



**SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL
HYDRAZONE DERIVATIVES FOR THE TREATMENT
OF ALZHEIMER'S DISEASE**

PhD Thesis

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PhD THESIS

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JURY AND INSTITUTE APPROVAL

Sazan Jameel HAJI ALI's thesis titled "SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL HYDRAZONE DERIVATIVES FOR THE TREATMENT OF ALZHEIMER'S DISEASE" was evaluated by the following jury on 13/06/2024 and in accordance with the relevant articles of the "Anadolu University Graduate Education Institute and Examination Regulation", in the Department of Pharmaceutical Chemistry. It was accepted as a PhD thesis.

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ABSTRACT

SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL HYDRAZONE DERIVATIVES FOR THE TREATMENT OF ALZHEIMER'S DISEASE

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Supervisor: Prof. Dr. Zafer Asım KAPLANCIKLI

In recent years, Alzheimer's disease has been addressed as a silent epidemic neurodegenerative disorder. Due to its complex pathophysiology, there is significant scientific interest in developing effective treatments that go beyond symptomatic relief. The goal is to raise the patient's quality of living and reduce the mortality rate associated with Alzheimer's, a goal that has not yet been fully realized, thus keeping Alzheimer's research in focus.

In this PhD thesis, we designed a set consisting of 27 hybrid molecules based on the molecular structure of donepezil, as an acetylcholinesterase inhibitor (AChEI). The hybrid molecules feature dihydrothiazolyl hydrazone and phenyl piperidine moieties. The produced compounds were assessed for their biological activity and cytotoxicity using IR, NMR, and HRMS spectroscopy. The biological activity studies comprise that the compound coded **D1.f** was the most active one among the other 27 derivatives against AChE enzyme. The inhibitory activities (IC_{50} values) of this compound on the relevant enzymes ranges from **0.039 ± 0.001** μ M, and its IC_{50} values in the NIH3T3 fibroblast cell line value as **3.324 ± 0.155** μ M with the highest value of selectivity index SI **85.231** among other derivatives. Among all derivatives, **D1.f** demonstrated the best AChEI activity and optimal docking poses and stability in the molecular dynamics studies. based on obtained data, molecule **D1.f** was a candidate for further investigations.

Keywords: Acetylcholine Esterase Inhibitors, Cytotoxicity, Alzheimer's Disease, Piperidine, Thiazole ring, Hantzsch method.

ÖZET

ALZHEIMER HASTALIĞININ TEDAVİSİNDE YÖNELİK YENİ HİDRAZON TÜREVLERİNİN SENTEZİ VE BİYOLOJİK AKTİVİTELERİNİN DEĞERLENDİRİLMESİ

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Danışman: Prof. Dr. Zafer Asım KAPLANCIKLİ

Son yıllarda Alzheimer hastalığı, sessiz salgın nörodejeneratif bir hastalık olarak ele alınmaktadır. Karmaşık patofizyolojisi nedeniyle, semptomatik iyileşmenin ötesine geçen etkili tedavilerin geliştirilmesine yönelik önemli bir bilimsel ilgi vardır. Alzheimer araştırmalarının odak noktasında hastanın yaşam kalitesini yükseltmek ve hastalığının ilerlemesi sonucu meydana gelebilen Alzheimer'a bağlı ölüm oranını azaltmak yer almaktadır.

Bu doktora tezinde asetilkolinesteraz inhibitörü (AChEI) olan donepezilin moleküler yapısını temel alarak 27 hibrit molekülden oluşan bir bileşik serisi tasarlanmıştır. Hibrit moleküller, dihidrotiyazolil hidrazon ve fenil piperidin kısımlarına sahiptir. Üretilen bileşiklerin yapıları IR, NMR ve HRMS spektroskopisi kullanılarak doğrulanmış ardından biyolojik aktiviteleri ve sitotoksisiteleri değerlendirilmiştir. Biyolojik aktivite çalışmaları **D1.f** kodlu bileşiğin AChE enzimine karşı diğer 27 türev arasında en aktif türev olduğunu göstermektedir. Bu bileşiğin ilgili enzimler üzerindeki inhibitör aktiviteleri (IC_{50} değerleri) $0,039 \pm 0,001 \mu M$ arasında değişmektedir ve NIH3T3 fibroblast hücre hattındaki IC_{50} değerleri $3,324 \pm 0,155 \mu M$ olup, diğer türevler arasında SI 85,231 ile en yüksek seçicilik indeksine sahiptir. Tüm türevler arasında **D1.f**, moleküler dinamik çalışmalarında en iyi AChEI aktivitesini, optimal kenetlenme pozlarını ve stabiliteyi göstermiştir. Elde edilen verilere dayanarak **D1.f** molekülü daha ileri araştırmalara aday konumdadır.

Anahtar Kelimeler: Asetilkolin Esteraz İnhibitörleri, Sitotoksiste, Alzheimer Hastalığı, Piperidin, Tiyazol halkası, Hantzsch yöntemi.

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Finally, I merely wish to say thank you to all of my friends who have helped me along the way.

This thesis is dedicated to all those who have helped me along the way. Thank you for being part of this journey.

DECLARATION OF COMPLIANCE WITH ETHICAL RULES AND PRINCIPLES

I certify that I am the author of this thesis and its my original work. I have followed scientific ethical guidelines and standards at every turn of this investigation, from planning to gathering data to analyzing and presenting findings. Every source of data and material utilized in this work has been appropriately acknowledged, and the sources are listed in the bibliography. This study has been screened using the " scientific program for detecting plagiarism " utilized by Anadolu University, and it has been confirmed that it does not contain any plagiarism. I acknowledge and accept all moral and legal consequences that may arise if any violation of this statement is discovered at any point.

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Sazan Jameel HAJI ALI

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LIST OF ABBREVIATIONS

Abbreviations	Full Name
ACh	:Acetylcholine
AChE	:Acetylcholinesterase
AChEI	:Acetyl Choline Esterase Inhibitor
AChR	:Acetylcholine Receptor
AD	:Alzheimer's Disease
ADMET	:Absorption, Distribution, Metabolism, Excretion and Toxicity
AGEs	:Glycation end products
ALS	:Amyotrophic Lateral Sclerosis
APP	:Amyloid Precursor Protein
ATR	:Attenuated Total Reflectance
A β	:Amyloid β
A β F	:A β Fibrils
A β O	:A β Oligomers
BACE1	:Beta Secretase 1
BBB	:Blood Brain Barrier
BChE	:Butyrylcholine esterase
CAS	:Catalytic Active Site
DPPH	:1,1-Diphenyl-2-picrylhydrazyl
ER	:Endoplasmic Reticulum
ESI	:Electron spray ionization
FDA	:Federation of Food And Drug Administration
Glu	:Glutamate
Glu	:Glutamic acid
HD	:Huntington's disease
HIA	:Human Intestinal Absorption
His	:Histidine

MAPT	:Microtubule-Associated Protein Tau
MPTP	:Mitochondrial Permeability Transition Pore
MS	:Multiple Sclerosis
MT	:Microtubule
MTDLs	:Multi-Target Directed Ligands
NFT	:Neurofibrillary Tangle
NMDA	:N-methyl-D-aspartate receptors
NMDAR	:N-Methyl D-Aspartate Receptor
OD	:Optical Density
PAS	:Peripheral Anionic Domain
PD	:Parkinson's disease
PDB	:Protein Data Bank
Phe	:Phenylalanine
P-tau	:Hyperphosphorylated Tau
ROS	:Reactive Oxygen Species
Ser	:Serine
SFPQ	:Splicing Factor Proline And Glutamine-Rich
Thr	:Threonine
Trp	:Tryptophan
Tyr	:Tyrosine
WHO	:World Health Organization
β S	: β -Secretase
γ S	: γ -Secretase

1. INTRODUCTION

The National Institutes of Health NIH defines neurodegenerative disorders as a class of illnesses in which specific cells in a region of the central nervous system either die off or cease to function [1]. Neurodegenerative disorders tend to worsen gradually over time and often lack a cure. Neurodegenerative disorders may be genetic or caused by predisposing factors [2]. Neurodegenerative disorders are marked by the continuous and gradual deterioration of specific types of neurons, primarily seen in conditions like Alzheimer's disease (AD), multiple sclerosis, Amyotrophic Lateral Sclerosis (ALS) Huntington's disease (HD), Parkinson's disease (PD) [3].

Different brain processes, including movement and physical activity as well as memory and cognition, can be impacted by neurodegenerative diseases. Nevertheless, the therapeutic options for these disorders are limited to symptomatic treatment and improving the quality of life to a degree only. As of right now, the available medications only improve symptoms rather than treat the underlying problem [2, 4].

Among those at age 65 and over, AD is the most common form of dementia among neurodegenerative illnesses [5]. German psychiatrist Alois Alzheimer initially recognized Alzheimer's disease in 1906. He identified and diagnosed the clinical-pathological disorder that would eventually bear his name [6].

Over a hundred years after Alois Alzheimer originally described the neurodegenerative illness that would bear his name, the pathological diagnosis of the condition still depends on the hallmarks of his description neurofibrillary tangles and amyloid plaques [6, 7]. The global status report by WHO 2021 indicates that the prevalence of AD and the number of dementia cases are projected to rise significantly in the next decades, increasing from 55 million in 2019 to an estimated 139 million by 2050 [8–10]. According to data from the NIH Statistics, in one decade starts from 2000, the number of deaths associated with prostate cancer, heart disease, and stroke dropped by 16%, 23%, and 8%, respectively, but the number of deaths associated with Alzheimer's disease grew by 68% [11].

Alzheimer's disease remains a complex health issue and continues to be an incurable condition in modern medicine [12]. The mystery surrounding the disease's causes, both in terms of health and social and economic dimensions, makes it one of the most challenging issues of our day [13].

This problem in the treatment of dementia is caused by many different factors, such as unclear pathophysiology, use of erroneous differential diagnoses, and variations in symptoms among individuals. Dementia symptoms are not experienced by two individuals in the same manner, so that it is difficult to provide a consistent treatment approach [5, 14].

Along with a range of neuropsychiatric symptoms, the most prominent symptoms of AD are changes in mood and behavior memory loss, cognitive impairment, inability to perform familiar tasks, and impairments of physical activities [10, 15–17]. The illness lasts for a long time roughly eight to ten years during which the patient experiences difficulties carrying out daily tasks until memory loss and immobility reach their ultimate stages [5, 18, 19].

The causes of neurodegeneration in AD remain unknown to this day, this uncertainty is the main cause in preventing the progression of effective and curative drugs. The etiology of AD, can be stemmed from various influences. The beta-amyloid proteins aggregation ($A\beta$) [20, 21], the damage and destruction of cholinergic neurons [21, 22], neuroinflammation [21, 23], mitochondrial damage [10], oxidative stress [24], and tau protein breakdown are the main risk factors for the development of AD [21, 25].

A prominent neuronal abnormality linked to AD is a decrease in the concentration of cholinergic neurotransmitters in the cortex and hippocampus, including acetylcholine (ACh) [26, 27]. Severe degeneration was found in the brains AD patients, with a significant reduction in cholinergic neurons and a significant ACh deficiency according to clinical data available in recent years. After serious injury to patients with AD, the ACh transferase activity is significantly decreased, which further indicates the damage to the cholinergic system [26, 28]. Thus, the most crucial approach in treating AD is to suppress the acetylcholine esterase enzyme (AChE) in order to stop ACh from being hydrolyzed. The only benefits of the currently utilized acetylcholine esterase inhibitors (AChEI) medications are

symptom relief and an improvement in the life quality for patients with mild to moderate cases. There isn't currently an AChEI to stop the condition from getting worse [26, 29]. There is still much to learn about the etiology of AD, and modern treatment options typically involve anticholinesterases, antioxidants, alpha- and beta-secretase inhibitors, so as NMDS receptors antagonists [30].

Based on the cholinergic theory, several AChEIs were developed [31], such as donepezil (1997), rivastigmine (2000) and galantamine (2001), currently, they are three authorized drugs that have been marketed for the treatment of AD. However, since these treatments diminishing the AD symptoms but cannot halt the disease's progression, their therapeutic value is constrained [31, 32]. Apart from the cholinergic theory of AD, there is strong evidence that memantine, a voltage-gated, uncompetitive NMDA antagonist, can shield neurons from excitotoxicity [33]. In 2003, the FDA gave it approval to treat Alzheimer's patients in the moderate to severe stages of the illness [34].

In addition to symptomatic treatment, neuroprotective, anti-amyloid, and neuron-restorative treatments have the potential to change the way the illness progresses [35]. In all phases of AD, donepezil is the medication that is prescribed the most and has the best response to treatment. Given that it interacts with the peripheral active site PAS and catalytic active site CAS domains of the AChE enzyme; it exhibits a robust inhibitory profile. In case of drug development and designing new molecules and treatment modalities, the donepezil binding property to the enzyme active site should be taken into account.

This doctoral thesis aims to design, synthesize, investigate the biological effects, and assess molecular modeling studies of new compounds containing three different set (Table 1.1) of dihydrothiazolyl hydrazone derivatives while accounting for the donepezil molecule's chemical structure as AChE inhibitors (Figure 1.1 and Scheme 1.1).

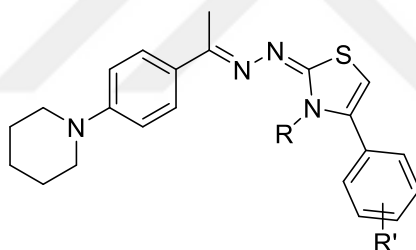
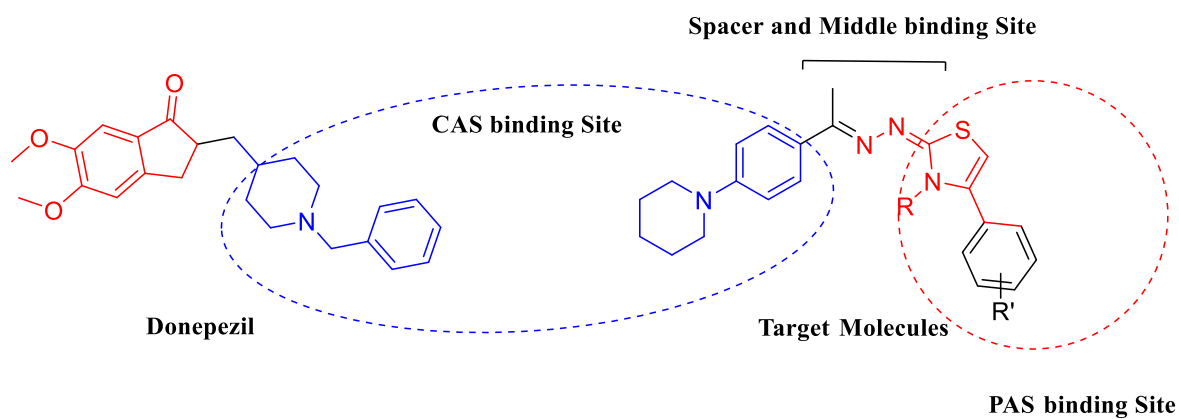
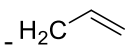
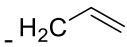
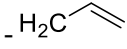
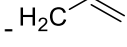
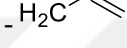
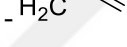
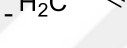
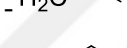
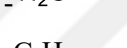


Figure.1.1. Structural formula of the designed targeted compounds.

Table.1 1. Derivatives of the designed chemicals.

Entry	R	R'
D1.a	-CH ₃	-CH ₃
D1.b	-CH ₃	-F
D1.c	-CH ₃	-NO ₂
D1.d	-CH ₃	-Cl
D1.e	-CH ₃	-OCH ₃
D1.f	-CH ₃	-CN
D1.g	-CH ₃	2',4'-Di-Chloro
D1.h	-CH ₃	3',4'-Di-Chloro

Table.1 1. Derivatives of the designed chemicals. (cont....)

D1.i	-CH ₃	2',4'-Di-Methyl
D2.a		-CH ₃
D2.b		-F
D2.c		-NO ₂
D2.d		-Cl
D2.e		-OCH ₃
D2.f		-CN
D2.g		2',4'-Di-Chloro
D2.h		3',4'-Di-Chloro
D2.i		2',4'-Di-Methyl
D3.a	-C ₂ H ₅	-CH ₃
D3.b	-C ₂ H ₅	-F
D3.c	-C ₂ H ₅	-NO ₂
D3.d	-C ₂ H ₅	-Cl
D3.e	-C ₂ H ₅	-OCH ₃
D3.f	-C ₂ H ₅	-CN
D3.g	-C ₂ H ₅	2',4'-Di-Chloro
D3.h	-C ₂ H ₅	3',4'-Di-Chloro
D3.i	-C ₂ H ₅	2',4'-Di-Methyl

2. REVIEW OF LITERATURES

2.1. Definition Alzheimer's Disease

The results from the autopsy of Auguste Deter's brain, a 55-year-old lady suffering from presenile dementia, were revealed by Dr. Alois Alzheimer on 3 November 1906 [36]. Though the audience was initially suspicious of the idea that physical lesions could cause psychiatric symptoms, Alzheimer publicly stated that aberrant diseased lesions in her brain, specifically fibrils inside her neurons (neurofibrillary tangles) and miliary bodies (senile plaques), were the cause of her condition. With the help of his mentor Emil Kraeplin, he stressed the relationship between pathological characteristics and clinical findings, and he came up with the term "Alzheimer's disease" (AD). It was discovered in the 1960s and 1970s that dementia of the garden kind and age-related dementia had the same pathology in their brains. This disorder, initially described in 1907 as a single case study, gained the name "Alzheimer's disease" in 1911 as Alzheimer documented more cases [36, 37].

The signs of the disease were described in full in the first paper authored by Alzheimer in 1907, when Auguste Deter was 51 years old and her family first became aware of her illness. Many scientists had made comments about that case and AD years later. The patient discussed by Alzheimer at 51 years old exhibited progressive presenile dementia and general cortical atrophy. In 1907 [38], Alzheimer likely used the Bielschowsky method, exploring the novel function of neurofibrils said Ramon y Cajal in 1910 [38]. However, electron microscopy 50 years later exposed neurofibrils as fixation artifacts said Peters in 1976 [38]. It is now acknowledged that aging is often associated with the presence of neuritic plaques and neurofibrillary tangles. cerebral cortex, developing in connection with degenerating neurons and as a normal aging process said Walton, 1981[38]. Senile plaques, core-formed by amyloid, characterize AD, with cerebral vascular amyloid containing beta/A4 protein often found in the cortex [38]. The current inclusion of patients over 65 in AD diagnoses creates an overlap with senile dementias, deviating from Alzheimer's initial description. Limited autopsies today make providing a differential diagnosis for the re defined AD nearly impossible. Alzheimer's conclusions from 1907 remain relevant, showcasing his foresight and enduring powers of observation and deduction [38].

According to popular definitions found in literature, acute and progressive memory loss is a hallmark of AD. The usual neuropathological characteristics of this neurodegenerative disorder include the presence of neurofibrillary tangle, amyloid plaques, and changes in neurotransmitter levels. ACh depletion in the cortex and hippocampus is remarkable and contributes to the unique clinical presentations of the disease [39].

Alzheimer's disease is categorized into three stages as per the of symptom progression. The first stage is the preclinical phase or mild stage, occurring before noticeable changes in cognition and daily activities. This stage is often marked by emotional changes. The second stage is mild AD related cognitive impairment, which is characterized by confusion, difficulty recalling names of objects, and the use of paraphrased or unconventional language. As mentioned in Alzheimer's paper, individuals may substitute terms, such as using "milk-pourer" instead of "cup." importantly, physical activities remain largely unimpaired during this stage [38]. The third stage which is the worse and last stage of the disease, is characterized dementia in which daily activity and cognition totally impaired [40].

There are two main pathogenic characteristics that define AD. The first characteristic is the build-up of beta-amyloid protein, which causes neurotic plaques to form. The tau protein when hyper-phosphorylated, drives the development of neurofibrillary tangles in the brains of those suffering from AD, is the second important pathogenic feature [41, 42].

2.2. Factors Leading to Alzheimer's Disease

The etiology of AD is unclear but it's known as age-related disease so one of the most predominant factor could be age. The incidence of AD rises most in those over 65, but early-onset AD, which can develop in anybody under 65, is also a possibility [43].

Another risk factor belongs to genetic factors such as mutation in various genes that enhance the production of beta-amyloid protein from amyloid precursor protein [44]. Genetic epidemiology has confirmed that homozygote carriers of the ApoE ϵ 4 allele have a 10- to 12-fold raised risk of developing AD [45]. Some other secondary predisposing risk factors are increasing the incidence of the disease such as inflammation, atherosclerosis [46], cardiovascular diseases [47], head injury, low educational level, elevated serum

homocysteine and cholesterol levels [48], low socioeconomic status, episodic depression, and female gender (estrogen deficit) [44, 49].

2.3. Mechanisms of AD development

As mentioned in the etiology section, AD is pathologically characterized by three main hypotheses, formation of senile plaques due to aggregations of A β protein, hyperphosphorylation of tau protein (neurofibrillary tangles), an irreversible degeneration of cholinergic neurons and neuro-inflammatory hypothesis (Figure 2.1) [50]. There are a number of theories as to what causes AD. This section focuses on three main hypotheses in detail and briefly the other secondary hypotheses such as; oxidative stress, neuro inflammation, and mitochondrial damage.

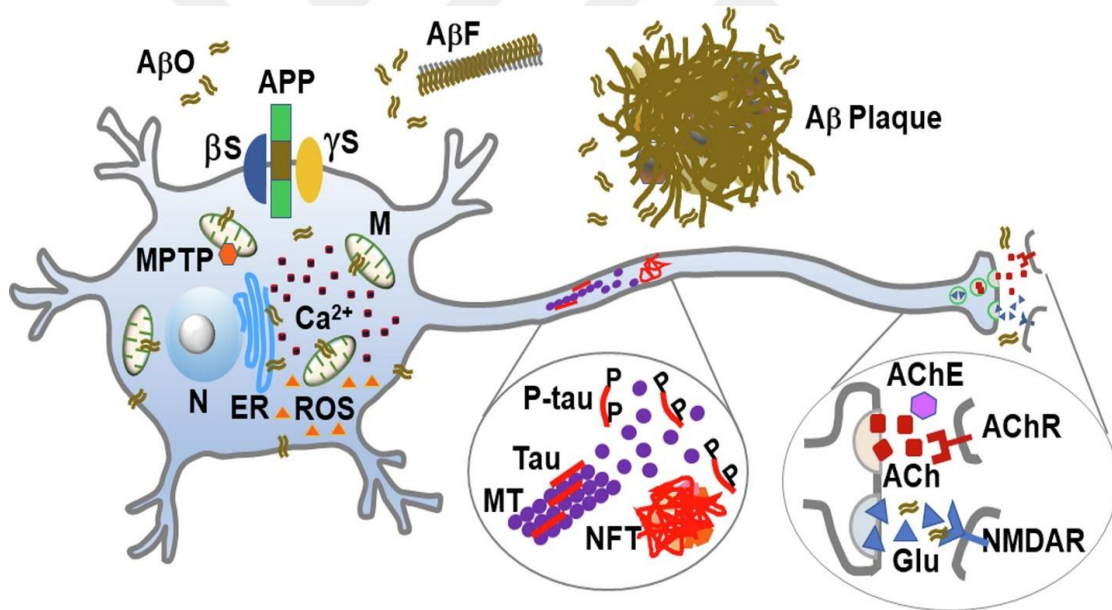


Figure 2.1. Illustration of the well-known neuropathogenic pathways implicated in Alzheimer's disease (AD) [51].

2.3.1. Beta-amyloid (A β) hypotheses

The pathophysiology of AD is still thought to be influenced by the A β peptide accumulation [44, 49]. Although the exact roles of amyloid A β and amyloid precursor protein APP are uncertain, it is thought that they play a part in the survival and function of neurons (Figure 2.2) [52]. A β is formed from its precursor APP, when it is released into the cytosol as a soluble peptide of 39–46 residues that is cleaved consecutively with the help of the

enzymes β and Gamma secretase. $A\beta_{-40}$ and $A\beta_{-42}$ are the most often generated isoforms as a consequence of App breakdown. The formation of fibrils and clusters is attributed to these two isoforms. The postmortem brains of AD patients had a higher ratio of $A\beta_{-42}$ than $A\beta_{-40}$ [53]. Several neuronal impairments, including excitotoxicity, synaptic disruption, oxidative stress, and mitochondrial malfunction result in neuronal death, which is brought on by the overproduction of $A\beta_{-42:40}$, this causes $A\beta$ to build up and senile plaques to form in the extracellular regions [54].

Then the developed senile plaques became the main cause of neuronal death through precipitating to form neurofibrils and oligomeric forms of $A\beta$ [55]. $A\beta$ oligomers may be much more harmful than fibrils or plaques when they build up in the brain of an AD patient [56]. In contrast to plaques, soluble oligomers seem to accumulate differently and manifest early in the pathogenesis [56]. According to evidence-based research, neurodegeneration and impairments of synaptic function in AD are greatly related accumulation of soluble $A\beta$ oligomers [57]. Both $A\beta_{-40-42}$ which is make up the major component of the senile plaque [58] and $A\beta$ oligomers were outlined as substantially increased in the soluble fraction of brain extracts from AD. with the fact that the level of oligomer is 20-fold higher in aqueous compared with detergent extracts [59].

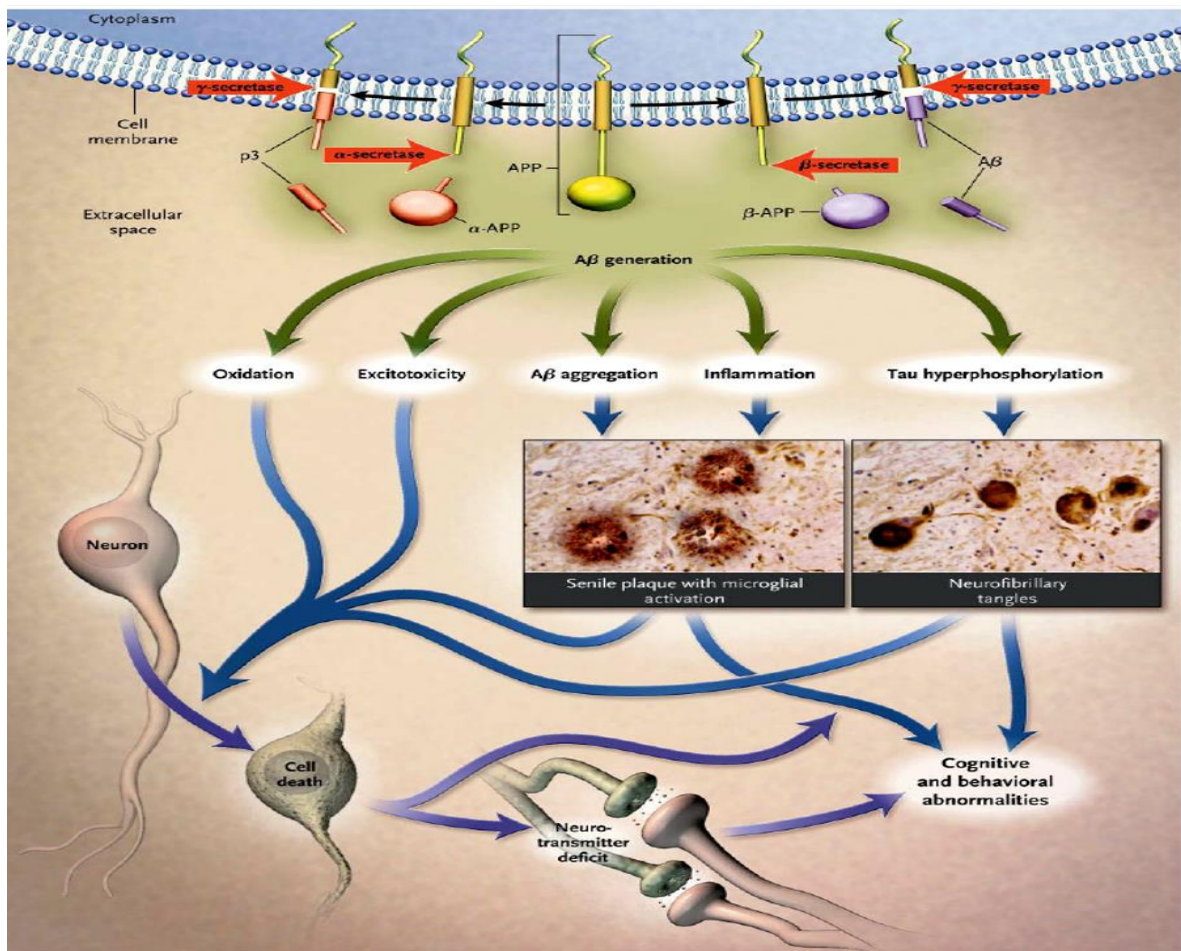


Figure 2. 2. Proposed amyloid cascade. This hypothesis suggests that the amyloid cascade begins with the production of the A β peptide from APP, proceeds through several subsequent stages, and ultimately leads to cell death [60, 61].

2.3.2. Tau or tangle hypothesis

The tubulin assemblies are stabilized and regulated by microtubule-associated proteins known as tau proteins. Two primary isoforms of this protein, 3R and 4R, which are mostly found in the axons of mature neurons as a microtubules binding sites [62]. According to the tau hypothesis, tau is the main ingredient that causes AD and is the main constituent of neurofibrillary tangle NFTs [39, 63]. Tau protein undergoes a configurational shift as a consequence of the phosphorylation of the amino acids Ser202, Thr205, and Ser208, which accelerates the development of tau filaments and NFTs [39].

The early event in the formation of NFTs started with shortening the tau caspases 3 repeat or 4 repeat. Recent research indicates that the tau hypothesis in the pathophysiology of AD is linked to the alteration and deregulation of the splicing factors proline and glutamine-rich (SFPQ) [64], the ensuing DNA abnormalities, and aberrant dynamics of T cell-restricted intracellular antigen 1 (TIA-1) positive stress granules [65]. Irregular phosphorylation and shortening of the sequences of the tau protein make it up a pathological entity that results in aggregation to form oligomers and NFTs, phosphorylation irregularly of tau has impact on its aggregation and development of AD (Figure 2.3) [66]. Recent studies showed that hyperphosphorilation of Ser208 at different stages of the disease has great impact on the pathology of the disease and strongly contribute to tau protein aggregation that results in neurodegeneration and synaptic impairments [62, 66].

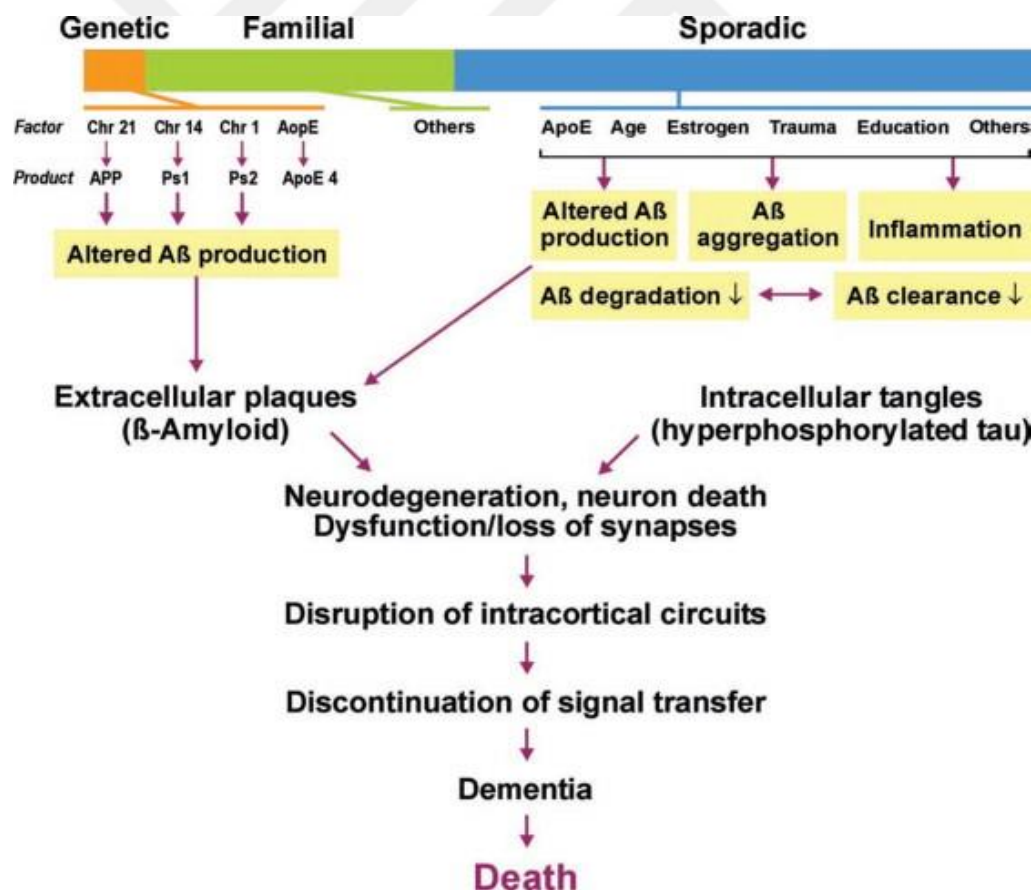


Figure 2.3. Major pathogenic factors and steps causing Alzheimer's disease [49].

As mentioned above about the tau NFTs and A β plaque hypothesis, there is a connection between both hallmarks and their impacts on AD. According to recent studies there are several findings about this correlation but the theory is resembling to the logic of egg or chicken [39, 67]. Research has shown that the protein-protein interaction between tau and A β is essential for their propagation and harmful effects [68]. According to the studies several models of interaction between these proteins have been mentioned as the following; The presence of A β enhances the seeding of toxic tau, Tau must be present for A β to be detrimental; the toxicity of A β and tau is increased by one another. Another model of interaction suggested one more potential explanation for the hyper phosphorylation of tau and the formation of NFTs is soluble oligomeric A β . The production of hyper phosphorylated tau aggregates and their interneuronal transmission are accelerated in the presence of amyloid plaques [69].

Studies done on post-mortem human brain tissues with AD showed that there is a high amount of both A β and tau protein when hyper phosphorylated in the frontal cortex striatum and hippocampus occurred [70]. Recent studies showed that A β aggregation through a sequence of actions that causes to hyper phosphorylation of tau and initiation of AD symptoms are all mediated by A β oligomer. Soluble A β oligomer are now suggested to cause neuronal damage [15]. Recent findings corroborate the theories that A β promotes tau seeding and enhances intracellular tau aggregation, and that A β oligomer increases tau pathology by increasing tau spreading triggered by paired helical filament *in vivo* [71, 72].

2.3.3 Acetylcholine hypothesis

The neurochemical results of AD are related to the clinical manifestations of the disease and pertain to the development of tau and A β pathology patterns, the formation of NFTs and A β plaques due to tau hyper phosphorylation, and the alterations in the neurotransmitter system especially in cholinergic systems (Figure 2.4). Cholinergic system is among one of the most focused hypothesis (Figure 2.5) causing AD due to the impact of cholinergic system on learning and cognitive function of brain [73]. The only medications that are now licensed to treat mild to moderate AD were developed as a result of the cholinergic hypothesis, which was the first explanation put up to explain AD [28, 74].

In memory generation, almost all parts of the CNS cooperate, and one of the most essential parts is the hippocampus. The cholinergic neuronal system from many brain parts controls and regulates the hippocampus function [75]. Learning and memory rely significantly on the cholinergic neuronal system from the prefrontal cortex, intervals, basal forebrain, and medial septum plays an essential role in learning and memory [75]. ACh was the first neurotransmitter identified as an executor of the cholinergic system and controlling learning and cognition [26]. The recognition of the importance of cholinergic activity in the brain for learning and memory dates back over fifty years, since it was shown that cholinergic antagonists, notably antimuscarinic drugs, might affect memory in rats [76].

Many investigations have demonstrated a strong association between age-related AD and cholinergic neuronal degeneration in the hippocampus region [75]. In the CNS, the neuronal function tremendously played by cholinergic system. A β ₁₋₄₂ and A β ₁₋₁₆ cause synapsin-1 to become dephosphorylated at physiological concentrations, which activates calcineurin and expands the pool of synaptic vesicles [77]. Through an increase in neurotransmitter release at the presynaptic synapse, this process improves neuroplasticity and memory formation [77]. Cholinergic signal transmission is essential for cognitive function as mentioned above, when the concentration of ACh is reduced by any factor the degeneration of cholinergic neurons will happen and results in impaired learning, memory, sleep regulation and attention [78]. In the basal forebrain, the pathological mechanism involves degeneration of cholinergic neuron, particularly in the Meynert nucleus, provided support for the cholinergic hypothesis over other alternative theories. This deficiency in

cholinergic transmission leads to memory deficit and a decline in learning in AD patients [75].

The function of cholinergic neurons in the CNS of people with mild cognitive impairment (MCI) or early AD should be further investigated as more advanced *in vivo* imaging techniques become accessible. It's interesting to note that the cholinergic theory seems to be supported by a number of *in vivo* imaging investigations done on AD patients to date. For instance, cortical level of AChE activity is indeed lower in AD patients, according to PET investigations employing *N*-methylpiperidin-4-yl-propionate [79]. Cortical nicotinic receptor impairments are substantially associated with the degree of cognitive impairment, as demonstrated by nicotine-based PET investigations, suggesting that these receptor abnormalities are an early event in AD [80]. Additional PET investigations using the nonselective muscarinic ligands *N*-methyl-4-piperidyl benzilate and quinuclidinyl benzilate show declines in binding in neocortical areas associated with both aging and AD [81]. Furthermore, single photon emission computed tomography (SPECT) investigations using benzovesamicol binding have shown that in early onset AD patients, the transporter of vesicular acetylcholine is decreased throughout the cerebral cortex and hippocampus entirely [82].

On the base of the mentioned theory, a series of experiments revealed that increasing the central concentration of ACh through ACh receptor agonists or AChEIs improves the memory ability and cognitive function of the brain. Acetylcholine esterase were approved in the 1980s and became the most recommended group of drugs (Donepezil, Galantamine and Rivastigmine) to improve the diseases' symptoms and ameliorate the life's quality of individuals with AD [83, 84]. Nowadays, raising the level of ACh in the CNS remain the main strategy against AD symptoms [85].

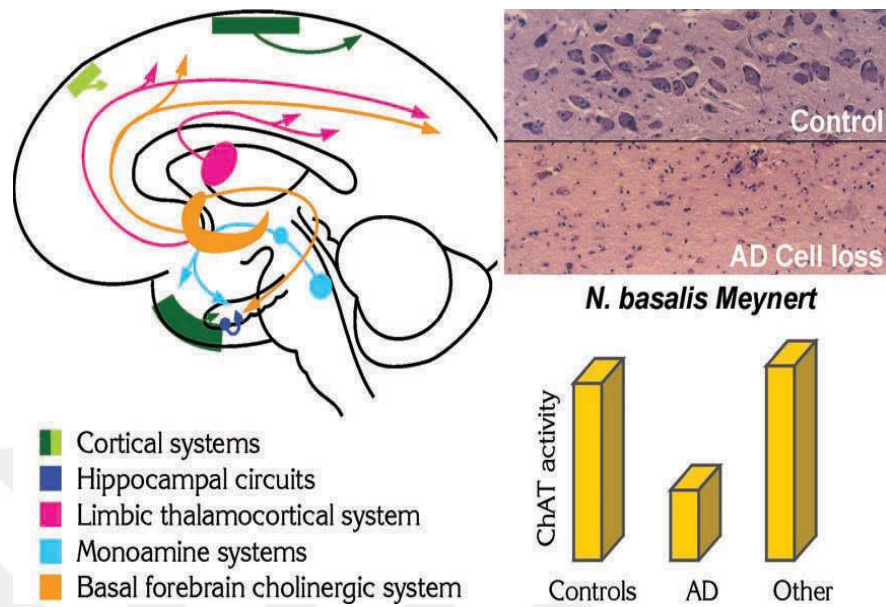


Figure 2.4. Participation of key neurotransmitter systems in AD [49].

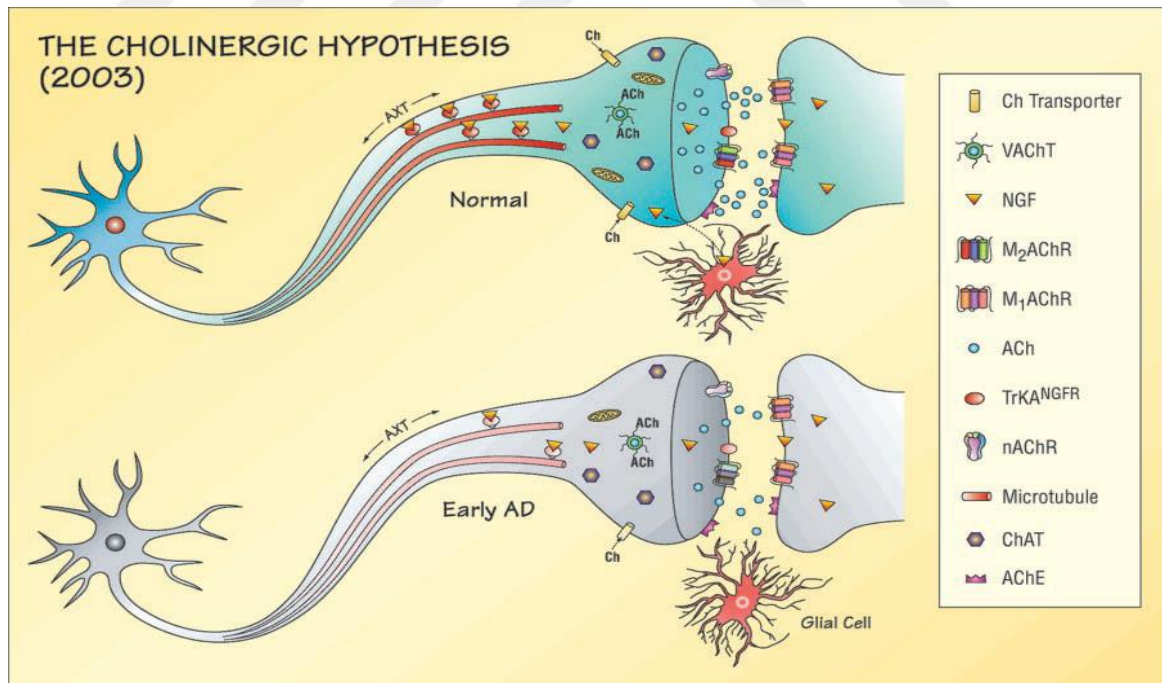


Figure 2.5. Schematic depiction of the known and proposed alterations in cholinergic neurons in the aged and early Alzheimer's disease (AD) brain compared to healthy young neurons [86].

AChE contributes to the progression of AD through two potential mechanisms. First, the AChE enzyme at CAS or PAS site degrades ACh. The cholinergic hypothesis indicates that patients with AD exhibit an average 55% reduction in cholinergic signaling compared to healthy individuals. Since cholinergic neurotransmission is crucial for memory consolidation and learning, modulating this pathway could be beneficial for AD treatment. Furthermore, A β and the PAS combine to create a stable complex, promoting the oligomerization of A β peptides and the formation of senile plaques. When AChE is incubated with A β peptide, the aggregation is three times greater than with A β alone [87].

2.3.4 Oxidative stress (free radical) hypothesis.

The widespread influence of oxidative stress in neurodegenerative diseases is increasingly being acknowledged. There is substantial evidence that oxidative stress affects brain tissue in AD patients as the condition advances (Figure 2.6). Generation of reactive oxygen species (ROS) and antioxidative defense are two main indicators of oxidative stress, are thought to play a worthy of attention in the aging-related neurodegeneration and cognitive decline process [88–90].

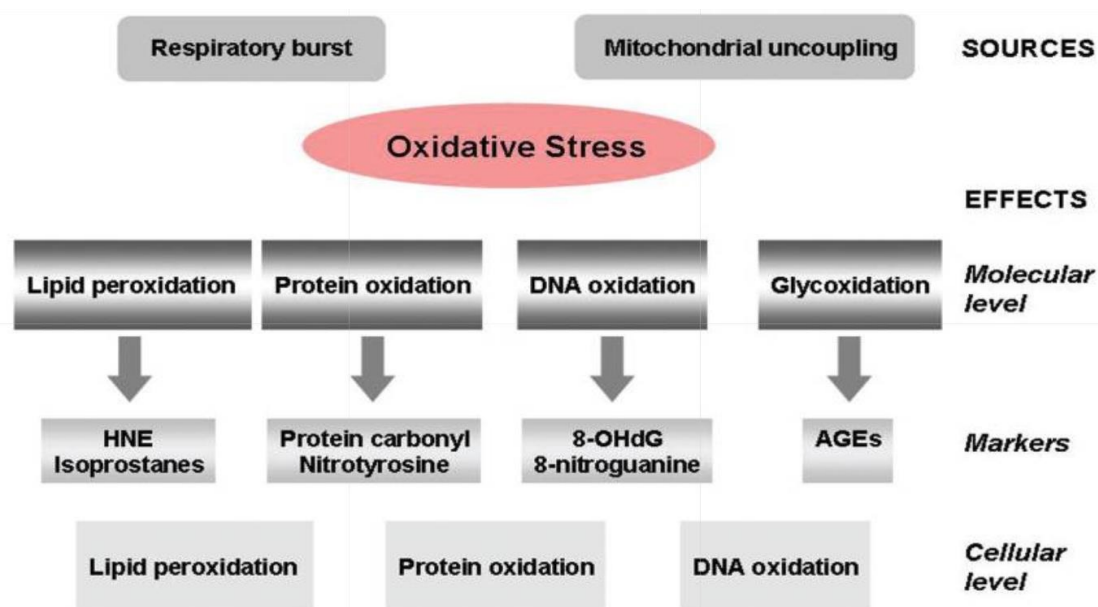


Figure 2.6. Origins of oxidative stress and its cellular and molecular effects [91].

Additionally, there is findings that oxidative stress, including the oxidation of proteins, lipids, DNA, and glycooxidation, impacts AD patients' brain tissue as the disease worsens. Amyloid plaques in AD contain advanced glycation end products (AGEs), which may be the result of rapid oxidation of glycated proteins. These end products accumulate extracellularly. AGEs have a role in the production of free radicals. both directly (chemically) and indirectly (cellularly) during neuronal death (Figure 2.7), which raises oxidative stress [91].

The presence of harmful compounds such as alcohols, peroxides, aldehydes, and ketones, along with oxidative modifications in mitochondrial and nuclear DNA, as well as cholestenone, serve as markers of oxidative stress in AD. Additionally, increased levels of oxidized proteins, lipid peroxidation end products, and advanced glycation end products also serve as indicators [92]. The introduction of medications for AD offers new prospects by halting the destructive cycles of oxidative stress and neurodegeneration. These therapeutic approaches, aimed at interrupting the generation of free radicals, encompass antioxidants, AGE inhibitors, and anti-inflammatory drugs [85, 91].

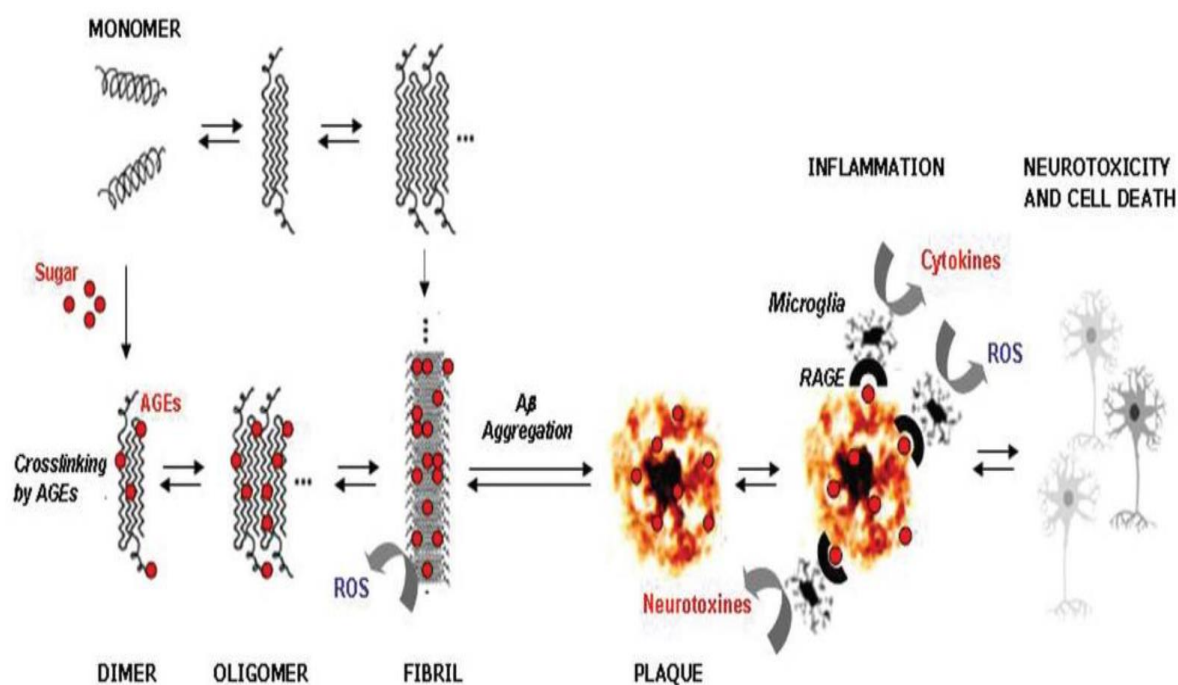


Figure 2.7. Direct and indirect effects of advanced glycation end products through crosslinking of Aβ peptide [91].

2.3.5 Excitotoxicity

It has also been suggested that excitotoxicity is a significant factor in neuronal death. The primary excitatory neurotransmitter, glutamate, or NMDA, is what causes it to become activated over time [93]. Excitatory glutamatergic neurotransmission has an impact on neuronal survival and synaptic formation which is mediated by the *N*-methyl-*D*-aspartate receptor (NMDAR). On the other hand, increased NMDAR activity increases cell death and excitotoxicity, which may be a predisposing factor to the neurodegeneration associated with AD [94]. Over activation of NMDA leads to disruptions in metabolic processes and cellular ion homeostasis (Ca^{2+} , K^+ , and Na^+). Dysregulation of Ca^{2+} is thought to be the most significant because elevated Ca^{2+} levels have the potential to permanently depolarize postsynaptic neurons, resulting in the production of reactive species and other compounds that are known to cause cell death [56, 95].

2.3.6 Neuroinflammatory hypothesis

In AD, inflammatory processes have long been recognized, but the inflammation hypothesis is relatively recent. According to this concept accumulating proteins (such as tau and amyloid beta) have a cascading effect that leads to inflammation. Since the 1980s, studies have reported cells around amyloid plaques and immune-related antigens in AD patients' brains. In the 1990s, the identification of activated complement factors, cytokines, and related receptors prompted the development of the concept of neuroinflammation within the CNS in AD [96]. Neuroinflammation, seen as a consequence in the amyloid hypothesis, involves $\text{A}\beta$ amyloid activating microglia, triggering a pro-inflammatory cascade (Figure 2.8 and 2.9) [97]. This cascade releases potentially neurotoxic substances, leading to degenerative changes in neurons. Microglial activation has also been linked to tau phosphorylation and the formation of neurofibrillary tangles [98, 99]. Supporting the inflammation hypothesis are epidemiological observations, such as lower AD prevalence in rheumatoid disease patients on long-term anti-inflammatory therapy. Additional observational research indicates that anti-inflammatory therapies may prevent the onset of AD [100, 101].

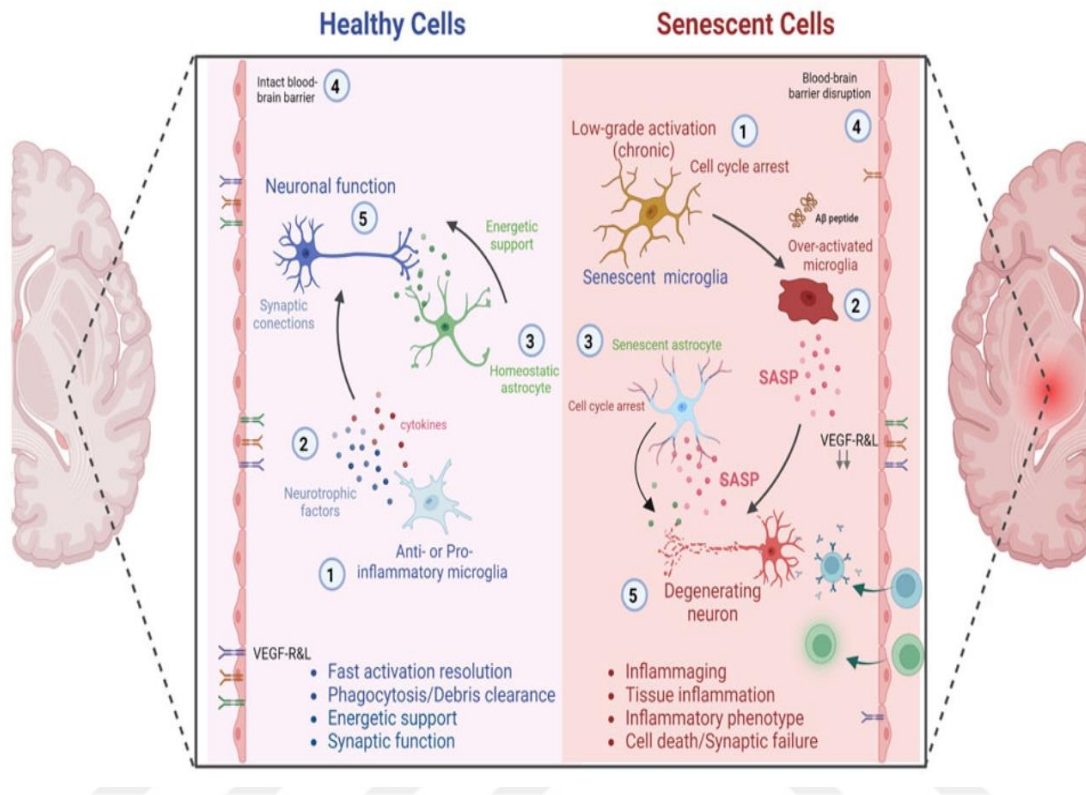


Figure 2.8. Effect of neuroinflammation and SNCs on Alzheimer's disease and neuronal degeneration. Microglia and astrocytes, among other young, healthy cells, release growth factors, pro- and anti-inflammatory mediators, and mediators of bioenergetic pathways that support brain homeostasis, neuronal survival, and cognitive functioning. In addition to the blood-brain barrier's integrity, which is guaranteed to be selective to certain substances and cells based on a particular receptor ratio. Senescent astrocytes and microglia, on the other hand, exhibit low resolution of the inflammatory response and persistently activate at a level greater than basal, undergo cell cycle arrest, and stay hyperactivated in response to various stimuli [99].

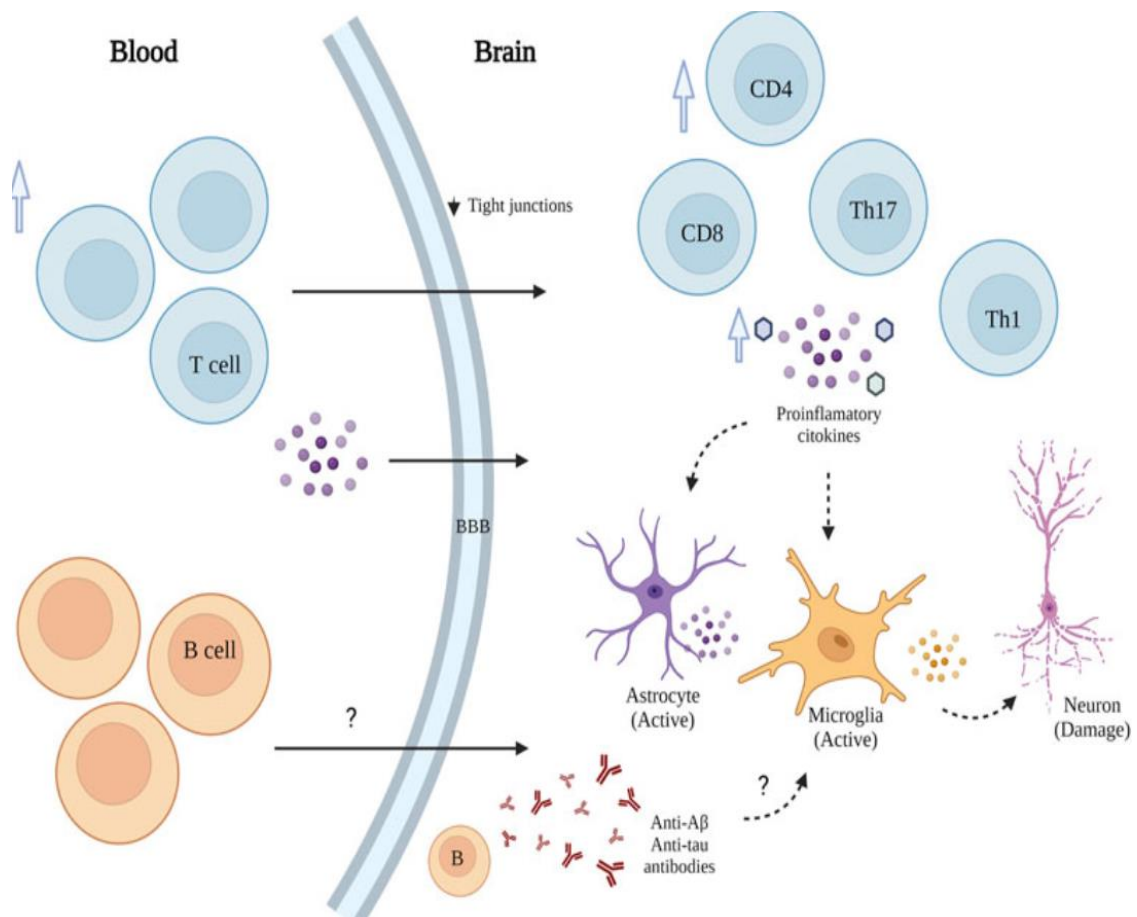


Figure 2.9. Diagram illustrating the function of T and B cells in AD. Increased neuroinflammation and accelerated neuronal death are caused by T and B cell infiltration into the brain.

2.4 The Intricate Journey Involved in Drug Developing for AD.

Existing drugs:

The prevalence of AD is rising as the world's population ages. Drugs that can stop AD from happening, postpone its beginning, decrease its course, or improve its symptoms must be found immediately. It has proven to be extremely challenging to produce drugs for AD. Following the 2003 approval of memantine, Lecanemab, is an experimental monoclonal antibody aims to specifically target amyloid beta ($A\beta$) aggregates, which are characteristic features of AD. Its development is focused on potentially slowing down the advancement of AD by diminishing the buildup of $A\beta$ plaques in the brain.

Clinical trials have been undertaken to assess both its safety and effectiveness in individuals with early AD and mild cognitive impairment caused by AD. It was authorized in 2023 [102]. Despite this, there is still no proven cure for the illness. The approaches that have been developed are intended to address impaired cognitive regulation, both in the prevention and treatment of illness. Antioxidant supplements, estrogen therapy, nonsteroidal anti-inflammatory drugs, amyloid accumulation inhibitors, muscarinic and nicotinic cholinergic agonists, cholesterol synthesis inhibitors, α -secretase activators, β and γ -secretase inhibitors, metal chelators, and MAO-B inhibitors are some of the approaches that are employed and still being developed [85].

The mainstays of AD pharmacotherapy today are drugs designed to raise brain acetylcholine levels, which facilitates cholinergic neurotransmission by inhibiting cholinesterase activity. For the symptomatic treatment of AD, there were only four approved medications for more than 20 years: memantine, galantamine, rivastigmine, and donepezil [3]. Based on clinical trials demonstrating a minor symptomatic effect on cognitive, behavioral, and global assessments, the FDA first approved these medications in 1995 under the name of acetylcholinesterase inhibitors (AChEIs). Later on the NMDA receptor antagonist that was approved by FDA with the name of memantine to treat dementia symptoms in patients with moderate to severe AD [103]. In therapeutic settings, memantine and AChEIs may be administered concurrently, these medicines lack disease-modifying efficacy but are acceptable and reduce the progression of neurological symptoms (such as

mood, memory, attention, irritability, and delusion) in individuals with specific stages of AD [51]. However, the absence of efficacious disease-modifying drugs that have come to light in consequence of these findings the challenges associated with developing a therapeutic agent that can alter the progression of a disease as complicated as AD. Over the past ten years, the majority of researches regarding AD has been directed on disease-modifying therapies, which aim to alter the course of the illness rather than just treat its symptoms [104], as the FDA authorized aducanumab, a monoclonal anti body (mAb) against aggregation of A β , in June 2021, making it the first medication that may modify AD illness [51].



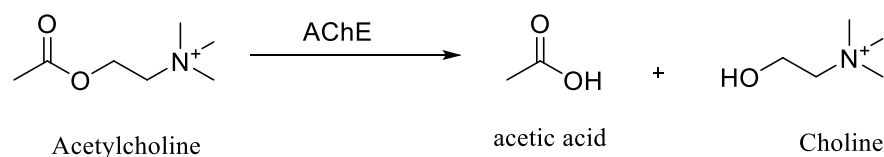
2.5. Targets of AD disease

Treatment strategies for AD are essentially split into two categories:

2.5.1 Cholinergic approaches

The medications most frequently used in therapy of AD are aimed to increase ACh neurotransmitter as regulation of ACh has direct impact on cognition. There are a several strategies to increase ACh concentration and enhance its neuronal transmission [105]. One of the most efficient approaches is through inhibition of AChE enzyme. ACh released from the presynaptic into the synaptic cleft during neurotransmission at the cholinergic synapsis. There, it binds to nicotinic and muscarinic receptors on the post-synapse. The breakdown of ACh is catalyzed by AChE, which terminates this nerve impulse transmission at the cholinergic synapsis [106]. When ACh attaches to AChE, it is broken down into choline and acetate (Scheme 2.1), hence increasing the neurotransmitter's function.

Furthermore, the catalytic activity of the enzyme exhibits enhanced at low ACh concentrations. The toxicity of amyloid fibrils both *in vitro* and *in vivo* appears to be modulated by the amyloidogenic factor AChE. When AChE is incorporated into Alzheimer's amyloid aggregates, stable complexes are formed. These complexes alter the enzyme's pharmacological and biochemical characteristics, increasing amyloid fibrils' neurotoxicity. This suggests that by influencing the processes leading to amyloid toxicity and the start of the disease, AChE may have a pathogenic function in AD [85, 107]. AChE inhibitors prevent the degradation of ACh that occurs after it exits cholinergic synapses. AChE inhibitors appear to both lessen symptoms and maybe slow down the disease's progression. Substrate binding is hindered by AChE's PAS, which is located near the mouth of the active site gorge. Medications that are frequently used to treat AD, such as galantamine, donepezil, and rivastigmine, function by preventing AChE activity [108].



Scheme.2.1. Degradation of ACh by AChE.

2.5.1.1. Active binding sites of acetylcholine esterase enzyme

The enzyme isolated from *Torpedo californica* (TcAChE) provided the initial crystal structure of AChE, which was published in 1991. This structure exposes a long, narrow gorge in the active site that extends from the catalytic site to the enzyme's surface, roughly 20 Å in depth and 5 Å in width. The CAS, which is in the bottom of the valley, and the PAS, which is nearer the entrance, are the two unique ligand-binding sites found in this gorge (Figure 2.10). The catalytic triad His440-Glu327-Ser200 and the crucial aromatic residue Trp84 are both present in the CAS. Trp279 is another noteworthy aromatic residue found in the PAS. The side-chains of Phe330 and Tyr121 cause the gorge to narrow between these two locations, creating a bottleneck that only permits water molecules to flow past. Larger cross-sectioned substrates and inhibitors would not be able to reach the catalytic site if the enzyme were stiff.

Nonetheless, other AChEs, such as those from mice and humans [109], share this structural characteristic because of their significant sequence similarity. The active-site gorge's large electrostatic dipole and high aromatic concentration are two other notable characteristics. The base and sides of the canyon are lined with aromatic components, and its axis is traversed by the dipole formed by the dispersion of charged residues. Positively charged ligands are drawn to the catalytic site by this dipole, and ligand mobility is facilitated by the aromatic residues' low-affinity binding sites. Because of this combination, positively charged substrates are drawn toward the catalytic site for quick processing, creating a "vacuum cleaner" effect [110].

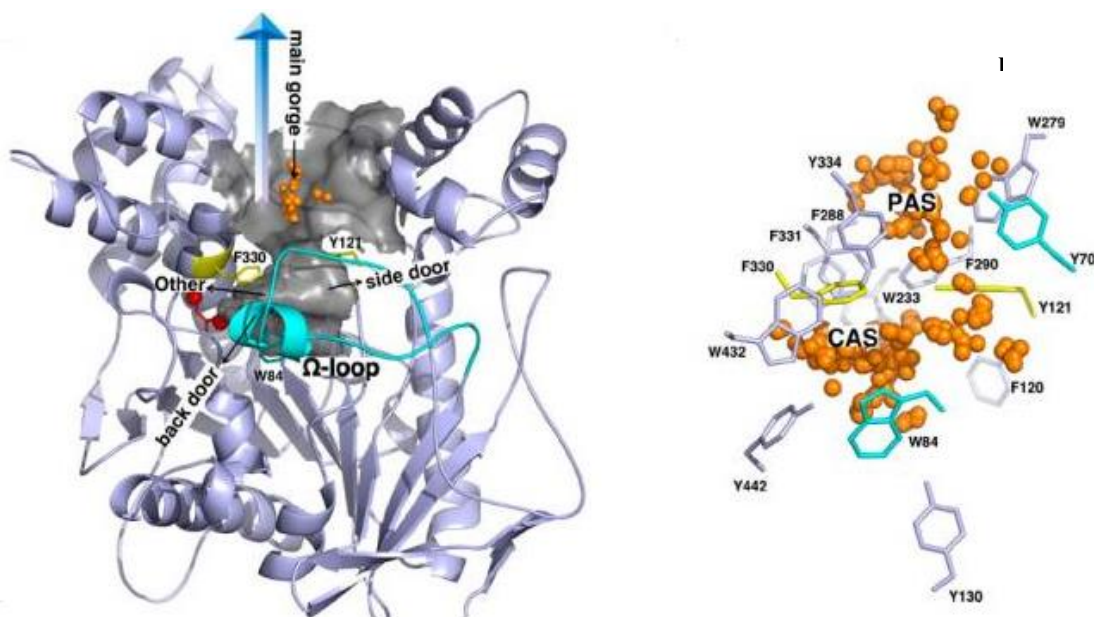


Figure 2.10. The crystal structure of TcAChE (PDB code: 2c5g) provides an illustration of the structure and arrangement of AChE. This illustration shows the active-site gorge and three possible routes for ligand trafficking. PyMol was used to create the gorge's intermolecular surface, and orange balls stood in for the probes that were used to locate it. Important residues such as Tyr121, Phe330, and Trp84 are shown as sticks, while the catalytic triad (His440-Glu327-Ser200) is represented as spheres. An arrow that is light blue indicates the dipole's orientation. The pocket-generated orange balls indicate that TcAChE's active-site gorge, which also includes 14 aromatic residues, is home to two ligand-binding sites, PAS and CAS [110].

2.5.1.2. Reversible acetylcholinesterase inhibitors in Alzheimer's disease treatment

- **Donepezil**

One of the second generation of AChEIs, donepezil is a derivative of indanone benzyl piperidine (Figure 2.11) that is widely used to treat AD [111]. A reversible selective inhibitor of AChE, donepezil binds to the PAS to produce both symptomatic and beneficial effects in the treatment of AD by delaying the formation of amyloid plaque [108]. Pharmaceutical companies Eisai and Pfizer manufacture the medication, which is marketed as Aricept. While donepezil is typically as a palliative treatment for mild-to-moderate AD is used, some clinical studies have demonstrated that it also improves cognitive performance in individuals with severe AD [112, 113].

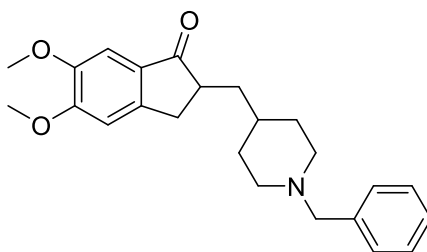


Figure 2.11. The structure of Donepezil molecule.

- ***Rivastigmine.***

Rivastigmine (Figure 2.12) was licensed in 2000 for mild to moderate treatment of AD, and it has been described as "pseudo-irreversible" [114]. Furthermore, this medication has been used to treat dementia associated with Parkinson's disease. Rivastigmine binds to the active sites of AChE and BChE, although its mechanism of action not clear precise. It was proposed that rivastigmine may increase cholinergic function by inhibiting the metabolism of ACh [115].

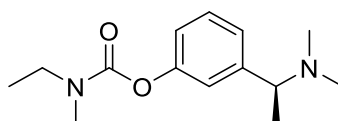


Figure 2.12. The structure of Rivastigmine molecule.

- ***Galantamine.***

Galantamine, is an alkaloid derived from heterocyclic phenanthridines (Figure 2.13). It has been extracted from the Amaryllidaceae family's bulbs and blossoms of daffodil flowers and *Galanthus woronowii* [116]. Galantamine exhibits various neuronal effects via diverse action mechanisms. Its historical use in Europe spans decades, particularly in addressing conditions like myasthenia, myopathy, and sensory as well as motor deficits linked with the central nervous system [117]. Galantamine's selective inhibition of central AChE with minimal peripheral effects is a key attribute that renders it effective in treating

AD related dementia. This unique characteristic allows Galantamine to target the central nervous system specifically, making it a suitable therapeutic option for this neurodegenerative condition[118]. In the light of these properties, galantamine was developed as a pro-drug under the name of Memogain, it was developed for the particular reason [119].

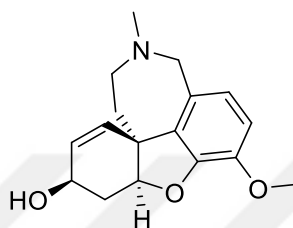


Figure 2.13. The structure of Galantamine molecule.

2.5.2. Targeting the Glutamatergic System

Excitatory glutamatergic neurotransmission, mediated in part by NMDA receptors, is crucial for neuronal survival and plasticity [94, 120]. However, excessive activation of NMDA receptors can result in glutamatergic excitotoxicity, contributing to neuronal cell death and having a significant impact on the etiology of neurodegenerative illnesses like AD [121]. Consequently, NMDA receptors have garnered significant attention recently due to their involvement in excitotoxicity and their impact on different forms of brain plasticity. Memantine, a non-competitive antagonist of glutamatergic NMDA receptors, is frequently prescribed for AD (Figure 2.14). It has demonstrated the ability to reduce A β levels in neuronal cultures and in *in vivo* animal model studies. Memantine alters the APP endocytosis pathway, which is essential for β -secretase to cleave the protein. Consequently, Memantine decreases brain concentration of APP, thereby reducing the risk of AD [122]. Since 2002, memantine has been sold under the Ebixa® commercial brand name [6].

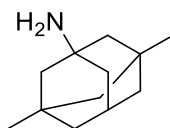


Figure 2.14. The structure of Memantine molecule.

2.6. Delve into Research On Ache Inhibition for Treating Alzheimer's Disease.

2.6.1. Piperidine nucleus as a potentially effective scaffold for Alzheimer's disease: current state of affairs and prospective outlook.

In modern therapy for the treatment of AD, the key goal is to preserve the level of ACh to improve the cerebral acetylcholine levels by facilitating cholinergic neurotransmission through inhibition of ChE enzymes such as AChE and BChE [123]. One of the most prescribed piperidine derivatives as AChEIs is donepezil in market right now. Due to the promising nature of this functional group, researchers extensively concentrate on it to discover its more potent derivatives.

Heterocycles and their derivatives play a crucial role in medicinal chemistry owing to their extensive pharmacological and therapeutic significance, as well as their broader implications in drug development and design. Among them, a heterocyclic moiety containing nitrogen atom, such as piperidine, showcases a diverse range of pharmacological characteristics [124]. The potential of piperidine derivatives is investigated in this section in combating AD. Given the pressing need for effective treatments, significant efforts are underway to develop medicinal agents for this disease. The focus here is on recent advancements in both natural and synthetic piperidine compounds and their efficacy against AD, particularly over the past 15 years. This section sheds light on the structures of the most promising compounds and their biological actions concerning AD. The encouraging results observed with these piperidine-based compounds underscore their potential as promising candidates for drug development, making them of great interest to researchers involved in providing neuroprotective agents [124].

Based on the active binding site of cholinesterase enzyme PAS and CAS, the benzyl piperidine moiety is an essential group for effectively inhibiting cholinesterase enzymes, similar to donepezil. The benzyl piperidine group exhibits strong binding to the catalytic site CAS, where it interacts with Trp84, Trp279, Phe330, and Phe331 [125]. Incorporation of different substituents on the benzyl-piperidine [125] and phenyl [126] residue could be a well-established strategy in synthesizing new active agents for treating AD. Anti-neuronal disease medications based on piperidines have progressed in development, and certain

important therapeutic compounds holding piperidines that have a critical target site have shown substantial activities.

A series of new indolyl piperidines base on the dual ligands NP61 and donepezil, have been designed by Chierrito et al., 2018. The targeted compounds were synthesized and biologically tested as AChEIs. They discovered very potent hBChEI, with acceptable drug-likeness properties and compatible BBB penetration. Among the compounds in the developed series, the one depicted in Figure 2.15, had a high level of BChE inhibitory effect, with an IC_{50} value (0.25 nM) when compared to reference compounds, donepezil and NP61, which had hBChE inhibition activities of 9.14 μ M and 2.9 nM, respectively. This finding may be used in the design of new specific BChEI in the future. As a result, compound shown in Figure 2.15 exhibited elongation in its interaction with AChE and a different conformation in its interaction with BChE [127].

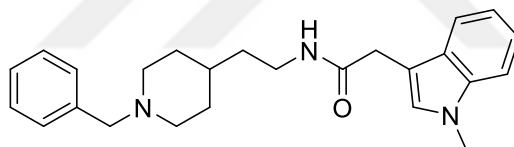


Figure 2.15. Molecular structure of *N*-(2-(1-Benzylpiperidin-4-yl)ethyl)-2-(1-methyl-1H-indol-3-yl)acetamide which designed by Chierrito et al., 2018.

Working on this field was expanded by Cai et al., 2019, with 4-*N*-phenylaminoquinoline derivatives through incorporation of piperidine as a biologically active functional moiety [128, 129]. Incorporation of piperidine moiety to their previously designed compounds [129] showed tremendously greater activity as AChEs inhibitor along with improved brain exposure. Additionally, these compounds exhibited metal-chelating and antioxidant properties.

Cai et al., 2019, have designed set of novel derivatives based on 4-*N*-phenylaminoquinoline. Following their synthesis, the proposed compounds were evaluated for their biological activity and impacts on metal-chelating characteristics, 1,1-Diphenyl-2-

picrylhydrazyl (DPPH) radical scavenging activity, and anticholinesterase activity. Among derivatives, the molecule as shown in (Figure 2.16) exhibited inhibitory potential comparable to the reference drug galantamine against both BChE and AChE enzymes, in regards to the obtained values of IC_{50} ($1.20\ \mu\text{M}$) against AChE and ($18.52\ \mu\text{M}$) against BChE, respectively, it showed the strongest inhibition against BChE and AChE. Furthermore, it was shown by molecular docking studies and kinetic study of AChE inhibition that this chemical functioned as a dual inhibitor, binding to AChE's PAS and CAS at the same time.

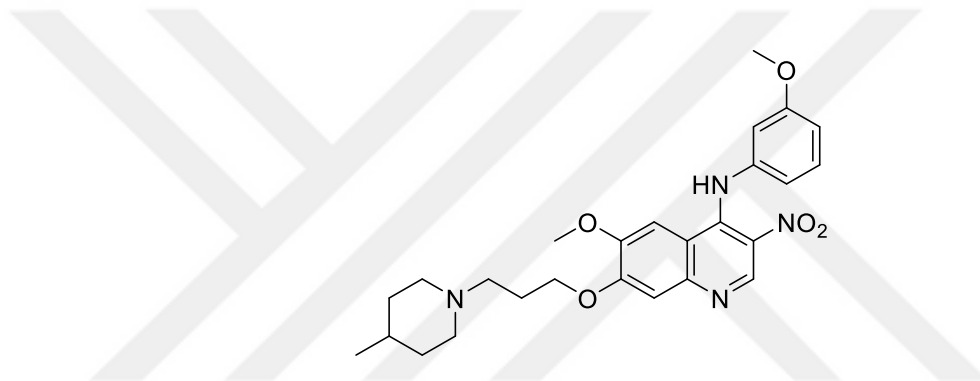


Figure 2.16. Molecular structure of 6-Methoxy-N-(3-methoxyphenyl)-7-(3-(4-methylpiperidin-1-yl)propoxy)-3-nitroquinolin-4-amine piperidine derivative compound designed by Cai et al.

More information regarding the piperidine moiety's ChE inhibitory activity, based on structure-based drug discovery methodologies to create human BChE inhibitors that are strong, reversible, and selective. Kořak et al., in 2018 designed a set of selective BChE inhibitors as seven different groups. Among them the group with introduced piperidine showed the most selective BChE inhibition activity [130]. Moreover, the authors performed the detailed study about the structural activity relationship SAR of the active compound, revealed that for best selectivity and inhibitory impact, nitrogen atoms were fundamental. The most potent derivative shown below (Figure 2.17) demonstrated picomolar inhibition of BChE, attributed to strong cation- π interactions observed in its complex crystal structure with human BChE. Additionally, this active compound effectively inhibited BChE in *in vivo* settings and showed no cytotoxicity. *In vitro* pharmacokinetic studies confirmed its high protein binding, permeability, and metabolic stability. Crucially, it exhibited sufficient

permeability to penetrate the BBB and notably enhanced cognition, learning abilities and memory in an induced dementia mouse model. Consequently, this identified chemical presents a prospective alternative for therapeutic development aimed at alleviating the symptoms caused by cholinergic deficiency in individuals with progressed AD [130].

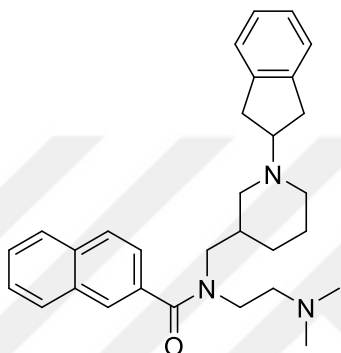


Figure 2.17. Structure of *N-((1-(2,3-Dihydro-1H-inden-2-yl)piperidin-3-yl)methyl)-N-(2-(dimethylamino)ethyl)-2-naphthamide* compound designed by Kořak et al.2018 as BChE inhibitor.

Building upon the success of previous researches, Wichur et al, 2020 [126], proposed a multi-targeted strategy for treating AD. They introduced novel derivatives of 1-Benzylpyrrolidine-3-amine with piperidine groups, which exhibited both antiaggregatory and antioxidant effects alongside their anticholinesterase effects. Additionally, the proposed active compound as shown *vide infra* (Figure 2.18) targeted β -secretase enzyme as well. The active compounds as depicted in Figure 2.18 was the strongest inhibitors of BChE with IC_{50} of 1.94 μ M. These multifunctional ligands exhibit encouraging *in vitro* activity along with their original scaffold, serve as an intriguing initial point for more investigations aimed at developing effective treatments for AD [126].

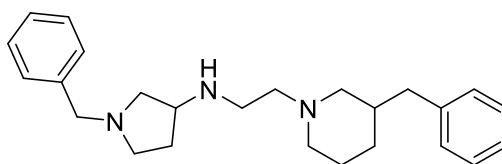


Figure 2.18. Molecular formula of dual cholinesterase inhibitor *1-Benzyl-N-(2-(3-benzylpiperidin-1-yl)ethyl)pyrrolidin-3-amine*.

El-Sayed et.al., 2019, designed and investigated a set of new pyrrolizine-based compounds as possible AChEI drugs [131]. The majority of the created compounds successfully prevented *in vitro* self-induced aggregation of A β and showed selectivity for hAChE over hBChE. Compound **A**, as depicted in Figure 2.19, exhibited a high degree of selectivity toward hAChE ($1.47 \pm 0.63 \mu\text{M}$ for hAChE and $40.15 \pm 3.31 \mu\text{M}$ for hBChE). In contrast, molecule **B** (Figure 2.19) showed a dual inhibitory effect against both hAChE and hBChE (0.40 ± 0.03 and $0.129 \pm 0.009 \mu\text{M}$, respectively). Furthermore, compounds **A** and **B** showed cytotoxicity levels that were either lower than or comparable to donepezil when tested against human neuroblastoma (SH-SY5Y) and normal human hepatic (THLE2) cell lines. *In vivo* studies further confirmed that both compounds effectively alleviated cognitive loss in induced AD mice [131].

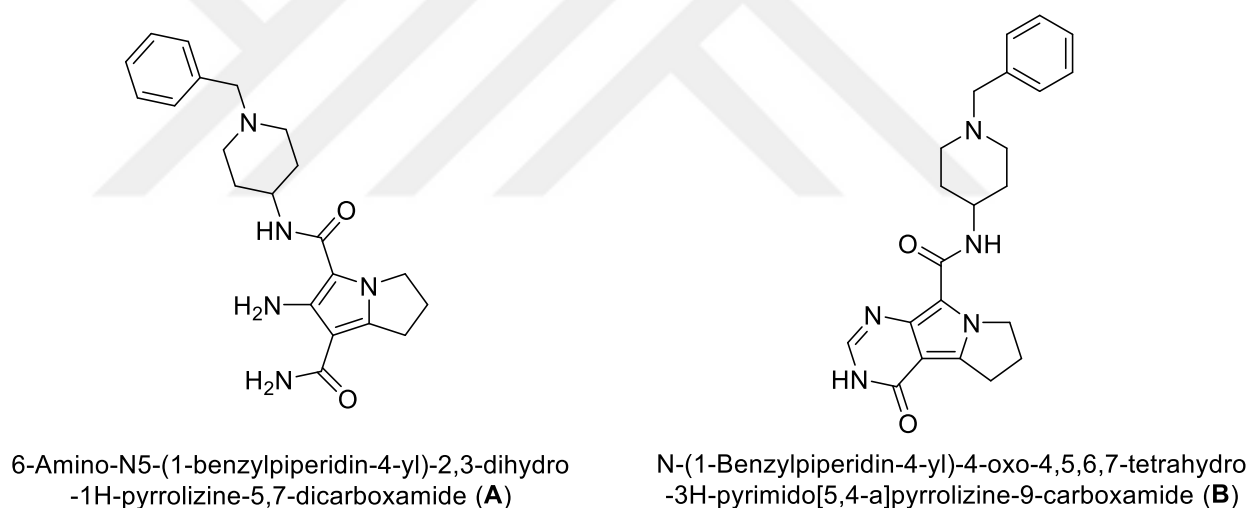


Figure 2.19. Molecular structure of most active compounds designed by El-Sayed et al., 2019.

Meena et al. (2015) [132], developed and described a class of Polypharmacological ligands as a potentially effective strategy to managing the intricate nature of AD. The series of benzylpiperidin, piperazin and benzhydrylpiperazin derivatives was evaluated for their biological activity as CAhEIs, A β self-aggregation, and anti oxidant. Interestingly, the compound as found below (Figure 2.20), demonstrated strong AChE inhibition (IC_{50} value of 2.13 nM) and significant AChE selectivity (around 38-fold). Kinetic analysis and docking studies indicate that this compound binds concurrently to the AChE active binding sites. Additionally, it demonstrating highest inhibition (88.81%) of self-induced A β_{1-42}

aggregations among them. The ADMET studies indicated that this compound holds promising derivative for further investigation and development of drugs targeting AD.

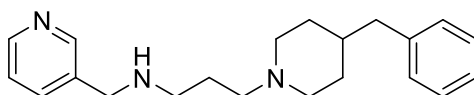


Figure 2.20. Molecular structure of 3-(4-Benzylpiperidin-1-yl)-n-(pyridin-3-ylmethyl)propan-1-amine compounds designed by Meena et.al., 2015.

A set of newly designed indanone derivatives was created, developed, and tested for their potential to treat AD by Meng et.al.,2012. Among designed compound, the molecule shown here, which features a piperidine group connected to an indanone ring (Figure 2.21), exhibited the IC_{50} of 0.0018 μ M value for AChE which indicating that this derivative has the strongest inhibitory activity. This compound's inhibition of AChE was 14 times more potent than donepezil, a common medication for AD. Additionally, these compounds demonstrated excellent metal-chelating properties, indicating their potential use in managing metal-related pathologies in AD [133].

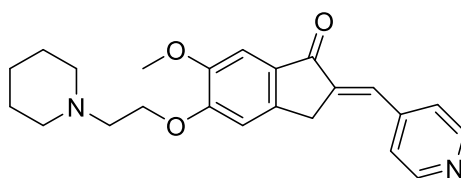


Figure 2.21. Molecular structure of 6-Methoxy-5-(2-(piperidin-1-yl)ethoxy)-2-(pyridin-4-ylmethylene)-2,3-dihydro-1H-inden-1-one compounds designed by Meng et.al.,2012.

A group of researchers (Żurek et.al., 2013), synthesized several derivatives of donepezil and explored their AChE inhibitory potential. To create these compounds, they combined indanone derivatives with the hydrazine nicotinate group. The potency and efficacy of derivatives was assessed by calculating their IC_{50} values and measuring their selectivity for BChE and AChE. The findings demonstrated that, in comparison to donepezil,

all of the chemicals exhibited a reduced interaction for AChE and a larger potency for BChE. Among all derivatives, the compound demonstrated in Figure 2.22, manifested the greatest selectivity for AChE [134].

