SYNTHEIS OF NEW COMPOUNDS
BEARING METHYLSULFONYL
PHARMACOPHORE AND EVALUATION
OF THEIR SELECTIVE
COX-2 INHIBITORY ACTIVITY

Master Thesis

Taha Almarsoomi

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SYNTHESIS of NEW COMPOUNDS BEARING METHYL SULFONYL PHARMACOPHORE and EVALUATION of THEIR SELECTIVE COX-2 INHIBITORY ACTIVITY

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ABSTRACT

SYNTHESIS OF NEW COMPOUNDS BEARING METHYL SULFONYL PHARMACOPHORE AND EVALUATION OF THEIR SELECTIVE COX-2 INHIBITORY ACTIVITY

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Department of Pharmaceutical Chemistry

Anadolu University, Health Sciences Institute, January 2023

Supervisor: Prof. Dr. Zafer Asım Kaplancıklı

Cyclooxygenase, also known as prostaglandin H2 synthase (PGH2), is one of the most important enzymes in pharmacology because inhibition of COX is the mechanism of action of most nonsteroidal anti-inflammatory drugs.

In this study, ten thiazole derivative compounds had synthesized. The analysis of the obtained compounds was performed by 1H NMR (Proton Nuclear Magnetic Resonance) and 13C NMR (Carbon-13 Nuclear Magnetic Resonance) methods. By this method, the obtained compounds could be elucidated. The inhibitory effect of the obtained compounds on cyclooxygenase (COX) enzymes investigated. The encoded compounds **5a**, **5b**, and **5c** were found to be the most potent compared to the reference compounds ibuprofen, celecoxib, and nimesulide. The inhibitory activity of **5a**, **5b**, and **5c** is approximate, but the 5a derivative proved to be the most active in the series with an IC_{50} value of 0.180 ± 0.002 µM.

The most potent COXs inhibitor was **5a**, which further investigated for its potential binding mode by molecular docking study. Compound **5a** found to be localized at the active site of the enzyme, similar to celecoxib, which has a remarkable effect on COXs enzymes.

Keywords: Thiazole, Cyclooxygenase, Dithiocarbamate.

ÖZET

METİL SÜLFONİL FARMAKOFOR TAŞIYAN YENİ BİLEŞİKLERİN SENTEZİ VE SEÇİCİ COX-2 İNHİBİTÖR AKTİVİTELERİNİN DEĞERLENDİRİLMESİ

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Prostaglandin H2 sentetaz (PGH2) olarak da bilinen siklooksijenaz (COX), organizmadaki önemli enzimlerden biridir. COX inhibisyonu çoğu nonsteroidal antiinflamatuar ilacın etki mekanizmasını oluşturmaktadır.

Bu çalışmada; tiyazol türevi 10 yeni bileşik sentezlenmiştir. Elde edilen bileşiklerin analizi 1H-NMR ve 13C-NMR yöntemleri ile yapılarak yapıları aydınlatılmıştır.

Elde edilen bileşiklerin siklooksijenaz (COXs) enzimleri üzerindeki inhibisyon etkisi araştırılmıştır. **5a**, **5b** ve **5c** kodlu bileşiklerin, referans bileşikler Ibuprofen, Selekoksib ve Nimesulid ile karşılaştırıldığında etkili olduğu bulunmuştur. **5a** Türevi 0.180±0.002 µM IC_{50} değeri ile serideki en aktif olduğu belirlenmiştir.

En aktif COX inhibitor etkiye sahip olan **5a** bileşiğinin enzim aktif yöresi ile etkileşimi incelenmiş ve selekoksib’in COX enzim yöresi ile olan etkileşime benzer bir etkileşim sergilediği gözlenmiştir.

Anahtar Kelimeler: Tiyazol, Ditiyokarbamat, Siklooksijenaz.

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LIST OF ABBREVIATIONS

13C-NMR	: Carbon-13 Nuclear Magnetic Resonance
1H-NMR	: Proton Nuclear Magnetic Resonance
AA	: Arachidonic Acid
BCS	: Biopharmaceutical Classification System
COXs	: Cyclooxygenase
DP	: PGD receptor
EIA	: Enzyme immunoassay
EP1, EP2, EP3, EP4	: PGE receptor
FP	: PGF receptor
IC	: Inhibitory Concentration
IP	: PGI receptor
kDa	: kilo Dalton
NSAID	: Nonsteroidal Anti-inflammatory Drugs
PGD2	: Prostaglandin D2
PGE2	: Prostaglandin E2
PGF2	: Prostaglandin F2\
PGG2	:Hydroperoxy endoprtoxide
PGH2	: Hydroxy endoperoxide
PGI2	: Prostacyclin
PGs	: Prostaglandins
PGs	: Prostaglandins
POX	: Peroxidase
SI	: Selectivity Index
TLC	: Thin-layer chromatography
TP	: Thromboxane A2 receptor
TXA2	: Thromboxane A2

1. INTRODUCTION AND OBJECTIVE

The human body has its own natural system in response to injury, infection, and irritation known as inflammation. Inflammation; It is a reaction that triggers by damage caused by physical, chemical, and inflammatory factors caused by microorganisms and parasites and occurs in all organs. An inflammatory response required for survival against injurious stimuli. Impaired perfusion and tissue damage in terminal capillaries underlie this response mechanism, which manifests itself as erythema, edema, hyperthermia, and functional impairment. As a result, plasma escapes into the extracellular space due to increased capillary permeability. As a result, the mediators released from the damaged tissue, pain receptors are stimulated; mediators like histamine and serotonin., prostaglandins, and kinins are released and increases the sensitivity of pain sensors and strengthens the perception of pain [1–3].

Under normal conditions, it is confined to the affected tissue; however, in some cases, it can lead to uncontrolled conditions. Cyclooxygenase (COX) is the primary mediator in inflammation. Nonsteroidal anti-inflammatory medications (NSAIDs), often known as COX inhibitors, are used to treat uncontrolled diseases [4].

COXs, is an enzyme responsibe for the synthesis of inflammatory mediators known as prostanoids, derived from arachidonic acid (AA), such as thromboxanes and prostaglandins (PGs). COX enzyme also familiar as prostaglandin endoperoxide synthase (PTGS) [5].

Eicosanoids are short-lived endogenous substances containing a 20-carbon atoms. They are released from membrane phospholipids and derived from arachidonic acid. Arachidonic acid, the precursor of eicosanoid biosynthesis, is an unsaturated fatty acid with 20 carbons and 4 double bonds and is released as a result of the hydrolysis of cell membrane phospholipids [6].

The COX enzyme has two isoenzymes: COX-1 and COX-2. Both catalyze pGs that cause pain, inflammation, and fever, but only COX-1 creates prostaglandins that protect the intestinal, stomach mucosa, and activate platelets. [7]. Eventhough both COX isoforms convert AA to PGs, but there are tremendous differences in their distribution

and their roles and effects on body. The most important differences are in the genes for COX-1 and COX-2 and their regulation [8].

As a result of the reactions induced by COX enzymes via cyclooxygenase, prostaglandin, prostacyclin, and thromboxane species are produced. The COX enzyme, which was thought to be the only form until 1990, has been found to have two isoforms [9]. In 2002, the third isoform was detected [1]. Studies on the structure of the COX-3 enzyme continue. Although COX-1 and COX-2 enzymes play a role in prostaglandin biosynthesis, they have different functions in terms of structure and function.

The inflammatory mediators known as prostaglandins, through reversible binding to G-protein-coupled membrane receptors, cause a series of pathological conditions. While levuloglandins, a new generation of prostaglandins that irreversibly through making a covalent bond bind to G-protein receptors, are recognized as new prostaglandin agents.

Inhibition of COX enzymes results in diminutions of inflammation, reducing induced fever, anti-thrombotic, reducing neurodegeneration, and have positive effects oncologic diseases. Aspirin was synthesized roughly one hundred years ago by Hoffman for the first time as an agent that would reduce gastrointestinal irritation as a side effect of salicylates while maintaining their efficacy [10].

All conventional NSAIDs do not selectively inhibit COXs at standard doses. The beneficial effects of anti-inflammatory and analgesic drugs arise from selective inhibition of COX-2, whereas gastrointestinal damage arises from simultaneous inhibition of COX -1 [8].

This study aimed to design, synthesize and investigate the biological activities of new compounds of COX-2 inhibitor containing methylsulfonyl moiety.

2. LITERATURE REVIEW

2.1. Cyclooxygenase

In 1976, the COX enzyme was initially isolated, and in 1988, it was cloned. The main role of COX enzymes is involved in the synthesis of PGs through an oxidation-reduction pathway of arachidonic acid AA to hydroperoxy endoperoxide (PGG2) and hydroxy endoperoxide (PGH2) subsequently. The PGH2 is converting to the primary prostanoids by a number of enzymatic and non-enzymatic mechanisms [11]. The COX-2 discovery in the early 1990s was the most important event in the development of new anti-inflammatory drugs [12].

2.1.1. Cyclooxygenase Chemical Structure

The homodimers of COX-1 and COX-2 are 70-kilo Dalton (kDa) subunits. Their catalytic activity and structural stability depend on dimerization. A cyclooxygenase and a peroxidase (POX) active site can be found in each subunit. The catalytic domain is home to the COX and peroxidase active sites, with the prosthetic heme group situated at the base of the peroxidase site. [13]. Three structural domains make up each monomer of COX: a small N-terminal epidermal growth factor domain, a membrane-binding domain, and a large globular catalytic domain. Four amphipathic R-helices make up cyclooxygenase's membrane-binding domain. The last helix (helix D), which protrudes into the catalytic domain, is the only one that does not share a plane with the other three helices (Figure 2.1)[14]. One face of the lipid bilayer interacts with the surface produced by the hydrophobic action. The majority of the COX monomer made up of the catalytic domain, which is also, where NSAID activity and substrate binding take place. [15]. These two isoforms, which are very similar in structure, differ in amino acid sequence.

The components needed for COX-1's catalytic activity are likewise present in COX-2. Positions 434 and 523 of COX-1's isoleucine have been swapped out for valine in COX-2. Compared to the 523rd position of COX-1, the smaller valine structure in COX-2 contributes to the selectivity of COX-2 by creating a side pocket in the substrate channel, allowing inhibitor entry.

In addition, due to the "gate" effect formed as a result of the valine/isoleucine exchange in the 434th position, more space is provided for the entry of the compounds in COX-2 due to the less steric hindrance of the less voluminous valine [1].

COX-2 is about 20-25% larger than COX-1. The steric inhibition of Ile in COX-1, and the gap created by the valine-isoleucine exchange in the residential area of COX-2 make this difference. Another variant is that COX-2 is 17 amino acids shorter at the N-terminal end and 18 amino acids more at the C-terminal end.

This variant has led to a difference in numbering between the two isoforms. So much so that the serine in which aspirin acetates is located as Ser530 in COX-1, while in COX-2 this serine coincides with the Ser516 sequence.

Although the amino acids responsible for maintaining catalytic activity are the same, COX-2's capacity to take up larger fatty acids makes COX-1 more specific to fatty acid substrates than COX-2. At these sites, COX-1 mainly metabolizes arachidonic acid, while COX-2 can metabolize all 18-/20-carbon fatty acid substrates.

The carboxylic acid group of selective/non-selective COX-1 inhibitors attaches to Arg-120, which is used by arachidonic acid COOH and to which selective inhibitors of COX-2 bind. Arginine, which can interact with polar parts, is found in COX-2 instead of histamine in the 513th position of COX-1, and this amino acid difference does not change the way of drug interaction but changes the chemical environment considerably [1].

The main difference between COX-1 and COX-2 therapeutically appears to be in physiological roles rather than structure. COX-1 consistently expressed in almost every tissue; It mediates the synthesis of prostaglandins, which control the normal functioning of platelet functions and renal functions in preserving the natural structure of the gastrointestinal tract. COX-2 is too low to be detected at the basal level in most tissues. It is an inducible enzyme from inflammation that stimulated by mitogenic effects such as cytokines, endotoxins, and growth factors. It provides the formation of prostaglandins in the area of inflammation. It is thought to be the target enzyme in the treatment approach to inflammatory diseases [16–18].

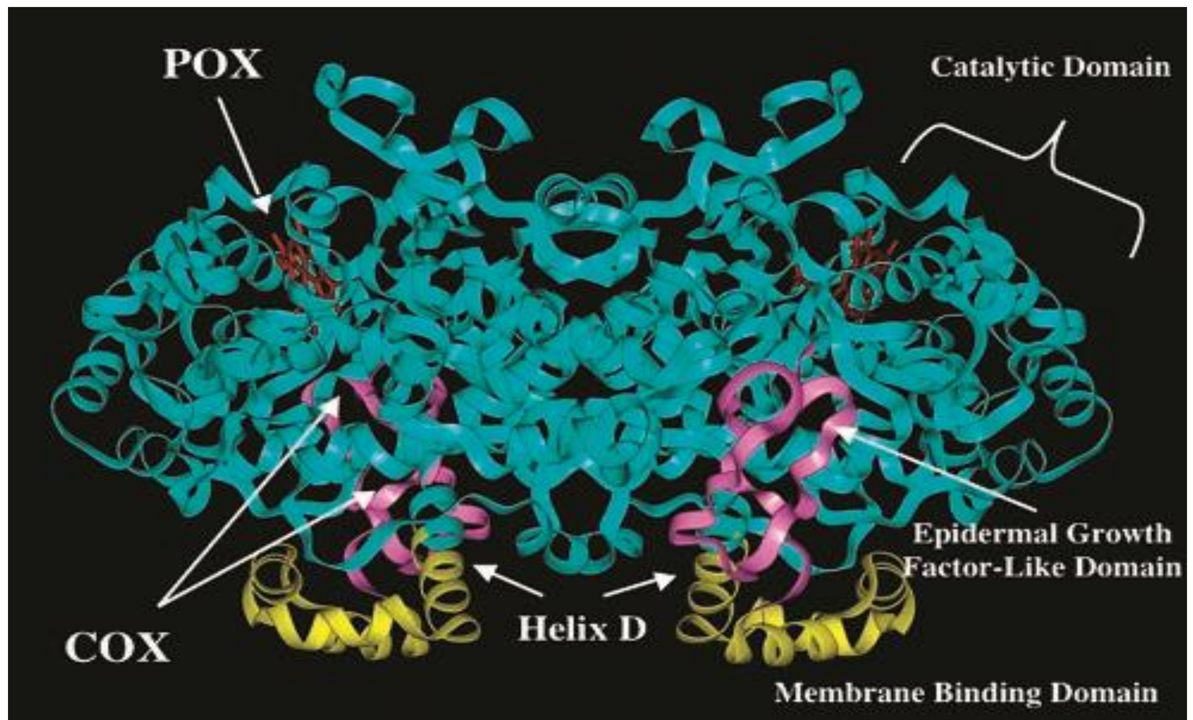

Figure 2.1. *Structural representation of the murine COX-2 dimer [13].*

2.2. Prostaglandins and Thromboxane

PGs are a complex group of oxygenated fatty acids that have been detected in all tissues of mammals. They are among the most effective natural substances important for bioregulation. Prostaglandin D2 (PGD2), prostaglandin E2 (PGE2), prostaglandin F2 (PGF2), and prostacyclin are the four bioactive forms of PGs (PGI2)[19]. By acting as lipid mediators in the body's autocrine and paracrine systems, they keep local homeostasis in check. In response to inflammation, the level of PG production changes remarkably. PG production suddenly increases in case of inflammation by induced COX enzyme which is known as COX-2, in contrast, in uninflamed tissues the level of PG is at the lowest level [20].

2.2.1. Prostaglandins and Thromboxane Chemical Structure

Prostaglandins found in all tissues got this name because they were first found in the secretion of the prostate [1]. Prostanoids are a group of COX metabolites. consist of twenty carbon atoms of unsaturated fatty acids known as prostaglandins and thromboxanes. the building block of prostaglandins is prostanoic acid, and they are separated from each other by the differences in their structural features. Firstly, differentiation in the functional groups at positions 9 and 11 of the cyclopentane ring. The other classification is based on the number of double bonds in the side chains, which distinguish them into three series1, 2, and 3. From its name, thromboxane A2 distinguished from prostaglandin in which an oxane ring has replaced the cyclopentane ring instead. These prostanoids act on cell surface receptors specific to each type [1,21].

2.2.2. Prostaglandins and Thromboxane Biosynthesis

PGs are not stored in tissues but synthesized because of membrane perturbations leading to the release of free fatty acids, generally AA, from esterified lipid sources. The released AA from plasma membrane both PGs and thromboxane A2 (TXA2) are formed by the aid of phospholipases and COXs enzymes [20]. The membrane-binding domain's base is where the COX active site is accessible, and from there, a lengthy hydrophobic channel enters the innermost region of the catalytic domain [15]. Through catalysis of two oxygen molecules of AA, COX enzyme induces PGG2. PGG2 diffuses to the active site POX and reduced to PGH2. Cell-specific synthases enzymes convert PGH2 into miscellaneous PGs and TxA2 [20].

Arachidonic acid is an unsaturated fatty acid ester found in membrane phospholipids and a precursor of prostaglandins. The first step in prostaglandin synthesis is the breakdown of phospholipids by phospholipase A2, resulting in arachidonic acid. The second step; catalyzed by COX enzymes and is the rate limiter of the synthesis. Prostaglandin G2 (PGG2), an unstable intermediate produced by the oxygenation of arachidonic acid, is converted to prostaglandin H2 (PGH2) thanks to the peroxidase activity of COX enzymes. With the effect of tissue-specific isomerases, PGH2 transforms into different prostaglandins and thromboxane [1]. Although their biosynthesis can be performed in almost any tissue, they are to some extent cell-specific. It is formed from

PGH2 wit isomerization results by the action of prostaglandin isomerase without any change in the cell step. Their secretion is neuronal stimulation, histamine intermediaries, gastrin, etc. It is initiated by gastrointestinal hormones [9].

