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Design and Synthesis of N-Succinyl-L,L-2.6-Diaminopimelic Acid Desuccinylase Inhibitors as Potential Novel Antibiotics

Thomas Dipuma

LOYOLA UNIVERSITY CHICAGO

DESIGN AND SYNTHESIS OF N-SUCCINYL,L,L-2.6-DIAMINOPIMELIC ACID DESUCCINYLASE INHIBITORS AS POTENTIAL NOVEL ANTIBIOTICS

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For my mom and sister, Bonnie & Gina.

But now I knew: I wanted to be a chemist.

—Oliver Sacks

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LIST OF ABBREVIATIONS

ACN	Acetonitrile
Boc	tert-butyloxycarbonyl
ANOVA	Analysis of Variance
CDC	Centers for Disease Control and Prevention
$CDCl_3$	Deuterated chloroform
Cs_2CO_3	Daily Life Stressors Scale
DAP	L,L-diaminopimelate
DapE	*N*-succinyl-L,L-diaminopimelic acid desuccinylase
DCM	Dichloromethane
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
EA	Ethyl acetate
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
Et_2O	Diethyl ether
Et_3N	Triethylamine
FDA	Food and Drug Administration
HCl	Hydrochloric acid
*Hi*DapE	*Haemophilus influenzae N*-succinyl-diaminopimelic acid dessucinylase enzyme
HTS	High-throughput screening

HOBt	Hydroxybenzotriazole
HPLC	High-performance liquid chromatography
kDa	kilodalton
L,L-SDAP	*N*-succinyl-L,L-diaminopimelic acid
m-DAP	*meso*-diaminopimelate
MDR	Multiple drug resistance
MeOH	Methanol
$MgSO_4$	Magnesium sulfate
MOE	Molecular Operating Environment
Na_2SO_4	Sodium sulfate
NaHCO3	Sodium bicarbonate
*Nm*DapE	*Neisseria meningitidis N*-succinyl-diaminopimelic acid dessucinylase enzyme
NMR	Nuclear magnetic resonance
PDB	Protein Data Bank
Pet. Ether	Petroleum ether
RT	Room temperature
T3P	Propylphosphonic anhydride
TFA	Trifluoroacetic acid
TLC	Thin layer chromatography
TMD	Targeted Molecular Dynamics
TMS	Tetramethylsilane
UHP	Urea-hydrogen Peroxide
UTIs	Urinary tract infections

UV	Ultra-violet
WHO	World Health Organization

ABSTRACT

N-Succinyl-L,L-2,6-Diaminopimelic Acid Desuccinylase (DapE) is a bacterial enzyme located in the lysine biosynthetic pathway of all Gram-negative and most Gram-positive species of bacteria including the notorious ESKAPE pathogens, or pathogenic strains of bacteria that have developed significant levels of resistance to currently available antibiotics. As a means to combat this challenge of growing antimicrobial resistance, we have identified the bacterial enzyme DapE as a conserved, novel enzymatic target within these resistant bacterial strains, as DapE is responsible for the production of *m*-DAP and lysine which are ultimately employed in bacterial cell wall synthesis. The overall aim of this research is to design and synthesize small molecule inhibitors of DapE as potential new broad-spectrum antibiotics.

CHAPTER ONE

THE NOVELTY OF THE *N*-SUCCINYL-L,L-DIAMINOPIMELIC ACID DESSUCINYLASE ENZYME

The Threat of Bacterial resistance and Need for Novel Antibiotics

The persistent abuse of antibiotics has exacerbated the emergence of multidrug resistance (MDR) in bacteria, rendering current treatments less and less effective at the global expense of the health of hospitalized patients.[1] In the United States alone, more than 35,000 deaths and 2.8 million cases of antibiotic resistant bacterial infections are reported each year, reinforcing the critical importance of identifying, designing, and synthesizing inhibitory compounds toward novel antibiotic targets.[2] There is an urgent need for antimicrobial agents with new mechanisms of action due to the lack of new antibiotics on the market. In fact, on average, it takes about ten years for a drug to reach the market.[3] Despite the rapid rise of antimicrobial resistance, there has been no development of novel antibiotics targeting bacterial infections in the last thirty years.[4]

Antibiotics are among the most routinely, and often injudiciously, used therapeutic drugs worldwide.[5] Antimicrobial agents have saved countless lives from bacterial infections. However, bacterial species continue to adapt defensive modes of survival exacerbated by overuse. The Infectious Disease Society of America (IDSA) coined the acronym ESKAPE pathogens referring to six bacterial species that have escaped the ability to be treated by existing antibiotics.[6] The bacterial pathogens are *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter*

species.[7] These multidrug resistant bacteria can carry more than one resistance plasmid, via horizontal gene transfer,[8] making them extremely pathogenic and life-threatening especially towards those who are immunocompromised.[5] As a result, the ISDA issued a call to action for developing sustainable antibacterial research in response to current resistance trends.[7] The increasing occurrence of MDR bacteria remains one of the major global threats to human health.[9]

In response, we have identified a previously uncharted enzymatic bacterial target known as *N*-Succinyl-L,L-diaminopimelic acid desuccinylase (DapE) toward the discovery of novel antibiotics. Collectively, we have characterized the structural integrity of this enzyme as well as made significant strides towards elucidating the mechanism of action showcased through protein crystallography and molecular dynamics. This chapter focuses on the foundation of the DapE project through proven studies built on the validation that this enzymatic target is not only essential for bacterial viability but also absent in mammals. Thus, DapE is a novel antibiotic target in the fight against antibiotic resistance.

DapE as a novel antibiotic target

In our research targeting novel bacterial biosynthetic pathways, we have selected within the lysine biosynthetic pathway the bacterial enzyme, *N*-Succinyl-L,L-diaminopimelic acid desuccinylase (DapE), given the key role this enzyme plays in the latter stages of the pathway.[10] Interest is upheld with the conservation of this enzyme in all strains of pathogenic gram negative and most gram positive bacteria.[11] DapE is responsible for the production of *m*-DAP, as well as lysine in which the *m*-DAP is ultimately recruited for bacterial peptidoglycan synthesis.[12] There are three metabolic pathways in which a majority of bacteria can incorporate L-aspartate to form *m*-DAP, as a precursor to lysine including the acetylase, dehydrogenase, and succinylase

pathways.[11] DapE is of peculiar interest as a potential antibiotic target due to the enzyme being conserved in the succinylase pathway across pathogenic strains of bacteria. Similar pathways are also absent in mammals, thus, eliminating the threat of mechanism-based toxicity in humans.[12]

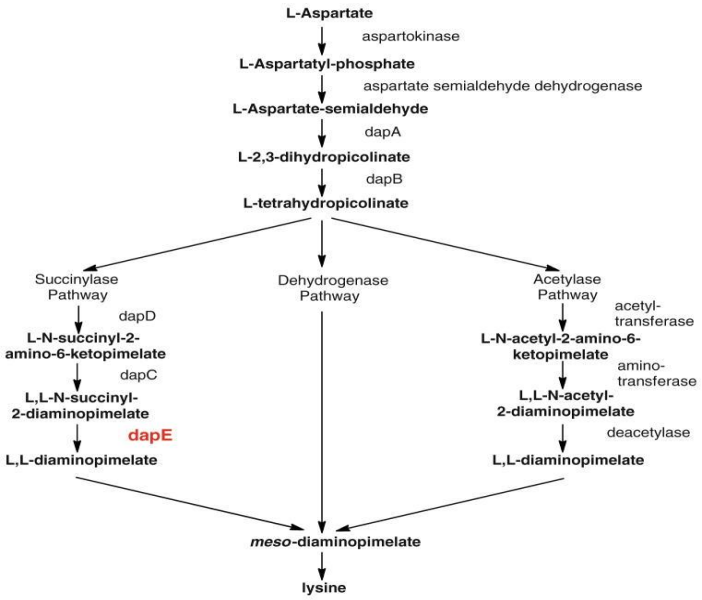
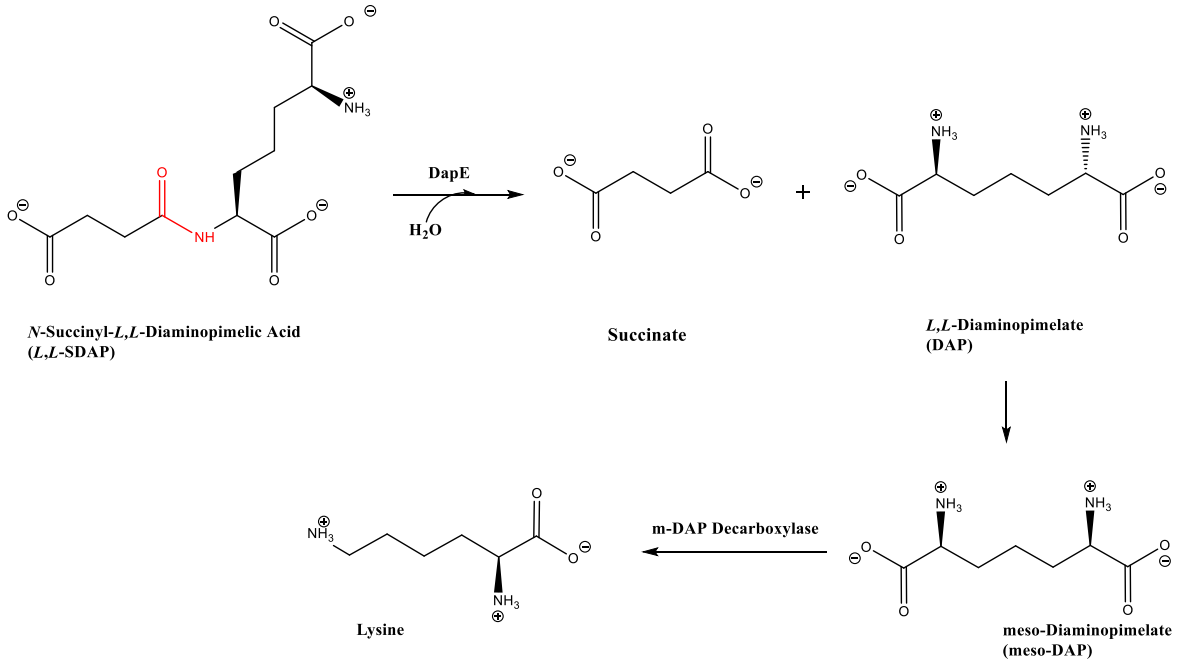


Figure 1. Lysine and *m*-DAP biosynthesis via bacterial succinylase pathway.