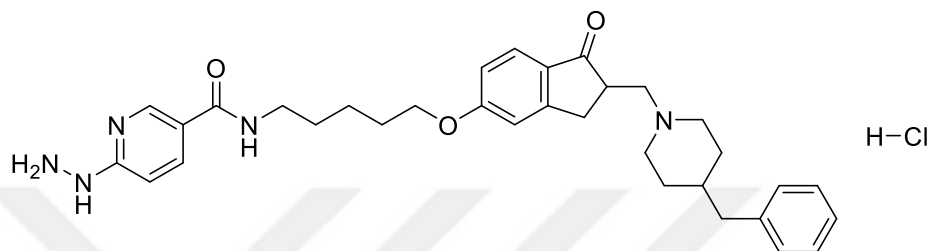
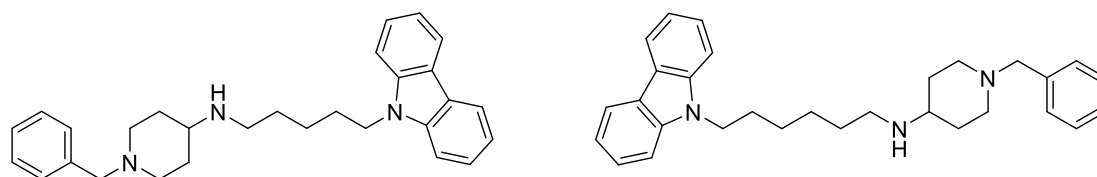


Figure 2.22. Molecular structure of *N*-{5-[2-(4-Benzylpiperidin-1-ylmethyl)-1-oxoindan-5-yloxy]-pentyl}-6-hydrazinonicotinamide hydrochloride compounds designed by Żurek et.al., 2013.

A multi-target-directed ligand strategy shows promise as a novel approach to treat AD, gaining significant attention. In order to determine how well a variety of carbazole-benzylpiperidine hybrids could inhibit BChE and AChE, researchers Sadeghian et al., 2020, developed, synthesized, and assessed the hybrids. A few of these substances were also examined for their ability to chelate metals and their anti- β -secretase (BACE1) action. Molecule **A** and **B** as shown below, were determined as the most potent inhibitors (Figure 2.23). Molecule **A** exhibited IC_{50} values of 16.5 μ M for AChE and 0.59 μ M for BChE, while compound **B** had IC_{50} values of 26.5 μ M for AChE and 0.18 μ M for BChE.

Notably, both compounds demonstrated interactions with Zn^{2+} ions. These substances function as non-competitive inhibitors, according to additional kinetic and docking tests, which raises the possibility that they may interact with acetylcholinesterase's peripheral anionic site PAS in addition to its CAS. This ability to interact with multiple sites concurrently could explain their strong inhibitory effect and potential efficacy in targeting key factors associated with AD [135].



N-(5-(9H-carbazol-9-yl) pentyl)-1-benzylpiperidin-4-amine

N-(6-(9H-carbazol-9-yl) hexyl)-1-benzylpiperidin-4-amine

Figure 2.23. Molecular structure of most active compounds designed by Sadeghian et al., 2020.

Promising treatment therapeutics for AD are developing in the form of multi-target-directed ligands. Coumarin, a versatile backbone with a range of bioactivities, has been employed to design new anti-neurodegenerative compounds, making it a suitable foundation for constructing MTDLs. Researchers Liu et al. (2022) investigated and synthesized a number of novel coumarin derivatives in this study, testing their inhibitory effects on key targets related to AD. Among the synthesized compounds, the molecule as shown here (Figure 2.24) stood out for its multifunctional profile. It demonstrated inhibitory activity potentially against AChE with an ($IC_{50} = 1.313 \pm 0.099 \mu M$) and SI toward BChE showed (selectivity index = 24.623), and inhibition of BACE1 ($IC_{50} = 1.227 \pm 0.112 \mu M$). Additionally, compound shown in Figure 2.24, exhibited low to moderate cytotoxicity in various cell lines, including HepG2 and SH-SY5Y, and low cytotoxicity in HL-7702, indicating a favorable safety profile. The evaluations of BBB permeability, suggesting its potential to reach the brain. Docking studies revealed that this chemical acts as a competitively inhibit the enzyme, indicating its binding to the active site. Experiments on acute toxicity showed that at 1000 mg/kg, it was not harmful. It also demonstrated acceptable physicochemical qualities, which strengthened its drug-like qualities. Overall, our results point to this drug as a favorable multifunctional option for treating AD because of its special mix of advantageous qualities, which include strong brain permeability, low toxicity, and specific enzyme inhibition [136].

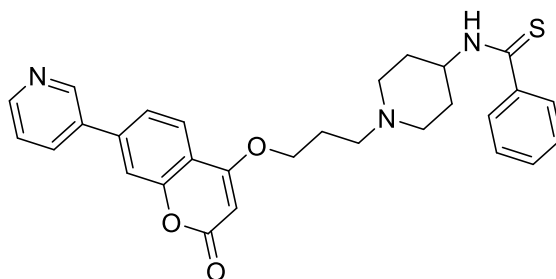


Figure 2.24. Molecular structure of 7-(Pyridin-3-yl)-4-(3-(4-((thiophen-3-ylmethyl)amino)piperidin-1-yl)propoxy)-2H-chromen-2-one compounds designed by Liu et.al,2022.

In order to study the biological effects of tacrine-2-amide derivatives on AChE inhibition, a number of them were produced. Less than 100 nM demonstrated good potency for all produced compounds. A comparison of substances (Figure 2.25) with tacrine (94.69 ± 4.88) was shown to have good potency towards inhibition of acetylcholinesterase, in an IC_{50} value of 23.66 nM. These substances exhibited a strong binding affinity for the enzyme's CAS and PAS, according to an *in-silico* study. The results of the docking research provided insights into the binding enthalpy, showing that π - π stacking interactions are essential to the binding process between proteins and ligands.

The binding energy of -24.03 kcal/mol and glide score of -10.38 kcal/mol indicate that the active chemical has good affinity for AChE. The compound in the following figure was considered to be the best one, as it had a superior glide score of -12.81 and an affinity binding of -59.90 kcal/mol [137].

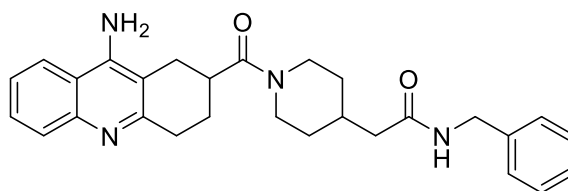


Figure 2.25. Molecular structure of 2-(1-(9-amino-1,2,3,4-tetrahydroacridine-2-carbonyl)piperidin-4-yl)-N-benzylacetamide compounds designed by Dogga et.al., 2022

Kwon et al. (2007) developed a series of piperidine derivatives to function as an AChE and A β peptide aggregation dual inhibitors in an attempt to create treatments for AD [138]. A second aromatic group was inserted in the design for the PAS, and an ester containing an aromatic group was included for binding to the CAS of enzyme. Something long and linear with a lipophilic component was considered to intercalate with A β oligomerization. These novel compounds were subjected to inhibition tests for A β ₁₋₄₂ peptide aggregation, which included AChE, BChE, and AChE-induced A β ₁₋₄₂ aggregations. The results indicated that compound in Figure 2.26, exhibited the best inhibitory potency and selectivity for AChE, but lower activity against BChE in comparison to other derivatives. Additionally, the investigation showed inhibitory actions against A β ₁₋₄₂ aggregations as well as A β aggregation produced by AChE. The 4-chlorobenzene group at the base of the AChE gorge is shown by docking models to be in parallel π - π stacking with Trp84's indole ring. Furthermore, the benzhydryl moiety developed a funnel-shaped covering over the peripheral site of AChE, which resulted in high AChE inhibitory impact and the capacity to prevent A β ₁₋₄₂ peptides aggregation induced by AChE.

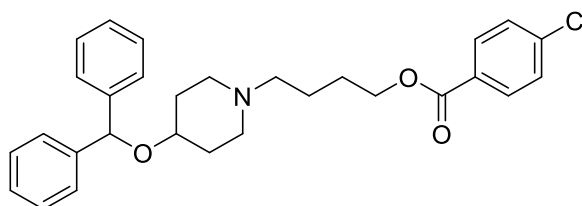


Figure 2.26. Chemical structure of 4-(4-(Benzhydryloxy)piperidin-1-yl)butyl 4-chlorobenzoate designed by Kwon, et. Al., 2007.

Van Greunen et. al., 2017, in the quest to find new treatments for AD, a series of twenty-seven acetylcholinesterase inhibitors were designed and synthesized, inspired by the molecular structure of donepezil, a drug used to manage Alzheimer's from mild to severe stages [139]. This study explored previously unexamined chemical variations around donepezil's core structure. Researchers explored two key modifications: substituting the piperidine ring in donepezil with various nitrogen-containing rings of varying sizes and

incorporating different linkers between the indanone and these new rings. Among the synthesized compounds, the analogue shown here (Figure 2.27) emerged as the most potent. The compound demonstrated a remarkable IC_{50} value of $0.03 \pm 0.07 \mu M$ against AChE, with no cytotoxicity observed ($IC_{50} > 100 \mu M$ in the SH-SY5Y cell line). In contrast, donepezil had an IC_{50} of $0.05 \pm 0.06 \mu M$ but exhibited significantly higher cytotoxicity, with an IC_{50} of $15.54 \pm 1.12 \mu M$. Molecular modeling demonstrated a strong correlation between the biological activity of the compounds and *in silico* studies.

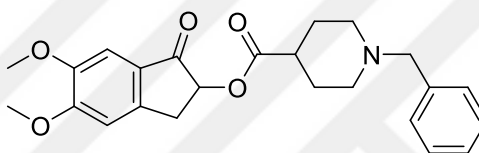
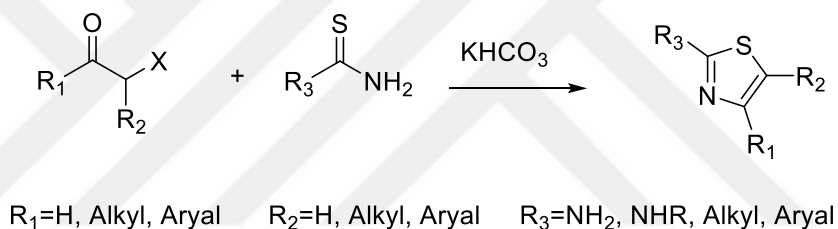


Figure 2. 27. 5,6-Dimethoxy-1-oxo-2,3-dihydro-1H-inden-2-yl 1-benzylpiperidine-4- carboxylate.

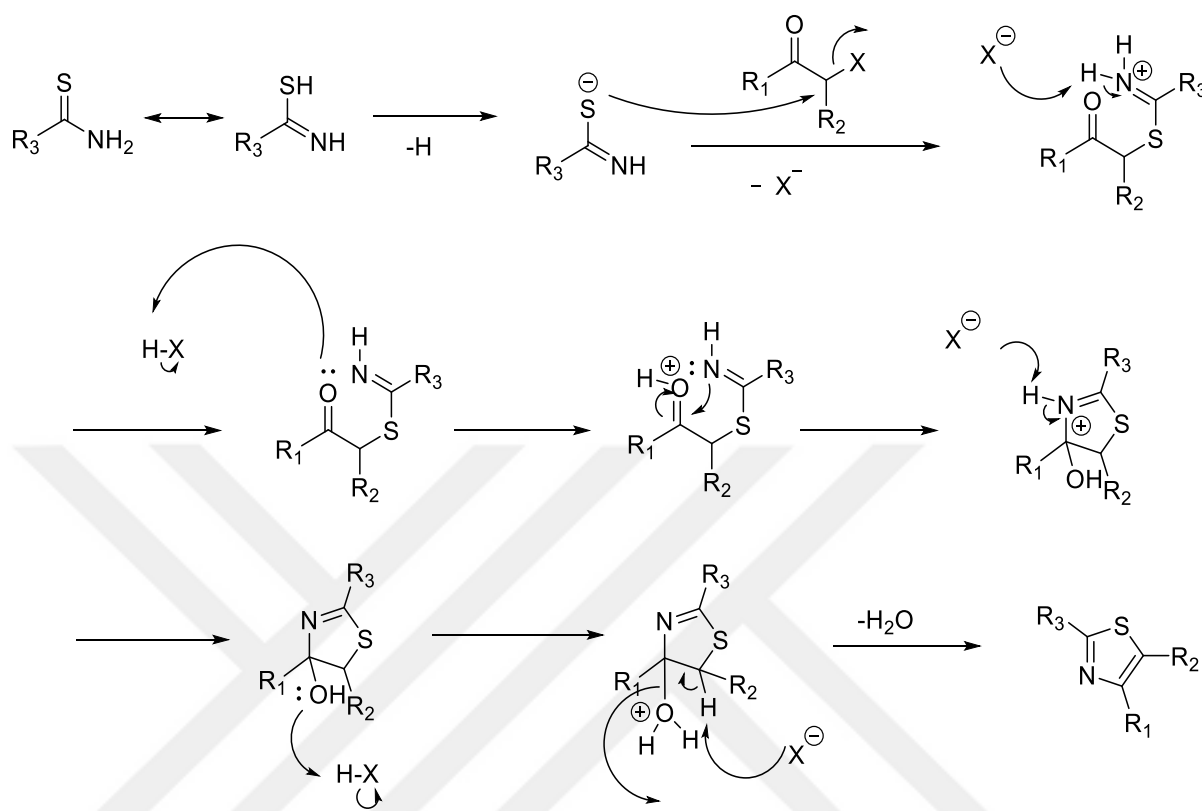
2.6.2. Thiazole nucleus as a promising scaffold for Alzheimer's disease: Future Perspectives and Current Landscape

2.6.2.1. *Synthetic strategies for hydrazinyl-thiazole derivatives*

Hantzsch and Weber synthesized hydrazinyl-thiazoles for the first time in 1887, using a process known as Hantzsch Thiazole Synthesis [140]. This method involved condensing α -haloketone and thiosemicarbazone to create derivatives of hydrazinyl-thiazoles. The intermediate had been separated at a low temperature during a multi-step process. Thiosemicarbazone or thiourea's sulfur atom has a higher nucleophilicity, which causes the reaction. Scheme 2.2 depicts the reaction path, while Scheme 2.3 shows the suggested mechanism [141].



Scheme.2. 2. Hantzsch Thiazole Synthesis.



Scheme.2 3. Mechanism of Hantzsch Thiazole Synthesis[141].

2.6.2.2. Thiazole-bearing compounds in pre-clinical investigation

The aromatic five-membered ring of thiazoles, a kind of azole heterocycles, has one nitrogen and one sulfur atom. Recently, these compounds and their derivatives have gained considerable interest due to their broad applications across various industries such as industrial processes, agrochemicals, and photographic sensitizers. Thiazoles also have significant pharmacological and biological activities, exhibiting antiretroviral effects (like ritonavir), antifungal actions (like abafungin), anti-inflammatory, antimicrobial properties (like sulfazole), anticancer properties (like tiazofurin), as well as antidiabetic, anti-Alzheimer, antioxidant, antihypertensive, and hepatoprotective characteristics [142]. Given that thiazole-based drugs have shown success in treating a wide array of conditions, there has been a noticeable uptick in interest around the thiazole structure in recent years [143, 144]. This focus has prompted numerous review articles that highlight the role of the thiazole ring

in developing new bioactive drug candidates. As a result, researchers worldwide have begun to explore thiazole-containing molecules for creating innovative anti-Alzheimer's drugs.

According to the molecular structure of donepezil, Sağlık et al. (2020) created several novel indan-thiazolyl hydrazone derivatives, which were then synthesized and evaluated for their anti-AD activity. The substances were evaluated for the inhibitory potential ability on A β plaque and AChE enzyme. The most active agent among the produced compounds was determined to be the molecules as shown below (Figure 2.28), has an IC₅₀ value of 0.026 μ M. According to molecular docking experiments, this chemical has the strongest binding affinity to AChE enzyme, which allows it to prevent A β aggregation and prevent the development of plaque. In addition, ADME parameters were evaluated and showed that the active compound has drugability like properties and it was candidate for further studies [145].

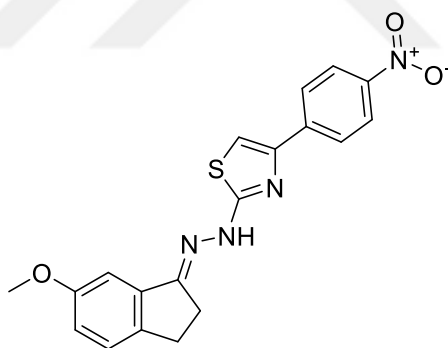


Figure 2.28. Molecular structure of 2-(2-(6-Methoxy-2,3-dihydro-1H-inden-1-ylidene)hydrazineyl)-4-(4-nitrophenyl)thiazole compounds designed by Sağlık et al., 2020.

In order to potentially treat AD, Davis and Eckroat (2021) have created a novel family of isatin-linked to dihydrothiazole derivatives. The most potent inhibitor among the designed compounds shown in Figure 2.29, with an IC₅₀ of 18.2 μ M. This substance performed similarly to galantamine and better than rivastigmine, both of which are prescribed as AChE inhibitors in clinical settings. Moreover, kinetic investigations of this drug revealed a non-competitive inhibitory pattern, whereas a molecular docking research anticipated a concomitant interaction with the enzyme active sites. The preferred molecule showed

promising drug-like characteristics *in silico* studies. The demonstrated compound deserves more research as AChE inhibitor in light of the data and findings that have been acquired [146].

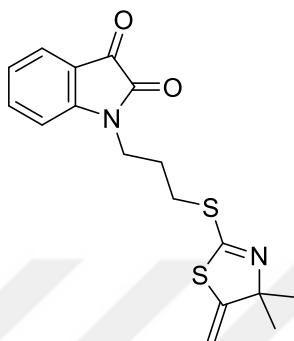


Figure 2. 29. Molecular structure of 1-(3-((4,4-Dimethyl-5-methylene-4,5-dihydrothiazol-2-yl)thio)propyl)indoline-2,3-dione compounds designed by Davis and Eckroat, 2021.

A new set of thiazole-piperazine hybrids, have been designed and synthesized by Osmaniye et al., 2019 targeted against AD. The potential inhibitory assay of the synthesized compounds as AChEI showed that the compound shown in Figure 2.30, was the most potent inhibitor based on its bind mode on AChE active binding sites. None of the chemicals, however, showed any discernible inhibition of the BChE enzyme [147].

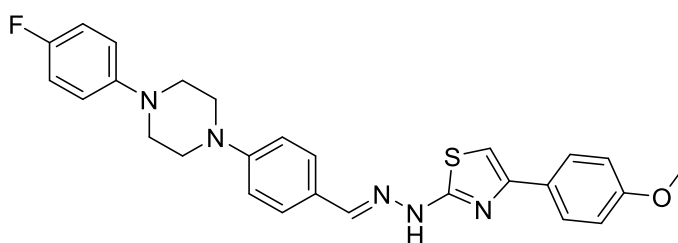


Figure 2.30. Molecular structure of 2-(2-(4-(4-(4-Fluorophenyl) piperazin-1-yl) benzylidene)hydrazineyl)-4-(4-methoxyphenyl)thiazole compounds designed by Osmaniye et al., 2019.

Based on the 7-benzyloxycoumarin pharmacophore, Amin et al. (2021) created and synthesized a set of new compounds containing a range of bioactive chemical moieties. The produced compounds shown a strong AChE inhibitory activity in the AChE inhibitory assay. The compound shown below (Figure 2.31) ($IC_{50} = 0.451 \mu M$) showed promising AChE inhibitory action, outperforming donepezil ($IC_{50} = 0.711 \mu M$) in *in vitro* evaluations. The candidate compound functions as a dual-type inhibitor, able to bind to the AChE enzyme's

active sites, according to a kinetic analysis of the proposed compounds. Scopolamine-induced memory impairment model in tested mice has remarkably improved by these compounds in *in-vivo* evaluation assays. In addition, computer-based analyses were conducted on the synthesized compounds. These analyses also predicted ADMET properties and other physicochemical characteristics. The docking results often correlated to the IC₅₀ values of the tested compounds, displaying a binding pattern similar to donepezil. As a result, the compound shown in Figure 2.31, was selected as a promising agent for further evaluations and investigations [148].

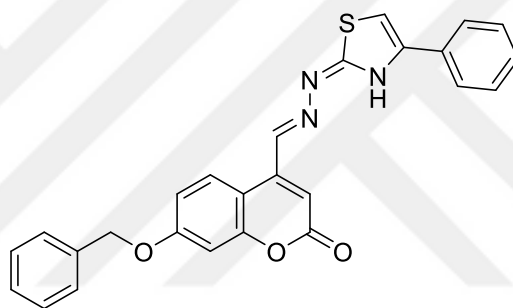


Figure 2.31. Molecular structure of 7-Benzyloxy-4-[[[(4-phenylthiazol-2(3H)ylidene)hydrazono]methyl]-2H-chromen-2-one compounds designed by Amin et al., 2021.

Dawood et al., 2024, designed, synthesized, and characterized a series of seven new benzimidazole–thiazole hybrid derivatives as AChE and BChE inhibitors. The *in vitro* evaluation demonstrated that several of these derivatives exhibit dual inhibitory activity against enzymes. Among them the compound as demonstrated below (Figure 2.32), showed the best results with an IC₅₀ 4.55 μ M against AChE and IC₅₀ 3.50 μ M against BChE. The potential molecule's antioxidant and neuroprotective qualities were also assessed. With a cell survival percentage of $67.42 \pm 7.90\%$, the results showed that the candidate chemical performed potential neuroprotection against SH-SY5Y cells damage induced by H₂O₂. With strong docking scores, the investigated compounds fit both enzymes' active sites quite well. The synthesized benzimidazole–thiazole hybrids have good physicochemical and pharmacokinetic qualities without going against Lipinski's rule, according to the *in silico*

investigation. These results imply that the substance depicted in Figure 2.32, may provide a novel model for the future development of more powerful cholinesterase inhibitors. [149].

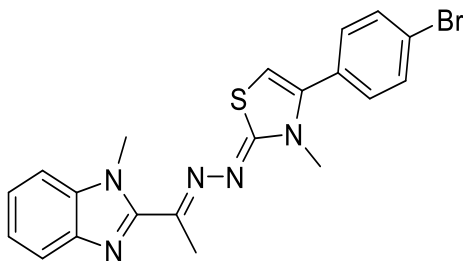


Figure 2.32. Molecular structure of 2-[4-(4-Bromophenyl)-3-methylthiazol-2(3H)-ylidene]-1-[1-(1-methyl-1H-benzo[d]imidazol-2-yl)ethylidene]hydrazine compounds designed by Dawood et al., 2024.

Again Sağlık et al., 2020, based on the cholinergic hypothesis, designed a set of new thiazolyldiazine derivatives which consisted of ten molecules. The enzymes inhibition assay of derivatives *in vitro* were evaluated. The results showed that each chemical had a negligible effect as BChEIs. On the contrary hand, the majority of the substances exhibited some level of AChE-inhibiting activity.

The most active agent in the series was determined to be the candidate compound (Figure 2.33), which showed an inhibitory profile at a rate comparable to that of the donepezil (IC_{50} value: $0.028 \pm 0.001 \mu M$). The results of a molecular docking study indicate that this substance adhered securely and in the optimum conformation to the AChE enzyme's active site [150].

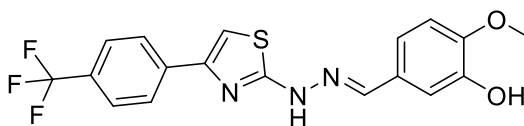


Figure 2.33. Molecular structure of 2-Methoxy-5-[(2-{4-[4-(trifluoromethyl)phenyl]thiazol-2-yl}hydrazineylidene)methyl]phenol compounds designed by Sağlık et al., 2020.

In 2013, Yurttaş et al. synthesized a novel anticholinesterase-active thiazole compound by incorporating piperazine into its structure. The biological activity was performed as both AChEIs and BChEI. The most active compound against AChE as shown in Figure 2.34, showed an IC_{50} of $0.011 \mu M$, while the reference donepezil had an IC_{50} of $0.054 \mu M$. Structural assessment demonstrated that the inclusion of a piperazine-substituted benzyl

group was vital for attaining high anticholinesterase activity. Nevertheless, there was no discernible inhibitory activity of the produced compounds against BChE [151].

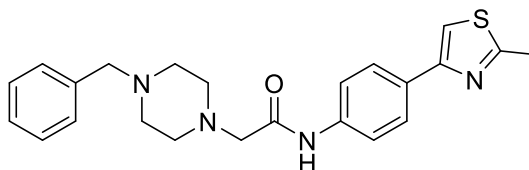


Figure 2.34. Molecular structure of 2-(4-Benzylpiperazin-1-yl)-N-(4-(2-methylthiazol-4-yl)phenyl)acetamide compounds designed by Yurttas et al., 2013.

In 2020, Ghotbi et al. created and synthesized a number of unique compounds including pyridinium and thiazole moieties. The suggested compounds' inhibitory potencies against AChE and A β were evaluated. The synthesized compounds displayed in (Figure 2.35) had IC₅₀ values of 0.40 and 0.69 μ M, respectively, indicating their effectiveness as AChE inhibitors within a sub micro molar concentration range. The strongest compounds (refer to Figure 2.34), which demonstrate interactions with both PAS and CAS sites in docking data. Moreover, both drugs had higher A β -self-assembly inhibitory effects (20.38 and 42.66%, respectively) at a 10 μ M dose than donepezil (14.70%).

Furthermore, in anti oxidant assay on PC12 cells, the highlighted compound was demonstrated to be an effective neuroprotective agent, with results nearly identical to those seen with donepezil. Using the PAMPA technique, it was shown that this compound can cross the BBB, making it a promising candidate for further investigation. The findings from this work provide valuable insights for developing new anti-Alzheimer medications [152].

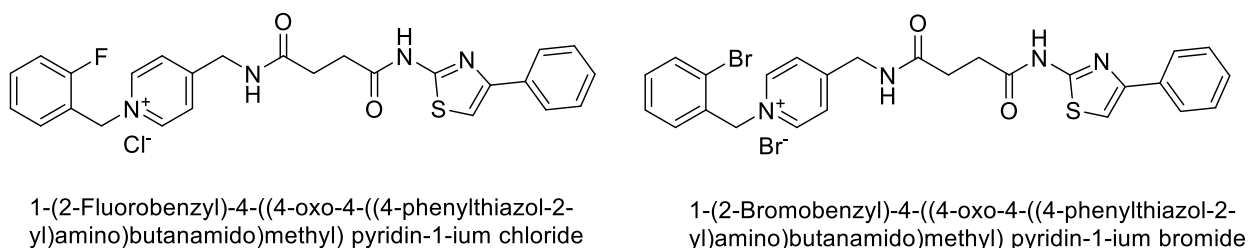


Figure 2.35. Molecular structure of most active compounds designed by Ghotbi et al., 2020.

Building on their previous work, Ibrar et al. (2018) synthesized and created a new set of coumarin-thiazole derivatives to evaluate their inhibitory effect against AChE and BChE. The chemical depicted in Figure 2.36, proved to be the most efficacious among those produced; its IC_{50} against AChE was $0.87 \pm 0.09 \mu M$, as demonstrated in (Figure2.36). Compounds from the coumarin-thiazole series with strong AChEI activity, like the one below, interacted with the AChE active site and obtained a Molecular Operating Environment (MOE) score of -11.19, according to the molecular docking experiments [153].

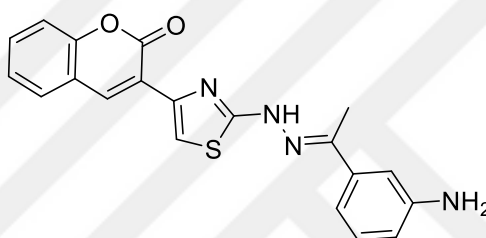


Figure 2.36. Molecular structure of 3-(2-(2-(1-(3-aminophenyl)ethylidene)hydrazineyl)thiazol-4-yl)-2H-chromen-2-one compounds designed by Ibrar et al., 2018.

Mumtaz et al., 2018 developed a variety of thioureas using aniline, potassium thiocyanate (KSCN), and acidic chloride as starting materials. These thioureas were subsequently transformed into newly synthesized thiazolidine derivatives (Figure 2.37). Upon examining the biological properties of these thiazolidine derivatives, the shown chemical here emerged as the most active against both AChE and BChE. Additionally, the most effective drugs' manner of inhibition against these enzymes was examined. Furthermore, molecular docking experiments were carried out to obtain an understanding of the interactions between these chemicals and the enzymes' active regions [154].

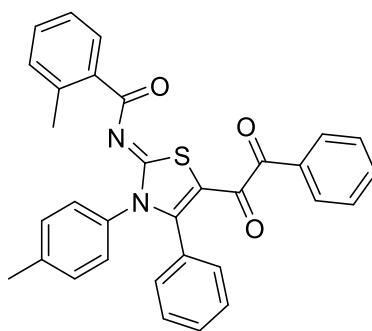


Figure 2.37. Molecular structure of 2-Methyl-N-(5-(2-oxo-2-phenylacetyl)-4-phenyl-3-(p-tolyl)thiazol-2(3H)-ylidene)benzamide compounds designed by Mumtaz et al., 2018.

Rahim et al., 2015, have developed a series of new compounds as AChE inhibitor for treatment of AD. A series of thirty thiazole analogs synthesized by reacting different acetophenone/benzaldehyde derivatives with thiosemicarbazide, followed by refluxing to obtain the final products. The inhibitory potential of these produced compounds against BChE and AChE was assessed and the IC_{50} values of $1.59 \pm 0.01 \mu M$ and $21.3 \pm 0.50 \mu M$, respectively was obtained. The analog highlighted here (Figure 2.38) had potent inhibitory action against ChEs among the produced compounds [155].

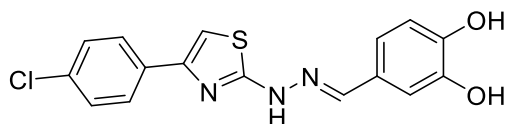


Figure 2.38. Molecular structure of (E)-4-((2-(4-(4-chlorophenyl)thiazol-2-yl)hydrazono)methyl)benzene-1,2-diol compounds designed by Rahim et al., 2015.

To develop multitarget-directed ligands for Alzheimer's disease treatment, Shidore et al. (2016) combined the pharmacophoric properties of diarylthiazole with the cholinesterase inhibitor donepezil, creating a novel class of hybrid molecules. These compounds exhibited significant in vitro anticholinesterase activity. Among them, the compound shown in Figure 2.39 was the most potent, with IC_{50} values of $0.30 \pm 0.01 \mu M$ for AChE and $1.84 \pm 0.03 \mu M$ for BChE. Enzyme kinetic tests revealed that this compound demonstrated mixed inhibition of AChE. Additionally, several of these hybrid compounds displayed notable antioxidant and

antiapoptotic effects *in vitro*, as well as moderate to strong inhibition of AChE-induced A β ₁₋₄₂ aggregations. The candidate compound also exhibited significant *in vivo* properties, including neuroprotection by reducing abnormal levels of A β ₁₋₄₂, phosphorylated Tau, cleaved caspase-3, and cleaved PARP proteins, thereby decreasing A β ₁₋₄₂-induced toxicity. These findings suggest that the compound has considerable potential for further development and use in Alzheimer's disease treatment [156].

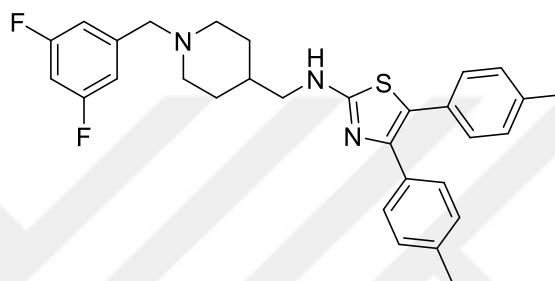


Figure 2.39. Molecular structure of *N*-[(1-(3,5-Difluorobenzyl)piperidin-4-yl)methyl]-4,5-bis(*p*-tolyl)thiazol-2-ylamine compounds designed by Shidore et al., 2016.

A new group of thiazole acetamides (Figure 2.40) was synthesized by Sun et al., 2016. By measuring these chemicals' *in vitro* ChEs assay, their potential use in AD has been examined. Among the synthesized compounds, the shown molecule here has great activity against AChE with an IC₅₀ of 3.14 ± 0.16 μ M and a SI of 2.94 against BChE. The derivatives were also evaluated for their ability to inhibit β -secretase and A β aggregation, demonstrating significant inhibitory activity, indicating their multi-target potential for AD treatment. Further studies on the candidate compound were carried out to explore its pharmacological profile as a potential lead drug against AD, including toxicity and docking studies, reinforcing its viability as a candidate for further research and development in the context of AD therapy [157].

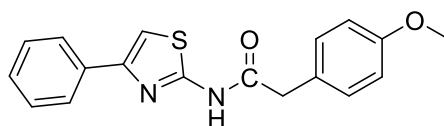


Figure 2.40. Molecular structure of 2-(4-methoxyphenyl)-*N*-(4-phenylthiazol-2-yl)acetamide compounds designed by Sun et al., 2016.

Hemaid et al. (2021) employed several analytical and spectral approaches to design, synthesize, and confirm the structures of nineteen new thiazole-based derivatives. Acetylcholinesterase (AChE) inhibition tests conducted *in vitro* revealed that the compound as shown here (Figure 2.41) exhibited the strongest inhibitory activity among the synthesized compounds, surpassing other compounds and comparable to the known AChE inhibitor donepezil (IC_{50} 103.24 nM). Molecular docking studies revealed that these compounds exhibit a similar binding orientation to donepezil at the AChE active site, implying high predicted binding affinities. As a result, the compound shown in Figure 2.41, was proposed as a promising candidate for the development of new, potentially more effective AChE inhibitors [158].

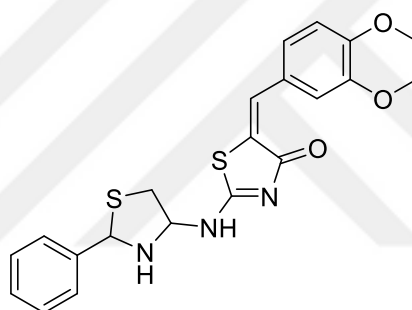


Figure 2.41. Molecular structure of 5-(3,4-Di-methoxybenzylidene)-2-((2-phenylthiazolidin-4-yl)amino)thiazol-4(5H)-one compounds designed by Hemaid et al. (2021).

3. MATERIAL

3.1. Required Materials

Table 3.1. List of required chemical material used during this thesis.

1-Piperidine	:Fluka, Germany
4-Flouro benzaldehyde	:Fluka, Germany
Methyl isothiocyanate	:Alfa Aesar, Germany
Ethylisothiocyanate	:Alfa Aesar, Germany
Allylisothiocyanate	:Alfa Aesar, Germany
2-Bromo-1-(p-tolyl)ethan-1-one	:Sigma-Aldrich, USA
2-Bromo-1-(4-fluorophenyl)ethan-1-one	:Sigma-Aldrich, USA
2-Bromo-1-(4-chlorophenyl)ethan-1-one	:Sigma-Aldrich, USA
2-Bromo-1-(4-nitrophenyl)ethan-1-one	:Sigma-Aldrich, USA
4-(2-Bromoacetyl)benzonitrile	:Sigma-Aldrich, USA
2-Bromo-1-(4-methoxyphenyl)ethan-1-one	:Sigma-Aldrich, USA
2-Bromo-1-(4-methoxyphenyl)ethan-1-one	:Sigma-Aldrich, USA
2-Bromo-1-(2,4-dimethylphenyl)ethan-1-one	:Sigma-Aldrich, USA
2-Bromo-1-(2,4-dichlorophenyl)ethan-1-one	:Sigma-Aldrich, USA
2-Bromo-1-(3,4-dichlorophenyl)ethan-1-one	:Sigma-Aldrich, USA
Hydrazine hydrate	:Sigma-Aldrich, Germany
Hydrochloric acid	:Merck, Germany
Potassium carbonate	:Merck, Germany
Acetone	:Sigma-Aldrich, Germany
Ethanol	:Tekim, Türkiye

Table 3.1. List of required chemical material used during this thesis. (cont...)

Petroleum ether	:Merck, Germany
Ethyl acetate	:Sigma-Aldrich, Germany
Methanol	:Merck, Germany
Ether	:Merck, Germany
TLC silica gel 60 F254 Aluminium sheet	:Merck, Germany
DMSO-d ₆	:Merck, Germany
DMSO	:Sigma, Germany
Donepezil	:Sigma, Germany
Tacrine	:Sigma, Germany
AChE-E.C.3.1.1.7, electric eel	:Sigma, Germany
BChE-E.C. 3.1.1.8, equine serum	:Sigma, Germany
Acetylthiocholine iodide (ATC)	:Fluka, Germany
Butyrylthiocholine iodide (BTC)	:Fluka, Germany

3.2. Required Equipment

Table 3.2. List of required equipment used during this thesis.

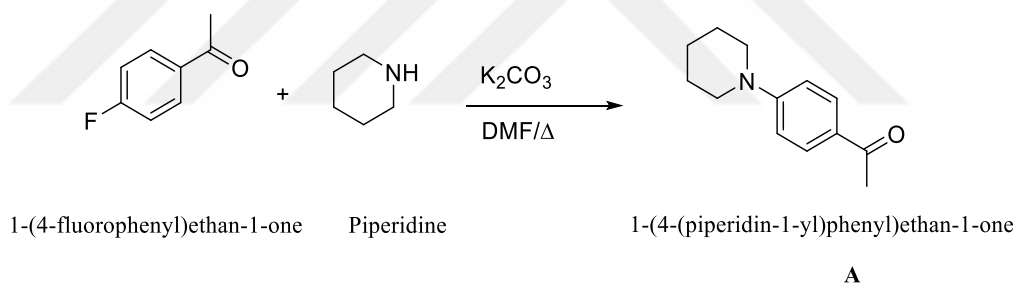
Electronic Scales	: Shimadzu, Libror EB-330 HU, Japan
Magnetic Base Heater Stirrer	: Heidolph, MR 3003, Germany
Ultraviolet Lamp	: Camag, Cabinet, Switzerland
Melting Point Determination Device	: Mettler Toledo-MP90 Melting Point System
Infrared Spectrophotometer IR	: Shimadzu-IR Affinity-1S, Japonya
Nuclear Magnetic Resonance Spectrometer NMR	: Bruker, UltraShield 300 MHz, ABD
Mass Spectrometer HRMS	: Shimadzu, LCMS-IT-TOF, Japonya
Sterile Cabinet	: Class II TypeA2 (CHC-222A2-60), Kore
Incubator	: Heraeus, Almanyia
Robotic Pipetting Table	: BioTek-Preccision XS ABD
Microplate Reader	: BioTek-Synergy H1 ABD
Vaccum Oven	:JEIO TECH, Korea
Vortex	: Wisemix, Kore

4. METHODS

4.1. Synthesis Methods

4.1.1 Synthesis of 1-(4-(piperidin-1-yl) phenyl)ethan-1-one (starting material A).

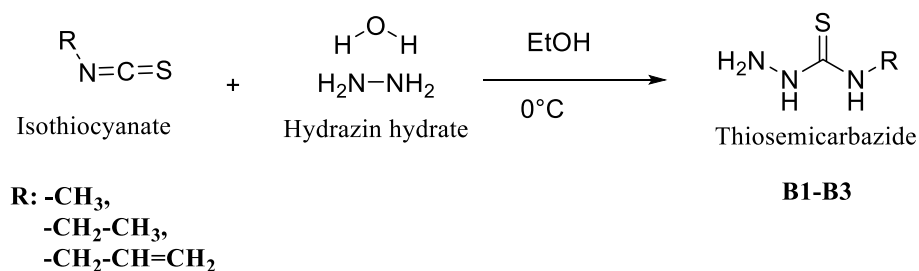
To synthesize the target compounds, the starting material **A** 1-(4-(piperidin-1-yl) phenyl)ethan-1-one (Scheme 4.1) was initially produced by reacting piperidine with 1-(4-Fluorophenyl)ethan-1-one. The process started with 10 grams (117.64 mmol) of piperidine, which was heated with 14.60 grams (117.64 mmol) of 1-(4-Fluorophenyl)ethan-1-one under reflux conditions for about 24 hours. Progress was monitored using thin-layer chromatography (TLC). After the reaction concluded, the mixture had been cooled and transferred to a beaker contained ice water, causing a precipitate to form. The resulting solid was filtered, purified into a yellow powder of 1-(4-(piperidin-1-yl) phenyl)ethan-1-one, and then recrystallized with ethanol.



Scheme 4.1. Synthesis of 1-(4-(piperidin-1-yl) phenyl)ethan-1-one (starting material **A**).

4.1.2. Synthesis of Three Different Series of Thiosemicarbazides (**B1-B3**)

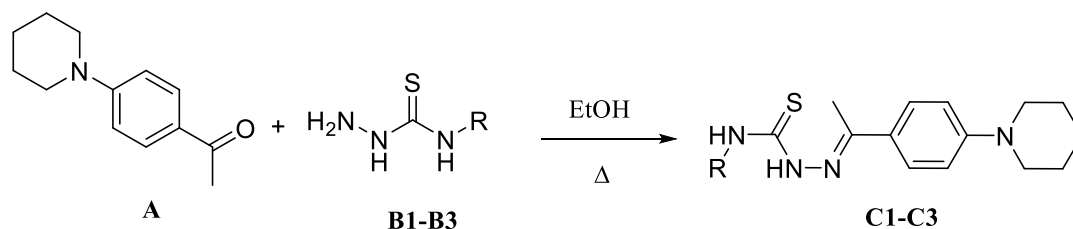
The synthesis of the second starting material **B1-B3** (Scheme 4.2) began with three different sets of: methyl, ethyl, and allyl isothiocyanates. The reaction was carried out in an ice bath, with hydrazine hydrate (4 eq.) being added drop-by-drop to isothiocyanates (1 eq.) while stirring continuously for approximately 2 hours. Upon completion of the reaction, the resulting white precipitate was separated and washed with cold ethanol to produce pure white crystals. The crystals were dried and then used for the subsequent step in the synthesis of the designed intermediate compounds.



Scheme 4. 2. Synthesis of thiosemicarbazides (second starting material **B1-B3**).

4.1.3. Synthesis of *N*-(*R*)-2-(1-(4-(piperidin-1 yl)phenyl)ethylidene)hydrazine-1-carbothioamide derivatives.

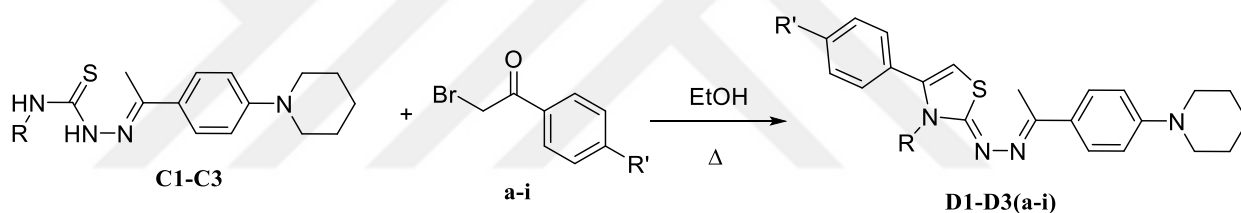
The intermediate compound **C1-C3** as shown in (Scheme 4.3) was synthesized from the previously obtained starting materials **A** and **B1-B3**. This step involved dissolving 5 grams (1 eq., 26.45 mmol) of compound **A** in ethanol. Afterward, different amounts (1 eq., 26.45 mmol) of compounds **B1-B3** were added to the flask, and the mixture was refluxed overnight. A few drops of hydrochloric acid were used as a catalyst to speed up the reaction. The reaction's progress was monitored using TLC. Once the reaction was complete, the intermediate **C1-C3** was obtained as an off-white powder, which was purified for use in the synthesis of the target compounds.



Scheme 4.3. Synthesis of *N*-(*R*)-2-(1-(4-(piperidin-1 yl)phenyl)ethylidene)hydrazine-1-carbothioamide derivatives (Intermediate material **C1-C3**).

4.1.4. Synthesis of the 3-R -2-((-1-(4-(piperidin-1 yl) phenyl) ethylidene) hydrazineylidene)-4-(R)-2,3-dihydrothiazole derivatives (D1-D3(a-i)).

The compounds in question were designed as new thiazole derivatives based on Hantzsch's synthesis method, as depicted in (Scheme 4.4) [142] . The intermediates, designated as **C1-C3**, were purified as three distinct derivatives. These intermediates were then refluxed with bromo acetophenone derivatives in ethanol. In this process, compounds **C1-C3** (1 eq.each) were refluxed with bromo acetophenone (1 eq.) derivatives (Table 4.1) in ethanol overnight. The reaction progress was monitored using TLC. The resulting precipitates were filtered and recrystallized in ethanol to obtain pure crystals of the desired products.



Scheme 4.4. Synthesis of the 3-R -2-((-1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-4-(R)-2,3-dihydrothiazole as target derivatives (Da-i) based on Hantzsch's synthesis method.

Table 4.1. The substituted of the 3-R -2-((-1-(4-(piperidin-1 yl) phenyl) ethylidene) hydrazineylidene)-4-(R)-2,3-dihydrothiazole derivatives (**D1-D3(a-i)**)..

Entry	R	R'
D1.a	-CH ₃	-CH ₃
D1.b	-CH ₃	-F
D1.c	-CH ₃	-NO ₂
D1.d	-CH ₃	-Cl
D1.e	-CH ₃	-OCH ₃
D1.f	-CH ₃	-CN
D1.g	-CH ₃	2',4'-Di-Chloro
D1.h	-CH ₃	3',4'-Di-Chloro
D1i	-CH ₃	2',4'-Di-methyl
D2.a	-H ₂ C=CH ₂	-CH ₃
D2.b	-H ₂ C=CH ₂	-F
D2.c	-H ₂ C=CH ₂	-NO ₂
D2.d	-H ₂ C=CH ₂	-Cl
D2.e	-H ₂ C=CH ₂	-OCH ₃
D2f	-H ₂ C=CH ₂	-CN
D2.g	-H ₂ C=CH ₂	2',4'-Di-Chloro
D2.h	-H ₂ C=CH ₂	3',4'-Di-Chloro
D2i	-H ₂ C=CH ₂	2',4'-Di-methyl
D3.a	-C ₂ H ₅	-CH ₃
D3.b	-C ₂ H ₅	-F
D3.c	-C ₂ H ₅	-NO ₂
D3.d	-C ₂ H ₅	-Cl
D3.e	-C ₂ H ₅	-OCH ₃
D3.f	-C ₂ H ₅	-CN

Table 4.2. The substituted of the 3-R -2-((-1-(4-(piperidin-1 yl) phenyl) ethylidene) hydrazineylidene)-4-(R)-2,3-dihydrothiazole derivatives (**D1-D3(a-i)**).(cont..).

D3.g	-C ₂ H ₅	2',4'-Di-Chloro
D3.h	-C ₂ H ₅	3',4'-Di-Chloro
D3.i	-C ₂ H ₅	2',4'-Di-methyl

4.2. Investigations Using Thin Layer Chromatography and Rf Value Calculation

With the use of TLC, the reactions at each stage of the synthesis were observed. Aluminum plates coated with silica gel 60 F254 and solvent combinations created specifically for this purpose were utilized in the TLC analysis. Capillary tubes were used to apply ethanol solutions containing the starting ingredients, as well as samples obtained at different stages of the reaction, onto the plates, which then developed in the selected mobile phases. Ultraviolet light with wavelengths of 254 nm and 366 nm was used to detect and examine the spots on the plates. The decision to halt or continue the reactions was based on the results from the TLC. Experiments were conducted to determine the appropriate solvent combinations for TLC applications throughout the thesis. The mobile phases that proved suitable for the control of the synthesis steps were petroleum ether: ethyl acetate in ratios of 3:1 and 1:1.

The retention factor, or R_f , is a quantitative measure used in thin-layer chromatography (TLC) to describe the relative distance a compound travels on a TLC plate compared to the distance traveled by the solvent front. It is defined as shown below:

$$R_f = \frac{\text{distance traveled by the compound}}{\text{distance traveled by the solvent front}}$$

4.3. Determination of Melting Points

The Mettler Toledo-MP90 Melting Point System was used to ascertain the melting points of the produced compounds. The synthesized compounds were placed into capillary tubes, which were inserted into the apparatus's chambers with one end open and to a depth of approximately $\frac{1}{2}$ centimeter. The melting point system then heated the samples in a controlled manner. Once the process was complete, the melting points of the compounds were determined by reviewing the recorded videos from the apparatus. These videos showed the change in state of the compounds, indicating when they transitioned from solid to liquid, which marks the melting point. This method allows for accurate and reliable determination of melting points, which is crucial for identifying and characterizing compounds in a synthesis process.

4.4. Determination of Infrared Spectrum IR

Using an attenuated total reflectance (ATR) attachment, the infrared (IR) fingerprints of the obtained chemicals were analyzed by using Shimadzu-IR Affinity-1S spectrophotometer. This method is commonly used for recording IR spectra, especially for solid samples like powders. For this process, the synthesized compounds in their powdered form are applied directly onto the ATR attachment of the IR spectrophotometer. The attachment typically contains a crystal, which can be made from materials like diamond, zinc selenite, or germanium, depending on the application and the required wavelength range. In ATR-IR spectroscopy, infrared light is focused on the crystal, and it is internally reflected multiple times. The sample in contact with the crystal absorbs a portion of this light, creating a distinctive spectrum based on its molecular structure.

4.5. Nuclear Magnetic Resonance Spectra (^1H and ^{13}C -NMR).

The proton ^1H and carbon ^{13}C -NMR spectra were acquired using a Bruker Ultra Shield 300 MHz NMR spectrometer. Chemical shifts (δ) were reported in parts per million (ppm) relative to the NMR solvent signals (DMSO- d_6 2.5 and 3.33-3.45 ppm for protons, δ_c 39.5 ppm for ^{13}C) as an internal standard tetramethylsilane (TMS) were used.

4.6. Analysis of Mass Spectroscopy

The compounds' mass spectra were acquired in both positive and negative modes. This was accomplished by using the electron spray ionization (ESI) technique to inject sample acetonitrile/methanol solutions into the LCMS-IT-TOF (Shimadzu, Kyoto, Japan) apparatus. ESI is a common ionization method used in mass spectrometry. It works by generating charged droplets of the sample in solution. As these droplets evaporate, the ions are released and can be analyzed by the mass spectrometer. The positive and negative modes indicate whether the ions are positively or negatively charged, providing flexibility in the analysis of different types of compounds. In this case, the use of acetonitrile/methanol as a solvent, combined with the LCMS-IT-TOF system, allowed for efficient and accurate detection of the mass-to-charge ratios of the compounds in both ionization modes, providing a comprehensive analysis of their mass spectra. This technique is often used in analytical

chemistry for identifying and characterizing chemical compounds in a variety of fields, including pharmaceuticals, biochemistry, and environmental science.

4.7. Determination of Biological Activity

The potential for the produced compounds' inhibition was evaluated through using an enzyme inhibition assay on the AChE enzyme. Distilled water from the Millipore/Milli-Q purification apparatus was utilized at every stage of the biological activity investigations during the evaluation process. In less than a week, all of the solutions required for the biological investigation were meticulously prepared and manufactured from scratch. A "BioTek-Precision Power (United States)" robotic pipetting system was utilized to distribute the prepared solutions and apply the test compounds and enzyme-substrate solutions to 96-well plates. The "BioTek-Synergy H1 Microplate Reader (United States)" was employed to establish and follow the enzyme protocol and to perform spectrophotometric measurements. Solutions of the compounds were prepared at two different concentrations, 10^{-3} M and 10^{-4} M, using dimethylsulfoxide (DMSO) as 2% solution, and these were used in the biological activity studies. Activity values were determined, ranging from 0% to 100% for each compound.

4.7.1. Anticholinesterase enzyme activity studies

The potential of inhibition of the obtained molecules on the AChE and BChE enzymes were examined using a variation of the Ellman method [145, 159].

4.7.1.1. Preparation of AChE and BChE enzyme solution

First, a 1% gelatin solution was made, subsequently the lyophilized AChE and BChE enzymes were dissolved in this solution. To create a new enzyme solution, AChE and BChE enzymes were added at a concentration of 500 U/mL to the gelatin solution. Following this, 1 mL of the created enzyme solution was transferred to a 100 mL volumetric flask, and water was added to the flask to reach a total volume of 100 mL. This process resulted in a stock enzyme solution with a concentration of 5 U/mL. After that, the recently made stock enzyme solution was separated into 0.7 mL aliquots and kept cold at -20°C . To ensure the concentrations were appropriate for the procedure, the stock enzyme solutions were first brought to room temperature. Each solution was then diluted with water to a final volume of

1.4 mL before beginning the activity experiments. Enzyme activity tests were conducted using the resulting 2.5 U/mL enzyme solutions.

4.7.1.2. Preparation of (0.075 M) acetylthiocholine iodide (ATC) solution.

To prepare the ATC solution, 0.217 g of ATC was first dissolved in a small volume of water. After the compound was completely dissolved, the solution was diluted by adding 10 mL of water. The prepared solution was then divided into smaller portions, with each portion containing 0.4 mL, and stored at -20°C until needed for use.

4.7.1.3. Preparation of (0.075 M) butyrylthiocholine iodide (BTC) solution.

BTC (0.237 g) was weighed first, and it was subsequently dissolved in water. Once the dissolution process was finished, water was added to make up to 10 mL. The prepared solution was separated into 0.4 mL portions and stored at -20 °C before to use.

4.7.1.4. Preparing a 0.01 M solution of 5-5-dithiobis (2-nitrobenzoic acid) (DTNB).

Prior to dissolving in water, DTNB (0.396 g) was weighed. NaHCO₃ (0.15 g) was added to this solution once the dissolution process was finished, and the volume was finished to 100 mL with water. Before being used, the produced solution was kept in 3 mL portions at -20 °C.

4.7.1.5. Preparing the phosphate buffer (pH=8.0).

An amount of potassium dihydrogen phosphate (13.61 g) were dissolved in one liter of water following the weighing process. Using 0.1 N NaOH solution and a pH meter, the solution was adjusted carefully to pH 8.0 ± 0.1. To produce a buffer solution appropriate for activity research, the modified buffer solution was subsequently passed through disposable filters featuring a 0.22 µm pore diameter. Before being used, the prepared solution was kept at 4°C.

4.7.1.6. AChE and BChE inhibition study

The developed compounds' investigation of their enzyme inhibitory activity was conducted by evaluating the enzyme inhibition using the modified colorimetric Ellman method as described above[160–162]. Before starting the inhibition studies, all solutions

were brought to 20-25 °C. The studies were performed using 96-well plates. Each well was brought to a final volume of 210 μ L using two different test solutions.

The first test solution for each well contained:

- 20 μ L of enzyme
- 20 μ L of DTNB
- 70 μ L of phosphate buffer

The second test solution for each well contained:

- 10 μ L of ATC/BTC
- 70 μ L phosphate buffer

To achieve a final volume of 210 μ L in each well, the following were added:

- 10 μ L of ATC/BTC
- 20 μ L of enzyme
- 20 μ L of inhibitor
- 20 μ L of DTNB
- 140 μ L of phosphate buffer

Using the Biotek Precision XS (United States) robotic equipment, 110 μ L of the first test solution and 20 μ L of inhibitor chemicals at varying doses were initially applied to 96-well plates. Four duplicates of each inhibitory chemical concentration were used. The BioTek-Synergy H1 (United States) microplate reader was filled with the prepared plates. The plates were first mixed for five minutes, and then they were incubated at 25°C for fifteen minutes.

After the 15-minute incubation, 80 μ L of the second test solution was added to each well from the dispenser chamber of the microplate reader. This was followed by 30 seconds of mixing. The first absorbance reading was then taken at a wavelength of 412 nm.

The second absorbance reading was obtained after a 5-minute mixing process to ensure the continuation of the reaction. The percentage inhibition rates were calculated using the differences between the two absorbance values obtained from the readings.

To calculate the percentage inhibition, the following formula was used:

$$\% \text{ Inhibition} = \left(\frac{[(A(C) - A(B)) - (A(I) - A(B))]}{(A(C) - A(B))} \right) \times 100$$

B: Blank (well without inhibitor and substrate)

C: Control (well containing inhibitor compound only)

A(B): The reading variation between absorption value of the blind well

A(C): The reading variation between absorption value of the control well

A(I): Reading difference between absorbance values of inhibitory substances

This formula was applied to determine the inhibition rates based on the differences between the initial and final absorbance values recorded at 412 nm. The IC₅₀ values of the given compounds were computed using Microsoft Office Excel 2013. The inhibition curves were constructed using the sigmoid dose-response model through the program's non-linear regression analysis feature. This method allowed for the accurate determination of the concentration of the compounds required to inhibit 50% of the enzyme activity.

4.8. Cytotoxicity Effect Studies

4.8.1. Proliferation of NIH3T3 cells

To maintain the mouse embryonic fibroblast cell line (NIH3T3) used in the study, routine passaging was performed every two to three days. Before starting the procedure, the cell culture bottle was gently shaken to dislodge any dead cells into the medium. The old medium was then removed with a sterile pipette and discarded. To wash the cells, 5 mL of phosphate buffer was added to the culture bottle, which was then drained of the buffer.

Next, a trypsin EDTA solution was added to detach the cells from the culture surface. The amount of trypsin EDTA used depended on the bottle size: 3-5 mL for 75 cm² bottles,

and 1-3 mL for 25 cm² bottles. The culture bottle was gently shaken to ensure even distribution of the trypsin EDTA, then placed in an incubator for about 5 minutes at 37°C with 5% CO₂ and 95% humidity.

After the incubation period, 20-25 mL of fresh medium was added to the bottle to neutralize the trypsin and suspend the cells. The cell suspension was then divided into new culture bottles at ratios of 1:2 or 1:3, depending on the desired confluence, and placed back in the incubator at the same conditions (37°C, 5% CO₂, and 95% humidity).

4.8.2. Seeding NIH3T3 cells on plates

To ensure consistency, a sterile pipette was used to gently mix the dead cells in the liquid of a cell culture flask that had been removed from the incubator. After that, the medium was taken out and thrown. A volume of 3-5 mL (1-3 mL per 25 cm²) of trypsin-EDTA solution was added to the culture bottle (75 cm² culture surface area). After giving the flask a little shake, it was incubated at 37°C, 5% CO₂, and 95% humidity for roughly five minutes. Following incubation, the trypsin was neutralized by adding fresh media to the culture bottle, and a pipette was used to move the cell suspension onto a centrifuge tube. After mixing the cell suspension, 10 µL was obtained and counted using an automated cell counter. A 96-well cell culture plate was filled with the leftover cell suspension at a ratio of 200 µL per well (1,104 cells/100 µL). The plate was incubated at 37°C, 5% CO₂, and 95% humidity for a duration of 24 hours.

4.8.3. MTT cytotoxicity test applications to NIH3T3 cells.

The plates were inverted to extract the medium after the 24-hour incubation period. Positive controls and serial quantities of the chemicals (made as stock solutions in DMSO, ranging from 0.000316 mM to 1 mM) were added to the plates. An additional twenty-four hours were spent incubating the plates at 37°C, 5% CO₂, and 95% humidity. The plates were inverted to remove the medium following this incubation time. Phosphate buffer was used to wash the cells, and the washing solution was disposed of. 100 µL of a 1:10 combination of medium and MTT solution (5 mg/mL) was made, and this mixture was then applied to each well. For three hours, the plates were incubated at 37°C, 5% CO₂, and 95% humidity.

The plates were inverted to extract the fluid following the incubation. The optical density (OD) readings at 540 nm were measured using an ELISA reader after 100 μ L of DMSO was added to each well. For every test substance concentration, the % inhibition values were computed. Non-linear regression analysis was used to calculate the inhibitory concentration IC₅₀ values, and the cytotoxic characteristics of the compounds were evaluated [163, 164].

4.9. Computational Docking Analysis

A method based on structure *in silico* docking was employed to identify the potential interaction and binding sites of the **D1.f** compound, which emerged as the most effective against AChE and BChE, enzymes among synthesized derivatives in this thesis. Protein-ligand interaction analysis for the AChE enzyme was conducted using its crystal structure (PDB: 4EY7) as a reference [165].

First, the “Protein Preparation Wizard” protocol in Schrödinger Suite 2015 [166] was utilized to prepare the crystal structure for docking studies. The potential charges of the atoms on the charged amino acids were then automatically ascertained under the given environmental conditions, and bond lengths were adjusted using the OPLS 2005 force field. Compounds for molecular docking studies were prepared using the LigPrep 3.8 module [167]. The grid creation and docking studies were performed using Glide 7.1 [168] with single precision (SP).

4.10. Molecular Dynamics Studies

An essential computational method for assessing the ligand's time-dependent stability within the active site of a drug-receptor complex is a molecular dynamics (MD) simulation. In this study, MD simulations were performed for compound **D1.f** over a duration of 100 ns to ensure the stability of the complexes identified from the docking results. In the dynamic research, Desmond [169, 170] used a 3-point (TIP3P) water model, then, the OPLS3e force field was used to minimize the complex's energy and perform a conventional Schrödinger Suite with a transferable intermolecular potential. In order to neutralize the system and represent the physiological concentration of monovalent ions, the simulations incorporated a final salt concentration of 0.15 M with Na⁺ and Cl⁻ ions [170]. The simulations were

conducted at a constant temperature of 310.55 K and a pressure of 1.01325 bar using the NPT ensemble class. The equations of motion were integrated using the RESPA integrator with NH thermostats maintaining constant temperature and the MTK method controlling pressure. The PME approach was utilized to compute long-range electrostatic interactions, while 9.0 Å was established as the threshold for van der Waals and short-range electrostatic interactions. System equilibration was achieved using Desmond's default protocol, involving a series of constrained minimization and MD simulations to gradually relax the system. After setting up the system, MD simulations were conducted with the specified parameters. The radius of gyration (Rg), and root mean square deviation (RMSD) values were calculated using the Desmond application.

4.11. Prediction of Physicochemical and Pharmacokinetics

The determined of physicochemical and pharmacokinetic properties of the synthesized compounds were done through using computational methods. pkCSM, a web-based application from Cambridge (available at <http://biosig.unimelb.edu.au/pkcsml/prediction>), was used to predict pharmacokinetic parameters and the Drug Metabolism and Excretion (DMET) profile [171]. The smiles (Simplified Molecular Input Line Entry Specification) of the compounds were obtained from ChemDraw 17.0 software in order to do additional drug-likeness research. The pkCSM platform offers insights into various pharmacokinetic parameters, such as absorption indicators like Caco-2 permeability, oral bioavailability, and human intestinal absorption (HIA); distribution parameters like BBB permeability and plasma protein binding (PPB); metabolism factors like inhibition of cytochrome P450 2D6 (CYP2D6) and cytochrome P450 3A4 (CYP3A4); and excretion-related data like renal clearance. It also evaluates toxicity risks such as organ toxicity and genomic toxicity. Furthermore, the pharmacological characteristics and drug-likeness of the compounds were assessed using the SwissADME online tool (available at <http://www.swissadme.ch/>) [172].

5. RESULTS AND DISCUSSIONS

5.1. Synthesis Studies

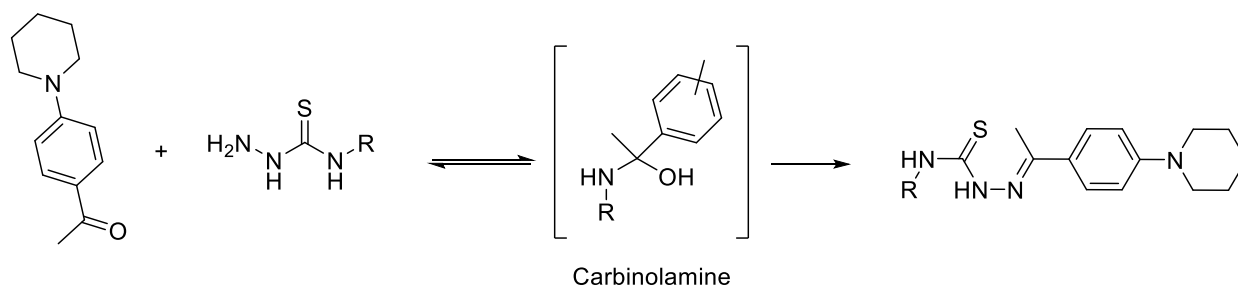
The synthesized compounds in this study are designed based on donepezil molecular structure, as an AChE inhibitor commonly used in Alzheimer's disease treatment. The compounds are designed to be suitable to interact with the active sites of AChE enzyme PAS and CAS. The piperidine moiety represents the same moiety as present in donepezil while instead of indanone moiety the compounds were designed to have five membered heterocyclic dihydrothiazole moiety. The synthesis was involved in three chemical reaction steps:

1. Synthesis of thiosemicarbazide derivatives

In this step three derivatives of isothiocyanate were used to synthesis the second precursor of the designed compounds in the presence of hydrazine hydrate through a nucleophilic reaction mechanism.

2. Synthesis of imines or Thiosemicarbazone

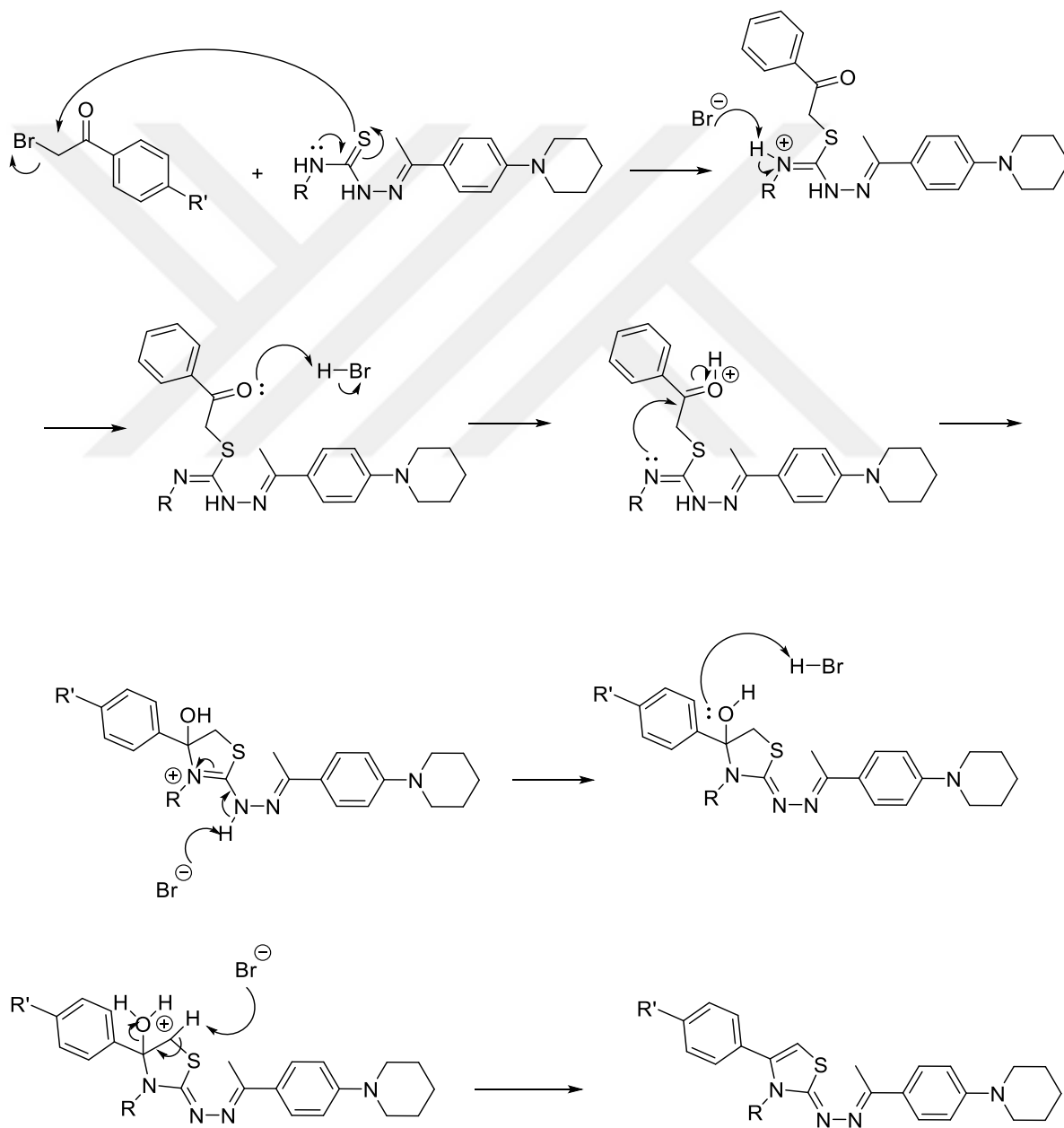
The most common method for the preparation of imines is the original reaction reported by Hugo Schiff in 1864 [173]. It is usually formed by the condensation of an aldehyde or ketone with a primary amine and elimination of water molecule. In this step the 1-(4-(piperidin-1-yl)phenyl)ethan-1-one are condensed with the synthesized thiosemicarbazide to form imine which is a reversible reaction in one step flask reaction and the dehydration of carbinolamine generally takes place under acid or base catalysis, or upon heating as shown in Scheme 5.1.



Scheme 5.1. Transition state and mechanism of Imine synthesis

3. Hantzsch synthesis method of dihydrothiazole moiety.

Synthesis of the last molecules **D1-D3** were done based on Hantzsch synthesis method which involves in several steps such as condensation, cyclization, reduction and substitution reaction as shown below (Scheme 5.2). In the cyclization transition step acid or base catalyst were used to facilitate the reaction [174].



Scheme 5.2. Mechanism of the synthesis of designed molecules according to Hantzsch synthesis method.

Overall, these synthesis studies aimed to generate new derivatives of donepezil with potential therapeutic applications in Alzheimer's disease.

5.1.1. 3-Methyl-2-((-1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-4-(p-substituted)-2,3-dihydrothiazole

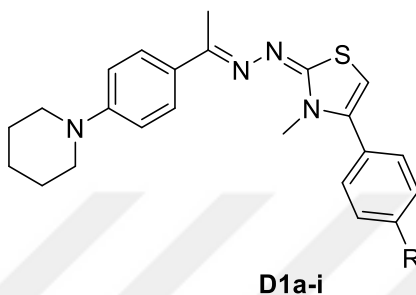


Figure 5.1. 3-Methyl-2-((-1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-4-(p-substituted)-2,3-dihydrothiazole.

D1.a-i was synthesized as mentioned in 4.1, based on Hantzsch Thiazole Synthesis method the reaction mechanism suggested as mentioned above:

5.1.1.1. 3-Methyl-2-((-1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-4-(p-tolyl)-2,3-dihydrothiazole

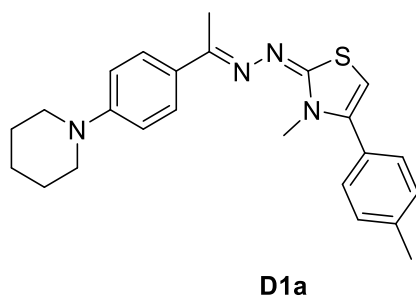


Figure 5.2. Molecular structure of 3-Methyl-2-((-1-(4-(piperidin-1 yl)phenyl)ethylidene)hydrazineylidene)-4-(p-tolyl)-2,3-dihydrothiazole.

Yield: 75%,

Melting point: 223°C.

IR Maximum Velocity (cm^{-1}): 2931 (C=C-H stretching band), 2812(C-H stretching band), 1589 (C=N stretching band), 1556 (C=C band), 1234 (C-N band), 812 (4-substituted phenyl out-of-plane deformation band).

^1H -NMR (300 MHz, $\text{DMSO}-d_6$): 1.58 (6H, s, piperidine-H), 2.33-2.36 (6H, s, Methyl-H), 3.21 (4H, s, piperidine-H), 3.30(3H, s, Methyl-H), 6.25 (1H, s, dihydrothiazole-H), 6.91-6.94(2H, d, $J=8.06$ Hz, Ar-H), 7.29-7.40(4H, m, Ar-H), 7.67-7.69 (2H, d, $J= 8.26$ Hz, Ar-H).

^{13}C -NMR (75 MHz, $\text{DMSO}-d_6$): $\delta = 14.38, 21.34, 24.43, 25.48, 33.83, 49.35, 99.21, 115.11, 127.22, 128.35, 128.71, 128.95, 129.78, 139.08, 140.91, 152.09, 154.57, 168.08$.

HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{24} \text{H}_{28} \text{N}_4 \text{S}$ $[\text{M}+\text{H}]^+$: 405.2107; found: 405.2091.

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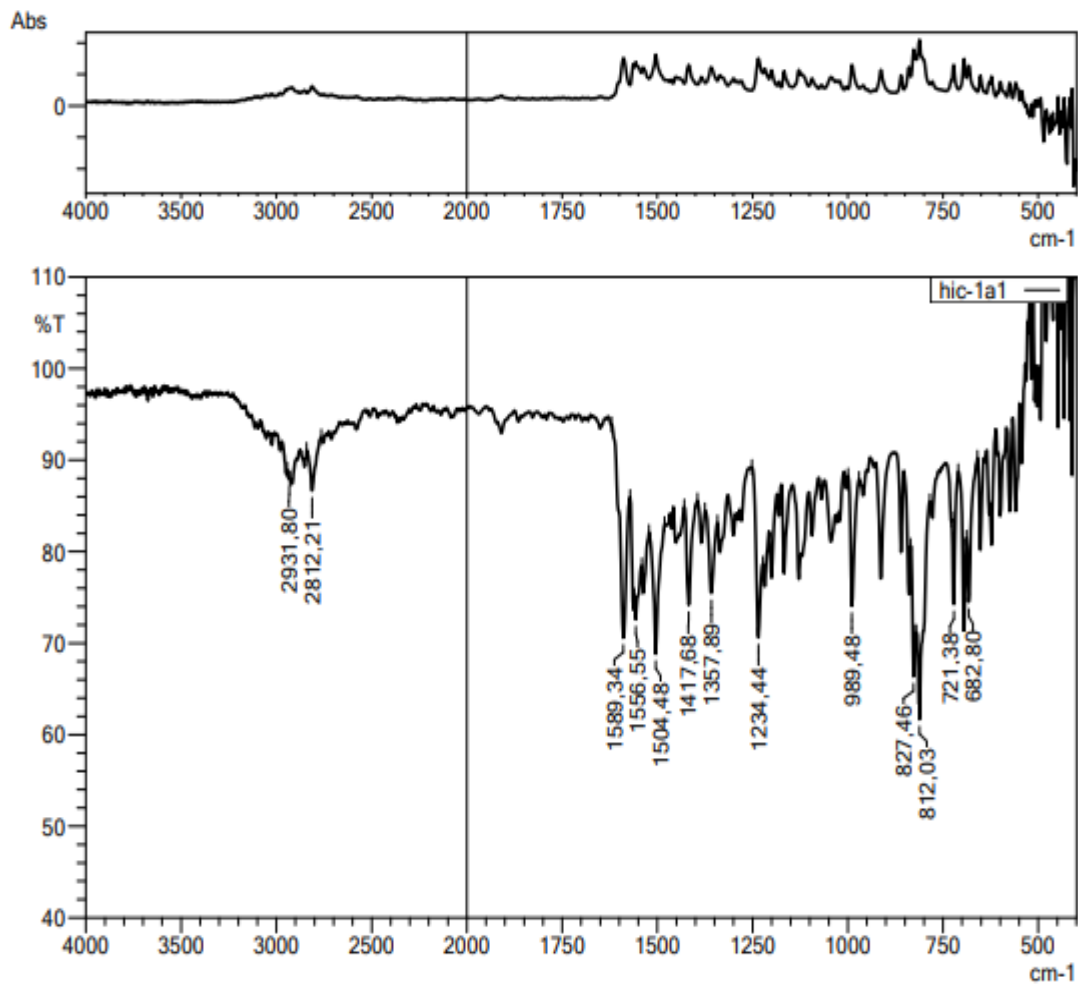


Figure 5. 3. IR fingerprint of D1.a.

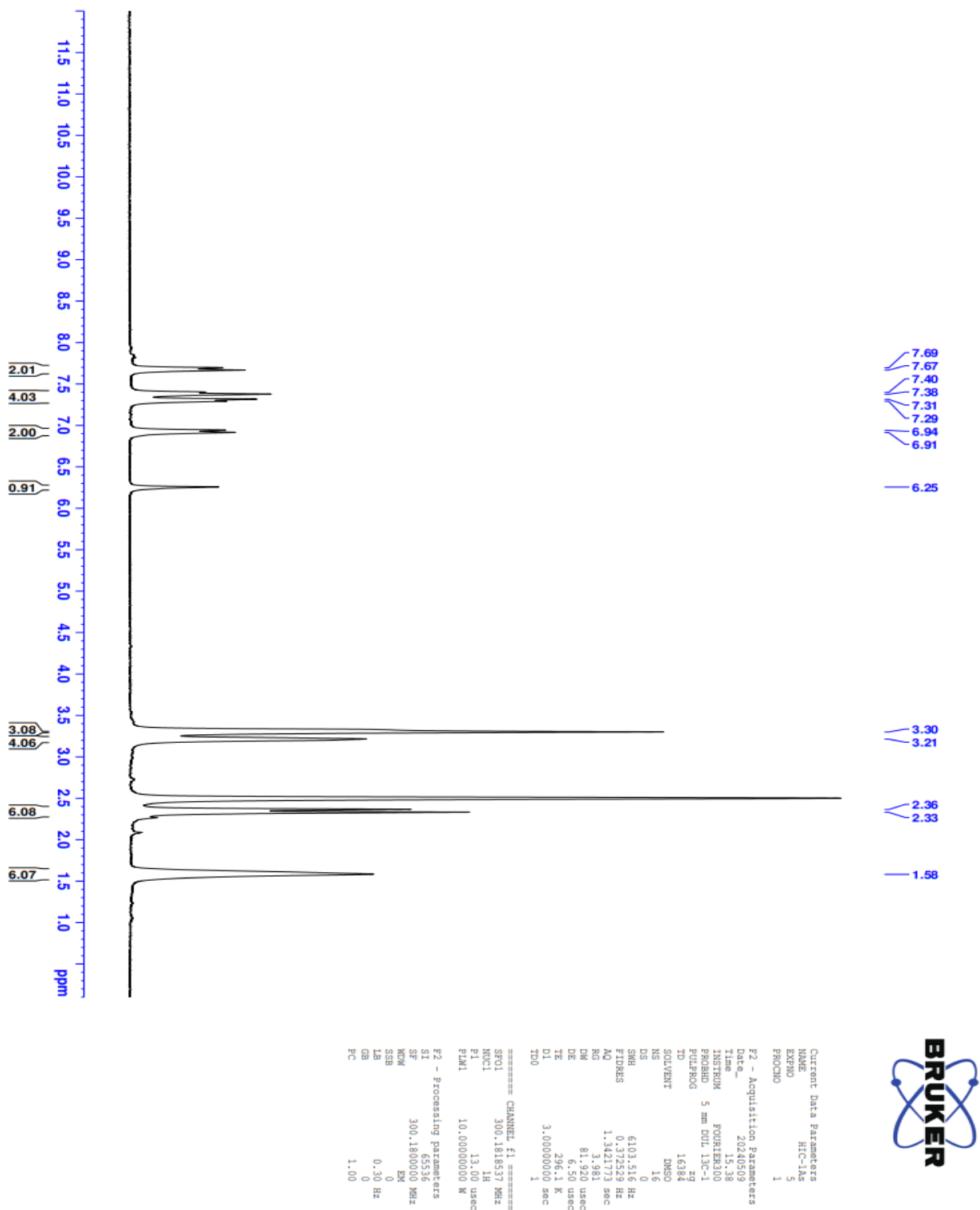


Figure 5.4. ^1H NMR signals of D1.a.

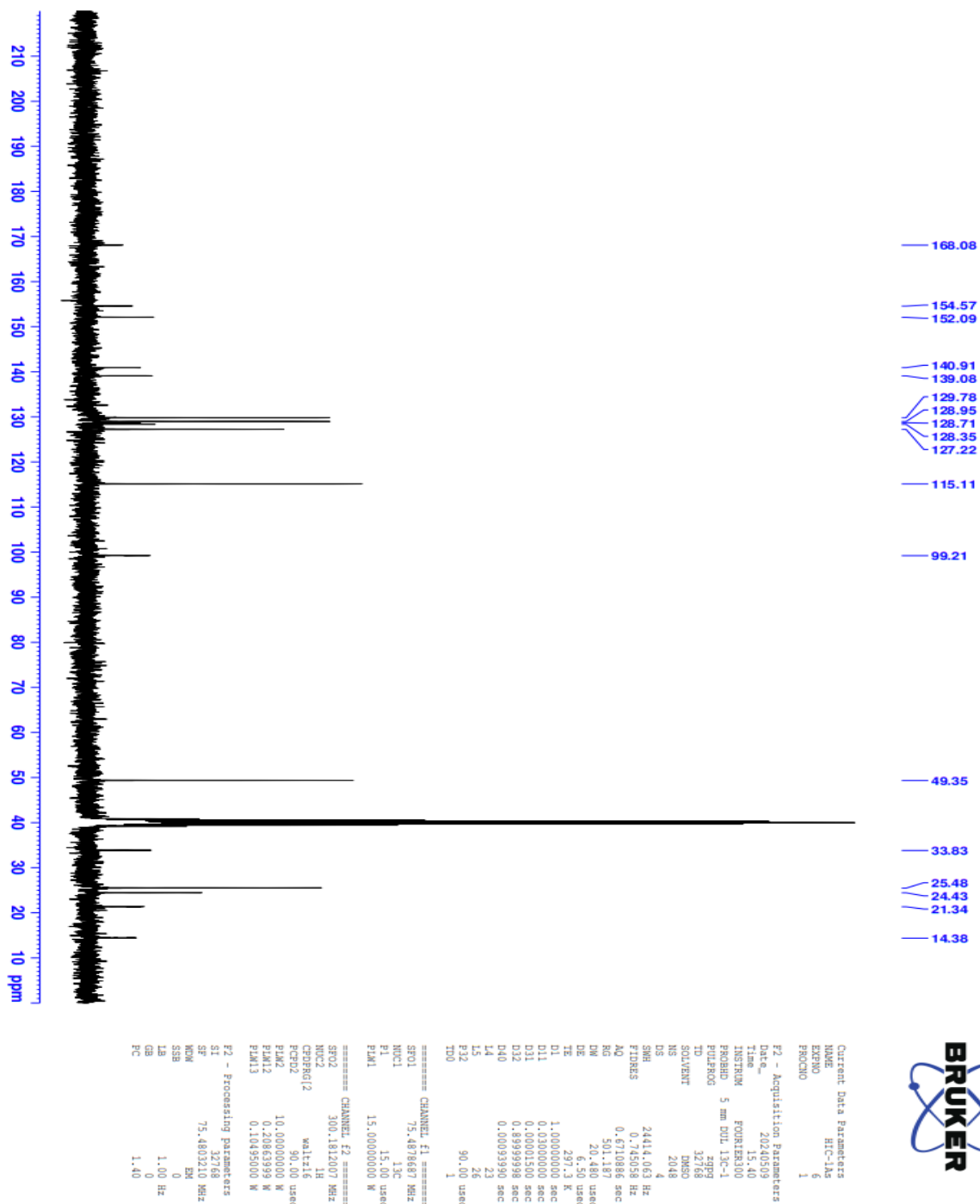


Figure 5.5. ^{13}C NMR signals of D1.a.

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C	4	4	32	F	1	0	0	Cl	1	0	0	Pd	2	0	0	Na
N	3	0	6	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5

HC Ratio: unlimited

Max Isotopes: 3

MSn Iso RI (%): 10.00

DBE Range: 0.0 - 30.0

Apply N Rule: no

Isotope RI (%): 1.00

MSn Logic Mode: AND

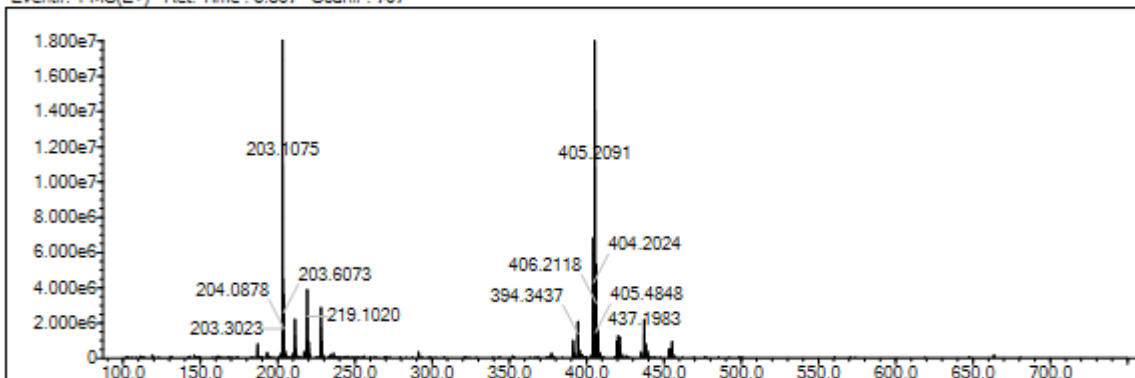
Electron Ions: both

Use MSn Info: yes

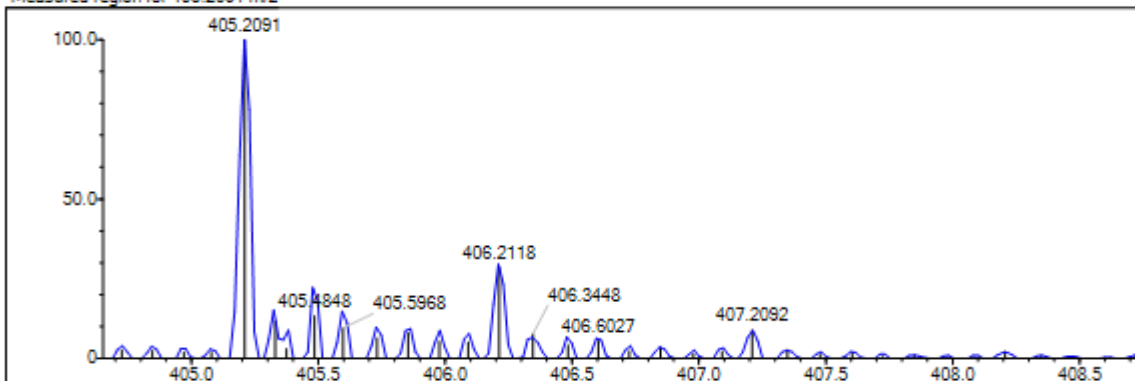
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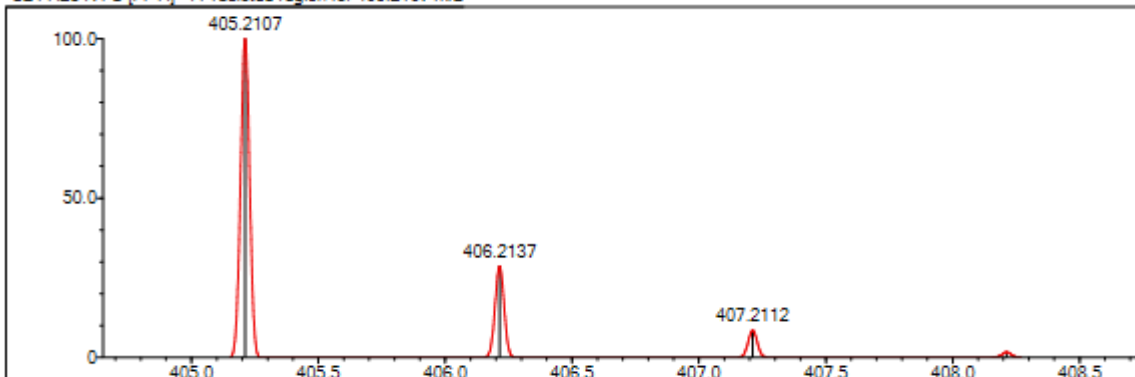
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Measured region for 405.2091 m/z



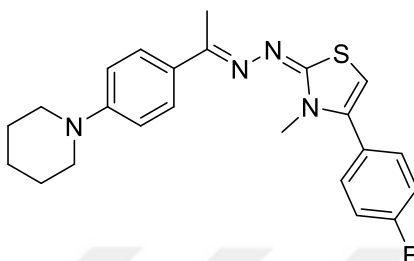
C24 H28 N4 S [M+H]⁺ : Predicted region for 405.2107 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
4	82.34	C24 H28 N4 S	[M+H] ⁺	405.2091	405.2107	-1.6	-3.95	88.89	13.0

Figure 5.6. HRMS spectrogram of D1.a.