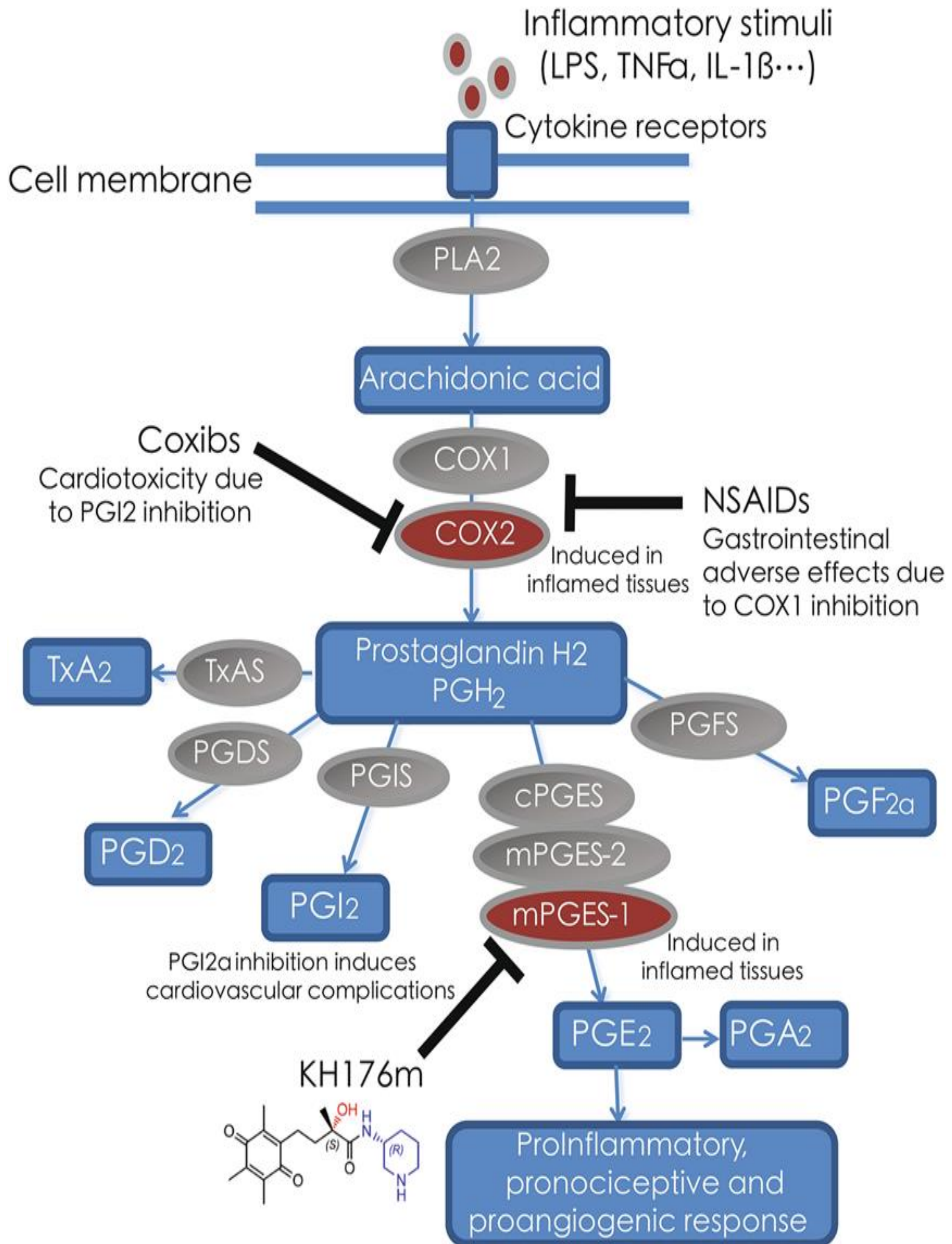

Figure 2.2. *Prostaglandins and thromboxane biosyntheis by the action of COXs [22]*

2.3. Non-steroidal Anti-inflammatory Drugs / Cyclooxygenase Inhibitors

The NSAIDs career continues from the 1900s to this day as COX enzyme inhibitors to treat inflammation. These include acetylsalicylic acid, ketorolac, celecoxib, etoricoxib, naproxen, indomethacin and etc. [23]. For all that, during its use, several side effects occurred such as vasomotor disturbances, bronchospasm, renal dysfunction, angioedema and meningeal syndrome, bone marrow depression, headache, and dizziness [23–25]. Thus, researchers are greatly concerned lately about finding selective COX-2 inhibitors to increase potency and safety. According to the kinetics of their interactions with COX-1 and COX-2, NSAIDs are also categorized into three classes: irreversible inhibitors, time-dependent and slowly reversible inhibitors, and freely reversible inhibitors [26].

2.3.1. COX Inhibitors

The most widely used therapeutics is the painkiller and anti-inflammatory drugs specifically NSAIDs. From the historical background, aspirin was the first beneficial NSAID has introduced more than one hundred years ago from now. each year about 50 000 tons of aspirin are produced and this amount represents the therapeutically benefits obtained from this pharmaceutic even today[27] . A scientific breakthrough happened in the 1970s, five decades ago, when the molecular mechanism of aspirin and other NSAIDs was revealed. Vane, Samuelson, and Bergstrom were successful in demonstrating the mechanism by which these anti-inflammatory drugs work to block prostaglandin production and biosynthesis (PGs)[28].

Aspirin is a COX inhibitor with the chemical structure $CH_3COOC_6H_4COOH$. Aspirin dissimilar to other NSAIDs, inhibit COX enzymes irreversibly. It also irreversibly blocks thromboxane A2 and preventing platelet aggregation [29]. At a sufficient dose required for analgesic action, aspirin act non selectively on COX enzymes. At the same time, is a selective COX-1 inhibitor at a smaller dose about 81mg to prevent platelet aggregation, and preclude an adverse cardiovascular events. Aspirin reduces inflammation at higher doses by blocking COX-2, although its ability to block COX-1 is stronger. There is growing evidence in the literature that low-dose aspirin decreases

TXA2's prothrombotic effects without reducing PGI2 synthesis's antithrombotic component. [30].

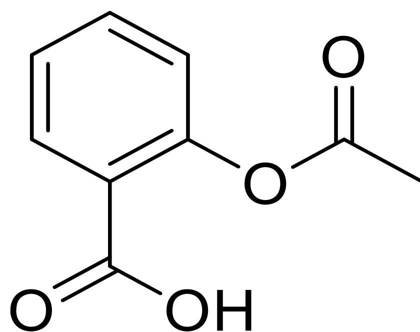

Figure 2.3. *Chemical structure of Aspirin.*

2.3.2. COX-2 Inhibitors

Until the end of the last few decades, it was commonly believed that there is a direct relation between the amount of available AA and the formed PGs. Although, investigations made by Needleman in 1988 advocated that this view was not acceptable scientifically. He found that COX protein concentration was shown to be remarkably higher in inflamed than in normal tissues [31].

Unlike COX-1, COX-2 expression is induced by inflammatory stimuli largely in cells such as synoviocytes, macrophages, and endothelial cells [28]. Thus, the development of selective COX-2 inhibitors will provide a tremendous service in the health sector to humanity.

Nowadays, scientists in this scope focus on finding and developing selective COX-2 inhibitors to obtain the most promising field of anti-inflammatory agents. Over the past few decades, up to 500 COX-2 inhibitors have been developed, but sadly, only celecoxib and rofecoxib have been made available as selective COX-2 inhibitors. [32].

2.3.2.1. *Celecoxib*

Celecoxib was chemically designate as $C_{17}H_{14}F_3N_3O_2S$ and is a di-aryl-substituted pyrazole. Celecoxib classified as selective COX-2 inhibitor by FDA based on its mechanism of action. Celecoxib's anti-inflammatory, antipyretic, and analgesic actions are due to this pharmacological activity. Since celecoxib only slightly inhibits COX-1, it might have fewer adverse effects on platelet function than aspirin. [33].

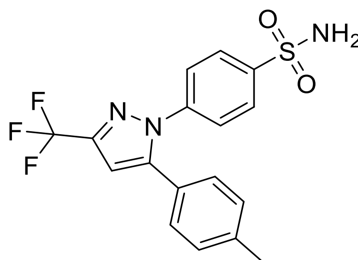

Figure 2.4. *Chemical structure of Celecoxib.*

2.3.2.2. *Valdecoxib*

Valdecoxib a diaryl-substituted isoxazole, chemically designated $C_{16}H_{14}N_2O_3S$ 4-(5-methyl-3-phenylisoxazol-4- yl) benzenesulfonamide), is an NSAID used to treat, osteoarthritis, rheumatoid arthritis and dysmenorrhea pain [34].

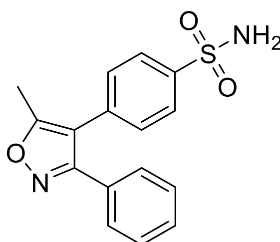

Figure 2.5. *Valdecoxib chemical structure.*

2.3.2.3. Rofecoxib

Rofecoxib, with the chemical formula $C_{17}H_{14}O_4S$, is a new-generation NSAIDs, in a dose-dependent manner, rofecoxib exhibits anti-inflammatory action through the inhibition of COX-2 selectively with insignificant action on the COX-1 isozyme in 1g dose evaluation. The pharmacokinetic studies of rofecoxib showed the following information; the oral bioavailability after a single dose of rofecoxib is 93%. Strong plasma protein binding of rofecoxib causes it to be broken down into inactive metabolites by the cytosolic reductase enzyme and eliminated via hepatic metabolism.[35].

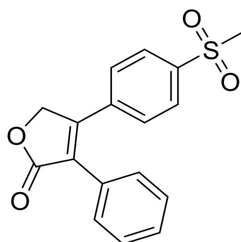

Figure 2.6. *Chemical structure of Rofecoxib*

2.3.2.4. Etoricoxib

An NSAID of the oxicam class with the chemical name 5-chloro-6-methyl-3-[4-(methylsulfonyl)phenyl)phenyl]-2,3-bipyridine [36]. Etoricoxib is a (COX)-2-selective inhibitor used to treat pain and inflammation associated with osteoarthritis, rheumatoid arthritis, acute gouty arthritis, dental procedures, and chronic pelvic pain. In contrast to other NSAIDs, Etoricoxib has a lower possibility of gastrointestinal toxicity in patients with osteoarthritis. Therefore, etoricoxib has greater potential to be used as an analgesic in the elderly population [37].

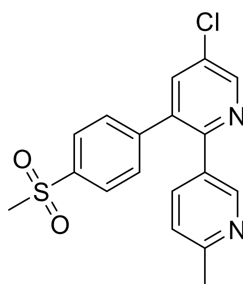

Figure 2.7. The molecular structures of etoricoxib

2.3.3. Non Selective Cox Inhibitors

2.3.3.1. *Ibuprofen*

Ibuprofen $C_{13}H_{18}O_2$, is one of the NSAIDs, that non-selectively inhibit COX enzymes and reducing the production of prostaglandin mediators that are responsible for pain and inflammation, by its anti-inflammatory impact [38][39]. There are many modified derivatives of ibuprofen have been prepared, to improve its analgesic and anti-inflammatory effects with minimum gastrointestinal side effects [40].

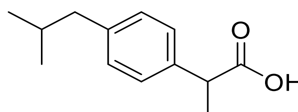

Figure 2.8. *Chemical structure of ibuprofen.*

2.3.3.2. *Ketoprofen*

The nonsteroidal anti-inflammatory drug ketoprofen, also known as 2-(3-benzoylphenyl) propionic acid, it is frequently used to treat inflammatory diseases. In recent decades, the skin has become a well-known site of administration for topical and systemic drug delivery [41].

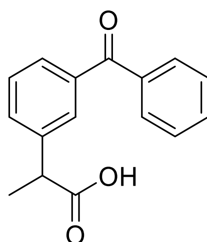

Figure 2.9. *Ketoprofen chemical structure.*

2.3.3.3. *Flurbiprofen*

Flurbiprofen, a NSAID is a phenylalkanoic acid derivative (2-(2-fluorobiphenyl-4-yl) propanoic acid) with molecular formula of $C_{15}H_{13}FO_2$ [10]. Flurbiprofen is commercially available as a racemic mixture. Flurbiprofen is an inhibitor of prostaglandin synthesis by inhibiting COX-enzyme, at the cellular level, drug's anti-inflammatory effect may partially explain [41].

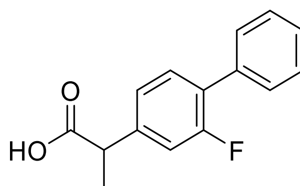

Figure 2.10. *Flurbiprofen chemical structure.*

2.3.3.4. *Indomethacin*

Indomethacin is one of the non-steroidal anti-inflammatory drugs, used for symptomatic relief of stiffness and pain in rheumatic diseases. The mechanism of action

is attributed to the non-selectively inhibition of COX-enzymes, resulting in decreased production of PGs, lipid mediators of inflammation [42].

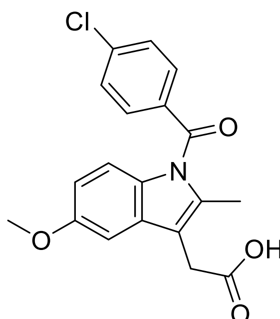

Figure 2.11. *Indomethacin chemical structure.*

2.3.3.5. *Diclofenac sodium*

Diclofenac sodium is traditional NSAIDs that it used for the treatment of rheumatic diseases and as post-surgery analgesia [43,44].

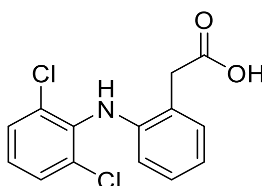

Figure 2.12. *Diclofenac sodium chemical structure.*

2.3.3.6. *Piroxicam*

Piroxicam is a NSAID, it is one of the potent COX-2 inhibitors to exhibit it is effect as analgesic, antipyretic and anti-inflammatory. It also used in the treatment protocol of brain stroke with other agents because of its inhibitory effect on aquaporin-4 [45].

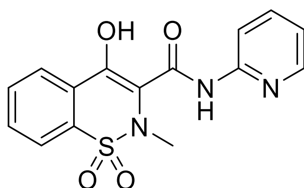

Figure 2.13. *Piroxicam chemical structure.*

2.3.4. New Synthesized of COX-2 Inhibitors

2.3.4.1. *Compounds Having Central Pyrazoline and Pyrazolone ring*

pyrazole heterocycles are pharmacologically active buildings in the synthesis and development of selective COX-2 inhibitors. clinically used medicines consisting of pyrazole scaffold are the following agents; celecoxib, antipyrin, aminopyrine, and metamizole [46]. In the past decade, number of pyrazole derivatives reported and screened for their COX-2 inhibition and anti-inflammatory activity has dramatically increased.

2014 had seen the development and synthesis of a brand-new chemical with pyrazole moiety by Bansal et al. [47]. With an inhibitory concentration (IC50) 0.31 mmol/L, a selectivity index (SI) > 222], and possible anti-inflammatory activity with an ED50 of 74.3 mg/kg in a carrageenan-induced rat paw edema model, the compound depicted in Figure 2.14 demonstrated exceptional selective inhibition of COX-2. Furthermore, an investigation on gastric acidity showed a better acid-cramping profile in contrast to aspirin and other non-selective NSAIDs. Molecular docking study revealed that the target compound had a greater binding affinity with COX-2 pockets than COX-1 active binding sites.

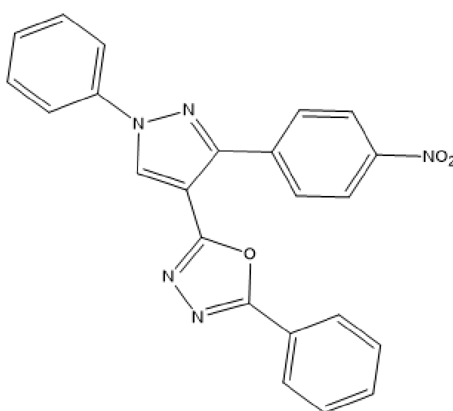

Figure 2.14. *Bansal et al.*

A group of 1,5-diphenylpyrazoles that El-Sayed et al. developed and synthesized have potential applications. The substances demonstrated strong COX -2 inhibitory activity and selectivity, with IC50 values of 0.45 mmol/L and SI values of 111.1, respectively. In the carrageenan-induced rat raw paw edema experiment, the compounds likewise shown remarkable anti-inflammatory action (ED50 118 and 120 mg/kg), in comparison to diclofenac (ED50 114 mg/kg) [48].

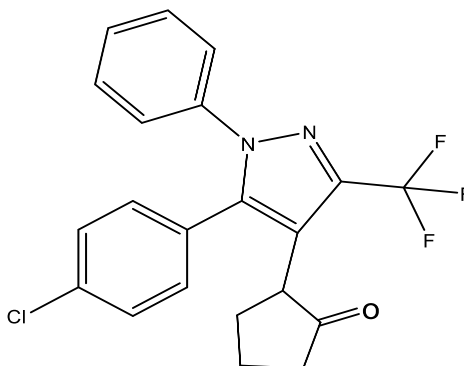

Figure 2.15. *El-Sayed et al.*

Abdel-Aziz et al created N-benzene sulfonamide-1 H-pyrazole analogs containing an aryl sulfonyl moiety at position 4. In vitro tests revealed that the most effective COX-2 inhibitor was a molecule having a p-toluyl sulfonyl group at position 4, whereas the methyl group's absence decreased the product's selectivity. The drug was also significantly less ulcerogenic than celecoxib (UI = 0.92 0.2) and showed a strong anti-inflammatory impact in vivo (ED50 = 51 0.7 m/kg at 3 hours).[49].

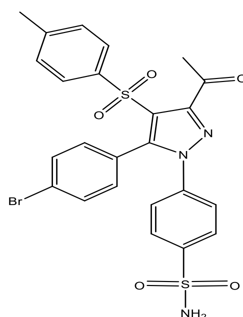

Figure 2.16. *Abdel-Aziz et al.*

A series of benzofuran-substituted pyrazole rings had designed by Hassan et al. as selective COX-2 inhibitors. The substance in Figure 2.17, which has a pyridine ring substituted at position 3 of a pyrazole, has shown to be just as active as celecoxib's trifluoromethyl group. Its ability to protect the GIT had demonstrated by the biological analysis of the targeted chemical, which revealed no inhibitory effect on the COX-1 isozyme. The methoxy and hydroxyl groups of benzofuran interacted with COX-2 via hydrogen bonding, according to molecular docking experiments. [50].

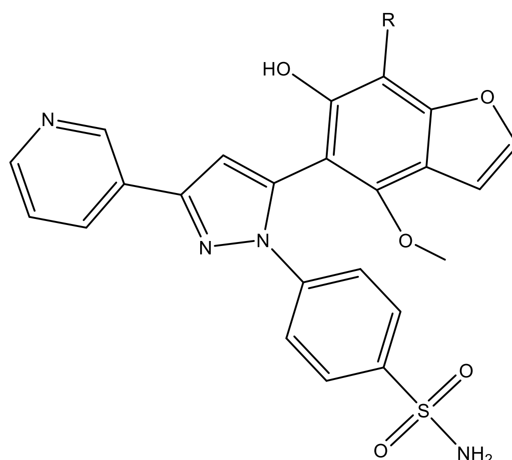

Figure 2.17. *Hassan et al.*

In 2014, Dube et al. synthesized a series of pyrazolone derivative and investigate their COX inhibitory effect of each compound. The shown compound in Figure 2.18 was selected as a candidate derivative as it exhibited excellent COX-2 inhibitory effect with the (IC_{50} = 1 μ M) and excellent SI (100) respectively. The sulfonamide establishes hydrogen bonds with Ser353 and Gln192 of the COX -2 active binding sites, according to molecular docking studies. Pyrazoline and the phenyl group interacted with COX-2 as well [51] .

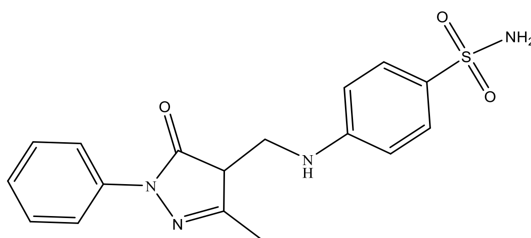

Figure 2.18. Dube et al.

In 2009, Rathish et al. designed the showed compound in Figure 2.19 and its derivatives as tri substituted pyrazoline pharmacophore. The compounds had constructed with the pyrazololine moiety as a selective COX-2 inhibitor. The COX-2 inhibitory activity of the derivatives was changed according to the substitutions on aryl groups. Unsubstituted aryl showed greater potency as anti-inflammatory agent in comparable to substituted derivatives. The target compound as shown here, showed no effect on gastric lining with greater anti-inflammatory activity in this study [52].

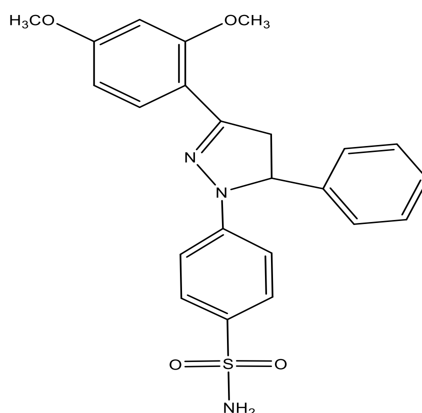

Figure 2.19. *Rathish et al.*

Abdellatif and his coworkers, tried to design and develop selective COX-2 inhibitor by synthesizing a series of pyrazoline derivatives as shown in this study. Derivatives with a benzene sulfonamide moiety at position 1 had greater activity than the derivatives with carboxyl group. The target molecule as shown in figure 2.20, exhibited excellent anti-inflammatory effect with selectively inhibition of COX-2 enzyme [53].

