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Synthesis, Characterization and Preliminary Pharmacological Evaluation of Naproxen Containing Pyrazoline Derivatives

A Thesis

Submitted to the Department of Pharmaceutical Chemistry and the Committee

of Graduate Studies of the College of Pharmacy-University of Al-Mustansiriyah in Partial Fulfillment

of the Requirement for the Degree of Master in Pharmacy

"Pharmaceutical Chemistry"

By

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بسم الله الرحمن الرحيم

اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ ﴿١﴾ خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ ﴿٢﴾ اقْرَأْ وَرَبُّكَ الْأَكْرَمُ ﴿٣﴾ الَّذِي عَلَّمَ بِالْقَلَمِ ﴿٤﴾ عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ ﴿٥﴾

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We certify that this thesis, **"Synthesis, Characterization and Preliminary Pharmacological Evaluation of Naproxen Containing Pyrazoline Derivatives"**, was prepared under our supervision at the Department of pharmaceutical Chemistry, College of Pharmacy- University of AL-Mustansiriyah as a partial fulfillment of the requirements for the degree of Master in Pharmacy (Pharmaceutical chemistry).

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Dedication

To.......

My parents

My lovely daughter

My sisters and brothers

With all my love

Noor

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List of Abbreviations

^{1}H-NMR	*Proton nuclear Magnetic Resonance*
AA	*Arachidonic Acid*
ANOVA	*Analysis of Variance*
Arg.	*Arginine*
ATP	*Adenosine Triphosphate*
CNS	*Central Nervous System*
COX	*Cyclooxygenase*
DCC	*Dicyclohexyl Carbodiimide*
DCM	*Dichloromethane*
DCU	*Dicyclohexylurea*
DMSO	*Dimethyl Sulfoxide*
FT-IR	*Fourier Transform Infrared Spectroscopy*
GAA	*Glacial Acetic Acid*
GI	*Gastrointestinal*
Gly.	*Glycine*
His.	*Histidine*
i.p.	*Intraperitoneal*
ILe	*Isoleucine*
Nap.	*Naproxen*
NSAIDs	*Non-Steroidal Anti-Inflammatory Drugs*
P	*Probability*
PG	*Prostaglandin*
PTZ	*Pentylenetetrazole*

SEM	*Standard Error of Mean*
Ser.	*Serine*
S_N2	*Second order nucleophilic substitution reaction*
Tb	*Tuberculosis*
TLC	*Thin Layer Chromatography*
Tyr.	*Tyrosine*

ABSTRACT

Non-steroidal anti-inflammatory drugs (NSAIDs) are the competitive inhibitors of cyclooxygenase (COX), the enzyme which mediates the bioconversion of arachidonic acid to inflammatory prostaglandins. Their use is associated with the side effects such as gastrointestinal problems. The therapeutic anti-inflammatory action of NSAIDs is produced by the inhibition of COX-2, while the undesired side effects arise from inhibition of COX-1 activity. Thus, it was thought that selective COX-2 inhibitors would have reduced side effects.

Therefore, pyrazoline ring derivatives as a pharmacophore were incorporated to the naproxen; to increase its size were synthesized and preliminarly evaluated as potential anti-inflammatory agents with expected selectivity toward COX-2 enzyme.

Synthesis of the target compounds (V_{a-f}) has been successfully achieved. Purity, characterization, and identification of the synthesized compounds were detected by determination of physical properties (melting points & R_f values), Fourier transform infrared spectroscopy (FT-IR) and ^{1}H-Nuclear magnetic resonance (^{1}H-NMR) spectroscopy.

In vivo potent anti-inflammatory effects of the final compounds were evaluated in rats using egg-white induced edema model of inflammation. The tested compounds (V_{a-f}) and the reference drug (naproxen) produced significant reduction of paw edema with respect to the effect of control group (propylene glycol 50% v/v). However, Compound (V_d) and (V_e) show comparable effect to naproxen at all experimental time while compounds (V_a), (V_b) and (V_c) produced significantly lower inhibitory effect than naproxen at time (120-240

min.). Furthermore, compound (V_f) exert significantly higher paw edema reduction than naproxen at (60-240min.).

Also the antibacterial activities of the final compounds were evaluated by Well Diffusion Method. All tested compounds exert significant antibacterial activity against gram positive and gram negative bacteria especially *Bacillus, Staphylococcus aureus, Pseudomonas Aeroginosa* and *Escherichia coli* in comparison to DMSO as control group, and parent compound naproxen. In comparison the antibacterial results among the tested compounds (V_e) may regard the best one and (V_c) the lower one.

These results encourage further evaluation of these compounds to demonstrate or identify their selectivity toward COX-2 isoenzyme and other antimicrobial activities.

CHAPTER ONE

INTRODUCTION

Introduction

1.1. General Aspects of Inflammation and Inflammatory Response:

|It is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. Inflammation is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue [1].

Inflammation can be classified as either acute or chronic. Acute inflammations describe the rapid response of innate immune components to a challenge. Acute inflammation starts rapidly and quickly becomes severe like: acute bronchitis, sore throat from a cold or flu, acute appendicitis, acute dermatitis, acute tonsillitis and acute sinusitis.

While chronic inflammation may arise because of susceptibility in the individual to perpetuate inflammatory response, failure to eradicate the agents or factors triggering inflammation (e.g. foreign body embedded in injured tissue), persistent microbial infection (e.g. TB, or continuing tissue damage) and pro-inflammatory stimuli, such as those encountered in the atherosclerotic plaque [2, 3].

Several pathways lead to the development of an inflammatory response, depending on the etiological agent (type of microorganism, tissue damage), the affected organ and the genetic and general background of the individual. An inflammatory response is characterized by: 1) vasodilatation of arterioles and capillaries, which causes an increased blood flow to the affected tissue; 2)increased vascular permeability; 3) recruitment of innate immunity cells (in acute inflammation) and adaptive immune cells (in chronic inflammation); and 4) fever.

These changes are caused by soluble inflammatory mediators: cytokines and chemokines, plasma proteases, lipid mediators, neuropeptides, amines, nitric oxide, acute phase proteins and leptin, among others; some of these mediators are stored in intracellular compartments and are released in response to inflammatory stimuli [4].

Almost, steroids namely: prednisolone, dexamethasone, betamethasone, triamcinolone and hydrocortisone are front-line, highly efficacious anti-inflammatory agents when used alone or in conjunction with other therapies. Their use, however, is limited due to their severe side effects [5]; these have been more or less replaced by much safer and better tolerated non-steroidal anti –inflammatory drugs (NSAIDs) [6].

1.2. Non-Steroidal Anti-Inflammatory Drugs:

Non- steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used medications in the world, ow

ing to their analgesic, anti-inflammatory, and antipyretic properties [7,8]. However, the use of "traditional" NSAIDs results in serious upper gastrointestinal (GI) adverse events in nearly one fourth of patients [9].

Recent years, epidemiological study indicate that NSAIDs are neuroprotective [10], so prolong used reduce the risk of Alzheimer [11].

Furthermore, clinical study provides evidence that the derivatives of NSAIDs are promising to give anticancer activity [12].

The most common side effects are the propensity of NSAIDs to induce gastric or intestinal ulceration. Thus patients who use NSAIDs on chronic basis have about three times greater relative risk for serious adverse GI events compared to the population of non-user [13,14] .There is therefore a need for anti-inflammatory and analgesic drugs that will provide symptom relief without causing GI injury [15].

1.3. Mode and Therapeutic Action of NSAIDs:

The NSAIDs are chemically diverse, most being organic acids. Despite their structural heterogeneity, NSAIDs possess a common mode of action, which block prostaglandin(PG) synthesis largely though their inhibition of the enzyme cyclooxygenase (COX), which catalyze the transformation of arachidonic acid to prostaglandins and thromboxanes [16] as shown in Figure (1-1).
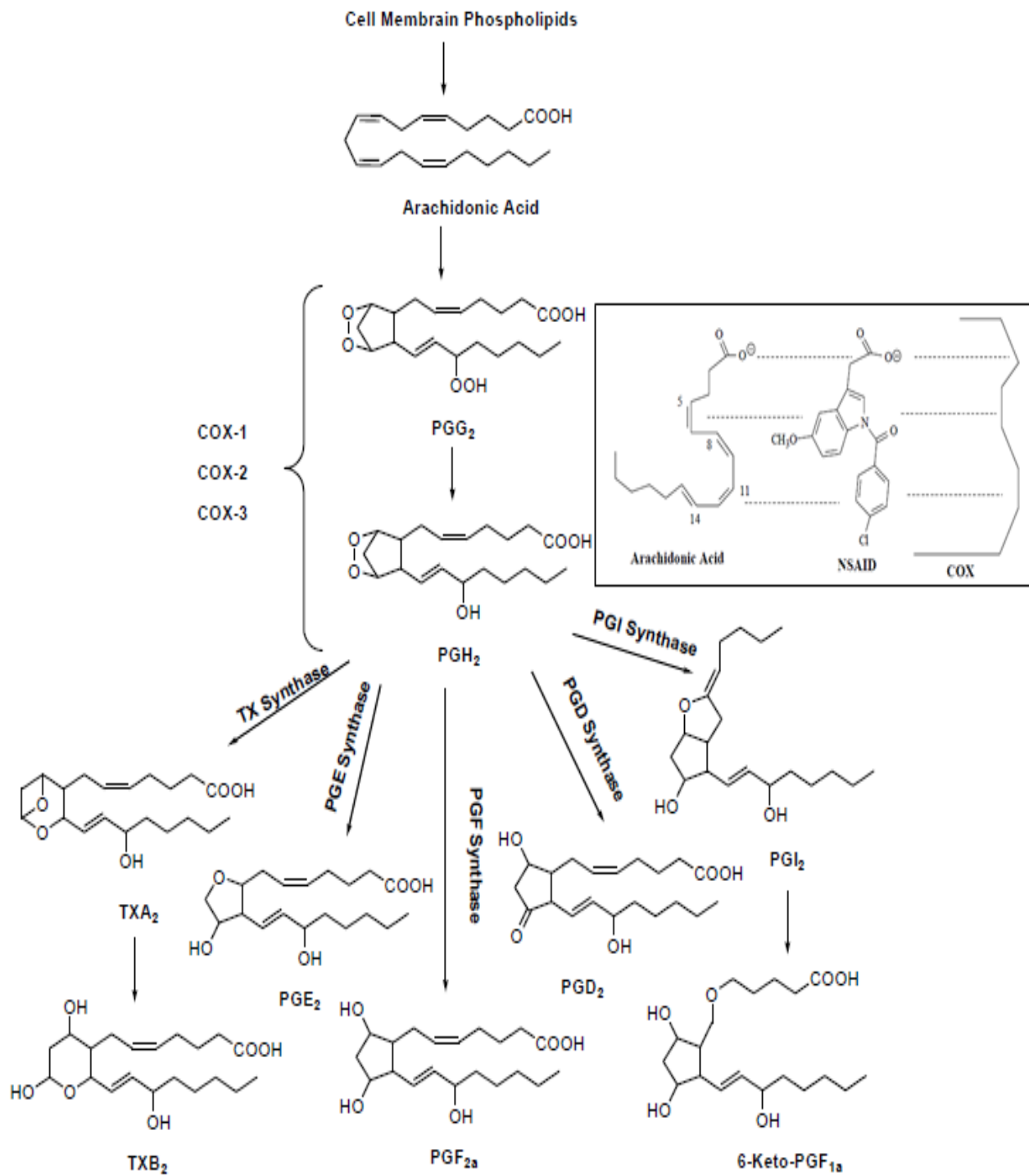
Figure (1-1): Enzymatic Pathway of Prostaglandin (PG) Formation from Arachidonic Acid [17, 18]

The NSAIDs have three major pharmacologically desirable actions, stemming from the suppression of prostanoid synthesis in inflammatory cells through inhibition of COX isoform [19]. These are:

1) Anti-Inflammatory Effect:

NSAIDs exert their anti-inflammatory effect through inhibition of prostaglandin synthase, or cyclooxygenase. Some work suggests that activation of endothelial cells and expression of cell adhesion molecules play a role in targeting circulating cells to inflammatory sites. NSAIDs may inhibit expression of these cell adhesion molecules and may directly inhibit activation and function of neutrophils [20].

2) Analgesic Effect:

Although they are classified as mild analgesics, NSAIDs have a more significant effect on pain resulting from the increased peripheral sensitization that occurs during inflammation and leads nociceptors to respond to stimuli that are normally painless. In particular, it is believed that inflammation leads to a lowering of the response threshold of polymodal nociceptors [21]

3) Antipyretic Effect:

NSAIDs reduce the body temperature in febrile states. The fact that selective COX-2 inhibitors are effective antipyretic agents indicates that the COX-2 predominantly involved in thermoregulation [22].

1.4. Types of COX Enzymes:

Three different COX enzymes existed, known as COX-1, COX-2 and COX-3: The distribution and roles of COX-1 and COX-2 in the body are shown in Figure (1-2).

COX-1 is a constitutive isoform found in most normal cells and tissues [23]. It is stimulated by growth factor and hormones and it has been called the housekeeping enzyme [24]. The COX-1 plays fundamental roles in the generation of PGs in homoeostasis[25], and several other physiological functions including gastric protection and control of renal blood flow [26].Moreover ,COX-1 regulate physiologic processes of platelet aggregation[24]. The door remains open, also, to potential roles for COX-1 as a primary player in pathogenesis; for example, COX-1 was implicated in a specific instance of carcinogenesis in the human ovary [27].

COX-2 is the readily inducible form of the enzyme and is commonly associated with several pathological conditions as showing in Figure (1-2), COX-2 is found in the heart [28],spinal cord [29], vascular endothelium, brain, kidney, bone and female reproductive system and is also involved in certain physiological processes [30,31]. However, it is induced by inflammatory stimuli such as bacterial endotoxin and cytokines [32,33]. For example, up regulation of COX-2 in arthritic joints contributes to the classical symptoms of rheumatoid arthritis (RA). Increased levels of COX-2 have also been seen in diseases such as Alzheimer's disease (AD), systemic lupus erythematous, colon, breast and pancreatic cancer, as well as diabetic neuropathy and premature labor [30,32].

COX-3 is a splice variant/isoenzyme of COX-1 and, more suitably, may have been named COX-1b. It was initially reported to be expressed in canine cerebral cortex. In humans COX-3 mRNA is found in highest concentrations in the brain and heart [34]. The importance of COX-3 is that it could explain the pharmacological actions of drugs such as acetaminophen and other antipyretic analgesic which are weak inhibitors of COX-1 and COX-2, but penetrate easily into the central nervous system. Non-steroid anti-inflammatory drugs such as diclofenac or ibuprofen are also potent inhibitors of COX-3 expressed in cultured cells, but being highly polar they are didn't to reach brain COX-3 in effective concentrations [35].
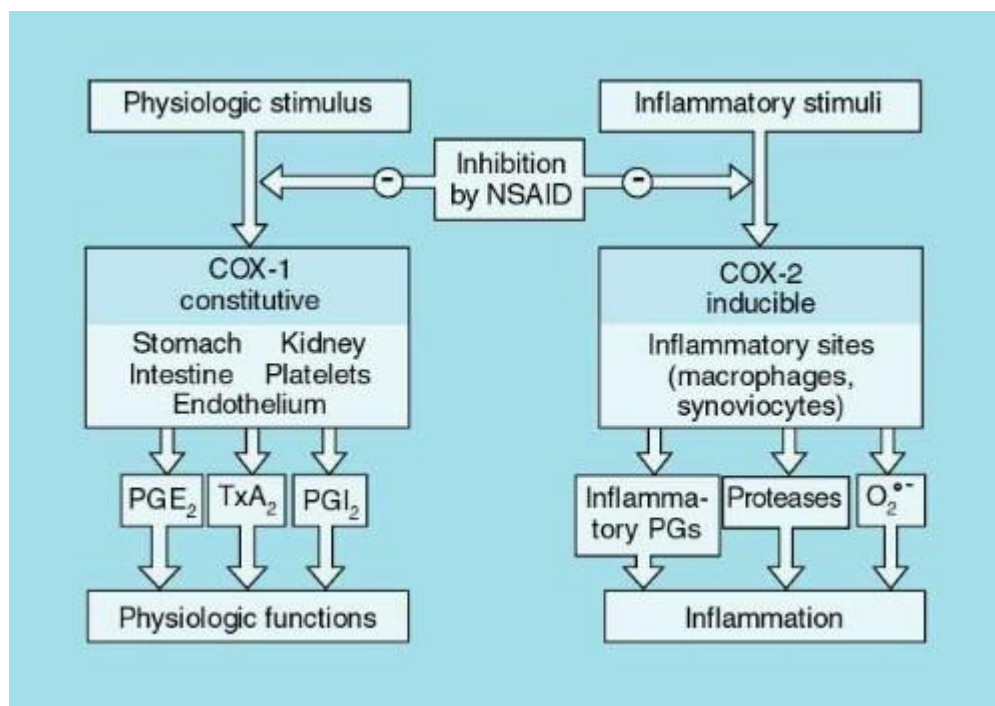
Figure (1-2): Roles of COX-1 and COX-2 [36]

1.5. Enzymatic Structure:

The COX isoenzymes are membrane-bound enzymes in the endoplasmic reticulum (ER). The crystal structures of the COX isoforms are quite structurally homologous and consistent with a high sequence identity. The overall structures of COX-1 and COX-2 are highly conserved. The COX monomer consists of three structural domains: an N-terminal epidermal growth factor (EGF)-like domain, a membrane binding domain (MBD) of about 48 amino acids in length which anchors the protein to one leaflet of the lipid bilayer, and a large C-terminal globular catalytic domain with the COX active site which accommodates the substrate or the inhibitors and the peroxidase one which contains the heme cofactor. These sites are distinct but functionally and structurally interconnected [37] Figure (1-3).
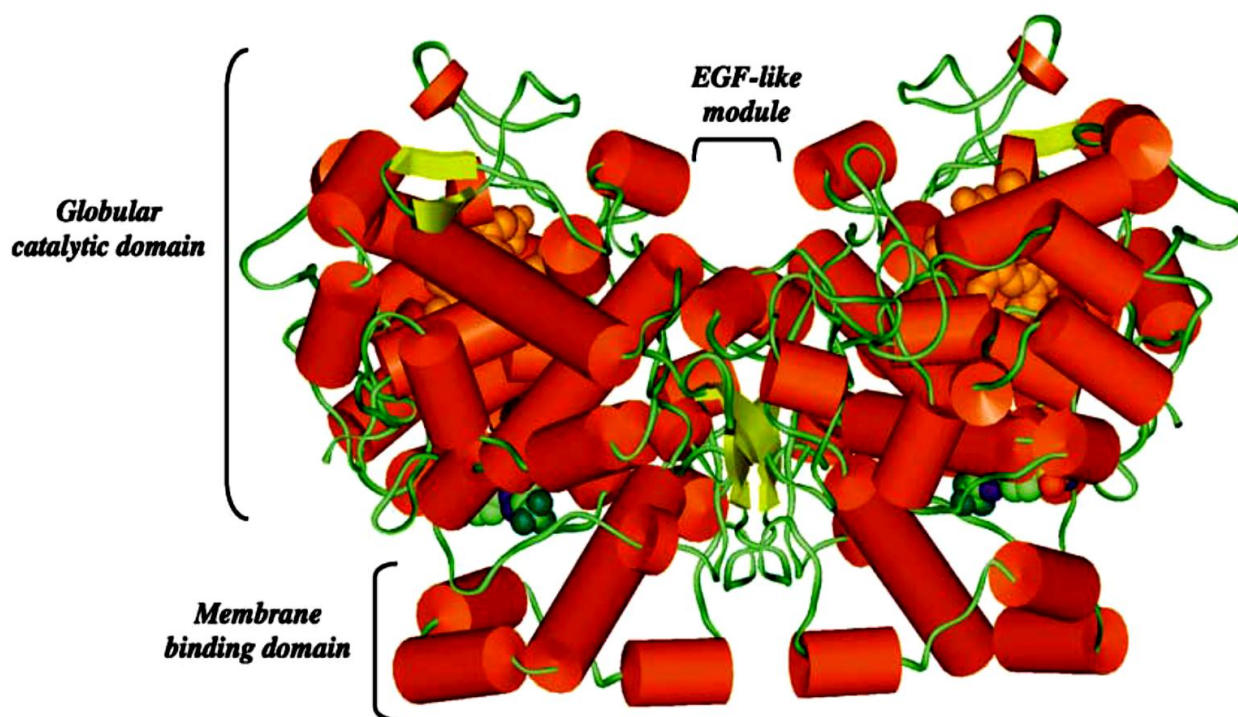
Figure (1-3): Structure of Mouse COX-2 Homodimer [38]

The cyclooxygenase active site is created by a long hydrophobic channel that is the site of non-steroidal anti-inflammatory drug binding. This active site extends from the membrane-binding domain (the lobby) to the core of the catalytic domain [39, 40].

The arachidonate-binding site is located in the upper half of the channel, from Arg-120 to near Tyr-385. Ser- 530, positioned in the middle of the channel, is the site of acetylation by aspirin [41].

Three amino acid differences result in a larger (about 20%) and more accessible channel, in COX-2. The exchange of a valine at position of 523 in COX-2 for a relatively bulky isoleucine (Ile) residue in COX-1 at the same position of the active site of the enzyme causes a structural modification. This modification in the COX-2 enzyme allows the access to an additional side pocket, which is a pre-requisite for COX-2 drug selectivity. Access to this side pocket is restricted in the case of COX-1. In addition, the exchange of Ile-434 for a valine in COX-2 allows a neighboring residue phenylalanine-518 to swing out of the way, increasing further access to the side cavity. Additional essential amino acid difference between these two isoforms is the presence of an arginine within the side pocket of COX-2, in place of histidine-513 (His-513) in COX-1, which can interact with polar moieties. These differences between the COX active sites have major implications for the selectivity profile of inhibitors [38, 42, and 43] as shown in Figure (1-4).
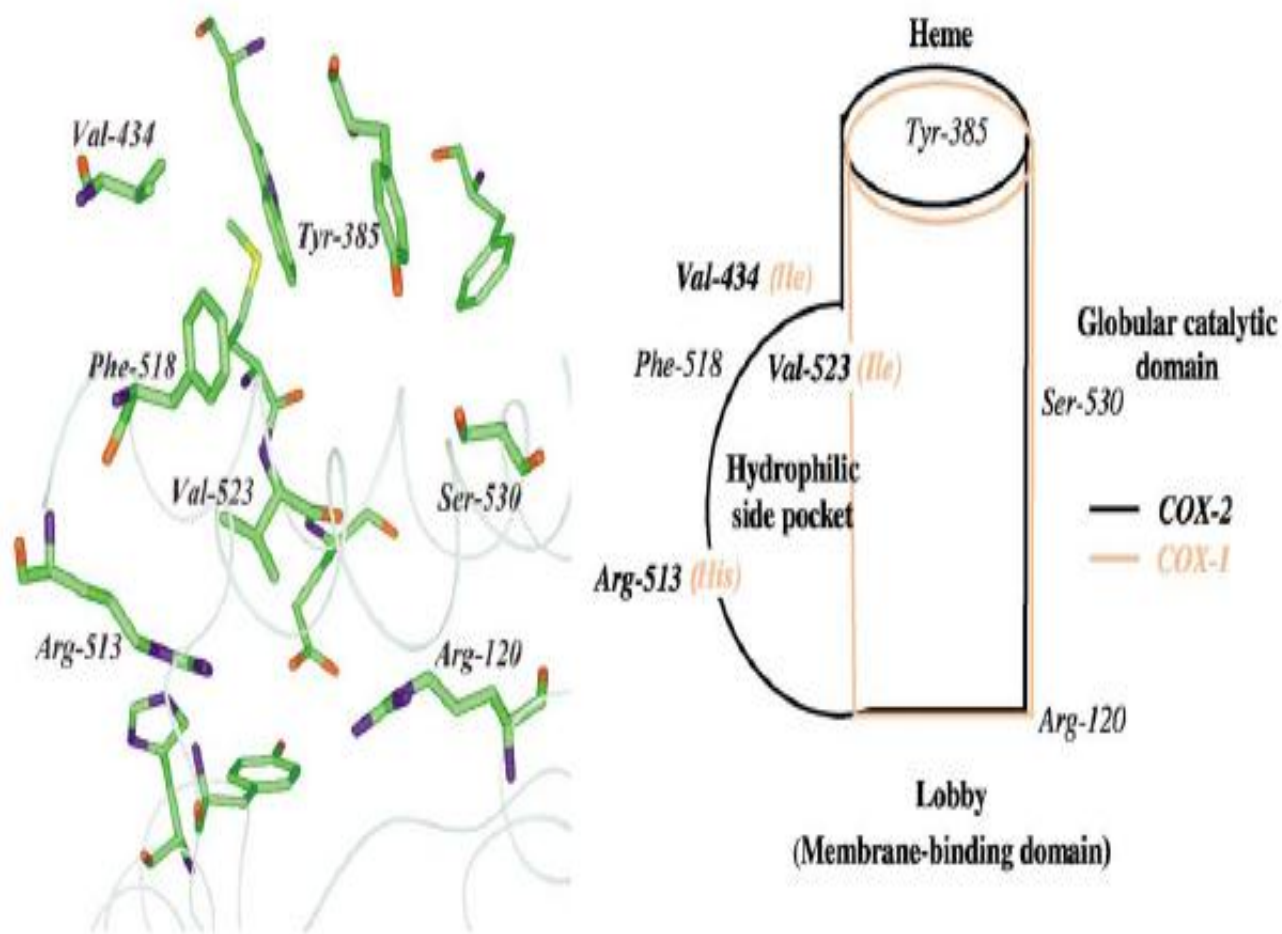
Figure (1-4): The COX-2 Active Site and Its Schematic Representation [44]

1.6. Classification of Non-Steroidal Anti-Inflammatory Drugs:

They are a chemically heterogeneous group of organic acids sharing certain therapeutic action and adverse effects. Aspirin is considered a prototype of NSAIDs to which anti-inflammatory, analgesic and antipyretic activities of other and newer NSAIDs are compared [45].

These agents can be classified in different ways:

First: Selectivity of NSAIDs:

NSAIDs are classified according to their selectivity to inhibit COX enzymes into:

1) Conventional COX Enzyme Inhibitors:

The Conventional COX enzyme inhibitors are not selective and inhibiting all types of COX enzyme, and cause peptic ulceration and dyspepsia. It is believed that such lack of selectivity and direct irritation of the gastric mucosa by NASIDs of a carboxylic acid type, are caused the "dual-insult" of NSAIDs. Prostaglandins have a protective role in the GI tract, preventing acid-insult to the mucosa [46].

2) Novel COX Enzyme Inhibitors:

The discovery of a second cyclooxygenase isoform COX-2 in the early 1990 created a new scenario where selective COX-2 inhibitors (coxibs) [47] represented a second generation NSAIDs, with a reduction in undesirable side effects of (GI) damage [48].

Because COX-2 is usually specific to inflamed tissue, there is much less gastric irritation associated with COX-2 inhibitors, with a decreased risk of peptic ulceration [49].

Selective COX-2 inhibitors elicit less clinically significant GI damage and bleeding than conventional NSAIDs [50, 51].

In addition, several other clinical uses of selective COX-2 inhibitors have been investigated [52], some of these indications including the treatment of the neurodegenerative disease like Alzheimer [53] and Parkinson disease [54] are now under clinical trials to validate the therapeutic possibilities of COX-2 inhibitors [55]. Moreover studies show that the COX-2 enzyme would be an interesting target in the treatment of some cancers [56]. These classes include celecoxib (1) and valdecoxib (2) [57].
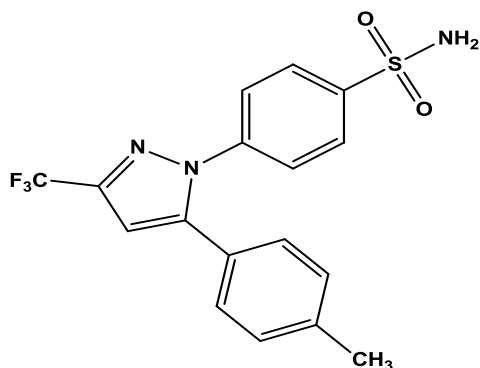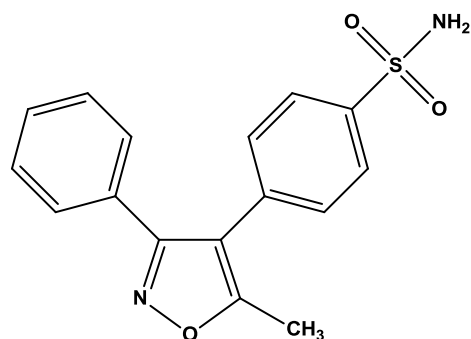
(1)   (2)

Second: Reversibility of COX Inhibitors:

NSAIDs can be classified according to their action on the cyclooxygenase enzyme in to:

1) *Irreversible Inhibitors of COX-1 or COX-2:*

Where aspirin (3) is the only medically used agent covalently and irreversibly acetylates the enzyme COX-1, so inactivating it, while acetylating of the enzyme COX-2 by aspirin will not inactivating this enzyme but leading to modification of

their products. The 2-(hex-1-ynylthio) phenyl acetate (4) is a selective irreversible COX-2 inhibitor which has the ability to inactivate this enzyme [58].
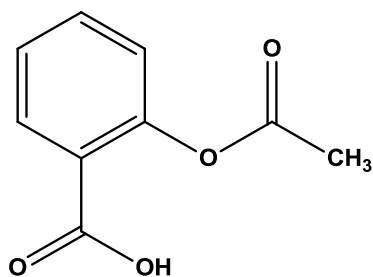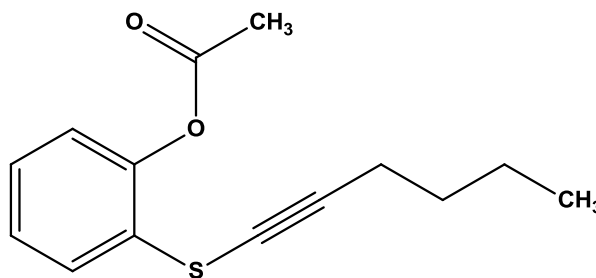
(3)   (4)

2) Reversible, Competitive Inhibitors of COX-1 and COX-2:

Inhibitors such as ibuprofen (5), compete with AA to bind to the catalytic center of COX [59].
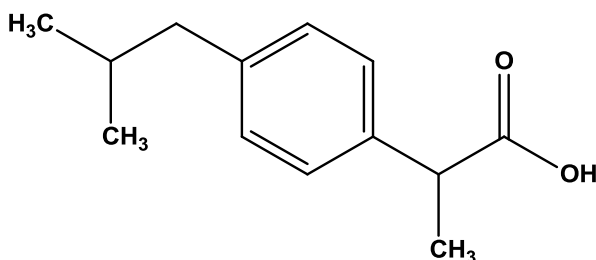
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3) Slow, Time-Dependent, Reversible Inhibitors of COX-1 and COX-2:

Acting through ionic interactions between a carboxylic moiety on the inhibitor and an arginine residue of COX, this group of NSAIDs, such as indomethacin (6) and flurbiprofen (7), seem to influence the helix D region of COX protein rendering it less flexible and thus less active [60].
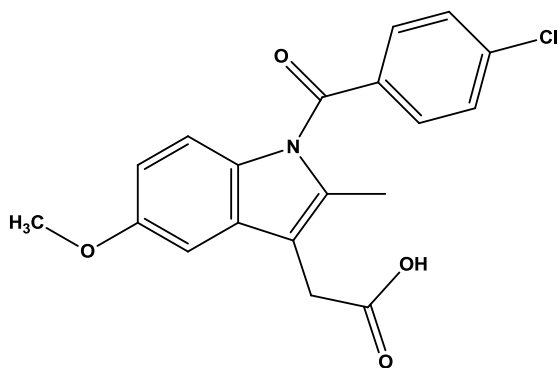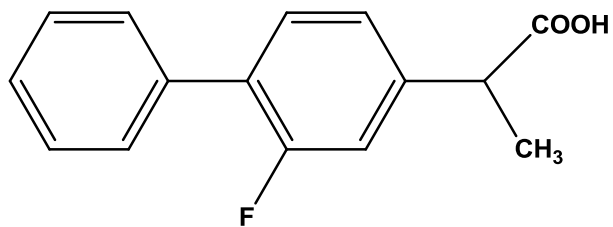
(6)   (7)

4) Slow, Time-Dependent Irreversible Inhibitors of COX-2:

Representatives of this group are selective COX-2 inhibitors such as celecoxib (1), rofecoxib (8) and others. They are weak competitive inhibitors of COX-1, but inhibit COX-2 in a slow time-dependent process [61].
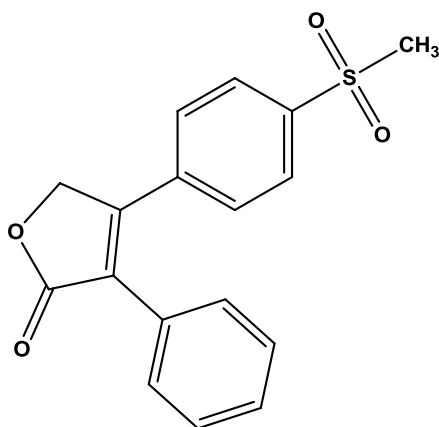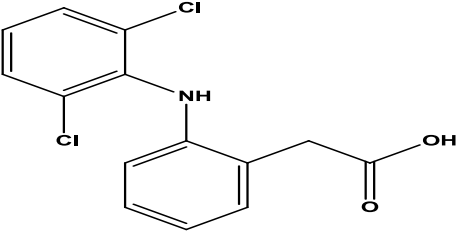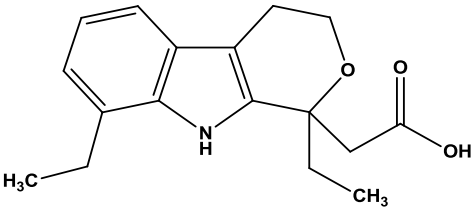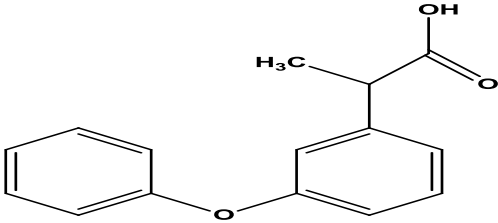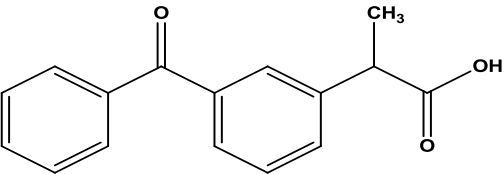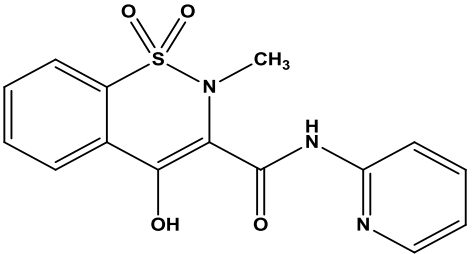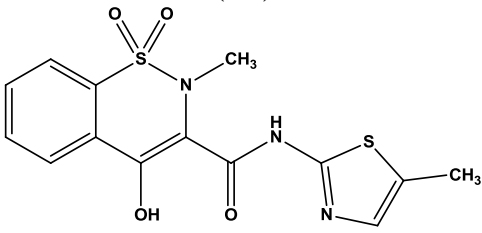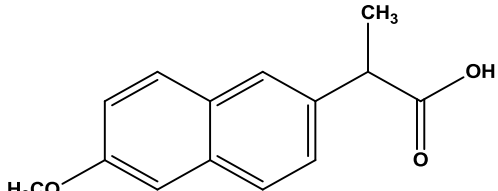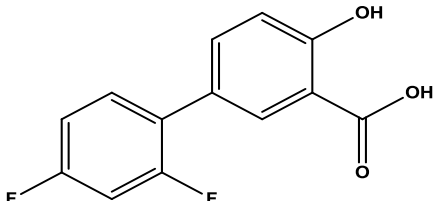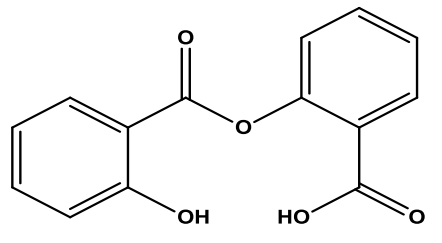
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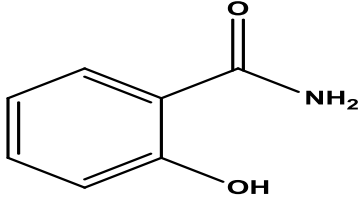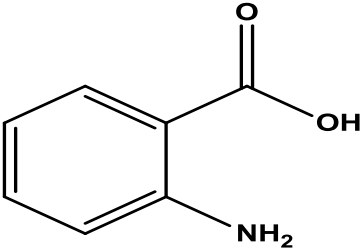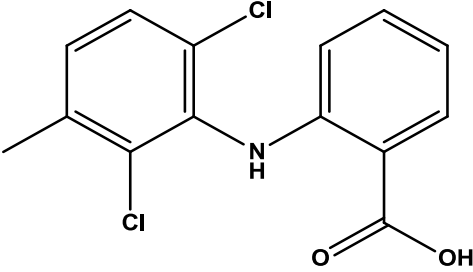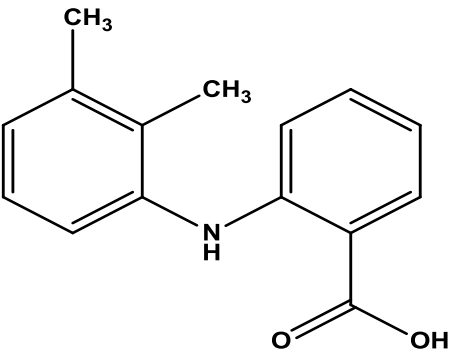
Third: Basic Chemical Structures:

NSAIDs can be classified according to their basic chemical structures as shown in Table (1-1):

Table (1-1): Classification of NSAIDs According to their Chemical Structure

Classes	Examples	Chemical Structure
Aryl acetic Acid Analogues	Diclofenac (9) [62,63]	(9)
Heteroarylacetic Acid Analogues	Indomethacin (6) Etodolac (10) [64]	(10)
Aryl propionic acid Analogues	Ibuprofen (5)[64] Flurbiprofen (7)[65] Fenoprofen (11) [66] ketoprofen (12) [67]	(11) (12)

Classes	Examples	Chemical Structure
Oxicams (Enolic Acids)	Piroxicam (13) Meloxicam (14)[68]	(13) (14)
Naphthalene Acetic Acid Analogues	Naproxen (15) [6,69]	(15)
Salicylic Acid Analogues	Diflunisal (16) [70] Salsalate (17)	(16) (17)

Classes	Examples	Chemical Structure
	Salicylamide (18)	 (18)
Anthranilates	Anthranilic acid(19) [71] Meclofenamic acid (20) Mefenamic acid (21)	 (19) (20) (21)

1.7. Selective COX-2 Inhibitors:

The differences in the effectiveness with which a particular NSAID inhibits an isoform of cyclooxygenase may affect both its activity and toxicity. It has been proposed that the perfect NSAID would inhibit the inducible COX-2 isoform (thereby decreasing inflammation) without having any effect on the constitutive COX-l isoform; such an agent would maximize effectiveness, without inducing toxicity, particularly gastro duodenal erosions [72].