5.1.1.2. 4-(4-Fluorophenyl)-3-methyl-2-((1-(4-(piperidin-1 yl)phenyl) ethylidene) hydrazineylidene)-2,3-dihydrothiazole.



D1b

Figure 5.7. Molecular structure of 4-(4-Fluorophenyl)-3-methyl-2-((1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-2,3-dihydrothiazole.

Yield: 86%,

Melting point: 233.2°C.

IR Maximum Velocity (cm^{-1}): 3109 (C=C-H stretching band), 2937 (C-H stretching band), 1585 (C=N stretching band), 1504 (C=C voltage band), 1240 (C-N band), 813 (4-substituted phenyl out-of-plane deformation band).

^1H -NMR (300 MHz, $\text{DMSO}-d_6$): 1.57 (6H, s, piperidine-H), 2.33 (3H, s, Methyl-H), 3.21 (4H, s, piperidine-H), 3.30 (3H, s, Methyl-H), 6.37 (1H, s, dihydrothiazole-H), 6.91-6.94 (2H, d, $J=8.14$ Hz, Ar-H), 7.54-7.55 (4H, q, $J=5.30$ Hz, Ar-H), 7.67-7.70 (2H, d, $J=8.69$ Hz, Ar-H).

^{13}C -NMR (75 MHz, $\text{DMSO}-d_6$): δ = 14.15, 24.37, 25.64, 29.72, 33.43, 50.02, 99.61, 115.32, 115.71, 115.99, 127.18, 127.50, 129.61, 130.55, 130.66, 139.62, 152.30, 155.83, 161.39, 164.69.

HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{23} \text{H}_{25} \text{N}_4 \text{FS}$ $[\text{M}+\text{H}]^+$: 409.1857; found: 409.1837.

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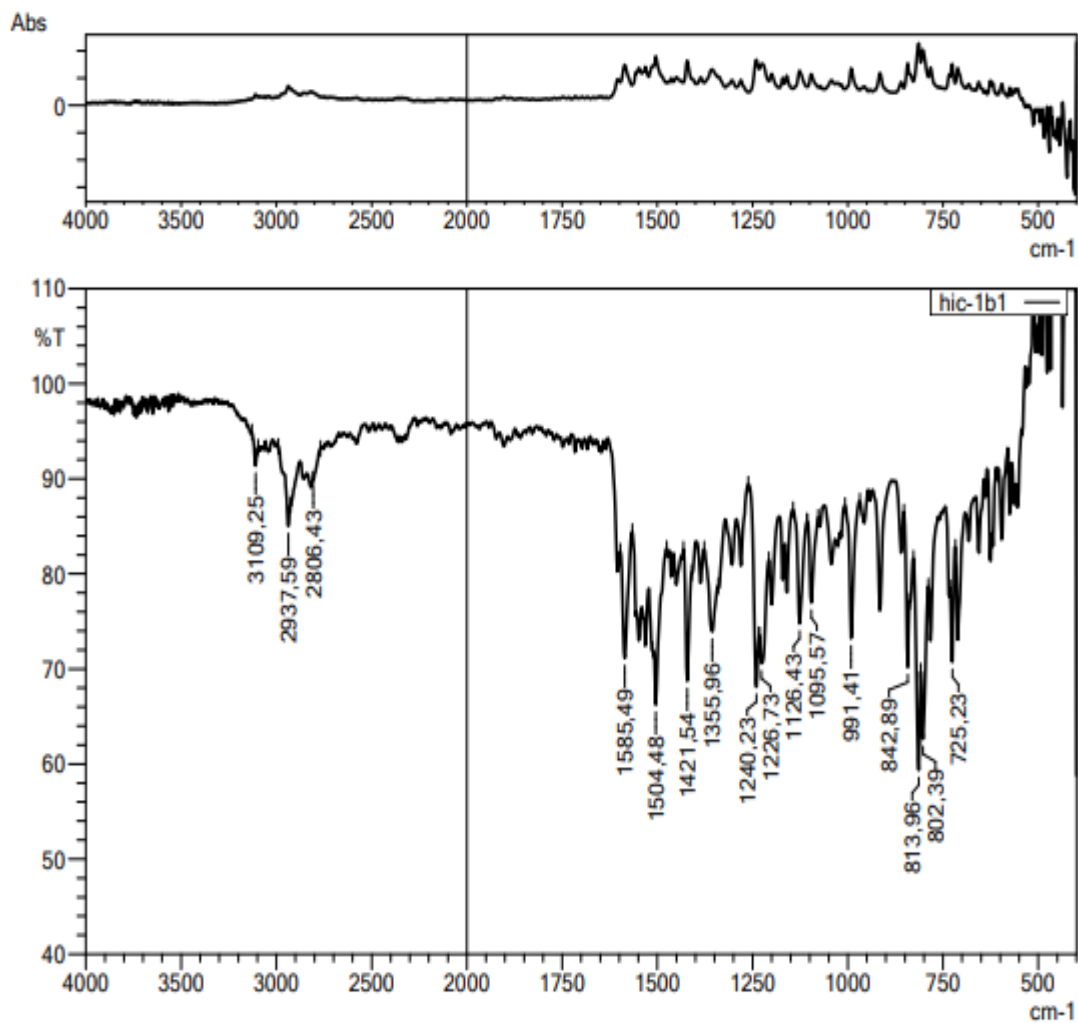


Figure 5.8. IR fingerprint of D1.b.

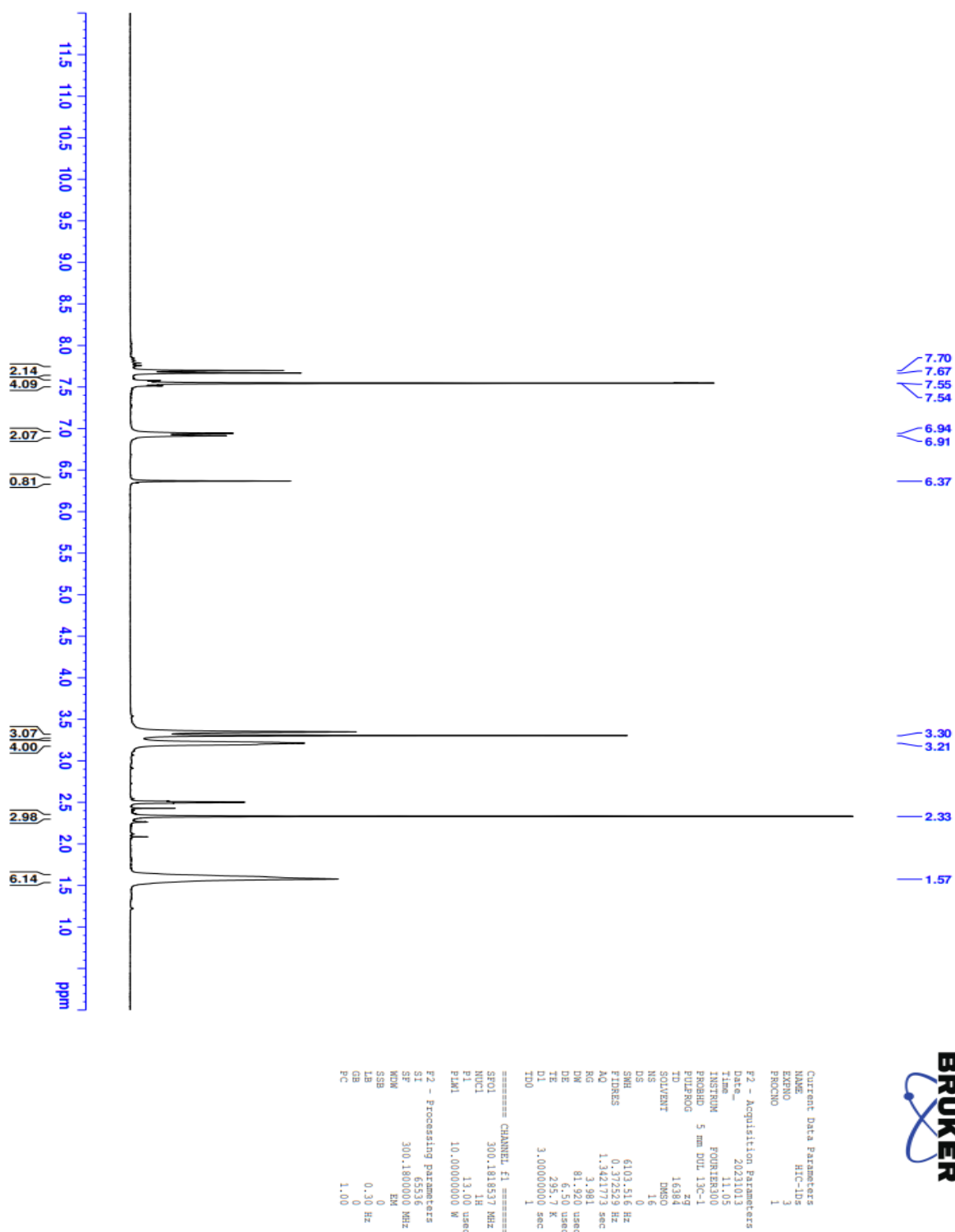


Figure S9. ¹H NMR signals of D1.b.

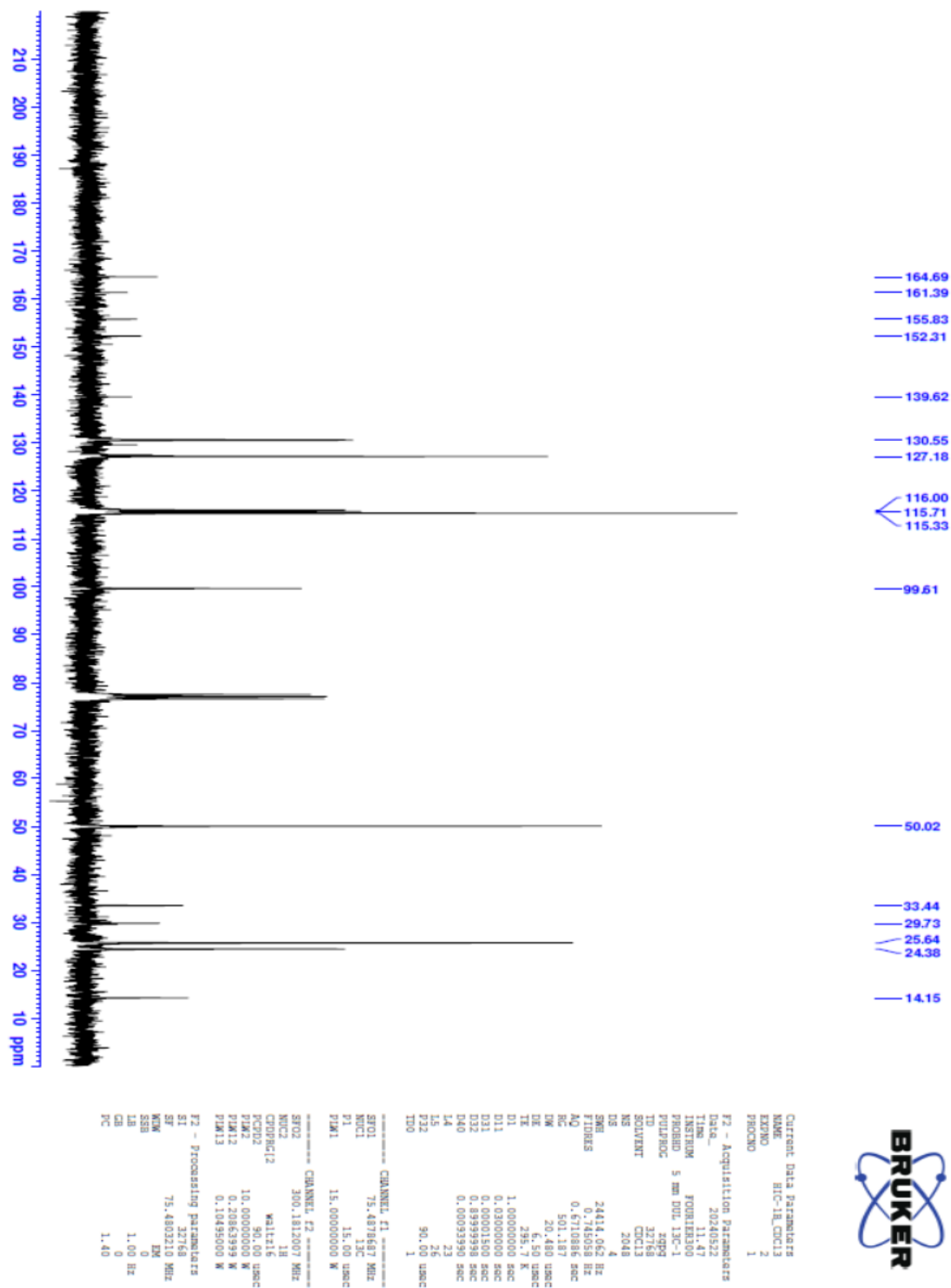


Figure 5. 10. ^{13}C NMR signals of D1.b.

Data File: C:\LabSolutions\Data\Analiz\derya\HIC-18 UST_578.lcd

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C	4	4	32	F	1	1	1	Cl	1	0	0	Pd	2	0	0	Na
N	3	0	6	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5

HC Ratio: unlimited

Max Isotopes: 3

MSn Iso RI (%): 10.00

DBE Range: 0.0 - 30.0

Apply N Rule: no

Isotope RI (%): 1.00

MSn Logic Mode: AND

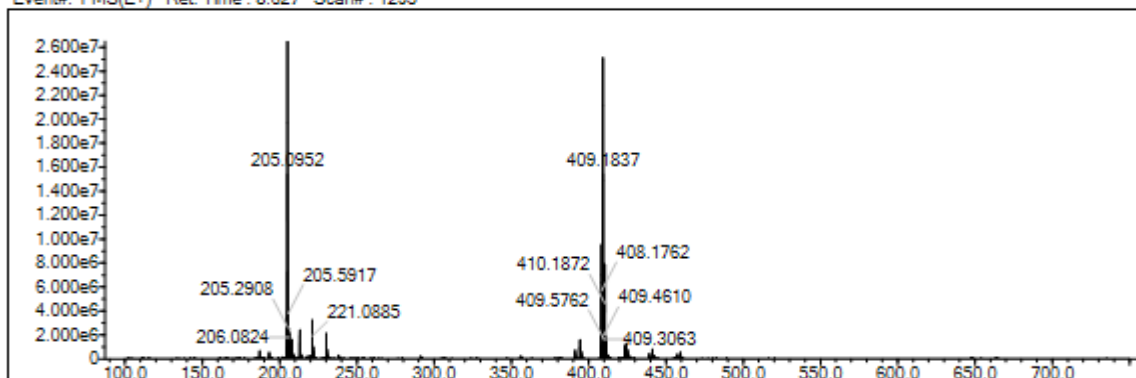
Electron Ions: both

Use MSn Info: yes

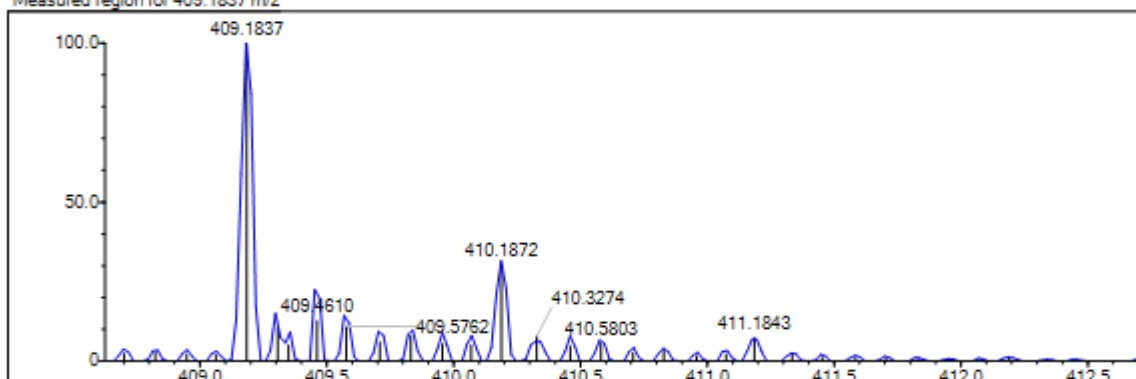
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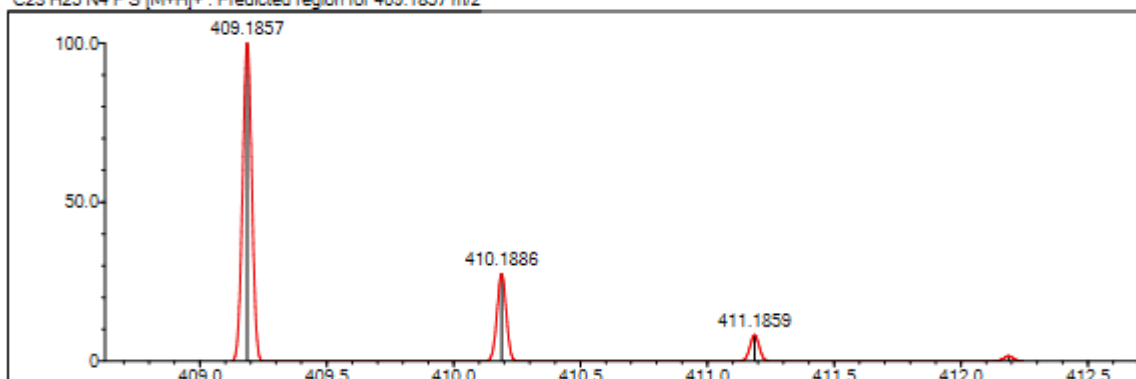
Event#: 1 MS(E+) Ret. Time: 8.627 Scan#: 1295



Measured region for 409.1837 m/z



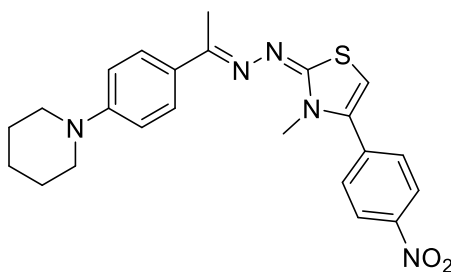
C23 H25 N4 F S [M+H]⁺ : Predicted region for 409.1857 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
6	85.88	C23 H25 N4 F S	[M+H] ⁺	409.1837	409.1857	-2.0	-4.89	95.13	13.0

Figure 5.11. HRMS spectrogram of D1.b

5.1.1.3. 3-Methyl-4-(4-nitrophenyl)-2-((1-(4-(piperidin-1-yl)phenyl) ethylidene) hydrazineylidene) -2,3-dihydrothiazole



D1c

Figure 5.12. 3-Methyl-4-(4-nitrophenyl)-2-((1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-2,3-dihydrothiazole.

Yield: 90%

Melting point: 257-259.4°C.

IR Maximum Velocity (cm^{-1}): 3103 (C=C-H stretching band), 2933 (C-H stretching band), 1587 (C=N stretching band), 1516 (C=C band), 1240 (C-N stretching band), 823 (4-substituted phenyl out-of-plane deformation band).

^1H -NMR (300 MHz, $\text{DMSO}-d_6$): 1.58 (6H, s, piperidine-H), 2.33 (3H, s, Methyl-H), 3.21 (4H, s, piperidine-H), 3.30 (3H, s, Methyl-H), 6.35 (1H, s, dihydrothiazole-H), 6.90-6.93 (2H, d, $J=8.56$ Hz, Ar-H), 7.54 (4H, s, Ar-H), 7.67-7.84 (2H, $J=8.63$ Hz, Ar-H).

HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{23} \text{H}_{25} \text{N}_5 \text{O}_2 \text{S}$ $[\text{M}+\text{H}]^+$: 436.1802; found: 436.1795.

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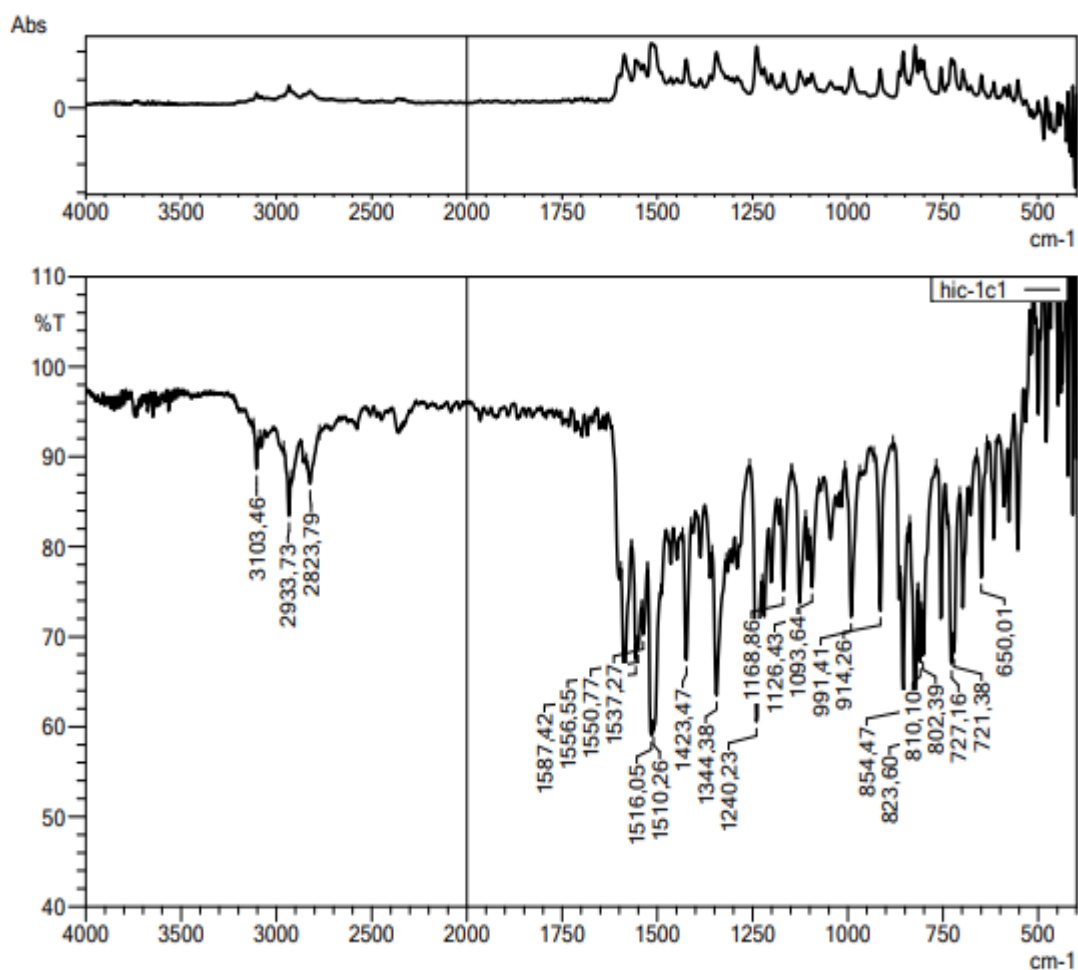


Figure 5.13. IR fingerprint of D1.c.

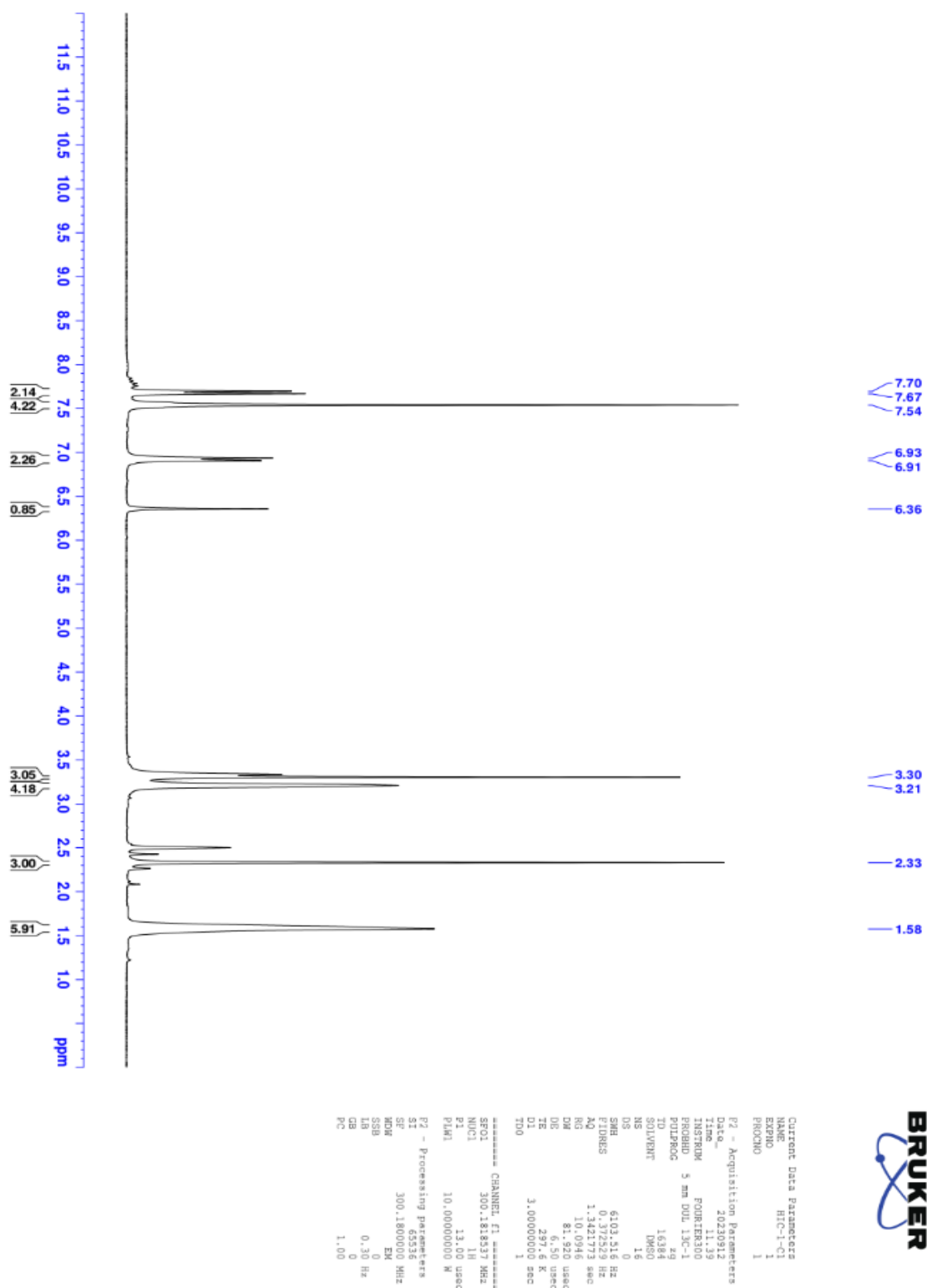


Figure 5. 14. ¹H NMR signals of D1.c.

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C	4	4	32	F	1	0	0	Cl	1	0	0	Pd	2	0	0	Na
N	3	0	6	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5

HC Ratio: unlimited

Max Isotopes: 3

MSn Iso RI (%): 10.00

DBE Range: 0.0 - 30.0

Apply N Rule: no

Isotope RI (%): 1.00

MSn Logic Mode: AND

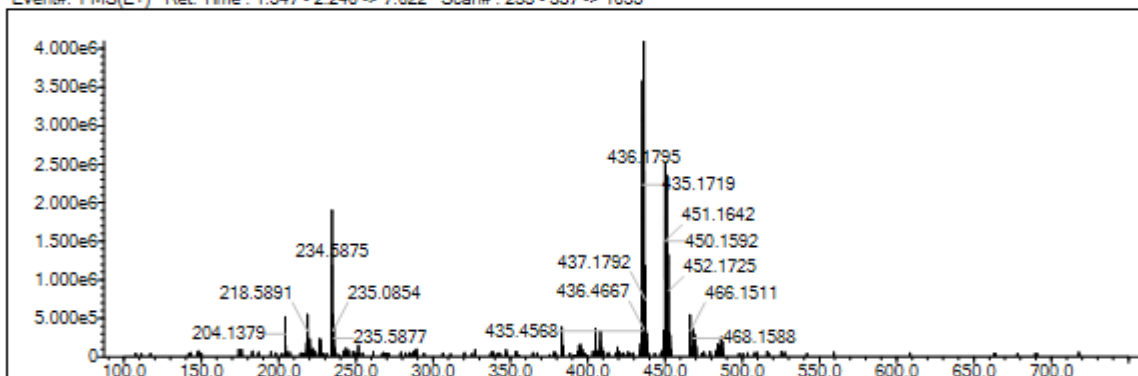
Electron Ions: both

Use MSn Info: yes

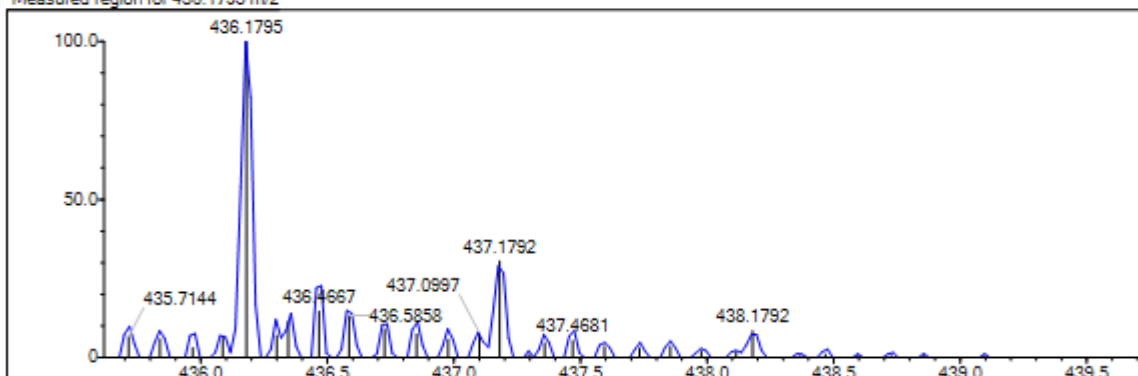
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Max Results: 50

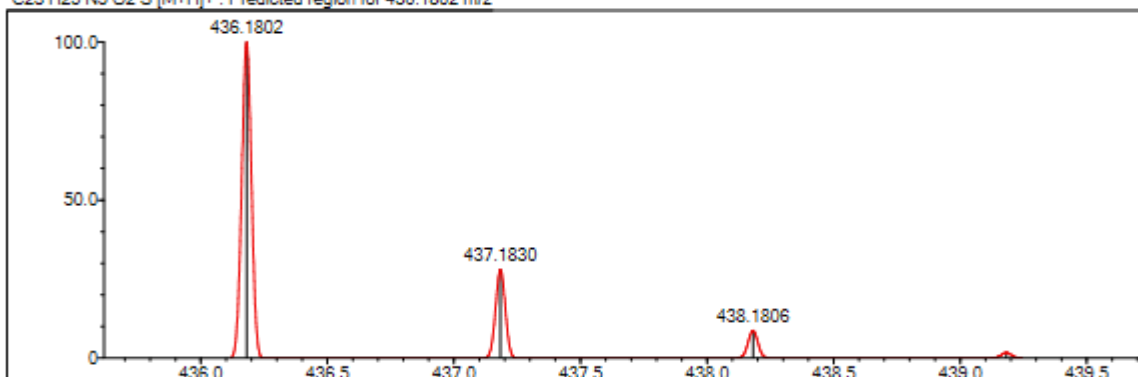
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Measured region for 436.1795 m/z



C23 H25 N5 O2 S [M+H]⁺ : Predicted region for 436.1802 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	91.44	C23 H25 N5 O2 S	[M+H] ⁺	436.1795	436.1802	-0.7	-1.60	92.83	14.0

Figure 5. 15. HRMS spectrogram of D1.c.

5.1.1.4. 4-(4-Chlorophenyl)-3-methyl-2-((1-(4-(piperidin-1-yl)phenyl) ethylidene) hydrazineylidene)-2,3-dihydrothiazole

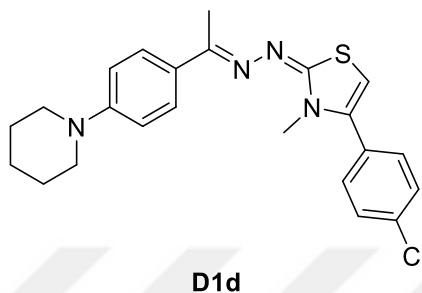


Figure 5.16. 4-(4-Chlorophenyl)-3-methyl-2-((1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-2,3-dihydrothiazole.

Yield: 80%

Melting point: 216.6°C.

IR Maximum Velocity (cm^{-1}): 3103 (C=C-H stretching band), 2937 (C-H stretching band), 1593 (C=N stretching band), 1537 (C=C band), 1236 (C-N stretching band), 827 (4-substituted phenyl out-of-plane deformation band).

^1H -NMR (300 MHz, $\text{DMSO}-d_6$): 1.57 (6H, s, piperidine-H), 2.33 (3H, s, Methyl-H), 3.21 (4H, s, piperidine-H), 3.30 (3H, s, Methyl-H), 6.37 (1H, s, dihydrothiazole-H), 6.91-6.94 (2H, d, $J=8.87$ Hz, Ar-H), 7.54-7.55 (4H, m, Ar-H), 7.67-7.70 (2H, d, $J=8.90$ Hz, Ar-H).

^{13}C -NMR (75 MHz, $\text{DMSO}-d_6$): δ = 14.41, 24.41, 25.46, 33.88, 49.36, 100.56, 113.37, 115.13, 127.26, 128.16, 129.28, 129.73, 130.01, 130.84, 134.24, 139.69, 154.82, 168.10.

HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{23} \text{H}_{25} \text{N}_4 \text{S Cl}$ $[\text{M}+\text{H}]^+$: 425.1561; found: 425.1544.

Item	Value
Acquired Date&Time	2.05.2024 14:17:43
Acquired by	System Administrator
Filename	C:\Users\dopnalab\Desktop\MASAUŠTŮ\sazan\hic\hic-1d1.ispd
Spectrum name	hic-1d1
Sample name	hic-1d
Sample ID	
Option	
Comment	
No. of Scans	30
Resolution	4 [cm ⁻¹]
Apodization	Happ-Genzel

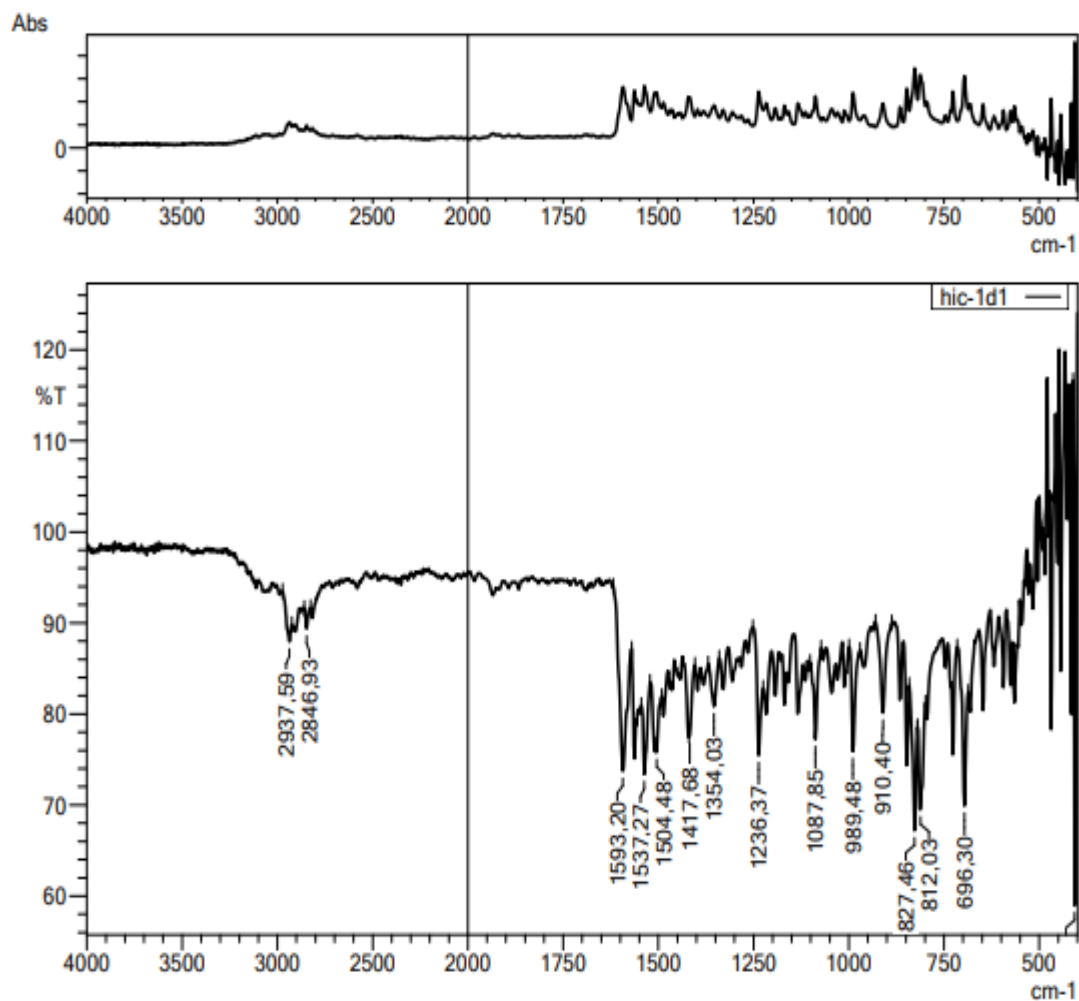
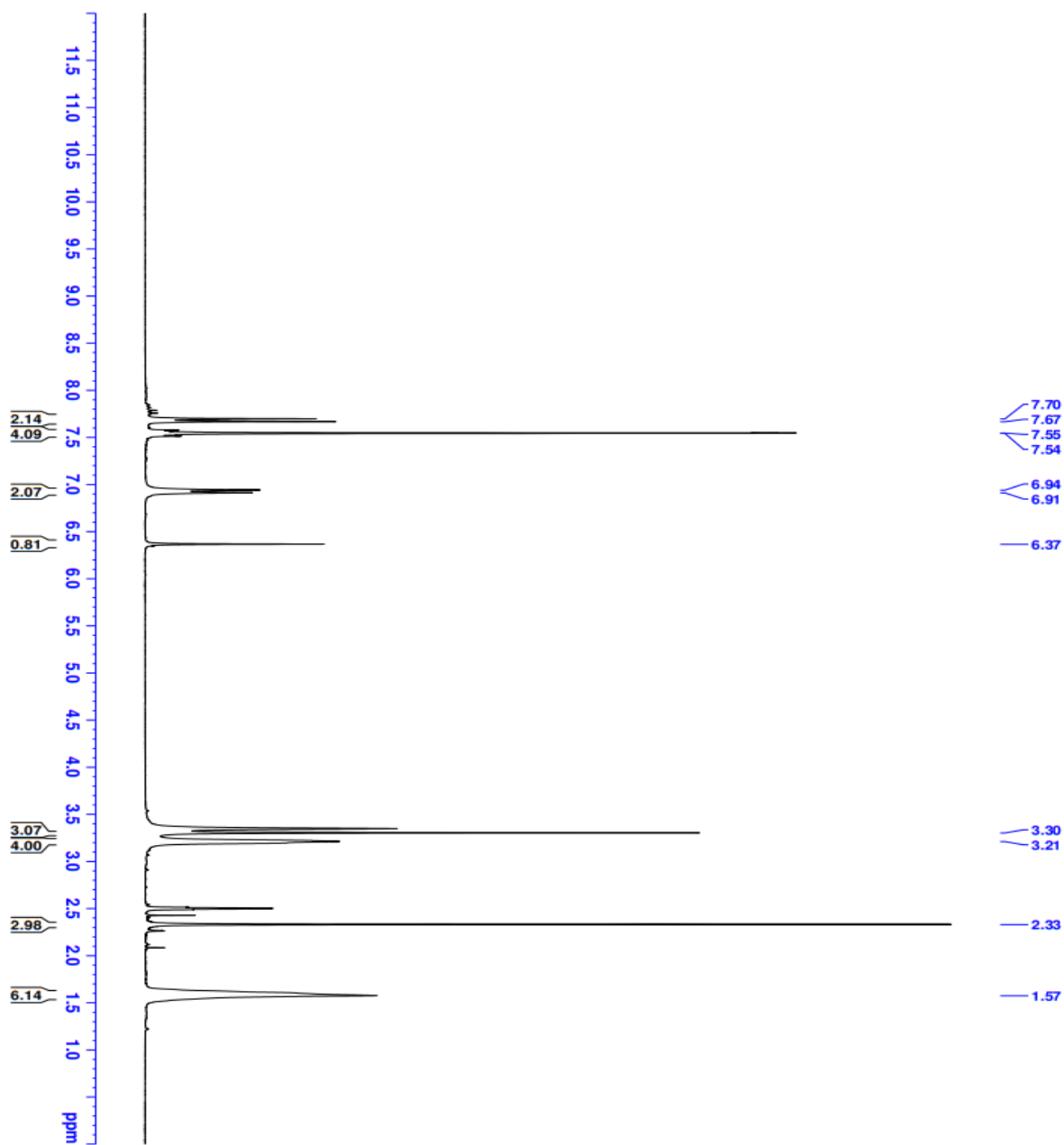


Figure 5.17. IR fingerprint of D1.d.



Current Data Parameters
 NAME: HIC-103
 EXPNO: 1
 PROCNO: 1
 F2 - Acquisition Parameters
 Date_: 20231013
 Time: 11:05
 INSTRUM: FOURIER300
 PROCBD: 5 mm DUL 13C-1
 PULPROG: zg
 TD: 16384
 SOLVENT: DMSO
 NS: 16
 DS: 0
 SWH: 6103.516 Hz
 FIDRES: 0.172529 Hz
 AQ: 1.3421773 sec
 RG: 3.981
 RW: 81.920 usec
 DE: 6.450 usec
 TE: 29.20 K
 D1: 3.0000000 sec
 TDO: 1
 ===== CHANNEL f1 =====
 SFO1: 300.181837 MHz
 NUC1: 1H
 P1: 13.00 usec
 PWM1: 10.0000000 W
 F2 - Processing parameters
 SI: 65536
 SF: 300.1800000 MHz
 NQW: EM
 SSB: 0
 LB: 0.30 Hz
 GB: 0
 PC: 1.00



Figure 5.18. ¹H NMR signals of D1.d.

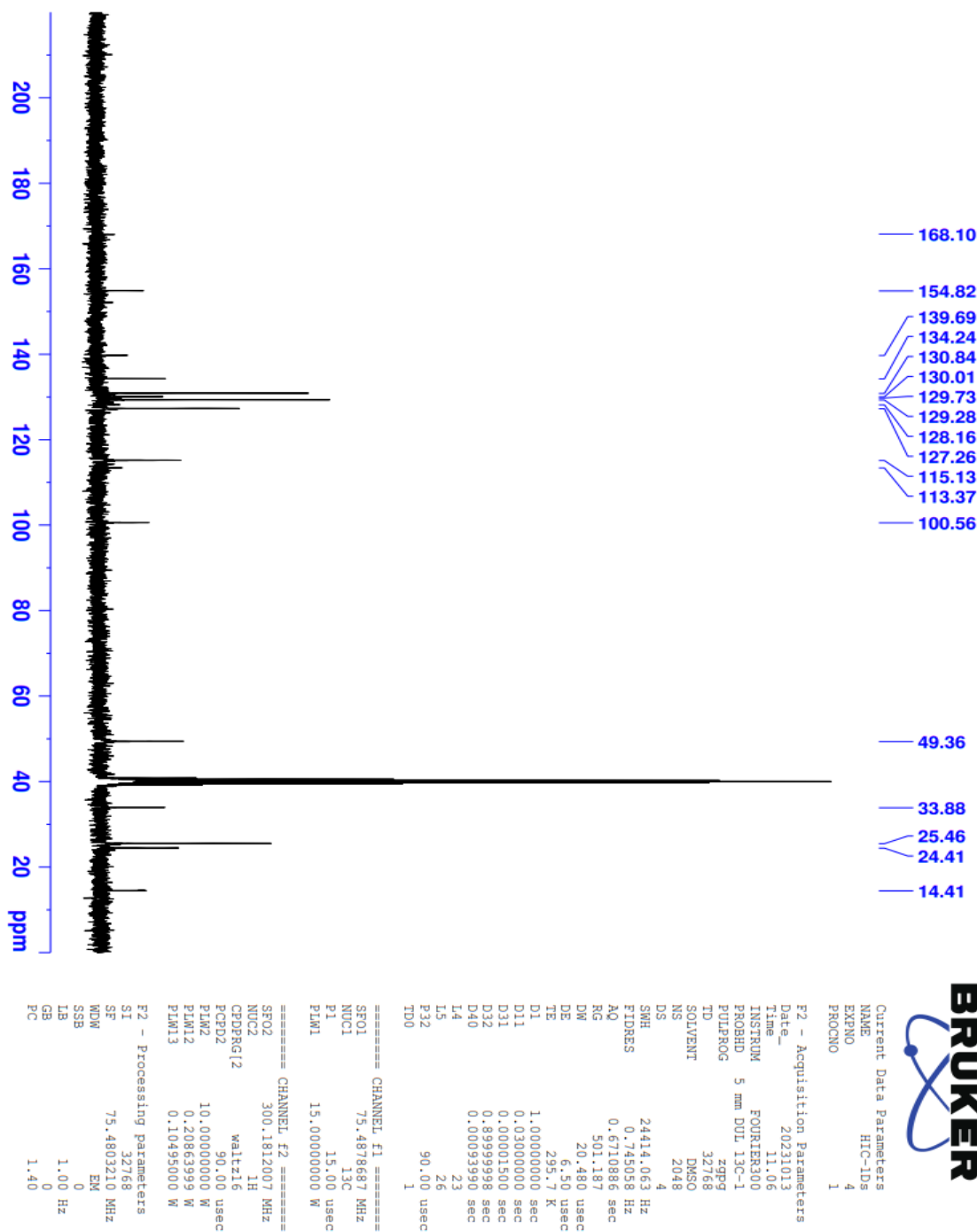


Figure 5. 19. ^{13}C NMR signals of D1.d.

Data File: C:\LabSolutions\Data\Analiz\derya\HIC-1E_582.lod

Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	8	33	O	2	0	3	S	2	0	2	Ru	2	0	0	H
C	4	4	32	F	1	0	0	Cl	1	1	1	Pd	2	0	0	Na
N	3	0	6	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5

HC Ratio: unlimited

Max Isotopes: 3

MSn Iso RI (%): 10.00

DBE Range: 0.0 - 30.0

Apply N Rule: no

Isotope RI (%): 1.00

MSn Logic Mode: AND

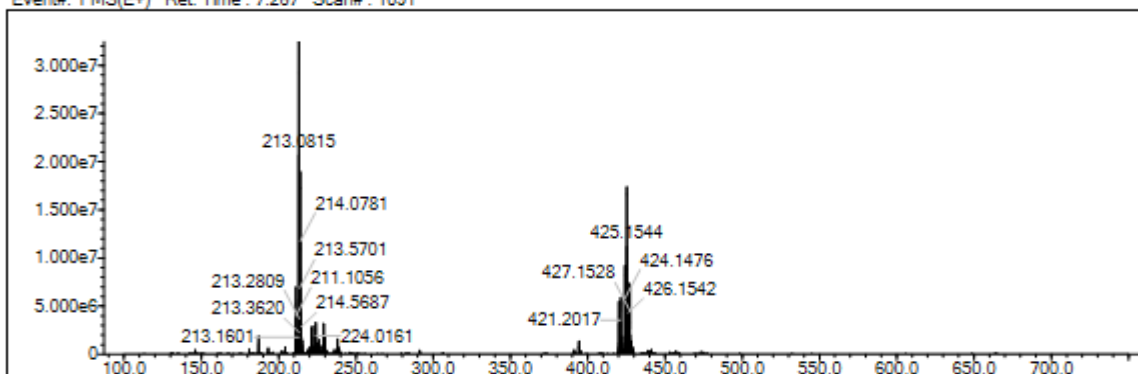
Electron Ions: both

Use MSn Info: yes

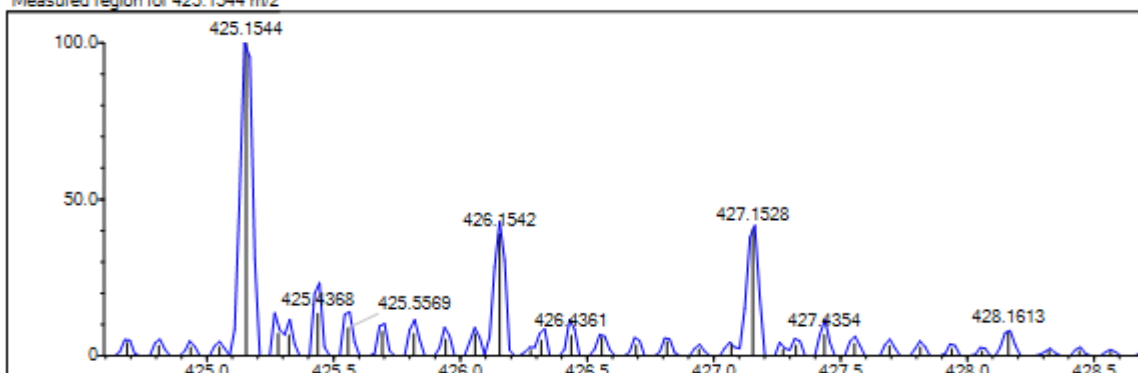
Isotope Res: 9000

Max Results: 50

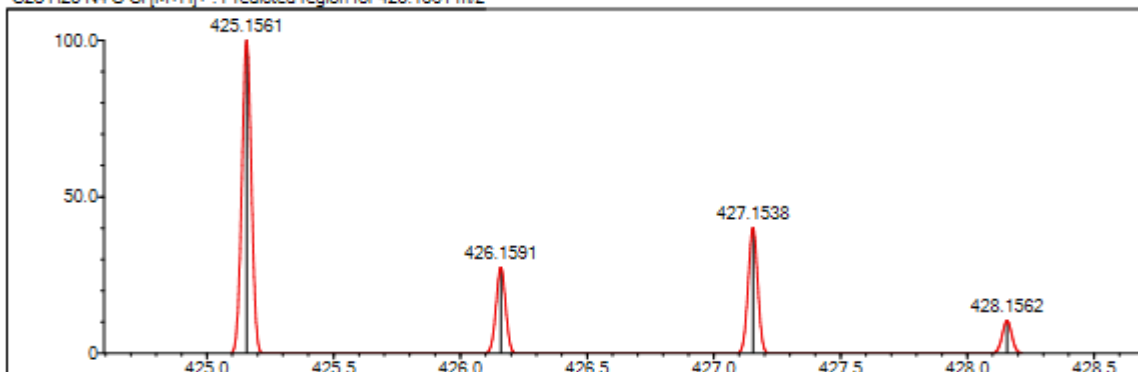
Event#: 1 MS(E+) Ret. Time : 7.267 Scan#: 1091



Measured region for 425.1544 m/z



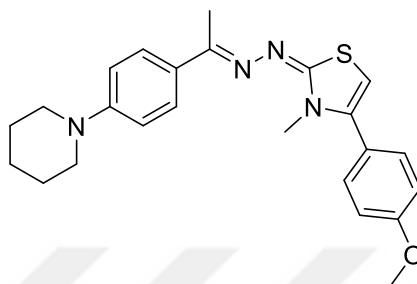
C23 H25 N4 S Cl [M+H]⁺ : Predicted region for 425.1561 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
3	59.29	C23 H25 N4 S Cl	[M+H] ⁺	425.1544	425.1561	-1.7	-4.00	64.10	13.0

Figure 5. 20. HRMS spectrogram of D1.d.

5.1.1.5. 4-(4-Methoxyphenyl)-3-methyl-2-((1-(4-(piperidin-1-yl)phenyl) ethylidene) hydrazineylidene)-2,3-dihydrothiazole



D1e

Figure 5.21. 4-(4-Methoxyphenyl)-3-methyl-2-((1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-2,3-dihydrothiazole.

Yield: 65%

Melting point: 217.6°C.

IR Maximum Velocity (cm^{-1}): 2943 (C=C-H stretching band), 2918 (C-H stretching band), 1591 (C=N stretching band), 1537 (C=C band), 1236 (C-N stretching band), 819 (4-substituted phenyl out-of-plane deformation band).

^1H -NMR (300 MHz, $\text{DMSO}-d_6$): 1.59 (6H, s, piperidine-H), 2.32 (3H, s, Methyl-H), 3.20 (4H, s, piperidine-H), 3.28(3H, s, Methyl-H), 3.80(3H, s, Methoxy-H), 6.17 (1H, s, dihydrothiazole), 6.90-6.92(2H, d, $J=7.52$ Hz, Ar-H), 7.01-7.03(2H, d, $J= 7.29$ Hz, Ar-H). 7.39-7.41(2H, d, $J= 7.26$ Hz, Ar-H), 7.67-7.70(2H, d, $J= 7.52$ Hz, Ar-H).

^{13}C -NMR (75 MHz, $\text{DMSO}-d_6$): $\delta = 14.13, 24.38, 25.65, 33.47, 50.02, 55.42, 98.68, 115.13, 115.40, 123.73, 129.84, 130.10, 140.52, 152.19, 155.43, 160.10, 168.70$.

HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{24} \text{H}_{28} \text{N}_4\text{O S}$ $[\text{M}+\text{H}]^+$: 421.2057; found: 421.2043.

Item	Value
Acquired Date&Time	2.05.2024 14:21:51
Acquired by	System Administrator
Filename	C:\Users\dopnalab\Desktop\MASAUSTU\sazan\hic\hic-1e1.ispd
Spectrum name	hic-1e1
Sample name	hic-1e
Sample ID	
Option	
Comment	
No. of Scans	30
Resolution	4 [cm-1]
Apodization	Happ-Genzel

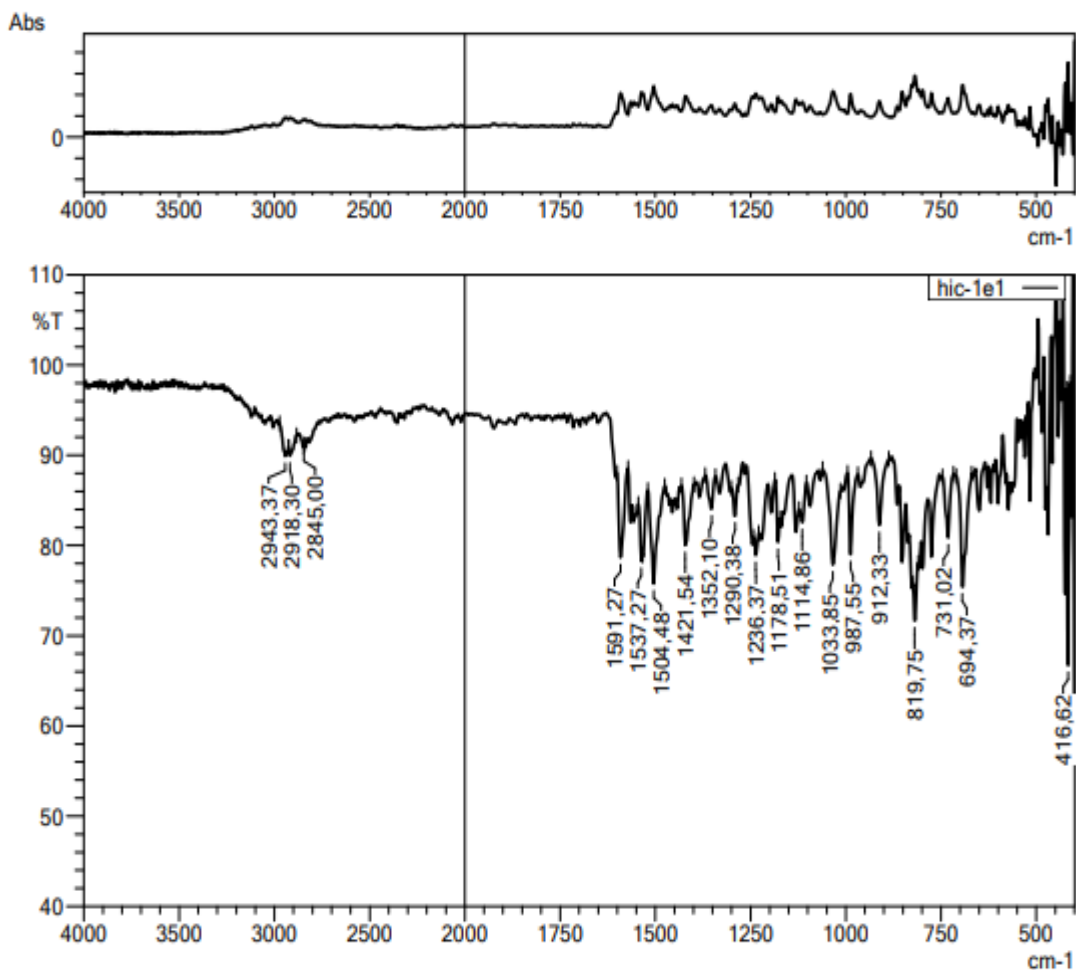


Figure 5.22. IR fingerprint of D1.e.

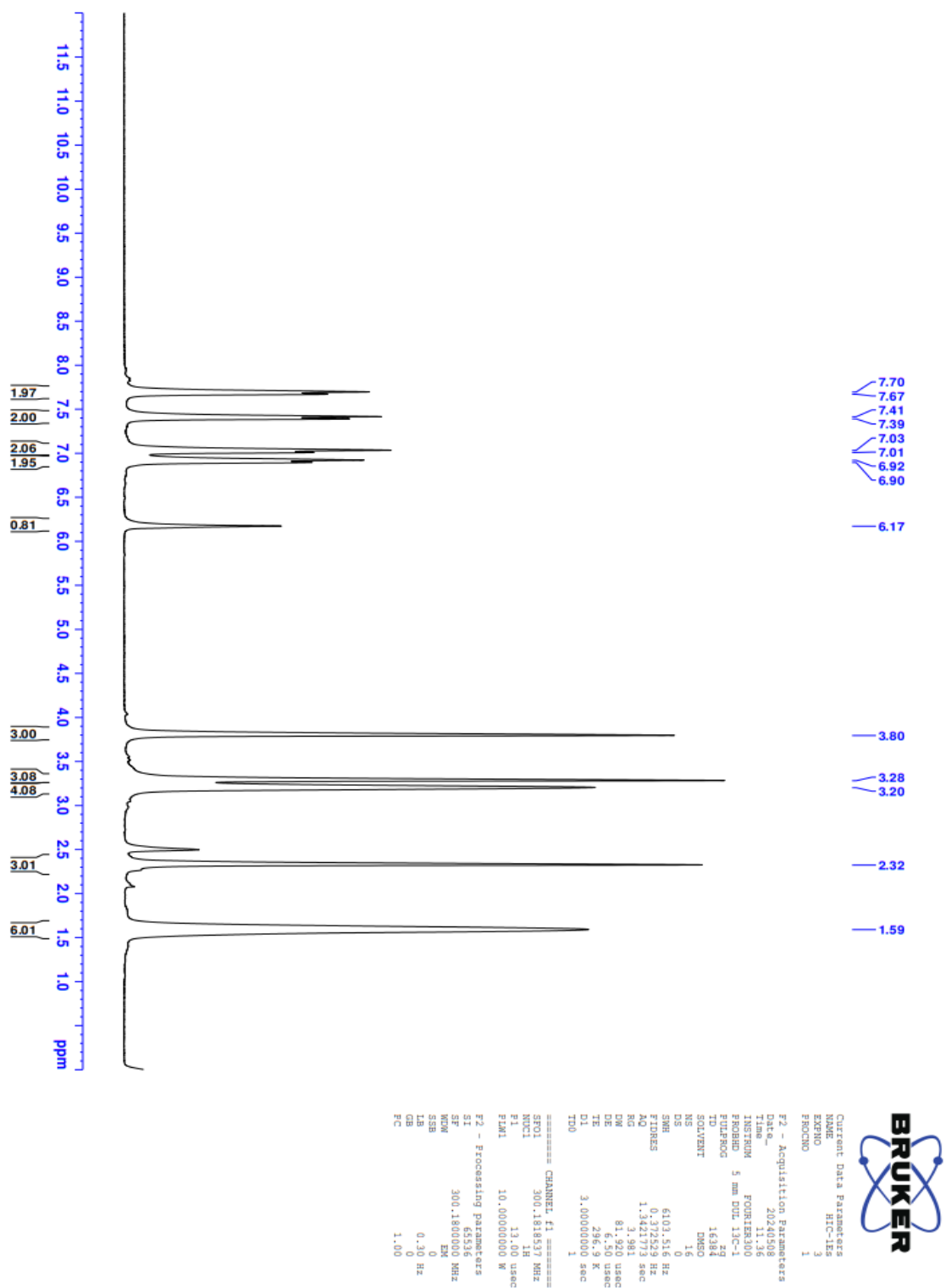
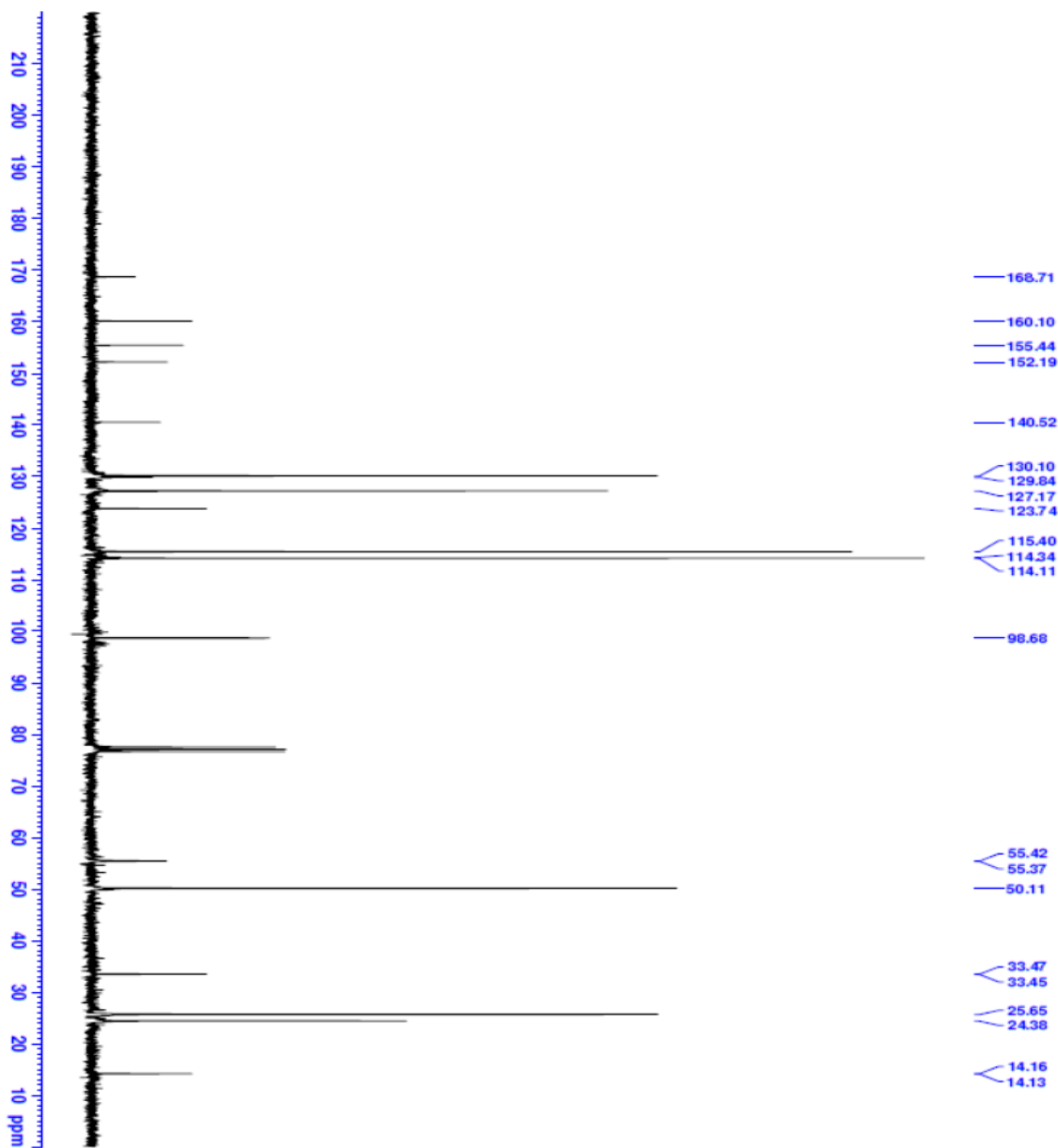


Figure 5.23. ¹H NMR signals of D1.e.



Current Data Parameters
NAME: HIC-1E.COC13
EXPNO: 2
PROCNO: 1

F2 - Acquisition Parameters
Date_: 20240522
Time: 10.47
INSTRUM: YOUNIUS30
PROBHD: 5 mm DUL 13C-1
PULPROG: zgpg30
TD: 32768
SOLVENT: CDCl3
NS: 1995
DS: 4
SWH: 24414.062 Hz
FIDRES: 0.145058 Hz
AQ: 0.610386 sec
RG: 501.187
DM: 20.480 sec
DE: 8.20 sec
TE: 300.2 K
D1: 1.00000000 sec
D11: 0.03000000 sec
D31: 0.00015000 sec
D32: 0.89999998 sec
D40: 0.00039900 sec
L4: 23
L5: 26
P32: 90.00 sec
T00: 1

CHANNEL F1
SFO1: 75.487687 MHz
NUC1: 13C
P1: 15.00 sec
PLN1: 15.00000000 W

CHANNEL F2
SFO2: 300.161007 MHz
NUC2: 1H
CROSSPC12: waltz16
PCPD2: 90.00 sec
PLN2: 10.00000000 W
PLN12: 0.20863999 W
PLN13: 0.10495000 W

F2 - Processing parameters
SI: 32768
SF: 75.480210 MHz
WDW: EM
SSB: 0
LB: 1.00 Hz
GB: 0
PC: 1.40



Figure 5. 24. ¹³CNMR signals of D1.e.

Data File: C:\LabSolutions\Data\Analz\data\HIC-1F UST_583.lod

Elmt	Val	Min	Max	Elmt	Val	Min	Max	Elmt	Val	Min	Max	Elmt	Val	Min	Max	Use Adduct
H	1	8	33	O	2	0	3	S	2	0	2	Ru	2	0	0	H
C	4	4	32	F	1	0	0	Cl	1	0	0	Pd	2	0	0	Na
N	3	0	6	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5

HC Ratio: unlimited

Max Isotopes: 3

MSn Iso RI (%): 10.00

DBE Range: 0.0 - 30.0

Apply N Rule: no

Isotope RI (%): 1.00

MSn Logic Mode: AND

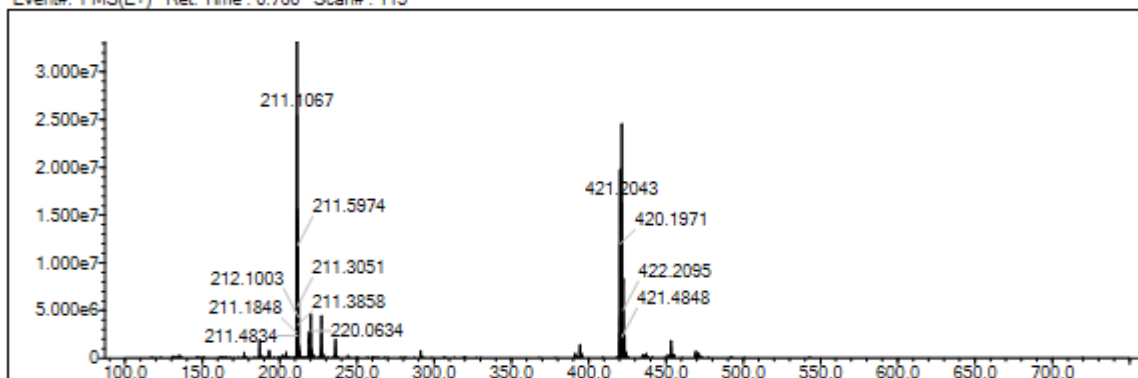
Electron Ions: both

Use MSn Info: yes

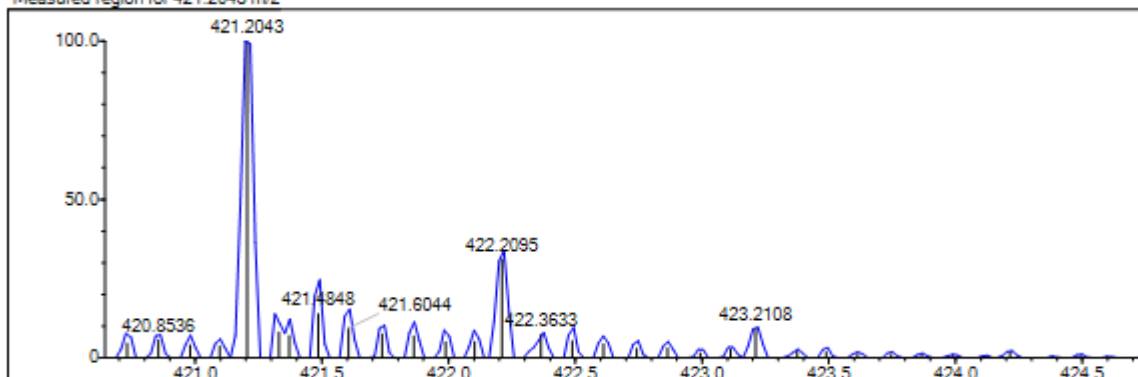
Isotope Res: 9000

Max Results: 50

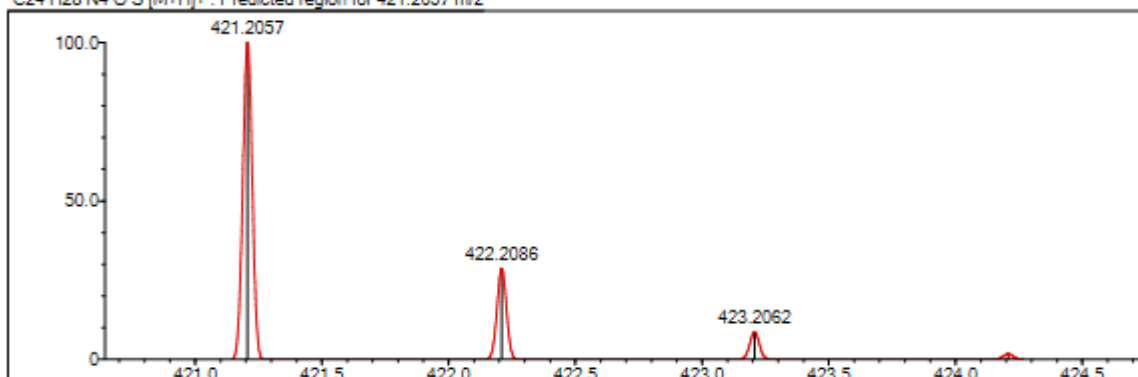
Event#: 1 MS(E+) Ret. Time : 0.760 Scan#: 115



Measured region for 421.2043 m/z



C24 H28 N4 O S [M+H]⁺ : Predicted region for 421.2057 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	82.91	C24 H28 N4 O S	[M+H] ⁺	421.2043	421.2057	-1.4	-3.32	88.02	13.0

Figure 5.25..HRMS spectrogram of D1.e.

5.1.1.6. 4-(-3-Methyl-2-((1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-2,3-dihydrothiazol-4-yl)benzonitrile

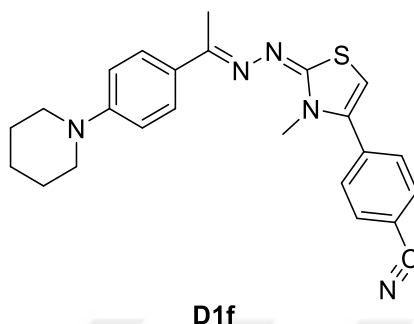


Figure 5. 26. 4-(-3-Methyl-2-((1-(4-(piperidin-1-yl) phenyl) ethylidene) hydrazineylidene)-2,3-dihydrothiazol-4-yl) benzonitrile.

Yield: 85%.

Melting point: 201.1°C.

IR Maximum Velocity (cm^{-1}): 3107 (C=C-H tension band), 2935 (C-H stretching band), 2229 (Nitrile-CN), 1604 (C=N tension band), 1585 (C=C voltage band), 1238 (C-N voltage band), 815 (4-substituted phenyl out-of-plane deformation band).

^1H -NMR (300 MHz, $\text{DMSO}-d_6$): 1.56 (6H, s, piperidine-H), 2.33 (3H, s, Methyl-H), 3.20 (4H, s, piperidine-H), 3.32 (3H, s, Methyl-H), 6.51 (1H, s, dihydrothiazole-H), 6.89-6.92 (2H, d, $J=8.97$ Hz, Ar-H), 7.66-7.72 (4H, t, $J=16.35$ Hz, Ar-H), 7.92-7.95 (2H, d, $J=8.35$ Hz, Ar-H).

^{13}C -NMR (75 MHz, $\text{DMSO}-d_6$): δ = 14.43, 14.47, 24.43, 25.48, 34.16, 49.30, 102.60, 111.84, 115.06, 118.98, 127.29, 128.51, 129.60, 133.14, 135.56, 139.45, 152.16, 155.14, 167.91.

HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{24}\text{H}_{25}\text{N}_5\text{S}$ $[\text{M}+\text{H}]^+$: 416.1903; found: 416.1891.

Item	Value
Acquired Date&Time	2.05.2024 14:26:20
Acquired by	System Administrator
Filename	C:\Users\dopnalab\Desktop\MASAÜSTÜ\isazan\hic\hic-1f1.ispd
Spectrum name	hic-1f1
Sample name	hic-1f
Sample ID	
Option	
Comment	
No. of Scans	30
Resolution	4 [cm ⁻¹]
Apodization	Happ-Genzel

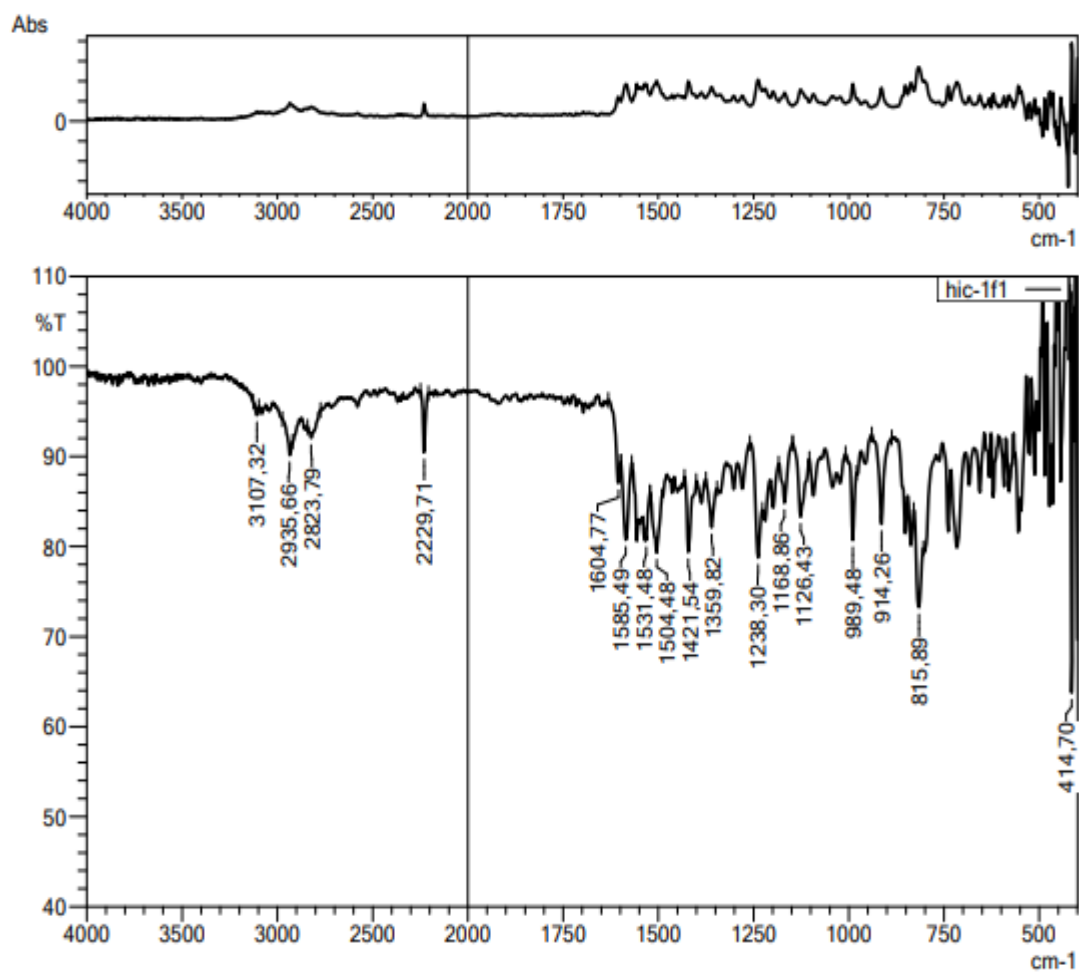


Figure 5.27. IR fingerprint of D1.f.