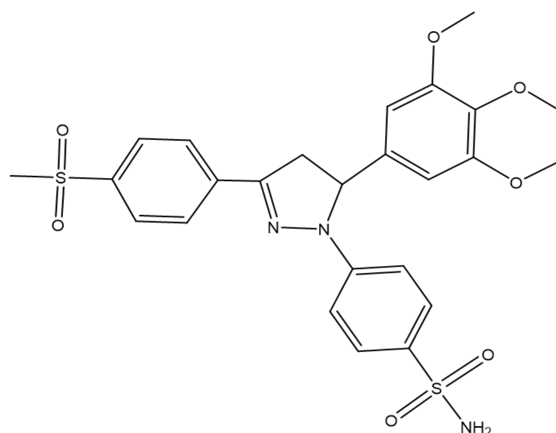

Figure 2.20. *Abdellatif et al.*

A relatively selective COX -2 inhibitory effect had demonstrated by the compounds illustrated in Figure 2.21, which Gopi's team developed using a chromogenic test (IC_{50}, 16.8 and 14.3 mmol/L, SI 0.5100 and 0.4400, respectively). The targeted ligand and COX-2 enzyme active binding site pockets interacted most effectively, according to a docking analysis. [54].

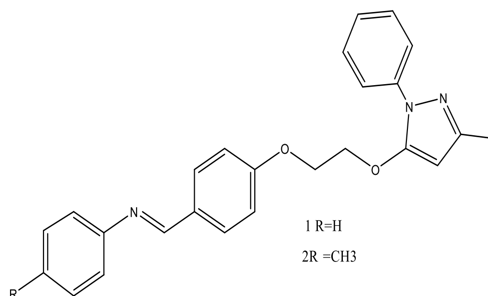

Figure 2.21. *Gopi et al.*

Mohammed et al created a new class of pyrazole compounds with an acylamino spacer. With an acknowledged selectivity and an IC_{50} of 1.76 mmol/L, the derivative demonstrated COX-2 inhibition. Synthesized compound (Figure 2.22) showed lower gastric ulceration effect and potent and anti-inflammatory than indomethacin [55].

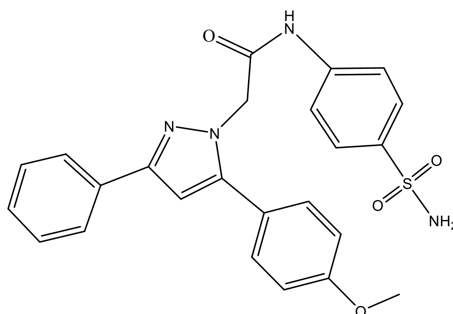

Figure 2.22. *Mohammed et al.*

2.3.4.2. Compounds Having an Indole Ring.

Kaur and his coworkers, deigned a series of substituted indole derivative, which, with an IC_{50} of 0.32 mmol/L and a SI of greater than 312, demonstrated selective COX -2 inhibitory action. A crucial hydrogen bond formed between the derivative depicted in Figure 2.23 and the active binding region of the COX-2 enzyme as a result of the docking investigation.[56].

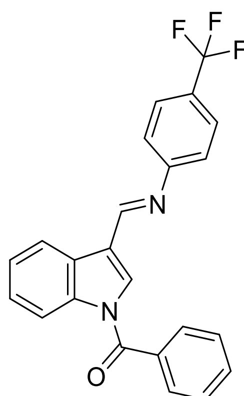

Figure 2.23. *Kaur et al.*

Indole pharmacophore in another study was also examined to its selectivity and affinity to COX-2 isozyme by Bhat's *et al*. The designed and synthesized target compound shown in Figure 2.24 processed selectively inhibition of COX -2 and required gastral safety profile. This research gave important data for the investigation of gastroprotective COX-2 inhibitors. [57].

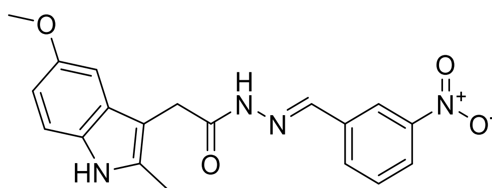

Figure 2.24. *Bhat et al.*

As COX-2 inhibitors, Singh et al. created a number of novel compounds having tosyl and dipeptide groups at the N-1 and C-3 positions, respectively. Among the reported derivatives, the compound in Figure 2.25 showed corresponding anti-inflammatory activity to diclofenac *in vivo* according to electivity index but, had greater COX -2 inhibitory activity IC_{50} of 0.54 mmol/L [58].

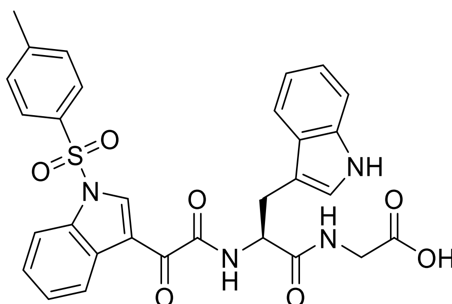

Figure 2.25. *Singh et al.*

In this study, indomethacin had redesigned to improve electivity and safety profile as NSAIDs. The synthesized derivative had obtained from indomethacin with several changes at different positions. Introducing sulfonamide moiety instead methoxy group at position 5 Figure 2.26, exhibited greater selectivity toward COX-2 isozyme and better safety profile. The target derivate was developed by Esteva˜o et al and investigated as a promised selective COX-2 inhibitor [59].

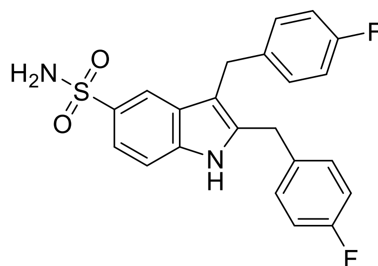

Figure 2.26. *Esteva˜o et al.*

Jung's group synthesized and designed some new indole analogs (Figure 2.27) that combines the structural motifs of anti-inflammatory metabolites of ascidians, the herdmanins. Based on bioisosteric design strategy, the acid moiety of indomethacine were replaced with hydrazine moiety to improve its biological activity and selectivity index. According to this modification, two esential hydrogen bonds with Tyr355 and Arg120 were formed. Indomethacin became a prototype of indole-containing analogs. Replacement of the C-3 acetic acid moiety in indomethacin with various substitutes is also an effective strategy to improve their activity and selectivity [60].

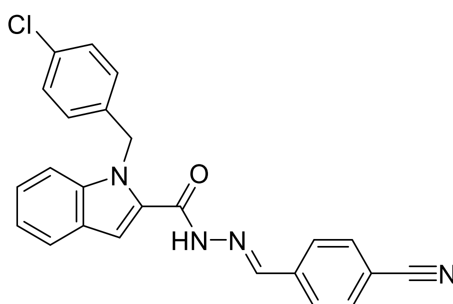

Figure 2.27. *Jung et al.*

2.3.4.3. *Compounds Having a Thiazole Ring.*

With a 4-chloro and 2-hydroxy substituted molecule, Hofmann's team created a novel thiazole analogue that inhibited COX-2 effectively and selectively, with an activity of 9.1 and a COX-2 product production rate of 1.1% at a concentration of 10 mmol/L. they had less ulcerogenic properties than celecoxib and comparable anti-inflammatory efficacy to celecoxib at various time points. Image 2.28 [61].

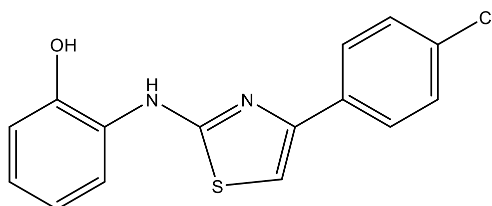

Figure 2.28. *Hofmann et al.*

Saglık et al. synthesized and designed new derivatives with thiazolyl-hydrazine-methylsulfonyl moiety as COX-2 inhibitors. *In vitro* assay studies showed that compound in Figure 2.29 had remarkable potency toward COX-2 inhibition selective similar to

nimesulide and celecoxib. According to the molecular docking results, the compound in Figure 2.29 bound in a similar manner to the enzyme COX -2 as celecoxib [62].

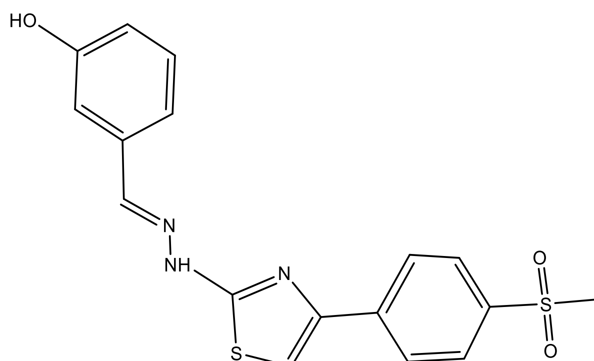

Figure 2.29. *Saglık et al.*

Abdel- Aziz et al. developed a number of novel derivatives using the acetamide moiety spacer to combine pyrimidine-5-carbonitrile with 2-amino-4-aryl-1,3-thiazole. The derivatives were synthesized aimed to improve anti-inflammatory with cardiac and gastric safety profiles as selective COX-2 inhibitors. Compared to celecoxib, the compound in Figure 2.30 showed good and specific COX-2 inhibitions. [63].

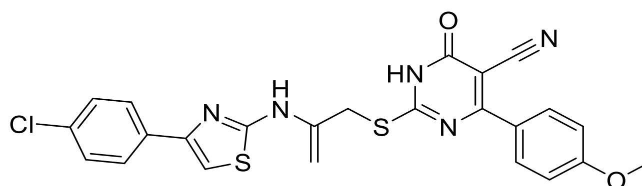

Figure 2.30. *Abdel- Aziz et al.*

2.3.4.4. ***Compounds Having a Tetrazole Ring.***

A tetrazole-containing drug with a high COX-2 inhibition and an IC50 value of 2.0 mmol/L was reported by Al-Hourani et al. [64], however its SI value (SI 2110) was lower than celecoxib's (SI 313).

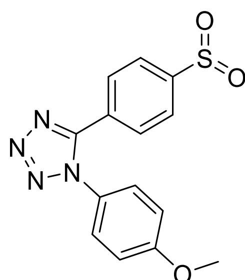

Figure 2.31. *Al-Hourani et al.*

After few years, same group Al-Hourani et al. recommended for further modifications on the previously designed derivatives as mentione at section 2.3.4.4. they synthesized, further 1,5-diaryl-substituted tetrazoles by further methylsulfonyl unit modifications. The biological study of the derivative showed in figure 2.32 revealed that it ha moderate COX-2 inhibitory activity and selectivity. The obtained results indicated that the novel derivatives are more active towards the COX-2 enzyme due to the presence of the methyl sulfonyl unit, methylene spacer at C-1, and longer linker [65].

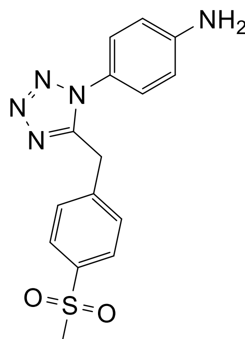

Figure 2.32. *Al-Hourani et al.*

3. MATERIALS

3.1. Materials and Equipment That Used in Synthesis of Compound.

Tables (3-1) and (3-2) demonstrate materials and equipment used in Synthesis of Compound respectively.

Table 3.1. *Material used in Synthesis of Compound.*

Materials	Manufacturing company
Pyrrolidine	Acros, Usa
Piperidine	Acros, Usa
4-Methylpiperidine	Merck, Germany
4-Methylpiperazine	Acros, Usa
4-Ethylpiperazine	Acros, Usa
4-Cyclohexylpiperazine	Acros, Usa
4-Phenylpiperazine	Acros, Usa
4-Methoxyphenylpiperazine	Acros, Usa
4-Nitrophenylpiperazine	Acros, Usa
4-Trifluoromethylpiperazine	Acros, Usa

Table 3.2. *Equipment used in Synthesis of Compound.*

Equipment	Company
Electronic balance	Japan, Shimadzu, libror eb-330 hu
Melting point system	Mettler toledo-mp90 melting point system
Incubator	Germany, Heraeus,
Infrared spectrophotometer	Japan, Shimadzu-IR affinity-1s
Mass spectrometer	Japan, Shimadzu, lcms-it-tof
Magnetic base heater stirrer	Germany, Heidolph, mr 3003
Nuclear magnetic resonance spectrometer	USA, Bruker, ultrashield 300 mhz
Sterile cabinet class ii typea2	Korea, (chc-222a2-60)
Ultraviolet lamp	Switzerland, Camag, cabinet
Vortex	Korea, Wisemix
Micro plate reader	USA, Biotek-synergy h1
Robotic pipetting table	USA, Biotek-preccision xs

4. METHODS

4.1. The Targeted Compounds' Synthesis

4.1.1. *Synthesis of 2-bromo-1-(4-(methylsulfonyl)phenyl)ethane-1-one (1).*

1-(4-(Methylsulfonyl)phenyl)ethane-1-one (5 g, 0.025 mol) was dissolved in acetic acid. HBr added to the reaction medium as a catalyst and placed in an ice bath. A solution of bromine (1.3 ml, 0.025 mol) in acetic acid was prepared and placed in a separatory funnel. The bromine solution added dropwise to the mixture. The reaction was followed by thin layer chromatography to check progressions. Once the reaction has finished, the mixture spilled in to ice; the precipitated substance extracted from the suitable solvent, filtered, dried, and crystallized.

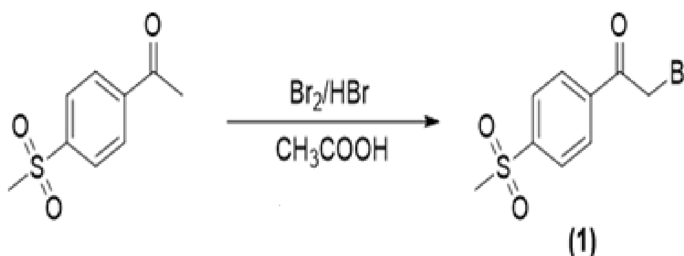

Figure 4.1. *Synthesis of 2-Bromo-1-(4-(methyl sulfonyl) phenyl) ethan-1-one (1).*

4.1.2. *Synthesis of 4-(4-(Methylsulfonyl)phenyl)thiazol-2-amine (2).*

2-Bromo-1-(4-(methylsulfonyl)phenyl)ethane-1-one (5 g, 0.018 mol) was dissolved in ethanol (100 mL) and thiourea (1.37 g, 0.018 mol) was added to the mixture. For eight hours, the reaction mixture had refluxed. TLC method had used to check the reaction progress. Once the reaction was finished, it was cooled, and the precipitated product was worked up further to obtain pure dried powder.

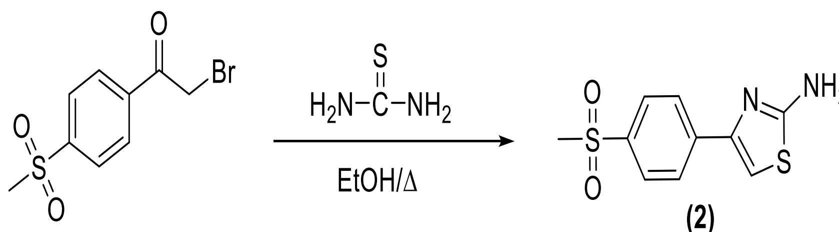

Figure 4.2. *Synthesis of 4-(4-(Methylsulfonyl)phenyl)thiazol-2-amine (2).*

4.1.3. *Synthesis of 2-chloro-N-(4-(4-(methylsulfonyl)phenyl)thiazol-2-yl)acetamide (3).*

4-(4-(Methylsulfonyl)phenyl)thiazole-2-amine (3.9 g, 0.015 mol) was dissolved in tetrahydrofuran (150 mL) and triethylamine (2.3 mL, 0.016 mol,) was added to the reaction medium as a catalyst. Chloroacetyl was added once the liquid was cooled. chloride (1.3 mL, 0.015 mol). After acidification with chloroacetyl chloride drop wisely, for a further hour, the reaction mixture was stirred at room temperature. After finishing the reaction, THF was removed by using the rotavapor, after being crystallized from ethanol, the raw material was rinsed with water.

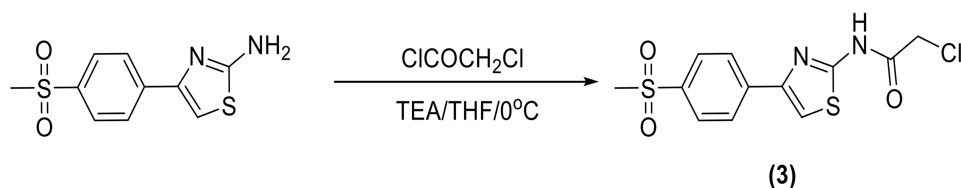

Figure 4.3. *Synthesis of 2-Chloro-N-(4-(4-(methylsulfonyl)phenyl)thiazol-2-yl)acetamide (3).*

4.1.4. *Synthesis of Dithiocarbamate Derivatives (4a-4j).*

Absolute ethanol and NaOH dissolved. Secondary amine derivatives (0.009 mol) were added to the reaction medium. Dropwise additions of CS2 (0.009 mol) in ethanol were made to the reaction medium. Thin layer chromatography method was used to follow up the reaction progression. After completion of the chemical reaction, the precipitated product filtered off and crystallized from ethanol.

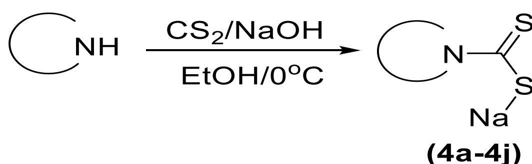

Figure 4.4. *Synthesis of Dithiocarbamate Derivatives (**4a-4j**).*

4.1.5. *Synthesis of Target Compounds (5a-5j).*

2-Chloro-N-(4-(4-(methylsulfonyl)phenyl)thiazol-2-yl)acetamide (3) was dissolved in acetone in an amount of (0.001 mol). Dithiocarbamate derivatives were added to the reaction medium (0.001 mol) and stirred for 12 hours at room temperature. Acetone was then removed from the mixture, the remaining substance was washed with water, and the substance was recrystallized by using ethanol.

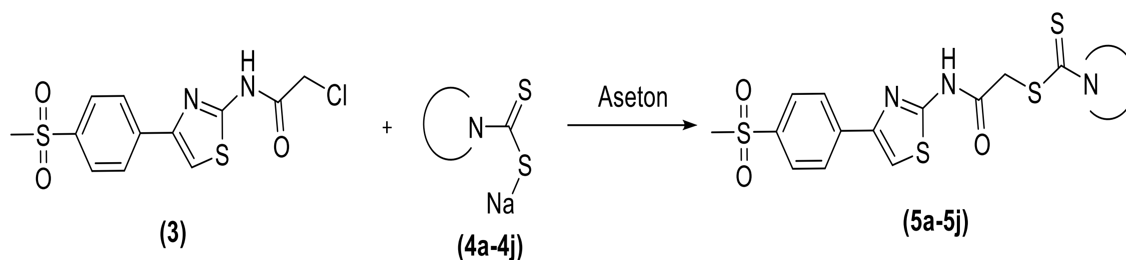

Figure 4.5. *Synthesis of Target Compounds (**5a-5j**).*

Table 4.1. *Synthesis of target compounds.*

Compound	Secondary Amine
5a	pyrrolidine
5b	Piperidine
5c	4-Methylpiperidine
5d	4-Methylpiperazine
5e	4-Ethylpiperazine
5f	4-Cyclohexylpiperazine
5g	4-Phenylpiperazine
5h	4-Methoxyphenylpiperazine
5i	4-Nitrophenylpiperazine
5j	4-Trifluorophenylpiperazine

4.2. 1H-NMR for Proton Nuclear Magnetic Resonance

The molecular structure of the designed compounds was proved by checking hydrogen atom isotopes by the application of 1-H-NMR spectroscopic analysis.

4.3. 13C-NMR for Carbon-13 Nuclear Magnetic Resonance

By mean of 13C-NMR the ^{13}C isotype of carbon can be identified, and clarify the synthesized product.

