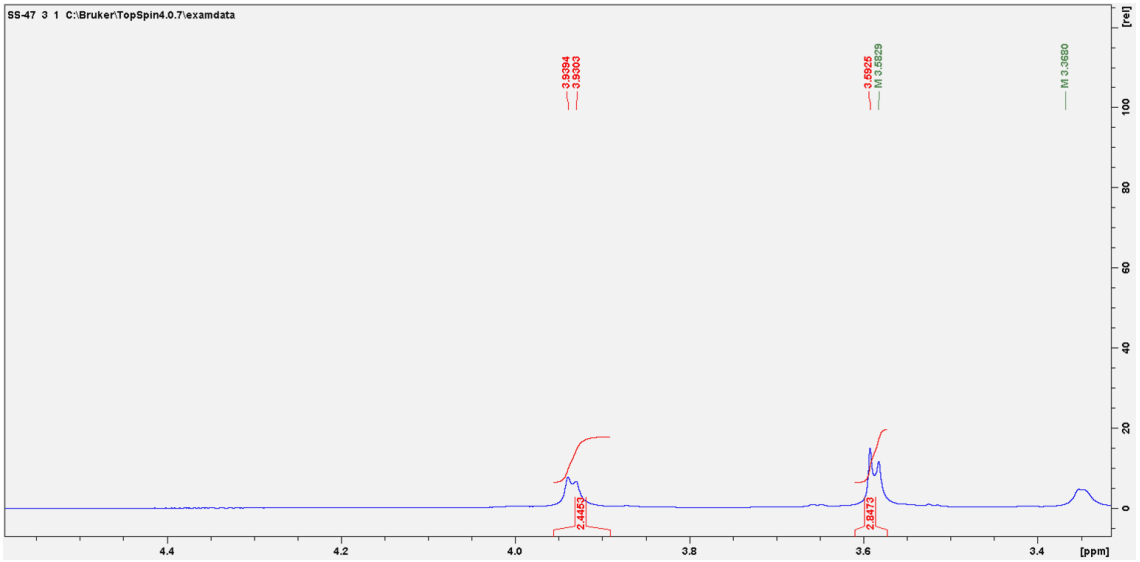
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M 3.3680
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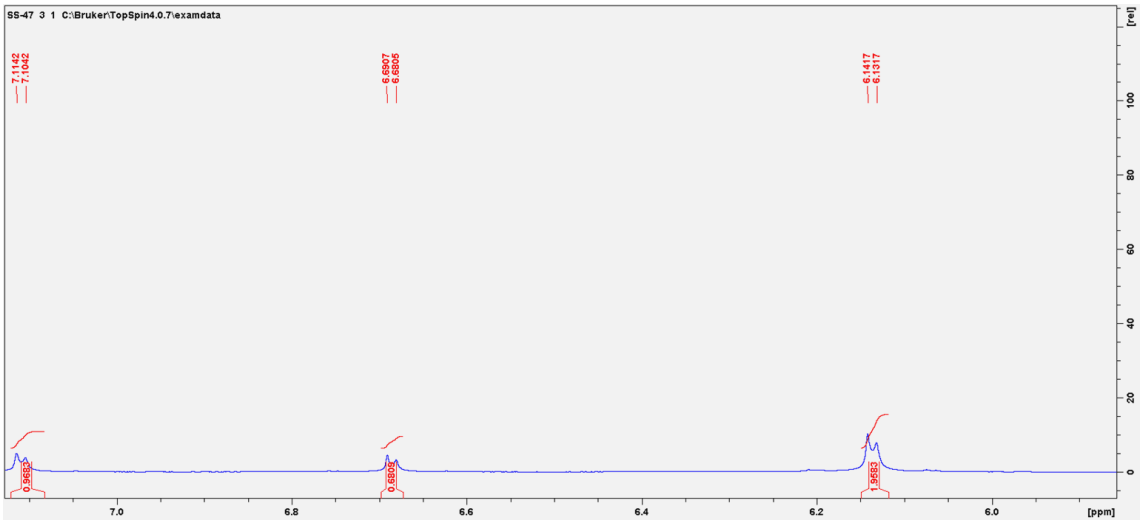
4.4
4.2
4.0
3.8
3.6
3.4
[ppm]
[rel]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6.6806
6.1417
6.1317
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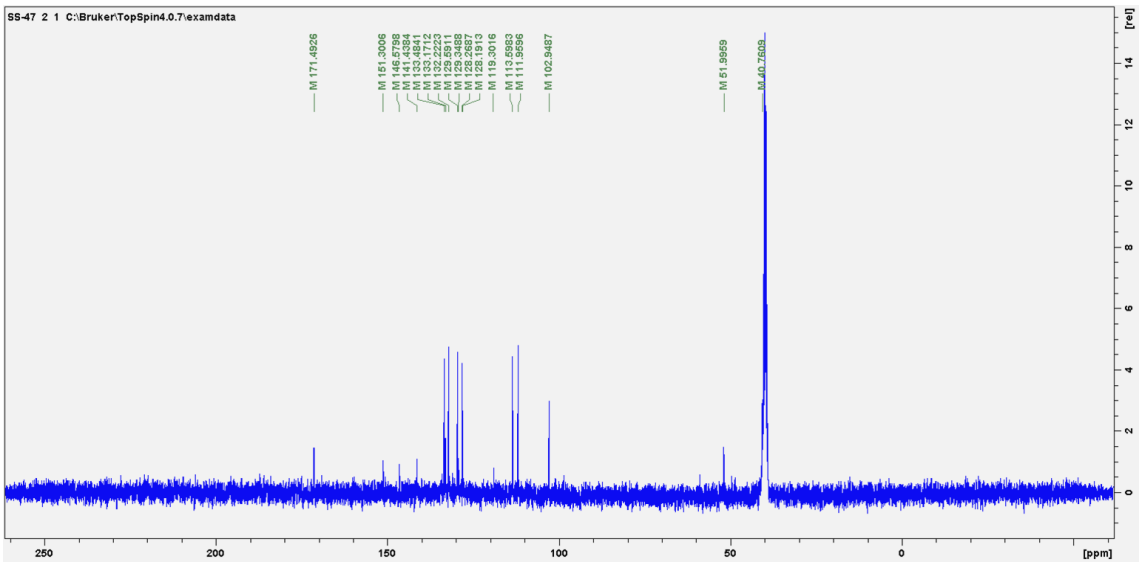
7.0
6.8
6.6
6.4
6.2
6.0
[ppm]
[rel]

C-NMR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M 151.3006
M 146.5788
M 143.4881
M 133.4841
M 133.1712
M 132.2223
M 129.6511
M 129.3488
M 128.2687
M 128.1913
M 119.3016
M 113.5583
M 111.9596
M 102.9487
M 51.9959
M 40.7609
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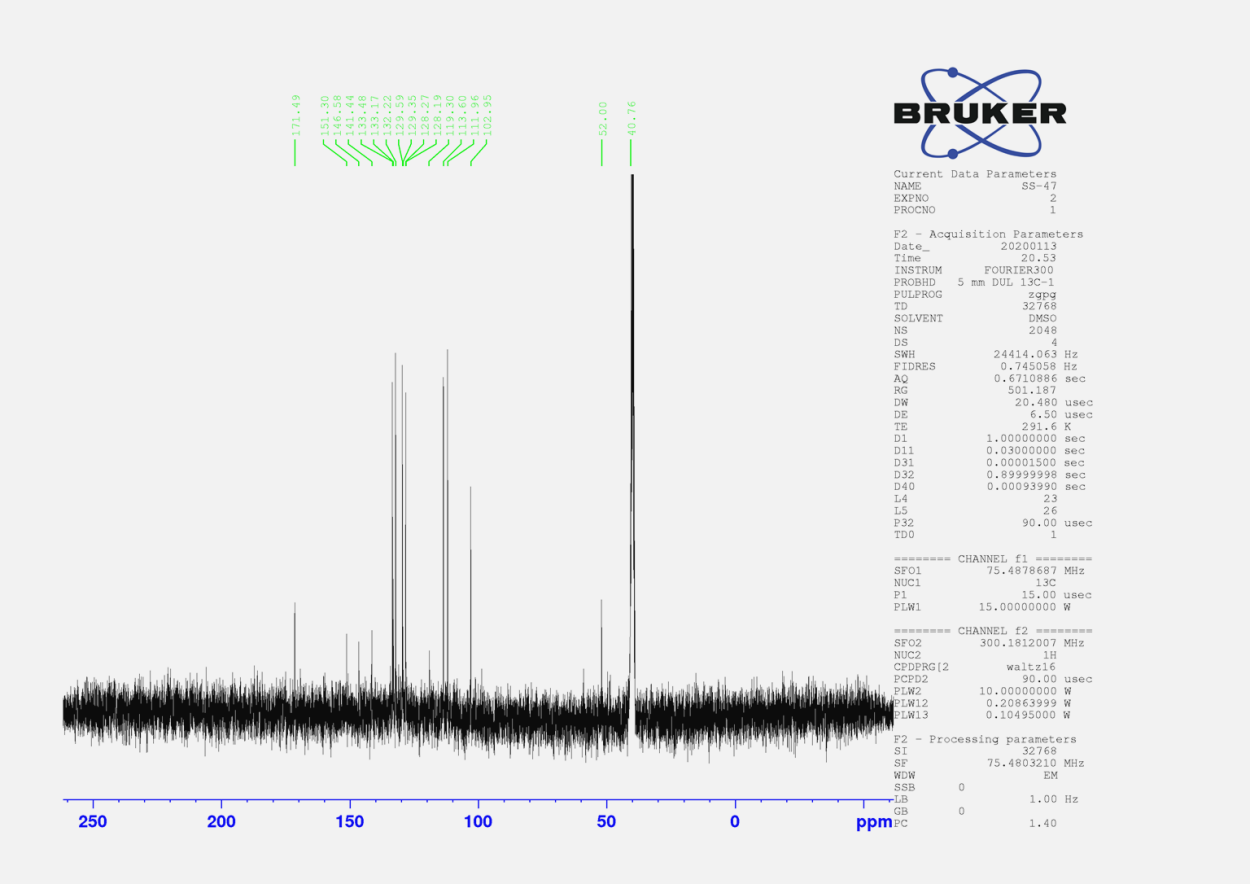
200
150
100
50
0
[ppm]
[rel]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146.58
141.44
133.48
133.11
132.22
129.59
129.35
128.27
128.19
119.30
113.60
111.96
102.95
52.00
40.76
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FIDRES 0.745058 Hz
AQ 0.6710886 sec
RG 501.187
DW 20.480 usec
DE 6.50 usec
TE 291.6 K
D1 1.00000000 sec
D11 0.03000000 sec
D31 0.00001500 sec
D32 0.89999998 sec
D40 0.00093390 sec
L4 23
L5 26
P32 90.00 usec
TD0 1
======== CHANNEL f1 ========
SFO1 75.4878687 MHz
NUC1 13C
P1 15.00 usec
PLW1 15.00000000 W
======== CHANNEL f2 ========
SFO2 300.1812007 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 10.00000000 W
PLW12 0.20863999 W
PLW13 0.10495000 W
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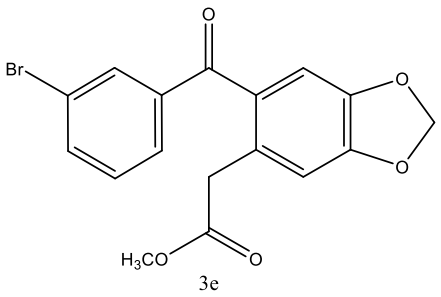
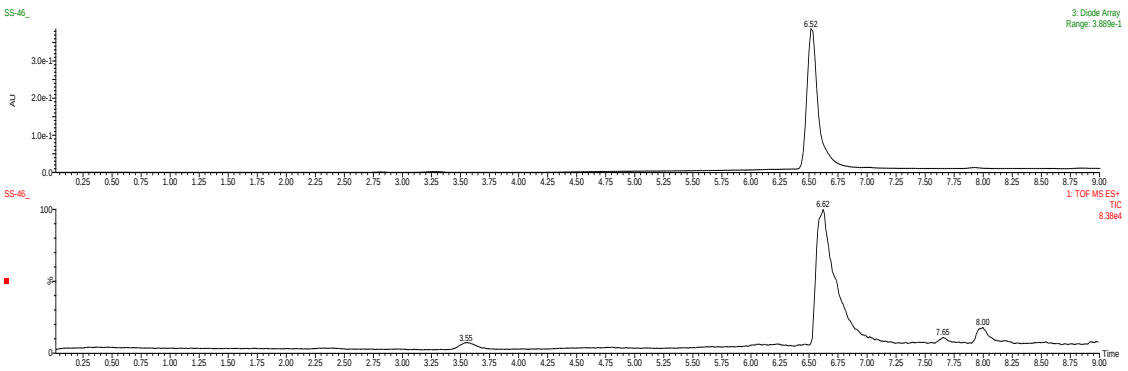
200
150
100
50
0
ppm

3e


Br
O
O
O
H3CO
O

3e

HRMS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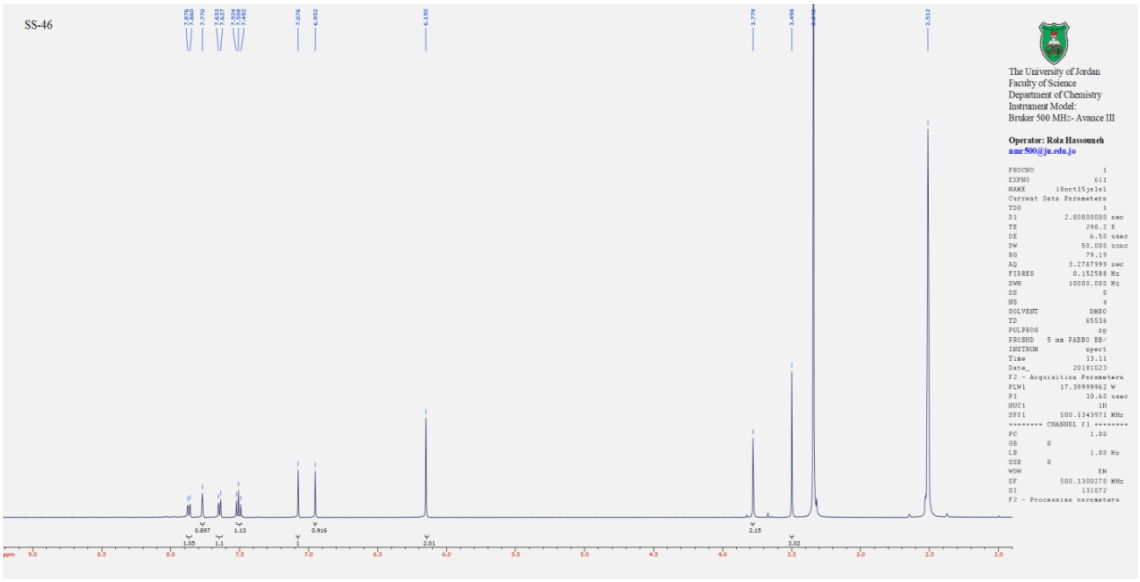
6.52
AU
SS-46_
1: TOF MS ES+
TIC
8.38e4
6.62
3.55
7.65
8.00
Time

H-NMR



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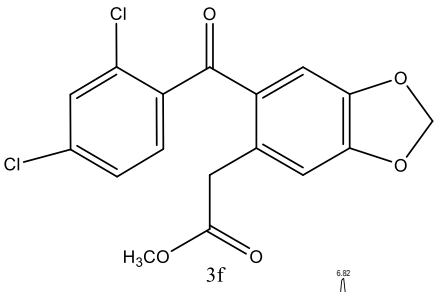
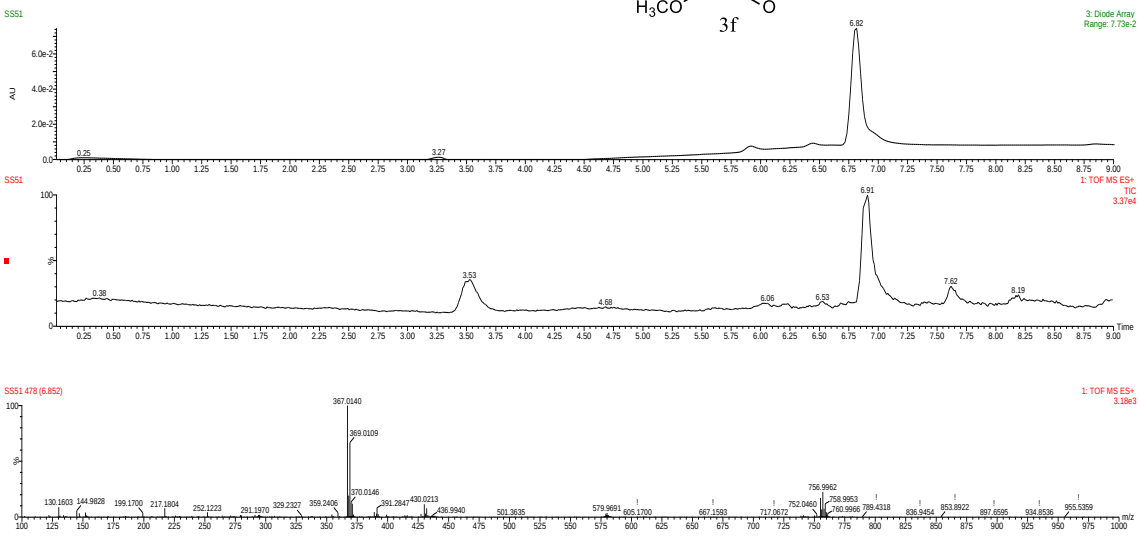