Selective COX-2 inhibitors differ from traditional NSAIDs in two major ways, Coxibs are less likely to result in NSAID-induced gastropathy, and they do not inhibit platelet function [73]. As a result, the major benefits of coxibs are the reduction in gastric ulcer formation and bleeding from those ulcers [74]. Another benefit of the platelet sparing coxibs is their use as analgesic and anti-inflammatory agents in situations in which bleeding may limit the use of the traditional NSAIDs, such as in trauma and surgical procedures [75, 76].

1.8. Chemical Classification of Selective COX-2 Inhibitors:

The large number of newly developed COX-2 inhibitors demonstrates how promising this field of anti-Inflammatory agents is expected to be. The chemical structures of COX-2 inhibitors are heterogenic. Contrary to the classical NSAIDs, this new class of enzyme inhibitors lacks a carboxylic acid group, thus effecting COX-2 affinity by a different orientation within the enzyme without formation of a salt bridge in the hydrophobia channel of the enzyme [77].

Selective COX-2 inhibitors can be classified in to:

1.8.1. Diaryl- or Aryl-Heteroaryl Ether and Thioether Derivatives:

One of the first COX-2 inhibitors was compound NS-398 (22) which has a completely different structure from classic NSAIDs. The compound showed inhibition of prostaglandin synthesis in inflammatory cells and was largely free of unwanted GI effects in animal models. Moreover, NS-398 did not affect prostaglandin production in the stomach or kidney [78].

On recognizing that NS-398 was a more or less preferential selective inhibitor of COX-2, new interest in this class of anti-inflammatory agents evolved. Nimesulide (23) and flosulide (24) are two other compounds with diaryl ether and thioether structure, respectively, which bears a methansulfonanilide moiety [79].

Flosulide is similar to nimesulide. The main difference between them is the incorporation of the electron- withdrawing substituent into the five-membered carbocyclic ring [80]. The thioether analogue of Flosulide L-745337 (25) was reported to have higher COX-2 specificity, better bioavailability, improved *in vivo* potency and greater GI safety than Flosulide [81].
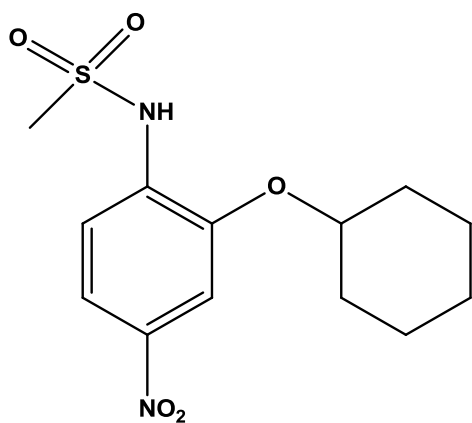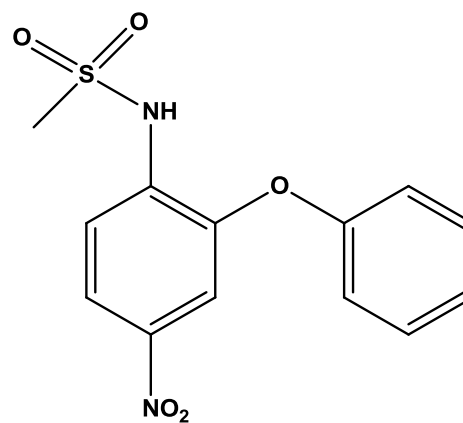
(22)   (23)
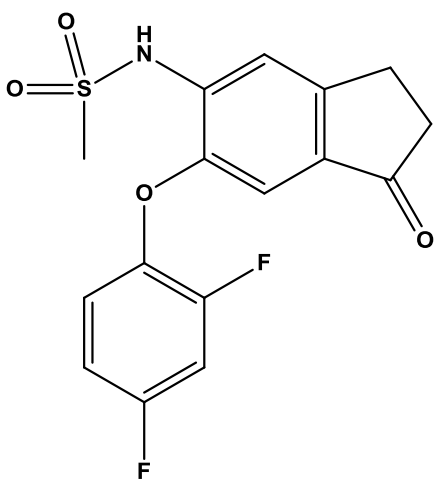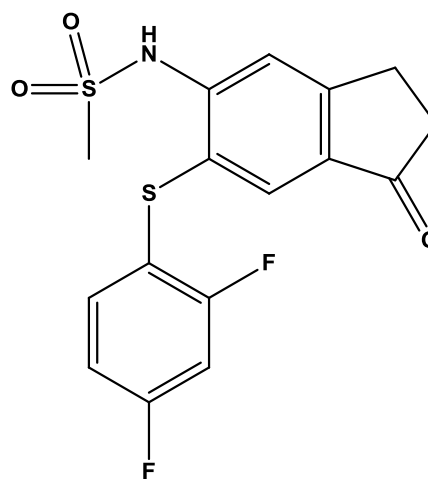
(24)   (25)

1.8.2. 1, 2-Diarylethylene Derivatives:

First examples of COX-2 inhibitors without central ring are compounds (26) and (27). Compound (26) was obtained by reduction of the furanone analogue to the corresponding diol. Results obtained with these two compounds demonstrated that both of them were selective COX-2 inhibitors and potent in the classical inflammation models [82].
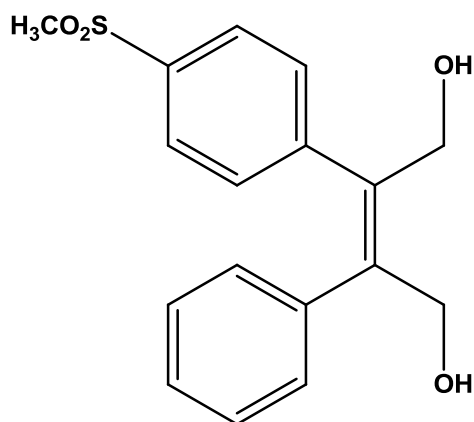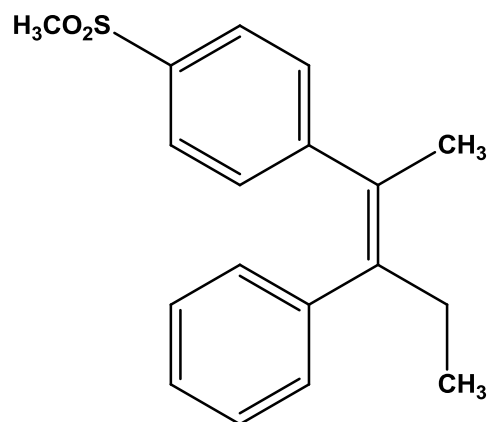
(26)   (27)

1.8.3. Carbocycles and Heterocycles with Vicinal Aryl Substitution:

These compounds represent the most important group of COX-2 inhibitors. DuP-697 (28) is the prototype of this class of compounds that is called Coxibs [83]. Clinical data of Dup-697 (28) showed selective inhibitory activity against COX-2 [84], but showed very long plasma half-life of (242 hr.) in human and because of its intrahepatic recirculation; it was unacceptable for further evaluation.

All Coxibs characterized by having a central carbocyclic or heterocyclic five membered ring system bearing tow vicinal aryl moieties, such as, cyclobutenone (29), pyrazole [85] Celcoxib(1), 2(5H)furanone [86] Refecoxib(8) and isoxazole [87] Valdecoxib (2). Some Coxibs have a six-membered ring as the central heterocycle such as the pyridine derivative Etoricoxib (30).

A novel class of 6-alkylthio-substituted six membered lactone (pyranone-2-one) ring (31) has been reported to exhibit very good *in vitro* COX-2 inhibitory potency and selectivity [88].

Structure activity relationship studies(SAR) of Coxibs showed that, substitution at position 4-of one of the aromatic ring system with a sulfonamide or a methylsulfonyl group is essential for optimum COX-2 selectivity and inhibitory potency and the presence of a *p*-F substituent on a non-sulfonyl vicinal phenyl ring improve *in vivo* activity [89].
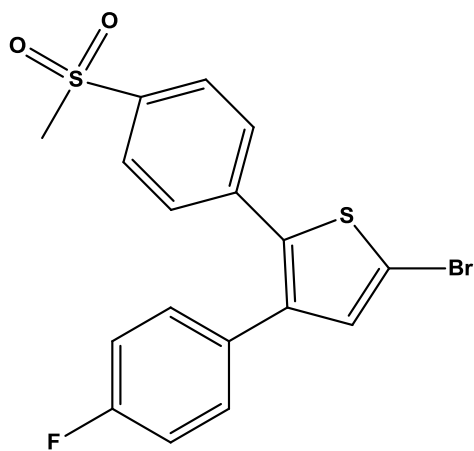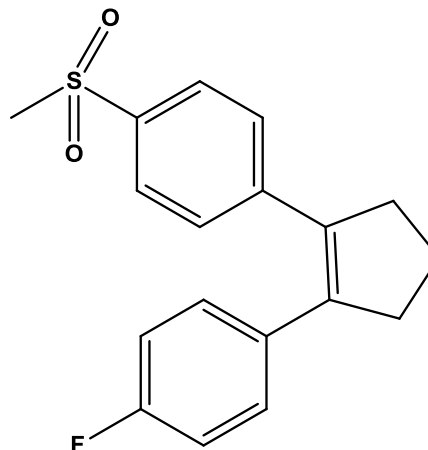
(28)   (29)
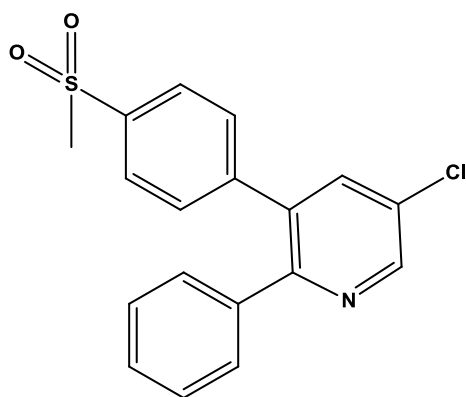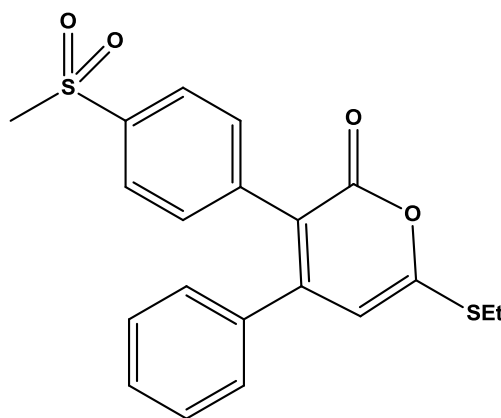
(30)   (31)

1.8.4. Compounds with Antioxidative Moieties:

Since COX enzyme catalysis involves radical intermediates, a radical scavenging moiety such as a di-*tert*-butylphenol interferes with COX reaction. Accordingly a series of compounds carrying this functional group was prepared and it was found that the thiazole derivative (32) was the most potent one [81].
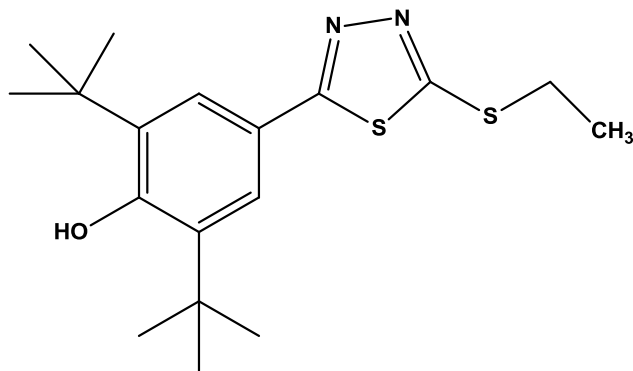
(32)

1.8.5. Aryl -Heteroaryl Ketones:

The ketone function link between an aryl ring and a heterocycle is extended known in the class of anti-inflammatory drugs, such as zomepirac (33), basically a COX-1 selective drug. The desired COX-2 selectivity was achieved by replacing the acetic acid group by other moieties such as a pyridazinone ring to yield RS-57067 (34) or by an N-acyl aminosulfonyl phenyl group to yield RS-1048934 (35) as shown in Figure (1-5) [90].
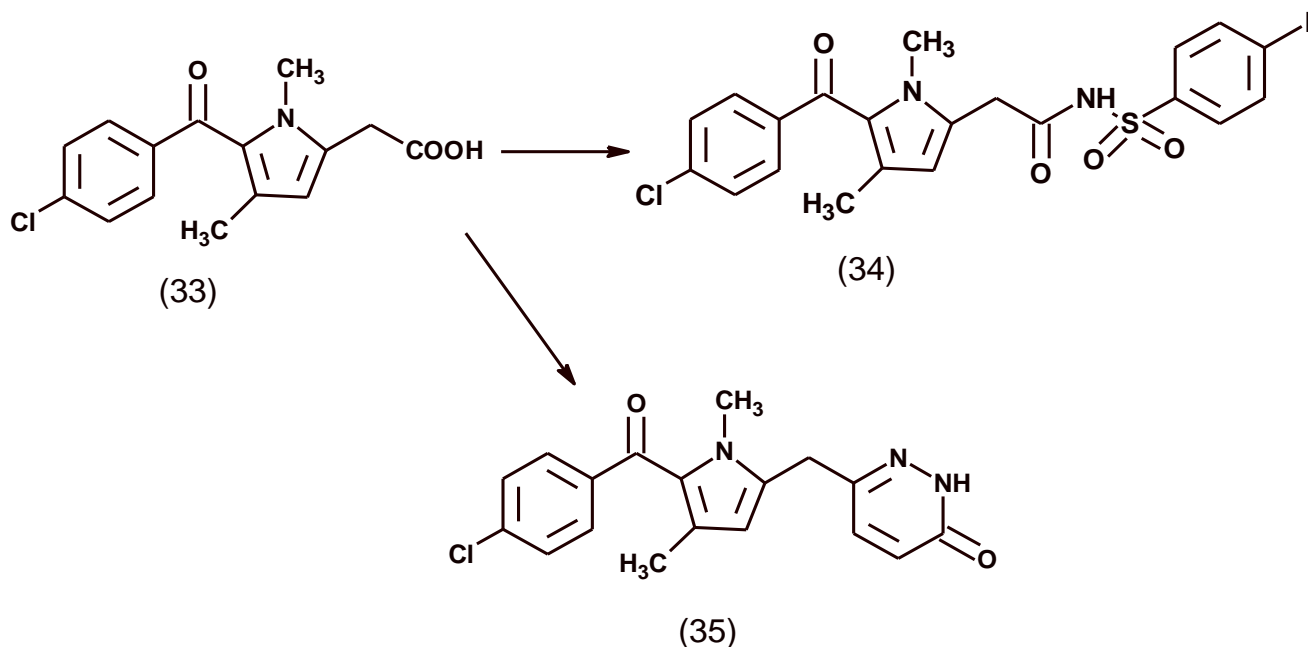
Figure (1-5): Conversion of Zomepirac to COX-2 Selective Inhibitors

1.8.6. Structurally Modified NSAIDs:

Many efforts have been made in search of novel selective COX-2 inhibitors by functional group modifications of well-known classical NSAIDs [91].

Flurbiprofen (7) has been successfully modified by using comparative computer modeling studies of the X-ray crystal structures of COX-1 and COX-2. Optimal selectivity was conferred by a 3-atom lipophilic substitution at the 3 position of the unsubstituted phenyl ring. The most effective analog was obtained by introducing two ethoxy groups at the 3 and 5position of flurbiprofen, yielding a compound (36), which has 77-fold greater selectivity than the parent compound [92].
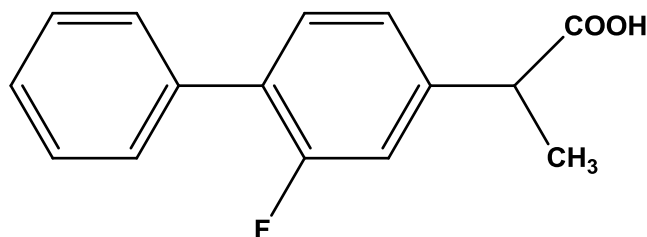
(7)
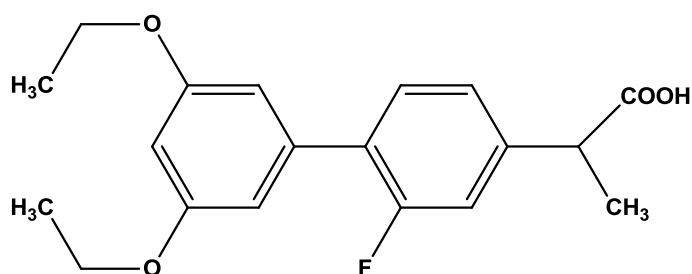
(36)

Indomethacin (6) possesses both COX-1 and COX-2 inhibitory activity. Introduction of larger substituents as trichlorobenzoyl moiety and altering the side chain by a beta-branched butyric acid afforded compounds L-748780 (37) and L-761066 (38) respectively with high potency and remarkable activity [93].However it was reported that esterification or amide formation of the arylacetic acid moiety of indomethacin gave compound (39) capable of binding tightly to COX-2 but not to COX-1 as shown in Figure (1-6) [94].

25

(6)

(37)

(39)

(38)
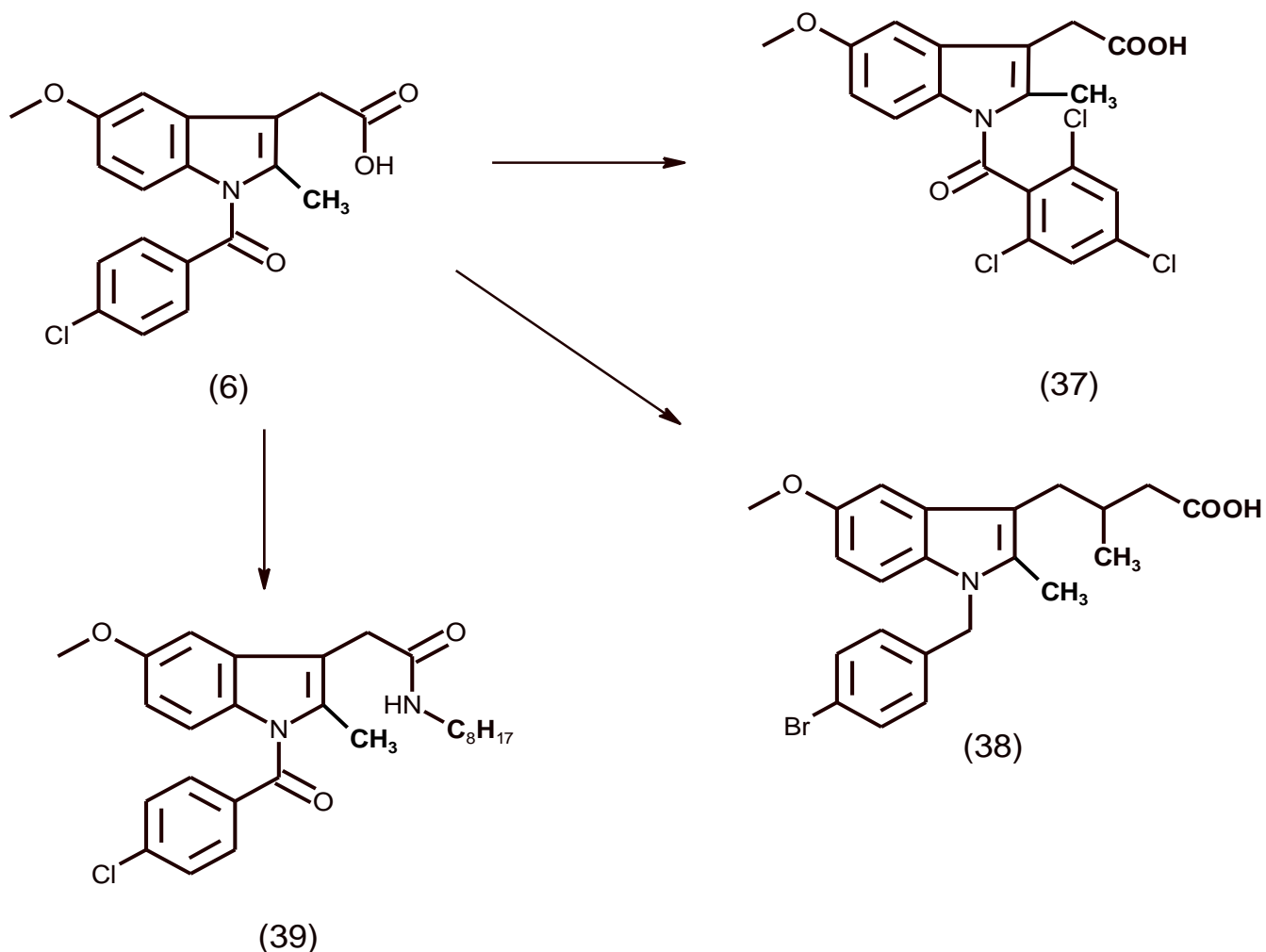
Figure (1-6): Conversion of Indomethacin to Selective COX-2 Inhibitors

The *N*-acetyl-2-carboxybenzesulfonamides (40) were synthesized by isosteric replacement of acetoxy group of aspirin (3) by $SO_2NHCOMe$ moiety and was identified as potent non-selective inhibitor of COX isozymes than aspirin [95]. *N*Acetyl- 2 carboxybenzesulfonamide analogs having substituted phenyl ring at C-4/C-5 position were also synthesized and it was observed that compounds (41) and (42) were potent and selective COX-2 inhibitors.

The compound (42) was less potent but more selective inhibitor than celecoxib, whereas (40) exhibited better *in vivo* anti-inflammatory activity in a carrageenan-induced rat paw edema assay although the latter was more potent and selective inhibitor of COX-2 *in vitro* as shown in Figure (1-7) [91].
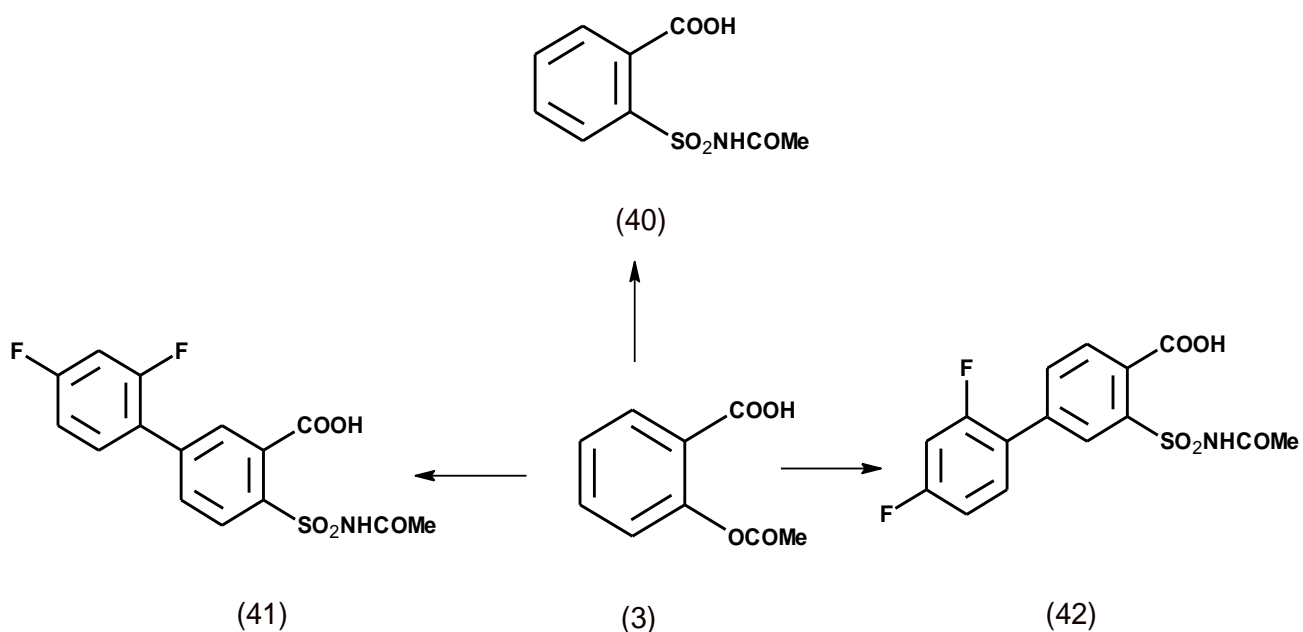
Figure (1-7): Conversion of Aspirin to Selective COX-2 Inhibitors

1.9. Pyrazoline:

There are numerous biologically active molecules which contain various heteroatoms such as nitrogen, sulphur and oxygen, always drawn the attention over the years mainly because of their biological importance [96].

Pyrazoline (43) is five-membered heterocyclic having two adjacent nitrogen atoms within the ring. It has only one endocyclic double bond and is basic in nature [97]. It plays a crucial role in the development of theory in heterocyclic chemistry and is also extensively used as useful synthons in organic synthesis [98].
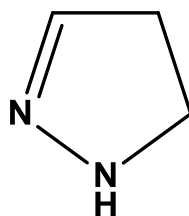
(43)

1.10. Biological Activities of Pyrazoline:

Compounds bearing pyrazoline moiety have been of great interest to synthetic and medicinal chemists for a long time due to their unique chemical and biological properties [99].

Pyrazolines nucleus and their derivatives have been found to have diverse pharmacological activities such as anticonvulsant, anti-inflammatory, antimicrobial, antiviral, anticancer, anti-helicobacter pylori, antitubercular, antiamoebic, antiandrogenic, hypotensive, and antihistaminic, antidiabetic, analgesic and antipyretic action [100, 101].

Biological Activities of Pyrazoline Include:

1.10.1 Anticancer Activity:

In vitro anticancer activity of thiazolone-based compounds containing the 5-aryl-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl framework were tested by the National Cancer Institute and most of them displayed anticancer activity on leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancer cell lines and the most efficient anticancer compound (44) was found to be active with selective influence on colon cancer cell lines [102]. Synthesis of substituted pyrazolines e.g compound (45) and evaluated the anticancer activity show their ability to inhibit P-glycoprotein mediated multidrug resistance by direct binding to a purified protein domain containing an ATP-binding site and a modulator interacting region compounds found to bind to P-glycoprotein with greater affinity [103].
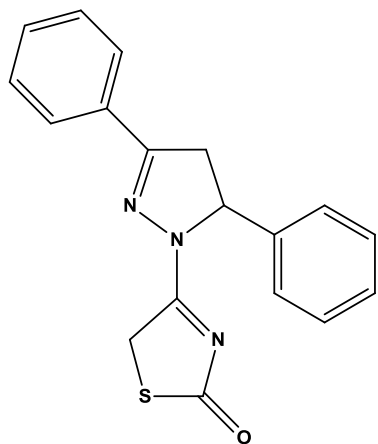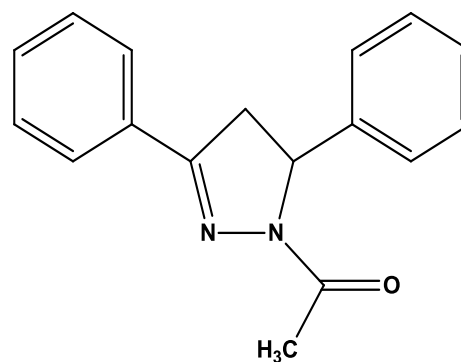
(44)   (45)

1.10.2. Analgesic Activity:

A series of 4-(5-substituted aryl-4,5-dihydropyrazole-3-yl-amino) phenols Figure (1-8) were synthesized by treating substituted aryl- N-chalconyl amino phenols with hydrazine hydrate. The compounds were investigated for analgesic activity by Glassman's analgesic model and anti-inflammatory activity by Carrageenan induced paw edema method in rat model. Observed increased in analgesic and anti-inflammatory activity can be attributed to the presence of 2-OH, 4-NO$_2$, and 4-Cl in the phenyl group in position 5 of the pyrazoline ring of synthesized compounds [104].

R= C$_6$H$_5$, 2-furyl, 4-NO$_2$C$_6$H$_4$,
4-OCH$_3$C$_6$H$_4$, 2-OHC$_6$H$_4$, 4-ClC$_6$H$_4$
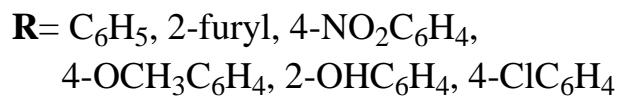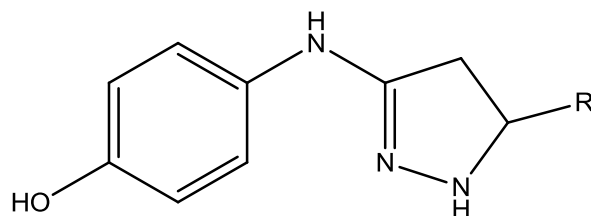
Figure (1-8): Derivatives of Pyrazoline with Analgesic Activity

1.10.3. Antitubercular Activity:

Syntheses of new 3-Pyrazoline derivatives from 3-β-picolinoylamino azomethyl-5-aromatic substituted-1-thioamide-3-pyrazoline and evaluated for antitubercular activity results in occurrence of compounds (46) and (47) which show good antitubercular activity [105].
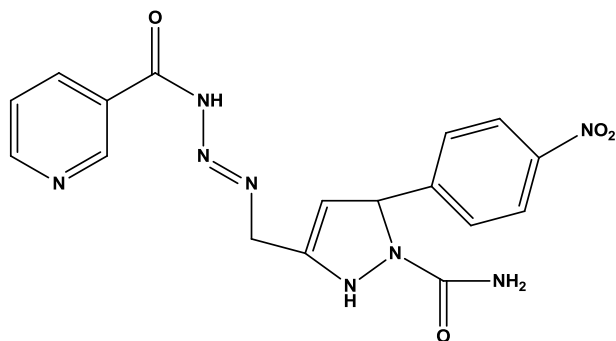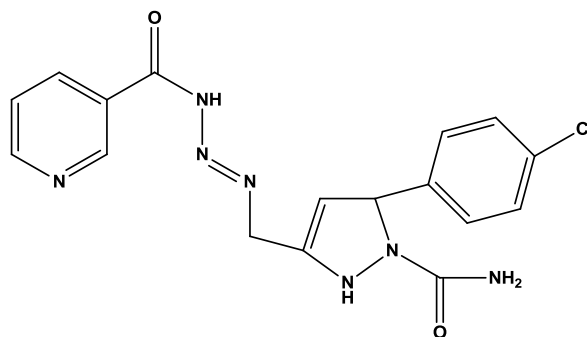
                     (46)   (47)

1.10.4. Anticonvulsant Activity:

A series of N-(4-(1,5-diphenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl) acetamide were synthesized and evaluated for their anticonvulsant activity. Compound (48) exhibited anticonvulsant activity, which was reflected by 30-80% protection observed against PTZ-induced seizures [106].
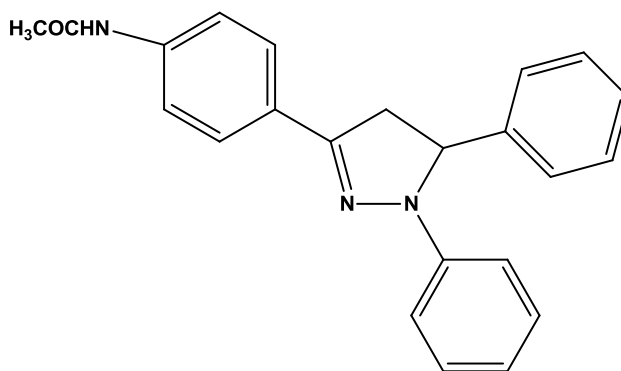
(48)

1.10.5. Hypotensive Activity:

Synthesis of some 1-(4-arylthiazol-2-yl)-3,5-diaryl-2-pyrazoline derivatives Figure (1- 9) and investigated their hypotensive activity by the tail-cuff method using clonidine as reference standard, showed appreciable hypotensive activities [97].