The bacterial enzyme DapE is responsible for catalyzing the hydrolysis of *N*-succinyl-L,L-diaminopimelic acid (L,L-SDAP) to succinic acid and L,L-diaminopimelate (DAP) as seen in Scheme **1**. As a result, the transformation of DAP to *m*-DAP and ultimately lysine which are incorporated into the bacterial cell wall.[13] Additionally, it has been shown that deletion of the dapE gene is lethal to *Helicobacter pylori* and *Mycobacterium smegmatis* species.[14, 15] In the presence of lysine supplemented media the *Helicobacter pylori* mutant was unable to grow. Growth was only possible in the presence of *m*-DAP supplemented media, in which the mutant was able to survive.[14] This reinforces the notion that DapE is essential for prokaryotic growth, proliferation, and that the lysine biosynthetic pathway is the primary pathway that supplies sources of *m*-DAP and lysine in most bacteria.[16] Alternatively, mammals including humans do

not have a lysine biosynthetic pathway as lysine is not synthesized but rather obtained from diet. Thus, DapE is an attractive potential drug target for two reasons: the mechanism-based selectivity towards pathogenic bacteria rather than the human host,[17] and the conservation of DapE across the ESKAPE pathogen species including the life-threatening *Mycobacterium tuberculosis*, *Escherichia coli*, *Vibrio chloerae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* species.[16]



Scheme 1. Enzymatic hydrolysis of L,L-SDAP to succinate and DAP by DapE.

Within the drug discovery process, protein crystallography provides pivotal insight regarding the structural integrity of a target enzyme. An enzyme crystal structure serves as a static image, illuminating key amino acid residues responsible for the catalysis, potential inhibitor binding, and substrate identification. In 2005, the first X-ray crystal structure of an apo DapE from *Neisseria meningitidis* (*Nm*DapE) was reported.[18] This reported protein crystal

structure contained no metal ion in the active site, and provided limited structural knowledge of DapE enzymes, as metal ion-bound structures are critical in determining the spatial arrangements of key amino acid residues involved in catalysis. Since that time additional distinctive X-ray crystal structures of DapE enzymes,[19] allowing us to gain a better understanding of the enzyme's mechanism of action, providing critical information in the fight against antibiotic resistance. Thus, for medicinal chemists, these crystal structures are essential tools enabling in the development of small molecule inhibitors.

X-ray crystal structures and active site architecture of DapE enzymes

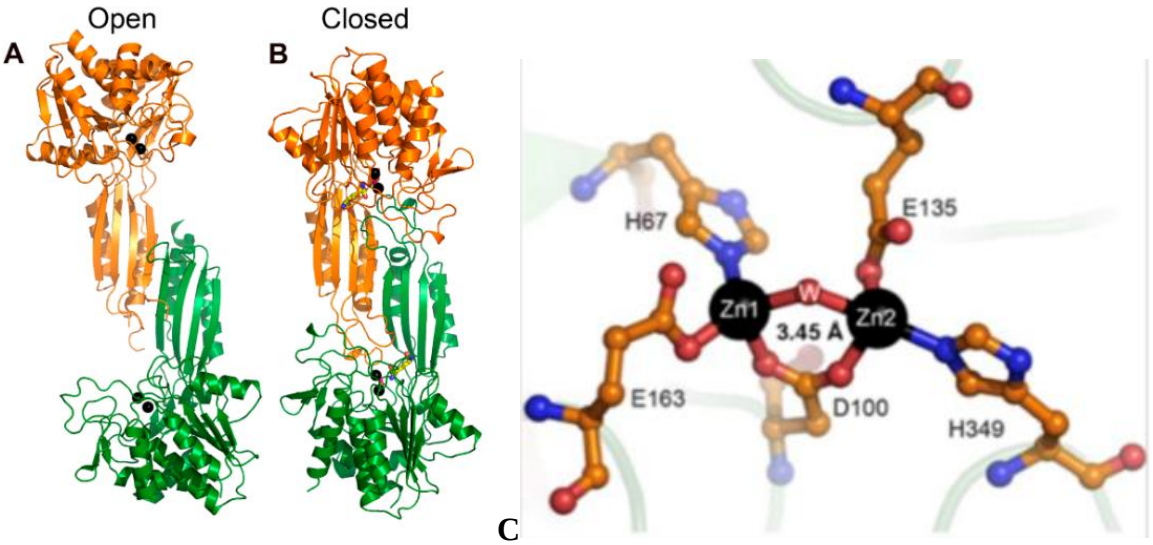


Figure 2. (A) Open conformation of the *Hi*DapE enzyme, (B) closed conformation of *Hi*DapE products-bound structure, and (C) the di-zinc metal center of the active site.

DapE is a hydrolase enzyme with a di-nuclear center consisting of two Zn(II) ions. Consistent with the larger family of M20 metalloenzymes, DapE is homodimeric (M_r = 41.6 kDa) with each subunit composed of a catalytic domain and dimerization domain, both playing a critical role in catalysis.[20] The catalytic domain houses the di-metallo active site of the DapE

enzyme with each Zn(II) ion adopting a distorted tetrahedral geometry. Histidine and glutamate residues coordinate each Zn(II) ion while aspartate and a water/hydroxide molecule bridge the two Zn(II) ions.[19] Elucidating structural components of the active site through X-ray crystal structures of *Haemophilus influenza* (*Hi*DapE)[19] showcased the substrate-enzyme interactions resulting in the observed dramatic conformational change upon substrate binding as seen in Figure **2**. Thus, binding of the substrate induces the dimer superstructure to flex and twist during catalysis.[20] This resulting conformational change allows the enzyme to transform from the open conformation (PDB: 3ICI)[19] to the closed conformation (PDB: 5VO3)[20] closing off access to the active site and driving the hydrolysis of the substrates scissile amide bond.

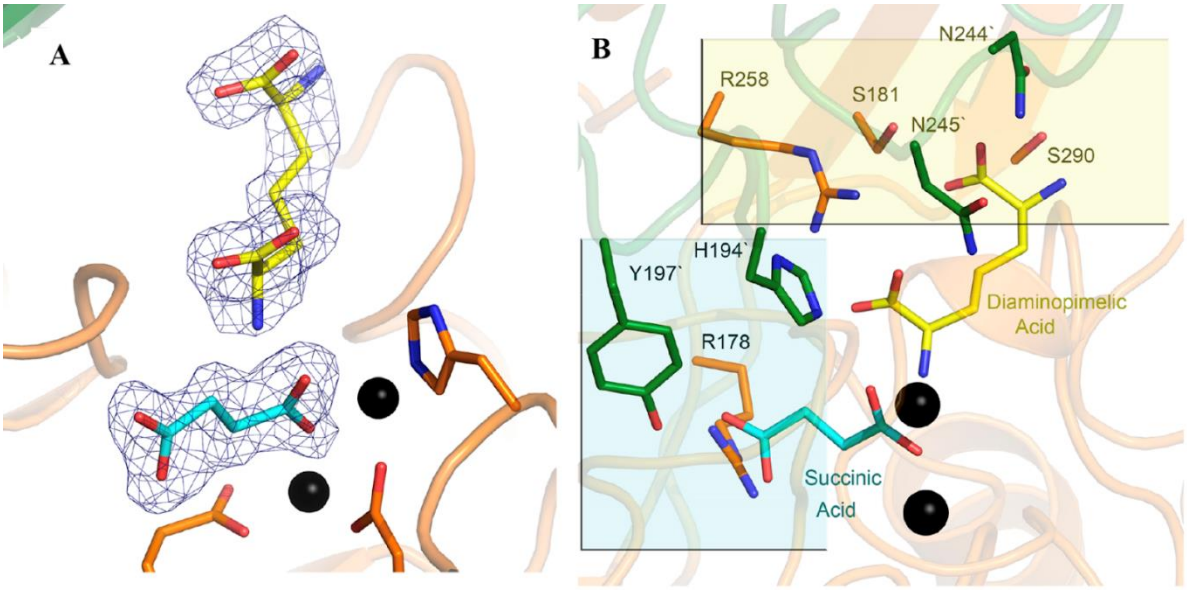


Figure 3. (A) succinic acid (cyan) and L,L-diaminopimelic acid (yellow) binding regions are highlighted to show the individual binding pockets. (B) Amino acid side chains interacting with the products of hydrolysis. Black spheres represent zinc ions.