3f

HRMS



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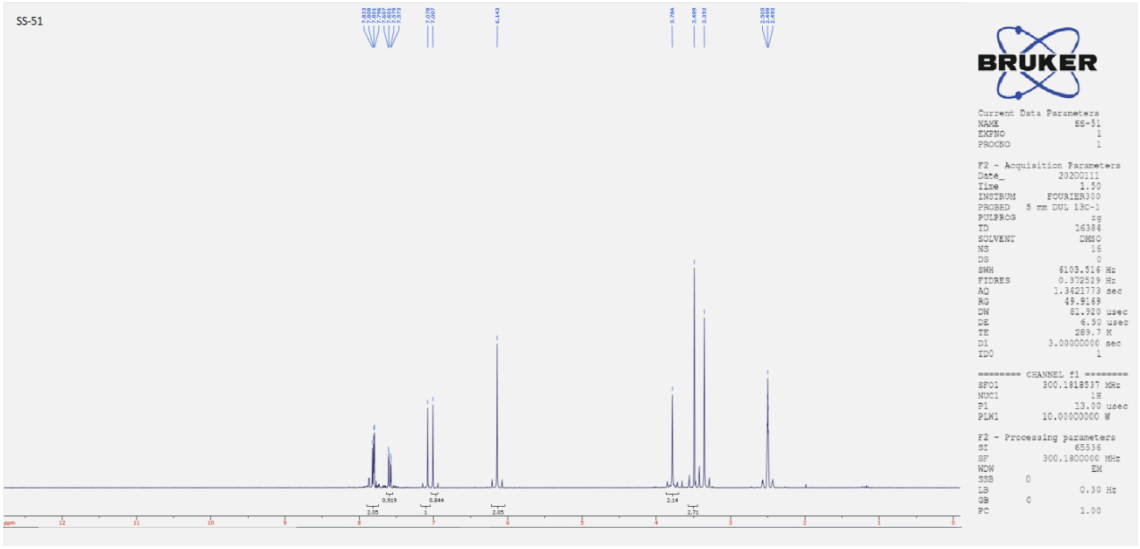
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FIDRES 0.372529 Hz
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RG 49.9169
DW 82.020 usec
DE 6.50 usec
TE 289.7 K
D1 3.00000000 sec
TD0 1
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NUC1 1H
P1 13.00 usec
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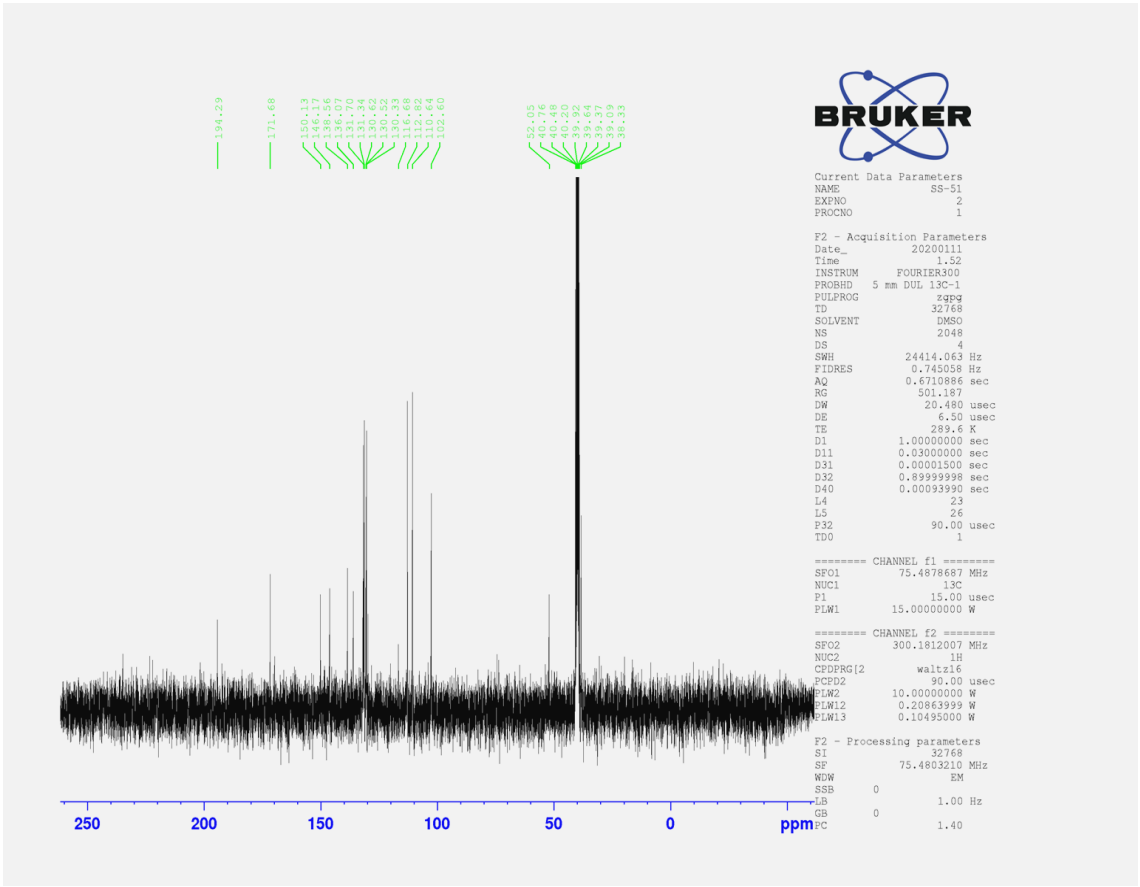
C-NMR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130.33
115.68
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110.64
102.60
52.05
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40.20
39.92
39.64
39.37
39.09
38.33
BRUKER
Current Data Parameters
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TD 32768
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NS 2048
DS 4
SWH 24414.063 Hz
FIDRES 0.745058 Hz
AQ 0.6710886 sec
RG 501.187
DW 20.480 usec
DE 6.50 usec
TE 289.6 K
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D32 0.89999998 sec
D40 0.00093990 sec
L4 23
L5 26
P32 90.00 usec
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NUC1 13C
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SFO2 300.1812007 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
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PLW12 0.20863999 W
PLW13 0.10495000 W
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200
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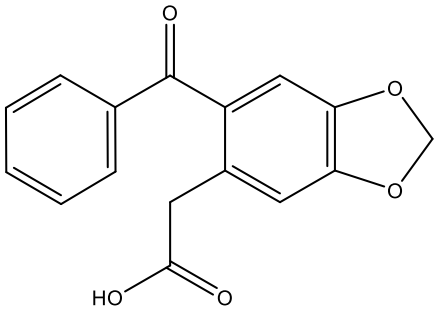
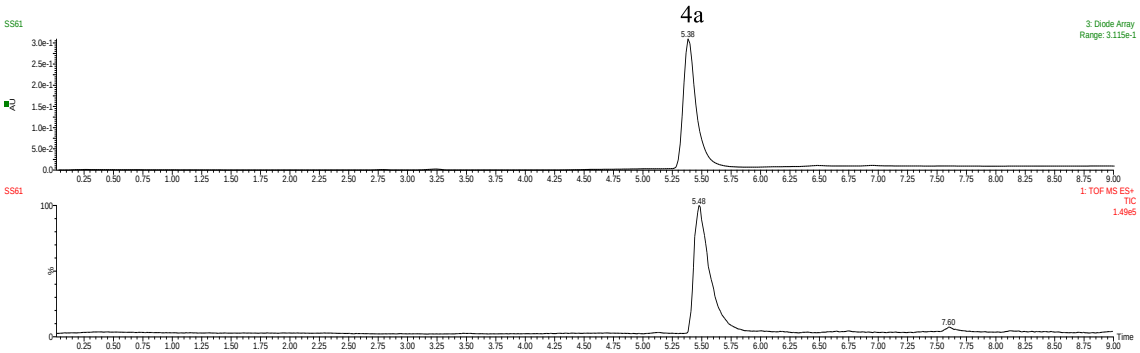
50
0
ppm

4a


O
O
O
HO
O

HRMS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TIC
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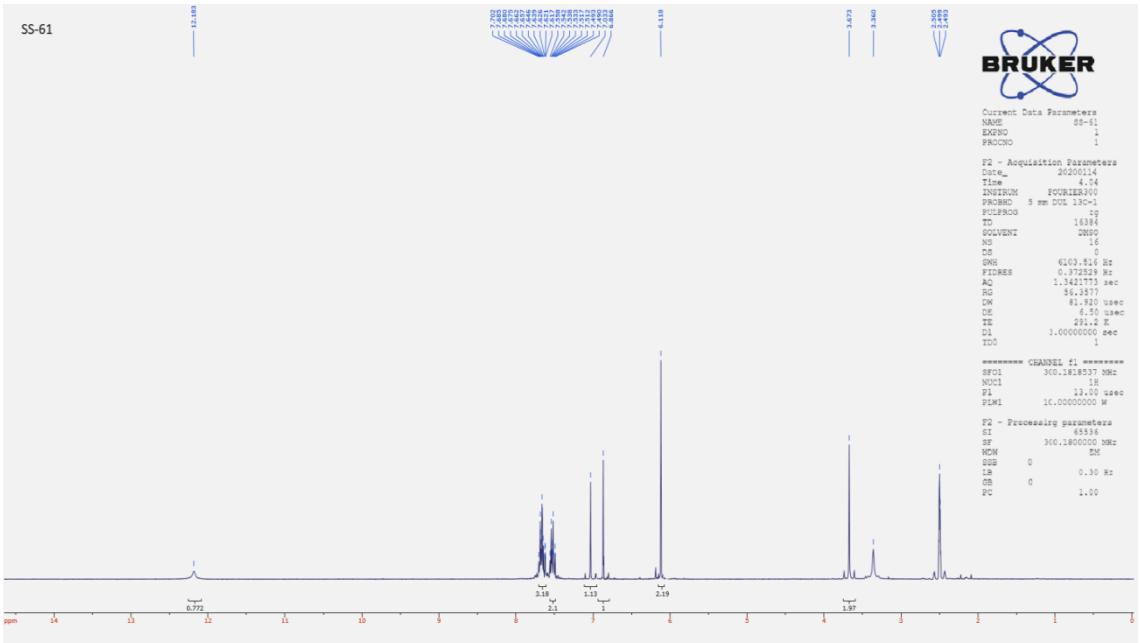
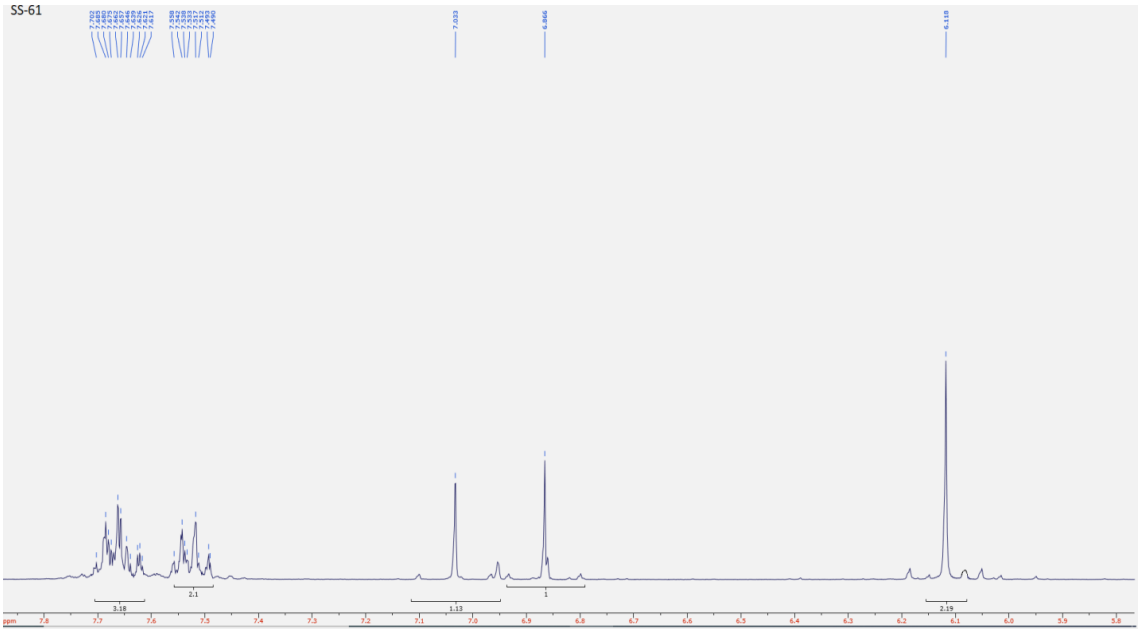
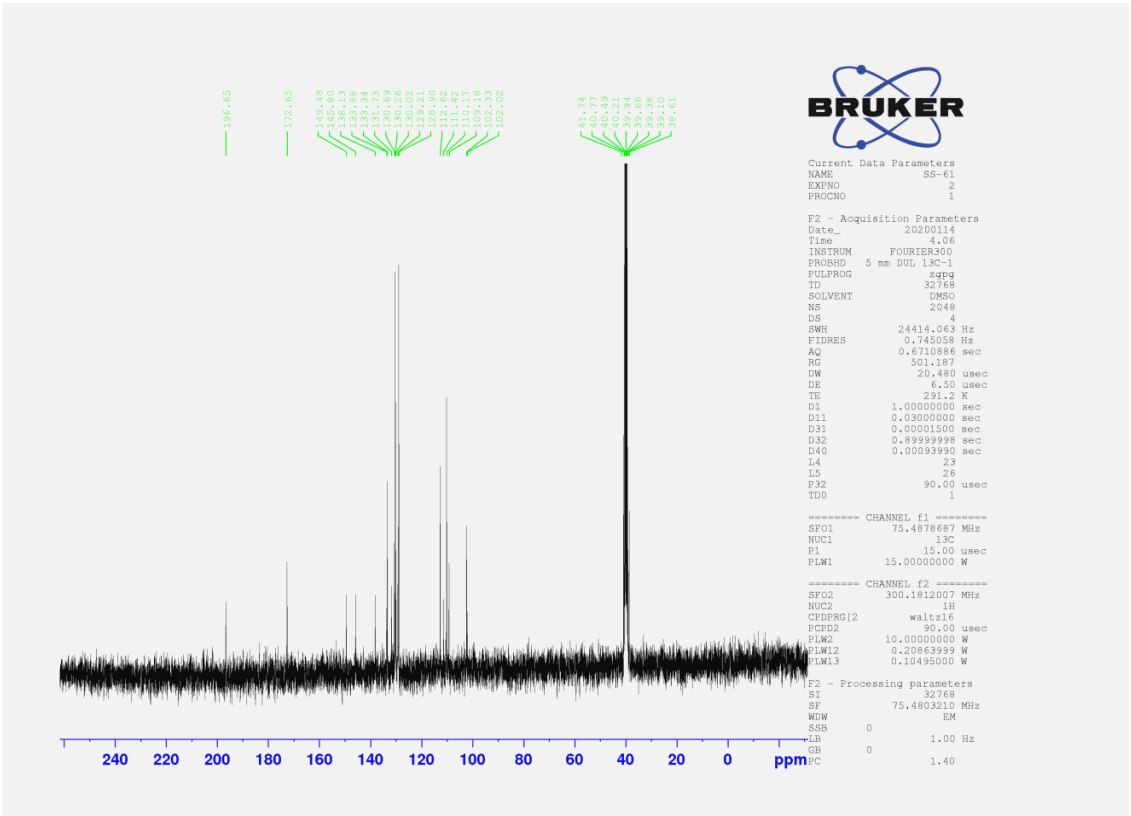
5.48
7.60
Time