4.4. In Vitro COX-1 and COX-2 Inhibition Assay

Fluorometric COX-1 and COX-2 inhibitor screening kits (Biovision, Swit zerland) was used to check the inhibitory potency of the synthesized compounds in vitro assay studies according to the instructions of the kit manufacturer. [66,67]. The evaluation was based on the detection on an intermediate product produced by COX enzyme which known a prostaglandin G2. Using the available fluorometric approach, the in vitro COX inhibition test was carried out, and the percentages and IC50 values of the obtained compounds were computed as previously described by our research team. [68, 69]

4.5. Molecular Docking Study

Molecular docking studies were performed using in-silico method to define the binding modes of compound **5a** (active compound) in the active binding sites of enzymes X-ray crystal structures of COX-2 (PDB ID:3LN1) [70], were retrieved from Protein Data Bank server (www.pdb.org, accessed 01 Dec 2022). For molecular docking research, the Schrödinger Maestro interface [71], was employed, and the Protein Preparation Wizard protocol of the Schrödinger Suite 2020 was used to prepare the enzyme crystals. The Ligprep module was used to prepare the ligands.[72], to accurately assign the atom kinds and protonation states. The structures were given bond order assignments and hydrogen atoms. The Glide module was used to create the grid [42], and docking runs were performed in standard precision docking mode (SP).

5. RESULTS AND DISCUSSION

5.1. Clarifying the Structures of Target Compounds

The goal of this thesis was to create new compounds with methyl sulfonyl and phenyl thiazol groups. Ten new compounds were developed for this purpose using five-step reaction techniques. The first compound produced was 2-Bromo-1-(4-(methylsulfonyl) phenyl) ethan-1-one. Then, 4-(4-(4-(methylsulfonyl)phenyl)thiazol-2-amine was synthesized. The third stage was the development of 2-chloro-N-(4-(4-(methylsulfonyl) phenyl) thiazol-2-yl) acetamide, and the fourth was the creation of derivatives of dithiocarbamate (**4a-4j**). The target compounds were produced in the last stage by dissolving 2-chloro-N-(4-(4-(4-(methylsulfonyl)phenyl)thiazol-2-yl) acetamide in acetone and adding dithiocarbamate derivatives to the reaction medium. By using 1H-NMR and 13C-NMR, the structures of the discovered compounds were validated. The chemicals listed below have NMR data.

5.1.1. *2-((4-(4-(Methylsulfonyl)phenyl)thiazol-2-yl)amino)-2-oxoethyl pyrrolidine-1-carbodithioate (5a)*

Yield: 88%, MP: 229-231℃

1H-NMR (300 MHz, *DMSO-d6*) : δ = 1.90-1.97 (2H, m, pyrrolidine), 2.02-2.09 (2H, m, pyrrolidine), 3.25 (3H, s, -SO_2CH_3) , 3.69 (2H, t, *J*=6.8 Hz, pyrrolidine), 3.76 (2H, t, *J*=6.9 Hz, pyrrolidine), 4.39 (2H, s, -CH_2-), 7.92 (1H, s, Thiazole), 7.99 (2H, d, *J*=8.6 Hz, 1,4-Disubstitutedbenzene), 8.16 (2H, d, J=8.6 Hz, 1,4-Disubstitutedbenzene), 12.65 (1H, s, -NH).

13 C-NMR (75 MHz, DMSO-*d6*): δ =24.29, 26.19, 44.03, 51.14, 55.74, 111.88, 126.72, 128.12, 139.20, 140.01, 147.59, 158.80, 166.91, 190.25.

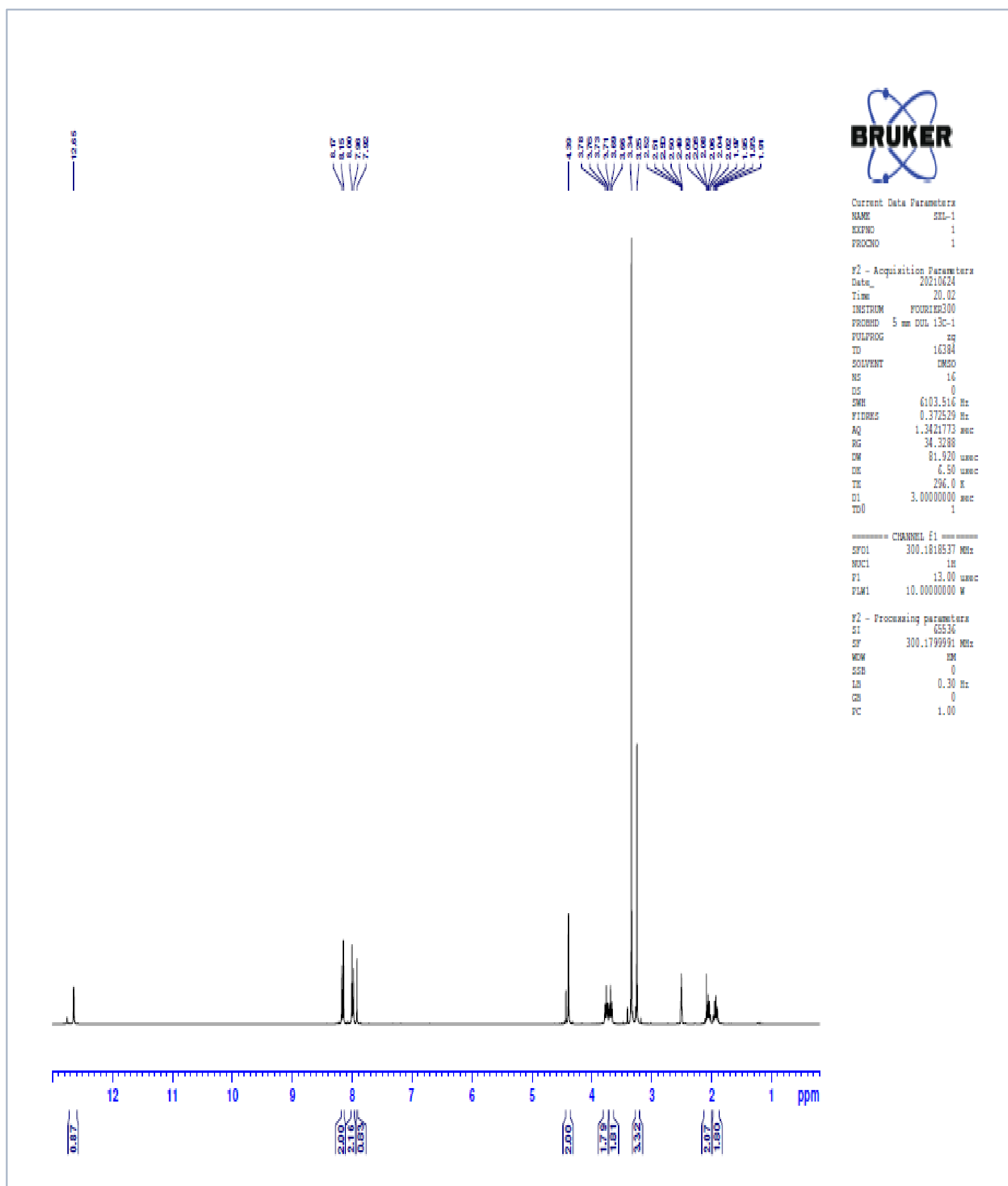

Figure 5.1. *1H-NMR spectrum of Compound* ***5a***

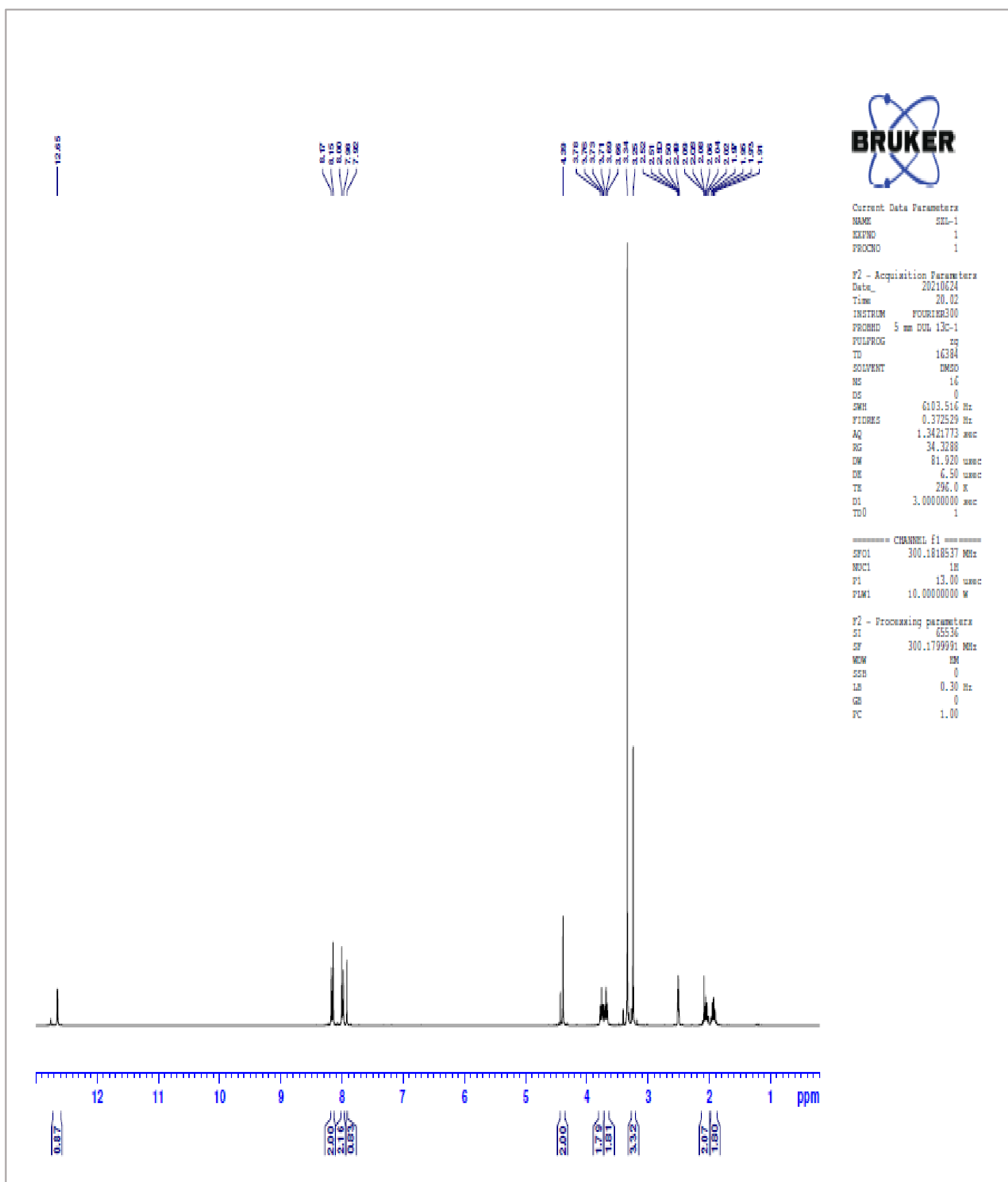

Figure 5.2. *13C-NMR spectrum of Compound **5a**.*

5.1.2. ***2-((4-(4-(Methylsulfonyl)phenyl)thiazol-2-yl)amino)-2-oxoethyl piperidine-1-carbodithioate (5b)***

Yield: 83 %., MP: 228-229.5℃

1H-NMR (300 MHz, *DMSO-d6*): δ = 1.61-1.65 (6H, m, piperidine), 3.25 (3H, s, -SO_2CH_3), 3.94 (2H, y, piperidine), 4.19 (2H, y, piperidine), 4.39 (2H, s, -CH_2-), 7.92 (1H, s, Thiazole), 7.99 (2H, d, *J*=8.6 Hz, 1,4-Disubstitutedbenzene), 8.16 (2H, d, *J*=8.6 Hz, 1,4-Disubstitutedbenzene), 12.67 (1H, s, -NH).

13 C-NMR (75 MHz, *DMSO-d6*): δ =23.91, 25.62, 26.33, 44.02, 51.60, 53.11, 111.85, 126.72, 128.12, 139.21, 140.00, 147.59, 158.82, 166.94, 193.14.

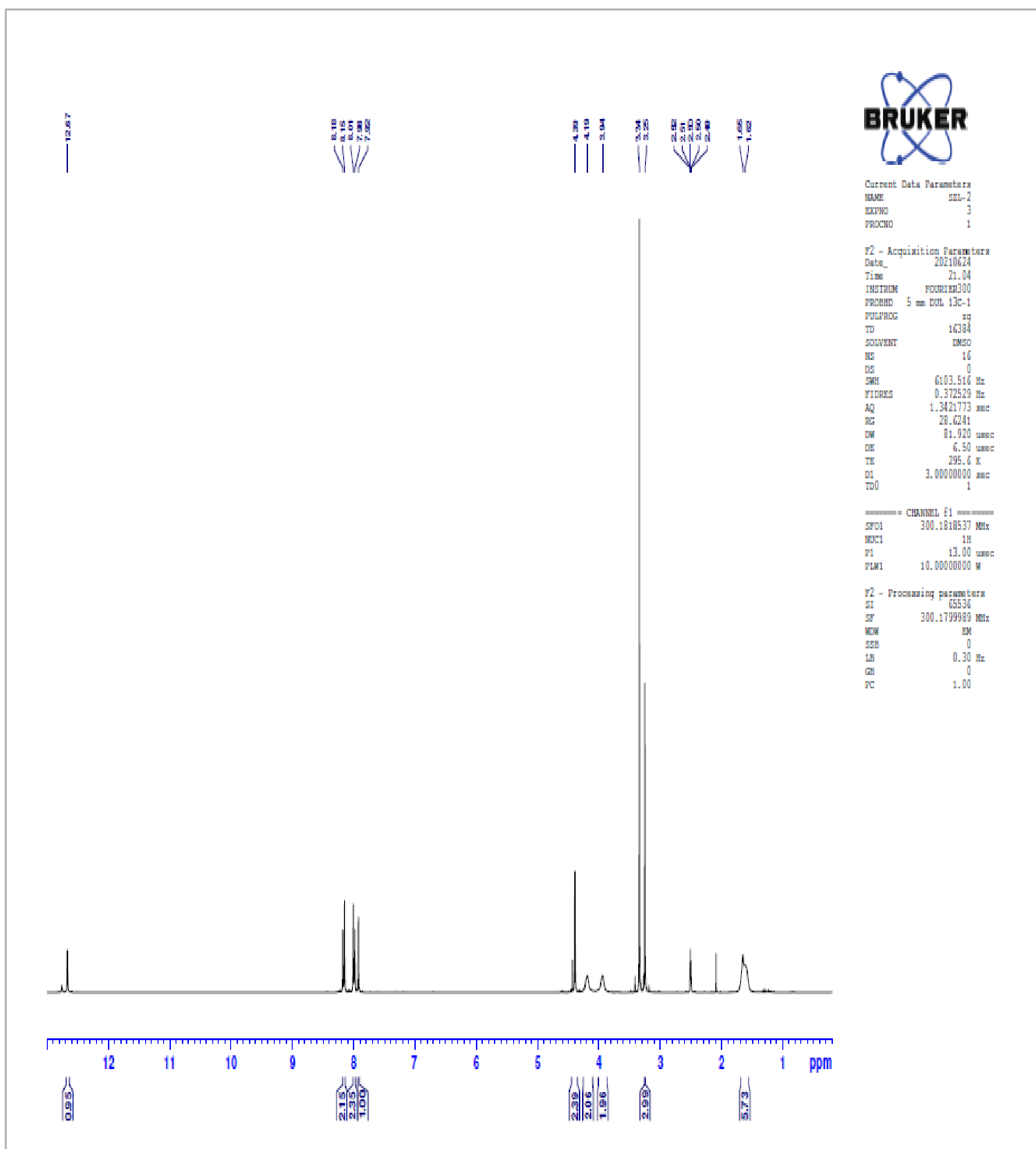

Figure 5.3. *1H-NMR spectrum of Compound **5b**.*

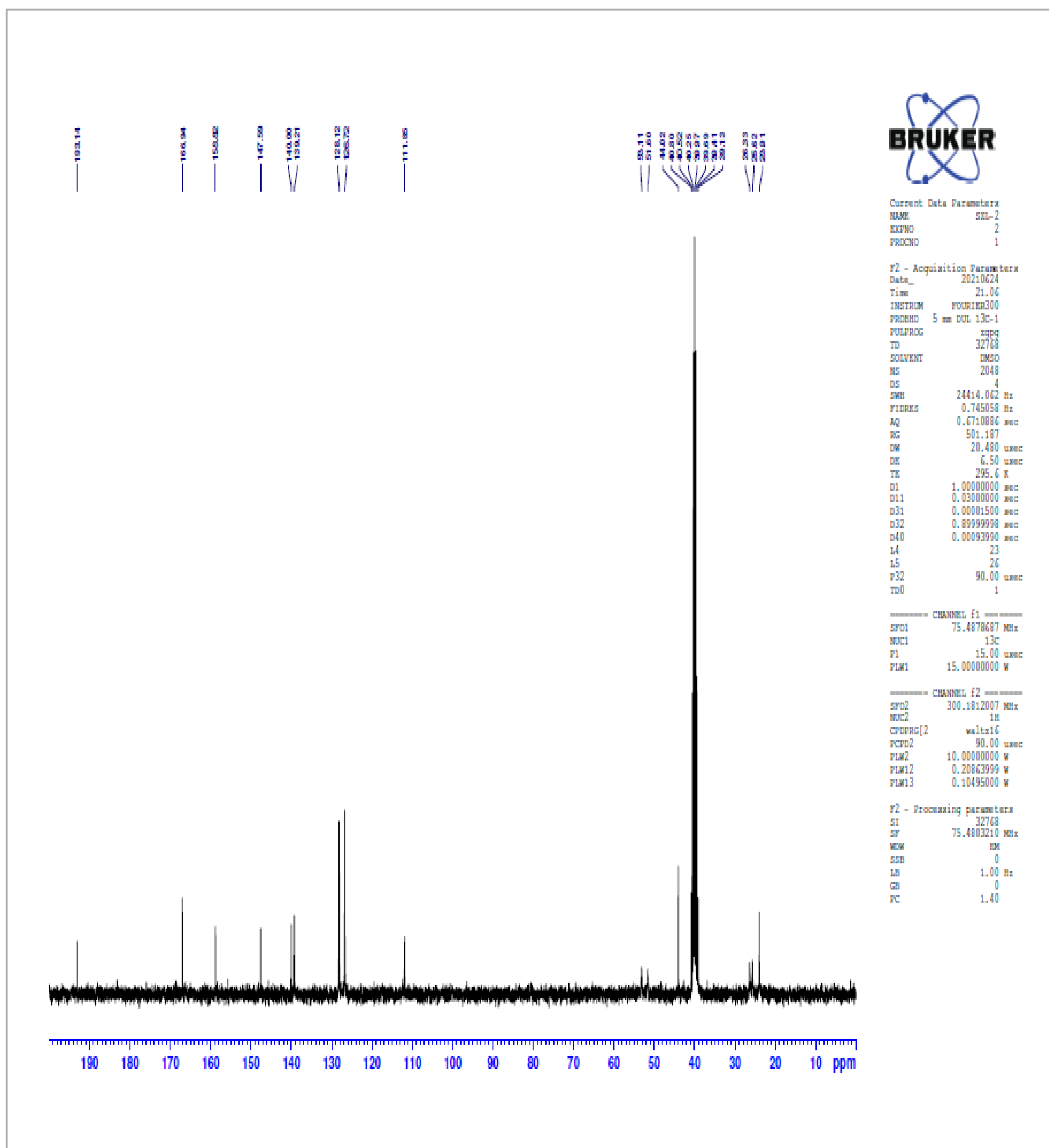

Figure 5.4. *13C-NMR spectrum of Compound* ***5b****.*

5.1.3. ***2-((4-(4-(Methylsulfonyl)phenyl)thiazol-2-yl)amino)-2-oxoethyl 4-methylpiperidine-1-carbodithioate (5c)***

Yield: 85%., MP: 197-198.7℃

1H-NMR (300 MHz, DMSO-*d6*) : δ = 0.93 (3H, d, *J*=6.1 Hz, -CH3), 1.12-1.18 (2H, m, piperidine), 1.72-1.82 (3H) , m, piperidine), 3.25 (1H, y, piperidine), 3.25 (3H, s, $-SO_2CH_3$), 4.38 (2H, s, $-CH_2-$), 4.43 (1H, y, piperidine), 4.45-4.50 (1H) , m, piperidine), 5.18-5.22 (1H, m, piperidine), 7.92 (1H, s, Thiazole), 7.99 (2H, d, *J*=8.6 Hz, 1,4-Disubstitutedbenzene), 8.16 (2H, d, *J*=8.6 Hz, 1,4-Disubstitutedbenzene), 12.67 (1H, s, -NH).

