ABSTRACT

Design and Synthesis of Functionalized Small-Molecule Inhibitors of Cathepsins L and K

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Inhibition of a class of cysteine proteases known as the cathepsins has received increasing attention in recent years, since these enzymes play key roles in cancer metastasis. Cathepsins B, K, and L have been of particular interest to this research study. These proteases have also been implicated in various disease states, including Alzheimer's disease, osteoporosis, and the severe acute respiratory syndrome (SARS). Cathepsin L is a ubiquitous cysteine protease found primarily within the lysosomes of most tissues; it is involved in normal protein turnover. The increased expression of this enzyme in cancer cells and increased secretion into the extracellular matrix aids in cancer growth and invasion. Within this study, a small library of inhibitors containing the thiosemicarbazone moiety was designed, synthesized, and evaluated for inhibition against these cathepsin proteases. The thiosemicarbazone moiety was contained on the thiochromanone, tetralone, chromanone, and 2,3-dihydroquinolinone molecular frameworks. Several of these analogues demonstrated potent inhibition of cathepsin L or cathepsin K. For example, 6-bromo- 2,3-dihydroquinolinone thiosemicarbazone strongly inhibited cathepsin L (IC_{50} = 164 nM), while 6-isopropylthiochromanone

thiosemicarbazone proved to be a potent inhibitor of cathepsin K (IC_{50} = 21 nM). Each of the thiosemicarbazone analogues in this study was inactive against cathepsin B (IC_{50} > 10,000 nM). This study further elucidates the structure activity relationship between these small molecules and the active sites of these enzymes.

Design and Synthesis of Functionalized Small-Molecule Inhibitors of Cathepsins L and K

by

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A Dissertation

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Patrick J. Farmer, Ph.D., Chairperson

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LIST OF ABBREVIATIONS

AcOH.....acetic acid

Bid .....BH3 interacting-domain death agonist

br..... broad (NMR)

°C .....degrees Celsius

d..... doublet (NMR)

DMSO.....dimethyl sulfoxide

δ ..... chemical shift in ppm

EtOH..... ethanol

EtOAc .....ethyl acetate

Et_2O .....diethyl ether

h .....hours

HPLC .....high performance liquid chromatography

Hz .....hertz

IC_{50} ..... inhibitory concentration

i-pr .....isopropyl

J ..... coupling constant

LCMS ..... liquid chromatography mass spectrometry

MeOH .....methanol

min..... minutes

m..... multiplet (NMR)

MMP..... matrix metalloprotease

NMR ..... nuclear magnetic resonance

p ..... pentet (NMR)

PCC..... pyridinium chlorochromate

PPA ..... polyphosphoric acid

ppm ..... parts per million

p-TSA ..... *para*-toluenesulfonic acid

q ..... quartet (NMR)

RT ..... room temperature

s..... singlet (NMR)

t ..... triplet (NMR)

TLC..... thin layer chromatography

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To my mom and best friend, Becky Jones
She has held my hand when I needed it, pushed me when I needed it, and helped me understand that nothing worthwhile in this life comes easy.

"I hope you don't mind
I hope you don't mind that I put down in words
How wonderful life is while you're in the world"

-Elton John

CHAPTER ONE

Introduction

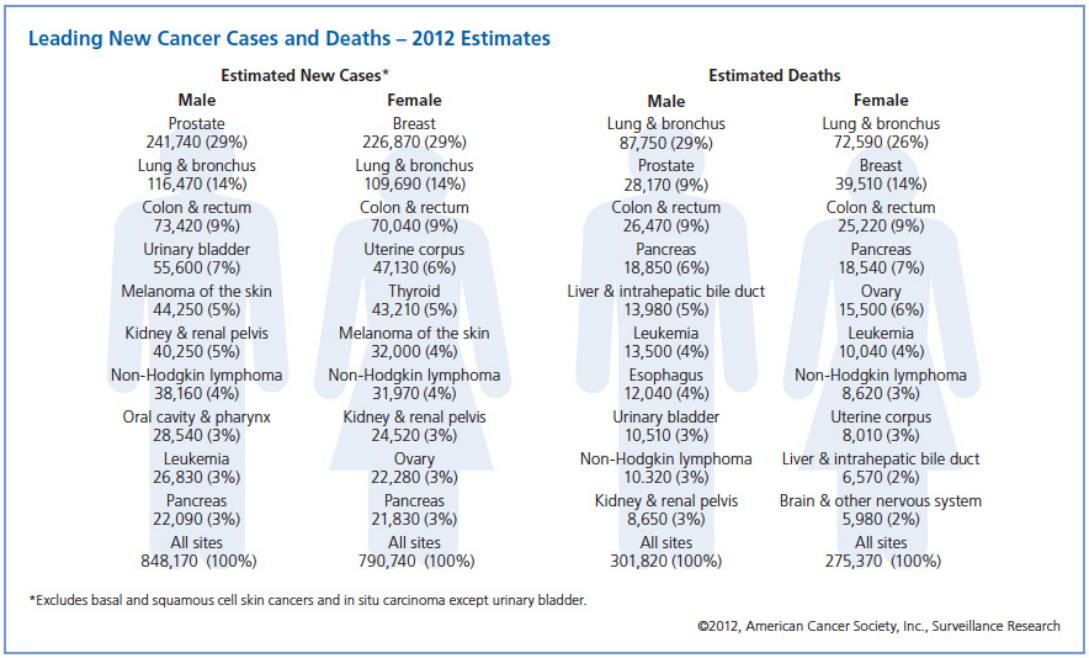
Importance of Drug Discovery

Drug discovery is an essential field, as medical issues affect everyone at some point in their life. Chemists are crucial to the process of finding solutions to the diseases that plague our lives. The field of medicinal chemistry relies on synthetic organic chemists to synthesize, purify, and characterize novel compounds. In addition to knowing synthetic techniques, organic medicinal chemists need to have a broader understanding of how a compound will interact with a biological target to better design new drugs. Synthesis of small molecules plays a role in probing unknown biological systems for better understanding of disease, as well as preparing novel compounds for the treatment of disease.[1]

Cancer is the leading cause of death behind heart disease. Over seven and a half million people died of cancer worldwide in 2008 and that number is projected to rise to over 13 million by 2030.[2] Unsurprisingly, the scope of people affected by this disease attracts much attention in the field of medical research. Cancer is the term used for a family of diseases that are described by the uncontrolled spread of abnormal cells. Several factors both internal and external and often in combination are known to cause cancer.[2,3] Internal causes can include genetic mutations and hormones, while numerous external factors can include physical, chemical, and biological carcinogens.[2]

Cancer can affect any part of the body and leading cancers vary in different populations. Figure 1.1 shows current estimates for cancer cases based on gender. One

major feature that makes cancer so dangerous is the ability for the abnormal cells to multiply rapidly and spread past the normal boundaries, invading other parts of the body. This is known as metastasis.[2]



Leading New Cancer Cases and Deaths – 2012 Estimates

Estimated New Cases*		Estimated Deaths	
Male	**Female**	**Male**	**Female**
Prostate 241,740 (29%)	Breast 226,870 (29%)	Lung & bronchus 87,750 (29%)	Lung & bronchus 72,590 (26%)
Lung & bronchus 116,470 (14%)	Lung & bronchus 109,690 (14%)	Prostate 28,170 (9%)	Breast 39,510 (14%)
Colon & rectum 73,420 (9%)	Colon & rectum 70,040 (9%)	Colon & rectum 26,470 (9%)	Colon & rectum 25,220 (9%)
Urinary bladder 55,600 (7%)	Uterine corpus 47,130 (6%)	Pancreas 18,850 (6%)	Pancreas 18,540 (7%)
Melanoma of the skin 44,250 (5%)	Thyroid 43,210 (5%)	Liver & intrahepatic bile duct 13,980 (5%)	Ovary 15,500 (6%)
Kidney & renal pelvis 40,250 (5%)	Melanoma of the skin 32,000 (4%)	Leukemia 13,500 (4%)	Leukemia 10,040 (4%)
Non-Hodgkin lymphoma 38,160 (4%)	Non-Hodgkin lymphoma 31,970 (4%)	Esophagus 12,040 (4%)	Non-Hodgkin lymphoma 8,620 (3%)
Oral cavity & pharynx 28,540 (3%)	Kidney & renal pelvis 24,520 (3%)	Urinary bladder 10,510 (3%)	Uterine corpus 8,010 (3%)
Leukemia 26,830 (3%)	Ovary 22,280 (3%)	Non-Hodgkin lymphoma 10.320 (3%)	Liver & intrahepatic bile duct 6,570 (2%)
Pancreas 22,090 (3%)	Pancreas 21,830 (3%)	Kidney & renal pelvis 8,650 (3%)	Brain & other nervous system 5,980 (2%)
All sites 848,170 (100%)	All sites 790,740 (100%)	All sites 301,820 (100%)	All sites 275,370 (100%)

*Excludes basal and squamous cell skin cancers and in situ carcinoma except urinary bladder.

Figure 1.1. Cancer distribution among men and women. Reproduced from the American Cancer Society, *Cancer Facts & Figures 2012*.[3]

Treatment programs for cancer can include surgery, radiation, and chemotherapy.[2] Early detection is critical since finding cancer early gives the patient the best chance of survival. Increased awareness and screenings have made a difference in recent times. Since the introduction of the Pap test in the 1950's cervical cancer rates have decreased seventy percent.[3] Another impressive trend to note is the reduction of breast cancer mortality since 1990 due to mammography and increased screenings.[3]

Part of the success of early detection has to do with maintaining the cancer as well as destroying it. Metastatic cancer has a high mortality rate and treatment is focused on

controlling growth.[4,5] Although capable of spreading to nearly any location in the body, cancer mostly metastasizes to the lungs, bones and liver.[5] The mechanism of metastatic spread is illustrated in Figure 1.2. The process begins by cancer cells invading normal surrounding tissue and entering into the lymphatic system and bloodstream through a process called intravasation.[6] Once cancer cells are in these systems they can travel throughout the body. Cancer cells can then adhere to the walls of the vessels in a new location and extravasate out of the walls of the blood vessels into new tissue.[6] At this point the cancer cells can multiply and motivate the growth of new blood vessels to the tumor in process known as angiogenesis.[4]

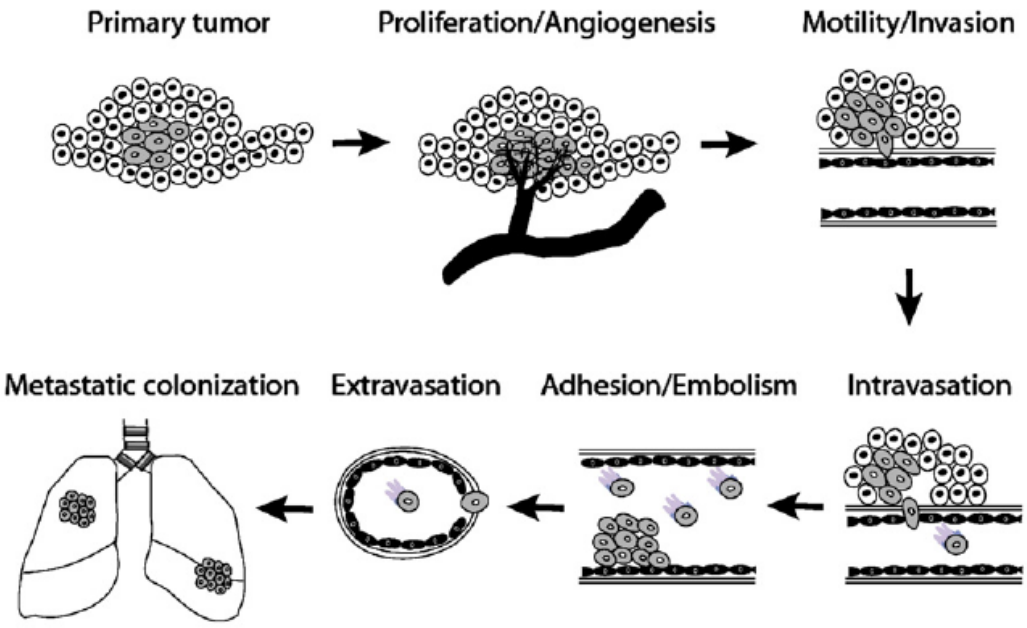


Figure 1.2. Process of tumor metastasis as the primary tumor invades the surrounding tissue, intravasates the blood vessles, extravasates at a different location, and colonizes in a different location in the body.[6]

Treatments for metastatic cancer are the same as those for primary cancers. Research is focused on stopping the spreading of cancer at various steps within the

metastatic progression.[4] The ability to inhibit the proteases that allow cancer cells to grow, move, and invade could lead to a successful therapy to slow or stop metastasis. One class of such enzymes is the cysteine proteases known as the cathepsins. The research presented herein addresses one member of this family, cathepsin L, an enzyme which has been implicated in metastasis.[7] This role in metastasis makes cathepsin L an interesting target for medicinal research aimed at discovering new anti-cancer therapeutics.[7,8,9]

Inhibitors of these cathepsins are also being investigated as therapeutics for other disease states. For example, cathepsin K has been advanced as an important target in the treatment of osteoporosis and other bone related diseases.[10] According to the International Osteoporosis Foundation, 200 million women around the world are affected by osteoporosis.[11] Fractures related to osteoporosis will affect one in three women over fifty years of age, and one in five men over fifty.[11] This frequency of occurrence makes osteoporosis a significant problem and in turn brings cathepsin K to the forefront of bone related drug research.

Proteases

Proteases have emerged as important biological targets in drug discovery over the past twenty years.[7] Proteases are enzymes that function by breaking down proteins through a hydrolysis reaction of peptide bonds. These proteolytic enzymes can be exopeptidases, meaning they cleave at the N or C terminus of a protein substrate, or they can be endopeptidases, which can cleave proteins in the middle. Some proteases can exhibit activity of both types.[9] There are five main categories of proteases that include

matrix metalloproteases (MMPs), cysteine proteases, serine proteases, aspartic proteases, and threonine proteases.[7,9]

Figure 1.3 details the reaction that takes place in the active site of a cysteine protease. The active site cysteine residue forms a covalent bond with the carbonyl carbon of the peptide bond producing a tetrahedral intermediate. The peptide bond is broken leaving a thio ester intermediate. A water molecule is deprotonated by the histidine residue, allowing nucleophilic attack on the carbonyl of the thio ester forming a second tetrahedral intermediate. This intermediate collapses, releasing the carboxylic acid product and free enzyme.

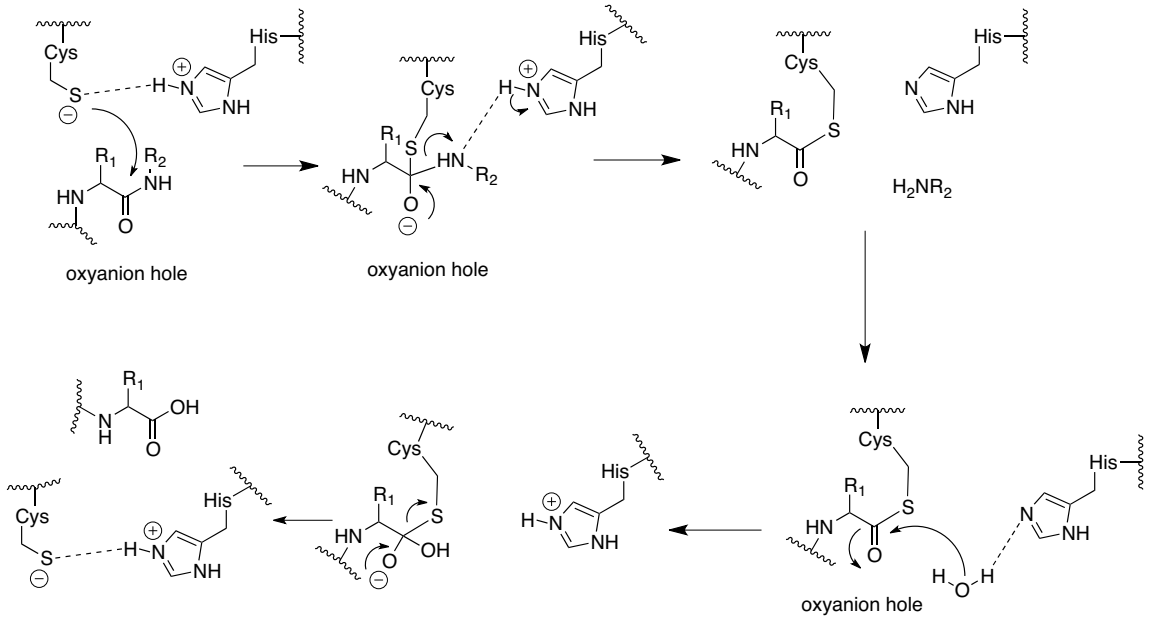


Figure 1.3. Reaction mechanism between a cysteine protease and peptide substrate

Protease activity has long been known to be important in digestion and protein turnover, but ongoing research has demonstrated that proteases are involved in many physiological processes that include cell-cycle progression, immune response, and wound

healing.[9] Understanding how proteases are involved in normal biological processes is crucial to discovering their potential utility as drug targets. To target a particular protease, knowing its normal healthy function versus how it might be modified in a disease state is necessary.[9]

Proteases may be receiving increased attention, but the first drugs used to inhibit the activity of proteases were discovered in the 1950's. Heparin and warfarin are two clinically relevant examples. These drugs indirectly prevent protease activity. Widespread research in academic and industrial settings is being done to directly suppress protease activity. Abnormal activity of various enzymes has been found to play a major role in cancer, cardiovascular disease, inflammation, neurodegenerative disorders, as well as bacterial, viral, and parasitic diseases.[9]

MMPs have received much attention as biological targets because they are involved in inflammation, cancer metastasis, arthritis, and heart disease.[12] Several MMP inhibitors entered clinical trials. Structures of some of these inhibitors are shown in Figure 1.4. Batimastat (I) from British Biotech,[13] Ilomastat (II),[14] marimastat (III)[15] which is also from British Biotech, and prinomastat (IV)[16] from Aguron are all hydroxamates. Two non-hydroxamates that have entered clinical trials are rebimastat (V)[17] from Bristol-Myers Squibb and tanomastat (VI)[17] from Bayer. Unfortunately, all MMP inhibitors have so far failed in clinical trials due to severe side effects or no clinical benefit.[9]

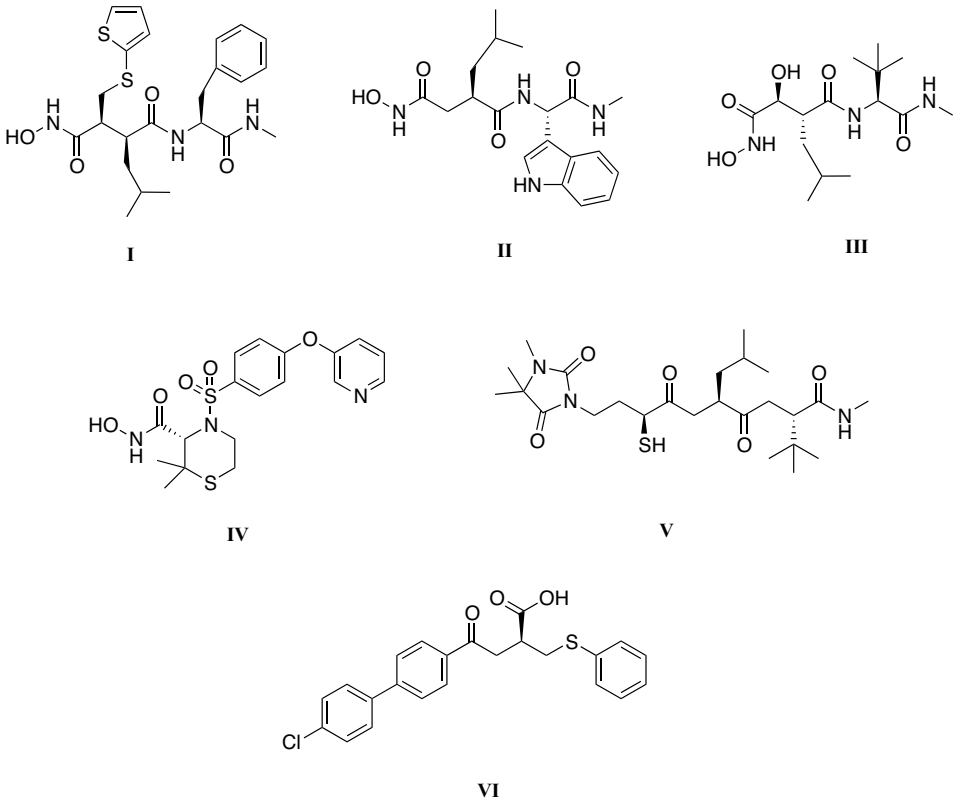


Figure 1.4. Inhibitors of matrix metalloproteases. (I) Batimastat[18] (II) Ilomastat[14] (III) Marimastat[15] (IV) Prinomastat[16] (V) Rebimastat[17] and (VI) Tanomastat[17]

Cathepsins

The cathepsins are a group of cysteine proteases. There are eleven cysteine cathepsins known, which include B, C, F, H, K, L, O, S, V, W, and X.[7,19] Cathepsins are part of the papain family of enzymes and are found throughout the body. Appropriately, the word cathepsin originates from the Greek word that means "to digest".[10] The cathepsins are most often located in the lysosomes, where their main function is protein degradation.[19]

All of the cathepsins consist of two domains, the left and the right. The active site is found in the cleft formed by these two domains.[8] A common characteristic of the cathepsins in the papain family is the active site residues which are Cys25 and His159.[8,20]

Cathepsins are synthesized as inactive preprocathepsins. The prepeptide is removed after synthesis. The propeptide segment wraps around the enzyme blocking off the active site as seen in Figure 1.5. The propeptide begins at the N-terminus of the mature enzyme, stretches up and over the active site, and ends in an alpha helix above the catalytic site.[20]

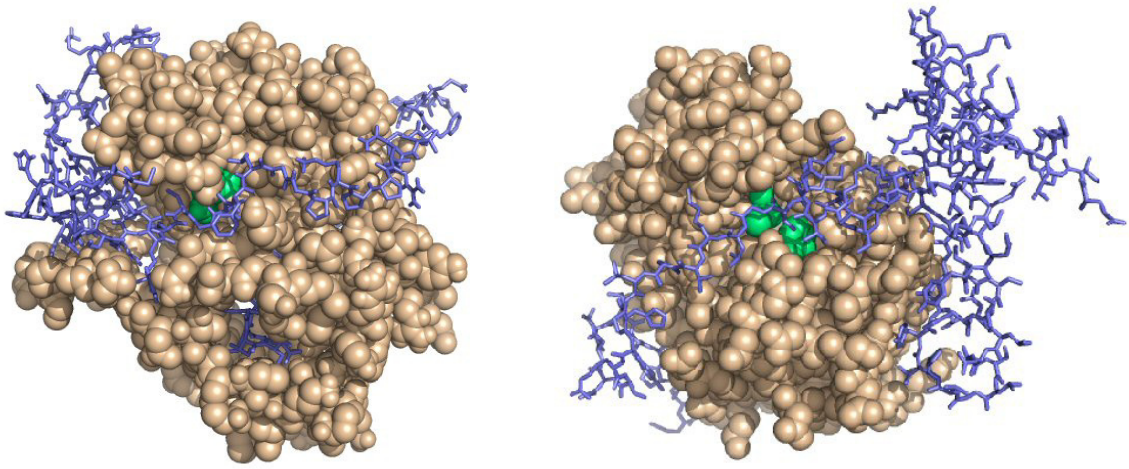


Figure 1.5. On the left, procathepsin B, on the right procathepsin K. The mature enzyme is tan, the propeptide is shown in blue-purple and the active cysteine residue is green. These images were reproduced with the permission of Dr. Ray Fort, University of Maine.[21]

Protein degradation is an important event, but it must be regulated. Cathepsins are regulated in a variety of ways. Localization within lysosomes is one mechanism that protects against uncontrolled proteolysis.[19] There are also endogenous inhibitors that are produced within the body that regulate cathepsin activity. Since cathepsins are first synthesized as preproenzymes, the propeptide portion must be cleaved to become the mature active form. This can be carried out by different proteases such as pepsin, cathepsin D and other cysteine proteases.[20] The pH of the intra or extracellular

environment can also be a critical factor in not only the activation, but for ideal enzymatic function.[20]

Cathepsin L

Cathepsin L, like many other cathepsins, starts off as preprocathepsin L. The procathepsin form is moved by the Golgi apparatus to the lysosomes where it is stored in it is mature form.[7] Cathepsin L is a ubiquitous enzyme that is involved in many critical processes within healthy cells. Since cathepsin L functions by degrading various proteins, it is critical for biological processes such as cell cycling, immune response, and postnatal central nervous system development.[7]

Cathepsin L knockout mice have shed even more light on the importance of this enzyme.[5,7,22,23] These are mice that have been engineered to not produce cathepsin L. These mice suffer from hair loss, epidermal hyperplasia, which is a thickening of the skin, abnormal spermatogenesis, and cardiomyopathy.[5,7,22] However, these severe effects should not discourage research on cathepsin L inhibitors as therapeutics because much of these negative effects are due to complete lack of the enzyme from the earliest stages of development. The importance of cathepsin L in early development was seen in mice that were lacking both cathepsins B and L. These mice died in the second through fourth weeks of life because of severe brain atrophy.[7] Even though critical to biological function, partial and temporary inhibition of cathepsin L, as it is associated with disease states, could be therapeutic. This is encouraging because of the role cathepsin L has in cancer.

Increased expression of the enzyme has been shown in several cancer types including breast, lung, colon, head, neck, gastrointestinal, melanomas and gliomas.[5,7]

The severity of the cancer has also been shown to be proportional to the level of cathepsin L expression.[7] Because expression is increased in cancer cells, cathepsin L may be a viable target. Much of drug discovery and design tries to take advantage of differences in diseased tissue versus healthy tissue.

The role of cathepsin L in cancer invasion and metastasis is fairly obvious because of its function degrading proteins. The increased expression outside of the cell allows it to break down extracellular matrix proteins and membrane proteins, which allows cancer cells to grow and spread. Cathepsin L has also been implicated in two other major processes that are crucial to cancer development, angiogenesis and apoptosis.[7] The roles of cathepsin L in cancer are outlined in Figure 1.6.

Apoptosis is the process of self-programmed cell death. Apoptosis is an important function that allows the body to get rid of damaged cells and is often seen in connection with genetic mutations or deletions. There are genes that control apoptosis and it is, overall, a process that protects. Successful cancer cells however have mutations in those genes. These mutations allow cancer cells to avoid apoptosis and thrive, being uncontrolled by normal apoptosis mechanisms. Lysosomes happen to be one of the major players in apoptosis. Destabilization of this organelle can stimulate the release of cathepsin L out of the lysosome and into the cytosol of the cell.[24] It is here that cathepsin L can cleave the protein BH3 interacting-domain death agonist (Bid).[25] Bid is a pro-apoptotic protein. Once activated by a protease, Bid initiates a chain of events involving the mitochondria that leads to apoptosis of the cell.[7] This relationship may indicate that upregulation of cathepsin L could be utilized to increase apoptosis.

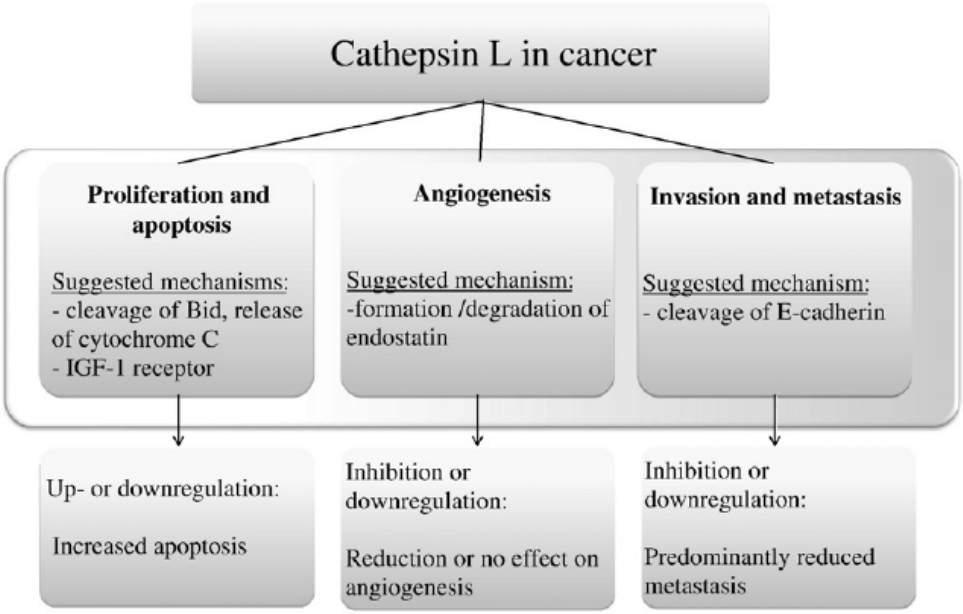


Figure 1.6. The role of cathepsin L in apoptosis, angiogensis, and metastasis.[7]

Another study showed that downregulation of the enzyme might be linked to an increase in apoptosis.[5,7] The study, using cathepsin L knockout mice, saw an increase in apoptosis in tumor cells. A suggested reasoning for this phenomenon has to do with a link between cathepsin L expression and the responsiveness of insulin-like growth factor-1 (IGF-1) receptors.[26] Decreasing cathepsin L led to a decrease in IGF-1 receptor response, which in turn led to increased apoptosis.[7.26] These varying results seem to indicate that there is a relationship between cathepsin L and the apoptosis mechanism,[7,27] but more research is required to fully understand this relationship.

Once a tumor reaches a certain size (1-2 mm diameter), it requires its own vasculature to sustain itself.[28] The process of this blood vessel development is known as angiogenesis.[7,29] The results of studies to understand the relationship between cathepsin L and angiogenesis also provide some contradictions. One study using cathepsin

knockout mice showed no significant reduction in angiogenesis.[30] Another study on mice showed that a broad-spectrum cathepsin inhibitor called JPM-OEt reduced angiogenesis. However, this reduction could be the effect of inhibition of other cathepsins, thus these results are inconclusive.[31] A third study utilized mice that were given human melanoma cells that were transfected with an anti-cathepsin L single-chain variable fragment.[32] The transfected cells expressed the anti-cathepsin L fragment, which bound to and inactivated the cathepsin L enzyme both inside and outside of the cell. This study showed a significant decrease in angiogenesis in the tumors in the mice.[32] More research is required to full understand the extent, if any, of the relationship between cathepsin L and angiogenesis.[7]

As previously mentioned, the correlation between cathepsin L and cancer invasion and metastasis is better understood. As the expression of cathepsin L outside of the cell increases, degradation of the basal lamina and the surrounding extracellular matrix decreases the cell-to-cell adhesion. In particular, the cleavage of E-cadherin decreases cell-cell adhesion.[5,7] Cadherins are a family of transmembrane proteins that mediate cell adhesion. E-cadherin is the member of this family that is found in epithelial tissue. The ability to cleave E-cadherin becomes particularly important to cancer cells that are trying to invade through the epithelial cells that line the outside of blood and lymph vessels.[7] The link between the cathepsin L enzyme and this invasive process has been studied in multiple ways, by using genetic modification and by utilizing cathepsin L inhibitors.[7]

Research thus far indicates that cathepsin L inhibitors could prevent metastasis and control tumor growth.[7,9,20] They will likely be used in conjunction with other

therapies designed to kill the cancer. The use of a cathepsin inhibitor has potential in slowing the cancer growth to the point that allows other chemotherapeutics to be effective at a potentially lower dose, which is significant due to the severe side effects associated with many chemotherapeutic agents.[7] It is important to further investigate these inhibitors and to understand the role of cathepsin L inhibition to best design therapies based upon this target.

The discovery of potent, selective, small-molecule inhibitors of cathepsin L is a growing focal point in research aimed toward the development of new anti-cancer therapeutic agents. Amos Smith and coworkers have published the structures of thiocarbazate and oxocarbazate inhibitors of cathepsin L.[33,34] Their research is focused on the link between cathepsin L and the SARS-CoV infection and Ebola virus. Research from Robert Marquis and coworkers has identified a small library of azepanone inhibitors of cathepsin L.[35] This group finds interest in the link that cathepsin L might have with bone resorption, as the role of cathepsin K has been more thoroughly studied.[35] Kiplin Guy and coworkers have been studying inhibitors of the trypanosomal cathepsin TbcatB in an effort to find therapeutics for African trypanosomiasis. Through their research they evaluated activity against similar human cysteine proteases to address selectivity issues and identified a purine nitrile inhibitor of cathepsin L.[36] A selection of the inhibitors found by these groups are shown in Figure 1.7.

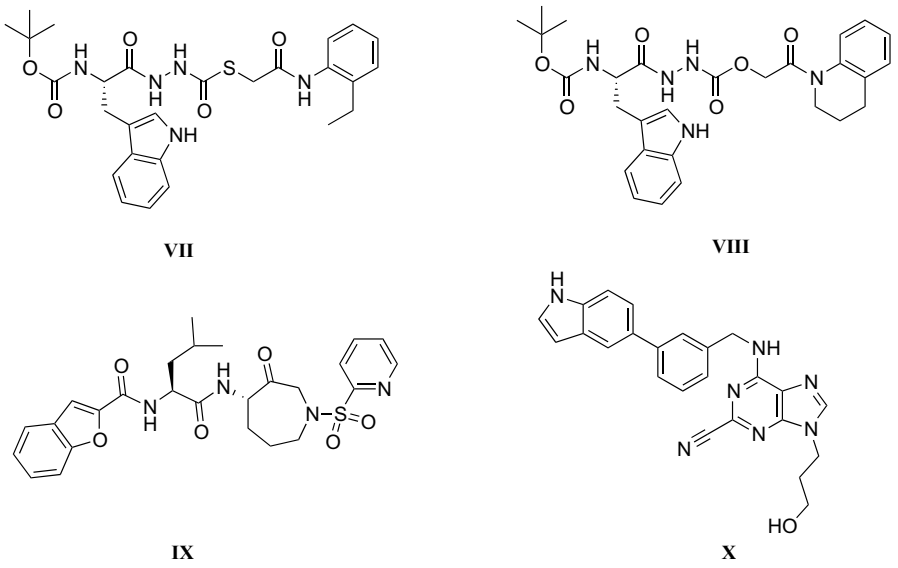


Figure 1.7. Representative inhibitors of cathepsin L. VII) thiocarbazate inhibitor[34] VIII) oxocarbazate inhibitor[33] IX) azapanone inhibitor[35] X) purine nitrile inihibitor[36]

Cathepsin B

Another member of interest in the cathepsin family is cathepsin B. Cathepsin B is one of the most extensively characterized cysteine proteases.[37] Cathepsin B is found in most tissue types and is present inside and outside of the cell. Intracellular cathepsin B is found in the lysosomes and is involved in the degradation of proteins. Outside of the cell, cathepsin B takes on many physiological roles.[37]

While most of the cathepsins are endopeptidases, meaning they can cleave peptide bonds that are not on the terminal end of the protein substrate, cathepsin B is an exopeptidase that can also exhibit some endopeptidase activity. It is a specific exopeptidase called a peptidyldipeptidase, meaning it can cleave the C-terminal dipeptide from the end of the protein substrate.[38] The structure of cathepsin B provides insight into its ability to cleave the terminal end of a protein.[38,39] A structural feature of cathepsin B absent from other members of its family is an eighteen residue portion known as the

occluding loop.[37] The occluding loop controls access into the active site of the enzyme. There are two histidine residues in this segment of the enzyme that provide a place for the carboxylic group at the C-terminal end of the substrate to bind.[37,38] The occluding loop is flexible at times to allow for endopeptidase activity. Whether the enzyme acts as an endo- or exopeptidase is highly pH dependent. The acidic pH in the lysosomes allows for intracellular cathepsin B to perform an exopeptidase role, whereas the neutral pH found outside the cell supports endopeptidase function.[37,40]

Extracellular cathepsin B can be found in two forms; soluble, free cathepsin B and as a bound enzyme, bound to the plasma membrane or extracellular matrix proteins.[37] It is known to be involved in several functions such as thyroxine synthesis, which plays a role in metabolic processes, and the cleavage of human prorenin, which regulates blood pressure.[37] Cathepsin B has also been implicated in many disease states, including, but not limited to, Alzheimer's disease, inflammation, and tumor metastasis.[37] The upregulation of cathepsin B has been reported for various cancers, such as prostate, colorectal, breast, colon, esphogeal, oral, gastric, lung, ovarian, thyroid, and melanoma.[37] In healthy cells, cathepsin B is regulated by endogenous inhibitors including the stefins and cystatins. The balance that exists between cathepsin B and these inhibitors in healthy tissue is askew in cancer cells. This imbalance adds to the invasive nature of a growing tumor.[37]

Cathepsin K

Specific cathepsins are recognized for high expression in certain tissue types. Cathepsin K is found in various tissues in the body at lower levels, but has notably high expression in osteoclasts.[40,41] Osteoclasts are bone cells that control bone resorption.

Cathepsin K is the major enzyme controlling this process by degrading the bone matrix proteins collagen, osteopontin and osteonectin.[41] The role of cathepsin K in bone resorption is detailed in Figure 1.8.[10] The connection between cathepsin K and bone resorption was discovered in 1996. A genetic disorder associated with severe bone growth defects called pycnodysostosis was linked to a mutation in the gene that codes for cathepsin K.[9,41]

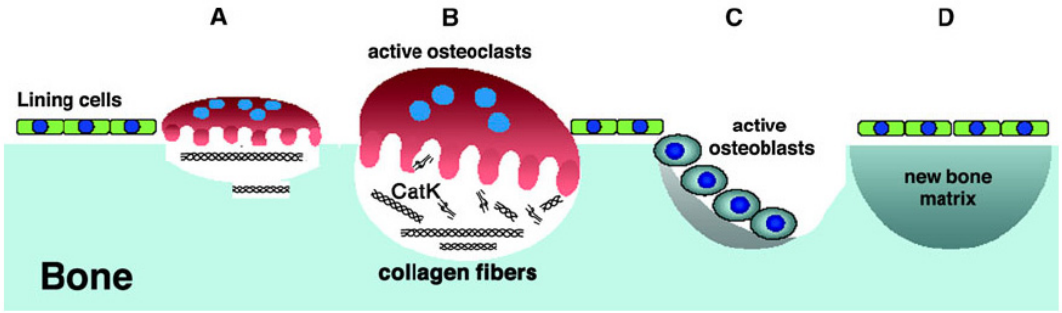


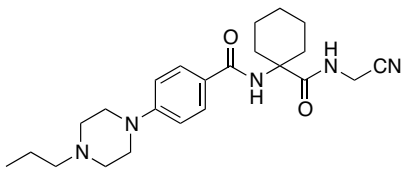
Figure 1.8. The role of cathepsin K in bone resorption. A) The osteoclast recognizes a site to repair. B) Cathepsin K is involved in demineralization and organic matrix resorption that forms the resorption pit. C) Osteoblasts occupy the resorption pit which initiates the deposition of new bone matrix. D) Finally, the resorption pit is filled in with new bone matrix.[10]

The importance of cathepsin K in bone resorption makes the enzyme a target of particular interest in the drug design of therapeutics for osteoporosis. Bones undergo continuous remodeling, which is a balance of resorption by the osteoclasts and formation in the osteoblasts. When resorption is upregulated bone density is lost which can lead to problems such as fractures. One important regulatory hormone is estrogen. Estrogen downregulates cathepsin K expression. As estrogen levels decrease cathepsin K levels will increase, leading to more bone resorption.[10]

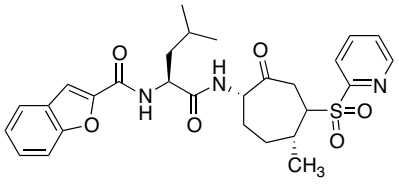
Extensive research has been done in the pharmaceutical industry on cathepsin K inhibitors.[9,10] Animal models are very important in drug research and rodent cathepsin K was discoved to be too different from the human enzyme to be useful, but the cathepsin K found in non-human primates was similar enough for GlaxoSmithKline to construct a testing model using monkeys.[9]

The only inhibitors of any cathepsins to enter clinical trials have been inhibitors of cathepsin K. A main advantage of this type of drug over other known classes is that the inhibition of cathepsin K not only prevents bone loss in the bone resorption process, but also does not interfere in the bone formation process.[9,10] The current, most commonly used treatment for osteoporosis is a class of compounds called bisphosphonates. The bisphosphonates suppress accelerated bone resorption, but also interfere with bone formation. Drugs with increased efficacy and safety are desirable and the focus of current research efforts.

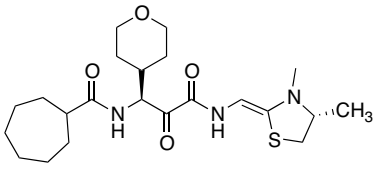
Four cathepsin K inhibitors have entered clinical trials to date (Figure 1.9). An inhibitor from ONO Pharmaceutical in Japan called ONO-5334 has shown positive results in phase II trials.[10,41] Balicatib, developed by Novartis, was withdrawn after phase II clinical trials.[10,43] Relicatib, an inhibitor from GlaxoSmithKline, showed unfavorable drug-drug interactions in phase I trials.[10,44] Odanacatib from Merck is the only inhibitor to reach phase III trials, the results of which are projected to be available in July 2012.[10]



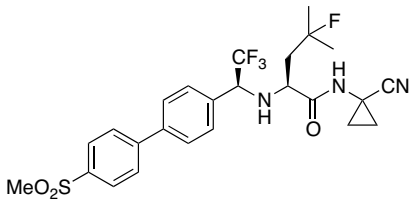
XI



XII



XIII



XIV

Figure 1.9. Cathepsin K inhibitors, Balicatib (XI),[43] Relacatib (XII),[44] ONO-5334 (XIII),[42] and Odanacatib (XIV)[43]

Thiosemicarbazone Moiety

The thiosemicarbazone moiety (Figure 1.10) is a functionality that has been researched extensively due to the variety of exhibited biological activity.[45,46] It has been broadly studied for its antitumor activity, as an antiviral, and as a therapeutic for certain parasitic ailments.[47] By the 1960's, a thiosemicarbazone drug, Marboran, was released to treat small pox.[46] More recently Triapine entered into clinical trials for the treatment of several types of cancer.[46] Much of the activity attributed to the thiosemicarbazone

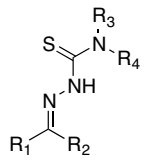


Figure 1.10. Generic structure of a thiosemicarbazone. R_1, R_2, R_3, and R_4 can be hydrogen, alkyl or aryl groups.

functionality is due to its ability to coordinate metal ions.[42] The coordinated thiosemicarbazone analogues sometimes display superior biological activity as compared to non-coordinated derivatives.[46,48]

Many of these metal complexes have been evaluated as antibacterial and antifungal agents. One example is a group of 2-acetylpyridine thiosemicarbazone derivatives that demonstrated inhibitory activity against *Neisseria gonorrhoeae, Neisseria meningitides, Staphylococcus faecalis,* and *Streptococcus faecalis.*[45,48] Metal complexes have also been studied for antitumor activity.[46,48] As early as 1956, a thiosemicarbazone complex with anti-leukemic effects was reported in the literature.[49]

Thiosemicarbazone derivatives have been complexed with many different transition metals, including iron, manganese, cobalt, nickel, copper, and zinc.[46] Depending on the overall structure, these thiosemicarbazone analogues can complex metal in different ways. Two such examples are illustrated in Figure 1.11.

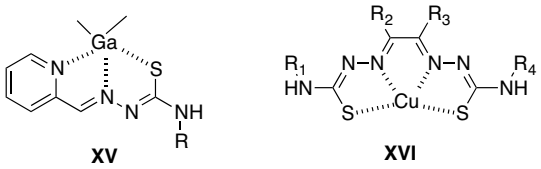


Figure 1.11. Varying metal thiosemicarbazone complexes. XV is a pyridine thiosemicarbazone Gallium complex and XVI represents a *bis*-thiosemicarbazone complexed with copper.[46]

As early as the 1970's, the thiosemicarbazones were shown to inhibit parasitic activity, primarily against *Plasmodium falciparum* and *Trypanosoma cruzi*.[47,50] These parasites are the origins of malaria and Chagas' disease. More recently, this activity against these parasites has become better understood.[47] Thiosemicarbazone compounds

have proven to be inhibitors of the cysteine proteases falcipain and cruzain, which are critical enzymes in the *P. falciparum* and *T. cruzi* parasites, respectively.[47,51,52,53]

These uncomplexed inhibitors of the cysteine protease, cruzain, have paved the way for interesting research of thiosemicarbazones as inhibitors of other cysteine proteases such as the human cathepsin L enzyme. Inhibitors containing this functional group have been reported by Pinney and coworkers from Baylor University,[54] as well as Kiplin Guy and coworkers from the University of California.[36] A representative group of thiosemicarbazone containing compounds, not complexed to a metal, that act as inhibitors of various cysteine proteases are illustrated in Figure 1.12.

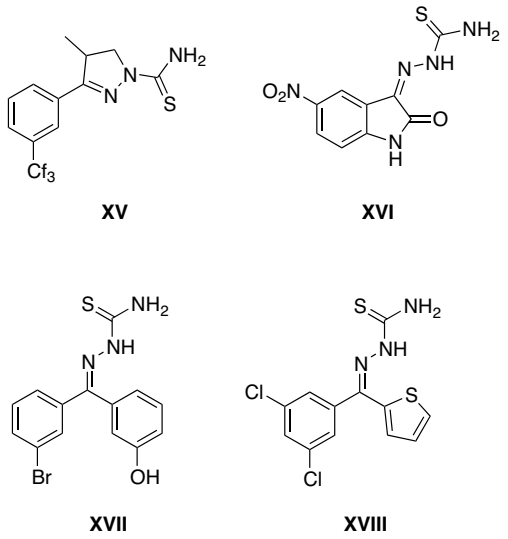


Figure 1.12. Cruzain inhibitor (XV),[52] falcipain inhibitor (XVI),[53] cathepsin L inhibitors (XVII)[36] and (XVIII).[54]

One challenge presented with the thiosemicarbazone functionality is the existence of *E* and *Z* isomers. Several studies concerning biologically active thiosemicarbazone structures have reported these *E/Z* isomers.[54,55,56,57] One such study reported that a highly active anticancer thiosemicarbazone, Bp4eT, exists mainly in the *Z* conformation but in

an aqueous solvent existed as both isomers in equilibrium.[56] Studying thiosemicarbazone *E/Z* isomers can be difficult because they are not always separable and even if one isomer is obtained, interconversion to the other isomer can occur.[56] Continued research is required to better understand this isomerism and its effects on enzyme activity.

Drug Design Rationale

The history behind the design of cathepsin inhibitors in the Pinney group started with a project that was focused on Chagas' disease. This disease is known as American trypanosomiasis and was discovered in 1909 by Dr. Carlos Chagas.[58] It is found only in the Americas, predominately in Latin America. This disease is caused by the protozoan parasite *Trypanosoma cruzi*.[59] This epidemic has spread by kissing bugs, contact with already infected blood, and congenitally, from mother to baby. It is indeed an epidemic as the Center for Disease Control estimates that eight to eleven million people in Mexico, Central and South America are infected. When diagnosed in early stage disease, there are two effective treatment agents, known as nifurtimox and benznidazole.[58] Many people do not realize, however, that they are infected, which is a problem because left untreated this disease is life threatening.[58,59]

In an effort to find therapeutics for this disease, the cysteine protease, cruzain, was identified as a viable biological target. Cruzain is necessary not only for the initial infection, but also replication and metabolism that allow the parasite to thrive in the host cells.[59] As the thiosemicarbazone moiety had already been shown to inhibit cruzain, a small-library of novel inhibitor containing that functionality was synthesized and tested against cruzain.[56]

As there are many essential human cysteine proteases, it is important that viable therapeutic inhibitors be selective for cruzain. In an effort to look at selectivity, this small-library of compounds was also tested against human cathepsin L. During this study, two compounds in particular emerged as potent inhibitors of cathepsin L. These results encouraged further investigation of the importance of cathepsin L and its inhibitors. These two lead compounds, a benzophenone thiosemicarbazone derivative and a thiochromanone thiosemicarbazone derivative, are shown in Figure 1.13.

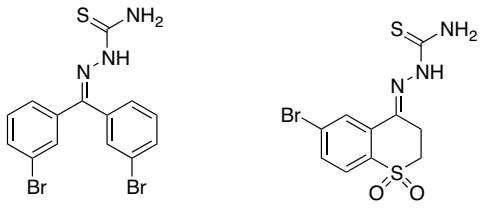


Figure 1.13. Lead thiosemicarbazone inhibitors of cathepsin L identified during research on Chagas' disease.[59,60]

A small-library of compounds based on the benzophenone structure was designed and synthesized by the Pinney group, some of which have been published in 2010 in *Bioorganic and Medicinal Chemistry Letters*.[54,55] A portion of a library designed around the thiochromanone scaffold is described in the following chapters. One strategy involves varying the functionality on the aromatic ring. Bromine is replaced with isopropyl aliphatic groups at the 6-position as well as the trifluoromethoxy functional group in the 6-position and the 8-position. Analogues with sulfide and sulfone functionalities in the 1-position were synthesized where possible.

Another strategy was to change the sulfone to study that aspect of the structure activity relationship with cathepsin L. Variations include carbon, oxygen, and nitrogen

analogues. Preparation of the nitrogen analogues required a different synthetic strategy and the details of that project will be detailed in the discussion.

CHAPTER TWO

Methods and Materials

General Methods

Chemicals and reagents used in the synthetic procedures were obtained from various suppliers (Sigma Aldrich Chem. Co., Acros Chem. Co., Alfa Aesar, Fisher Scientific, EMD Chemicals, Fluorochem, Ltd., and VWR). Thin layer chromatography (TLC) plates (pre-coated glass plates with silica gel 60 F254, 0.25 mm thickness, EMD Chemicals, VWR) were used to monitor reactions. Silica gel (200 – 400 mesh, 60Å) used for column chromatography was obtained from either Silicycle Inc. or VWR. Purification of intermediates and products was carried out using manual flash column chromatography with silica gel or with a Biotage® Isolera™ flash purification system using Biotage® KP-Sil SNAP columns. Intermediates and products were characterized by ^{1}H-NMR (Varian operating at 500 MHz or Bruker operating at 300 MHz) and ^{13}C-NMR (Varian operating at 125 MHz or Bruker operating at 75 MHz). Deuterated $CDCl_3$ (with 0.03 % TMS as internal standard), acetone-d_6, and DMSO-d_6 were used as common solvents for the NMR studies. Each of the chemical shifts is expressed in ppm (δ). The coupling constants (*J*) are expressed in Hz and peak patterns are reported as broad (br), singlet (s), doublet (d), triplet (t), quartet (q), and multiplet (m). High-resolution mass spectra (HRMS) were obtained using Electrospray Ionization (ESI) technique on a Thermo Scientific LTQ Orbitrap Discovery Mass Spectrometer in the Baylor University Mass Spectrometry Center. Purity of the final compounds was further analyzed using an Agilent Technologies 1200 series HPLC system with a Diode Array and Multiple

Wavelength Detector SL, equipped with an Agilent Eclipse XDB-C18 column (4.6 mm ID X 250 mm (5 μm) 80 Å), T = 30 °C; eluents solvent A, water, solvent B, acetonitrile; gradient 90 % A 10 % B →0 % A 100 % B over 0 to 20 min, hold for 2 min; flow rate 1.5 mL/min; injection volume 20 μL; monitored at 300 and 320 nm wavelength.