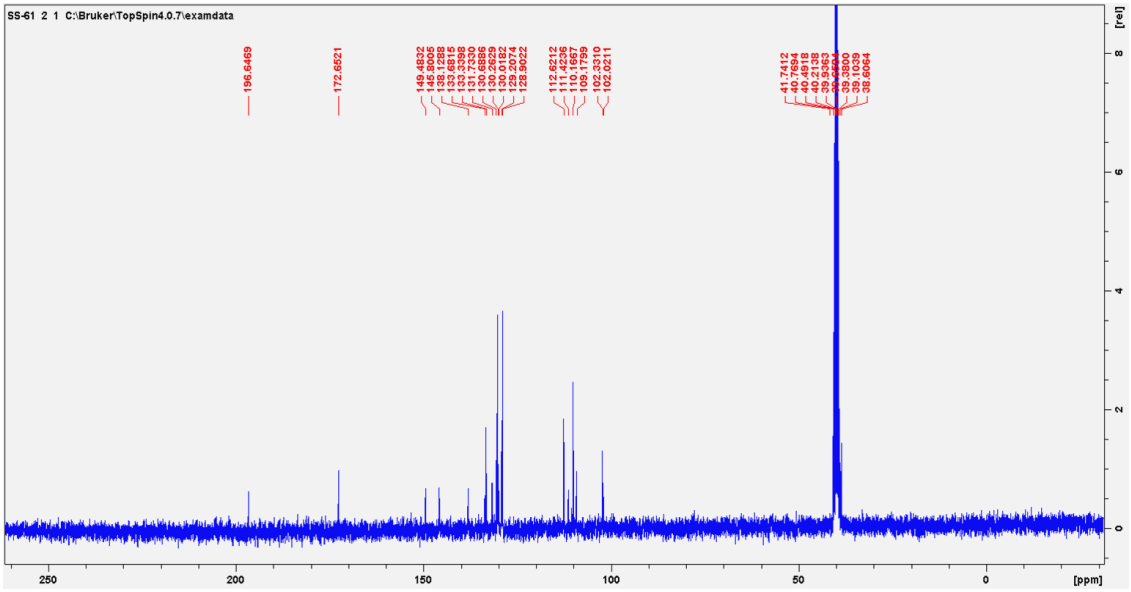
H-NMR

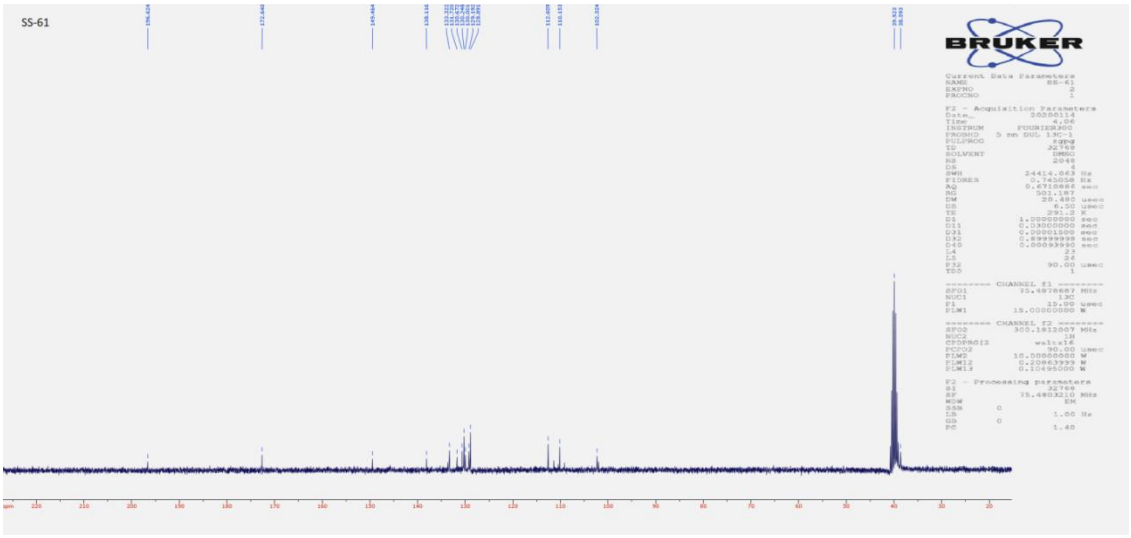

SS-61
BRUKER


SS-61

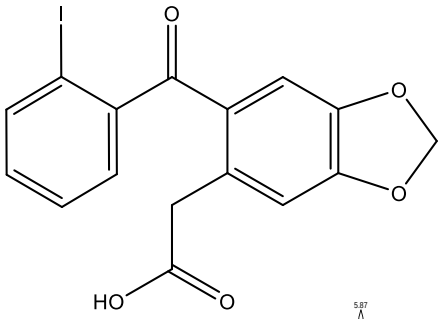
C-NMR


196.65
172.65
149.48
145.80
138.13
133.68
131.44
131.73
130.69
130.26
130.22
129.21
128.90
112.62
111.42
110.17
103.18
102.38
102.02
41.74
40.77
40.49
40.21
39.94
39.66
39.38
39.10
38.81
240 220 200 180 160 140 120 100 80 60 40 20 0 ppm
BRUKER
Current Data Parameters
NAME SS-61
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200114
Time 4.06
INSTRUM FOURIER300
PROBHD 5 mm DUL 13C-1
PULPROG zgpg
TD 32768
SOLVENT DMSO
NS 2048
DS 4
SWH 24414.063 Hz
FIDRES 0.745058 Hz
AQ 0.6710886 sec
RG 501.187
DW 20.480 usec
DE 6.50 usec
TE 291.2 K
D1 1.00000000 sec
D11 0.03000000 sec
D31 0.00001500 sec
D32 0.89999998 sec
D40 0.00093990 sec
L4 23
L5 26
P32 90.00 usec
TD0 1
======== CHANNEL f1 ========
SFO1 75.4878687 MHz
NUC1 13C
P1 15.00 usec
PLW1 15.00000000 W
======== CHANNEL f2 ========
SFO2 300.1812007 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 10.00000000 W
PLW12 0.20863999 W
PLW13 0.10495000 W
F2 - Processing parameters
SI 32768
SF 75.4803210 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40


SS-61 2 1 C:\Bruker\TopSpin4.0.7\examdata
196.6469
172.6521
149.4832
145.8005
138.1288
133.6815
133.3368
131.7330
130.6886
130.3229
130.0182
129.2074
128.9022
112.6212
111.4256
110.1457
109.1799
102.3310
102.0211
41.7412
40.7694
40.4918
40.2138
39.9358
39.4564
39.3800
39.1039
38.8264
[ppm]

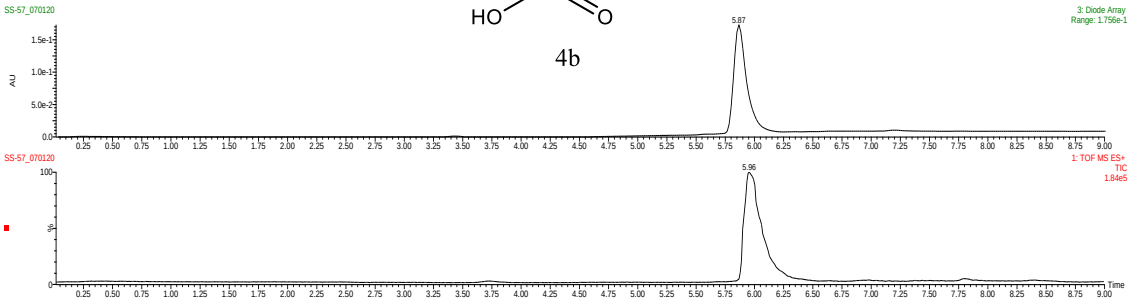

SS-61
BRUKER

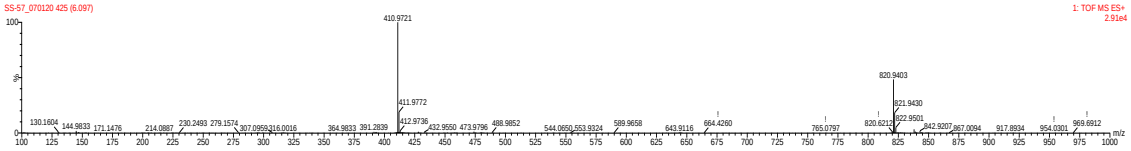
4b

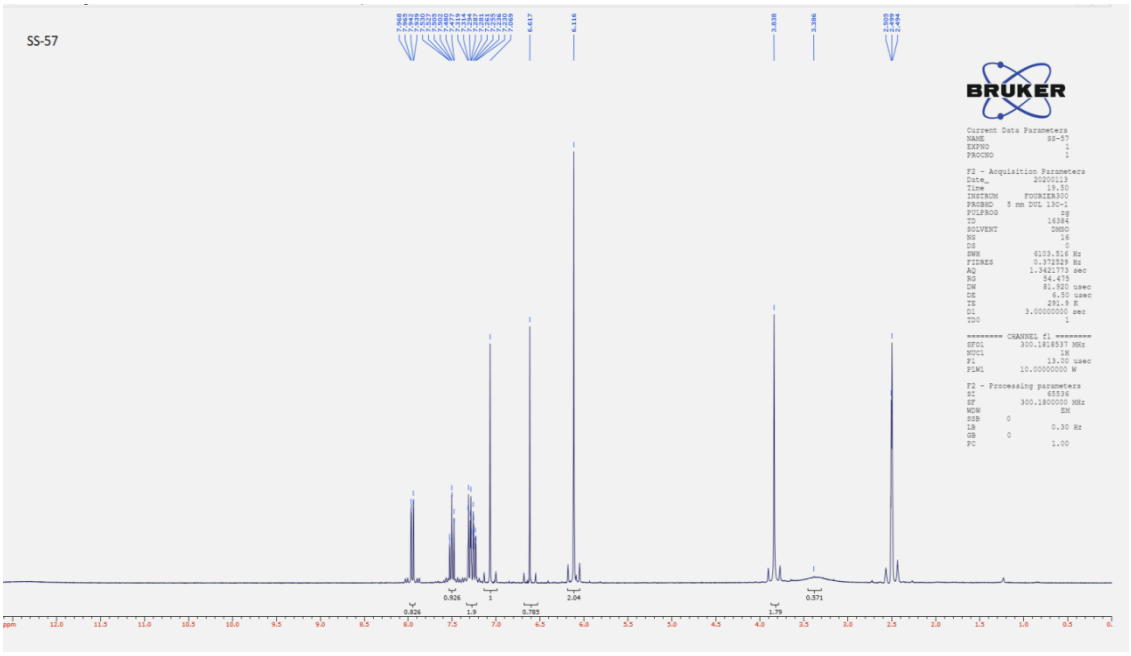

I
O
O
O
HO
O

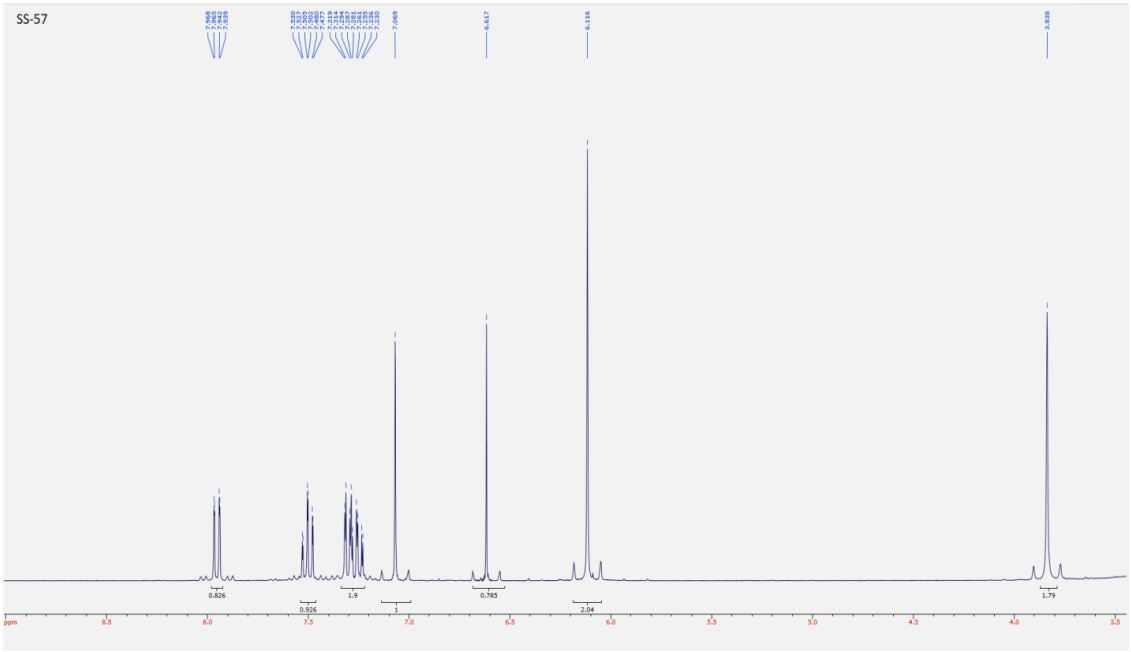
4b

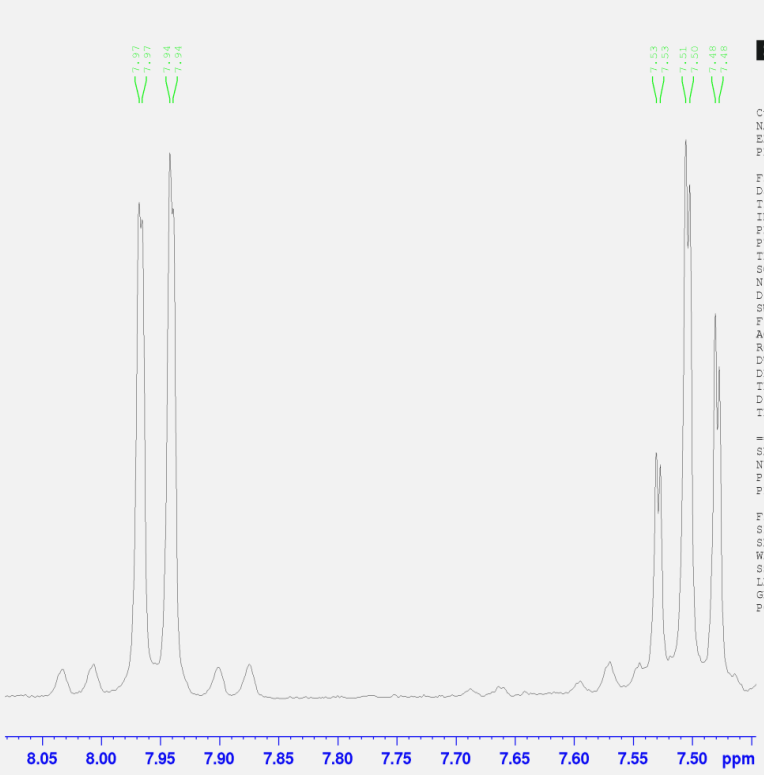
HRMS


SS-57_070120
3: Diode Array
Range: 1.756e-1
5.87
AU
SS-57_070120
1: TOF MS ES+
TIC
1.84e5
5.96
Time


SS-57_070120 425 (6.097)
1: TOF MS ES+
2.91e4
410.9721
411.9772
412.9736
820.9403
821.9430
822.9501
m/z

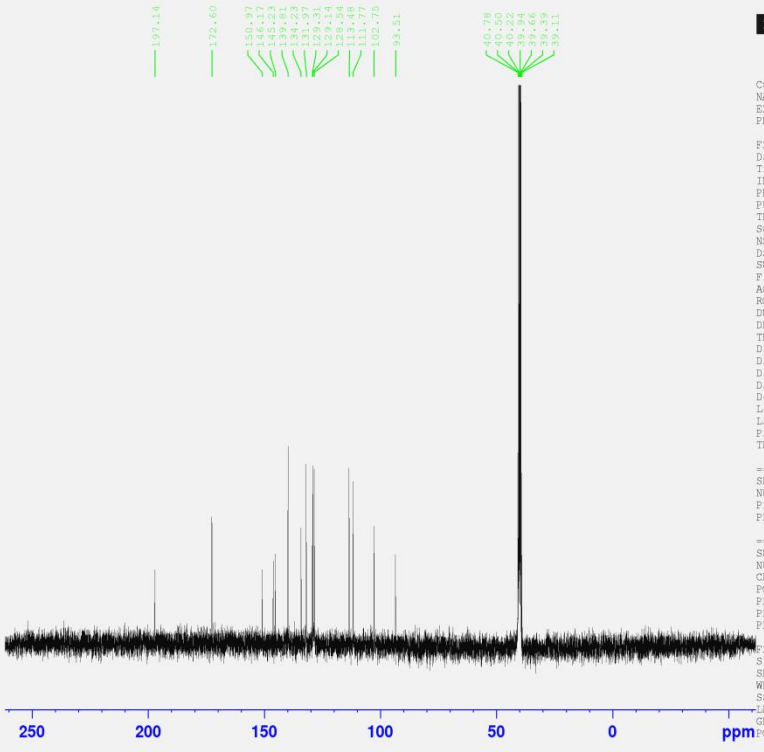

SS-57
BRUKER


SS-57



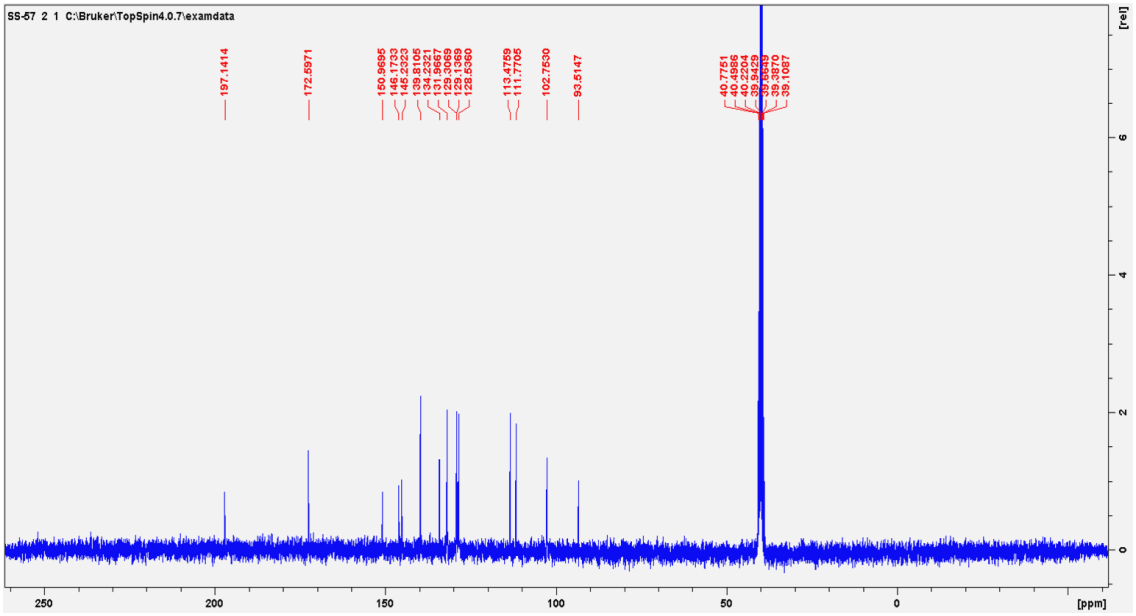
BRUKER

```
Current Data Parameters
NAME                  SS-57
EXPNO                     1
PROCNO                    1

F2 - Acquisition Parameters
Date_              20200113
Time                  19.50
INSTRUM         FOURIER300
PROBHD     5 mm DUL 13C-1
PULPROG                zg
TD                    16384
SOLVENT                DMSO
NS                       16
DS                        0
SWH                6103.516 Hz
FIDRES             0.372529 Hz
AQ                 1.3421773 sec
RG                    54.475
DW                   81.920 usec
DE                    6.50 usec
TE                   291.9 K
D1                3.00000000 sec
TD0                       1

======== CHANNEL f1 ========
SFO1             300.1818537 MHz
NUC1                     1H
P1                    13.00 usec
PLW1            10.00000000 W

F2 - Processing parameters
SI                    65536
SF               300.1800000 MHz
WDW                      EM
SSB          0
LB                    0.30 Hz
GB           0
PC                    1.00
```