Additional assessment of the active site from the closed DapE structure bound to the hydrolytic products illustrates that the substrate binding pocket can be divided into succinic acid and L,L-DAP binding regions.[20] Furthermore, the architecture of the active site has built in specificity for the L,L-isoform of SDAP.[19] The DapE structures suggest that the first step in catalysis for DapE is the recognition of the native substrate, L,L-SDAP, by the crescent-shaped cavity adjacent to the dinuclear Zn(II) cluster when the enzyme is in the open conformation. This substrate binding induces stark conformational changes, the resultant closed DapE structure, which positions the amide carbonyl oxygen of L,L-SDAP adjacent to a zinc atom and triggers the formation of an oxyanion hole by shifting His194.B from the dimerization domain of the B protein strand into the active site. Formation of a strong hydrogen bond between His194.B and the amide carbonyl oxygen facilitates reorganization of the Zn atom coordination sphere, thus displacing the bridging water molecule onto the adjacent zinc atom and activating the scissile carbonyl carbon for nucleophilic attack. Deprotonation of the zinc-bound water molecule by Glu134.A forms a nucleophilic hydroxide moiety. Once the zinc-bound hydroxide is formed, it can attack the activated carbonyl carbon of the substrate and form a tetrahedral TS complex. As observed for similar M20 metalloenzymes, such as the aminopeptidase from *Aeromonas proterlytica* (AAP), and further confirmed by the [ZnZn(*Hi*DapE)] product-bound structure, Glu134.A serves as a general acid-base catalyst, providing a proton to the amino nitrogen returning it to its ionized state. Upon cleavage of the amide, the tethering interaction of the products that maintains the closed enzyme conformation is disrupted. Release of the products is entropy-driven, facilitating re-formation of the open DapE conformation and release of the hydrolyzed products with the addition of a bridging water molecule.[20]

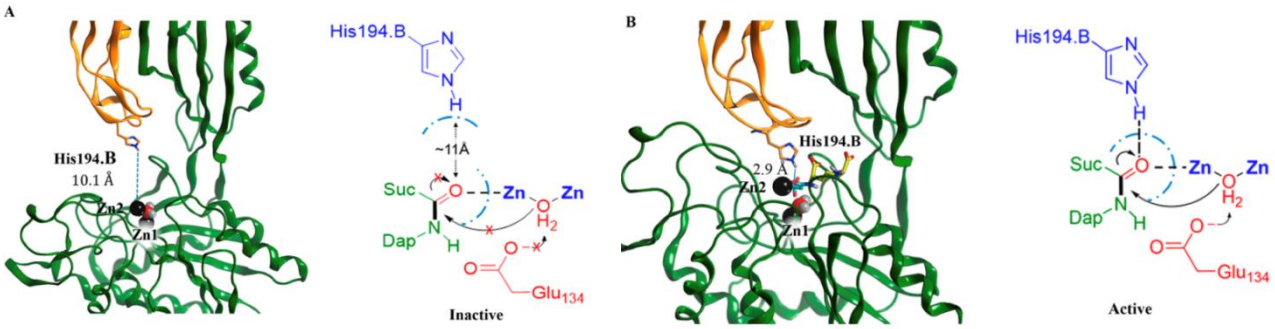


Figure 4. (A) the position of His194.B (orange) is shown in the open conformation of *Hi*DapE. (B) Structure of proposed oxyanion hole constitutes with His194.B and a Zn (II) ion in the *Hi*DapE closed conformation.

Conclusion

In collaboration with Dr. Ken Olsen, we are incorporating molecular dynamics (MD) to investigate the conformational changes of the DapE enzyme from the open conformation to the closed conformation in the presence of substrate using NAMD/VMD[21], MOE,[22] and Targeted Molecular Dynamics.[23] Thus, these X-ray crystallographic structures of bound products combined with computational analysis will aid us in the rational design and development towards lead optimization of more potent novel DapE inhibitors.

CHAPTER TWO

DESIGN AND SYNTHESIS OF TETRAZOLE & PYRAZOLE INHIBITORS OF *N*-SUCCINYL-L,L-DIAMINOPIMELIC ACID DESSUCINYLASE

Lead identification and synthetic strategies of DapE inhibitors

Hit molecules generated by a high-throughput screen (HiTs) served as the dominant discovery form of new lead structures in DapE inhibition. This was accomplished by screening 33,000 compounds from the ChemBridge corporation using an enzyme-coupled assay with a selection criterion of >20% inhibition of DapE at 12 μM inhibitor concentration resulting in the identification of five lead molecules classified into four distinct chemical classes: an amide with a beta-sulfone, an *N*-difluoromethyl sulfonamide, two *N*-acyl-sulfonamide indolines, and a phenyltetrazole thioether. These four distinct series of inhibitors satisfy Lipinski's rule of five and are in accordance with Veber's rules for drug likeness containing ≤ 10 rotatable bonds and polar surface area of ≤ 140 $Å^2$.[24] To our benefit, the lead molecules have potential zinc binding groups such as amides, sulfonamides and sulfones. All five hits are free of PAINS (pan assay interference compounds) structural motifs.[25] PAINS are compounds that are not selective towards a particular target and, as a result, give rise to false positive results in a high-throughput screen.[26] As medicinal chemists, we are tasked with designing and synthesizing analogs of these HiTs-derived lead molecules to improve drug-like properties including potency, selectivity, oral bioavailability, and penetration through the blood brain barrier.

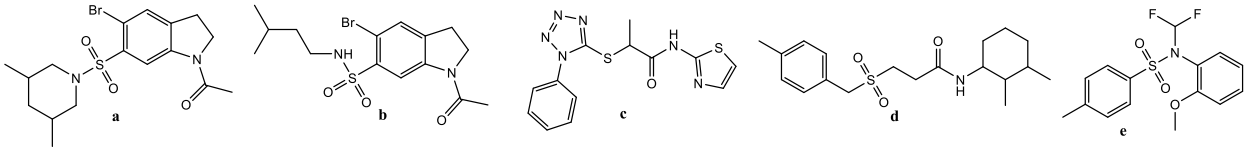


Figure 5. DapE hit compounds with calculated physiochemical properties.

These lead compounds were synthesized by our previous group members: Dr. Cory Reidl (**a**), Dr. Tahirah Heath (**b**), and Dr. Thahani Habeeb (**e**). One of my contributions to the project was successfully synthesizing compound (**c**) after examination of past data revealing the desired lead was not isolated. All five hit molecules (**a**-**e**) were tested using our novel ninhydrin-based assay and the inhibitory potencies of our lead compounds were established by the calculated IC_{50} values shown in Figure **5**. [27] Guided by molecular docking with MOE, we aim to further improve the potency of the hit-derived tetrazole analogs. The docking results should predict the interactions between the inhibitor molecules and the active site residues and the di-zinc center of the DapE enzyme. The inhibitory potencies of the synthesized inhibitors against DapE will be obtained by our ninhydrin-based biochemical assay.

Design, Synthesis of Tetrazole- and Pyrazole-based DapE Inhibitors

Tetrazoles are an aromatic five membered heterocylic ring containing four nitrogen atoms and one carbon atom. These planar heterocycles are often used as a bioisostere of a carboxylic acid and is metabolically stable.[28] Tetrazoles are found in 23 FDA approved drugs, [29] as tetrazole drugs have been implemented to improve oral bioavailability as well as decrease lipophilicity.[30] Moreover, the utility of tetrazoles as synthetic scaffolds in medicinal chemistry

have shown increased application as a result of increased opportunities for ligand-receptor interactions with biological targets, and favorable physiochemical properties.[28]

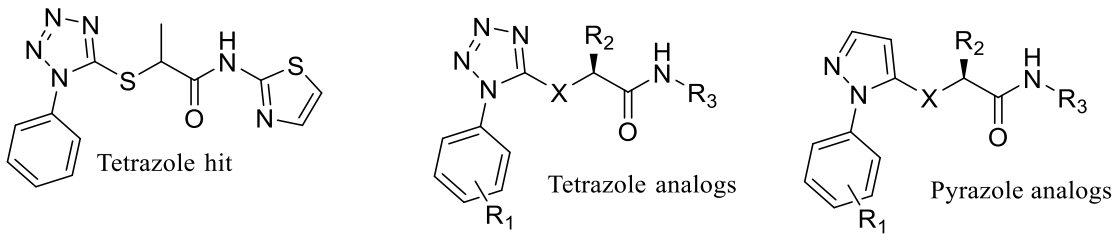


Figure 6. Plausible point derivations of tetrazole and pyrazole derived DapE inhibitors.

In parallel, we have designed an analogous series of the tetrazole hit in which the sulfur atom of the thioether is replaced with a nitrogen atom to enable synthesis from commercially available amino acids. Additionally, we designed pyrazole analogs as tetrazole isosteres to enhance the drug likeness of the inhibitor molecules with increased solubility, oral bioavailability, and they are expected to provide increased interactions and tighter binding in the active site.[31, 32]

Both tetrazole (**A**) and pyrazole (**B**) scaffolds enable three-point functional group derivatizations as shown in Figure **6** to drive the SAR in expanding our series in synthesizing more potent and efficacious drug candidates. Selected heterocyclic moieties can replace the thiazole ring at the R_1 position providing H-bond acceptors, whereas the R_3 position can be modified with substituted phenyl groups. Additionally, for the N-linked series, we are incorporating a range of amino acids with hydrophobic side chains at R_2, including Val, Ile, and Phe.

Utilizing MOE, the docking of the tetrazole hit with *Hi*DapE suggests enantioselective binding to the active site with a preference for the (*R*)-enantiomer (ΔG = - 8.59 kcal/mol) over the (*S*)-enantiomer (ΔG = - 7.74 kcal/mol).[20] Figure **7** shows the binding interactions of the 5-aminotetrazole and the initial pyrazole analogs with active site residues and the di-zinc metal center.