13 C-NMR (**75 MHz, DMSO-*d6***): δ =21.52, 30.38, 33.64, 34.23, 44.02, 50.75, 52.30, 111.86, 126.72, 128.13, 139.21, 140.00, 147.59, 158.81, 166.94, 193.26.

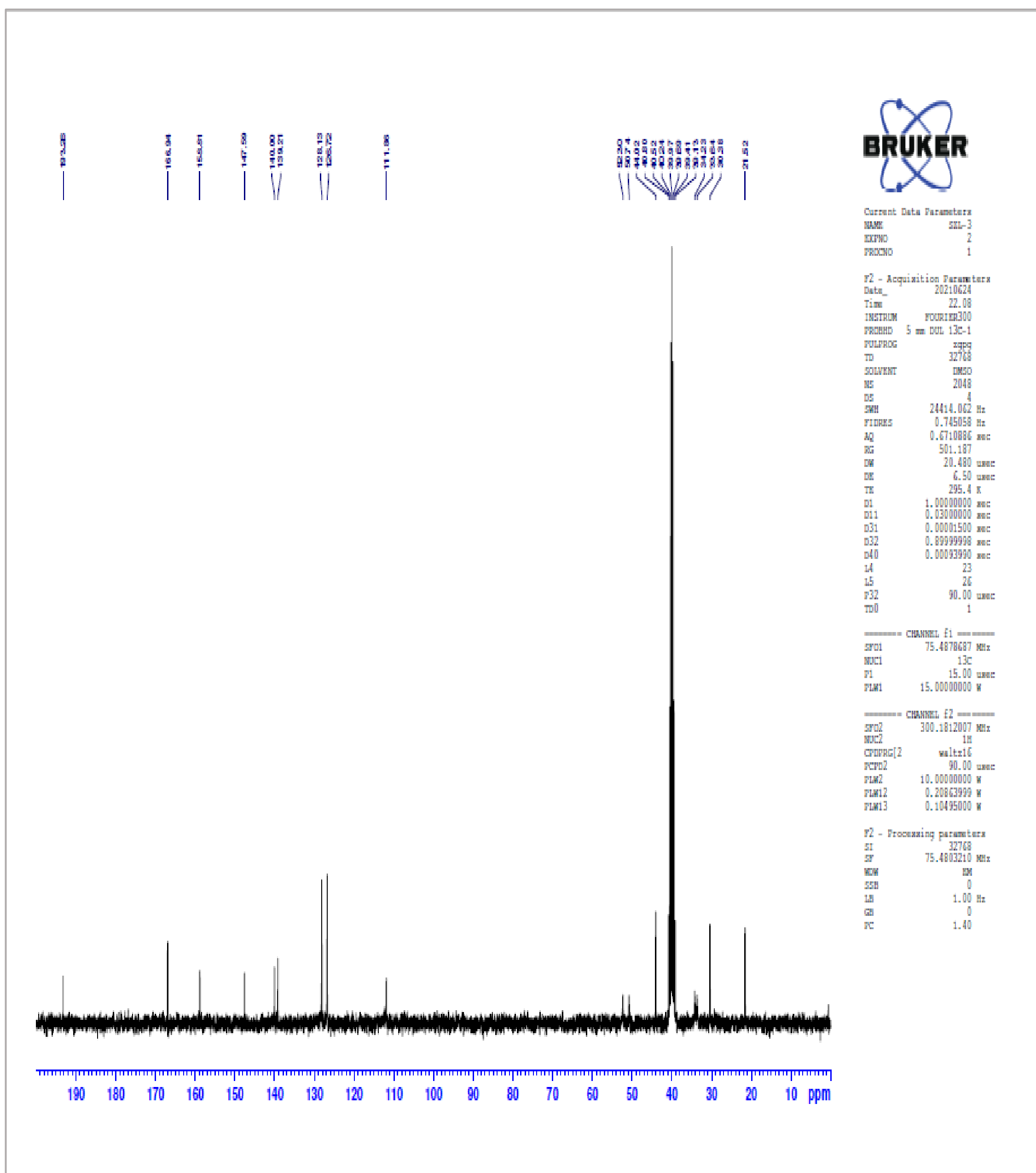

Figure 5.5. *1H-NMR spectrum of Compound* ***5c****.*

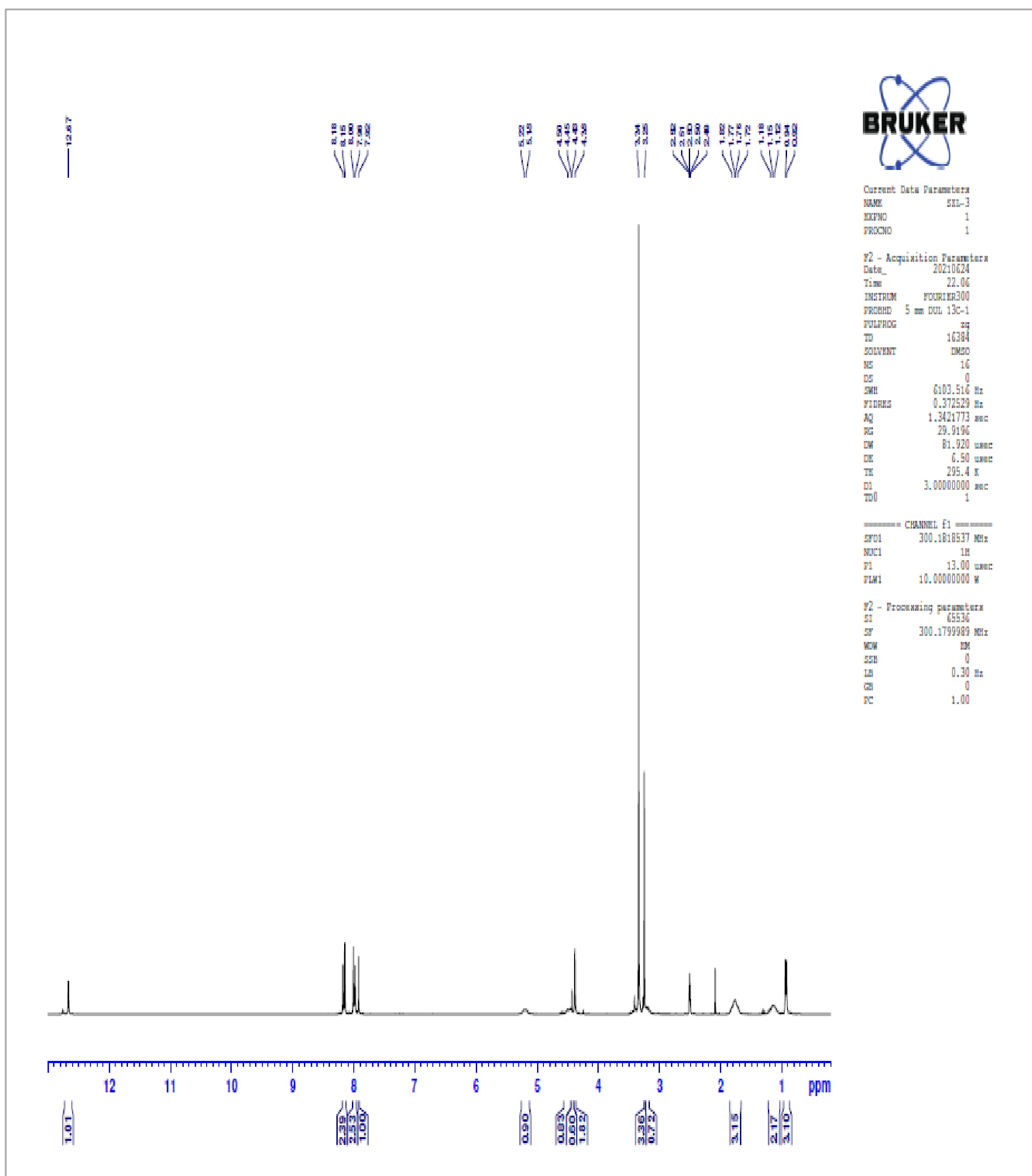

Figure 5.6. *13C-NMR spectrum of Compound* ***5c***.

5.1.4. ***2-((4-(4-(Methylsulfonyl)phenyl)thiazol-2-yl) amino)-2-oxoethyl 4-methylpiperazine-1-carbodithioate (5d)***

Yield: 89 %., MP: 214.8-216.3℃

1H-NMR (300 MHz, DMSO-*d6*): δ = 2.24 (3H, s, -CH3), 2.45 (4H, y, piperazine), 3.24 (3H, s, -SO_2CH_3), 3.95 (2H, y, piperazine), 4.19 (2H, y, piperazin), 4.39 (2H, s, -CH_2-), 7.92 (1H, s, Thiazol), 7.99 (2H, d, *J*=8.5 Hz, 1,4-Disubstitutedbenzene), 8.16 (2H, d, *J*=8.5 Hz, 1,4-Disubstitutedbenzene), 12.68 (1H, s, -NH).

13C-NMR (75 MHz, DMSO-d_6): δ =40.13, 42.76, 44.02, 45.42, 50.10, 51.42, 54.31, 111.88, 126.72, 128.12, 139.19, 140.01, 147.60, 158.80, 166.81, 194.65.

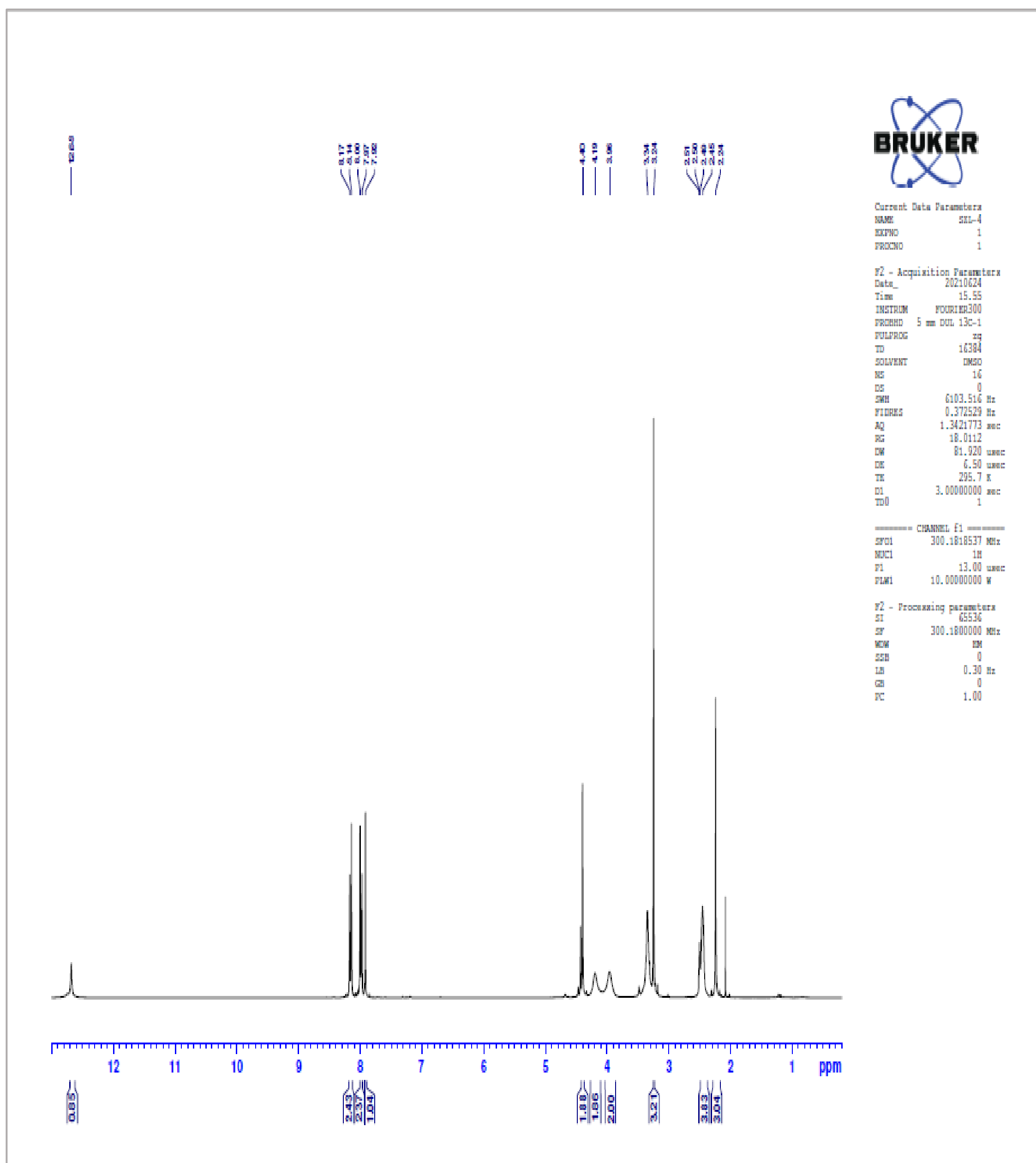

Figure 5.7. *1H-NMR spectrum of Compound* ***5d****.*

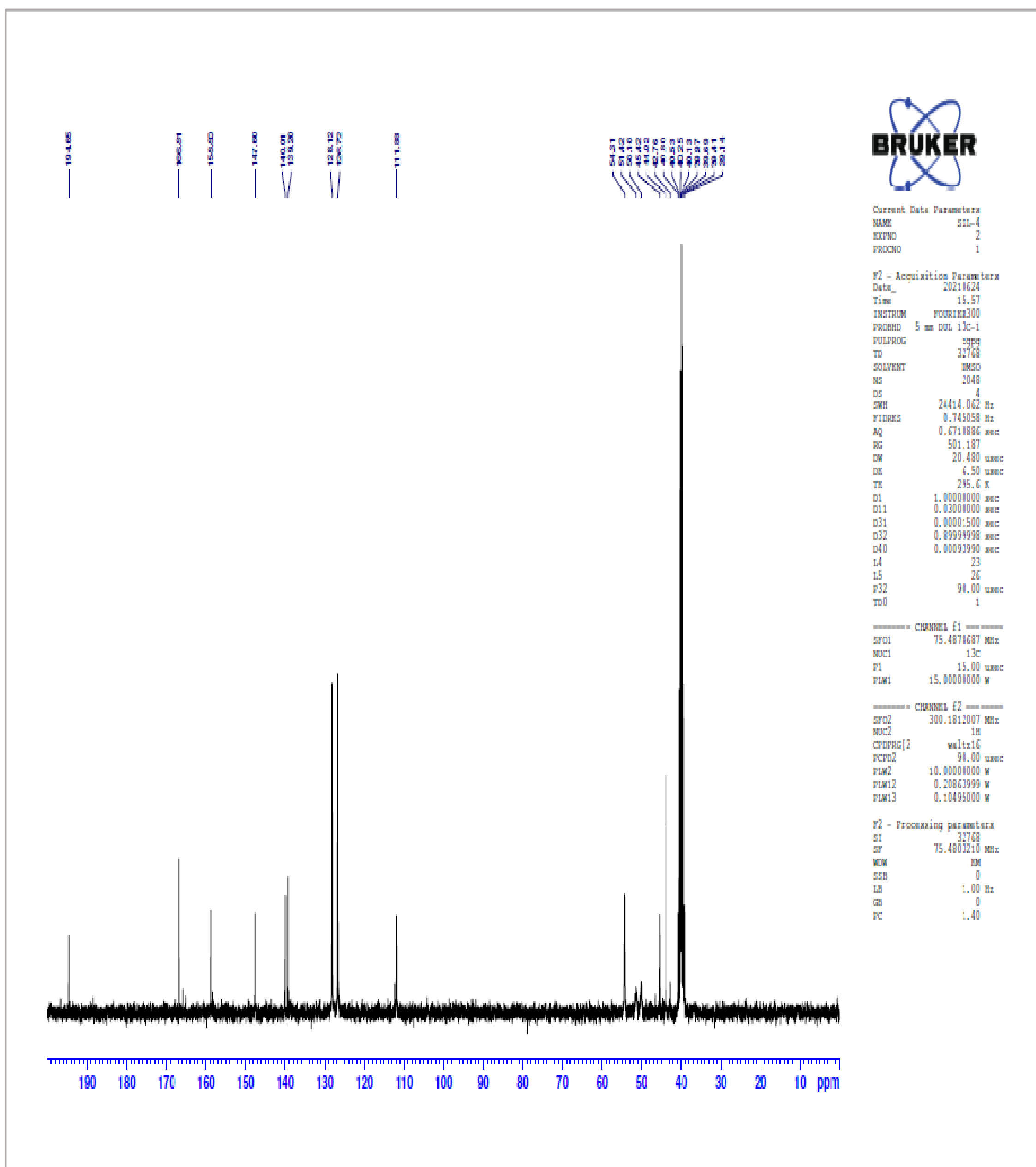

Figure 5.8. *13C-NMR spectrum of Compound* ***5d***

5.1.5. ***2-((4-(4-(Methylsulfonyl)phenyl)thiazol-2-yl)amino)-2-oxoethyl 4-ethylpiperazine-1-carbodithioate (5e)***

Yield: 87 %., MP: 220-221.7℃

1H-NMR (300 MHz, DMSO-*d6*): δ = 1.02 (3H, t, *J*=7.2 Hz, $-CH_3$), 2.39 (2H, q, *J*=6.9 Hz, $-CH_2-$), 2.48-2.50 (4H, m, piperazine), 3.24 (3H, s, $-SO_2CH_3$), 3.95 (2H, y, piperazine), 4.19 (2H, y, piperazine), 4.39 (2H, s, -CH2-), 7.92 (1H, s, Thiazole), 7.99 (2H, d, *J*=8.6 Hz, 1,4-Disubstitutedbenzene), 8.16 (2H, d, *J*=8.5 Hz, 1,4-Disubstitutedbenzene), 12.68 (1H, s, -NH).

13 C-NMR (75 MHz, DMSO-*d6*): δ =12.29, 40.10, 44.03, 50.31, 51.47, 52.10, 111.87, 126.72, 128.12, 139.20, 140.01, 147.60, 158.80, 166.83, 194.44.