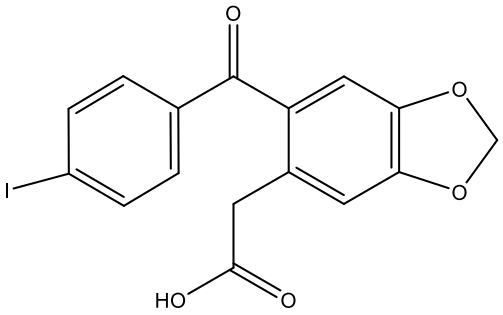


BRUKER

```
Current Data Parameters
NAME                  SS-57
EXPNO                     2
PROCNO                    1

F2 - Acquisition Parameters
Date_              20200113
Time                  19.52
INSTRUM         FOURIER300
PROBHD     5 mm DUL 13C-1
PULPROG             zgpg
TD                    32768
SOLVENT                DMSO
NS                     2048
DS                        4
SWH               24414.063 Hz
FIDRES             0.745058 Hz
AQ                 0.6710886 sec
RG                   501.187
DW                   20.480 usec
DE                    6.50 usec
TE                   291.9 K
D1                1.00000000 sec
D11               0.03000000 sec
D31               0.00001500 sec
D32               0.89999998 sec
D40               0.00093990 sec
L4                        23
L5                        26
P32                  90.00 usec
TD0                       1

======== CHANNEL f1 ========
SFO1              75.4878687 MHz
NUC1                     13C
P1                    15.00 usec
PLW1            15.00000000 W

======== CHANNEL f2 ========
SFO2             300.1812007 MHz
NUC2                     1H
CPDPRG[2              waltz16
PCPD2                 90.00 usec
PLW2            10.00000000 W
PLW12            0.20863999 W
PLW13            0.10495000 W

F2 - Processing parameters
SI                    32768
SF               75.4803210 MHz
WDW                      EM
SSB          0
LB                    1.00 Hz
GB           0
PC                    1.40
```

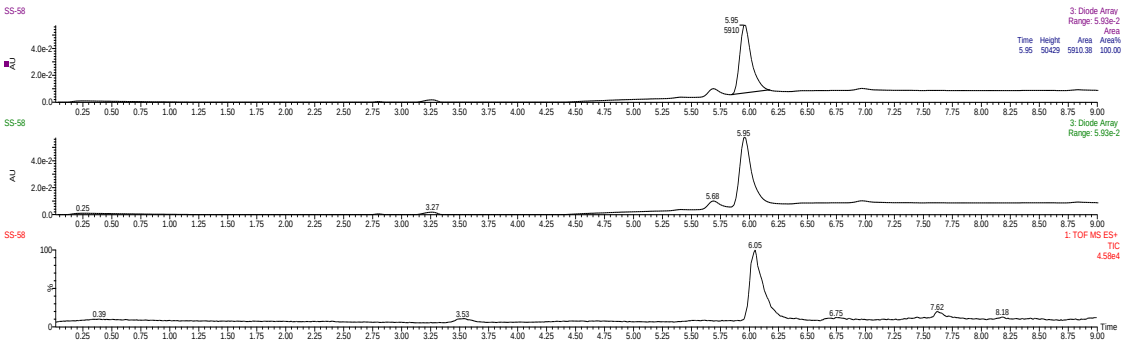

SS-57 2 1 C:\Bruker\TopSpin4.0.7\examdata
197.1414
172.6971
150.9695
146.7723
146.2323
139.8105
134.2321
131.9687
129.3969
129.1369
128.5360
113.4759
111.7705
102.7530
93.5147
40.7751
40.4886
40.2204
40.0522
39.5343
39.3870
39.1087
[rel]
[ppm]

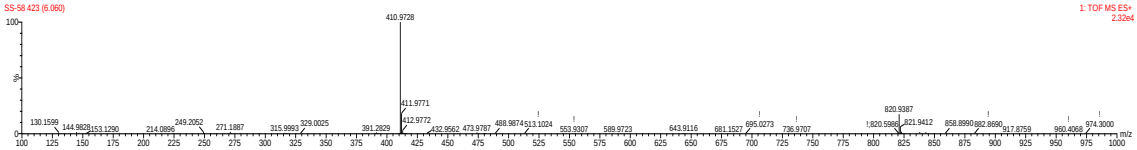
4c


O
O
O
I
HO
O

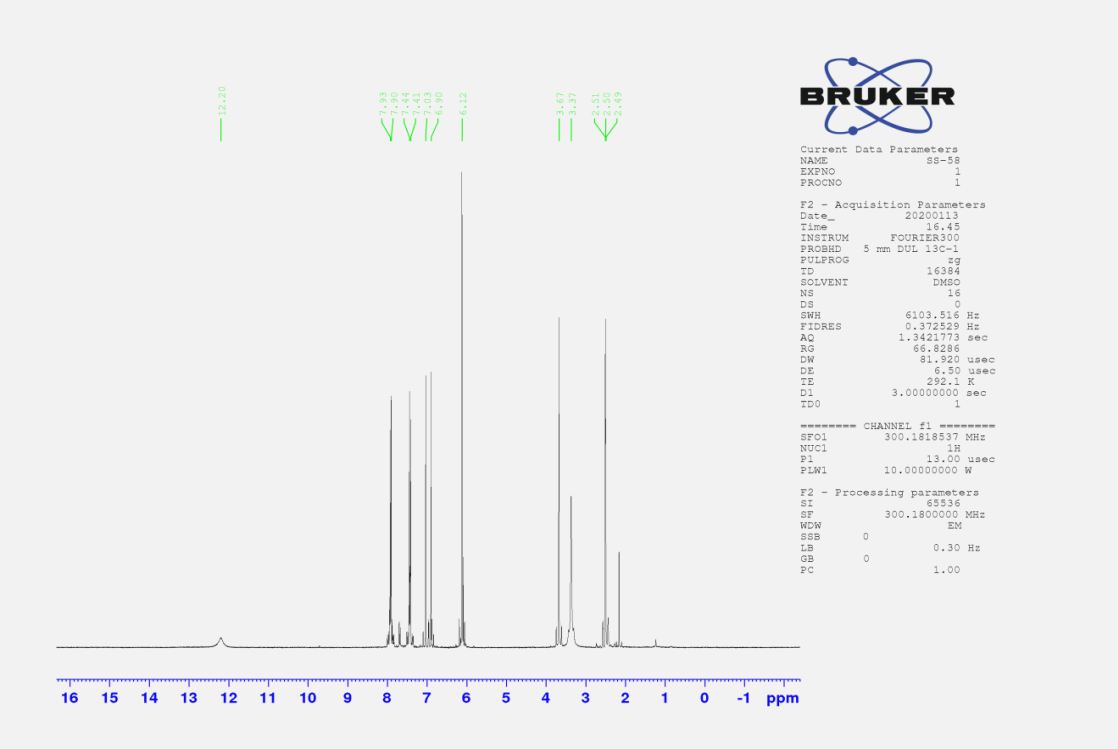
4c

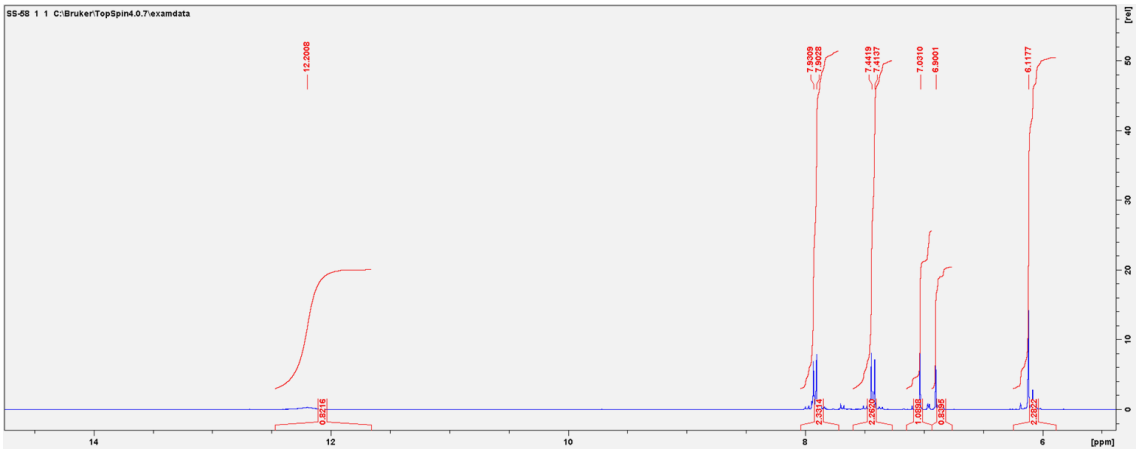
HRMS

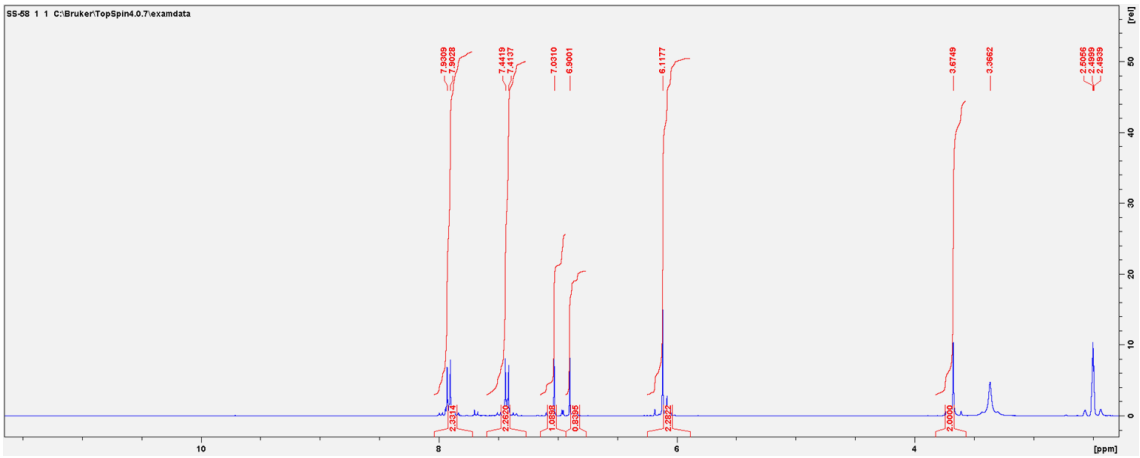

SS-58
5.95
5910
3: Diode Array
Range: 5.93e-2
Area
Time Height Area Area%
5.95 50429 5910.38 100.00
AU
SS-58
5.95
5.68
3.27
0.25
SS-58
1: TOF MS ES+
TIC
4.58e4
6.05
0.39
3.53
6.75
7.62
8.18
Time

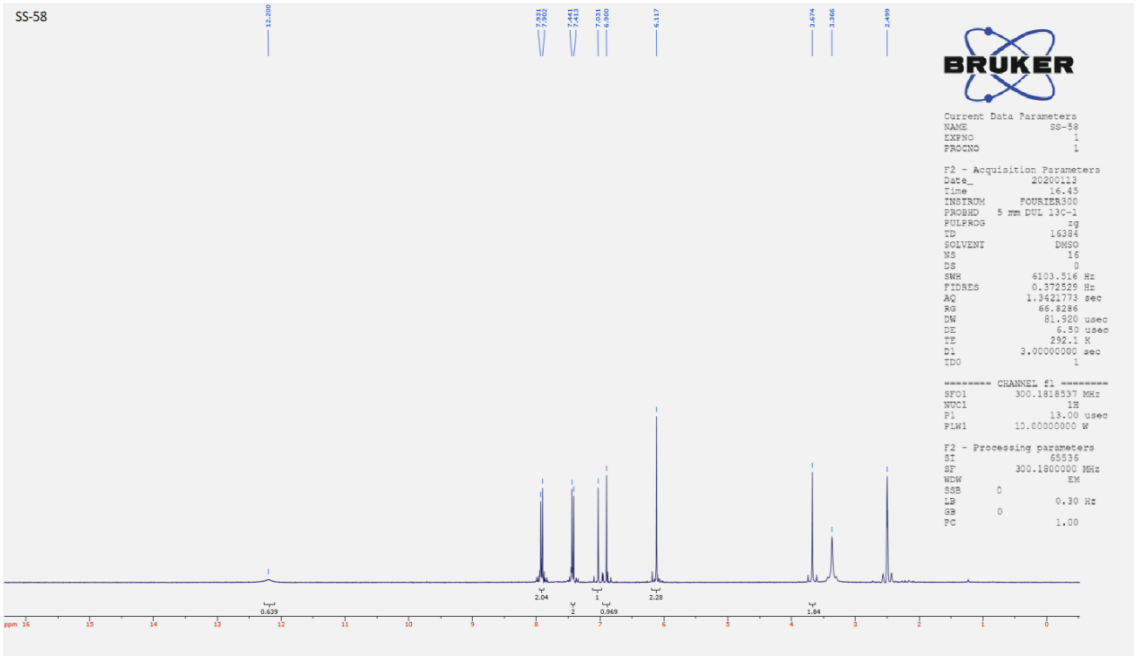

SS-58 423 (6.060)
1: TOF MS ES+
2.32e4
410.9728
411.9771
412.9772
820.9387
%
m/z

H-NMR

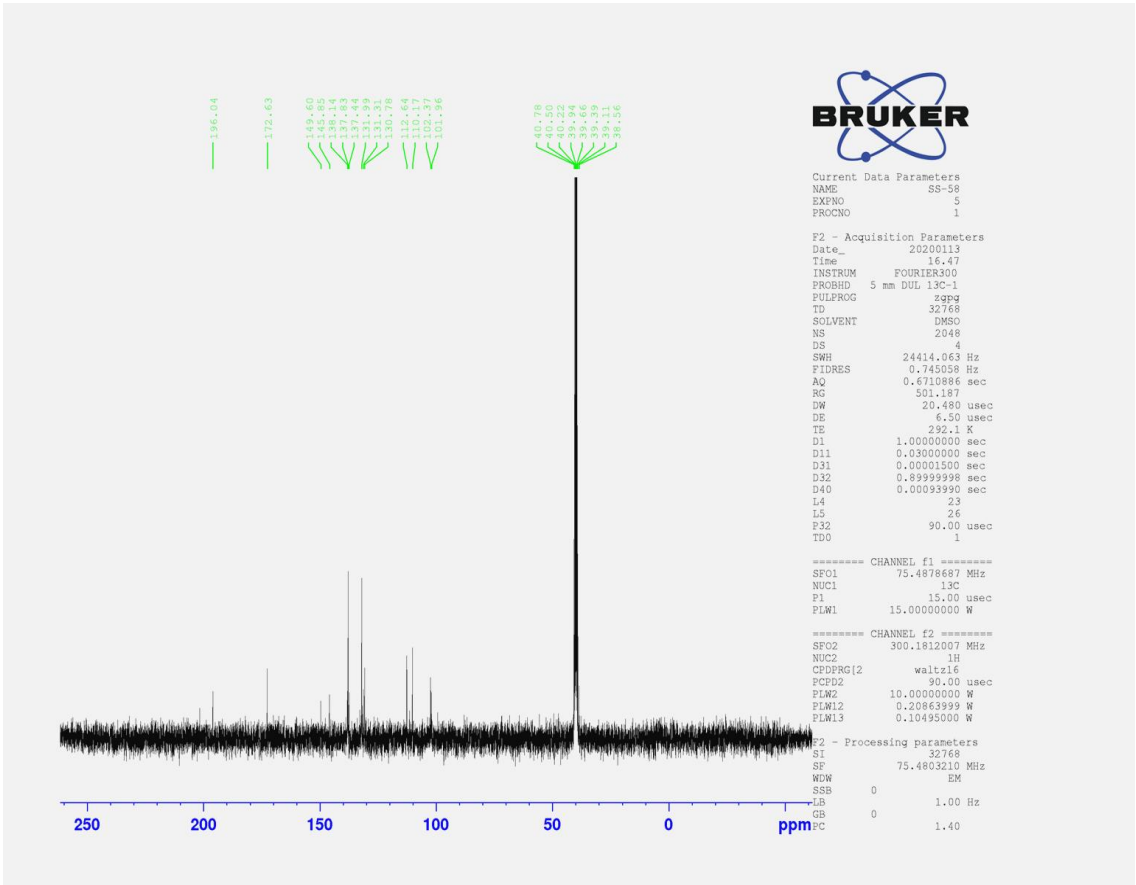

12.20
7.93
7.90
7.44
7.41
7.03
6.90
6.12
3.67
3.37
2.51
2.50
2.49
BRUKER
Current Data Parameters
NAME SS-58
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200113
Time 16.45
INSTRUM FOURIER300
PROBHD 5 mm DUL 13C-1
PULPROG zg
TD 16384
SOLVENT DMSO
NS 16
DS 0
SWH 6103.516 Hz
FIDRES 0.372529 Hz
AQ 1.3421773 sec
RG 66.8286
DW 81.920 usec
DE 6.50 usec
TE 292.1 K
D1 3.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 300.1818537 MHz
NUC1 1H
P1 13.00 usec
PLW1 10.00000000 W
F2 - Processing parameters
SI 65536
SF 300.1800000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 ppm