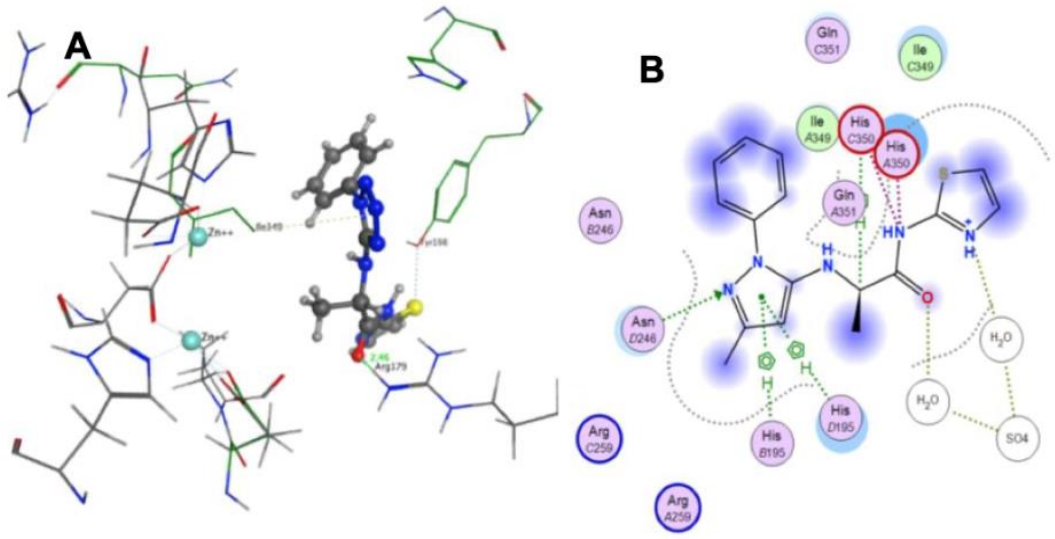
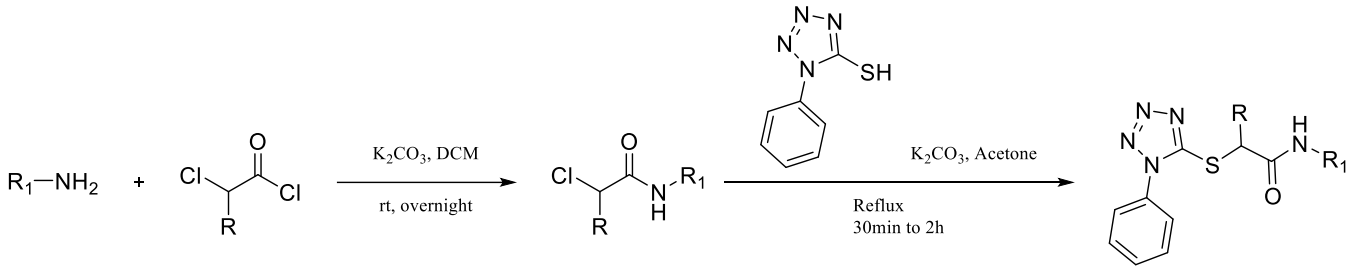


Figure 7. Hit-inspired (A) tetrazole and (B) pyrazole analogs bound to the DapE active site.

During the docking experiments performed using MOE, the tetrazole original hit-derived N-linked pyrazole analog was bound to the DapE active site. These results indicated the significance of the pyrazole ring as that forms a π-hydrogen interaction with the imidazole NH of H195. The hydrogen bond between the pyrazole nitrogen and Asn-246 suggests that a hydrogen bond acceptor at this position would be critical in binding. The amide moiety of the inhibitor could be beneficial in binding as it provides two favorable hydrogen bonds through a hydrogen bond with the NH and His-350, and a hydrogen bond between the amide oxygen and water

molecule. The 2-aminothiazole ring is housed within a hydrophobic pocket, and the corresponding nitrogen atom could form a hydrogen bond interaction with the active site residues. Furthermore, these docking experiments encouraged derivations incorporating various heterocyclic amine moieties in replace of the thiazole ring.

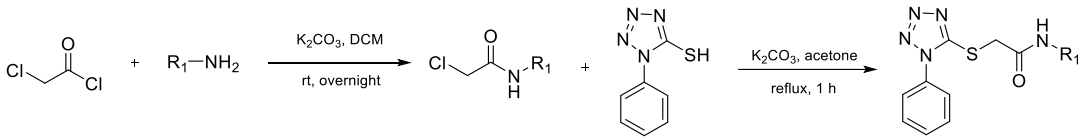
Synthesis and Inhibitory Potencies



Scheme 2. General Synthesis for tetrazole analogs.

In preparation of the S-linked tetrazole and pyrazole series of inhibitors, our synthetic route takes advantage of the robust nucleophilic substitution between the alpha-halo amide intermediates and the respective tetrazole and pyrazole thiols. The synthesis of the tetrazole analogs is achieved by two successive base-mediated coupling reactions following the route illustrated in Scheme **2**. First, the desired heterocyclic amine is reacted with chloroacetyl chloride in DCM at room temperature in the presence of potassium carbonate where R_2 is a proton and with alpha-chloropropionyl chloride where R_2 is a methyl group providing the corresponding alpha-halo amide intermediate with commercially available tetrazole thiol in acetone affording the corresponding phenyl tetrazole thio-linked analogs.

Table 1. Synthesis of tetrazole-glycine analogs.



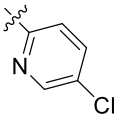
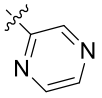
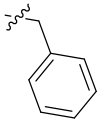
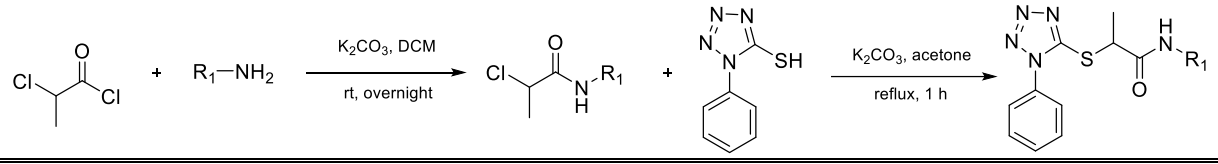
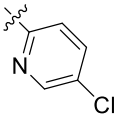
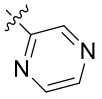
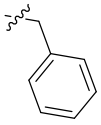
Compound	R_1	Compound	R_1
1a		1e	
1b		1f	
1c		1g	
1d			

Table 2. Synthesis of tetrazole-alanine analogs.



Compound	R_1	Compound	R_1
2a		2e	
2b		2f	
2c		2g	
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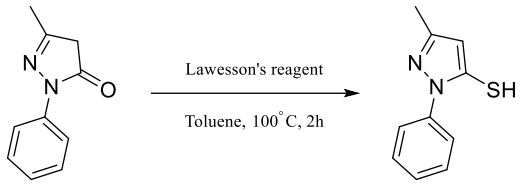
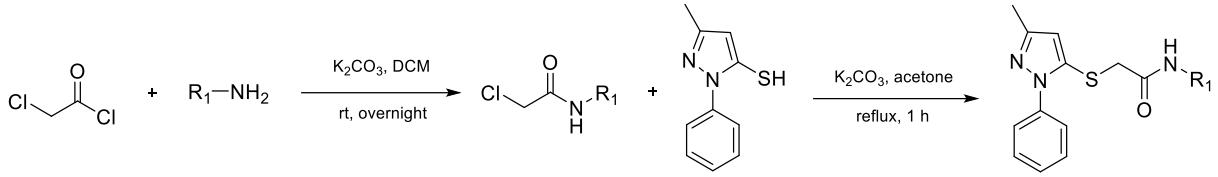


Scheme 3. Synthesis of pyrazole thiols.[33]

Table 3. Synthesis of pyrazole-glycine analogs.



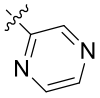
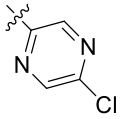
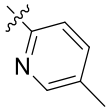
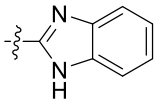
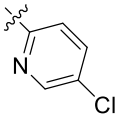
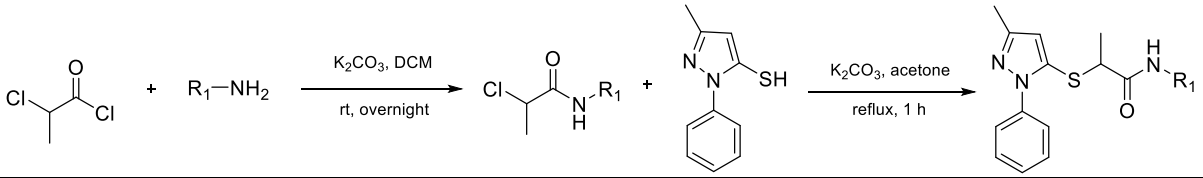
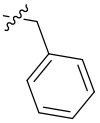
Compound	R_1	Compound	R_1
3a		3g	
3b		3h	
3c		3i	
3d		3j	*n-$(CH_2CH_3)_2$
3e		3k	
3f			

Table 4. Synthesis of pyrazole-alanine analogs.