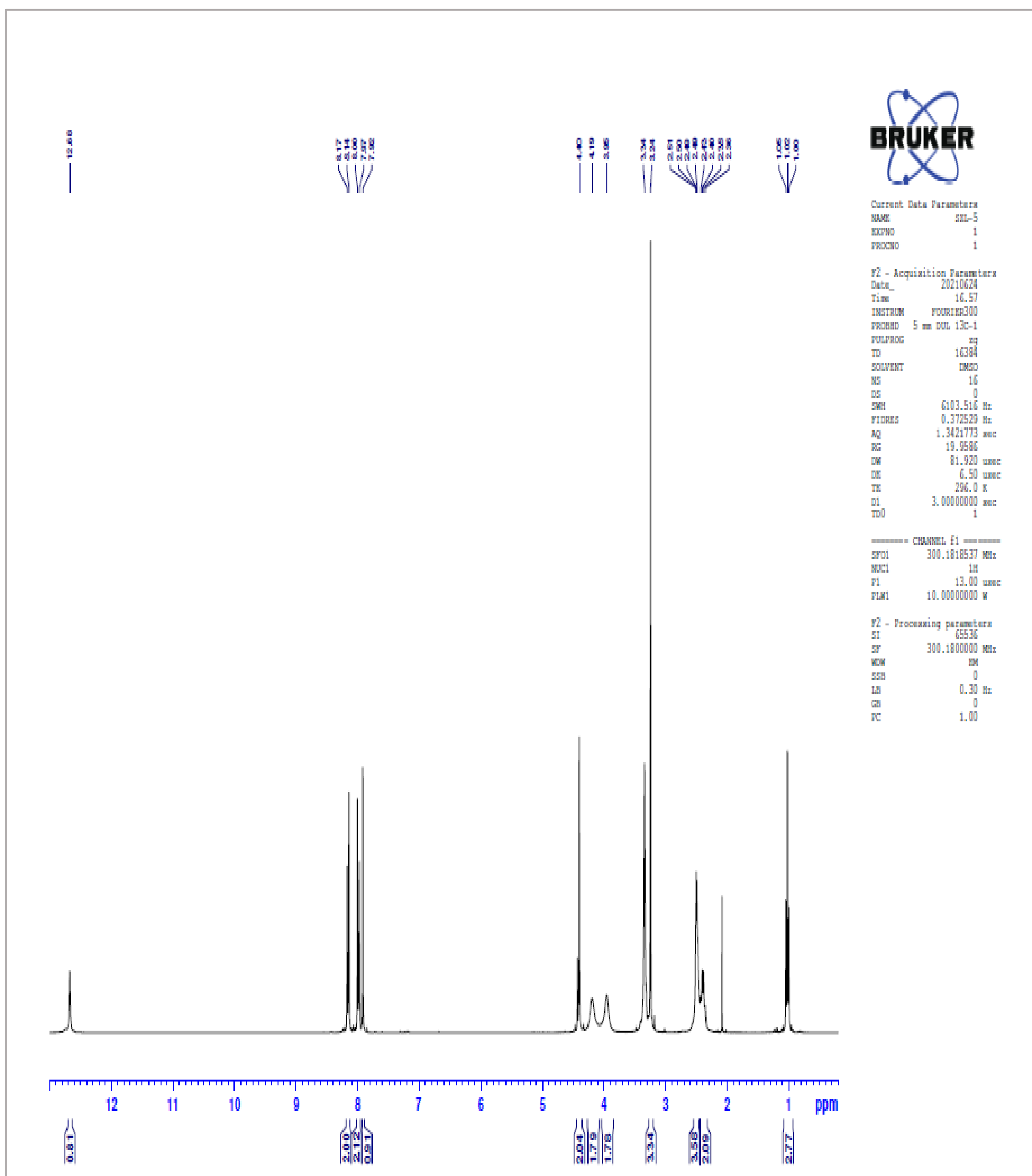

Figure 5.9. *1H-NMR spectrum of Compound* ***5e****.*

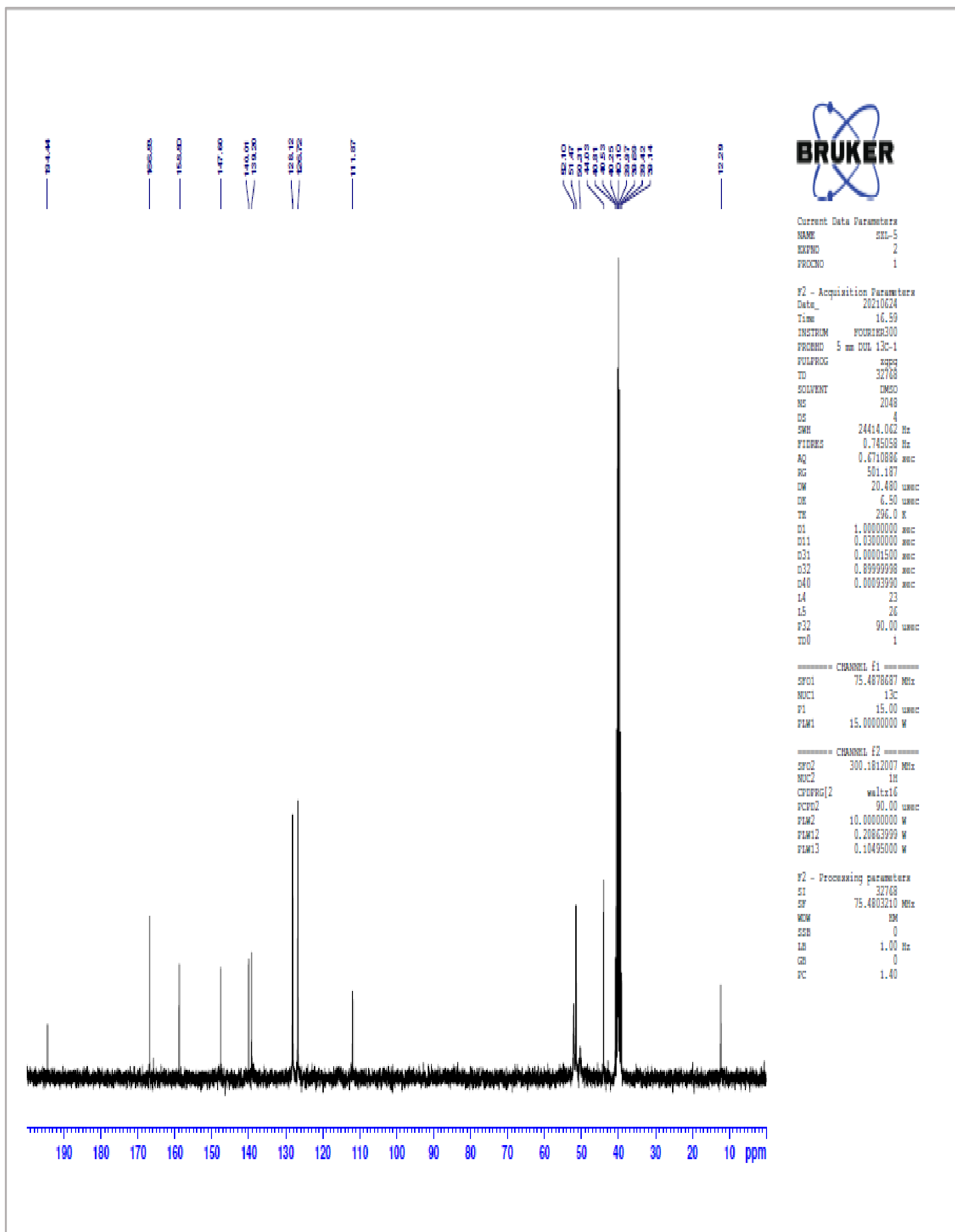

Figure 5.10. *13C-NMR spectrum of Compound* ***5e****.*

5.1.6. ***2-((4-(4-(Methylsulfonyl)phenyl)thiazol-2-yl)amino)-2-oxoethyl 4-cyclohexylpiperazine-1-carbodithioate (5f)***

Yield: 90%., MP: 212-214.3℃

1H-NMR (300 MHz, DMSO-*d6*): δ = 1.16-1.19 (5H, m, cyclohexyl), 1.54-1.59 (1H, m, cyclohexyl), 1.74 (4H, y, cyclohexyl), 2.28-2.29 (1H, m, cyclohexyl), 2.59 (4H, y, piperazine), 3.24 (3H, s, $-SO_2CH_3$), 3.92 (2H, y, piperazine), 4.16 (2H, y, piperazine), 4.39 (2H, s, -CH2-), 7.92 (1H, s, Thiazole), 7.99 (2H, d, *J*=8.5 Hz, 1,4-Disubstitutedbenzene), 8.16 (2H, d, *J*=8.5 Hz, 1.4) -Disubstitutedbenzene), 12.67 (1H, s, -NH).

13 C-NMR (75 MHz, DMSO-*d6*): δ =25.69, 26.26, 28.75, 44.03, 48.52, 50.87, 52.19, 62.73, 111.91, 126.71, 128.12, 139.20, 140.01, 147.60, 158.80, 166.85, 194.14.

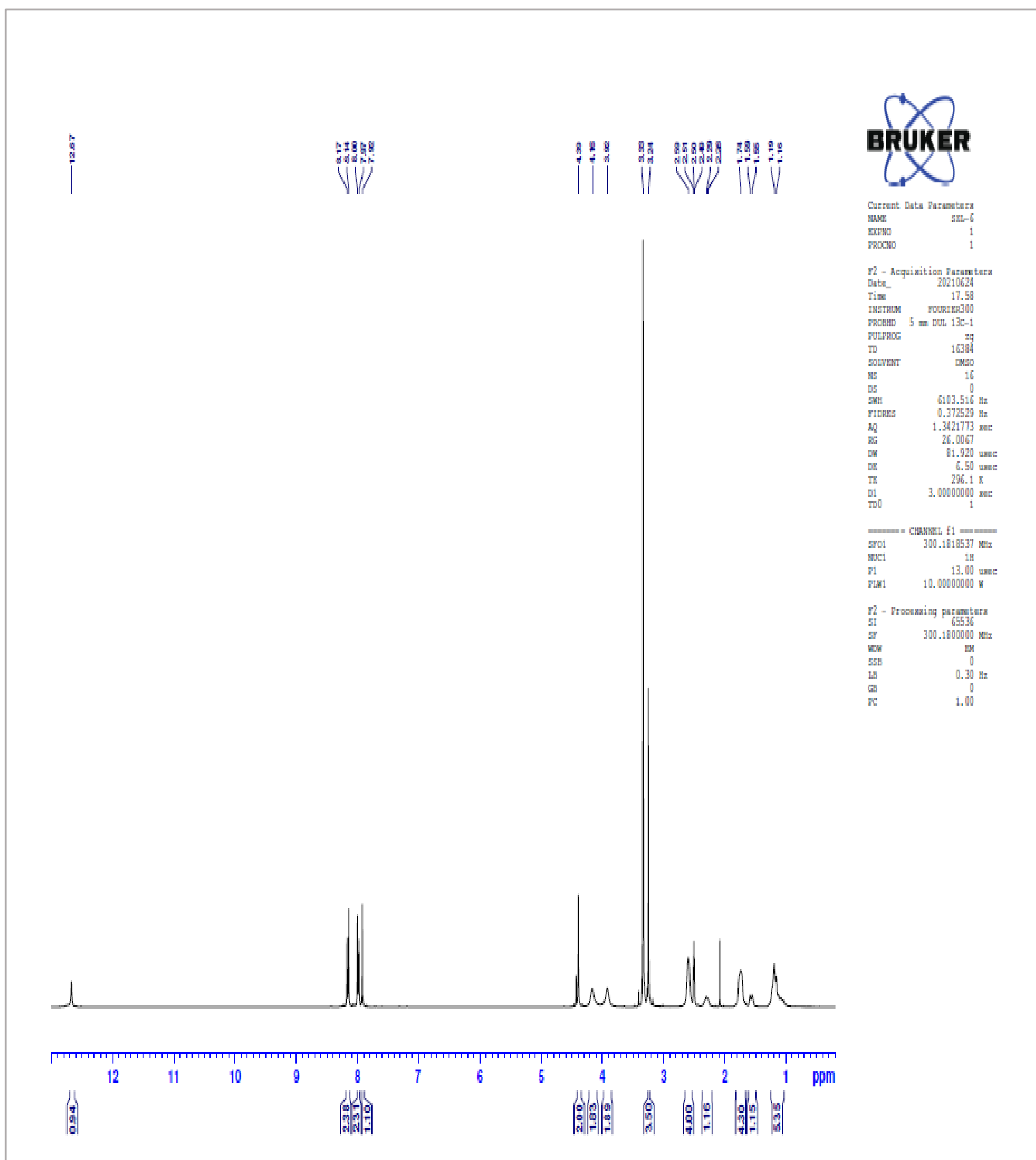

Figure 5.11. *1H-NMR spectrum of Compound **5f**.*

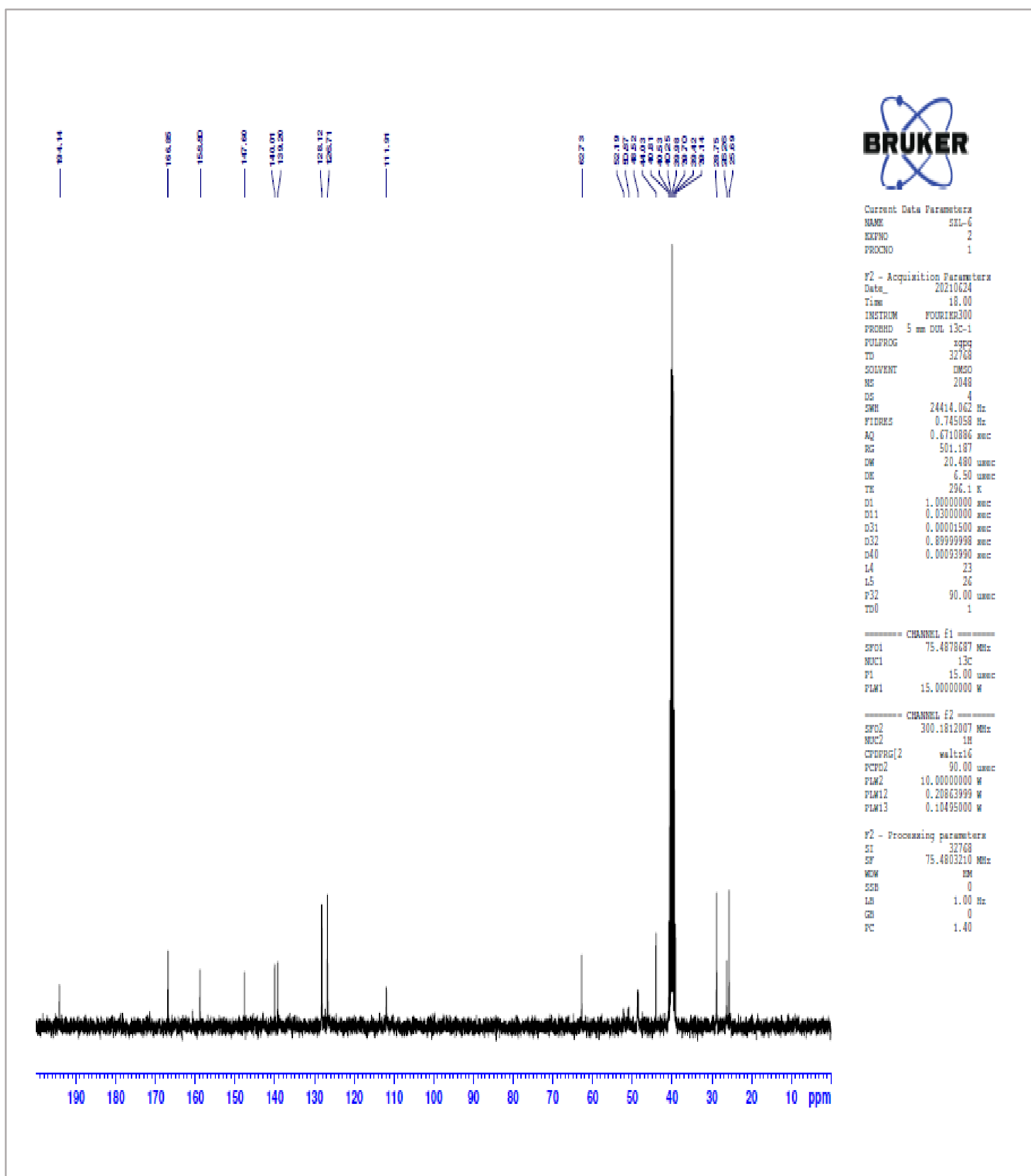

Figure 5.12. *13C-NMR spectrum of Compound* ***5f****.*

5.1.7. ***2-((4-(4-(Methylsulfonyl)phenyl)thiazol-2-yl)amino)-2-oxoethyl 4-phenylpiperazine-1-carbodithioate (5g)***

Yield: 85%., MP: 215-216.8℃

1H-NMR (300 MHz, DMSO-*d6*): δ = 2.69 (1H, y, piperazine), 3.17 (1H, y, piperazine), 3.24 (3H, s, -SO_2CH_3), 3.27-3.31 (4H, m, piperazine), 4.12 (1H, y, piperazine), 4.33 (1H, y, piperazine), 4.43 (2H, s, -CH_2-), 6.79-6.84 (1H, m, Monosubstitutedbenzene), 6.92- 6.97 (2H, m, Monosubstitutedbenzene), 7.22-7.27 (2H, m, Monosubstitutedbenzene), 7.92 (1H, s, Thiazole), 7.97-8.00 (2H, m, 1,4-Disubstitutedbenzene), 8.16 (2H, d, *J*=8.5 Hz, 1,4-Disubstitutedbenzene), 12.69 (1H, s, -NH).

13C-NMR (75 MHz, DMSO-*d6*): δ =40.81, 42.76, 44.02, 48.02, 48.64, 52.94, 111.93, 115.95, 119.76, 126.73, 128.13, 129.53, 139.20, 140.01, 147.61, 150.48, 156.80, 80.80, 139.20, 140.01, 147.61, 150.48, 166.80, 80.80, 194.68.