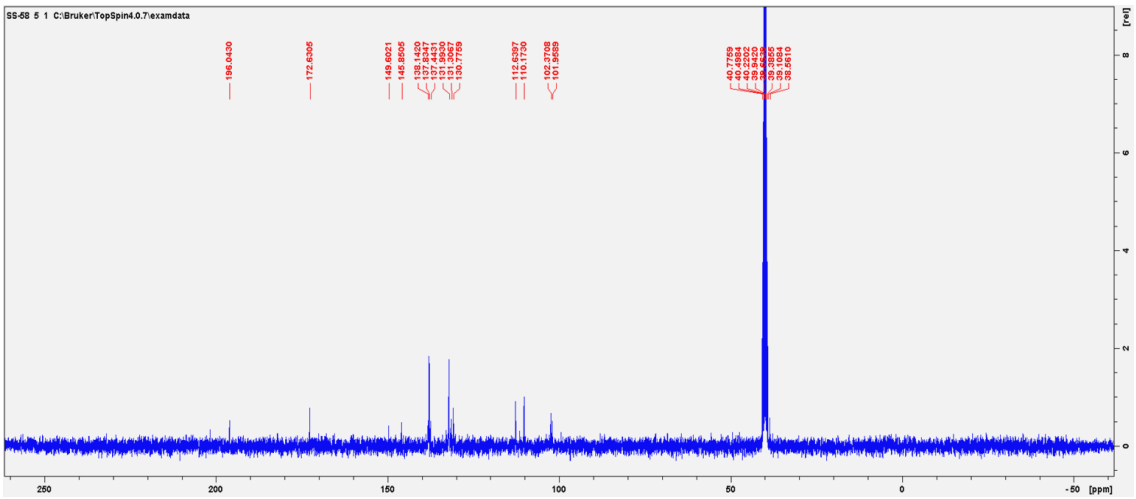

SS-58 1 1 C:\Bruker\TopSpin4.0.7\examdata
12.2008
7.9309
7.9028
7.4419
7.4137
7.0310
6.9001
6.1177
[rel]
[ppm]

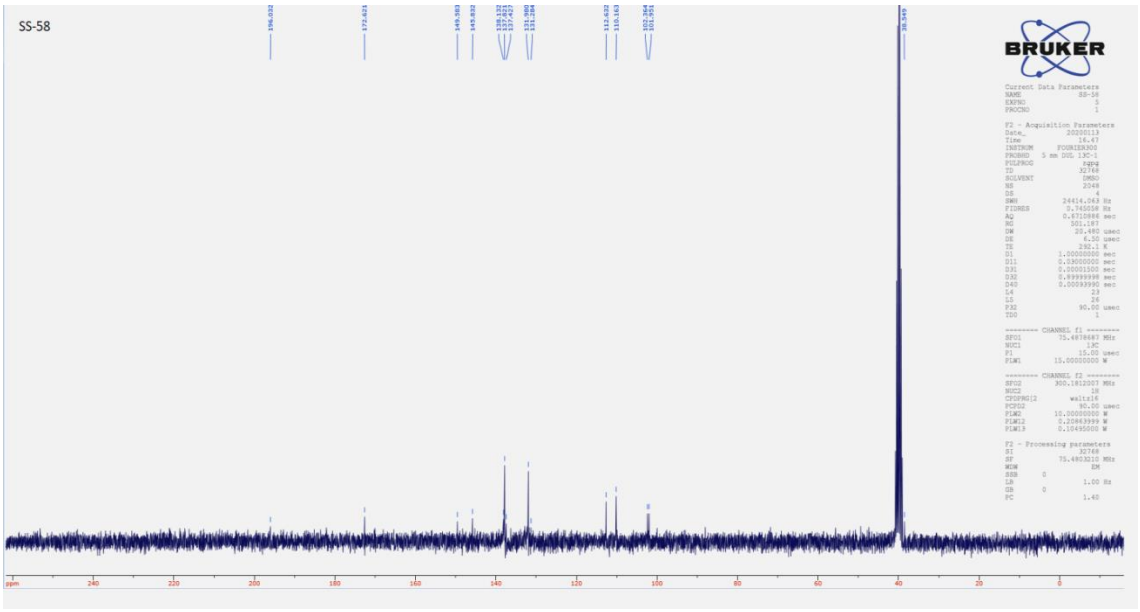

SS-58 1 1 C:\Bruker\TopSpin4.0.7\examdata
7.9309
7.9028
7.4419
7.4137
7.0310
6.9001
6.1177
3.6749
3.3662
2.5056
2.4839
[rel]
[ppm]


SS-58
BRUKER
Current Data Parameters
NAME SS-58
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200113
Time 16.45
INSTRUM FOURIER300
PROBHD 5 mm DUL 13C-1
PULPROG zg
TD 16384
SOLVENT DMSO
NS 16
DS 0
SWH 6103.516 Hz
FIDRES 0.372528 Hz
AQ 1.3421773 sec
RG 66.8286
DW 81.920 usec
DE 6.50 usec
TE 292.1 K
D1 3.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 300.1818537 MHz
NUC1 1H
P1 13.00 usec
PLW1 15.00000000 W
F2 - Processing parameters
SI 65536
SF 300.1800000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

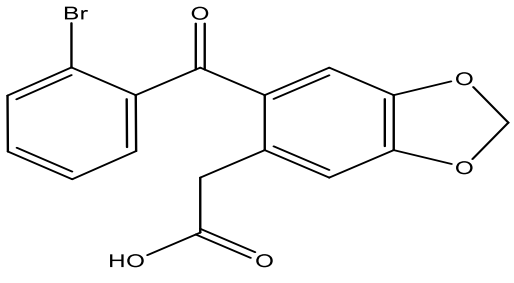
C-NMR


BRUKER
Current Data Parameters
NAME SS-58
EXPNO 5
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200113
Time 16.47
INSTRUM FOURIER300
PROBHD 5 mm DUL 13C-1
PULPROG zgpg
TD 32768
SOLVENT DMSO
NS 2048
DS 4
SWH 24414.063 Hz
FIDRES 0.745058 Hz
AQ 0.6710886 sec
RG 501.187
DW 20.480 usec
DE 6.50 usec
TE 292.1 K
D1 1.00000000 sec
D11 0.03000000 sec
D31 0.00001500 sec
D32 0.89999998 sec
D40 0.00093990 sec
L4 23
L5 26
P32 90.00 usec
TD0 1
======== CHANNEL f1 ========
SFO1 75.4878687 MHz
NUC1 13C
P1 15.00 usec
PLW1 15.00000000 W
======== CHANNEL f2 ========
SFO2 300.1812007 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 10.00000000 W
PLW12 0.20863999 W
PLW13 0.10495000 W
F2 - Processing parameters
SI 32768
SF 75.4803210 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
196.04
172.63
149.60
145.85
138.14
137.83
137.44
131.39
131.31
130.78
112.64
110.17
102.37
101.96
40.78
40.50
40.22
39.94
39.66
39.39
39.11
38.56
250
200
150
100
50
0
ppm


SS-68 5 1 C:\Bruker\TopSpin4.0.7\examdata
196.0430
172.6305
149.6021
145.8505
138.1420
137.6347
137.4181
131.9930
131.3767
130.7769
112.6397
110.1730
102.3708
101.9889
40.7759
40.4884
40.2302
39.9720
39.7138
39.4556
39.1974
38.9392
38.6810
[rel]
250
200
150
100
50
0
-50 [ppm]