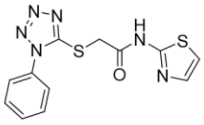
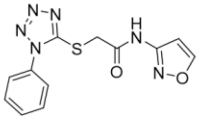
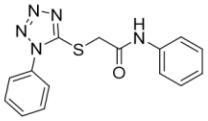
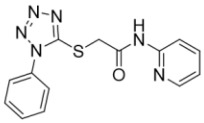
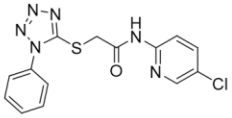
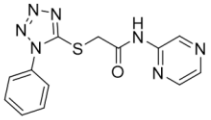
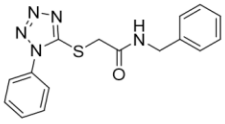


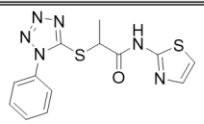
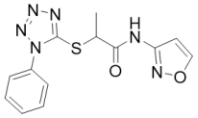
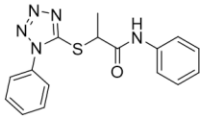
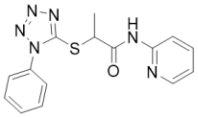
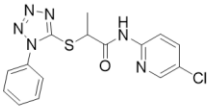
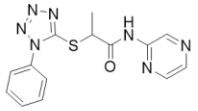
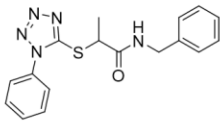
Compound	R_1	Compound	R_1
4a		4d	
4b		4e	
4c			

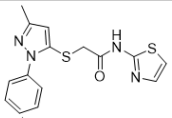
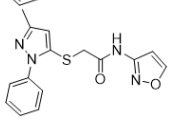
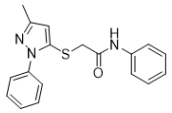
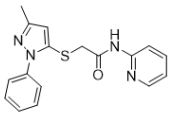
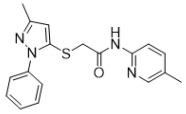
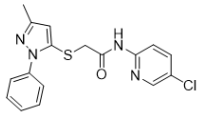
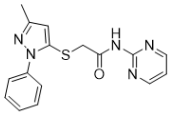
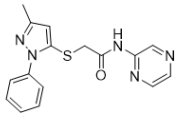
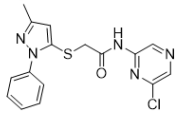
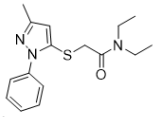
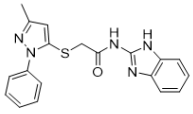
Inhibitory potencies of tetrazole analogs tested against DapE at 200 μM are listed in the SAR table. According to the data, the tetrazole analogs with the alpha-protons demonstrated a significantly greater potency and percent inhibition in comparison to the tetrazole analogs with the alpha-methyl groups. Compound **1a** exhibts 95.1% inhibition at 200 μM, where the corresponding racemic alpha-methyl tetrazole analog **2a** inhibits DapE only by 60.3% at the same concentration. This trend is observed between compounds **1e** (92.4%) and **2c** (75.6%), which is indicative of a possible steric clash of the alpha-methyl group with the amino acid residues in the active site of DapE. This suggests that an alkyl group at the alpha position of the tetrazole analogs might not be essential in inhibitor binding. When comparing the inhibitory potencies of the pyrazole analogs with the corresponding tetrazole parent compounds, an increased percent inhibition of DapE was observed as in pyrazole **4a** (79.4%) vs tetrazole **2a** (60.3%). A moderate preference observed for the pyrazole moiety over the tetrazole validates the importance of the nitrogen atom at the second position of the pyrazole ring in inhibitor binding.

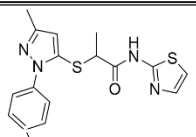
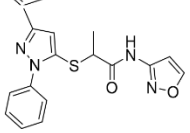
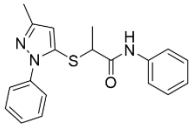
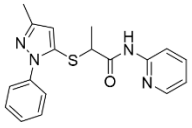
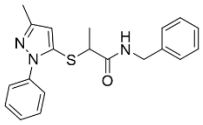
In contrast, tetrazole nitrogen atoms in the third and fourth position might not participate in ligand binding since the activity was not lost when the tetrazole was replaced with pyrazole moiety. Interestingly, with the current inhibitory data, pyrazoles are somewhat more potent than the respective tetrazoles. IC_{50} values of inhibitors showed greater than 90% inhibition at 200 μM as the remaining analogs are currently being investigated using the ninhydrin assay.

Table 5. Tetrazole & Pyrazole analogs.

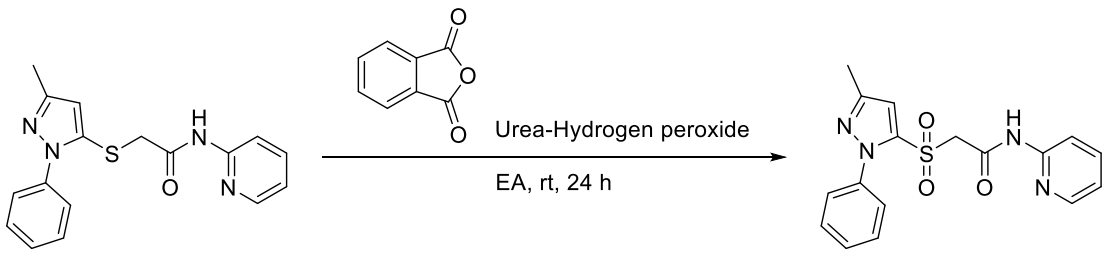
Compound	Structure	Molecular Weight (mg)	LogP	Melting Point (°C)	% Inhibition at 200 μM
1a		318.4	0.4	214-215	95.1
1b		302.3	0.9	179-180	67.4
1c		311.4	1.8	162-164	92.4
1d		312.4	1.3	158-160	>99
1e		346.8	1.9	183-185	39.5 @75 mM
1f		313.3	2.0	154-155	TBD
1g		325.4	2.0	103-104	TBD

Compound	Structure	Molecular Weight (mg)	LogP	Melting Point (°C)	% Inhibition at 200 µM
2a		332.4	0.7	188-190	60.3
2b		316.3	1.2	139-141	TBD
2c		325.4	2.1	123-125	75.6
2d		326.4	1.6	141-143	27.7 @ 100 mM
2e		360.8	2.2	116-117	TBD
2f		325.4	1.3	137-139	TBD
2g		339.4	2.3	74-76	TBD

Compound	Structure	Molecular Weight (mg)	LogP	Melting Point (°C)	% Inhibition 200 uM
3a		330.4	2.7	157-159	TBD
3b		314.4	2.3	119-121	TBD
3c		323.4	3.3	Oil	TBD
3d		324.4	2.7	Oil	TBD
3e		338.4	3.0	114-115	TBD
3f		358.8	3.2	72-73	TBD
3g		325.4	2.2	159-161	TBD
3h		325.4	1.9	Oil	TBD
3i		359.8	2.5	Oil	TBD
3j		303.4	3.0	Oil	TBD
3k		363.4	3.0	174-176	TBD

Compound	Structure	Molecular Weight (mg)	LogP	Melting Point (°C)	% Inhibition at 200 µM
4a		344.5	2.3	Oil	79.4
4b		328.4	2.8	98-100	61.4
4c		337.4	3.8	132-134	TBD
4d		338.4	3.3	130-132	94.6 @100 mM
4e		351.5	3.9	97-99	TBD

Additionally, propelling SAR in efforts to optimize our most potent inhibitors, these analogs are currently being oxidized to their corresponding sulfoxide & sulfone species with respect to the awaited inhibitory data from our in house ninhydrin assay. Following the procedure of our past group member, Dr. Marlon Lutz, the selected pyrazole analog is reacted with urea-hydrogen peroxide in the presence of pthalic anhydride for 24 h at room temperature overnight to yield the resultant transformation of the fully desired oxidized sulfone species. Currently, the sulfoxide species are being investigated guided by literature procedures using the multi-faceted oxidiant reagent, potassium peroxymonosulfate (Oxone).



Scheme 4. Oxidation of tetrazole analogs.

Experimental

Materials and Methods

All solvents were distilled prior to use or purchased as anhydrous grade. All reagents were used without further purification unless otherwise noted. Molecular sieves were activated at 300-350 °C under vacuum unless otherwise stated. Chloroacetyl chloride, 2-chloropropionyl chloride, amino heterocycles, amines, and potassium carbonate were purchased from Sigma-Aldrich. Synthesis of 3-methyl-1-phenyl-1*H*-pyrazole-5-thiol was conducted according to literature. All synthetic reactions were conducted under an atmosphere of nitrogen. Silica gel 60 Å, 40-75µm (200 x 400 mesh) was used for column chromatography. Aluminum-backed silica gel 200 µm plates purchased from Sorbtech were used for TLC. ^{1}H NMR spectra were obtained using a 500 MHz spectrometer with tetramethylsilane (TMS) as the internal standard. ^{13}C NMR spectra were obtained using a 75 or 125 MHz spectrometer. The purity of all compounds was determined to be ≥95% (unless otherwise noted) by high performance liquid chromatography (HPLC) employing a mobile phase A = 5% acetonitrile B in water and a mobile phase B = 0.1% TFA in acetonitrile with a gradient of 60% B increasing to 95% over 10 min, holding at 95% B for 5 min, then returning to 60% B and holding for 5 min. HRMS spectra were measured on a TOF instrument by electrospray ionization (ESI). HRMS spectra were collected using a Waters Acquity I class UPLC and Xevo G2-XS QT of mass spectrometer with Waters Acquity BEH C18 column (1.7 µm, 2.1 x 50 mm).