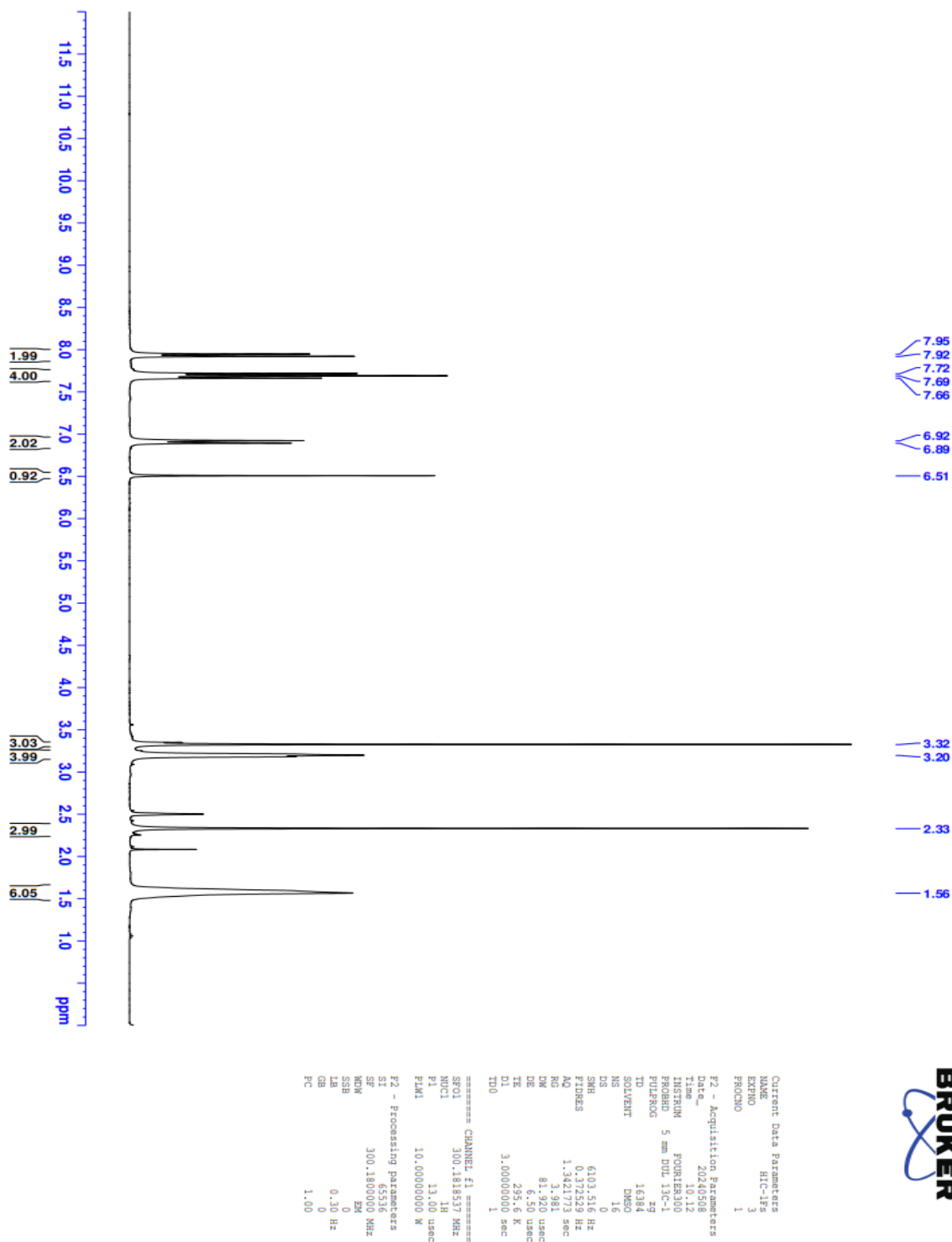
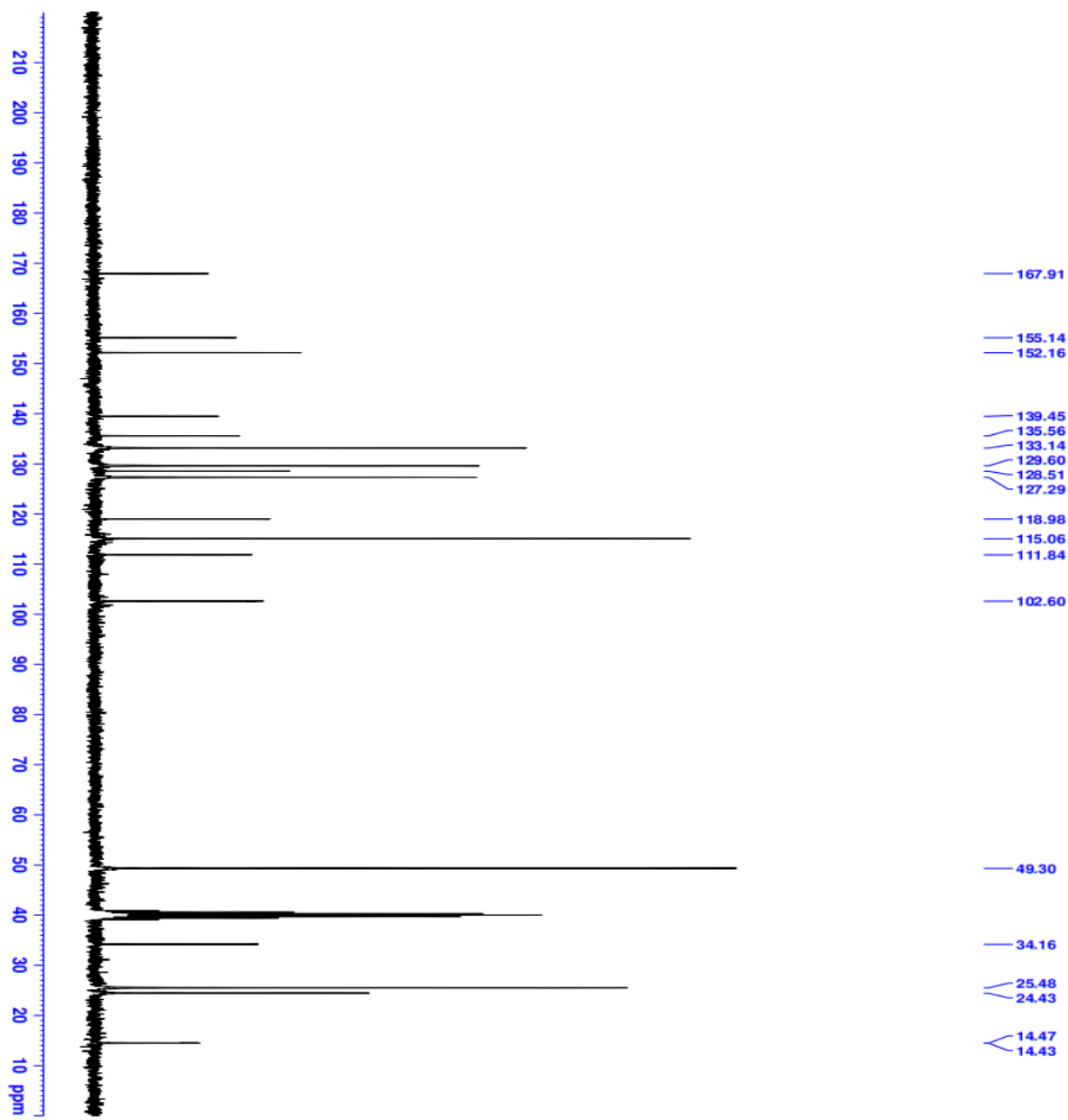