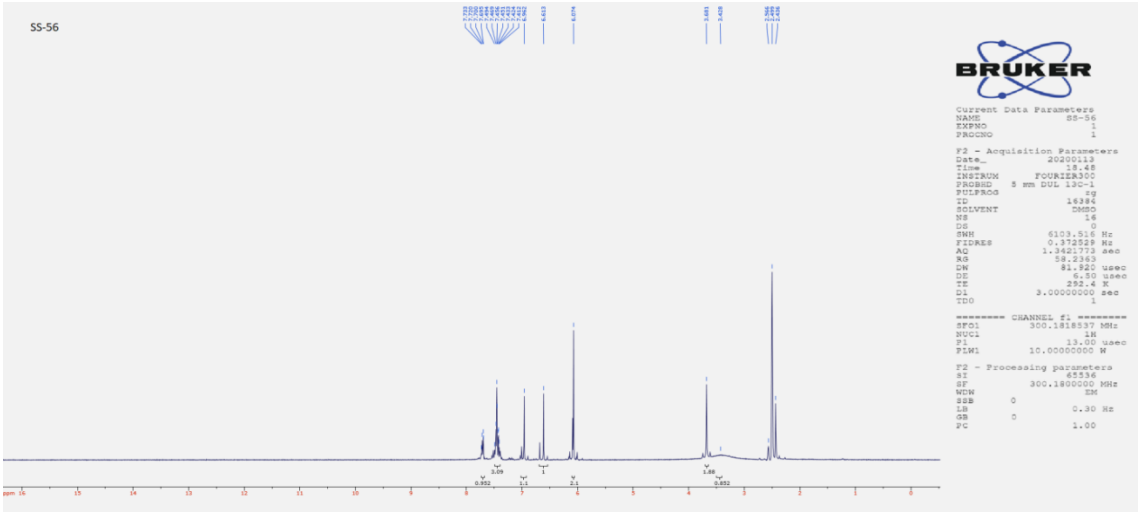

SS-58
BRUKER

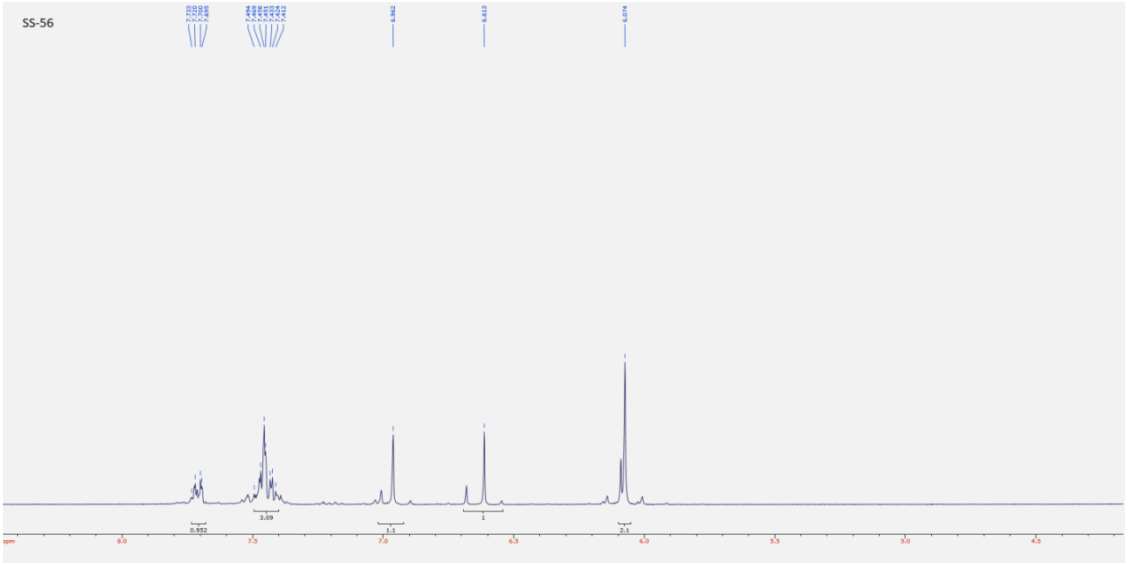
4d

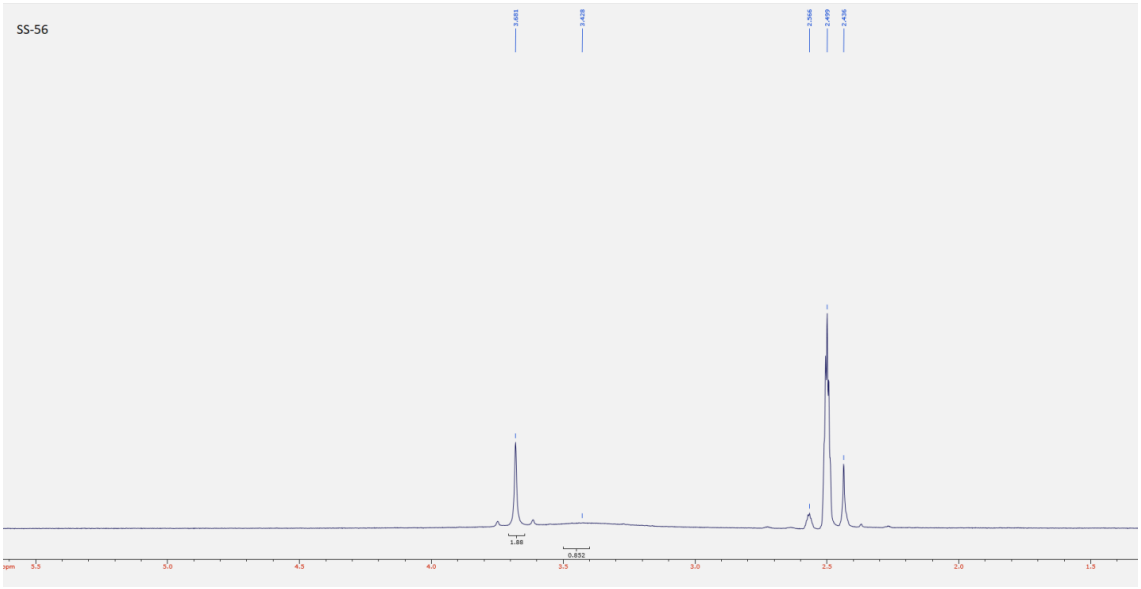

Br
O
O
O
HO
O

4d

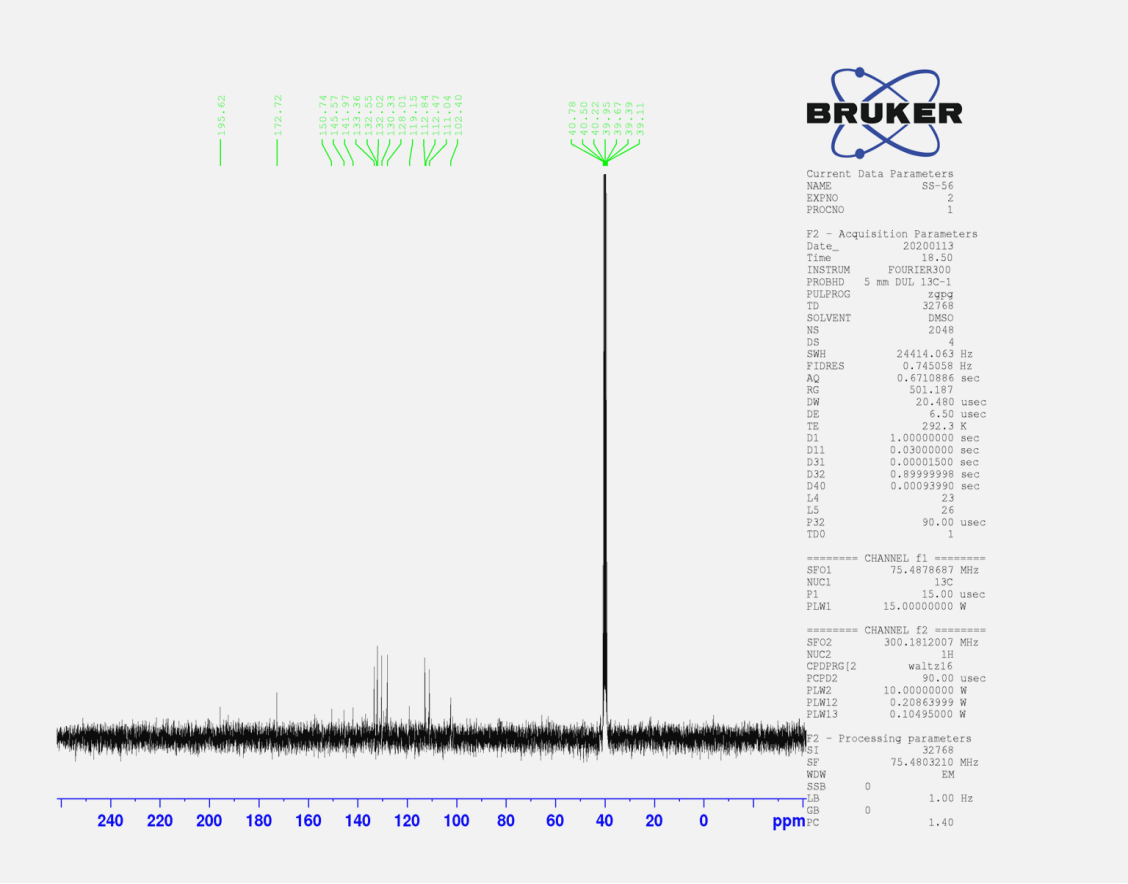
H-NMR

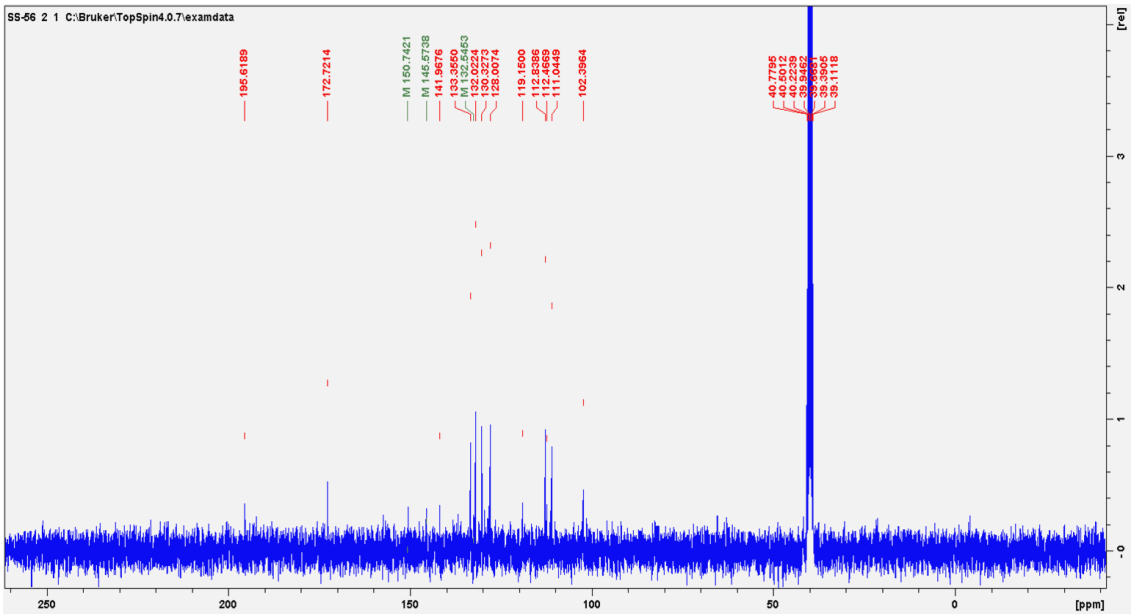






C-NMR


195.62
172.72
150.74
145.97
141.97
133.36
132.55
132.02
130.33
128.01
119.15
112.84
112.47
111.04
102.40
40.78
40.50
40.22
39.95
39.67
39.39
39.11
240 220 200 180 160 140 120 100 80 60 40 20 0 ppm
BRUKER
Current Data Parameters
NAME SS-56
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200113
Time 18.50
INSTRUM FOURIER300
PROBHD 5 mm DUL 13C-1
PULPROG zgpg
TD 32768
SOLVENT DMSO
NS 2048
DS 4
SWH 24414.063 Hz
FIDRES 0.745058 Hz
AQ 0.6710886 sec
RG 501.187
DW 20.480 usec
DE 6.50 usec
TE 292.3 K
D1 1.00000000 sec
D11 0.03000000 sec
D31 0.00001500 sec
D32 0.89999998 sec
D40 0.00093990 sec
L4 23
L5 26
P32 90.00 usec
TD0 1
======== CHANNEL f1 ========
SFO1 75.4878687 MHz
NUC1 13C
P1 15.00 usec
PLW1 15.00000000 W
======== CHANNEL f2 ========
SFO2 300.1812007 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 10.00000000 W
PLW12 0.20863999 W
PLW13 0.10495000 W
F2 - Processing parameters
SI 32768
SF 75.4803210 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40


SS-56 2 1 C:\Bruker\TopSpin4.0.7\examdata
195.6189
172.7214
M 150.7421
M 145.5738
141.9676
133.3550
M 132.5463
132.0234
130.3273
128.0074
119.1500
112.8386
112.4683
111.0449
102.3964
40.7785
40.5017
40.2239
39.9462
39.6684
39.3905
39.1118
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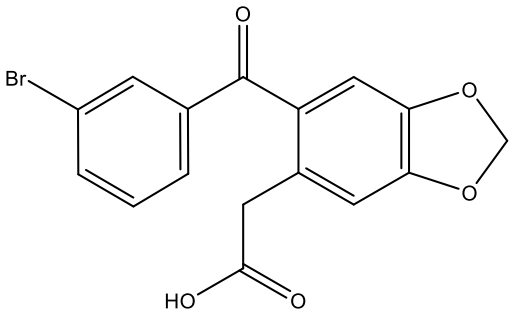
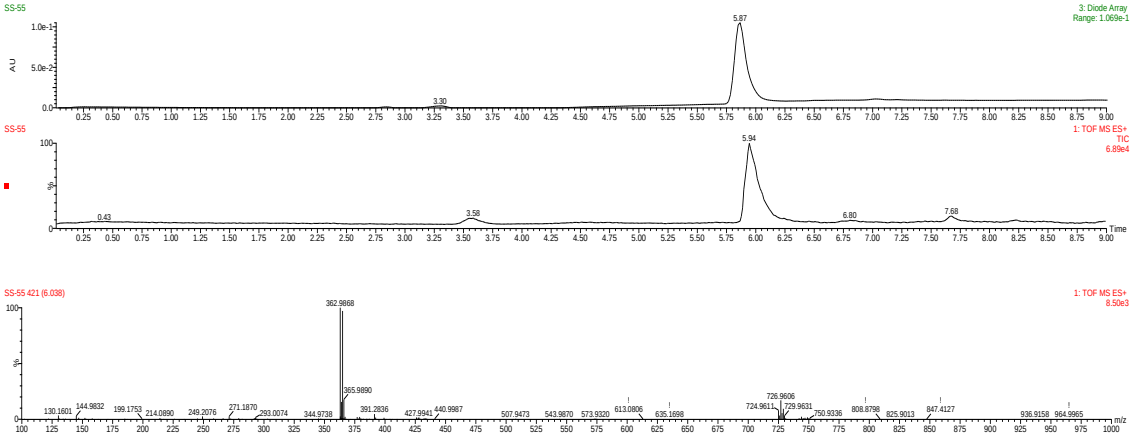
[rel]