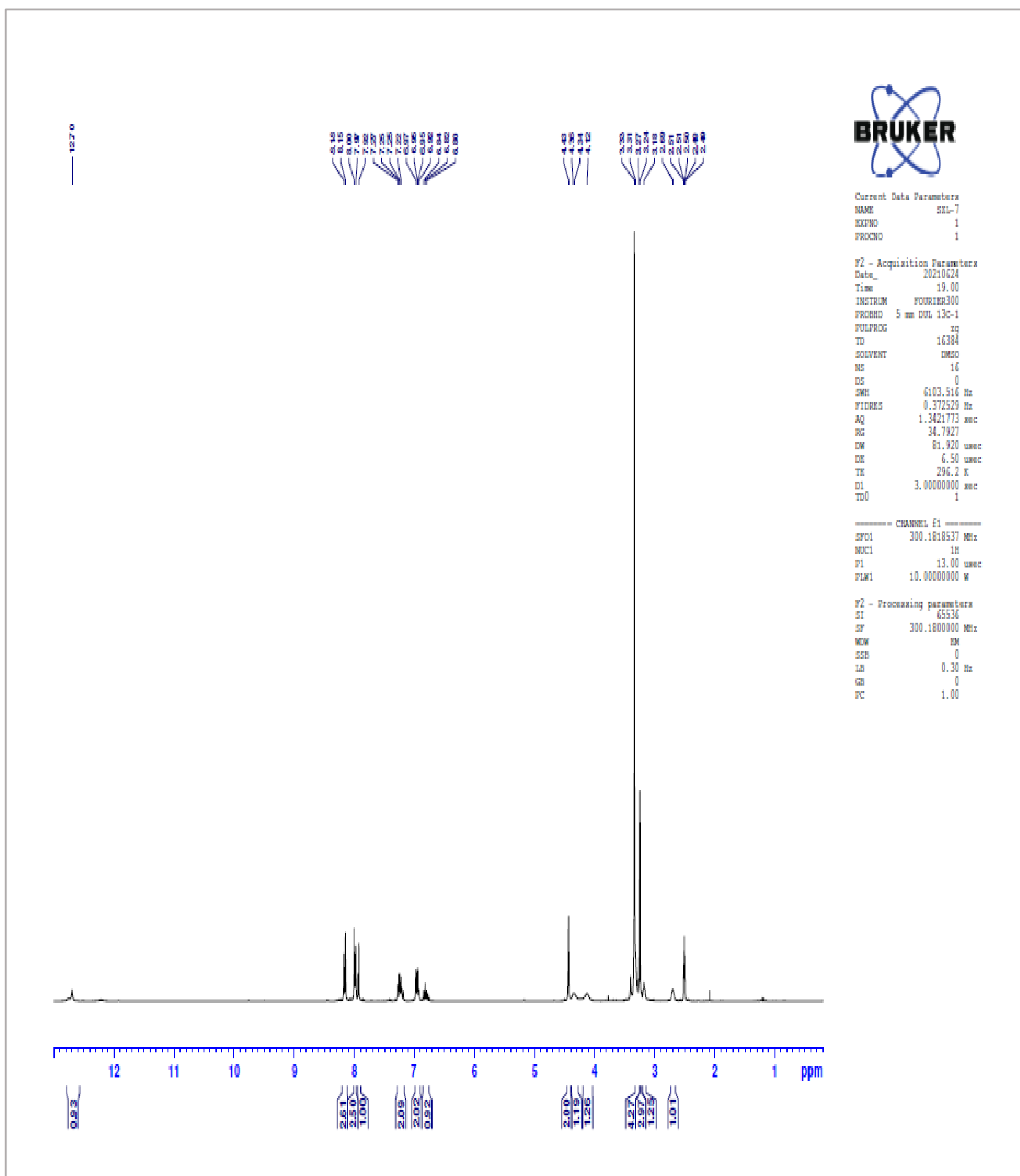

Figure 5.13. *1H-NMR spectrum of Compound* ***5g****.*

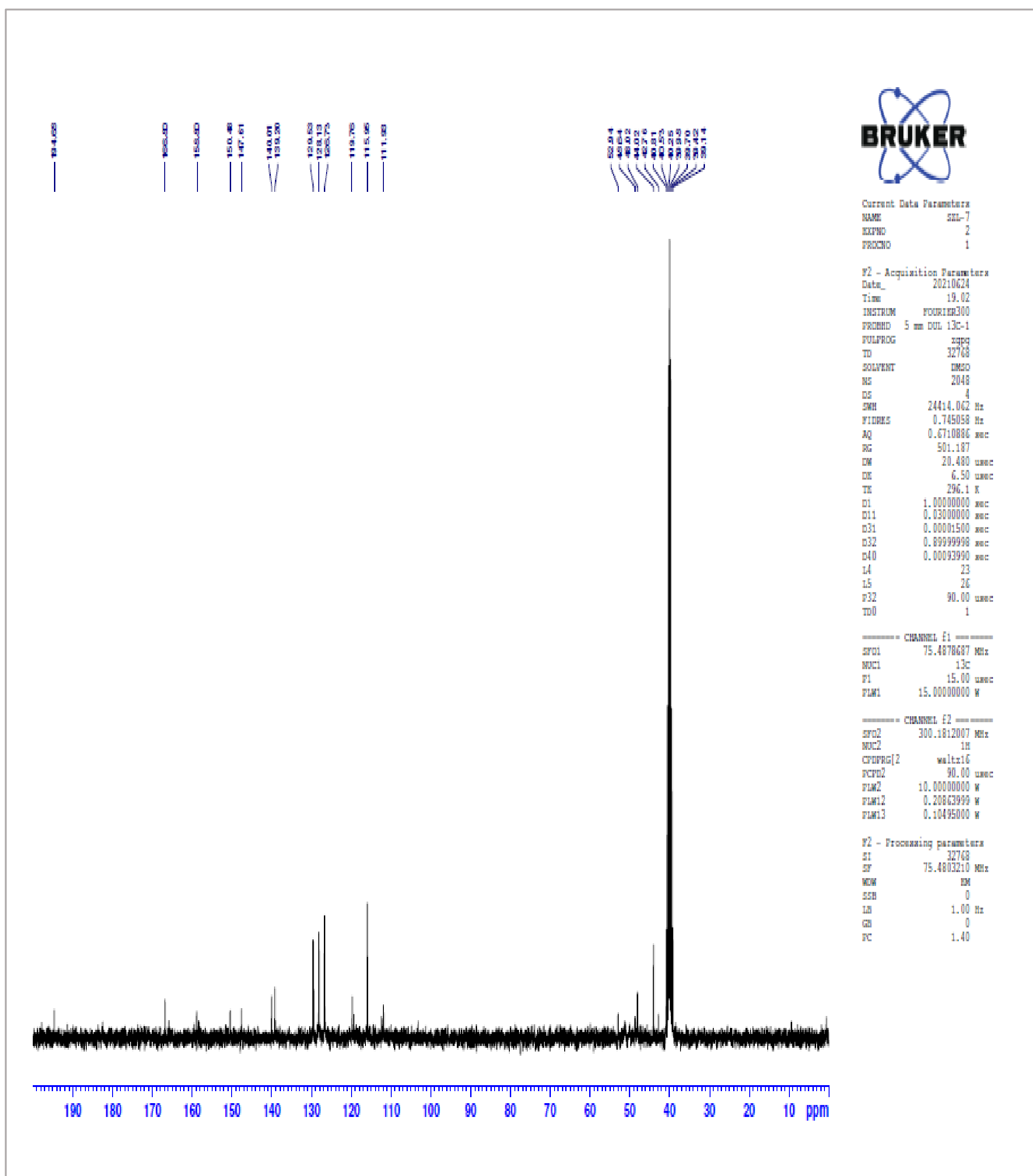

Figure 5.14. *13C-NMR spectrum of Compound **5g***.

5.1.8. ***2-((4-(4-(Methylsulfonyl)phenyl)thiazol-2-yl)amino)-2-oxoethyl 4-(4-methoxyphenyl)piperazine-1-carbodithioate (5h)***

Yield: 79%., MP: 223-224℃

1H-NMR (300 MHz, DMSO-*d6*): δ =3.15 (4H, y, piperazine), 3.25 (3H, s, -SO_2CH_3), 3.69 (3H, s, -OCH_3), 4.11 (2H, y, piperazine), 4.34 (2H, y, piperazine), 4.43 (2H, s, -CH2-), 6.85 (2H, d, *J*=9.2 Hz, 1,4-Disubstitutedbenzene), 6.94 (2H, d) , *J*=9.2 Hz, 1.4-Disubstitutedbenzene), 7.92 (1H, s, Thiazole), 7.99 (2H, d, *J*=8.6 Hz, 1.4-Disubstitutedbenzene), 8.16 (2H, d, *J*=8.6 Hz) , 1,4-Disubstitutedbenzene), 12.70 (1H, s, -NH).

13C-NMR (75 MHz, DMSO-*d6*): δ =40.11, 44.02, 49.81, 51.56, 55.68, 111.88, 114.82, 118.40, 126.73, 128.13, 139.20, 140.01, 144.86, 147.61, 153.89, 158.81, 166.81, 194.67

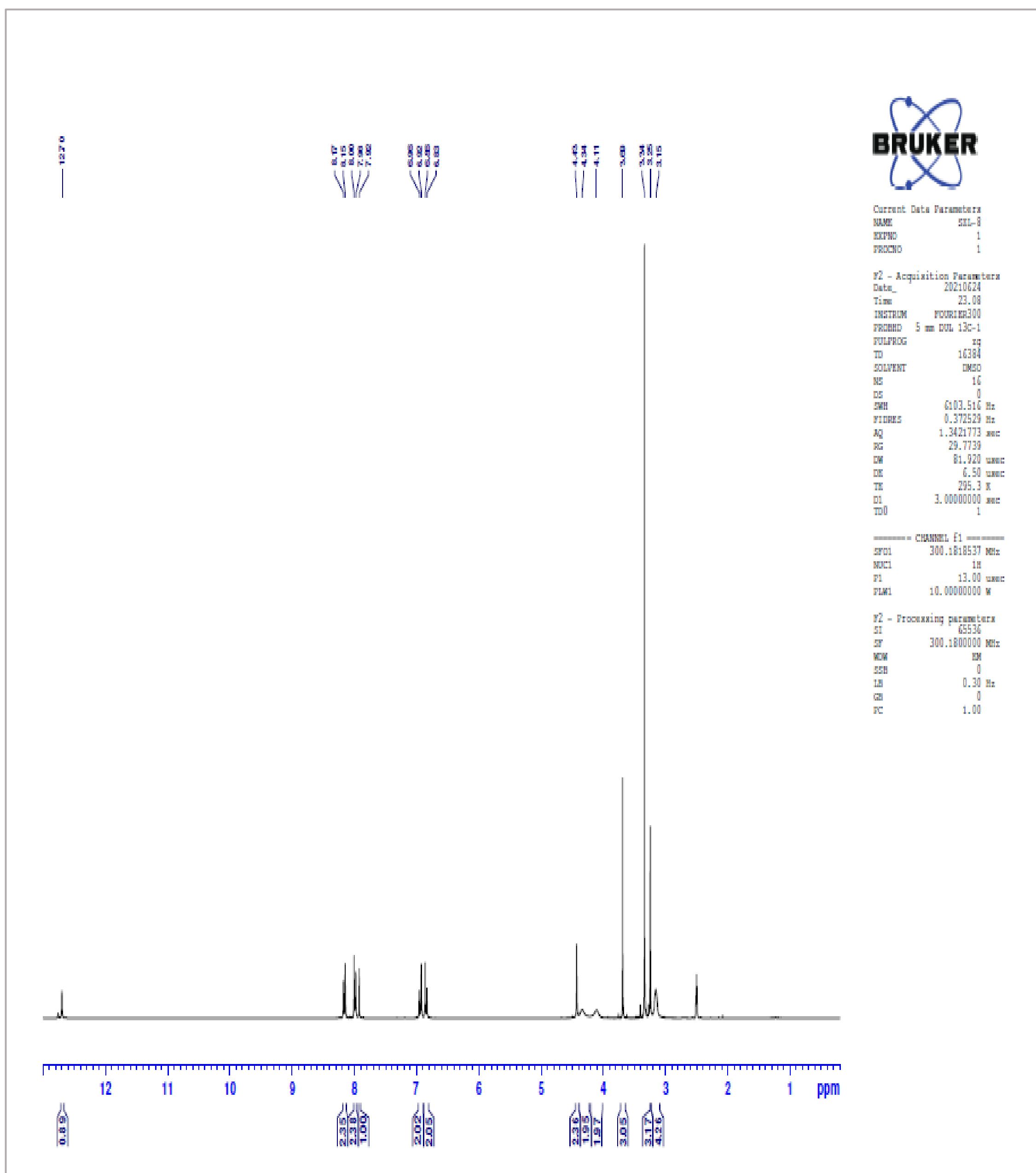

Figure 5.15. *1H-NMR spectrum of Compound* ***5h.***

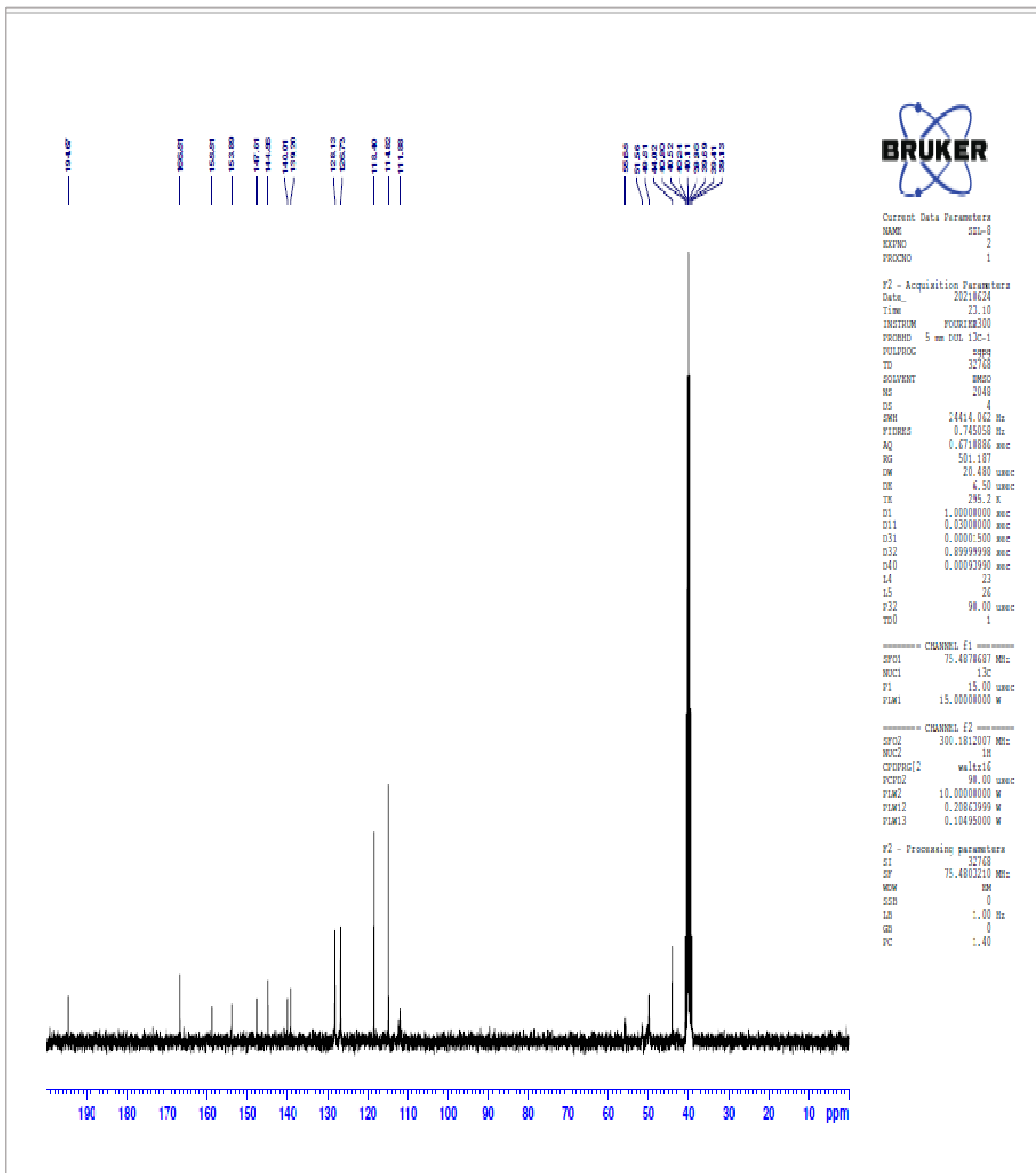

Figure 5.16. *13C-NMR spectrum of Compound **5h**.*

5.1.9. ***2-((4-(4-(Methyl sulfonyl)phenyl)thiazol-2-yl)amino)-2-oxoethyl 4-(4-nitrophenyl)piperazine-1-carbodithioate (5i)***

Yield: 82%., MP: 244-251℃

1H-NMR (300 MHz, DMSO-*d6*): δ =3.25 (3H, s, $-SO_2CH_3$), 3.74 (4H, y, piperazine), 4.17 (2H, y, piperazine), 4.34 (2H) , y, piperazine), 4.44 (2H, s, $-CH_2-$), 6.95 (2H, d, *J*=9.5 Hz, 1,4-Disubstitutedbenzene), 7.92 (1H, s, Thiazole), 7.99 (2H, d, *J*=8.5 Hz, 1,4-Disubstitutedbenzene), 8.10 (2H, d, *J*=9.4 Hz, 1,4-Disubstitutedbenzene), 8.16 (2H, d, *J*=8.6 Hz, 1,4-Disubstitutedbenzene), 12.70 (1H, s, -NH).

13C-NMR (75 MHz, DMSO-*d6*): δ =44.02, 45.08, 48.95, 50.63, 111.94, 112.30, 126.28, 126.73, 128.13, 137.29, 139.19, 140.02, 147.61, 154.15, 158.80, 166.75, 194.82.

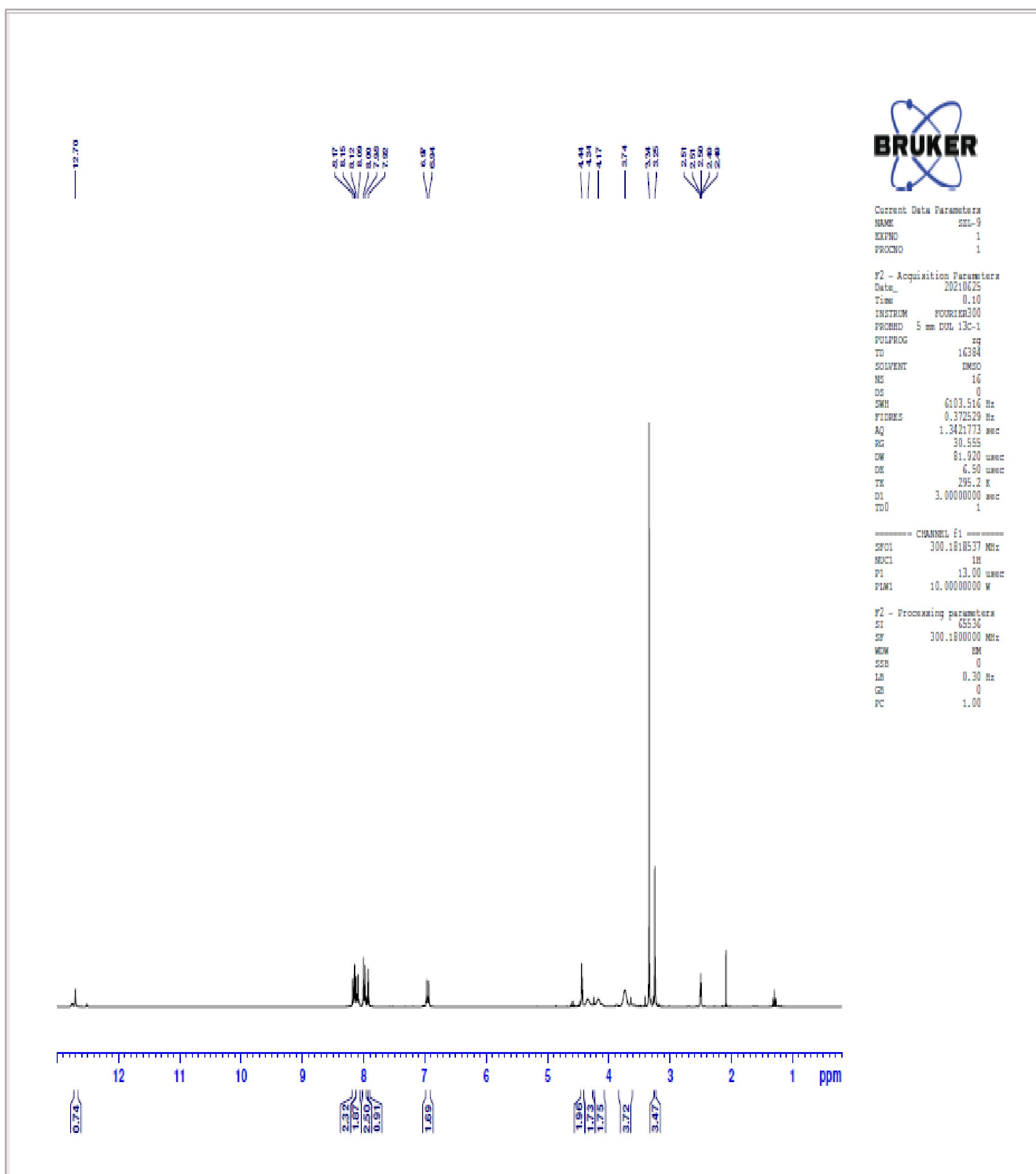

Figure 5.17. *1H-NMR spectrum of Compound **5i**.*

Figure 5.18. *13C-NMR spectrum of Compound **5i.***

5.1.10. ***2-((4-(4-(Methylsulfonyl)phenyl)thiazol-2-yl)amino)-2-oxoethyl 4-(4-trifluoromethylphenyl)piperazine-1-carbodithioate (5j)***

Yield: 81 %., MP: 237-239.5℃

1H-NMR (300 MHz, DMSO-*d6*): δ =3.25 (3H, s, $-SO_2CH_3$), 3.53 (4H, y, piperazine), 4.14 (2H, y, piperazine), 4.34 (2H) , y, piperazine), 4.44 (2H, s, $-CH_2-$), 7.04 (2H, d, *J*=8.8 Hz, 1,4-Disubstitutedbenzene), 7.54 (2H, d, *J*=8.8 Hz, 1.4-Disubstitutedbenzene), 7.92 (1H, s, Thiazole), 7.99 (2H, d, *J*=8.5 Hz, 1,4-Disubstitutedbenzene), 8.16 (2H, d, *J*=8.5 Hz, 1,4-Disubstitutedbenzene), 12.70 (1H, s, -NH).