Synthesis of Benzophenone Derivatives

Bis(3-bromophenyl)methanol (**1**)

Magnesium (1.51 g, 62.2 mmol) was taken up in anhydrous ether (20 mL). A pinch of I_2 was also added. The reaction was stirred at room temperature under nitrogen. Six drops of 1,3-dibromobenzene were added and allowed to stir until the mixture became cloudy. The rest of the 1,3-dibromobenzene (3.15 mL, 27.0 mmol) was added and the reaction was left to stir until all of the Mg was consumed. The reaction was brought to 0°C in an ice bath and an ethereal solution containing 3-bromobenzaldehyde (7.17 mL, 59.4 mmol) was added. The reaction was brought back to room temperature and stirred for 1h. The reaction was quenched with water (11.8 mL) and then acidified with 12M HCl(4.5 mL). The product was extracted with ether (2 × 20 mL). The combined organic layers were washed with brine, dried of Na_2SO_4, and concentrated under reduced pressure. Purification of the crude mixture by flash column chromatography (silica gel, hexanes: ethyl acetate, 90:10) afforded pure alcohol **1** as a yellow oil (3.30 g, 9.64 mmol, 36% yield).

^{1}H NMR ($CDCl_3$, 500 MHz) 7.49 (2H, t, J = 1.5 Hz), 7.38 (2H, dt, J = 8.0 Hz, 1.5 Hz), 7.22 (2H, d, J = 7.5 Hz), 7.17 (2H, t, J = 8.0 Hz), 5.66 (1H, s), 2.77 (1H, br s).

Bis(3-bromophenyl) ketone (**2**)

A suspension of PCC (3.12 g, 14.5 mmol) and Celite (3.0 g) in dry dichloromethane (50 mL) was stirred under nitrogen at 0°C for 10 min. A solution of alcohol **1** (3.30 g, 9.64 mmol) in dry dicholormethane (20 mL) was added. After stirring at room temperature for 2h, the reaction was quenched with ether (15 mL). The reaction mixture was filtered through floursil-silica gel and rinsed with ether. The filtrant was concentrated under reduced pressure and dried under house vacuum overnight, yielding pure ketone **2** as an off-white solid (2.66 g, 7.82 mmol, 81%).

^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.93 (2H, t, J = 1.5 Hz), 7.74 (2H, ddd, J = 8.5 Hz, 2.0 Hz, 1.0 Hz), 7.69 (2H, d, J = 7.5 Hz), 7.38 (2H, t, J = 8.0 Hz).

Bis(3-bromophenyl) ketone thiosemicarbazone (**3**)

Ketone **2** (2.66 g, 7.82 mmol) was dissolved in anhydrous methanol (90 mL). The reaction was heated at reflux for 15 min, and then thiosemicarbazide (0.854 g, 9.38 mmol) and a catalytic amount of *p*-toluenesulfonic acid were added. After 30 h at reflux, the solvent was removed under reduced pressure. Purification of the crude mixture by flash column chromatography (silica gel, hexanes: ethyl acetate, 80:20) afforded thiosemicarbazone **3** as an off-white solid 1.40 g, 3.39 mmol, 43% yield).

^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.57 (1H, s), 7.73 (1H, ddd, J = 8.0 Hz, 2.0 Hz, 1.0 Hz), 7.71 (1H, t, J = 2.0 Hz), 7.55 (1H, ddd, J = 7.5 Hz, 1.5 Hz, 1.0 Hz), 7.49 (1H, t, J = 8.0 Hz), 7.41-7.40 (2H, m), 7.32 (1H, dt, J =8.0 Hz, 1.0 Hz), 7.23 (2H, t, J = 8.0 Hz).

^{13}C NMR (DMSO, 125 MHz) δ 178.8, 146.1, 139.1, 133.9, 133.4, 132.8, 132.3, 131.6, 130.9, 129.7, 128.1, 127.4, 123.4, 122.7.

HRMS (ESI) calculated for $C_{14}H_{12}Br_2N_3S$ $(M+H)^+$ 413.9098, found 413.9100

HPLC retention time 14.37 min.

Synthesis of thiochromanone thiosemicarbazone derivatives

6-Bromothiochroman-4-one (**4a**)[61]

Aluminum trichloride (1.62 g, 12.2 mmol) was added to an oven dried flask and diluted with dry dichloromethane (5 mL). The suspension was stirred under nitrogen at 0 °C for 10 min. A solution of thiochroman-4-one (1.00 g, 6.09 mmol) in dry dichloromethane (2 mL) was added and allowed to stir for 10 min. A solution of bromine (0.328 mL, 6.39 mmol) in dry dichloromethane (2 mL) was added over 30 min. The reaction was stirred for 4.5 h. Ice cold water was added, first a few drops slowly and then 40 mL. The product was extracted with dichloromethane (3 x 30 mL), washed with brine, and dried over Na_2SO_4. Solvent was removed under reduced pressure. Purification of the crude mixture by flash column chromatography (silica gel, hexanes: ethyl acetate, gradient 95:5 to 80:20) provided a mixture of 6-bromothiochromanone **4a** and 8-bromothiochromanone **4b**, as pale yellow oil (0.287 g, 1.18 mmol).

6-Bromo-1,1-dioxothiochroman-4-one (**5**)[61]

The crude mixture of ketones **4a** and **4b** (0.287 g, 1.18 mmol) was dissolved in glacial acetic acid (9 mL) and 35% w/w hydrogen peroxide solution (0.202 mL). After 2 h at reflux, the solution was cooled to room temperature, water (6 mL) was added and the product was extracted with dichloromethane (3 × 25 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. Purification using flash chromatography (silica gel, hexanes: ethyl acetate, gradient 80:20

to 40:60) afforded pure sulfone **5** as a white solid (0.107 g, 0.390 mmol, 6%, calculated for two steps, beginning with bromination of thiochroman-4-one. See ketone **4**).

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.25 (1H, d, *J* = 1.9 Hz), 7.95 (1H, dd, *J* = 8.4 Hz, 2.0 Hz), 7.88 (1H, d, *J* = 8.3 Hz), 3.72-3.68 (2H, m), 3.46-3.41 (2H, m).

6-Bromo-1,1-dioxothiochroman-4-one thiosemicarbazone (**6**)[62]

Sulfone **5** (0.400 g, 1.45 mmol) was dissolved in anhydrous methanol (25 mL). The solution was heated at reflux for 15 min, and thiosemicarbazide (0.138 g, 1.52 mmol) and a catalytic amount of *p*-toluenesulfonic acid were added. After 12 h at reflux, the resulting precipitate was collected by filtration and washed by methanol. After drying under high vacuum, thiosemicarbazone **6** was obtained as a white solid (0.218 g, 0.630 mmol, 43% yield).

^{1}H NMR (500 MHz, DMSO-d_6): δ 10.59 (1H, s), 8.78 (1H, d, *J* = 2.0 Hz), 8.57 (1H, s), 8.52 (1H, s), 7.81 (1H, dd, *J* = 8.5 Hz, 2.0 Hz), 7.75 (1H, d, *J* = 8.0 Hz), 3.69 (2H, t, *J* = 6.0 Hz), 3.28 (2H, t, 6.0 Hz).

^{13}C NMR (DMSO, 125 MHz) δ 180.0, 139.3, 137.2, 135.0, 133.3, 129.7, 127.7, 125.1, 46.1, 25.0.

HRMS (ESI) calculated for $C_{10}H_9BrN_3O_2S_2$ $(M-H)^-$ 345.9325, found 345.9332

HPLC retention time 7.21 min.

3-((4-Isopropylphenyl)thio)propanoic acid (**7**) **(**also commercially available**)**

A solution of sodium carbonate (0.696 g, 6.57 mmol) and 3-bromopropionic acid (1.01 g, 6.57 mmol) in ice-cold water (10 mL) was added to a solution of sodium hydroxide (0.262 g, 6.57 mmol) and 4-isopropylthiophenol (1.02 mL, 6.57 mmol) in ice-

cold water (10 mL). The reaction was heated at reflux for 1 h, and then cooled to room temperature. In a separatory funnel, the reaction mixture was washed with ethyl acetate (25 mL) to remove the unreacted starting material. The water layer was acidified with hydrochloric acid (10% aq) and extracted with ethyl acetate (2 × 25 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and the solvent was removed under reduced pressure. After drying by house vacuum, propanoic acid **7** was obtained as a white solid (1.29 g, 5.76 mmol, 88% yield).

^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.33 (2H, d, J = 8.0 Hz) 7.17 (2H, d, J = 9.0Hz), 3.12 (2H, t, J = 7.0 Hz), 2.89 (1H, septet, *J* = 7.0 Hz), 2.67 (2H, t, J = 7.0 Hz), 1.24 (6H, d, J = 7.0 Hz).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 178.0, 148.1, 131.4, 131.2, 127.3, 34.3, 33.7, 29.4, 23.9.

3-((4-(Trifluoromethoxy)phenyl)thio)propanoic acid (**8**)[63]

A solution of sodium carbonate (0.273 g, 2.58 mmol) and 3-bromopropionic acid (0.395 g, 2.58 mmol) in ice-cold water (10 mL) was added to a solution of sodium hydroxide (0.103 g, 2.58 mmol) and 4-trifluoromethoxythiophenol (0.500 g, 2.58 mmol) in ice-cold water (10 mL). The reaction heated at reflux for 2 h, and then cooled to room temperature. In a separatory funnel, the reaction mixture was washed with ethyl acetate (25 mL) to remove the unreacted starting material. The water layer was acidified with hydrochloric acid (10% aq) and was extracted with ethyl acetate (2 × 25 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, and the solvent was removed under reduced pressure. After drying under house vacuum, propanoic acid **8** was obtained as a white solid (0.600 g, 2.25 mmol, 87% yield).

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.40 (2H, d, *J* = 9.0 Hz), 7.16 (2H, d, *J* = 8.0 Hz), 3.16 (2H, t, *J* = 7.0 Hz), 2.68 (2H, t, *J* = 7.0 Hz).

3-((2-(Trifluoromethoxy)phenyl)thio)propanoic acid (**9**)

A solution of sodium carbonate (0.273 g, 2.58 mmol) and 3-bromopropionic acid (0.395 g, 2.58 mmol) in ice-cold water (10 mL) was added to a solution of sodium hydroxide (0.103 g, 2.58 mmol) and 2-trifluoromethoxythiophenol (0.500 g, 2.58 mmol) in ice-cold water (10 mL). The reaction was heated at reflux for 1.5 h, and then cooled to room temperature. In a separatory funnel, the reaction mixture was washed with ethyl acetate (25 mL) to remove the unreacted starting material. The water layer was acidified with hydrochloric acid (10% aq) and was extracted with ethyl acetate (2 × 25 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, and the solvent was removed under reduced pressure. After drying under house vacuum, propanoic acid **9** was obtained as a white solid (0.355 g, 1.33 mmol, 52% yield).
^{1}H-NMR ($CDCl_3$, 500 MHz) δ 11.22 (1H, bs), 7.43-7.41 (1H, m), 7.27-7.25 (3H, m), 3.20-3.16 (2H, m), 2.71-2.68 (2H, m).

6-Isopropylthiochroman-4-one (**10**)[64]

Polyphosphoric acid (10.0 g) was added to a flask containing propanoic acid **7** (1.00 g, 4.46 mmol). The reaction mixture was heated at 60°C for 2 h using overhead stirring. The reaction was quenched with ice and the mixture was extracted with ethyl acetate (3 × 25 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and the solvent was removed under reduced pressure. Purification of the crude

mixture by flash column chromatography (silica gel, hexanes: ethyl acetate, gradient 95:5 to 60:40) afforded pure ketone **10** as a yellow oil (0.351 g, 1.70 mmol, 38% yield).
^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.99 (1H, d, J = 2.0 Hz), 7.27 (1H, dd, J = 8.0 Hz, 2.0 Hz), 7.21 (1H, d, J = 8.5 Hz), 3.22 (2H, m), 2.97 (2H, dd, J = 8.0 Hz, 6 Hz), 2.90 (1H, m) 1.24 (6H, m).

6-(Trifluoromethoxy)thiochroman-4-one (**11**)[63]

Polyphosphoric acid (6.00 g) was added to a flask containing propanoic acid **8** (0.600 g, 2.26 mmol). The reaction mixture was heated at 60 °C for 3.5 h using overhead stirring. The reaction was quenched with ice and the product was extracted with ethyl acetate (3 × 25 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and the solvent was removed under reduced pressure. Purification of the crude mixture by flash column chromatography (silica gel, hexanes: ethyl acetate, gradient 95:5 to 70:30) afforded pure ketone **11** (0.211 g, 0.851 mmol, 63% yield).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.97 (1H, d, J = 2.1 Hz), 7.33 (1H, d, J = 8.4 Hz), 7.26-7.23 (1H, m), 3.28-3.24 (2H, m), 3.02-2.97 (2H, m).

8-(Trifluoromethoxy)thiochroman-4-one (**12**)

Polyphosphoric acid (3.00 g) was added to a flask containing propanoic acid **9** (0.285 g, 1.07 mmol). The reaction mixture was heated at 60 °C for 1 h using overhead stirring. The reaction was quenched with ice and a solid crashed out. After filtration, pure ketone **12** was obtained as a white solid (0.178 g, 0.715 mmol, 67% yield).
^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.08 (1H, d, J = 8.0 Hz), 7.39 (1H, d, J = 7.0 Hz), 7.21 (1H, td, J = 8.0 Hz, 1.0 Hz), 3.25 (2H, t, J = 5.5 Hz), 2.99 (2H, t, J = 5.5 Hz)

6-Isopropylthiochroman-4-one thiosemicarbazone (**13**)

Ketone **10** (0.100 g, 0.485 mmol) was dissolved in anhydrous methanol (12 mL). The reaction was heated at reflux under nitrogen for 30 min, and then thiosemicarbazide (0.0463 g, 0.509 mmol) and a catalytic amount of *p*-toluenesulfonic acid were added. After 36 h at reflux, the resulting precipitate was collected by filtration and washed with methanol. After drying under house vacuum, thiosemicarbazone **13** was obtained as a white solid (0.0357g, 0.128 mmol, 26% yield).

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.75 (1H, s), 7.81 (1H, s), 7.37 (1H, bs), 7.22 (1H, d, J = 8.5 Hz), 7.16 (1H, dd, J = 8.0 Hz, 2.0 Hz), 6.47 (1H, bs), 3.05-3.02 (2H, m), 2.96-2.94 (2H, septet, J = 7.0 Hz), 2.93-2.87 (1H, m), 1.25 (6H, d, J = 6.5 Hz).

^{13}C NMR (125 MHz, $CDCl_3$): δ 179.3, 146.7, 145.8, 134.2, 130.6, 128.7, 128.2, 124.5, 33.9, 28.4, 25.9, 23.9.

HRMS (ESI) calculated for C13H17N3S2H (M+H)+ 280.0937, found 280.0937.

HPLC retention time 12.67 min.

6-(Trifluoromethoxy)thiochroman-4-one thiosemicarbazone (**14**)

Ketone **11** (0.041 g, 0.165 mmol) was dissolved in anhydrous methanol (10 mL). The reaction was heated at reflux for 15 min, and then thiosemicarbazide (0.0157 g, 0.173 mmol) and a catalytic amount of *p*-toluenesulfonic acid were added. After 24 h at reflux, the solvent was removed under reduced pressure. Purification of the crude mixture by flash column chromatography (silica gel, hexanes: ethyl acetate, gradient 95:5 to 80:20) afforded thiosemicarbazone **14** as a white solid (0.016 g, 0.041 mmol, 30% yield).

^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.80 (1H, s), 7.82 (1H, d, J = 2.0 Hz), 7.32 (1H, s), 7.29 (1H, d, J = 8.5 Hz), 7.13 (1H, dd, J = 8.5 Hz, 1.0 Hz), 6.51 (1H, s), 3.07 (2H, t, J = 6.0 Hz), 2.98 (2H, t, J = 6.5 Hz).

^{13}C NMR (125 MHz, DMSO-d_6): 179.5, 147.1, 143.9, 135.7, 132.3, 130.0, 122.4, 120.4 (1C, q, $J_{C\text{-}F}$ = 256.4 Hz), 118.9, 27.7, 25.7.

HRMS (ESI) calculated for $C_{11}H_{10}F_3N_3OS_2H$ $(M + H)^+$ 322.0290, found 322.0290.

HPLC retention time 11.82 min.

8-(Trifluoromethoxy)thiochroman-4-one thiosemicarbazone (**15**)

Ketone **12** (0.150 g, 0.604 mmol) was dissolved in anhydrous methanol (10 mL). The reaction was heated at reflux for 15 min, and then thiosemicarbazide (0.057 g, 0.634 mmol) and a catalytic amount of *p*-toluenesulfonic acid was added. After 12 h at reflux, the resulting precipitate was filtered, and pure thiosemicarbazone **15** was obtained as a white solid (0.020 g, 0.062 mmol, 10% yield).

^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.34 (1H, s), 8.44 (1H, d, J = 8.5 Hz), 8.41 (1H, s), 8.09 (1H, s), 7.35 (1H, d, J = 8.0 Hz), 7.21 (1H, t, J = 8.0 Hz), 3.07-3.02 (4H, m).

^{13}C NMR (DMSO-d_6, 125 MHz) δ 179.62, 145.06, 144.32, 133.81, 131.11, 126.68, 125.64, 121.64, 120.68 (q, $J_{C\text{-}F}$ = 256.4 Hz) 27.47, 24.62.

HRMS (ESI) calculated for $C_{11}H_{10}F_3N_3OS_2H$ $(M + H)^+$ 322.0290, found 322.0291.

HPLC retention time 11.61 min.

6-Isopropyl-1,1-dioxothiochroman-4-one (**16**) (also commercially available)

Ketone **10** (0.238 g, 1.15 mmol) was dissolved in glacial acetic acid (6 mL) and 35% w/w hydrogen peroxide solution (0.197 mL). After 2 h at reflux, the solution was

cooled to room temperature, water (10 mL) was added and the product was extracted with dichloromethane (2 × 25 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. Purification using flash chromatography (silica gel, hexanes: ethyl acetate, gradient 80:20 to 40:60) afforded pure sulfone **16** as a white solid (0.180 g, 0.754 mmol, 65% yield).
^{1}H NMR(500 MHz, $CDCl_3$): δ 7.98 (1H, d, *J* = 1.5 Hz), 7.94 (1H, d, *J* = 8.0 Hz), 7.68 (1H, dd, *J* = 8.5 Hz, 2.0 Hz), 3.68 (2H, dd, *J* = 7.5 Hz, 6.0 Hz), 3.42 (2H, dd, *J* = 7.0 Hz, 5.5 Hz), 3.06-3.03 (1H, m), 1.29 (6H, d, *J* = 7.0 Hz).

6-(Trifluoromethoxy)-1,1-dioxothiochroman-4-one (**17**)

Ketone **11** (0.162 g, 0.653 mmol) was dissolved in glacial acetic acid (5 mL) and 35% w/w hydrogen peroxide solution (5 mL). After 3 h at reflux, the solution was cooled to room temperature, water (10 mL) was added, and the product was extracted by dichloromethane (2 × 25 mL). The combined organic layers were concentrated under reduced pressure. Purification using flash chromatography (silica gel, hexanes: ethyl acetate, 60:40) afforded sulfone **17** (0.142 g, 0.507 mmol, 78% yield).
^{1}H NMR (500 MHz, $CDCl_3$): δ 8.10 (1H, d, *J* = 8.5 Hz), 7.94 (1H, dd, *J* = 2.0 Hz, 1.0 Hz), 7.64 (1H, ddd, *J* = 8.5 Hz, 2.5 Hz, 0.5 Hz), 3.73-3.71 (2H, m), 3.48-3.46 (2H, m).

6-Isopropyl-1,1-dioxothiochroman-4-one thiosemicarbazone (**18**)

Sulfone **16** (0.180 g, 0.754 mmol) was dissolved in anhydrous methanol (12 mL). The solution was heated at reflux for 15 min, and then thiosemicarbazide (0.072 g, 0.791 mmol) and a catalytic amount of *p*-toluenesulfonic acid was added. After 36 h at reflux, the resulting precipitate was collected by filtration and washed with methanol. After

drying under high vacuum, thiosemicarbazone **18** was obtained as a white solid (0.113 g, 0.362 mmol, 48% yield).

^{1}H NMR (500 MHz, DMSO-d_6): δ 10.54 (1H, s), 8.57 (1H, s), 8.31 (1H, s), 8.25 (1H, s), 7.76 (1H, d, J = 8.0 Hz), 7.53 (1H, d, J = 8.0 Hz), 3.63 (2H, t, J = 6.0 Hz), 3.28 (2H, t, J = 6.5 Hz), 3.07 (1H, m), 1.23 (6H, d, J = 7.0 Hz).

^{13}C NMR (125 MHz, DMSO-d_6): δ 179.9, 154.5, 140.9, 135.9, 133.0, 128.1, 125.6, 123.3, 46.3, 34.0, 25.1, 23.9.

HRMS (ESI) calculated for $C_{13}H_{17}N_3O_2S_2H$ $(M+H)^+$ 312.0835, found 312.0836.

HPLC retention time 9.19 min.

6-(Trifluoromethoxy)-1,1-dioxothiochroman-4-one thiosemicarbazone (**19**)

Sulfone **17** (0.060 g, 0.214 mmol) was dissolved in anhydrous methanol (10 mL). The solution was heated to reflux for 15 min, and then thiosemicarbazide (0.021 g, 0.225 mmol) and a catalytic amount of *p*-toluenesulfonic acid were added. After 36 h at reflux, the resulting precipitate was collected by filtration and washed with methanol. After drying under high vacuum, thiosemicarbazone **19** was obtained as a white solid (0.005 g, 0.014 mmol, 7% yield).

^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.65 (1H, s), 8.61 (1H, s), 8.59 (1H, d, J = 2.5 Hz), 8.49 (1H, s), 7.97 (1H, d, J = 8.5 Hz), 7.61 (1H, dd, J = 8.5 Hz, 1 Hz), 3.73 (2H, t, J = 5.0 Hz), 3.30 (2H, t, J = 5.0 Hz).

^{13}C NMR (DMSO-d_6, 125 MHz) δ 180.1, 151.8, 139.3, 137.1, 136.2, 126.1, 122.6, 120.36 (1C, q, J = 258, CF_3), 120.35, 46.1, 25.1.

HRMS (ESI) calculated for $C_{10}H_9N_3O_3S_2$ (M-H)- 352.0043, found 352.0049.

HPLC retention time 9.11 min.

3-(Naphthalen-2-ylthio)propanoic acid (**20**)[65]

A solution of sodium carbonate (0.265 g, 2.50 mmol) and 3-bromopropionic acid (0.383 g, 2.50 mmol) and in ice-cold water (4 mL) was added to a solution of sodium hydroxide (0.100 g, 2.50 mmol) and naphthalene-2-thiol (0.400 g, 2.50 mmol) in ice-cold water (5 mL). The reaction was heated at reflux for 1.5 h, and then cooled to room temperature. In a separatory funnel, the reaction mixture was washed with ethyl acetate (25 mL) to remove unreacted starting material. The water layer was then acidified with hydrochloric acid (10% aq) and was extracted with ethyl acetate (2 × 25 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, and the solvent was removed under reduced pressure. After drying under house vacuum, propanoic acid **20** was obtained as a white solid (0.480 g, 2.07 mmol, 83% yield).

^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.82-7.75 (4H, m), 7.50-7.43 (3H, m), 3.25 (2H, t, J = 7.0 Hz), 2.71 (2H, t, J = 7.0 Hz).

2,3-Dihydro-1H-benzo[f]thiochromen-1-one (**21**)[66]

Polyphosphoric acid (4.00 g) was added to a flask containing propionic acid **20** (0.450 g, 1.94 mmol). The reaction mixture was heated at 60 °C for 2 h using overhead stirring. The reaction was quenched with ice and the product was collected by filtration. Purification of the crude product by flash column chromatography (silica gel, hexanes: ethyl acetate, gradient 90:10 to 0:100) afforded pure ketone **21** as a white solid (0.200 g, 0.935 mmol, 48% yield).

^{1}H NMR (500 MHz, $CDCl_3$): 9.16 (1H, d, J = 9.0 Hz), 7.77 (1H, d, J = 8.5 Hz), 7.74 (1H, d, J = 8.0 Hz), 7.60 (1H, td, J = 7.0 Hz, 1.5 Hz), 7.46-7.43 (1H, m), 7.28 (1H, d, J = 9.0 Hz), 3.31-3.28 (2H, m), 3.12-3.10 (2H, m).

2,3-Dihydro-1H-benzo[f]thiochromen-1-one thiosemicarbazone (**22**)[66]

Ketone **21** (0.107 g, 0.500 mmol) was dissolved in anhydrous methanol (10 mL). The solution was heated to reflux for 15 min, and then thiosemicarbazide (0.048 g, 0.525 mmol) and a catalytic amount of *p*-toluenesulfonic acid were added. The reaction was refluxed for 18 h and then cooled to room temperature. The precipitate was filtered and recrystallized from methanol, affording pure thiosemicarbazone **22** as a white solid (0.035 g, 0.122 mmol, 25% yield).

^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.93 (1H, s), 8.55 (1H, d, J = 8.0 Hz), 7.79 (1H, d, J = 7.5 Hz), 7.70 (1H, d, J = 8.5 Hz), 7.49 (1H, td, J = 6.5 Hz, 1.0 Hz), 7.46-7.43 (1H, m), 7.37 (1H, d, J = 8.5 Hz), 7.30 (1H, s), 6.41 (1H, s), 3.19 (2H, t, J = 6.5 Hz), 3.05 (2H, t, J = 6.5 Hz) .

^{13}C NMR ($CDCl_3$, 125 MHz) δ 179.5, 145.8, 138.6, 133.1, 131.7, 129.6, 128.5, 128.0, 127.4, 126.6, 125.9, 125.6, 32.1, 26.9.

HRMS (ESI) calcd for $C_{14}H_{13}N_3S_2H$ $(M + H)^+$ 288.0624, found 288.0624.

HPLC retention time 1.61 min.

Synthesis of tetralone derivatives

7-Methoxy-tetral-4-one thiosemicarbazone (**23**)

7-Methoxy-1-tetralone (0.250 g, 1.42 mmol) was dissolved in anhydrous methanol (10 mL). The solution was heated to reflux for 10 min, and then thiosemicarbazide (0.136 g, 1.49 mmol) and a catalytic amount of *p*-toluenesulfonic acid were added. After 8 h at reflux, the resulting precipitate was collected by filtration and

washed with methanol. After drying under high vacuum, thiosemicarbazone **23** was obtained as a white solid (0.256 g, 1.02 mmol, 72% yield).

^{1}H NMR (DMSO, 500 MHz) δ 10.04 (1H, s), 8.27 (1H, s), 8.03 (1H, s), 7.73 (1H, d, *J* = 3.0 Hz), 7.08 (1H, d, *J* = 8.5 Hz), 6.86 (1H, dd, *J* = 8.0 Hz, 2.5 Hz), 3.77 (3H, s), 2.67-2.64 (4H, m), 1.77 (2H, p, *J* = 6.0 Hz).

^{13}C NMR (DMSO, 125 MHz) δ 179.1, 158.2, 148.2, 133.2, 133.0, 129.9, 116.8, 109.3, 55.8, 28.6, 26.2, 22.1.

HRMS (ESI) calcd for $C_{12}H_{15}N_3OS$ $(M + H)^+$ 250.1014, found 250.1009.

HPLC retention time: 9.48 min.

6-Methoxy-tetral-4-one thiosemicarbazone (**24**)

6-Methoxy-1-tetralone (0.500 g, 2.84 mmol) was dissolved in anhydrous methanol (15 mL). The solution was heated to reflux for 20 min, and then thiosemicarbazide (0.271 g, 2.98 mmol) and a catalytic amount of *p*-toluenesulfonic acid were added. After 4 h at reflux, the resulting precipitate was collected by filtration and washed with methanol. After drying under high vacuum, thiosemicarbazone **24** was obtained as an off-white solid (0.512 g, 2.05 mmol, 72% yield).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.71 (1H, s), 7.94 (1H, d, *J* = 15.0 Hz), 7.36 (1H, br s), 6.79 (1H, dd, *J* = 14.5 Hz, 4.5 Hz), 6.67 (1H, d, *J* = 4 Hz), 6.30 (1H, br s), 3.83 (3H, s), 2.77 (2H, t, *J* = 9.5 Hz), 2.58 (2H, t, *J* = 10.5 Hz), 1.98 (2H, p, *J* = 10.5 Hz).

^{13}C NMR ($CDCl_3$, 75 MHz) δ 178.8, 161.0, 148.0, 142.3, 126.4, 124.1, 113.4, 112.7, 55.3, 29.6, 25.3, 21.5.

HRMS (ESI) calcd for $C_{12}H_{15}N_3OS$ $(M + H)^+$ 250.1014, found 250.1010.

HPLC retention time: 5.42 min.

5-Methoxy-tetral-4-one thiosemicarbazone (**25**)

5-Methoxy-1-tetralone (0.250 g, 1.42 mmol) was dissolved in anhydrous methanol (10 mL). The solution was heated to reflux for 10 min, and then thiosemicarbazide (0.135 g, 1.49 mmol) and a catalytic amount of *p*-toluenesulfonic acid were added. After 8 h at reflux, the resulting precipitate was collected by filtration and washed with methanol. After drying under high vacuum, thiosemicarbazone **25** was obtained as a white solid (0.252 g, 1.01 mmol, 71% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.13 (1H, s), 8.25 (1H, s), 7.93 (1H, s), 7.90 (1H, d, J = 8.5 Hz), 7.15 (1H, t, J = 8.0 Hz), 6.93 (1H, d, J = 8.0 Hz), 3.78 (3H, s), 2.64 (4H, t, J = 6.0 Hz), 1.76 (2H, p, J = 6.5 Hz).

^{13}C NMR (125 MHz, DMSO-d_6): δ 179.2, 156.6, 148.4, 133.3, 129.0, 126.7, 117.8, 111.1, 55.9, 25.7, 22.2, 21.2.

HRMS (ESI) calcd for $C_{12}H_{15}N_3OS$ $(M + H)^+$ 250.1014, found 250.1008.

HPLC retention time: 7.85 min.

5-Hydroxy-tetral-4-one thiosemicarbazone (**26**)

5-Hydroxy-1-tetralone (0.250 g, 1.54 mmol) was dissolved in anhydrous methanol (10 mL). The solution was heated to reflux for 10 min, and then thiosemicarbazide (0.147 g, 1.62 mmol) and a catalytic amount of *p*-toluenesulfonic acid were added. After 18 h at reflux, the resulting precipitate was collected by filtration and washed with methanol. The crude product was recrystallized first from methanol, then from ethanol. After drying under high vacuum, thiosemicarbazone **26** was obtained as an off-white solid (0.018 g, 0.080 mmol, 5% yield).

^{1}H NMR (500 MHz, DMSO-d_6): δ 10.08 (1H, s), 9.40 (1H, s), 8.21 (1H, s), 7.87 (1H, s), 7.74 (1H, d, J = 7.5 Hz), 6.98 (1H, t, J = 7.5 Hz), 6.78 (1H, dd, J = 7.5 Hz, 0.5 Hz), 2.64-2.60 (4H, m), 1.75 (2H, p, 6.0 Hz).

^{13}C NMR (125 MHz, DMSO-d_6): δ 179.1, 154.6, 148.8, 133.4, 127.6, 126.5, 116.5, 115.4, 25.8, 22.3, 21.4.

6-Amino-tetral-4-one thiosemicarbazone (**27**)

6-amino-chroman-4-one (0.300 g, 1.86 mmol) was dissolved in anhydrous methanol (25 mL). The solution was heated to reflux for 10 min, and then thiosemicarbazide (0.177 g, 1.95 mmol) and a catalytic amount of *p*-toluenesulfonic acid were added. After 6 h at reflux, the reaction mixture was cooled to room temperature and the product crashed out overnight in the freezer. The product was collected by filtration and washed with methanol. The crude product was recrystallized from methanol. After drying under high vacuum, thiosemicarbazone **27** was obtained as a yellow solid (0.087 g, 0.370 mmol, 20% yield).

^{1}H NMR (500 MHz, DMSO-d_6): δ 9.85 (1H, s), 8.04 (1H, s), 7.94 (1H, d, J = 8.5 Hz), 7.72 (1H, s), 6.40 (1H, dd, J = 8.5 Hz, 2.0 Hz), 6.27 (1H, d, J = 2.0 Hz), 5.43 (2H, s), 2.59-2.53 (4H, m), 1.73 (2H, p, J = 5.5 Hz).

^{13}C NMR (125 MHz, DMSO-d_6): δ 178.3, 150.5, 149.9, 141.9, 127.2, 120.0, 113.1, 112.3, 29.7, 26.2, 22.0.

HPLC retention time: 5.64 min.

2-Tetralone thiosemicarbazone (**28**)

2-Tetralone (0.250 g, 1.71 mmol) was dissolved in anhydrous methanol (8 mL). The solution was heated to reflux for 10 min, and then thiosemicarbazide (0.163 g, 1.80 mmol) and a catalytic amount of *p*-toluenesulfonic acid were added. After 4 h at reflux, the solution was cooled to room temperature and concentrated under reduced pressure. Crude product was recrystallized from ethyl acetate. Additional purification using flash chromatography (silica gel, hexanes: ethyl acetate, 60:40) afforded thiosemicarbazone **28** (0.042 g, 0.192 mmol, 11% yield) as two isomers in a 1:1.43 ratio (The more abundant isomer will be denoted with an "A" and the secondary isomer will be "B").

^{1}H NMR (500 MHz, DMSO): δ 10.06 (1H, s, isomer B), 9.95 (1H, s, isomer A), 8.12 (1H, s, isomer B), 8.08 (1H, s, isomer A), 7.65 (1H, s, isomer B), 7.61 (1H, s, isomer B), 7.23-7.13 (8H, m, isomers A and B), 3.80 (2H, s, isomer B), 3.54 (2H, s, isomer A), 2.86-2.82 (4H, m, isomers A and B), 2.60 (2H, t, J = 7.0 Hz, isomer A), 2.51 (2H, t, J = 6.5 Hz, isomer B).

^{13}C NMR (125 MHz, DMSO): δ 179.1, 179.1, 155.6, 154.5, 138.5, 137.4, 135.6, 133.6, 128.9, 128.4, 127.6, 127.5, 126.91, 126.87, 126.86, 126.5, 38.2, 32.2, 31.4, 28.9, 27.5, 26.6.

HPLC retention time: 5.46 min.

1-Benzosuberone thiosemicarbazone (**29**)

1-Benzosuberone (0.250 g, 1.56 mmol) was dissolved in anhydrous methanol (10 mL). The solution was heated to reflux for 10 min, and then thiosemicarbazide (0.149 g, 1.64 mmol) and a catalytic amount of *p*-toluenesulfonic acid were added. After 12 h at reflux, after the solution cooled to room temperature the product crashed out and was

collected by filtration and washed with methanol. After drying under high vacuum, thiosemicarbazone **29** was obtained as a white solid (0.180 g, 0.771 mmol, 49% yield).

^{1}H NMR (500 MHz, DMSO): δ 10.10 (1H, s), 8.24 (1H, s), 7.72 (1H, s), 7.56 (1H, d, *J* = 8.0 Hz), 7.30 (1H, td, *J* = 7.5 Hz, 1.5 Hz), 7.22 (1H, td, *J* = 7.5 Hz, 1.0 Hz), 7.15 (1H, d, *J* = 7.5 Hz), 2.69-2.65 (4H, m), 1.69 (2H, p, *J* = 7.0 Hz), 1.52 (2H, p, *J* = 6.0 Hz).

^{13}C NMR (500 MHz, DMSO): δ 179.5, 155.9, 139.3, 138.2, 129.6, 129.0, 128.5, 126.8, 31.3, 28.1, 25.7, 21.4.

HPLC retention time: 9.73 min.

Synthesis of Chromanone Derivatives

6-Chloro-chroman-4-one thiosemicarbazone (**30**)

6-chloro-chroman-4-one (0.300 g, 1.64 mmol) was dissolved in anhydrous methanol (10 mL). The solution was heated to reflux for 10 min, and then thiosemicarbazide (0.179 g, 1.97 mmol) and a catalytic amount of *p*-toluenesulfonic acid were added. After 23 h at reflux, after the solution cooled to room temperature the product crashed out and was collected by filtration and washed with methanol. After drying under high vacuum, thiosemicarbazone **30** was obtained as a white solid (0.335 g, 1.31 mmol, 79% yield).

^{1}H NMR (500 MHz, DMSO-d_6): δ 10.31 (1H, s), 8.39 (1H, d, *J* = 2.5 Hz), 8.31 (2H, br s), 7.78 (1H, dd, *J* = 8.5 Hz, 2.5 Hz), 6.90 (1H, d, *J* = 9 Hz), 4.21 (2H, t, *J* = 6.0 Hz), 2.89 (2H, t, *J* = 6.5 Hz).

^{13}C NMR (125 MHz, DMSO-d_6): δ 179.3, 156.2, 141.3, 131.0, 126.0, 125.2, 122.3, 119.6, 65.2, 25.6.

2-Phenyl-chroman-4-one thiosemicarbazone (**31**)

2-Phenyl-chroman-4-one (0.300 g, 1.34 mmol) was dissolved in anhydrous methanol (15 mL). The solution was heated to reflux for 10 min, and then thiosemicarbazide (0.128 g, 1.41 mmol) and a catalytic amount of *p*-toluenesulfonic acid were added. After 8 h at reflux, after the solution cooled to room temperature the product crashed out and was collected by filtration and washed with methanol. After drying under high vacuum, thiosemicarbazone **31** was obtained as a white solid (0.275 g, 0.920 mmol, 69% yield).

^{1}H NMR (500 MHz, DMSO): δ 8.70 (1H, s), 7.98 (1H, dd, *J* = 10 Hz, 2.5 Hz), 7.47-7.45 (7H, m), 7.03 (2H, m), 6.42 (1H, br s), 5.15 (1H, dd, *J* = 15.0 Hz, 4.0 Hz), 3.09 (1H, dd, *J* = 15.0 Hz, 3.0 Hz), 2.77 (1H, dd, *J* = 15.0 Hz, 9.0 Hz).

^{13}C NMR (125 MHz, DMSO): δ 179.1, 157.7, 142.8, 138.8, 132.2, 128.95, 128.92, 126.0, 124.3, 122.0, 119.1, 118.3, 77.2, 32.5.

Synthesis of 2-3-dihydroquinolinone Derivatives

3-(Phenylamino)propanoic acid (**32**)

Aniline (1.36 mL, 15.0 mmol), methyl acrylate (1.35 mL, 15.0 mmol), and acetic acid (0.081 mL, 1.5 mmol) were combined in a 5 mL microwave safe sealed vial. The mixture was heated in a microwave reactor to 200 °C for 20 min. The crude mixture was quenched over ice. Crude product precipitated out and was collected by filtration. This crude intermediate was then dissolved in a solution of water (30 mL), methanol (30 mL), and NaOH (3.20 g, 80 mmol). The reaction mixture was heated at 70 °C for 2.5 days. After cooling to room temperature, the mixture was acidified to pH 6 using concentrated HCl. The product was extracted with ether (4 × 20 mL), washed with brine, dried over

Na_2SO_4, and concentrated under reduced pressure. Purification using flash chromatography (silica gel, CH_2Cl_2:MeOH:Et_3N, 80:20:0.1) afforded propanoic acid **32** as a yellow oil (0.230 g, 1.39 mmol, 9% yield).

^{1}H NMR (500 MHz, DMSO): δ 7.16 (2H, t, *J* = 8.0 Hz), 6.70 (1H, t, *J* = 7.5 Hz), 6.63 (2H, d, *J* = 8.0 Hz), 3.41 (2H, br s), 2.60 (2H, t, *J* = 6.0 Hz).

2,3-Dihydroquinolin-4(1H)-one (**33**)

Polyphosphoric acid (4.00 g) was added to a flask containing propanoic acid **32** (0.100 g, 0.605 mmol). The reaction mixture was heated at 50 °C for 3.5 h using overhead stirring. The reaction was quenched with ice and pure product crashed out. Filtration afforded pure ketone **33** (0.044 g, 0.296 mmol, 49% yield).

^{1}H NMR (500 MHz, DMSO): δ 7.85 (1H, d, *J* = 8.0 Hz), 7.30 (1H, t, *J* = 7.0 Hz), 6.74 (1H, t, *J* = 7.0 Hz), 6.66 (1H, d, *J* = 8.0 Hz), 4.36 (1H, s), 3.60-3.57 (2H, m), 2.71 (2H, t, *J* = 7.0 Hz).

2,3-Dihydroquinolin-4(1H)-one thiosemicarbazone (**34**)

Ketone **33** (0.040 g, 0.272 mmol) was dissolved in anhydrous methanol (10 mL). The solution was heated to reflux for 10 min, and then thiosemicarbazide (0.026 g, 1.05 mmol) and a catalytic amount of *p*-toluenesulfonic acid were added. After 36 h at reflux, the solution was cooled to room temperature and concentrated under reduced pressure. Purification using flash chromatography (silica gel, hexanes:ethyl acetate, 80:20 to 0:100) afforded thiosemicarbazone **34** (0.058 g, 0.260 mmol, 97% yield).

^{1}H NMR (500 MHz, DMSO): δ 10.07 (1H, s), 8.13 (1H, s), 8.05 (1H, d, *J* = 7.5 Hz), 7.86 (1H, s), 7.05 (1H, td, *J* = 8.0 Hz, 1.5 Hz), 6.63 (1H, d, *J* = 7.5 Hz), 6.55 (1H, t, *J* = 8.0 Hz, 1.0 Hz), 6.13 (1H, s), 3.16 (2H, t, *J* = 6.0 Hz), 2.69 (1H, t, *J* = 6.5 Hz).

^{13}C NMR (125 MHz, DMSO): δ 178.8, 149.3, 146.5, 130.8, 125.9, 117.7, 117.1, 115.8, 39.9, 25.9.

HRMS (ESI) calcd for $C_{12}H_{15}N_3OS$ $(M + H)^+$ 221.0861, found 221.0856.

HPLC retention time: 6.85 min.

3-((4-Bromophenyl)amino)propanoic acid (**35**)

4-Bromoaniline (0.500 g, 2.91 mmol), acrylic acid (0.199 mL, 2.91 mmol), and 20% acetic acid (2 mL) were combined in a round bottom flask. The reaction mixture was heated to reflux with stirring for 18 h. The crude product was obtained as a brown oil at this point and taken on to the cyclization without purification.

6-Bromo-2,3-dihydroquinolin-4(1H)-one (**36**)

Crude propanoic acid **35** (4.1 mmol based on theoretical yield) was dissolved in Eaton's reagent (12.3 g) in a ratio of Eaton's reagent (3 g) per mmol of carboxylic acid). Note, Eaton's reagent is 7.7% phosphorous pentoxide in methanesulfonic acid. The solution was heated to 55°C overnight. After cooling to room temperature, the reaction was quenched over ice. The product was extracted with ethyl acetate (3 × 25 mL), washed with brine, dried over Na_2SO_4. Purification using flash chromatography (silica gel, hexanes:ethyl acetate, 83:17 to 0:100) afforded pure ketone **36** (0.029 g, 0.130 mmol, 3% yield calculated for two steps, beginning with propanoic acid synthesis See propanoic acid **35**).

^{1}H NMR (500 MHz, DMSO): δ 7.94 (1H, d, *J* = 2.5 Hz), 7.35 (1H, dd, *J* = 8.5 Hz, 2.5 Hz), 6.58 (1H, d, *J* = 8.5 Hz), 4.50 (1H, br s), 3.58 (2H, t, *J* = 7.0 Hz), 2.69 (7.0 Hz).
^{13}C NMR (125 MHz, DMSO): δ 192.4, 150.7, 137.6, 130.0, 120.5, 117.7, 110.2, 42.0, 37.6.

6-Bromo-2,3-dihydroquinolin-4(1H)-one thiosemicarbazone (**37**)

Ketone **36** (0.028 g, 0.124 mmol) was dissolved in anhydrous methanol (5 mL). The solution was heated to reflux for 10 min, and then thiosemicarbazide (0.012 g, 0.130 mmol) and a catalytic amount of *p*-toluenesulfonic acid were added. After 6 h at reflux, the solution was cooled to room temperature and concentrated under reduced pressure. Purification using flash chromatography (silica gel, hexanes:ethyl acetate, 85:15 to 0:100) afforded thiosemicarbazone **37** as a yellow solid (0.028 g, 0.092 mmol, 76% yield).
^{1}H NMR (500 MHz, DMSO): δ 10.09 (1H, s), 8.26 (1H, d, *J* = 2.0 Hz), 8.18 (1H, s), 8.14 (1H, s), 7.17 (1H, dd, *J* = 9.0 Hz, 2.5 Hz), 6.61 (1H, d, *J* = 8.5 Hz), 6.36 (1H, s), 3.17 (1H, t, *J* = 6.5 Hz), 2.69 (1H, t, *J* = 6.5 Hz).
^{13}C NMR (125 MHz, DMSO): δ 178.9, 148.2, 145.1, 133.1, 127.8, 119.5, 117.8, 108.8, 39.5, 25.4.

3-((4-Bromophenyl)(methyl)amino)propanoic acid (**38**)

4-Bromo-*N*-methylaniline (0.338 mL, 2.69 mmol), acrylic acid (0.184 mL, 2.69 mmol), and 20% acetic acid (2 mL) were combined in a round bottom flask. After heating at reflux for 18 h, the reaction was quenched with water (25 mL) and extracted with ethyl acetate (3 × 20 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. Purification using flash

chromatography (silica gel, hexanes:ethyl acetate:AcOH, 70:30:<1) afforded propanoic acid **38** (0.438 g, 1.70 mmol, 63% yield).

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.29 (2H, d, *J* = 8.5 Hz), 6.60 (2H, d, *J* = 9.0 Hz), 3.63 (2H, t, *J* = 7.0 Hz), 2.90 (3H, s), 2.59 (2H, t, 7.0 Hz).

^{13}C NMR (125 MHz, $CDCl_3$): δ 178.6, 178.5, 147.5, 132.0, 114.49, 114.47, 109.2, 48.5, 38.4, 31.5.

6-Bromo-1-methyl-2,3-dihydroquinolin-4-one (**39**)

Eaton's reagent (4.65 g) was added to a flask containing propanoic acid **38** (0.400 g, 1.55 mmol) and heated to 55°C for 18 h. After cooling to room temperature, the reaction mixture was quenched over ice and extracted with ethyl acetate (3 × 25 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. Purification using flash chromatography (silica gel, hexanes:ethyl acetate, 8812 to 0:100) afforded pure ketone **39** as a yellow solid (0.050 g, 0.208 mmol, 13% yield).

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.99 (1H, d, *J* = 2.5 Hz), 7.45 (1H, dd, *J* = 9.0 Hz, 6.0 Hz), 6.61 (1H, d, *J* = 9.0 Hz), 3.47 (2H, t, *J* = 7.0 Hz), 2.98 (3H, s), 2.73 (2H, t, *J* = 7,0 Hz).

^{13}C NMR (500 MHz, $CDCl_3$): δ 192.4, 151.4, 137.8, 130.4, 121.0, 115.2, 109.7, 51.2, 39.3, 38.0.

6-Bromo-N-methyl-2,3-dihydroquinolin-4-one thiosemicarbazone (**40**)

Ketone **39** (0.050 g, 0.208 mmol) was dissolved in anhydrous methanol (5 mL). The solution was heated to reflux for 10 min, and then thiosemicarbazide (0.020 g, 0.218

mmol) and a catalytic amount of *p*-toluenesulfonic acid were added. After 6 h at reflux, solid yellow product had crashed out. Upon filtration, pure thiosemicarbazone **40** was obtained as a yellow solid (0.011 g, 0.035 mmol, 17% yield).

^{1}H NMR (500 MHz, DMSO): δ 10.08 (1H, s), 8.35 (1H, d, *J* = 2.0 Hz), 8.20 (1H, s), 8.17 (1H, s), 7.31 (1H, dd, *J* = 9.0 Hz, 2.5 Hz), 6.67 (1H, d, *J* = 9.0 Hz), 3.15 (2H, t, *J* = 6.5 Hz), 2.83 (3H, s), 2.79 (2H, t, *J* = 6.5 Hz).

^{13}C NMR (500 MHz, DMSO): δ 178.9, 148.8, 144.8, 133.4, 127.7, 121.4, 115.4, 109.8, 49.1, 39.5, 26.1.

3-((2-Benzylphenyl)amino)propanoic acid (**41**)

2-benzylaniline (1.00 g, 5.45 mmol) was added to a round bottom flask with acrylic acid (0.374 mL, 5.45 mmol) and 20% acetic acid (4 mL). The reaction mixture was stirred at reflux for 9 h. The mixture was made basic with 1M NaOH and washed with ether (10 mL) to remove any remaining starting material. The aqueous layer was acidified to pH 7 with 10% by volume HCl solution. The product was extracted with ether (3 × 40 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. Crude brown oil **41** (0.930 g) was taken on to the cyclization without further purification.

8-Benzyl-2,3-dihydroquinolin-4(1H)-one (**42**)

Eaton's reagent (10.9 g) was added to a round bottom flask containing crude propanoic acid **41** (0.930 g, 3.64 mmol) and heated to 50 °C for 12 h. The reaction was quenched with ice and the product was extracted with ethyl acetate (4 × 20 mL). The combined organic layers with washed with brine, dried over Na_2SO_4, and concentrated

under reduced pressure. Purification using flash chromatography (silica gel, hexanes: ethyl acetate, 88:12 to 0:100) afforded pure ketone **42** as a yellow oil (0.118 g, 0.496 mmol, 13% yield).

^{1}H NMR (500 MHz, DMSO): δ 7.83 (1H, dd, *J* = 8.0 Hz, 1.5 Hz), 7.33-7.16 (6H, m), 6.73 (1H, t, *J* = 8.0 Hz), 4.29 (1H, s), 3.89 (2H, s), 3.46 (2H, t, *J* = 8.0 Hz), 2.64 (2H, t, *J* = 7.0 Hz).

8-Benzyl-2,3-dihydroquinolin-4(1H)-one thiosemicarbazone (**43**)

Ketone **42** (0.118 g, 0.496 mmol) was dissolved in anhydrous methanol (10 mL). The solution was heated to reflux for 10 min, and then thiosemicarbazide (0.047 g, 0.521 mmol) and a catalytic amount of *p*-toluenesulfonic acid were added. After 12 h at reflux, the solution was cooled to room temperature and concentrated under reduced pressure. Purification using flash chromatography (silica gel, hexanes: ethyl acetate, 88:12 to 0:100) afforded thiosemicarbazone **43** as a yellow solid (0.055 g, 0.178 mmol, 36% yield).

^{1}H NMR (500 MHz, DMSO): δ 10.05 (1H, s), 8.12 (1H, s), 8.00 (1H, d, *J* = 7.5 Hz), 7.84 (1H, s), 7.29-7.17 (5H, m), 6.84 (1H, d, *J* = 6.5 Hz), 6.52 (1H, t, *J* = 7.5 Hz), 5.73 (1H, s), 3.81 (2H, s), 3.19 (2H, t, *J* = 6.0 Hz), 2.69 (2H, t, *J* = 6.5 Hz).

^{13}C NMR (125 MHz, DMSO): δ 178.7, 147.1, 146.9, 140.2, 131.6, 129.2, 128.7, 126.4, 126.2, 124.3, 118.1, 116.8, 40.2, 36.2, 25.8.