Figure 5.28. ^1H NMR signals of D1.f.



```

Current Data Parameters
NAME      HIC-1fs
EXPNO     4
PROCNO    1

F2 - Acquisition Parameters
Date_     20240508
Time      10.14
INSTRUM   FOURIER300
PROBHD    5 mm DUL 13C-1
PULPROG   zgpg
TD         32768
SOLVENT   DMSO
NS         2048
DS         4
SWH        24414.063 Hz
FIDRES     0.745058 Hz
AQ         0.6710886 sec
RG         501.187
DW         20.480 usec
DE         6.50 usec
TE         295.6 K
D1         1.00000000 sec
D11        0.03000000 sec
D12        0.0001500 sec
D13        0.89999998 sec
D14        0.00093990 sec
L4         23
L5         26
F32        90.00 usec
TD0        1

===== CHANNEL f1 =====
SFO1       75.487687 MHz
NUC1       13C
P1         15.00 usec
PLM1       15.00000000 W

===== CHANNEL f2 =====
SFO2       300.1812007 MHz
NUC2       1H
PCPDPRG12  waltz16
PCPDPRG2   90.00 usec
FREQ2      10.00000000 W
FREQ2      0.20865959 W
FREQ3      0.10435000 W

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

Figure 5.29. ^{13}C NMR signals of D1.f.

Data File: C:\LabSolutions\Data\Analiz\dera\HIC-1F ALT_584.lcd

Elmt	Val	Min	Max	Elmt	Val	Min	Max	Elmt	Val	Min	Max	Elmt	Val	Min	Max	Use Adduct
H	1	8	33	O	2	0	3	S	2	0	2	Ru	2	0	0	H
C	4	4	32	F	1	0	0	Cl	1	0	0	Pd	2	0	0	Na
N	3	0	6	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5

HC Ratio: unlimited

Max Isotopes: 3

MSn Iso RI (%): 10.00

DBE Range: 0.0 - 30.0

Apply N Rule: no

Isotope RI (%): 1.00

MSn Logic Mode: AND

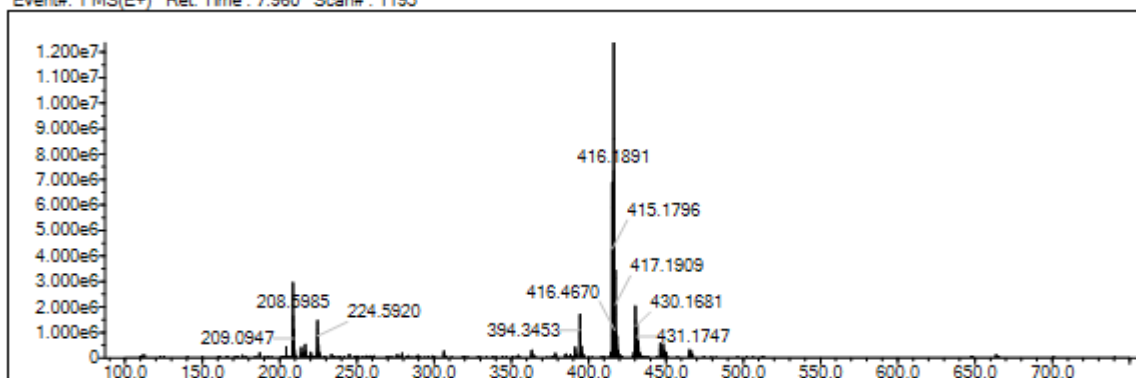
Electron Ions: both

Use MSn Info: yes

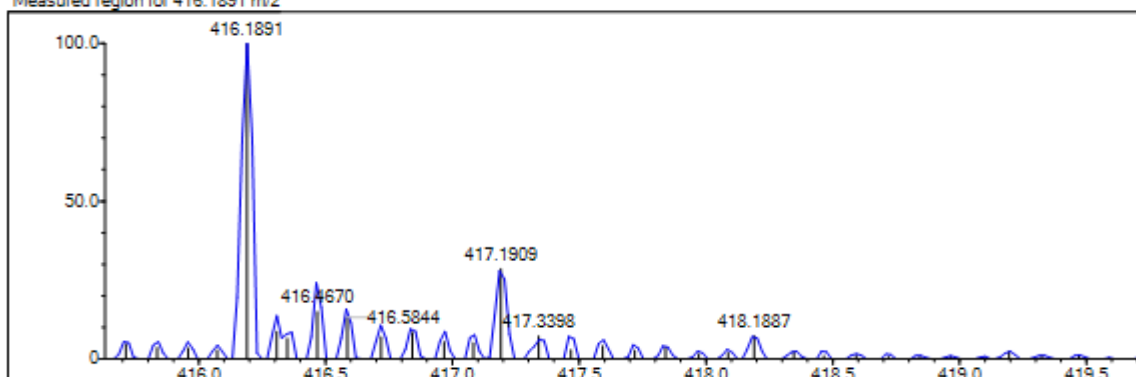
Isotope Res: 9000

Max Results: 50

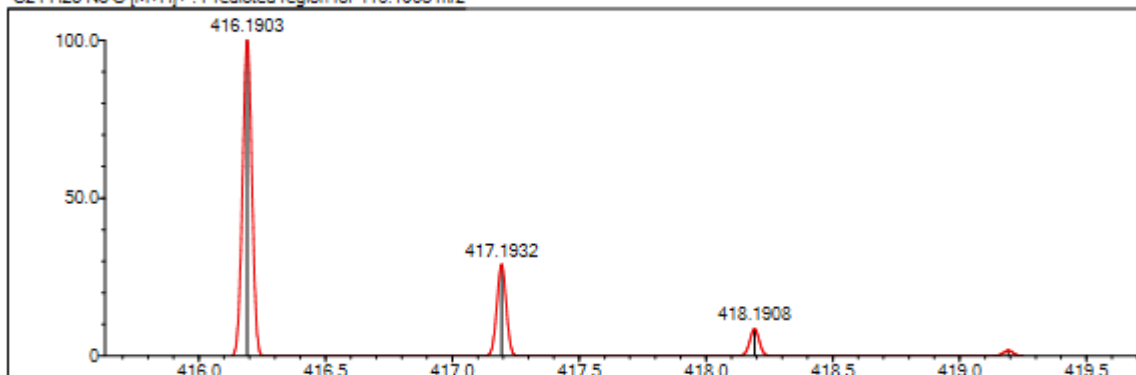
Event#: 1 MS(E+) Ret. Time: 7.960 Scan#: 1195



Measured region for 416.1891 m/z



C24 H25 N5 S [M+H]⁺: Predicted region for 416.1903 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	94.20	C24 H25 N5 S	[M+H] ⁺	416.1891	416.1903	-1.2	-2.88	98.84	15.0

Figure 5.30. HRMS spectrogram of D1.f.

5.1.1.7. 4-(2,4-Dichlorophenyl)-3-methyl-2-((1-(4-(piperidin-1-yl)phenyl) ethylidene) hydrazineylidene)-2,3-dihydrothiazole

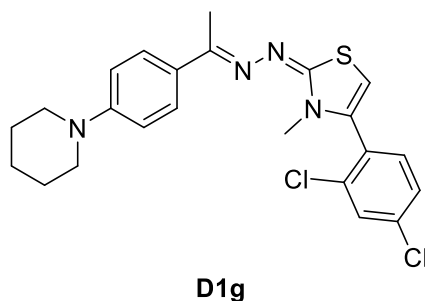


Figure 5.31. 4-(2,4-Dichlorophenyl)-3-methyl-2-((1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-2,3-dihydrothiazole.

Yield: 75%.

Melting point: 179.4°C.

IR Maximum Velocity (cm^{-1}): 3100 (C=C-H stretching band), 2929 (C-H stretching band), 1591 (C=N stretching band), 1562 (C=C band), 1238 (C-N voltage band), 815 (4-substituted phenyl out-of-plane deformation band).

^1H -NMR (300 MHz, $\text{DMSO}-d_6$): 2.26(6H, s, piperidine-H), 2.99 (3H, s, Methyl-H), 3.32 (3H, s, Methyl-H), 3.86-3.90 (4H, m, piperidine-H), 7.59-7.62(2H, d, $J=8.86$ Hz, Ar-H), 7.90 (1H, s, dihydrothiazole-H), 8.21-8.25(1H, m, Ar-H), 8.34-8.37(3H, m, Ar-H), 8.59-8.62(1H, d, $J=8.59$ Hz, Ar-H).

^{13}C -NMR (75 MHz, $\text{DMSO}-d_6$): δ = 14.39, 24.43, 25.50, 29.86, 48.25, 49.28, 93.08, 115.02, 127.27, 127.84, 128.57, 131.08, 131.67, 132.85, 134.53, 137.88, 152.18, 155.72, 166.26.

HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{23} \text{H}_{24} \text{N}_4 \text{S Cl}_2$ $[\text{M}+\text{H}]^+$:459.1171; found: 459.1164.

Item	Value
Acquired Date&Time	2.05.2024 14:32:01
Acquired by	System Administrator
Filename	C:\Users\dopnalab\Desktop\MASAU\STU\Isazan\hic\hic-1h1.ispd
Spectrum name	hic-1h1
Sample name	hic-1h
Sample ID	
Option	
Comment	
No. of Scans	30
Resolution	4 [cm-1]
Apodization	Happ-Genzel

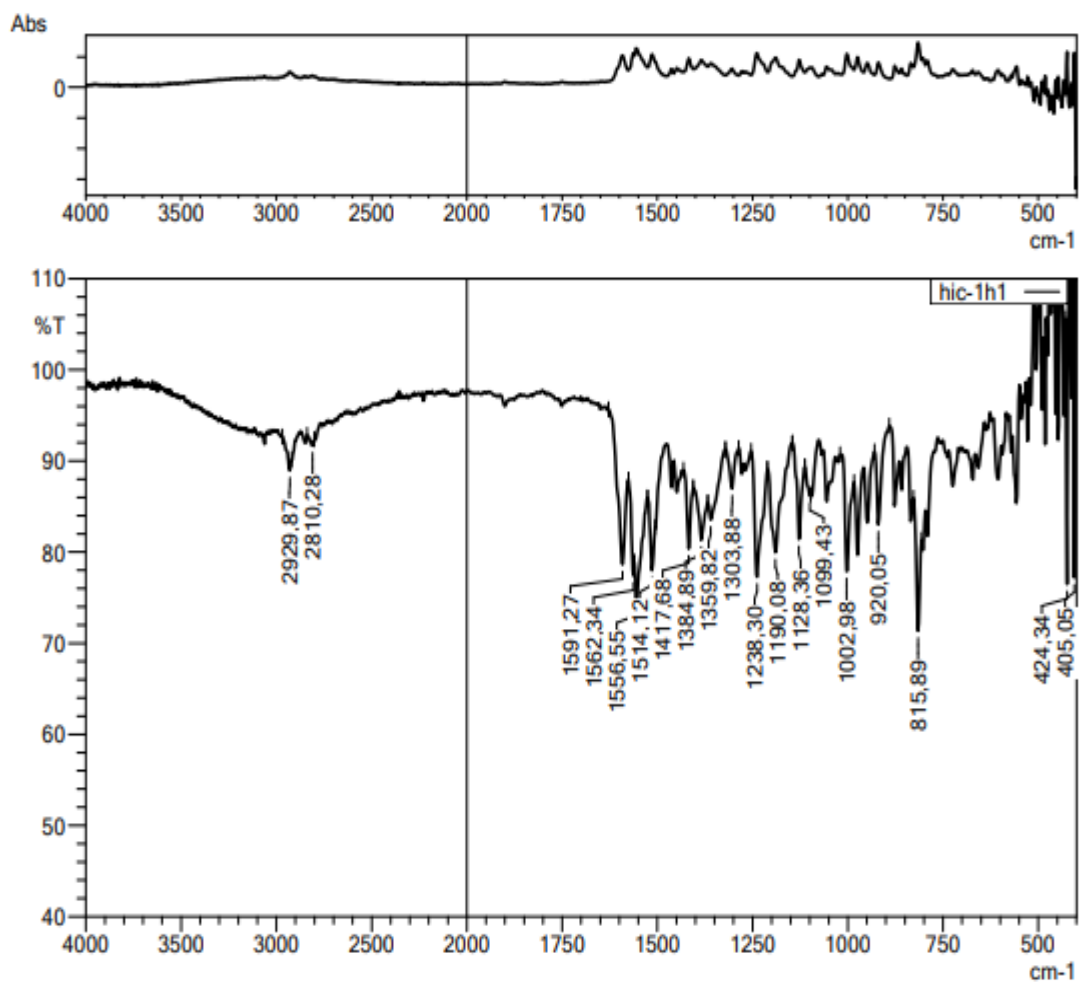


Figure 5.32. IR fingerprint of D1.g.

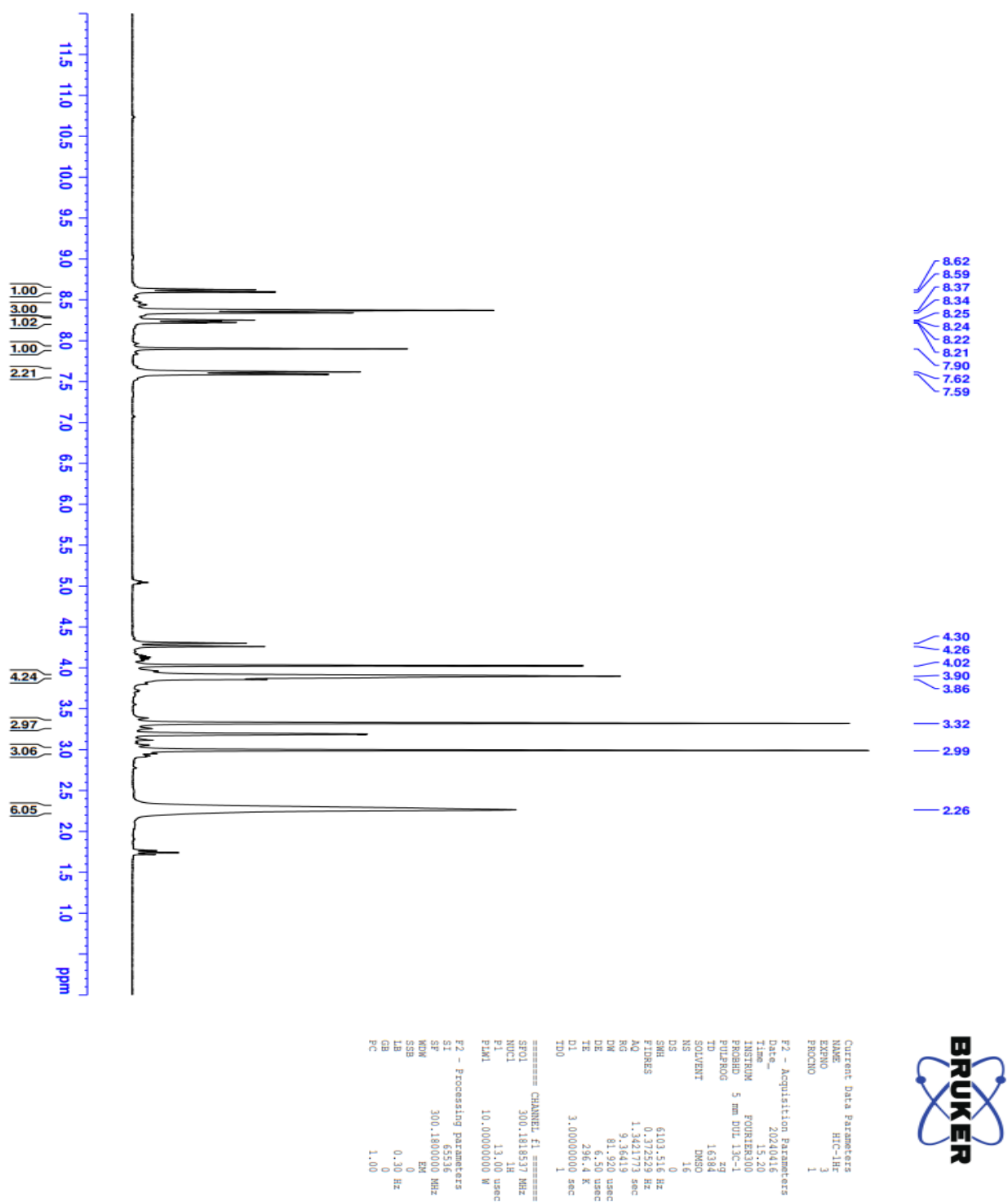


Figure 5.33. ^1H NMR signals of D1.g.



Figure 5. 34. ^{13}C NMR signals of 1Dg.

Data File: C:\LabSolutions\Data\Analiz\derya\HIC-11 ALT_587.lcd

Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	8	33	O	2	0	3	S	2	0	2	Ru	2	0	0	H
C	4	4	32	F	1	0	0	Cl	1	2	2	Pd	2	0	0	Na
N	3	0	6	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5

HC Ratio: unlimited

Max Isotopes: 3

MSn Iso RI (%): 10.00

DBE Range: 0.0 - 30.0

Apply N Rule: no

Isotope RI (%): 1.00

MSn Logic Mode: AND

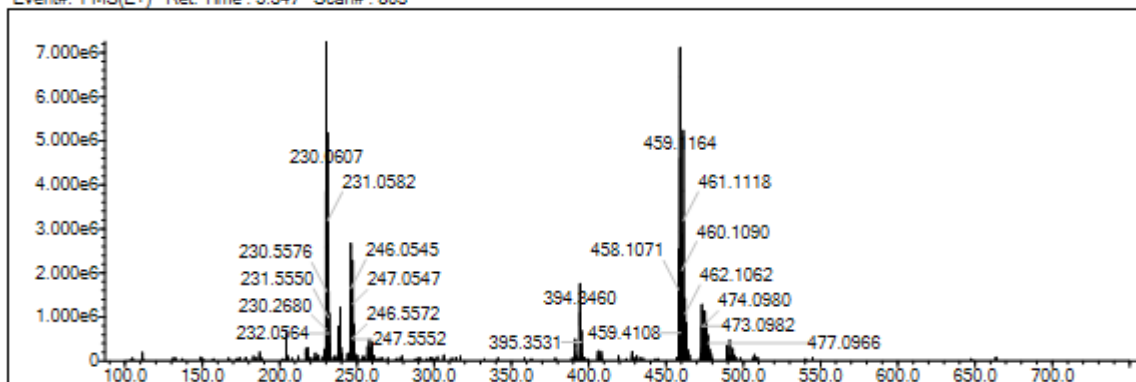
Electron Ions: both

Use MSn Info: yes

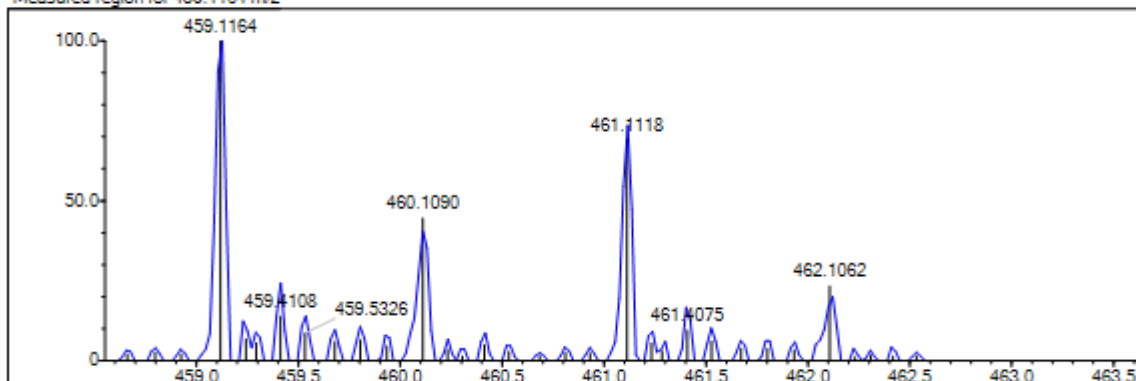
Isotope Res: 9000

Max Results: 50

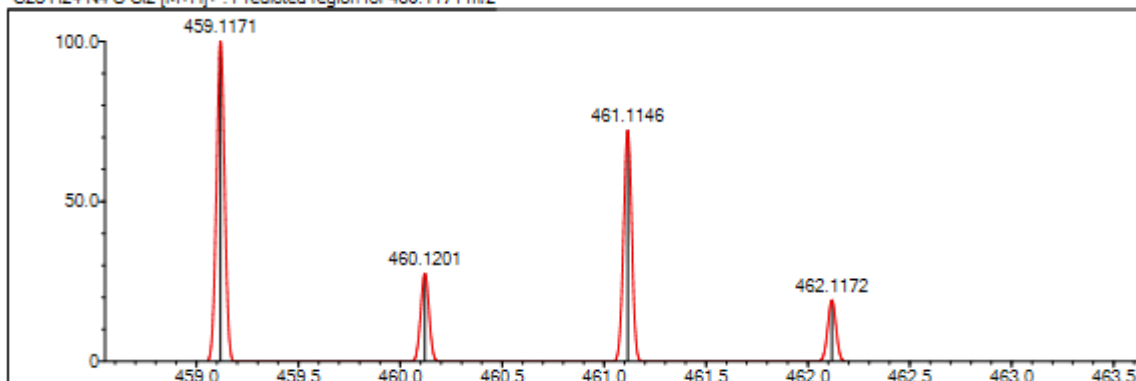
Event#: 1 MS(E+) Ret. Time: 5.347 Scan#: 803



Measured region for 459.1164 m/z



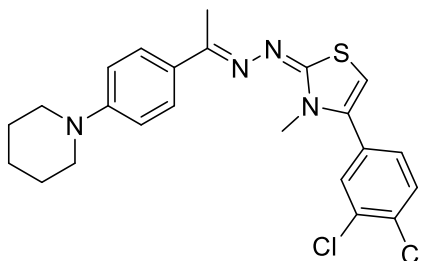
C23 H24 N4 S Cl2 [M+H]⁺ : Predicted region for 459.1171 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	54.47	C23 H24 N4 S Cl2	[M+H] ⁺	459.1164	459.1171	-0.7	-1.52	55.19	13.0

Figure 5.35. HRMS spectrogram of D1.g.

5.1.1.8. 4-(3,4-Dichlorophenyl)-3-methyl-2-((1-(4-(piperidin-1-yl)phenyl) ethylidene) hydrazineylidene)-2,3-dihydrothiazole



D1h

Figure 5.36. 4-(3,4-Dichlorophenyl)-3-methyl-2-((1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-2,3-dihydrothiazole.

Yield: 70%.

Melting point: 175°C.

IR Maximum Velocity (cm^{-1}): 3093 (C=C-H tension band), 2931 (C-H stretching band), 1589 (C=N stretching band), 1556 (C=C band), 1234 (C-N stretching band), 821 (3,4-substituted phenyl out-of-plane deformation band).

^1H -NMR (300 MHz, $\text{DMSO}-d_6$): 1.57(6H, s, piperidine-H), 2.33 (3H, s, Methyl-H), 3.21 (4H, m, piperidine-H), 3.33(3H, s, Methyl-H), 6.46(1H, s, dihydrothiazole-H), 6.90-6.93(2H, d, $J=8.61$ Hz, Ar-H), 7.50-7.53(1H, d, $J_1\&J_2=8.31$ Hz, Ar-H), 7.66-7.69(2H, d, $J=8.47$, Hz, Ar-H), 7.73-7.76 (1H, d, $J=8.27$ Hz, Ar-H), 7.82 (1H, s, Ar-H).

^{13}C -NMR (75 MHz, $\text{DMSO}-d_6$): $\delta = 14.42, 24.43, 25.48, 33.89, 49.31, 101.65, 115.07, 127.27, 129.22, 131.33, 131.68, 132.02, 138.48, 152.13, 154.98, 167.80$.

HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{23} \text{H}_{24} \text{N}_4 \text{S Cl}_2$ $[\text{M}+\text{H}]^+$:459.1171; found: 459.1166.

Item	Value
Acquired Date&Time	2.05.2024 14:43:58
Acquired by	System Administrator
Filename	C:\Users\dopnalab\Desktop\MASAUŠTŮ\sazan\hic\hic-1i2.ispd
Spectrum name	hic-1i2
Sample name	hic-1i
Sample ID	
Option	
Comment	
No. of Scans	30
Resolution	4 [cm-1]
Apodization	Happ-Genzel

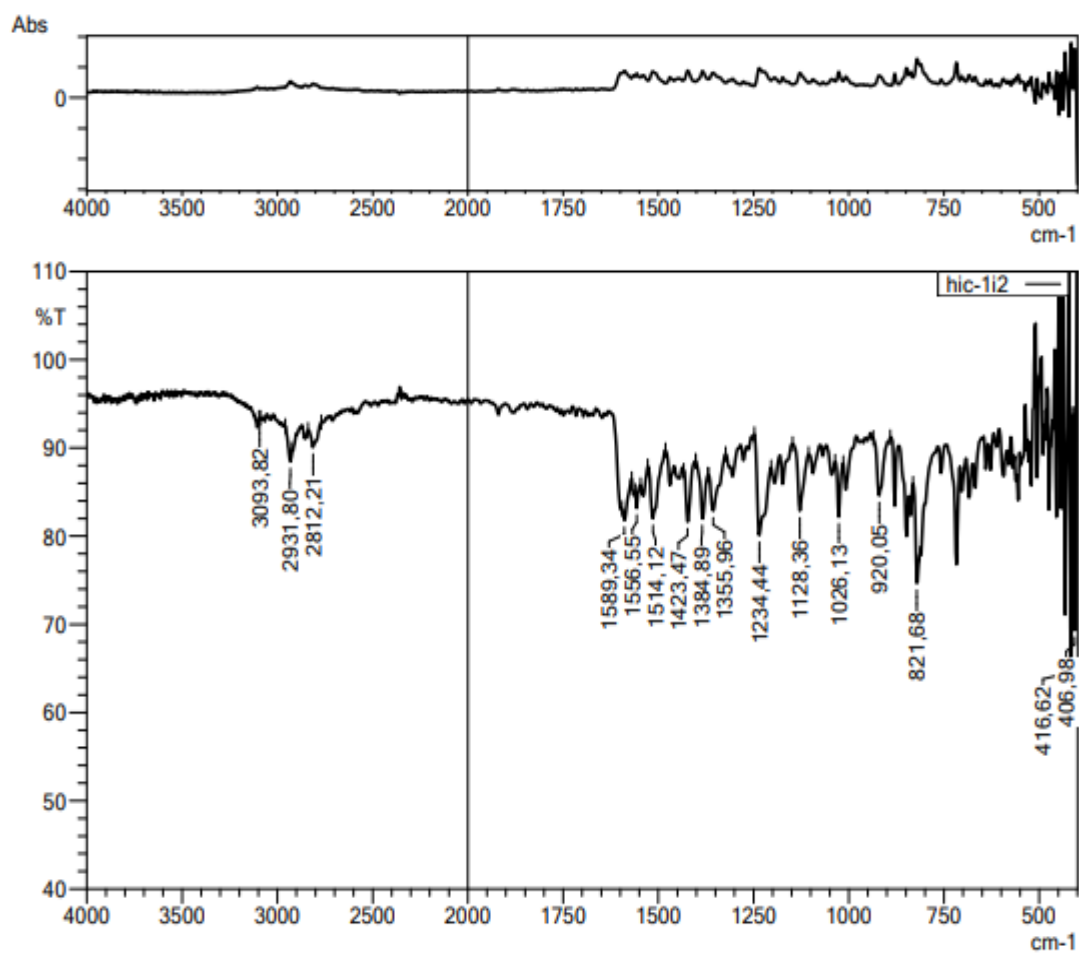


Figure 5.37. IR fingerprint of D1.h.

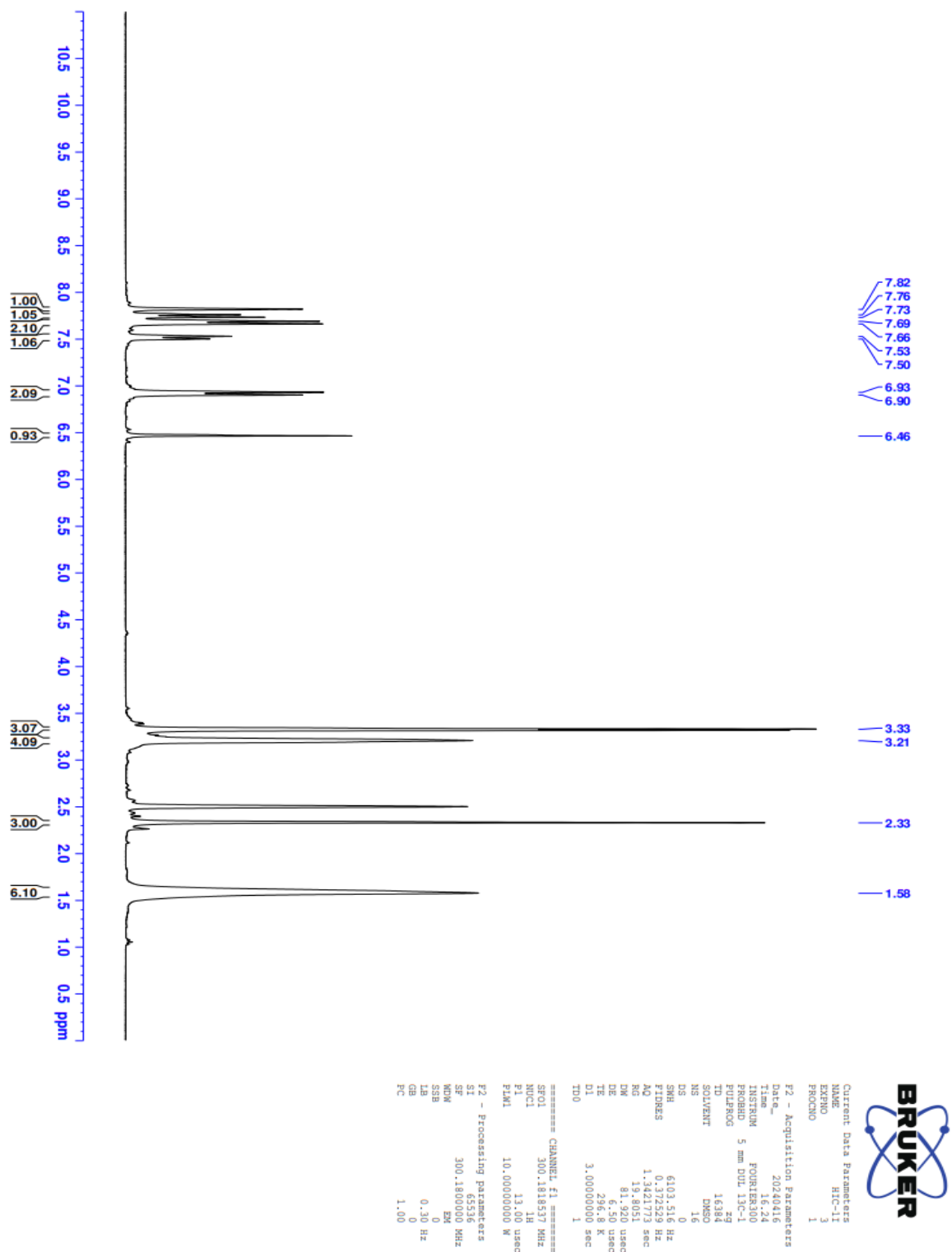


Figure 5.38. ^1H NMR signals of D1.h.

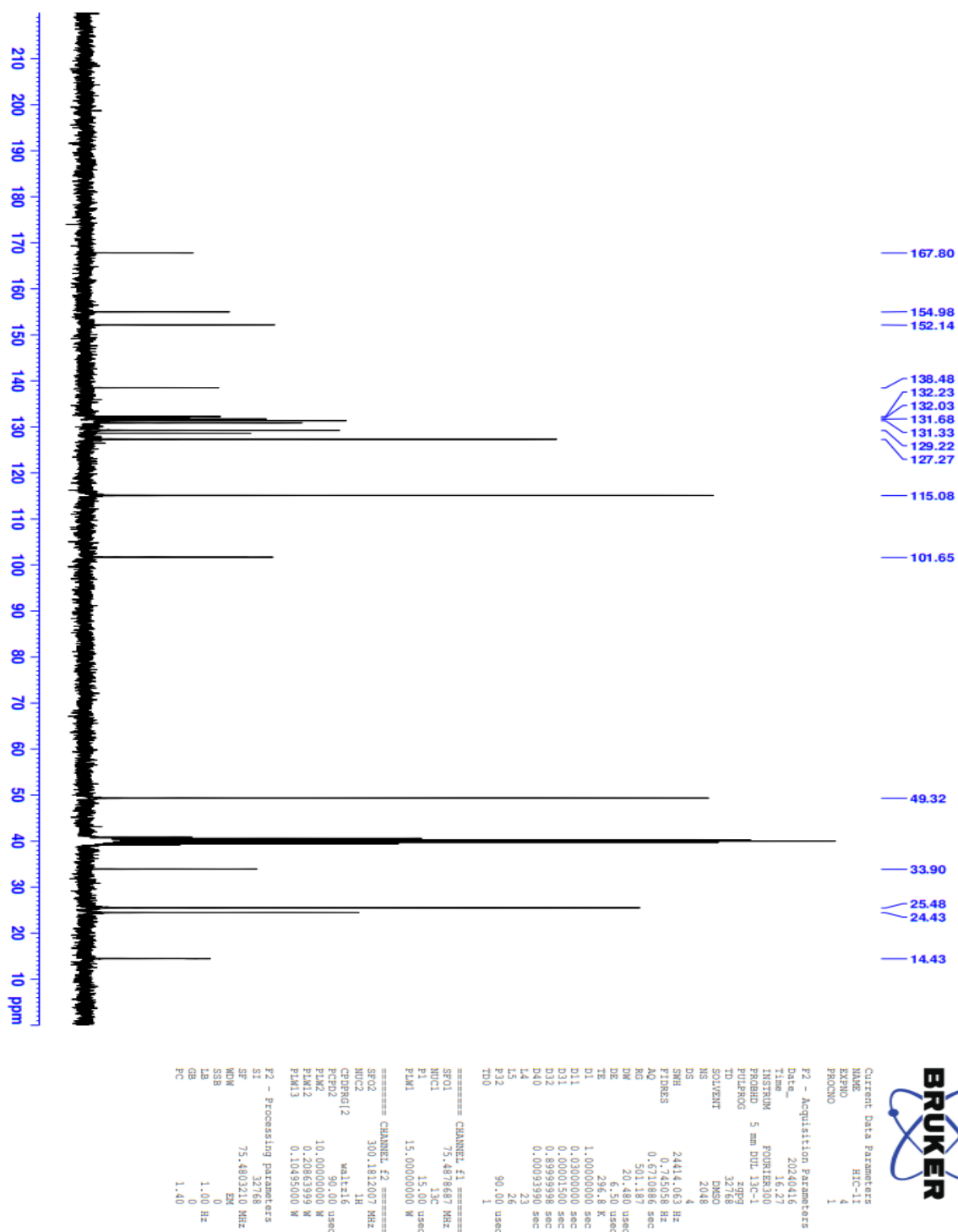


Figure 5.39. ^{13}C NMR signals of D1.h.

Data File: C:\LabSolutions\Data\Analiz\denya\HIC-11 UST_588.lcd

Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	8	33	O	2	0	3	S	2	0	2	Ru	2	0	0	H
C	4	4	32	F	1	0	0	Cl	1	2	2	Pd	2	0	0	Na
N	3	0	6	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5

HC Ratio: unlimited

Max Isotopes: 3

MSn Iso RI (%): 10.00

DBE Range: 0.0 - 30.0

Apply N Rule: no

Isotope RI (%): 1.00

MSn Logic Mode: AND

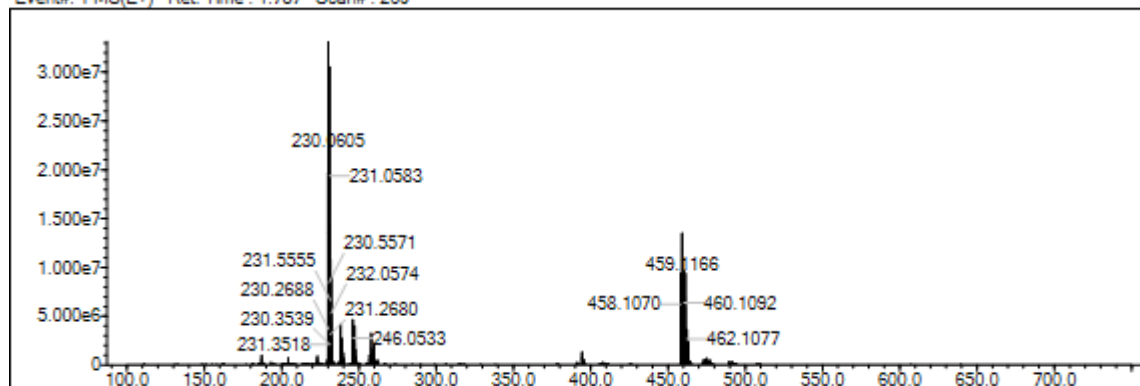
Electron Ions: both

Use MSn Info: yes

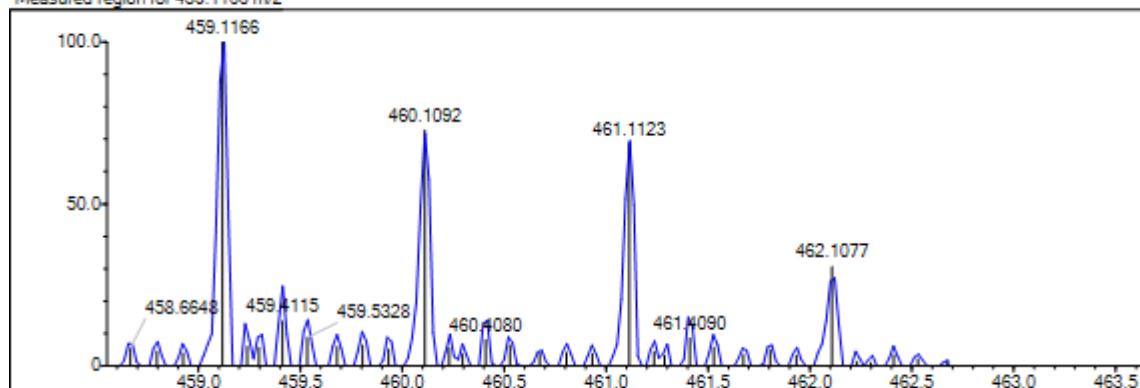
Isotope Res: 9000

Max Results: 50

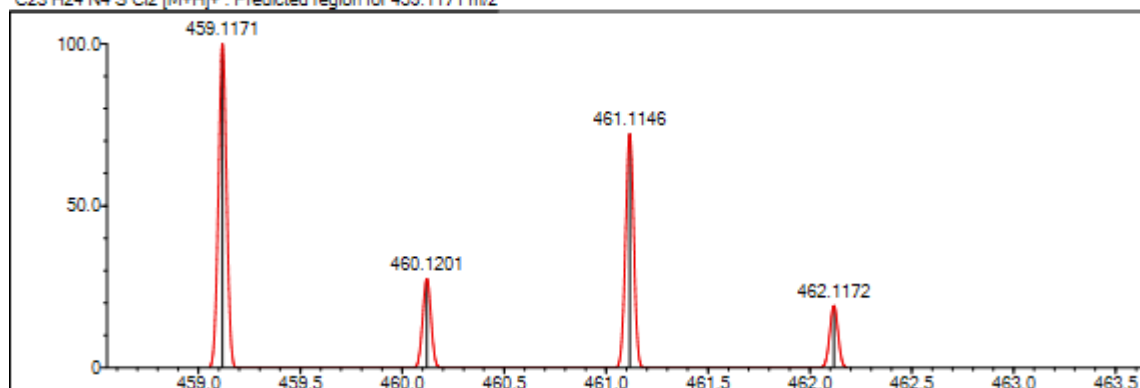
Event#: 1 MS(E+) Ret. Time : 1.787 Scan#: 269



Measured region for 459.1166 m/z



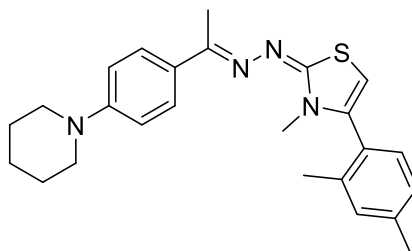
C23 H24 N4 S Cl2 [M+H]⁺ : Predicted region for 459.1171 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
8	26.59	C23 H24 N4 S Cl2	[M+H] ⁺	459.1166	459.1171	-0.5	-1.09	26.65	13.0

Figure 5.40. HRMS spectrogram of D1.h.

5.1.1.9. 4-(2,4-Dimethylphenyl)-3-methyl-2-((1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-2,3-dihydrothiazole



D1i

Figure 5.41. 4-(2,4-Dimethylphenyl)-3-methyl-2-((1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-2,3-dihydrothiazole.

Yield: 60%

Melting point: 175°C.

IR Maximum Velocity (cm^{-1}): 2935 (C=C-H stretching band), 2918 (C-H stretching band), 1587 (C=N stretching band), 1562 (C=C band), 1236 (C-N stretching band), 833 (2,4-disubstituted benzene out-of-plane deformation band).

^1H -NMR (300 MHz, $\text{DMSO}-d_6$): 1.57(6H, s, piperidine-H), 2.16 (3H, s, Methyl-H), 2.32 (6H, s, Methyl-H), 3.04 (3H, s, Methyl-H), 3.18-3.20 (4H, m, piperidine-H), 6.15 (1H, s, dihydrothiazole-H), 6.90-6.93(2H, d, $J=8.94$ Hz, Ar-H), 7.09-7.18 (3H, m, Ar-H), 7.66-7.69 (2H, d, $J=8.89$ Hz, Ar-H).

^{13}C -NMR (75 MHz, $\text{DMSO}-d_6$): δ = 13.51, 15.21, 18.77, 23.85, 25.50, 31.64, 33.49, 49.35, 51.14, 100.27, 114.05, 116.20, 126.10, 128.02, 128.28, 128.76, 129.72, 131.84, 132.34, 137.59, 139.42, 139.50, 152.05, 154.40, 167.48.

HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{25} \text{H}_{30} \text{N}_4 \text{S}$ $[\text{M}+\text{H}]^+$: 419.2264, found: 419.2255.

Item	Value
Acquired Date&Time	2.05.2024 14:48:21
Acquired by	System Administrator
Filename	C:\Users\dopnalab\Desktop\MASAUSTU\isazan\hic\hic-1j1.ispd
Spectrum name	hic-1j1
Sample name	hic-1j
Sample ID	
Option	
Comment	
No. of Scans	30
Resolution	4 [cm-1]
Apodization	Happ-Genzel

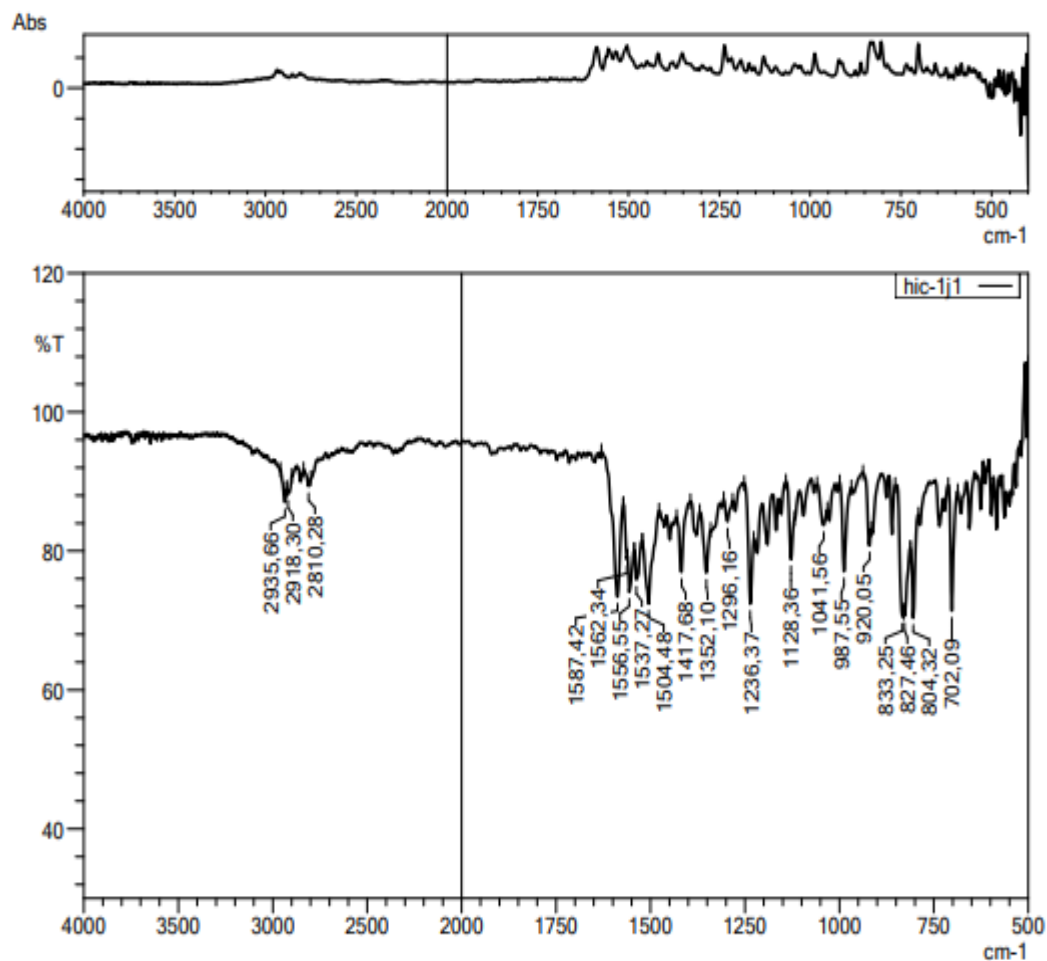


Figure 5.42. IR fingerprint of D1.i.

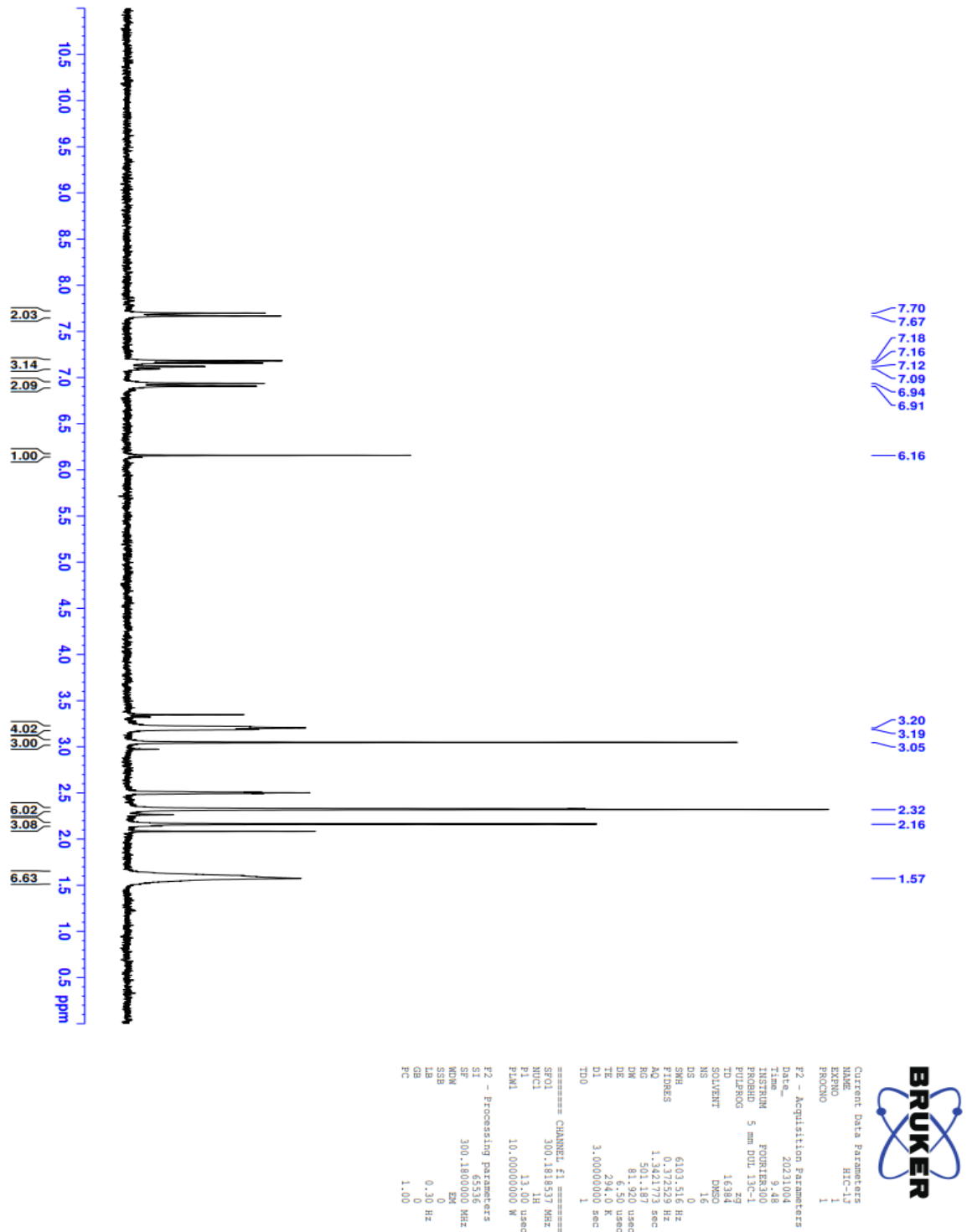
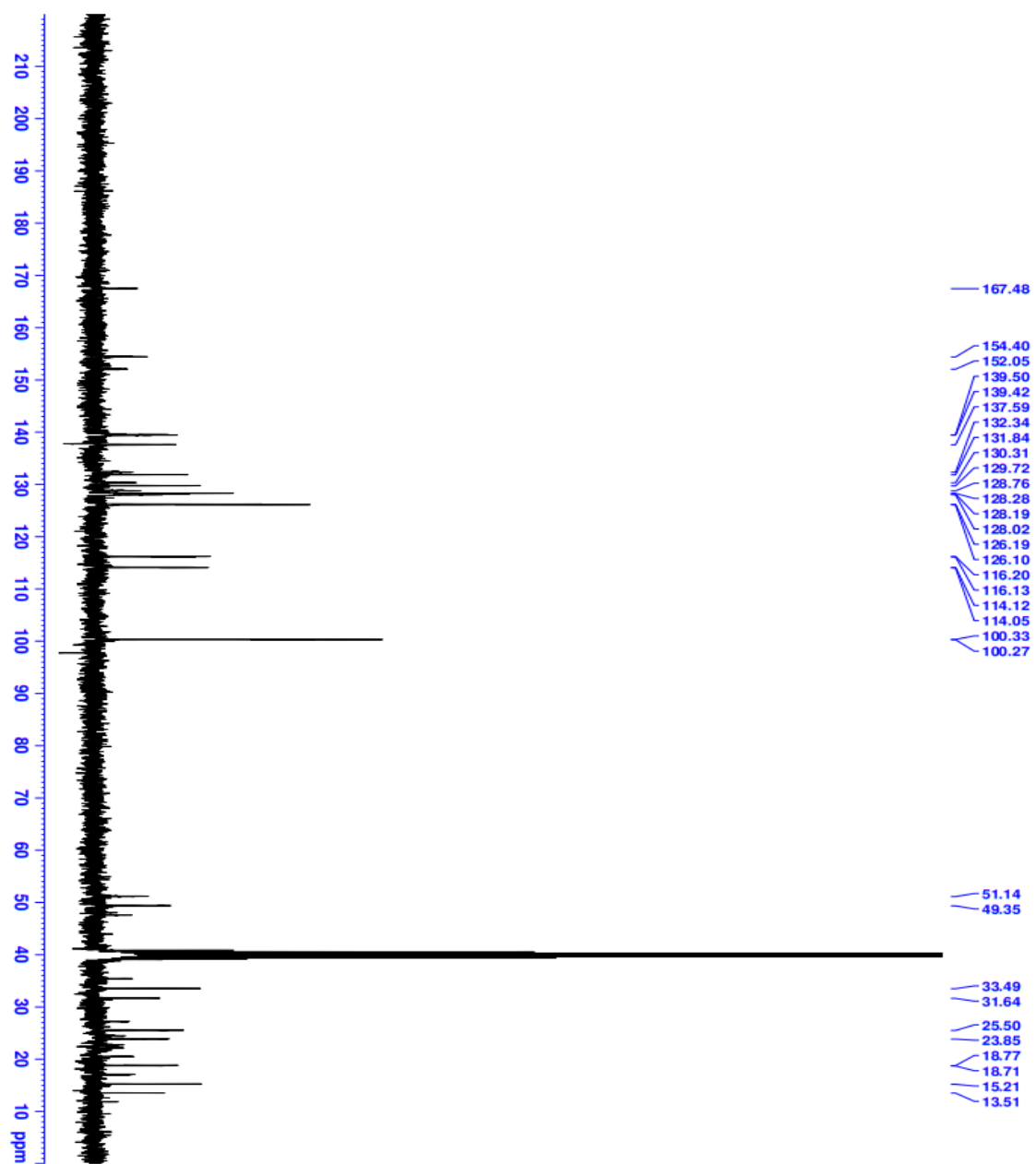


Figure 5.43. ¹H NMR signals of D1.i.



Current Data Parameters
NAME RIC-1J
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20231004
Time 9:50
INSTRUM FOURIER300
PROBHD 5 mm DUL 13C-1
PULPROG zgpg30
TD 32768
SOLVENT DMSO
NS 2048
DS 4
SWH 24414.063 Hz
FIDRES 0.745058 Hz
AQ 0.6710986 sec
RG 501.187
DM 201.187
DE 14.530 sec
TE 294.0 K
TD 1.00000000 sec
D1 0.03000000 sec
D11 0.00001500 sec
D32 0.8999998 sec
D40 0.00093990 sec
L4 23
L5 26
P32 90.00 usec
TD0 1

===== CHANNEL f1 =====
SFO1 75.481887 MHz
NUC1 13C
P1 15.00 usec
PLM1 15.00000000 W

===== CHANNEL f2 =====
SFO2 300.1812007 MHz
NUC2 1H
CPCPRG12 waltz16
PCPD2 90.00 usec
PLM2 10.00000000 W
PLW2 0.20863999 W
PLW3 0.10435000 W

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

Figure 5.44. ¹³CNMR signals of D1.i.

Data File: C:\LabSolutions\Data\Analiz\Idea\HIC-1J ALT_591.lcd

Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	8	33	O	2	0	3	S	2	0	2	Ru	2	0	0	H
C	4	4	32	F	1	0	0	Cl	1	0	0	Pd	2	0	0	Na
N	3	0	6	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5

HC Ratio: unlimited

Max Isotopes: 3

MSn Iso RI (%): 10.00

DBE Range: 0.0 - 30.0

Apply N Rule: no

Isotope RI (%): 1.00

MSn Logic Mode: AND

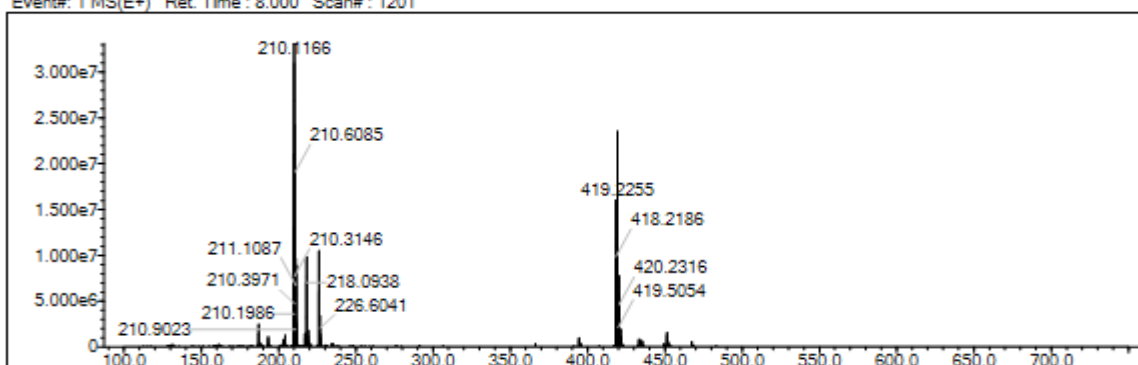
Electron Ions: both

Use MSn Info: yes

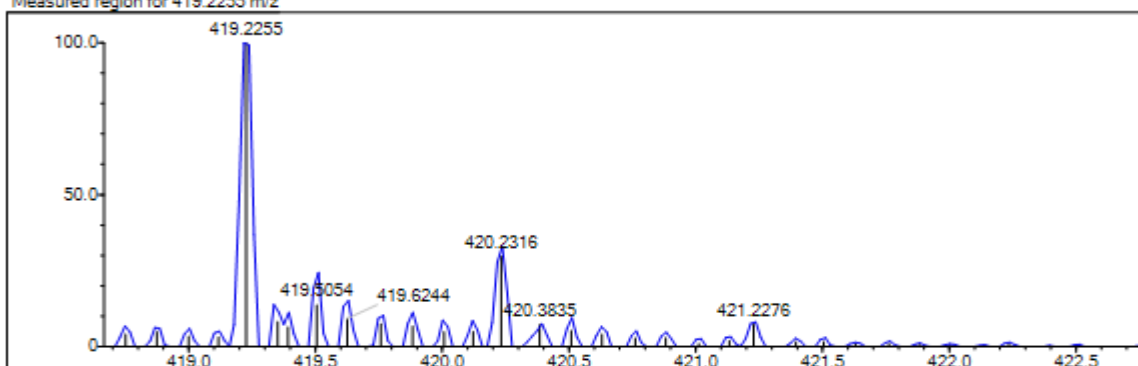
Isotope Res: 9000

Max Results: 50

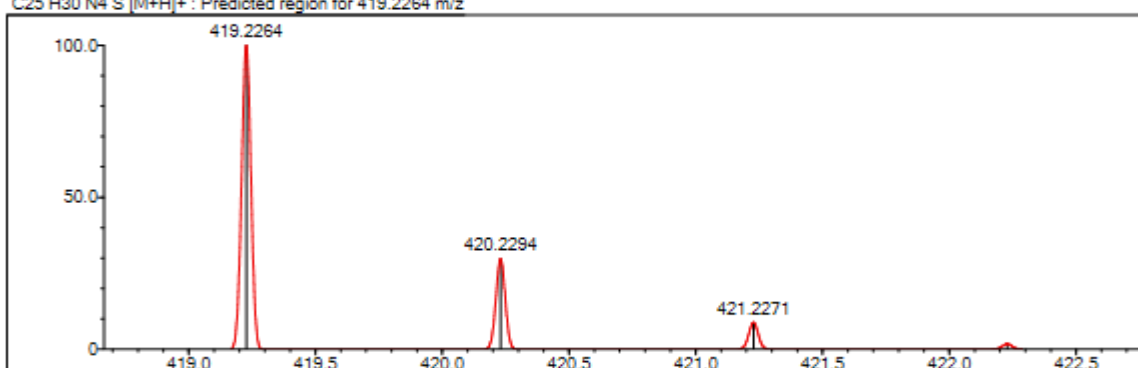
Event#: 1 MS(E+) Ret. Time: 8.000 Scan#: 1201



Measured region for 419.2255 m/z



C25 H30 N4 S [M+H]⁺: Predicted region for 419.2264 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	85.65	C25 H30 N4 S	[M+H] ⁺	419.2255	419.2264	-0.9	-2.15	88.19	13.0

Figure 5.45. HRMS spectrogram of D1.i.

5.1.2 Synthesis of 3-Allyl-2-((1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-4-(p-substituted)-2,3-dihydrothiazole derivatives

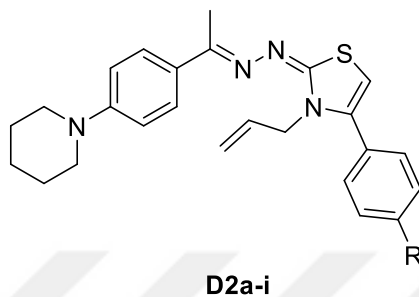


Figure 5.46. 3-Allyl-2-((1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-4-(p-substituted)-2,3-dihydrothiazole derivatives.

The D2.a-i derivatives were synthesized according to the same method and mechanism as mentioned in section 4.1 and 5.1.1 (*vide supra*)

5.1.2.1. 3-Allyl-2-((1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-4-(p-tolyl)-2,3-dihydrothiazole

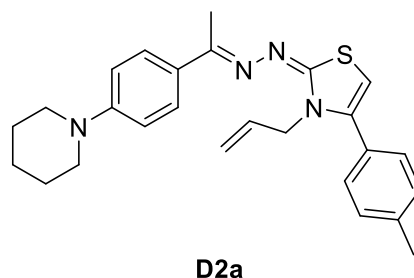


Figure 5.47. 3-Allyl-2-((1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-4-(p-tolyl)-2,3-dihydrothiazole.

Yield: 78%.

Melting point: 129.4°C.

IR Maximum Velocity (cm^{-1}): 2933 (C=C-H stretching band), 2787 (C-H stretching band), 1591 (C=N stretching band), 1504 (C=C band), 1234 (C-N stretching band), 815 (4-substituted benzene out-of-plane deformation band).

¹H-NMR (300 MHz, DMSO-*d*₆): 1.57(6H, s, piperidine-H), 2.29 (3H, s, Methyl-H), 2.34 (3H, s, Methyl-H), 3.20 (4H, m, piperidine-H), 4.38(2H, s, Allyl-H), 4.90-4.96(1H, d, *J*=17.64 Hz, Allyl-H), 5.08-5.12(1H, d, *J*=10.36 Hz, Allyl-H), 5.76-5.87 (1H, m, Allyl-H), 6.24 (1H, s, dihydrothiazole-H), 6.90-6.93(2H, d, *J*=8.64 Hz, Ar-H), 7.25-7.34 (4H, q, *J*=10.44 Hz, Ar-H), 7.65-7.69 (2H, d, *J*=8.55 Hz, Ar-H).

¹³C-NMR (75 MHz, DMSO-*d*₆): δ =14.42, 21.30, 24.42, 25.50, 47.99, 49.31, 99.69, 115.07, 116.96, 127.26, 128.42, 128.60, 128.99, 129.68, 133.26, 139.13, 140.60, 152.11, 154.95, 167.15.

HRMS (*m/z*): [M+H]⁺ calcd for C₂₆ H₃₀ N₄ S [M+H]⁺: 431.2264; found: 431.224.

Item	Value
Acquired Date&Time	3.05.2024 10:32:16
Acquired by	System Administrator
Filename	C:\Users\dopnab\Desktop\MASAU\TÜ\isazan\hic\hic-2a1.ispd
Spectrum name	hic-2a1
Sample name	hic-2a
Sample ID	
Option	
Comment	
No. of Scans	30
Resolution	4 [cm-1]
Apodization	Happ-Genzel

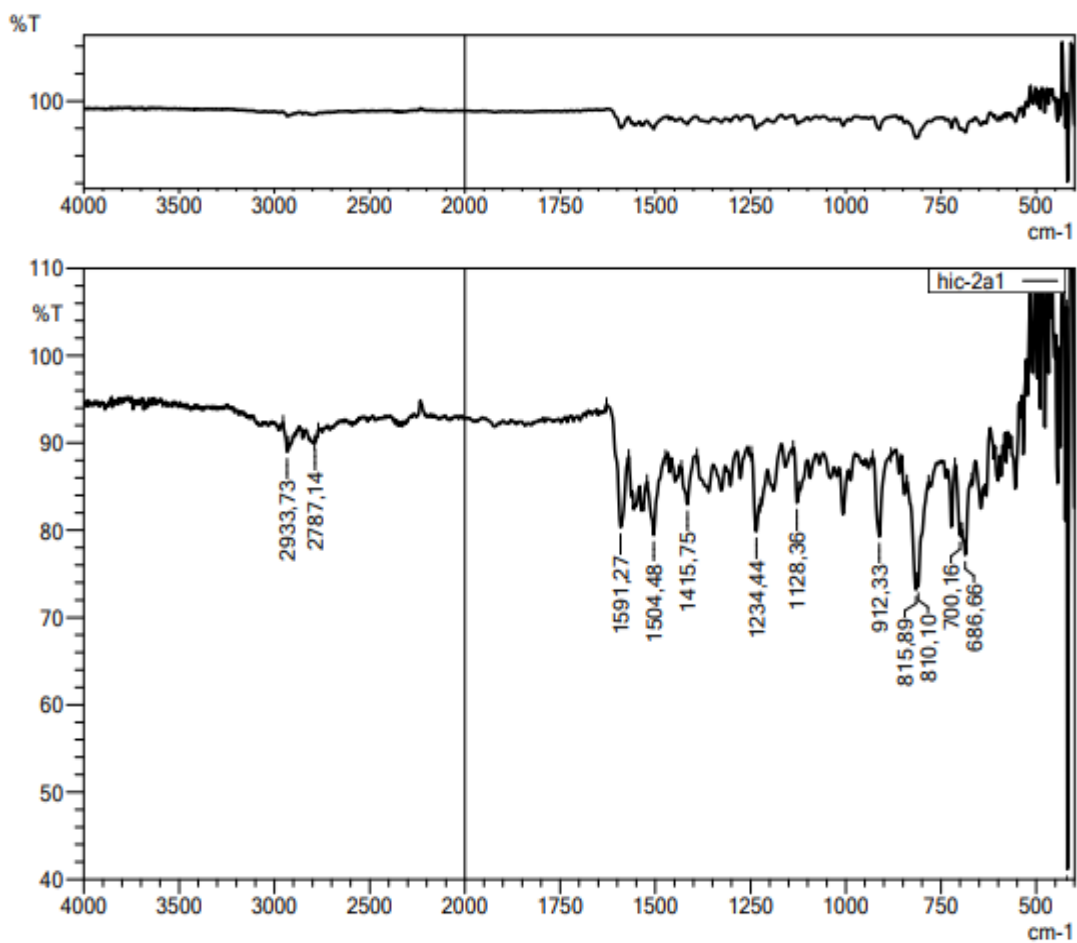


Figure 5.48. IR fingerprint of D2.a.

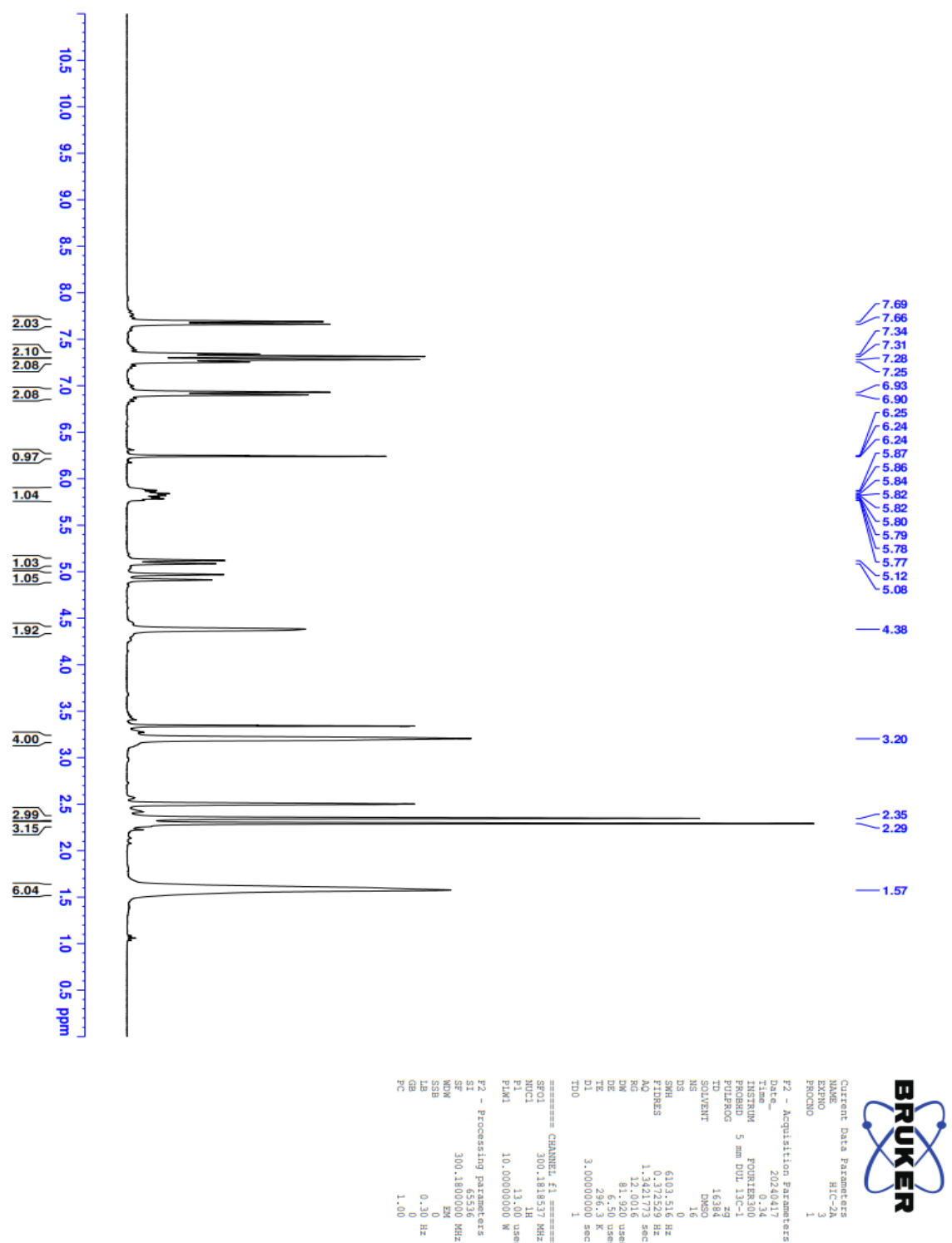
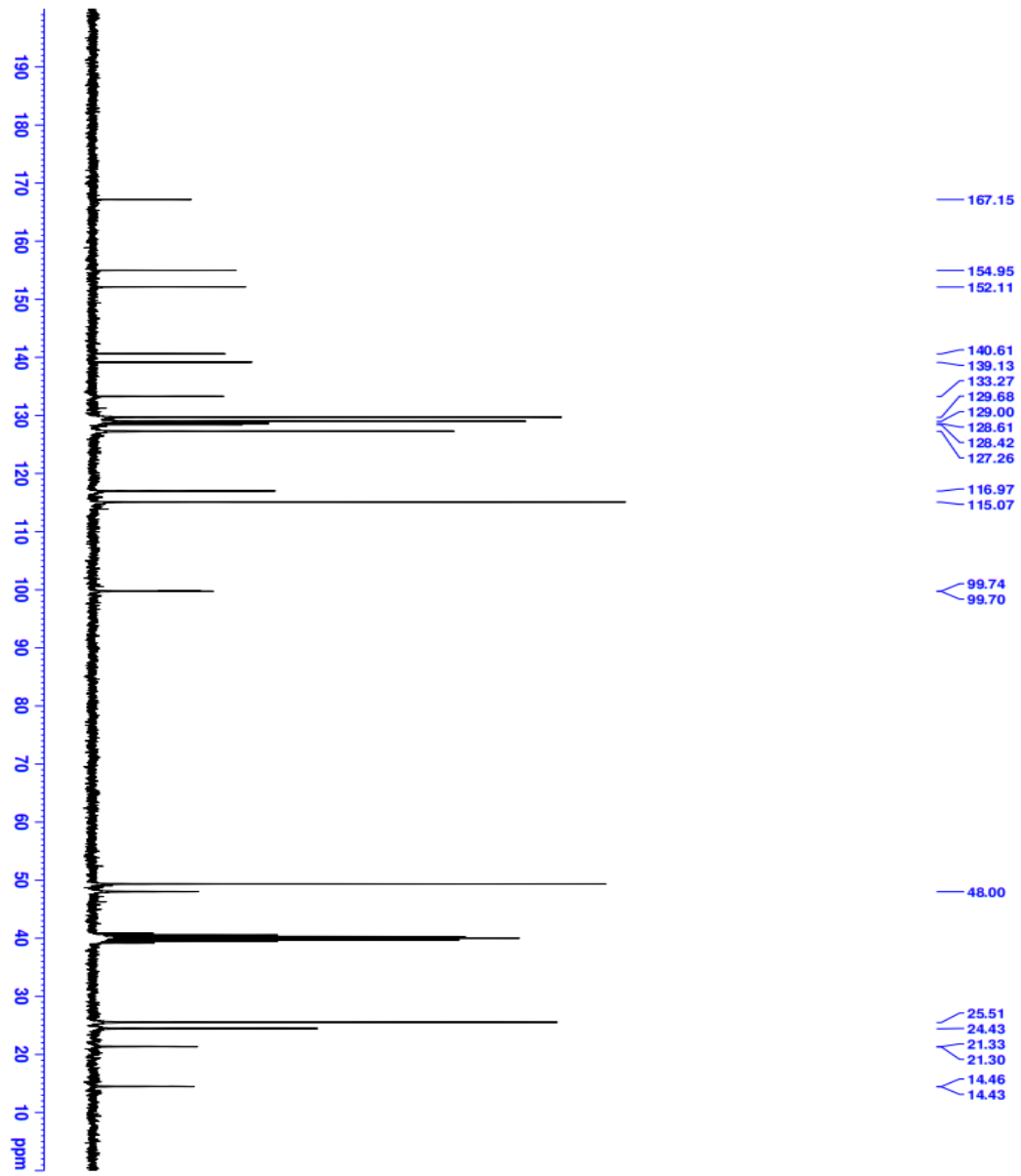


Figure 5.49. ¹H NMR signals of D2.a.



Current Data Parameters
 NAME HIC-23
 EXNO 4
 PROCNO 1

F2 - Acquisition Parameters
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 Time_ 10:08:36
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 PROBRD 5 mm BBL 1H-1
 PULPROG zgpg30
 TD 32768
 SOLVENT DMSO
 NS 2048
 DS 4
 SWH 24414.623 Hz
 FIDRES 0.1735544 Hz
 AQ 0.6710886 sec
 RG 501.187
 DW 20.480 usec
 DE 6.50 usec
 TE 296.2 K
 D1 1.00000000 sec
 D11 0.00000000 sec
 D12 0.0001500 sec
 D13 0.8999998 sec
 D40 0.0003990 sec
 L4 23
 L5 26
 L12 90.00 usec
 L13 1
 L14 1

===== CHANNEL f1 =====
 SFO1 75.481687 MHz
 NUC1 13C
 P1 15.00 usec
 PL1 15.00000000 W

===== CHANNEL f2 =====
 SFO2 300.1812007 MHz
 NUC2 1H
 P2 15.00 usec
 PL2 15.00000000 W

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

Figure 5.50. ¹³CNMR signals of D2.a.

Data File: C:\LabSolutions\Data\Analysis\deriva\HIC-2B ALT_579.lcd

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H	1	8	33	O	2	0	3	S	2	0	2	Ru	2	0	0	H
C	4	4	32	F	1	0	0	Cl	1	0	0	Pd	2	0	0	Na
N	3	0	6	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5

HC Ratio: unlimited

Max Isotopes: 3

MSn Iso RI (%): 10.00

DBE Range: 0.0 - 30.0

Apply N Rule: no

Isotope RI (%): 1.00

MSn Logic Mode: AND

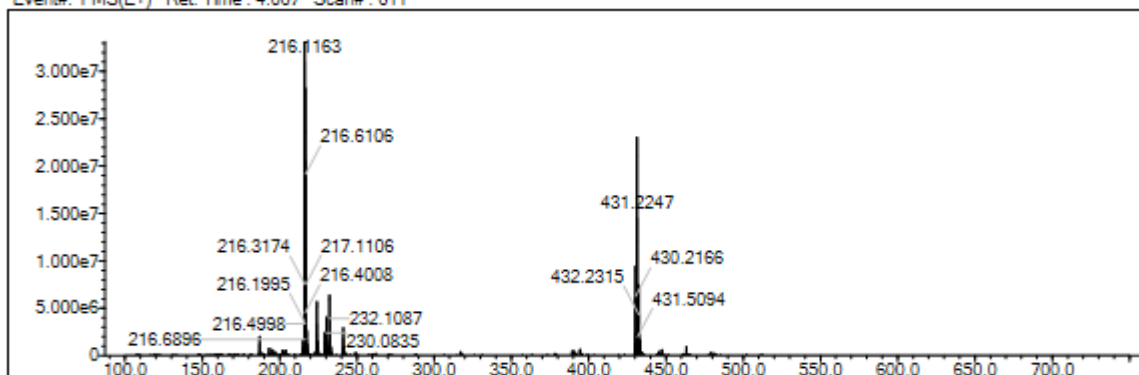
Electron Ions: both

Use MSn Info: yes

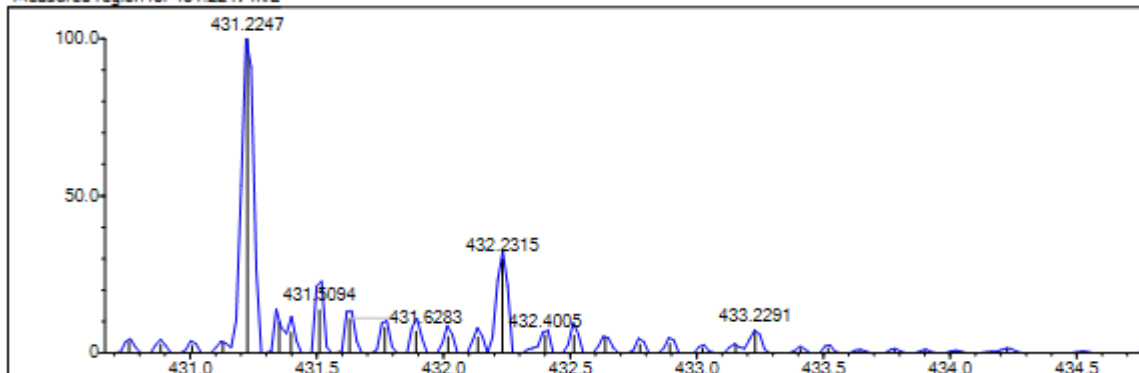
Isotope Res: 9000

Max Results: 50

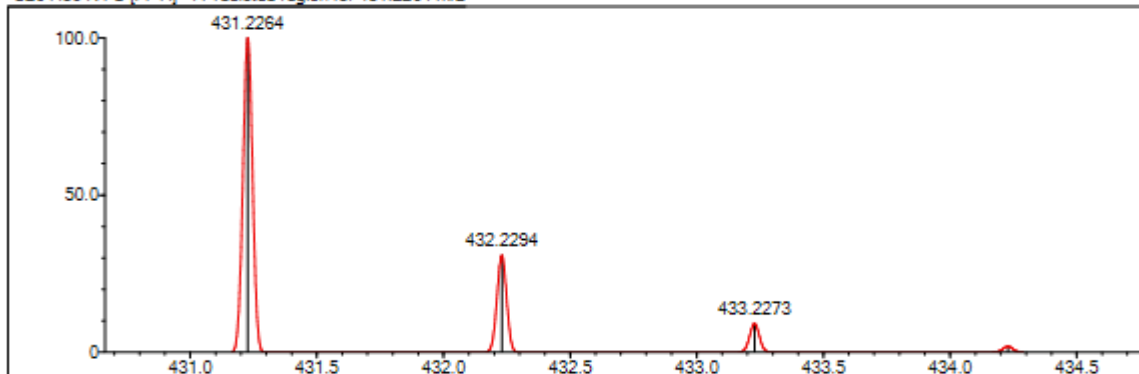
Event#: 1 MS(E+) Ret. Time: 4.067 Scan#: 611



Measured region for 431.2247 m/z



C26 H30 N4 S [M+H]⁺: Predicted region for 431.2264 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	92.30	C26 H30 N4 S	[M+H] ⁺	431.2247	431.2264	-1.7	-3.94	99.62	14.0

Figure 5.51. HRMS spectrogram of D2.a.

5.1.2.2. 3-Allyl-4-(4-fluorophenyl)-2-((1-(4-(piperidin-1yl) phenyl) ethylidene)hydrazineylidene)-2,3-dihydrothiazole

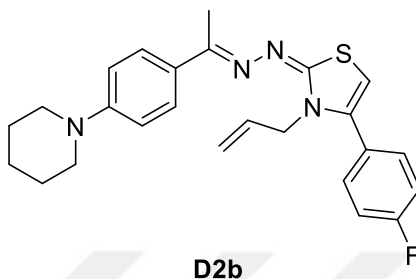


Figure 5.52. 3-Allyl-4-(4-fluorophenyl)-2-((1-(4-(piperidin1yl)phenyl)ethylidene)hydrazineylidene)-2,3-dihydrothiazole.

Yield: 65%.

Melting point: 116°C.

IR Maximum Velocity (cm^{-1}): 3076 (C=C-H stretching band), 2931 (C-H stretching band), 1591 (C=N stretching band), 1535 (C=C band), 1220 (C-N stretching band), 817 (4-substituted benzene out-of-plane deformation band).

^1H -NMR (300 MHz, $\text{DMSO}-d_6$): 1.60 (6H, s, piperidine-H), 2.30 (3H, s, Methyl-H), 3.22 (4H, br.s, piperidine-H), 4.38-4.39 (2H, s, Allyl-H), 4.91-4.96 (1H, d, $J=17.20\text{Hz}$, Allyl-H), 5.07-5.11 (1H, d, $J=10.29\text{ Hz}$, Allyl-H), 5.78-5.86 (1H, m, Allyl-H), 6.32 (1H, s, dihydrothiazole-H), 6.92-6.94 (2H, d, $J=8.71\text{ Hz}$, Ar-H), 7.30-7.34 (2H, t, $J=8.70\text{ Hz}$, Ar-H), 7.49-7.53 (2H, m, Ar-H), 7.67-7.69 (2H, d, $J=8.65\text{ Hz}$, Ar-H).

^{13}C -NMR (75 MHz, $\text{DMSO}-d_6$): δ = 14.46, 24.43, 25.50, 47.97, 49.30, 100.49, 115.06, 116.02, 116.23, 117.06, 127.28, 128.55, 131.44, 131.53, 133.18, 139.45, 152.14, 155.13, 167.01.

HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{25}\text{H}_{27}\text{N}_4\text{FS}$ $[\text{M}+\text{H}]^+$: 435.2013; found: 435.2009

Item	Value
Acquired Date&Time	3.05.2024 10:37:27
Acquired by	System Administrator
Filename	C:\Users\dopnalab\Desktop\MASAÜSTÜ\isazan\hic\hic-2b1.ispd
Spectrum name	hic-2b1
Sample name	hic-2b
Sample ID	
Option	
Comment	
No. of Scans	30
Resolution	4 [cm-1]
Apodization	Happ-Genzel

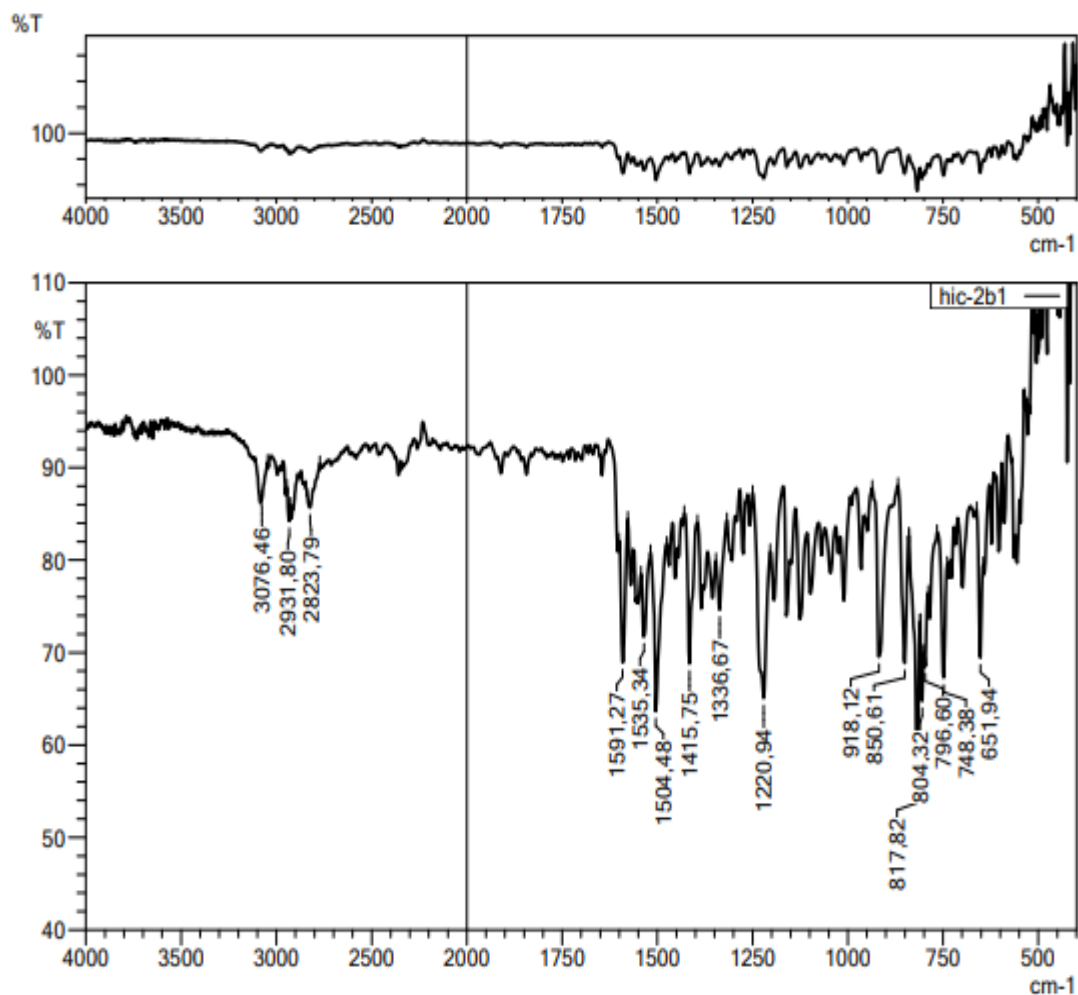


Figure 5.53. IR fingerprint of D2.b.

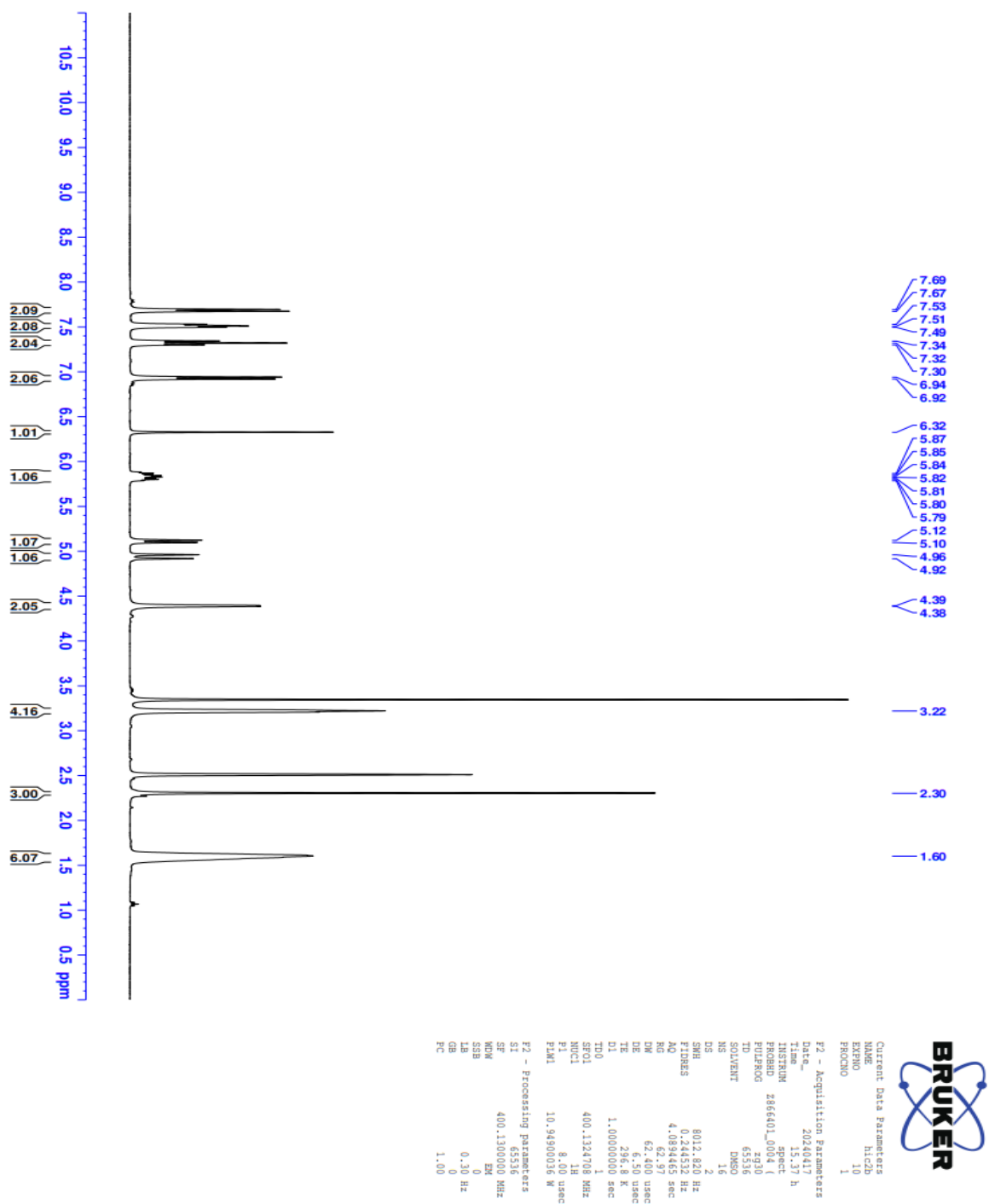


Figure 5.54. ^1H NMR signals of D2.b.

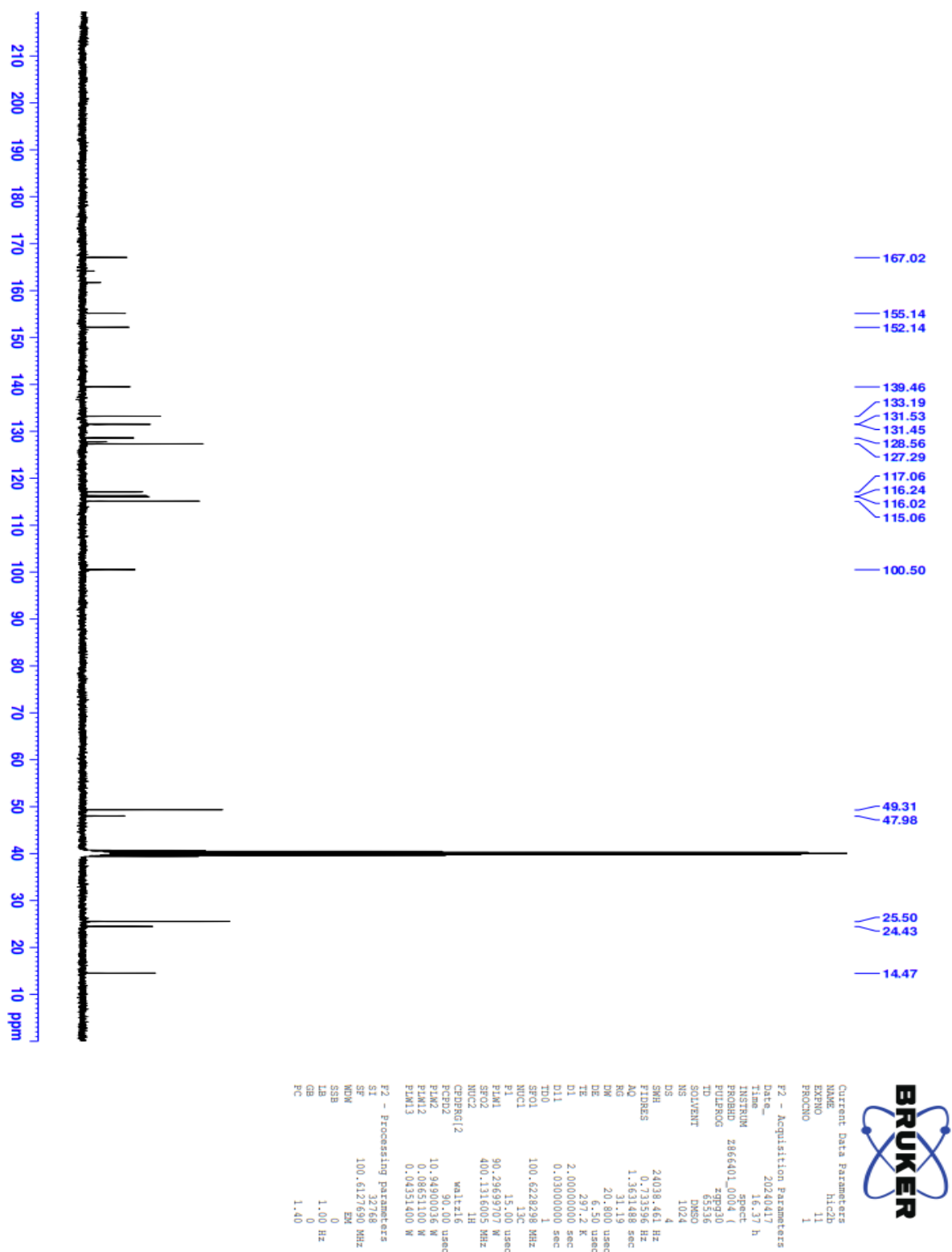


Figure 5.55. ^{13}C NMR signals of D2.b.

Data File: C:\LabSolutions\Data\Analiz\denya\HIC-2C UST_580.lod

Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	8	33	O	2	0	3	S	2	0	2	Ru	2	0	0	H
C	4	4	32	F	1	1	1	Cl	1	0	0	Pd	2	0	0	Na
N	3	0	6	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5

HC Ratio: unlimited

Max Isotopes: 3

MSn Iso RI (%): 10.00

DBE Range: 0.0 - 30.0

Apply N Rule: no

Isotope RI (%): 1.00

MSn Logic Mode: AND

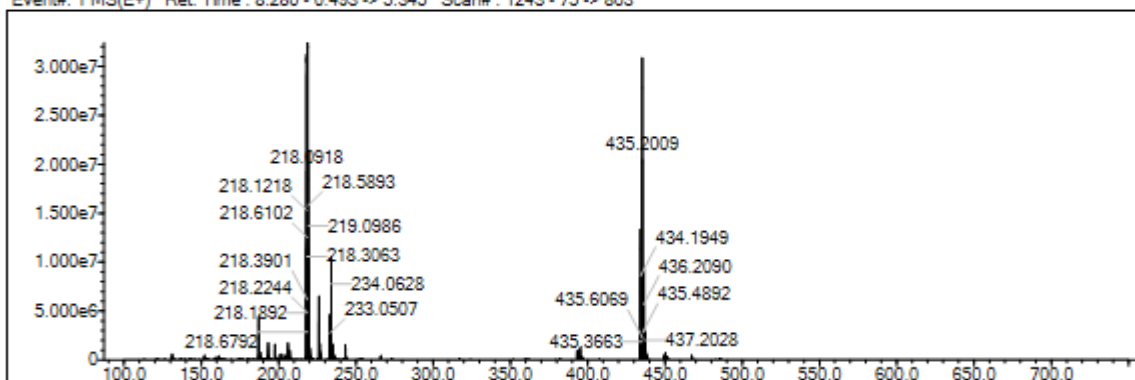
Electron Ions: both

Use MSn Info: yes

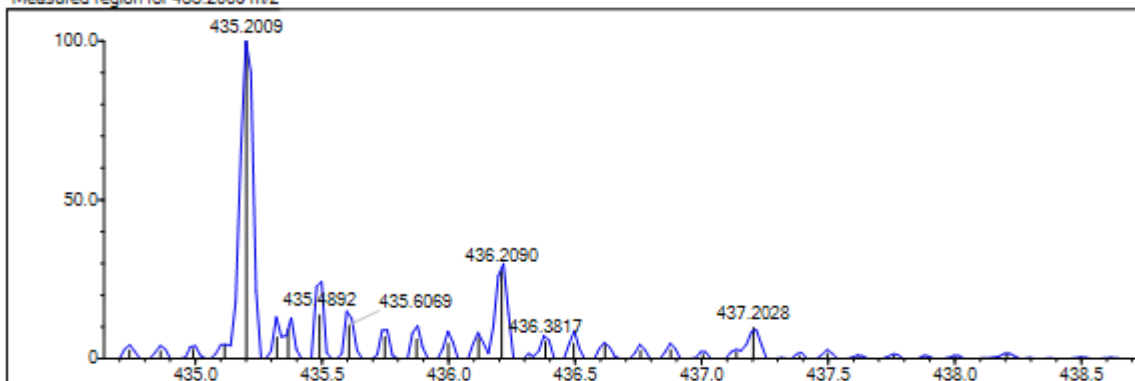
Isotope Res: 9000

Max Results: 50

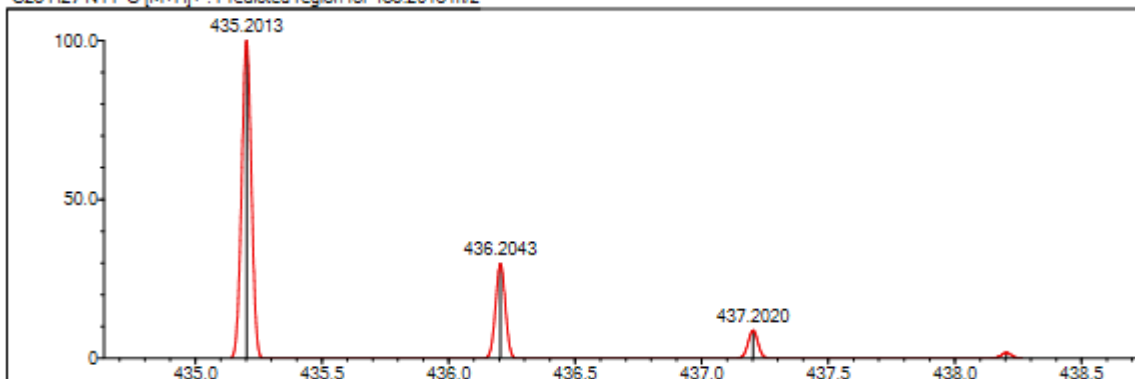
Event#: 1 MS(E+) Ret. Time : 8.280 - 0.493 -> 5.345 Scan#: 1243 - 75 -> 803



Measured region for 435.2009 m/z



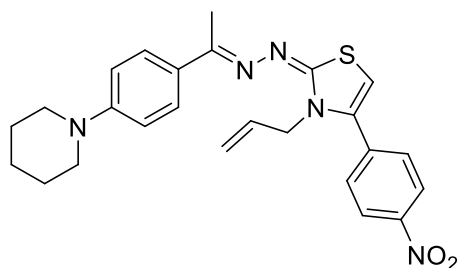
C25 H27 N4 F S [M+H]⁺ : Predicted region for 435.2013 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	90.52	C25 H27 N4 F S	[M+H] ⁺	435.2009	435.2013	-0.4	-0.92	90.52	14.0

Figure 5.56. HRMS spectrogram of D2.b.

5.1.2.3. 3-Allyl-4-(4-nitrophenyl)-2-((1-(4-(piperidin-1-yl)phenyl) ethylidene) hydrazineylidene)-2,3-dihydrothiazole



D2c

Figure 5.57. 3-Allyl-4-(4-nitrophenyl)-2-((1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-2,3-dihydrothiazole.

Yield: 85%.

Melting point: 155.6°C.

IR Maximum Velocity (cm^{-1}): 3080 (C=C-H stretching band), 2933(C-H stretching band), 1587 (C=N stretching band), 1552 (C=C band), 1236 (C-N stretching band), 819 (4-substituted benzene out-of-plane deformation band).

^1H -NMR (300 MHz, $\text{DMSO}-d_6$): 1.58(6H, s, piperidine-H), 2.30 (3H, s, Methyl-H), 3.21(4H, br.s, piperidine-H), 4.47 (2H, s, Allyl-H), 4.92-4.98(1H, d, $J=17.23\text{Hz}$, Allyl-H), 5.09-5.13(1H, d, $J=10.41\text{Hz}$, Allyl-H), 5.79-5.90 (1H, m, Allyl-H), 6.60-6.61(1H, s, br, dihydrothiazole-H), 6.91-6.94(2H, d, $J=7.89\text{ Hz}$, Ar-H), 7.66-7.76 (4H, dd, $J_1\&J_2=5.43, 7.23\text{ Hz}$, Ar-H), 8.29-8.32 (2H, d, $J=7.20\text{ Hz}$, Ar-H).

^{13}C -NMR (75 MHz, $\text{DMSO}-d_6$): δ =14.53, 24.42, 25.48, 48.33, 49.25, 103.67, 115.03, 117.32, 124.35, 127.36, 128.32, 130.00, 133.10, 137.55, 138.74, 147.88, 152.20, 155.73, 167.07.

HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{25}\text{H}_{27}\text{N}_5\text{O}_2\text{S}$ $[\text{M}+\text{H}]^+$: 462.1958; found: 462.1947.

Item	Value
Acquired Date&Time	3.05.2024 10:42:36
Acquired by	System Administrator
Filename	C:\Users\dopnalab\Desktop\MASAÜSTÜsazan\hic\hic-2c1.jspd
Spectrum name	hic-2c1
Sample name	hic-2c
Sample ID	
Option	
Comment	
No. of Scans	30
Resolution	4 [cm-1]
Apodization	Happ-Genzel

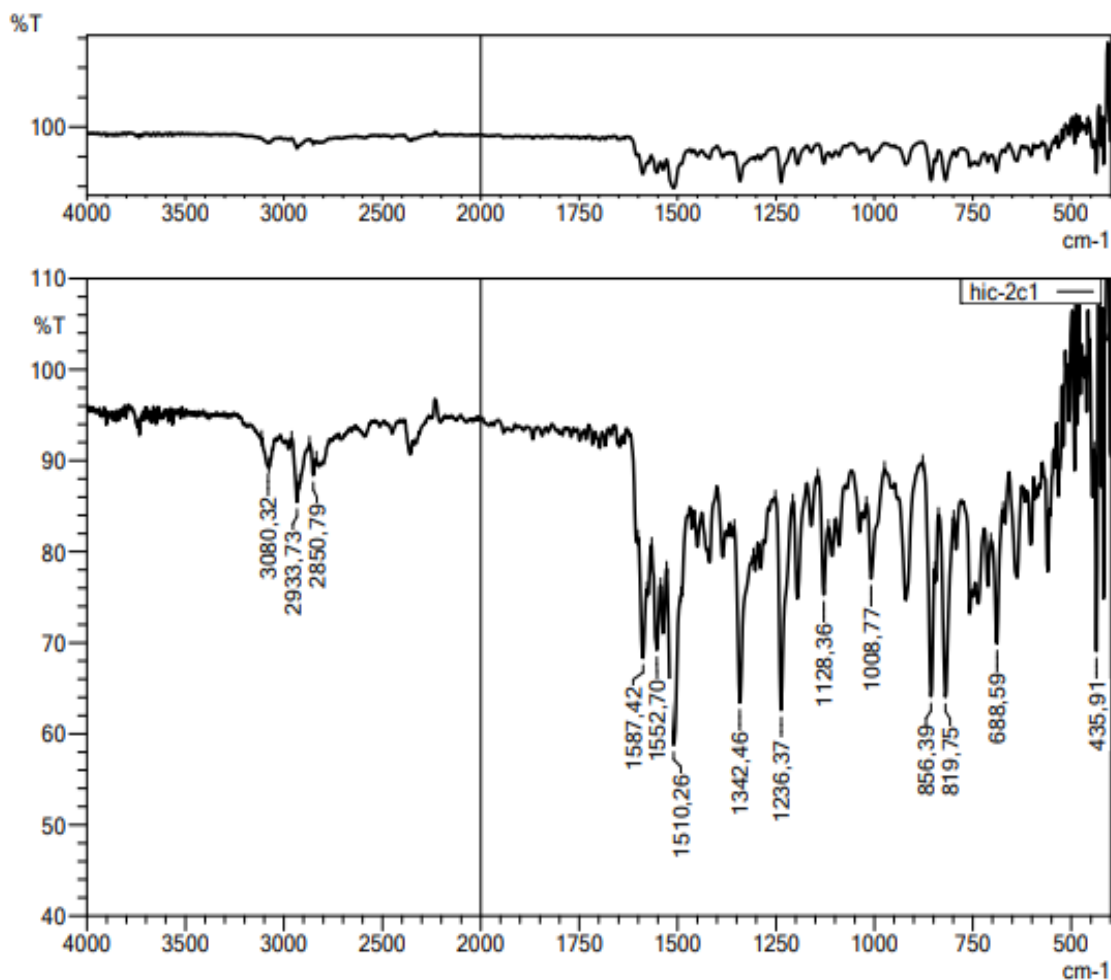


Figure 5.58. IR fingerprint of D2.c.

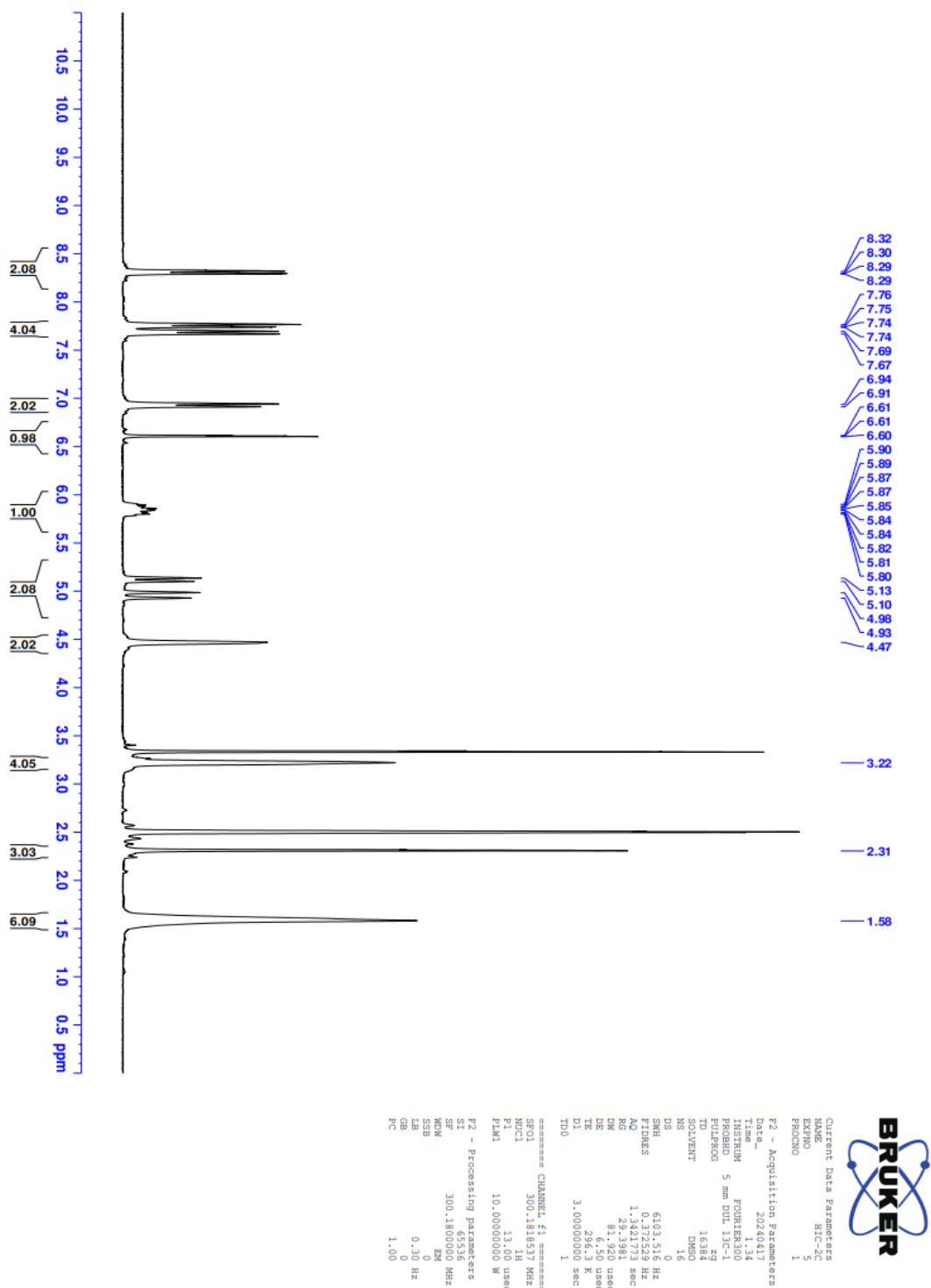


Figure 5.59. ¹H NMR signals of D2.c.

Data File: C:\LabSolutions\Data\Analiz\derya\HIC-2C ALT_581.lcd

Elmt	Val	Min	Max	Elmt	Val	Min	Max	Elmt	Val	Min	Max	Elmt	Val	Min	Max	Use Adduct
H	1	8	33	O	2	0	3	S	2	0	2	Ru	2	0	0	H
C	4	4	32	F	1	0	0	Cl	1	0	0	Pd	2	0	0	Na
N	3	0	6	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5

HC Ratio: unlimited

Max Isotopes: 3

MSn Iso RI (%): 10.00

DBE Range: 0.0 - 30.0

Apply N Rule: no

Isotope RI (%): 1.00

MSn Logic Mode: AND

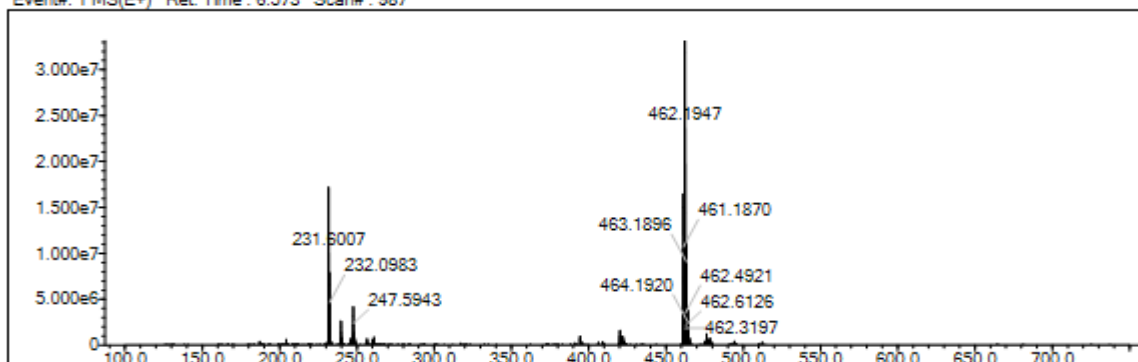
Electron Ions: both

Use MSn Info: yes

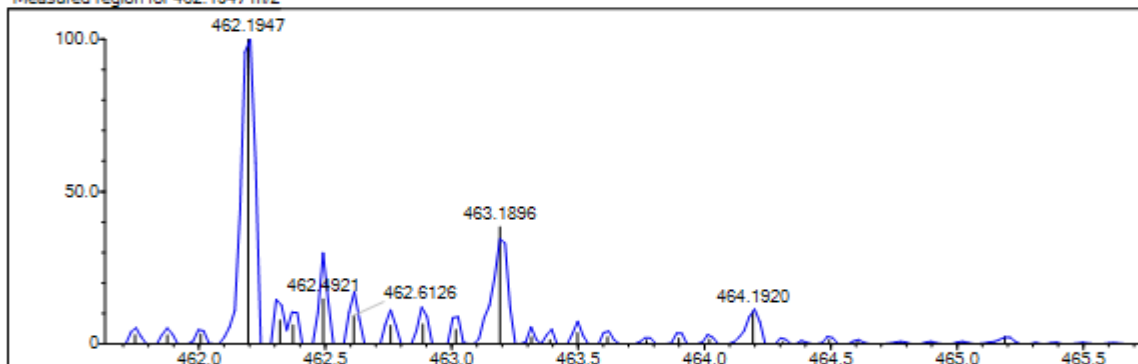
Isotope Res: 9000

Max Results: 50

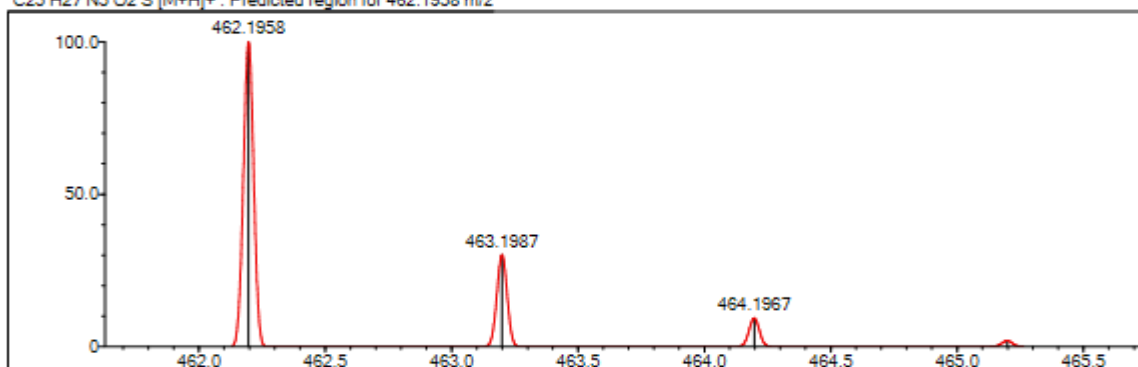
Event#: 1 MS(E+) Ret. Time: 6.573 Scan#: 987



Measured region for 462.1947 m/z



C25 H27 N5 O2 S [M+H]⁺ : Predicted region for 462.1958 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	73.79	C25 H27 N5 O2 S	[M+H] ⁺	462.1947	462.1958	-1.1	-2.38	76.42	15.0

Figure 5.61. HRMS spectrogram of D2.c.

5.1.2.4. 3-Allyl-4-(4-chlorophenyl)-2-((1-(4-(piperidin-1-yl)phenyl) ethylidene) hydrazineylidene) -2,3-dihydrothiazole

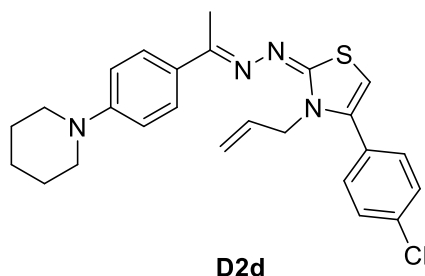


Figure 5.62. 3-Allyl-4-(4-chlorophenyl)-2-((1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-2,3-dihydrothiazole.

Yield: 75%.

Melting point: 145.2°C.

IR Maximum Velocity (cm^{-1}): 3080 (C=C-H stretching band), 2931 (C-H stretching band), 1591 (C=N stretching band), 1556 (C=C band), 1232 (C-N stretching band), 817 (4-substituted benzene out-of-plane deformation band).

^1H -NMR (300 MHz, $\text{DMSO}-d_6$): 1.60(6H, s, piperidine-H), 2.29 (3H, s, Methyl-H), 3.22(4H, br.s, piperidine-H), 3.80 (3H, s, Methyl-H), 4.38(2H, s, Allyl-H), 4.93-4.97(1H, d, $J=17.20\text{Hz}$, Allyl-H), 5.10-5.13(1H, d, $J=10.26\text{ Hz}$, Allyl-H), 5.80-5.88 (1H, m, Allyl-H), 6.22(1H, s, dihydrothiazole-H), 6.92-6.94(2H, d, $J=8.41\text{ Hz}$, Ar-H), 7.02-7.04(2H, d, $J=8.10\text{ Hz}$, Ar-H), 7.37-7.39 (2H, d, $J=8.15\text{Hz}$, Ar-H), 7.67-7.69 (2H, d, $J=8.30\text{ Hz}$, Ar-H).

^{13}C -NMR (75 MHz, $\text{DMSO}-d_6$): δ =14.45, 24.43, 25.50, 47.96, 49.32, 55.72, 99.30, 114.54, 115.08, 116.95, 123.48, 127.26, 130.59, 133.30, 140.39, 152.11, 154.89, 160.27, 167.10.

HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{25}\text{H}_{27}\text{N}_4\text{S Cl}$ $[\text{M}+\text{H}]^+$: 451.1718; found: 451.1716.

Item	Value
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Acquired by	System Administrator
Filename	C:\Users\dopnalab\Desktop\MASAÜSTÜ\isazani\hic\hic-2d1.ispd
Spectrum name	hic-2d1
Sample name	hic-2d
Sample ID	
Option	
Comment	
No. of Scans	30
Resolution	4 [cm-1]
Apodization	Happ-Genzel

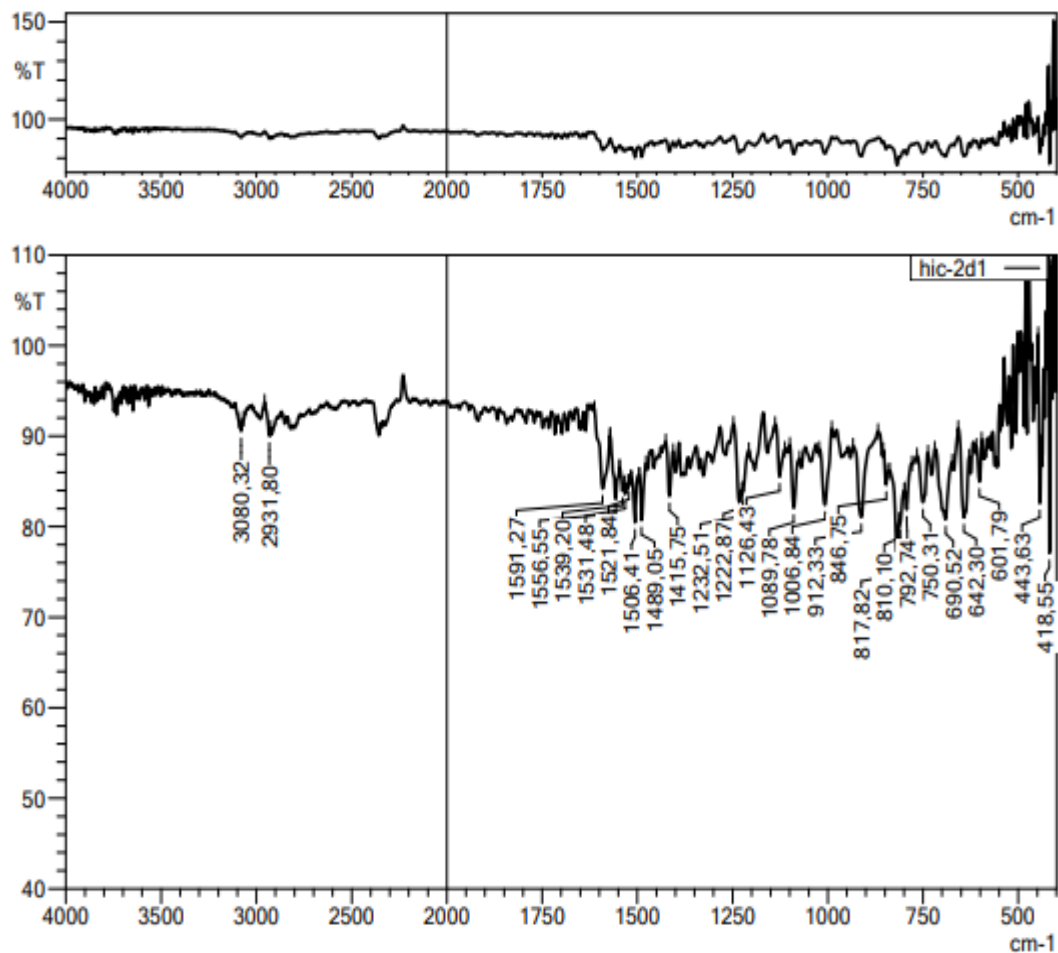


Figure 5.63. IR fingerprint of D2.d.

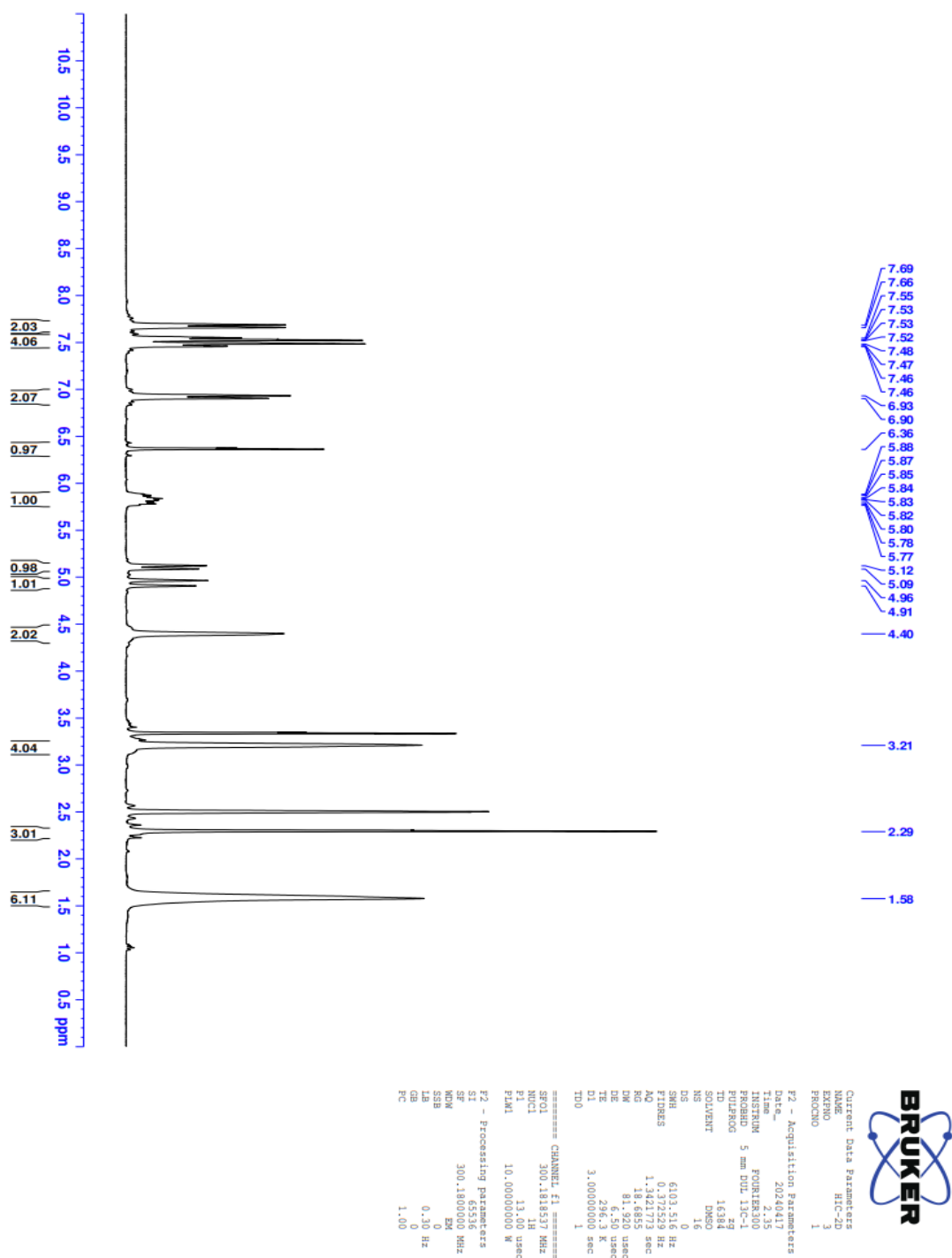
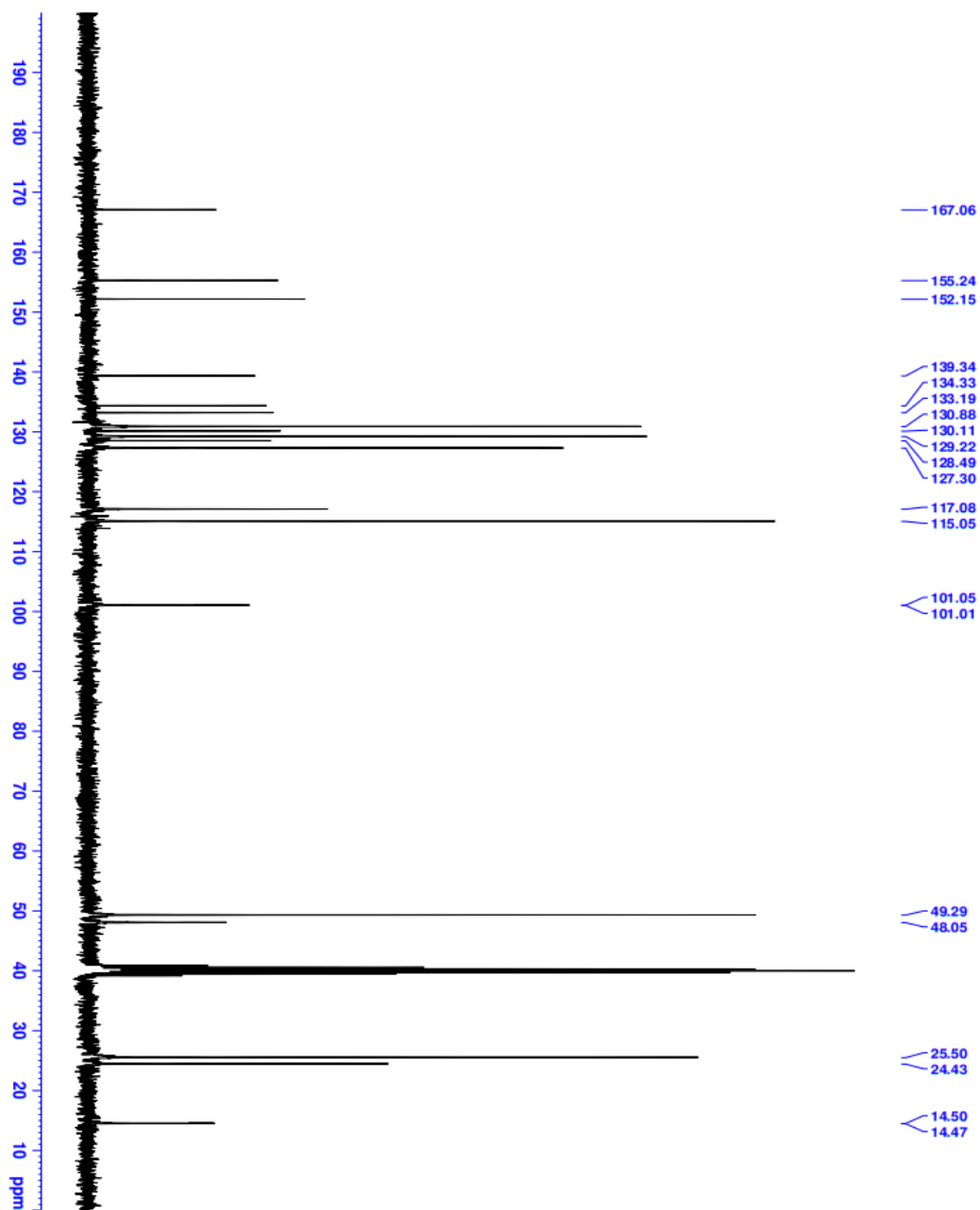


Figure 5.64. :¹HNMR signals of D2.d.



Current Data Parameters
NAME HIC-2D
EXPNO 4
PROCNO 1

F2 - Acquisition Parameters
Date_ 20240117
Time 12:22:11
INSTRUM PULPROB
PROBHD 5 mm DUL 13C-1
PULPROG zgpg
TD 32768
SOLVENT DMSO
NS 2048
DS 4
SWH 24414.065 Hz
FIDRES 0.745058 Hz
AQ 0.6710886 sec
RG 501.187
DW 20.480 usec
DE 6.50 usec
TE 298.2 K
D1 1.0000000 sec
D11 0.0300000 sec
D31 0.0001500 sec
D32 0.8999998 sec
D40 0.0093990 sec
L4 23
L5 26
T2 90.00 usec
T20 1

===== CHANNEL f1 =====
SFO1 75.487887 MHz
NUC1 13C
P1 15.00 usec
PL1 15.0000000 W

===== CHANNEL f2 =====
SFO2 300.1812007 MHz
NUC2 1H
CPRG12 waltz16
PCPD2 90.00 usec
PL12 10.0000000 W
PL13 0.1045500 W
PL14 0.1045500 W

F2 - Processing parameters
SI 32768
SF 75.4803210 MHz
WDW EM
SSB 0
GB 1.0 Hz
PC 1.40

Figure 5.65. ^{13}C NMR signals of D2.d.

Data File: C:\LabSolutions\Data\Analiz\derya\HIC-2E UST_583.lod

Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	8	33	O	2	0	3	S	2	0	2	Ru	2	0	0	H
C	4	4	32	F	1	0	0	Cl	1	1	1	Pd	2	0	0	Na
N	3	0	6	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5

HC Ratio: unlimited

Max Isotopes: 3

MSn Iso RI (%): 10.00

DBE Range: 0.0 - 30.0

Apply N Rule: no

Isotope RI (%): 1.00

MSn Logic Mode: AND

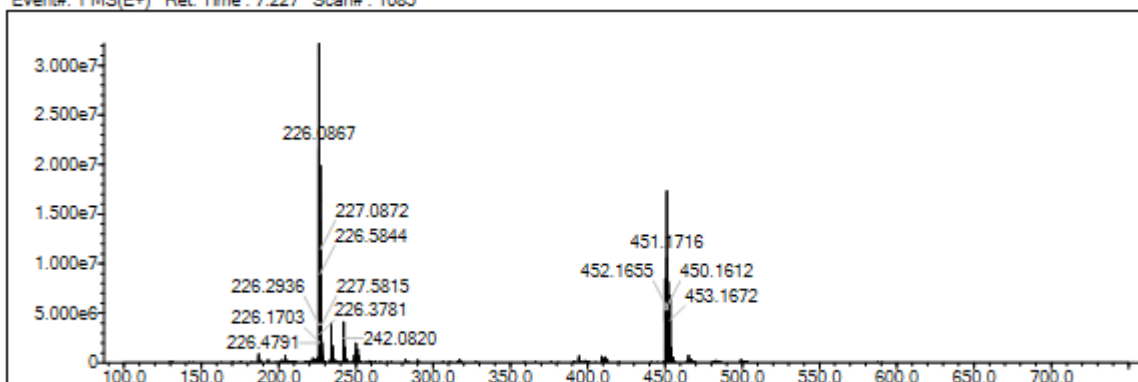
Electron Ions: both

Use MSn Info: yes

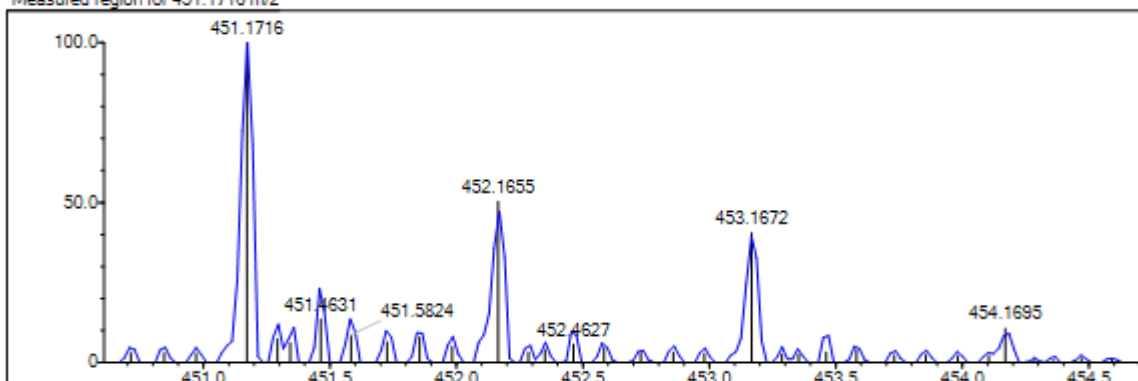
Isotope Res: 9000

Max Results: 50

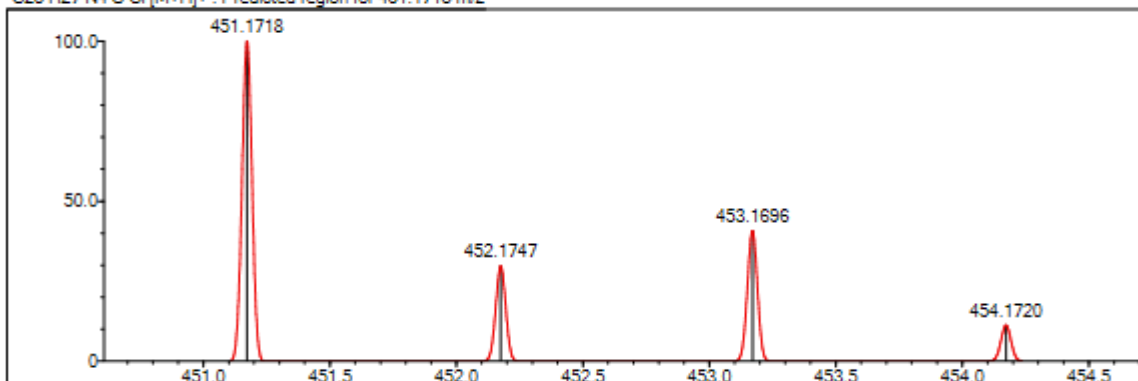
Event#: 1 MS(E+) Ret. Time : 7.227 Scan#: 1085



Measured region for 451.1716 m/z



C25 H27 N4 S Cl [M+H]⁺ : Predicted region for 451.1718 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	49.89	C25 H27 N4 S Cl	[M+H] ⁺	451.1716	451.1718	-0.2	-0.44	49.89	14.0

Figure 5.66. HRMS spectrogram of D2.d.

5.1.2.5. 3-Allyl-4-(4-methoxyphenyl)-2-((1-(4-(piperidin-1-yl)phenyl) ethylidene) hydrazineylidene)-2,3-dihydrothiazole

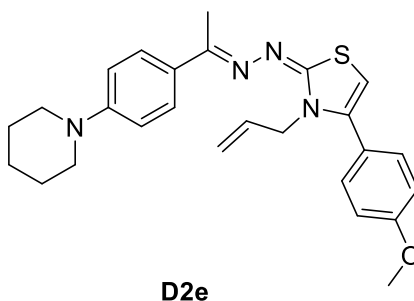


Figure 5.67. 3-Allyl-4-(4-methoxyphenyl)-2-((1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-2,3-dihydrothiazole..

Yield: 65%ç

Melting point: 145.2°C.

IR Maximum Velocity (cm^{-1}): 3080 (C=C-H stretching band), 2918(C-H stretching band), 1587 (C=N stretching band), 1504 (C=C band), 1232 (C-N stretching band), 821 (4-substituted benzene out-of-plane deformation band).

^1H -NMR (300 MHz, $\text{DMSO}-d_6$): 1.60(6H, s, piperidine-H), 2.29 (3H, s, Methyl-H), 3.22(4H, br.s, piperidine-H), 3.80 (3H, s, Methyl-H), 4.38(2H, s, Allyl-H), 4.93-4.97(1H, d, $J=17.20\text{Hz}$, Allyl-H), 5.10-5.13(1H, d, $J=10.26\text{ Hz}$, Allyl-H), 5.80-5.88 (1H, m, Allyl-H), 6.22(1H, s, dihydrothiazole-H), 6.92-6.94(2H, d, $J=8.41\text{ Hz}$, Ar-H), 7.02-7.04(2H, d, $J=8.10\text{ Hz}$, Ar-H), 7.37-7.39 (2H, d, $J=8.15\text{Hz}$, Ar-H), 7.67-7.69 (2H, d, $J=8.30\text{ Hz}$, Ar-H).

^{13}C -NMR (75 MHz, $\text{DMSO}-d_6$): $\delta = 14.45, 24.43, 25.50, 47.96, 49.32, 55.72, 99.30, 114.54, 115.08, 116.95, 123.48, 127.26, 130.59, 133.30, 140.39, 152.11, 154.89, 160.27, 167.10$.

HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{26}\text{H}_{30}\text{N}_4\text{O S}$ $[\text{M}+\text{H}]^+$: 447.2213; found: 447.2213.

Item	Value
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Acquired by	System Administrator
Filename	C:\Users\dopnalab\Desktop\MASAÜSTÜsazan\hic\hic-2e1.ispd
Spectrum name	hic-2e1
Sample name	hic-2e
Sample ID	
Option	
Comment	
No. of Scans	30
Resolution	4 [cm-1]
Apodization	Happ-Genzel

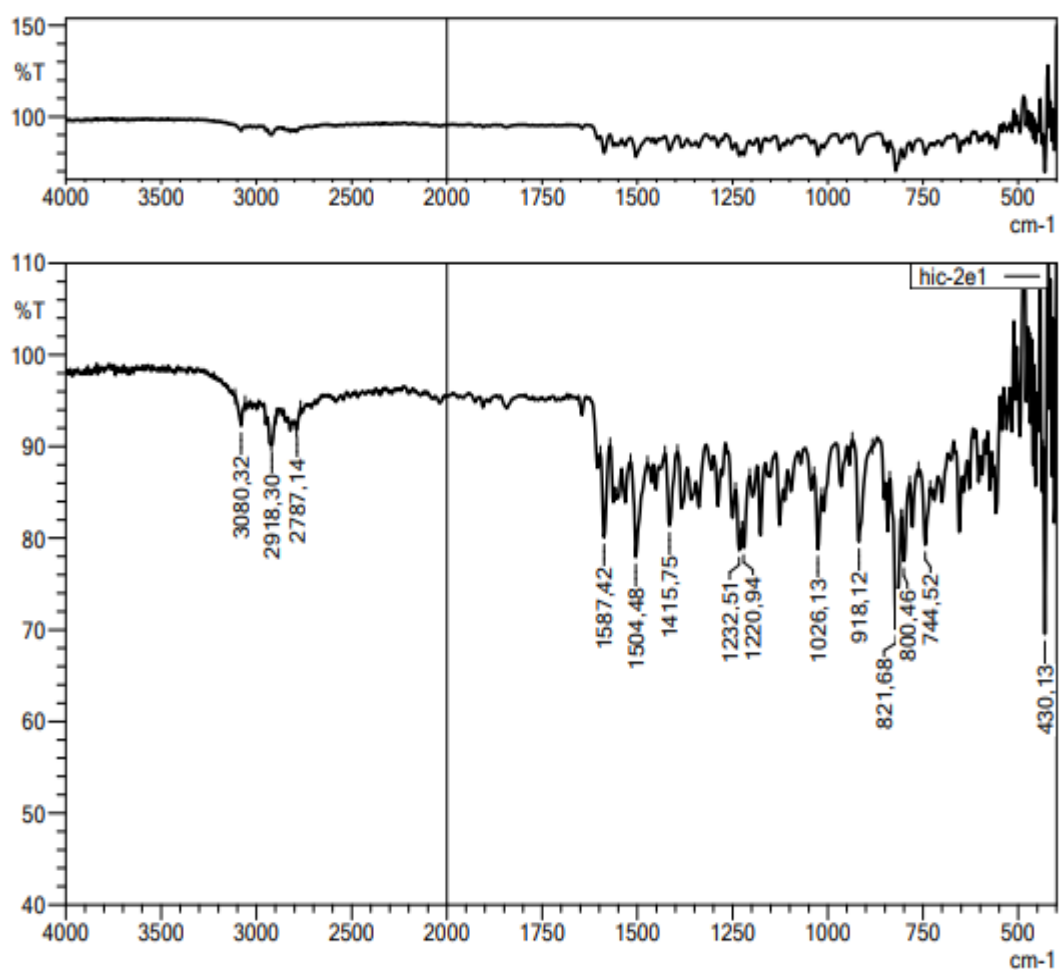
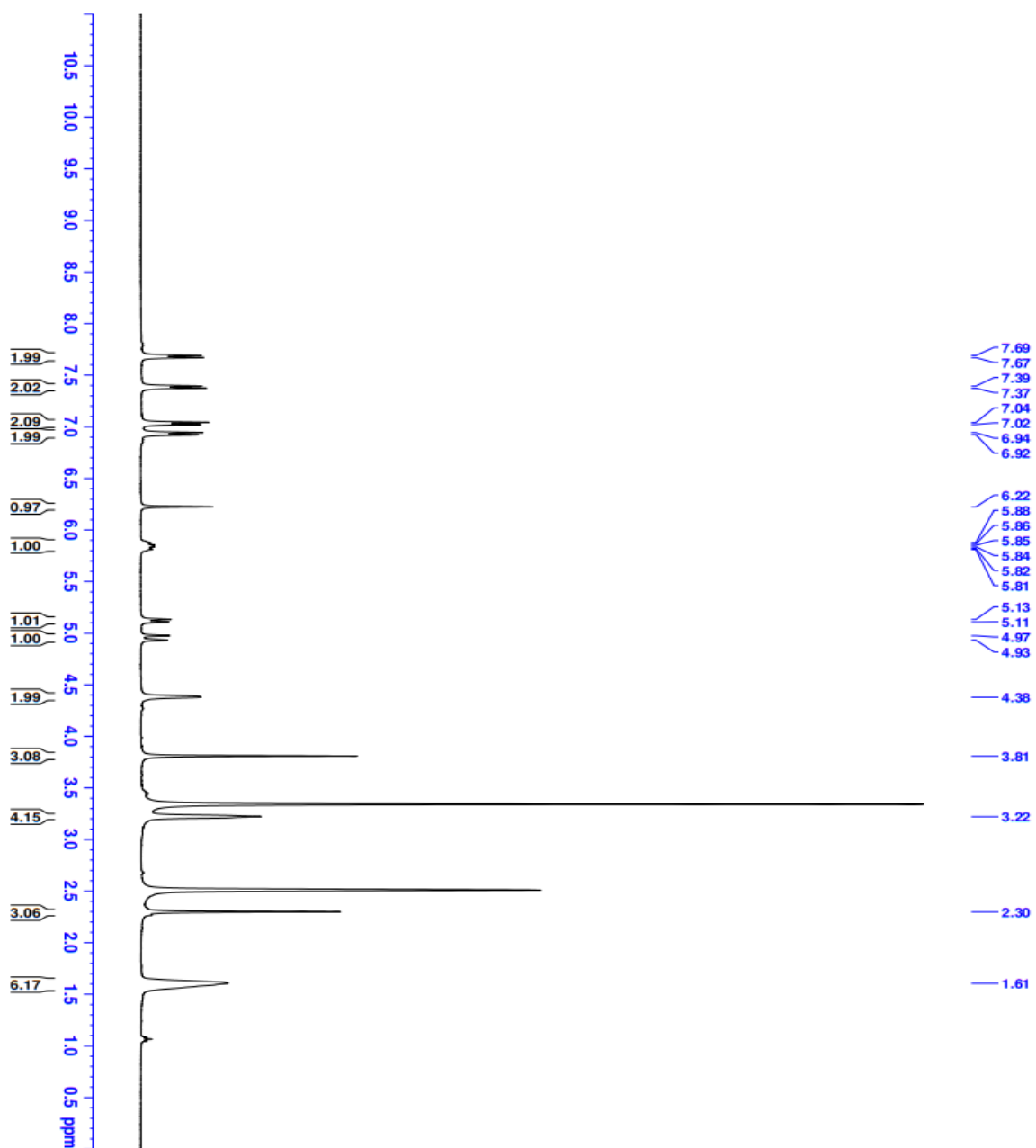


Figure 5.68. IR fingerprint of D2.e.

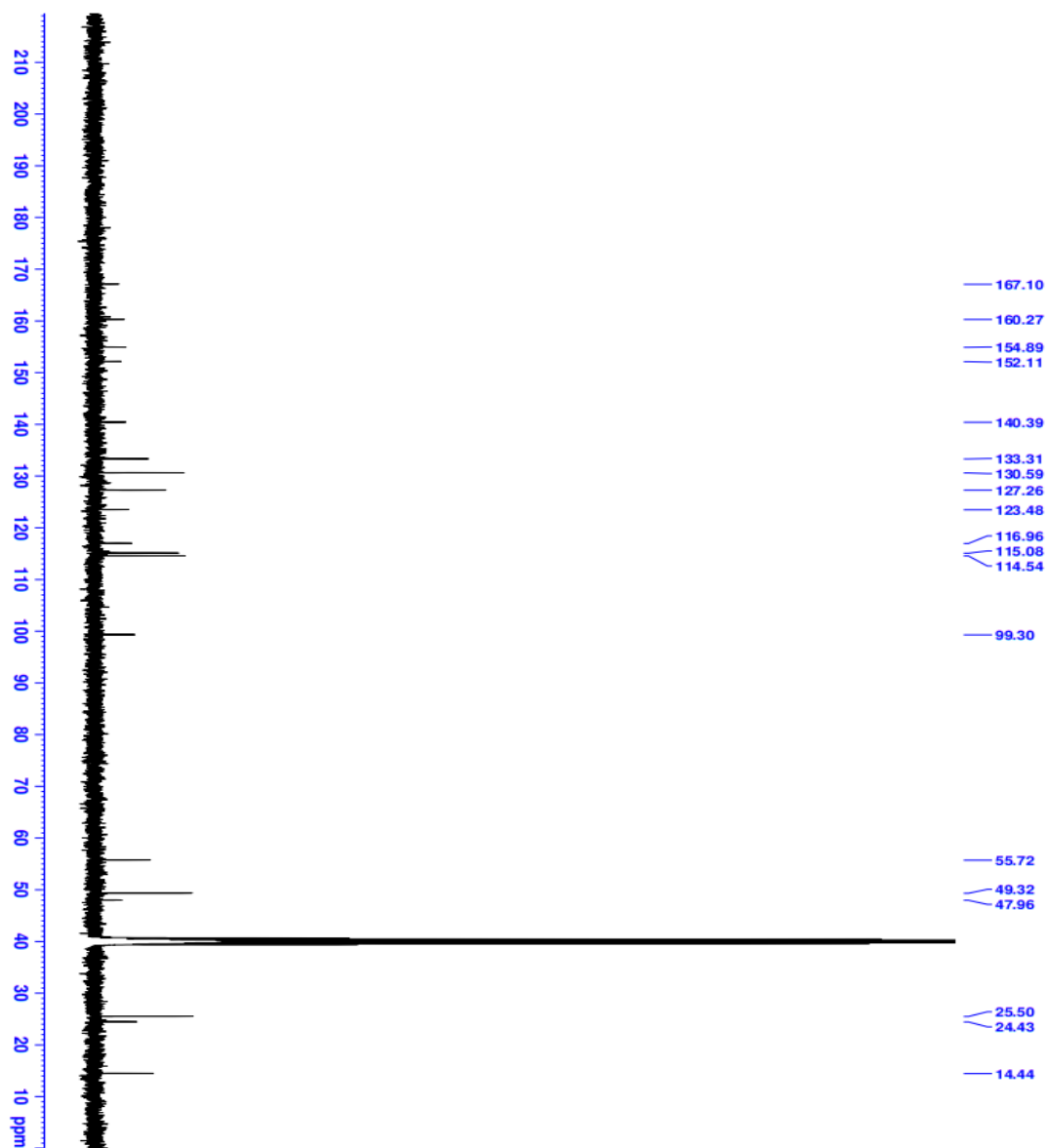


Current Data Parameters
NAME h1c2e
EXPNO 10
PROCNO 1

F2 - Acquisition Parameters
Date_ 20220417
Time 11.10 h
INSTRUM spect
PROBHD zgpg30
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 62.97
DM 62.400 usec
DE 6.50 usec
TE 298.2 K
D1 1.0000000 sec
TD0 1
SFO1 400.1324708 MHz
NUC1 1H
P1 8.00 usec
PL1 10.9490036 W

F2 - Processing parameters
SI 65536
SF 400.1300000 MHz
WDW EM
SSB 0
GB 0.30 Hz
PC 1.00

Figure 5.69. ¹H NMR signals of D2.c.



BRUKER

Current Data Parameters

NAME	h2c
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20240417
Time	14.30 h
INSTRUM	spec
PROBHD	286401_0094
PULPROG	zgpg30
TD	65536
SOLVENT	DMSO
NS	1024
DS	4
SWH	24038.461 Hz
FIDRES	0.73356 Hz
AQ	1.3631488 sec
RG	42.75
DM	20.800 usec
DE	15.500 usec
TE	297.2 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1
SFO1	100.628298 MHz
NUC1	13C
P1	15.00 usec
PLM1	90.2959707 W
SCANS	400
NUC2	400.116003 MHz
CEPRG12	waltz16
PCPD2	90.00 usec
PLM2	10.94900036 W
PLM12	0.08651100 W
PLM13	0.04351400 W

F2 - Processing parameters

SI	32768
SF	100.617658 MHz
RGW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

Figure 5.70. ^{13}C NMR signals of D2.e.

Data File: C:\LabSolutions\Data\Analz\data\HIC-2F UST_585 lod

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H	1	8	33	O	2	0	3	S	2	0	2	Ru	2	0	0	H
C	4	4	32	F	1	0	0	Cl	1	0	0	Pd	2	0	0	Na
N	3	0	6	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5

HC Ratio: unlimited

Max Isotopes: 3

MSn Iso RI (%): 10.00

DBE Range: 0.0 - 30.0

Apply N Rule: no

Isotope RI (%): 1.00

MSn Logic Mode: AND

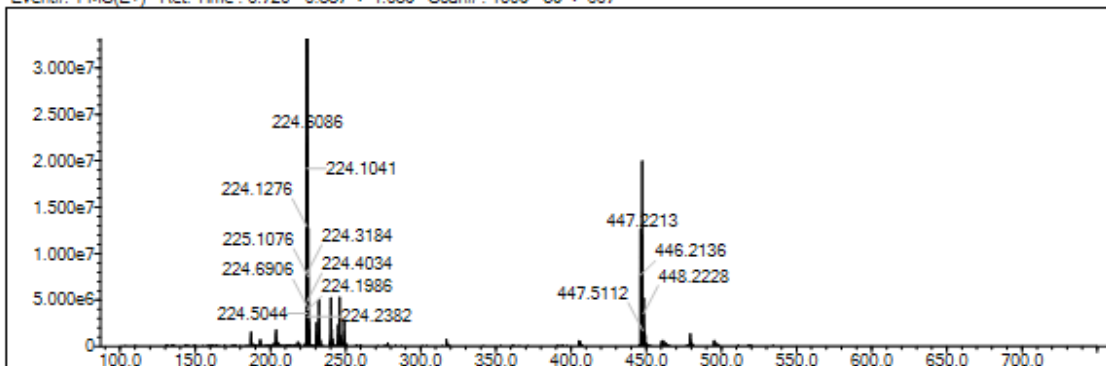
Electron Ions: both

Use MSn Info: yes

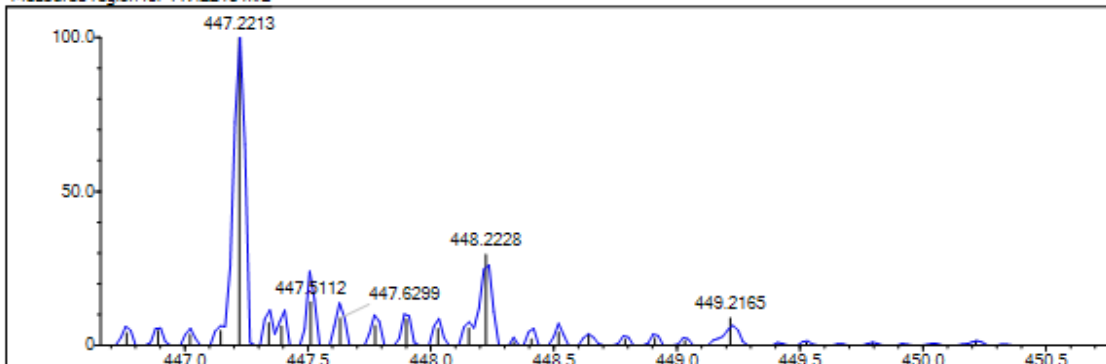
Isotope Res: 9000

Max Results: 50

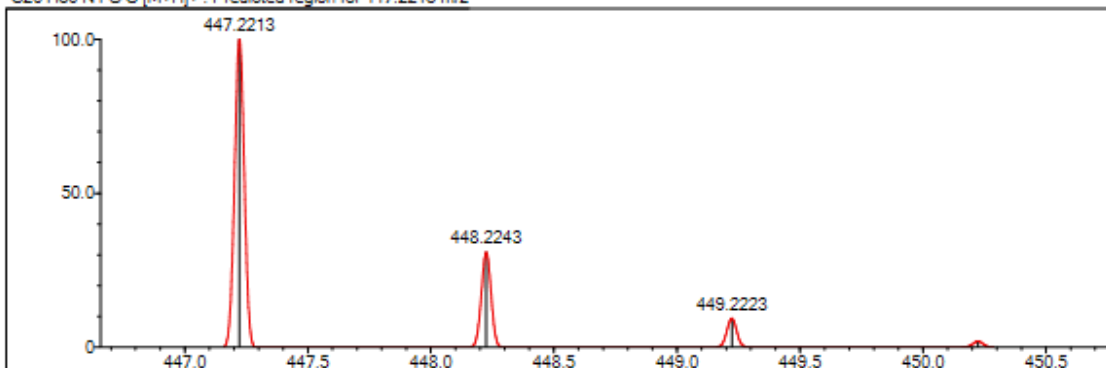
Event#: 1 MS(E+) Ret. Time : 6.720 - 0.387 -> 4.039 Scan#: 1009 - 59 -> 607



Measured region for 447.2213 m/z



C26 H30 N4 O S [M+H]⁺ : Predicted region for 447.2213 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	67.97	C26 H30 N4 O S	[M+H] ⁺	447.2213	447.2213	-0.0	0.00	67.97	14.0

Figure 5.71. HRMS spectrogram of D2.e.

5.1.2.6. 4-(3-Allyl-2-((1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-2,3-dihydrothiazol-4-yl)benzonitrile

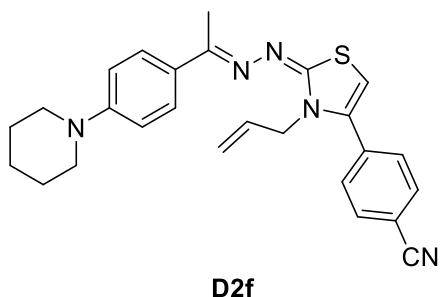


Figure 5.72. 4-(3-Allyl-2-((1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-2,3-dihydrothiazol-4-yl)benzonitrile.

Yield: 90%.

Melting point: 195.1°C.

IR Maximum Velocity (cm^{-1}): 3379 (C=C-H tension band), 2931 (C-H stretching band), 2243 (Nitrile-CN stretching band), 1595 (C=N stretching band), 1571 (C=C band), 1236 (C-N stretching band), 817 (4-substituted benzene out-of-plane deformation band).

^1H -NMR (300 MHz, $\text{DMSO}-d_6$): 1.58-1.59(6H, br.s, piperidine-H), 2.29 (3H, s, Methyl-H), 3.20-3.22(4H, br.s, piperidine-H), 3.64-3.76(2H, m, Allyl-H), 4.96-5.01(2H, m, Allyl-H), 5.75-5.85 (1H, m, Allyl-H), 6.91-6.93(2H, d, $J=8.59$ Hz, Ar-H), 7.18(1H, s, dihydrothiazole-H), 7.67-7.69(2H, d, $J=6.33$ Hz, Ar-H), 7.72-7.75 (2H, d, $J=6.03$ Hz, Ar-H), 7.87-7.89 (2H, d, $J=8.07$ Hz, Ar-H).

^{13}C -NMR (75 MHz, $\text{DMSO}-d_6$): δ =14.53, 24.42, 25.51, 41.98, 47.35, 49.24, 94.34, 111.56, 114.97, 116.88, 119.07, 127.40, 127.92, 128.38, 134.71, 148.20, 152.28, 156.87, 165.37.

HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{26} \text{H}_{27} \text{N}_5 \text{S}$ $[\text{M}+\text{H}]^+$: 442.2060; found: 442.2051.

Item	Value
Acquired Date&Time	3.05.2024 10:57:59
Acquired by	System Administrator
Filename	C:\Users\dopnlab\Desktop\MASAU\STU\sazan\hic\hic-2f1.ispd
Spectrum name	hic-2f1
Sample name	hic-2f
Sample ID	
Option	
Comment	
No. of Scans	30
Resolution	4 [cm-1]
Apodization	Happ-Genzel

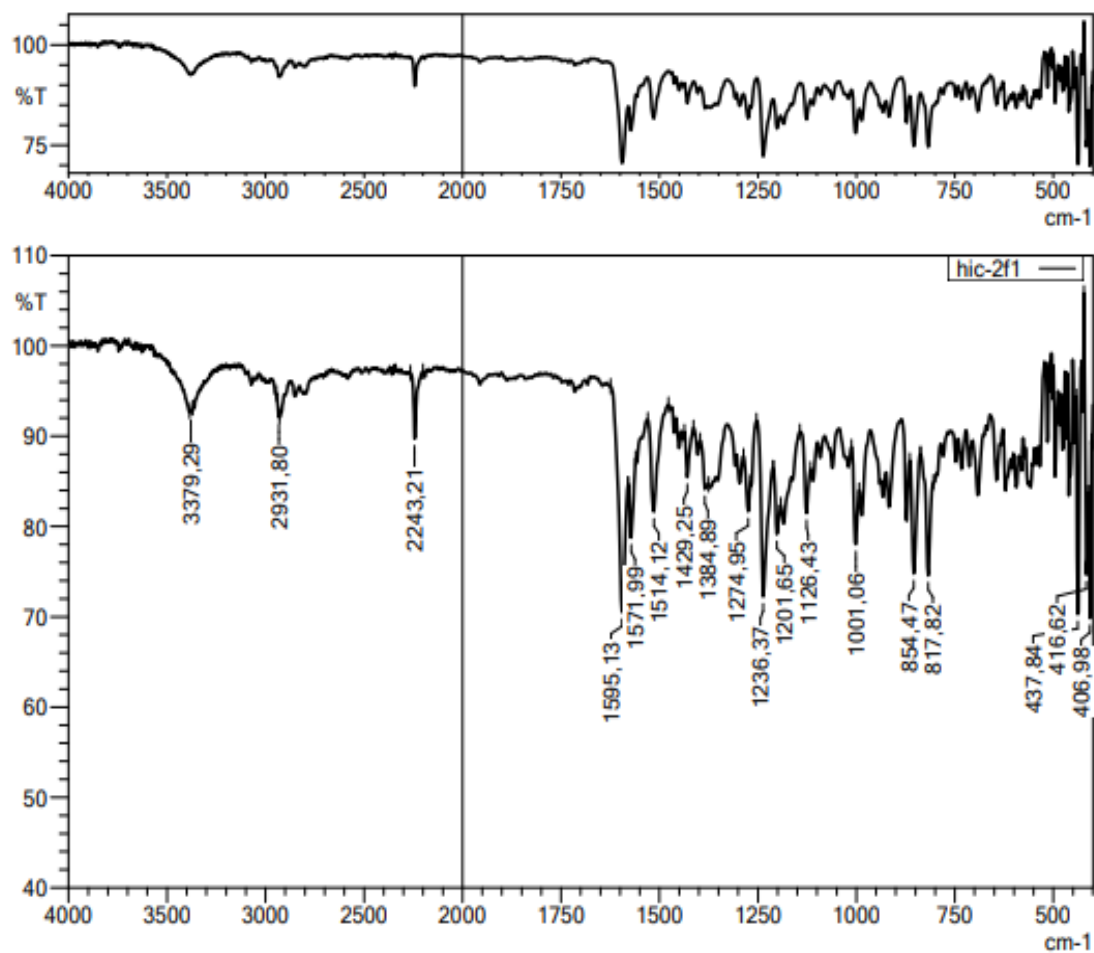


Figure 5.73. IR fingerprint of D2f.

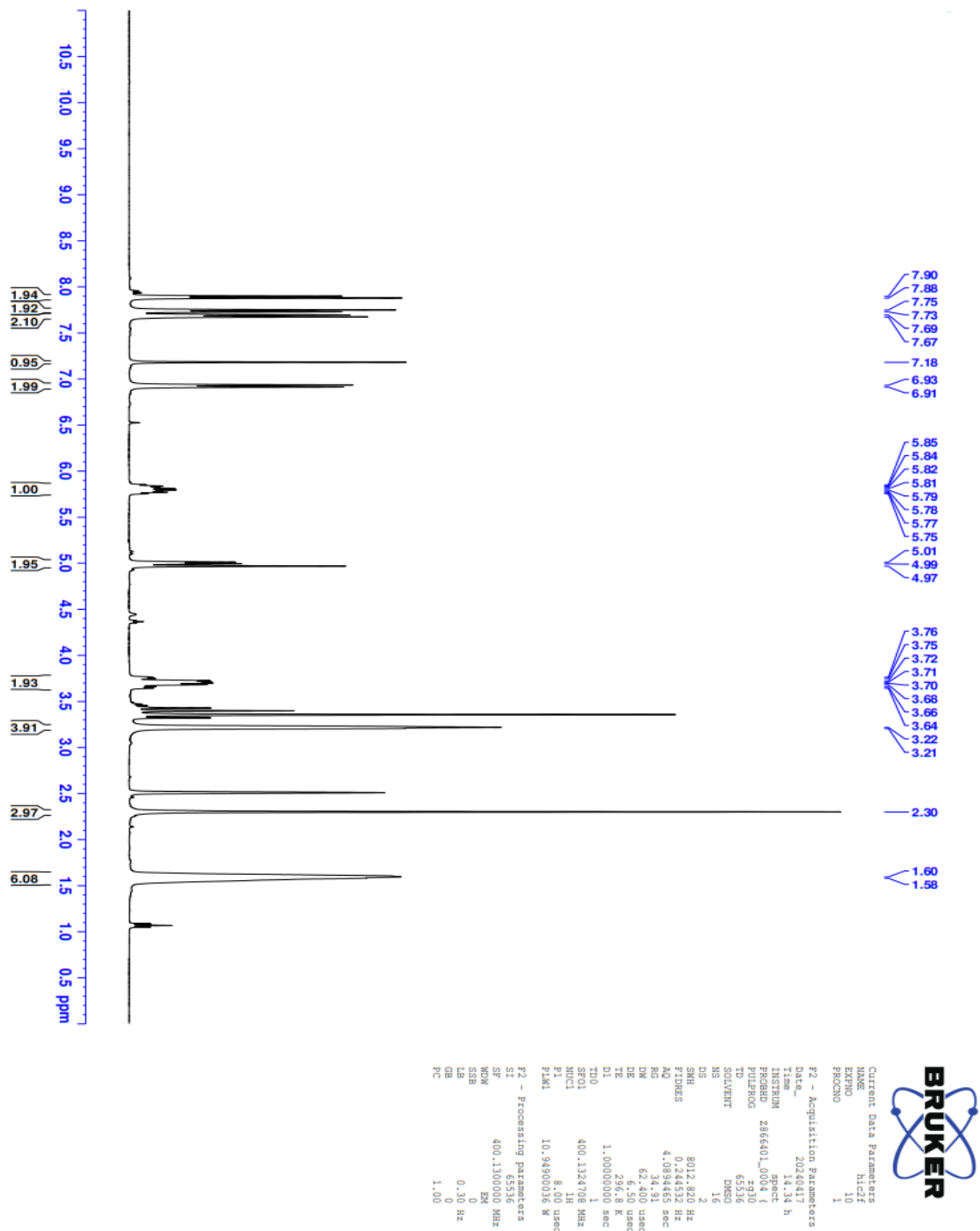


Figure 5.74. ¹H NMR signals of D2f.

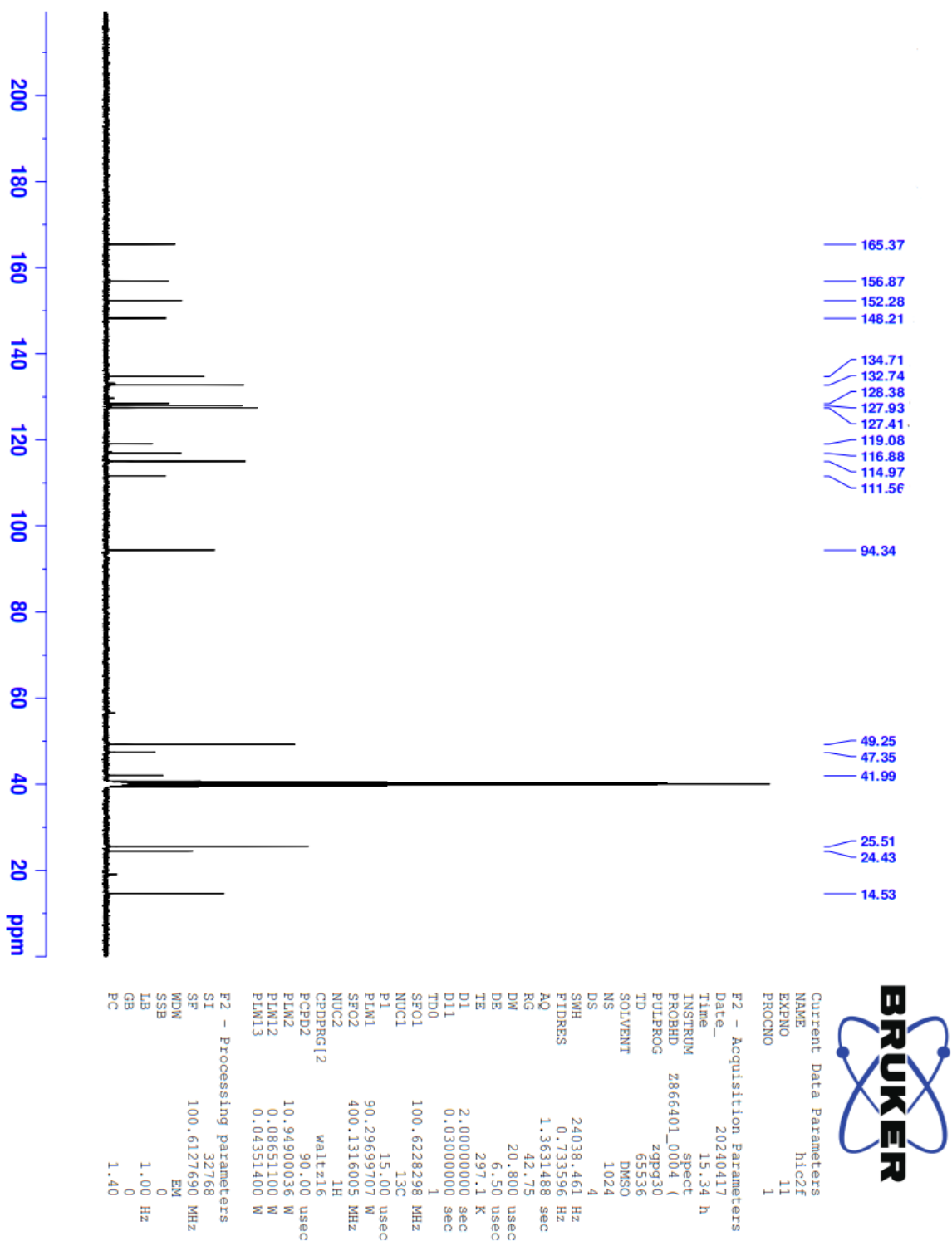


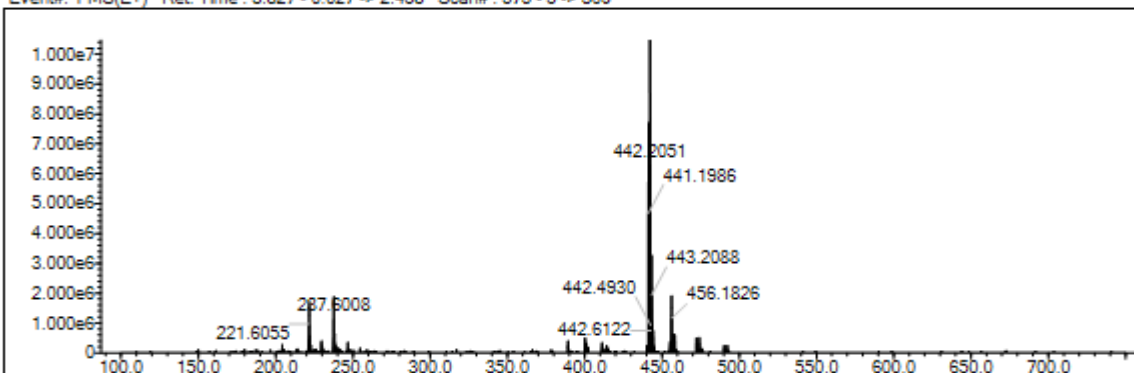
Figure 5.75. ^{13}C NMR signals of D2f.

Data File: C:\LabSolutions\Data\Analiz\denya\HIC-2\ ALT_587.lcd

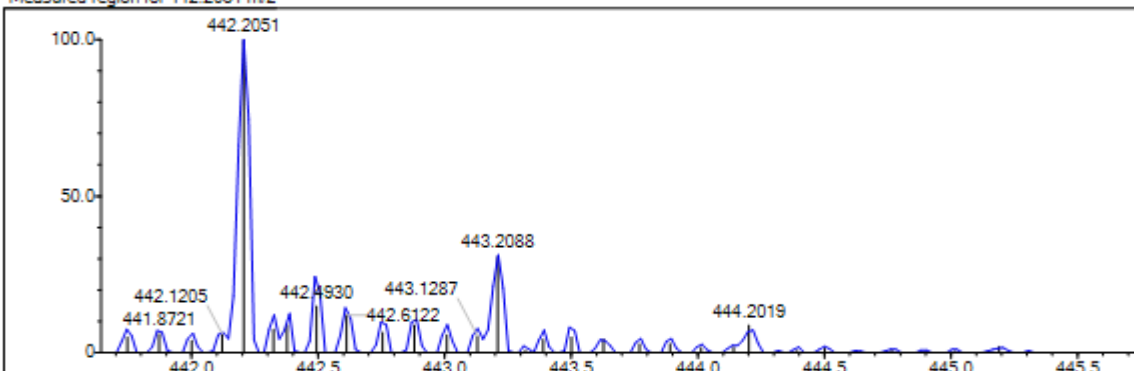
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H	1	8	33	O	2	0	3	S	2	0	2	Ru	2	0	0	H
C	4	4	32	F	1	0	0	Cl	1	0	0	Pd	2	0	0	Na
N	3	0	6	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5
 HC Ratio: unlimited
 Max Isotopes: 3
 MSn Iso RI (%): 10.00
 DBE Range: 0.0 - 30.0
 Apply N Rule: no
 Isotope RI (%): 1.00
 MSn Logic Mode: AND
 Electron Ions: both
 Use MSn Info: yes
 Isotope Res: 9000
 Max Results: 50

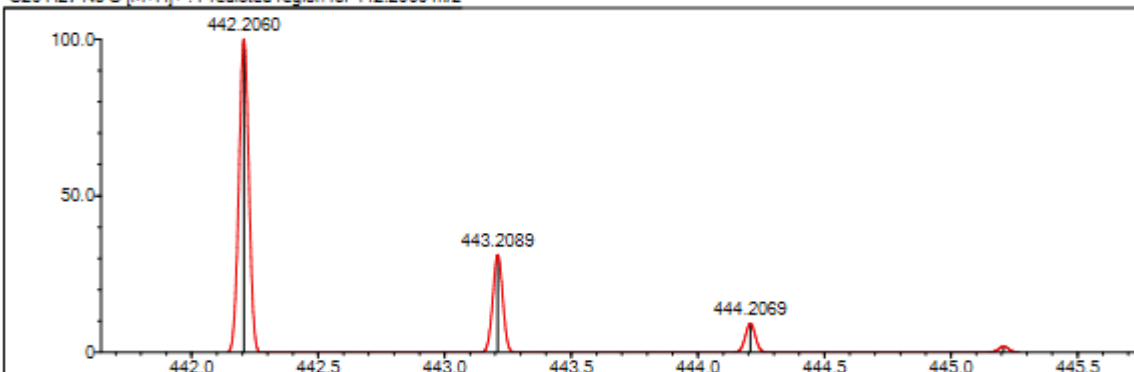
Event#: 1 MS(E+) Ret. Time : 3.827 - 0.027 -> 2.458 Scan#: 575 - 5 -> 369



Measured region for 442.2051 m/z



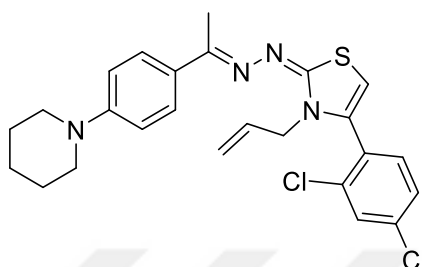
C26 H27 N5 S [M+H]⁺ : Predicted region for 442.2060 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
3	73.73	C26 H27 N5 S	[M+H] ⁺	442.2051	442.2060	-0.9	-2.04	75.70	16.0

Figure 5.76. HRMS spectrogram of D2f.

5.1.2.7. 3-Allyl-4-(2,4-dichlorophenyl)-2-((1-(4-(piperidin-1-yl)phenyl) ethylidene) hydrazineylidene)-2,3-dihydrothiazole



D2g

Figure 5.77. 3-Allyl-4-(2,4-dichlorophenyl)-2-((1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-2,3-dihydrothiazole.

Yield: 86%.

Melting point: 115.5°C.

IR Maximum Velocity (cm^{-1}): 3078 (C=C-H stretching band), 2935 (C-H stretching band), 1589 (C=N stretching band), 1573 (C=C band), 1236 (C-N stretching band), 823 (2,4-disubstituted benzene out-of-plane deformation band).

^1H -NMR (300 MHz, $\text{DMSO}-d_6$): 1.57(6H, s, piperidine-H), 2.28 (3H, s, Methyl-H), 3.20-3.24(4H, br.s, piperidine-H), 3.67-3.71 (2H, br.s, Allyl-H), 4.78-4.89(2H, m, , Allyl-H), 5.71-5.82(1H, m, Allyl-H), 6.90-6.93(2H, d, $J=8.8$ Hz, Ar-H), 7.32(1H, s, dihydrothiazole-H), 7.51-7.55(1H, d, $J=8.5$ Hz, Ar-H), 7.64-7.68(3H, m, Ar-H), 7.91-7.94(1H, d, $J=8.6$ Hz, Ar-H).

^{13}C -NMR (75 MHz, $\text{DMSO}-d_6$): δ =14.46, 24.42, 25.52, 31.15, 47.03, 49.28, 92.92, 115.00, 116.78, 127.31, 127.61, 128.52, 130.95, 131.49, 133.48, 134.50, 134.59, 138.13, 152.20, 156.14, 165.60.

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Sample name	hic-2h
Sample ID	
Option	
Comment	
No. of Scans	30
Resolution	4 [cm-1]
Apodization	Happ-Genzel

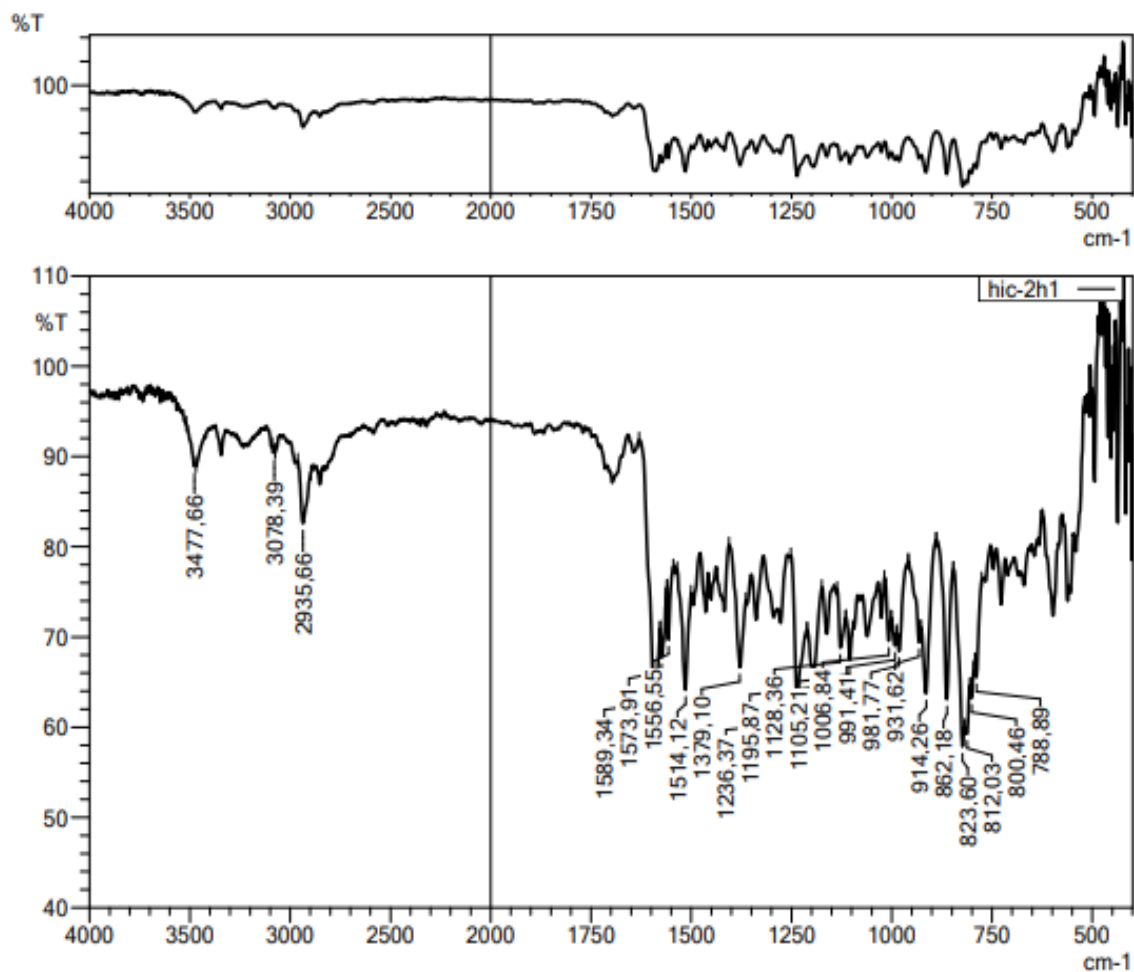


Figure 5.78. IR fingerprint of D2.g.

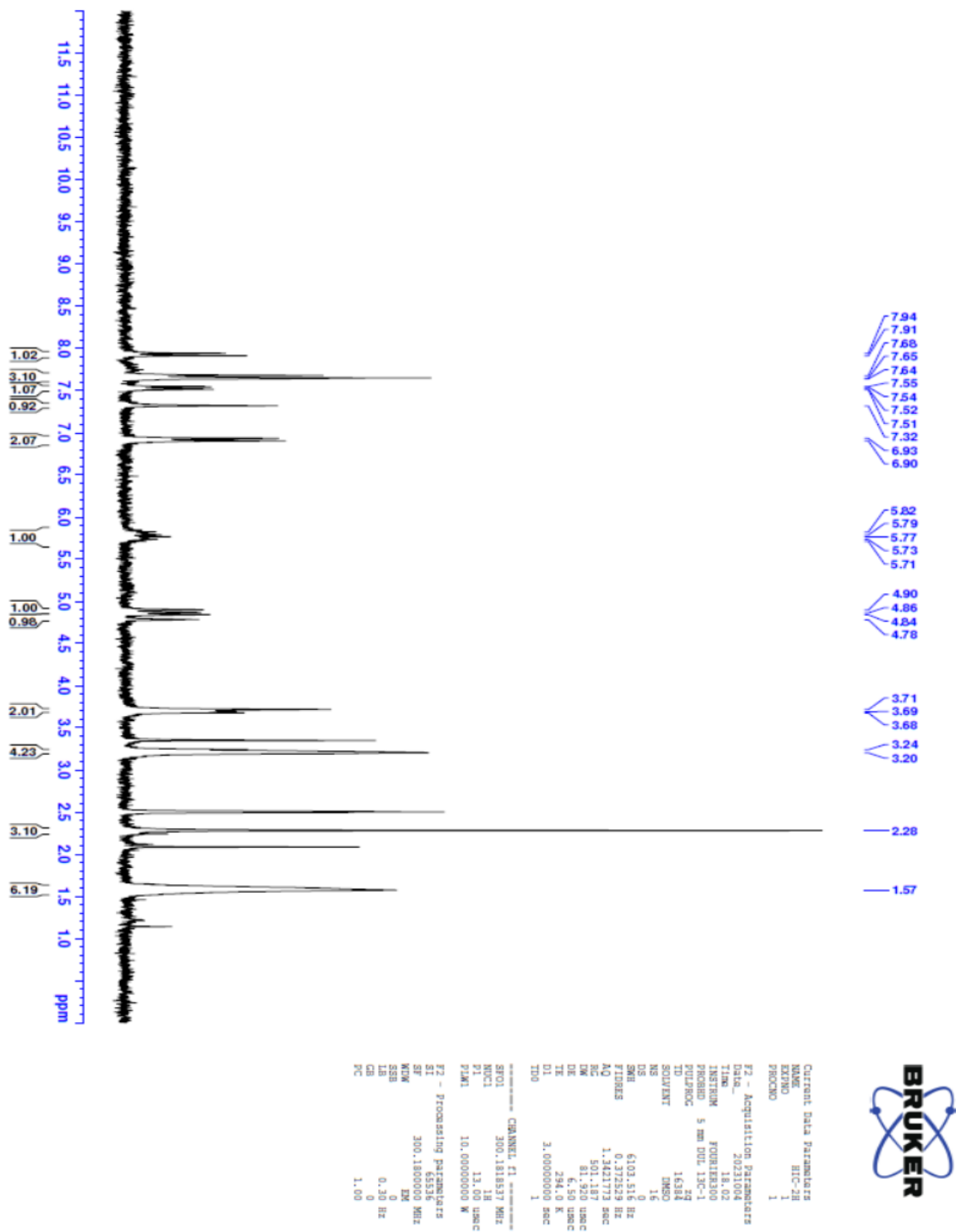


Figure 5.79. ¹H NMR signals of D2.g.

5.1.2.8. 3-Allyl-4-(3,4-dichlorophenyl)-2-(((E)-1-(4-(piperidin-1-yl) phenyl) ethylidene)hydrazineylidene)-2,3-dihydrothiazole

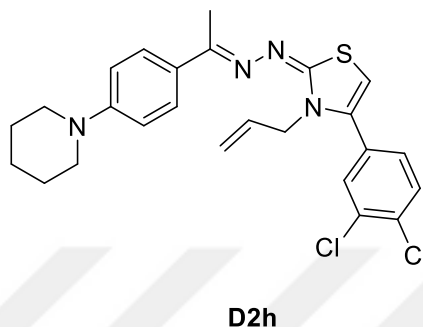


Figure 5.81. 3-Allyl-4-(3,4-dichlorophenyl)-2-(((E)-1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-2,3-dihydrothiazole.

Yield: 65%.

Melting point: 115.5°C.

IR Maximum Velocity (cm^{-1}): 3082 (C=C-H stretching band), 2924 (C-H stretching band), 1593 (C=N stretching band), 1504 (C=C band), 1232 (C-N stretching band), 821 (3,4-disubstituted phenyl out-of-plane deformation band).

^1H -NMR (300 MHz, $\text{DMSO}-d_6$): 1.60(6H, s, piperidine-H), 2.30 (3H, s, Methyl-H), 3.22(4H, br.s, piperidine-H), 4.42-4.43 (2H, br.s, Allyl-H), 4.94-4.99(1H, d, $J=17.20$ Hz, Allyl-H), 5.12-5.15(1H, d, $J=10.34$ Hz, Allyl-H), 5.81-5.90 (1H, m, Allyl-H), 6.48(1H, s, dihydrothiazole-H), 6.91-6.94(2H, d, $J=8.32$ Hz, Ar-H), 7.45-7.48(1H, d, $J=8.30$ Hz, Ar-H), 7.67-7.69 (2H, d, $J=8.25$ Hz, Ar-H), 7.73-7.76 (2H, d, $J=7.50$ Hz, Ar-H).

^{13}C -NMR (75 MHz, $\text{DMSO}-d_6$): δ = 14.50, 24.43, 25.49, 48.20, 49.28, 102.18, 115.04, 117.13, 127.32, 128.43, 129.20, 130.85, 131.13, 131.76, 131.90, 132.33, 138.10, 152.18, 155.47, 166.94.

HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{25} \text{H}_{26} \text{N}_4 \text{S Cl}_2$ $[\text{M}+\text{H}]^+$: 485.1328; found: 485.1310.

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Sample name	hic-2i
Sample ID	
Option	
Comment	
No. of Scans	30
Resolution	4 [cm ⁻¹]
Apodization	Happ-Genzel

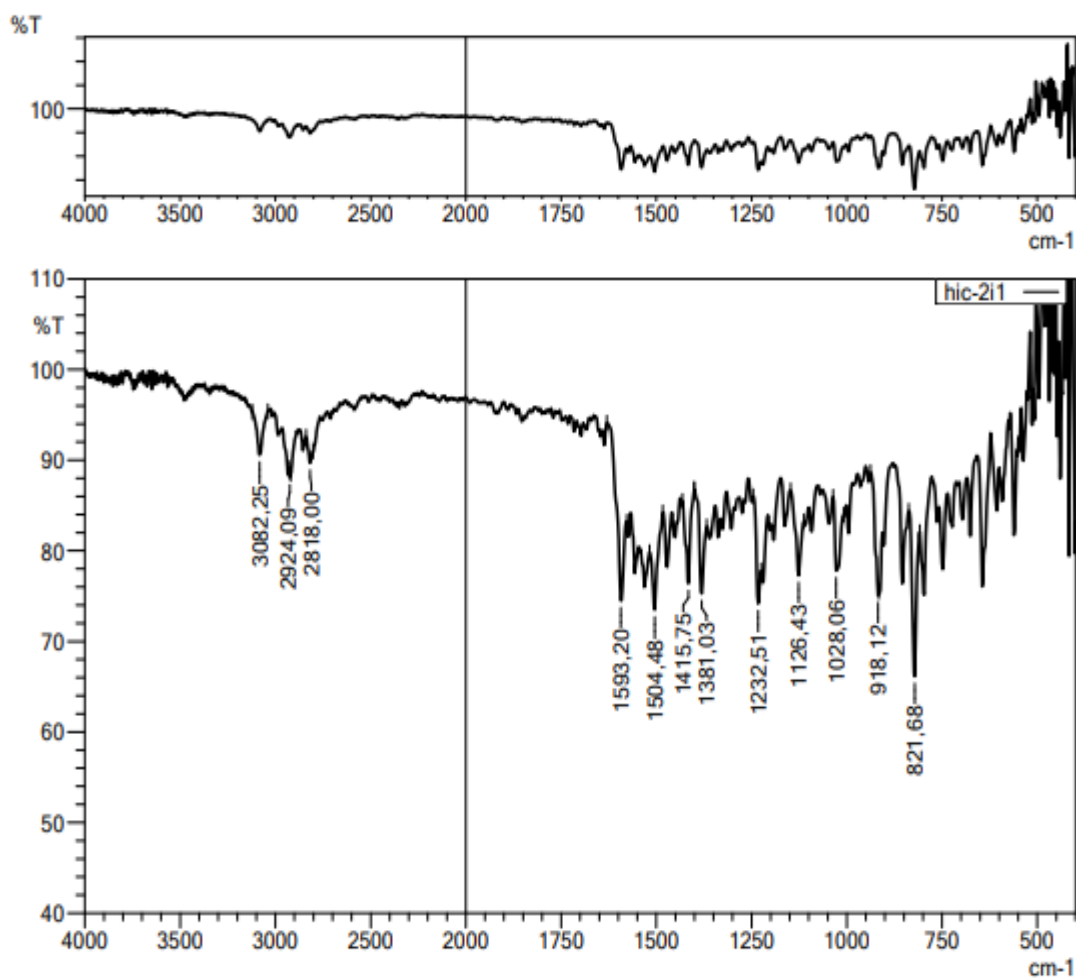


Figure 5.82. IR fingerprint of D2.h.

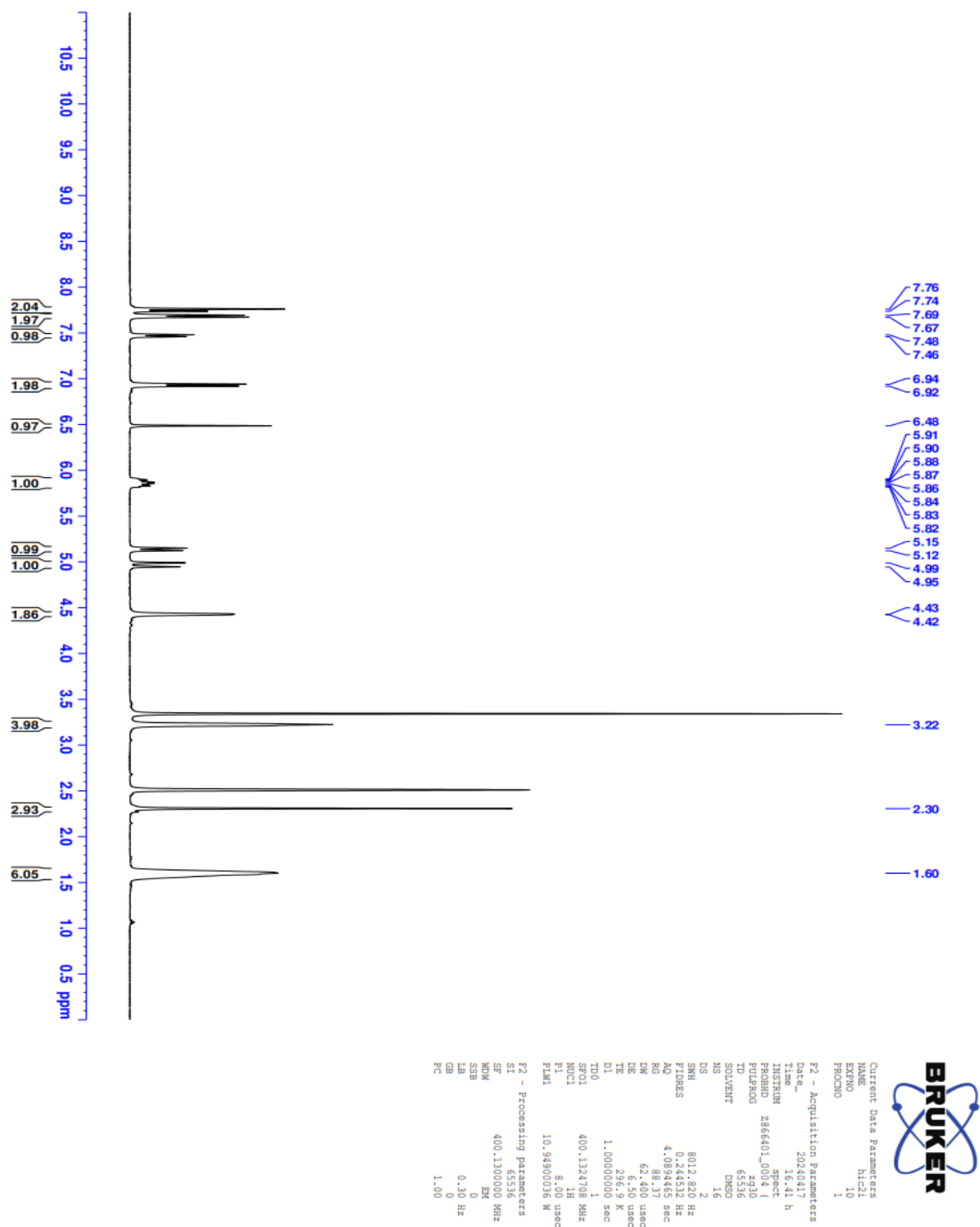


Figure 5.83. ¹H NMR signals of D2.h.

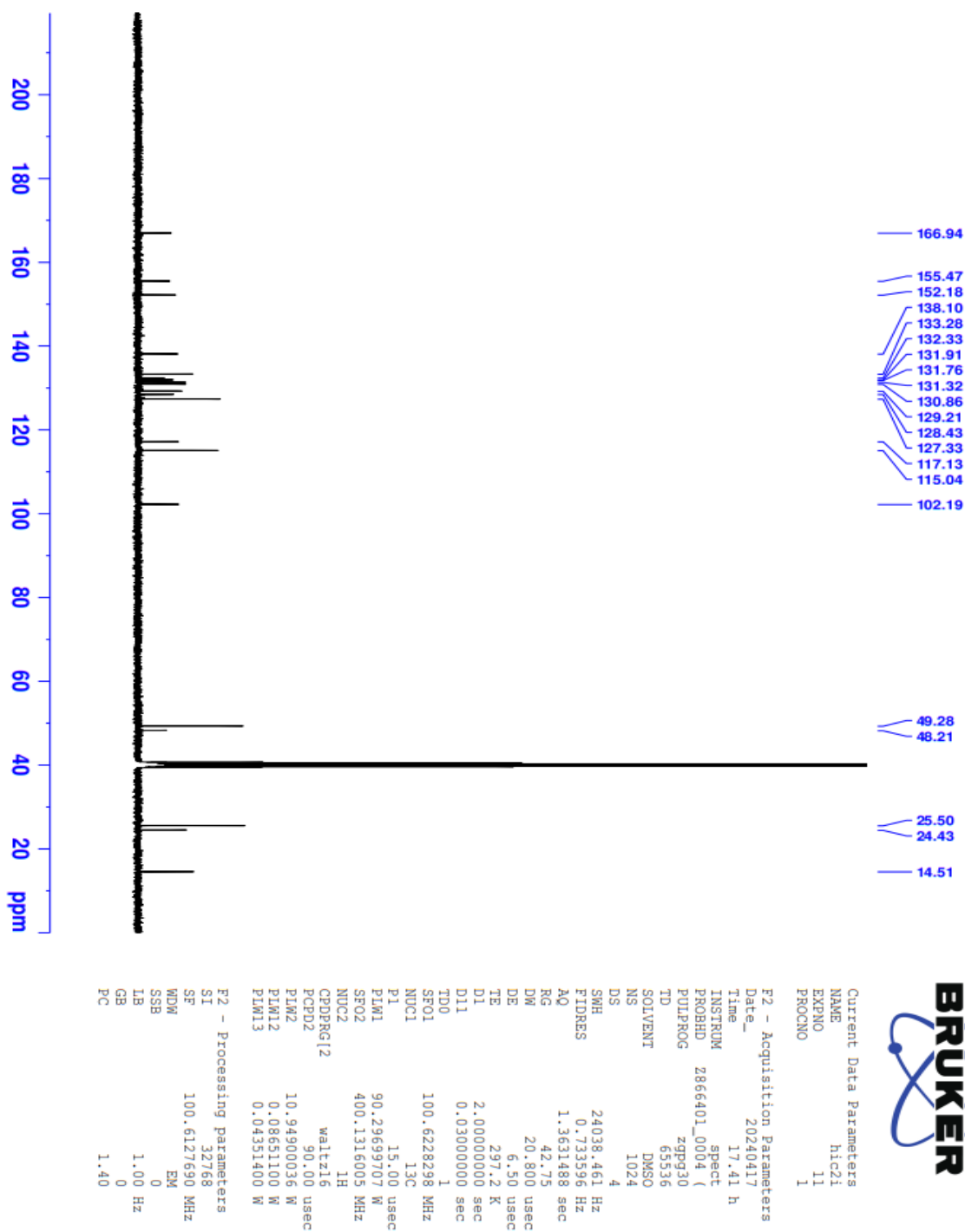


Figure 5.84. ^{13}C NMR signals of D2.h.

Data File: C:\LabSolutions\Data\Analiz\iderya\MEOH_589.lod

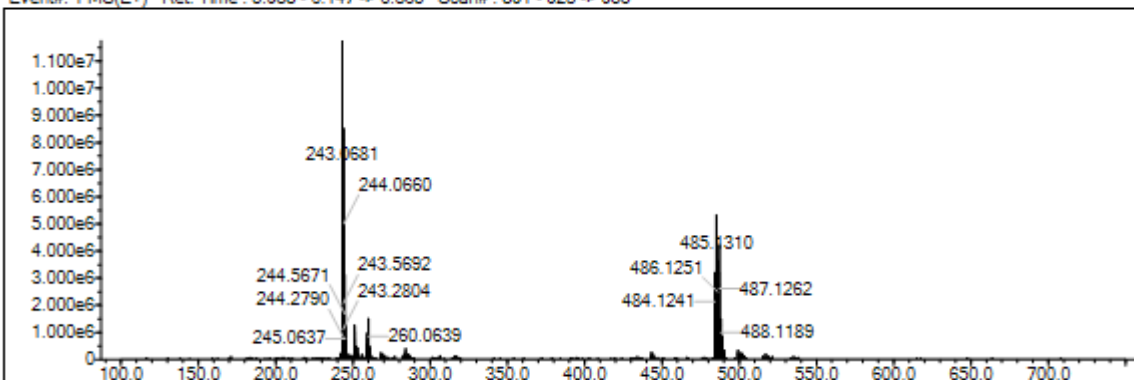
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	8	33	O	2	0	3	S	2	1	1	Ru	2	0	0	H
C	4	4	32	F	1	0	0	Cl	1	2	2	Pd	2	0	0	Na
N	3	0	4	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5
 HC Ratio: unlimited
 Max Isotopes: 3
 MSn Iso RI (%): 10.00

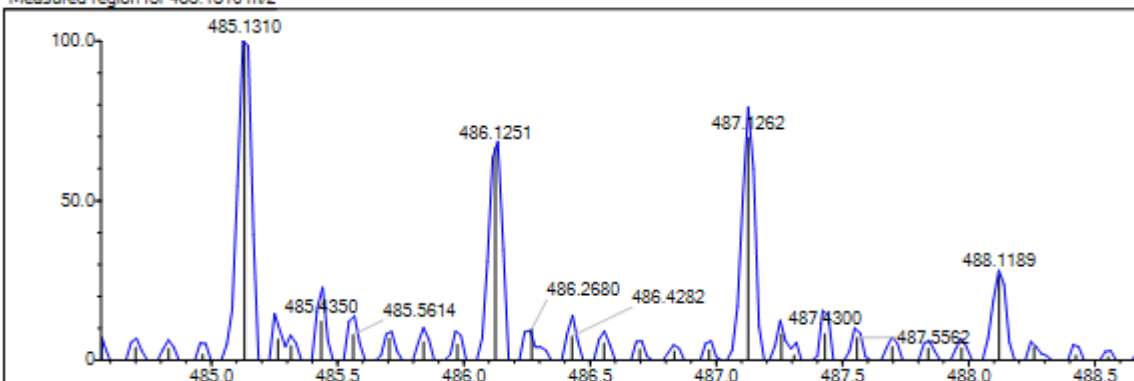
DBE Range: 0.0 - 30.0
 Apply N Rule: no
 Isotope RI (%): 1.00
 MSn Logic Mode: AND

Electron Ions: both
 Use MSn Info: yes
 Isotope Res: 9000
 Max Results: 50

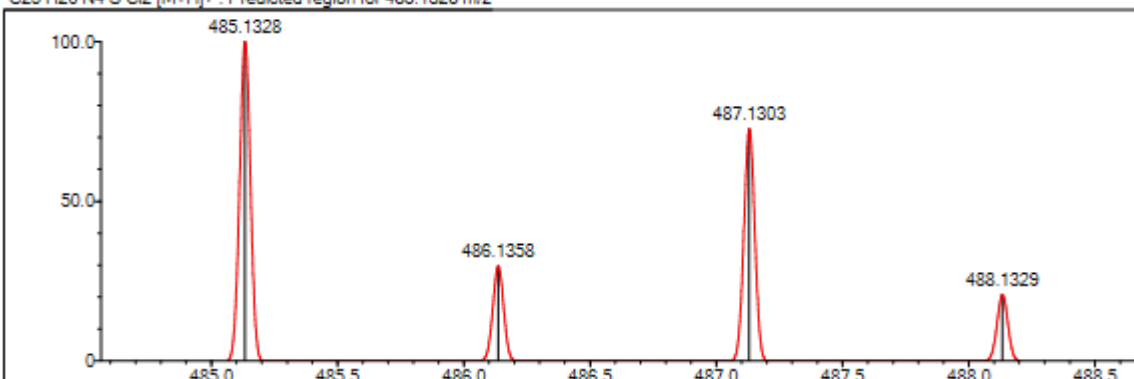
Event#: 1 MS(E+) Ret. Time : 5.933 - 6.147 -> 6.365 Scan#: 891 - 923 -> 955



Measured region for 485.1310 m/z



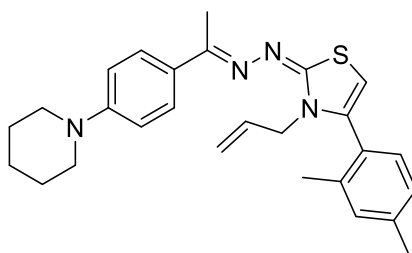
C25 H26 N4 S Cl2 [M+H]⁺ : Predicted region for 485.1328 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
4	25.69	C25 H26 N4 S Cl2	[M+H] ⁺	485.1310	485.1328	-1.8	-3.71	27.56	14.0

Figure 5.85. HRMS spectrogram of D2.h.

5.1.2.9. 3-Allyl-4-(2,4-dimethylphenyl)-2-(((E)-1-(4-(piperidin-1-yl)phenyl) ethylidene) hydrazineylidene)-2,3-dihydrothiazole



D2i

Figure 5.86. 3-Allyl-4-(2,4-dimethylphenyl)-2-((1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-2,3-dihydrothiazole.

Yield: 70%.

Melting point: 176.6°C.

IR Maximum Velocity (cm^{-1}): 3100 (C=C-H stretching band), 2935 (C-H stretching band), 1591 (C=N stretching band), 1552 (C=C band), 1236 (C-N stretching band), 823 (2,4-disubstituted phenyl out-of-plane deformation band).

^1H -NMR (300 MHz, $\text{DMSO}-d_6$): 1.57(6H, s, piperidine-H), 2.15 (3H, s, Methyl-H), 2.29 (3H, s, Methyl-H), 2.32 (3H, s, Methyl-H), 3.20(4H, br.s, piperidine-H), 4.13 (2H, s, Allyl-H), 4.80-4.86(1H, d, $J=17.2$ Hz, Allyl-H), 5.02-5.05(1H, d, $J=10.2$ Hz, Allyl-H), 5.65-5.78 (1H, m, Allyl-H), 6.14(1H, s, dihydrothiazole-H), 6.90-6.93(2H, d, $J=8.52$ Hz, Ar-H), 7.06-7.15(3H, m, Ar-H), 7.66-7.69 (2H, d, $J=8.47$ Hz, Ar-H).

^{13}C -NMR (75 MHz, $\text{DMSO}-d_6$): δ =14.42, 19.72, 21.28, 24.43, 25.50, 47.32, 49.31, 99.55, 115.09, 117.23, 126.88, 127.22, 127.82, 128.66, 130.89, 131.28, 132.73, 137.69, 138.80, 139.49, 152.08, 154.74, 166.57.

HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{27} \text{H}_{32} \text{N}_4 \text{S}$ $[\text{M}+\text{H}]^+$: 445.2420, found: 445.2427.

Item	Value
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Sample name	hic-2\
Sample ID	
Option	
Comment	
No. of Scans	30
Resolution	4 [cm-1]
Apodization	Happ-Genzel

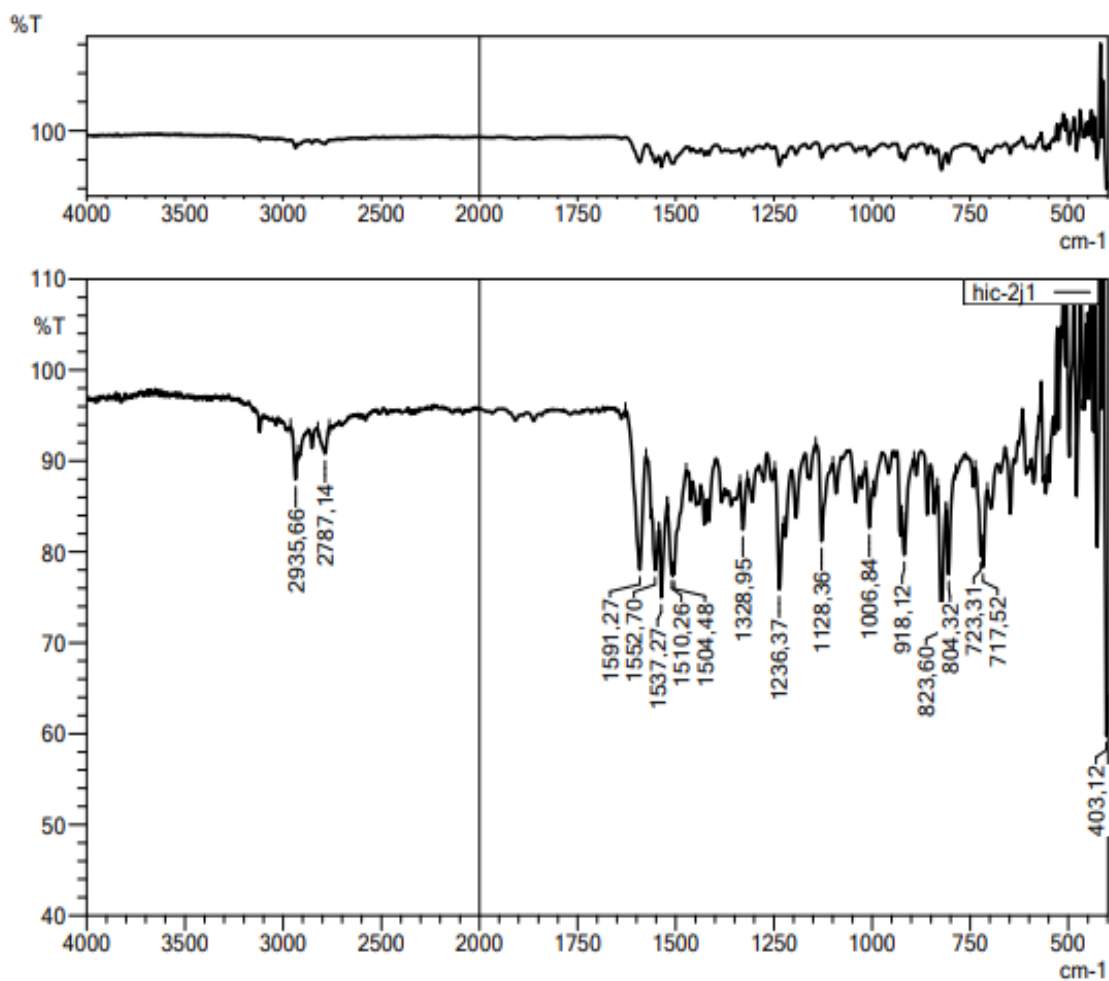


Figure 5.87. IR fingerprint of D2.i.

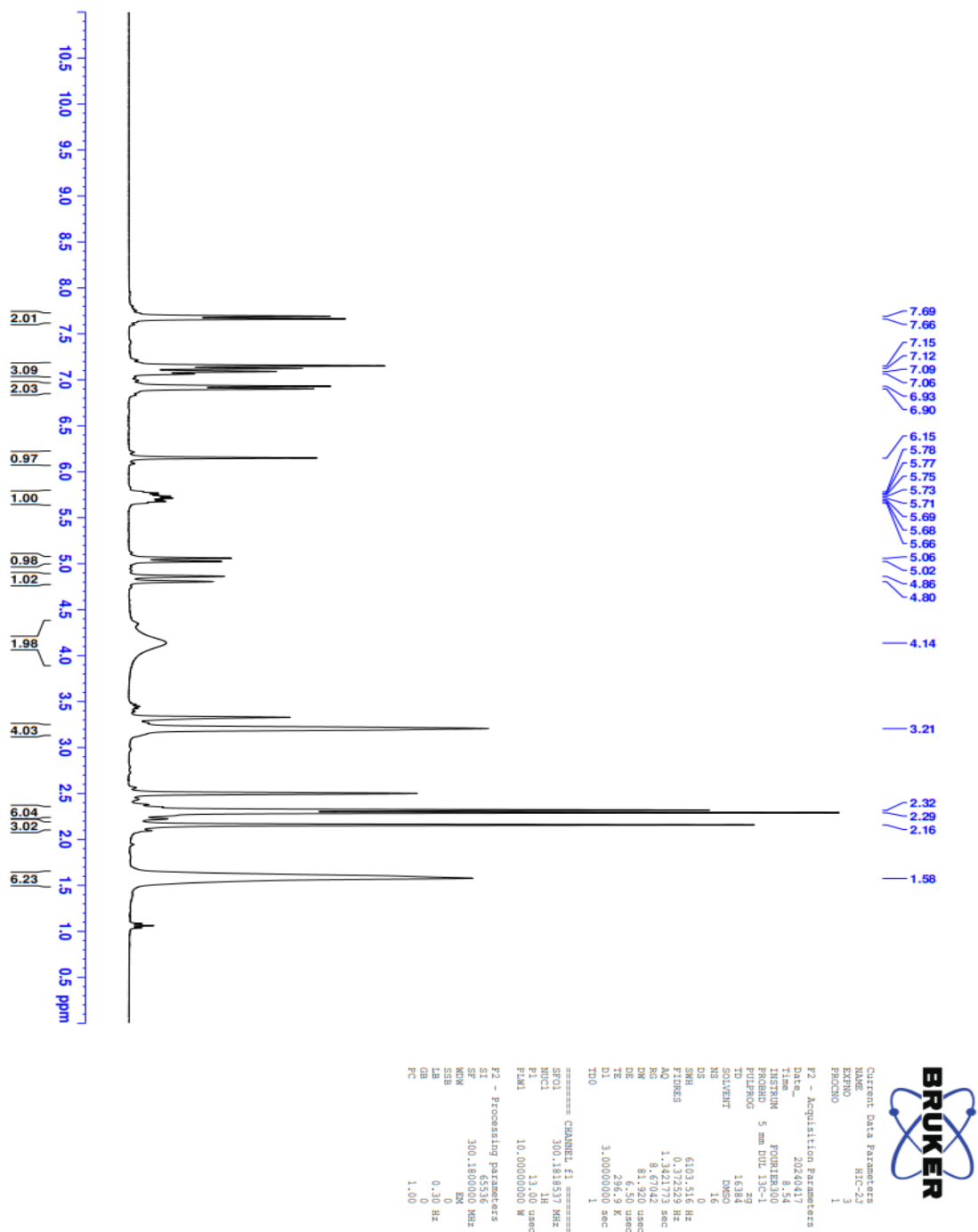


Figure 5.88. ¹H NMR signals of D2.i.

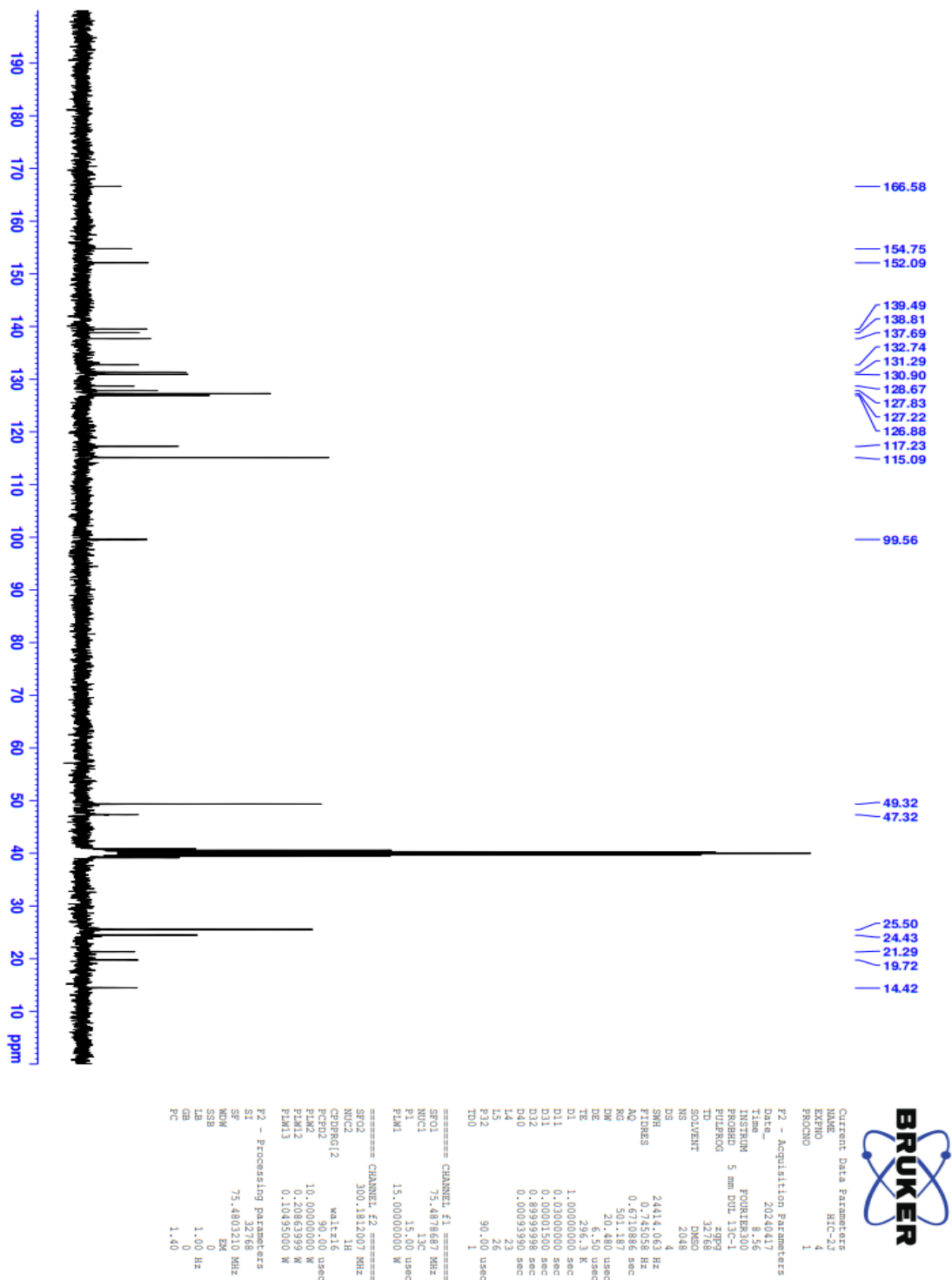


Figure 5.89. ^{13}C NMR signals of D2.i.

Data File: C:\LabSolutions\Data\Analiz\derya\MEOH_589.lcd

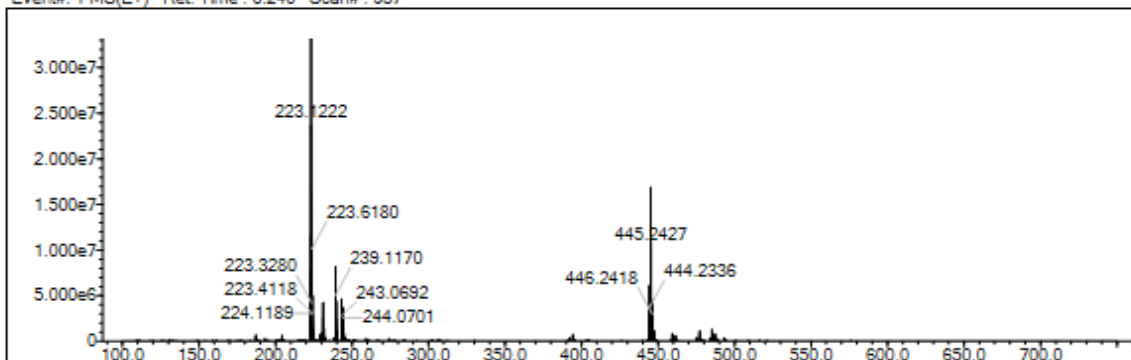
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H	1	8	33	O	2	0	3	S	2	0	2	Ru	2	0	0	H
C	4	4	32	F	1	0	0	Cl	1	0	0	Pd	2	0	0	Na
N	3	0	6	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5
 HC Ratio: unlimited
 Max Isotopes: 3
 MSn Iso RI (%): 10.00

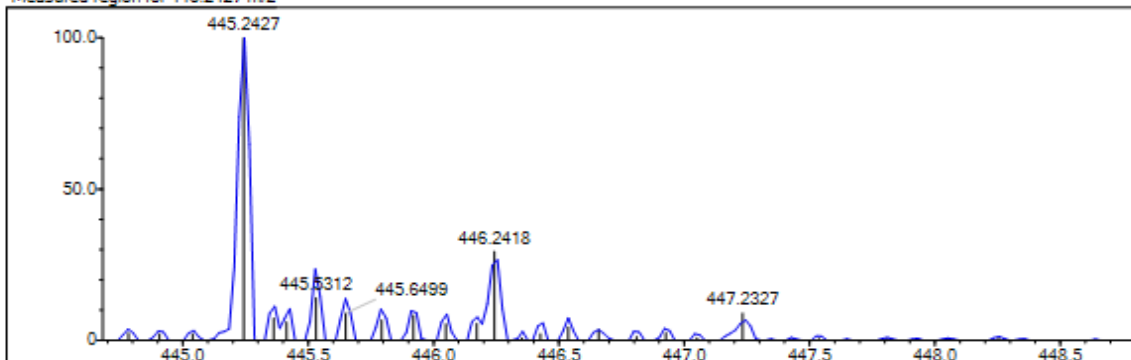
DBE Range: 0.0 - 30.0
 Apply N Rule: no
 Isotope RI (%): 1.00
 MSn Logic Mode: AND

Electron Ions: both
 Use MSn Info: yes
 Isotope Res: 9000
 Max Results: 50

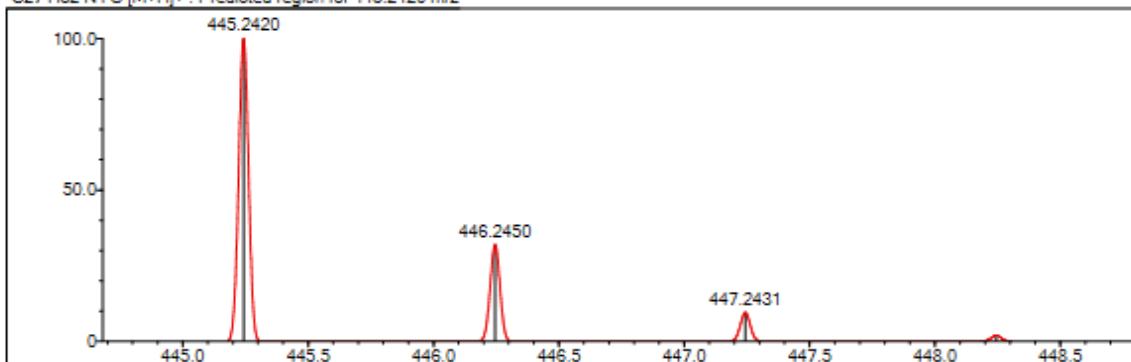
Event#: 1 MS(E+) Ret. Time : 6.240 Scan#: 937



Measured region for 445.2427 m/z



C27 H32 N4 S [M+H]⁺ : Predicted region for 445.2420 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	61.39	C27 H32 N4 S	[M+H] ⁺	445.2427	445.2420	0.7	1.57	62.28	14.0

Figure 5.90. HRMS spectrogram of D2.i.

5.1.3. Synthesis of 3-Ethyl-2-((1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-4-(p-substituted)-2,3-dihydrothiazole derivatives

A series consists of nine substances of 3-Ethyl-2-((1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-4-(p-substituted)-2,3-dihydrothiazole derivatives were synthesized according the same method as mentioned above (Section 4.1). The following derivatives are listed along with their characteristic analysis data.

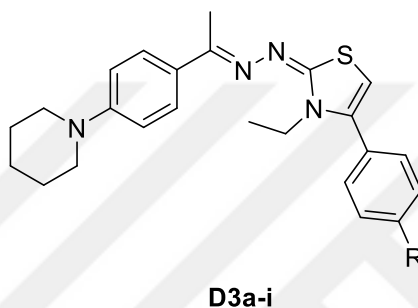


Figure 5.91. Synthesis of 3-Ethyl-2-((1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-4-(p-substituted)-2,3-dihydrothiazole derivatives

5.1.3.1. 3-Ethyl-2-((1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-4-(p-tolyl)-2,3-dihydrothiazole

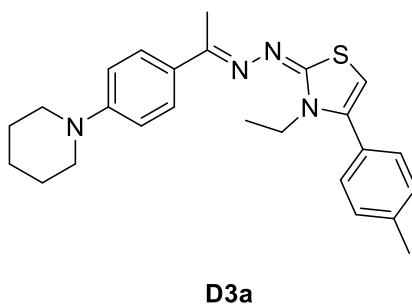


Figure 5.92. 3-Ethyl-2-((1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-4-(p-tolyl)-2,3-dihydrothiazole.

Yield: 75 %.

Melting Point: 164.2°C.

IR Maximum Velocity (cm^{-1}): 3100 (C=C-H stretching band), 2924(C-H stretching band), 1593 (C=N stretching band), 1585 (C=C band), 1234 (C-N stretching band), 827 (4-substituted phenyl out-of-plane deformation band).

^1H -NMR (300 MHz, DMSO- d_6): δ = 1.10-1.14 (3H,t, J = 6.88 Hz, methyl-H), 1.56 (6H, s, piperidine-H), 2.31 (3H, s, methyl-H), 2.35 (3H, s, methyl-H), 3.20 (4H, m, piperidine-H), 3.73-3.80 (2H, q, J = 6.6 Hz, methylene-H), 6.19 (1H, s, dihydrothiazole-H), 6.90-6.93(2H, d, J = 8.78 Hz, Ar-H), 7.27-7.35 (4H, q, J = 6.96 Hz, Ar-H), 7.66-7.69 (2H, d, J =8.70 Hz, Ar-H).

^{13}C -NMR (75 MHz, DMSO- d_6): δ = 13.47, 14.42, 21.32, 24.42, 25.50, 41.00, 49.32, 99.68, 100.96, 115.10, 126.13, 126.22,127.21, 128.63, 128.70, 129.07, 139.13, 140.48, 152.07, 154.53, 167.21.

HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{25} \text{H}_{30} \text{N}_4 \text{S}$ $[\text{M}+\text{H}]^+$: 419.2264; found: 419.2251.

Item	Value
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Sample ID	
Option	
Comment	
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Resolution	4 [cm ⁻¹]
Apodization	Happ-Genzel

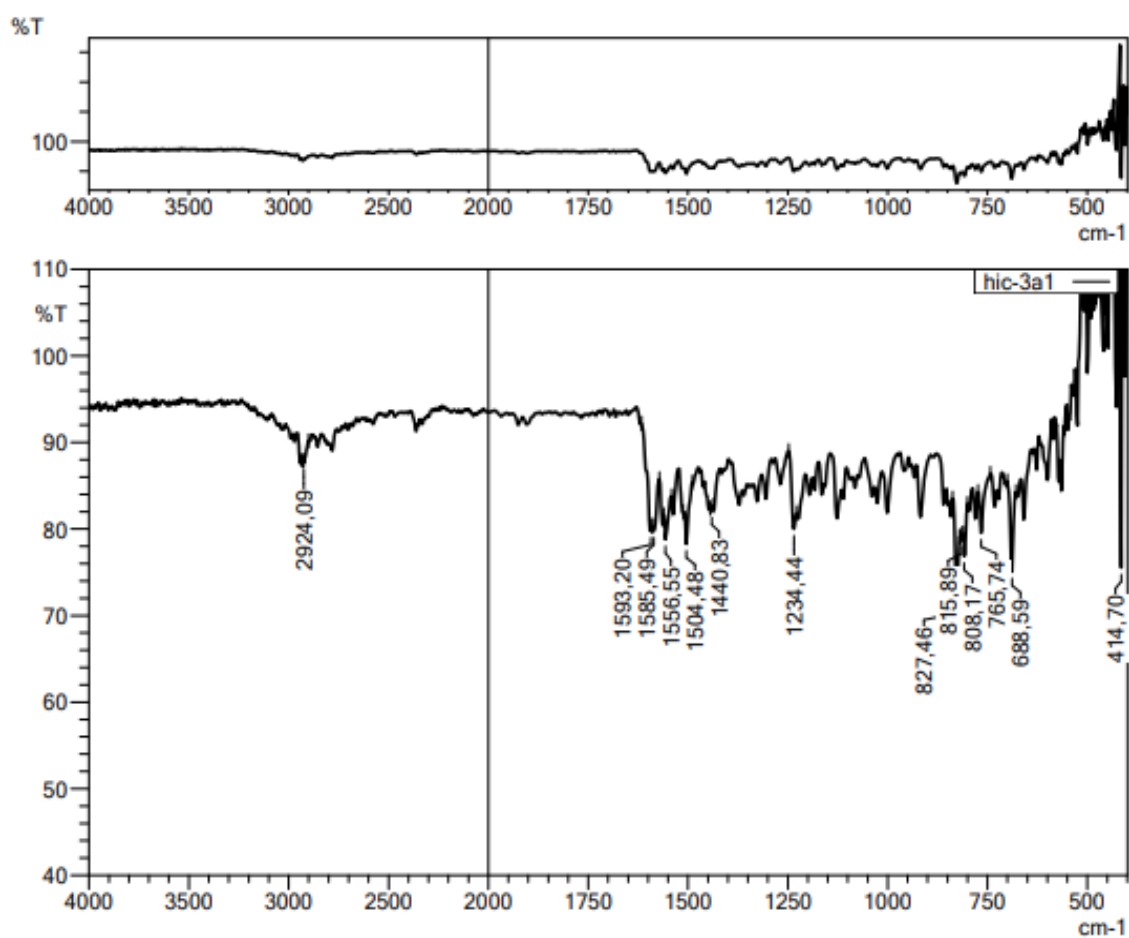


Figure 5.93. IR fingerprint of D3.a.

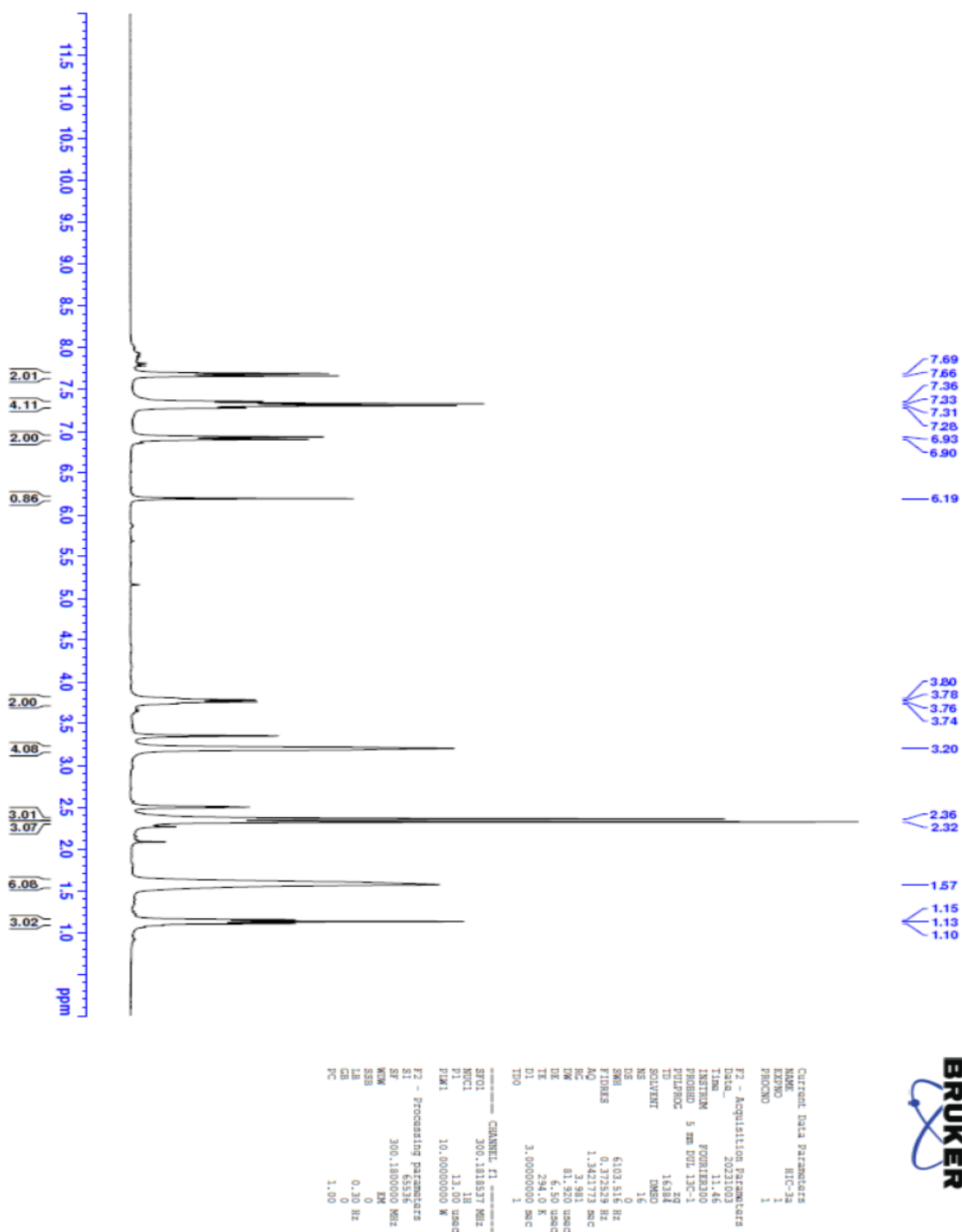


Figure 5. 94. ¹H NMR signals of D3.a.

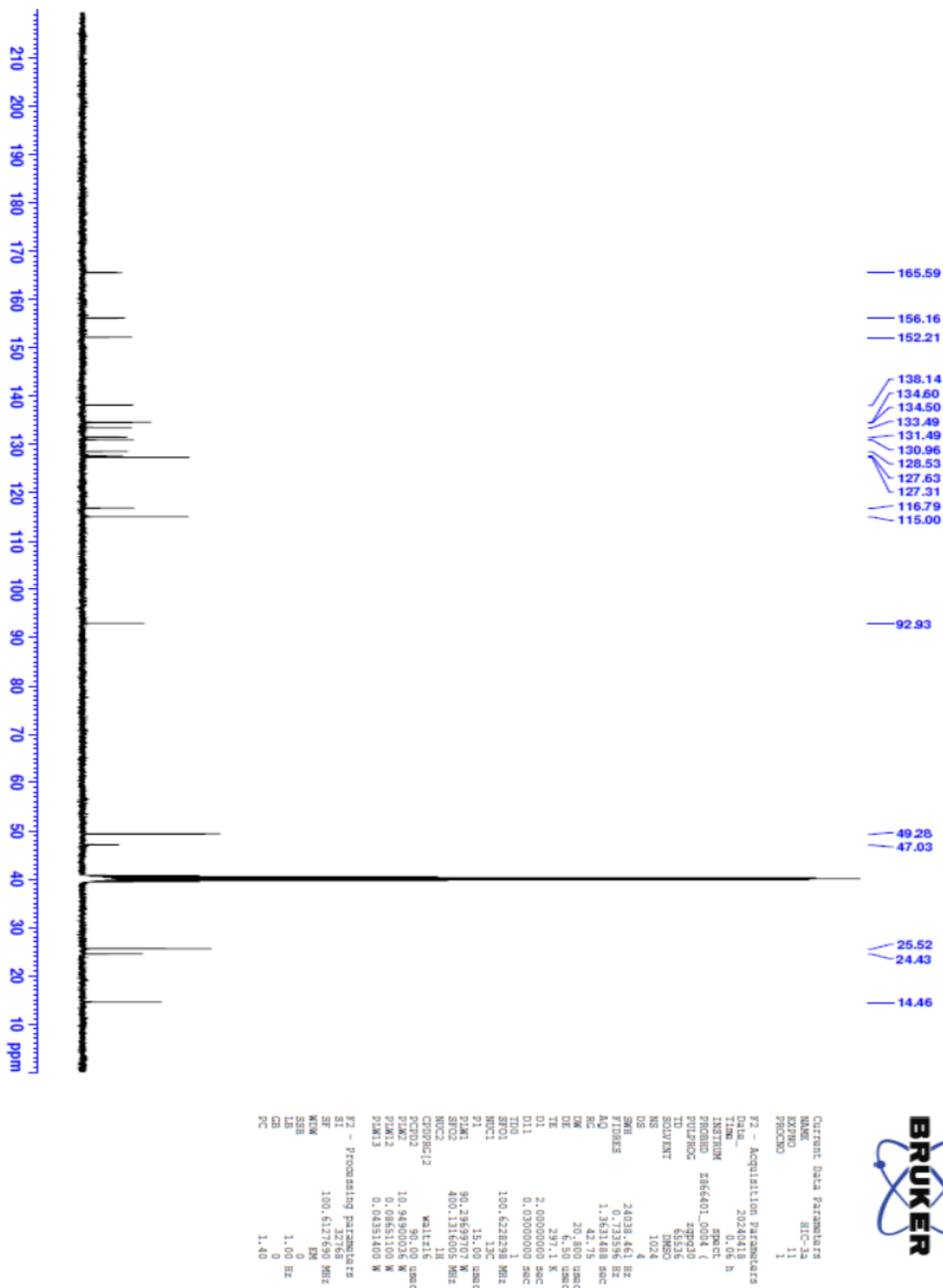


Figure 5.95. ^{13}C NMR signals of D3.a.

Data File: C:\LabSolutions\Data\Analiz\dera\HIC-3A_X_582.lod

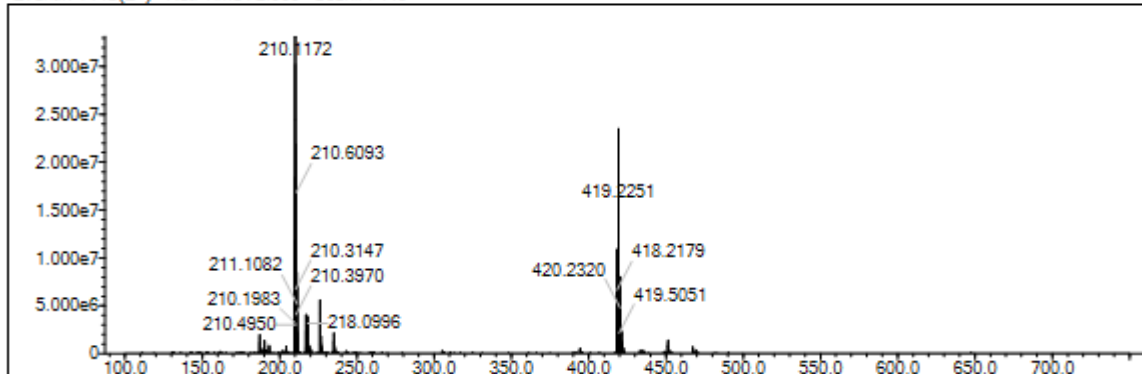
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C	4	4	32	F	1	0	0	Cl	1	0	0	Pd	2	0	0	Na
N	3	0	4	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5
 HC Ratio: unlimited
 Max Isotopes: 3
 MSn Iso RI (%): 10.00

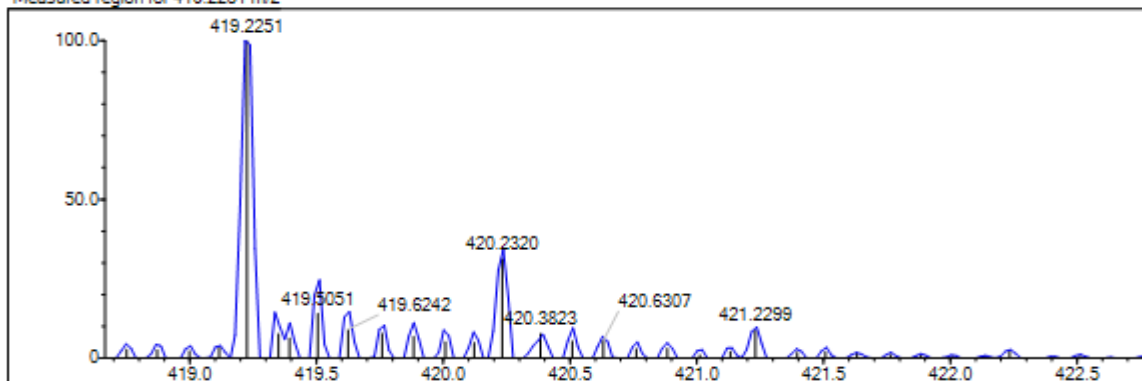
DBE Range: 0.0 - 30.0
 Apply N Rule: no
 Isotope RI (%): 1.00
 MSn Logic Mode: AND

Electron Ions: both
 Use MSn Info: yes
 Isotope Res: 9000
 Max Results: 50

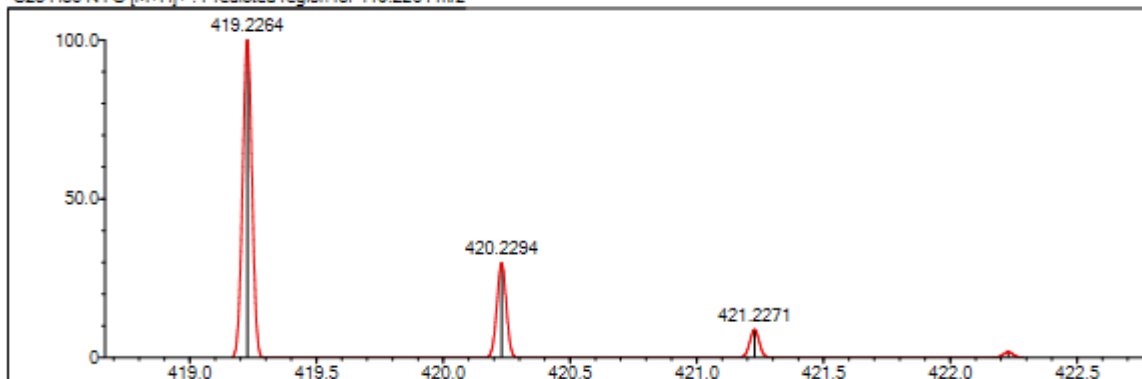
Event#: 1 MS(E+) Ret. Time : 2.960 Scan#: 445



Measured region for 419.2251 m/z



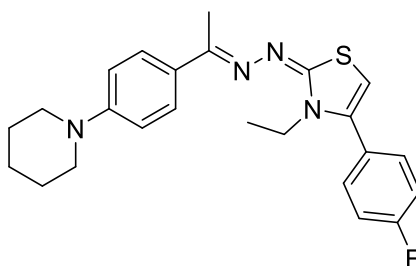
C25 H30 N4 S [M+H]⁺: Predicted region for 419.2264 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	90.34	C25 H30 N4 S	[M+H] ⁺	419.2251	419.2264	-1.3	-3.10	95.34	13.0

Figure 5.96. HRMS spectrogram of D3.a.

5.1.3.2. 3-Ethyl-4-(4-fluorophenyl)-2-((1-(4-(piperidin-1-yl)phenyl) ethylidene) hydrazineylidene)-2,3-dihydrothiazole



D3b

Figure 5.97. 3-Ethyl-4-(4-fluorophenyl)-2-((1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-2,3-dihydrothiazole.

Yield: 70 %.

Melting Point: 150°C.

IR Maximum Velocity (cm^{-1}): 3109 (C=C-H stretching band), 2937 (C-H stretching band), 1583 (C=N stretching band), 1548 (C=C band), 1238 (C-N stretching band), 815 (4-substituted phenyl out-of-plane deformation band).

^1H -NMR (300 MHz, $\text{DMSO}-d_6$): δ = 1.12-1.15 (3H, t, J = 6.95 Hz, methyl-H), 1.57-1.61 (6H, br.s, piperidine-H), 2.33 (3H, s, methyl-H), 3.20-3.22 (4H, q, J = 6.87 Hz, piperidine-H), 3.75-3.79 (2H, q, J = 7.19 Hz, methylene-H), 6.27 (1H, s, dihydrothiazole-H), 6.91-6.94 (2H, d, J = 8.79 Hz, Ar-H), 7.32-7.36 (2H, t, J = 8.74 Hz, Ar-H), 7.52-7.55 (2H, m, Ar-H), 7.67-7.70 (2H, d, J =8.69 Hz, Ar-H).

^{13}C -NMR (75 MHz, $\text{DMSO}-d_6$): δ = 13.58, 14.44, 24.43, 25.50, 49.33, 56.49, 100.41, 115.09, 116.15, 116.37, 127.25, 127.96, 128.66, 131.52, 131.60, 139.35, 152.11, 154.74, 161.32, 164.12, 167.04.

HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{24} \text{H}_{27} \text{N}_4 \text{FS}$ $[\text{M}+\text{H}]^+$: 423.2013; found: 423.1996.

Item	Value
Acquired Date&Time	3.05.2024 11:23:45
Acquired by	System Administrator
Filename	C:\Users\dopnalab\Desktop\MASAU\STU\sazan\hic\hic-3b1.ispd
Spectrum name	hic-3b1
Sample name	hic-3b
Sample ID	
Option	
Comment	
No. of Scans	30
Resolution	4 [cm-1]
Apodization	Happ-Genzel

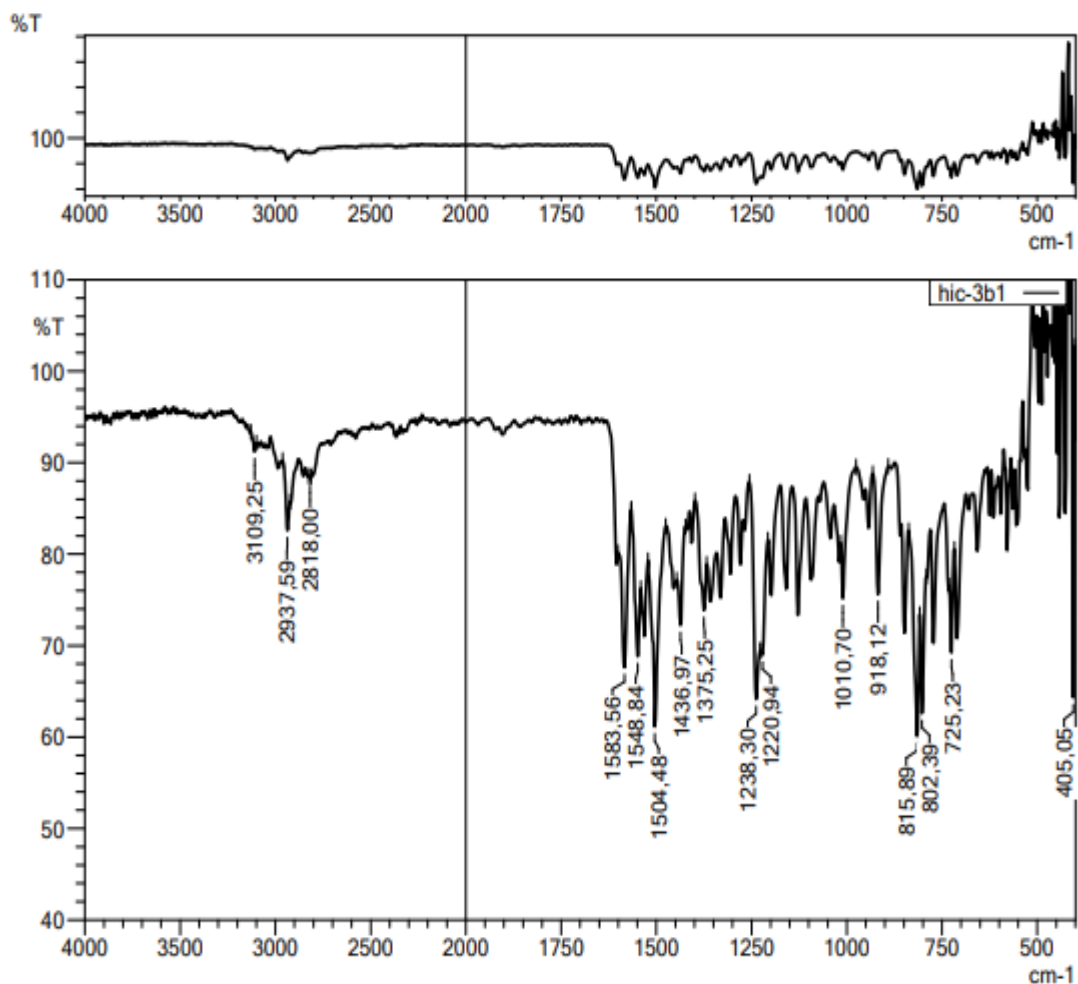


Figure 5.98. IR fingerprint of D3.b.

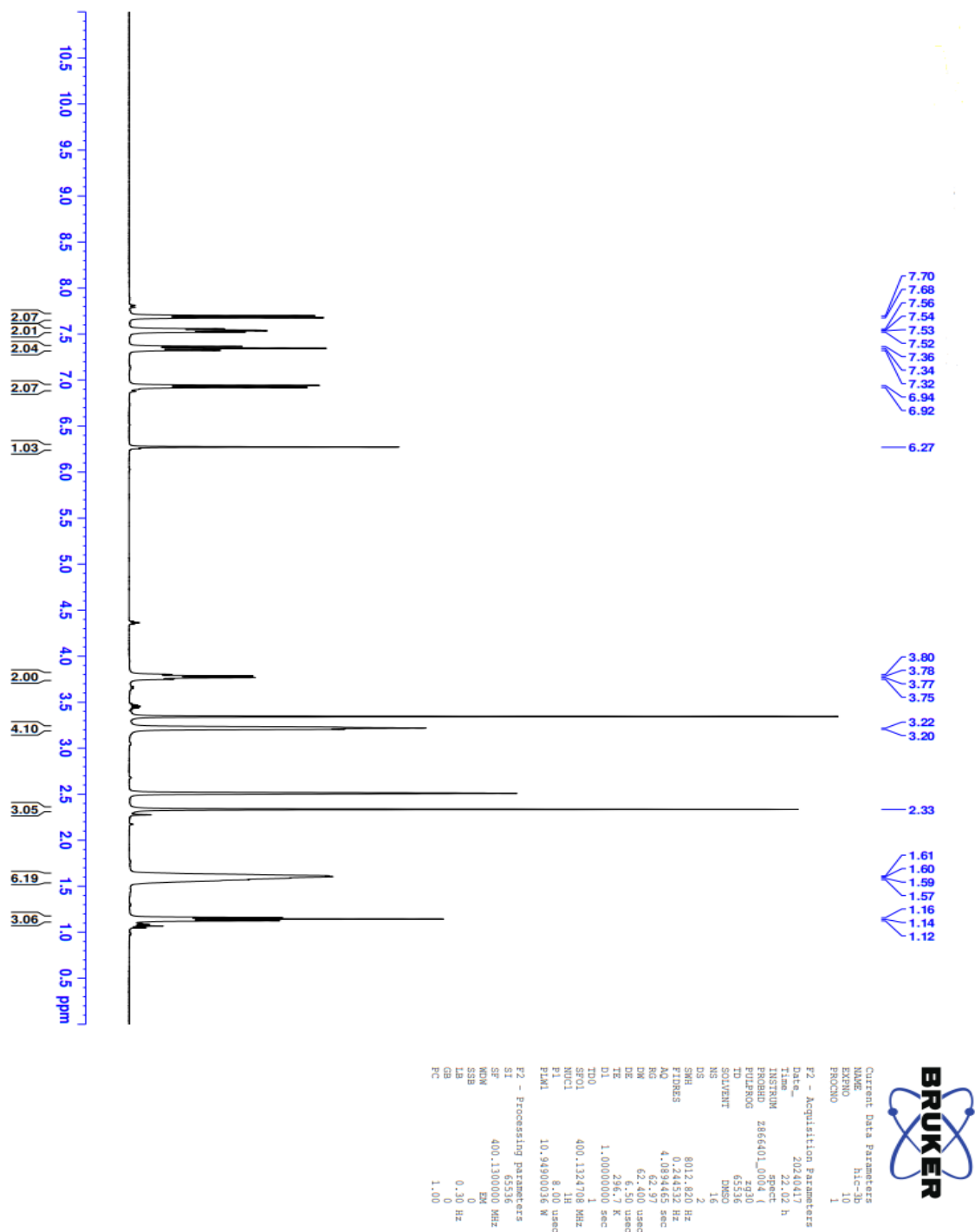
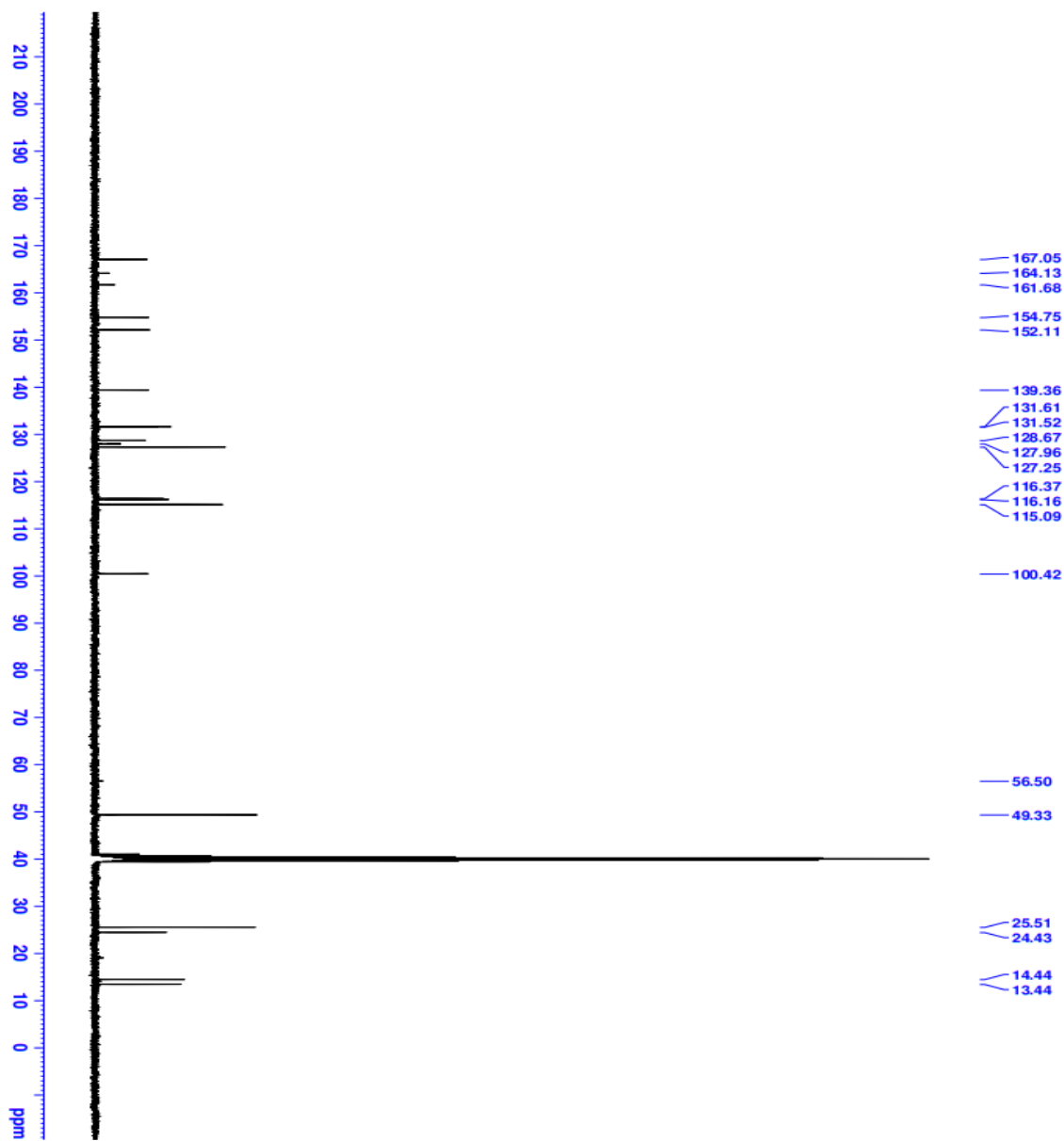


Figure 5.99. ¹H NMR signals of D3.b.



Current Data Parameters
 NAME h1c-30
 EXPNO 11
 PROCNO 1
 F2 - Acquisition Parameters
 Date_ 20240417
 Time 23.02 h
 INSTRUM spect
 PROBRD zgpg30
 PULPROG zgpg30
 TD 65536
 SFO2 1024
 SOLVENT DMSO
 NS 1024
 DS 4
 SMH 24038.461 Hz
 FIDRES 0.713596 Hz
 AQ 1.361488 sec
 RG 26.71
 RM 20.490 usec
 TD 65536
 TE 303.1 K usec
 DE 1.0000000 sec
 DI 2.0000000 sec
 D11 0.0300000 sec
 TDO 1
 SFO1 100.6228298 MHz
 NUC1 13C
 P1 15.00 usec
 PL1 90.2969707 W
 SFO2 400.116005 MHz
 NUC2 1H
 CPOPRG12 waltz16
 FCFD2 90.00 usec
 PLW2 10.9490036 W
 PLW12 0.0885109 W
 PLW13 0.04351409 W
 F2 - Processing parameters
 SI 32768
 SF 100.6127659 MHz
 WNW 0
 SSB 0
 LB 1.00 Hz
 GB 0
 PC 1.40



Figure 5.100. ^{13}C NMR signals of D3.b.

Data File: C:\LabSolutions\Data\Analiz\dera\HIC-3B_1.lod

Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	8	33	O	2	0	3	S	2	1	1	Ru	2	0	0	H
C	4	4	32	F	1	1	1	Cl	1	0	0	Pd	2	0	0	Na
N	3	0	4	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5

HC Ratio: unlimited

Max Isotopes: 3

MSn Iso RI (%): 10.00

DBE Range: 0.0 - 30.0

Apply N Rule: no

Isotope RI (%): 1.00

MSn Logic Mode: AND

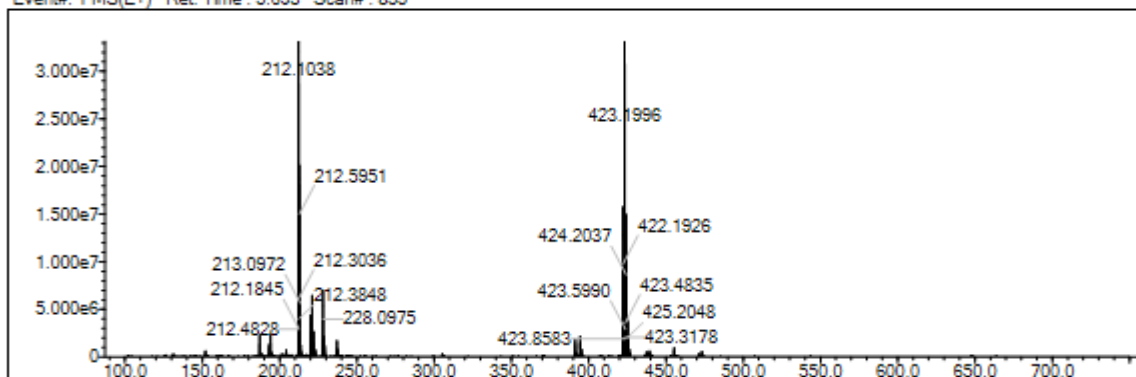
Electron Ions: both

Use MSn Info: yes

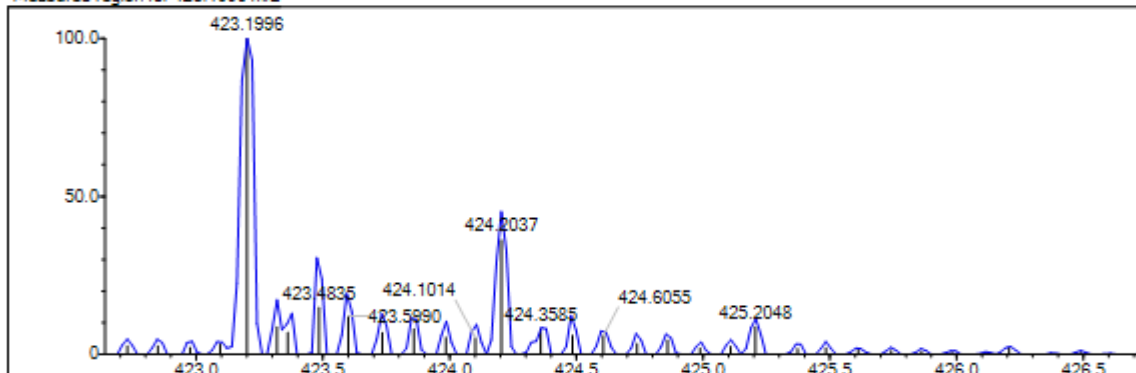
Isotope Res: 9000

Max Results: 50

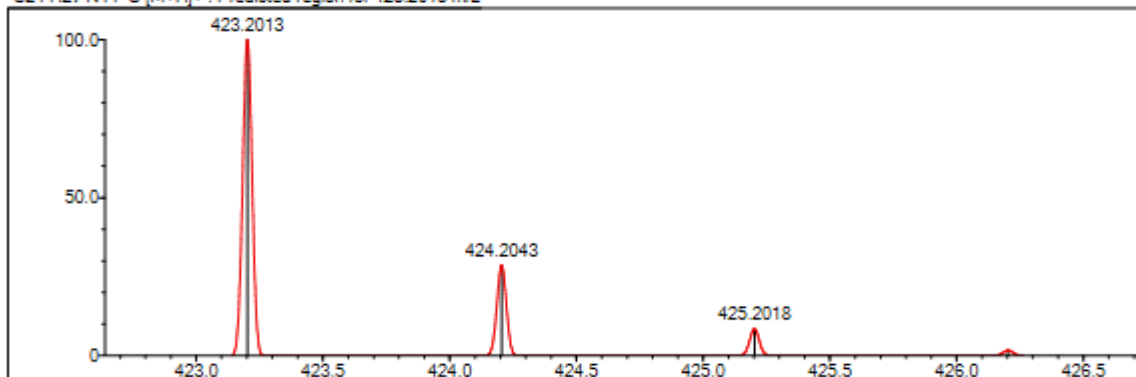
Event#: 1 MS(E+) Ret. Time: 5.693 Scan#: 855



Measured region for 423.1996 m/z



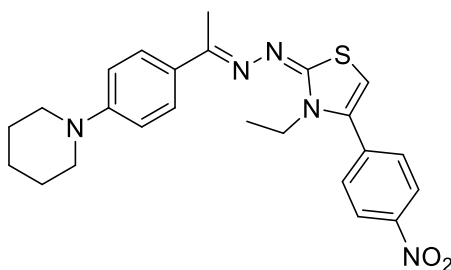
C24 H27 N4 F S [M+H]⁺: Predicted region for 423.2013 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	61.09	C24 H27 N4 F S	[M+H] ⁺	423.1996	423.2013	-1.7	-4.02	66.08	13.0

Figure 5.101. HRMS spectrogram of D3.b.

5.1.3.3. 3-Ethyl-4-(4-nitrophenyl)-2-((1-(4-(piperidin-1 yl)phenyl)ethylidene)hydrazineylidene)-2,3-dihydrothiazole



D3c

Figure 5.102. 3-Ethyl-4-(4-nitrophenyl)-2-((1-(4-(piperidin-1 yl)phenyl)ethylidene)hydrazineylidene)-2,3-dihydrothiazole.

Yield: 89%.

Melting Point: 166.5°C.

IR Maximum Velocity (cm^{-1}): 3124 (C=C-H stretching band), 2943(C-H stretching band), 1589 (C=N stretching band), 1504 (C=C band), 1234 (C-N stretching band), 823 (4-substituted phenyl out-of-plane deformation band).

^1H -NMR(300MHz, $\text{DMSO-}d_6$): 1.16-1.19(3H, t, $J= 6.93\text{Hz}$, methyl-H), 1.59-1.60(6H, s, piperidine-H), 2.09 (3H, s, methyl-H), 3.22-3.23(4H, s, piperidine-H), 3.82-3.87(2H, q, $J= 6.93\text{ Hz}$, methylene-H), 6.55(1H,s, dihydrothiazole-H), 6.93-6.95(2H, d, $J= 8.72\text{ Hz}$, Ar-H), 7.68-7.70(2H, d, $J= 8.62\text{ Hz}$, Ar-H), 7.78-7.80(2H, d, $J=8.52\text{ Hz}$, Ar-H), 8.33-8.35(2H, d, $J=8.65\text{Hz}$, Ar-H).

^{13}C -NMR (75 MHz, $\text{DMSO-}d_6$): $\delta = 13.51, 14.53, 24.43, 25.49, 49.27, 103.52, 115.06, 124.47, 127.33, 128.42, 130.11, 137.73, 138.63, 152.18, 155.37, 167.12$.

HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{24}\text{H}_{27}\text{N}_5\text{O}_2\text{S}$ $[\text{M}+\text{H}]^+$: 450.1958; found: 450.1949.

Item	Value
Acquired Date&Time	3.05.2024 11:28:37
Acquired by	System Administrator
Filename	C:\Users\ldopnab\l\Desktop\MASAU\T\isazan\hic\hic-3c1.ispd
Spectrum name	hic-3c1
Sample name	hic-3c
Sample ID	
Option	
Comment	
No. of Scans	30
Resolution	4 [cm-1]
Apodization	Happ-Genzel

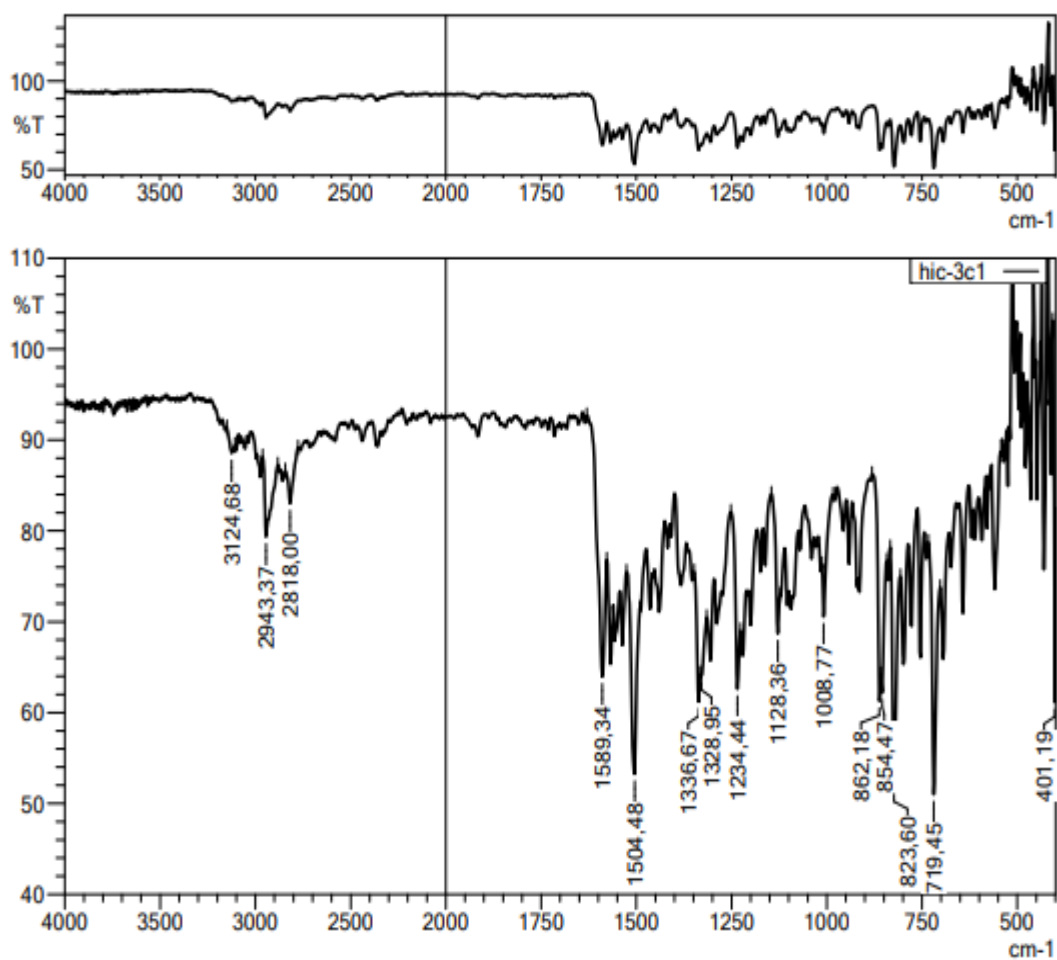


Figure 5.103. IR fingerprint of D3.c.

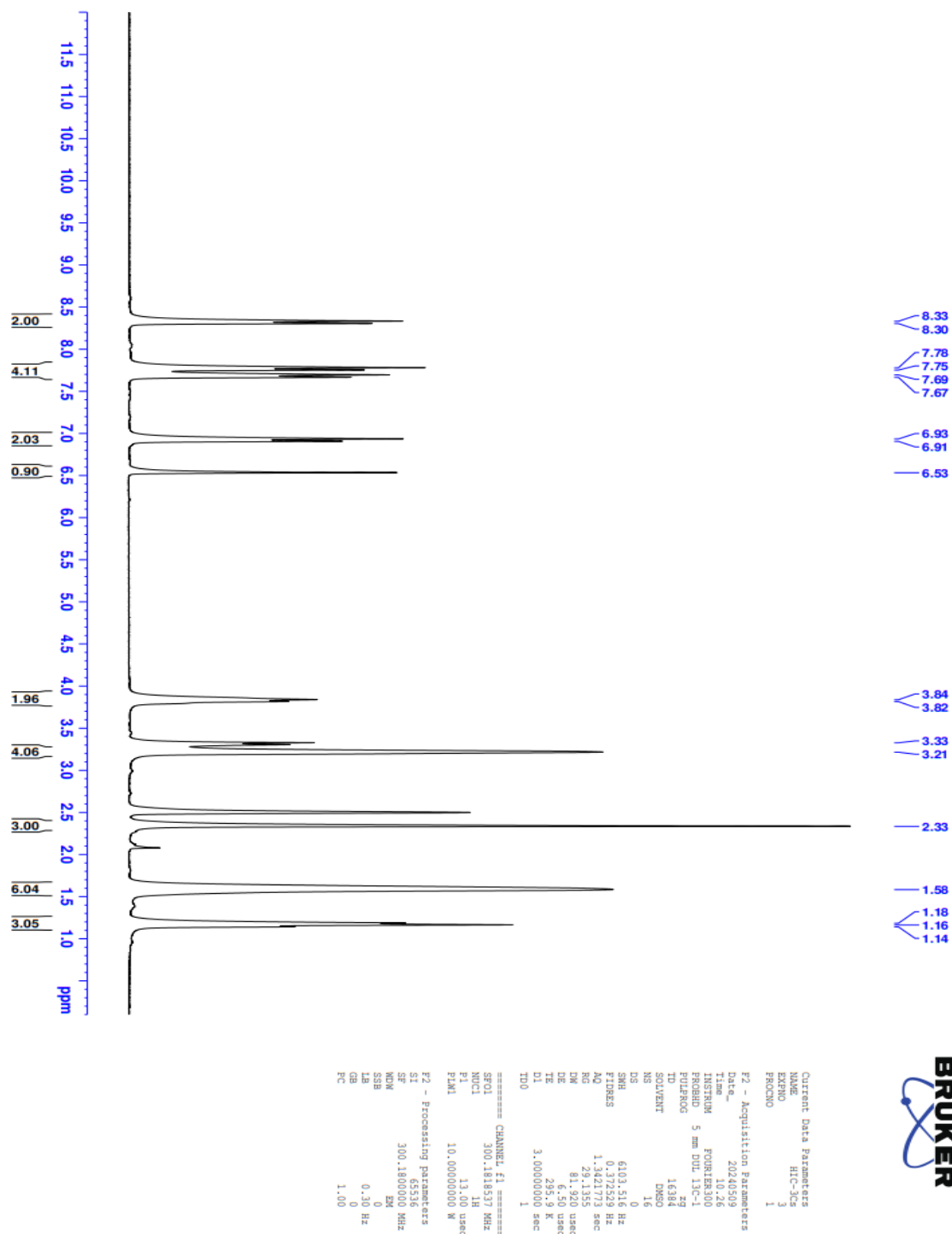
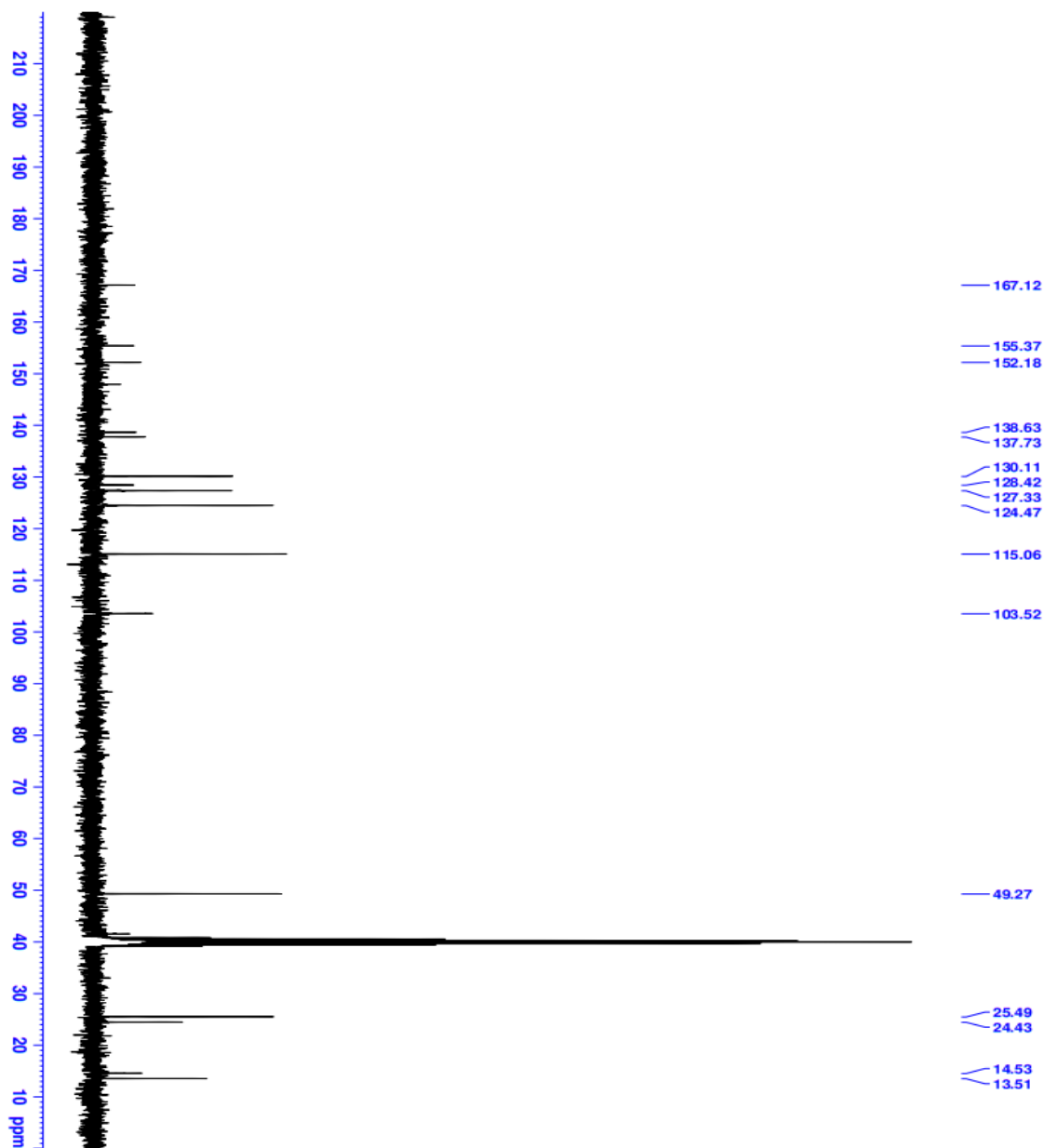


Figure 5.104. ^1H NMR signals of D3.c.