R$_1$=H   **R$_2$**= H, CH$_3$ **R$_3$**= H, OCH$_3$
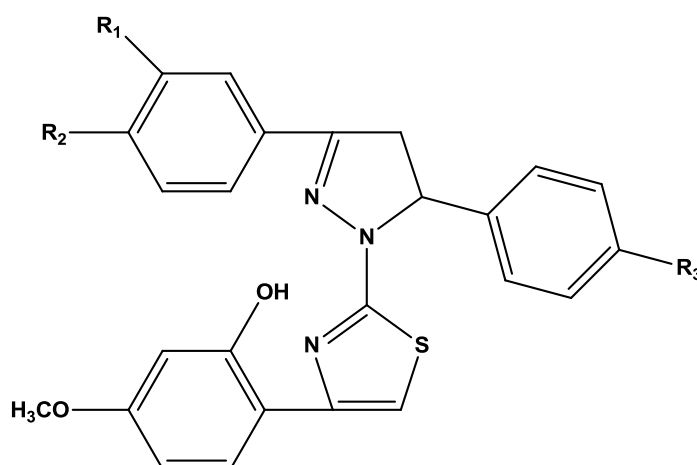
Figure (1-9): Derivatives of Pyrazoline with Hypotensive Activities

1.10.6. Anti-Helicobacter Pylori Activity:

A series of N-1-substituted3,5-diphenyl pyrazolines Figure (1-10) were synthesized and evaluated for their antibacterial activity. All the synthesized compounds showed little or no activity against different species of Gram +ve and Gram -ve bacteria of clinical relevance and against various strains, including those resistant to the reference compound metronidazole. Compounds containing N1-acetyl group and a 4- methoxy substituent in the 5-phenyl ring showed the best activity against H. pylori metronidazole resistant strains [99].
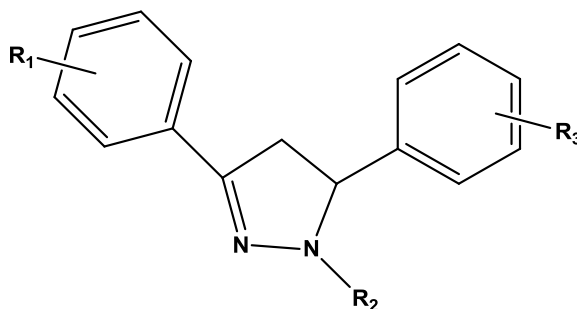
R₁= H, 2-OH, 4-OH, 2,4-OH, 6-OH
R₂= 4-Cl phenyl, C(O)CH$_3$
R₃= 4-Cl, 2-Cl, 4-OCH$_3$, 3,4-OCH$_3$

Figure (1-10): Derivatives of Pyrazoline with Anti-Helicobacter Activities

1.10.7. Antimicrobial Activity:

Some novel pyrazoline derivatives were synthesized containing sulfone and trifluoromethyl groups Figure (1-11) screened for antimicrobial activity against gram-positive, gram-negative and fungi. All the synthesized derivatives showed good activity when compared to the standard drugs [107].
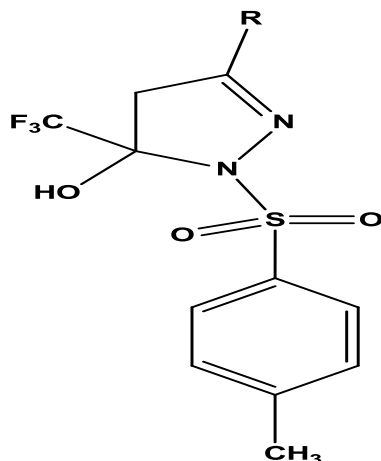
R= H, 4FPh, 4-MeOPh, 4-BrPh, Ph, Me

Figure (1-11): Pyrazoline Derivatives with Antimicrobial Activities

Some novel isonicotinoyl-pyrazoline derivatives synthesis by microwave assisted and evaluated their antimicrobial activity. Substituted 3-(benzylidene amino)-1-isonicotinoyl-1H-pyrazole-5(4H)-one derivatives Figure (1-12) were synthesized from the condensation of isonicotinohydrazide with ethyl-2-cyanoacetate. All the newly synthesized compounds were screened for their anti-microbial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas Aeroginosa* and fungi such as *Candida albicans* and they showed promising antifungal and antibacterial activities [108].
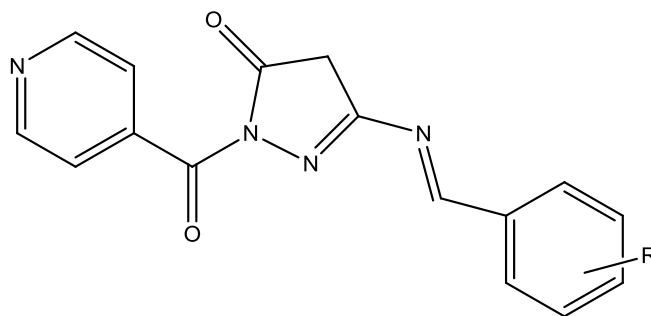
R= OCH$_3$, OH, CH$_3$, F

Figure (1-12): Isonicotinoyl Pyrazoline Derivatives Activities

1.10.8. Anti-Inflammatory Activity:

A novel series of 1-thiocarbamoyl-3-substituted phenyl-5-(2- pyrrolyl)-4, 5-dihydro-(1*H*)-pyrazole derivatives Figure (1- 13) were synthesized. The synthesized compounds were tested for their *in vivo* anti-inflammatory activity by two different bioassays, carrageenan-induced edema and acetic acid-induced increase in capillary permeability in mice. Compound with methoxy group and allyl group on the thiocarbamoyl moiety exhibited good anti-inflammatory activity comparable to that of indomethacin with no ulcerogenic effects [109].
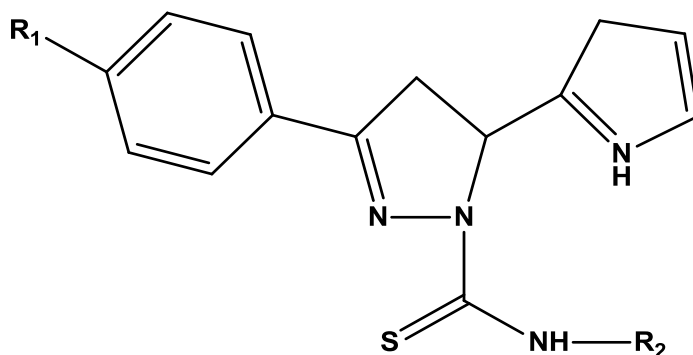
R_1= CH_3, Cl, OCH_3
R2= CH_3, C_2H_5, C_3H_5, C_6H_5

Figure (1-13): 1-thiocarbamoyl-3-substituted phenyl-5-(2- pyrrolyl)-4, 5 dihydro-(1H)-pyrazole Derivatives

A series of ethyl-5-amino-4-[(3,5-dialkyl-1H-pyrazol-1-yl) carbonyl]-3-methylthiophene-2-carboxylate Figure (1-14) were synthesized. The compounds were evaluated for anti-inflammatory, analgesic and ulcerogenic activities. Among the compounds studied, compounds containing the substituted hydrazide at C-3 position showed more potent anti-inflammatory activity than the standard drug (Indomethacin and Aspirin), without ulcerogenity. While other compounds showed moderate activities [110].

R= CH$_3$, C$_2$H$_5$, NH$_2$
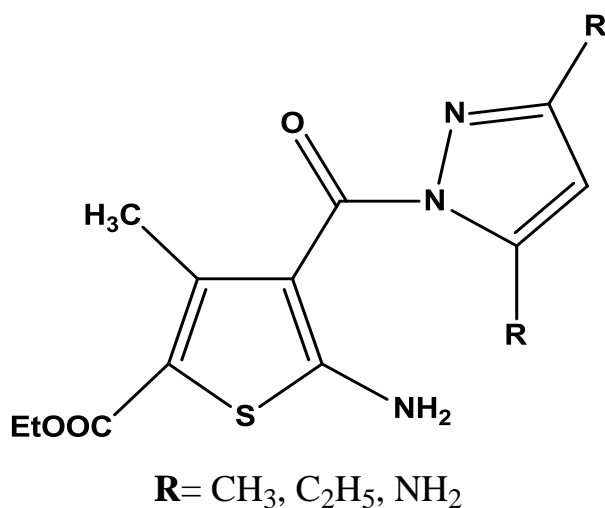
Figure (1-14): Ethyl-5-amino-4-[(3,5-dialkyl-1H-pyrazol-1-yl) carbonyl]-3-methylthiophene-2-carboxylate Derivatives

1.11. Strategy of the Work:

Naproxen is a well-known non-steroidal anti-inflammatory drug, it is available with low cost and the chemical structure of it has no additional functional groups that may undergo conversion to another intermediate throughout the overall reaction, so it will undergo straight line reaction.

The direction of the present work is to synthesis potential non-steroidal anti-inflammatory agents that are derivatives of naproxen by incorporating a group of pyrazoline pharmacophore in the carboxylate group of naproxen. Then evaluated them as anti-inflammatory agents.

These newly synthesized compounds may represent potent anti-inflammatory agents and exhibit expected selectivity towards COX-2 enzyme due to their large size than its parent naproxen compound and the fact of presence the side pocket near the base of the active site of COX-2 enzyme makes its site 20% larger than that of COX-1 so the active center of COX-2 can accommodate larger structures than those which are able to fit the active site of COX-1 [111]. As shown in Figure (1-15).
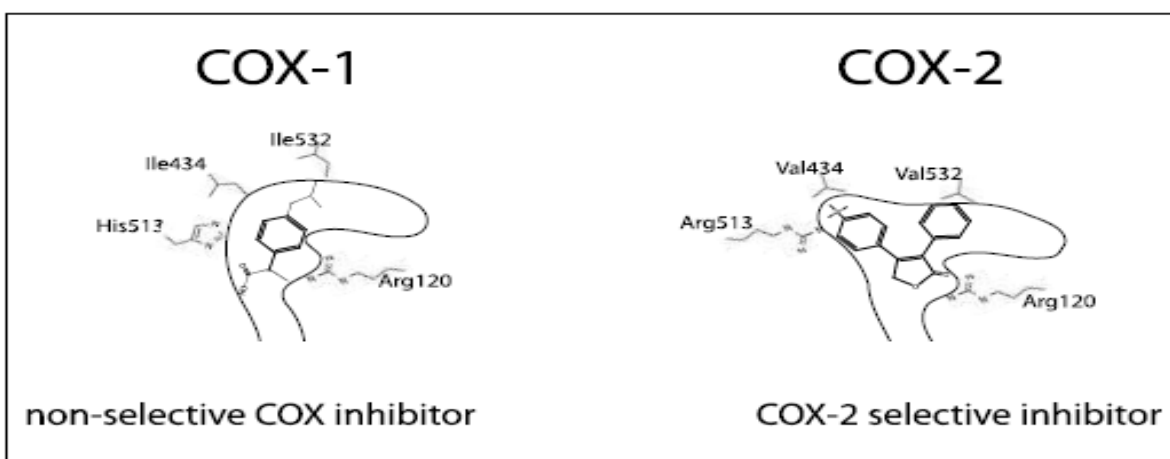
Figure (1-15): Difference between COX-1 and COX-2 in Size of Active Center [112]

1.12. Aim of the Work:

The aim of this work is to synthesis new naproxen derivatives with expected selectivity against COX-2 enzyme and evaluation of their anti-inflammatory activity. This task is to be done by linking the naproxen with the pyrazoline molecules with glycine as spacer arm in order to increase some of their desired activities. Evaluation of their antibacterial activity also aimed in our work since N_1-substituted 3,5-diphenyl pyrazoline have several pharmacological activities as explored in this introduction.

The general structure of these compounds:

R= H, Cl, NO$_2$, OH, OCH$_3$, N (CH$_3$)$_2$
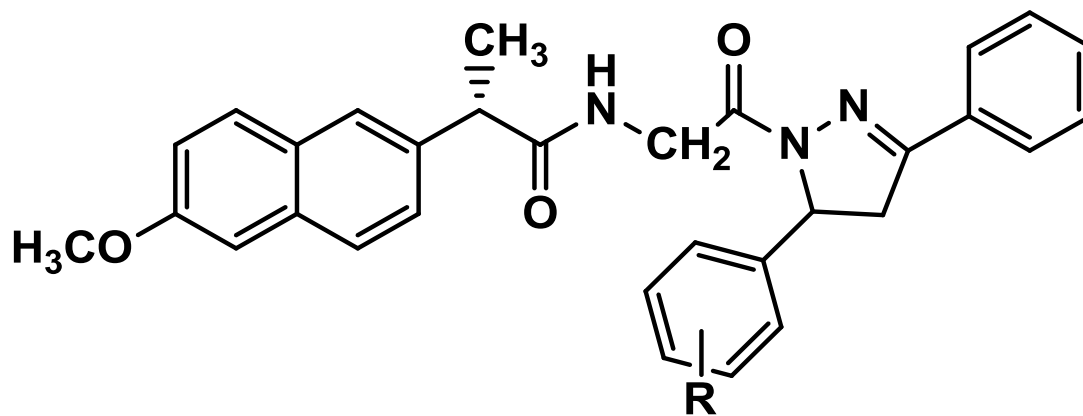
CHAPTER TWO
EXPERIMENTAL

Materials & Methods

2.1. Chemicals:

The specific chemicals used in this work are listed with their suppliers in Table (2-1).

Table (2-1): Chemicals with their Suppliers.

Materials	Company	Origin
Acetophenone 99.5%	Sigma-Aldrich	Germany
Benzaldehyde 99.5%	BDH	England
Dicyclohexylcarbodimide 99%	Sigma-Aldrich	Germany
Glacial acetic acid 90%	BDH	England
Glycine 98.5%	Fluka	Switzerland
Hydrazine hydrate 90%	Fluka	Switzerland
(2S)-Naproxen	SDI	Iraq
p-Chlorobenzaldehyde 97%	BDH	England
p-Dimethylaminobenzaldehyde 99%	BDH	England
p-Hydroxybenzaldehyde 97%	Himedia	India
p-Methoxybenzaldhyde 98%	Himedia	India
p-Nitrobenzaldehyde 98%	Himedia	India
Thionyl chloride 97%	BDH	England
Triethyl amine 99%	Avochem	England

All of the solvents and materials used were of analar type and used without further purification.

2.2. Equipment and Instruments:

The equipment and Instruments with their suppliers that are used in this work is listed in Table (2-2).

Table (2-2): Equipment and Instruments with their Suppliers.

Equipment	Company	Country
Electrical melting point apparatus	Stuart	UK
Ultrasonic bath	SB25-12 DTDN	China
FT- IR spectrophotometer	Shimadzu 8400s	Japan
^{1}H-NMR	Shimadzu Bruker 500 MHz	Japan

2.3. Method of Characterization and Identification:

General methods of identification of synthesized products including:

2.3.1. Thin Layer Chromatography:

Thin layer chromatography was run on TLC silica gel (60) $F_{254,}$ Merck (Germany), for checking the purity of the products as well as monitoring the progress of the reaction. Compounds were revealed upon irradiation with uv light.

Chromatograms were eluted by the following systems:

A: Benzene: carbon tetrachloride: Ethyl acetate (50:40:10) [113]

B: Chloroform: Methanol (85: 15) [114]

2.3.2. Melting Point:

Electro thermal melting point apparatus and open capillary tubes were used to determine the melting points and are uncorrected.

2.3.3. Infrared Spectra:

Infrared spectra were recorded as KBr disc by using FT- IR spectrophotometer, in college of pharmacy, AL-Mustansiriyah University.

2.3.4. ^{1}H-NMR Spectra:

The ^{1}H-NMR spectra was performed at The University of Jordan, Faculty of Science, and Department of Chemistry. Instrument Model: Bruker 500 MHz-Avanc III.

2.4. Chemical Synthesis:

The synthesis of target compounds (I-V$_{a-f}$) was achieved following procedures illustrated in Scheme (2-1).

	R
a	H
b	Cl
c	NO2
d	OH
e	OCH3
f	N(CH3)2
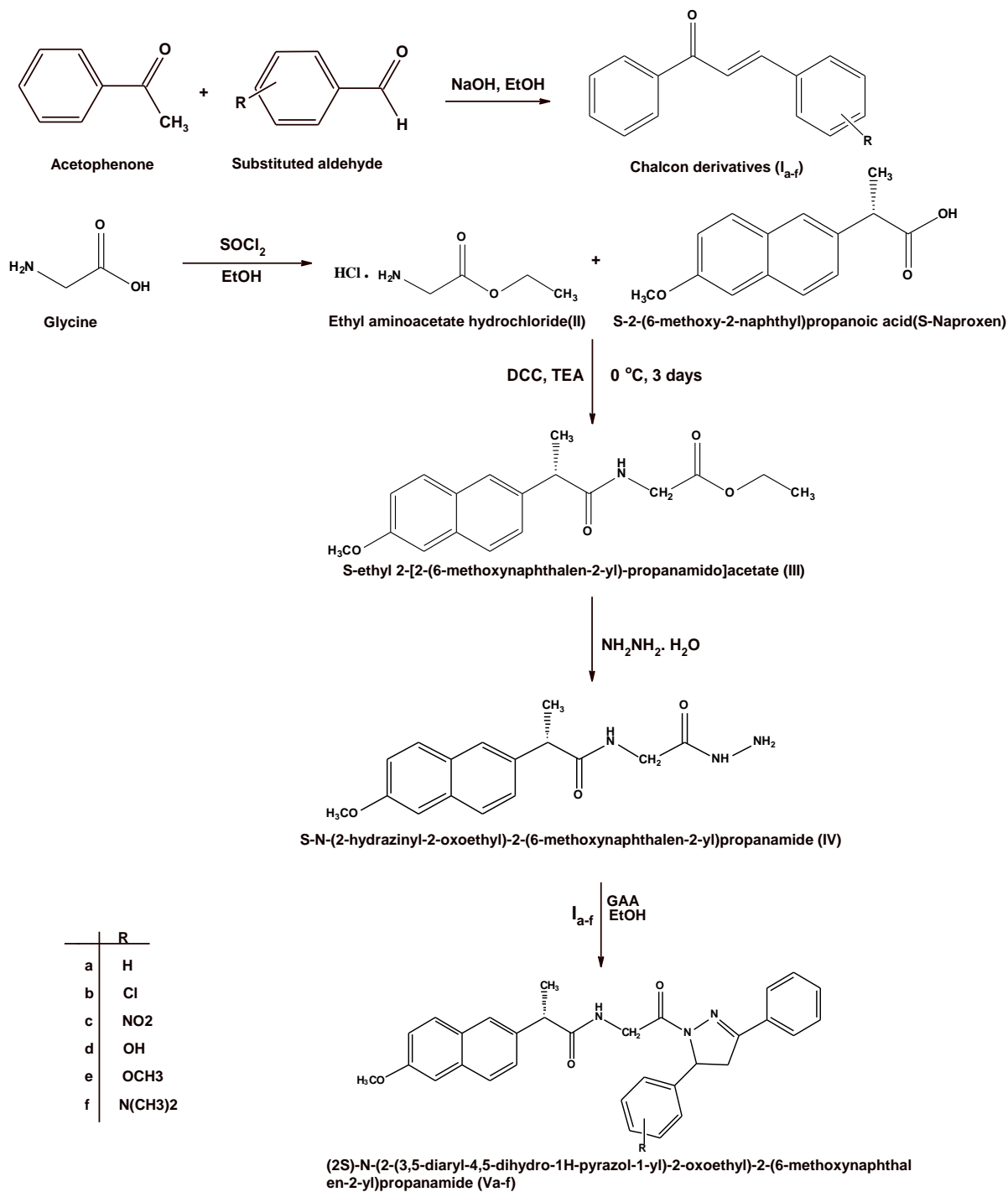
Scheme (2-1): Synthesis of Intermediates and Target Compounds

2.4.1. General Procedure for Synthesis of Chalcone Derivatives (I a-f):
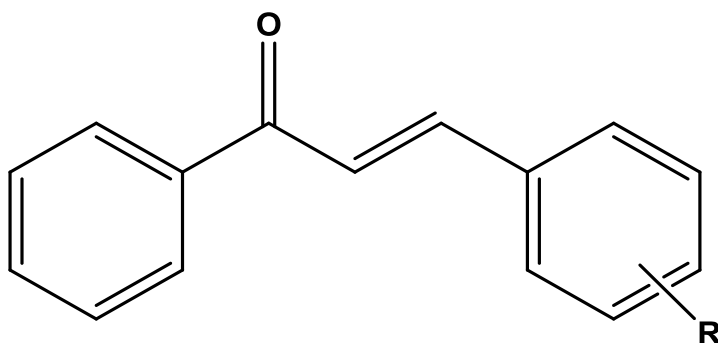
Acetophenone (1.18 mL, 10 mmole) and aromatic aldehyde derivatives (a-f) (10 mmole) were added to absolute ethanol (22 mL), and then sodium hydroxide (40%, 10 mL) solution was adding drop wise over (2 min.). The mixture was irradiated by an ultrasonic generator in a water bath at (30-35 $^{\circ}$C) for (25 min.) turbidity appeared in the mixture, which was then neutralized with 2N HCl. The solid product formed was filtered, washed with cold water and recrystallized by ethanol [115,116].

The percent yield, physical data and R_f values are given in Table (3-1).FT- IR spectra for these compounds are shown in Figures (3-3 to 3-8) and ^{1}H-NMR spectra for the compound(I a-f) is shown in Figure (3-18 to 3-21).

Table (2-3): Aromatic Aldehyde's Name and Products No.

No.	Aromatic Aldehyde's Name	Products No.	R	Quantity(gm)
a	Benzaldehyde	I_a	H	1.06
b	4-Chlorobenzaldehyde	I_b	Cl	1.40
c	4-Nitrobenzaldehyde	I_c	NO_2	1.51
d	4-Hydroxybenzaldehyde	I_d	OH	1.22
e	4-methoxybenzaldehyde	I_e	OCH_3	1.36
f	4-Dimethylaminobenzaldehyde	I_f	$N(CH_3)_2$	1.49

2.4.2. Synthesis of Ethyl-2-amino acetate hydrochloride (II):
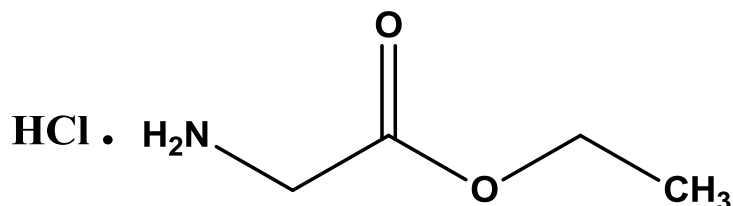
Thionyl chloride (0.8 mL, 11mmole) was added gradually to absolute ethanol (10 mL) cooled to (0°C). 2-Aminoacetic acid (0.75g, 10 mmole) was suspended in the reaction mixture and subjected to ultra-sonication at room temperature for (45 min.). On completion of the reaction, the solvent was removed under reduced pressure and the residue was purified by recrystallization from methanol: diethyl ether [117].

The percent yield, physical data and R_f values are given in Table (3- 1). FT- IR spectrum for this compound is shown in Figure (3-9).

2.4.3. Synthesis of (2S)-Ethyl-2-[2-(6-methoxynaphthalen-2-yl)-propanamido] acetate (III):
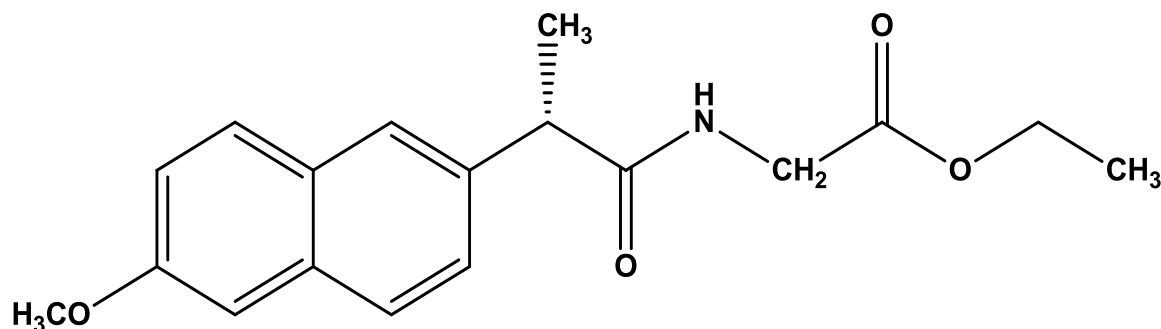
Compound (II) (2.8 g, 20 mmole), triethylamine (3 mL, 21 mmole) and (2S)-Naproxen (4.6 g, 20 mmole) were dissolved in dry DCM (40 mL). The reaction mixture was stirred at $(0^{\circ}C)$ for (30 min.). To this solution DCC (4.13 g, 20 mmole) in dry DCM (10 mL) was added slowly in a drop wise manner. Reaction mixture was stirred for 3 days at (0°C). Precipitated DCU was filtered off and the solvent was distilled off under reduced pressure. The product obtained was dissolved in ethyl acetate (30 mL) and filtered. Ethyl acetate layer was washed with 10% aqueous solution of sodium bicarbonate (3x30 mL) and distilled water (3x30 mL). Ethyl acetate layer was dried with anhydrous magnesium sulphate and filtered to get a clear solution of product in ethyl acetate. Solvent was evaporated under reduced pressure and the crude product was recrystallized by using hexane: ethyl acetate [118].

The percent yield, physical data and R_f values are given in Table (3-1). FT- IR spectrum for this compound is shown in Figure (3-10) and ^{1}H-NMR spectra for this compound is shown in Figures (3-22A &3-22B).

2.4.4. Synthesis of (2*S*)-*N*-(2-hydrazinyl-2-oxoethyl)-2-(6-methoxy-naphthalen-2-yl) propanamide (IV):
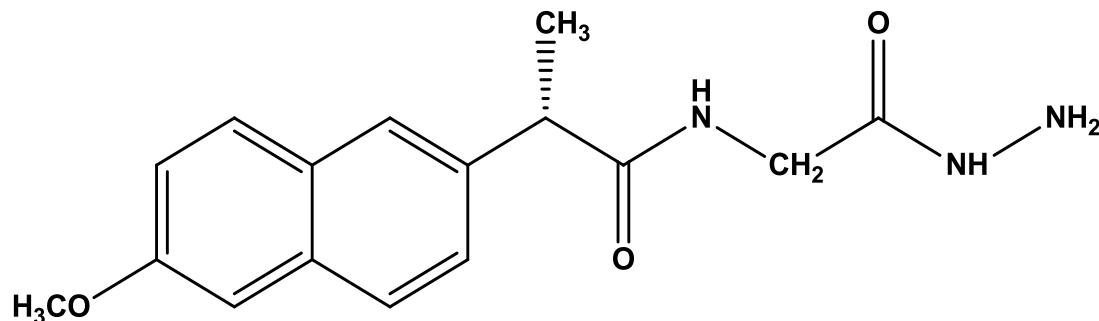
Compound (III) (1.00 g, 3 mmole) was dissolved in (15 mL) methanol and hydrazine hydrate (90%) (0.7 mL, 14 mmole) was added. The reaction mixture was stirred at (24°C) overnight. On the next day the solvent was removed under reduced pressure and the crude product was washed with ether under stirring to afford the product in pure state [118].

The percent yield, physical data and R_f values are given in Table (3-1). FT- IR spectrum for this compound is shown in Figure (3-11) and ^{1}H-NMR spectra for this compound is shown in Figures (3-23A & 3-23B).

2.4.5. General Procedure for Synthesis of (2S)-N-(2-(3, 5-diaryl-4-5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)-2-(6-methoxynaphthalen-2-yl) propanamid (V$_{a-f}$):
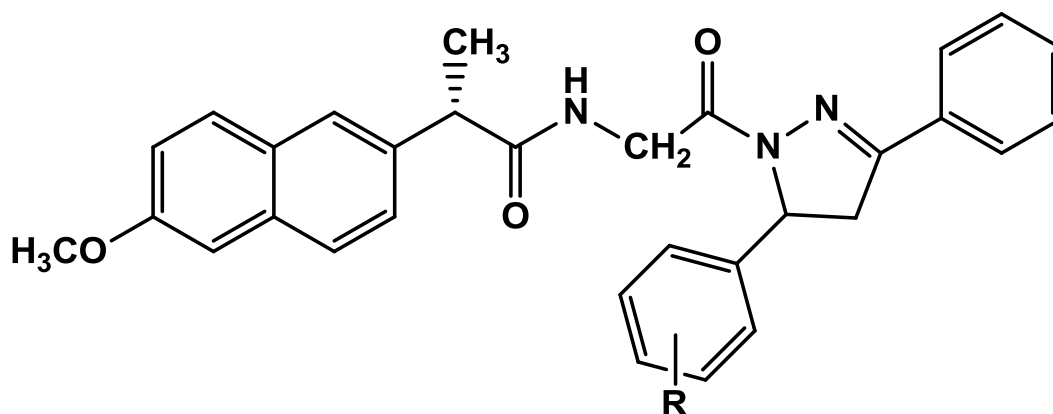
In a 100 mL rounded bottom flask a mixture of one chalcone derivatives was taken (I$_{a-f}$) (1 mmole) and compound (IV) (0.30 g,1 mmole) in (20 mL) of absolute ethanol, the mixture was refluxed, after (15 min.) a catalytic glacial acetic acid was added and the contents allowed getting reflux for (24 hrs.). The reaction time was considered by performing TLC to obtain single spot. The reacting mixture was cooled in to (20 mL) of cold water to precipitate out the product. The product was filtered and washed twice with cold water then the product was dried and re-crystallized from hot Ethanol [119].

The percent yield, physical data and R$_f$ values are given in Table (3-1). FT- IR spectra for these compounds are shown in Figures (3-12 to 3-17) and ^{1}H-NMR spectra for these compounds are shown in Figures (3-24 to 3-29).

2.5. Preliminary Pharmacological Studies:

2.5.1. Anti-Inflammatory Evaluation Study:

*In viv*o acute anti-inflammatory effects of the chemically synthesized compounds (V_{a-f}) were evaluated in egg-white induced paw edema [120]. Their evaluation for their ant-inflammatory activity based on measuring the decreases of paw thickness.

2.5.1.1. Methods:

A. Animals:

Albino rats of either sex weighing (170 ± 10 gm) were supplied by Iraqi center for cancer and medical genetic research and were housed in College of Pharmacy / University of Al-mustansiriyah under standardized conditions for 10 days for acclimatization. Animals were fed commercial chaw and had free access to water. Animals were brought to the laboratory, one hour before the experiment, and were divided into eight groups (each group consist of 6 rats) as follows:

Group A: six rats served as control and treated with the vehicle (propylene glycol 50% v/v).

Group B: six rats treated with (2S)-naproxen as reference substance in a dose of 50mg/kg suspended in propylene glycol [121].

Group C-H: six rats /group treated with the tested compounds (V_{a-f}) respectively in doses that determined below, also suspended in propylene glycol.

B. Calculations for Dose Determination:

M.Wt. of (2S)-Naproxen = 230.26

50mg / kg / 230 = Dose / M.Wt. of the tested compounds [122].

Table (2-4): Compounds with their Molecular Weight and Dose:

Compounds	Molecular Weight	Dose mg/ kg
(2S)-Naproxen	230.26	50.00
V_a	491.59	106.74
V_b	526.03	114.22
V_c	536.58	116.51
V_d	507.58	110.21
V_e	521.60	113.26
V_f	534.65	116.09

C. Experimental Design:

The anti-inflammatory activity of the tested compounds was studied using the egg-white induced edema model. The paw thickness was measured by vernea at seven time intervals (0, 30, 60, 120, 180, 240, and 300 min.) after drug administration. Acute inflammation was produced by a subcutaneous injection of (0.05 mL) of undiluted egg-white into the plantar side of the left hind paw of the rats; (30 min.) after intra-peritoneal administration of the drugs or their vehicle [123].

2.5.1.2. Statistical Analysis:

The data was expressed as the mean ± SEM and results were analyzed for statistical significance using student *t*-test (Two Sample Assuming Equal Variances)

for comparison between mean values. While comparisons between different groups were made using ANOVA: Two factors without replication. Probability (P) value of less than 0.05 was considered significant.

2.5.2. Antimicrobial Activity:

The antimicrobial activity of the target compounds was done in college of Pharmacy / University of Al-mustansiriyah. A preliminary antibacterial activity has been carried out according to Well Diffusion Method: The prepared compounds have been studied for their antimicrobial activity *in vitro* against four tested bacteria (*Staphylococcus aureus ,Bacillus Subtalus*, as gram positive bacteria and *Pseudomonas Aeroginosa, Escherichia coli*, as gram negative bacteria) were clinically activated and maintained on nutrient agar medium for testing antibacterial. (Ciprofloxacin) was used as a reference drug for antibacterial activity.

2.5.2.1. Sensitivity Assay: [124]

Well diffusion assay was carried out by using bacterial suspension of about $(1.5 \times 10^6$ CFU/ml) with turbidity standard (number 0.5). This was used to inoculate by swabbing the surface of Mueller Hinton agar (MHA) plates. Excess liquid was air-dried under a sterile hood. In each agar plate of tested bacteria four wells were made and (100 μl) of each concentration was added in it. The plates were incubated at (37 °C) for (24 hrs.). The assessment of antibacterial activity was based on measurement of the diameter of inhibition zone formed around the well, and show that the zone of inhibition mostly increased with the increasing of concentration of the tested compounds, as shown in Table (3-17).

CHAPTER THREE

RESULTS & DISCUSSION

Results & Discussion

The synthesis of the final compounds (V_{a-f}) through their intermediate will be discussed as well as the results of their characterization, identification and evaluation of compounds (V_{a-f}) as anti-inflammatory agents and anti-bacterial activity.

3.1. Synthetic Studies:

The synthetic procedures for the compounds ($I-V_{a-f}$) are illustrated in Scheme (2-1).The characterization and purity of these compounds and their intermediates (percent yields, melting points and R_f values) were given in Table (3-1).

The functional groups of the synthesized compounds were identified using FT-IR spectroscopy, as shown in Figures (3-3 to 3-17). The chemical structures were confirmed using ^{1}H-NMR spectroscopy as shown in Figures (3-18 to 3-29).

3.1.1. Synthesis of Chalcone Derivatives (I_{a-f}):

Acetophenone and aromatic aldehyde derivatives (a-f) were add to ethanol, sodium hydroxide (40%) solution was add drop wise over (2 min.). The mixture was exposed to an ultrasonic generator in a water bath at (35^oC) for (25 min.) turbidity appeared in the mixture, which was then neutralized with 2N HCl. The solid product formed was filtered, washed with cold water and recrystallized by ethanol.