Biochemical Evaluation

Final compounds were evaluated for inhibitory activity against cathepsins B, L and K. Our collaborators in the Trawick laboratory at Baylor University carried out all

biochemical evaluations. The assay conditions for cathepsin B and L have been previously reported by Pinney, Trawick and coworkers.[51,52]

Cathepsin B Assay for IC_{50} Determination

Human liver Cathepsin B (Calbiochem) was pre-incubated with inhibitors at various concentrations for 5 minutes at 37 °C. The assay was initiated by addition of substrate Z-Arg-Arg-aminomethylcoumarin (Bacchem) and the final assay conditions were 1.1 nM cathepsin B, 60 μM Z-R-R-AMC, 126 mM sodium potassium phosphate pH 6.0 (Fisher), 0.3 mM EDTA (Omnipure), 2.7 mM DTT (Omnipure), 0.004% Brij 35 (Sigma), and 2.0% DMSO (Acros) in a final volume of 200 μL. Inhibitors were serially diluted with DMSO and 0.01% Brij 35 to include a final concentration range of 20 μM to 10 pM. The reaction was monitored fluorometrically for 5 minutes at 37 °C using black 96 well Corning 3686 assay microplates with a Thermo Fluoroskan Ascent FL microplate reader at excitation and emission filter wavelengths of 355 nm and 460 nm, respectively. Data were analyzed to determine IC_{50} values utilizing GraphPad Prism 4.03 software with a minimum of a triplicate on the same microplate.

Several samples (16, 23, 29, and 34) were analyzed on the Fluoromax2. The assay conditions followed those noted for the microplate assay with the following modifications: 6 μM Z-R-R-AMC substrate and 250 μl final volume.

Cathepsin L Assay for IC_{50} Determination

Human liver Cathepsin L (Sigma) was pre-incubated with inhibitors at various concentrations for 5 minutes at 25 °C. The assay was initiated by addition of substrate Z-Phe-Arg-aminomethylcoumarin (Bacchem) and the final assay conditions were 1 nM

cathepsin L, 50 μM Z-F-R-AMC, 100 mM sodium acetate pH 5.5, 1 mM EDTA (Omnipure), 3 mM DTT (EMD), 0.01 % Brij 35 (Sigma), and 2.0 % DMSO (Acros). Inhibitors were serially diluted with DMSO and water to include a final concentration range of 10 μM to 10 pM. The reaction was monitored fluorometrically for 5 minutes at 25 °C using black 96 well Corning 3686 assay microplates with a Thermo Fluoroskan Ascent FL microplate reader at excitation and emission filter wavelengths of 355 nm and 460 nm, respectively. Data were analyzed to determine IC_{50} values utilizing GraphPad Prism 4.03 software with a minimum of a triplicate on the same microplate.

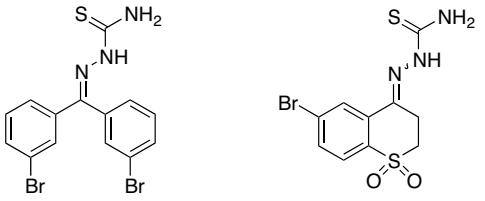
Cathepsin K Assay for IC_{50} Determination

Human procathepsin K (Enzo) was preactivated under acidic conditions, using 32.5 mM sodium acetate pH 3.5. Then, mature cathepsin K was pre-incubated with inhibitors at various concentrations for 5 minutes at 25 °C. The assay was initiated by addition of substrate Z-Phe-Arg-aminomethylcoumarin (Bacchem) and the final assay conditions were 1.5 nM cathepsin K, 50 μM Z-F-R-AMC, 150 mM sodium acetate pH 5.5, 2.5 mM EDTA (Omnipure), 2.5 mM DTT (EMD), 0.01 % Brij 35 (Sigma), and 4.0 % DMSO (Acros). Inhibitors were serially diluted with DMSO and water to include a final concentration range of 10 μM to 10 pM. The reaction was monitored fluorometrically for 5 minutes at 25 °C using black 96 well Corning 3686 assay microplates with a Thermo Fluoroskan Ascent FL microplate reader at excitation and emission filter wavelengths of 355 nm and 460 nm, respectively. Data were analyzed to determine IC_{50} values utilizing GraphPad Prism 4.03 software with a minimum of a triplicate on the same microplate.

CHAPTER THREE

Results and Discussion

The syntheses of various novel cathepsin L inhibitors containing the thiosemicarbazone moiety are detailed herein. The scale up resynthesis was required of two lead compounds that were previously discovered in the search for cruzain inhibitors to treat Chagas' disease for further biochemical evaluation in the area of the cathepsins.[59] These two lead compounds are shown in Figure 3.1. The synthesis of thiochromanone thiosemicarbazone analogues is detailed, followed by the synthesis of other derivatives based on variations of the thiochromanone scaffold. These derivatives are based upon replacing the sulfur at the 1-position of the thiochromanone scaffold with carbon in the tetralone scaffold, with nitrogen in the 2,3-dihydroquinolinone scaffold, and with oxygen in the chromanone scaffold. Explanations of synthetic strategies, as well as important observations and unexpected product formations will be described.

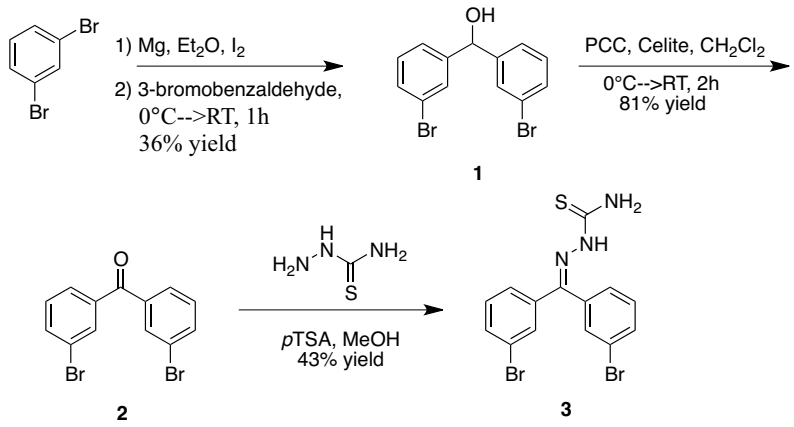


3 **6**

Figure 3.1. Lead compounds, dibromobenzophenone thiosemicarbazone **3** and 6-bromothiochromanone thiosemicarbazone **6**.

Scale Up of Previously Synthesized Lead Thiosemicarbazone Inihibitors

Scheme 3.1 details the synthesis of dibromobenzophenone thiosemicarbazone **3**, which was previously synthesized by Pinney and coworkers.[56] Compound **3** was originally synthesized as a potent inhibitor of cruzain (IC_{50} = 24 nM).[59] It was also screened against human cathepsin L and shown to be a potent inhibitor (IC_{50} = 1.5 nM).[60] In general, low (nM range) IC_{50} values (concentration at 50% inhibition of activity) are desirable in new small-molecule drug candidates. Encouraged by these results, a larger amount of thiosemicarbazone **3** was required for advanced biochemical and biological studies.



Scheme 3.1. Synthesis of dibromobenzophenone analogue **3**

The synthesis was carried out by combining 1,3-dibromobenzene and 3-bromobenzaldehyde in a classic Grignard reaction. The dibromobenzene was converted into the Grignard reagent through a reaction with magnesium. The benzaldehyde was added only after all of the magnesium was consumed. This reaction yielded alcohol **1** in a 36% yield. Alcohol **1** was oxidized using PCC and Celite, yielding ketone **2** in an 81% yield. During oxidation by PCC, reduced chromium salts, which can be dark and viscous, form and can make purification difficult. Adding Celite helps to solve this issue

because the salts will deposit on the Celite, where they are easily removed during filtration.[67] The resulting ketone **2** was then converted to the thiosemicarbazone in a condensation reaction with thiosemicarbazone in a 43% yield. This reaction required *p*-TSA as a catalyst.

This new batch of benzophenone thiosemicarbazone **3** was evaluated for its ability to inhibit cathepsin L and gave unexpected results. Dr. Rogelio Siles, a former member of the Pinney group, synthesized the original batch of compound **3**, known as RS-IV-35.[59,68] RS-IV-35 contained higher levels of impurities than the new batch, LMJ-I-006, as evidenced by NMR and HPLC. Use of LCMS, indicated that the main impurity was the analogue of compound **3** where one of the bromine atoms is replaced by a hydrogen atom as illustrated in Figure 3.2. That impurity is likely formed during the Grignard reaction. The impurity was previously evaluated,[59,68] and found to be inactive (IC_{50} > 10,000 nM).

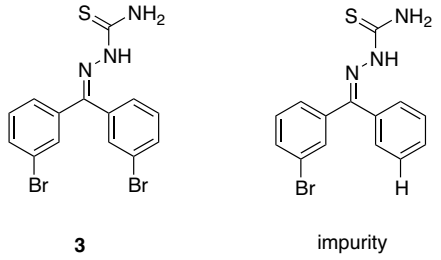


Figure 3.2. Benzophenone **3** and the predominant impurity formed during synthesis

One feasible explanation for the varying inhibitory activity of the different batches has to do with the solubility of the compound under the conditions of the biochemical assays. The IC_{50} values for cathepsin L are reported in Table 3.1. Collaborators in the Trawick laboratory adjusted parameters in the assay to further

understand the varying IC_{50} value results. The original conditions of the assay used 0.7% DMSO and a substrate concentration of 50 μM. Increasing the amount of DMSO to 3%, greatly improved the IC_{50} value (IC_{50} = 6.6 nM). This result is easily understood by considering the high solubility of these compounds in DMSO, so increasing the amount in the assay allows more inhibitor to be in solution and act on the enzyme. Using less substrate also improved inhibitory activity. Going from substrate concentrations of 50 μM to 5 μM decreased IC_{50} values at 3% DMSO assays (IC_{50} from 6.6 nM and 1.77 nM, respectively). Two percent DMSO and 5 μM substrate was used from then on in the cathepsin L assay,[54,55] which gave an IC_{50} value of 3.73 nM.

Table 3.1. Cathepsin L inhibitory activity of dibromobenzophenone thiosemicarbazone

Batch	Cathepsin L IC_{50} (nM)	% DMSO	Substrate (μM)
RS-IV-35	1.5	0.7	50
LMJ-I-006	65	0.7	50
LMJ-I-006	6.6	3.0	50
LMJ-I-006	3.73	2.0	5
LMJ-I-006	1.77	3.0	5

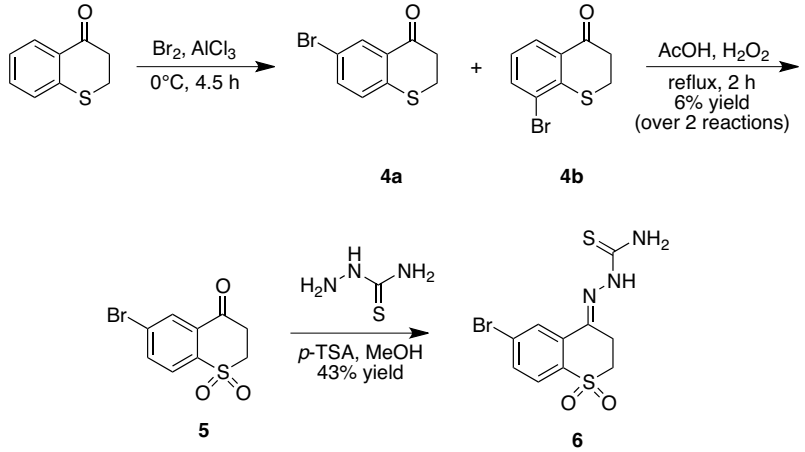
The second lead inhibitor (thiosemicarbazone **6**) that was identified by Pinney and coworkers to be potent against cathepsin L, was based on the thiochromanone scaffold.[59] Thiosemicarbazone **6** was originally found to be a fairly potent inhibitor of cruzain (IC_{50} = 210 nM).[59] This compound demonstrated high inhibitory activity against cathepsin L (IC_{50} = 1 nM) in the initial assay.[68] Analogue **6** was originally synthesized by Dr. Rogelio Siles and that first batch is referred to as RS-V-96. A larger amount of

compound **6** was also required for further studies and the new batch is referred to as LMJ-I-45. Similarly to the scale up of the benzophenone analogue **3**, the biochemical assay yielded unexpected results as displayed in Table 3.2. RS-V-96 was evaluated under the original assay conditions of 0.7% DMSO and substrate concentration of 50 μM. When LMJ-I-45 was evaluated under the new conditions of 2% DMSO and substrate concentration of 5 μM, the result was significantly decreased inhibition (IC_{50} = 574 nM).

Table 3.2. IC_{50} determination for 6-bromothiochromanone thiosemicarbazone analogue **6**

Batch	IC_{50} (nM)	% DMSO used in assay	Substrate (μM)
RS-V-96	1	0.7	50
LMJ-I-45	574	2.0	5

The synthesis of 6-bromothiochromanone thiosemicarbazone (compound **6**) was carried out as previously reported,[59] and is described in Scheme 3.2. Commercially available thiochroman-4-one was brominated using diatomic bromine and the Lewis acid, aluminum trichloride. The bromination resulted in more than one regioisomer; yielding



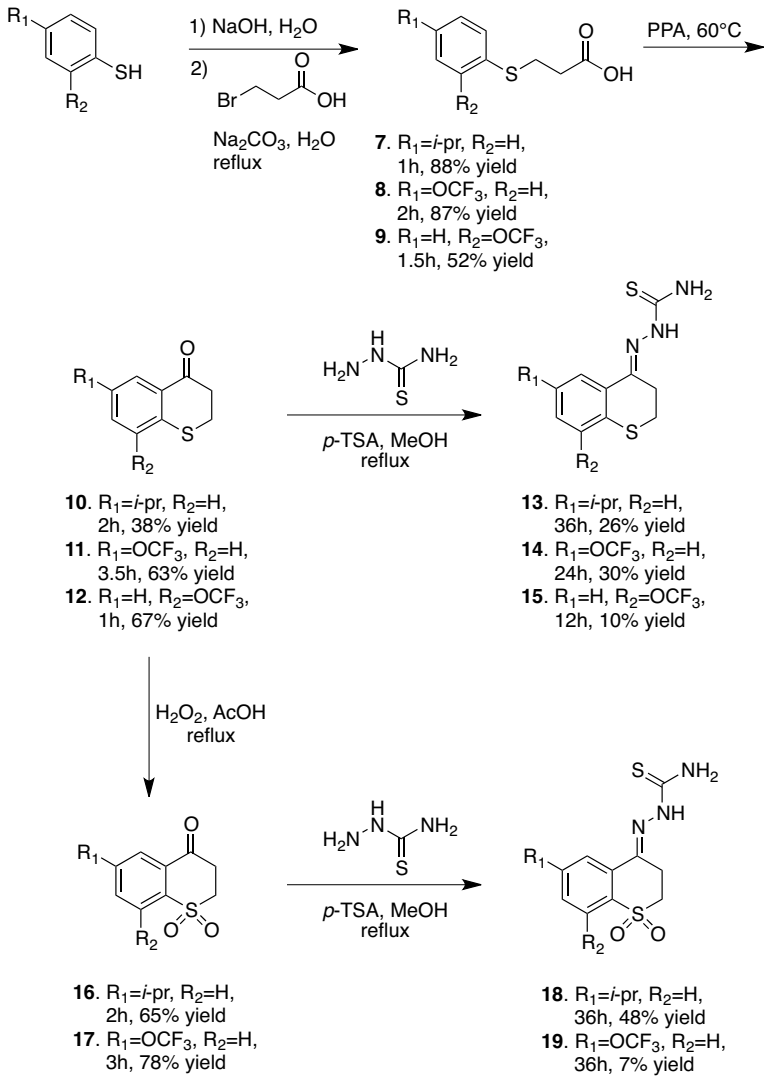
Scheme 3.2. Synthesis of 6-bromothiochromanone thiosemicarbazone

products with bromine at the 6-position (**4a**) and 8-position (**4b**). These regioisomers proved too difficult to separate at this step. After oxidizing the sulfur to a sulfone using glacial acetic acid and hydrogen peroxide, the desired 6-bromo-1,1-dioxo thiochromanone was more easily purified. The overall yield through both steps is quite low due to the lack of selectivity in the bromination step.

Synthesis of Thiochromanone Thiosemicarbazone Derivatives

The success of the 6-bromothiochromanone thiosemicarbazone **6** paved the way for the design of new derivates based on the thiochromanone scaffold. New derivatives were made to explore the importance of the bromine functional group on inhibitory activity. Isopropyl and trifluoromethoxy groups were introduced to the aromatic portion of the ring. The synthesis of these new functionalized thiochromanone and 1,1-dioxothiochromanone thiosemicarbazones are described in Scheme 3.3.

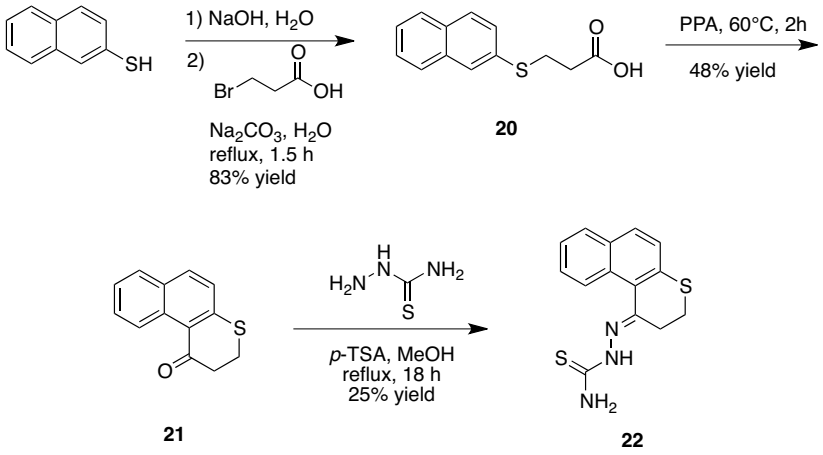
Commercially available, appropriately functionalized thiophenols were chosen as starting materials. 4-Isopropyl thiophenol, 6-trifluoromethoxy thiophenol, and 8-trifluoromethoxy thiophenol were each used to make analogues. The alkylation was carried out by deprotonating the thiol with NaOH and adding in a solution of 3-bromopropionic acid and Na_2CO_3. This solution was also basic to protect the carboxylic acid. The carboxylic acid products were cyclized using the viscous polyphosphoric acid. This reaction is performed without the use of a solvent, which is referred to as neat. Use of overhead stirring was helpful as the reagents are so viscous. Each reaction turned a shade of orange-red, which became yellow after being quenched over ice. These first two steps, the alkylation and cyclization, were adapted from van Vliet and coworkers.[69]



Scheme 3.3. Synthetic route for thiochromanone and 1,1-dioxo thiochromanone thiosemicarbazone analogues

Condensation with thiosemicarbazide was carried out as before with a portion of the ketone intermediates to yield the sulfide thiosemicarbazone derivatives **13**, **14**, and **15**. The 6-isopropyl and 6-trifluoromethoxy ketone analogues (**10** and **11**, respectively) were oxidized using glacial acetic acid and hydrogen peroxide. These two sulfone analogues (**16** and **17**) were then condensed with thiosemicarbazide to obtain analogues **18** and **19**.

Naphthalene-2-thiol was used as a starting material in the synthesis of compound **22**, as shown in Scheme 3.4. The alkylation was done as before in base with 3-bromopropionic acid.[69] The cyclization was carried out using PPA followed by quenching over ice; however, the product shown (compound **21**) was not the expected cyclization product. The carbonyl carbon bonded to the 1-position of the naphthalene ring as opposed to the anticipated 3-position. The formation of compound **21** can be explained by resonance. The carbocation intermediate that forms leading to the actual product has nine resonance structures, whereas the carbocation leading to the anticipated product would have only seven resonance structures (Figure 3.1). Resonance structures indicate the extent to which the positive charge on the carbocation intermediate can disperse throughout the molecule. The more that the charge can be dispersed, the more stable the carbocation intermediate will be. Therefore the cyclized product **21** is obtained because it forms a more stable intermediate carbocation. The final condensation was carried out using thiosemicarbazide and a catalytic amount of *p*-TSA as previously described.



Scheme 3.4. Synthesis of the fused ring thiochromanone thiosemicarbazone analogue

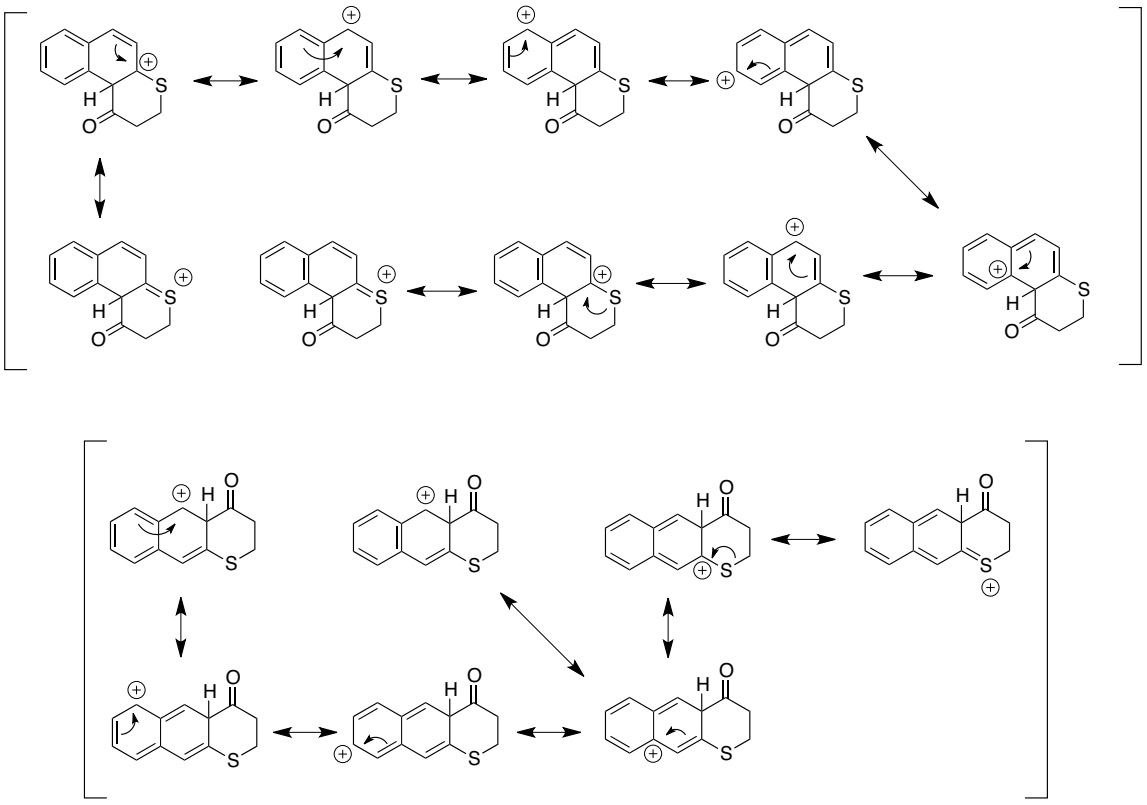


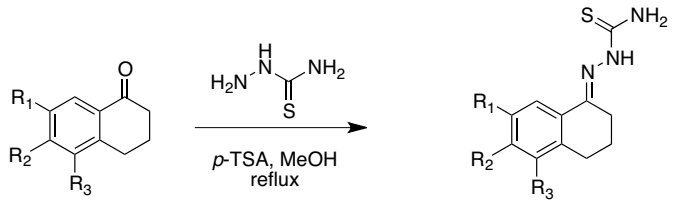
Figure 3.3. Resonance structures that support the formation of the cyclization product **21**

Synthesis of Tetralone Thiosemicarbazone Derivatives

To explore not only the importance of various functional groups on the aromatic ring but also the importance of the sulfur in the aliphatic ring, tetralone derivatives were synthesized to compare compounds with a carbon in place of a sulfur or sulfone. Commercially available tetralone starting materials with different functionality on the aromatic ring were condensed with thiosemicarbazide and a catalytic amount of *p*-TSA as described in Scheme 3.5.

Many of these syntheses were simple, one step reactions with pure product precipitating out of solution and needing no further purification beyond filtration. 7-Methoxy, 6-methoxy, and 5-methoxy tetralone analogues (**23**, **24**, and **25**, respectively)

were obtained with one-step condensation reactions that yielded pure product requiring no extensive purification.



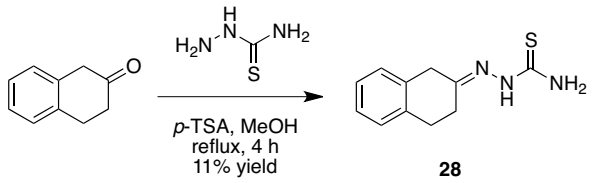
23. R_1=OCH_3, R_2=H, R_3=H, 8h, 72% yield
24. R_1=H, R_2=OCH_3, R_3=H, 4h, 72% yield
25. R_1=H, R_2=H, R_3=OCH_3, 8h, 71% yield
26. R_1=H, R_2=H, R_3=OH, 18h, 5% yield
27. R_1=H, R_2=NH_2, R_3=H, 6h, 20% yield

Scheme 3.5. Synthesis of tetralone thiosemicarbazone derivatives

5-Hydroxytetralone thiosemicarbazone (compound **26**) presented more difficulties during purification that led to a low yield of 5%. The product that precipitated out of the reaction was pure but contained a significant amount of ethyl acetate from the work up, as evidenced by NMR. Drying overnight under house vacuum did not remove the ethyl acetate. An Abderhalden drying pistol, which allows drying of a compound under reduced pressure at an elevated temperature, was also applied for several hours. As the percentage of ethyl acetate still did not decrease, as shown by NMR, recrystallization was used to free compound **26** of solvent. The first attempt was done using hot methanol as the recrystallizing solvent. The product that crystallized was free of ethyl acetate but persistently held on to a significant amount of methanol. A second recrystallization from ethanol proved successful in drying the hydroxyl derivative from solvent, the only downside being the considerably lower yield.

6-Amino tetralone thiosemicarbazone (compound **27**) also required recrystallization, but only from methanol as the recrystallizing solvent. The methanol did

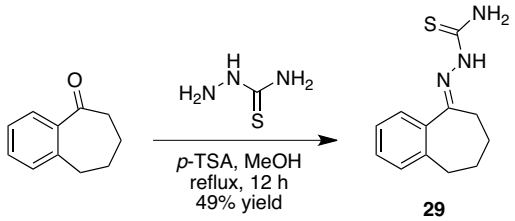
not stick to this compound and approximately six hours under high vacuum using an oil pump was enough to dry the final product. One round of recrystallization still resulted in a reduced yield (20%). However this yield was high when compared to the purification of 5-hydroxytetralone thiosemicarbazone analogue **26** which required two rounds of recrystallization utilizing two different solvents.



Scheme 3.6. Synthesis of 2-tetralone thiosemicarbazone

Another compound of interest was 2-tetralone thiosemicarbazone **28**. This compound was designed to explore the importance of the placement of the thiosemicarbazone moiety on the scaffold. The commercially available 2-tetralone was condensed with thiosemicarbazide as before with *p*-TSA as the catalyst. The purification was less straightforward as the TLC of the product showed many impurities. Recrystallization and column chromatography were both utilized, yielding isomers that were inseparable. This result was interesting because it is the only compound from this study to present two isomers, as evidenced by NMR. These *E/Z* isomers about the imine bond are in a 1:1.43 ratio. Thiosemicarbazone *E/Z* isomers were previously reported by Pinney and coworkers as well as others.[54,55,56,57] In some of those studies however isomers were seen by HPLC. When compound **28** was analyzed by HPLC, it showed only a single peak.

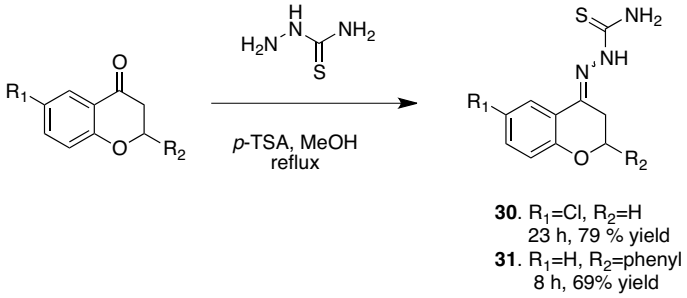
As a further effort to broaden the structure activity relationship associated with this small-library of compounds, benzosuberone analogue **29** was synthesized, as shown in Scheme 3.7. This analogue possesses a 7-membered aliphatic ring. This compound was obtained from the condensation of thiosemicarbazide with the commercially available 1-benzosuberone. The pure product precipitated out of the reaction and required no further purification.



Scheme 3.7 Synthesis of 1-benzosuberone thiosemicarbazone

Synthesis of Chromanone Thiosemicarbazone Derivatives

Similar to the tetralone derivatives, chromanone analogues shown in Scheme 3.8 were synthesized to observe the effect that an oxygen atom in the 1-position has on activity against cathepsin L. Commercially available chromanones were treated with



Scheme 3.8. Synthesis of chromanone analogues **30** and **31**.

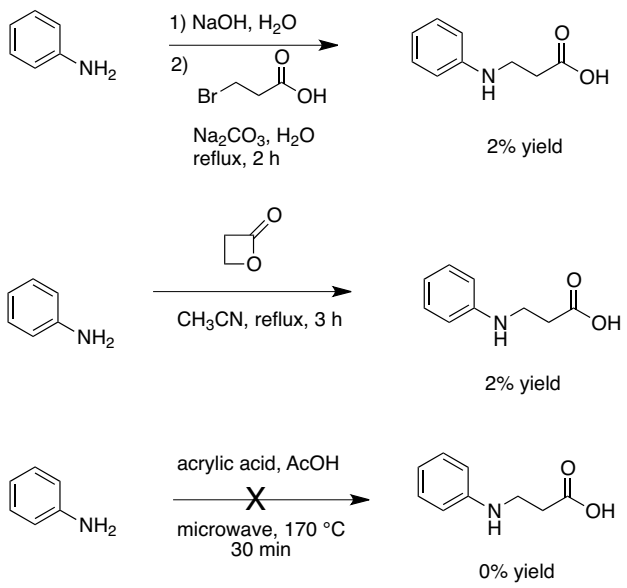
thiosemicarbazide and a catalytic amount of *p*-TSA in methanol to obtain analogues **30** and **31**.

Synthesis of 2,3-Dihydroquinolinone Derivatives

The final set of derivatives introduced nitrogen to the 1-position in place of the sulfur from the thiochromanone thiosemicarbazone derivatives. As well as providing more information about the importance of sulfur at the 1-position, having an amine in this position will provide opportunities to convert the compounds into HCl salts. Salt formation in pharmaceutical research has been of increased interest over the last 60 years because of the low solubility of new organic compounds.[70] Aqueous solubility is a key factor in viable drugs because the body does not provide the same organic solvents as the laboratory environment where the compounds are synthesized. It has been estimated that one-third of new organic compounds that are discovered display aqueous solubility of less than 10 μg/mL.[70] According to the rules outlined by Lipinski, solubility is one of the key factors that determines bioavailability. Solubility has been closely linked to permeability through membranes. For compounds with medium permeability, aqueous solubility must be at least 52 μg/mL to be a viable, bioavailable drug.[68] For this reason, aqueous solubility of organic compounds must be increased. Salt formation is a viable solution in many cases because salts generally exhibit higher aqueous solubility than their free basic or acidic forms.[70] According to the Handbook of Pharmaceutical Salts, two-thirds of all current drugs are salts.[71] This makes designing drugs with sites that lend themselves to salt formation very appealing.

The synthesis of these amine-containing compounds offered challenges. Many reactions were attempted before finding a route that worked as shown in Scheme 3.9. The first target was 2,3-dihydroquinolin-4(1H)-one thiosemicarbazone **34** (Scheme 3.10). The same route that was used to prepare the thiochromanone derivatives only resulted in

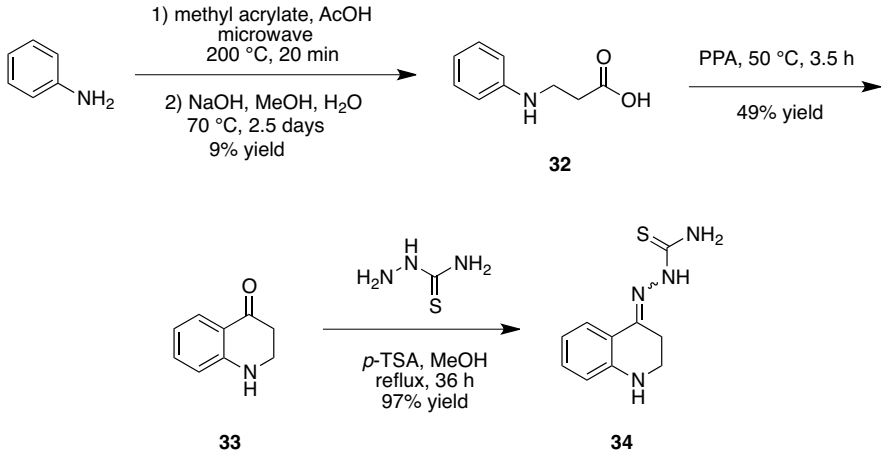
a 2% yield. This low yield is understood by considering the pKa values of aniline and thiophenol. An aryl thiol has a pKa of about 7; whereas the pKa of the amine in aniline is 25. In basic conditions the aryl thiol was deprotonated, encouraging nucleophilic attack of the bromopropionic acid. Aniline is already relatively basic and does not behave the same way under these conditions. As it is basic on its own, aniline does react somewhat but the yield was very low (2%). Another strategy was adapted from the literature that showed success with reacting amines with ß-propiolactone.[72] This reaction was tried several times with the highest yield also being 2%. The third route adapted from the literature included using acrylic acid and acetic acid under microwave conditions.[73] This reaction produced mostly a dark brown tarry product that had none of the desired product.



Scheme 3.9. Initial attempts to synthesize 3-(phenylamino)propanoic acid **32**

By changing some of the parameters of the microwave reaction conditions, yields of the desired propanoic acid intermediate **32** were increased enough to move on to

cyclization. Using methyl acrylate instead of acrylic acid and a higher temperature with shorter time in the microwave, a crude ester intermediate was synthesized. Hydrolysis of the ester produced intermediate **32** in a 9% yield. Once the propanoic acid was obtained, cyclization and condensation with thiosemicarbazide were carried out as before with ease.



Scheme 3.10. Synthetic route towards 2,3-hydroquinolinone derivative **34**

6-Bromo-2,3-dihydroquinolin-4(1H)-one thiosemicarbazone (**37**) was designed because of its similarity to the lead compound (**6**). The same technique using the methyl acrylate and microwave conditions did not yield any desired product. It was decided that conventional heating should be tried and acrylic acid was used to eliminate the hydrolysis step. The alkylation of the amine produced mono and dialkylated products as shown in Figure 3.4.

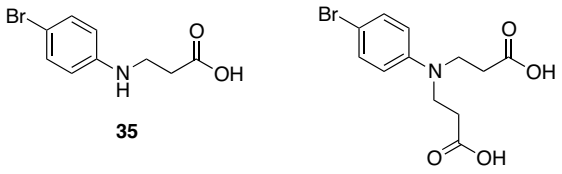
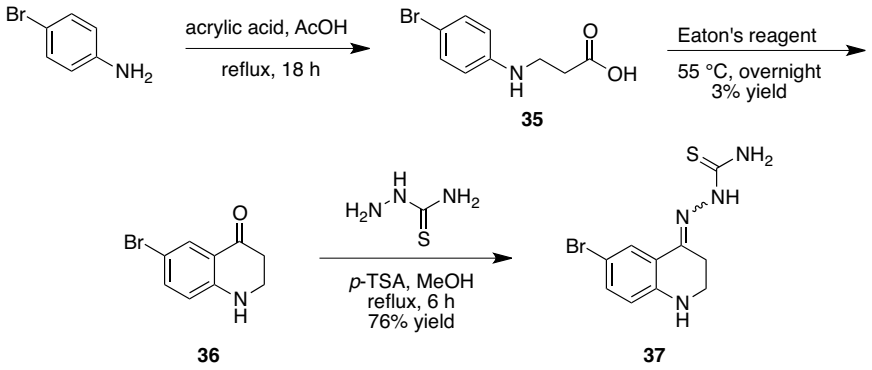


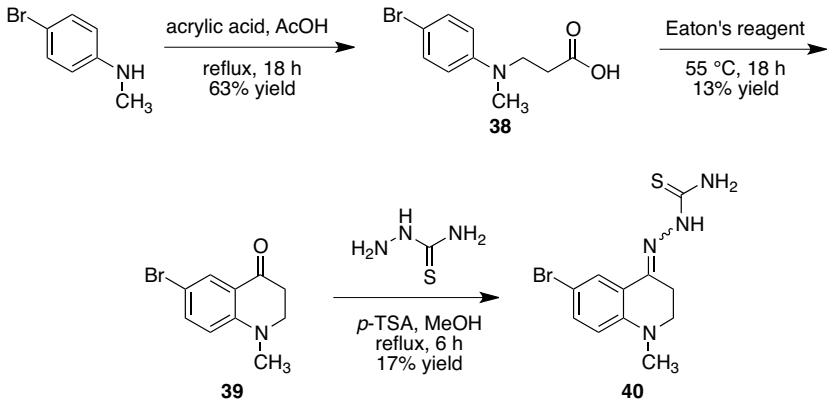
Figure 3.4. Desired mono alkylated product **35** and the dialkylated byproduct

After several unsuccessful purification attempts, the crude mixture of mono and dialkylated products was taken on to the cyclization step where it was possible to purify intermediate **36**. In this route, cyclization was carried out using Eaton's reagent, which is 7.7% phosphorous pentoxide in methanesulfonic acid. The ketone was condensed with thiosemicarbazide to obtain the bromo derivative **37**. This synthesis is illustrated in Scheme 3.11.



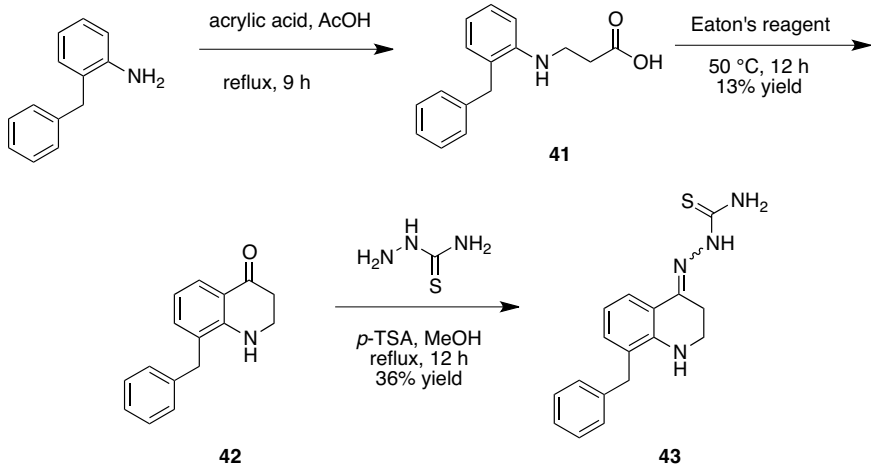
Scheme 3.11. Successful synthesis of analogue **37**

To eliminate the problem of the dialkylation, 1-bromo-*N*-methylaniline was used to synthesize compound **40**, as shown in Scheme 3.12. Using the secondary amine starting material improved yields significantly (63%). The cyclization and condensation were carried out as before. The yields of these two reactions were low, but enough product was obtained to continue.



Scheme 3.12. Synthetic route towards the synthesis of 6-bromo-1-methyl-2,3-dihydroquinolin-4-one thiosemicarbazone **40**

Finally, 2-benzylaniline was utilized to access a derivative with a different functionality on the aromatic ring. Mono and dialkylation products were seen as predicted and following cyclization of the crude product, desired cyclized product **42** was obtained in 13% yield. After condensation with thiosemicarbazide, final product **43** was obtained in 36% yield (Scheme 3.13).

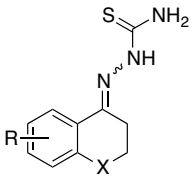


Scheme 3.13. Synthetic route towards analogue **43**

Biological Evaluation

All IC_{50} values were determined through collaboration with members of the Trawick group at Baylor University. Table 3.3 elucidates the results of assays involving thiochromanone thiosemicarbazone derivatives. Of this group, 6-trifluoromethoxy derivative **14** showed significant inhibition of cathepsin L (IC_{50} = 256 nM). 6-Isopropylthiochromanone thiosemicarbazone **13** was the most potent inhibitor of the entire set of analogues and this inhibitory activity was against cathepsin K (IC_{50} = 21 nM).

Table 3.3. IC_{50} values for thiochromanone analogues

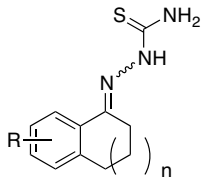


Compound	R	X	IC_{50} values (nM)		
			Cat B	Cat L	Cat K
6	6-Br	SO_2	>10,000	574	ND
13	6-*i*-pr	S	>10,000	>10,000	21
18	6-*i*-pr	SO_2	>10,000	>10,000	111
14	6-OCF_3	S	>10,000	256	179
19	6-OCF_3	SO_2	>10,000	3960	471
15	8-OCF_3	S	>10,000	>10,000	>10,000
22	Fused 5,6-phenyl	S	>10,000	>10,000	ND

ND – not determined

The tetralone analogues were also evaluated for activity against cathepsins B, L, and K. None of the tetralone derivatives exhibited any activity against the enzymes (IC_{50} >10,000 nM) as shown in Table 3.4.

Table 3.4. IC_{50} values for tetralone analogues

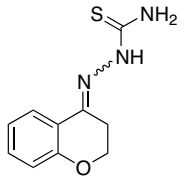


Compound	R	n	Thiosemicarbazone position	IC_{50} values (nM)		
				Cat B	Cat L	Cat K
23	7-OCH_3	1	1	>10,000	>10,000	ND
24	6-OCH_3	1	1	>10,000	>10,000	ND
25	5-OCH_3	1	1	>10,000	>10,000	ND
26	5-OH	1	1	>10,000	>10,000	>10,000
27	6-NH_2	1	1	>10,000	>10,000	>10,000
28	H	1	2	>10,000	>10,000	ND
29	H	2	1	>10,000	>10,000	>10,000

ND – not determined

The two chromanone derivatives were evaluated and the results are shown in Table 3.5. 6-Chlorochromanone thiosemicarbazone did show some activity against cathepsin L (IC_{50} = 369 nM).

Table 3.5. IC_{50} values for chromanone analogues

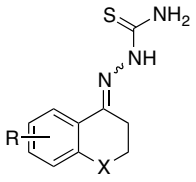


Compound	R	IC_{50} values (nM)		
		Cat B	Cat L	Cat K
30	6-Cl	>10,000	369	ND
31	2-phenyl	>10,000	>10,000	>10,000

ND – not determined

The final group of 2,3-dihydroquinolinone analogues was also tested for inhibition and the results are detailed in Table 3.6. None of the derivatives have been

Table 3.6. IC_{50} values for 2,3-dihydroquinolinone derivatives



Compound	R	X	IC_{50} values (nM)		
			Cat B	Cat L	Cat K
34	H	NH	>10,000	>10,000	ND
37	6-Br	NH	>10,000	164	ND
40	6-Br	NCH_3	ND	ND	ND
43	6-benzyl	NH	>10,000	ND	ND

ND – not determined

evaluated to date for inhibition against cathepsin K. Compound **40** has evaluated. 6-Bromo-2,3-dihydroquinolinone thiosemicarbazone **37** did show potent inhibition against

cathepsin L (IC_{50} = 164 nM), which is exciting. This compound was of particular interest because of its structural similarity to one of the lead compounds and was a challenge to obtain.

CHAPTER FOUR

Conclusions

Through the course of this research, understanding of synthetic strategies towards the thiochromanone and 2,3-dihydroquinolinone derivatives was furthered. Accessing these two similar molecular frameworks required a fairly different synthetic strategy. The cyclization and thiosemicarbazone condensation steps are well defined and successful for a wide variety of functionalized derivatives. This research could benefit from continued optimization of the synthesis of the 2,3-dihydroquinolinone as these preliminary routes have decidedly low yields.

The biochemical evaluations described herein have added to the understanding of the structure activity relationship of this class of compounds to cathepsins B, L, and K. Functionalizing the aromatic ring of the thiochromanone structure gave insight into the importance of the bromine atom in the lead thiosemicarbazone analogue **6**. The 6-trifluoromethoxy analogues (**14** and **19**) both exhibited activity against cathepsin L (IC_{50} = 256 nM and 3960 nM, respectively) with the thiochromanone demonstrating higher potency than the 1,1-dioxothiochromanone. In fact, the thiochromanone thiosemicarbazone analogues outperformed the corresponding 1,1-dioxothiochromanone thiosemicarbazone derivatives in the cathepsin K assays as well. Many of these functionalized thiochromanone thiosemicarbazone analogues were quite potent against cathepsin K with 6-isopropylthiochromanone thiosemicarbazone **13** demonstrating an IC_{50} value of 21 nM.

Replacing the sulfur atom with carbon as explored with the tetralone analogues, rendered compounds with no activity against cathepsins B, L or K (IC_{50} >10,000 nM). Of the two chromanone analogues, only the 6-chlorochromanone thiosemicarbazone analogue **30** showed activity against cathepsin L (IC_{50} = 369 nM). Placement of a halogen in the 6-position of these compounds appears to have a positive effect on their inhibitory ability.

2,3-Dihydroquinolinone thiosemicarbazone derivatives investigated the effects that nitrogen incorporation would have on activity. Not all of the compounds have been evaluated to date, but incorporation of a halogen in the 6-position (as seen in 6-bromo-2,3-dihydroquinolinone thiosemicarbazone **37**) again led to potent activity of this compound against cathepsin L (IC_{50} = 164 nM).

This small-library of nineteen new compounds is part of a larger library of thiosemicarbazone analogues synthesized in the Pinney laboratory. Additional information becomes apparent when looking at the whole library. To further study the SAR of these compounds with cathepsins K and L, analogues should be synthesized and evaluated that are directly comparable. New compounds that are directly comparable (in terms of functional group type or location) to the lead compounds discovered in the cruzain research, will be targeted for future synthesis. It is also important to design compounds that are directly comparable across the other scaffolds of interest: tetralone, chromanone, and 2,3-dihydroquinolinone structures. For instance, incorporating the same functional group in the equivalent position on each of these scaffolds would provide directly comparable data. Some of the new compounds discussed herein were targeted based on ease of synthesis. As we knew little of the inhibitory potency of tetralone and

chromanone derivatives, targets were chosen that had readily available starting materials. By choosing targets in this fashion, non-predictable results were obtained, such as the potent isopropyl inhibitor (compound **13**) of cathepsin K. This approach also limited the number of direct comparisons that could be made. Further design of new compounds should be guided by knowledge gained in these preliminary IC_{50} determinations, and will require the development of appropriate synthetic pathways.

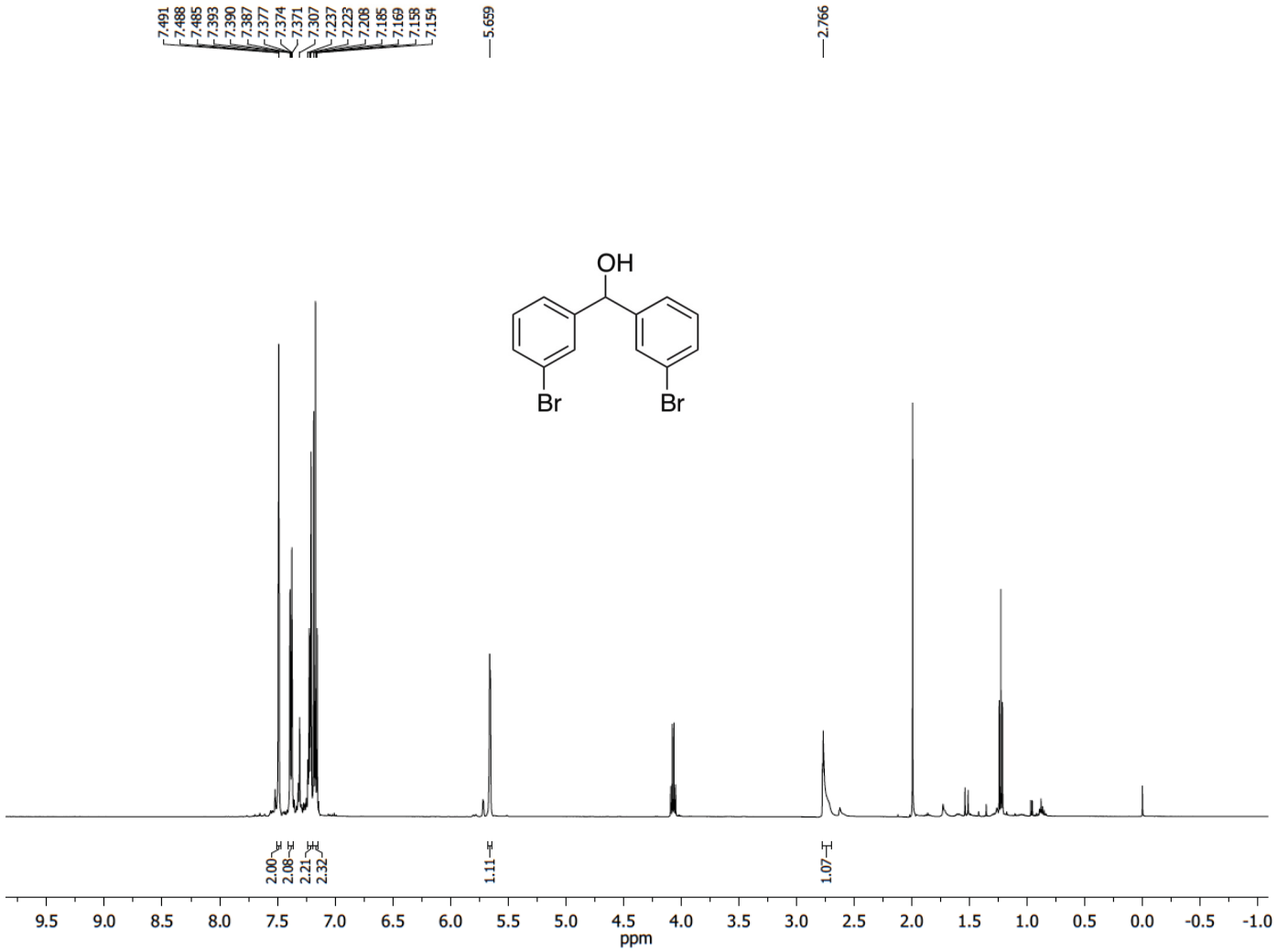
Another consideration for future work in this area relates to advanced biochemical and biological evaluations. Promising compounds should be evaluated in cell migration and invasion assays to assess their ability to hinder the cancer cells' mobility that is crucial for metastasis. Eventually, small animal studies will be important to assess an inhibitor's ability to impede metastatic capability *in vivo*. The best drug candidates will not only inhibit invasion in both of these studies, but will be bioavailable and easy to dose. This initial library of molecules and associated SAR studies provide an important foundation for future studies directed toward the discovery and development of effective, anti-metastatic, therapeutic agents.