```

Current Data Parameters
NAME          HIC-3Cs
EXPNO         4
PROCNO        1

F2 - Acquisition Parameters
Date_         20240509
Time          10.28
INSTRUM       PULPROG
PROBHD        5 mm DUL 13C-1
PULPROG       zgpg
TD            32768
SOLVENT       DMSO
NS            2048
DS            4
SWH           24444.063 Hz
FIDRES        0.745058 Hz
AQ            0.6710886 sec
RG            501.187
DM            20.480 usec
DE            28.480 usec
TE            300.2 K
D1            1.00000000 sec
D11           0.01000000 sec
D31           0.00001500 sec
D32           0.8999998 sec
D40           0.00093990 sec
I4            23
L5            26
P32           90.00 usec
TD0           1

===== CHANNEL f1 =====
SFO1          75.4878687 MHz
NUC1          13C
P1            15.00 usec
PLM1          15.00000000 W

===== CHANNEL f2 =====
SFO2          300.1812007 MHz
NUC2          1H
CPDPRG12     waltz16
PCPD2         90.00 usec
PLM2          10.00000000 W
PLM12         0.20863999 W
PLM13         0.10495000 W

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



Figure 5.105. ¹³CNMR signals of D3.c.

Data File: C:\LabSolutions\Data\Analiz\derya\HIC-3D ALT_3.lod

Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	8	33	O	2	0	3	S	2	1	1	Ru	2	0	0	H
C	4	4	32	F	1	0	0	Cl	1	0	0	Pd	2	0	0	Na
N	3	0	5	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5

HC Ratio: unlimited

Max Isotopes: 3

MSn Iso RI (%): 10.00

DBE Range: 0.0 - 30.0

Apply N Rule: no

Isotope RI (%): 1.00

MSn Logic Mode: AND

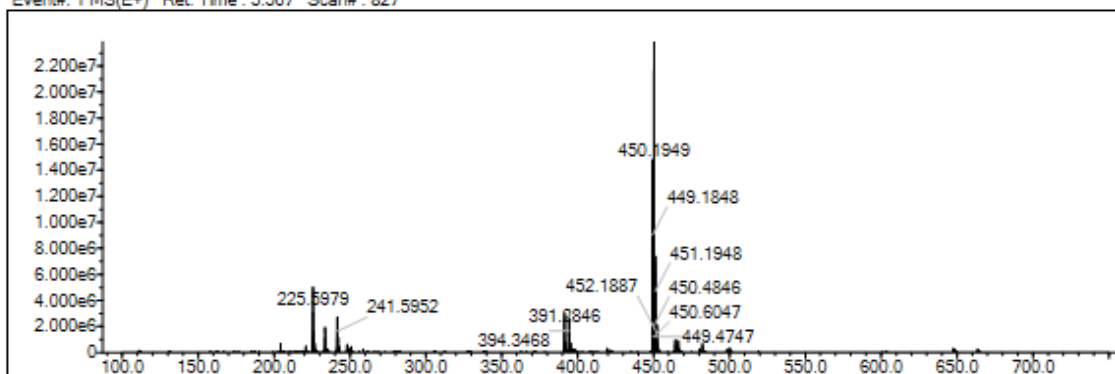
Electron Ions: both

Use MSn Info: yes

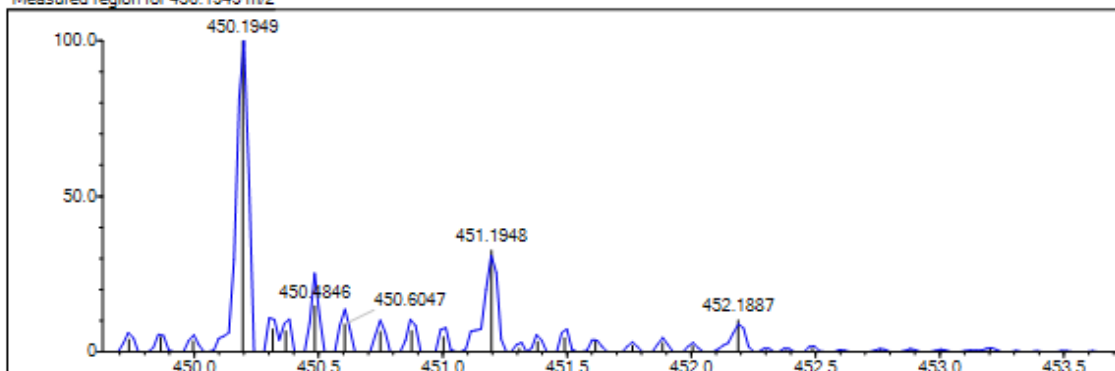
Isotope Res: 9000

Max Results: 50

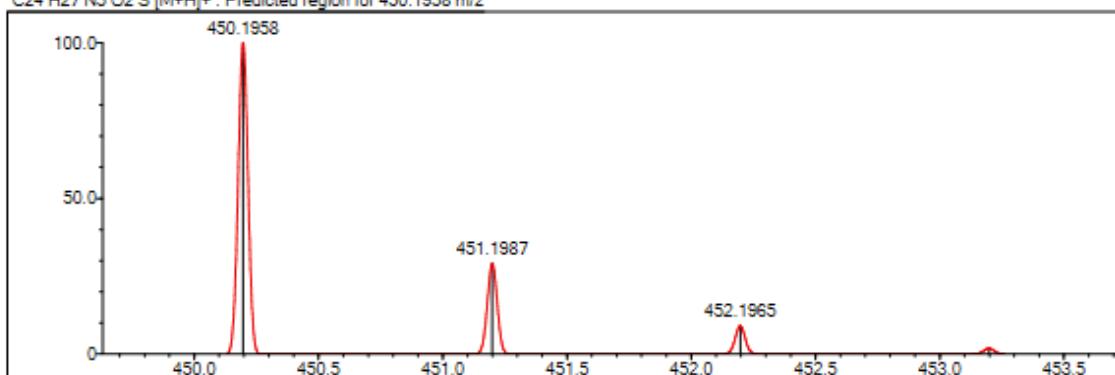
Event#: 1 MS(E+) Ret. Time : 5.507 Scan#: 827



Measured region for 450.1949 m/z



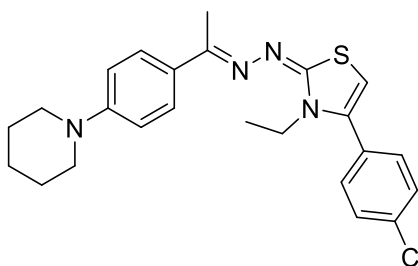
C24 H27 N5 O2 S [M+H]⁺ : Predicted region for 450.1958 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	73.22	C24 H27 N5 O2 S	[M+H] ⁺	450.1949	450.1958	-0.9	-2.00	75.10	14.0

Figure 5.106. HRMS spectrogram of D3.c.

5.1.3.4. 4-(4-Chlorophenyl)-3-ethyl-2-((1-(4-(piperidin-1-yl)phenyl) ethylidene) hydrazineylidene)-2,3-dihydrothiazole



D3d

Figure 5.107. 4-(4-Chlorophenyl)-3-ethyl-2-((1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-2,3-dihydrothiazole.

Yield: 74%.

Melting Point: 156.5°C.

IR Maximum Velocity (cm^{-1}): 3080 (C=C-H stretching band), 2931 (C-H stretching band), 1587 (C=N stretching band), 1562 (C=C band), 1236 (C-N stretching band), 812 (4-substituted phenyl out-of-plane deformation band).

^1H -NMR(300MHz, $\text{DMSO}-d_6$): 1.13-1.16 (3H, t, J = 6.82 Hz, methyl-H), 1.60(6H, s, piperidine-H), 2.33 (3H, s, methyl-H), 3.22(4H, br.s, piperidine-H), 3.77-3.79(2H, q, J = 6.92 Hz, methylene-H), 6.32(1H,s, dihydrothiazole-H), 6.92-6.94(2H, d, J = 8.65 Hz, Ar-H), 7.50-7.58(4H, m, Ar-H), 7.67-7.69(2H, d, J =8.54 Hz, Ar-H).

^{13}C -NMR (75 MHz, $\text{DMSO}-d_6$): δ = 13.47, 14.44, 24.42, 25.49, 41.12, 49.01, 49.30, 100.95, 113.91, 115.08, 127.26, 128.59, 129.34, 130.32, 130.98, 134.34, 139.21, 152.11, 154.85, 167.10.

HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{24}\text{H}_{27}\text{N}_4\text{S}\text{Cl}$ $[\text{M}+\text{H}]^+$: 439.1718; found: 439.1713.

Item	Value
Acquired Date&Time	3.05.2024 11:34:38
Acquired by	System Administrator
Filename	C:\Users\dopnalab\Desktop\MASAUŠTŪ\sazan\hic\hic-3d1.ispd
Spectrum name	hic-3d1
Sample name	hic-3d
Sample ID	
Option	
Comment	
No. of Scans	30
Resolution	4 [cm-1]
Apodization	Happ-Genzel

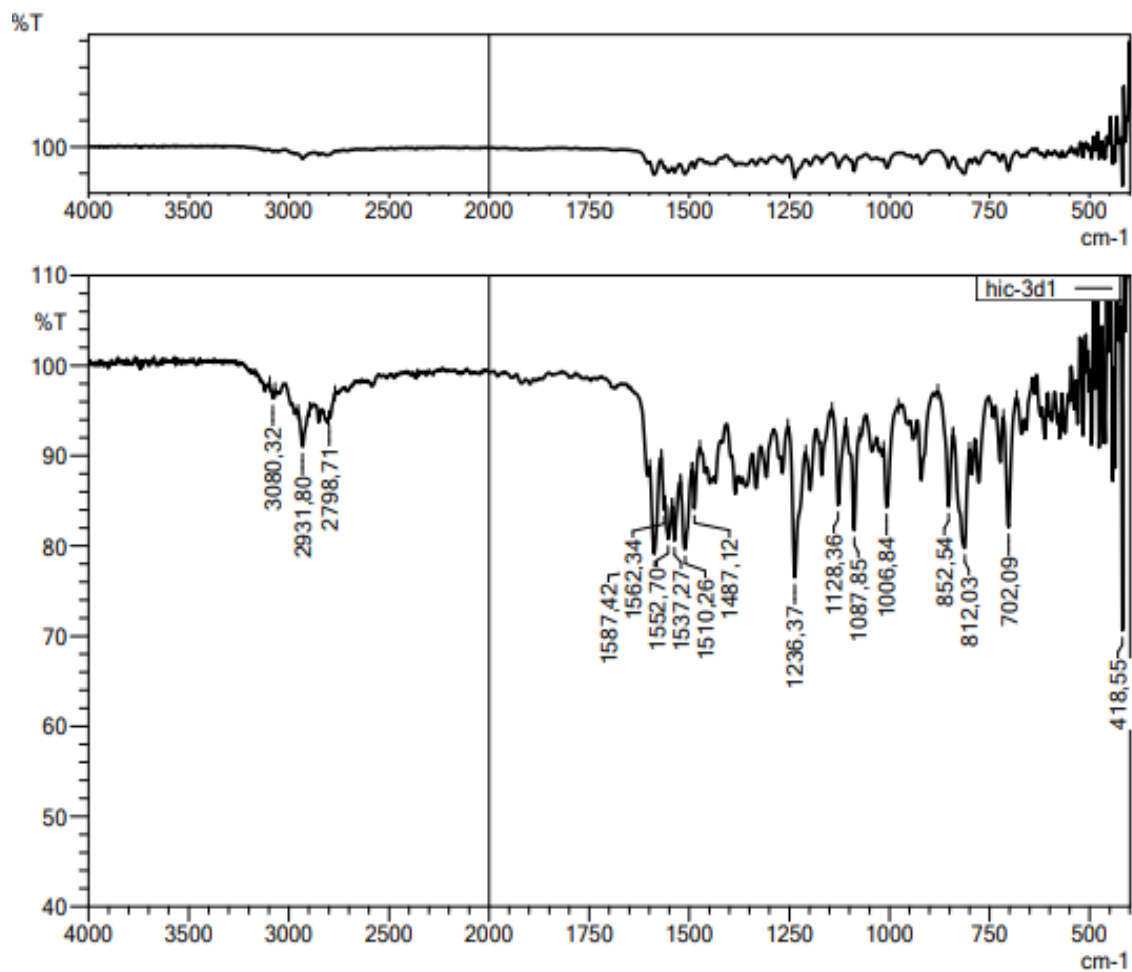


Figure 5.108. IR fingerprint of D3.d.

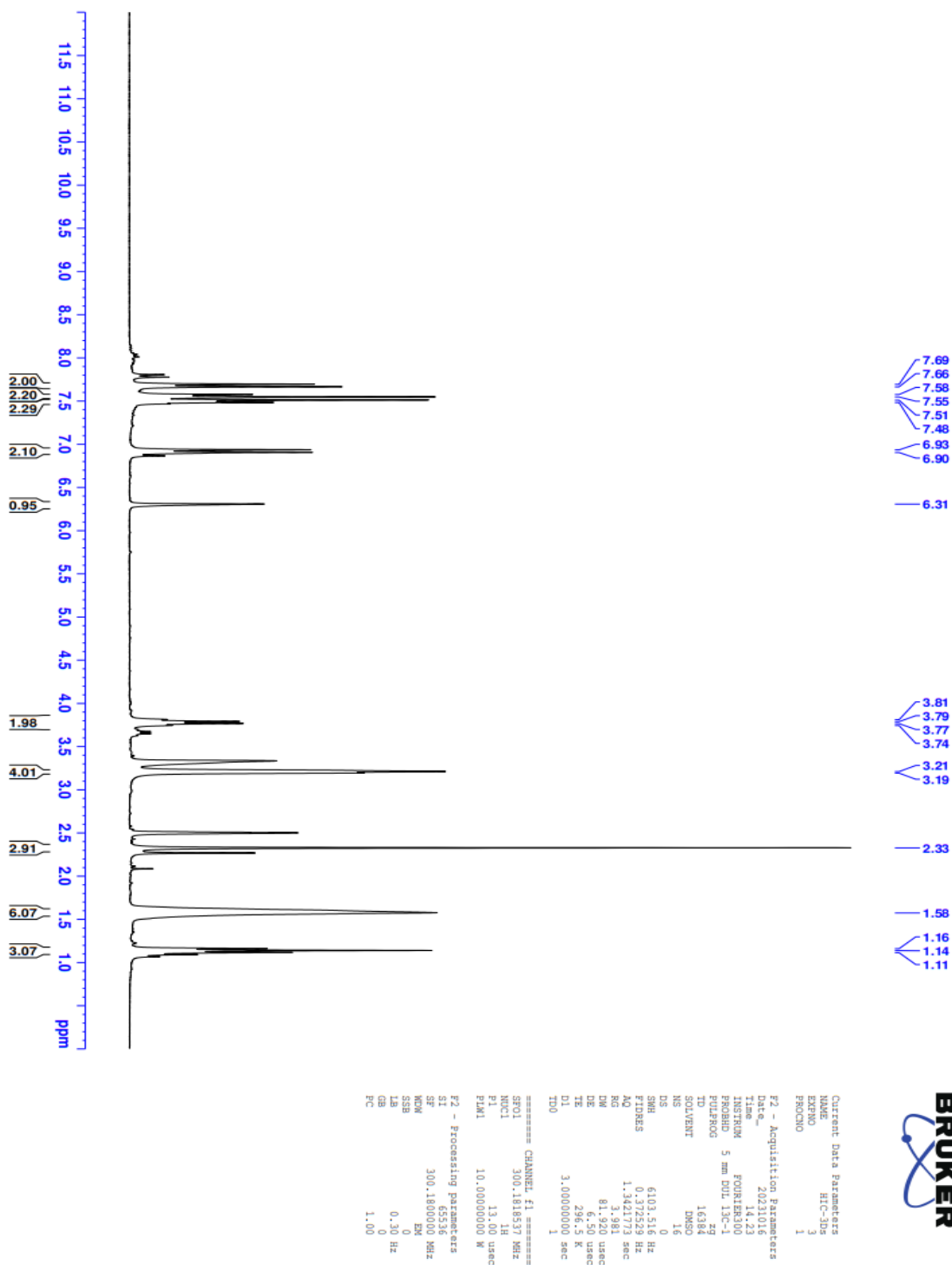


Figure 5.109. ¹H NMR signals of D3.d.

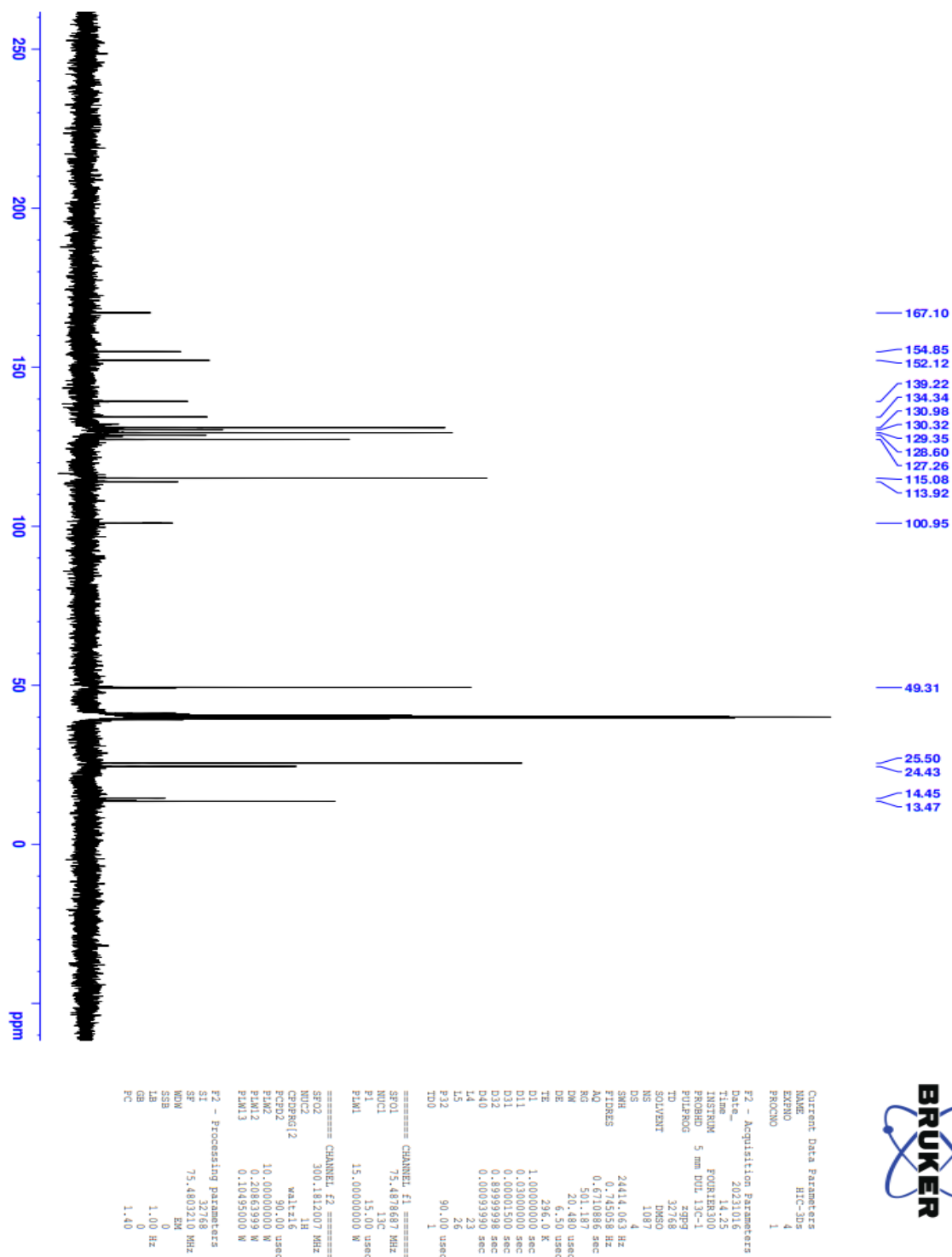


Figure 5.110. ^{13}C NMR signals of D3.d.

Data File: C:\LabSolutions\Data\Analiz\derya\HIC-3E_4.lod

Elmt	Val	Min	Max	Elmt	Val	Min	Max	Elmt	Val	Min	Max	Elmt	Val	Min	Max	Use Adduct
H	1	8	33	O	2	0	3	S	2	1	1	Ru	2	0	0	H
C	4	4	32	F	1	0	0	Cl	1	1	1	Pd	2	0	0	Na
N	3	0	5	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5

HC Ratio: unlimited

Max Isotopes: 3

MSn Iso RI (%): 10.00

DBE Range: 0.0 - 30.0

Apply N Rule: no

Isotope RI (%): 1.00

MSn Logic Mode: AND

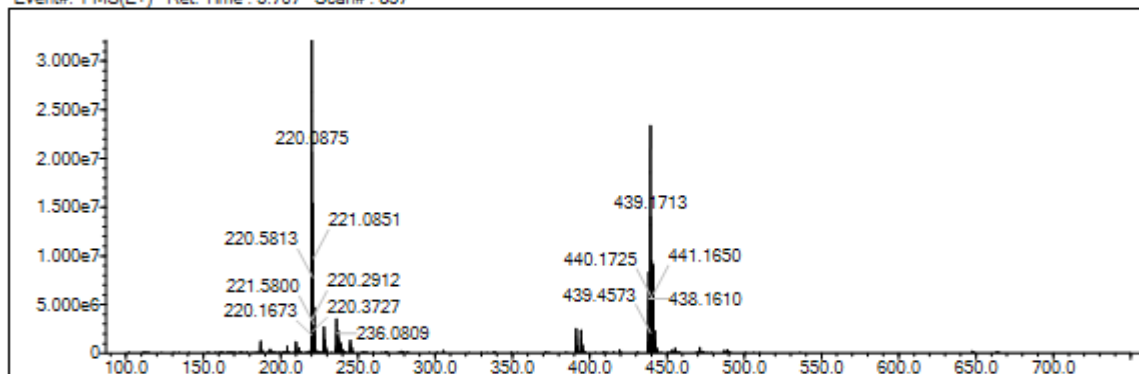
Electron Ions: both

Use MSn Info: yes

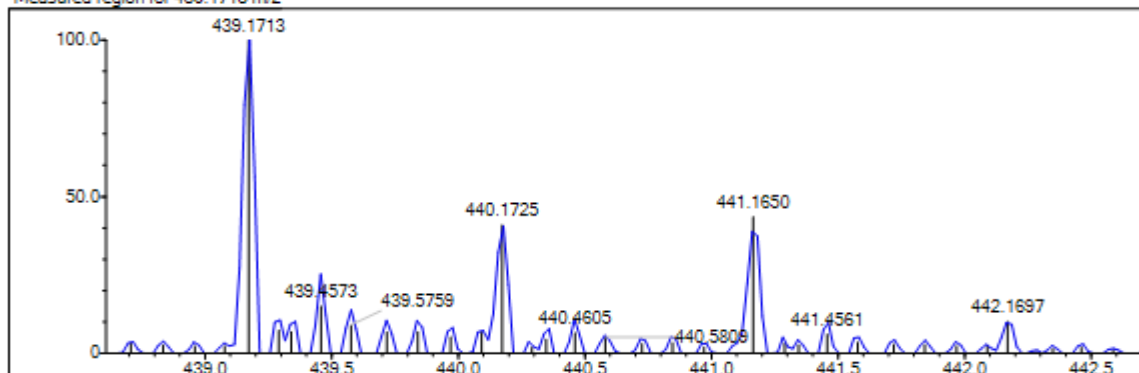
Isotope Res: 9000

Max Results: 50

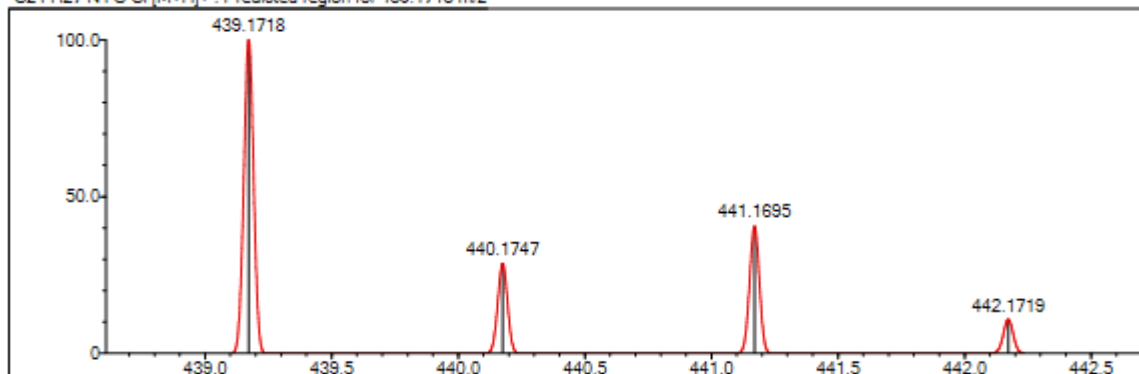
Event#: 1 MS(E+) Ret. Time : 5.707 Scan#: 857



Measured region for 439.1713 m/z



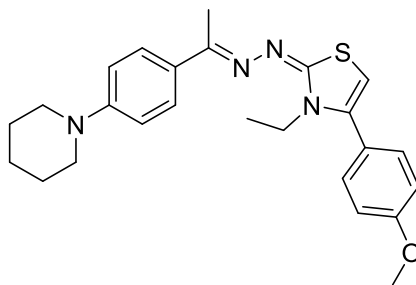
C24 H27 N4 S Cl [M+H]⁺ : Predicted region for 439.1718 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	74.65	C24 H27 N4 S Cl	[M+H] ⁺	439.1713	439.1718	-0.5	-1.14	74.91	13.0

Figure 5.111. HRMS spectrogram of D3.d.

5.1.3.5. 3-Ethyl-4-(4-methoxyphenyl)-2-((1-(4-(piperidin-1-yl)phenyl) ethylidene) hydrazineylidene)-2,3-dihydrothiazole



D3e

Figure 5.112. 3-Ethyl-4-(4-methoxyphenyl)-2-((1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-2,3-dihydrothiazole.

Yield: 90%.

Melting Point: 148.1°C.

IR Maximum Velocity(cm^{-1}): 3101 (C=C-H stretching band), 2929(C-H stretching band), 1587 (C=N stretching band), 1556 (C=C band), 1238 (C-N stretching band), 812 (4-substituted phenyl out-of-plane deformation band).

^1H -NMR(300MHz, $\text{DMSO}-d_6$): 1.12-1.16 (3H, t, J = 6.86 Hz, methyl-H), 1.60 (6H, s, piperidine-H), 2.33 (3H, s, methyl-H), 3.22 (4H, br.s, piperidine-H), 3.77 (2H, q, J = 6.98Hz, methylene-H), 3.78-3.8116 (3H, s, methyl-H), 6.16 (1H,s, dihydrothiazole-H), 6.92-6.94 (2H, d, J = 8.47 Hz, Ar-H), 7.04-7.06 (2H, d, J =8.08 Hz, Ar-H), 7.39-7.41 (2H, d, J =8.03 Hz, Ar-H), 7.67-7.69 (2H, d, J = 8.36 Hz, Ar-H).

^{13}C -NMR (75 MHz, $\text{DMSO}-d_6$): δ = 13.46, 14.41, 24.43, 25.51, 40.95, 49.35, 55.72, 99.29, 114.66, 115.10, 123.70, 127.22, 128.76, 130.65, 140.29, 152.08, 154.49, 160.24, 167.15.

HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{25} \text{H}_{30} \text{N}_4 \text{O S}$ $[\text{M}+\text{H}]^+$: 435.2213; found: 435.2193.

Item	Value
Acquired Date&Time	3.05.2024 11:38:53
Acquired by	System Administrator
Filename	C:\Users\dopnlab\Desktop\MASAUŞTU\sazan\hic\hic-3e1.ispd
Spectrum name	hic-3e1
Sample name	hic-3e
Sample ID	
Option	
Comment	
No. of Scans	30
Resolution	4 [cm-1]
Apodization	Happ-Genzel

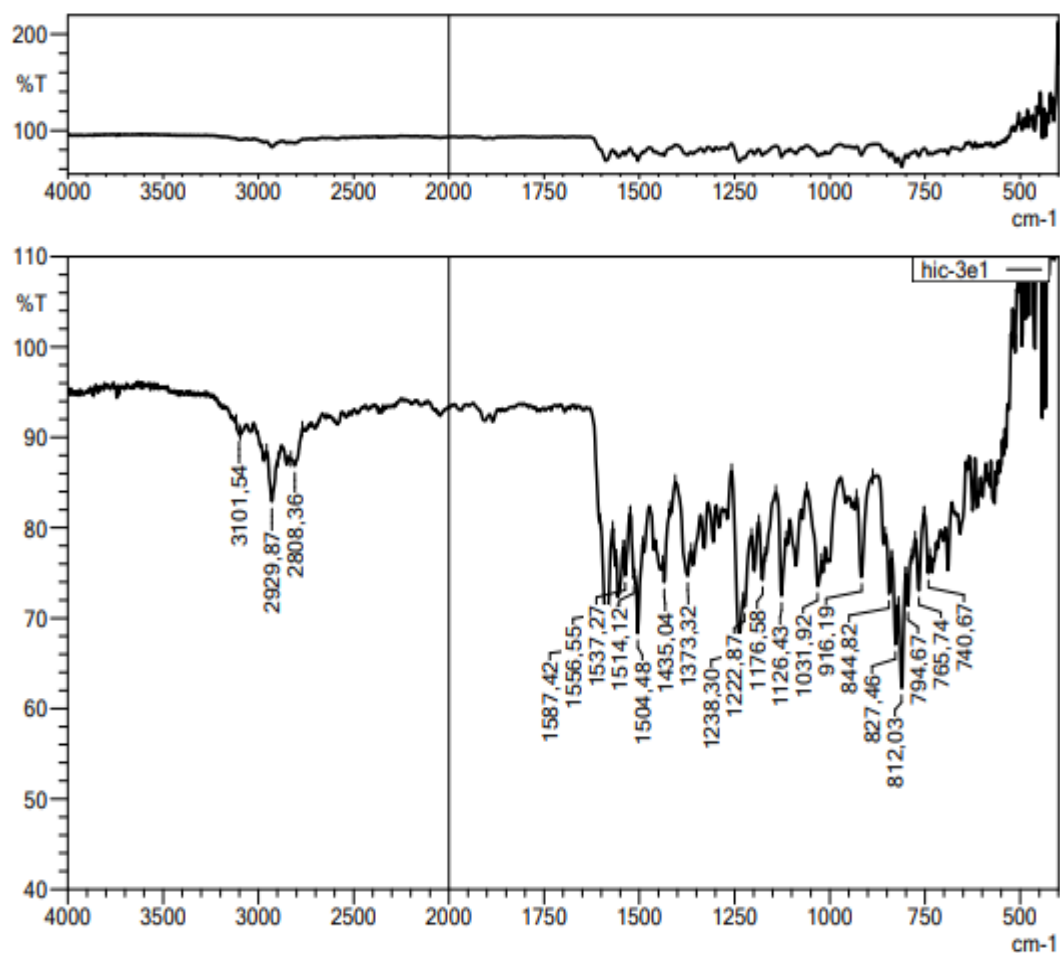


Figure 5.113. IR fingerprint of D3.e.

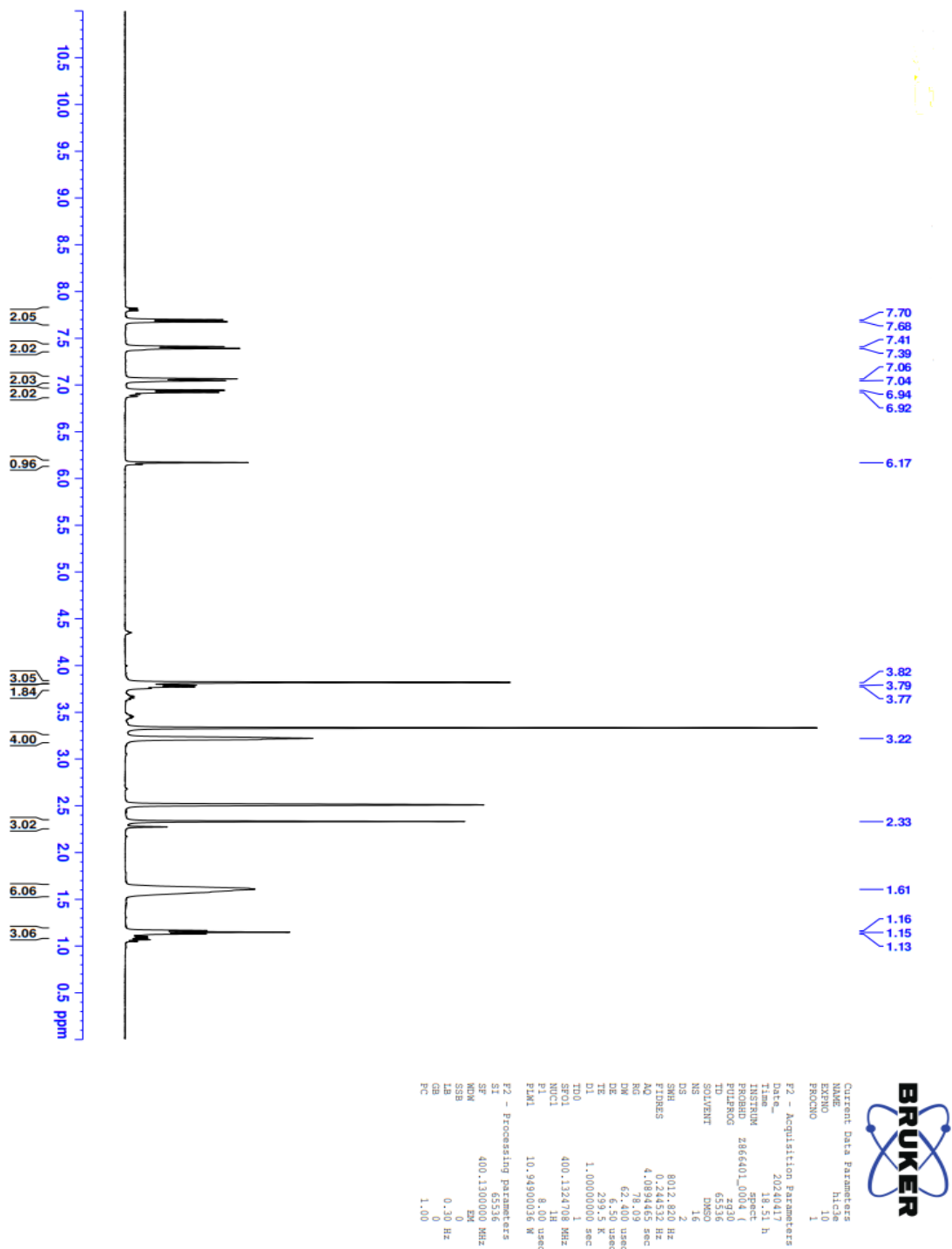


Figure 5.114. ¹H NMR signals of D3.e.

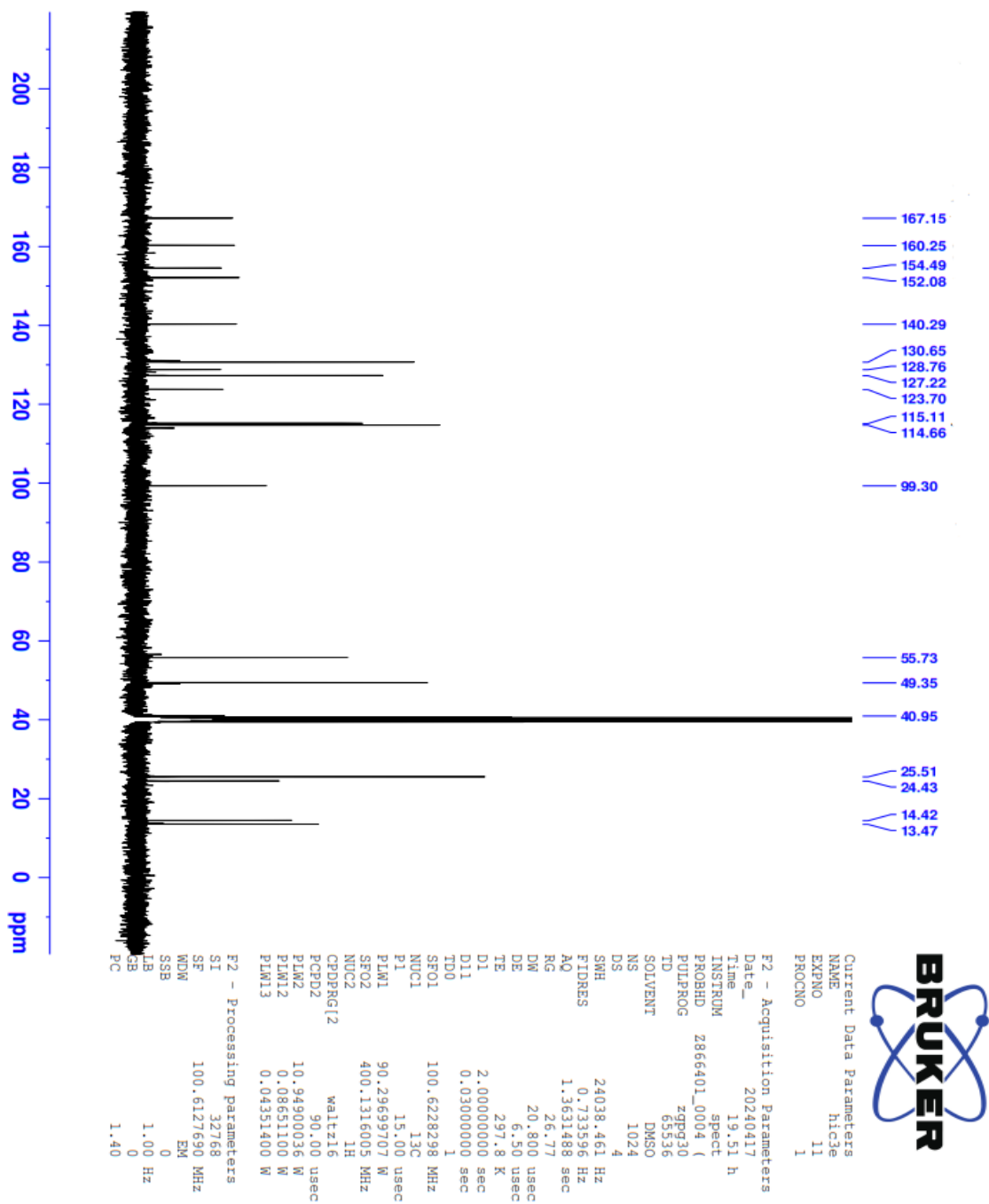


Figure 5.115. ¹³C NMR signals of D3.e.

Data File: C:\LabSolutions\Data\Analiz\denya\HIC-3E_4 lod

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H	1	8	33	O	2	0	3	S	2	1	1	Ru	2	0	0	H
C	4	4	32	F	1	0	0	Cl	1	0	0	Pd	2	0	0	Na
N	3	0	5	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5

HC Ratio: unlimited

Max Isotopes: 3

MSn Iso RI (%): 10.00

DBE Range: 0.0 - 30.0

Apply N Rule: no

Isotope RI (%): 1.00

MSn Logic Mode: AND

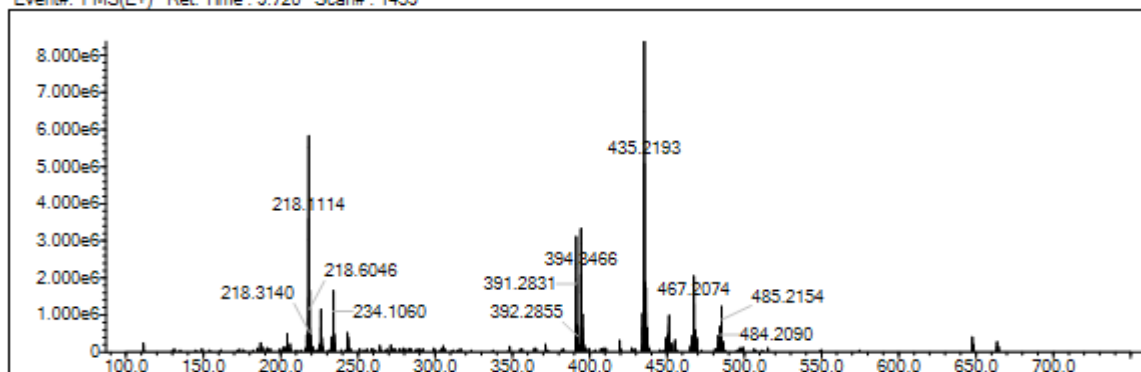
Electron Ions: both

Use MSn Info: yes

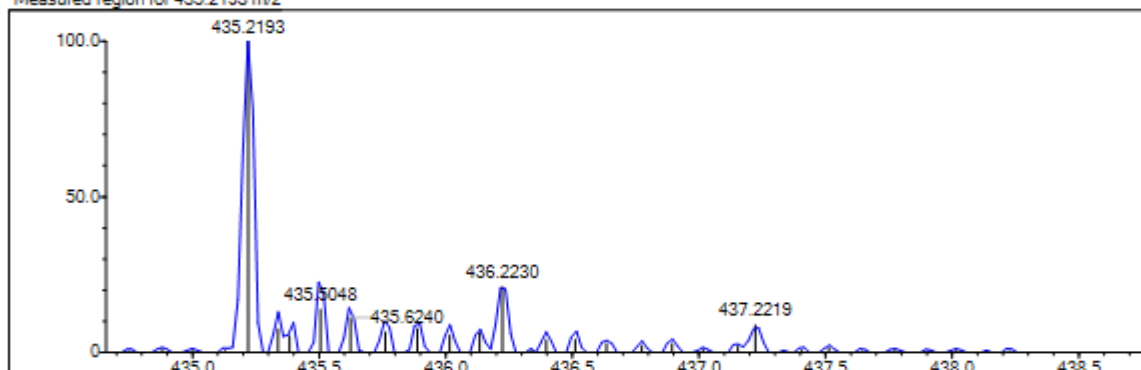
Isotope Res: 9000

Max Results: 50

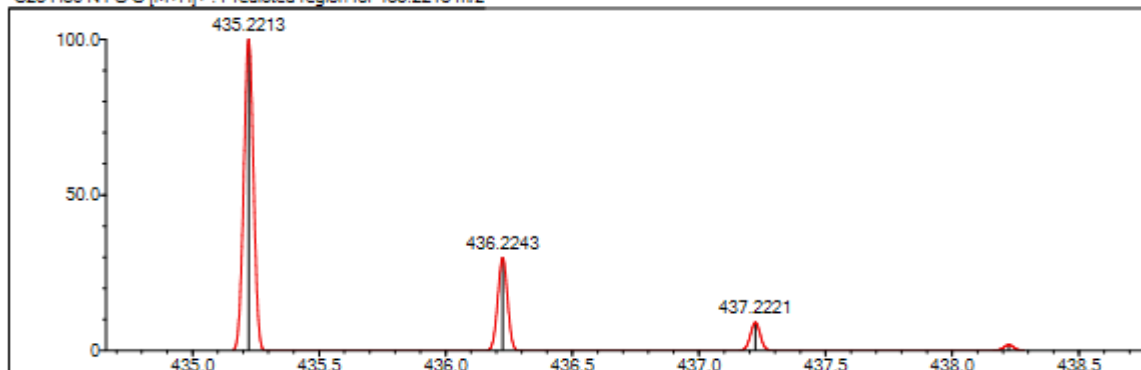
Event#: 1 MS(E+) Ret. Time : 9.720 Scan#: 1459



Measured region for 435.2193 m/z



C25 H30 N4 O S [M+H]⁺: Predicted region for 435.2213 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	73.23	C25 H30 N4 O S	[M+H] ⁺	435.2193	435.2213	-2.0	-4.60	80.48	13.0

Figure 5.116. HRMS spectrogram of D3.e.

5.1.3.6. 4-(-3-Ethyl-2-((-1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-2,3-dihydrothiazol-4-yl)benzonitrile

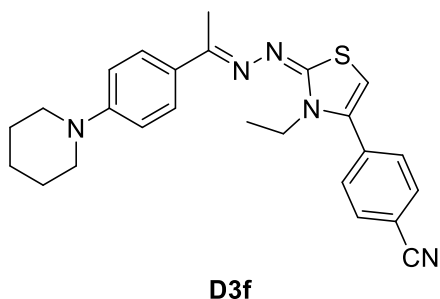


Figure 5.117. 4-(-3-Ethyl-2-((-1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-2,3-dihydrothiazol-4-yl) benzonitrile.

Yield: 75%.

Melting Point: 166.3°C.

IR Maximum Velocity (cm^{-1}): 3074 (C=C-H stretching band), 2929 (C-H stretching band), 2225 (Nitrile-CN stretching), 1585 (C=N stretching band), 1556 (C=C band), 1238 (C-N stretching band), 819 (4-substituted phenyl out-of-plane deformation band).

$^1\text{H-NMR}$ (300MHz, $\text{DMSO-}d_6$): 1.15 (3H, br.s, methyl-H), 1.60 (6H, s, piperidine-H), 2.34 (3H, s, methyl-H), 3.22 (4H, br.s, piperidine-H), 3.80-3.82 (2H, q, $J = 5.61$ Hz, methylene-H), 6.48 (1H, s, dihydrothiazole-H), 6.93-6.95 (2H, d, $J = 7.79$ Hz, Ar-H), 7.69 (4H, br.s, Ar-H), 7.97-7.99 (2H, d, $J = 6.95$ Hz, Ar-H).

$^{13}\text{C-NMR}$ (75 MHz, $\text{DMSO-}d_6$): $\delta = 12.64, 13.66, 14.33, 15.36, 23.79, 25.48, 41.40, 49.26, 51.10, 101.54, 112.03, 113.98, 116.14, 118.94, 119.00, 126.21, 128.39, 128.68, 130.86, 132.17, 134.42, 136.04, 138.96, 152.15, 155.21, 167.15$.

HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{25}\text{H}_{27}\text{N}_5\text{S}$ $[\text{M}+\text{H}]^+$: 430.2060; found: 430.2040.

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Sample ID	
Option	
Comment	
No. of Scans	30
Resolution	4 [cm-1]
Apodization	Happ-Genzel

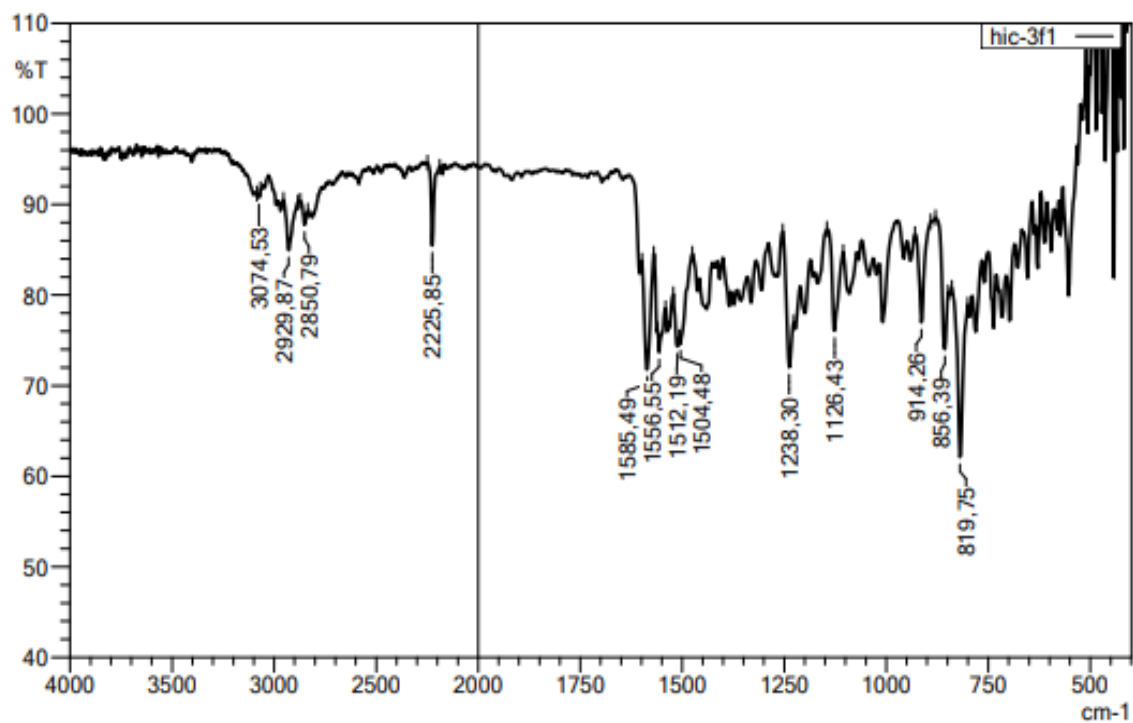
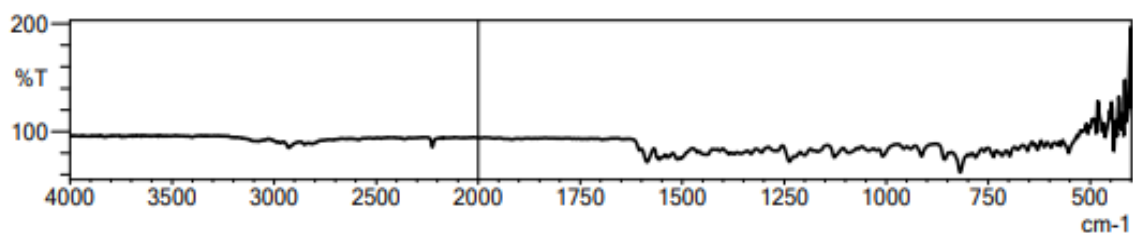


Figure 5.118. IR fingerprint of D3.f.

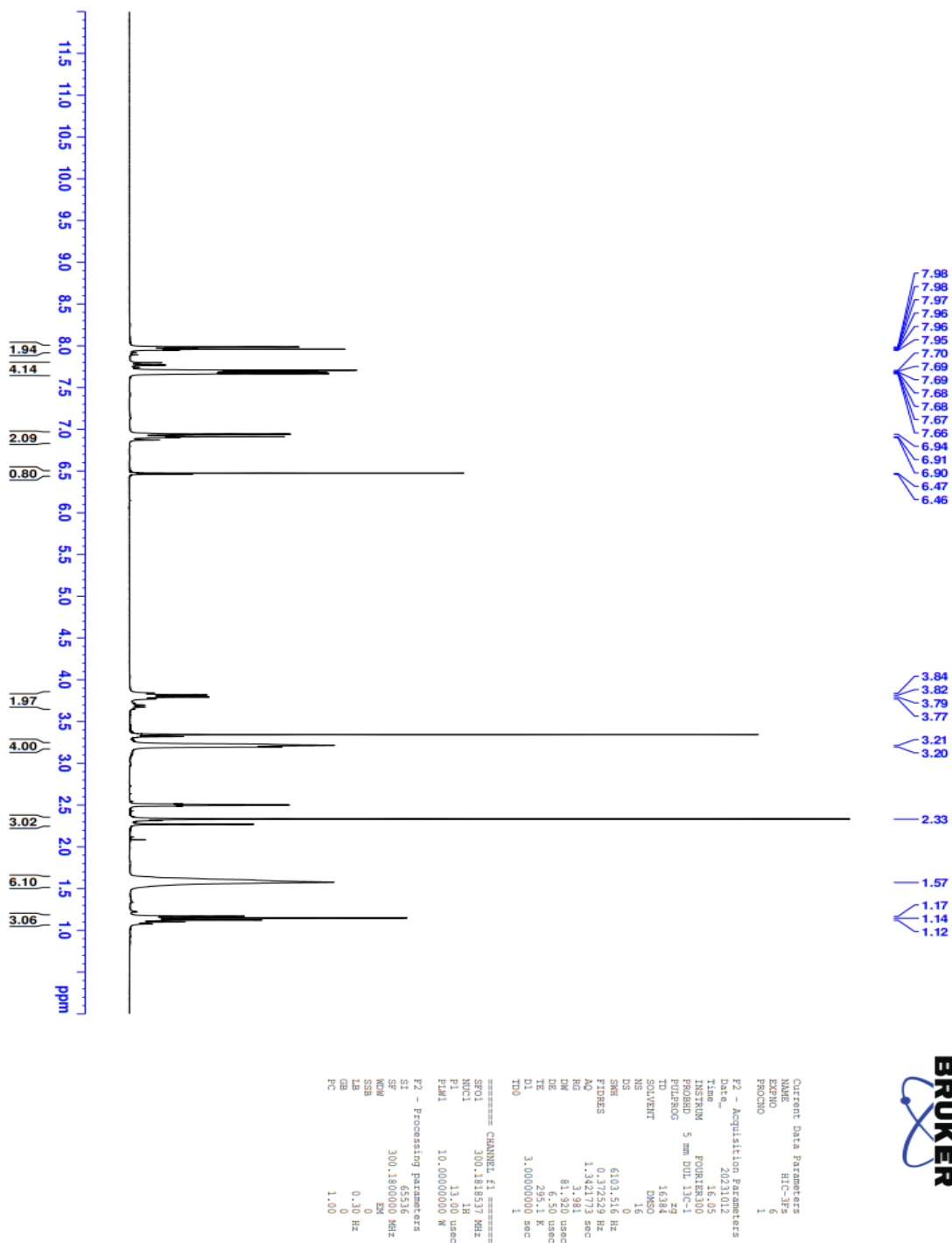
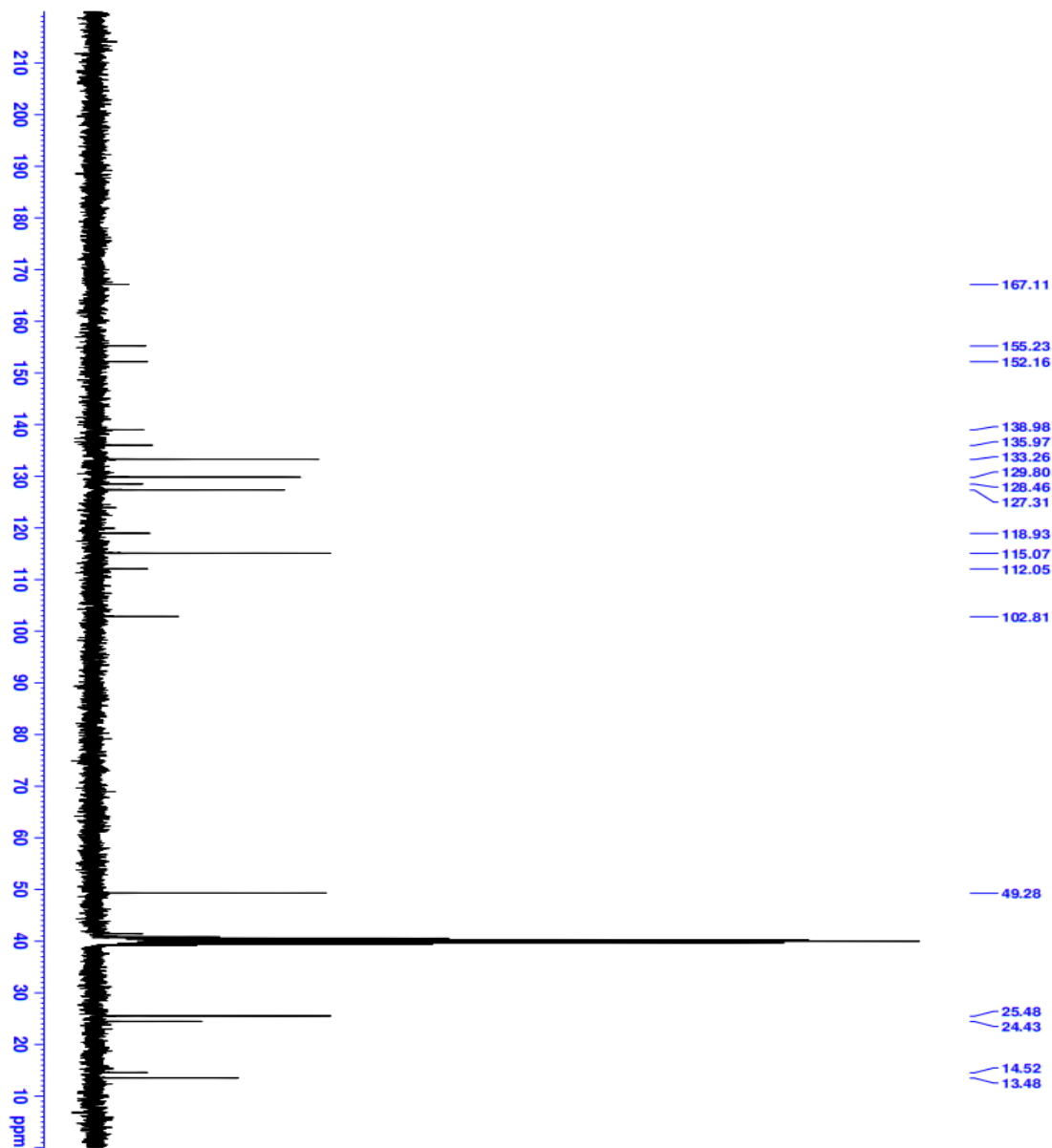