Scheme (3-1): shows the mechanism for the base-catalyzed aldol condensation between acetophenone and benzaldehyde which involves the following steps.

Step 1: Formation of enolate ion (acid-base reaction)

Is an acid-base reaction. Hydroxide functions as a base and removes an acidic α-hydrogen giving a reactive enolate.

Step 2: Alkoxide formation (nucleophilic addition)

The nucleophilic enolate attacks the carbonyl carbon of benzaldehyde in a nucleophilic addition process giving an intermediate alkoxide.

Step 3: Protonation of alkoxide

The alkoxide deprotonates a water molecule producing a hydroxide ion and a β-hydroxyketone, the aldol product.

Step 4: Dehydration

The hydroxide acts as a base and removes an acidic β-hydrogen giving the reactive enolates. The electrons associated with a negative charge of the enolate are used to form a carbon-carbon double bond (C=C) and displace a leaving group, regenerating the hydroxide giving the final product, the conjugated ketone.

The structure of chalcone compounds (I_{a-f}) were characterized by melting point and R_f values given in Table (3-1) as well as the appearance of the FT-IR characteristic absorption bands of $\upsilon_{C=O}$ group at 1660-1649 cm^{-1} and $\upsilon_{C=C}$ group at 1603-1599 cm^{-1} and 1574-1558 cm^{-1} as in Table (3-2) and disappearance the stretching of acetophenone and aldehydes carbonyl groups at 1696 cm^{-1} and 1710-1680 cm^{-1} respectively.

The ^{1}H-NMR showed multiplet for CH protons of CH=CH at 8.10-8.18(δ, ppm) as in Table (3-3 to 3-6).

Step 1: <u>Formation of enolate ion</u>

Step 2: <u>Alkoxide formation (neuclophilic addition)</u>

Step 3: <u>Protonation of alkoxide</u>
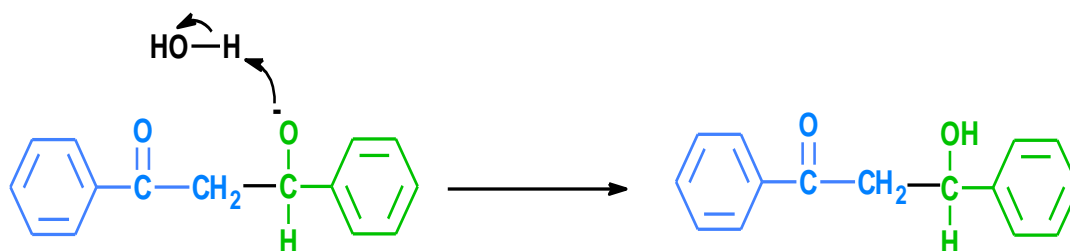
Step 4: <u>Dehydration</u>
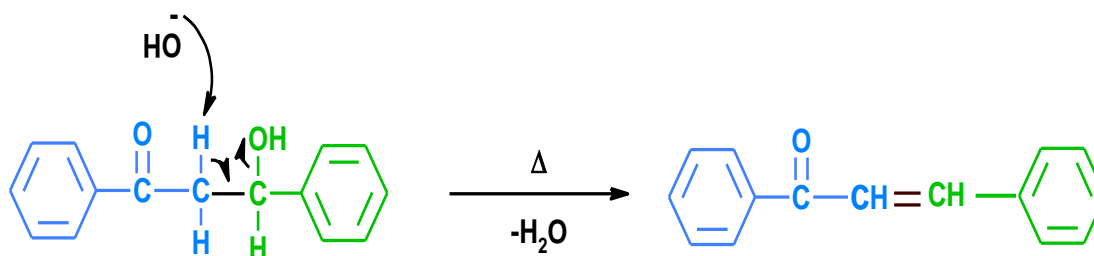
Scheme (3- 1): Mechanism of Chalcone Synthesis [125]

3.1.2. Synthesis of Ethyl-2-amino acetate hydrochloride (II):

The standard procedure for the synthesis of amino acid esters hydrochloride involves the refluxing of a reactants mixture of an amino acid, thionyl chloride and ethanol for about (2-4 hr.) or stirring of a mixture for over (24hr.) at room temperature in the present study, the esterification reactions have been carried out in an ultrasonic bath at ambient temperature. It has been demonstrated that the esterification can be significantly accelerated by the use of ultrasound.

The mechanism of esterification of amino acid with thionyl chloride in the presence of alcohol first takes place by S_N2 mechanism. The reaction occurs by nucleophilic acyl substitution pathway in which the carboxylic acid is converted into a chlorosulfite (-OSOCl) intermediate, thereby replacing the (OH) of the acid with a much better leaving group. The chlorosulfite then react with a nucleophilic chloride ion to produce the acyl chloride of the amino acid.

Second the acyl chloride undergoes addition reaction of hydroxyl group of alcohol at the carbonyl group followed by elimination of chloride ion to form an ester of amino acid(C-protected amino acid) as shown in Scheme (3-2).

The structure of compound(II) was identified by melting point and R_f values given in Table (3-1) as well as the appearance of the FT-IR characteristic absorption bands of $\upsilon_{C=O}$ stretching of ester at 1748 cm^{-1} and υ_{C-O-C} stretching of ester at1250 cm^{-1} as in Table (3-2).

Acyl chlorosulfite   Acetyl chloride

$$\bigcirc = NH_2CH_2-$$
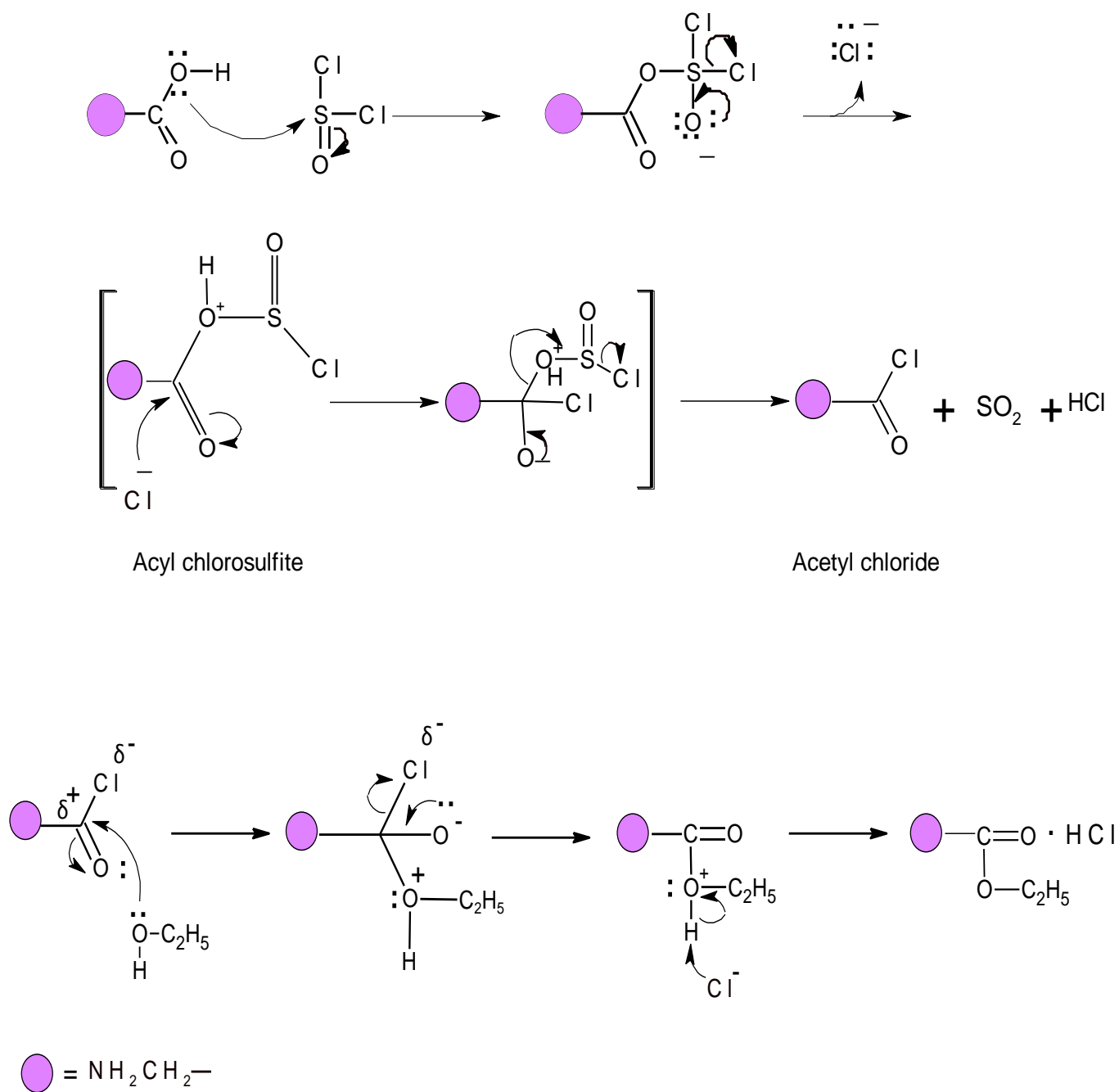
Scheme (3-2): Mechanism of Ethyl amino acetate hydrochloride Synthesis

(126,127)

3.1.3. Synthesis of Compound (III); Formation of Amide Bond:

Treatment of carboxylic acid (2S)-Naproxen and C-protected amino acid with DCC leads directly to amide formation. Mechanism below Scheme (3-3) shows how DCC promotes the condensation of an amine and the carboxylic acid to give an amide.

The structure of compound (III) was identified by melting point and R_f values given in Table (3-1) as well as the appearance of the FT-IR characteristic absorption bands of υ_{NH} stretching of amid at 3293 cm^{-1} and $\upsilon_{C=O}$ stretching of ester and amide at 1740 cm^{-1}and 1649 cm^{-1} respectively as in Table (3-2).

The ^{1}H-NMR spectra showed the broad singlet for NH amide proton at 8.40(δ, ppm) as in Table (3-7).

Mechanism:

Step 1: In the first stage of the reaction, the carboxylic acid adds to one of the double bonds of DCC to give an *o*-acylisourea.
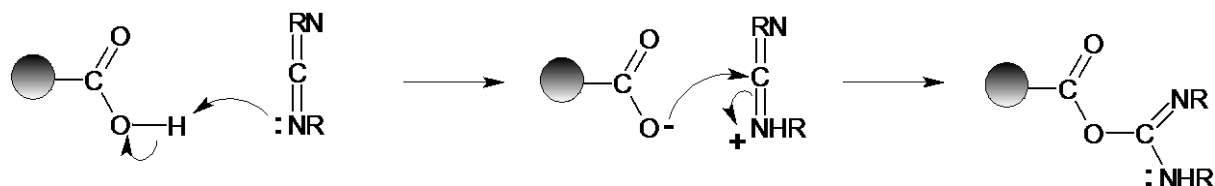
O-Acylisourea

Step 2: structurally, *o*-acylisoureas resemble carboxylic acid anhydride and are powerful acylating agents. In the reaction's second stage the amine adds to the carbonyl group of the *o*-acylisourea to give a tetrahedral intermediate.
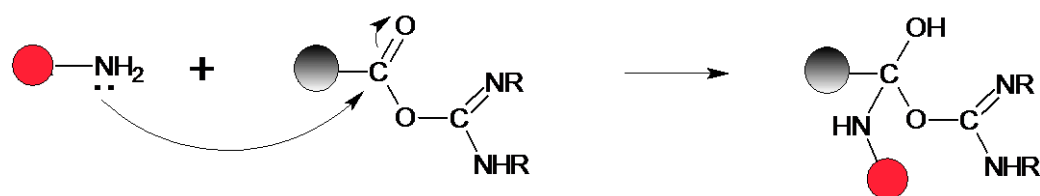
Tetrahedral intermediate

Step 3: The tetrahedral intermediate dissociates to an amide and *N, N' -*dicyclohexylurea.
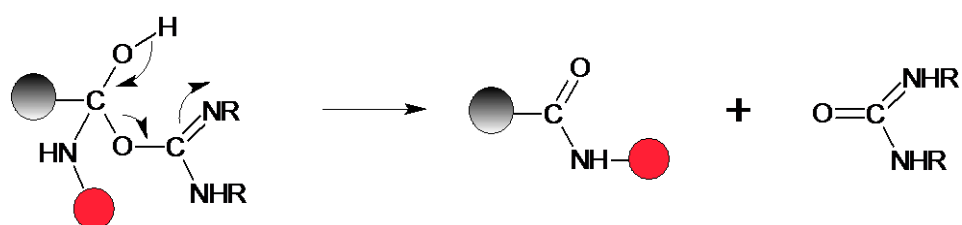
N, N' –Dicyclohexylurea

Scheme (3-3): Mechanism of Amide Synthesis [128]

3.1.4. Synthesis of Compound (IV); Formation of Hydrazide:

Compound (IV) was synthesized by the reaction of compound (III) with hydrazine hydrate (90%) in absolute methanol.

The reaction of hydrazine hydrate with ester is one of the most common reactions to synthesize the acid hydrazide; it is a tetrahedral nucleophilic substitution reaction. The mechanism of this reaction outlined as follow in Scheme (3-4).
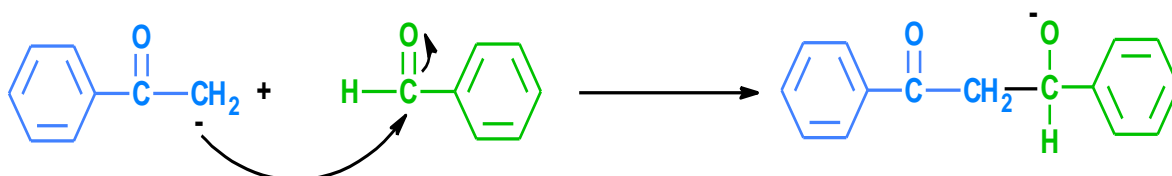
The structure of compound (IV) was identified by melting point and R_f values given in Table (3-1) as well as the appearance of the FT-IR characteristic absorption bands of υ_{NHNH2} stretching at 3339 cm^{-1} and 3277 cm^{-1} and $\upsilon_{C=O}$ stretching of amidic and amide at 1678 cm^{-1} and 1645 cm^{-1} respectively as in Table (3-2).

The ^{1}H-NMR spectra showed the broad singlet for NH$_2$ protons of hydrazide at 4.20(δ, ppm), broad singlet for NH amide proton at 8.20(δ, ppm) and singlet for NH proton of hydrazide at 9.01(δ, ppm) as in Table (3-8).
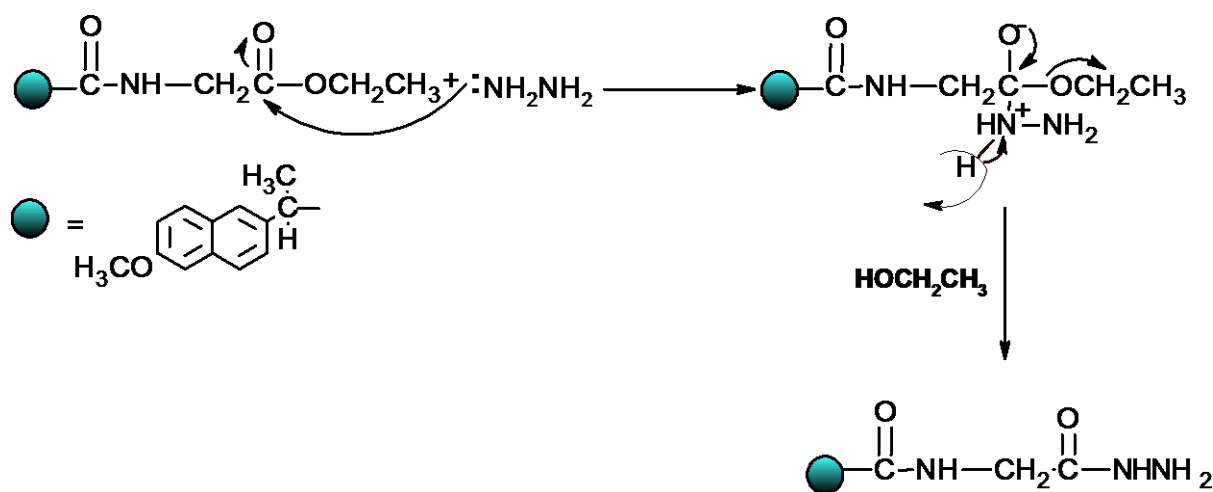
Scheme (3-4): Mechanism of Hydrazide Synthesis [129]

3.1.5. Synthesis of Compounds (V_{a-f}); Formation of Pyrazoline Ring:

Chalcone Derivatives (I_{a-f}) and compound (IV) in ethanol, the mixture was refluxed, after (15 min.) a catalytic glacial acetic acid was added and the contents allowed getting reflux for (24 hrs.). The reaction time was considered by performing TLC to obtain single spot. Cool the reacting mixture and transfer it in to (20 ml) of cold water to precipitate out the product. Filtered it, washed with cold water, dried and re-crystallized from hot ethanol.

The reaction of α, β- unsaturated carbonyl (chalcone) with hydrazine to form hydrazones as intermediates. These hydrazone intermediates on treatment with glacial acetic acid in ethanol isomerizes to pyrazolines the mechanism of this reaction outlined as follow in Scheme (3-5).

The structure of compounds (V_{a-f}) was identified by melting point and R_f values given in Table (3-1) as well as the appearance of the FT-IR characteristic absorption bands of $\upsilon_{C=O}$ stretching of amide at 1658-1649 cm^{-1}, $\upsilon_{C=N}$ stretching of pyrazoline ring at 1572-1523 cm^{-1} as in Table (3-2).

The ^{1}H-NMR spectra showed a multiplet due to overlap of CH$_2$ protons of pyrazoline and CH proton of naproxen at 3.49-3.71(δ, ppm) and a broad singlet for CH proton of pyrazoline ring at 5.20-5.38(δ, ppm) as in Table (3-9 to 3-14).
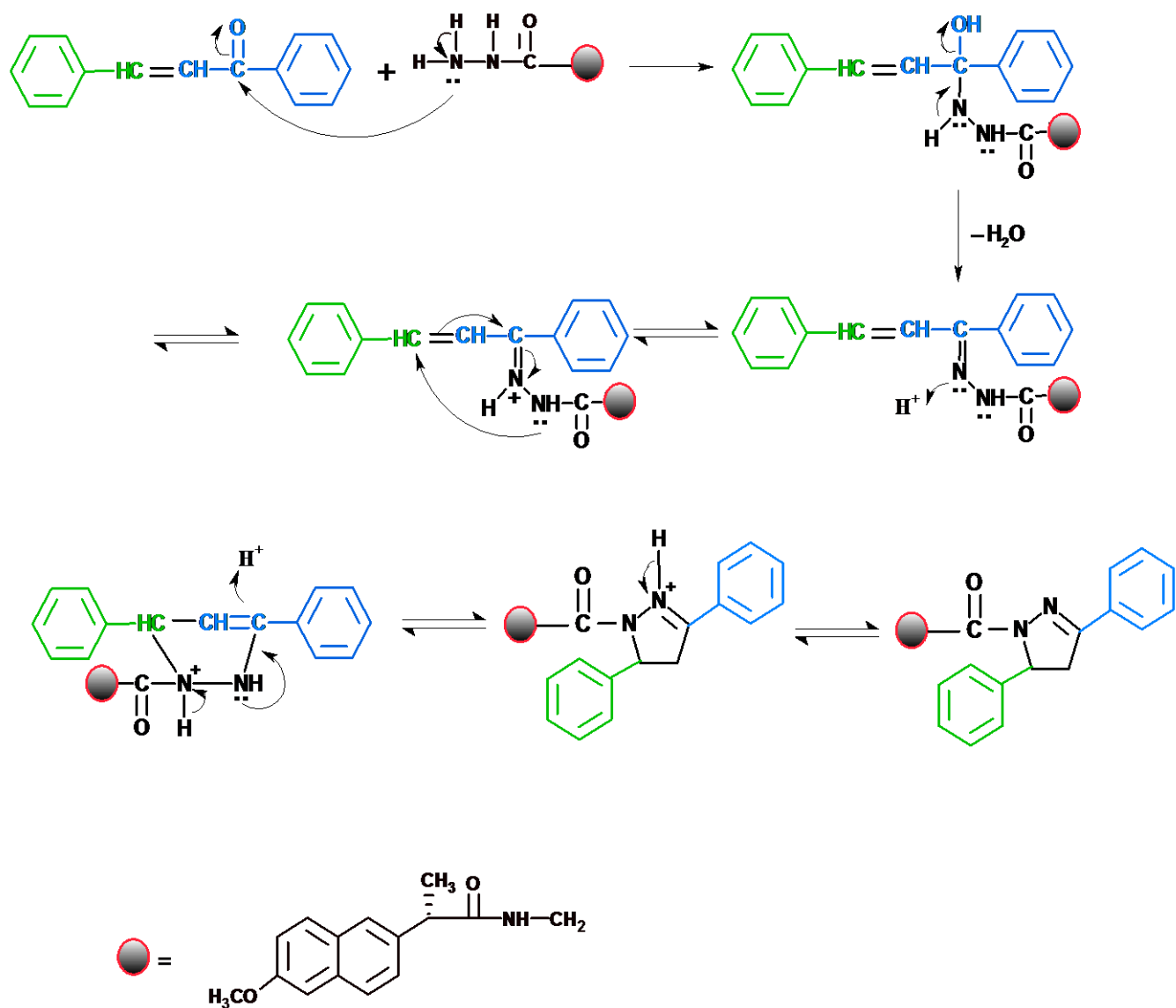
Scheme (3-5): Mechanism of Pyrazoline Ring Synthesis [109,130]

3.2. Characterization and Identification of the Target Compounds and their Intermediates:

The physical appearance, melting point and R_f values of the synthesized compounds and their intermediates were listed in Table (3-1). TLC was performed in two different solvent systems in order to follow up the reaction pattern and reveal purity of the synthesized compounds and their intermediates by the present of one spot with different R_f values.

3.2.1. Interpretation of the Results of Infrared Spectra:

The FT-IR spectra of the target synthesized compounds and their intermediates showed the characteristic absorption bands by which their functional groups were identified. The values of the characteristic bands of these spectra have been discussed according to the literature survey of analogous compounds [131-133] and references book [134], and summarized in Table (3-2).
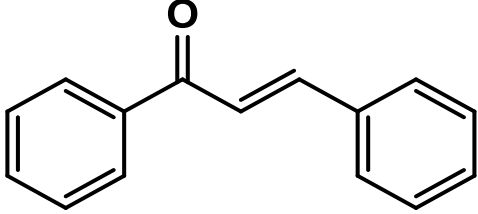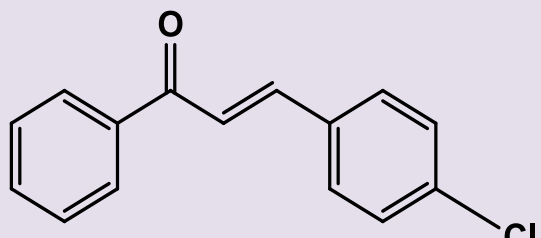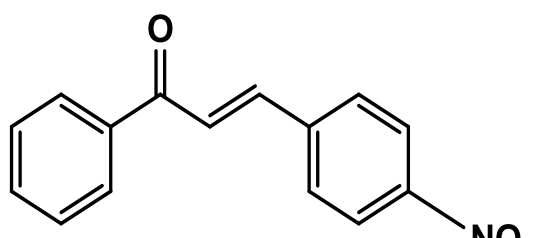
3.2.2. Interpretation of the Results of ^{1}H-NMR Spectra:
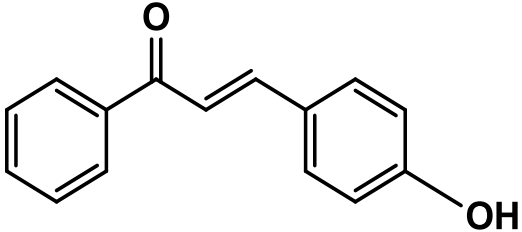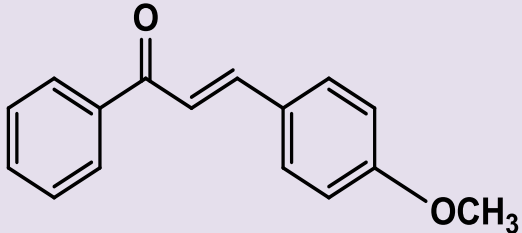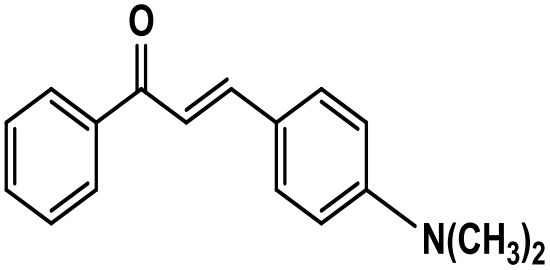
The[1]H-NMR analysis was used to identify the target compounds and their intermediates. The spectra were recorded in DMSO solvent. The values of characteristic chemical shifts have been discussed according to the literature survey of analogous compounds [131-133] and references book [134,135] and summarized in Tables (3-3to 3-14).
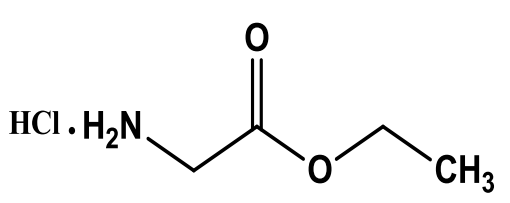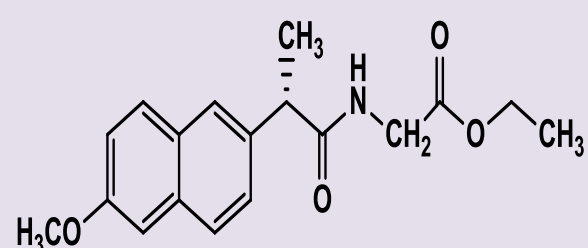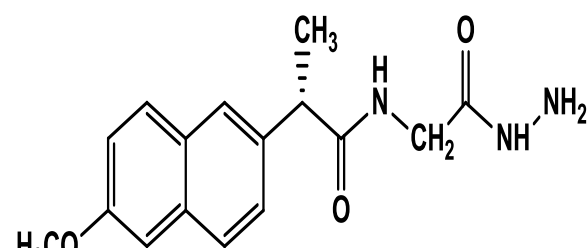
Table (3-1): The Characterization and Physical Parameters of the Target Compounds and their Intermediates

No.	Chemical name	Formula	M. Wt.	Description	% yield	M.P. (°C)	R_f
I_a	1,3-Diphenylpropenone	$C_{15}H_{12}O$	208	Pale yellow crystals	94	56-58	A=0.52 B=0.39
I_b	3-(4-chlorophenyl)-1-phenylprop-2-en-1-one	$C_{15}H_{11}OCl$	242	Off white crystals	78	112-114	A=0.57 B=0.46
I_c	3-(4-nitrophenyl)-1-phenylprop-2-en-1-one	$C_{15}H_{11}NO_3$	253	Orange powder	70	159-161	A=0.46 B=0.36
I_d	3-(4-hydroxyphenyl)-1-phenylprop-2-en-1-one	$C_{15}H_{12}O_2$	224	Green-yellowish powder	67	182-184	A=0.81 B=0.65
I_e	3-(4-methoxyphenyl)-1-phenylprop-2-en-1-one	$C_{16}H_{14}O_2$	238	Pale yellow crystals	75	73-75	A=0.54 B=0.44
I_f	3-(4-(dimethylamino)phenyl)-1-phenylprop-2-en-1-one	$C_{17}H_{17}NO$	251	Orange crystals	80	111-113	A=0.59 B=0.49
II	ethyl amino acetate hydrochloride	$C_4H_{10}NO_2Cl$	139	White crystals	90	145-147	A=0.55 B=0.48
III	(2S)-ethyl 2-(2-(6-methoxynaphthalen-2-yl)propanamido)acetate	$C_{18}H_{21}NO_4$	315	Off white powder	65	84-86	A=0.66 B=0.55
IV	(2S)-N-(2-hydrazinyl-2-oxoethyl)-2-(6-methoxynaphthalen-2-yl)propanamide	$C_{16}H_{19}N_3O_3$	301	yellow powder	78	158-160	A=0.75 B=0.50
V_a	(2S)-N-(2-(3,5-diphenyl-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)-2-(6-methoxynaphthalen-2-yl)propanamide	$C_{31}H_{29}N_3O_3$	491	Off white powder	70	68-70	A=0.61 B=0.49
V_b	(2S)-N-(2-(5-(4-chlorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)-2-(6-methoxynaphthalen-2-yl)propanamide	$C_{31}H_{28}N_3O_3Cl$	526	Off white powder	63	78-80	A=0.65 B=0.54
V_c	(2S)-2-(6-methoxynaphthalen-2-yl)-N-(2-(5-(4-nitrophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)propanamide	$C_{31}H_{28}N_4O_5$	536	Pale orange powder	61	85-88	A=0.55 B=0.45
V_d	(2S)-N-(2-(5-(4-hydroxyphenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)-2-(6-methoxynaphthalen-2-yl)propanamide	$C_{31}H_{29}N_3O_4$	507	Light brown powder	58	87-89	A=0.70 B=0.58
V_e	(2S)-2-(6-methoxynaphthalen-2-yl)-N-(2-(5-(4-methoxyphenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)propanamide	$C_{32}H_{31}N_3O_4$	521	Off white powder	68	100-102	A=0.62 B=0.51
V_f	(2S)-N-(2-(5-(4(dimethyl amino)phenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)-2-(6-methoxynaphthalen-2-yl)propanamide	$C_{33}H_{34}N_4O_3$	534	Orange powder	54	115-117	A=0.59 B=0.45
Nap.	(2S)-2-(6-methoxynaphthalen-2-yl) propanoic acid	$C_{14}H_{14}O_3$	230	White powder	-	157-158	A=0.67 B=0.15
Gly.	Aminoacetic acid	$C_2H_5NO_2$	75	White powder	-	233-234	A=0.75 B=0.38
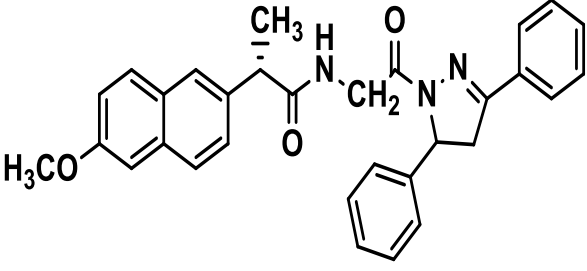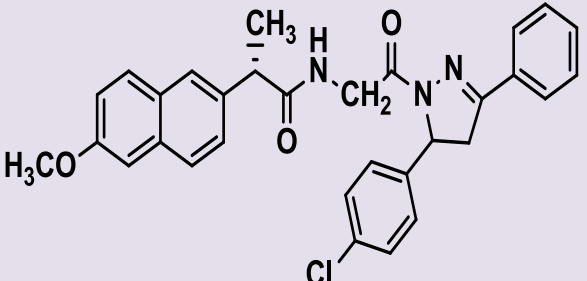
Table (3-2): Characteristic FT-IR Absorption Bands of the Synthesize Compounds
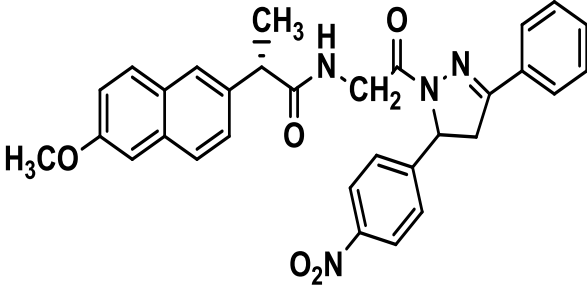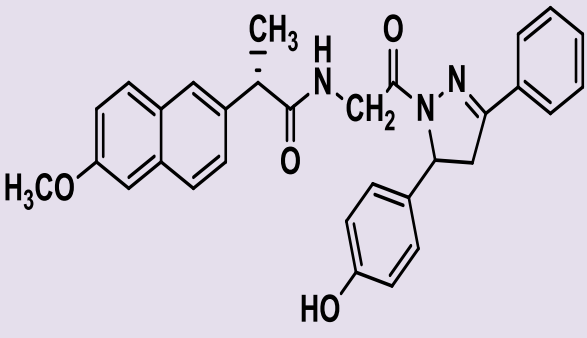
Compounds	Bands (cm^{-1})	Interpretation
I_a	3059	CH asymmetric stretching of aromatic
	3036	CH symmetric stretching of aromatic
	1660	C=O stretching
	1603 1573	Aromatic C=C stretching
I_b	3061	CH asymmetric stretching of aromatic
	3020	CH symmetric stretching of aromatic
	1658	C=O stretching
	1599 1570	Aromatic C=C stretching
	821	C-Cl
I_c	3074	CH asymmetric stretching of aromatic
	2951	CH symmetric stretching of aromatic
	1660	C=O stretching
	1603	Aromatic C=C stretching
	1516	NO$_2$ asymmetric stretching
	1336	NO$_2$ symmetric stretching
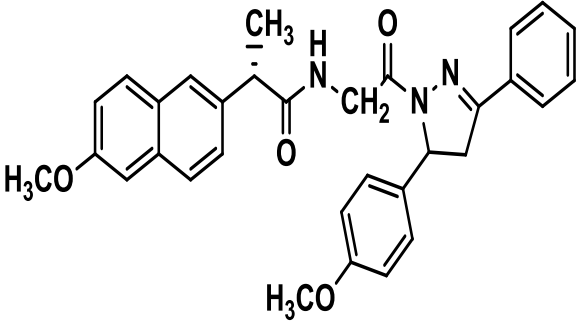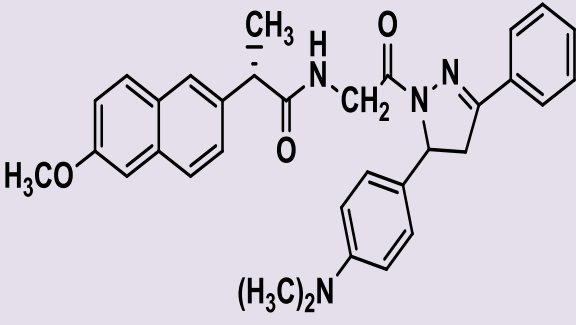
Compounds	Bands (cm^{-1})	Interpretation
I$_d$	3244	Phenolic O-H stretching
	3020	CH asymmetric stretching of aromatic
	2812	CH symmetric stretching of aromatic
	1649	C=O stretching
	1599 1558	Aromatic C=C stretching
	1348	O-H bending
	1215	C-OH stretching
I$_e$	3057	CH asymmetric stretching of aromatic
	3016	CH symmetric stretching of aromatic
	2953	CH asymmetric stretching of CH$_3$
	2902	CH symmetric stretching of CH$_3$
	1657	C=O stretching
	1601 1574	Aromatic C=C stretching
	1263	C-OCH$_3$ stretching
I$_f$	3086	CH asymmetric stretching of aromatic
	3051	CH symmetric stretching of aromatic
	2987	CH asymmetric stretching of CH$_3$
	2860	CH symmetric stretching of CH$_3$
	1649	C=O stretching
	1599 1562	Aromatic C=C stretching
	1172	N-CH$_3$ stretching