APPENDIX

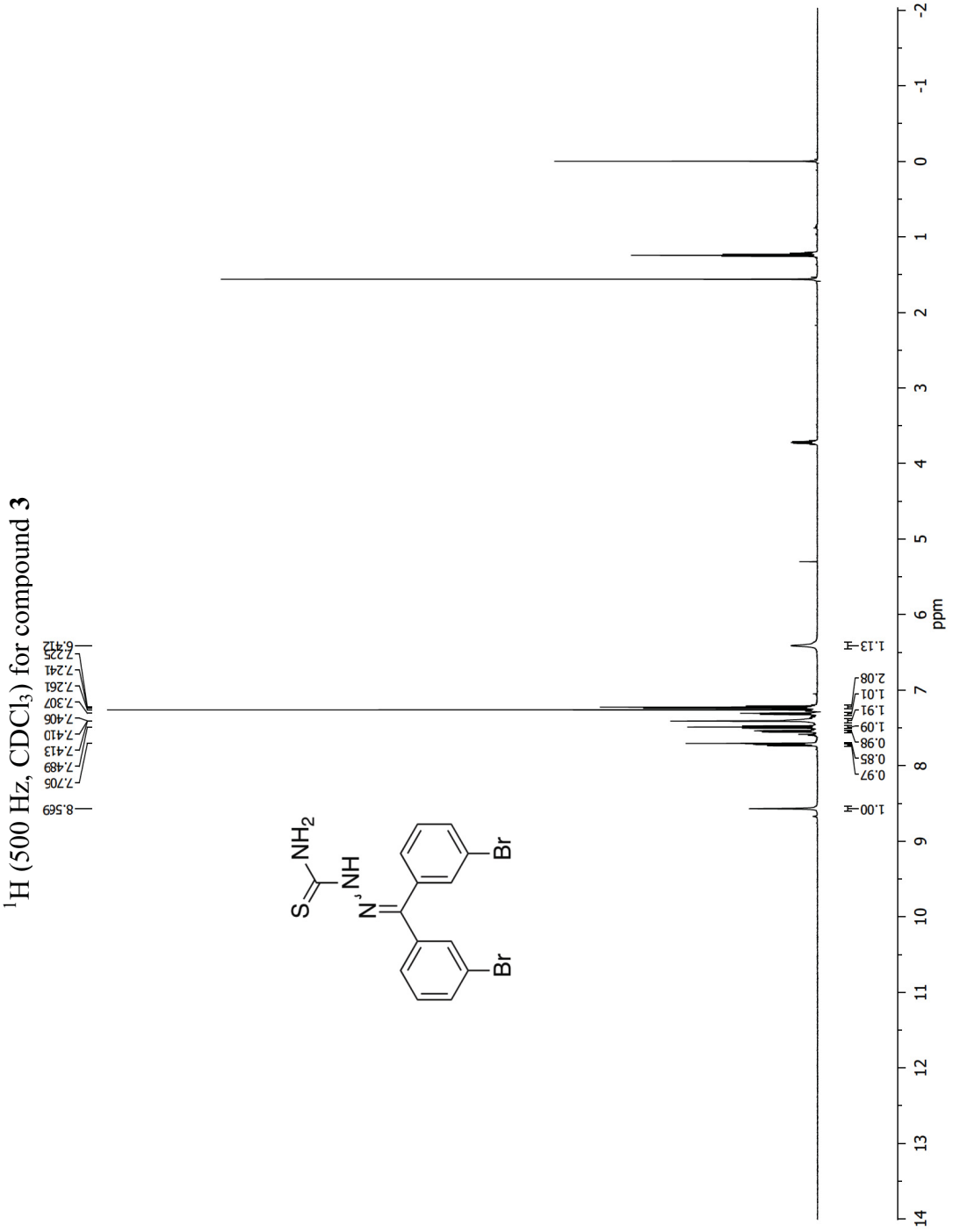
APPENDIX

NMR spectra

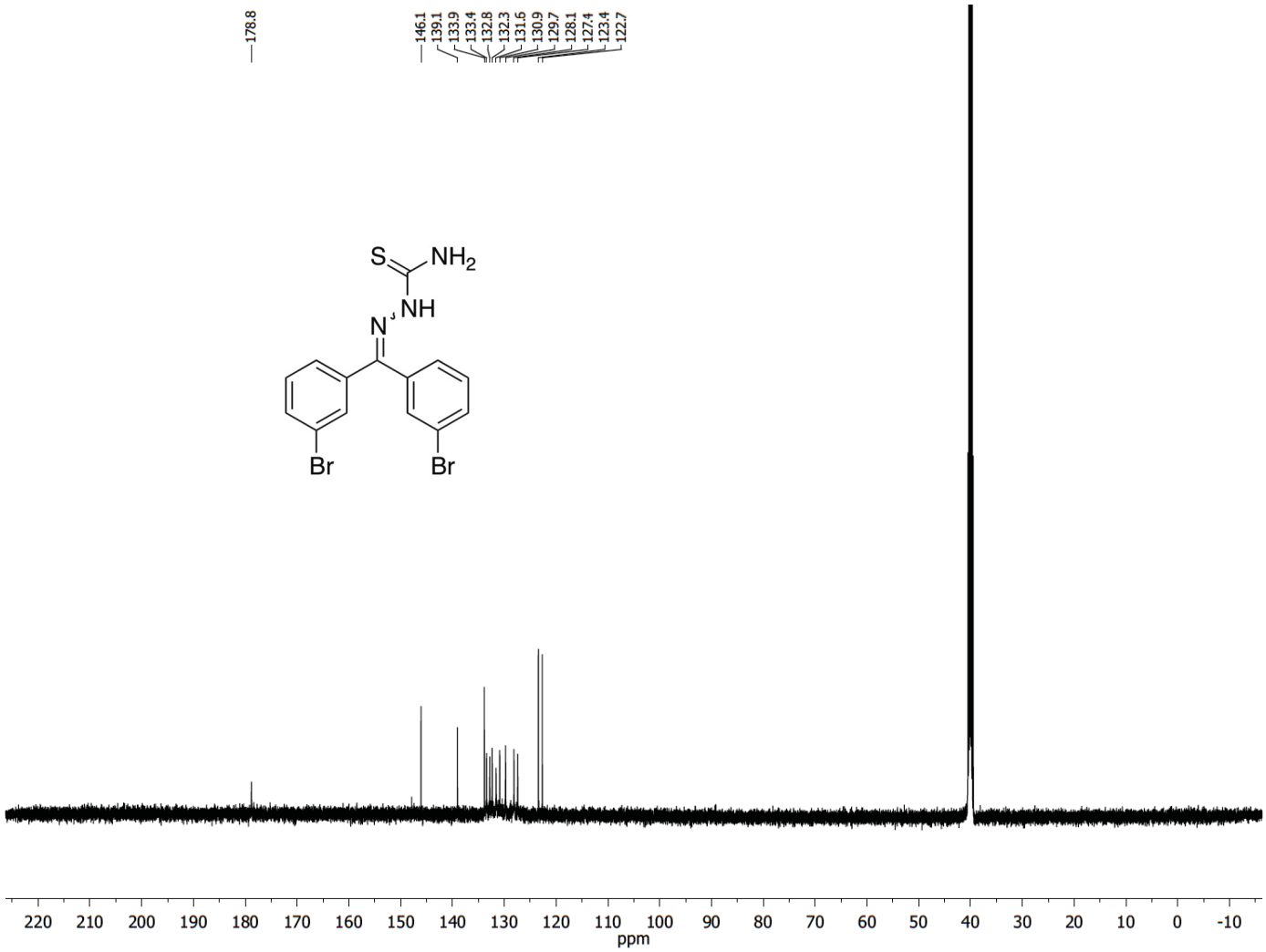
^{1}H (500 Hz, $CDCl_3$) for compound **1**



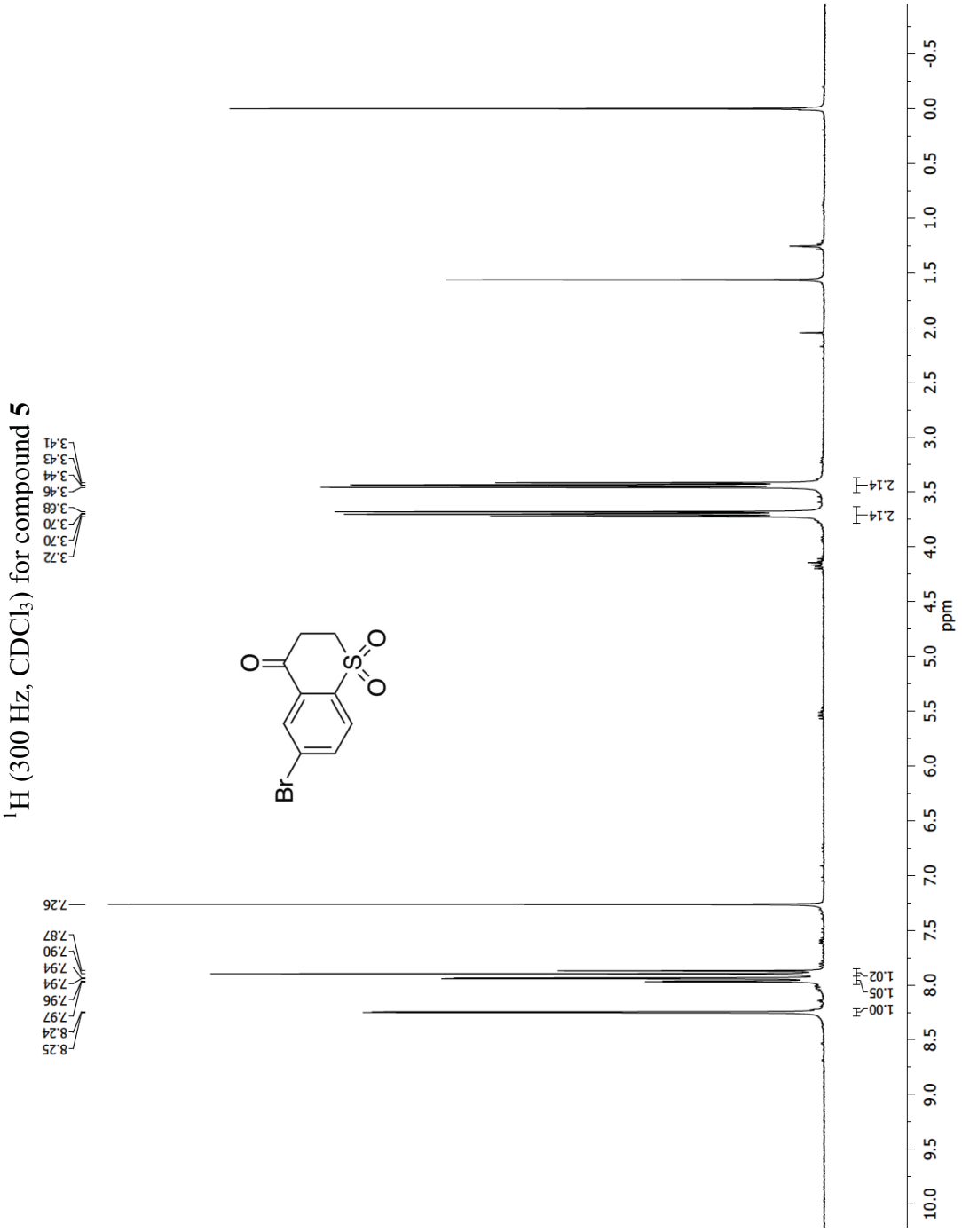
^{1}H (500 Hz, $CDCl_3$) for compound **3**



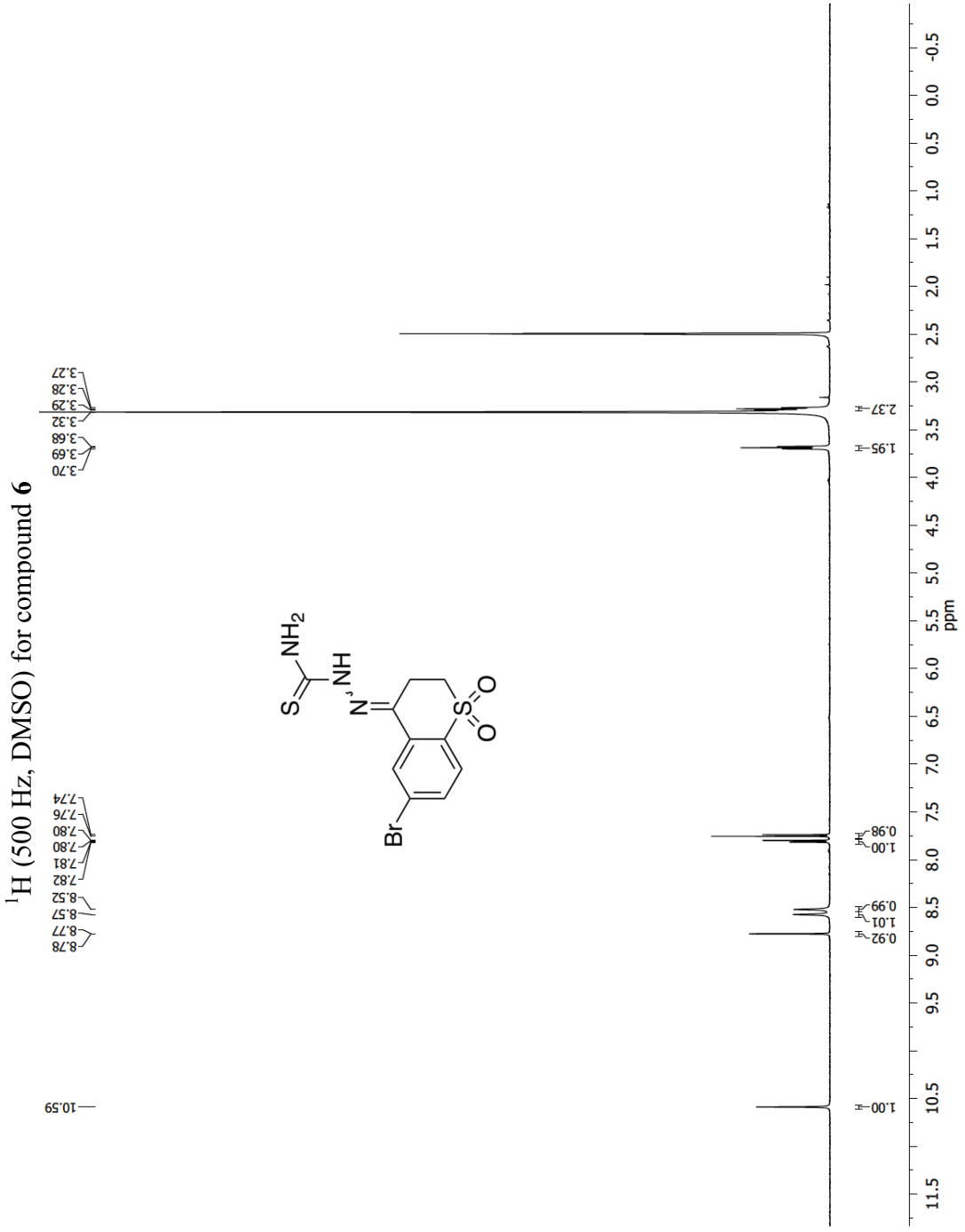
^{13}C (125 Hz, DMSO) for compound **3**



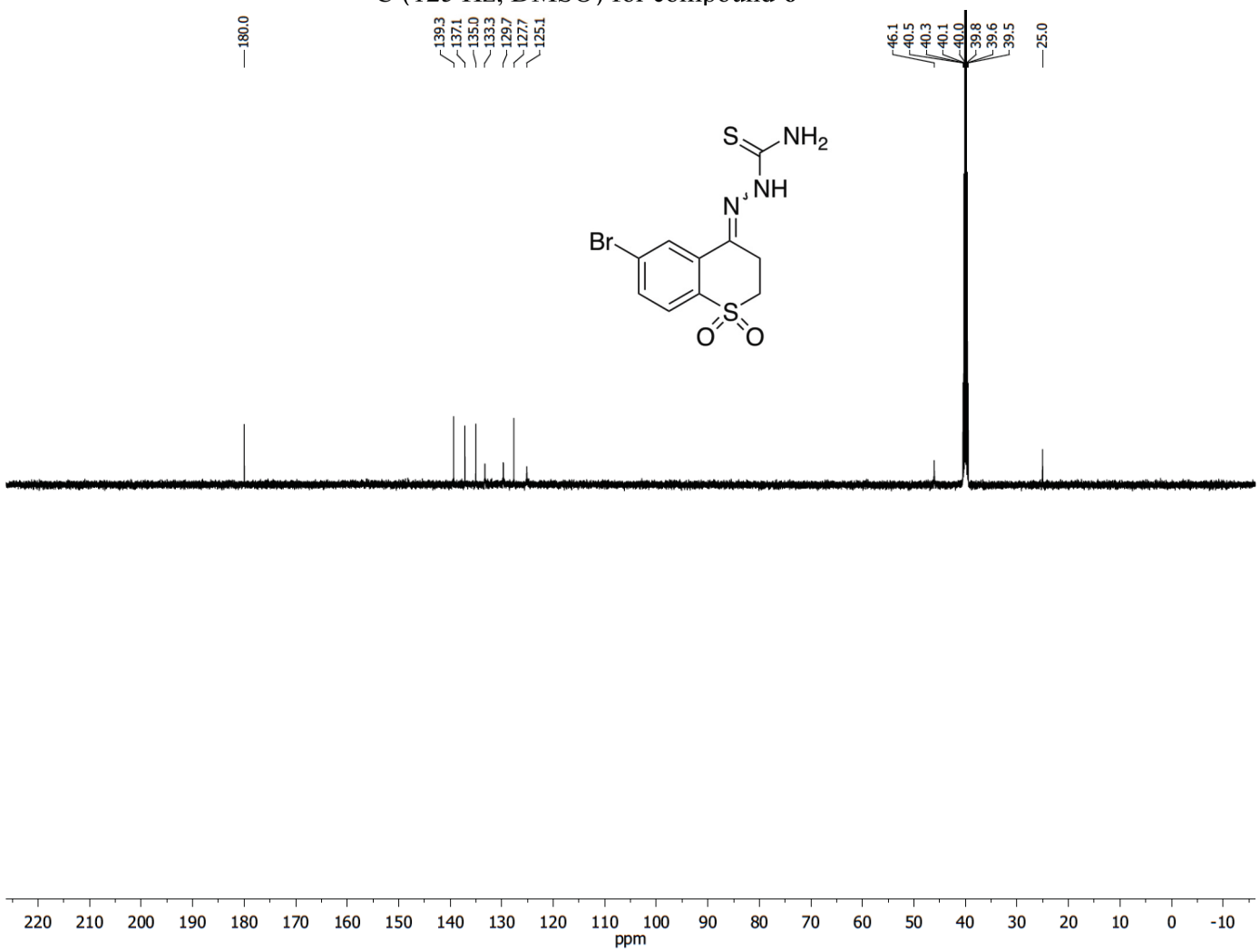
^{1}H (300 Hz, $CDCl_3$) for compound **5**



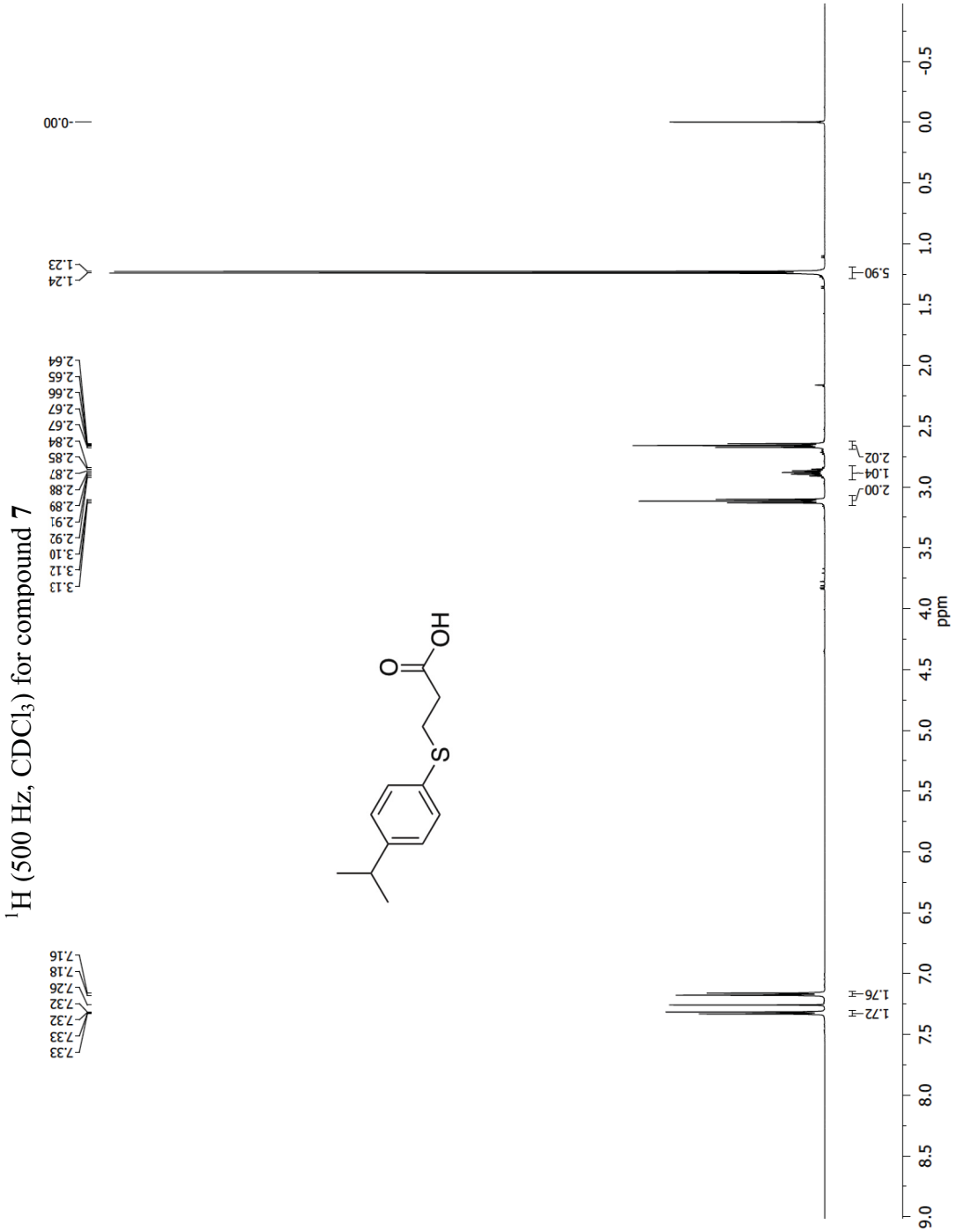
^{1}H (500 Hz, DMSO) for compound **6**



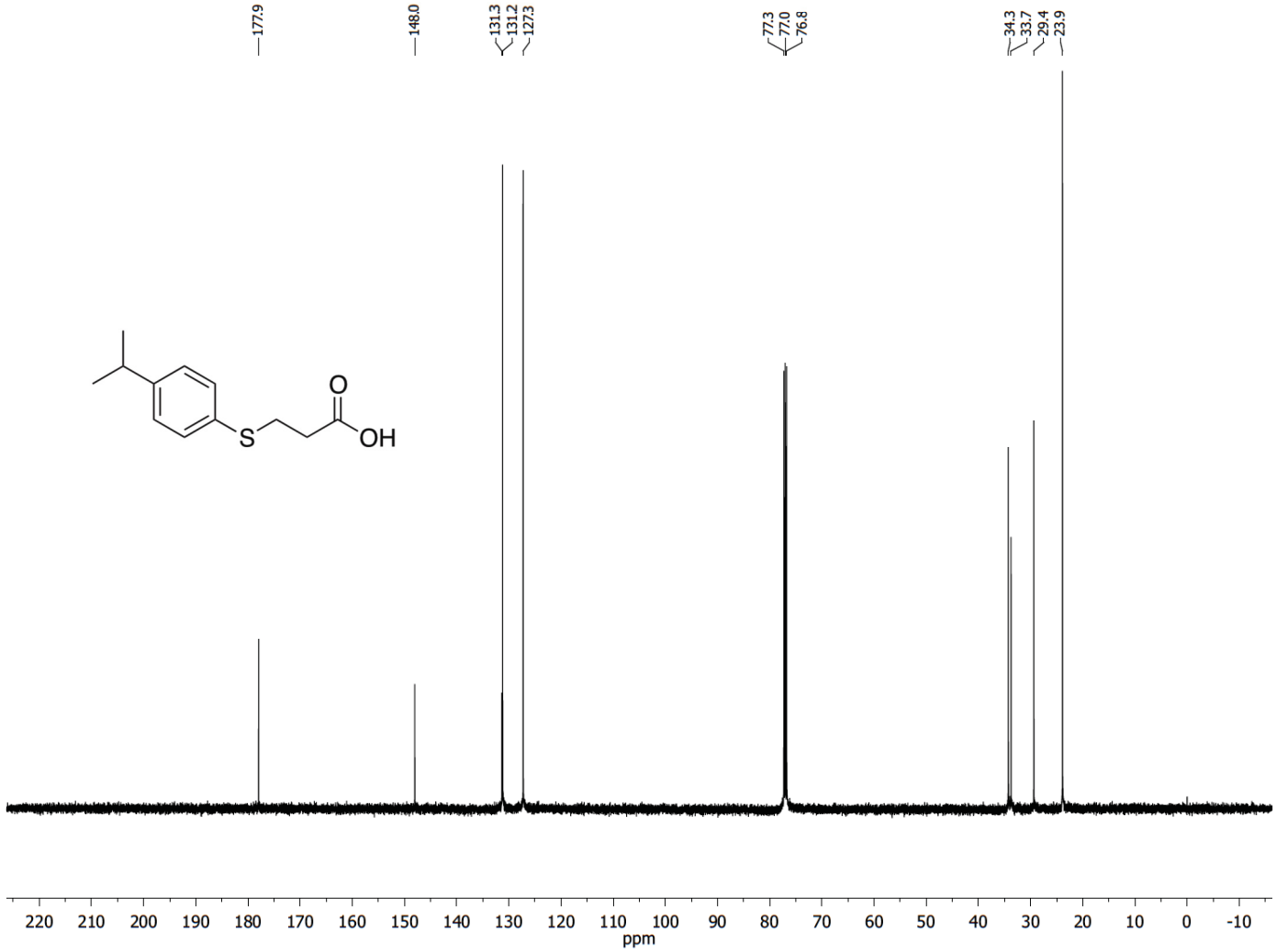
^{13}C (125 Hz, DMSO) for compound **6**



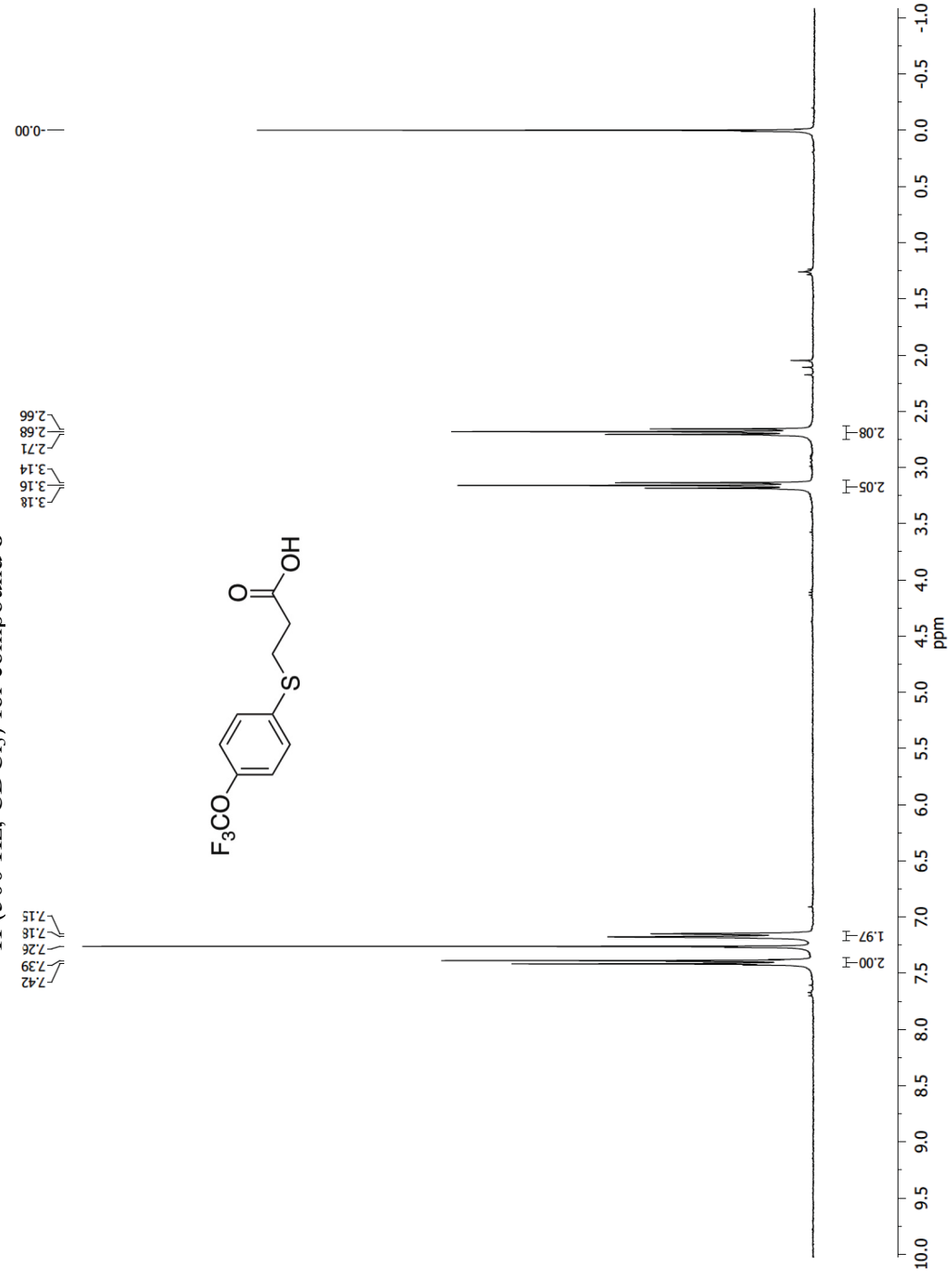
^{1}H (500 Hz, $CDCl_3$) for compound **7**



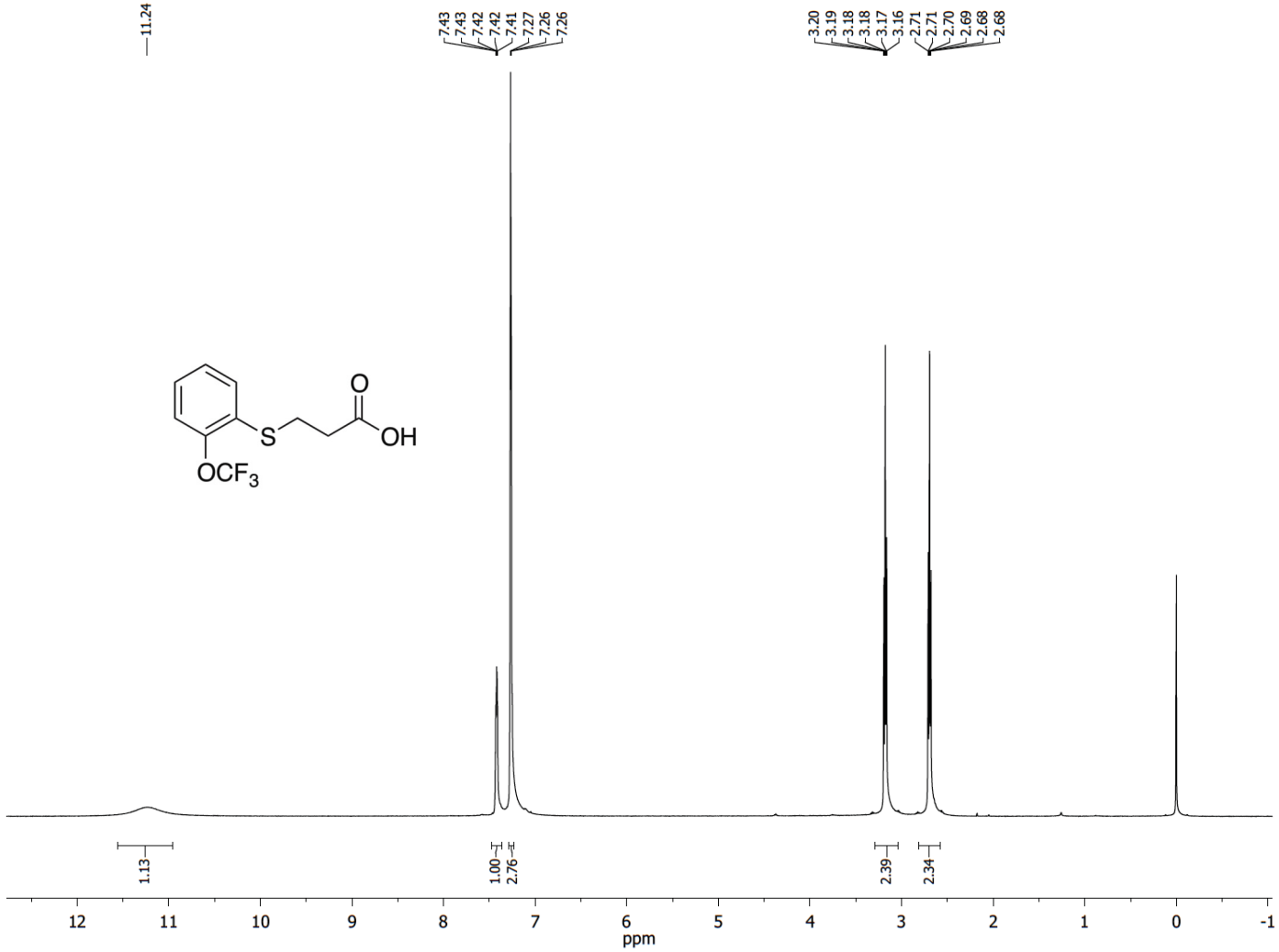
^{13}C (125 Hz, $CDCl_3$) for compound **7**



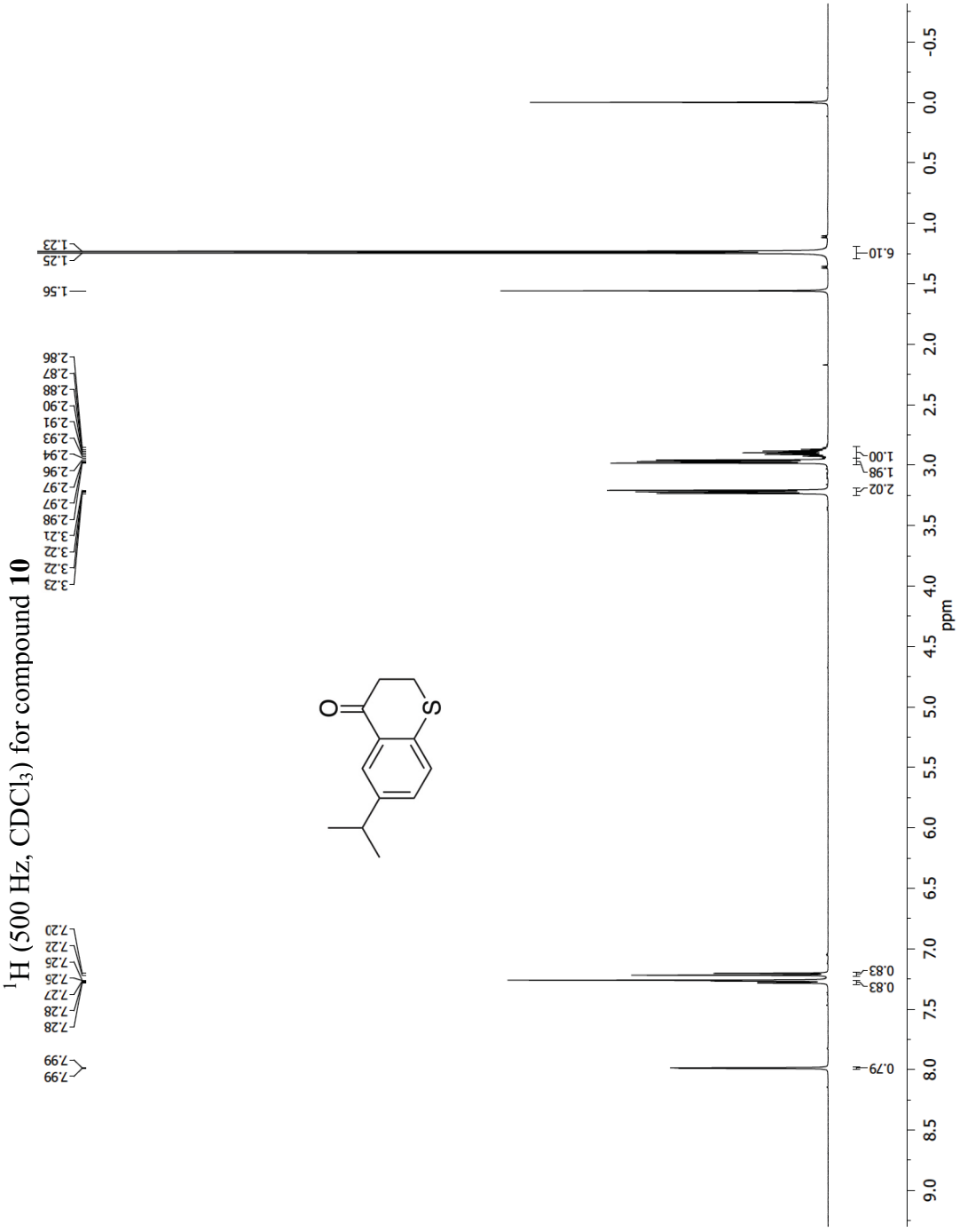
^{1}H (500 Hz, $CDCl_3$) for compound **8**



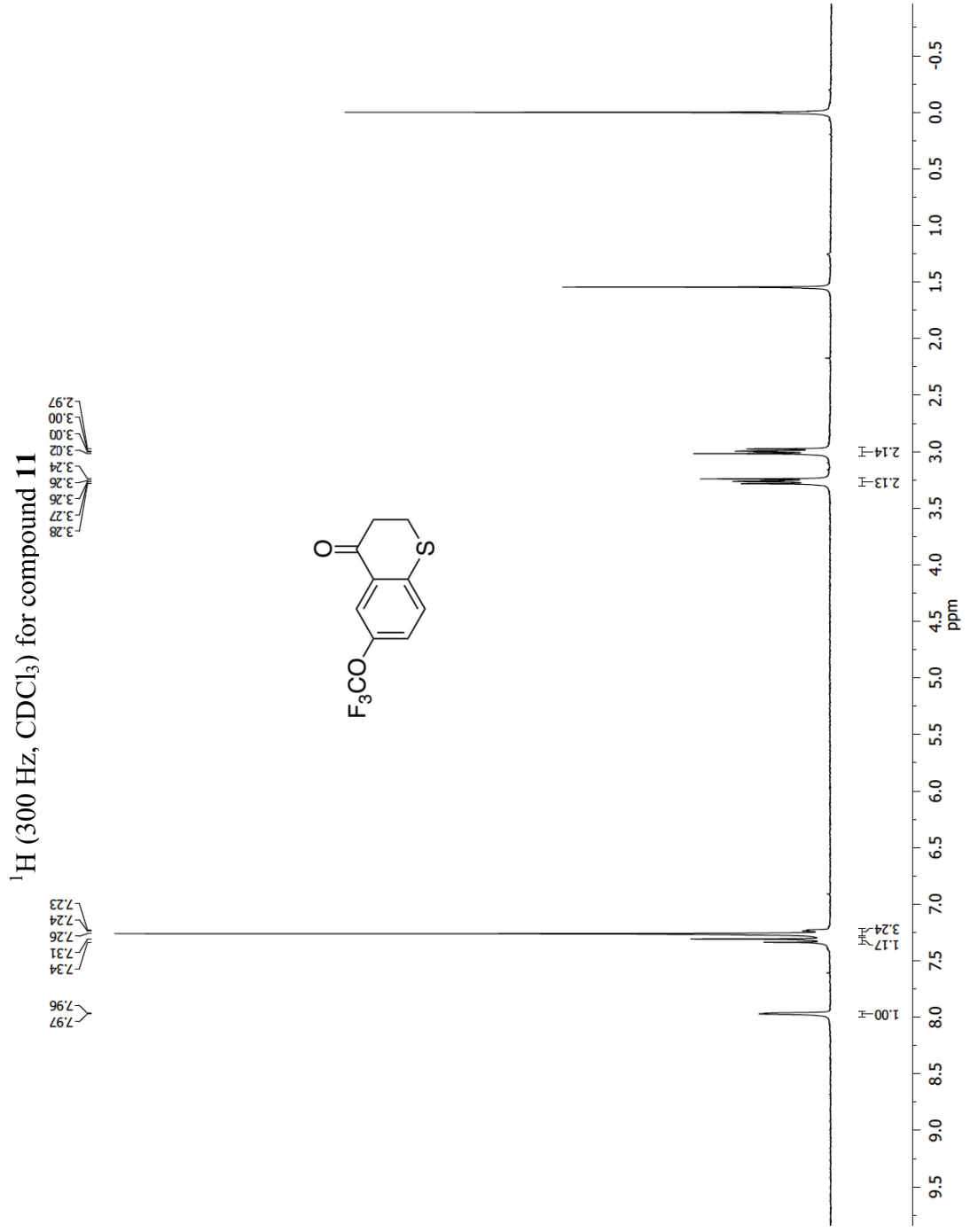
^{1}H (500 Hz, $CDCl_3$) for compound **9**



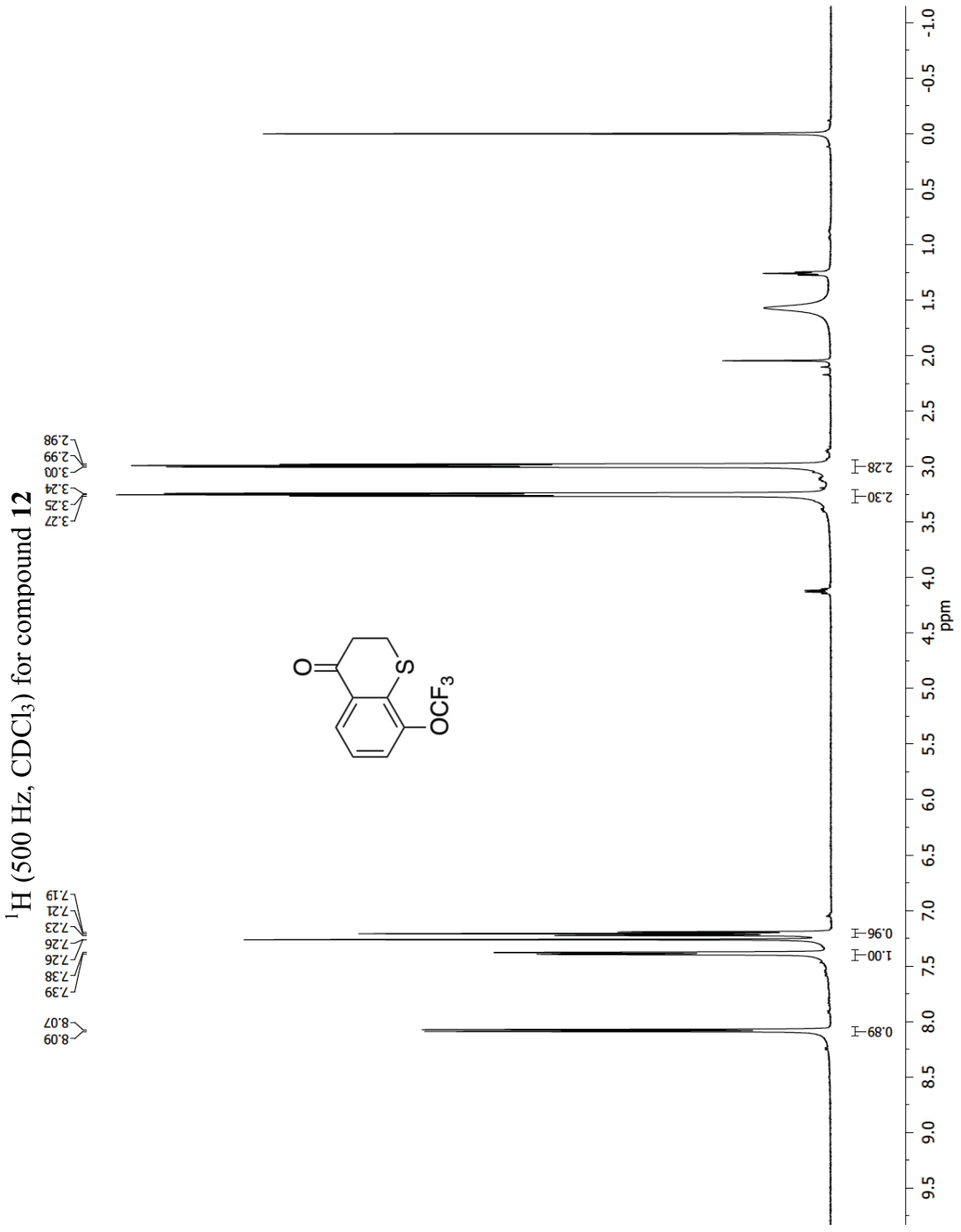
^{1}H (500 Hz, $CDCl_3$) for compound **10**



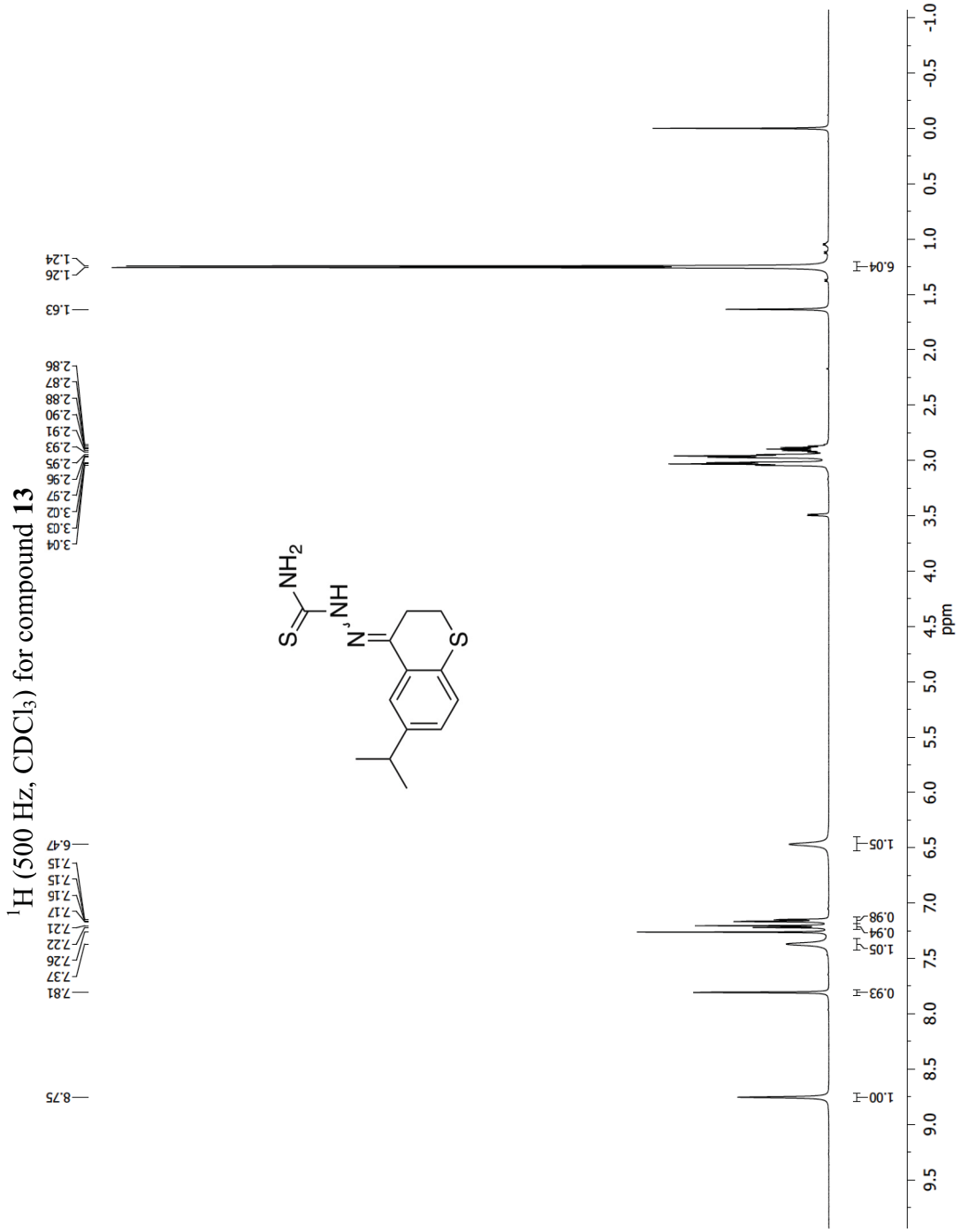
^{1}H (300 Hz, $CDCl_3$) for compound **11**



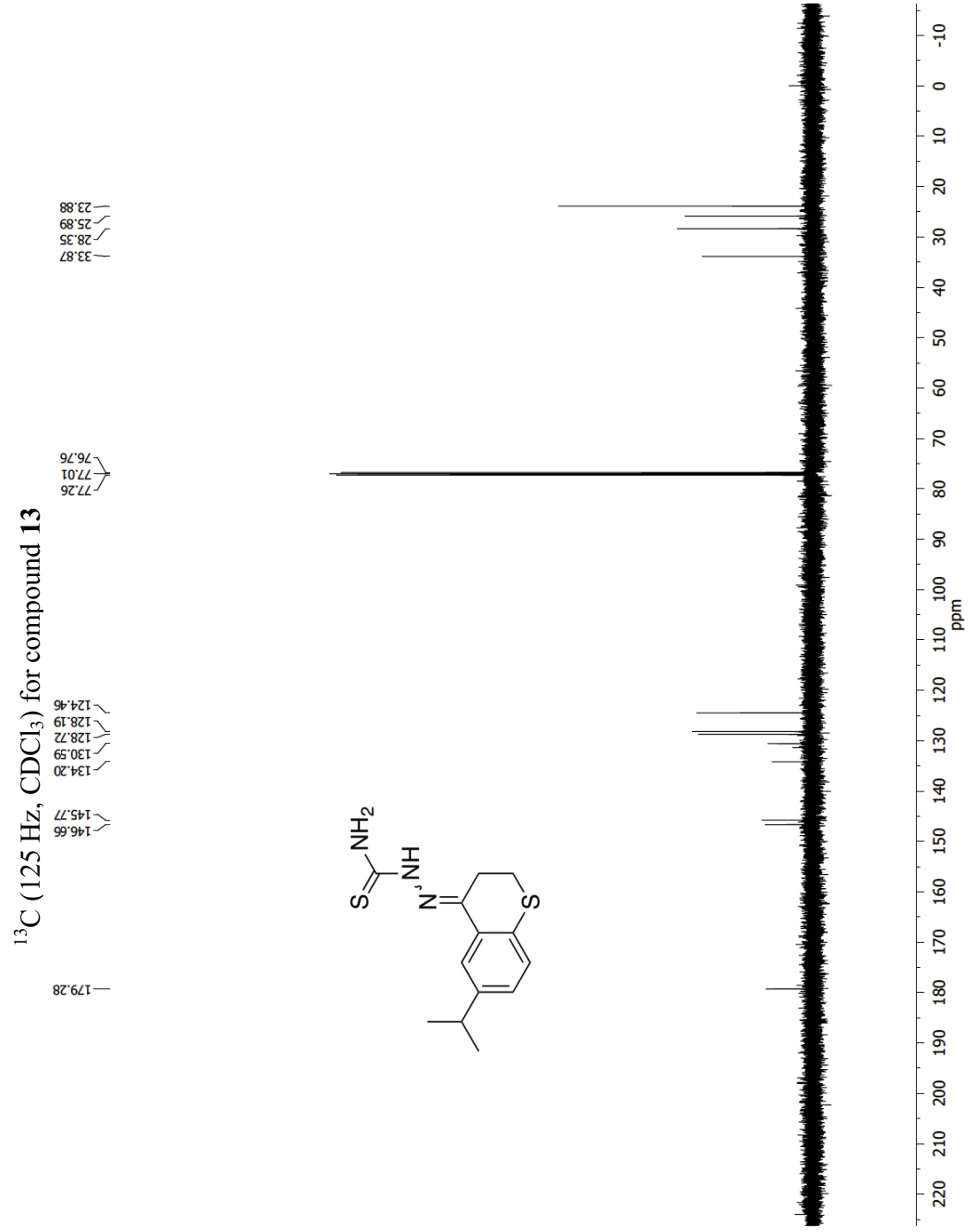
^{1}H (500 Hz, $CDCl_3$) for compound **12**