13C-NMR (75 MHz, DMSO-*d6*): δ =44.02, 46.19, 49.46, 50.99, 111.89, 114.13, 118.09, 119.19, 126.73, 126.83, 128.13, 139.19, 140.01, 147.61, 152.57, 158.80, 166.78,

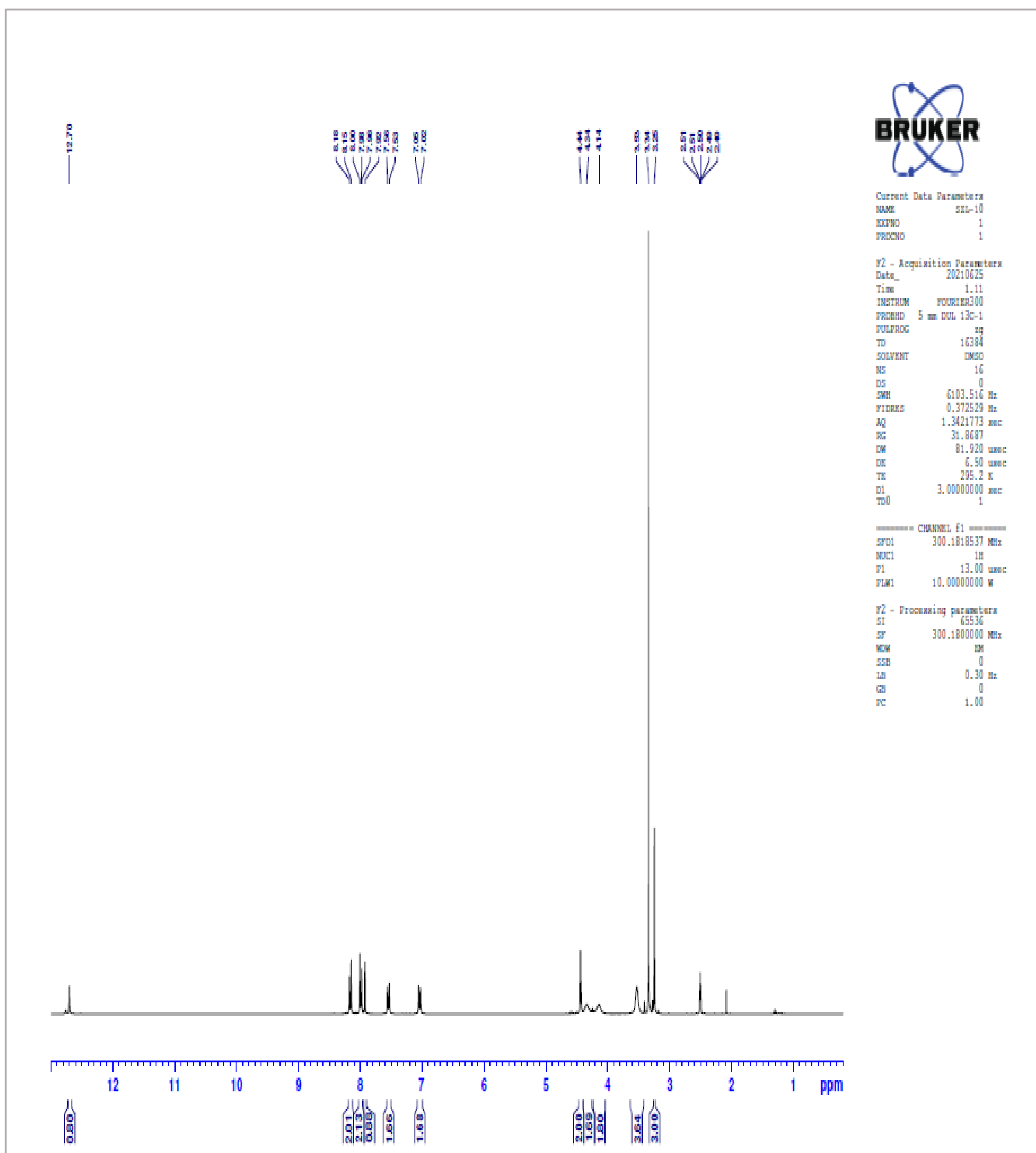

Figure 5.19. 1H-NMR spectrum of Compound **5j.**

Figure 5.20. 13C-NMR spectrum of Compound **5j**.

5.2. Evaluation of Spectral Data

The structures of 10 original compounds, coded **5a-5j**, which were synthesized, were clarified with 1HNMR and 13C-NMR methods.

5.2.1. Evaluation of 1H-NMR Spectra

As predicted, the synthesized compounds' protons produced peaks in their 1H-NMR spectra. The total hydrogen number in the acquired compound spectra was identified, and the anticipated number of peaks were seen

The pyrrolidin gave a peak in 1.90-1.97 and 2.02-2.09 in 5a coded compound while the piperidine gave the peak in 1.61-1.65 , 3.94 , and 4.19 in 5b and in 5c it gave the peak in a range of 1.12-1.18 , 1.72-1.82 , 4.45-4.50 and 5.18-5.22.

The chemical structures of the compounds share a piperazine ring, a benzothiazole structure, and a 1,4 disubstituted benzene ring, according to an analysis of the compound's 1H-NMR data and a chemical diagram. The table below lists the piperazine ring's protons.

Table 5.1. *The protons of the piperazine ring.*

Compound	PPM		
5d	2.45, 4H	3.95, 2H	4.19, 2H
5e	2.48-2,50, 4H	3.95, 2H	4.19, 2H
5f	2.59, 4H	3.92, 2H	4.16, 2H
5g	2.69, 1H	3.17, 1H 3.27-3.31, 4H	4.12, 1H
5h	3.15, 4H	4.11, 2H	4,34, 2H
5i	3.47, 4H	4.12, 2H	4.34, 2H
5j	3.33, 4H	4.14, 2H	4.34, 2H

According to 1H NMR results of the thiazole compounds in the structure (**5a-5j**), the striking details are similar with chemical shift equal to 7.92 in all structure. In the **5a** coded compound, 2 hydrogen multiples were observed between the values of 1.90- 1.97 and 2.20-2.09 ppm.

5.2.2. ***Evaluation of 13C-NMR spectra***

All of the expected Carbone atoms were present in the 13C-NMR spectrums of the synthetic chemical samples. Other aliphatic carbons provided a peak between 12.2 and 62.73 ppm, while aromatic carbons produced a peak between 111.85 and 194.82 ppm.

5.3. In vitro COX-1 and COX-2 Inhibition Assay

The results of substances' COX-1/COX-2 inhibitory efficacy determined by fluorometric assay. Synthesized and reference compounds prepared in inhibition experiments at 10^{-3} and 10^{-4} molar concentrations. After calculating the percent inhibition values at 10^{-3}-10^{-4} M concentrations, the IC_{50} values of the selected compounds were calculated with the help of the graph obtained by nonlinear regression analysis. The results show the highest COX-2 inhibition in **5c** (89.3±0.9 %, 10^{3}) and in comparison with (79.3±1.1 %, 10^{3}) for COX-1 inhibition, while, the reference compound (Ibuprofen, Celecoxib, Nimesulide) show (98.6±1.0, 92.9±1.8, 98.5±2.0 %)in 10^{3} M for COX-2 inhibition respectively. The inhibition potency of **5a** and **5b** are approximate to **5c.** With an IC50 of 0.1800.002 M, the **5a** derivative was found to be the most active in the series.

Table 5.2. *% Inhibition of the synthesized compounds, ibuprofen, celecoxib and nimesulide against COX-1 and COX-2 enzymes.*

Compounds	COX-1 % Inhibition		COX-2 % Inhibition	
	10^{-3} M	10^{-4} M	10^{-3} M	10^{-4} M
5a	81.6±1.9	24.1±1.3	**85.4±1.8**	**72.9±1.2**
	79.9±1.7	32.9±0.8	**86.2±1.5**	**62.9±1.0**
5c	79.3±1.1	44.4±1.0	**89.3±0.9**	**69.1±0.8**
5d	29.4±0.6	11.2±0.5	28.1±0.4	6.6±0.1
5e	57.7±1.9	10.7±0.4	57.1±1.1	18.7±1.2
5f	82.3±1.8	48.8±1.2	80.8±1.5	38.3±1.3
5g	84.4±1.4	47.3±1.4	82.3±1.7	49.9±1.2
5h	86.9±1.3	31.7±0.7	89.3±1.4	34.6±1.0
5i	83.2±1.3	47.9±0.7	84.7±2.0	48.3±1.6
5j	83.7±1.3	31.5±1.0	85.3±1.2	50.9±0.9
Ibuprofen	98.7±0.9	88.9±1.1	98.6±1.0	89.9±1.7
Celecoxib	-	-	92.9±1.8	86.1±1.0
Nimesulide	-	-	98.5±2.0	89.9±2.1

Table 5.3. *IC_{50} values of 5a, 5b, 5c, ibuprofen, celecoxib and nimesulide against COX-2.*

Compounds	IC_{50} (µM)
5a	**0.180±0.002**
5b	2.827±0.046
5c	5.769±0.354
Ibuprofen	5.589±0.278
Celecoxib	0.132±0.004
Nimesulide	1.692±0.077

5.4. Molecular Docking Study

To support the new synthetic derivatives' increased potency (**5a-5j**), Molecular docking was used to look at the most powerful inhibitor's potential method of binding (**5a**). Docking studies was performed on the COX-2 crystals (PDB ID:3LN1) [70]. For approved binding model. Compound **5a** and celecoxib's location in the COX-2 enzyme's active region depicted in Figure 5.21. (PDB ID: 3LN1). A careful examination of Figure 5.21 shows that compound **5a** localized to the enzyme active site in the same way as celecoxib. Even celecoxib and compound **5a** overlap.

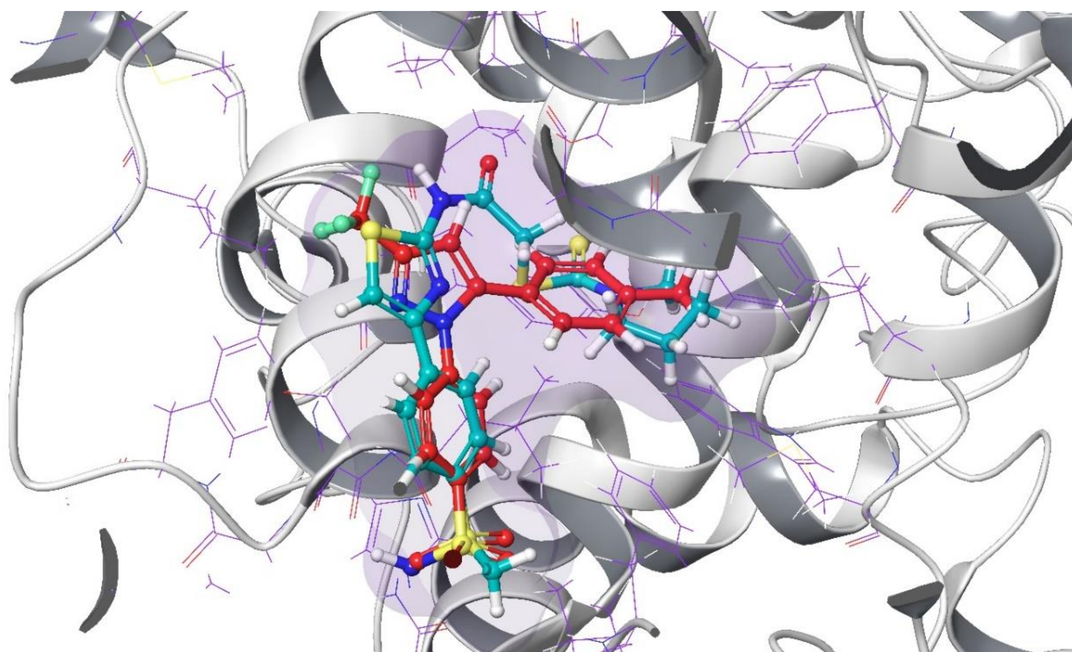

Figure 5.21. *The super imposition poses of celecoxib and compound 5a in the enzyme active site (PDB Code: 3LN1) (blue molecule:**5a**; red molecule:celecoxib).*

Figure 5.22 represents the two-dimensional placement of chemical **5a** in the COX-2 enzyme's active region (PDB ID: 3LN1). First, the amino group of Arg106 interacts with the thiadiazole ring of molecule **5a** to generate a π -cation connection. Additionally, phenyl ring forms a π-π interaction with hydroxyl group of Tyr341.

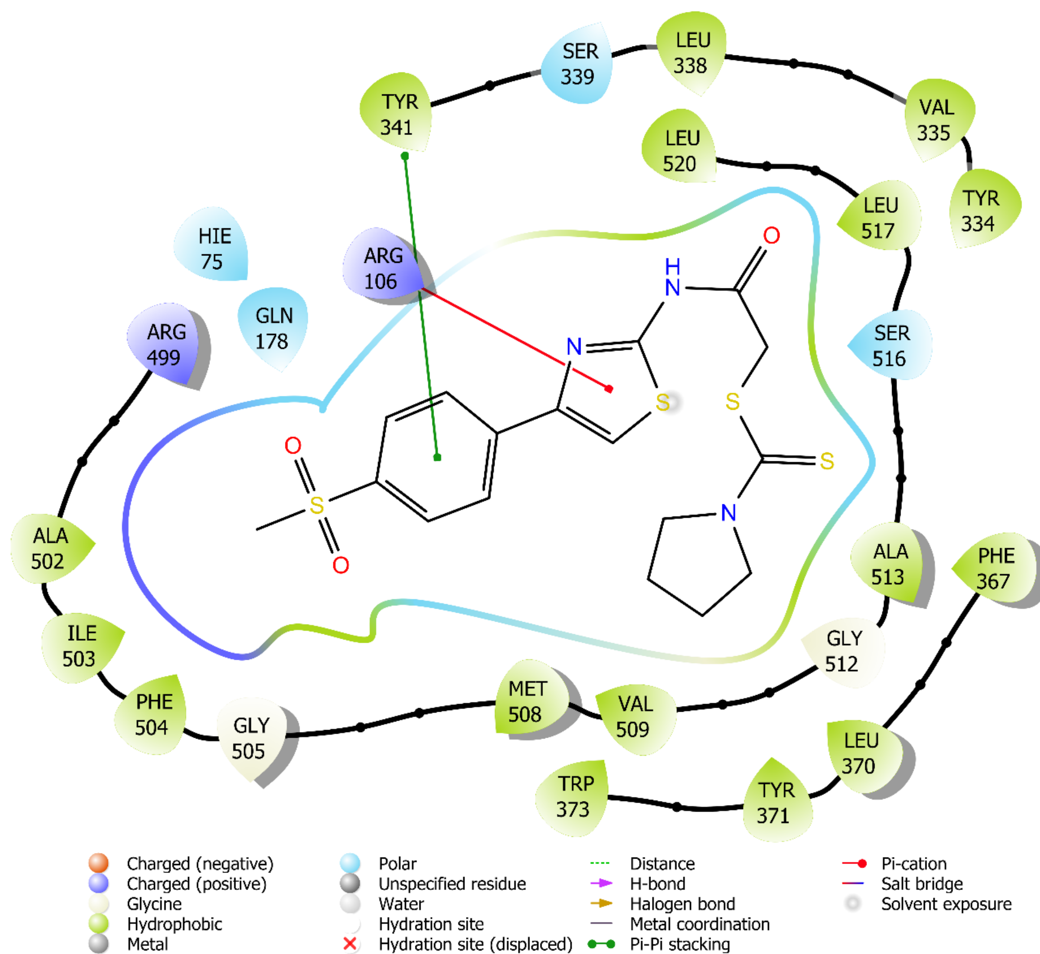

Figure 5.22. *The two-dimensional interacting mode of compound* ***5a*** *in the active region of COX-2 enzyme (PDB ID: 3LN1).*

Figure 5.23 depicts the three-dimensional location of chemical **5a** in the COX-2 enzyme's active site (PDB ID: 3LN1). In addition to the interactions seen in 2D poses, molecule **5a** also forms three aromatic hydrogen bonds when its three-dimensional positions are analyzed (Figure 5.23). The phenyl rings of compound **5a** and Tyr341 form the first of these aromatic hydrogen bonds. The second is created when the phenyl ring of Phe504 and its methyl sulfonyl group combine. Last but not least, an aromatic hydrogen bond was seen between the amino acid Hie75's imidazole ring and its methyl sulfonyl group (Figure 5.23). Compound **5a**'s three-dimensional interaction with the COX-2 enzyme's active area (PDB).

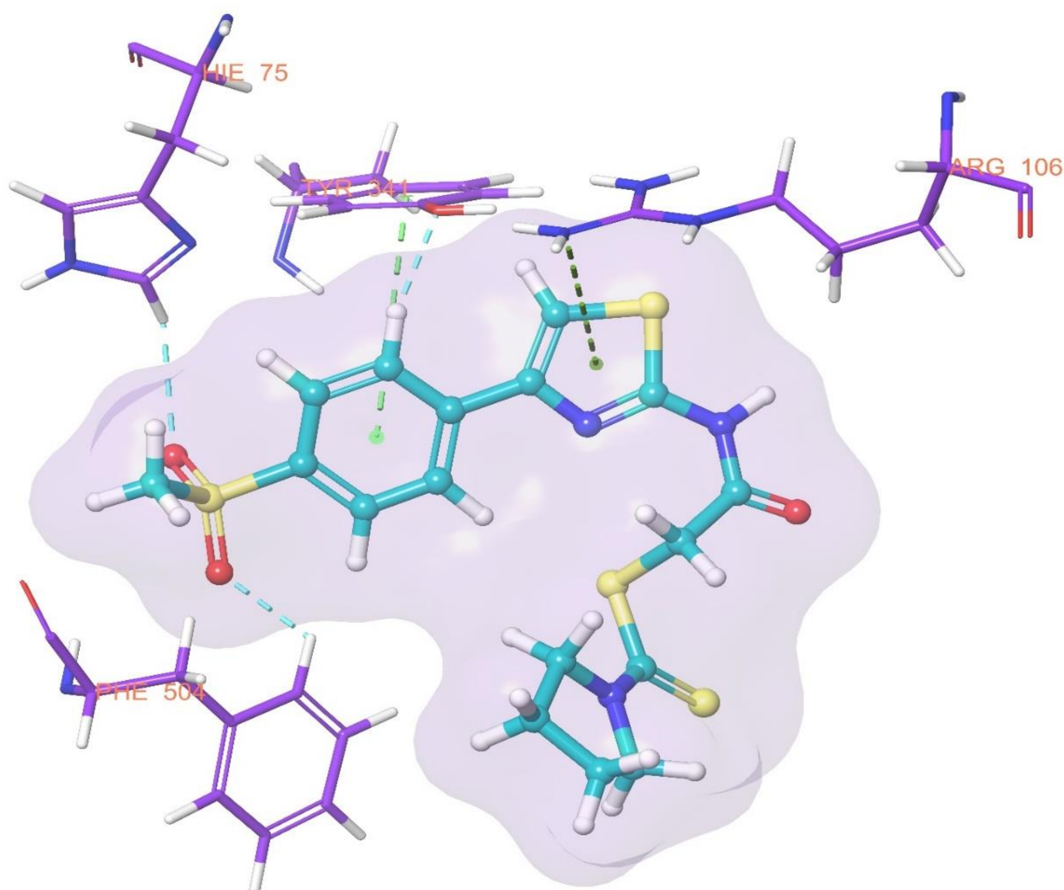

Figure 5.23. *The three-dimensional interacting mode of compound* ***5a*** *in the active region of COX-2 enzyme (PDB ID: 3LN1).*

6. CONCLUSIONS AND RECOMMENDATIONS

Ten derivatives were shown to be COX enzyme inhibitors in this study. The capacity of these substances to reduce COX-1 and COX-2 activity in vitro has been tested. In comparison to the reference drugs Ibuprofen, Celecoxib, and Nimesulide, the compounds **5a**, **5b**, and **5c** were found to be potent. The most effective COX-2 inhibitor was **5a**, which was further explored for its probable method of binding by molecular docking research, which revealed that **5a** is localized to the enzyme active site similarly to celecoxib.

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