Compounds	Bands (cm^{-1})	Interpretation
II	2978	Stretching vibration of primary amine salt
	2675	CH asymmetric stretching of CH$_3$
	2637	CH symmetric stretching of CH$_3$
	1748	C=O stretching of ester
	1250	C-O-C stretching of ester
	1136	C-N stretching
III	3293	NH stretching of amide
	3069	C-H stretching of aromatic
	2974	CH asymmetric stretching of CH$_3$
	2934	CH symmetric stretching of CH$_3$
	1740	C=O stretching of ester
	1649	C=O stretching of amide
	1610	Aromatic C=C stretching
	1261	Ar-O-C stretching of naproxen
IV	3339 3277	NHNH$_2$ stretching
	3036	C-H stretching of aromatic
	2986	CH asymmetric stretching of CH$_3$
	2939	CH symmetric stretching of CH$_3$
	1678	C=O stretching of amidic
	1645	C=O stretching of amide
	1606	Aromatic C=C stretching
	1265	Ar-O-C stretching of naproxen

Compounds	Bands (cm^{-1})	Interpretation
V$_a$	3296	NH stretching of amide
	3059	C-H stretching of aromatic
	2968	CH asymmetric stretching of CH$_3$
	2935	CH symmetric stretching of CH$_3$
	1653	C=O stretching of amide
	1604	Aromatic C=C stretching
	1533	C=N stretching of pyrazoline ring
	1267	Ar-O-C stretching of naproxen
V$_b$	3292	NH stretching of amide
	3059	C-H stretching of aromatic
	2974	CH asymmetric stretching of CH$_3$
	2935	CH symmetric stretching of CH$_3$
	1653	C=O stretching of amide
	1606	Aromatic C=C stretching
	1541	C=N stretching of pyrazoline ring
	1265	Ar-O-C stretching of naproxen
	813	C-Cl

Compounds	Bands (cm^{-1})	Interpretation
V$_c$	3294	NH stretching of amide
	3059	C-H stretching of aromatic
	2947	CH asymmetric stretching of CH$_3$
	2935	CH symmetric stretching of CH$_3$
	1658	C=O stretching of amide
	1604	Aromatic C=C stretching
	1572	C=N stretching of pyrazoline ring
	1516	NO$_2$ asymmetric stretching
	1342	NO$_2$ symmetric stretching
	1265	Ar-O-C stretching of naproxen
V$_d$	3298	NH stretching of amide
	3284	Phenolic O-H stretching
	3059	C-H stretching of aromatic
	2995	CH asymmetric stretching of CH$_3$
	2937	CH symmetric stretching of CH$_3$
	1649	C=O stretching of amide
	1604	Aromatic C=C stretching
	1545	C=N stretching of pyrazoline ring
	1340	O-H bending
	1267	Ar-O-C stretching of naproxen