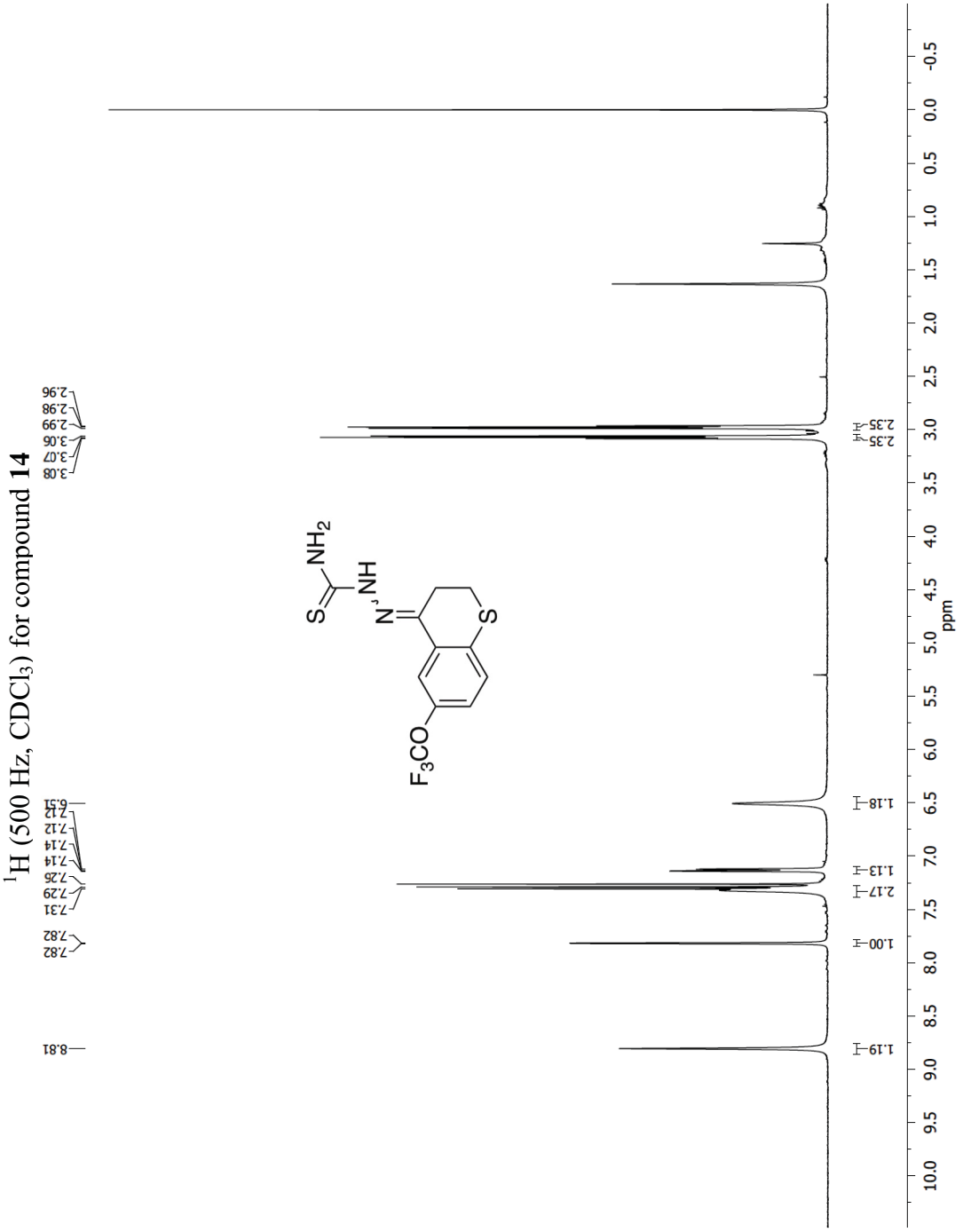
^{1}H (500 Hz, $CDCl_3$) for compound **13**



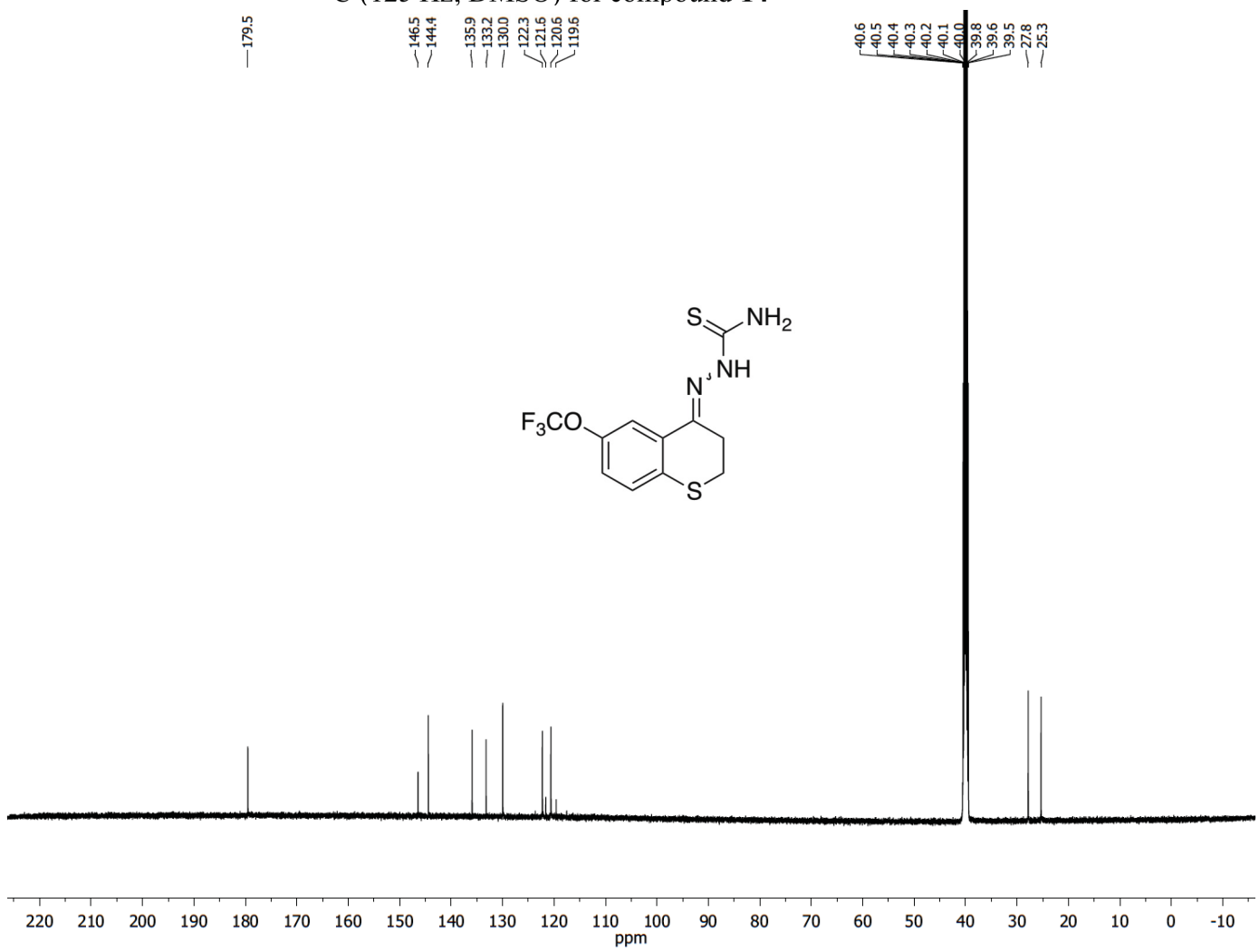
^{13}C (125 Hz, $CDCl_3$) for compound **13**



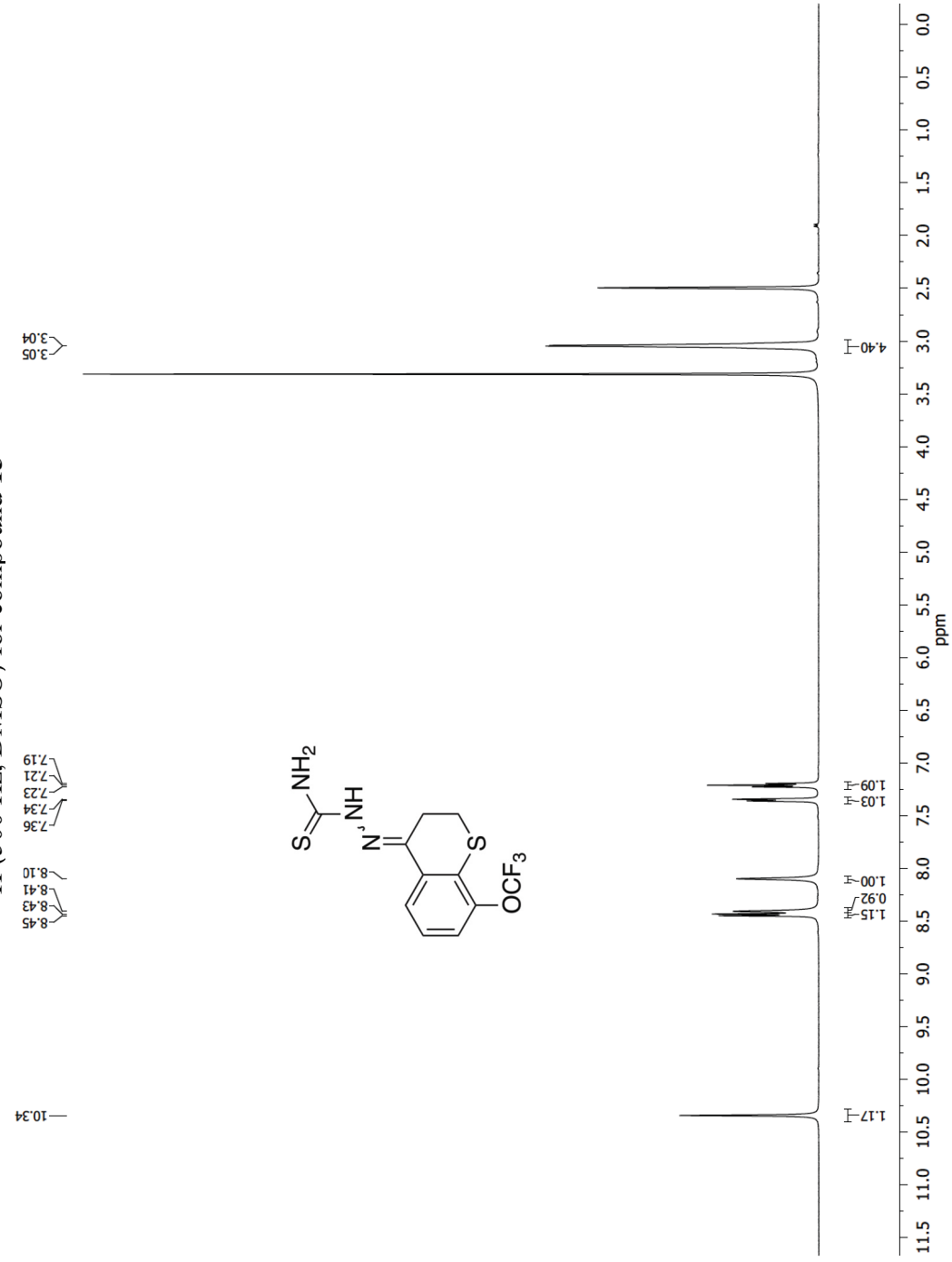
^{1}H (500 Hz, $CDCl_3$) for compound **14**



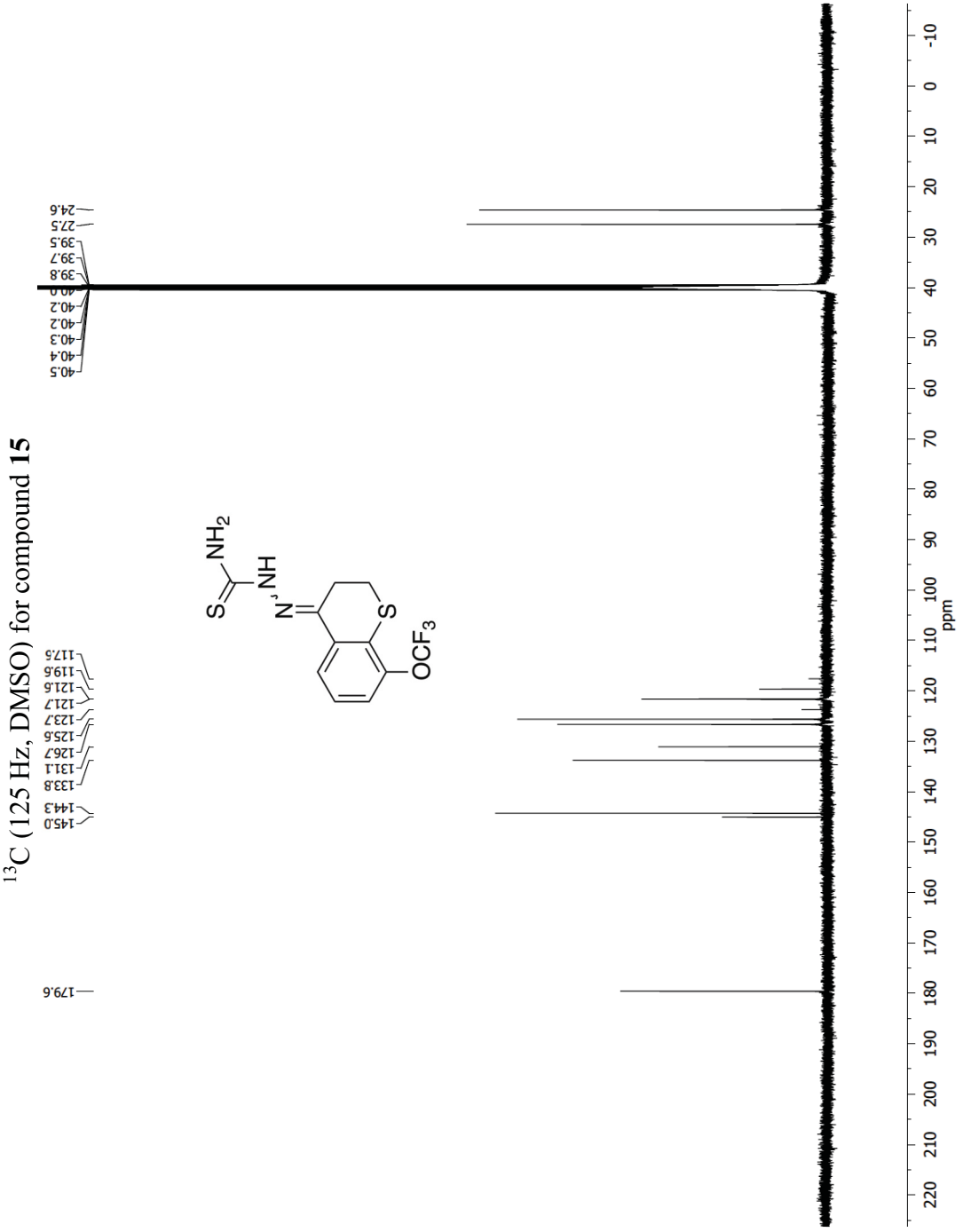
^{13}C (125 Hz, DMSO) for compound **14**



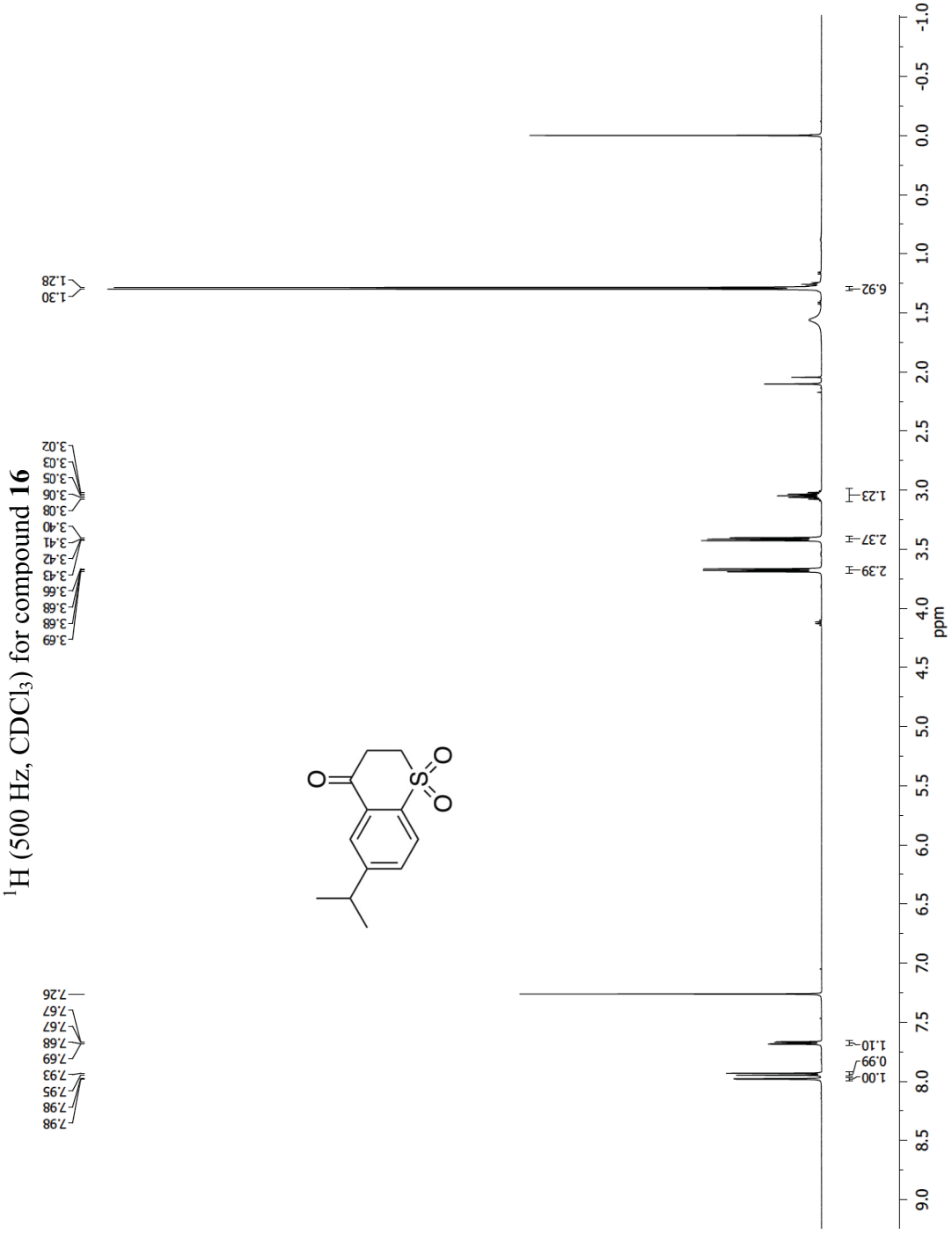
^{1}H (500 Hz, DMSO) for compound **15**



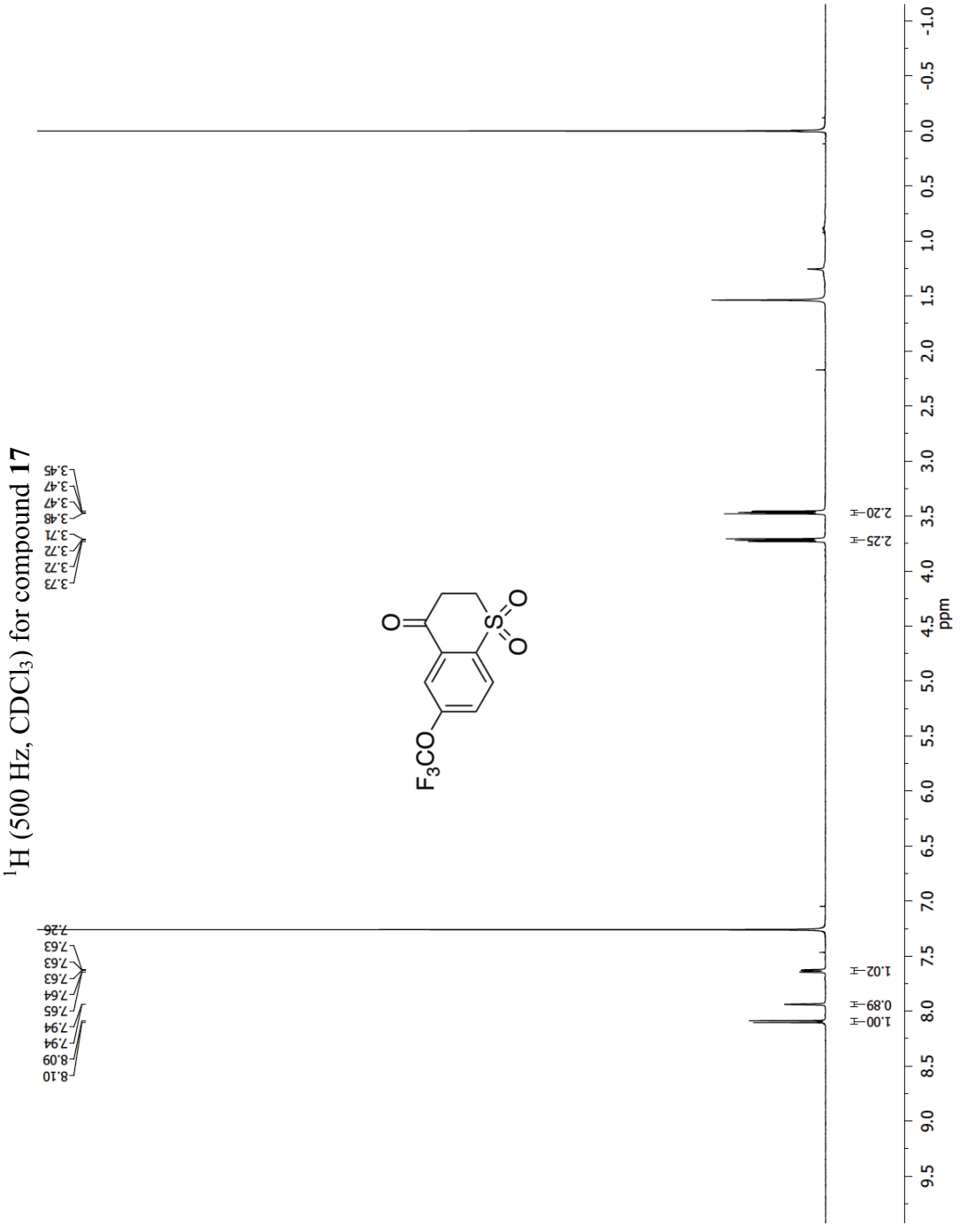
^{13}C (125 Hz, DMSO) for compound **15**



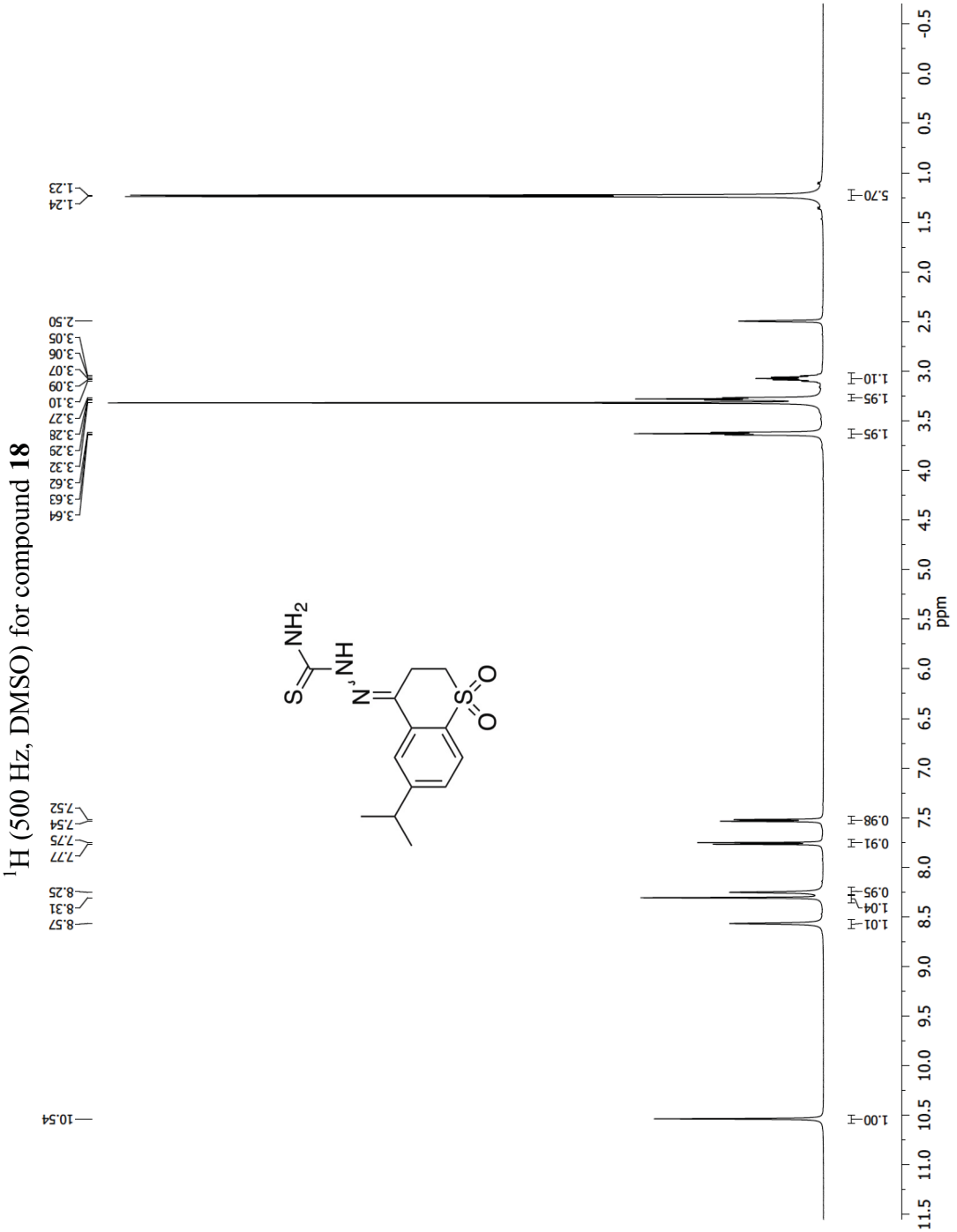
^{1}H (500 Hz, $CDCl_3$) for compound **16**



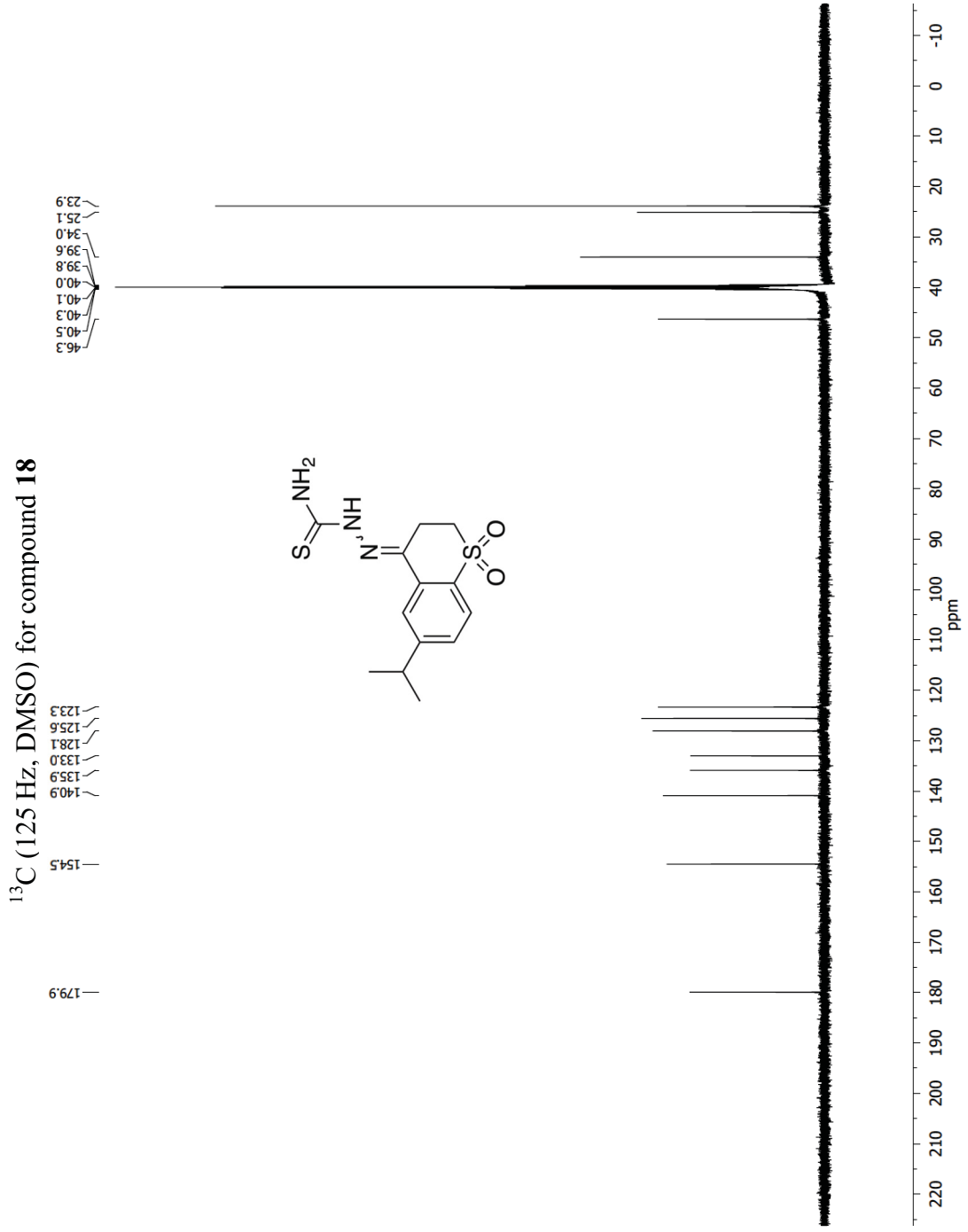
^{1}H (500 Hz, $CDCl_3$) for compound **17**



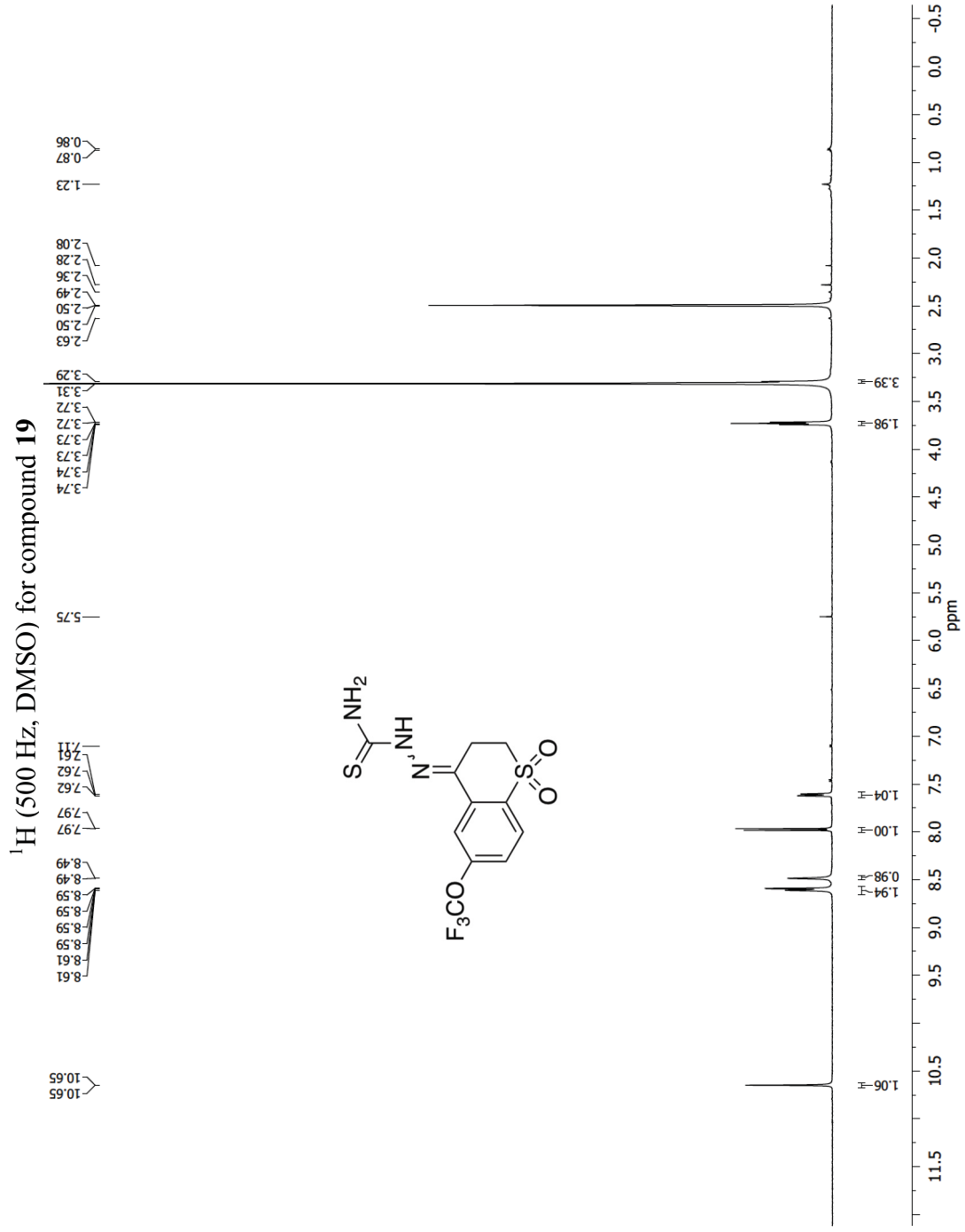
^{1}H (500 Hz, DMSO) for compound **18**



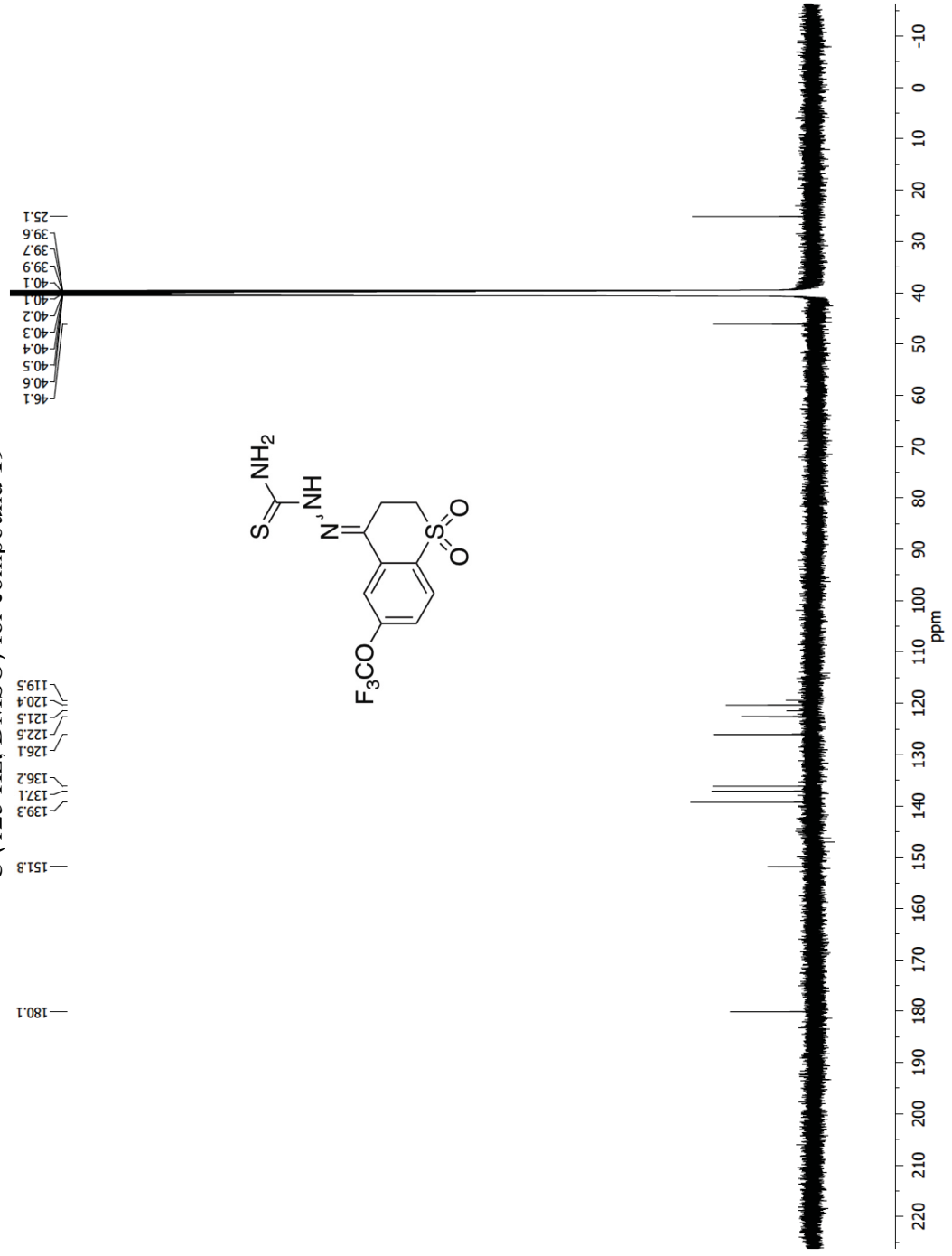
^{13}C (125 Hz, DMSO) for compound **18**



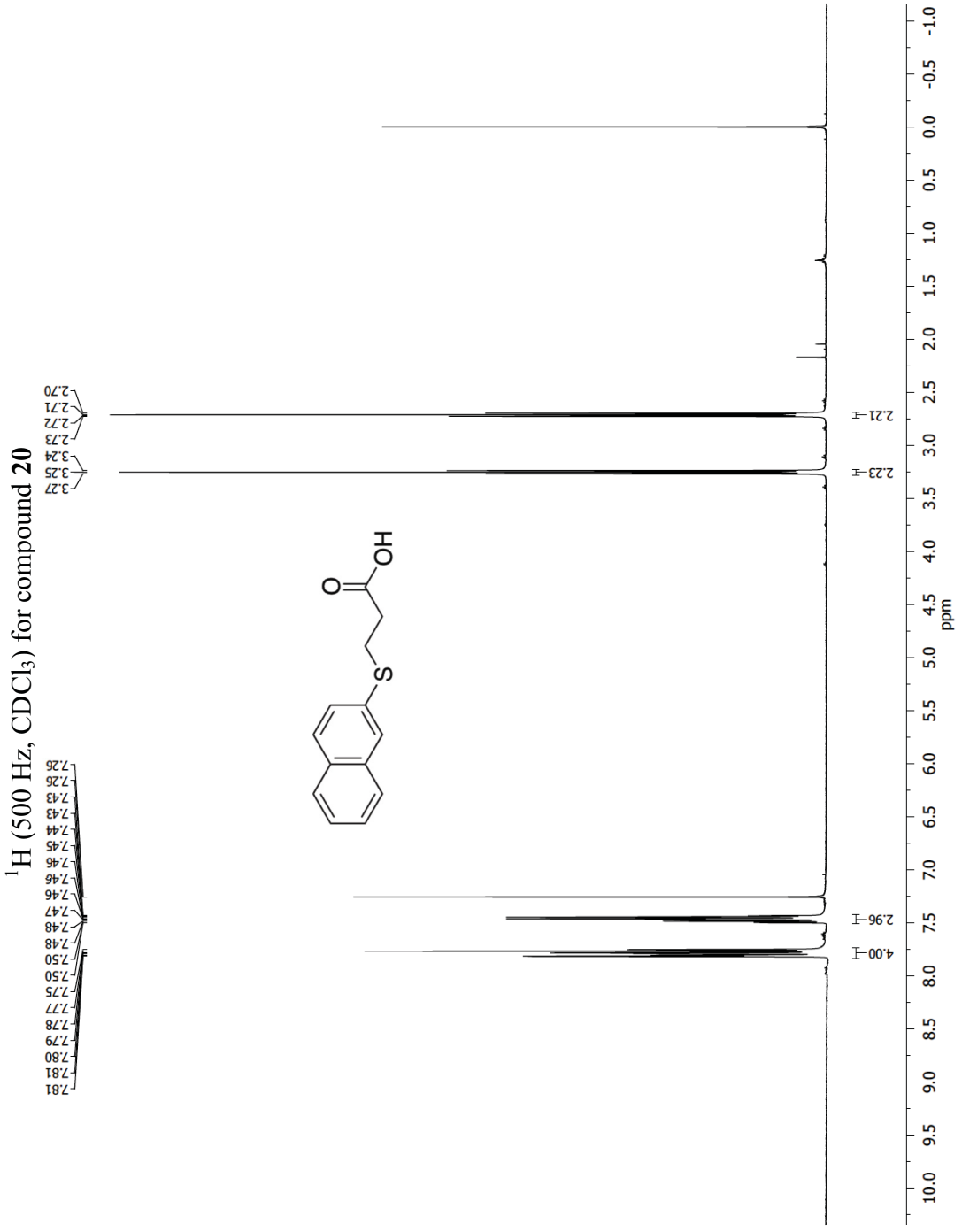
^{1}H (500 Hz, DMSO) for compound **19**



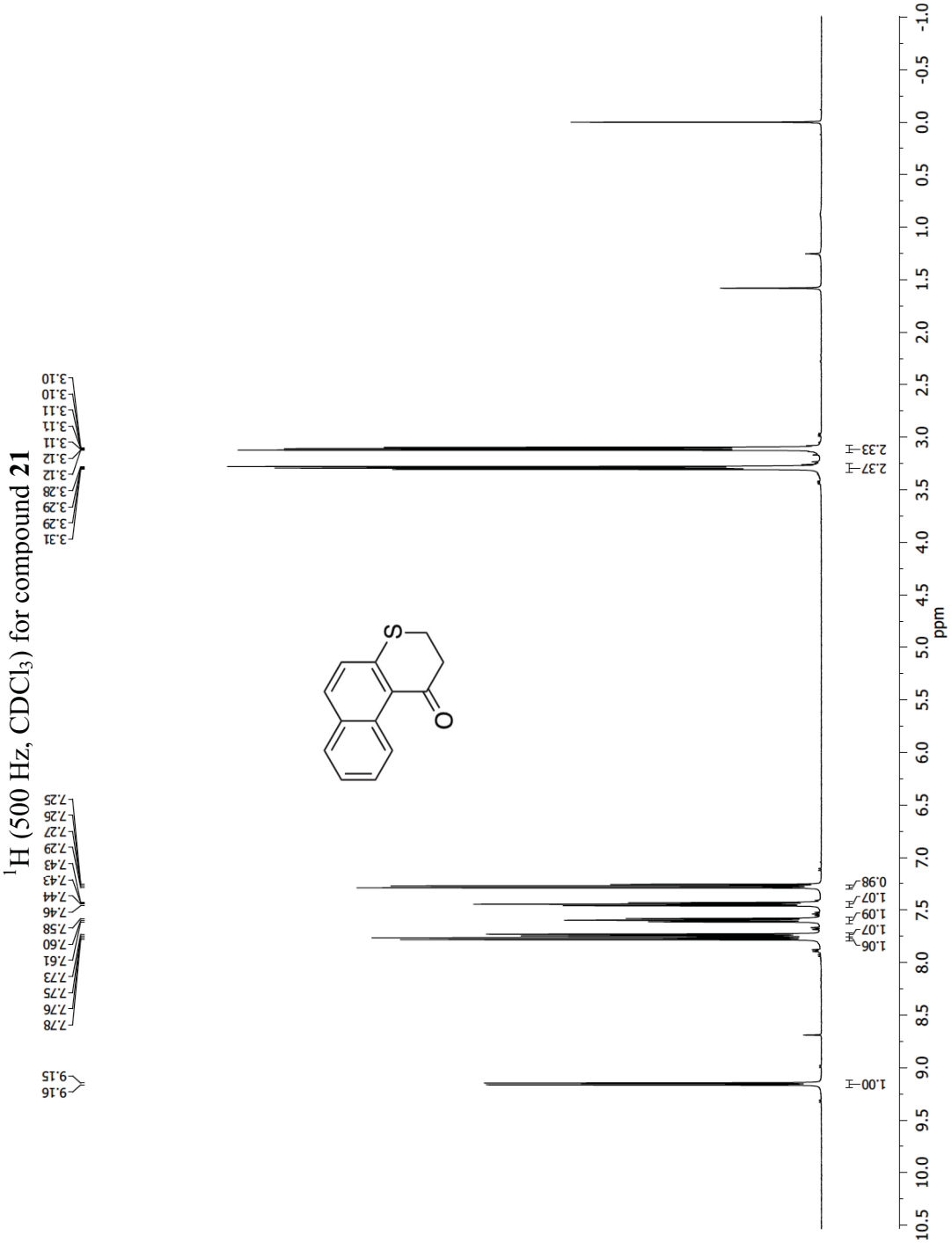
^{13}C (125 Hz, DMSO) for compound **19**



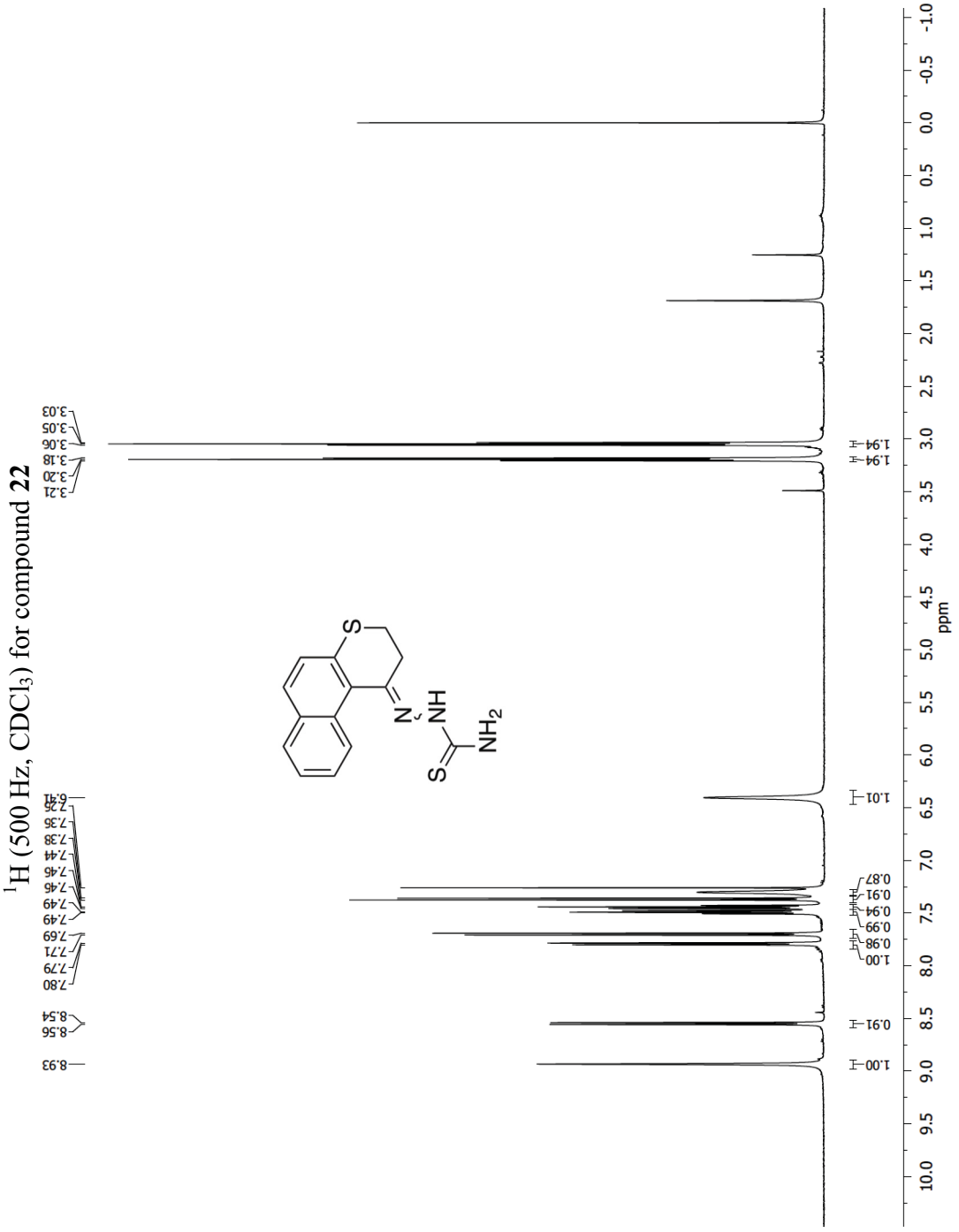
^{1}H (500 Hz, $CDCl_3$) for compound **20**



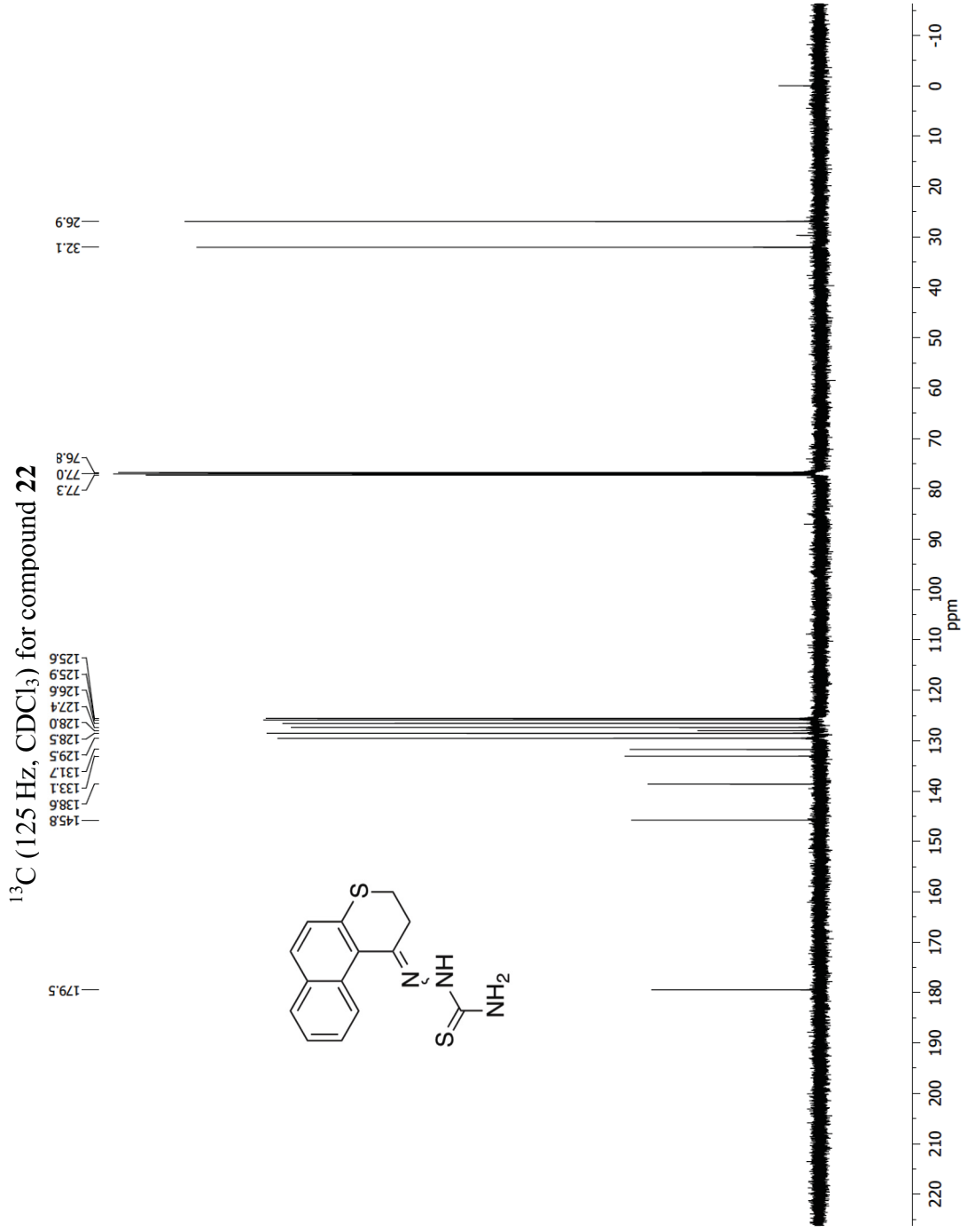
^{1}H (500 Hz, $CDCl_3$) for compound **21**