4e

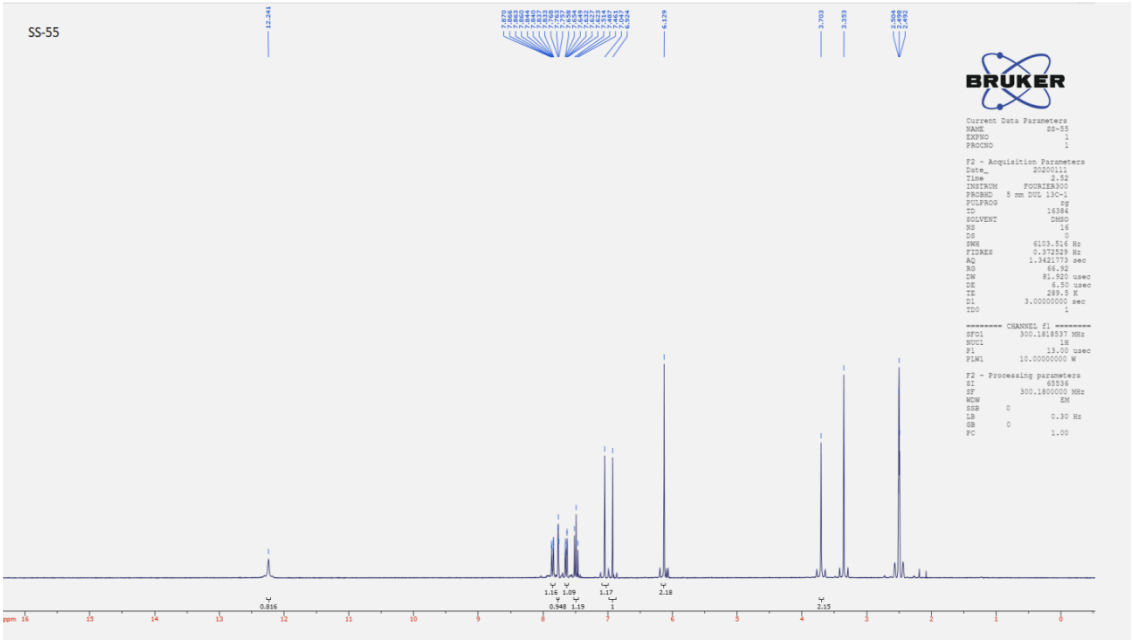


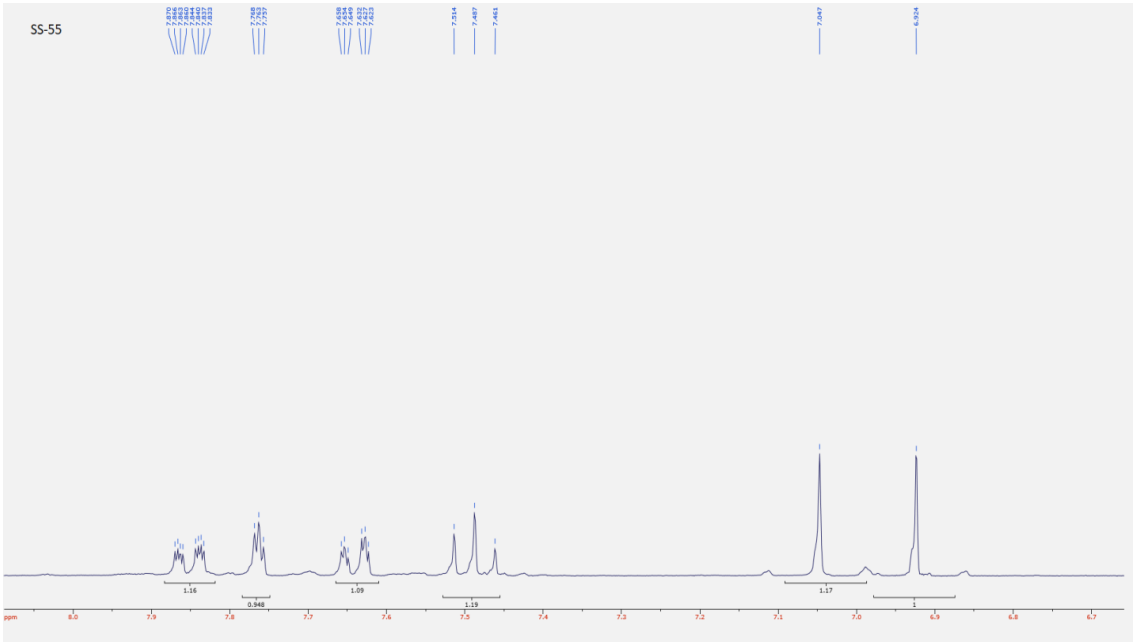
4e

HRMS

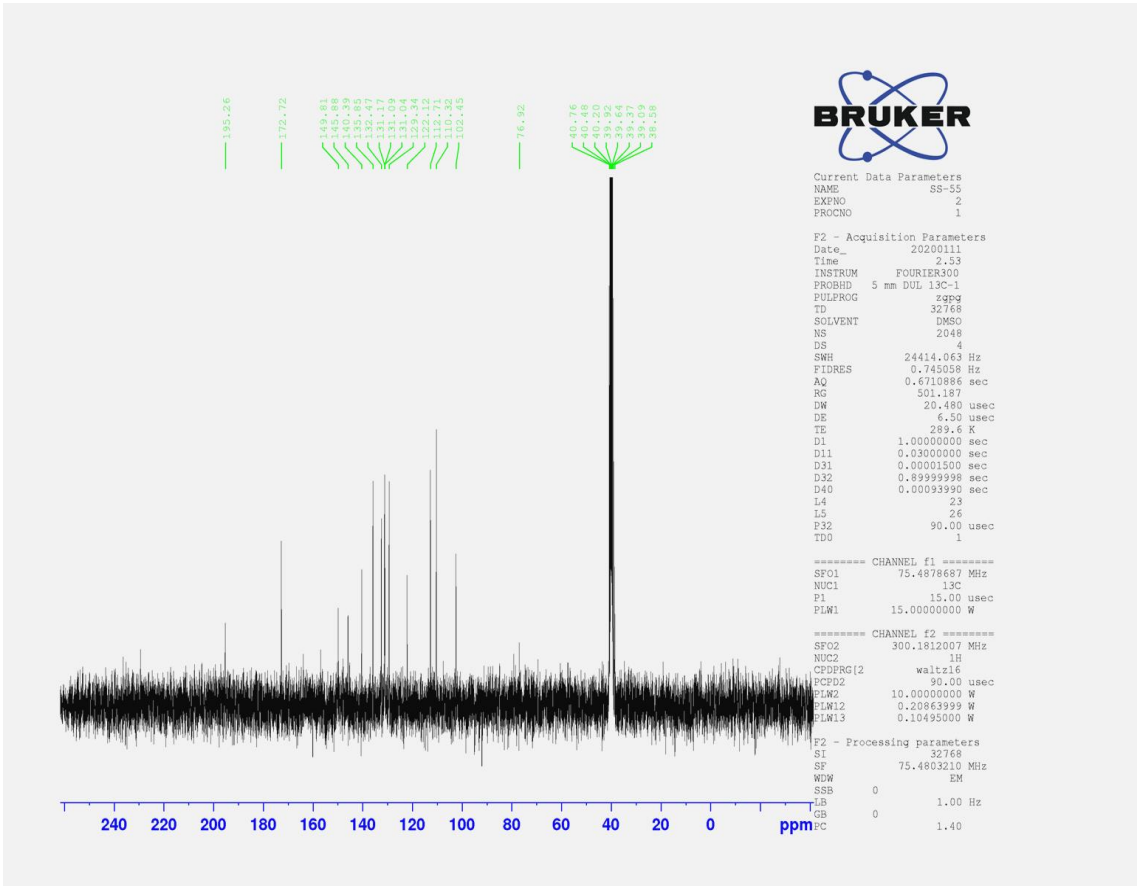


H-NMR




SS-55

C-NMR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PROCNO 1
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Time 2.53
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PROBHD 5 mm DUL 13C-1
PULPROG zgpg
TD 32768
SOLVENT DMSO
NS 2048
DS 4
SWH 24414.063 Hz
FIDRES 0.745058 Hz
AQ 0.6710886 sec
RG 501.187
DW 20.480 usec
DE 6.50 usec
TE 289.6 K
D1 1.00000000 sec
D11 0.03000000 sec
D31 0.00001500 sec
D32 0.89999998 sec
D40 0.00093990 sec
L4 23
L5 26
P32 90.00 usec
TD0 1
======== CHANNEL f1 ========
SFO1 75.4878687 MHz
NUC1 13C
P1 15.00 usec
PLW1 15.00000000 W
======== CHANNEL f2 ========
SFO2 300.1812007 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 10.00000000 W
PLW12 0.20863999 W
PLW13 0.10495000 W
F2 - Processing parameters
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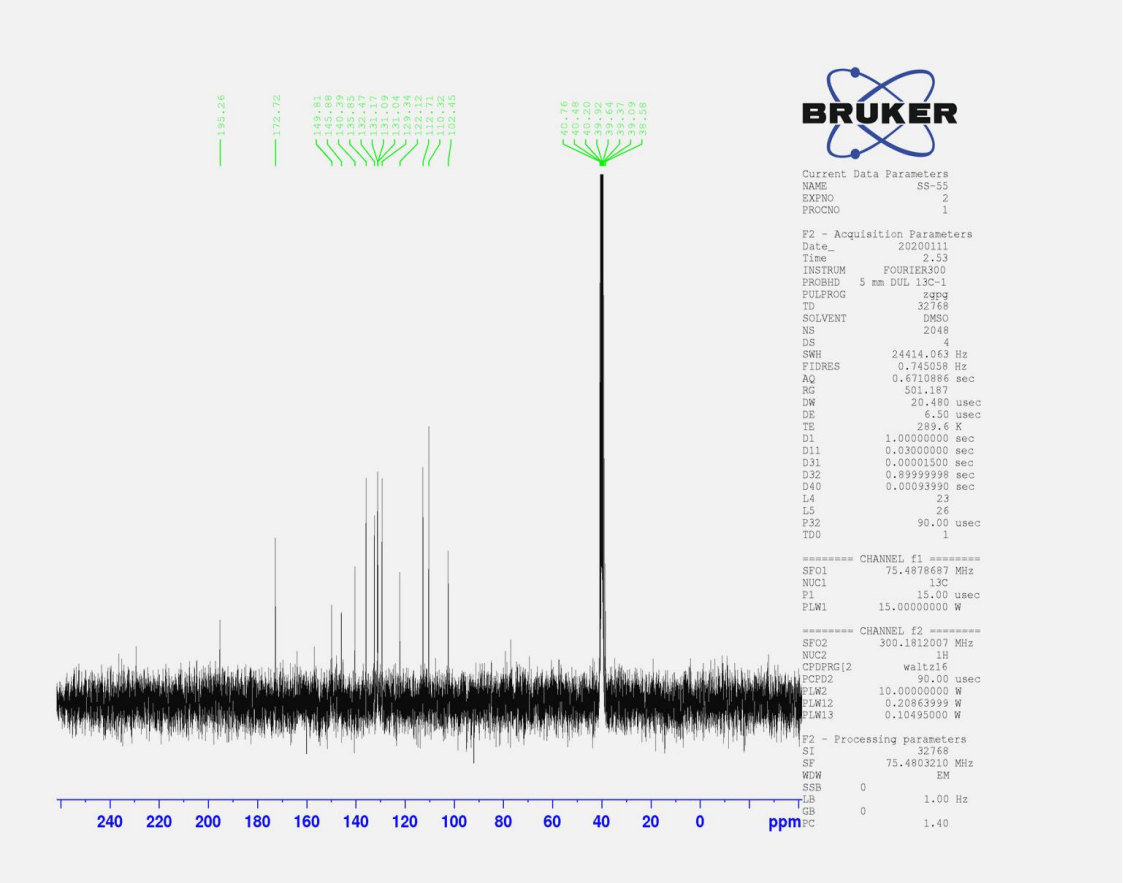
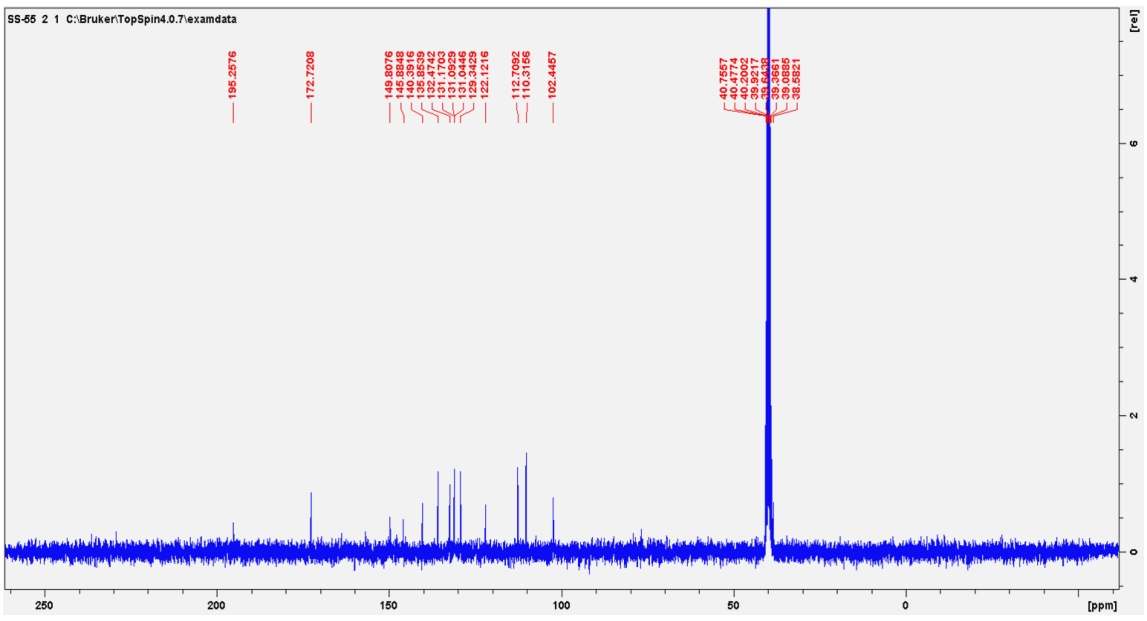
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
240 220 200 180 160 140 120 100 80 60 40 20 0 ppm


195.26
172.72
149.81
145.88
140.39
135.85
132.47
131.17
131.09
131.04
129.34
122.12
122.71
110.32
102.45
40.76
40.48
40.20
39.92
39.64
39.37
39.09
38.58
BRUKER
Current Data Parameters
NAME SS-55
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200111
Time 2.53
INSTRUM FOURIER300
PROBHD 5 mm DUL 13C-1
PULPROG zgpg
TD 32768
SOLVENT DMSO
NS 2048
DS 4
SWH 24414.063 Hz
FIDRES 0.745058 Hz
AQ 0.6710886 sec
RG 501.187
DW 20.480 usec
DE 6.50 usec
TE 289.6 K
D1 1.00000000 sec
D11 0.03000000 sec
D31 0.00001500 sec
D32 0.89999998 sec
D40 0.00093990 sec
L4 23
L5 26
P32 90.00 usec
TD0 1
======== CHANNEL f1 ========
SFO1 75.4878687 MHz
NUC1 13C
P1 15.00 usec
PLW1 15.00000000 W
======== CHANNEL f2 ========
SFO2 300.1812007 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 10.00000000 W
PLW12 0.20863999 W
PLW13 0.10495000 W
F2 - Processing parameters
SI 32768
SF 75.4803210 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
240 220 200 180 160 140 120 100 80 60 40 20 0 ppm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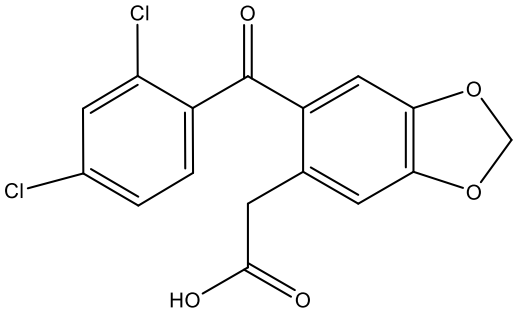
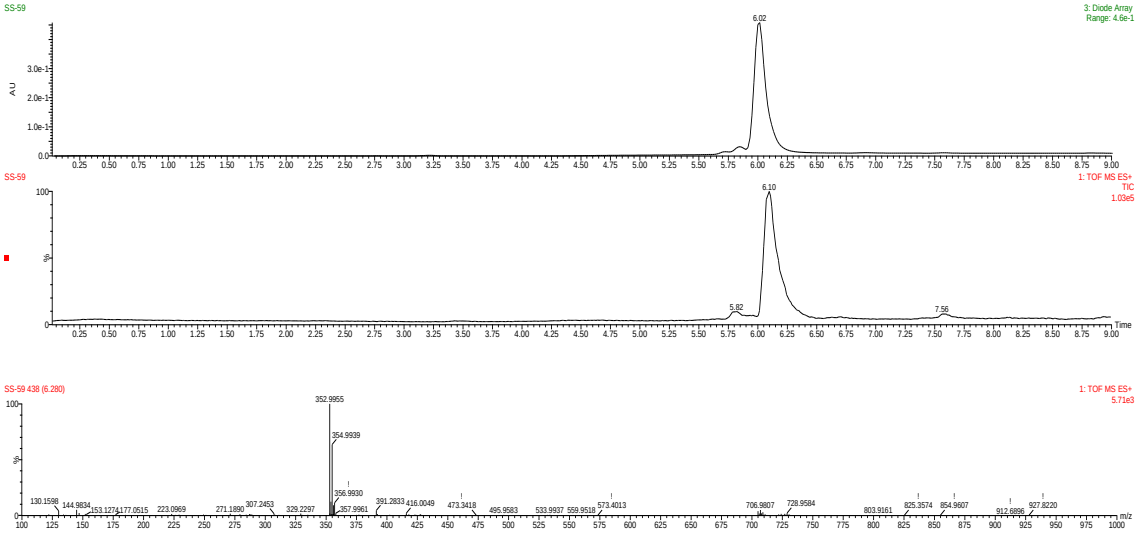
172.7208
149.8076
145.8848
140.3916
135.8558
132.4742
131.1703
131.0929
131.0446
129.3429
122.1216
112.7092
110.3166
102.4457
40.7557
40.4774
40.1992
39.9210
39.6428
39.3661
39.0895
38.8821
[rel]
250 200 150 100 50 0 [ppm]