Compounds	Bands (cm^{-1})	Interpretation
V$_e$	3298	NH stretching of amide
	3059	C-H stretching of aromatic
	2972	CH asymmetric stretching of CH$_3$
	2933	CH symmetric stretching of CH$_3$
	1655	C=O stretching of amide
	1604	Aromatic C=C stretching
	1537	C=N stretching of pyrazoline ring
	1261	Ar-O-C stretching of naproxen
V$_f$	3294	NH stretching of amide
	3057	C-H stretching of aromatic
	2974	CH asymmetric stretching of CH$_3$
	2933	CH symmetric stretching of CH$_3$
	1653	C=O stretching of amide
	1603	Aromatic C=C stretching
	1523	C=N stretching of pyrazoline ring
	1267	Ar-O-C stretching of naproxen
	1166	N-CH$_3$ stretching
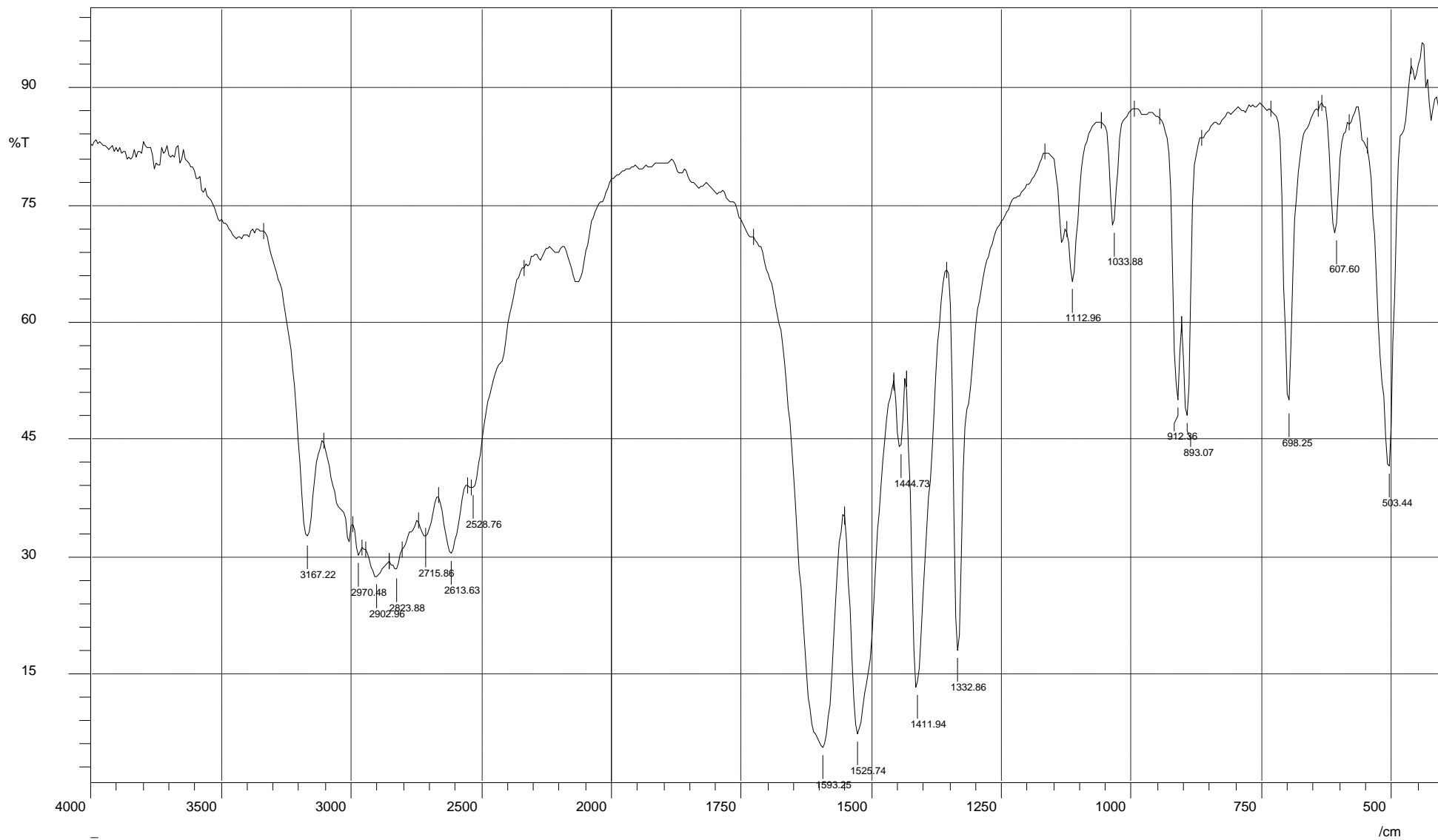
Figure (3-1): FT-IR Spectrum of Glycine Using KBr Disc
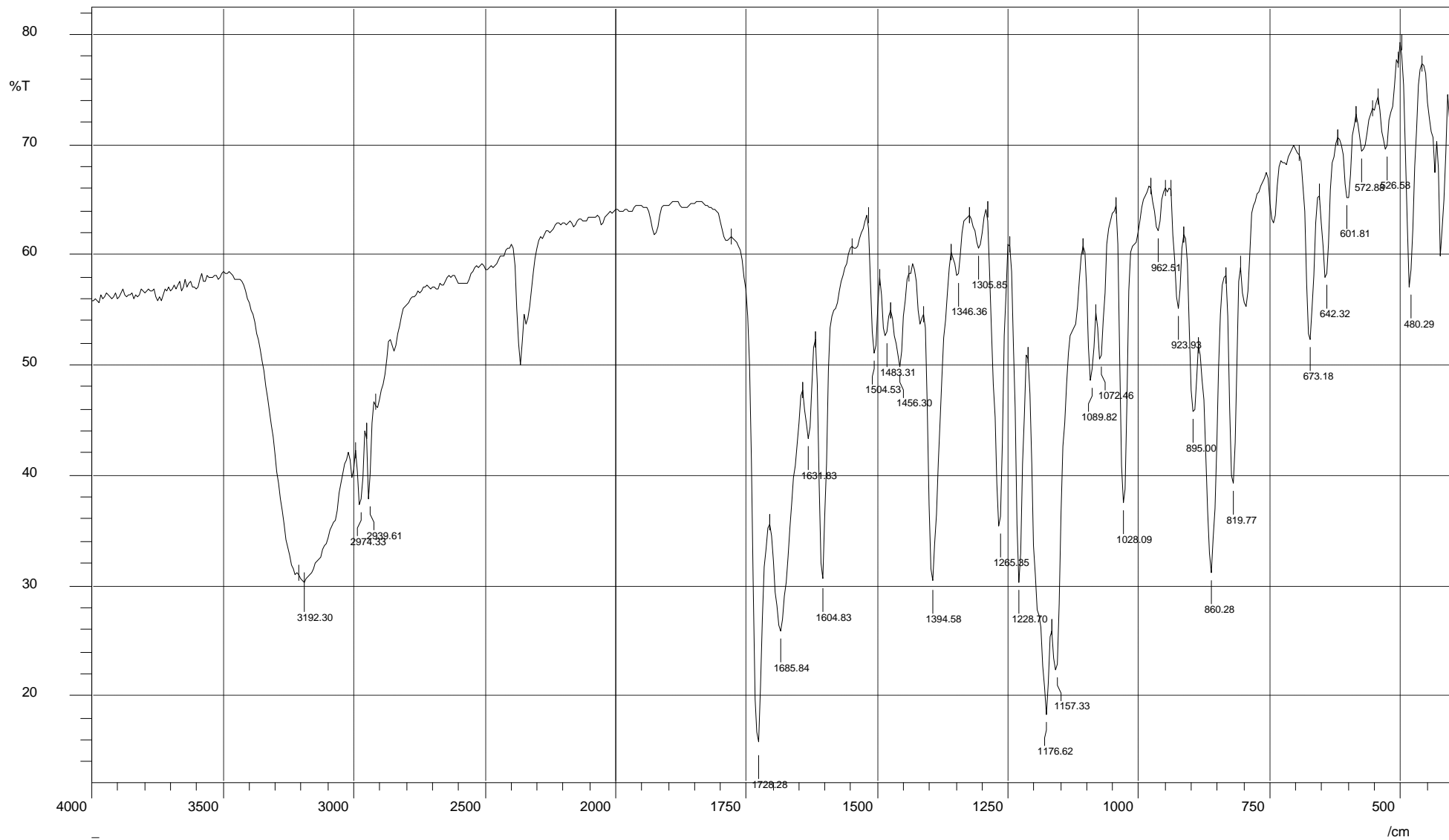
Figure (3-2): FT-IR Spectrum of (2S)-Naproxen Using KBr Disc
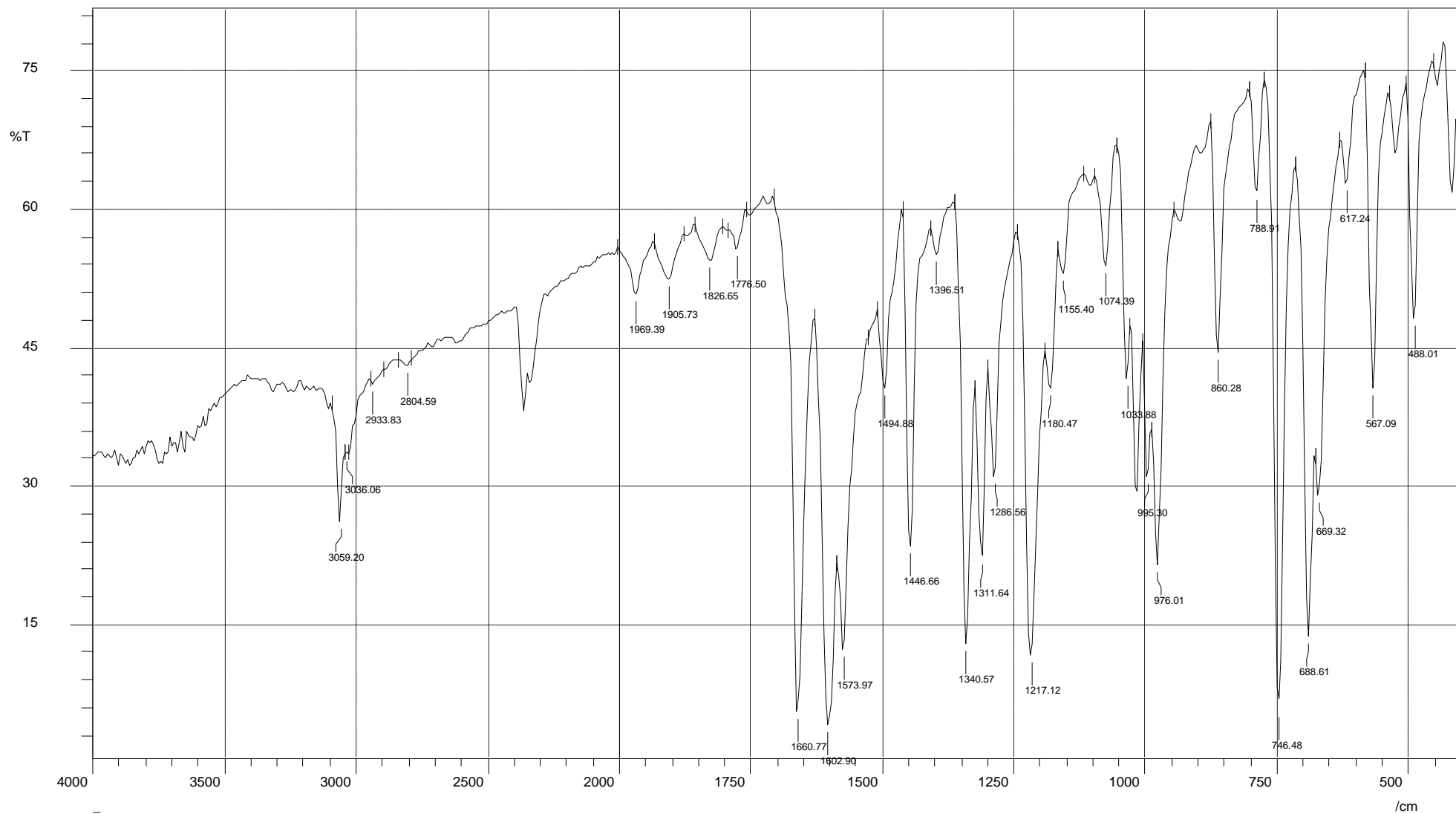
Figure (3-3): FT-IR Spectrum of Compound (I_a) Using KBr Disc
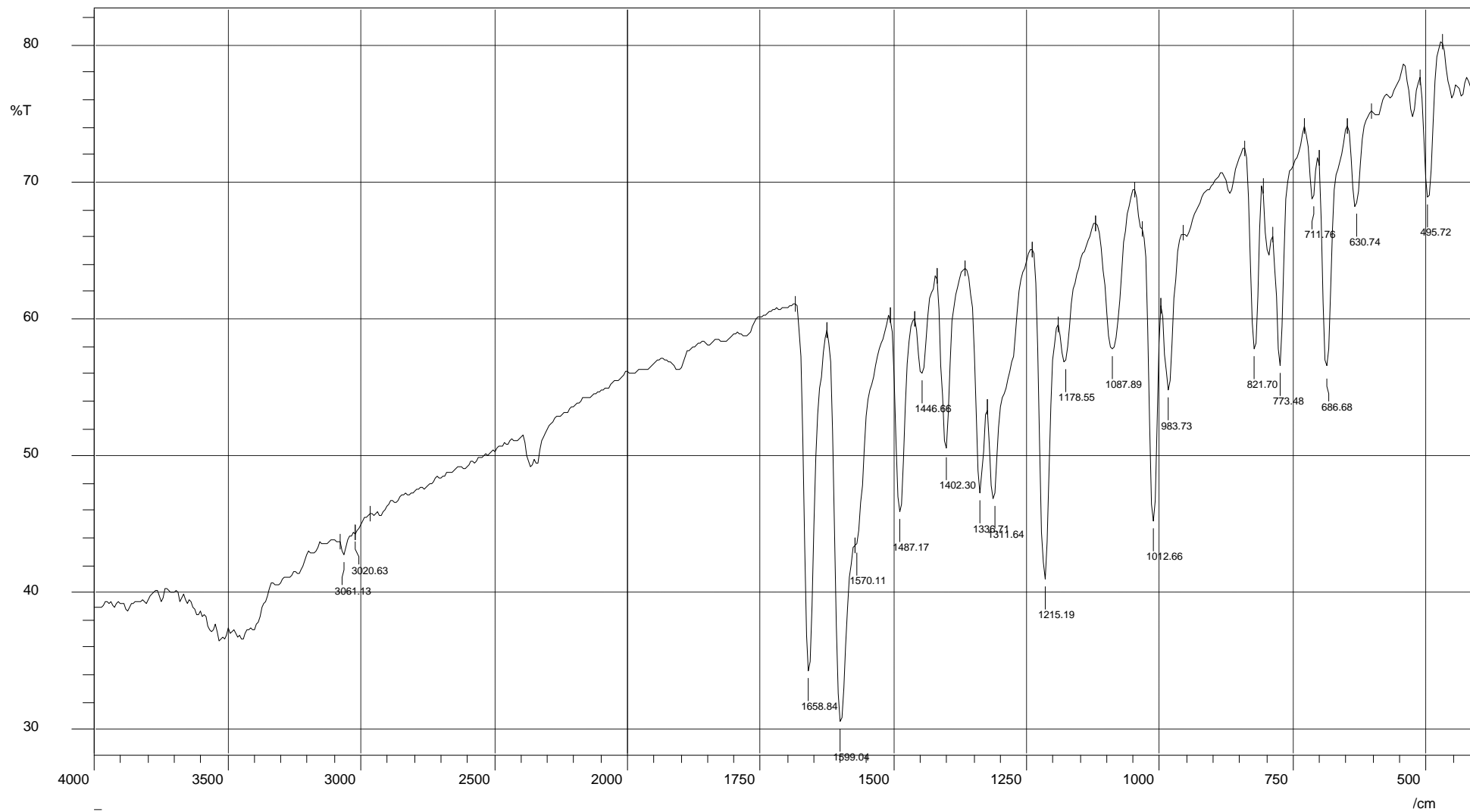
Figure (3-4): FT-IR Spectrum of Compound (I$_b$) Using KBr Disc
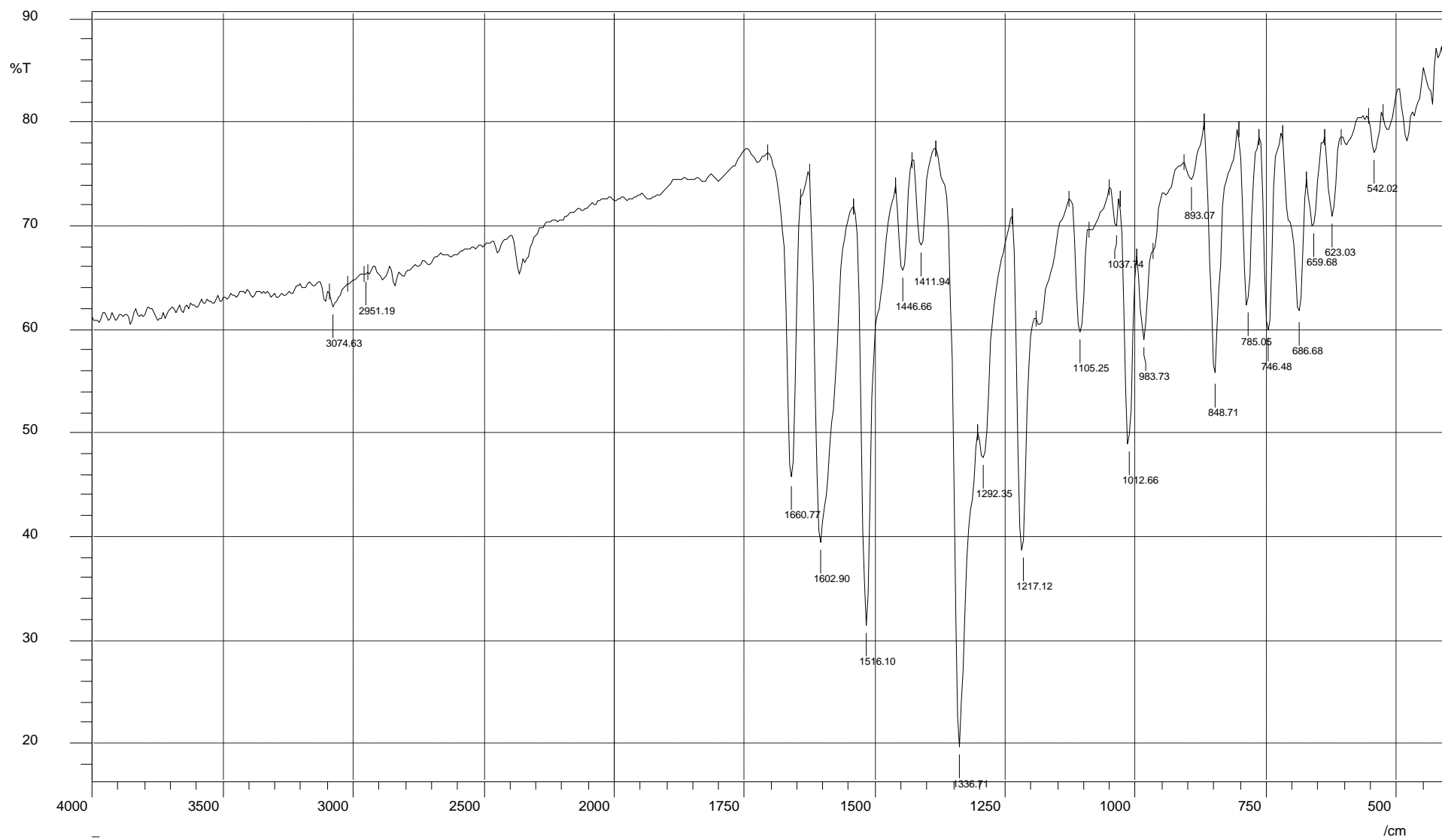
Figure (3-5): FT-IR Spectrum of Compound (I$_c$) Using KBr Disc
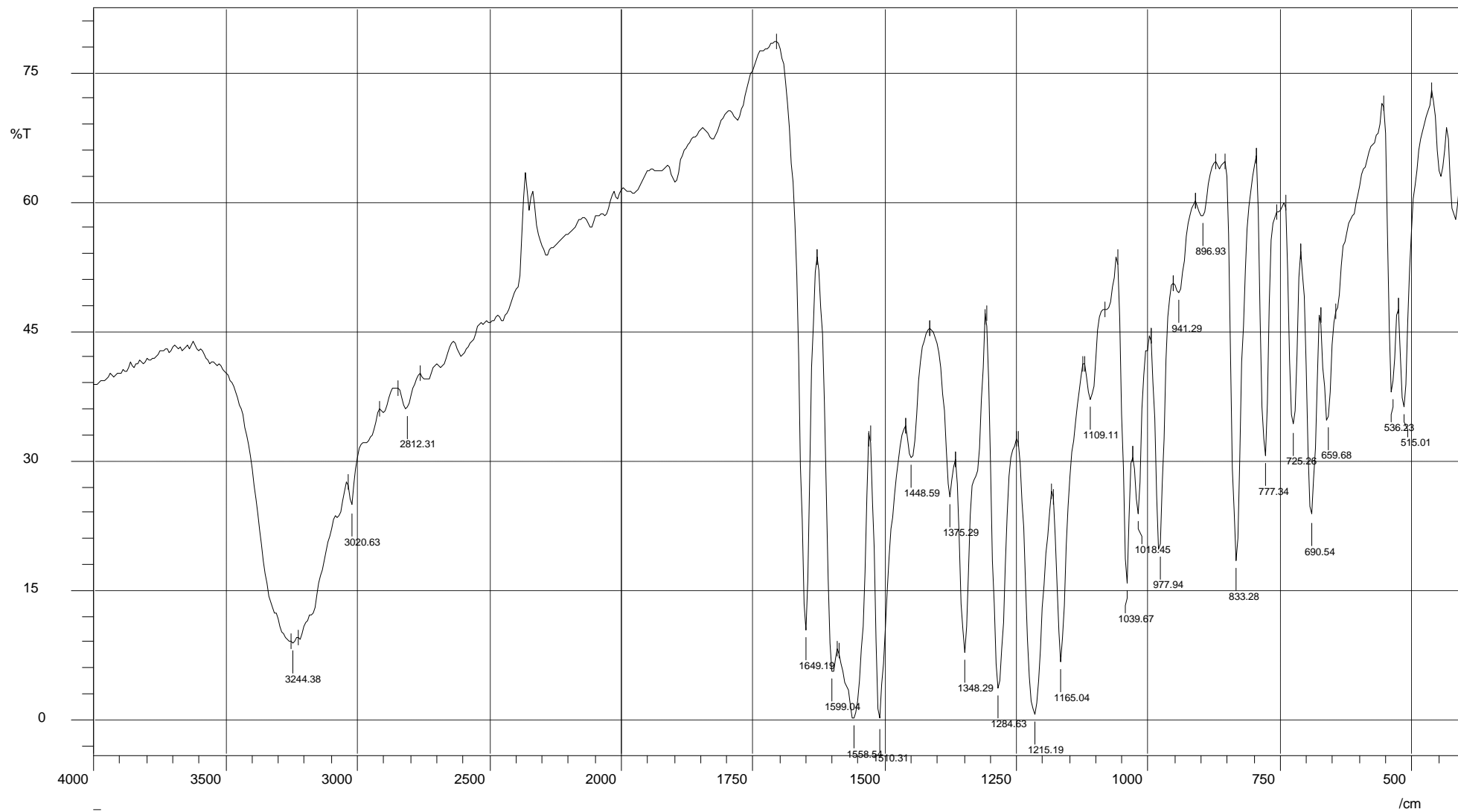
Figure (3-6): FT-IR Spectrum of Compound (I$_d$) Using KBr Disc
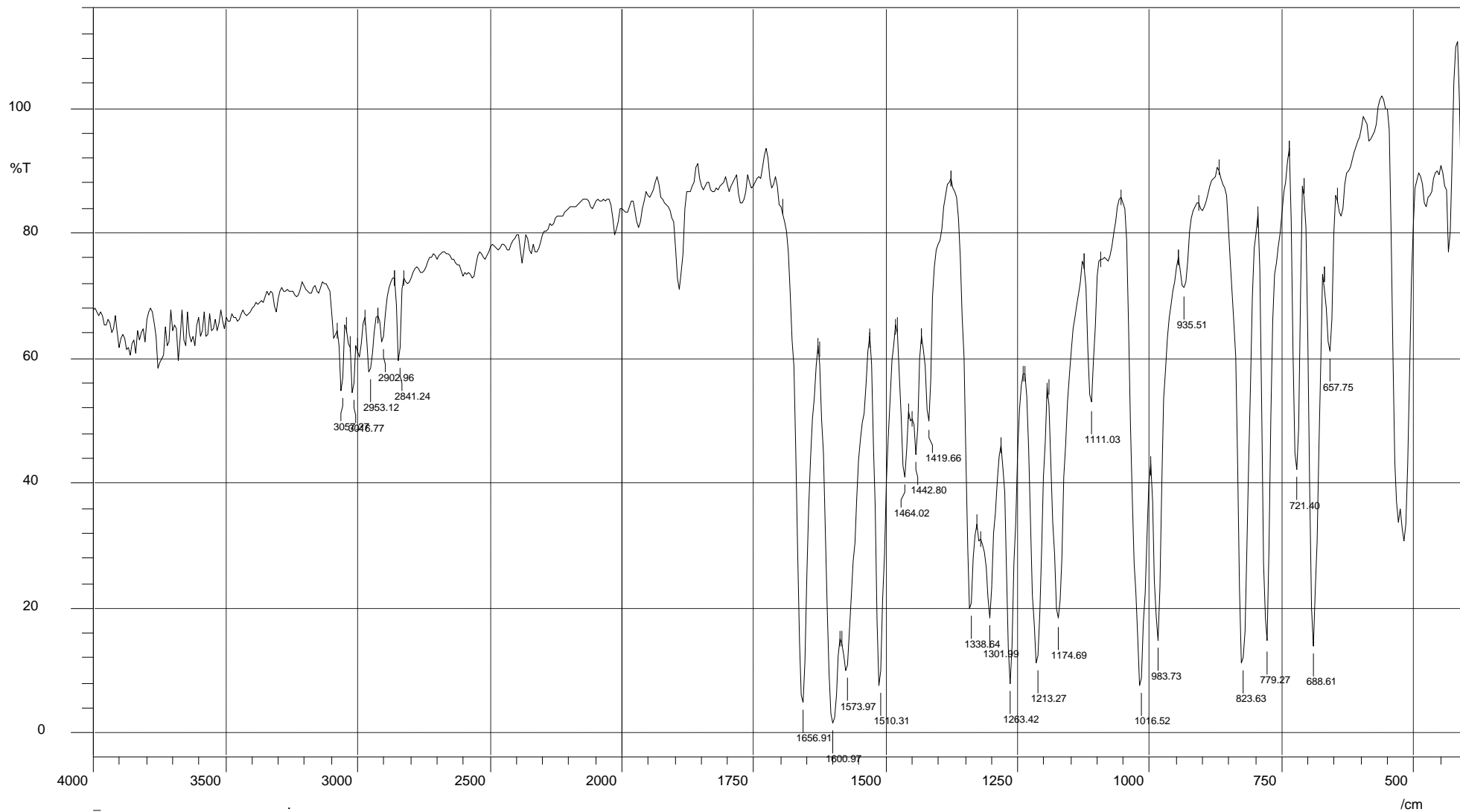
Figure (3-7): FT-IR Spectrum of Compound (I$_e$) Using KBr Disc
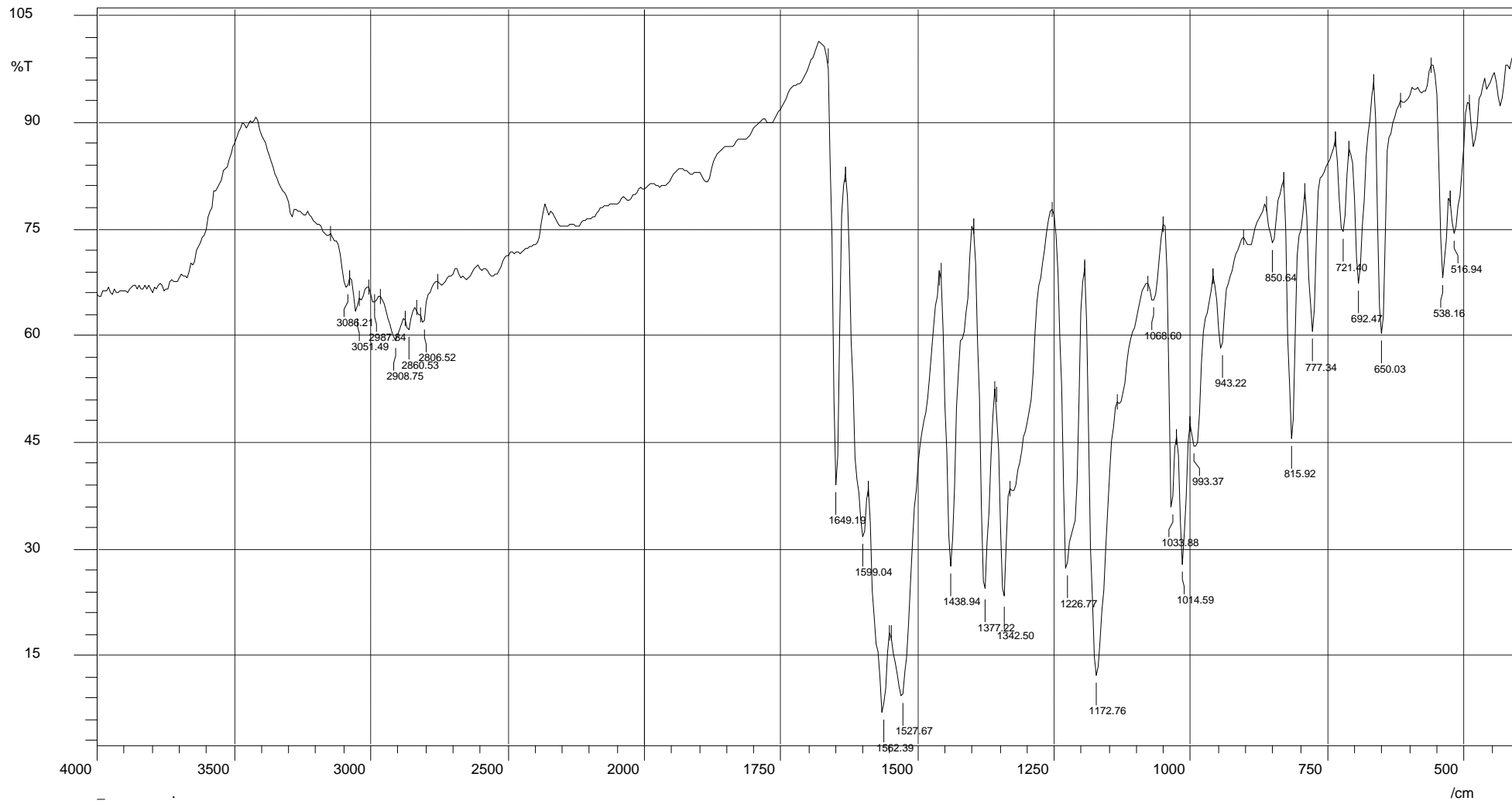
Figure (3-8): FT-IR Spectrum of Compound (I$_f$) Using KBr Disc
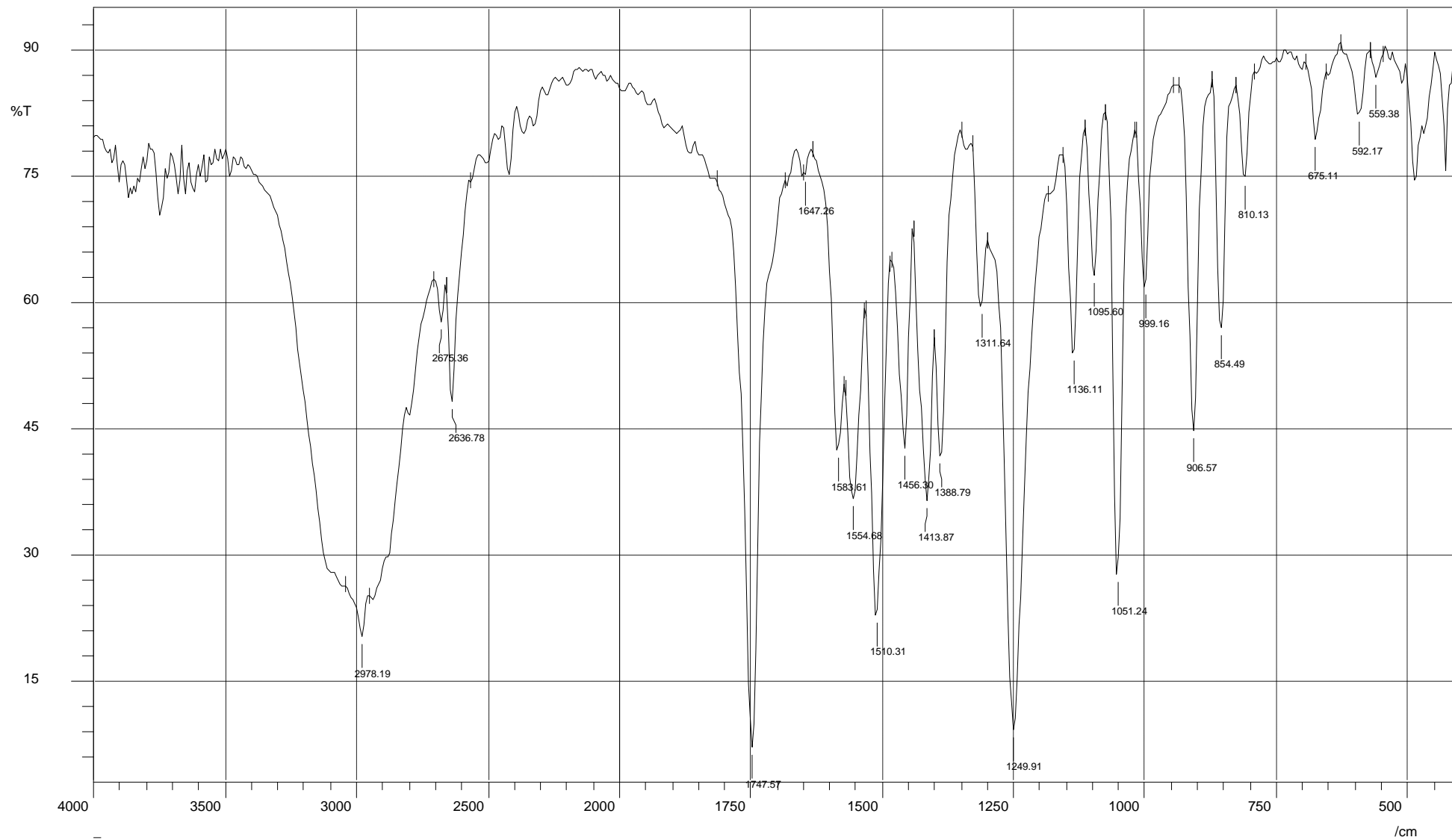
Figure (3-9): FT-IR Spectrum of Compound (II) Using KBr Disc
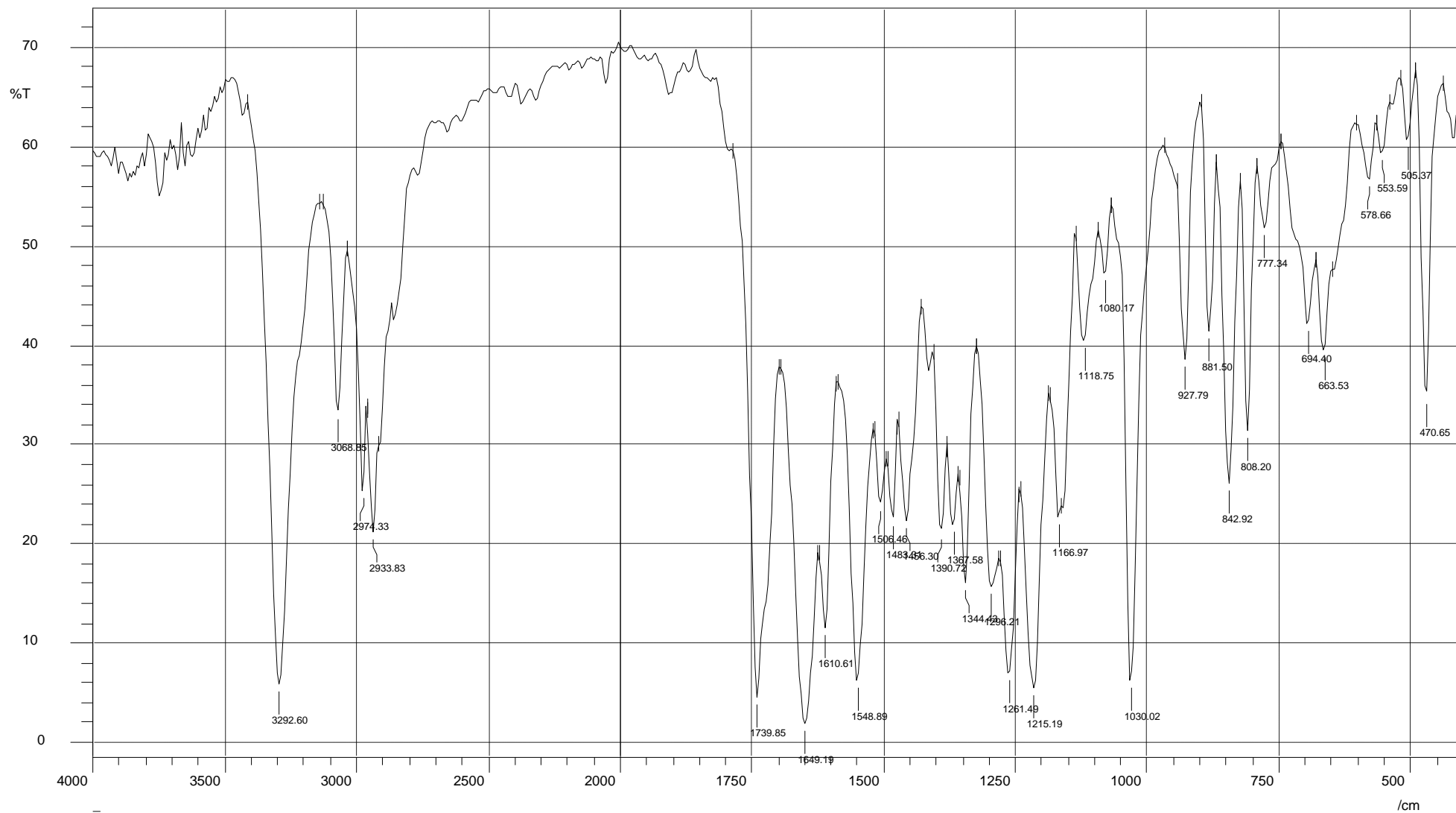
Figure (3-10): FT-IR Spectrum of Compound (III) Using KBr Disc
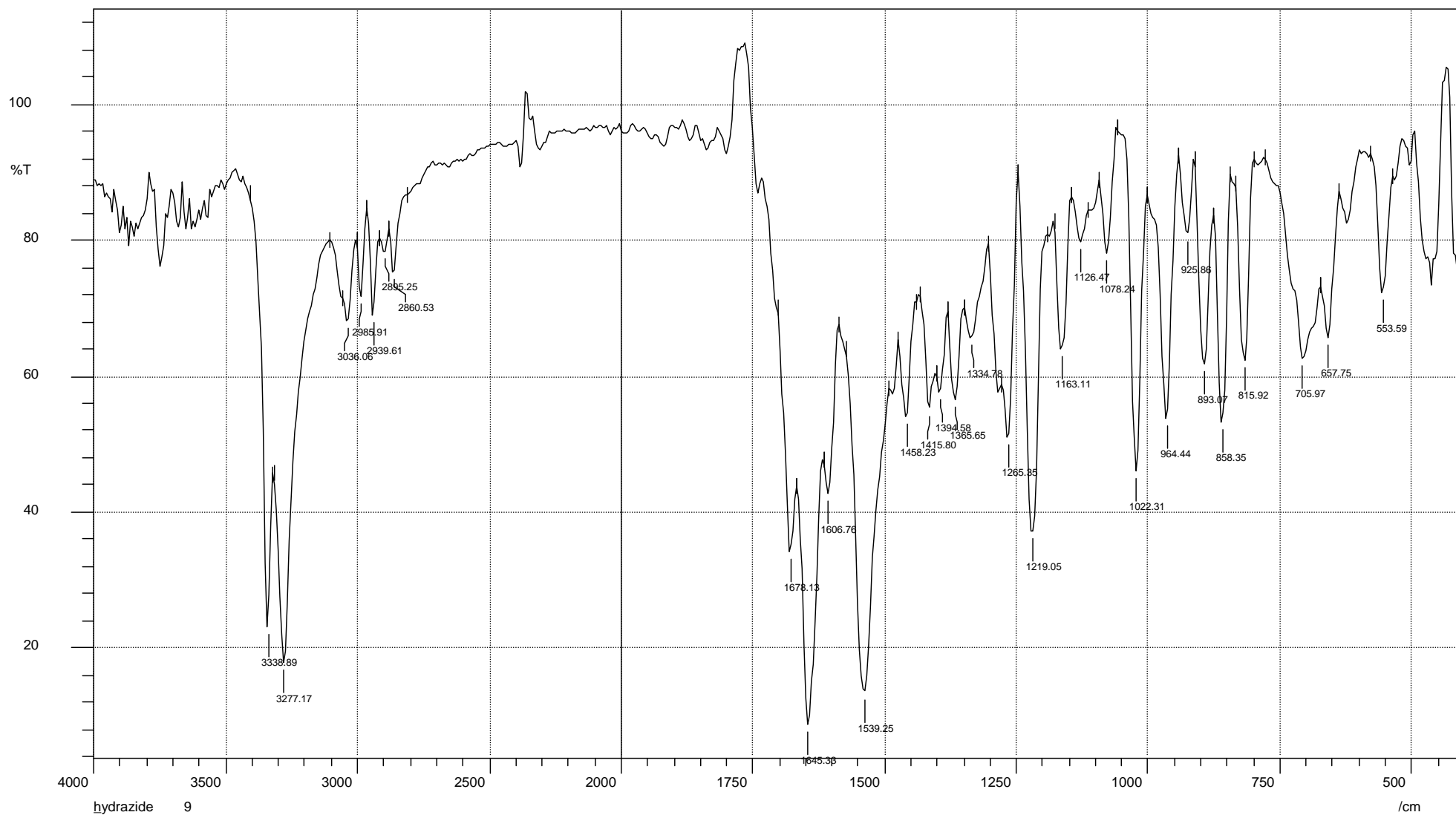
Figure (3-11): FT-IR Spectrum of Compound (IV) Using KBr Disc
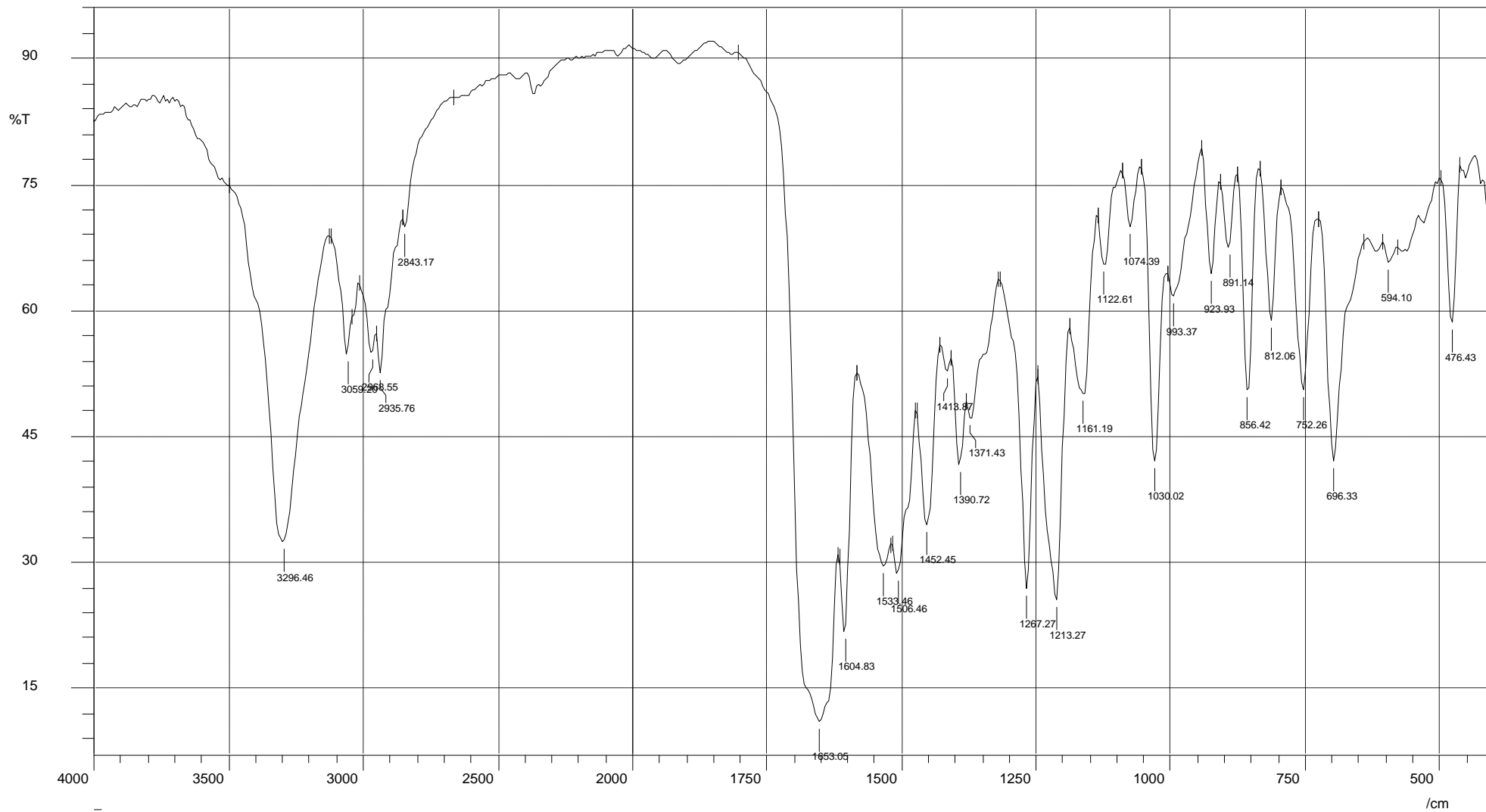
Figure (3-12): FT-IR Spectrum of Compound (V_a) Using KBr Disc
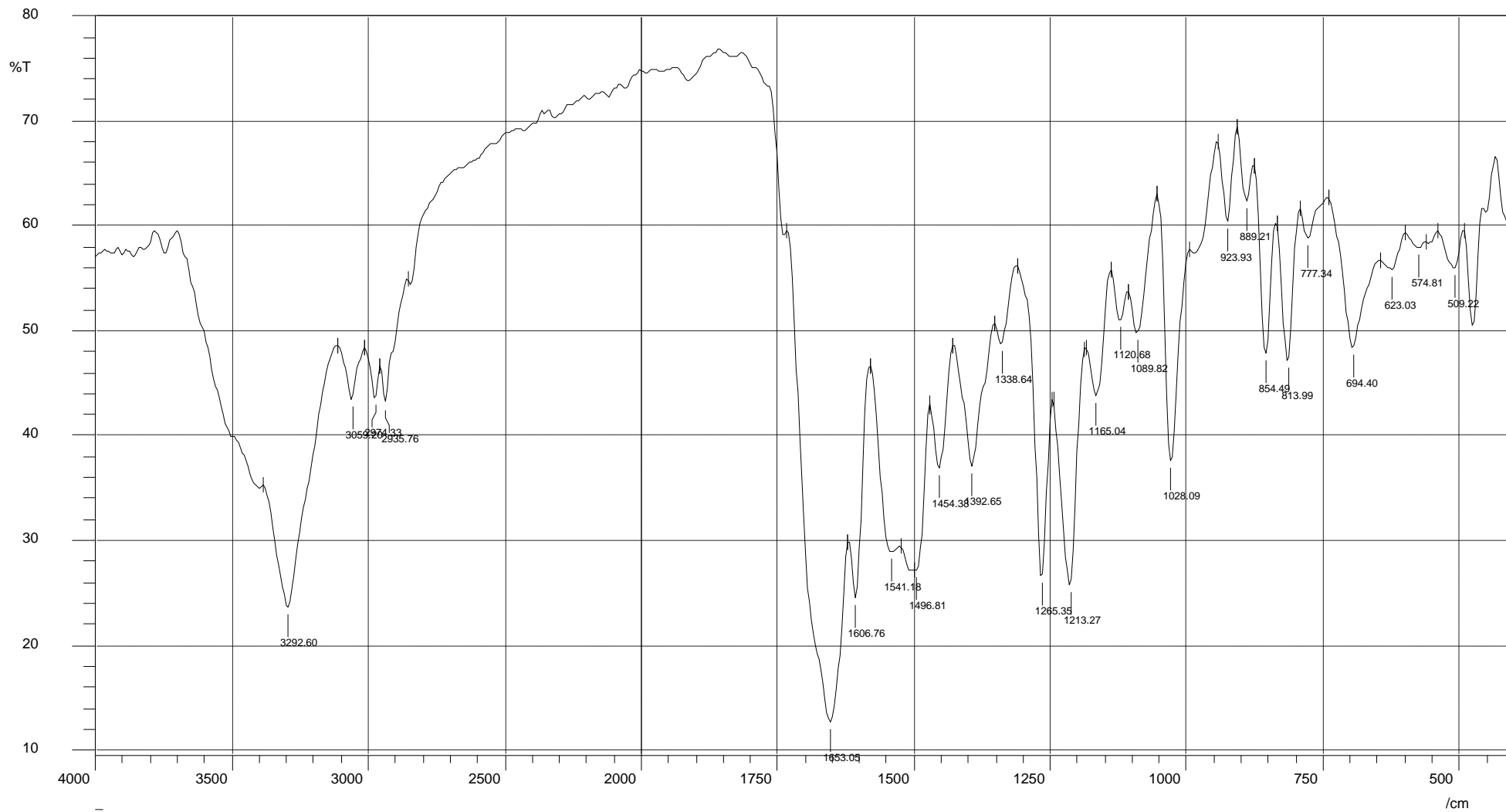
Figure (3-13): FT-IR Spectrum of Compound (V$_b$) Using KBr Disc
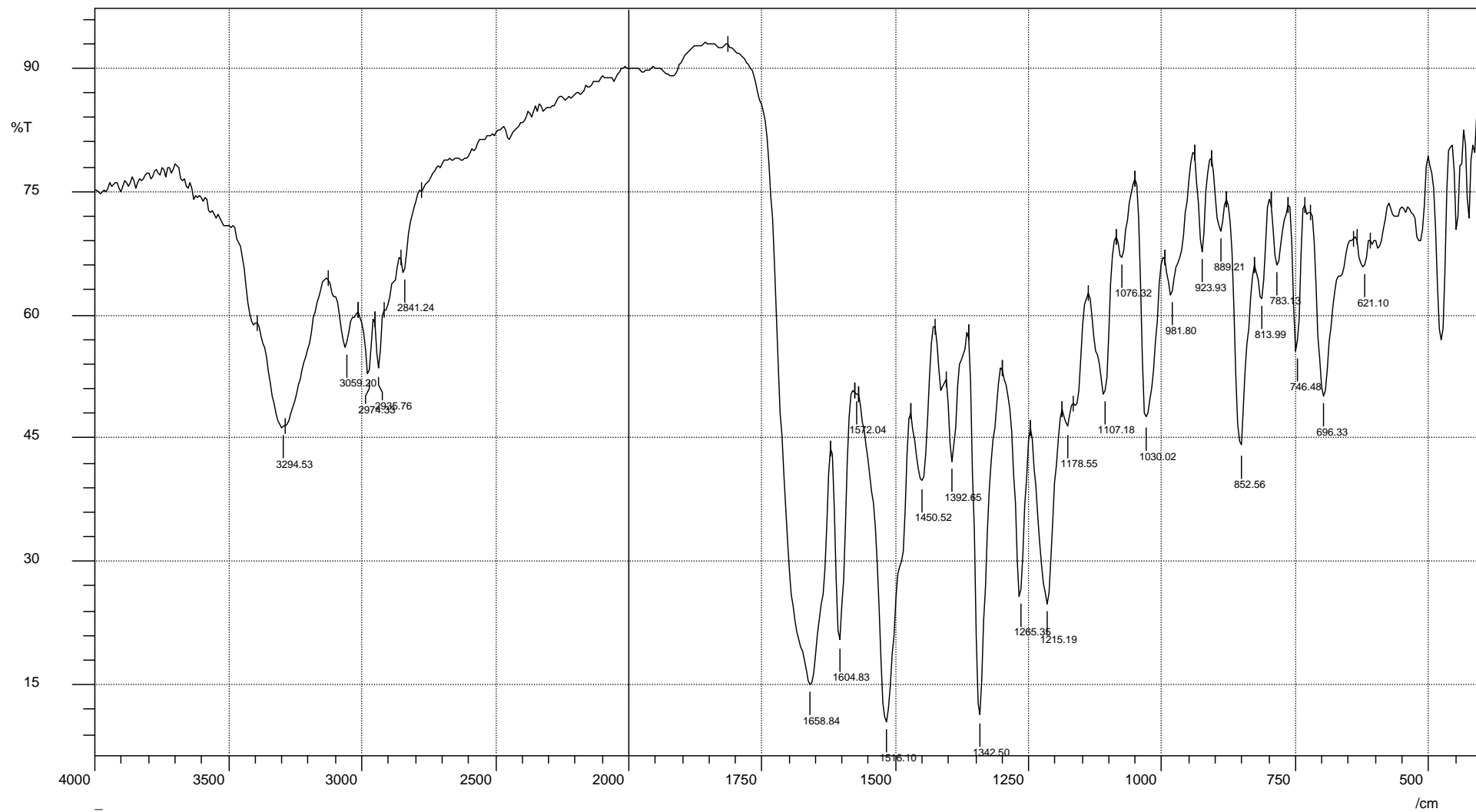
Figure (3-14): FT-IR Spectrum of Compound (V$_c$) Using KBr Disc
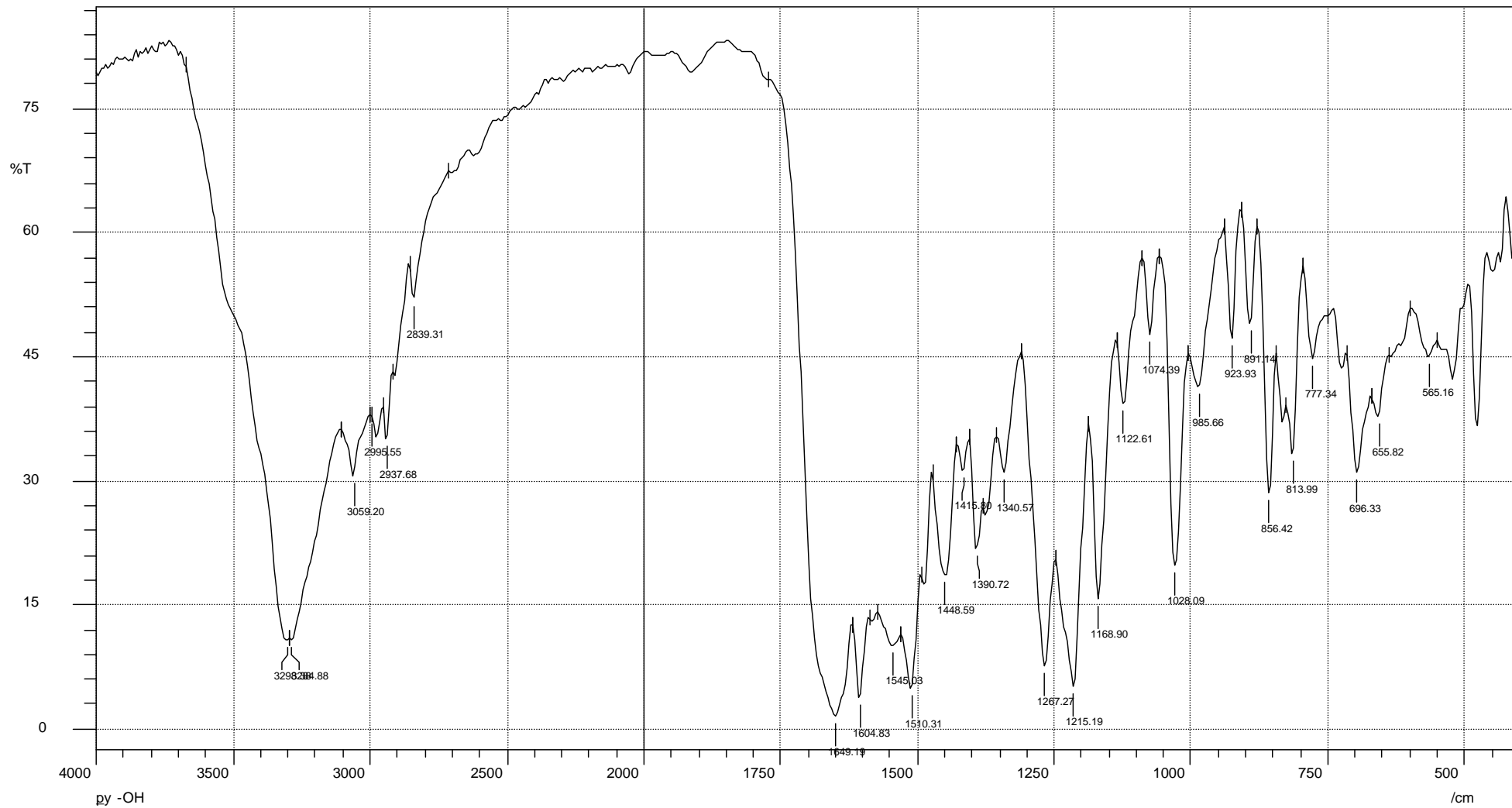
Figure (3-15): FT-IR Spectrum of Compound (V$_d$) Using KBr Disc
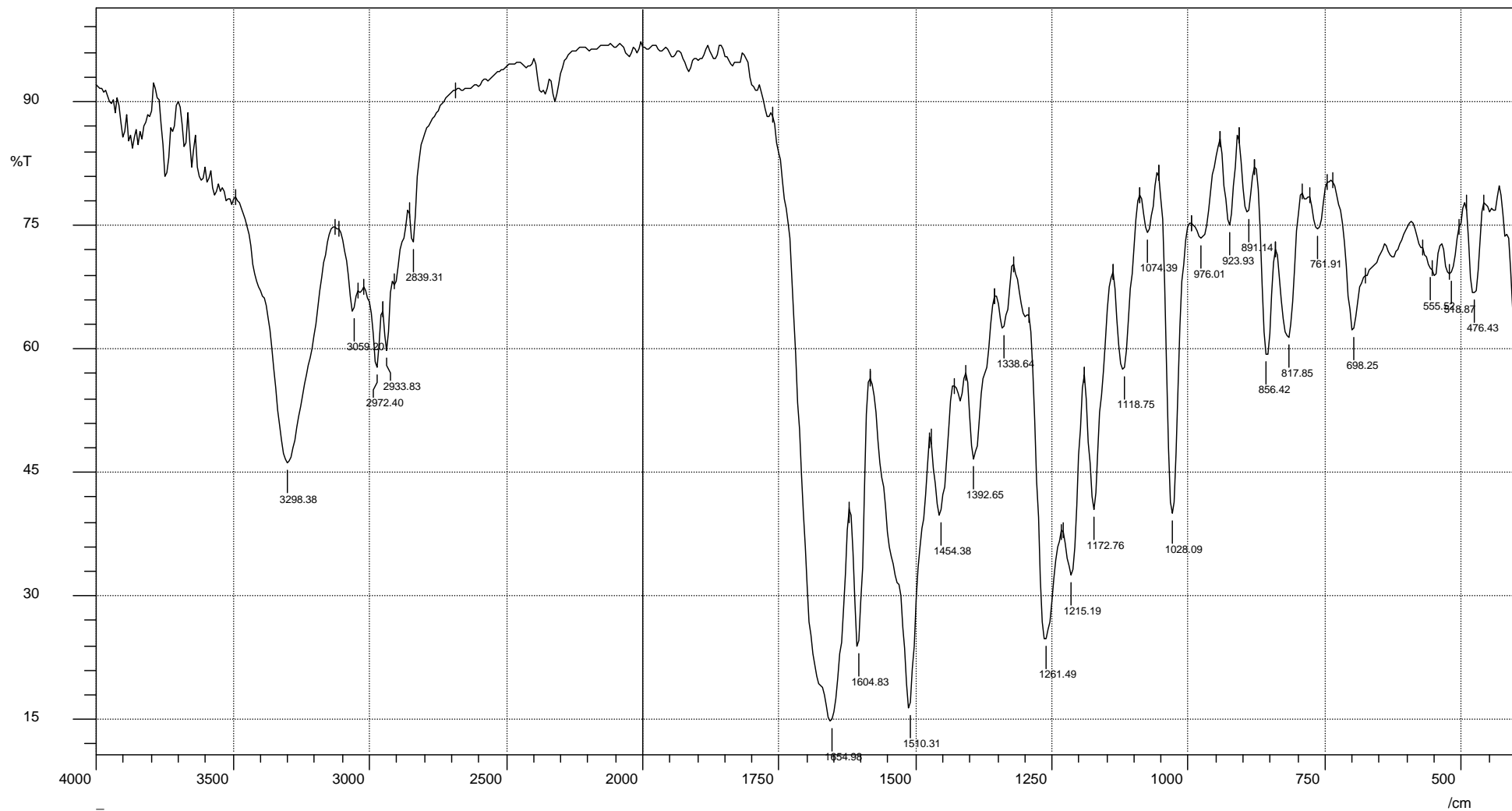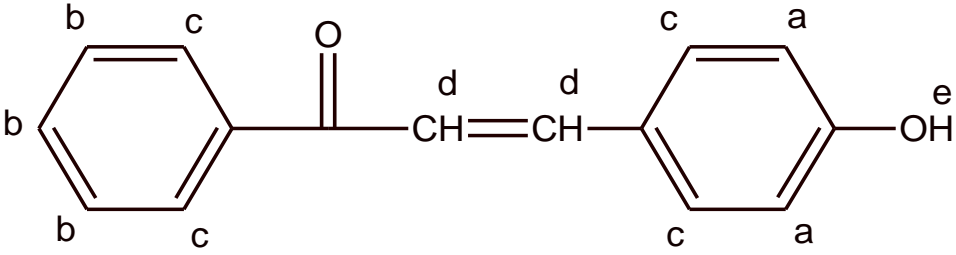
Figure (3-16): FT-IR Spectrum of Compound (V$_e$) Using KBr Disc
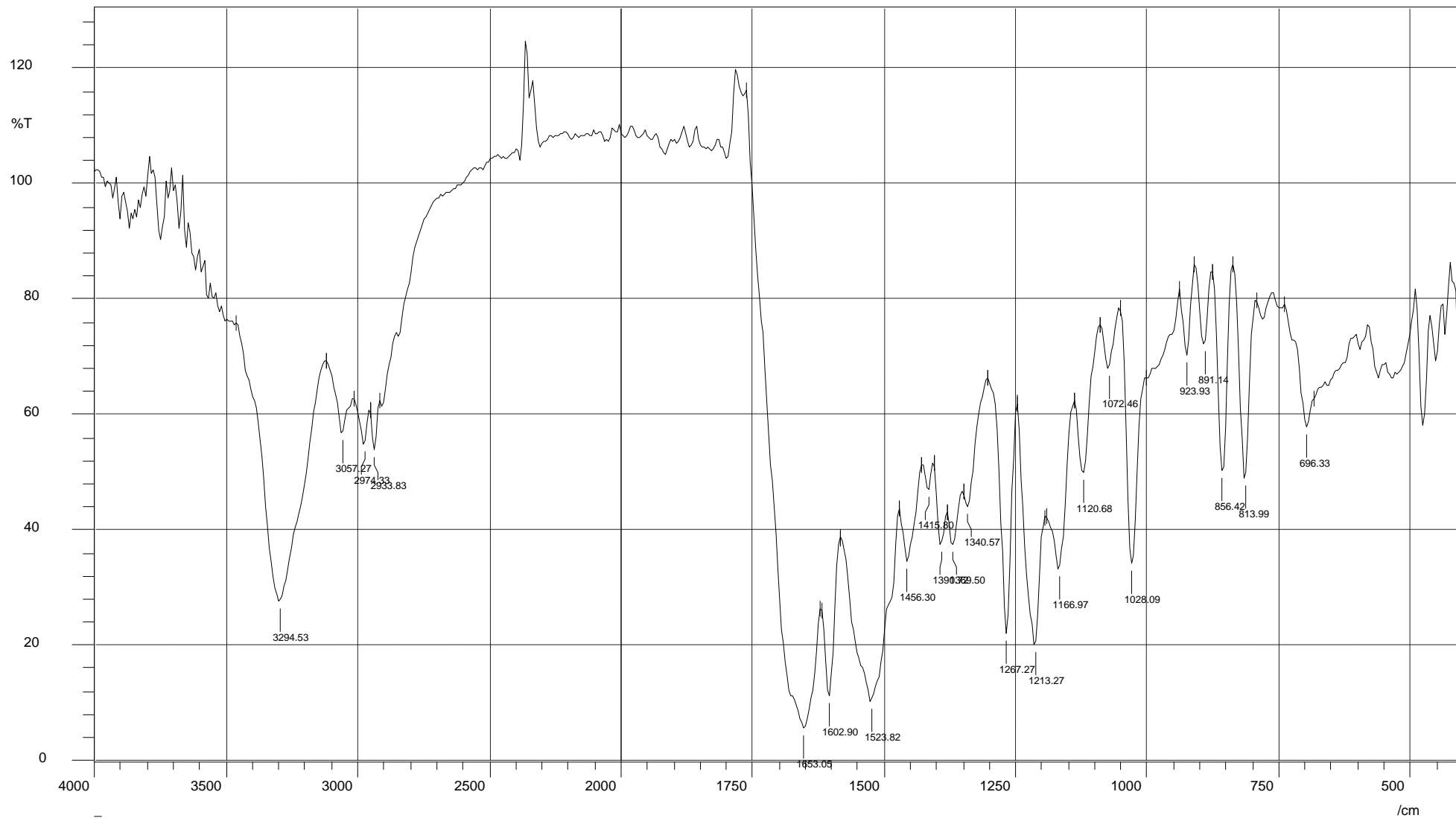
Figure (3-17): FT-IR Spectrum of Compound (V_f) Using KBr Disc