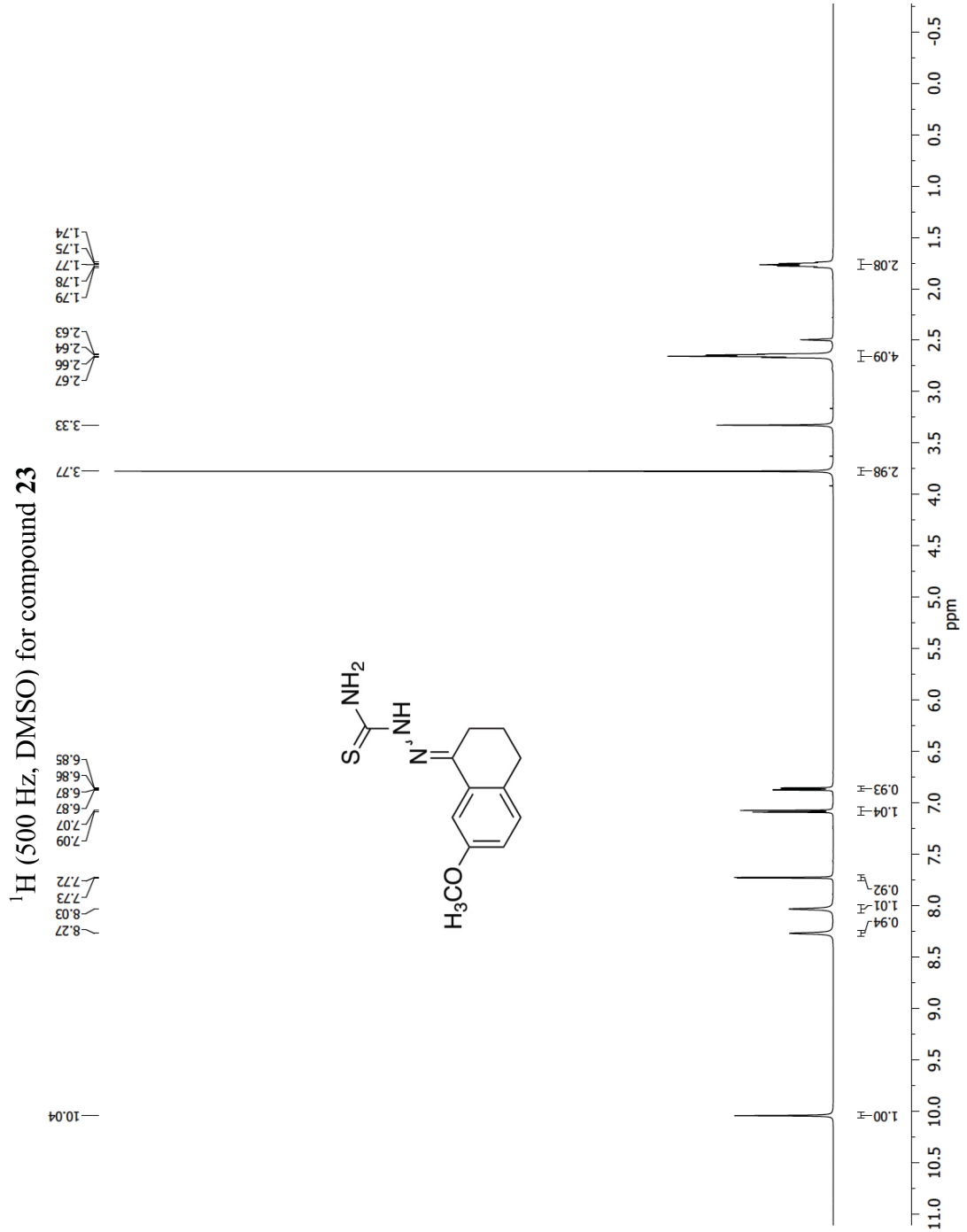
^{1}H (500 Hz, $CDCl_3$) for compound **22**



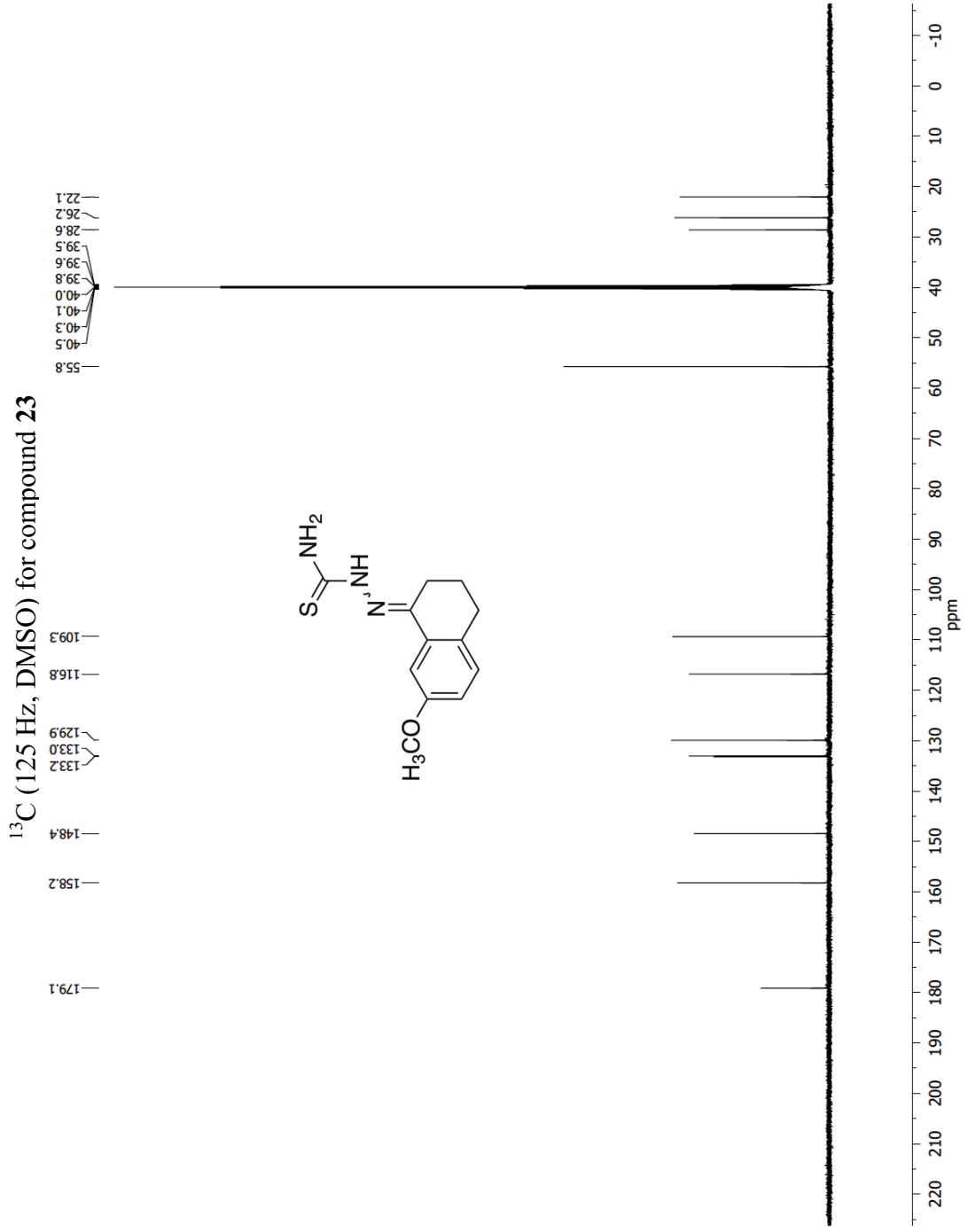
^{13}C (125 Hz, $CDCl_3$) for compound **22**



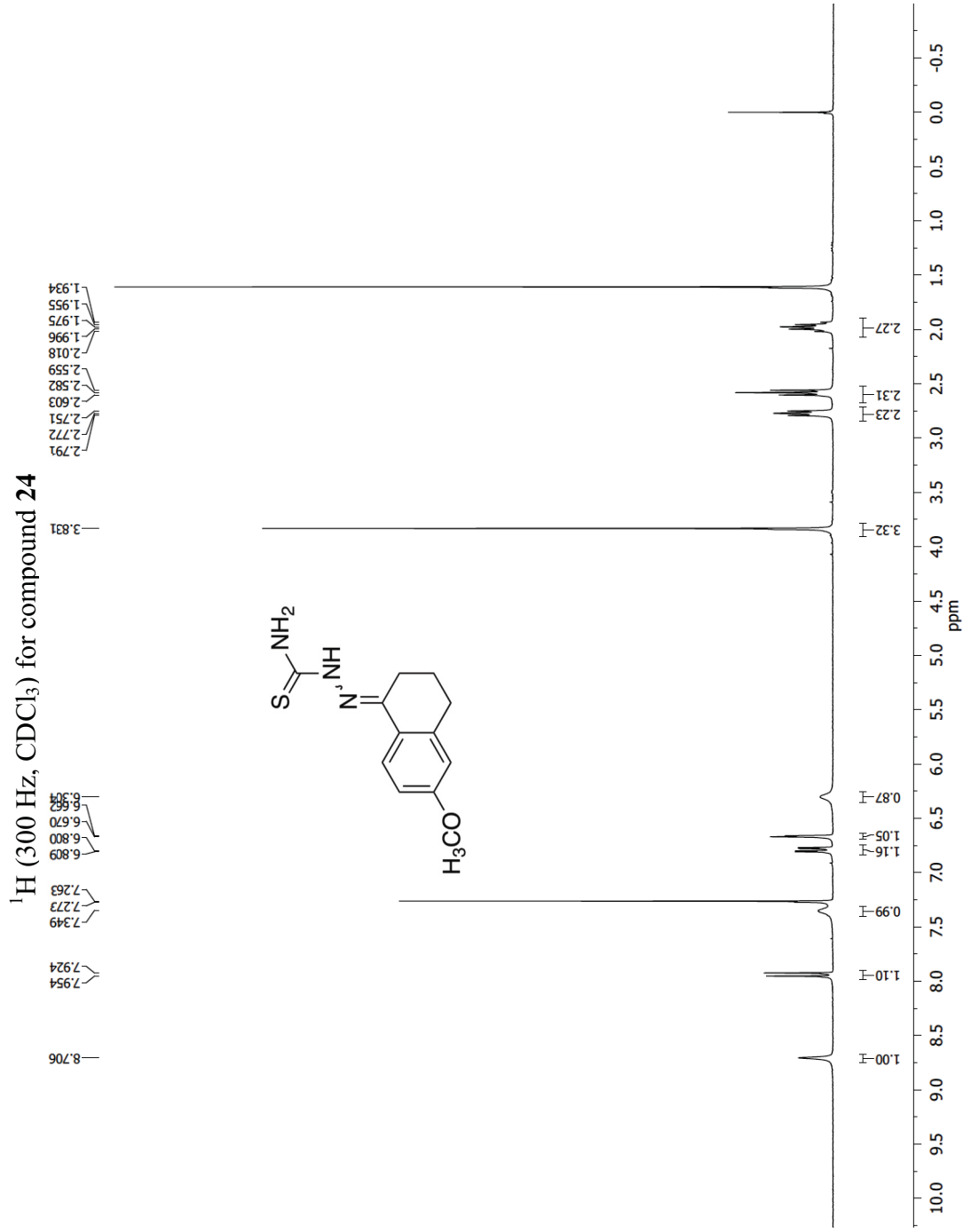
^{1}H (500 Hz, DMSO) for compound **23**



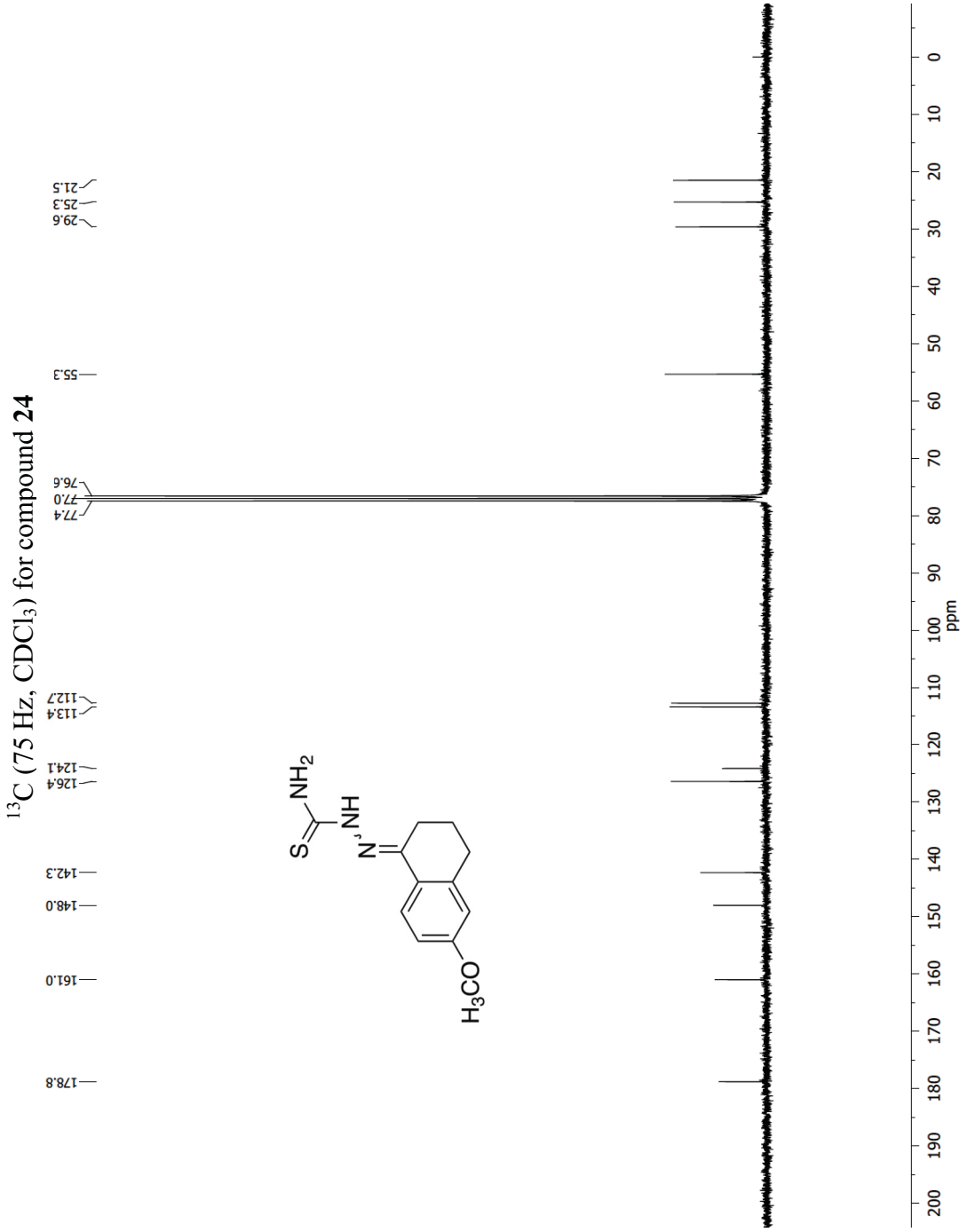
^{13}C (125 Hz, DMSO) for compound **23**



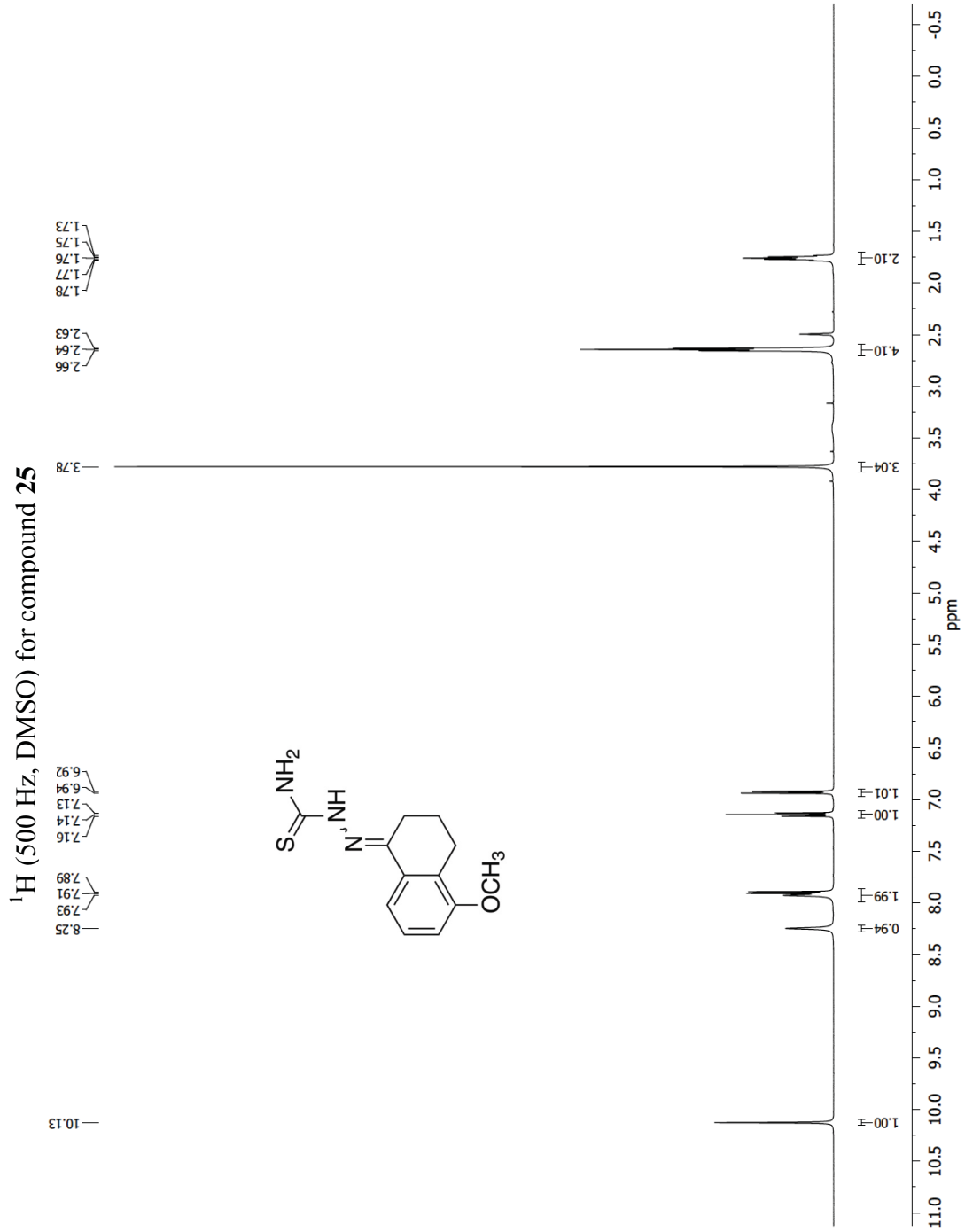
^{1}H (300 Hz, $CDCl_3$) for compound **24**

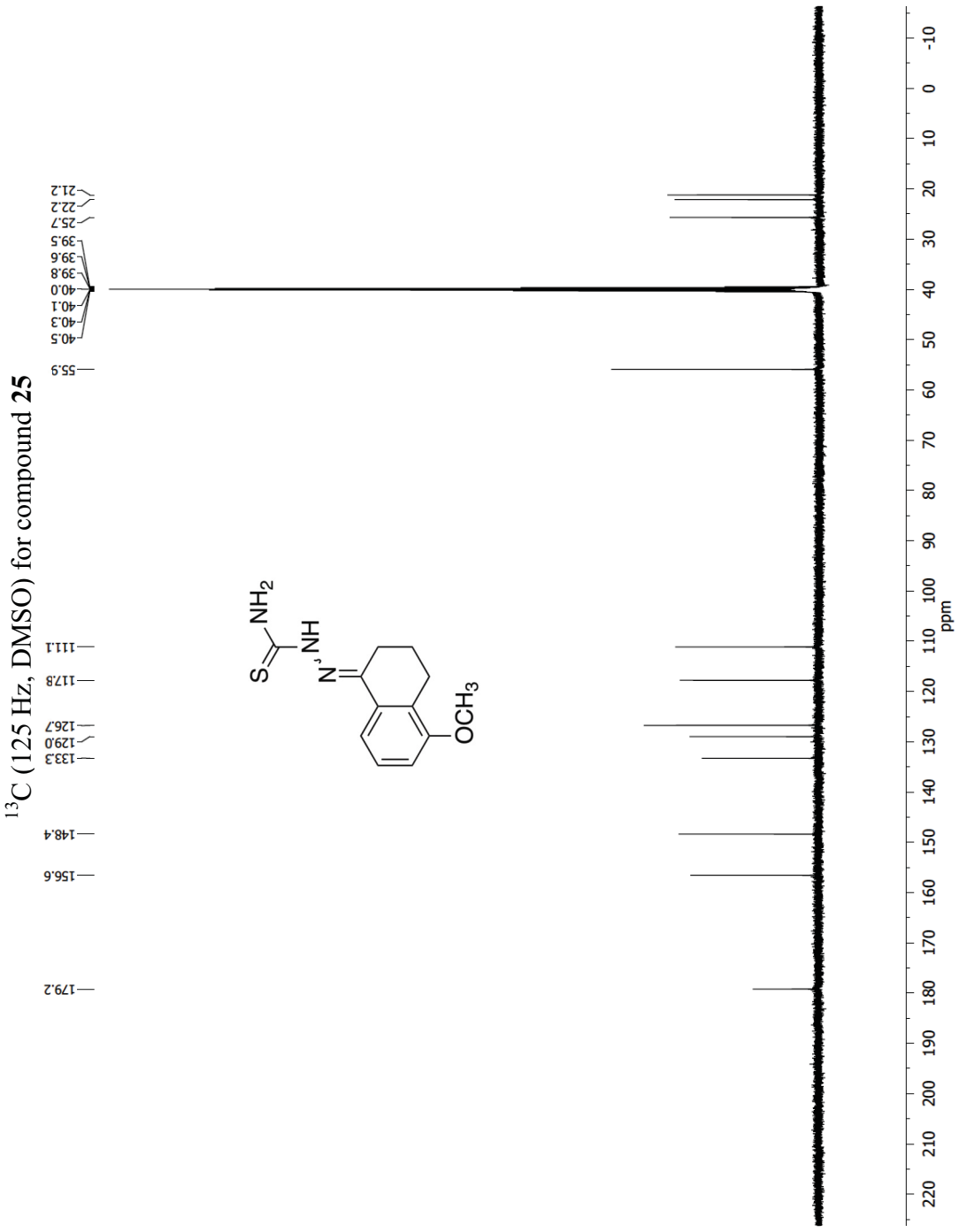


^{13}C (75 Hz, $CDCl_3$) for compound **24**

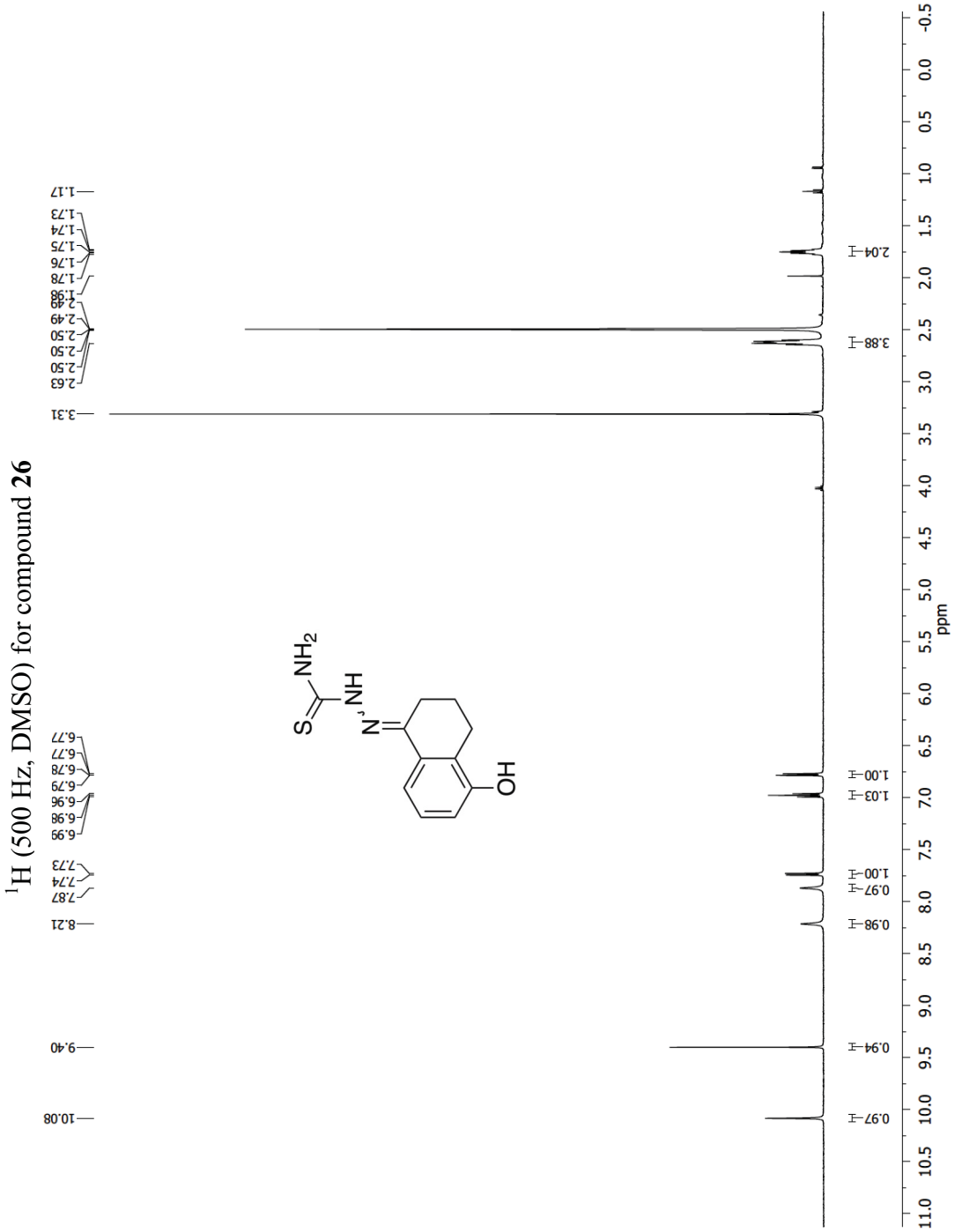


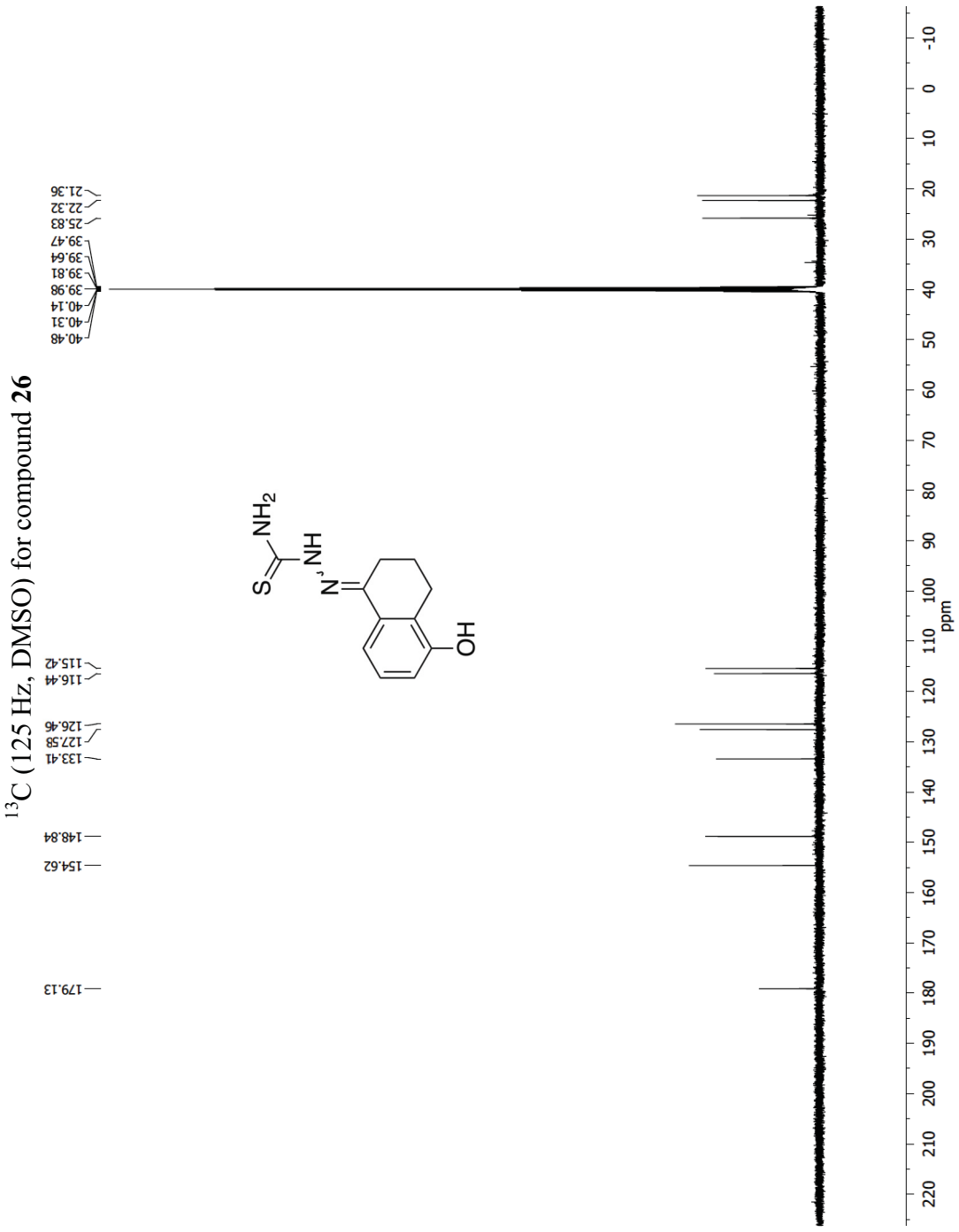
^{1}H (500 Hz, DMSO) for compound **25**



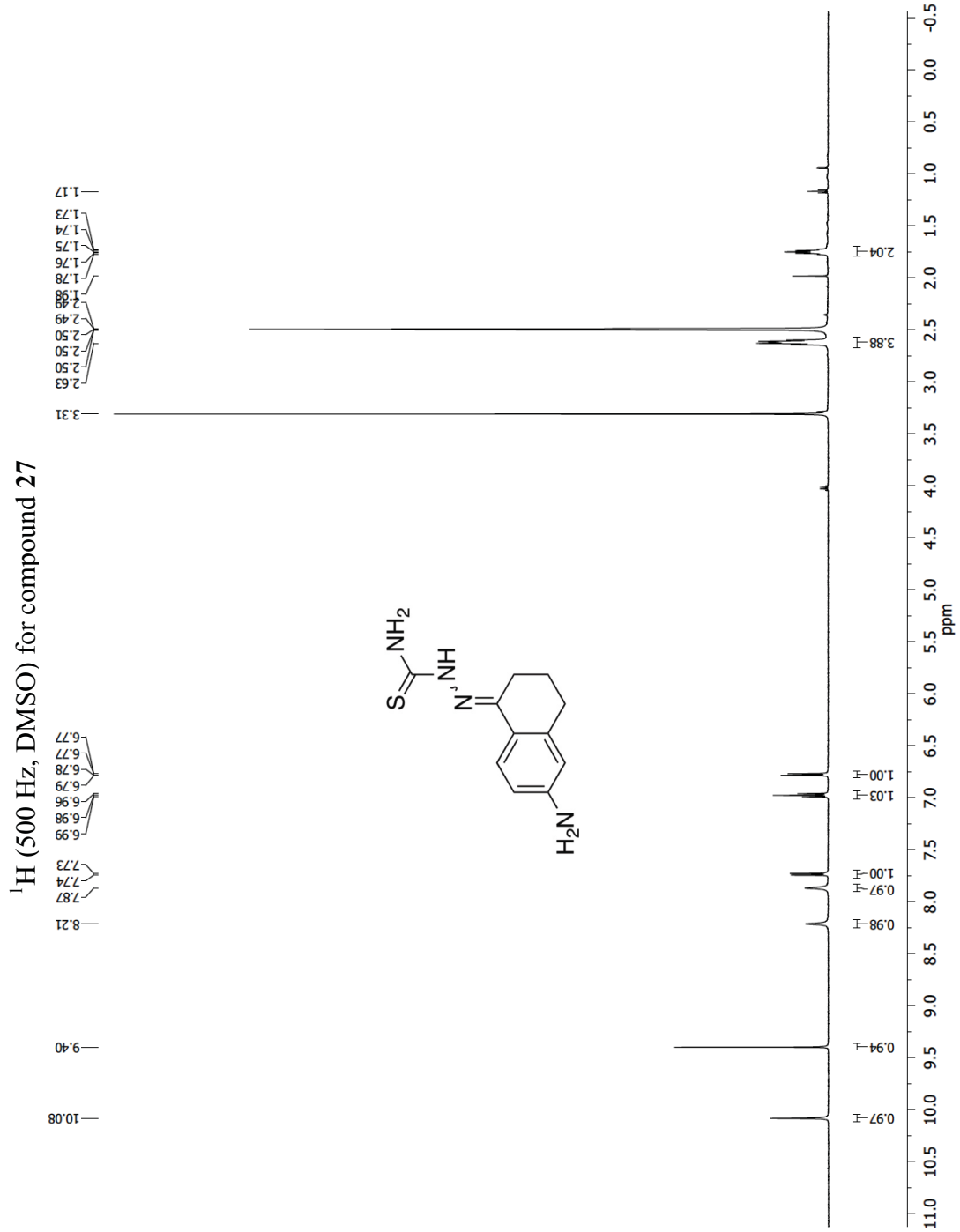

^{13}C (125 Hz, DMSO) for compound **25**
179.2
156.6
148.4
133.3
129.0
126.7
117.8
111.1
55.9
40.5
40.3
40.1
40.0
39.8
39.6
39.5
25.7
22.2
21.2
NH2
NH
S
N
OCH3
ppm

^{1}H (500 Hz, DMSO) for compound **26**

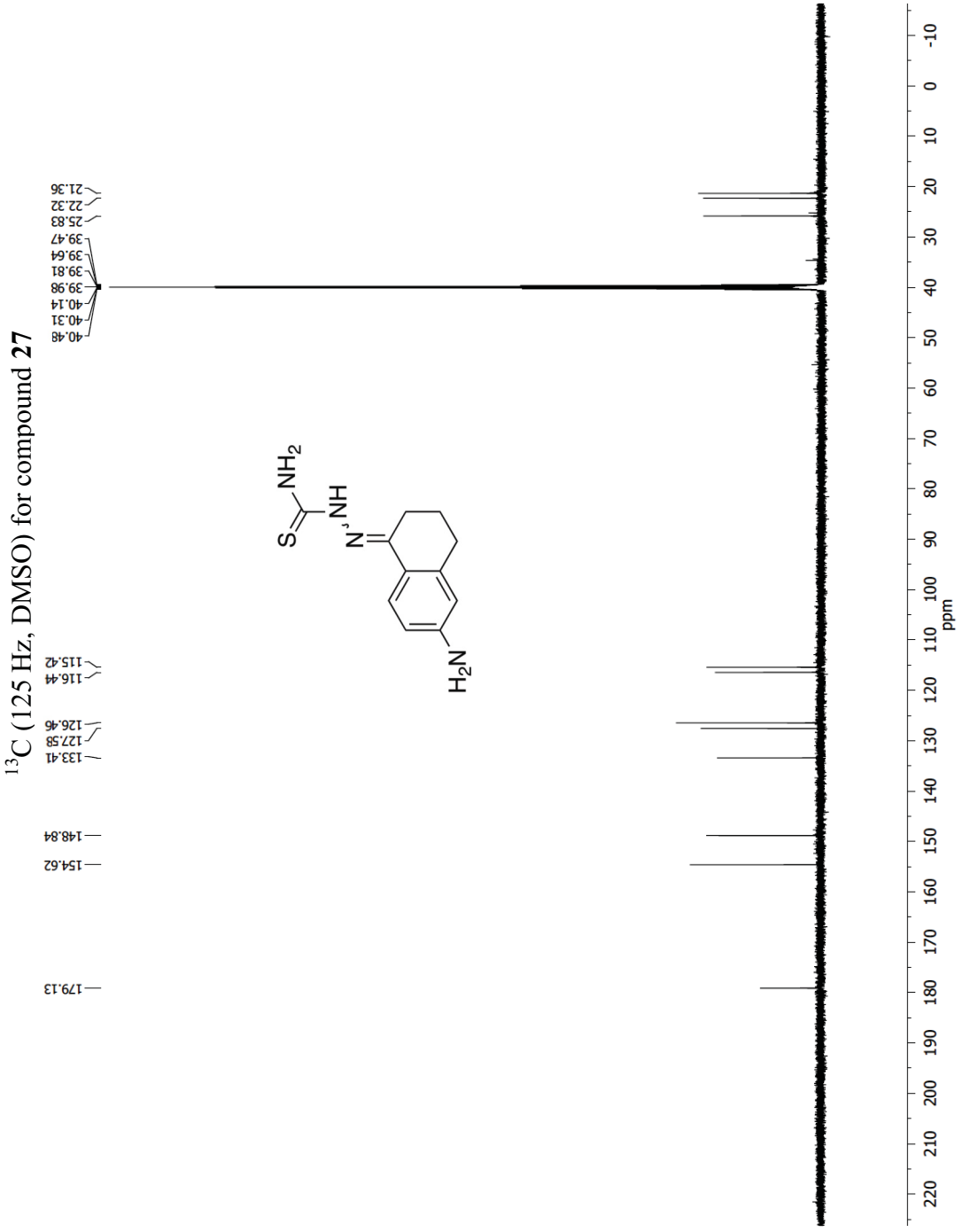



13C (125 Hz, DMSO) for compound 26
179.13
154.62
148.84
133.41
127.58
126.46
116.44
115.42
40.48
40.31
40.14
39.98
39.81
39.64
39.47
25.83
22.32
21.36
NH2
NH
N
S
OH
ppm

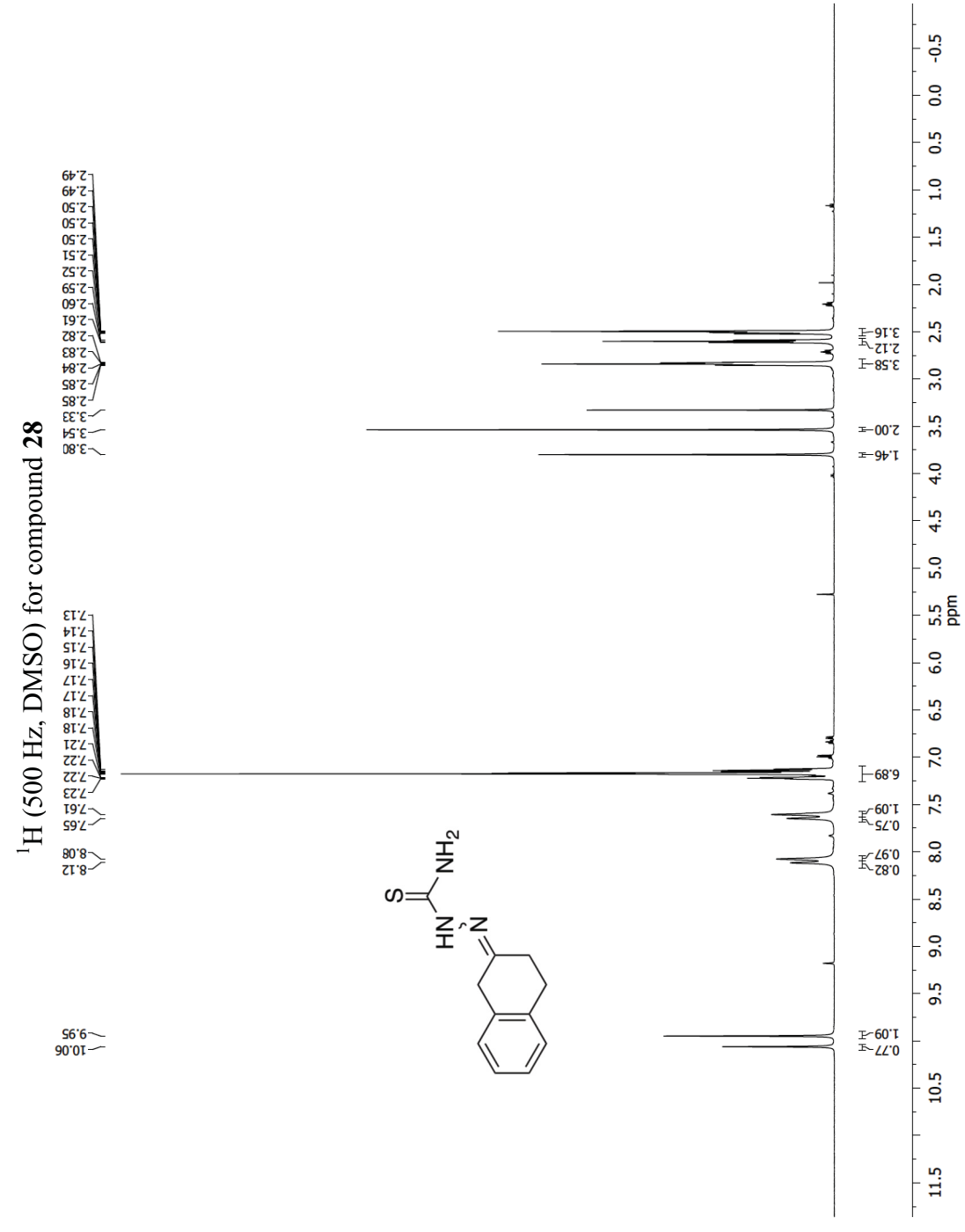
^{1}H (500 Hz, DMSO) for compound **27**



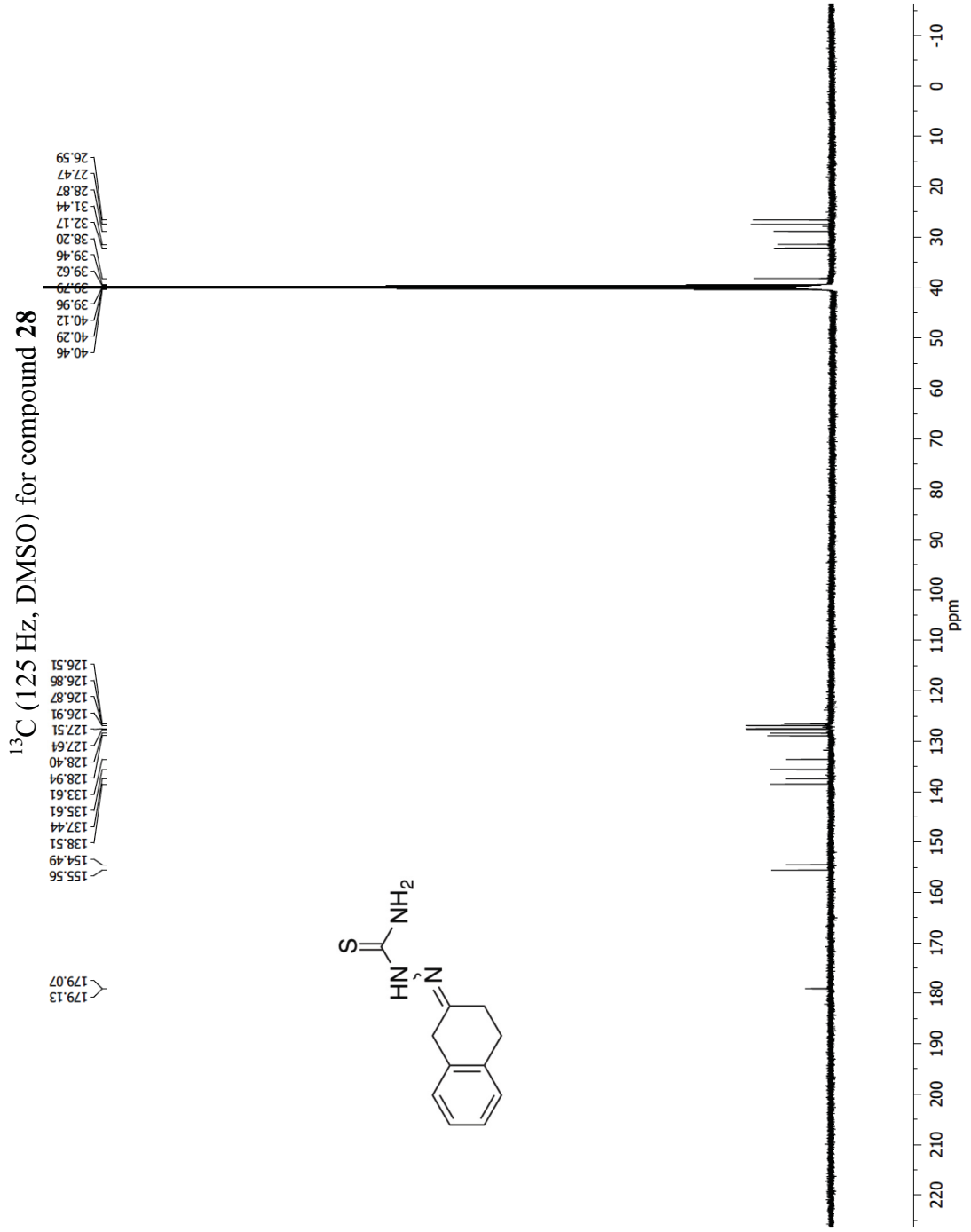
^{13}C (125 Hz, DMSO) for compound **27**



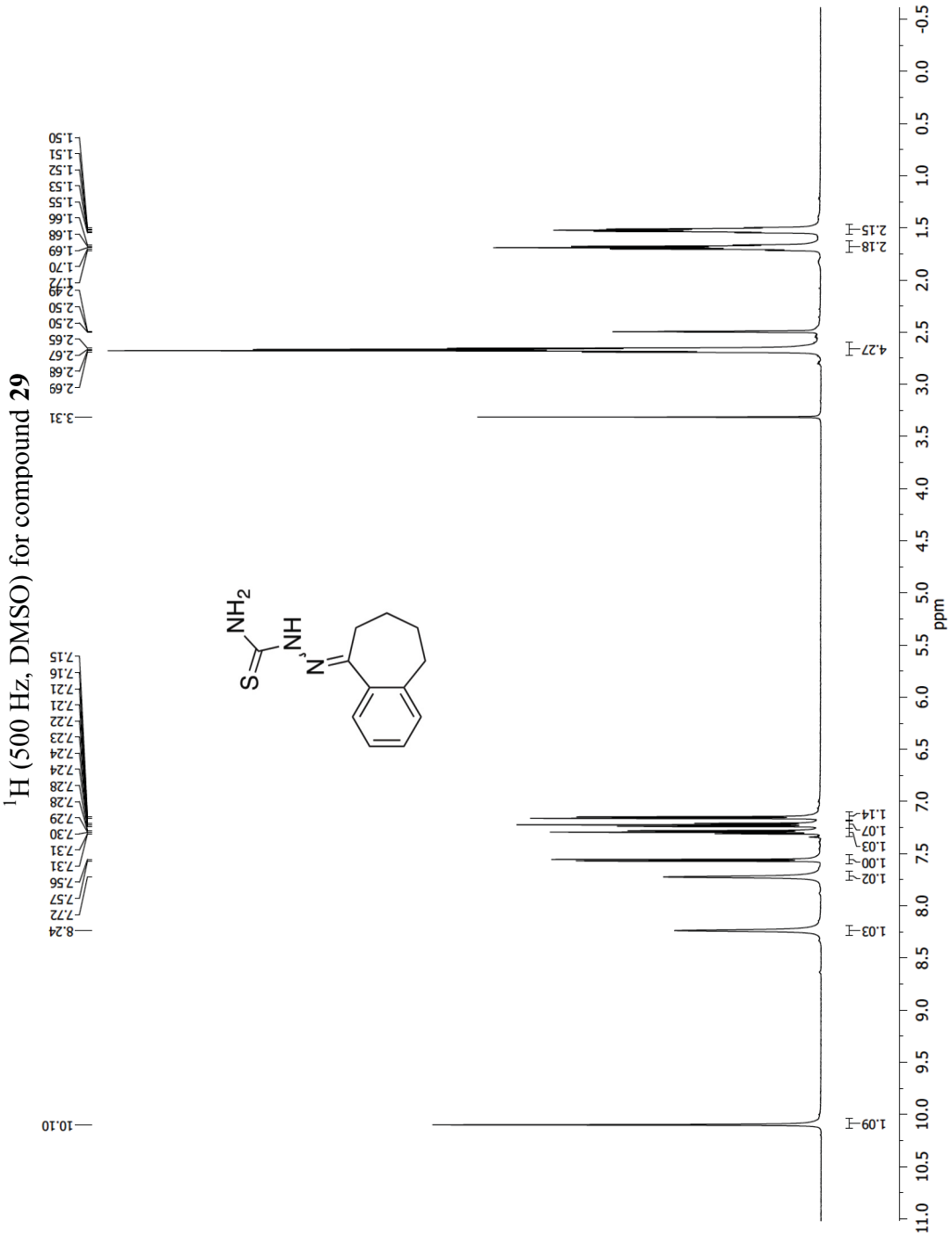
^{1}H (500 Hz, DMSO) for compound **28**