4f

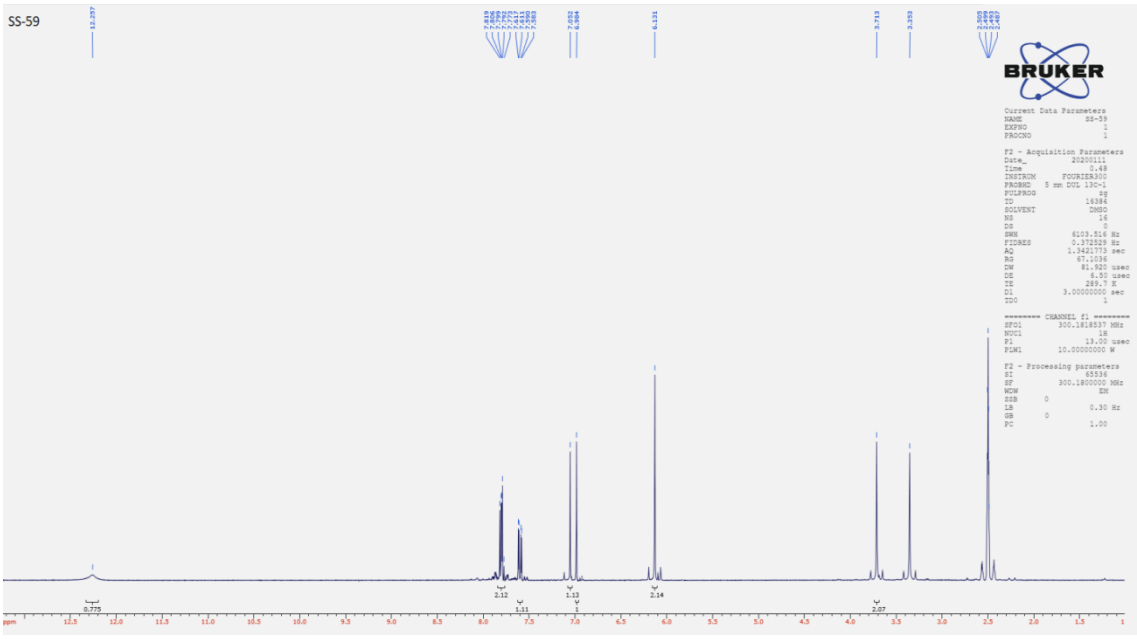


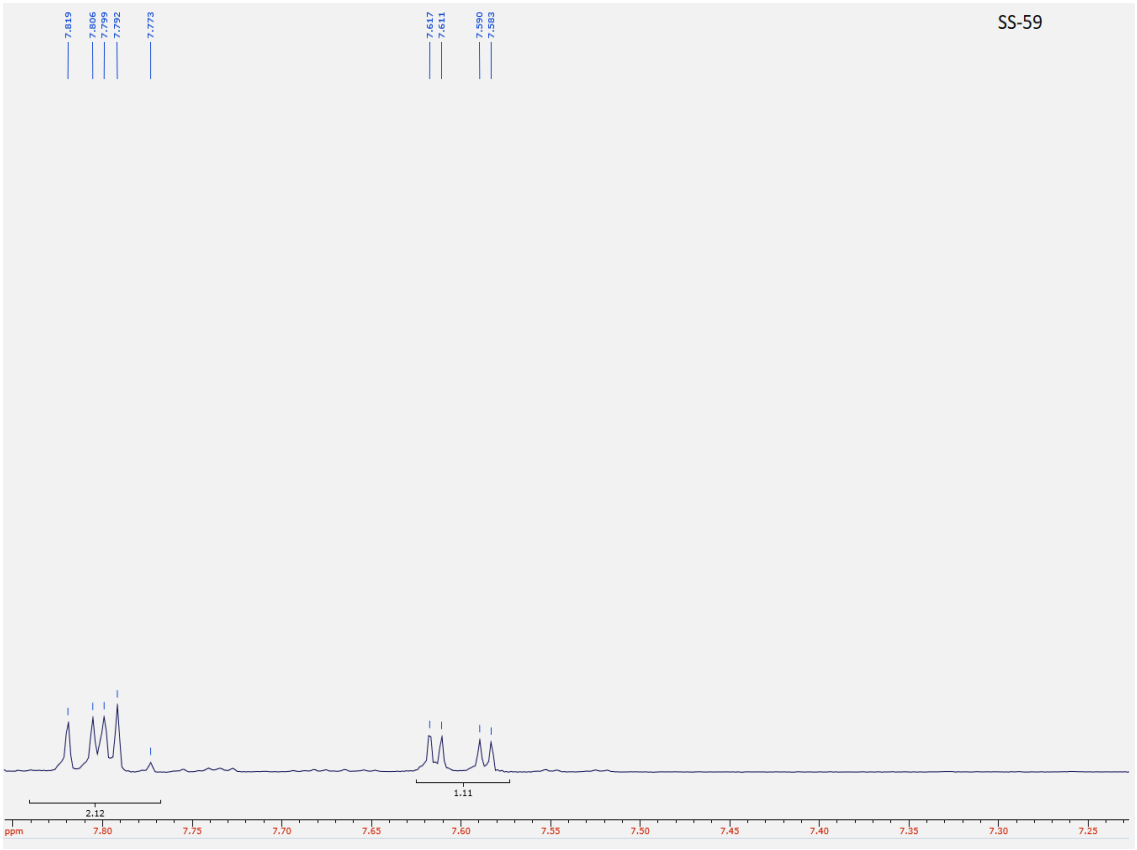
4f

HRMS



H-NMR

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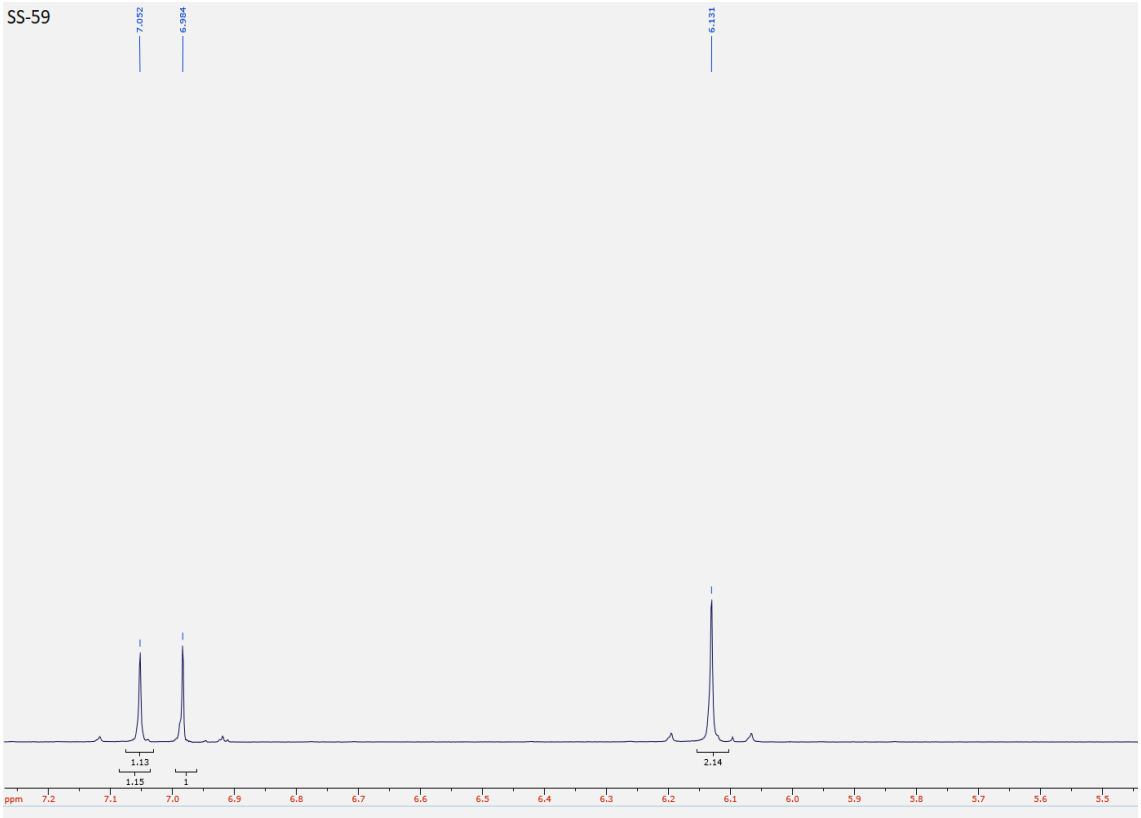
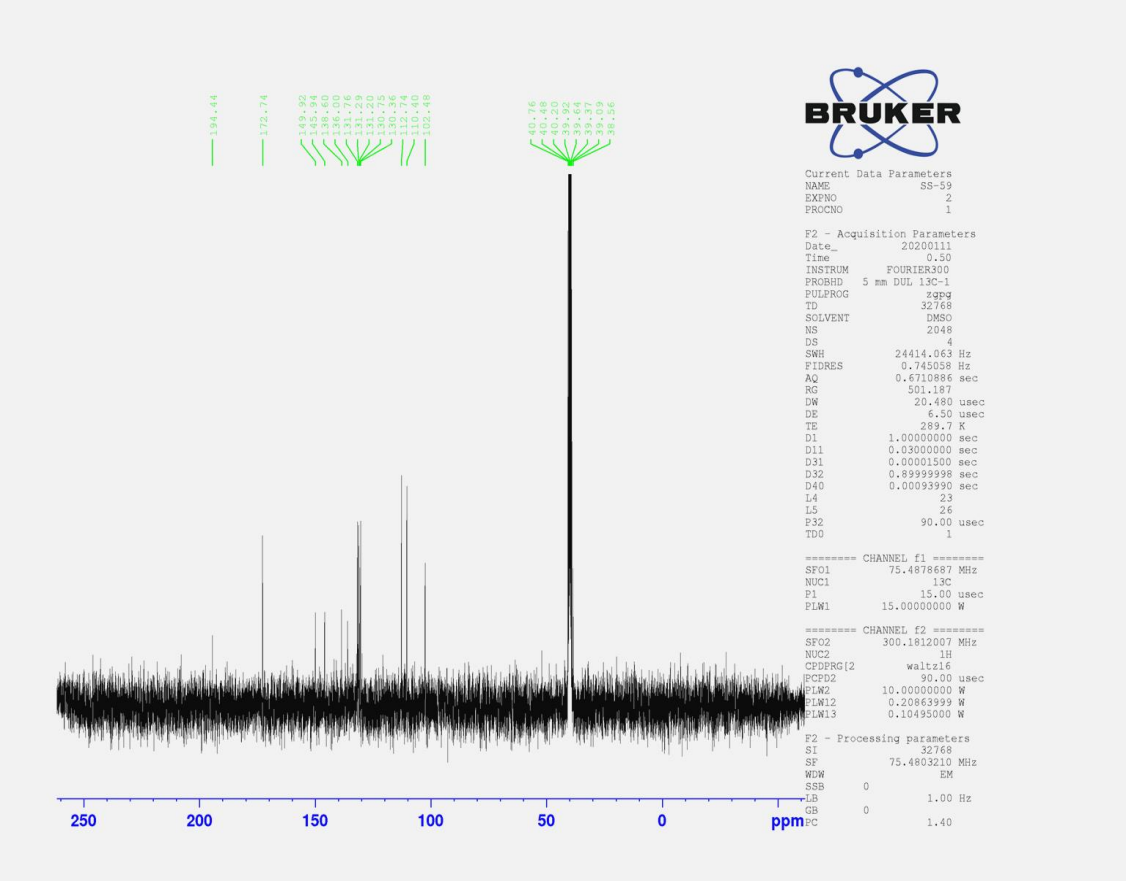
7.806
7.799
7.792
7.773
7.617
7.611
7.590
7.583
2.12
1.11
ppm
7.80
7.75
7.70
7.65
7.60
7.55
7.50
7.45
7.40
7.35
7.30
7.25


SS-59
7.052
6.984
6.131
1.13
1.15
1
2.14
ppm
7.2
7.1
7.0
6.9
6.8
6.7
6.6
6.5
6.4
6.3
6.2
6.1
6.0
5.9
5.8
5.7
5.6
5.5

C-NMR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172.74
149.92
145.94
138.60
136.00
131.76
131.29
131.20
130.75
130.36
123.74
110.40
102.48
40.76
40.68
40.20
39.92
39.64
39.37
39.09
38.56
BRUKER
Current Data Parameters
NAME SS-59
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PROCNO 1
F2 - Acquisition Parameters
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Time 0.50
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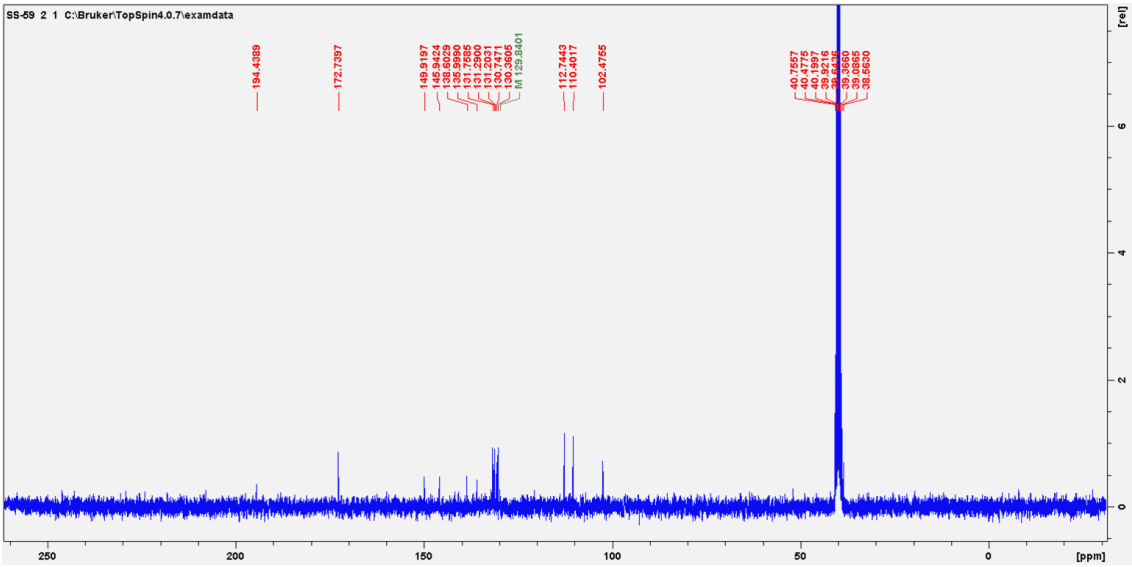
PROBHD 5 mm DUL 13C-1
PULPROG zgpg
TD 32768
SOLVENT DMSO
NS 2048
DS 4
SWH 24414.063 Hz
FIDRES 0.745058 Hz
AQ 0.6710886 sec
RG 501.187
DW 20.480 usec
DE 6.50 usec
TE 289.7 K
D1 1.00000000 sec
D11 0.03000000 sec
D31 0.00001500 sec
D32 0.89999998 sec
D40 0.00093990 sec
L4 23
L5 26
P32 90.00 usec
TD0 1
======== CHANNEL f1 ========
SFO1 75.4878687 MHz
NUC1 13C
P1 15.00 usec
PLW1 15.00000000 W
======== CHANNEL f2 ========
SFO2 300.1812007 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 10.00000000 W
PLW12 0.20863999 W
PLW13 0.10495000 W
F2 - Processing parameters
SI 32768
SF 75.4803210 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
250
200
150
100
50
0
ppm


SS-59 2 1 C:\Bruker\TopSpin4.0.7\examdata
194.4389
172.7397
149.9197
145.9424
138.6029
135.9990
131.7585
131.2990
131.2031
130.7471
130.3690
123.8401
112.7443
110.4017
102.4755
40.7557
40.4775
40.1987
39.9216
39.6436
39.3650
39.0865
38.5630
[rel]
6
4
2
0
250
200
150
100
50
0
[ppm]

Appendix B

Figures of Study's

Figure B.1

heterocyclic structures with therapeutic activitie

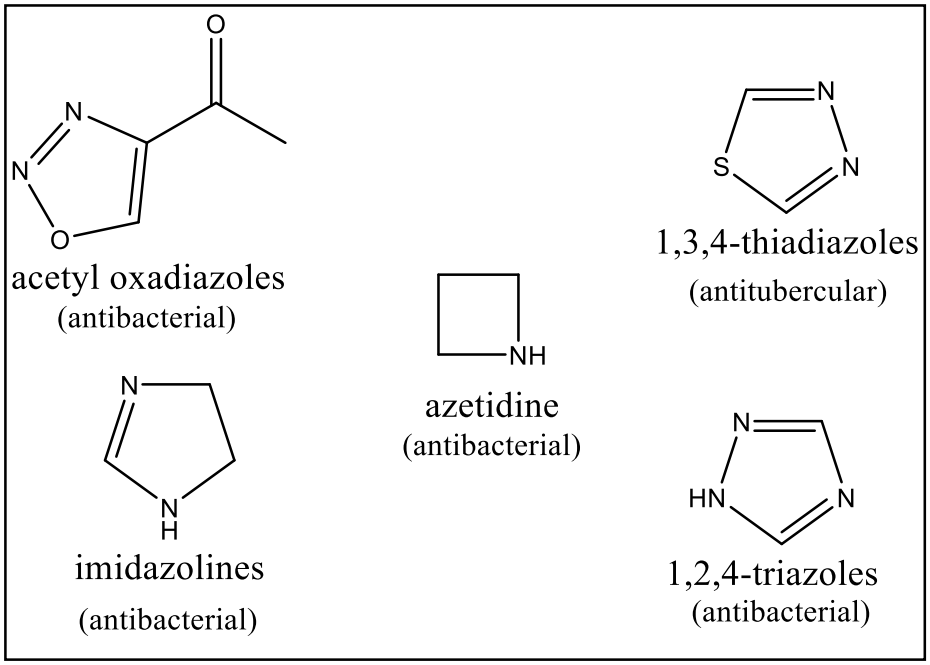


Figure B.2

Detail biochemistry of prostanoids and their pathway of formation

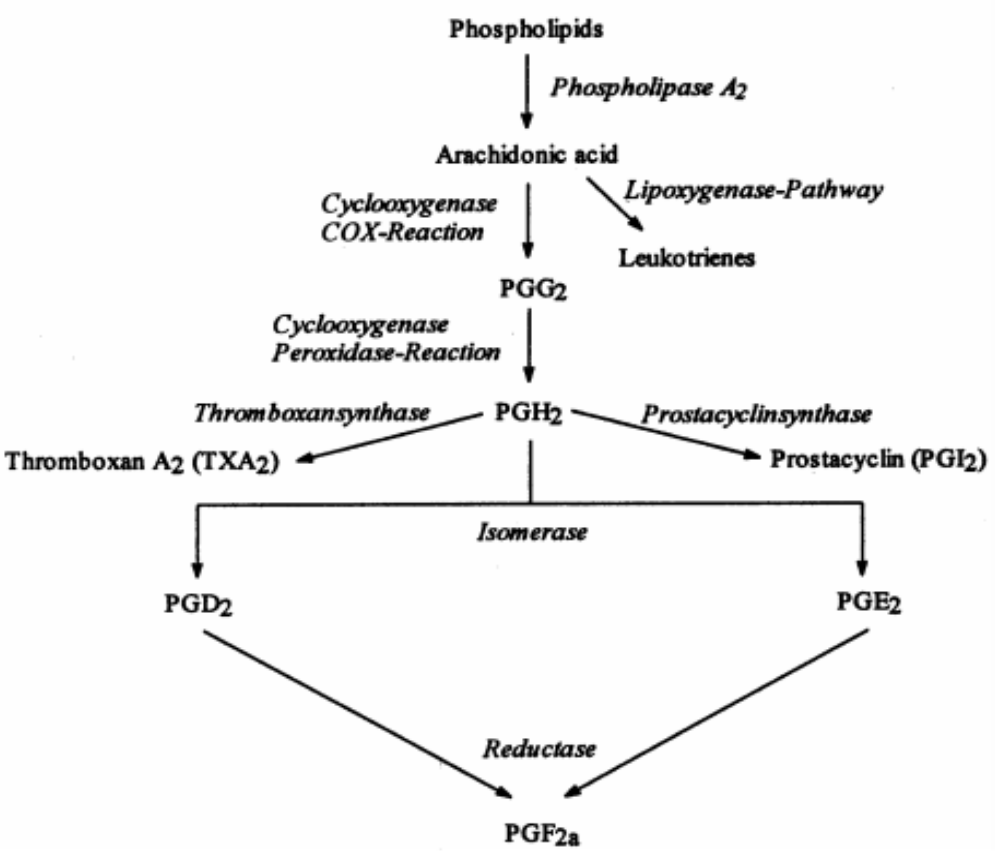
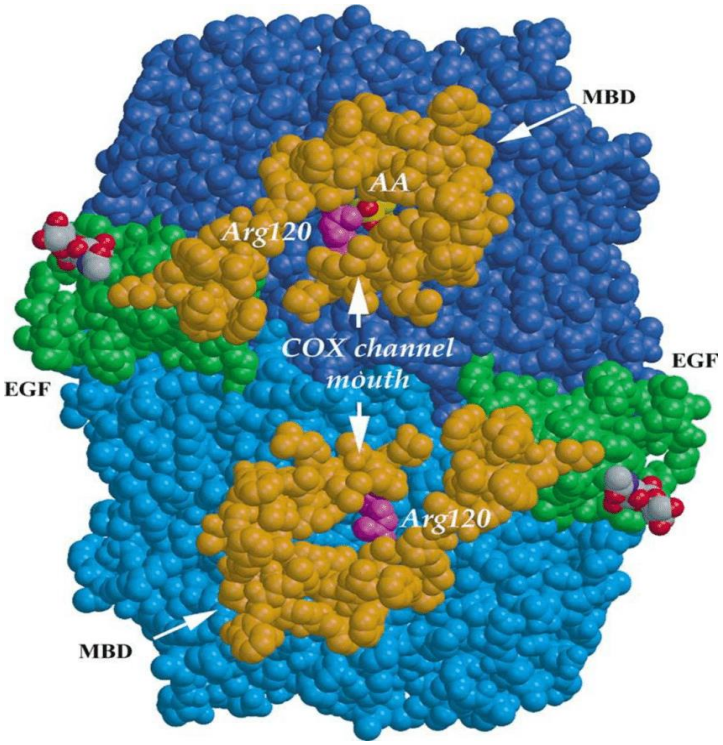


Figure B.4

domains of COX enzyme



جـامعـــة النجاح الوطنية

كليـــة الدراســـات العليـــا

تصنيع وتوصيف وتقييم الفعالية العلاجية لمركبات جديدة مشتقة من مركب 3،4– ميثيلين دايوكسي بنزين

إعداد

صبا محمود حميدي

إشراف

د. محمد هواش

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

2022

تصنيع وتوصيف وتقييم الفعالية العلاجية لمركبات جديدة مشتقة من مركب 3،4– ميثيلين دايوكسي بنزين

إعداد

صبا محمود حميدي

إشراف

د. محمد هواش

الملخص

العقاقير غير الستيرويدية المضادة للالتهابات (NSAIDs) هي ضمن العلاج الأكثر استخدامًا في جميع أنحاء العالم؛ فهي تثبط إنزيم (COX) التي تتوسط التحول الأحيائي لحمض الأراكيدونيك إلى البروستاجلاندين الالتهابي. في أطروحة الماجستير هذه، تم تصنيع مشتقات جديدة من البنزوديوكسول مع أريل أسيتات ومجموعات حمض أريل أسيتيك، وتم تحديدها وتقييمها من حيث فعاليتها وانتقائيتها تجاه COX-1 وCOX-2 باستخدام مجموعة اختبار تثبيط (COX) في المختبر. تم تقييم السمية الخلوية للمركب باستخدام فحص MTS ضد خط خلايا سرطان عنق الرحم (HeLa). استخدمنا تقنيات FTIR وHRMS و1 H-NMR و13 C-NMR للتعرف على المركبات. أوضحت النتائج أن أقوى مركب ضد إنزيم COX-1 كان 4f مع IC_{50} = 0.725 ميكرومتر. أظهر المركب 3b نشاطًا قويًا ضد كل من COX-1 و COX-2 مع IC=1.12 و1.3 ميكرومتر، على التوالي، وتم تحديد نسبة الانتقائية (0.862) لتكون أفضل من كيتوبروفين (0.196). في الجهة الأخرى، كان المركب 4d هو الأكثر انتقائية مع قيمة نسبة COX-1 /COX-2 البالغة 1.809 في ارتباط مع نسبة الكيتوبروفين. أظهرت جميع المركبات نشاطًا سامًا للخلايا ضد خط خلايا سرطان عنق الرحم هيلا عند نطاق تركيز عالٍ (0.219 – 1.94 ملي

مولار)، وكان أكثر المركبات السامة للخلايا هو 3e مع قيمة CC_{50} بقيمة 219 ميكرومتر. كان هذا أكثر بعشرة أضعاف من قيم IC_{50} البالغة 2.36 و2.73 ميكرومتر مقابل COX-1 وCOX-2، على التوالي. في الختام، تحتوي المكتبة المركبة على نشاط معتدل ضد كل من إنزيمات COX-1 و COX-2 وكانت المركبات المهلجنة Ortho أكثر فاعلية من تلك الموجودة في Meta.

كلمات مفتاحية: العقاقير غير الستيرويدية المضادة للالتهاب، البنزوديوكسول، هيلا او سرطان عنق الرحم، كيتوبروفين.