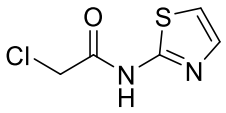
General procedure for 2-chloro-N-acetamides & 2-((1-phenyl-1H-tetrazol-5-yl)thio)-N-acetamides in thioether derivative synthesis

To a tall clear 4 dram vial, equipped with a magnetic stir rod/flea bar, the desired amine (1.0 eq) along with potassium carbonate (1.2 eq) was suspended in anhydrous dichloromethane (0.3 M) freshly distilled over calcium hydride. The reaction vessel was purged with nitrogen gas to ensure the transformation took place under inert atmospheric conditions. To the reaction vessel, chloroacetyl chloride or 2-chloropropionyl chloride (3.5 eq) was added dropwise under nitrogen at 0°C to room temperature, as the contents were set to stir at 250 rpm –paired with frequent de-gassing of the vessel being essential - with periodic HPLC monitoring. Upon completion of reaction, dichloromethane and excess acid chloride was concentrated under reduced pressure, and the resultant crude was suspended in DI water (2 mL). The organic product was extracted using ethyl acetate (3 x 2 mL), washed with brine (3 x 2 mL), and the combined organic layers were dried over anhydrous Na_2SO_4. Then the solvent was removed by evaporation under reduced pressure yielding a crude product mixture which was subject to purification through recrystallization with a di-solvent system of a 1:3 ratio of dichloromethane and hexane to afford the corresponding 2-chloro-*N*-acetamide which was isolated and reacted for the next step.

In a clear 2 dram vial: equipped with a magnetic stir rod/flea bar, the isolated amide intermediate (1.0 eq), phenyl-tetrazole thiol (1.0 eq), and potassium carbonate (1.2 eq) was suspended in anhydrous acetone (0.3 M) under nitrogen at reflux for 30 min to an hour with periodic HPLC monitoring. Upon completion, acetone was evaporated off under reduced pressure and the resultant crude was suspended in DI water (2 mL). The organic product was extracted using ethyl acetate (3 x 2 mL), washed with brine (3 x 2 mL), and the combined

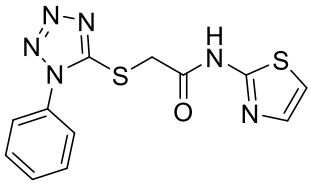
organic layers were dried over anhydrous Na_2SO_4. The solvent was then removed by evaporation under reduced pressure yielding a crude product mixture which was subjected to purification through recrystallization with a di-solvent system of a 1:3 ratio dichloromethane and hexane to afford the corresponding 2-((1-phenyl-1H-tetrazol-5-yl)thio)-*N*-acetamide.

2-Chloro-*N*-2-thiazolylacetamide



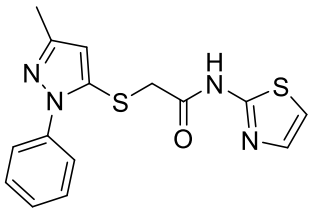
2-aminothiazole (100 mg, 0.933 mmol) was used in this reaction and followed the general procedure along with literature precedence to isolate the amide intermediate needed to be carried over for the next step.[34]

2-((1-phenyl-1*H*-tetrazol-5-yl)thio)-*N*-(thiazol-2-yl)acetamide (1a)



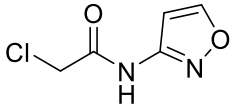
2-Chloro-*N*-2-thiazolylacetamide intermediate (30.0 mg, 0.163 mmol) was used in this reaction. The crude mixture of **1f** was purified with DCM/Hexane (1:3) recrystallization solvents to afford **1f** as a white crystalline solid (31.0mg, 62%): mp 214-215˚C. **^{1}H NMR** (500 MHz, DMSO-d_6) δ 12.55 (s, 1H), 7.74 – 7.63 (m, 5H), 7.51 (d, *J* = 3.5 Hz, 1H), 7.26 (d, *J* = 3.5 Hz, 1H), 4.47 (s, 2H). **^{13}C NMR** (126 MHz, DMSO) δ 165.64, 165.54, 158.14, 154.26, 138.31, 133.45, 131.22, 130.59, 130.33, 124.94, 114.38, 60.23, 40.50, 40.33, 40.17, 40.00, 39.83, 39.67, 39.50, 36.71, 21.25, 14.56. **HRMS (ESI):** $C_{12}H_{10}N_6OS_2Na$ $[M+Na]^+$: calcd.: 341.0255; found: 341.0260.

2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-(thiazol-2-yl)acetamide (3a)



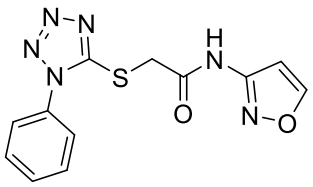
2-Chloro-*N*-2-thiazolylacetamide intermediate (30.0 mg, 0.163 mmol) was used in this reaction. The crude mixture of **3a** was purified with DCM/Hexane (1:3) recrystallization solvents to afford **3a** as a white solid (30.79 mg, 57%): mp 157-159˚C. **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 11.23 (s, 1H), 7.53 – 7.46 (m, 2H), 7.44 – 7.36 (m, 2H), 7.39 – 7.29 (m, 2H), 7.00 (d, *J* = 3.6 Hz, 1H), 6.34 (s, 1H), 3.55 (s, 2H), 2.28 (s, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 165.54, 158.63, 149.96, 139.03, 136.79, 132.29, 128.98, 128.07, 125.26, 125.13, 114.12, 112.02, 77.29, 77.04, 76.78, 39.11, 13.66. **HRMS (ESI):** $C_{15}H_{15}N_4OS_2$ $[M+H]^+$: calcd.: 331.0687; found: 331.0682.

2-Chloro-*N*-3-isoxazolylacetamide



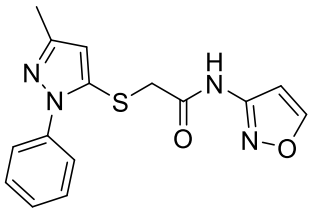
3-aminoisoxazole (100 mg, 1.18 mmol) was used in this reaction and followed the general procedure along with literature precedence to isolate the amide intermediate needed to be carried over for the next step.[35]

***N*-(isoxazol-3-yl)-2-((1-phenyl-1*H*-tetrazol-5-yl)thio)acetamide (1b)**



2-Chloro-*N*-3-isoxazolylacetamide intermediate (30.0 mg, 0.186 mmol) was used in this reaction. The crude mixture of **1b** was purified with DCM/Hexane (1:3) recrystallization solvents to afford **1b** as a white crystalline solid (38.2 mg, 68%): mp 179-180˚C. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 10.05 (s, 1H), 8.30 (d, *J* = 1.7 Hz, 1H), 7.64 – 7.54 (m, 5H), 7.02 (d, *J* = 1.7 Hz, 1H), 4.19 (s, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.36, 159.41, 159.12, 156.99, 153.74, 133.15, 130.65, 130.09, 123.70, 99.36, 99.04, 77.28, 77.03, 76.77, 42.48, 36.61. **HRMS (ESI):** $C_{12}H_{10}N_6O_2SNa$ $[M+Na]^+$: calcd.: 325.0484; found: 325.0488.

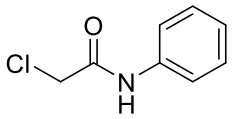
***N*-(isoxazol-3-yl)-2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)acetamide (3b)**



2-Chloro-*N*-3-isoxazolylacetamide intermediate (30.0 mg, 0.186 mmol) was used in this reaction. The crude mixture of **3b** was purified with DCM/Hexane (1:3) recrystallization solvents to afford **3b** as a white solid (44.8 mg, 77%): mp 119-121˚C. **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 9.15 (s, 1H), 8.29 (dd, *J* = 1.8, 0.6 Hz, 1H), 7.54 – 7.48 (m, 2H), 7.48 – 7.41 (m, 2H), 7.41 – 7.34 (m, 1H), 6.99 (d, *J* = 1.8 Hz, 1H), 6.32 (s, 1H), 3.50 (s, 2H), 2.29 (s, 3H), 1.31 –

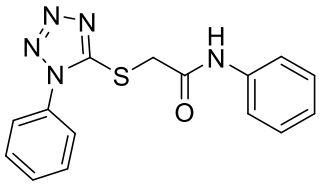
1.23 (m, 0H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 165.59, 159.10, 156.83, 150.03, 139.00, 132.32, 129.09, 128.26, 125.27, 111.38, 99.11, 77.28, 77.02, 76.77, 39.62, 13.65. **HRMS (ESI):** $C_{15}H_{15}N_4O_2S$ $[M+H]^+$: calcd.: 315.0916; found: 315.0909.

Chloroacetanilide



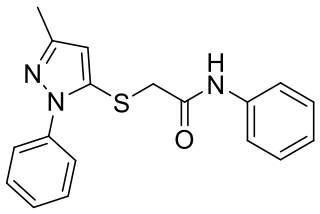
Aniline (100 mg, 1.07 mmol) was used in this reaction and followed the general procedure along with literature precedence to isolate the amide intermediate needed to be carried over for the next step.[36]

***N*-phenyl-2-((1-phenyl-1*H*-tetrazol-5-yl)thio)acetamide(1c)**



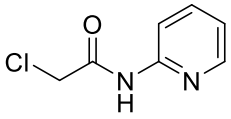
Chloroacetanilide intermediate (30.0 mg, 0.176 mmol) was used in this reaction.The crude mixture of **1e** was purified with DCM/Hexane (1:3) recrystallization solvents to afford **1c** as a fluffy white solid (42.3 mg, 77%): mp 162-164˚C. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 9.43 (s, 1H), 7.64 – 7.54 (m, 7H), 7.37 – 7.29 (m, 2H), 7.12 (tt, *J* = 7.4, 1.2 Hz, 1H), 4.08 (s, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.32, 154.55, 137.69, 133.13, 130.67, 130.09, 129.05, 124.69, 123.70, 119.83, 77.28, 77.02, 76.77, 36.99. **HRMS (ESI):** $C_{15}H_{13}N_5OSNa$ $[M+Na]^+$: calcd.: 334.0739; found: 334.0721.