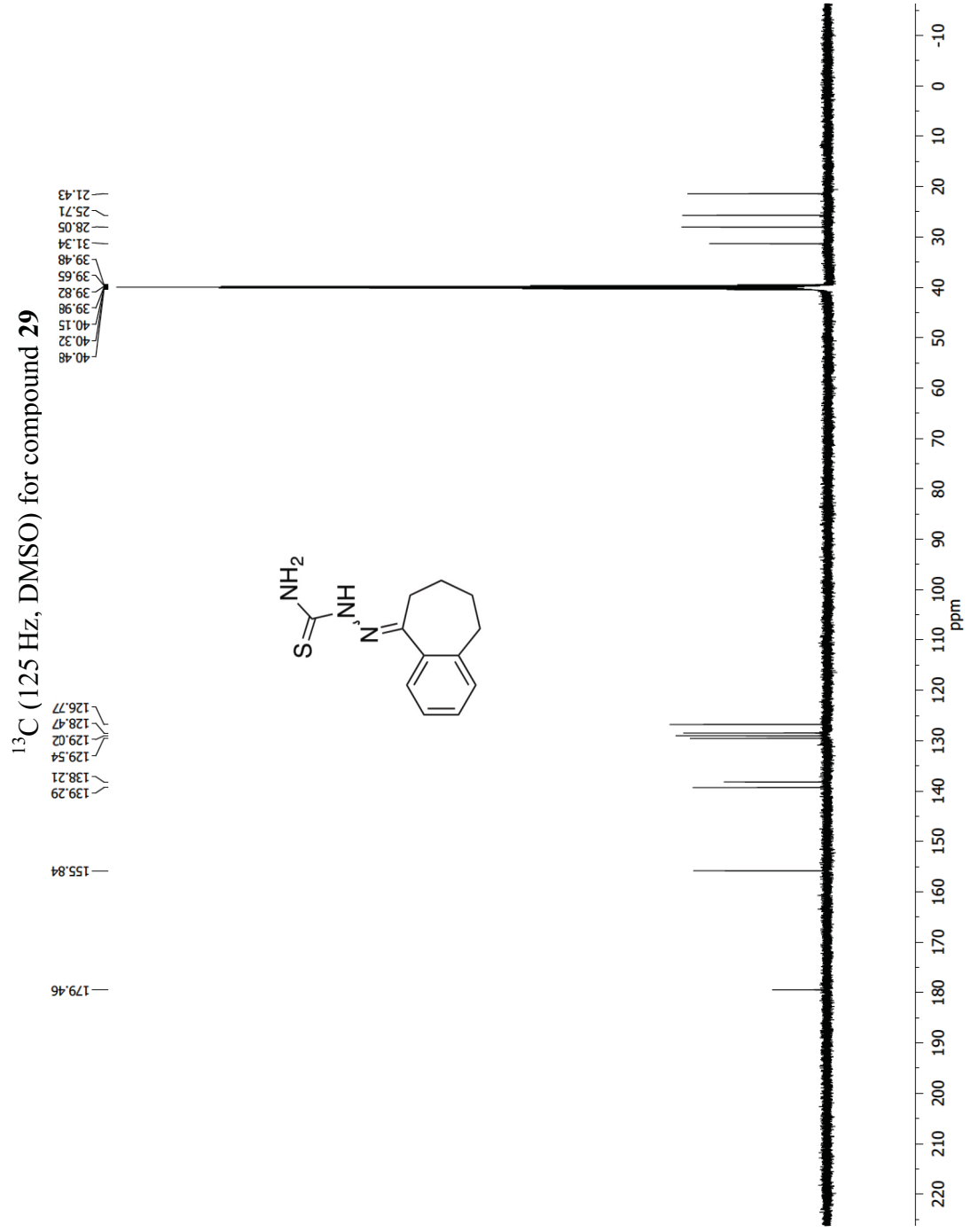
^{13}C (125 Hz, DMSO) for compound **28**



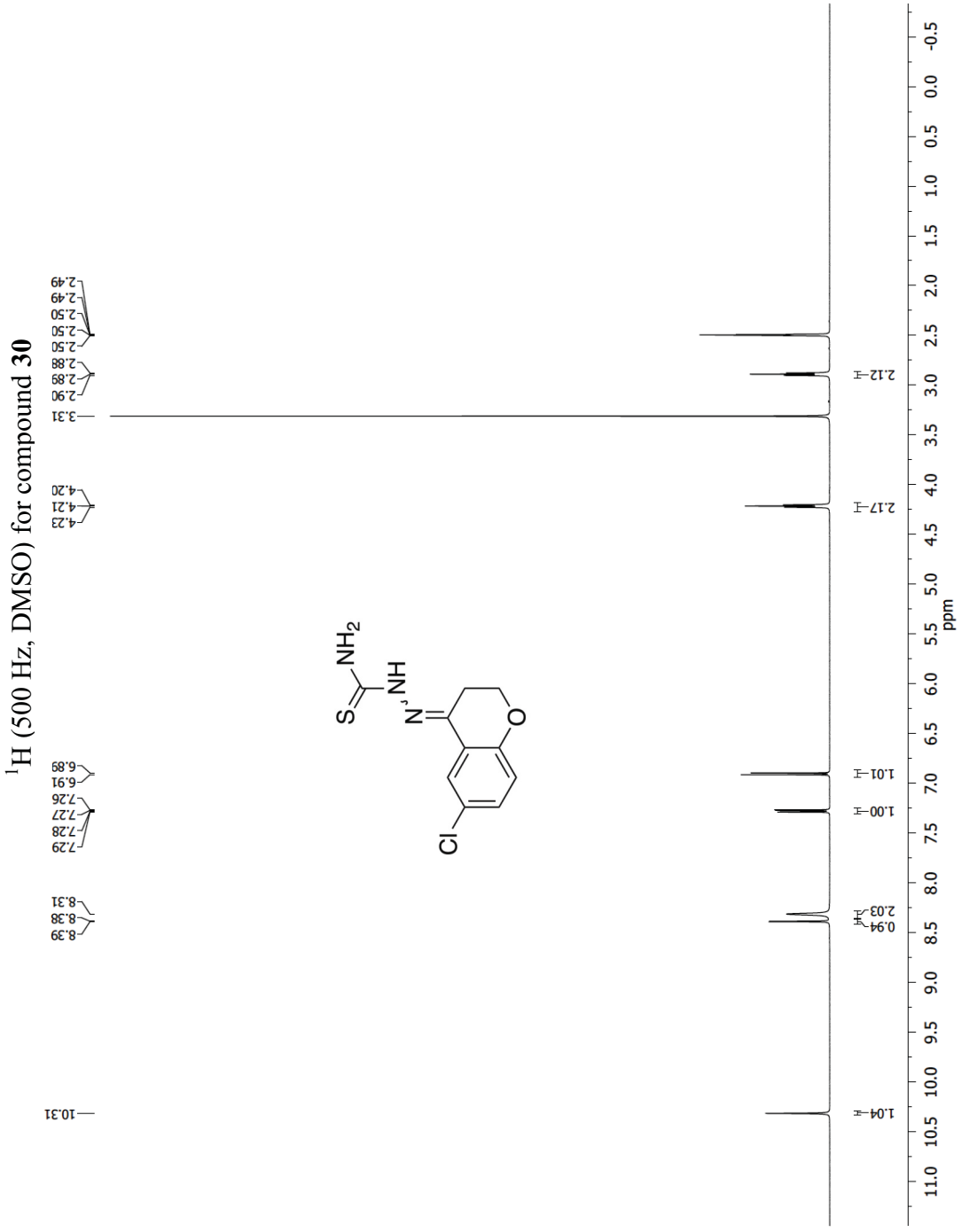
^{1}H (500 Hz, DMSO) for compound **29**



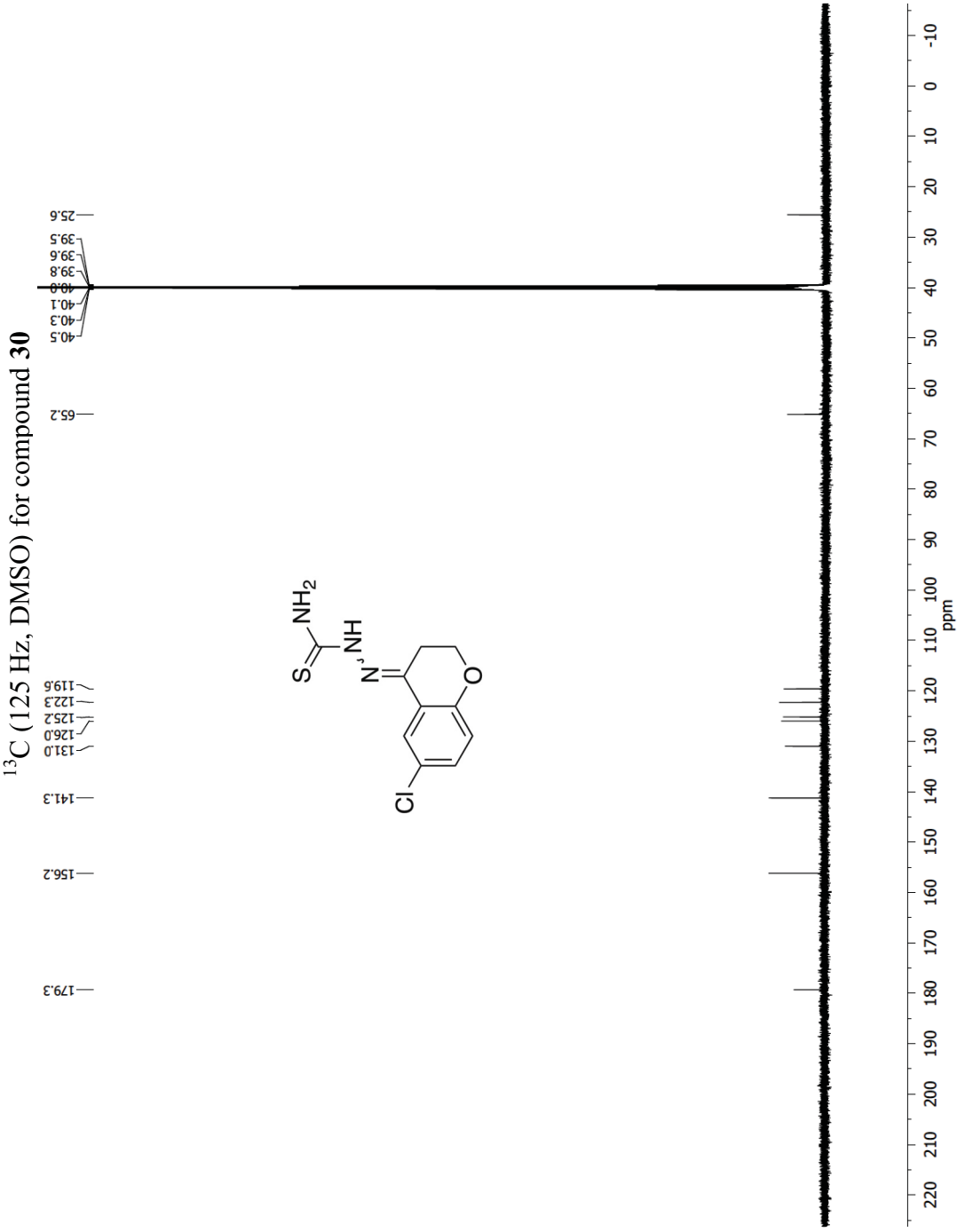
^{13}C (125 Hz, DMSO) for compound **29**



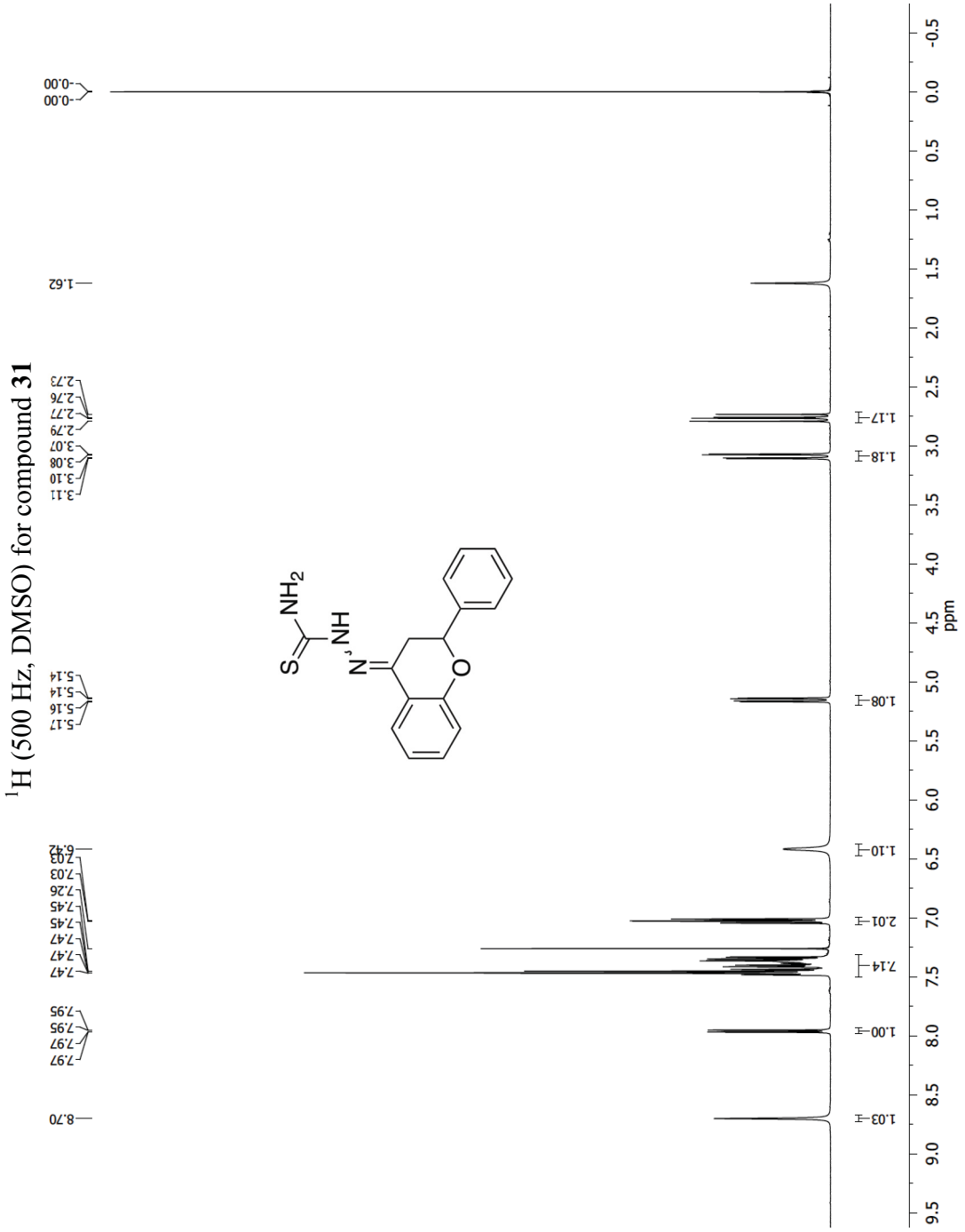
^{1}H (500 Hz, DMSO) for compound **30**



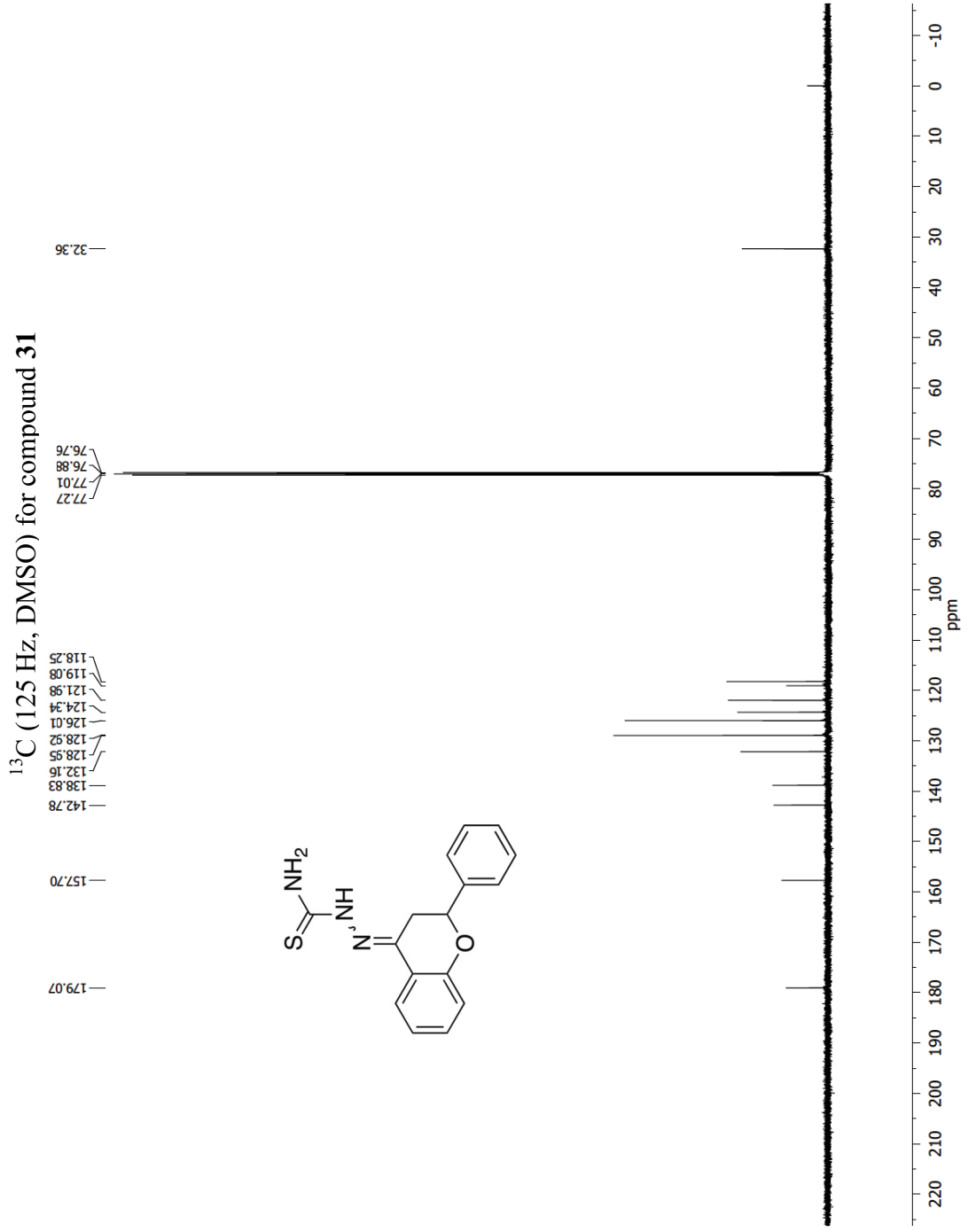
^{13}C (125 Hz, DMSO) for compound **30**



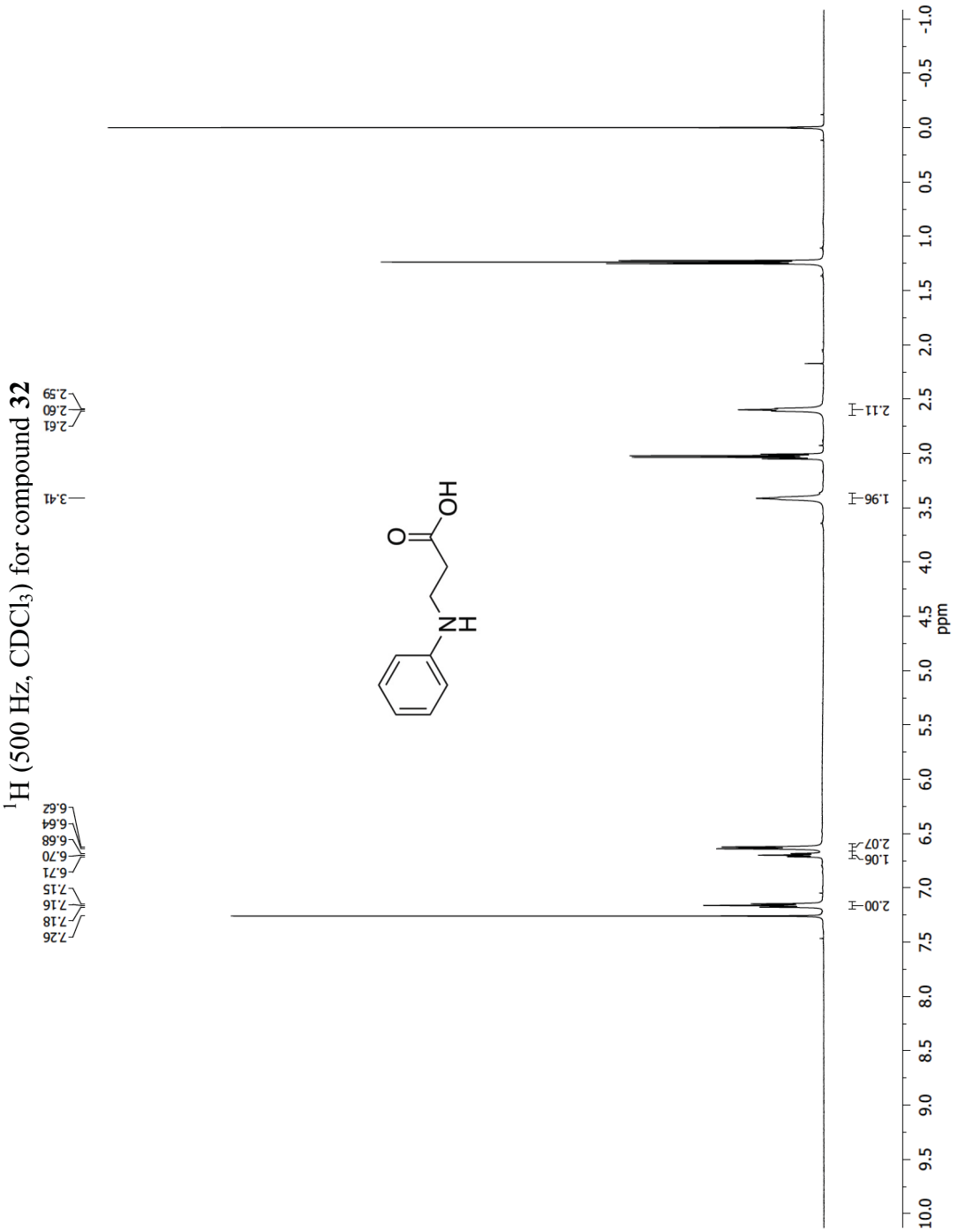
^{1}H (500 Hz, DMSO) for compound **31**



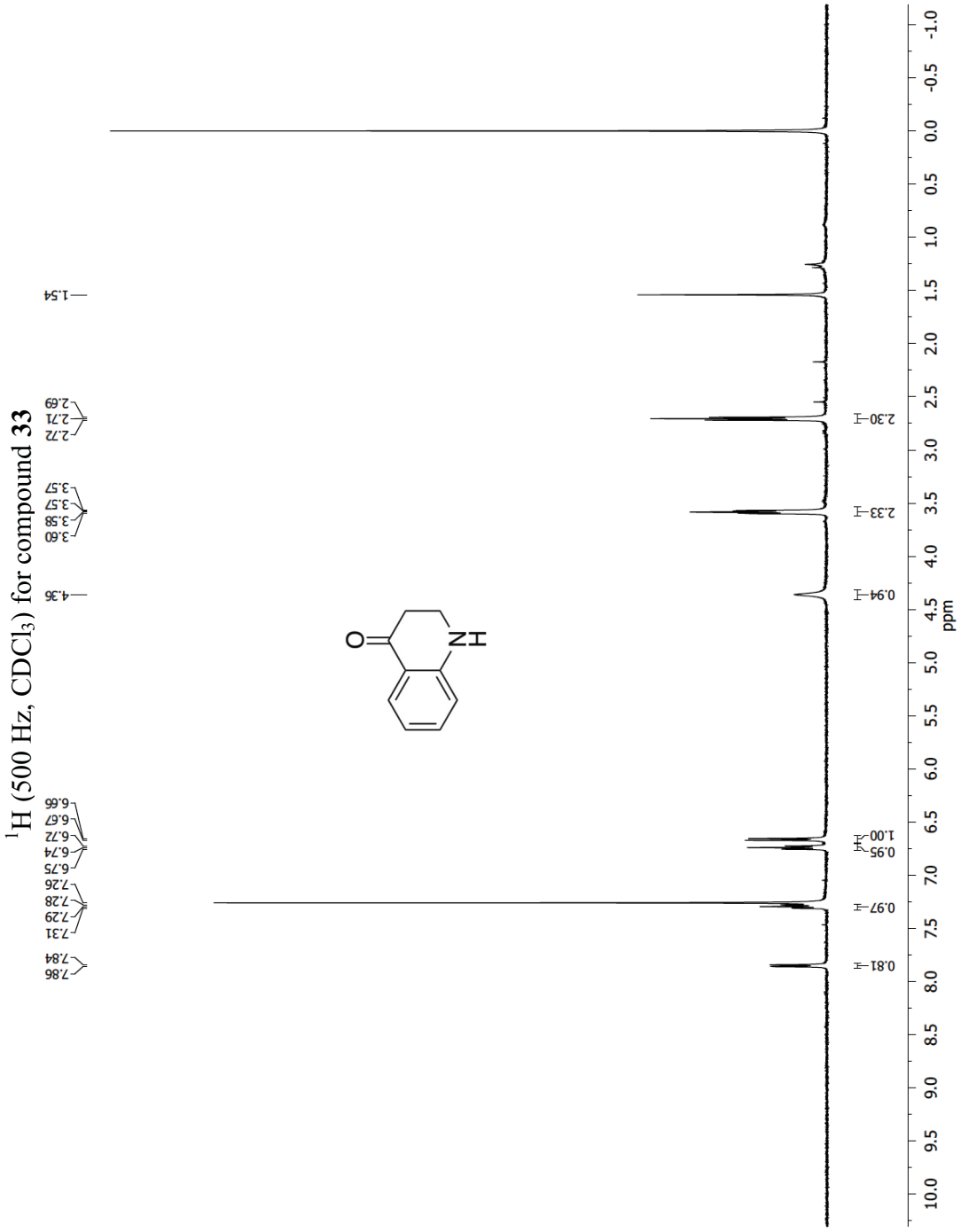
^{13}C (125 Hz, DMSO) for compound **31**



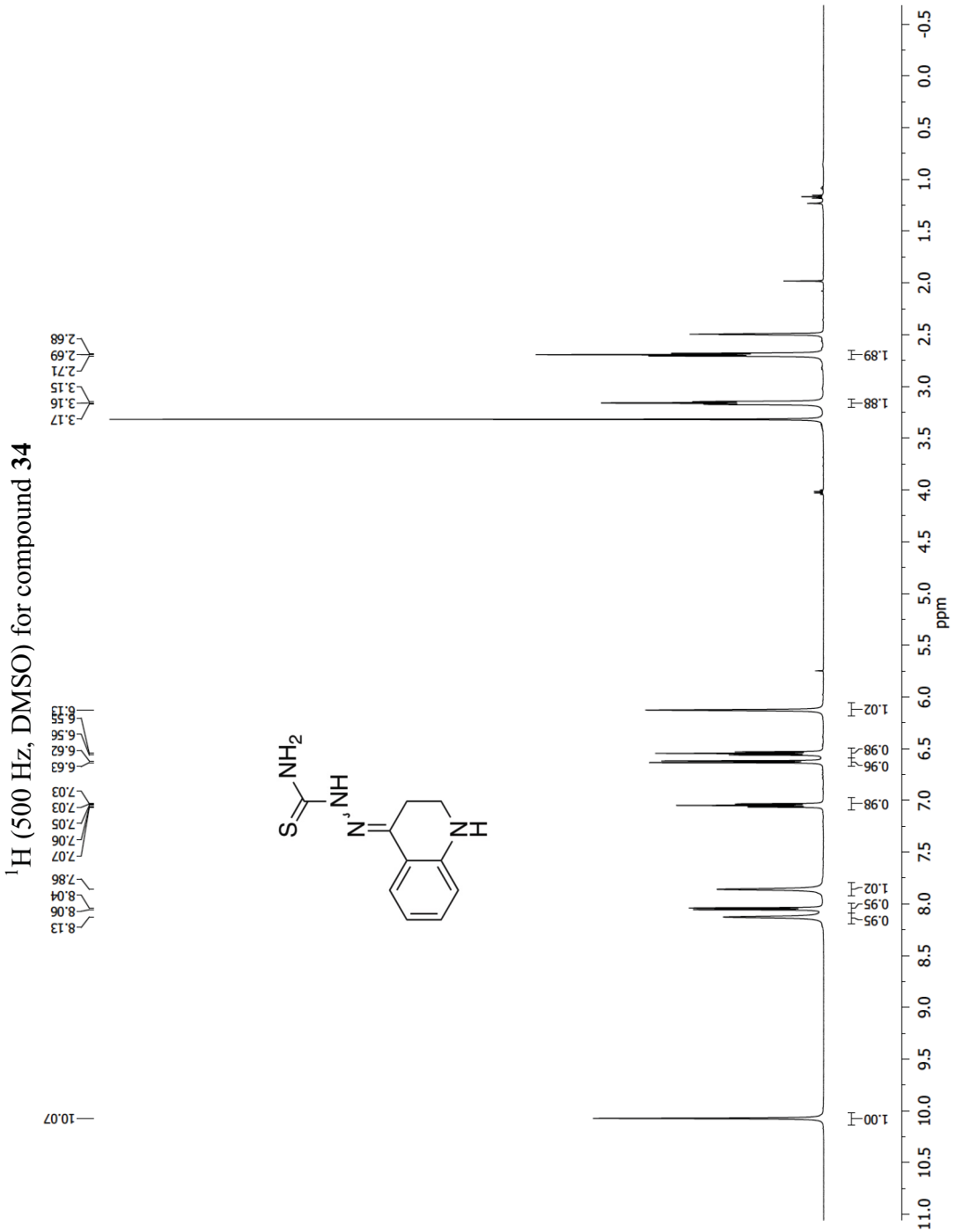
^{1}H (500 Hz, $CDCl_3$) for compound **32**



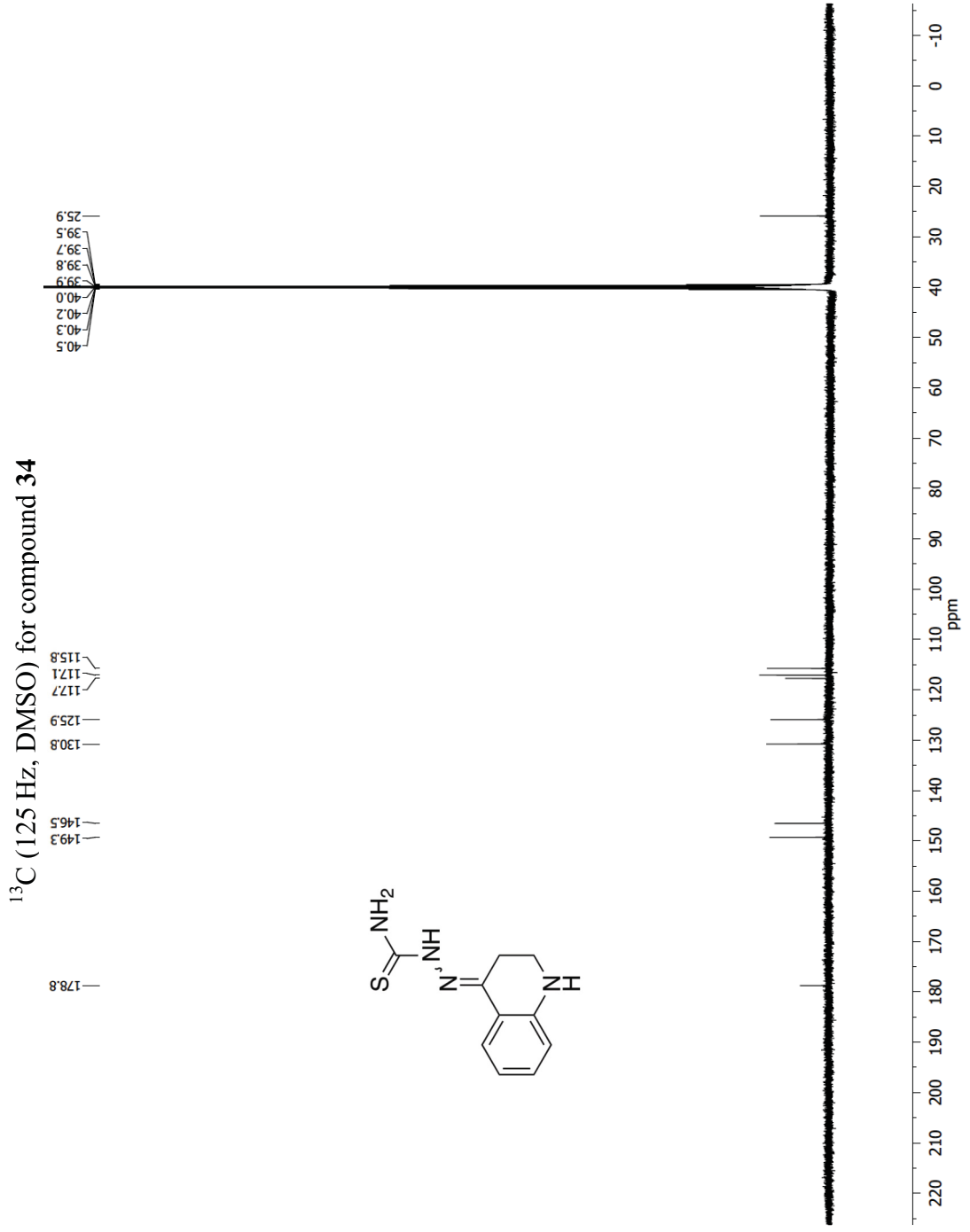
^{1}H (500 Hz, $CDCl_3$) for compound **33**



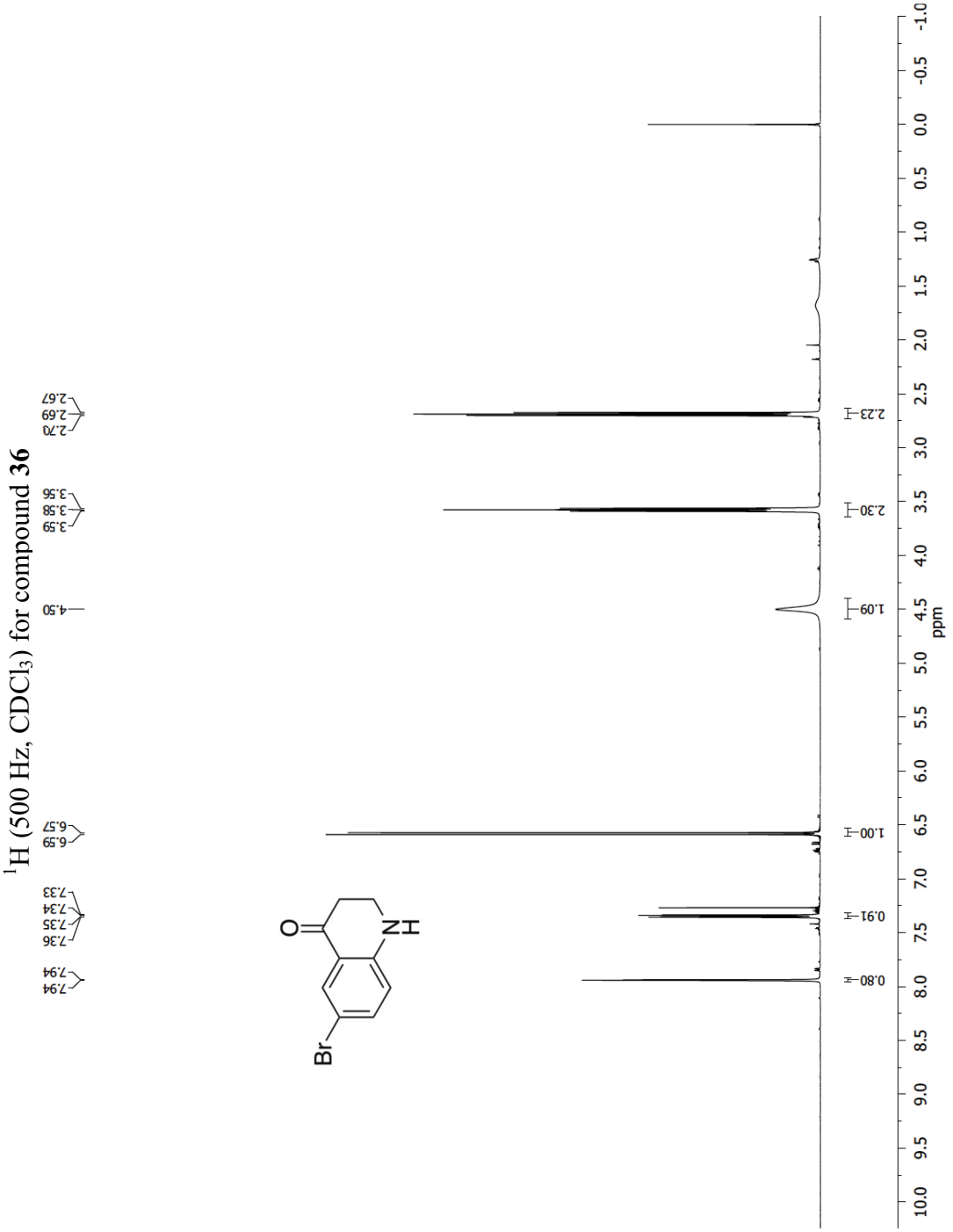
^{1}H (500 Hz, DMSO) for compound **34**

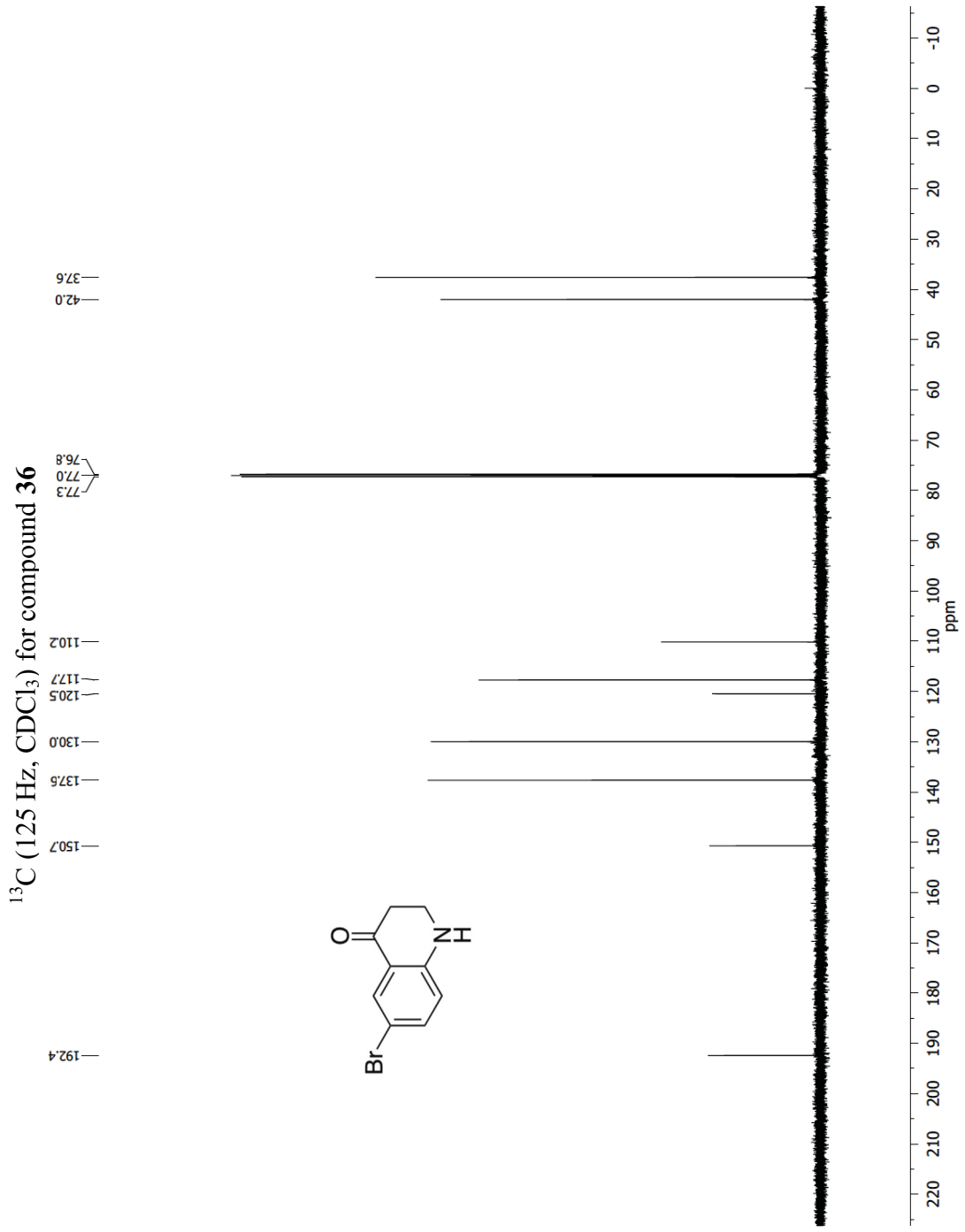


^{13}C (125 Hz, DMSO) for compound **34**

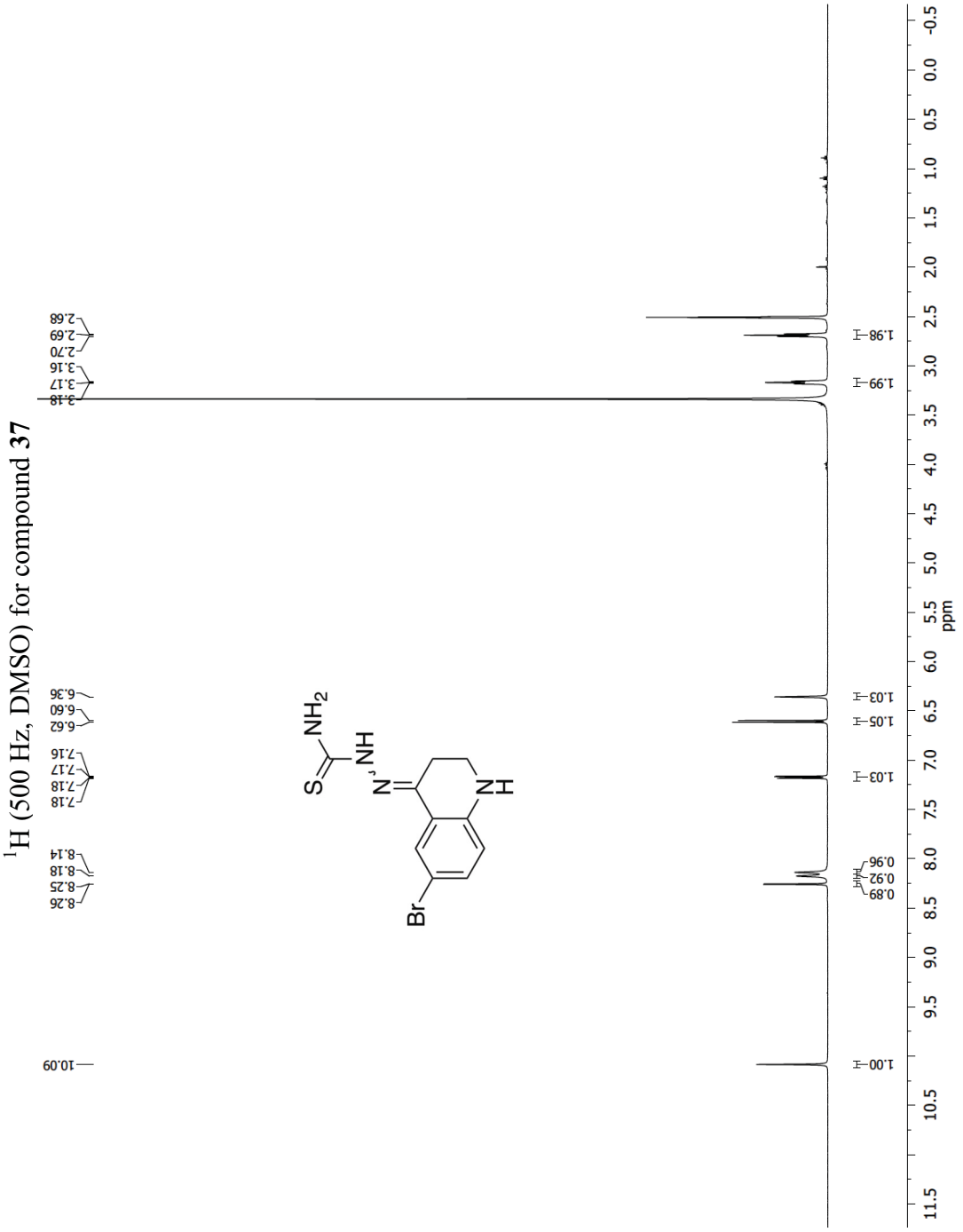


^{1}H (500 Hz, $CDCl_3$) for compound **36**

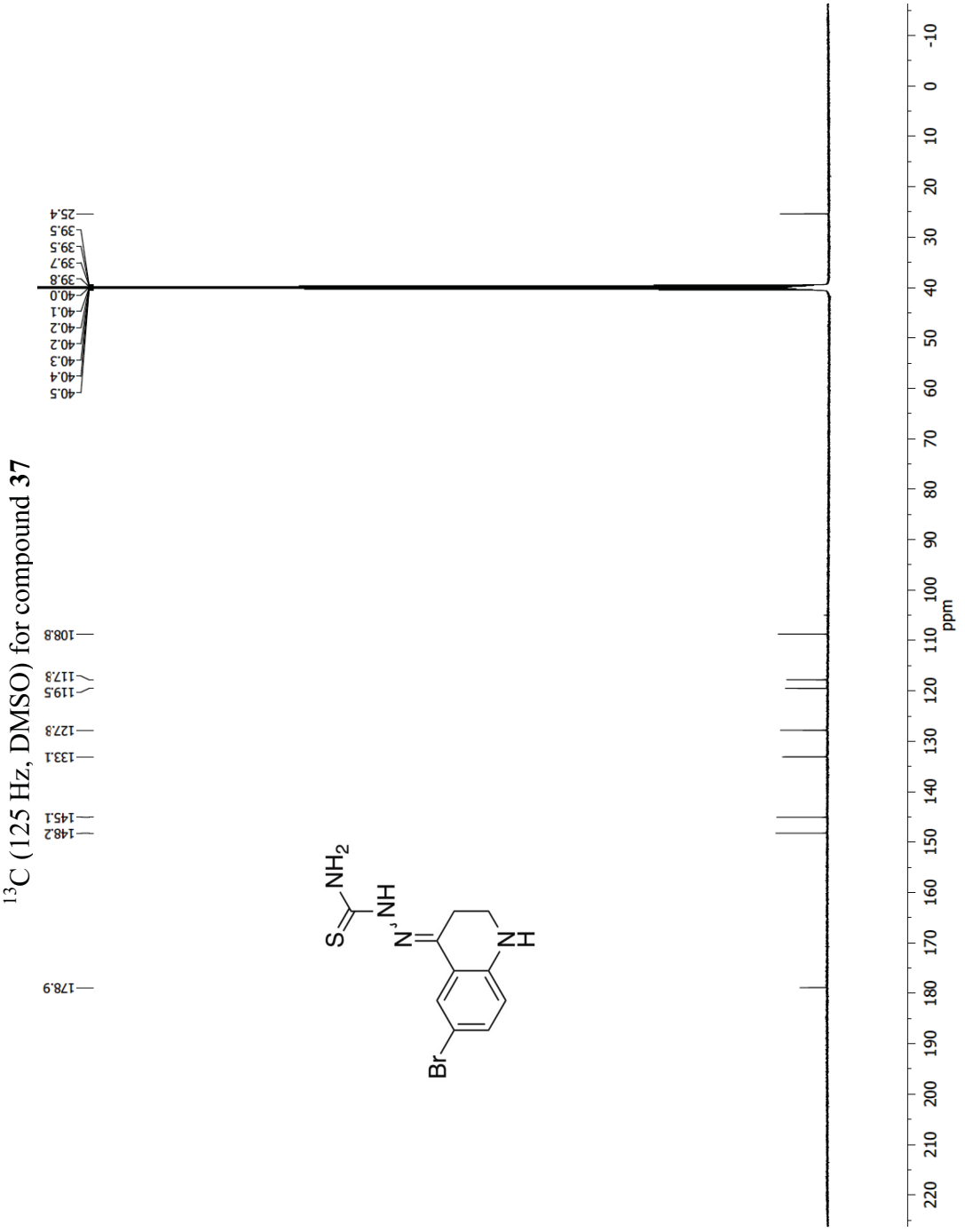



^{13}C (125 Hz, $CDCl_3$) for compound **36**
192.4
150.7
137.5
130.0
120.5
117.7
110.2
77.3
77.0
76.8
42.0
37.6
O
NH
Br
ppm

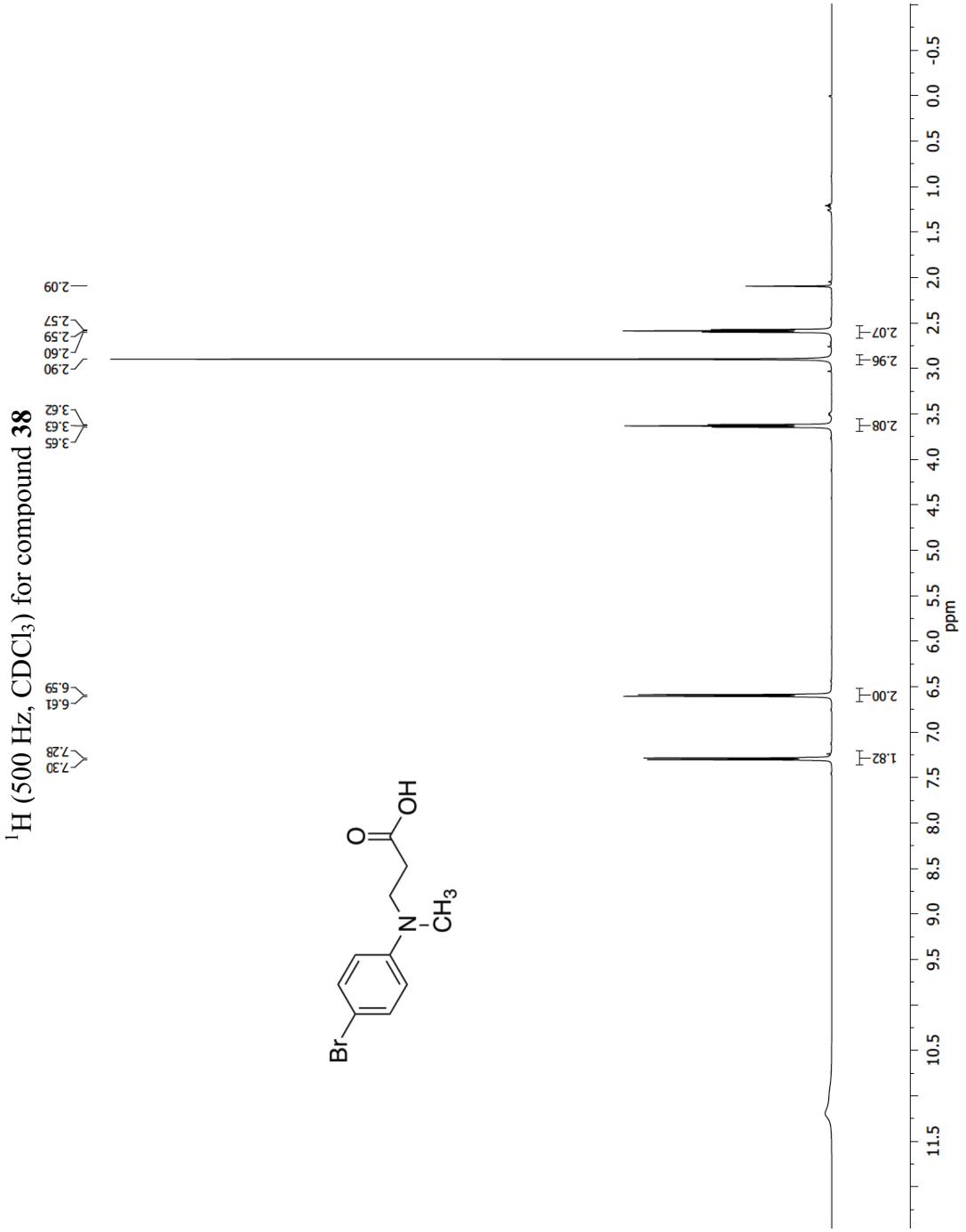
^{1}H (500 Hz, DMSO) for compound **37**