Figure 5.119. ¹H NMR signals of D3.f.



```

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EXPERNO   7
PROCNO    1

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PULPROG   zgpg30
TD        65536
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NS         2048
DS         4
SWH        24414.063 Hz
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AQ         0.6710886 sec
RG         501.187
DE         20.480 usec
TE         295.6 K
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D11        0.03000000 sec
D12        0.03000000 sec
D13        0.88000000 sec
D14        0.00093990 sec
L4         23
L5         26
L6         26
P12        90.00 usec
TD0        1

===== CHANNEL f1 =====
SFO1       75.487687 MHz
NUC1       13C
P1         15.50 usec
PL1        0.00000000 W

===== CHANNEL f2 =====
SFO2       300.1812007 MHz
NUC2       1H
P2         90.00 usec
PL2        0.00000000 W
PLW2       0.20863999 W
PLW3       0.10495000 W

F2 - Processing parameters
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RG         65536
DE         0
SSB        0
LB         1.00 Hz
GB         0
PC         1.40
  
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Figure 5.120. ¹³CNMR signals of D3.f.

Data File: C:\LabSolutions\Data\Analiz\derya\HIC-3F_7 lod

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C	4	4	32	F	1	0	0	Cl	1	0	0	Pd	2	0	0	Na
N	3	0	5	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5

HC Ratio: unlimited

Max Isotopes: 3

MSn Iso RI (%): 10.00

DBE Range: 0.0 - 30.0

Apply N Rule: no

Isotope RI (%): 1.00

MSn Logic Mode: AND

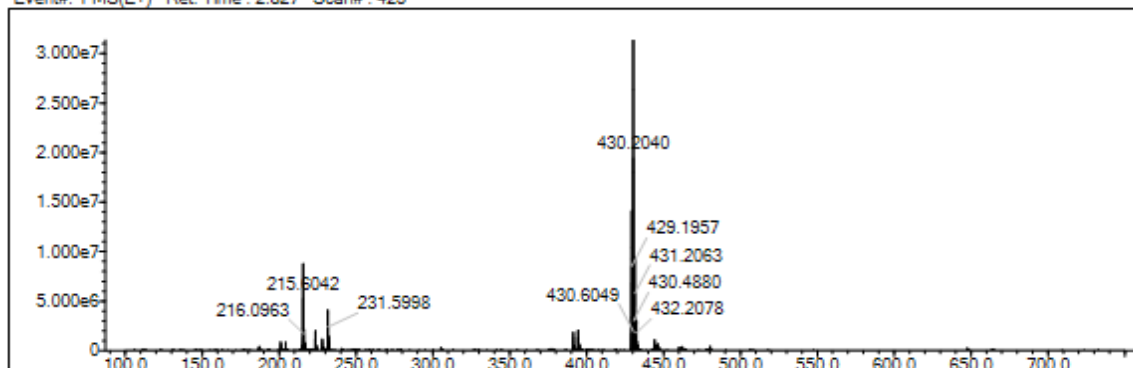
Electron Ions: both

Use MSn Info: yes

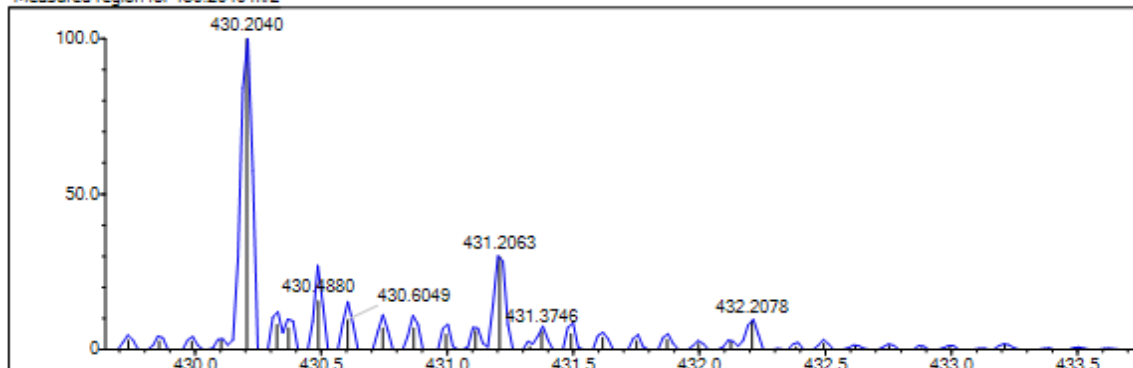
Isotope Res: 9000

Max Results: 50

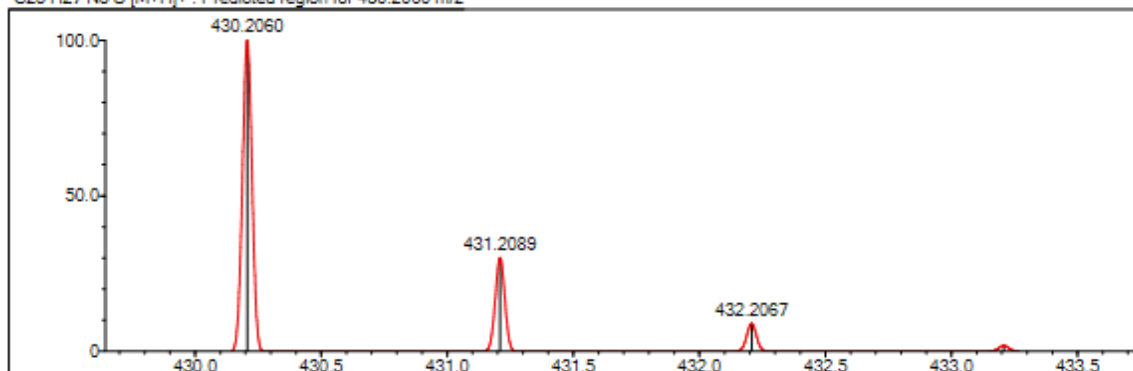
Event#: 1 MS(E+) Ret. Time : 2.827 Scan#: 425



Measured region for 430.2040 m/z



C25 H27 N5 S [M+H]⁺ : Predicted region for 430.2060 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
3	83.27	C25 H27 N5 S	[M+H] ⁺	430.2040	430.2060	-2.0	-4.65	91.63	15.0

Figure 5.121. HRMS spectrogram of D3.f.

5.1.3.7. 4-(2,4-Dichlorophenyl)-3-ethyl-2-((1-(4-(piperidin-1 yl)phenyl) ethylidene)hydrazineylidene)-2,3-dihydrothiazole

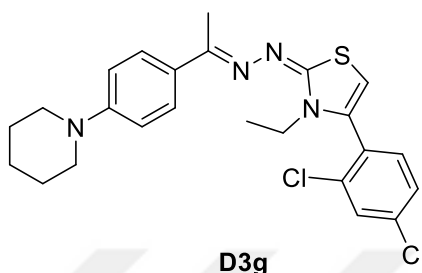


Figure 5.122. 4-(2,4-Dichlorophenyl)-3-ethyl-2-((1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-2,3-dihydrothiazole.

Yield: 80%.

Melting Point: 163.7°C.

IR Maximum Velocity (cm^{-1}): 3100 (C=C-H stretching band), 2922 (C-H stretching band), 1589 (C=N stretching band), 1573 (C=C band), 1234 (C-N stretching band), 812 (2,4-disubstituted phenyl out-of-plane deformation band).

$^1\text{H-NMR}$ (300MHz, $\text{DMSO-}d_6$): 1.02-1.06 (3H, t, $J= 6.90\text{Hz}$, methyl-H), 1.60 (6H, br.s, piperidine-H), 2.30 (3H, s, methyl-H), 3.21 (4H, s, piperidine-H), 3.17-3.20 (2H, m, methylene-H), 6.91-6.93 (2H, d, $J= 8.67\text{ Hz}$, Ar-H), 7.24(1H,s, dihydrothiazole-H), 7.54-7.56 (1H, m, Ar-H), 7.66-7.69 (3H, m, Ar-H), 7.93-7.96 (2H, d, $J= 8.58\text{ Hz}$, Ar-H).

$^{13}\text{C-NMR}$ (75 MHz, $\text{DMSO-}d_6$): $\delta = 13.46, 14.41, 24.43, 25.53, 49.29, 93.35, 115.02, 127.26, 127.75, 128.63, 128.84, 131.03, 131.55, 133.26, 134.50, 138.37, 152.16, 155.62, 165.54$.

HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{24}\text{H}_{26}\text{N}_4\text{S Cl}_2$ $[\text{M}+\text{H}]^+$: 473.1328; found:473.1319.

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Acquired by	System Administrator
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Spectrum name	hic-3h1
Sample name	hic-3h
Sample ID	
Option	
Comment	
No. of Scans	30
Resolution	4 [cm ⁻¹]
Apodization	Happ-Genzel

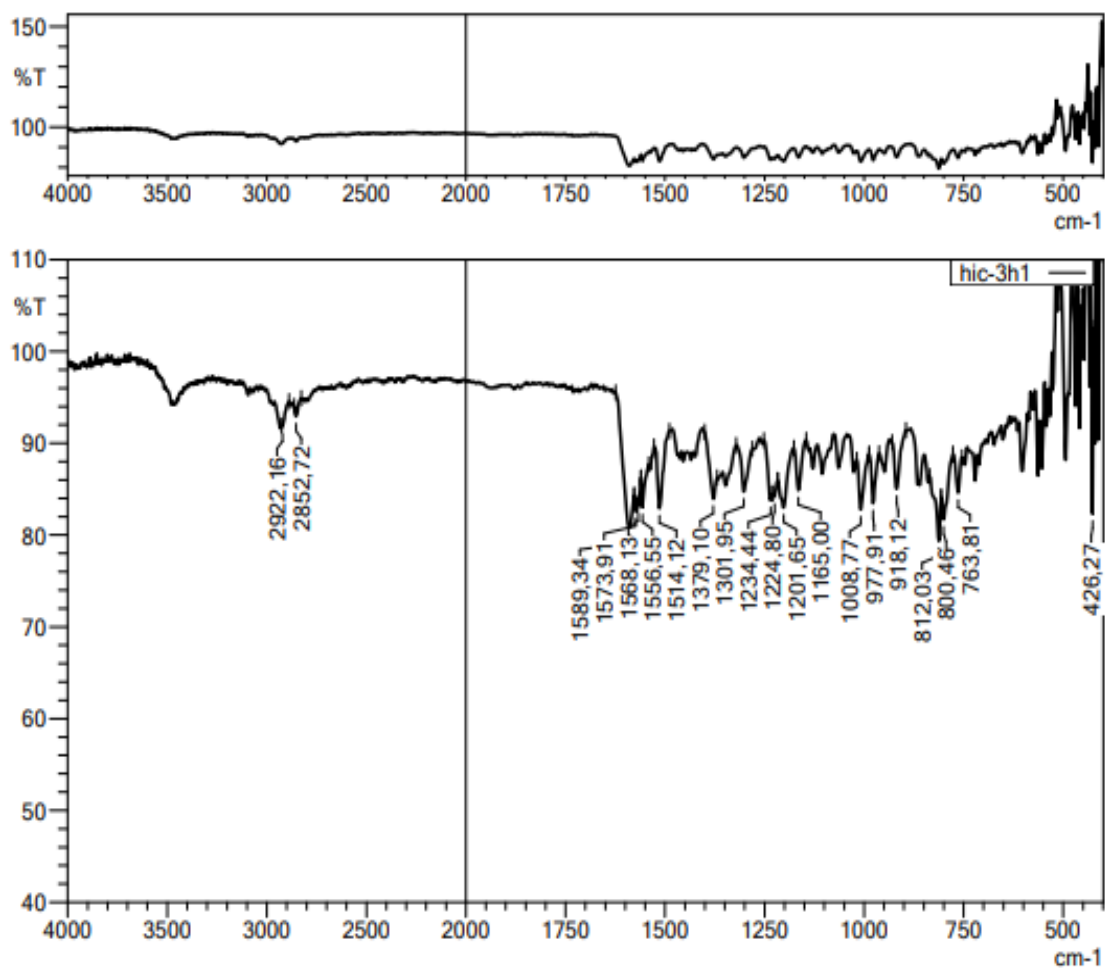


Figure 5.123. IR spectrum of compound D3.g.

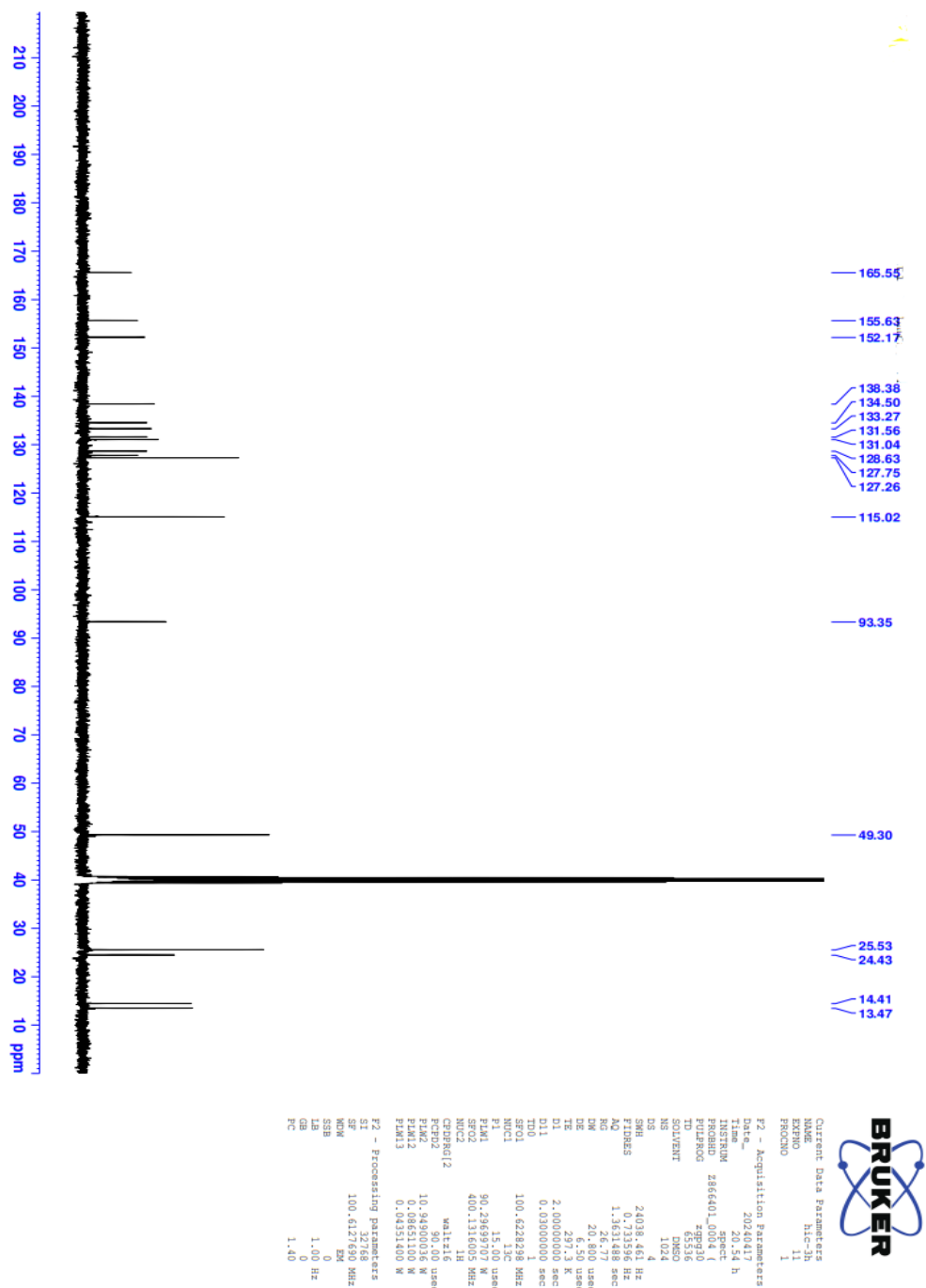


Figure 5.125. ¹³CNMR signals of compound D3.g.

Data File: C:\LabSolutions\Data\Analiz\derya\MEOH_581.lcd

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C	4	4	32	F	1	0	0	Cl	1	2	2	Pd	2	0	0	Na
N	3	0	5	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5

HC Ratio: unlimited

Max Isotopes: 3

MSn Iso RI (%): 10.00

DBE Range: 0.0 - 30.0

Apply N Rule: no

Isotope RI (%): 1.00

MSn Logic Mode: AND

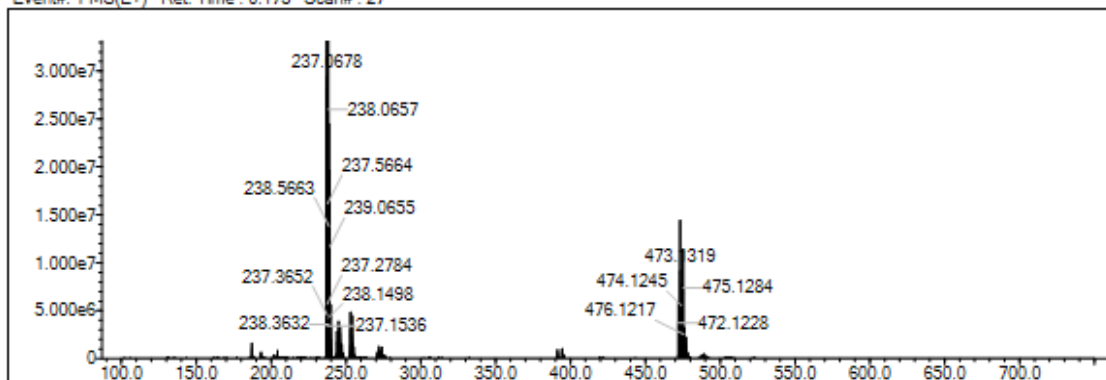
Electron Ions: both

Use MSn Info: yes

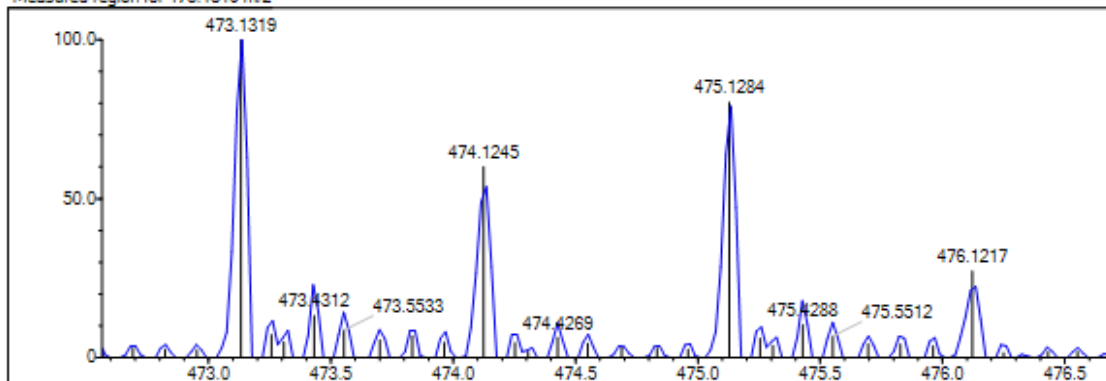
Isotope Res: 9000

Max Results: 50

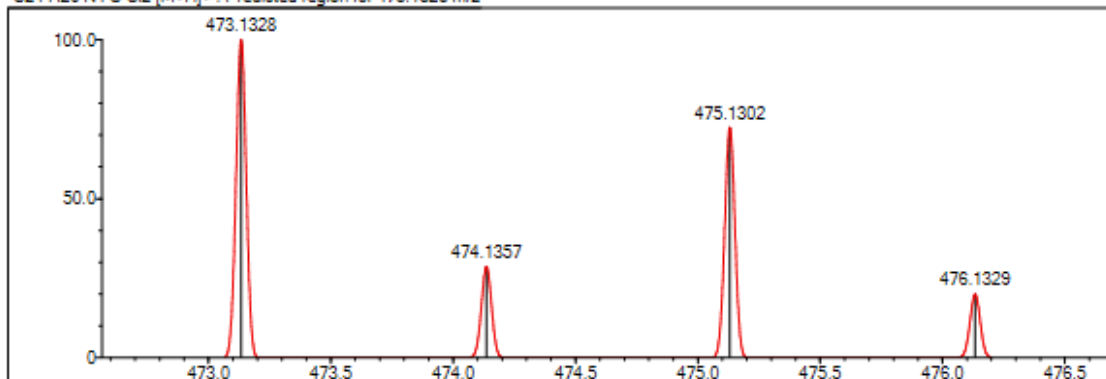
Event#: 1 MS(E+) Ret. Time : 0.173 Scan#: 27



Measured region for 473.1319 m/z



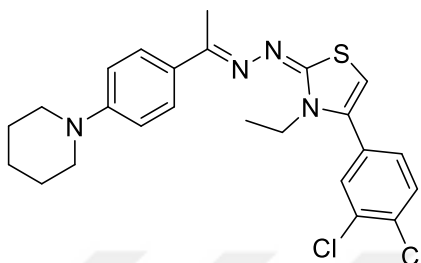
C24 H26 N4 S Cl2 [M+H]⁺ : Predicted region for 473.1328 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
3	35.30	C24 H26 N4 S Cl2	[M+H] ⁺	473.1319	473.1328	-0.9	-1.90	36.11	13.0

Figure 5.126. HRMS spectrogram of D3.g.

5.1.3.8. 4-(3,4-Dichlorophenyl)-3-ethyl-2-((1-(4-(piperidin-1 yl)phenyl)ethylidene)hydrazineylidene)-2,3-dihydrothiazole



D3h

Figure 5.127. 4-(3,4-Dichlorophenyl)-3-ethyl-2-((1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-2,3-dihydrothiazole.

Yield: 73%.

Melting Point: 138.36°C.

IR Maximum Velocity (cm^{-1}): 3099 (C=C-H stretching band), 2935 (C-H stretching band), 1591 (C=N stretching band), 1541 (C=C band), 1236 (C-N stretching band), 819 (3,4-disubstituted phenyl out-of-plane deformation band).

^1H -NMR(300MHz, $\text{DMSO}-d_6$): 1.11-1.16 (3H, t, $J = 6.99\text{Hz}$, methyl-H), 1.57 (6H, br.s, piperidine-H), 2.32 (3H, s, methyl-H), 3.21 (4H, br.s, piperidine-H), 3.76-3.82 (2H, q, $J = 6.83\text{Hz}$, methylene-H), 6.41(1H,s, dihydrothiazole-H), 6.90-6.93 (2H, d, $J = 7.49\text{ Hz}$, Ar-H), 7.46-7.49 (1H, d, $J = 8.28\text{ Hz}$, Ar-H), 7.66-7.69 (2H, d, $J = 8.63\text{ Hz}$, Ar-H), 7.74-7.78 (2H, d, $J = 13.4\text{ Hz}$, Ar-H).

^{13}C -NMR (75 MHz, $\text{DMSO}-d_6$): $\delta = 13.48, 14.46, 24.42, 25.50, 41.99, 49.30, 94.27, 102.03, 114.80, 115.06, 127.28, 128.07, 128.53, 129.31, 131.03, 131.40, 131.97, 132.38, 137.94, 144.34, 152.13, 152.23, 155.03, 156.30, 165.36, 166.97$.

HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{24}\text{H}_{26}\text{N}_4\text{S}\text{Cl}_2$ $[\text{M}+\text{H}]^+$: 473.1328; found: 473.1319.

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Sample ID	
Option	
Comment	
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Resolution	4 [cm-1]
Apodization	Happ-Genzel

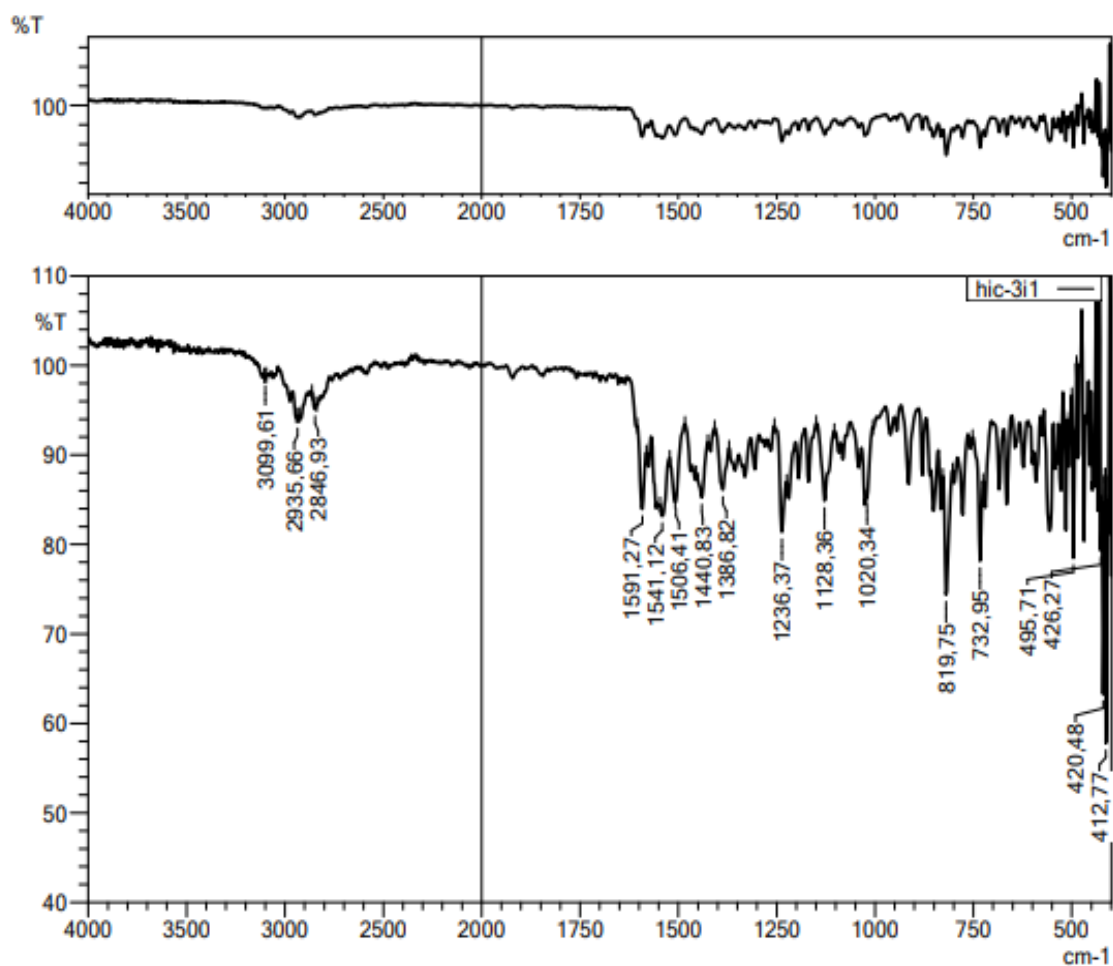


Figure 5.128. IR fingerprint of D3.h.

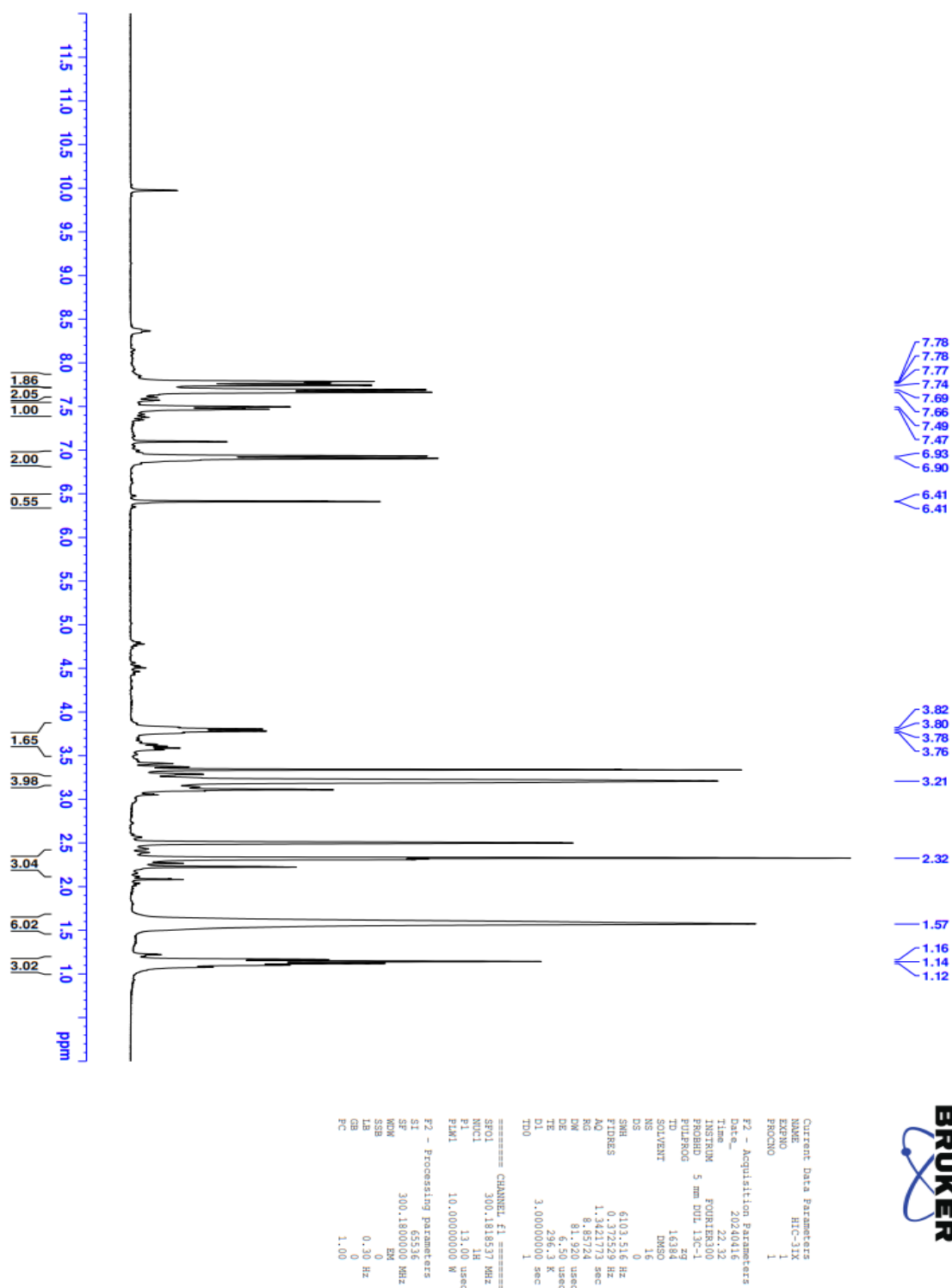


Figure 5.129. ¹H NMR signals of D3.h.

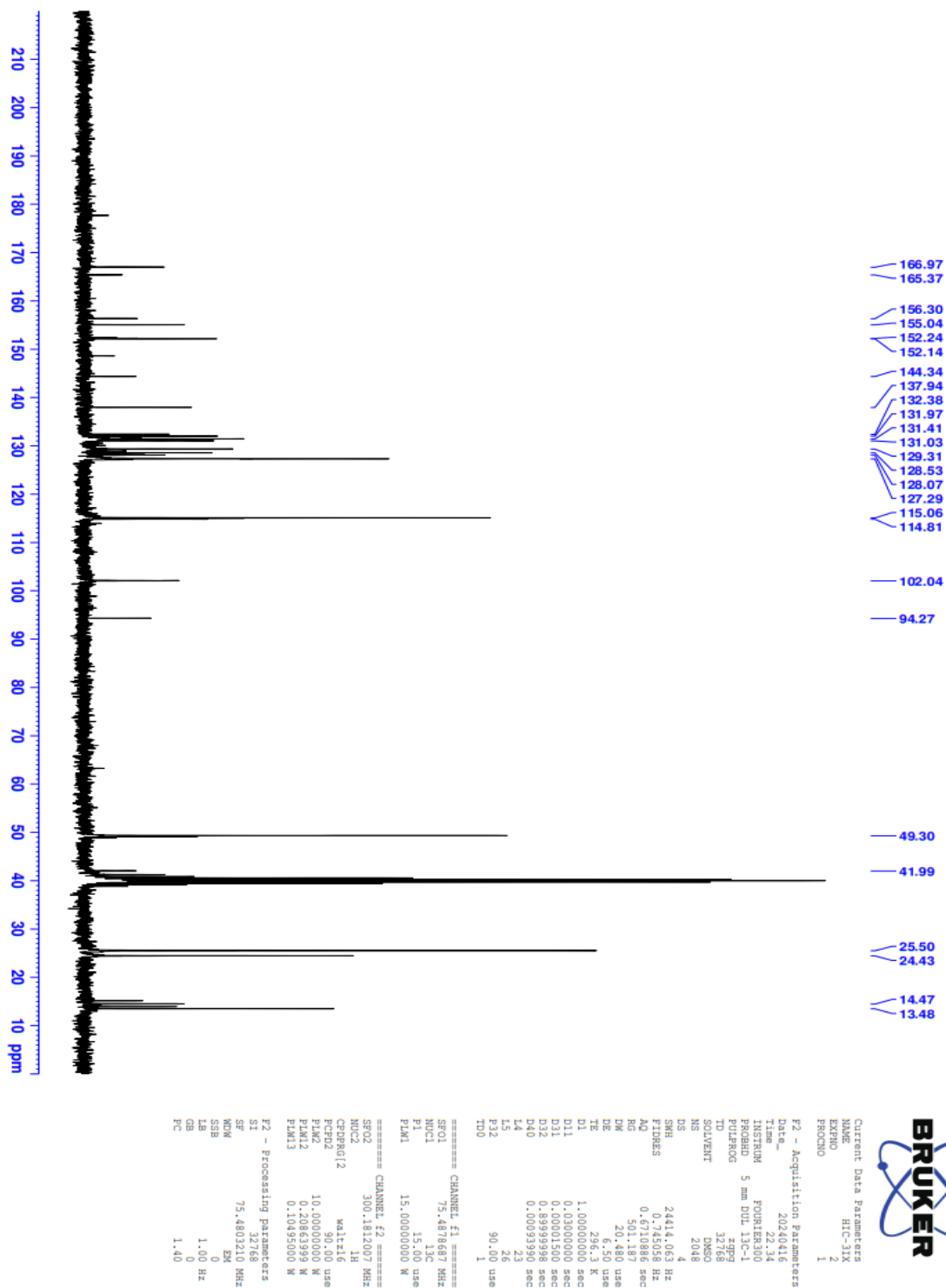


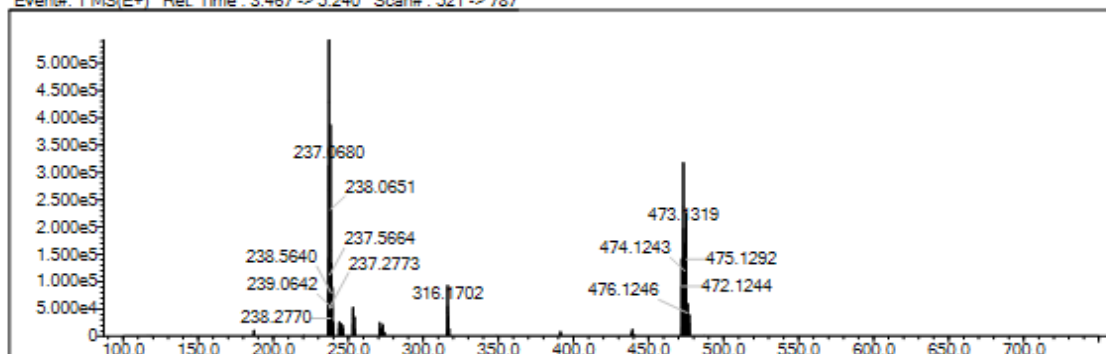
Figure 5.130. ^{13}C NMR signals of D3.h.

Data File: C:\LabSolutions\Data\Analiz\derya\MEOH_632.lcd

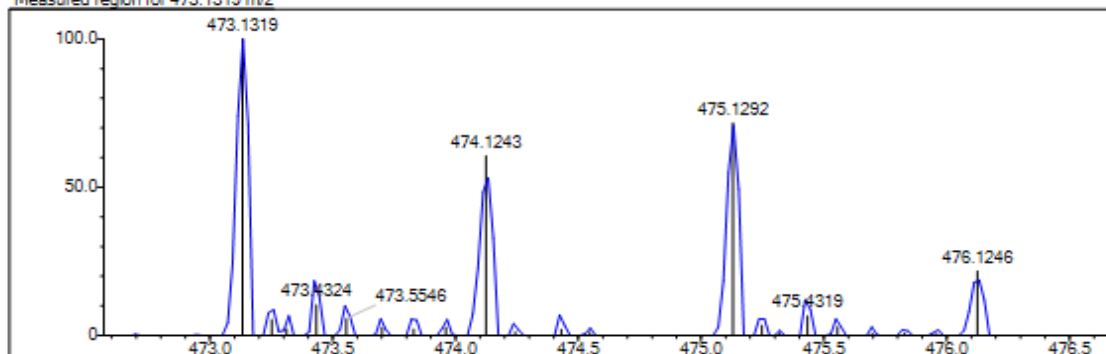
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C	4	4	32	F	1	0	0	Cl	1	2	2	Pd	2	0	0	
N	3	0	5	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5
 DBE Range: 0.0 - 20.0
 Electron Ions: both
 HC Ratio: unlimited
 Apply N Rule: no
 Use MSn Info: yes
 Max Isotopes: 3
 Isotope RI (%): 1.00
 MSn Iso RI (%): 10.00
 MSn Logic Mode: AND
 Isotope Res: 9000
 Max Results: 50

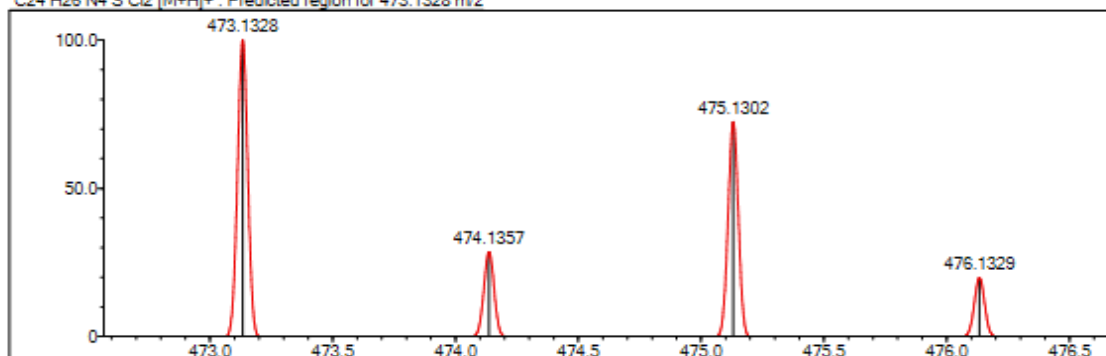
Event#: 1 MS(E+) Ret. Time : 3.467 -> 5.240 Scan#: 521 -> 787



Measured region for 473.1319 m/z



C24 H26 N4 S Cl2 [M+H]⁺ : Predicted region for 473.1328 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	39.70	C24 H26 N4 S Cl2	[M+H] ⁺	473.1319	473.1328	-0.9	-1.90	40.61	13.0

Figure 5.131. HRMS spectrogram of D3.h.

5.1.3.9. 4-(2,4-Dimethylphenyl)-3-ethyl-2-((1-(4-(piperidin-1-yl)phenyl) ethylidene) hydrazineylidene)-2,3-dihydrothiazole

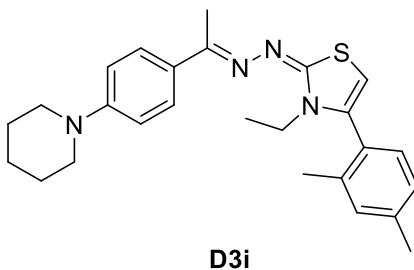


Figure 5.132. 4-(2,4-Dimethylphenyl)-3-ethyl-2-((1-(4-(piperidin-1-yl)phenyl)ethylidene)hydrazineylidene)-2,3-dihydrothiazole.

Yield: 68%.

Melting Point: 158.9°C.

IR Maximum Velocity (cm^{-1}): 3115 (C=C-H stretching band), 2935 (C-H stretching band), 1595 (C=N stretching band), 1573 (C=C stretching band), 1236 (C-N stretching band), 819 (2,4-disubstituted phenyl out-of-plane deformation band).

^1H -NMR(300MHz, $\text{DMSO}-d_6$): 0.98-1.02 (3H, t, $J = 6.83\text{Hz}$, methyl-H), 1.55-1.57 (6H, d, piperidine-H), 2.16 (3H, s, methyl-H), 2.26-2.31 (6H, s, methyl-H), 3.20 (4H, d, piperidine-H), 3.35 (2H, s, methylene-H), 6.11 (1H, s, dihydrothiazole-H), 6.84-6.93(2H, m, Ar-H), 7.11-7.18 (3H, m, Ar-H), 7.66-7.69 (1H, d, $J = 8.95$, Ar-H), 7.79-7.82 (1H, d, $J = 8.96$, Ar-H).

^{13}C -NMR (75 MHz, $\text{DMSO}-d_6$): $\delta = 13.56, 14.46, 24.42, 25.50, 48.27, 49.02, 49.33, 99.38, 113.91, 114.48, 115.11, 126.56, 127.06, 127.18, 127.61, 128.10, 128.78, 130.76, 131.03, 131.30, 132.57, 133.36, 135.80, 137.67, 138.61, 152.37, 154.35, 166.60$.

HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{26} \text{H}_{32} \text{N}_4 \text{S}$ $[\text{M}+\text{H}]^+$:433.2423; found:433.2420.

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Sample ID	
Option	
Comment	
No. of Scans	30
Resolution	4 [cm-1]
Apodization	Happ-Genzel

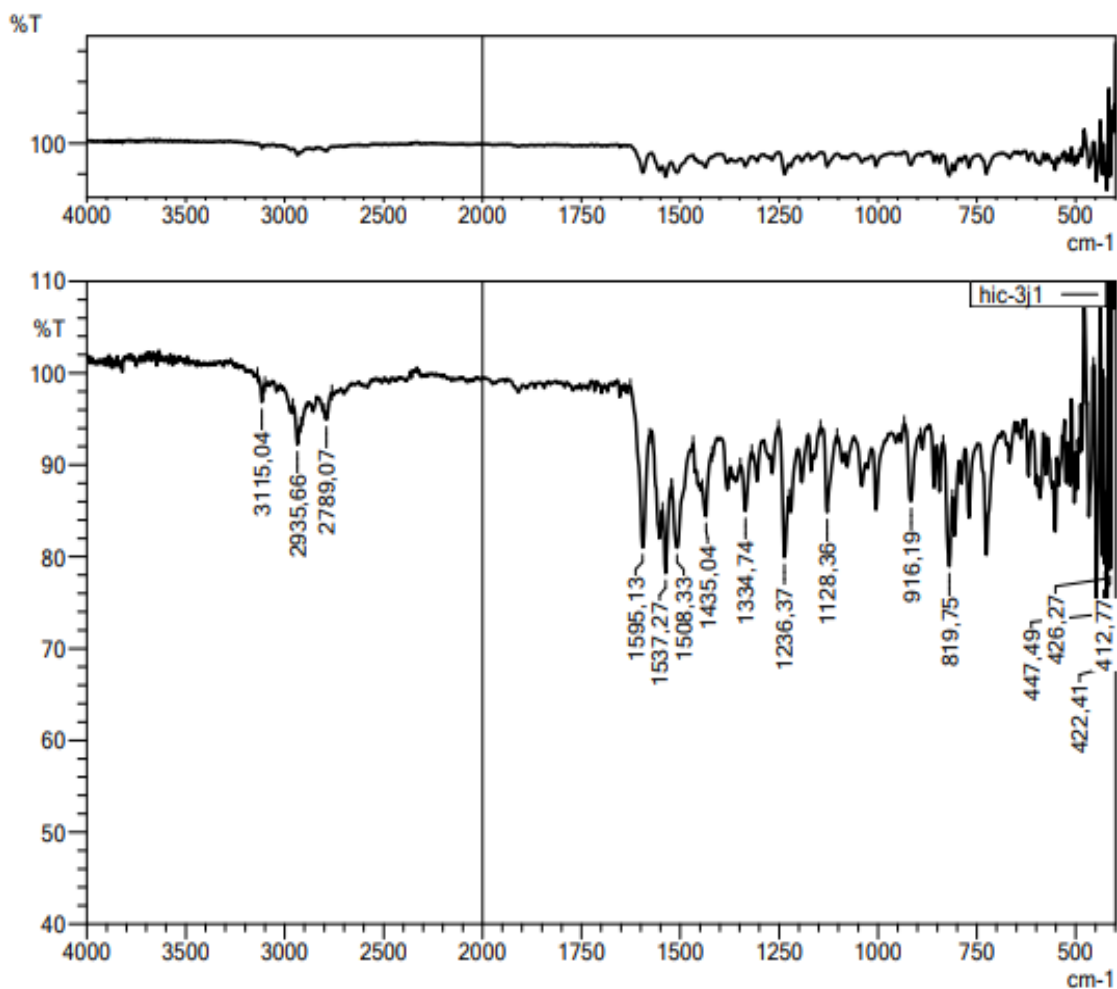


Figure 5.133. IR fingerprint of D3.i.

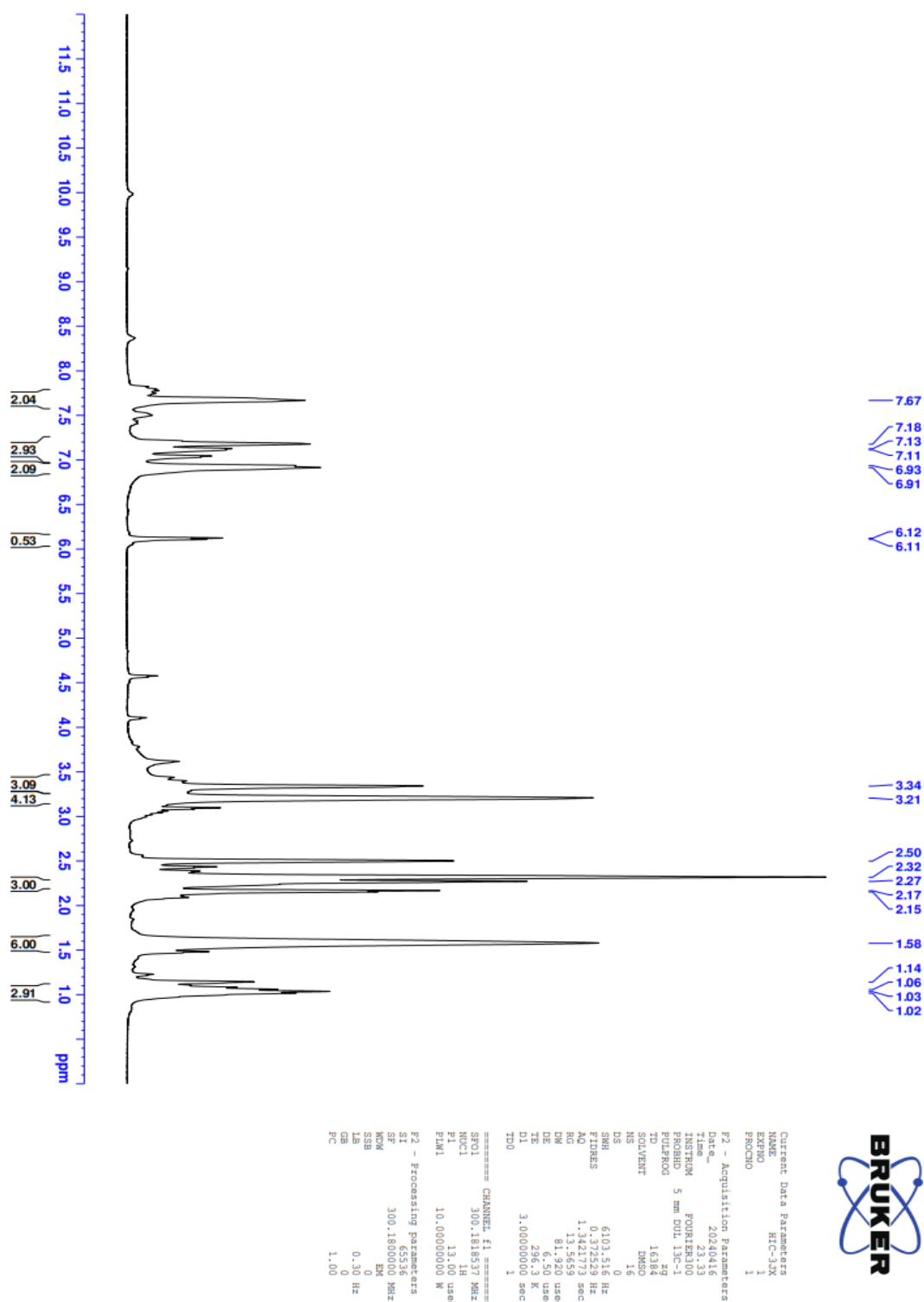


Figure 5.134. ¹H NMR signals of D3.i.



Figure 5.135. ^{13}C NMR signals of D3.i.

Data File: C:\LabSolutions\Data\Analz\data\HIC-3JX_621.lod

Elmt	Val	Min	Max	Elmt	Val	Min	Max	Elmt	Val	Min	Max	Elmt	Val	Min	Max	Use Adduct
H	1	8	33	O	2	0	4	S	2	0	2	Ru	2	0	0	H
C	4	4	30	F	1	0	0	Cl	1	0	0	Pd	2	0	0	
N	3	0	5	P	3	0	0	Br	1	0	0	I	3	0	0	

Error Margin (ppm): 5

HC Ratio: unlimited

Max Isotopes: 3

MSn Iso RI (%): 10.00

DBE Range: 0.0 - 50.0

Apply N Rule: no

Isotope RI (%): 1.00

MSn Logic Mode: AND

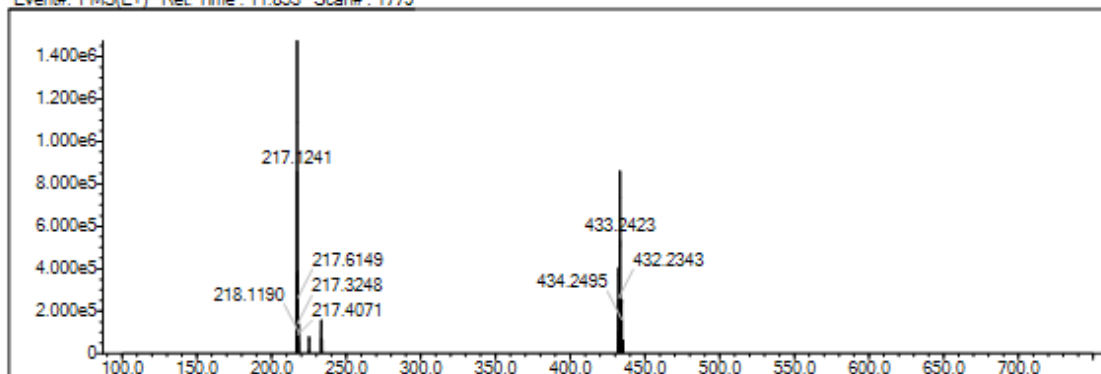
Electron Ions: both

Use MSn Info: yes

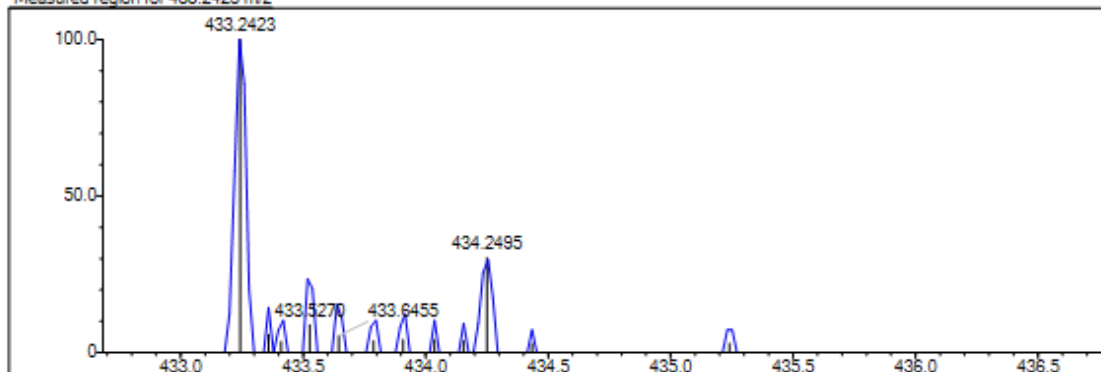
Isotope Res: 9000

Max Results: 50

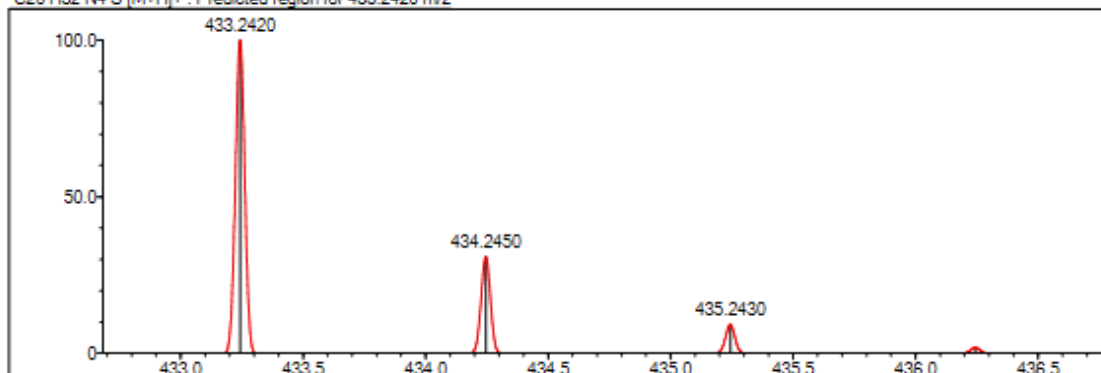
Event#: 1 MS(E+) Ret. Time : 11.853 Scan#: 1779



Measured region for 433.2423 m/z



C26 H32 N4 S [M+H]⁺ : Predicted region for 433.2420 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	90.38	C26 H32 N4 S	[M+H] ⁺	433.2423	433.2420	0.3	0.69	90.38	13.0

Figure 5.136. HRMS spectrogram of D3.i.

5.2. Spectroscopic Evaluation Study of the Synthesized Compounds D1-D3(a-i).

The infrared spectra analysis of the synthesized compounds confirms the successful condensation between the carbonyl functional group of ketone and the thiosemicarbazide derivatives. This is evidenced by the disappearance of absorption bands corresponding to both NH groups and carbonyl groups. The spectra also reveal characteristic stretching vibration absorption bands including C=N, N-N, C-N, and C=C bonds of both aliphatic and aromatic moieties.

Moreover, the compounds exhibit =C-H and C-H (sp^3 and sp^2) bands in the range of 3100-2800 cm^{-1} with medium intensity. The most prominent functional group is the C-N band, which appears as a sharp medium peak around 1250-1000 cm^{-1} in all synthesized compounds. Strong bands around 1650-1550 cm^{-1} indicate the presence of C=C bonds in both aliphatic and aromatic structures.

Furthermore, the appearance of a medium sharp peak around 1650-1500 cm^{-1} signifies the presence of C=N bonds directly bonded to the dihydrothiazole moiety. A significant observation is the disappearance of carbonyl functional group stretching bands around 1750-1700 cm^{-1} , originating from bromoacetophenones involved in cyclization with the thiosemicarbazone moiety.

The ^1H -NMR spectra of all compounds exhibit several distinctive features. Firstly, two broad single peaks are observed at 1.55-1.60 ppm, representing the presence of 6H atoms adjacent to the piperidine ring, and another broad single peak around 3.20-3.22 ppm, indicating the existence of 4H atoms proximal to the N atom of the piperidine ring.

One of the significant characteristic peaks is the presence of a single proton at a downfield region near the aromatic fields, attributed to the thiazole ring, typically occurring at 6-7 ppm. The aromatic proton peaks in all spectra manifest around 6.89-8.35 ppm.

Additionally, the three protons of the methyl group of the ethylidene moiety in most compounds resonate at 3.20-3.22 ppm. Notably, the most prominent disparity among the ^1H -NMR spectra lies in the R group linked to the N atom of the dihydrothiazole ring:

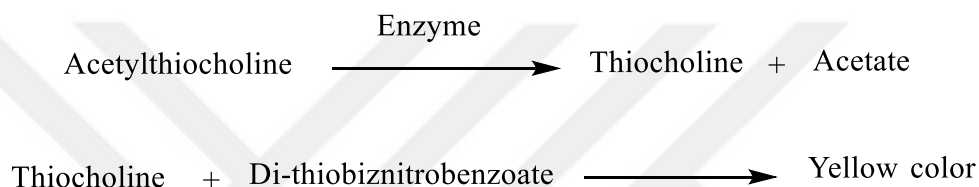
- In compounds **D1.a-i**, a methyl group appears as a single strong peak at 2.28-2.32 ppm.
- In **D2.a-i**, an allyl moiety is observed, represented by three distinct peaks around 4-5.5 ppm, each corresponding to different five hydrogen atoms.
- In compounds **D3.a-i**, an ethyl group is present, depicted by a triplet peak (3H atoms- CH_3) at 1.5 ppm and a quartet of peaks around 4 ppm representing (CH_2) atoms in all compounds.

The ^{13}C -NMR spectra of the compounds corroborate the infrared findings by indicating the absence of carbonyl carbon peak signals in the downfield region (170-200 ppm). This absence aligns with the successful condensation observed in the IR spectra, where the carbonyl functional groups were involved in the reaction and no longer present in the synthesized compounds. Furthermore, the remaining ^{13}C -NMR signals are consistent with their assigned structures, supporting the overall structural analysis conducted through both IR and ^1H -NMR spectroscopy. This coherence reinforces the robust synthesis and structural characterization of the compounds.

Additional confirmation was provided through mass spectrums (*vide supra*) for the produced compounds. The electron spray method was utilized to analyze the molecular m/z ratio (for ions) of the synthesized molecules, employing a positive ionization technique. Mass spectra primarily depict the molecular weight of cations and radical cations. Relative abundances were plotted against m/z values instead of obtaining their exact values. Since both the molecular ion and the recorded molecule divisions carry a +1 charge, m/z values correlate with molecular weight values. Consequently, peaks with numerical values greater than their molecular weight (molecular ion peaks; M+1 peaks) were anticipated in the spectra. Upon examination of the spectra, the expected M+1 peaks corresponding to the molecular weights of the derivatives were indeed observed, validating the analysis.

5.3. Evaluation of Biological Activity Studies.

Using the colorimetric approach developed by Ellman et al., used for examined the cholinesterase inhibitory activity of the produced chemicals in this thesis [161]. This technique relies on detecting changes in absorbance resulting from a chemical reaction (Scheme 5.3). In this method, the AChE enzyme hydrolyzes the substrate acetylthiocholine iodide, producing acetate and thiocholine. Thiocholine then reacts with DTNB, a specific chromogenic marker, generating nitrobenzoate and producing absorbance at 412 nm.



Scheme.5.3. Mechanism of Ellman Method Reaction.

The biological efficacy of the resultant chemicals as AChE and BChE inhibitors was evaluated and represented in Table 5.1. The enzyme inhibitory potential of the compounds was compared to both donepezil (as an AChEI) and tacrine (as a BChEI). As shown below, at a concentration of 10^{-3} M, donepezil's inhibitory potential was 99.156 ± 1.302 M, which is close to the inhibitory potential values obtained from some of the synthesized compounds. Among them, compounds **D1.f**, **D1.c**, **D1.e**, **D2.f**, **D2.c**, **D1.g**, **D1.i**, **D3.f**, **D3.a**, and **D1.d** showed inhibitory potentials of 96.755 ± 2.087 , 94.621 ± 2.351 , 92.584 ± 1.802 , 75.967 ± 2.281 , 67.012 ± 1.849 , 63.485 ± 1.964 , 59.330 ± 1.294 , 57.418 ± 1.721 , 56.234 ± 1.751 , and 55.668 ± 2.079 M, respectively. Among the 27 compounds tested, ten compounds exhibited more than 50% inhibition, and three of these compounds showed up to 80% enzyme inhibition.

At a concentration of 10^{-4} M, donepezil showed an inhibitory potential of 97.395 ± 1.255 . Among the synthesized compounds, only three exhibited significant inhibitions: **D1.f**, **D1.c**, and **D1.e**, with enzyme inhibition potential values of 91.204 ± 2.427 M, 90.326 ± 1.526 M, and 84.089 ± 2.150 M, respectively. When examining the IC_{50} values of the derivatives, donepezil had an IC_{50} value of 0.0201 ± 0.0014 μ M. Three of the synthesized compounds showed comparable results to donepezil. Compounds **D1.f**, **D1.c**, and **D1.e** had

the lowest IC₅₀ values among all the synthesized compounds, with values of 0.039 ± 0.001 μ M, 0.290 ± 0.010 μ M, and 0.613 ± 0.025 μ M, respectively. Specifically, compound **D1.f** IC₅₀ value (0.039 ± 0.001 μ M) against the AChE enzyme is comparable to that of the reference drug donepezil against the AChE enzyme. Reflecting on the obtained data, it is evident that **D1.f** is a promising agent among the designed compounds as an AChEI agent.

Regarding BChE enzyme inhibitory activity, none of the chemicals displayed inhibition levels surpassing 50%. The active compounds against AChE showed their BChE % inhibition against BChE as follows: compound **D1.f** exhibited 29.020 ± 1.241 M, compound **D1.c** exhibited 24.401 ± 0.941 M, and compound **D1.e** exhibited 27.636 ± 1.084 M, in comparison to tacrine, which exhibited 99.827 ± 1.378 M. the IC₅₀ of all compounds was greater than 1000 μ M.

As explained by several studies on AChE active binding sites, strong interactions with the active binding sites CAS and PAS are crucial. One significant finding is that the highest binding affinity was observed on more hydrophobic surfaces, where the surface concentration of AChE was directly proportional to surface hydrophobicity [175]. Based on this finding, molecules with higher hydrophobicity, such as those with para nitrile, nitro, and methoxy functional groups, demonstrated greater AChE binding potency. As shown in Table 5.1, the nitrile functionality in all sets showed good results in terms of AChE inhibition percentage.

Table 5.1. The % inhibition of AChE and BChE enzymes at 10⁻³ and 10⁻⁴ M concentrations of different bile acids and IC₅₀ (μ M).

Compounds	AChE % Inhibition		AChE IC ₅₀ (μ M)	BChE % Inhibition		BChE IC ₅₀ (μ M)
	10 ⁻³ M	10 ⁻⁴ M		10 ⁻³ M	10 ⁻⁴ M	
D1.a	40.512 ± 1.923	36.464 ± 1.723	>1000	32.523 ± 1.062	20.422 ± 0.984	>1000
D1.b	37.163 ± 1.541	31.058 ± 1.364	>1000	30.154 ± 0.936	19.564 ± 0.864	>1000
D1.c	94.621 ± 2.351	90.326 ± 1.526	0.290 ± 0.010	24.401 ± 0.941	21.613 ± 0.852	>1000

Table 5.1. The % inhibition of AChE and BChE enzymes at 10⁻³ and 10⁻⁴ M concentrations of different bile acids and IC₅₀ (μM). (cont...).

D1.d	55.668 ±2.079	29.637 ±1.227	>100	22.954 ±0.725	15.167 ±0.734	>1000
D1.e	92.584 ±1.802	84.089 ±2.150	0.613 ±0.025	27.636 ±1.084	17.884 ±0.710	>1000
D1.f	96.755 ±2.087	91.204 ±2.427	0.039 ±0.001	29.020 ±1.241	16.959 ±0.784	>1000
D1.g	63.485 ±1.964	16.862 ±0.778	>100	36.345 ±1.556	31.062 ±1.067	>1000
D1.h	27.564 ±1.178	14.719 ±0.646	>1000	31.487 ±1.310	24.303 ±0.925	>1000
D1.i	59.330 ±1.294	13.658 ±0.520	>100	40.859 ±1.864	29.474 ±0.846	>1000
D2.a	38.574 ±1.354	30.141 ±1.445	>1000	30.761 ±1.068	20.898 ±0.802	>1000
D2.b	47.195 ±2.168	41.667 ±1.961	>1000	38.232 ±1.662	27.411 ±1.248	>1000
D2.c	67.012 ±1.849	31.449 ±0.932	>100	35.010 ±1.141	30.664 ±1.151	>1000
D2.d	31.205 ±1.323	28.025 ±0.884	>1000	30.541 ±1.028	20.020 ±0.926	>1000
D2.e	39.638 ±1.757	25.338 ±1.057	>1000	25.667 ±0.963	17.235 ±0.634	>1000
D2.f	75.967 ±2.281	29.766 ±1.264	>100	21.928 ±0.855	16.489 ±0.578	>1000
D2.g	39.737 ±1.879	31.619 ±1.474	>1000	29.330 ±1.352	23.967 ±1.074	>1000
D2.h	31.520 ±1.040	20.337 ±0.989	>1000	36.163 ±1.361	31.484 ±0.963	>1000
D2.i	28.097 ±1.251	20.484 ±0.862	>1000	42.142 ±2.024	28.710 ±1.121	>1000
D3.a	56.234 ±1.751	37.536 ±0.921	>100	45.448 ±2.087	25.320 ±1.004	>1000
D3.b	40.158 ±1.848	35.848 ±1.246	>1000	32.557 ±1.459	22.635 ±0.964	>1000
D3.c	42.798 ±2.036	38.191 ±1.738	>1000	33.895 ±1.545	27.269 ±1.158	>1000
D3.d	32.320 ±1.441	23.258 ±1.053	>1000	37.461 ±1.462	21.348 ±1.066	>1000
D3.e	34.146 ±1.568	21.564 ±0.926	>1000	36.305 ±1.422	30.251 ±0.956	>1000

Table 5.1. The % inhibition of AChE and BChE enzymes at 10⁻³ and 10⁻⁴ M concentrations of different bile acids and IC₅₀ (μM). (cont...).

D3.f	57.418 ±1.721	25.366 ±0.856	>100	30.246 ±1.048	21.085 ±0.947	>1000
D3.g	26.655 ±1.152	20.930 ±0.884	>1000	33.528 ±1.264	26.787 ±1.062	>1000
D3.h	28.384 ±1.250	23.661 ±0.969	>1000	38.664 ±1.320	32.469 ±1.131	>1000
D3.i	40.874 ±1.587	22.148 ±0.962	>1000	31.537 ±1.036	24.145 ±0.858	>1000
Donepezil	99.156 ±1.302	97.395 ±1.255	0.0201 ±0.0014	-	-	-
Tacrine	-	-	-	99.827 ±1.378	98.651 ±1.402	0.0064 ±0.0002

5.4. Evaluation of Cytotoxicity Effect Studies

The synthesized chemicals displayed effectiveness against ACh enzyme in *in vitro* experiments. Cytotoxicity studies were conducted on these compounds using the NIH3T3 healthy fibroblast cell line from mice. After the experimental studies, the percentage inhibition values for each concentration of the test items were calculated (Table 5.2). The values of IC₅₀ of the chemicals were determined by nonlinear regression analysis, allowing the cytotoxic properties of the compounds to be interpreted.

As described above, molecules coded **D1.f** and **D1.c** were active against AChE enzymes. The inhibition potential (IC₅₀ values) of these derivatives on the relevant enzymes range from **0.039 ±0.001** to **0.290±0.010** μM, and their IC₅₀ values in the NIH3T3 fibroblast cell line range from **3.324±0.155** to **2.410±0.109** μM, as shown in (Table 5.2).

Based on the definition of SI compound **D1.f** can be selected as an ideal compound among all the synthesized compounds as showed high relative toxic concentration with the lowest active concentration as AChEI. In conclusion, it was determined that compound **D1.f** is not toxic at the IC₅₀ concentrations at which they are active against the relevant enzymes.

Table 5.2. Evaluation of IC₅₀ values of candidate compounds against AChE enzyme and NIH3T3 cells by MTT cytotoxicity test.

Molecule	AChE Enzyme	NIH3T3 cell line	Selectivity Index *
D1.c	0.290±0.010	2.410±0.109	8.310
D1.f	0.039±0.001	3.324±0.155	85.231

Selectivity Index * = IC₅₀(NIH3T3) / IC₅₀(AChE)

5.5. Computational Docking Analysis

To investigate the potential interactions of **D1.f**, the compound synthesized as part of this thesis with the highest AChE antagonistic activity, its interactions with the enzyme's catalytic core (PDB Code: 4EY7) [165] were analyzed. Using the Glide program, docking investigations were carried out on the crystal structure. The GlideScore SP method was used to create the poses that were most likely. The docking postures that were acquired by working with the AChE enzyme are shown in Figure 5.137. The functional moieties of compound **D1.f** such as 4-cyanobenzene and thiazole rings establish two π - π connections with the amino acid Trp286's indole ring, as seen in (Figure 5.137). Tyr341's phenyl group interacts π - π with the thiazole ring as well. These interactions show that compound **D1.f** significantly localizes to the PAS region of the AChE enzyme. They involve the 4-cyanobenzene and the neighboring thiazole ring. Additionally, a π - π interaction was manifested between Tyr337's phenyl ring and the phenyl ring next to the hydrazone group in the structure's center. With the interaction observed with the amino acid Tyr337, compound **D1.f** was found to bind very strongly and effectively to the CAS region of the AChE enzyme active site. The cyano group in the cyanobenzene ring forms a hydrogen bond with the amino group of Ser293. All of these findings collectively elucidate the potent blocking activity of compound **D1.f** on the enzyme *in vitro*.

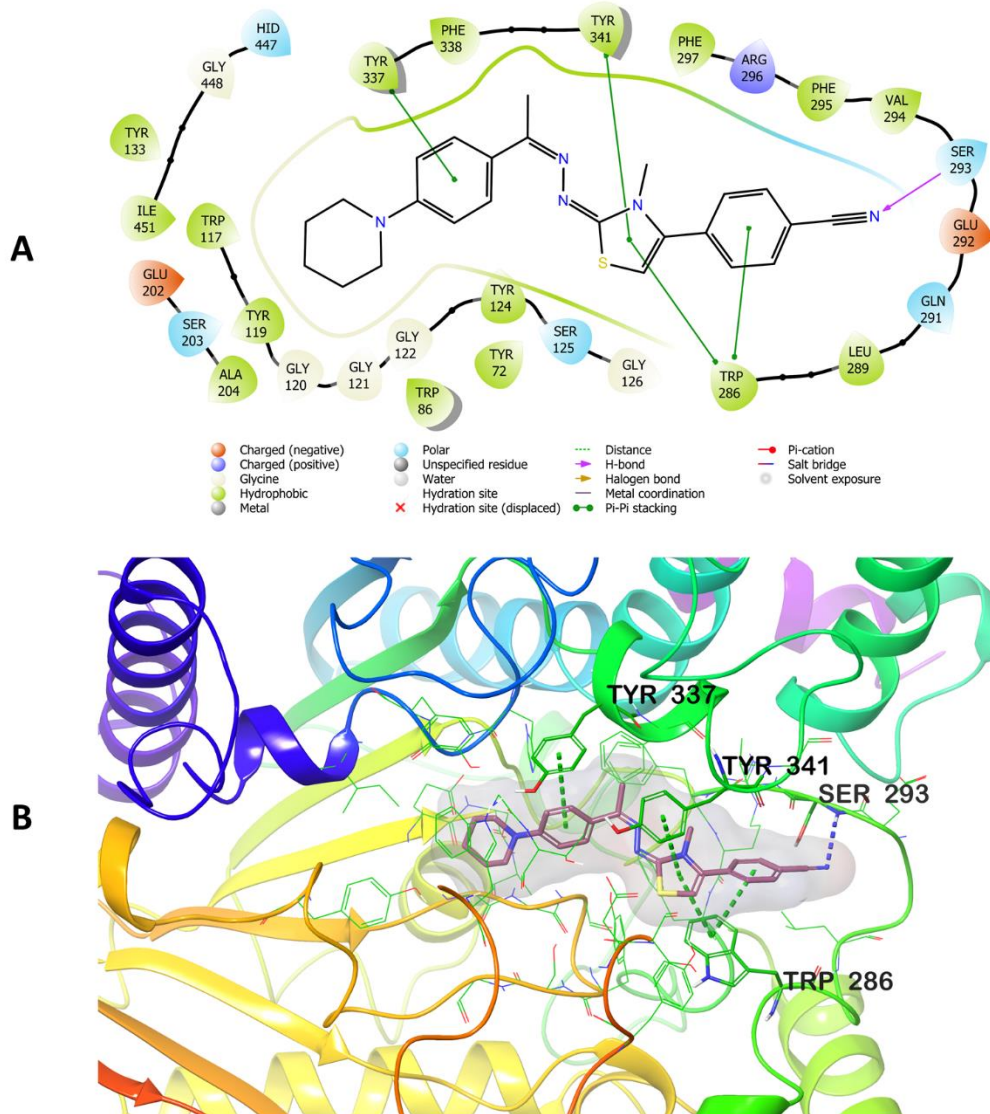


Figure 5.137. Two-dimensional (A) and three-dimensional (B) view of the interaction of compound **D1.f** with the AChE enzyme active site

5.6. Evaluation of Molecular Dynamics Studies

The MD simulation technique is commonly employed for probing the dynamic interactions of proteins or protein-ligand systems. In this thesis, a 100 ns MD simulation was conducted under an open hydration setup to assess the stability of the docking complex involving the potential compound **D1.f** and the enzyme AChE (PDB code: 4EY7) [165]. Figure 5.138 illustrates the outcomes pertaining to the **D1.f**-AChE enzyme complex. The

robustness of the model throughout the virtualization was assessed using RMSD. Figure 5.138 (A) displays the RMSD plot plotted against simulation time. With the RMSD values consistently falling within the specified range of 1-3 Å, and peaking at a maximum of 1.8 Å, it's appropriate to conclude that the stability of our model was effectively maintained throughout the simulation. The RMSD value showed an initial increase up to 11.20 nanoseconds, but then stabilized around 14.40 ns.

This analysis suggests that the **D1.f**-AChE complex protein was effectively stabilized during the simulation. Figures 5.138 (B, C, and D) delineate the interaction patterns between compound **D1.f** and amino acids within the catalytic domain of the enzyme. Specifically, Figure 5.138(B) highlights the amino acids that engage in interactions surpassing the 10% threshold within the enzyme's core. As per the analysis, compound **D1.f** engages in significant interactions within the enzyme's core and it forms π - π interactions predominantly with Trp286 (27% and 61%), Tyr124 (21%), Phe338 (12%), and Tyr341 (15%).

Additionally, hydrogen bonds are established with Tyr124 (11%) and Ser293 (19%). In Figure 5.138 (C), the interaction fractions by complex throughout the simulation are illustrated using color codes: blue represents water-mediated hydrogen bonds, green indicates regular hydrogen bonds, and purple signifies hydrophobic interactions. Here, interactions with amino acids crucial for the enzyme's active site are clearly observed. Specifically: hydrogen bonds involve the amino acids Tyr124, Trp286, His287, and Ser293, water-mediated hydrogen bonding involves the amino acids Asp74, Leu76, Tyr124, Val282, Asn283, Trp286, His287, Leu289, Pro290, Gln291, Glu292, Ser293, Tyr341, and Gly342, Ionic interactions are established with the amino acid Tyr341, and hydrophobic interactions were detected with amino acids Tyr72, Trp86, Tyr124, Trp286, His287, Val294, Phe297, Tyr337, Phe338, and Tyr341.

In Figure 5.138 (D), the interaction count versus residue plot reveals continuous interactions of compound **D1.f** with residues such as Trp286, Tyr337, Phe338, and Tyr341. The interactions commenced approximately at the 25th ns with Tyr72 and around the 43rd ns with Phe297, persisting predominantly uninterrupted thereafter. Indeed, all these amino acids have contributed significantly to maintaining stability. The continuous and uninterrupted interaction with crucial residues like Trp286, Tyr337, Phe338, and Tyr341, which play

pivotal roles in the AChE enzyme's core, underscores the compound's potential efficacy. Aromatic hydrogen bonds established over the period of the 100 ns simulation were identified by observing the MD simulation video. It was observed that the compound **D1.f** engages in aromatic hydrogen bonds with the following amino acids: Tyr72, Tyr124, Trp286, Leu289, Glu292, Arg296, Phe338, and Tyr341. Specifically, these interactions involve the OH group of Tyr72, Tyr341 and Tyr124, the C=O group of Trp286, Arg296 Phe338, and Leu289, the amino group of Glu292.



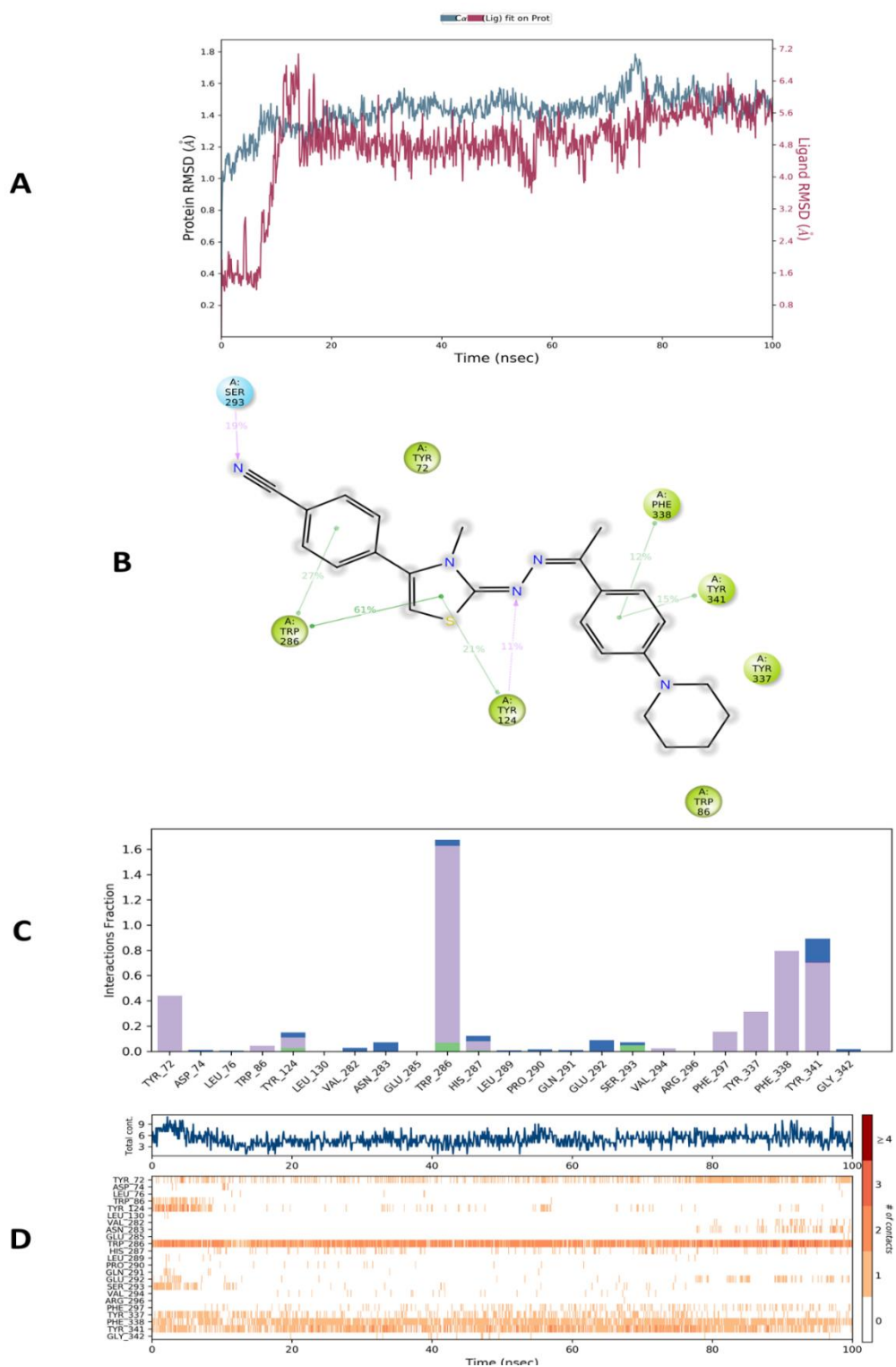


Figure 5.138. Presents the results of the MD simulation conducted with the compound **D1.f** -AChE complex.

5.7. Pharmacokinetic Studies

An optimal pharmacokinetic profile with high bioactivity and low toxicity is crucial for any compound considered for development as a drug. To minimize time and resource waste during drug design and discovery, computational tools can be used to forecast pharmacokinetic aspects such as absorption, distribution, metabolism, excretion, and toxicity (commonly known as ADMET) [176, 177]. By examining the molecular structure of synthesized compounds, pharmacokinetic parameters were estimated using software applications such as pkCSM and SwissADME, designed to assess these attributes. These programs offer insights into a compound's potential as a drug candidate. To evaluate the suitability of synthesized compounds, their pharmacokinetic parameters and physicochemical properties were predicted and analyzed, as summarized in (Table 5.3) below.

The physicochemical features of the **D1-D3** presumed using the SwissADME website, with the results summarized in (Table 5.3). These properties help evaluate key aspects such as solubility, lipophilicity, drug-likeness, and more, providing valuable insights into the compounds' potential as drug candidates.

Table 5. 3. Physicochemical properties of the synthesized compounds **D1-D3**

Molecule	H-bond acceptors	H-bond donors	Mr	Tpsa	Ilogp	Consensus log p	Silicos-it log p	Esol log s	Surfaces area
D1.a	2	0	127.83	61.13	4.44	5.15	6.59	-6.01	176.415
D1.b	3	0	122.82	61.13	4.29	5.12	6.49	-5.87	174.215
D1.c	4	0	131.68	106.95	4.29	5.12	6.49	-5.87	184.703
D1.d	2	0	127.87	61.13	4.37	5.33	6.71	-6.31	180.353
D1.e	3	0	129.35	70.36	4.46	4.8	6.13	-5.78	181.528
D1.f	3	0	127.58	84.92	4.06	4.58	6.11	-5.66	180.807
D1.g	2	0	132.88	61.13	4.62	5.86	7.34	-6.9	190.656
D1.h	2	0	132.88	61.13	4.62	5.86	7.34	-6.9	190.656
D1.i	2	0	132.79	61.13	4.54	5.45	7.12	-6.31	182.780
D2.a	2	0	136.97	61.13	4.54	5.45	7.12	-6.31	188.455
D2.b	3	0	131.96	61.13	4.54	5.45	7.12	-6.31	186.256
D2.c	4	0	140.82	106.95	4.54	5.45	7.12	-6.31	196.743
D2.d	2	0	137.01	61.13	4.54	5.45	7.12	-6.31	192.393
D2.e	3	0	138.49	70.36	4.54	5.45	7.12	-6.31	192.848
D2.f	3	0	136.72	84.92	4.54	5.45	7.12	-6.31	202.697
D2.g	2	0	142.02	61.13	4.54	5.45	7.12	-6.31	202.697
D2.h	2	0	142.02	61.13	4.54	5.45	7.12	-6.31	202.697
D2.i	2	0	141.93	61.13	4.54	5.45	7.12	-6.31	194.820
D3.a	2	0	132.63	61.13	4.54	5.45	7.12	-6.31	182.780
D3.b	3	0	127.63	61.13	4.54	5.45	7.12	-6.31	180.580
D3.c	4	0	136.49	106.95	4.54	5.45	7.12	-6.31	191.068
D3.d	2	0	132.68	61.13	4.54	5.45	7.12	-6.31	186.718

Table 5. 3. Physicochemical properties of the synthesized compounds **D1-D3** (cont..).

D3.e	3	0	134.16	70.36	4.67	5.14	6.52	-6.02	187.893
D3.f	3	0	132.38	84.92	4.12	4.88	6.5	-5.9	187.172
D3.g	2	0	137.69	61.13	4.12	4.88	6.5	-5.9	197.021
D3.h	2	0	137.69	61.13	4.12	4.88	6.5	-5.9	197.021
D3.i	2	0	137.6	61.13	4.12	4.88	6.5	-5.9	189.145

The evaluation of the synthesized compounds revealed that they exhibit considerable solubility in water, with Log S (solubility in mol/L) values ranging from -5.735 to -7.017 mol/L, indicating good aqueous solubility. Among these compounds, **D1.f** has the highest solubility in water. Regarding absorption, all compounds demonstrated high Caco-2 permeability, suggesting efficient absorption in the gastrointestinal tract. Notably, **D3.i** showed the highest absorption rate among all analogs, with up to 94% intestinal absorption, far exceeding the threshold for poorly absorbed drugs (less than 30% intestinal absorption).

In terms of distribution, all compounds exhibited a distribution volume within the optimal range (0.551 to 0.944), indicating effective drug distribution across tissues. Most of compounds can cross the BBB, with log BBB values ranging from -0.758 to 0.531, surpassing the -1 cutoff typically used to indicate limited penetration into the central nervous system. Regarding metabolism, the compounds showed no inhibition of CYP2D6, a key enzyme in drug metabolism. However, they are effective inhibitors of CYP3A4, suggesting a role in the metabolism of xenobiotic in the body. The clearance values for the compounds **D1-D3** suggest a favorable rate of drug elimination relative to their plasma concentration. These clearance values indicate that the compounds are likely to be efficiently removed from the body, reducing the risk of accumulation and associated toxicity.

Lastly, the predicted results showed no hepatotoxicity potential across most of the compounds. Overall, these findings from the ADMET analysis suggest that the synthesized compounds have promising pharmacokinetic properties, with good solubility, absorption, distribution, and low toxicity risks.

Table 5. 4. Pharmacokinetic parameters of the synthesized compounds D1-D3.

Compounds	Absorption			Distribution			Excretio	Toxicity	
	Water solubility ^a	Caco-2 permeability ^b	VDss (human) ^c	Intestin al absorption (human) ^d	CNS permeability ^e	BBB permeability ^f	Total clearance ^h	Hepatotoxici ty ^g	Max. tolerated dose (human) ⁱ
D1.a	-5.922	1.043	0.676	96.401	-1.323	0.531	-0.027	No	-0.554
D1.b	-6.023	1.082	0.639	95.899	-1.357	0.511	-0.232	No	-0.556
D1.c	-5.853	0.545	0.561	94.541	-0.755	0.032	-0.064	No	-0.064
D1.d	-6.221	1.041	0.645	94.942	-1.323	0.518	-0.101	No	-0.542
D1.e	-5.949	1.041	0.717	97.348	-1.43	0.387	-0.051	No	-0.522
D1.f	-5.735	1.027	0.551	97.46	-1.475	0.263	-0.002	No	-0.704
D1.g	-6.73	1.029	0.675	93.858	-1.322	0.457	-0.045	No	-0.507
D1.h	-6.807	1.035	0.753	94.607	-1.34	0.483	0.083	No	-0.511
D1.i	-6.157	1.033	0.74	96.775	-1.322	0.483	-0.069	No	-0.536
D2.a	-6.224	1.027	0.872	95.888	-1.353	0.429	-1.353	No	0.065
D2.b	-6.422	1.066	0.826	95.386	-1.387	0.409	-0.141	No	-0.2
D2.c	-6.203	0.526	0.719	94.039	-1.678	-0.762	0.027	No	-0.248
D2.d	-6.51	1.025	0.839	94.43	-1.353	0.416	-0.009	No	-0.198
D2.e	-6.34	1.025	0.896	96.835	-1.459	0.286	0.041	No	-0.173
D2.f	-6.05	1.011	0.742	96.947	-1.515	0.162	0.089	No	-0.371
D2.g	-6.988	1.013	0.874	93.008	-1.35	0.355	0.046	No	-0.154
D2.h	-7.056	1.016	0.937	94.095	-1.37	0.4	0.174	No	-0.169
D2.i	-6.448	1.017	0.944	95.925	-1.35	0.382	0.022	No	-0.182

Table 5.4. Pharmacokinetic parameters of the synthesized compounds **D1-D3**.(cont...)

D3.a	-6.143	1.032	0.809	95.774	-1.361	0.512	-0.022	yes	-0.453
D3.b	-6.288	1.069	0.768	95.272	-1.395	0.492	-0.227	No	-0.452
D3.c	-6.095	0.516	0.677	93.918	-1.624	-0.758	0.059	No	-0.421
D3.d	-6.439	1.028	0.776	94.315	-1.361	0.499	-0.096	No	-0.44
D3.e	-6.209	1.028	0.842	96.72	-1.47	0.369	-0.046	No	-0.415
D3.f	-5.971	1.014	0.68	96.832	-1.466	0.244	0.003	No	-0.602
D3.g	-6.942	1.139	0.822	93.172	-1.36	0.436	-0.04	No	-0.391
D3.h	-7.017	1.023	0.878	93.94	-1.376	0.467	0.088	No	-0.411
D3.i	-6.379	1.143	0.891	96.089	-1.36	0.462	-0.064	yes	-0.422

* (log mol/L)^a, (log Papp in 10⁻⁶ cm/s)^b, (log L/kg)^c, (% Absorbed)^d, (Fu)^e, (log PS)^f, (Yes/No)^g, (log ml/min/kg)^h, (log mg/kg/day)ⁱ[171]

As its obvious in Table 5.5, most of the compounds are inhibitor for cytochrome p450 isozymes such as; CYP3A4, CYP2C9, and CYP2C19 enzymes, it may hinder the body's capacity to metabolize other xenobiotic, such as medications and chemicals found in the environment.

Table 5. 5. Effect of the synthesized molecules on metabolism of other xenobiotic.

Molecule	Pgp substrate	CYP1A2 Inhibitor	CYP2C19 Inhibitor	CYP2C9 Inhibitor	CYP2D6 Inhibitor	CYP3A4 Inhibitor
D1.a	Yes	No	No	Yes	No	Yes
D1.b	No	No	Yes	Yes	No	Yes
D1.c	No	No	Yes	Yes	No	Yes
D1.d	No	No	Yes	Yes	No	Yes
D1.e	No	No	Yes	Yes	No	Yes
D1.f	No	No	Yes	Yes	No	Yes
D1.g	Yes	No	Yes	Yes	No	No
D1.h	Yes	No	Yes	Yes	No	No
D1.i	Yes	No	No	Yes	No	Yes
D2.a	Yes	No	No	Yes	No	Yes
D2.b	Yes	No	No	Yes	No	Yes
D2.c	Yes	No	No	Yes	No	Yes
D2.d	Yes	No	No	Yes	No	Yes
D2.e	Yes	No	No	Yes	No	Yes
D2.f	Yes	No	No	Yes	No	Yes
D2.g	Yes	No	No	Yes	No	Yes
D2.h	Yes	No	No	Yes	No	Yes
D2.i	Yes	No	No	Yes	No	Yes

Table 5. 5. Effect of the synthesized molecules on metabolism of other xenobiotic.(cont...)

D3.a	Yes	No	No	Yes	No	Yes
D3.b	Yes	No	No	Yes	No	Yes
D3.c	Yes	No	No	Yes	No	Yes
D3.d	Yes	No	No	Yes	No	Yes
D3.e	Yes	No	Yes	Yes	No	Yes
D3.f	No	No	Yes	Yes	No	Yes
D3.g	No	No	Yes	Yes	No	Yes
D3.h	No	No	Yes	Yes	No	Yes
D3.i	No	No	Yes	Yes	No	Yes

All analogs demonstrated the same bioavailability value, which is a positive indication regarding their pharmacological potential. The drug-likeness of these compounds, assessed by their physicochemical properties, helps to identify suitable oral drug candidates. The evaluation was based on five different rule-based filters [178] commonly used to determine drug-likeness. Table 5.6 presents the results of this drug-likeness evaluation for the hybrid compounds. This profiling offers promising insights into the compounds' potential as orally administered drugs.

All the test compounds demonstrated good drug resemblance, indicating they could be suitable candidates for further research (Table 5.6). Except for **D1.g** and **D1.h**, which had one violation in the Egan filter due to a higher surface area, the rest of the compounds had one violation of drug-likeness based on Lipinski and Ghose's criteria and no violations according to Veber's rules. According to the "Five Laws," a molecule is considered orally active/absorbent if it meets at least two of these guidelines [179]. All compounds break Brenk's rule may be to the presence of thiazole fragments. Despite this, they showed two violations in terms of lead-likeness because of their high molecular weight (greater than 350) and high XLOGP3 values, which should be more than 3.5. These factors can affect their suitability as lead candidates for drug development. Nonetheless, the overall results suggest that these compounds have potential and may warrant further investigation despite their violations.

Table 5.6. Drug likeness properties of synthesized compounds

Molecule	Lipinski Violations	Ghose Violations	Veber Violations	Egan Violations	Muegge Violations	Bioavailability Score	PAINS Alerts	Brenk Alerts	Leadlikeness Violations	Synthetic Accessibility
D1.a	1	0	0	0	1	0.55	0	1	2	3.8
D1.b	1	0	0	0	1	0.55	0	1	2	3.7
D1.c	1	0	0	0	1	0.55	0	1	2	3.7
D1.d	1	0	0	0	1	0.55	0	1	2	3.66
D1.e	0	0	0	0	1	0.55	0	1	2	3.75

Table 5.6. Drug likeness properties of synthesized compounds. (cont...)

D1.f	0	0	0	0	0	0.55	0	1	2	3.77
D1.g	1	2	0	1	1	0.55	0	1	2	3.72
D1.h	1	2	0	1	1	0.55	0	1	2	3.72
D1.i	1	1	0	0	1	0.55	0	1	2	3.95
D2.a	1	1	0	0	1	0.55	0	1	2	3.95
D2.b	1	1	0	0	1	0.55	0	1	2	3.95
D2.c	1	1	0	0	1	0.55	0	1	2	3.95
D2.d	1	1	0	0	1	0.55	0	1	2	3.95
D2.e	1	1	0	0	1	0.55	0	1	2	3.95
D2.f	1	1	0	0	1	0.55	0	1	2	3.95
D2.g	1	1	0	0	1	0.55	0	1	2	3.95
D2.h	1	1	0	0	1	0.55	0	1	2	3.95
D2.i	1	1	0	0	1	0.55	0	1	2	3.95
D3.a	1	1	0	0	1	0.55	0	1	2	3.95
D3.b	1	1	0	0	1	0.55	0	1	2	3.95
D3.c	1	1	0	0	1	0.55	0	1	2	3.95
D3.d	1	1	0	0	1	0.55	0	1	2	3.95
D3.e	0	1	0	0	1	0.55	0	1	2	3.91
D3.f	0	1	0	0	1	0.55	0	1	2	3.92
D3.g	0	1	0	0	1	0.55	0	1	2	3.92
D3.h	0	1	0	0	1	0.55	0	1	2	3.92
D3.i	0	1	0	0	1	0.55	0	1	2	3.92

*Lipinski: 1 violation: MLOGP>4.15, Leadlikeness No; 2 violations: MW>350, XLOGP3>3.5[172].

6. CONCLUSION AND RECOMMENDATIONS

Alzheimer's disease (AD) poses a considerable public health challenge due to its increasing incidence and significant mortality rates. The WHO projects that, by 2050, there will be 139 million people living with dementia globally, primarily as a result of AD. As a silent epidemic in Europe, and around the world in general, AD is one of the most common forms of dementia in older adults over age 65. Considering the current lack of effective treatments for AD, it's imperative to prioritize research aimed at finding solutions. The rising prevalence and high mortality associated with AD underscore the urgency and importance of advancing medical research to find effective therapies. AD stands out as a prevalent form of dementia. The mystery surrounding the disease's causes, both in terms of health and social and economic dimensions, makes it one of the most challenging issues of our day [13].

The most crucial approach in treating AD is to suppress the AChE enzyme in order to stop ACh from being hydrolyzed. The only benefits of the currently utilized AChEI medications are symptom relief and an improvement in patient's living standards during mild to moderate illness. There isn't currently an AChEI to stop the condition from getting worse [26, 29]. There is still much to learn about the etiology of AD, and modern treatment options typically involve anticholinesterases, antioxidants, alpha- and beta-secretase inhibitors, as well as antagonists of NMDA receptors [30].

In this doctoral thesis, we aimed to design, synthesize, and evaluate the bio efficacy of three different sets of hybrid molecules comprising dihydrothiazole and phenyl piperidine moieties as AChE inhibitors to alleviate AD symptoms. Totally, 27 new hydrazinyl dihydrothiazole derivatives potentially effective for treating AD were synthesized. The frameworks of the developed chemicals were confirmed using IR, ¹H NMR, ¹³C NMR, and mass spectroscopic methods. Biological activity studies and molecular modeling, alongside reference substances, were also conducted.

The differences were in the aliphatic moieties of thiosemicarbazide compounds to evaluate their effect on binding with enzymatic core of AChE. The hydrazinyl dihydrothiazole moiety was identified as a critical pharmacophore, essential for engaging the catalytic domain of AChE enzyme.

The comprehensive analysis of the synthesized compounds through various spectroscopic techniques confirms their successful synthesis and provides detailed insights into their structural characteristics. The results from IR, ^1H -NMR, ^{13}C -NMR, and mass spectrometry collectively confirm the successful synthesis and detailed structural characterization of the synthesized compounds. The disappearance of characteristic absorption bands, the appearance of newly detected peaks in IR and NMR spectra, and the observed molecular ion peaks in mass spectra all corroborate the anticipated chemical transformations and the final molecular structures.

The assessment of the synthesized chemicals reveals notable insights into their potential as inhibitors of AChE and BChE. The synthesized compounds, particularly **D1.f**, **D1.c**, and **D1.e**, show strong potential as AChE inhibitors with significant inhibitory activity and low IC_{50} values, comparable to the reference drug donepezil. Their lower activity against BChE suggests a selective inhibition profile, which is advantageous for targeting AChE specifically in therapeutic applications. These findings bring attention to the potential of these chemicals for further development as selective AChE inhibitors, which could be valuable in the treatment of diseases such as Alzheimer's.

Regarding the evaluation of the inhibitory effects of the synthesized molecules on the AChE enzyme and their cytotoxicity on the NIH3T3 cell line, as represented in section 5.4, reveals significant insights into their potential therapeutic use and selectivity. The data highlight compound **D1.f** as a superior candidate for further development as an AChE inhibitor due to its high inhibitory potency and low cytotoxicity, evidenced by its high Selectivity Index. The high selectivity of **D1.f** suggests that it is highly effective in targeting AChE while exhibiting minimal toxicity to normal cells. Conversely, while **D1.c** shows moderate inhibitory activity, its lower Selectivity Index indicates a higher level of cytotoxicity relative to its AChE inhibition, making it less favorable as a therapeutic candidate. Therefore, compound **D1.f** stands out as a promising lead for future studies aimed at developing potent and selective cholinesterase inhibitors for therapeutic use.

The detailed analysis and simulations conducted for compound **D1.f** provide robust evidence of its potential as a potent acetylcholinesterase (AChE) inhibitor. The conclusions derived from the study are as follows:

Compound **D1.f** displayed significant binding interactions with key residues within the enzymatic core of AChE (PDB Code: 4EY7). The key residue interactions are Trp286 and Tyr341: These residues showed continuous interactions throughout the simulation, underscoring their critical role in stabilizing the **D1.f** complex. The second important key residue is aromatic hydrogen bonds: the compound engaged in aromatic hydrogen bonds with residues such as Tyr72, Tyr124, Trp286, Leu289, Glu292, Arg296, Phe338, and Tyr341, further affirming its strong and stable binding within the AChE active site. In docking study, we conclude that compounds substituted with hydrogen bond acceptor functional groups such as CN, NO₂ and OCH₃ demonstrated significantly greater AChE inhibitory effects such as **D1.f**, **D1.c** and **D1.e** than other derivatives.

The combination of docking and MD simulation results illustrates that compound **D1.f** binds effectively and stably to active regions of AChE. This dual binding capability is significant as it can potentially inhibit AChE more effectively, reducing the breakdown of acetylcholine and potentially alleviating symptoms of diseases such as Alzheimer's. In summary, compound **D1.f** demonstrates exceptional potential as an AChE inhibitor, with strong and stable binding interactions within the enzyme's active site. The docking studies and MD simulations collectively highlight its efficacy, making it a promising candidate for next Phase in the treatment of AD.

The theoretical pharmacokinetic studies provide a comprehensive assessment of various physicochemical properties for a series of synthesized compounds. The key parameters examined shown in table 5.3, 5.4 and 5.5. The findings can be summarized and concluded that the compounds demonstrate properties that could be favorable for oral bioavailability, such as low molecular weight and moderate hydrogen bonding potential. However, their poor solubility, as indicated by negative Esol logS values, might limit their absorption and necessitate formulation optimization. The logP values suggest that these compounds have high lipophilicity, which is beneficial for membrane permeability but could pose challenges for solubility and distribution.

The synthesized compounds are likely to have good permeability but may require enhancement of solubility through prodrug approaches or formulation strategies to ensure effective bioavailability. The detailed characterization of these compounds lays a solid

foundation for further optimization and development, particularly focusing on improving solubility without compromising permeability. This balance is crucial for achieving optimal pharmacokinetic profiles for potential therapeutic applications.

Pharmacokinetic profile of the compounds generally exhibits favorable properties for absorption and distribution, with efficient intestinal absorption and moderate to high permeability across Caco-2 monolayers. However, their poor water solubility may necessitate formulation strategies to enhance bioavailability as mentioned above. The toxicity profile of the compounds demonstrates low hepatotoxicity and are associated with safe maximum tolerated doses, indicating a potentially favorable safety profile for further development.

In summary, the instrumental analysis, biological activity studies, cytotoxicity assessments, and pharmacokinetic properties of the compounds lay a strong groundwork for further research and potential applications as enzyme inhibitors. The findings particularly highlight **D1.f** as a promising drug candidate, warranting further investigation to optimize its solubility and evaluate its therapeutic efficacy and safety in preclinical and clinical studies.

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