Table (3-3): ^{1}H-NMR Data and their Interpretation of Copmound (I_a)
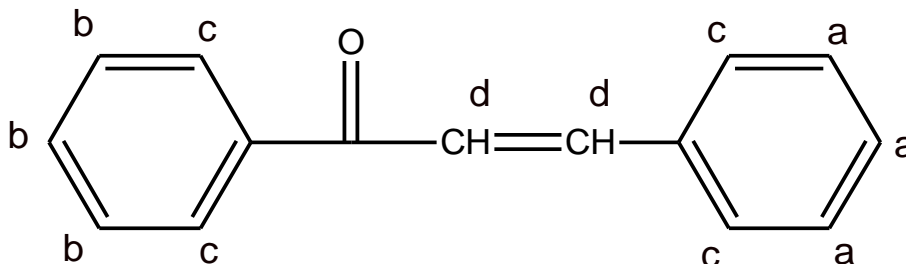
Group	Chemical shift ppm	No. of H	Interpretation
a	7.45-7.56	3	Multiplet, for aromatic protons
b	7.58-7.68	3	Multiplet, for aromatic protons
c	7.76-7.96	4	Multiplet, for aromatic protons
d	8.16-8.18	2	Multiplet, for CH protons of CH=CH

Table (3-4): ^{1}H-NMR Data and their Interpretation of Compound (I_d)

Group	Chemical shift ppm	No. of H	Interpretation
a	6.86-6.87	2	Multiplet, for aromatic protons
b	7.51-7.68	3	Multiplet, for aromatic protons
c	7.72-7.75	4	Multiplet, for aromatic protons
d	8.12-8.13	2	Multiplet, for CH protons of CH=CH
e	10.12	1	Broad singlet for OH proton
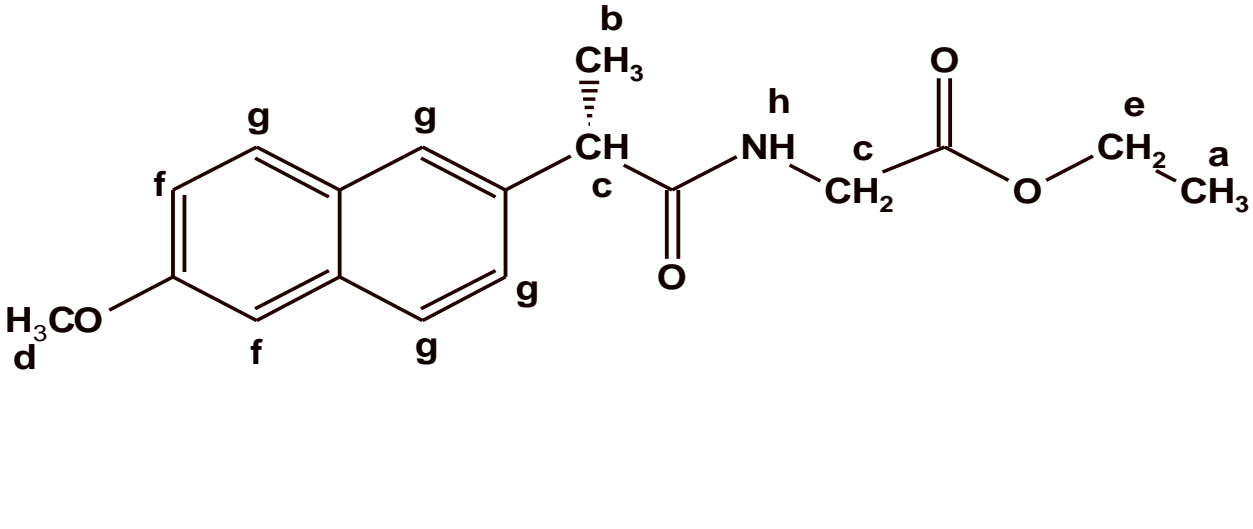
Table (3-5): ^{1}H-NMR Data and their Interpretation of Compound (I_e)

Group	Chemical shift ppm	No. of H	Interpretation
a	3.82	3	Singlet,for -OCH$_3$ protons
b	7.55-7.58	2	Multiplet, for aromatic protons
c	7.64-7.65	3	Multiplet, for aromatic protons
d	7.72-7.86	4	Multiplet, for aromatic protons
e	8.14-8.15	2	Multiplet, for CH protons of CH=CH
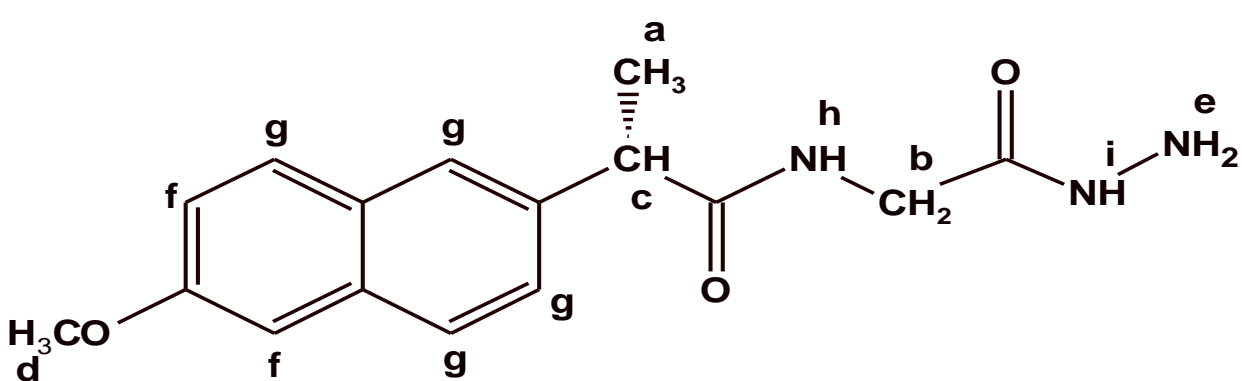
Table (3-6): ^{1}H-NMR Data and their Interpretation of Compound (I_f)

Group	Chemical shift ppm	No. of H	Interpretation
a	3.02	6	Singlet, for $N(CH_3)_2$ protons
b	6.75-6.76	2	Multiplet, for aromatic protons
c	7.55-7.65	3	Multiplet, for aromatic protons
d	7.68-7.72	4	Multiplet, for aromatic protons
e	8.10-8.11	2	Multiplet, for CH protons of CH=CH
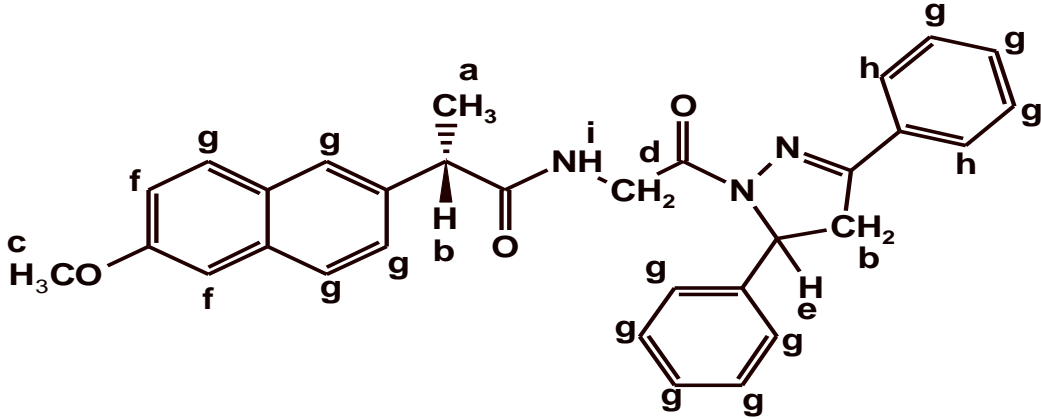
Table (3-7): ^{1}H-NMR Data and their Interpretation of Compound (III)

Group	Chemical shift ppm	No. of H	Interpretation
a	1.14	3	Triplet, for CH_3 protons of ester
b	1.24-1.26	3	Doublet, for CH_3 protonsof naproxen
c	3.80-3.84	3	Quartate, for CH proton of naproxen and CH_2protons of glycine
d	3.87	3	Singlet, for -OCH_3 protons of naproxen
e	4.04-4.08	2	Multiplet, for CH_2 protons of ester
f	7.14-7.16	2	Doublet, for CH protons ortho to methoxy
g	7.28-7.79	4	Multiplet, for naphthalene protons
h	8.40	1	Broad singlet for NH amide proton

Table (3-8): ^{1}H-NMR Data and their Interpretation of Compound (IV)
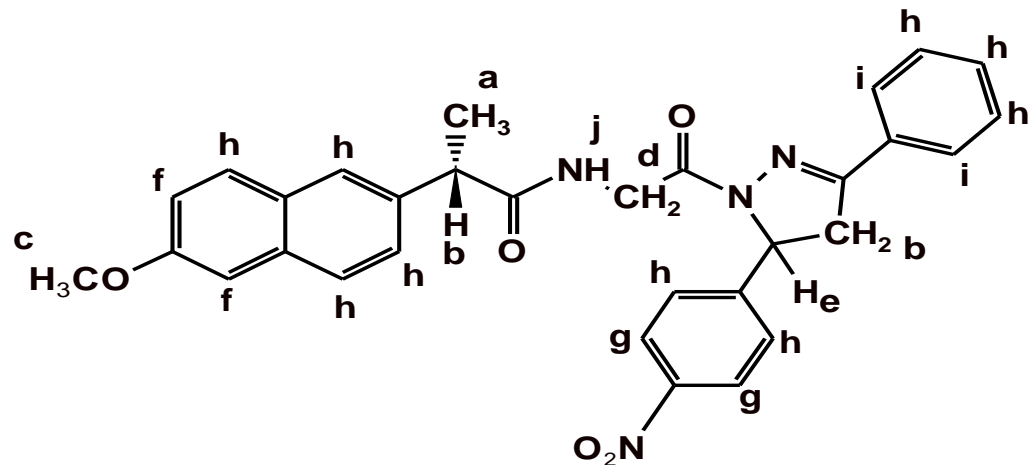
Group	Chemical shift ppm	No. of H	Interpretation
a	1.41-1.42	3	Doublet, for CH_3 protonsof naproxen
b	3.55-3.73	2	Doublet of Doublet, for CH_2 protons of glycine
c	3.81-3.87	1	Quartate, for CH proton of naproxen
d	3.90	3	Singlet, for $-OCH_3$ protons of naproxen
e	4.20	2	Broad singlet, for NH_2 protons of hydrazide
f	7.13-7.15	2	Doublet, for CH protons ortho to methoxy
g	7.27-7.83	4	Multiplet, for naphthalene protons
h	8.20	1	Broad singlet for NH amide proton
i	9.01	1	Singlet, for NH proton of hydrazide

Table (3-9): ^{1}H-NMR Data and their Interpretation of Compound (V_a)
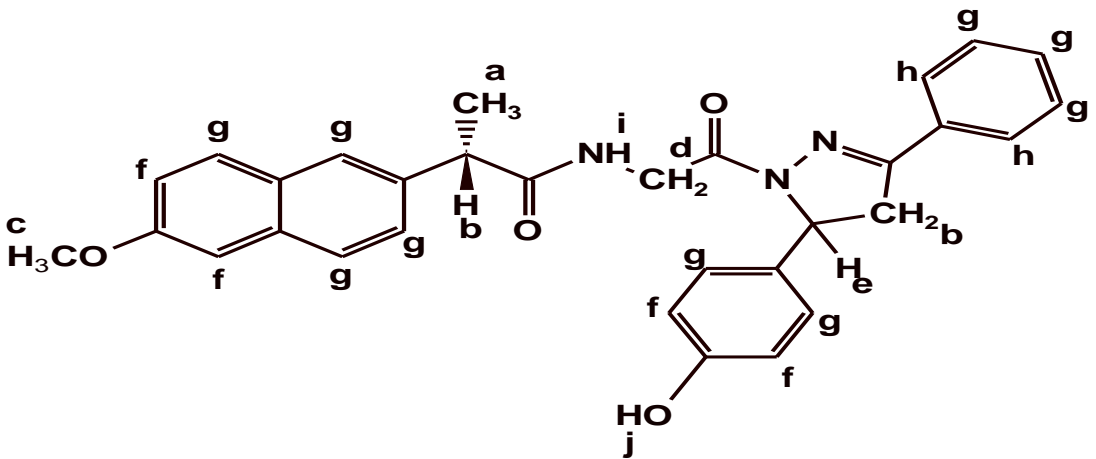
Group	Chemical shift ppm	No. of H	Interpretation
a	1.41-1.43	3	Doublet, for CH_3 protons of naproxen
b	3.49-3.73	3	Multiplet, due to overlap of CH_2 protons of pyrazoline and CH proton of naproxen
c	3.86	3	Singlet, for -OCH_3 protons of naproxen
d	4.23	2	Broad singlet, for CH_2 protons of glycine
e	5.28	1	Broad singlet, for CH proton of pyrazoline ring
f	7.13-7.15	2	Doublet, for CH protons ortho to methoxy
g	7.23-7.97	12	Multiplet, for naphthalene &aromatic protons
h	8.17-8.20	2	Multiplet, for aromatic protons
i	8.52	1	Broad singlet , for NH amide proton

Table (3-10): ^{1}H-NMR Data and their Interpretation of Compound (V_b)
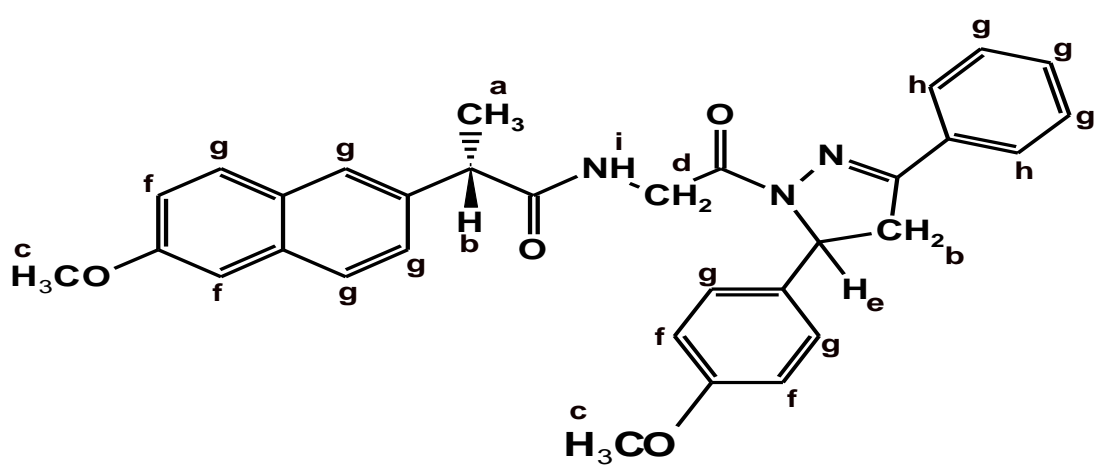
Group	Chemical shift ppm	No. of H	Interpretation
a	1.42-1.44	3	Doublet, for CH_3 protons of naproxen
b	3.52-3.71	3	Multiplet, due to overlap of CH_2 protons of pyrazoline and CH proton of naproxen
c	3.86	3	Singlet, for $-OCH_3$ protons of naproxen
d	4.00	2	Broad singlet, for CH_2 protons of glycine
e	5.38	1	Broad singlet, for CH proton of pyrazoline ring
f	7.13-7.15	2	Doublet, for CH protons ortho to methoxy
g	7.27-7.40	2	Doublet, for CH protons ortho to chloro
h	7.45-7.99	9	Multiplet, for naphthalene &aromatic protons
I	8.09-8.32	2	Multiplet, for aromatic protons
j	8.41	1	Broad singlet for NH amide proton

Table (3-11): ^{1}H-NMR Data and their Interpretation of Compound(V_c)
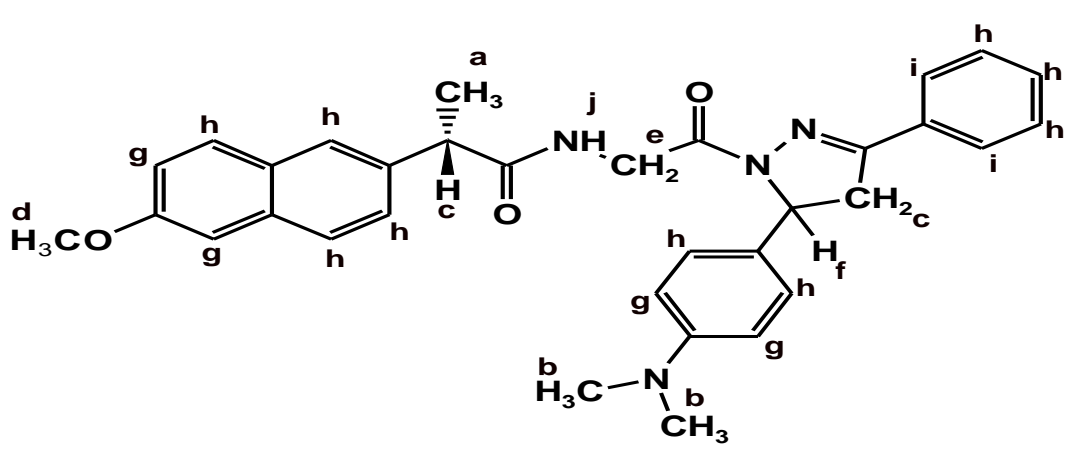
Group	Chemical shift ppm	No. of H	Interpretation
a	1.41-1.43	3	Doublet, for CH_3 protons of naproxen
b	3.48-3.71	3	Multiplet, due to overlap of CH_2 protons of pyrazoline and CH proton of naproxen
c	3.86	3	Singlet ,for -OCH_3 protons of naproxen
d	4.00	2	Broad singlet, for CH_2 protons of glycine
e	5.21	1	Broad singlet, for CH proton of pyrazoline ring
f	7.13-7.15	2	Doublet, for CH protons ortho to methoxy
g	7.27-7.37	2	Doublet, for CH protons ortho to nitro
h	7.47-8.08	9	Multiplet, for naphthalene &aromatic protons
i	8.13-8.30	2	Multiplet, for aromatic protons
J	8.41	1	Broad singlet for NH amide proton

Table (3-12): 1H-NMR Data and their Interpretation of Compound (V_d)

Group	Chemical shift ppm	No. of H	Interpretation
a	1.41-1.43	3	Doublet, for CH$_3$ protons of naproxen
b	3.57-3.74	3	Multiplet, due to overlap of CH$_2$ protons of pyrazoline and CH proton of naproxen
c	3.86	3	Singlet,for -OCH$_3$ protons of naproxen
d	4.27	2	Broad singlet, for CH$_2$ protons of glycine
e	5.20	1	Broad singlet, for CH proton of pyrazoline ring
f	6.66-6.87	4	Doublet, for CH protons ortho to methoxy and hydroxy
g	7.03-7.78	9	Multiplet, for naphthalene &aromatic protons
h	8.12-8.28	2	Multiplet, for aromatic protons
i	8.44	1	Broad singlet for NH amide proton
j	10.22	1	Broad singlet for OH proton

Table (3-13): 1H-NMR Data and their Interpretation of Compound (V_e)

Group	Chemical shift ppm	No. of H	Interpretation
a	1.42-1.44	3	Doublet, for CH_3 protons of naproxen
b	3.58-3.83	3	Multiplet, due to overlap of CH_2 protons of pyrazoline and CH proton of naproxen
c	3.86	6	Singlet, for -OCH_3 protons of naproxen
d	4.00	2	Broad singlet, for CH_2 protons of glycine
e	5.25	1	Broad singlet, for CH proton of pyrazoline ring
f	7.14-7.27	4	Doublet, for CH protons ortho to methoxy
g	7.33-7.90	9	Multiplet, for naphthalene &aromatic protons
h	8.11-8.29	2	Multiplet, for aromatic protons
i	8.41	1	Broad singlet for NH amide proton

Table (3-14): 1H-NMR Data and their Interpretation of Compound (V_f)

Group	Chemical shift ppm	No. of H	Interpretation
a	1.43-1.44	3	Doublet, for CH_3 protons of naproxen
b	2.99	6	Singlet, for $N(CH_3)_2$ protons
c	3.60-3.76	3	Multiplet, due to overlap of CH_2 protons of pyrazoline and CH proton of naproxen
d	3.86	3	Singlet, for -OCH_3 protons of naproxen
e	4.29	2	Broad singlet, for CH_2 protons of glycine
f	5.27	1	Broad singlet,for CH proton of pyrazoline ring
g	7.14-7.27	4	Doublet, for CH protons ortho to methoxy and N-dimethyl
h	7.47-7.86	9	Multiplet, for naphthalene &aromatic protons
i	8.13-8.30	2	Multiplet, for aromatic protons
j	8.47	1	Broad singlet for NH amide proton

1c
H1 NMR

Figure (3-18): ^{1}H-NMR Spectrum of Compound (I_a)

Figure (3-19): ¹H-NMR Spectrum of Compound (I_d)

Figure (3-20): ¹H-NMR Spectrum of Compound (Iₑ)

5c
H1 NMR

Figure (3-21): ^{1}*H-NMR Spectrum of Compound (I$_f$)*

A
H1 NMR

The University of Jordan
Faculty of Science
Department of Chemistry

Instrument Model:
Bruker 500 MHz–Avance III

Operator: Fatima Mustafa
nmr500@ju.edu.jo

Current Data Parameters
NAME   14oct15jalal
EXPNO   251
PROCNO   1

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PULPROG   zg
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FIDRES   0.154650 Hz
AQ   3.2331092 sec
RG   49.66
DW   49.333 usec
DE   6.50 usec
TE   298.2 K
D1   2.00000000 sec
TD0   1

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NUC1   1H
P1   10.60 usec
PLW1   17.39999962 W

F2 – Processing parameters
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SF   500.1300000 MHz
WDW   EM
SSB   0
LB   1.00 Hz
GB   0
PC   2.00

Figure (3-22) A: ¹H-NMR Spectrum of Compound (III)

A
H1 NMR

The University of Jordan
Faculty of Science
Department of Chemistry

Instrument Model:
Bruker 500 MHz−Avance III

Operator: Fatima Mustafa
nmr500@ju.edu.jo

Current Data Parameters
NAME   14oct15jalal
EXPNO   251
PROCNO   1

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DS   0
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FIDRES   0.154650 Hz
AQ   3.2331092 sec
RG   49.66
DW   49.333 usec
DE   6.50 usec
TE   298.2 K
D1   2.00000000 sec
TD0   1

======== CHANNEL f1 ========
SFO1   500.1344108 MHz
NUC1   1H
P1   10.60 usec
PLW1   17.39999962 W

F2 − Processing parameters
SI   131072
SF   500.1300000 MHz
WDW   EM
SSB   0
LB   1.00 Hz
GB   0

Figure (3-22) B: ^{1}H-NMR Spectrum of Compound (III)

103

h
H1 NMR

9.0106 8.4343 8.3247 8.2027 8.1914 8.1800 7.8333 7.7881 7.7702 7.7514 7.7342 7.4891 7.4707 7.4536 7.4508 7.2775 7.2730 7.1552 7.1503 7.1374 7.1325
4.3678 4.1980 4.0642 4.0393 4.0287 4.0078 3.9818 3.9714 3.9355 3.9070 3.8660 3.8423 3.8282 3.8143 3.7259 3.7140 3.6938 3.6819 3.5958 3.5847 3.5636 3.5526 3.3374 2.6457 2.5109 2.3720
1.4219 1.4079 1.2780

The University of Jordan
Faculty of Science
Department of Chemistry

Instrument Model:
Bruker 500 MHz–Avance III

Operator: Fatima Mustafa
nmr500@ju.edu.jo

Current Data Parameters
NAME   14oct15jalal
EXPNO   241
PROCNO   1

F2 – Acquisition Parameters
Date_   20141023
Time   9.18
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PROBHD   5 mm PABBO BB/
PULPROG   zg
TD   65536
SOLVENT   DMSO
NS   4
DS   0
SWH   10135.135 Hz
FIDRES   0.154650 Hz
AQ   3.2331092 sec
RG   49.66
DW   49.333 usec
DE   6.50 usec
TE   298.2 K
D1   2.00000000 sec
TD0   1

-------- CHANNEL f1 ========
SFO1   500.1344108 MHz
NUC1   1H
P1   10.60 usec
PLW1   17.39999962 W

F2 – Processing parameters
SI   131072
SF   500.1300000 MHz
WDW   EM
SSB   0
LB   1.00 Hz
GB   0
PC   2.00

1.00 1.01 1.06 0.58 1.45 1.07 1.08 1.02   2.06 3.11 1.13 2.08   3.23
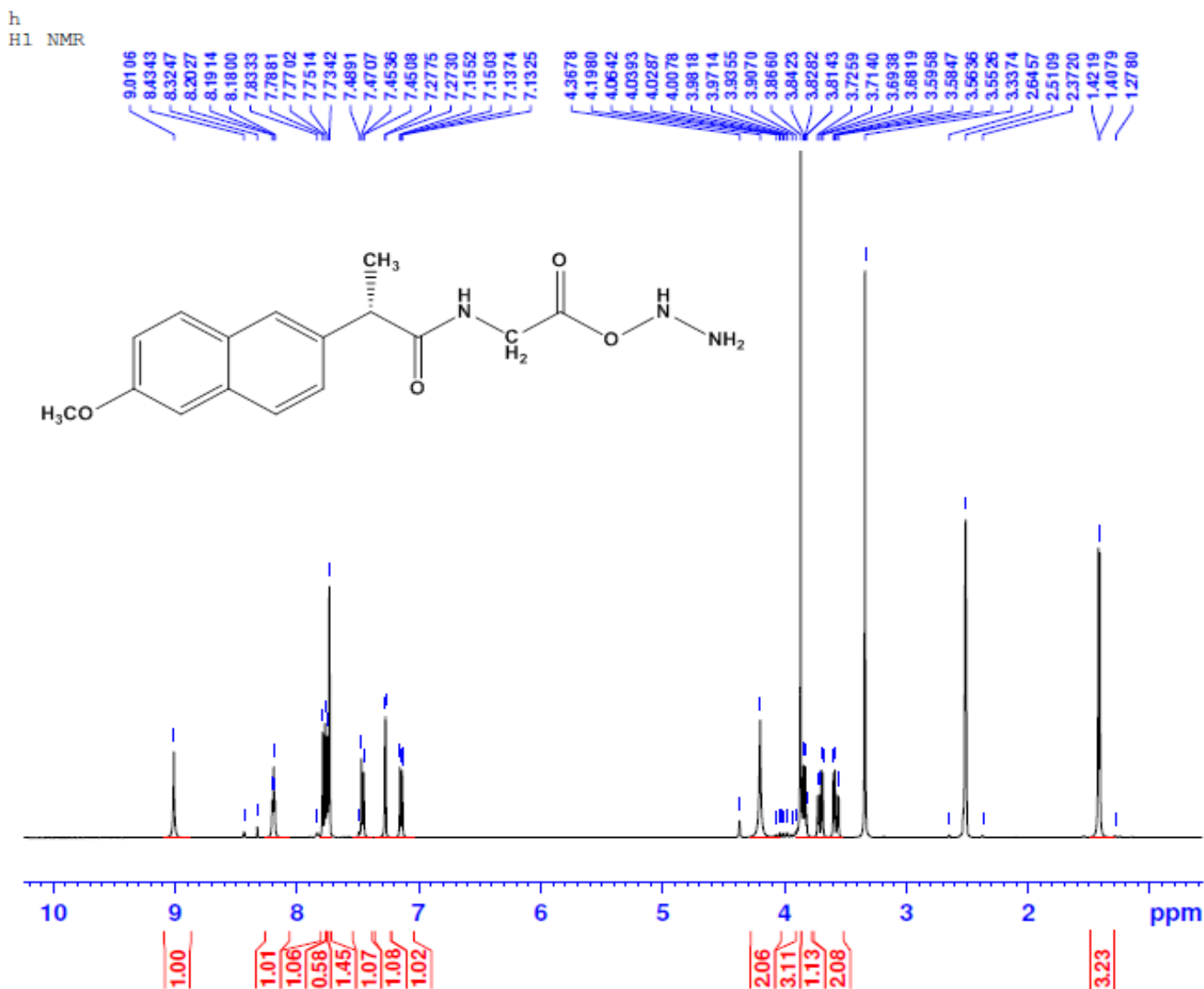
Figure (3-23) A: ¹H-NMR Spectrum of Compound (IV)

104

h
H1 NMR
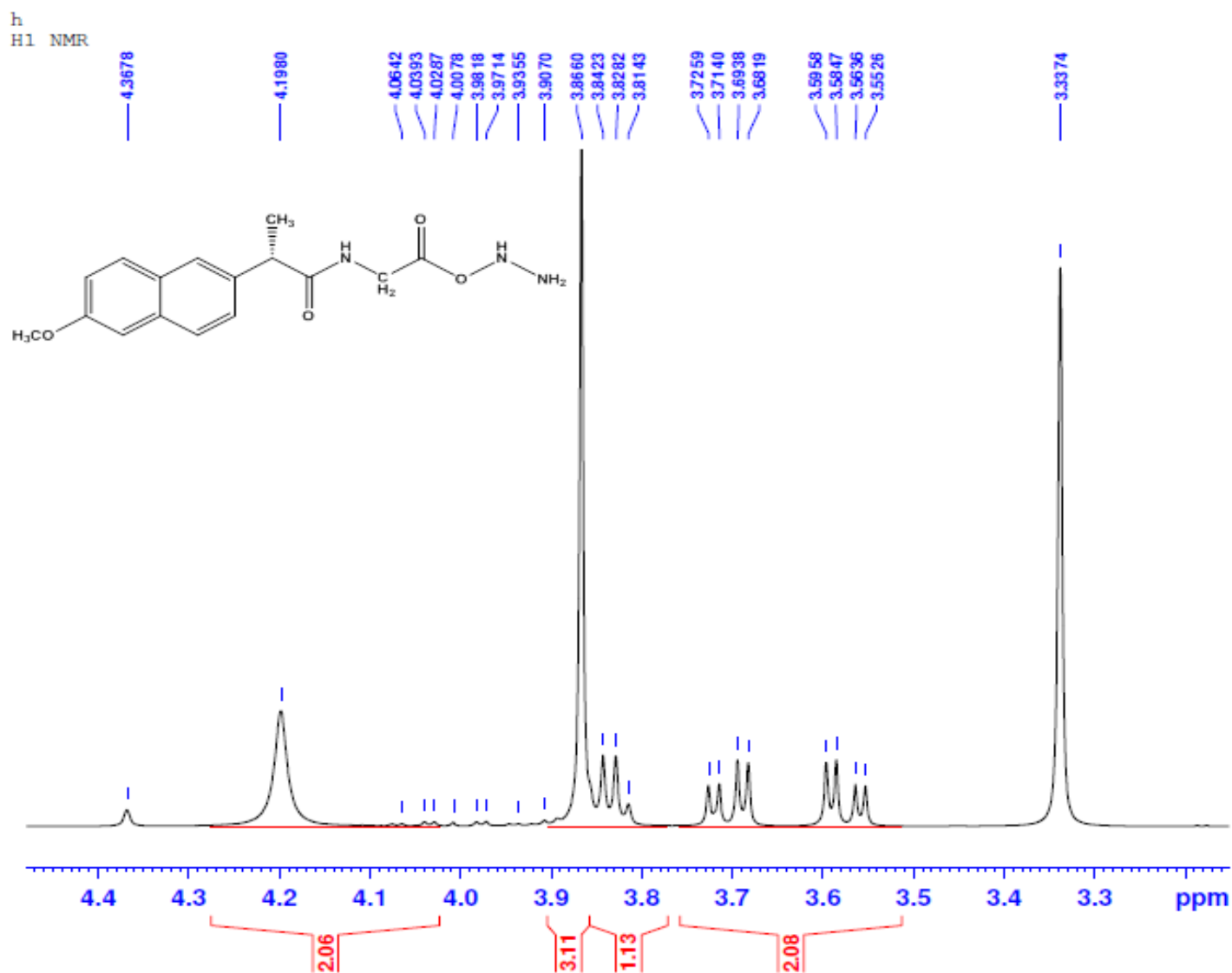
Figure (3-23) B: ^{1}H-NMR Spectrum of Compound (IV)

1p
H1 NMR

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Instrument Model:
Bruker 500 MHz–Avance III

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Current Data Parameters
NAME   15jan15jalal
EXPNO   541
PROCNO   1

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Time   9.40
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PULPROG   zg
TD   65536
SOLVENT   DMSO
NS   4
DS   0
SWH   10135.135 Hz
FIDRES   0.154650 Hz
AQ   3.2331092 sec
RG   20.14
DW   49.333 usec
DE   6.50 usec
TE   298.2 K
D1   2.00000000 sec
TDO   1

-------- CHANNEL f1 --------
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NUC1   1H
P1   10.60 usec
PLW1   17.39999962 W

F2 - Processing parameters
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PC   3.00
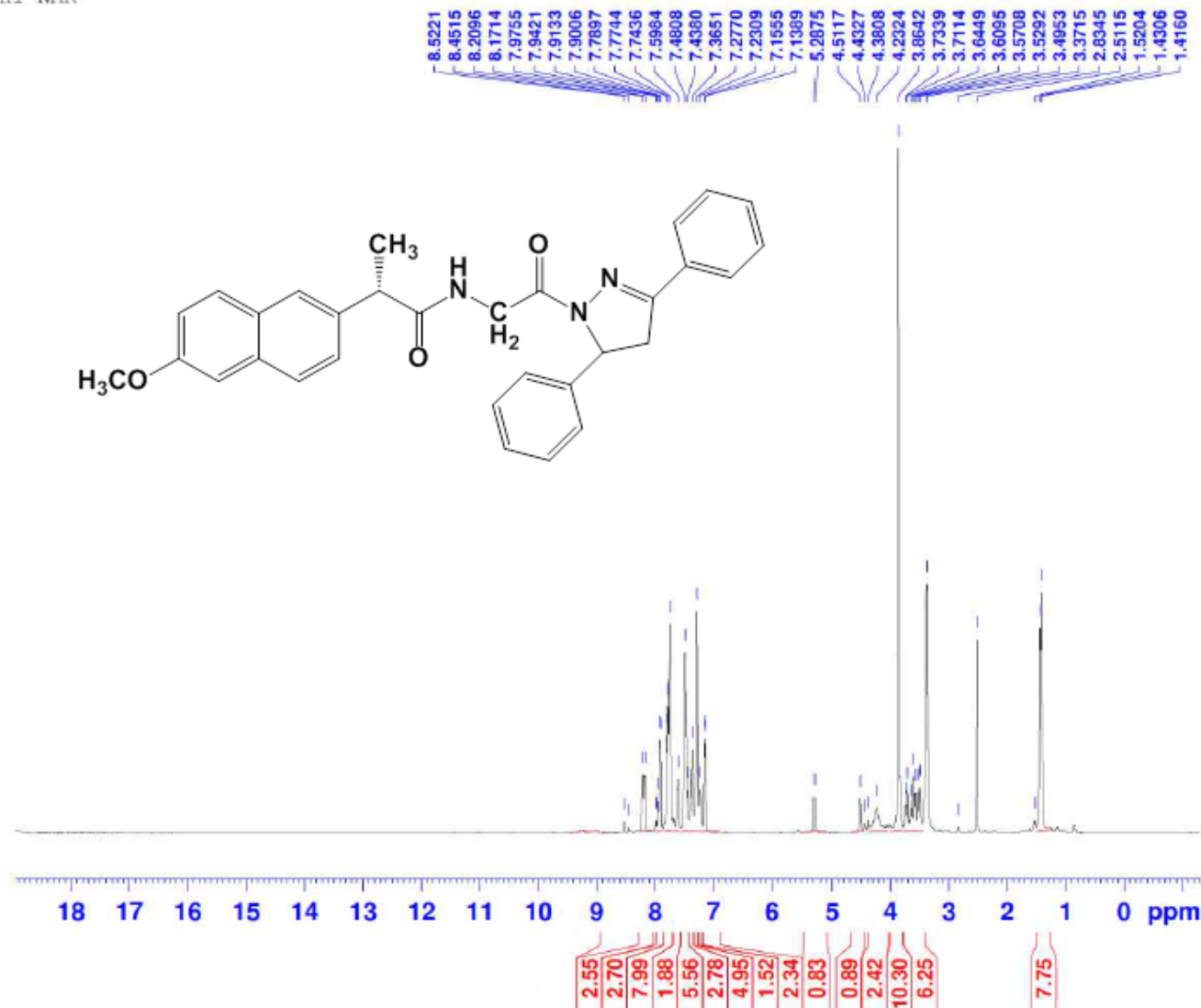
Figure (3-24): ¹H-NMR Spectrum of Compound (Vₐ)