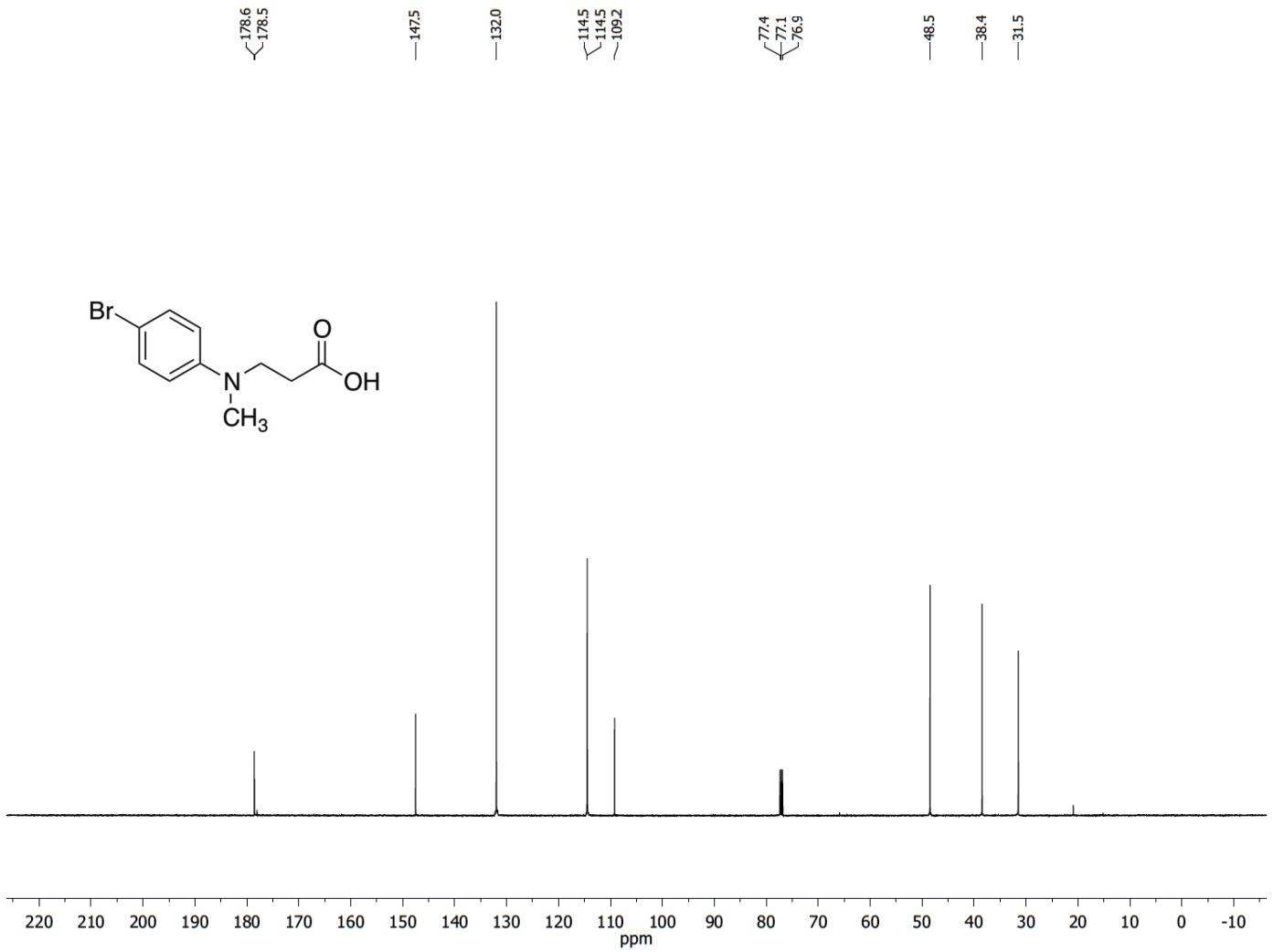
^{13}C (125 Hz, DMSO) for compound **37**



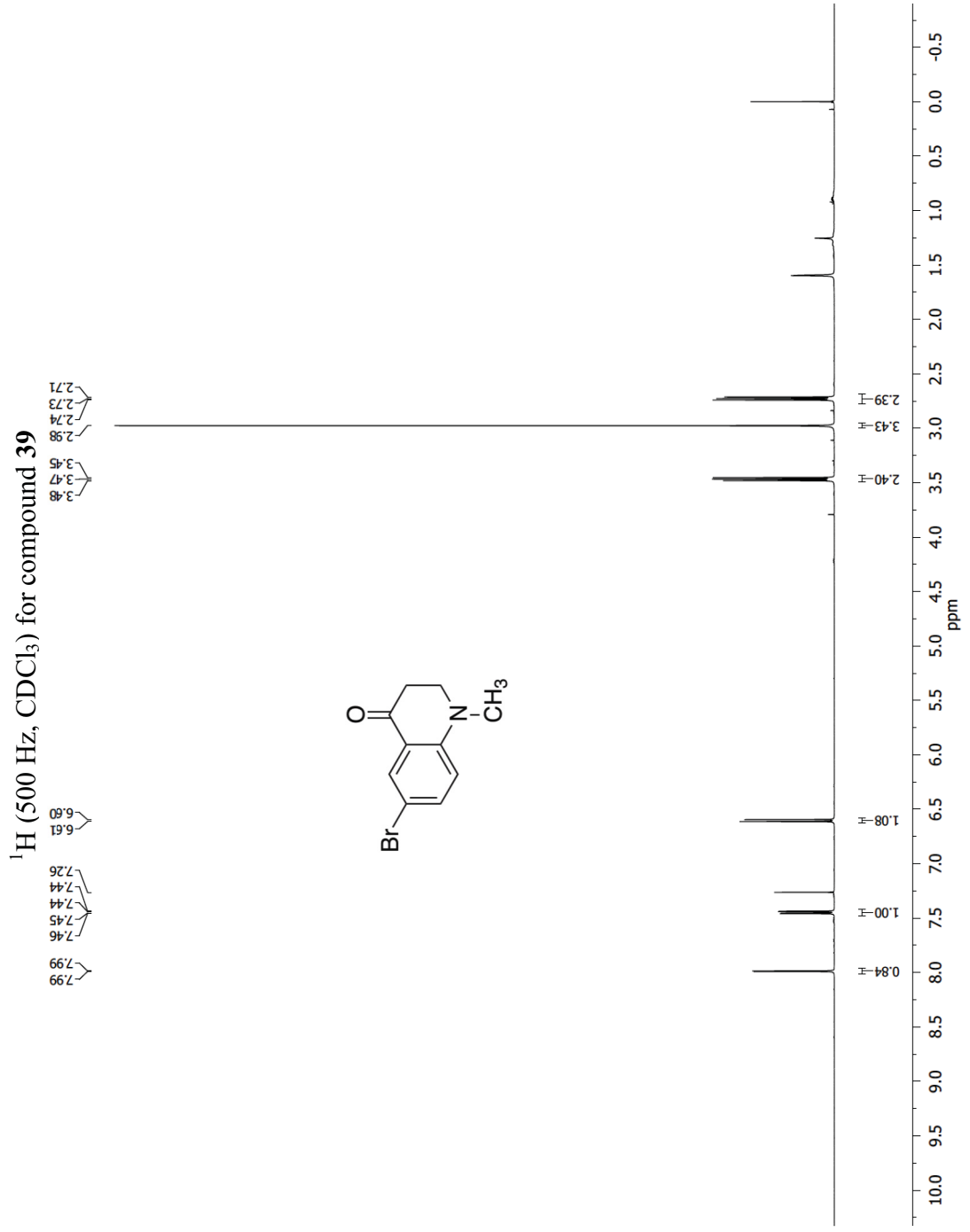
^{1}H (500 Hz, $CDCl_3$) for compound **38**

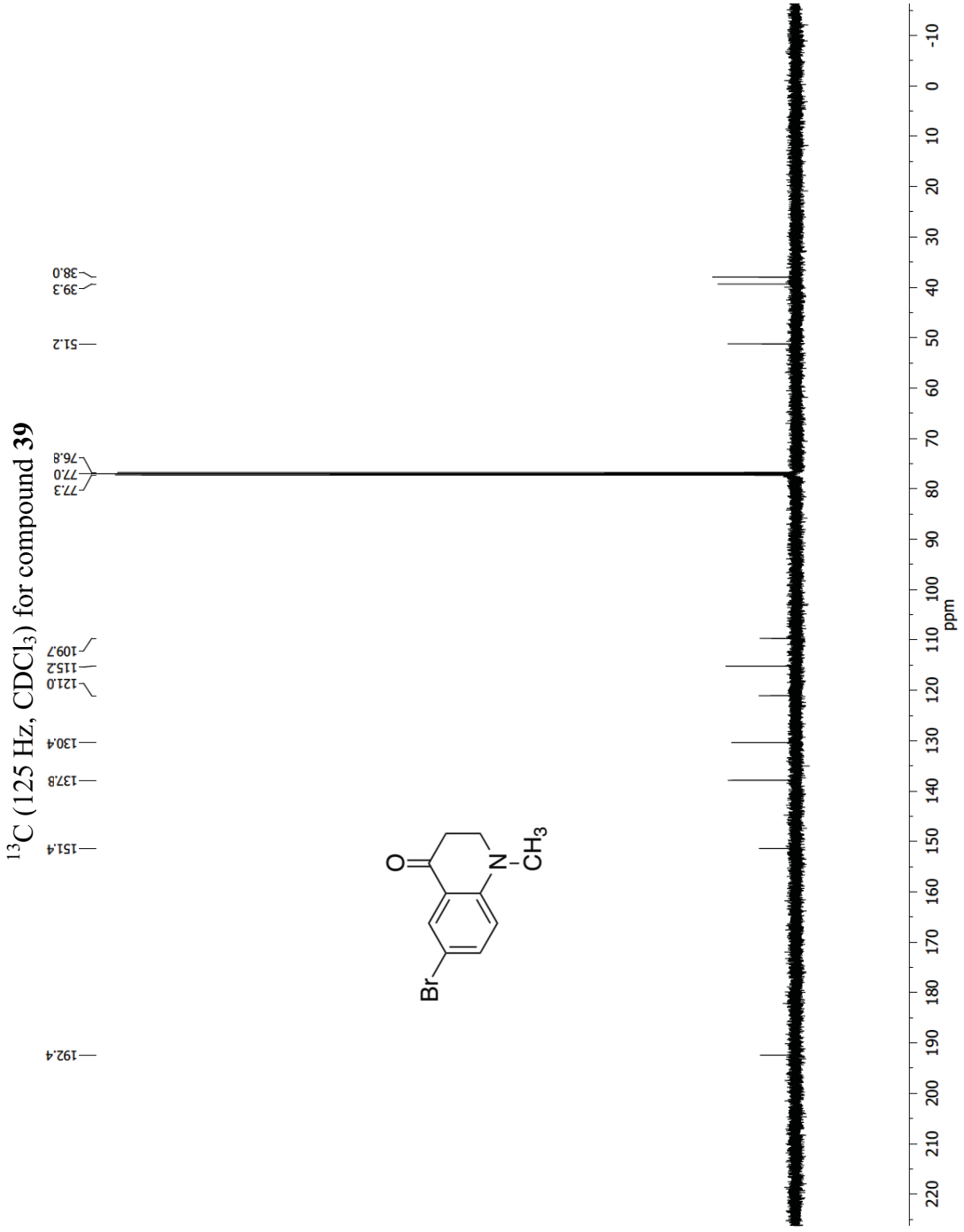


^{13}C (125 Hz, $CDCl_3$) for compound **38**

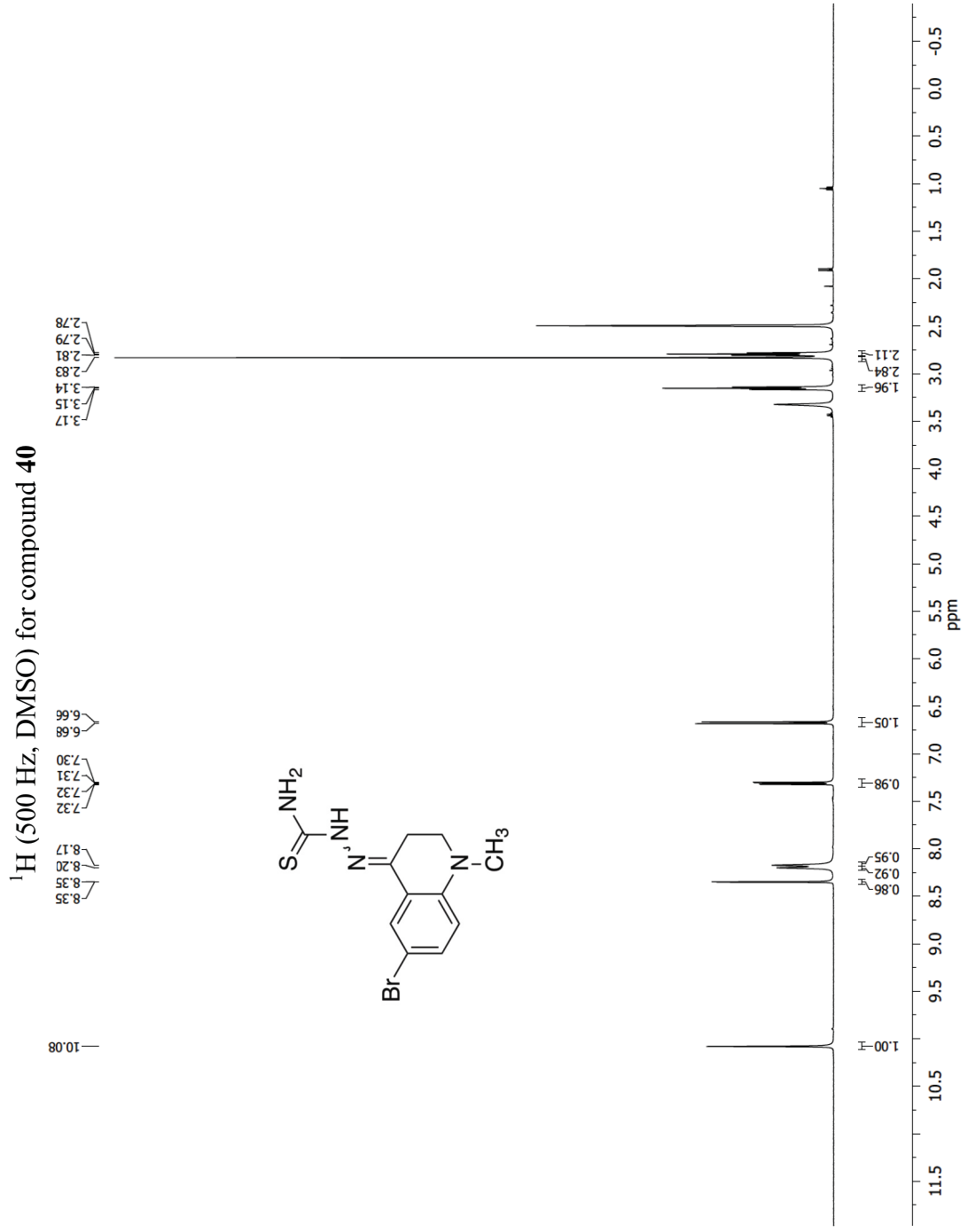


^{1}H (500 Hz, $CDCl_3$) for compound **39**

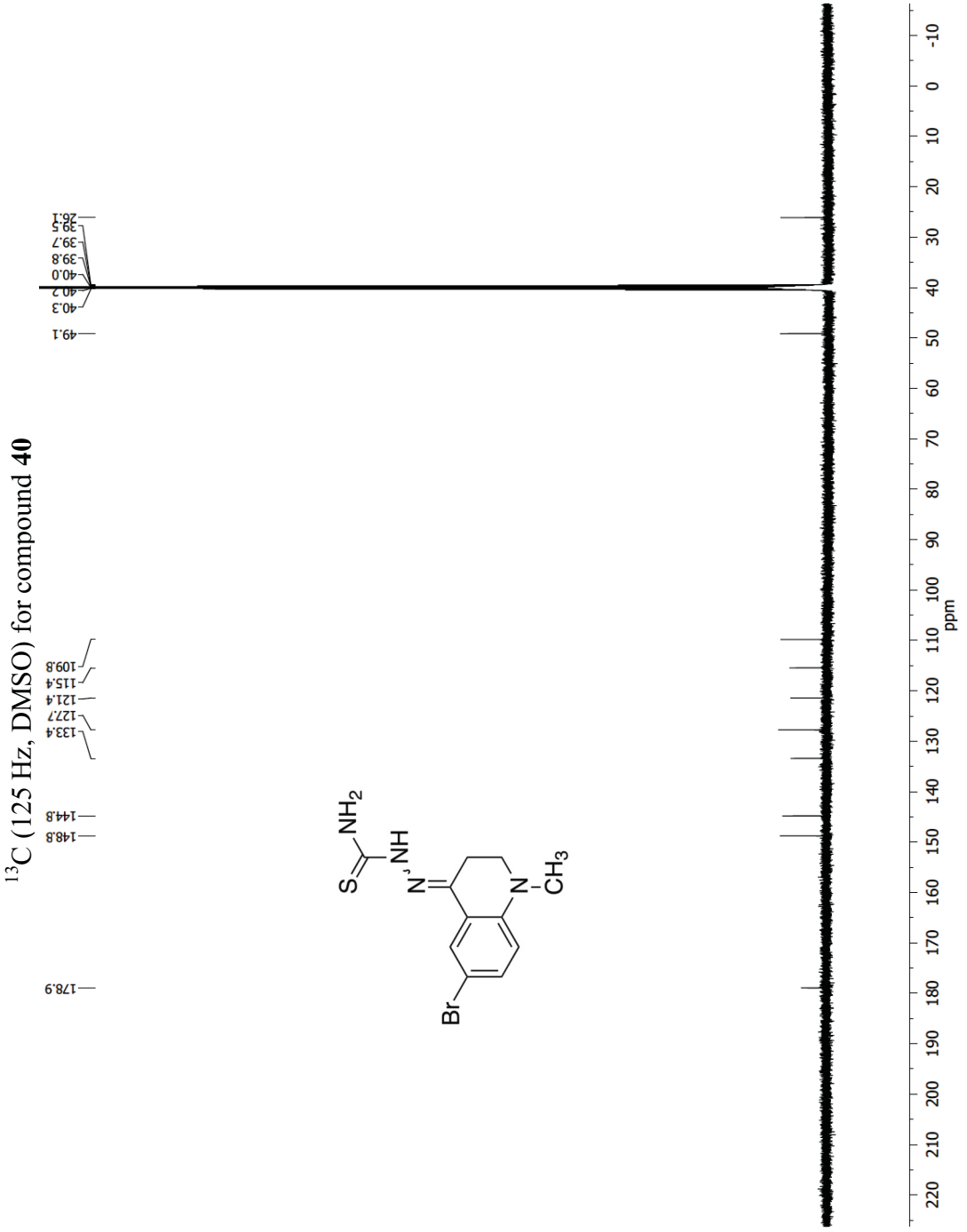



13C (125 Hz, CDCl3) for compound 39
192.4
151.4
137.8
130.4
121.0
115.2
109.7
77.3
77.0
76.8
51.2
39.3
38.0
O
N
CH3
Br
ppm

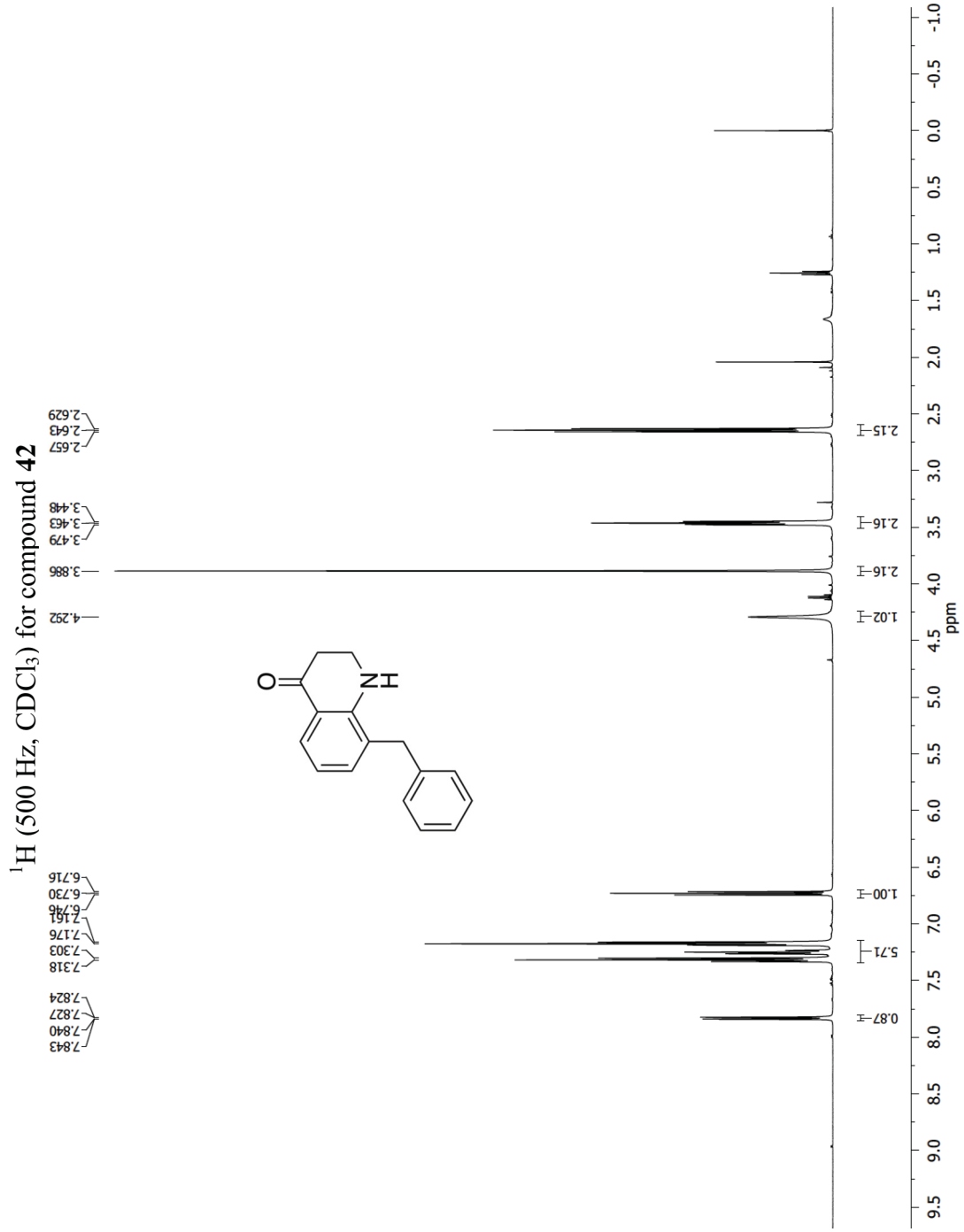
^{1}H (500 Hz, DMSO) for compound **40**



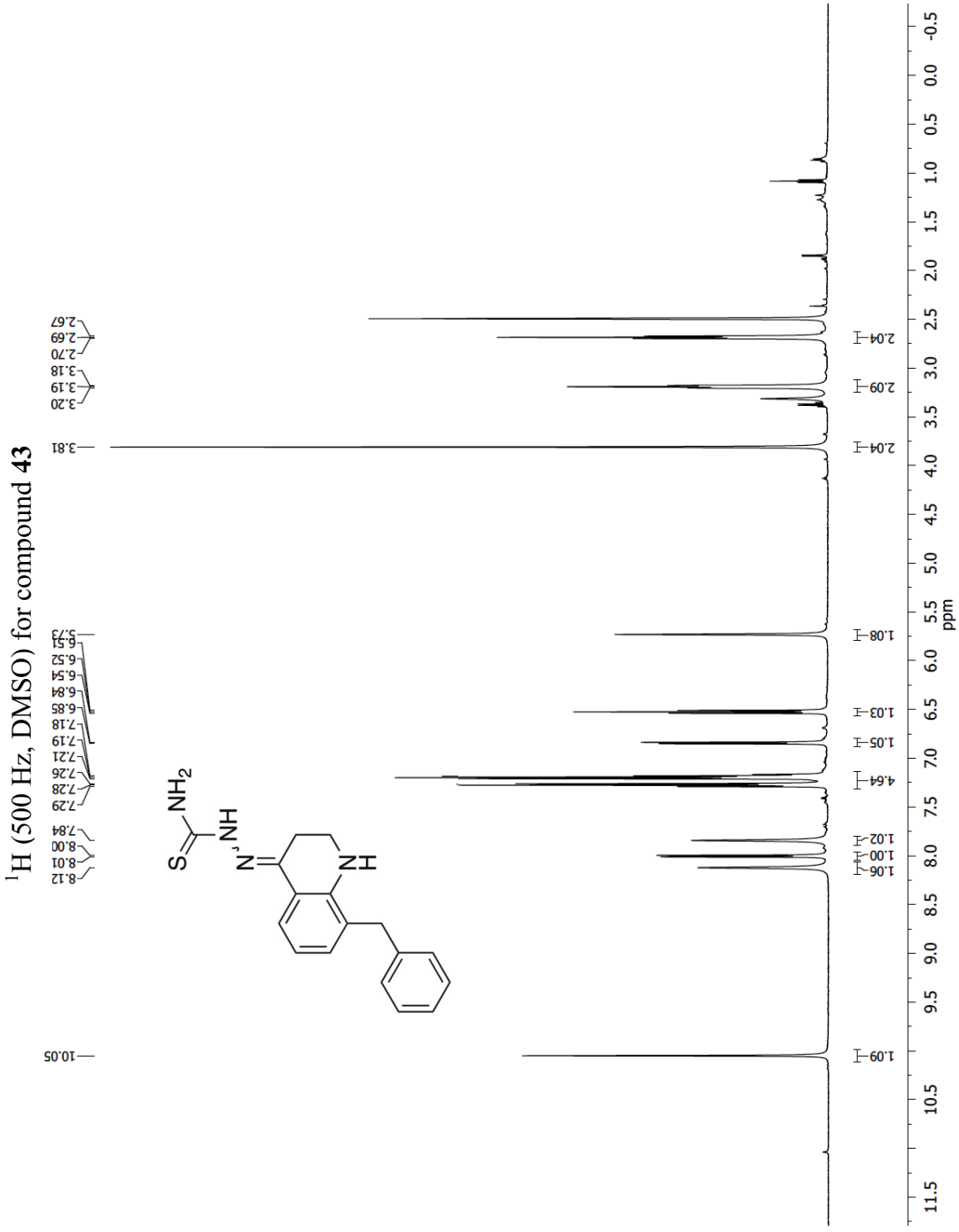
^{13}C (125 Hz, DMSO) for compound **40**



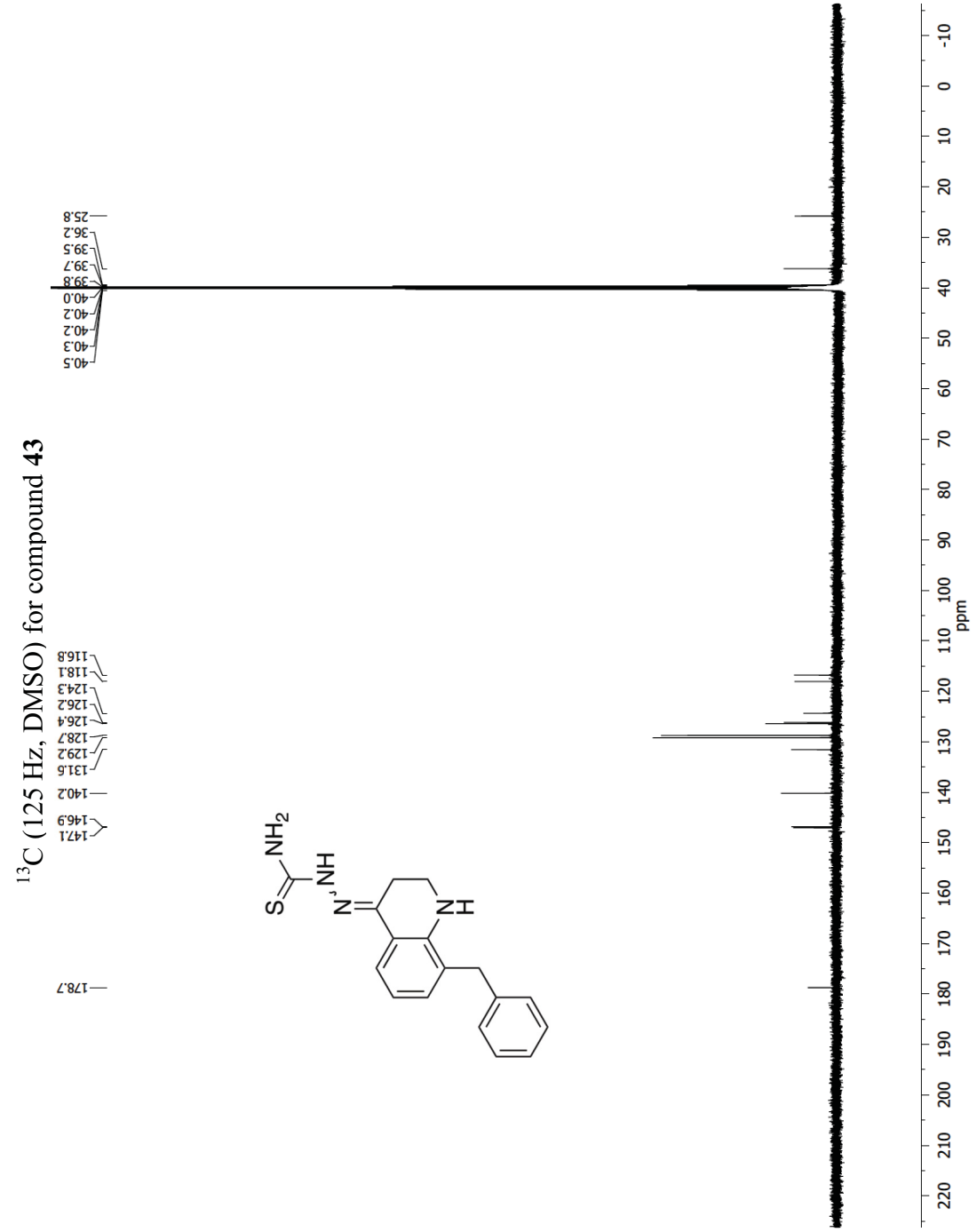
^{1}H (500 Hz, $CDCl_3$) for compound **42**



^{1}H (500 Hz, DMSO) for compound **43**



^{13}C (125 Hz, DMSO) for compound **43**



REFERENCES

1) Schreiber, S. L. Organic synthesis toward small-molecule probes and drugs. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 6699-6702.

2) World Health Organization. Cancer Fact Sheet. http://www.who.int/mediacentre/factsheets/fs297/en/index.html (accessed February 2012).

3) American Cancer Society. *Cancer Facts & Figures 2012*. Atlanta: American Cancer Society; 2012.

4) National Cancer Institute. Fact Sheet: Metastatic Cancer. http://www.cancer.gov/cancertopics/factsheet/Sites-Types/metastatic (accessed February 2012).

5) Gocheva, V.; Joyce, J. A. Cysteine cathepsins and the cutting edge of cancer invasion. *Cell Cycle* **2007**, *6*, 60-64.

6) Iilizumi, M.; Liu W.; Pal, S. K.; Furuta, E.; Watabe, K. Drug development against metastasis-related genes and their pathwars: a rationale for cancer therapy. *Biochim. Biophys. Acta* **2008**, *1786*, 87-104.

7) Lankelma, J. M.; Voorend, D. M.; Barwari, T.; Koetsveld, J.; Van der Spek, A. H.; De Porto, A. P. N. A.; Rooijen, G. V.; Van Noorden, C. J. F. Cathepsin L, target in cancer treatment? *Life Sciences*. **2010**, *86*, 225-233.

8) Turk, V.; Turk, B.; Turk, D. Lysosomal cysteine proteases: facts and opportunities. *EMBO J.* **2001**, *20*, 4629-4633.

9) Turk, B. Targeting proteases: successes, failures and future prospects. *Nat. Rev. Drug Discovery* **2006**, *5*, 785-799.

10) Bromme, D. Cysteine cathepsins and the skeleton. *Clinic Rev. Bone Miner. Metab.* **2011**, *9*, 83-93.

11) Internation Osteoporosis Foundation. Facts and statistics about osteoporosis and its impact. http://www.iofbonehealth.org/facts-and-statistics.html (accessed February 2012).

12) Saftig, P.; Reiss, K. The "A disintegrin and metalloproteases" ADAM10 and ADAM17: Novel drug targets with therapeutic potential? *Eur. J. Cell Biol.* **2011**, *90*, 527-535.

13) Ochi, Y.; Yamada, H.; Mori, H.; Nakanishi, Y.; Nishikawa, S.; Kayasuga, R.; Kawada, N.; Kunishige, A.; Hashimoto, Y.; Tanaka, M.; Sugitani, M.; Kawabata, K. Effects of ONO-5334, a novel orally-active inhibitor of cathepsin K, on bone metabolism. *Bone* **2011**, *49*, 1351-1356.

14) Marcq, V.; Mirand, C.; Decarme, M.; Emonard, H.; Hornebeck, W. MMPs inhibitors: New succinylhydroxamates with selective inhibition of MMP-2 over MMP-3. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2843-2846.

15) Tortorella, M. D.; Tomasselli, A. G.; Mathis, K. J.; Schnute, M. E.; Woodard, S. S.; Munie, G.; Williams, J. M.; Caspers, N.; Wittwer, A. J.; Malfait, A.-M.; Shieh, H.-S. Structural and inhibition analysis reveals the mechanism of selectivity of a series of aggrecanase inhibitors. *J. Biol. Chem.* **2009**, *284*, 24185-24191.

16) Holms, J.; Mast, K.; Marcotte, P.; Elmore, I.; Li, J.; Pease, L.; Glaser, K.; Morgan, D.; Michaelides, M.; Davidsen, S. Discovery of selective hydroxamic acid inhibitors of tumor necrosis factor-α converting enzyme. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2907-2910.

17) Rao. B. G. Recent developments in the design of specific matrix metalloproteinase inhibitors aided by structural and computational studies. *Curr. Pharm. Des.* **2005**, *11*, 295-322.

18) Botos, I.; Scapozza, L.; Zhang, D.; Liotta, L. A.; Meyer, E. F. Batismastat, a potent matrix metalloproteinase inhibitor, exhibits an unexpected mode of binding. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 2749-2754.

19) Palermo, C.; Joyce, J. A. Cysteine cathepsin proteases as pharmacological targets in cancer. *Trends Pharmacol. Sci.* **2008**, *29*, 22-28.

20) Turk, B.; Turk, D.; Turk, V. Lysosomal cysteine proteases: more than scavengers. *Biochim. Biophys. Acta* **2000**, *1477*, 98-111.

21) The cysteine proteases – IV. http://chemistry.umeche.maine.edu/CHY431/Peptidase10b.html (accessed January 2012).

22) Roth, W.; Deussing, J.; Botchkarev, V. A.; Pauly-Evers, M.; Saftig, P.; Hafner, A.; Schmidt, P.; Schmahl, W.; Scherer, J.; Anton-Lamprecht, I.; Von Figura, K.; Paus, R.; Peters, C.; Cathepsin L deficiency as molecular defect of furless: hyperproliferation of keratinocytes and perturbation of hair follicle cycling. *FASEB J.* **2000**, *14*, 2075-2086.

23) Wright, W. W.; Smith, L.; Kerr, C.; Charron, M. Mice that express enzymatically inactive cathepsin L exhibit abnormal spermatogenesis. *Biol. Reprod.* **2003**, *68*, 680-687.

24) Van Nierop, K.; Muller, F. J.; Stap, J.; Van Noorden, C. J.; van Eijk, M.; de Groot, C. Lysosomal destabilization contributes to apoptosis of germinal center B-lymphocytes. *J. Histochem. Cytochem.* **2006,** *54*, 1425–1435.

25) Stoka, V.; Turk, B.; Schendel, S. L.; Kim, T. H.; Cirman, T.; Snipas, S. J.; Ellerby, L.M.; Bredesen, D.; Freeze, H.; Abrahamson, M.; Brömme, D.; Krajewski, S.; Reed, J. C.; Yin, X. M.; Turk, V.; Salvesen G.S. Lysosomal protease pathways to apoptosis. Cleavage of bid, not pro-caspases, is the most likely route. *J. Biol. Chem.* **2001** *276*, 3149–3157.

26) Navab, R.; Pedraza, C.; Fallavollita, L,; Wang, N.; Chevet, E.; Auguste, P.; Jenna, S.; You, Z.; Bikfalvi, A.; Hu, J.; O'Connor, R.; Erickson, A.; Mort, J. S.; Brodt, P. Loss of responsiveness to IGF-I in cells with reduced cathepsin L expression levels. *Oncogene* **2008**, *27*, 4973–4985.

27) Kenig, S.; Frangez, R.; Pucer, A.; Lah, T. Inhibition of cathepsin L lowers the apoptotic threshold of glioblastoma cells by up-regulating p53 and transcription of capsases 3 and 7. *Apoptosis* **2011**, *16*, 671-682.

28) Pandya, N. M.; Dhalla, N. S.; Santani, D. D. Angiogenesis – a new target for future therapy. *Vasc. Pharmacol.* **2006**, *44*, 265-274.

29) Iilizumi, M.; Liu W.; Pal, S. K.; Furuta, E.; Watabe, K. Drug development against metastasis-related genes and their pathwars: a rationale for cancer therapy. *Biochim. Biophys. Acta* **2008**, *1786*, 87-104.

30) Strojnik, T.; Kavalar, R.; Trinkaus, M.; Lah, T. T.; Cathepsin L in glioma progression: comparison with cathepsin B. *Cancer Detect. Prev.* **2005**, *29*, 448-455.

31) Joyce, J. A.; Baruch, A.; Chehade, K.; Meyer-Morse, N.; Giraudo, E.; Tsai, F. Y.; Greenbaum, D.C.; Hager, J. H.; Bogyo, M.; Hanahan, D.; Cathepsin cysteine proteases are effectors of invasive growth and angiogenesis during multistage tumorigenesis. *Cancer Cell* **2004**, *5*, 443-453.

32) Guillaume-Rousselet, N.; Jean, D.; Frade, R. Cloning and characterization of anti-cathepsin L single chain variable fragment whose expression inhibits procathepsin L secretion in human melanoma cells. *Biochem. J.* **2002**, *367*, 219-227.

33) Shah, P. P.; Wang, T.; Kaletsky, R. L.; Myers, M. C.; Purvis, J. E.; Jing, H.; Huryn, D. M.; Greenbaum, D. C.; Smith III, A. B.; Bates, P.; Diamond, S. L. A small-molecule oxocarbazate inhibitor of human cathepsin L blocks severe acute respiratory syndrome and ebola pseudotype virus infection into human embryonic kidney 293T cells. *Mol. Pharmacol.* **2010**, *78*, 319-324.

34) Myers, M. C.; Shah, P. P.; Diamond, S. L.; Huryn, D. M.; Smith, III A. B. Identification and synthesis of a unique thiocarbazate cathepsin L inhibitor. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 210-214.

35) Marquis, R. W.; James, I.; Zeng, J.; Trout, R. E. L.; Thompson, S.; Rahman, A.; Yamashita, D. S.; Xie, R.; Ru, Y.; Gress, C. J.; Blake, S.; Lark, M. A.; Hwang, S.; Tomaszek, T.; Offen, P.; Head, M. S.; Cummings, M. D.; Veber, D. F. Azepanone-based inhibitors of human cathep-sin L. *J. Med. Chem.* **2005**, *48*, 6870-6878.

36) Mallari, J. P.; Shelat, A. A.; Kosinski, A.; Caffrey, C. R.; Connelly, M.; Zhu, F.; McKerrow, J. H.; Guy, R. K. Structure-guided development of selective TbcatB inhibitors. *J. Med. Chem.* **2009**, *52*, 6489-6493.

37) Frlan, R.; Gobec, S. Inhibitors of cathepsin B. *Curr. Med. Chem.* **2006**, *13*, 2309-2327.

38) Illy, C.; Quraishi, O.; Wang, J.; Purisima, E.; Vernet, T.; Mort, J. S. Role of the occluding loop in cathepsin B activity. *J. Biol. Chem.* **1997**, *272*, 1197-1202.

39) Tomoo, K. Development of cathepsin inhibitors and structure-based design of cathepsin B-specific inhibitor. *Curr. Top. Med. Chem.* **2010**, *10*, 696-707.

40) Mohamed, M. M.; Sloane, B. F. Cysteine cathepsins: multifunctional enzymes in cancer. *Nat. Rev. Cancer* **2006**, *6*, 764-775.

41) Xue, Y.; Cai, T.; Shi, S.; Wang, W.; Zhang, Y.; Mao, T.; Duan, X. Clinical and animal research findings in pycnodysostosis and gene mutations of cathepsin K from 1996 to 2011. *Orphanet J. Rare. Dis.* **2011**, *6*:20.

42) Ochi, Y.; Yamada, H.; Mori, H.; Nakanishi, Y.; Nishikawa, S.; Kayasuga, R.; Kawada, N.; Kunishige, A.; Hashimoto, Y.; Tanaka, M.; Sugitani, M.; Kawabata, K. Effects of ONO-5334, a novel orally-active inhibitor of cathepsin K, on bone metabolism. *Bone* **2011**, *49*, 1351-1356.

43) Gauthier, J. Y.; Chauret, N.; Cromlish, W.; Desmarais, S.; Duong, L. T.; Falgueyret, J.-P.; Kimmel, D. B.; Lamontagne, S.; Leger, S.; Leriche, T.; Li, C. S.; Masse, F.; McKay, D. J.; Nicoll-Griffith, D. A.; Oballa, R. M.; Palmer, J. T.; Percival, M. D.; Riendeau, D.; Robicaud, J.; Rodan, G. A.; Rodan, S. B.; Seto, C.; Therien, M.; Truong, V.-L.; Venuti, M. C.; Wesolowski, G.; Young, R. N.; Zamboni, R.; Black, W. C. The discovery of odanacatib (MK-0822), a selective inhibitor of cathepsin K. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 923-928.

44) Kumar, S.; Dare, L.; Vasko-Moser, J. A.; James, I. E.; Blake, S. M.; Rickard, D. J.; Hwang, S.-M.; Tomaszek, T.; Yamashita, D. S.; Marquis, R. W.; Oh, H.; Jeong, J. U.; Veber, D. F.; Gowen, M.; Lark, M. W.; Stroup, G. A highly potent inhibitor of cathepsin K (relacatib) reduces biomarkers of bone resorption both *in vitro* and in acute model of elevated bone turnover *in vivo* in monkeys. *Bone* **2007**, *40*, 122-131.

45) Dobek, A. S.; Klayman, D. L.; Dickson, E. T. Jr.; Scovill, J. P.; Tramont, E. C. Inhibition of clinically significant bacterial organisms in vitro by 2-acetylpyridine thiosemicarbazones. *Antimicrob. Agents Chemother.* **1980**, *18*, 27-36.

46) Pelosi, G. Thiosemicarbazone metal complexes: from structure to activity. *The Open Crystall. J.* **2010**, *3*, 16-28.

47) Greenbaum, D. C.; Mackey, Z.; Hansell, E.; Doyle, P.; Gut, J.; Caffrey, C. R.; Lehrman, J.; Rosenthal, P. J.; McKerrow, J. H.; Chibale, K. Synthesis and structure-activity relationships of parasiticidal thiosemicarbazone cysteine protease inhibitors against *Plasmodium falciparum, Trypanosoma brucei,* and *Trypanosoma cruzi. J. Med. Chem.* **2004**, *47*, 3212-3219.

48) Beraldo, H.; Gambino, D. The wide pharmacological versatility of semicarbazones, thiosemicarbazones and their metal complexes. *Mini-Rev. Med. Chem.* **2004**, *4*, 31-39.

49) Brockman, R. W.; Thomson, J. R.; Bell, M. J.; Skipper, H. E. Observations on the antileukemic activity of pyridine-2-carboxaldehyde thiosemicarbazone and thiocarbohydrazone. *Cancer Res.* **1956**, *16*, 167-170.

50) Wilson, H. R.; Revankar, G. R.; Tolman, R. L. In vitro and in vivo activity of certain thiosemicarbazones against *Trypanosoma cruzi*. *J. Med. Chem.* **1974**, *17*, 760-761.

51) Du, X.; Guo, C.; Hansell, E.; Doyle, P. S.; Caffrey, C. R.; Holler, T. P.; McKerrow, J. H.; Cohen, F. E. Synthesis and structure-activity relationship study of potent trypanocidal thiosemicarbazone inhibitors of the trypanosomal cysteine protease cruzain. *J. Med. Chem.* **2002**, *45*, 2695-2707.

52) Rodrigues, G. C.; Aguiar, A. P.; Vianez, Junior, J. L.; Macrae, A.; Nogueira de Melo, A. C.; Vermelho, A. B. Peptidase inhibitors as a possible therapeutic strategy for Chagas disease. *Curr. Enzyme Inhib.* **2010***, 6*, 183-194.

53) Ettari, R.; Bova, F.; Zappala, M.; Grasso, S.; Micale, N. Falcipain-2 inhibitors. *Med. Res. Rev.* **2010**, *30*, 136-167.

54) Kishore Kumar, G. D.; Chavarria, G. E.; Charlton-Sevcik, A. K.; Yoo, G. K.; Song, J.; Strecker, T. E.; Siim, B. G.; Chaplin, D. J.; Trawick, M. L.; Pinney, K. G. Functionalized benzophenone, thiophene, pyridine, and fluorine thiosemicarbazone derivatives as inhibitors of cathepsin L. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6610-6615.

55) Kishore Kumar, G. D.; Chavarria, G. E.; Charlton-Sevcik, A. K.; Arispe, W. M.; MacDonough, M. T.; Strecker, T. E.; Chen, S.; Siim, B. G.; Chaplin, D. J.; Trawick, M. L.; Pinney, K. G. Design, synthesis, and biological evaluation of potent thiosemicarbazone based cathepsin L inhibitors. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1415-1419.

56) Stariat, J.; Kovarikova, P.; Klimes, J.; Kalinowski, D. S.; Richardson, D. R. Development of an LC-MS/MS method for analysis of interconvertible *Z/E* isomers of the novel anticancer agent, Bp4eT. *Anal. Bioanal. Chem.* **2010**, *397*, 161-171.

57) Ota, A. T.; Temperini, M. L. A.; Areas, E. P. G.; Loos, M. Modeling of the interconversion between Z and E isomeric forms of pyridine-2-formyl thiosemicarbazone. *J. Mol. Struct.: THEOCHEM* **1998**, *451*, 269-275.

58) Center for Disease Control and Prevention. Parasites – American Trypanosomiasis (also known as Chagas Disease). http://www.cdc.gov/paracites/chagas (accessed February 2012).

59) Siles, R., Chen, S.; Zhou, M.; Pinney, K. G.; Trawick, M. L. Design, synthesis, and biochemical evaluation of novel cruzain inhibitors with potential application in the treatment of Chaga's disease. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4405-4409.

60) Siles, R. Design, synthesis, and biological evaluation of new anti-cancer nitrogen-containing combretastatins and novel cysteine protease inhibitors for the treatment of Chagas. Ph.D. Dissertation, Baylor University, Waco, TX, 2006.

61) Siles, R., Chen, S.; Zhou, M.; Pinney, K. G.; Trawick, M. L. Design, synthesis, and biochemical evaluation of novel cruzain inhibitors with potential application in the treatment of Chaga's disease. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4405-4409.

62) Siles, R.; Zhou, M.; Ackley, J. F.; Pinney, K. G.; Chen, S.-E.; Arispe-Angulo, W. M.; Trawick, M. L. Preparation of aryl (thio)semicarbazones as inhibitors of cysteine proteases for treatment of protozoan infections such as trypanosomiasis, malaria and leishmaniasis. U.S. Patent US 20090076076, Mar 19, 2009.

63) Masato, Y.; Nobuki, S.; Ayumu, S.; Hideki, F. Phenoxyethylamine derivatives as α1D adrenergic receptor antagonists. Eur. Patent WO 2010137620, Dec 2, 2010.

64) Ma, Z.; Zhang, X.; Wang, S.; He, Y.; Yang, G.; Li, B.; Yang, J.; Lu, Y.; Sun, J. Design and synthesis of and α,β-epoxyketones as new anticancer agents. *Chin. J. Chem.* **2011**, *29*, 757-764.

65) Gao, S.; Tseng, C.; Tsai, C. H.; Yao, C.-F. Fluoride ion-catalyzed conjugate addition for easy synthesis of 3-sulfanylpropionic acid from thiol and α,β-unsaturated carboxylic acid. *Tetrahedron* **2008**, *64*, 1955-1961.

66) Gerasimova, N. P.; Nozhin, N. A.; Moskichev, Y. A.; Alov, Y. M. Sythesis of 5,6-benzothiochroman-4-one and some of its derivatives. *Izv. Vyssh. Uchebn. Zaved., Khim. Khim. Tekhnol.* **2006**, *49*, 115-119.

67) Tojo, G.; Fernandez, M. Chapter 1.5. Pyridinium chlorchromate (PCC). In *Oxidation of alcohols to aldehydes and ketones: A guide to current common practices*; Tojo, G. Ed. Springer Science + Business Media, Inc.: New York, 2006, 46-68.

68) Lipinski, C. A. Drug-like properties and the cases of poor solubility and poor permeability. *J. Pharmacol. Toxicol. Methods* **2000**, *44*, 235-249.

69) Van Vliet, A.; Rodenhuis, N.; Dijkstra, D.; Wikstrom, H. Synthesis and pharmacological evaluation of thiopyran analogues of the dopamine D_3 receptor-selective agonist (4a*R*,10b*R*)-(+)-*trans*-3,4,4a,10b-tetrahydro-4-*n*-propyl-2*H*,5*H*-[1]benzopyrano[4,3-b]-1,4-oxazin-9-ol (PD 128907). *J. Med. Chem.* **2000**, *43*, 2871-2882.

70) Serajuddin, A. T. M. Salt formation to improve drug solubility. *Adv. Drug Delivery Rev.* **2007**, *59*, 603-616.

71) Maurin, M. B.; Grant, D. J. W.; Stahl, P. H. The physicochemical background: Fundamentals of ionic equilibria. In *Handbook of pharmaceutical salts*: Stahl, P. H.; Wermuth, C. G. Eds. Veriag Helvetica Chimica Acta: Zurich, Switzerland, 2002, p 9-19.

72) Atwal, M. S.; Bauer, L.; Dixit, S. N.; Gearien , J. E.; Morris, R. W. Analgesics. Some substituted 2,3-dihydro-4-quinolones. *J. Med. Chem.* 1965, 8, 566-71.

73) Amore, K. M.; Leadbetter, N. E.; Tyson, A.; Schmink, J. R. Fast, easy, solvent-free, microwave-promoted Michael addition of anilines to **α,β**-unsaturated alkenes: synthesis of N-aryl functionalized **β**-amino esters and acids. *Tet. Lett.* 2006, 47, 8583-8586.