2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-phenylacetamide (3c)



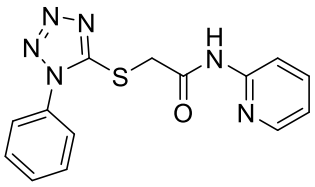
Chloroacetanilide intermediate (30.0 mg, 0.176 mmol) was used in this reaction. The crude mixture of **3c** was purified by column chromatography using ethyl acetate/hexane (30/70)to afford **3c** as a white solid (66%): mp oil. 1H NMR (500 MHz, Chloroform-d) δ 8.16 (s, 1H), 7.55 – 7.47 (m, 2H), 7.44 (dd, J = 8.6, 6.8 Hz, 2H), 7.40 – 7.24 (m, 5H), 7.12 (tt, J = 7.2, 1.4 Hz, 1H), 6.32 (s, 1H), 3.49 (s, 2H), 2.29 (s, 3H), 1.76 – 1.66 (m, 0H), 1.33 (s, 2H), 0.93 – 0.79 (m, 2H).13C NMR (126 MHz, CDCl3) δ 165.12, 150.16, 139.27, 139.04, 137.05, 133.09, 130.92, 129.10, 128.99, 128.81, 128.16, 125.22, 124.84, 119.83, 114.07, 111.05, 77.30, 77.05, 76.79, 66.22, 40.18, 38.85, 37.10, 36.64, 33.82, 33.20, 31.92, 30.15, 30.03, 29.69, 29.65, 29.51, 29.36, 29.16, 28.95, 27.08, 26.73, 25.90, 24.68, 23.38, 22.69, 14.13, 13.64, 10.89. **HRMS (ESI):** $C_{18}H_{18}N_3OS$ $[M+H]^+$: calcd.: 324.1171; found: 324.1165.

2-(Chloroacetylamino)pyridine



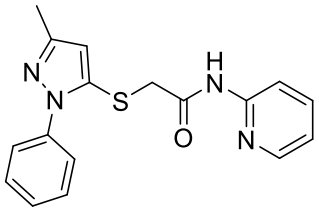
2-aminopyridine (100 mg, 1.06 mmol) was used in this reaction and followed the general procedure along with literature precedence to isolate the amide intermediate needed to be carried over for the next step.[37]

2-((1-phenyl-1H-tetrazol-5-yl)thio)-*N*-(pyridine-2-yl)acetamide (1d)



The 2-(Chloroacetylamino)pyridine intermediate (30.0 mg, 0.175 mmol) was used in the reaction. The crude mixture of **1d** was purified with DCM/Hexane (1:3) recrystallization solvents to afford **1d** as a white crystalline solid (21 mg, 38%): mp 158-160˚C. **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 9.49 (s, 1H), 8.33 (tdd, *J* = 4.9, 1.9, 0.9 Hz, 1H), 8.17 (dd, *J* = 22.6, 8.4 Hz, 1H), 7.72 (dddd, *J* = 19.3, 8.3, 7.3, 1.9 Hz, 1H), 7.62 – 7.53 (m, 4H), 7.14 – 7.03 (m, 1H), 4.22 (s, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 165.47, 153.71, 150.96, 148.14, 138.35, 133.28, 130.52, 130.03, 123.71, 120.31, 114.25, 77.28, 77.03, 76.77, 37.34. **HRMS (ESI):** $C_{14}H_{12}N_6OSNa$ $[M+Na]^+$: calcd.: 335.0691; found: 335.0715.

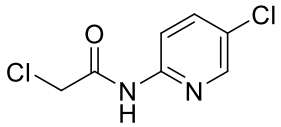
2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-(pyridine-2-yl)acetamide (3d)



2-(Chloroacetylamino)pyridine intermediate (30.0 mg, 0.175 mmol) was used in the reaction. The crude mixture of **3d** was purified by column chromatography using ethyl acetate/hexane

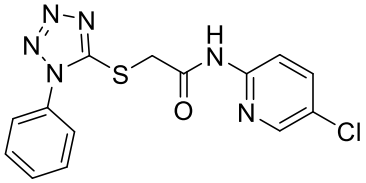
(30/70) to afford **3d** as a brown oil (17.59 mg, 31%): mp oil. **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 8.73 (s, 1H), 8.28 (ddd, *J* = 4.9, 2.0, 0.9 Hz, 1H), 8.11 (d, *J* = 8.4 Hz, 1H), 7.70 (ddd, *J* = 8.5, 7.3, 1.9 Hz, 1H), 7.55 – 7.49 (m, 2H), 7.43 (dd, *J* = 8.5, 7.0 Hz, 2H), 7.39 – 7.32 (m, 1H), 7.07 (ddd, *J* = 7.4, 4.9, 1.1 Hz, 1H), 6.33 (s, 1H), 3.49 (s, 2H), 2.28 (s, 3H), 1.26 (s, 1H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 165.83, 150.66, 149.98, 147.93, 139.08, 138.39, 132.78, 129.04, 128.09, 125.26, 120.29, 113.88, 111.14, 77.28, 77.02, 76.77, 40.04, 29.70, 13.66. **HRMS (ESI):** $C_{17}H_{17}N_4OS$ $[M+H]^+$: calcd.: 325.1123; found: 325.1124.

2-Chloro-*N*-(5-chloro-2-pyridinyl)acetamide



2-amino-5-chloropyridine (100 mg, 0.777 mmol) was used in this reaction and followed the general procedure along with literature precedence to isolate the amide intermediate needed to be carried over for the next step.[38]

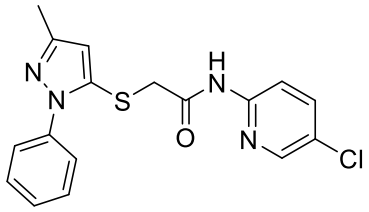
***N*-(5-chloropyridin-2-yl)-2-((1-phenyl-1*H*-tetrazol-5-yl)thio)acetamide (1e)**



2-Chloro-*N*-(5-chloro-2-pyridinyl)acetamide (30.0 mg, 0.146 mmol) was used in this reaction.The crude mixture of **1e** was purified with DCM/Hexane (1:3) recrystallization solvents to afford **1e** as a white solid (27 mg, 53%): mp 183-185˚C. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 9.56 (s, 1H), 8.19 (dd, *J* = 2.6, 0.8 Hz, 1H), 8.07 (d, *J* = 8.9 Hz, 1H), 7.59 (dd, *J* = 8.8, 2.6 Hz,

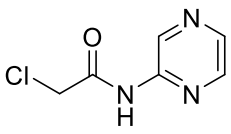
1H), 7.52 (s, 4H), 7.55 – 7.48 (m, 1H), 4.13 (s, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.58, 153.76, 149.25, 146.81, 137.92, 133.20, 130.60, 130.07, 127.33, 123.68, 114.83, 77.28, 77.02, 76.77, 37.13. **HRMS (ESI):** $C_{14}H_{12}ClN_6OS$ $[M+H]^+$: calcd.: 347.0482; found: 347.0495.

***N*-(5-chloropyridin-2-yl)-2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)acetamide (3f)**



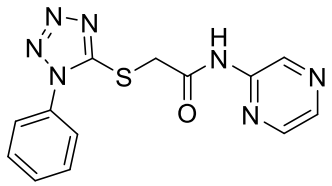
2-Chloro-*N*-(5-chloro-2-pyridinyl)acetamide (30.0 mg, 0.146 mmol) was used in this reaction. The crude mixture of **3f** was purified with DCM/Hexane (1:3) recrystallization solvents to afford **3f** as a white solid (17.48 mg 33%): mp 72-73˚C. **^{1}H NMR** (500 MHz, Chloroform-d) δ 8.70 (s, 1H), 8.22 (d, J = 2.5 Hz, 1H), 8.09 (d, J = 8.9 Hz, 1H), 7.66 (dd, J = 8.8, 2.6 Hz, 1H), 7.57 – 7.48 (m, 2H), 7.43 (dd, J = 8.7, 6.9 Hz, 2H), 7.39 – 7.32 (m, 1H), 6.32 (s, 1H), 3.48 (s, 2H), 2.29 (s, 3H). **^{13}C NMR** (126 MHz, CDCl3) δ 165.84, 150.02, 148.92, 146.76, 146.59, 139.02, 138.14, 137.99, 132.50, 129.07, 128.16, 127.33, 125.26, 114.55, 114.50, 111.34, 77.28, 77.03, 76.78, 42.70, 40.04, 13.65. **HRMS (ESI):** $C_{17}H_{16}ClN_4OS$ $[M+H]^+$: calcd.: 359.0733; found: 359.0759.

2-Chloro-*N*-2-pyrazinylacetamide



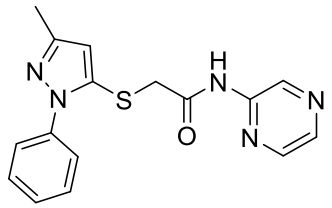
Aminopyrazine (100 mg, 1.05 mmol)was used in this reaction and followed the general procedure along with literature precedence to isolate the amide intermediate needed to be carried over for the next step.[39]

2-((1-phenyl-1*H*-tetrazol-5-yl)thio)-*N*-(pyrazin-2-yl)acetamide (1f)



2-Chloro-*N*-2-pyrazinylacetamide intermediate (30.0 mg, 0.174 mmol) was used in this reaction.The crude mixture of **1f** was purified with DCM/Hexane (1:3) recrystallization solvents to afford **1f** as a red/orange crystalline solid (31.2 mg, 57%): mp 143-145˚C. **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 9.79 (s, 1H), 9.48 (s, 1H), 8.37 (d, *J* = 2.5 Hz, 1H), 8.29 (dd, *J* = 2.6, 1.6 Hz, 1H), 7.64 – 7.54 (m, 5H), 4.21 (s, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 165.71, 153.86, 147.83, 142.37, 140.75, 137.06, 133.13, 130.69, 130.12, 123.66, 77.28, 77.02, 76.77, 36.84. **HRMS (ESI):** $C_{13}H_{11}N_7OSNa$ $[M+Na]^+$: calcd.: 336.0644; found: 336.0665.