Figure (3-25): ¹H-NMR Spectrum of Compound (Vᵦ)

Figure (3-26): ^{1}H-NMR Spectrum of Compound (V_c)

6P
H1 NMR
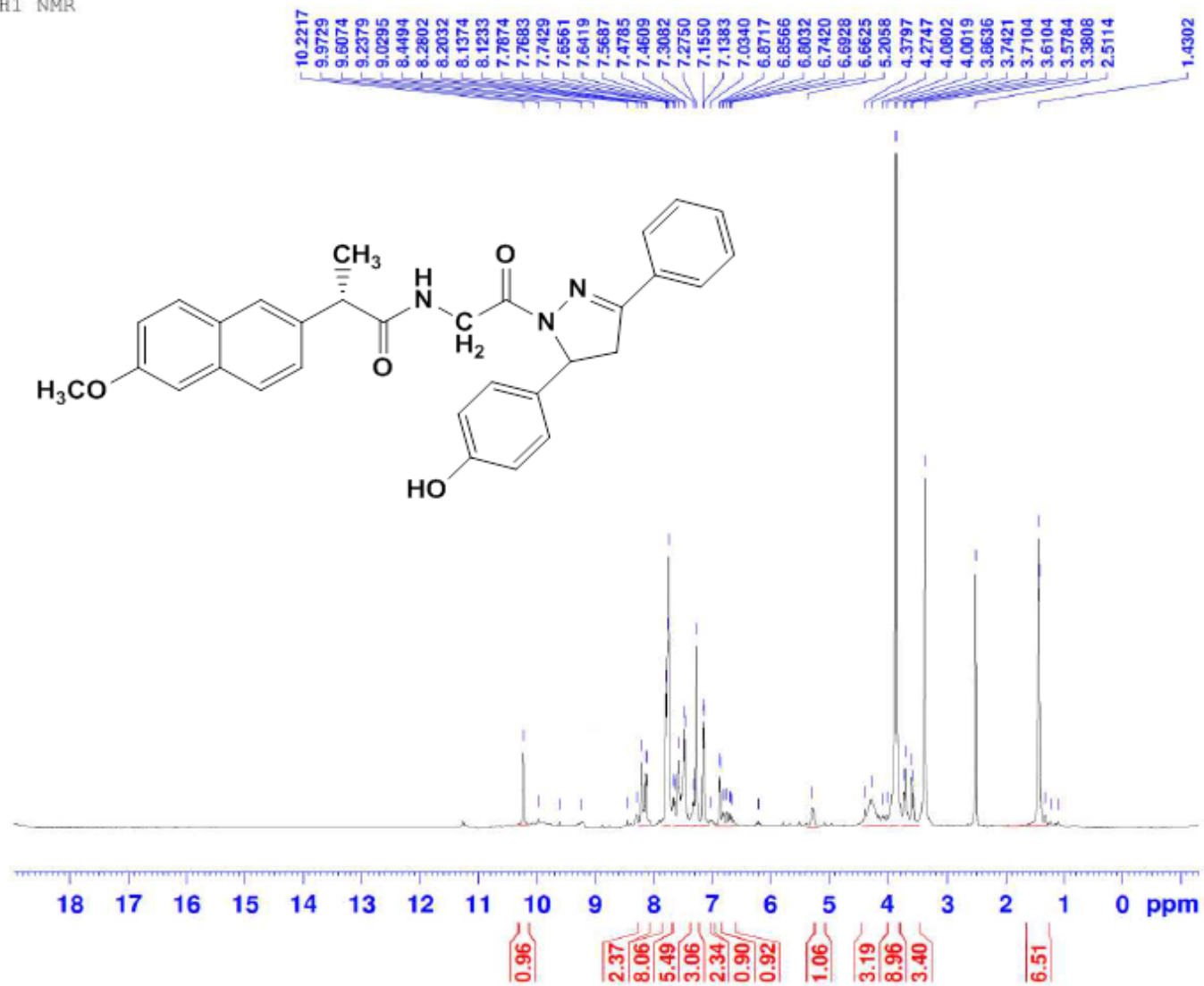
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Instrument Model:
Bruker 500 MHz–Avance III

Operator: Fatima Mustafa
nmr500@ju.edu.jo

Current Data Parameters
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EXPNO   461
PROCNO   1

F2 - Acquisition Parameters
Date_   20150121
Time   9.01
INSTRUM   spect
PROBHD   5 mm PABBO BB/
PULPROG   zg
TD   65536
SOLVENT   DMSO
NS   4
DS   0
SWH   10135.135 Hz
FIDRES   0.154650 Hz
AQ   3.2331092 sec
RG   25.04
DW   49.333 usec
DE   6.50 usec
TE   298.2 K
D1   2.00000000 sec
TD0   1

======== CHANNEL f1 ========
SFO1   500.1344108 MHz
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P1   10.60 usec
PLW1   17.39999962 W

F2 - Processing parameters
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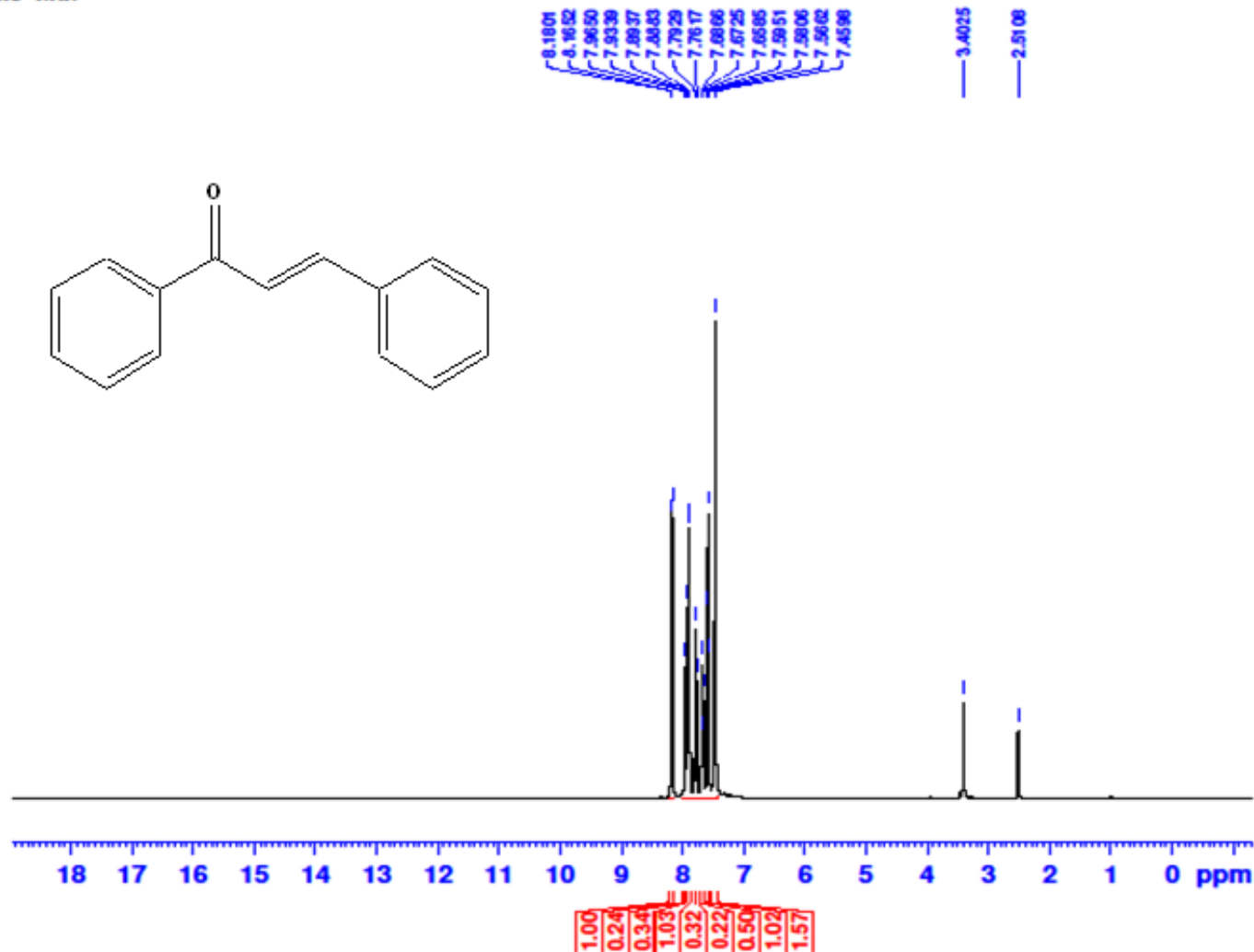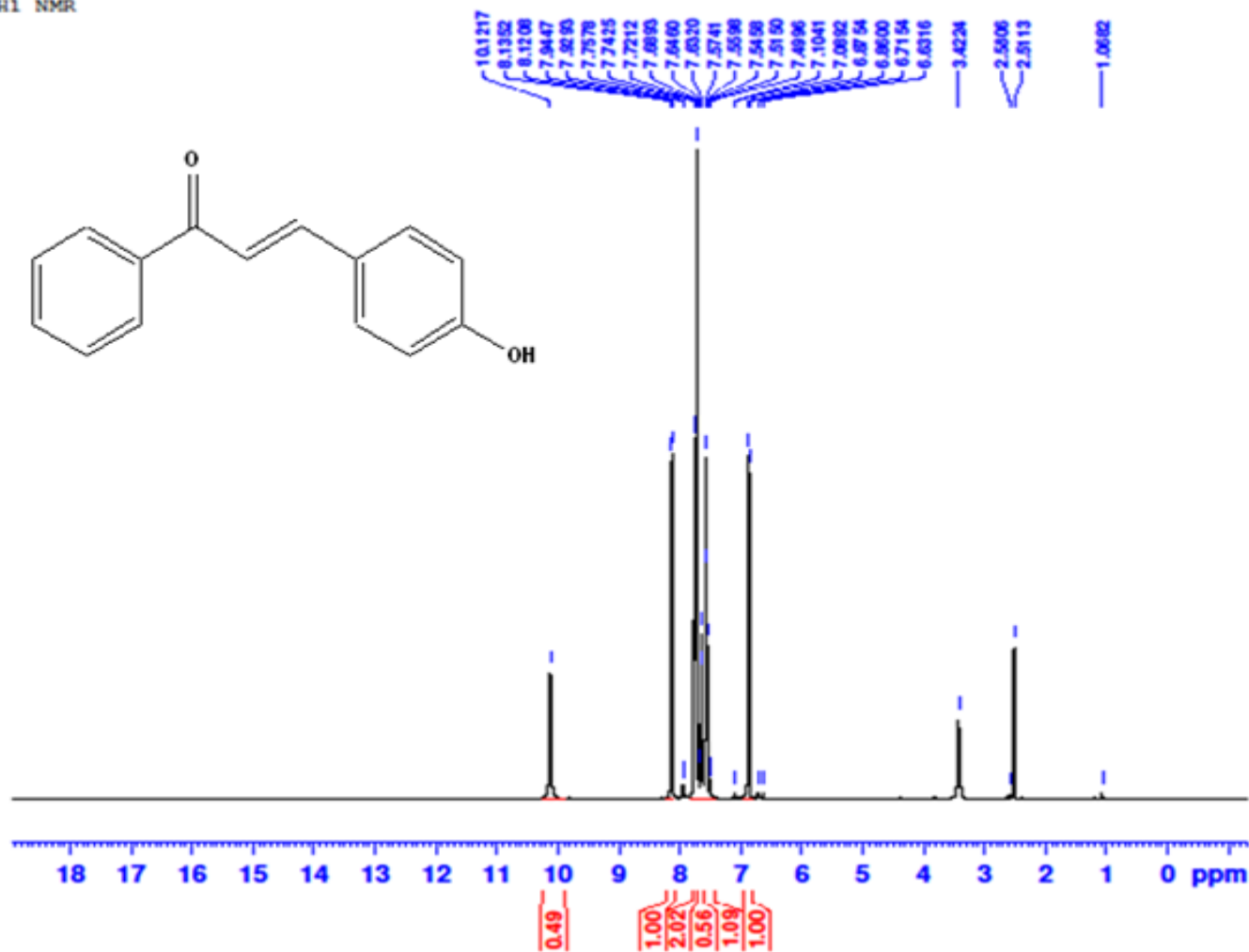WDW   EM
SSB   0
LB   1.00 Hz
GB   0
PC   1.50

Figure (3-27): 1H-NMR Spectrum of Compound (V$_d$)

Figure (3-28): 1H-NMR Spectrum of Compound (Vₑ)

5P
H1 NMR

Figure (3-29): 1H-NMR Spectrum of Compound (V_f)

111

3.3. Pharmacological Study:

3.3.1. Anti-Inflammatory Evaluation Study:

This section concerned with the results of preliminary pharmacological evaluation of tested compounds as anti-inflammatory agents using paw-edema method following intra-plantar injection of egg-white into rat hind paw.

3.3.1.1. Dose Determination of the Tested Compounds:

The determination of the dose of the newly synthesized compounds (V_{a-f}) were depending on the dose of the (2S)-Naproxen (reference compound) in which the tested compounds are derived from it.

Then according to molecular weight of the tested compound the dose was calculated using the following equation:

$$\frac{Dose\ of\ reference\ compound}{Molecular\ weight\ of\ reference\ compound} = \frac{Dose\ of\ tested\ compound}{Molecular\ weight\ of\ tested\ compound}$$

3.3.1.2. *In Vivo* Method for Evaluation of Anti-Inflammatory Activity:

The most widely used primary test to screen new anti-inflammatory agent's measures the ability of the compound to reduce local edema induced in the rat paw by injection of an irritant agent [136]. Many irritant agents have been used in the paw-edema method like dextran, egg-white and carrageenan solution. The paw edema induced by carrageenan has been expansively studied in the assessment of the anti-inflammatory action of steroidal and non-steroidal drugs involving several chemical mediators such as histamine, serotonin, bradykinin and prostaglandins [137].

Subcutaneous injection of carrageenan into the rat paw produces inflammation resulting from plasma extravasations, increased tissue water and plasma protein exudation along with neutrophil extravasations, all due to the metabolism of AA [138].

Carrageenan induced edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1-2 hr.) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotriene's, polymorph nuclear cells and prostaglandins produced by tissue macrophages [136].

3.3.1.3. Evaluation of the Anti-Inflammatory Activity of the Tested Compounds:

The anti-inflammatory activity of the tested compounds has been evaluated in comparison with their vehicle (control group) and (2S)-Naproxen. Table (3-15) explains the effect of tested compounds (V_{a-f}) in comparison to control and (2S)-Naproxen.

Figure (3-30) show the effect of all tested compounds with statistically significant ($P<0.05$) reduction in paw edema thickness.

Table 3-15: The Anti-Inflammatory Effect of Control, (2S)-Naproxen and Compounds (V_{a-f}) on Egg-White Induced Paw Edema in Rats

	Compounds	Time (min)						
		0	**30**	**60**	**120**	**180**	**240**	**300**
Paw Thickness (mm) / n=6	**Control**	4.85±0.05	5.83±0.06	6.58±0.06	6.96±0.03	6.81±0.06	6.71±0.02	5.39±0.01
	Naproxen	4.82±0.04	5.72±0.05	6.51±0.05	5.81±0.05^{*a}	5.44±0.06^{*a}	5.14±0.06^{*a}	4.93±0.02*
	V_a	4.87±0.06	5.74±0.02	6.56±0.06	6.31±0.01^{*b}	5.72±0.03^{*b}	5.43±0.02^{*b}	5.06±0.04*
	V_b	4.81±0.03	5.75±0.06	6.51±0.01	6.21±0.04^{*b}	5.76±0.06^{*b}	5.51±0.02^{*b}	4.99±0.05*
	V_c	4.81±0.02	5.75±0.01	6.50±0.03	6.17±0.06^{*b}	5.73±0.05^{*b}	5.47±0.06^{*b}	5.03±0.06*
	V_d	4.78±0.01	5.79±0.06	6.49±0.04	5.84±0.03^{*a}	5.46±0.02^{*a}	5.16±0.05^{*a}	5.06±0.06*
	V_e	4.82±0.06	5.74±0.02	6.49±0.03	5.91±0.02^{*a}	5.41±0.04^{*a}	5.15±0.01^{*a}	4.99±0.06*
	V_f	4.84±0.01	5.82±0.02	5.77±0.04*	5.43±0.05^{*c}	5.08±0.06^{*c}	4.86±0.03^{*c}	4.94±0.08*

Non-identical superscripts (a, b&c) among different tested compounds are considered significantly different ($P<0.05$); *significantly different compared to control ($P<0.05$).Data are expressed in mm paw thickness as mean ± SEM. n= number of animals. Time (0) is the time of i.p. injection of naproxen, tested compounds and propylene glycol. Time (30) is the time of injection of egg white (induction of paw edema).
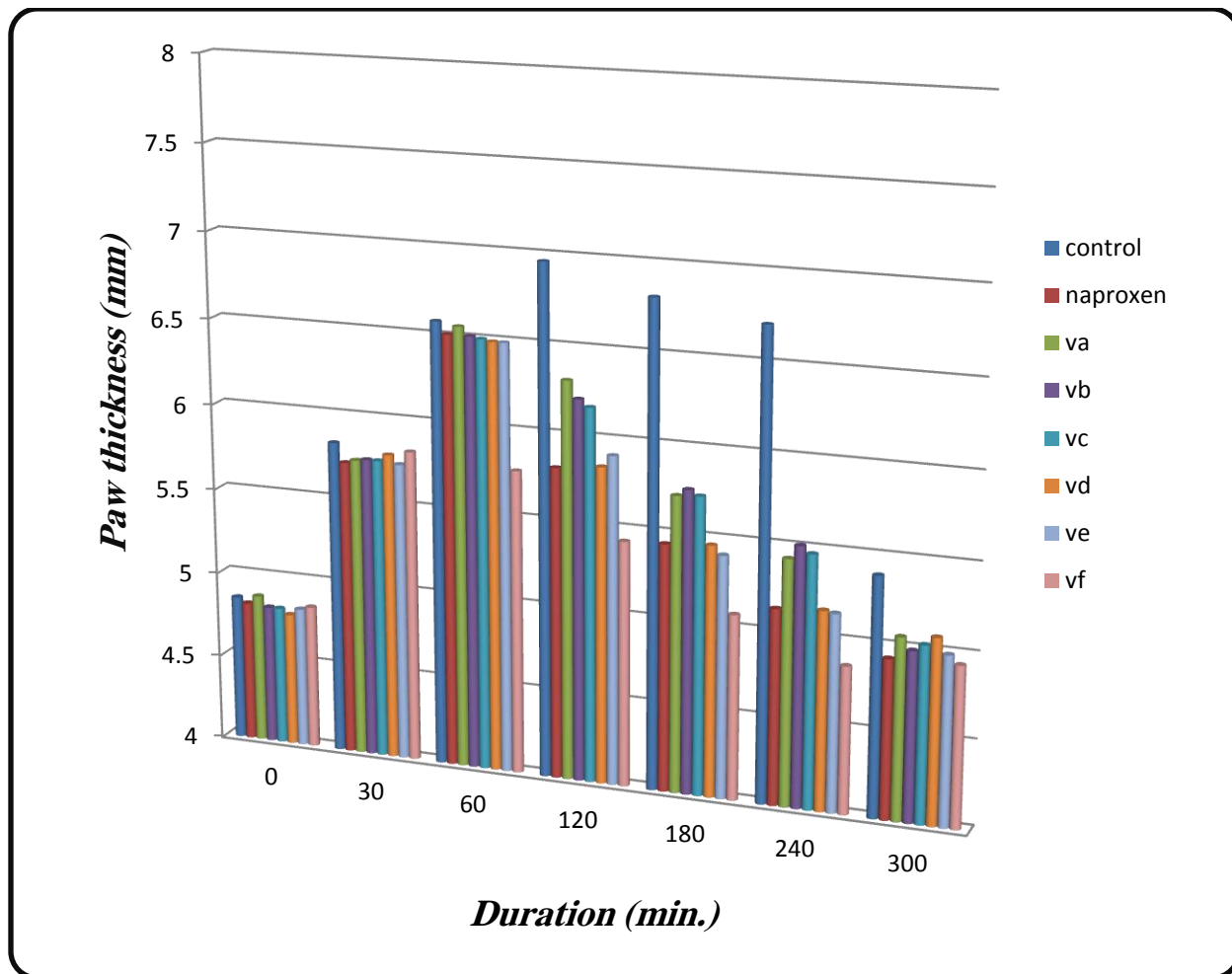
Figure (3-30): Effect of (2S)-Naproxen, Propylene glycol, Compounds (V_{a-f}) on Egg-White Induced Paw Edema in Rats.

3.3.1.4. Comparative Study:

❖ All tested compounds exerted significant reduction of paw edema in comparison to the effect of propylene glycol 50%v/v (control group).

❖ The effect of (2S)-Naproxen and all tested compounds started at time (120 min.) except (V_f) started at (60 min.) which indicate fast onset of action.

❖ The effect of tested compounds and (2S)-Naproxen continued until the end of experiment.

❖ Compound (V_d) and (V_e) show comparable effect to (2S)-Naproxen at all experimental time while compounds (V_a), (V_b) and (V_c) produced significantly lower inhibitory effect than (2S)-Naproxen at time (120-240 min.).

❖ Surprisingly compound (V_f) exert significantly higher paw edema reduction than (2S)-Naproxen at (60-240min.) since compound (V_f) have dimethyl amino group which is electron donating group that activate the ring.

❖ At time (300 min.) all tested compounds show comparable effect to that of (2S)-Naproxen.

3.3.1.5. Percent of Inhibition in Paw Edema Thickness:

The percent of inhibition of paw edema thickness at each time interval was calculated from the mean effect in control and treated animals according to the equation:

%inhibition= [Vc –Vt /Vc] ×100

Where Vc and Vt are the mean paw thickness of the control group and tested group (at time t-time zero) respectively [139-141].

The comparison among the (2S)-Naproxen, compounds (V_{a-f}) were shown in Table (3-16) and Figures (3-31).

Table (3-16): Percent of Inhibition of (2S)-Naproxen, Compounds (V_{a-f}) on Egg-White Induced Paw Edema in Rats.

		Treatment groups						
	Time (min.)	**Naproxen**	V_a	V_b	V_c	V_d	V_e	V_f
Percent of Inhibition	**60**	2.3%	2.3%	1.7%	3.2%	1.1%	3.4%	46.2%
	120	53.0 %	31.7%	33.6%	35.5%	49.7%	48.3%	72.0%
	180	68.3%	56.6%	51.5%	53.0%	65.3%	69.8%	87.7%
	240	83%	70%	62%	65%	80%	82%	99%
	300	80%	65%	67%	59%	48%	69%	81%
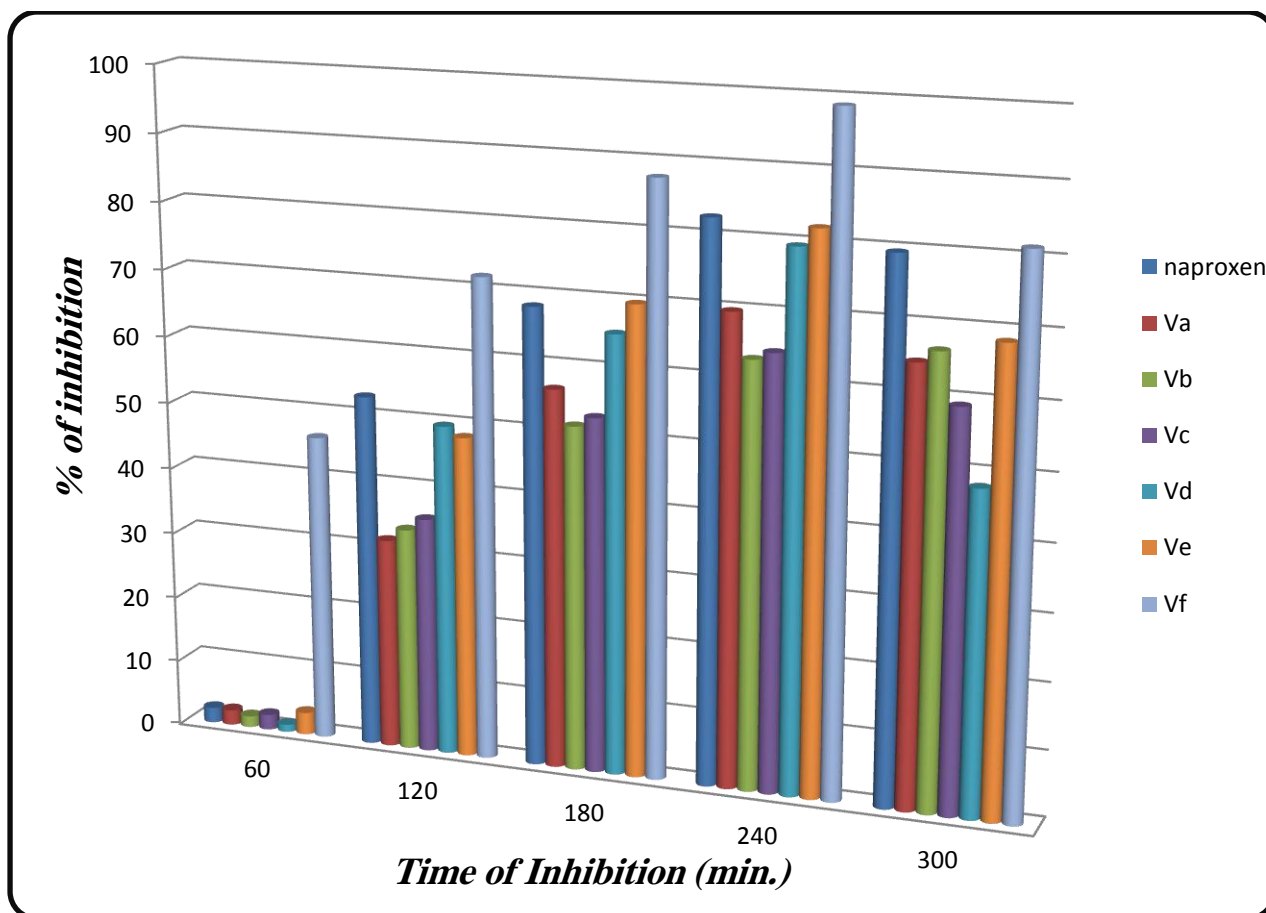
Figure (3-31): Percent of Inhibition Produced by (2S)-Naproxen and Compound (V$_{a-f}$) on Paw Edema.

3.3.2. The Antibacterial Activity:

(2S)-Naproxen and Ciprofloxacin as reference, DMSO as control and the synthesized compounds (V$_{a-f}$) were screened for their antibacterial activity studies against *Pseudomonas Aeroginosa*, *Escherichia coli*, *Bacillus* and *Staphylococcus aureus* at concentrations of (62.5,125, 250 and 500 µg/mL) except the control which used pure.

Table (3-17) illustrates the inhibition zone in (mm.) for each concentration of all tested compounds.

In general all tested compounds showed an interesting activity against gram positive and gram negative bacteria especially *Bacillus, Staphylococcus aureus, Pseudomonas Aeroginosa* and *Escherichia coli.* unlike the parent compound (2S)-Naproxen.

These tested compounds exert significant antibacterial activity in comparison to DMSO as control group. And these obtained results are compatible with many studies showed some NSAIDs have good antibacterial action especially ibuprofen [142], aspirin [143] and diclofenac sodium [144].

In comparison to standard compound (Ciprofloxacin), tested compound exert lower effect against *Escherichia coli* and nearly comparable effect against *Pseudomonas Aeroginosa*. Also the tested compounds showed a comparable antibacterial effect against *Bacillus* and lower activity against *Staphylococcus aureus*.

In comparison the antibacterial results among the tested compounds (V$_e$) may regard the best one and (V$_c$) the lower one which may lead to the conclusion that electron withdrawing substitutes have generally lower antibacterial effect than electron donating substitutes.

Table (3-17): Antibacterial Activity of Synthesized Compounds (V$_{a-f}$):

Sample Code and Standard	Concentration (µg/ml)	Zone of Inhibition (mm)			
		Gram Negative		Gram Positive	
		E. coli	*P.aeruginosa*	*Bacillus*	*Staph. aureus*
V$_a$	500.0	20	19	29	18
	250.0	7	19	24	10
	125.0	12	14	23	7
	62.5	-	-	23	-
V$_b$	500.0	16	20	23	21
	250.0	12	21	25	12
	125.0	7	10	19	-
	62.5	7	8	17	-
V$_c$	500.0	13	17	21	16
	250.0	10	14	20	8
	125.0	9	13	19	8
	62.5	10	12	15	-
V$_d$	500.0	15	19	24	15
	250.0	13	14	24	13
	125.0	11	10	26	10
	62.5	11	-	20	8
V$_e$	500.0	23	25	32	14
	250.0	15	15	20	7
	125.0	11	11	19	7
	62.5	11	7	16	-
V$_f$	500.0	16	22	22	20
	250.0	15	20	20	16
	125.0	13	15	20	14
	62.5	13	11	20	-
Naproxen	500.0	-	12	-	-
	250.0	-	-	-	-
	125.0	-	-	14	-
	62.5	-	-	-	-
Ciprofloxacin	500.0	35	18	26	25
	250.0	33	23	30	25
	125.0	30	19	26	25
	62.5	28	13	26	25
DMSO	pure	-	-	-	-

3.4. Conclusion:

1) The synthesis of the designed compounds has been successfully achieved.

2) Characterization and identification of the synthesized compounds were confirmed by determination of physical properties, FT-IR spectroscopy and ^{1}H-NMR spectra.

3) The Preliminary study of anti-inflammatory activity showed that Compound (V_d) and V_e show comparable effect to naproxen while compounds (V_a), (V_b) and (V_c) produced significantly lower inhibitory effect than (2S)-Naproxen and compound (V_f) exert significantly higher inhibitory effect than (2S)-Naproxen.

4) The Preliminary study of antibacterial activity of the prepared compounds showed significant activity against gram positive and gram negative bacteria especially *Bacillus, Staphylococcus aureus, Pseudomonas Aeroginosa* and *Escherichia coli.* unlike the parent compound (2S)-Naproxen.

5) The synthesized compounds may exhibit expected selectivity towards COX-2 enzyme due to their large size than its parent naproxen.

3.5. Further Study:

1) Determination of COX-2 selectivity of the tested compounds by assessing COX-2: COX-1 inhibitory ratio using human whole blood assay.

2) Evaluation of their anti-helicobacter, anticonvulsant, hypotensive and anticancer activity since N_1-substituted 3,5-diphenyl pyrazoline exhibited several pharmacological activities.

3) Study the ulcerogenic side effects of tested compounds.

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الخلاصـــــة

تعتبر الادوية المضادة للالتهابات غير الستيرويدية هي مثبطات تنافسية لانزيمات الاكسدة (الكوكس), الانزيم الذي يتوسط التحويل البايولوجي لحامض الاراكيدونيك الى البروستاجلاندين يرتبط استخدامها مع اثار جانبية مثل مشاكل الجهاز الهضمي.

الفعالية العلاجية لمضادات الالتهاب تنتج عن تثبيط انزيم كوكس-2 بينما تظهر الاثار الجانبية بسبب تثبيط انزيم كوكس-1 وهكذا فان مثبطات انزيم كوكس-2 الانتقائية قد خفضت الاثار الجانبية.

تم تخليق مشتقات البايرزولين الحلقية كمجموعة دوائية ادمجت الى النابروكسين لزيادة حجمه وقيمت كعوامل مؤثرة للالتهابات مع انتقائية مثبطة متوقعة نحو انزيم كوكس-2.

ان تخليق المركبات المستهدفة (V_{a-f}) قد انجز بنجاح. تم التاكد من نقاوة وخواص المركبات المخلقة وذلك بقياس الخواص الفيزيائية (درجة الانصهار ومعامل التعويق), وقياس اطياف الاشعة تحت الحمراء واطياف التردد النووي المغناطيسي.

تم تقييم تاثير مضادات الالتهاب للمركبات النهائية في الجسم الحي (الجرذان) باستخدام زلال البيض لاستحداث وذمة تحت الجلد كنموذج للالتهاب. ان المركبات المختبرة (V_{a-f}) والدواء المقارن (النابروكسين) اظهر انخفاض مؤثر للوذمة مقارنة مع البروبلين كلايكول كمجموعة ضابطة. مع ذلك اظهر المركبين (V_d) و (V_e) تاثير مشابه للنابروكسين في كل اوقت الاختبار بينما المركبات (V_a), (V_b) و (V_c) اظهرت تاثير تثبيطي اقل من النابروكسين ولفترة اختبار تراوحت من(120-240 دقيقة) علاوة على ذلك المركب (V_f) اظهر وبشكل كبير وملحوظ خفض للوذمة ولفترة اختبار تتراوح من(60-240 دقيقة).

تم تقييم فعالية المركبات النهائية كمضادات للجراثيم بطريق الانتشار الحسن. جميع المركبات المختبرة اظهرت نشاط مضاد للبكتريا كبير ضد بكتريا موجبة الجرام وسالبة الجرام خاصة *الباسلص, ستافلوكوكس اوريس, سيدومونس ايروجينوزا و الايكولاي* مقارنة مع (DMSO)

كمجموعة ضابطة والنابروكسين كدواء مقارن. مقارنة النتائج المضادة للجراثيم بين المركبات المختبرة اظهرت بان المركب (V_e) يعتبر هو الافضل والمركب (V_c) هو اقلهم. هذه النتائج تشجع على اجراء تقييمات اضافية على المركبات المخلقة لاظهار او تشخيص درجة انتقائيتها المثبطة للانزيم كوكس-2 وانشطتها الاخرى كمضادات للميكروبات.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

الجامعة المستنصرية

كلية الصيدلة

تخليق وتشخيص وتقيم دوائي اولي لنابروكسين يحتوي على مشتقات البايرزولين

رسالة مقدمه الى فرع الكيمياء الصيدلانية والى لجنة الدراسات العليا في كلية الصيدله ـ الجامعه المستنصرية كجزء من متطلبات الحصول على درجة الماجستير في الصيدلة (الكيمياء الصيدلانية)

من قبل

نور منير محمد

بكلوريوس صيدلة 2009

باشراف

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