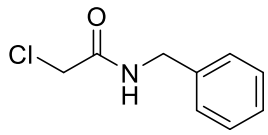
2-((3-methyl-1-phenyl-1H-pyrazol-5-yl)thio)-N-(pyrazin-2-yl)acetamide (3h)



2-Chloro-*N*-2-pyrazinylacetamide intermediate (30.0 mg, 0.174 mmol) was used in this reaction.The crude mixture of **3h** was purified by column chromatography using ethyl acetate/hexane (30/70) to afford **3h** as a brown oil (19.6mg, 34.54%): mp oil. **^{1}H NMR** (500 MHz, Chloroform-d) δ 9.42 (s, 1H), 8.63 (s, 1H), 8.37 (d, J = 2.6 Hz, 1H), 8.24 (dd, J = 2.6, 1.6 Hz, 1H), 7.55 – 7.48 (m, 2H), 7.42 (dd, J = 8.7, 7.0 Hz, 2H), 7.38 – 7.30 (m, 1H), 6.35 (s, 1H), 3.51 (s, 2H), 2.29 (s, 3H). **^{13}C NMR** (126 MHz, CDCl3) δ 165.85, 150.08, 147.38, 142.09,

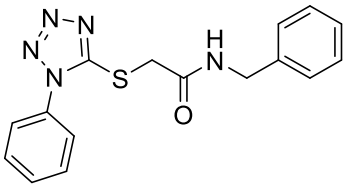
140.78, 138.99, 136.79, 132.23, 129.07, 128.13, 125.28, 111.56, 77.28, 77.03, 76.78, 65.86, 39.92, 31.93, 30.16, 29.70, 29.36, 22.70, 15.28, 14.13, 13.66. **HRMS (ESI):** $C_{16}H_{16}N_5OS$ $[M+H]^+$: calcd.: 326.1076; found: 326.1078.

***N*-Benzyl-2-chloroacetamide**



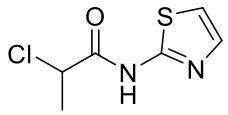
Benzylamine (100 mg, 0.933 mmol) was used in this reaction and followed the general procedure along with literature precedence to isolate the amide intermediate needed to be carried over for the next step.[40]

***N*-benzyl-2-((1-phenyl-1*H*-tetrazol-5-yl)thio)acetamide (1g)**



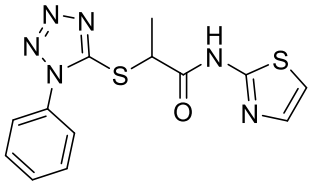
N-Benzyl-2-chloroacetamide intermediate (30.0 mg, 0.163 mmol) was used in this reaction. The crude mixture of **1g** was purified with DCM/Hexane (1:3) recrystallization solvents to afford **1g** as a fluffy white solid (41 mg,81%): mp 103-104˚C.**^{1}H NMR** (500 MHz, Chloroform-*d*) δ 7.62 – 7.52 (m, 5H), 7.35 (d, *J* = 6.7 Hz, 1H), 7.35 – 7.27 (m, 2H), 7.30 – 7.21 (m, 3H), 4.47 (d, *J* = 5.9 Hz, 2H), 4.01 (s, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 167.02, 154.08, 137.58, 133.23, 130.53, 130.02, 128.73, 127.63, 127.59, 123.71, 77.28, 77.02, 76.77, 44.05, 36.05. **HRMS (ESI):** $C_{13}H_{11}N_7OSNa$ $[M+Na]^+$: calcd.: 348.0895; found: 348.0916.

2-chloro-*N*-(thiazol-2-yl)propanamide



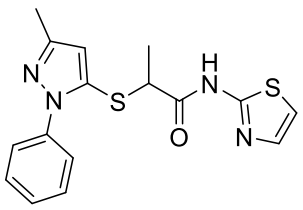
2-aminothiazole (100 mg, 0.933 mmol) was used in this reaction and followed the general procedure along with literature precedence to isolate the amide intermediate needed to be carried over for the next step.[41]

2-((1-phenyl-1*H*-tetrazol-5-yl)thio)-*N*-(thiazol-2-yl)propanamide (2a)



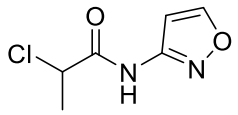
2-chloro-*N*-(thiazol-2-yl)propanamide intermédiate (30.0 mg, 0.157 mmol) was used in this reaction.The crude mixture of **2a** was purified with DCM/Hexane (1:3) recrystallization solvents to afford **2a** as a white solid (18.0 mg, 35%): mp 188-190˚C. **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 11.81 (s, 1H), 7.62 – 7.52 (m, 6H), 7.04 (d, *J* = 3.6 Hz, 1H), 4.88 (q, *J* = 7.2 Hz, 1H), 1.80 (d, *J* = 7.2 Hz, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 168.27, 158.13, 153.58, 137.72, 133.18, 130.57, 130.03, 123.73, 114.26, 77.28, 77.02, 76.77, 45.15, 17.40. **HRMS (ESI):** $C_{13}H_{13}N_6OS_2$ $[M+H]^+$: calcd.: 333.0592; found: 333.0609.

2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-(thiazol-2-yl)propanamide (4a)



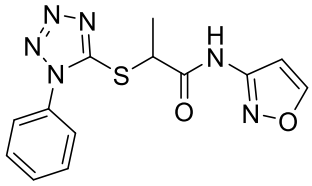
2-chloro-*N*-(thiazol-2-yl)propanamide intermédiate (30.0 mg, 0.157 mmol) was used in this reaction.The crude mixture of **4a** was purified with DCM/Hexane (1:3) recrystallization solvents to afford **4a** as an oil (31.32 mg, 60%): mp oil. **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 10.99 (s, 1H), 7.47 – 7.41 (m, 2H), 7.39 – 7.23 (m, 4H), 6.99 (d, *J* = 3.6 Hz, 1H), 6.34 (s, 1H), 3.61 (q, *J* = 7.1 Hz, 1H), 2.27 (s, 3H), 1.45 (d, *J* = 7.1 Hz, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 168.84, 158.59, 149.71, 139.02, 136.95, 130.18, 128.72, 127.85, 125.54, 114.36, 113.94, 77.28, 77.02, 76.77, 47.11, 31.59, 22.66, 17.21, 14.13, 13.64. **HRMS (ESI):** $C_{16}H_{17}N_4OS_2$ $[M+H]^+$: calcd.: 345.0844; found: 345.0856.

2-chloro-*N*-(isoxazol-3-yl)propanamide



3-aminoisoxazole (100 mg,, 1.18 mmol) was used in this reaction and followed the general procedure along with literature precedence to isolate the amide intermediate needed to be carried over for the next step.[42]

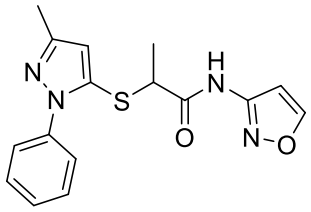
***N*-(isoxazol-3-yl)-2-((1-phenyl-1*H*-tetrazol-5-yl)thio)propanamide (2b)**



2-chloro-*N*-(isoxazol-3-yl)propanamide intermediate (30.0 mg, 0.206 mmol) was used in this reaction.The crude mixture of **2b** was purified with DCM/Hexane (1:3) recrystallization solvents to afford **2b** as a white solid (29.21 mg, 45%): mp 139-141˚C. **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 10.20 (s, 1H), 8.29 (d, *J* = 1.8 Hz, 1H), 7.63 – 7.52 (m, 5H), 7.03 (d, *J* = 1.8 Hz, 1H), 4.74

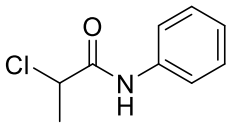
(q, *J* = 7.3 Hz, 1H), 1.73 (d, *J* = 7.3 Hz, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 168.67, 159.02, 157.06, 153.76, 133.16, 130.61, 130.04, 123.74, 99.38, 77.28, 77.03, 76.77, 44.96, 22.19, 16.77. **HRMS (ESI):** $C_{13}H_{12}N_6O_2SNa$ $[M+Na]^+$: calcd.: 339.0640; found: 339.0644.

***N*-(isoxazol-3-yl)-2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)propanamide (4b)**



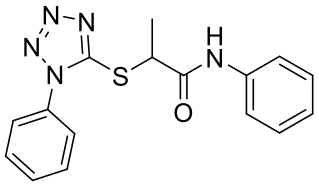
2-chloro-*N*-(isoxazol-3-yl)propanamide intermediate (30.0 mg, 0.206 mmol) was used in this reaction. The crude mixture of **4b** was purified with DCM/Hexane (1:3) recrystallization solvents to afford **4b** as a white solid (27.0 mg, 40%): mp 98-100˚C. **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 9.10 (s, 1H), 8.27 (d, *J* = 1.7 Hz, 1H), 7.51 – 7.44 (m, 2H), 7.43 – 7.36 (m, 2H), 7.36 – 7.30 (m, 1H), 6.97 (d, *J* = 1.8 Hz, 1H), 6.35 (s, 1H), 3.57 (q, *J* = 7.2 Hz, 1H), 2.29 (s, 3H), 1.43 (d, *J* = 7.2 Hz, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 169.22, 159.25, 158.89, 157.01, 149.78, 139.08, 130.78, 128.87, 128.07, 125.54, 113.63, 99.22, 99.08, 77.28, 77.03, 76.77, 48.11, 22.11, 17.25, 13.64. **HRMS (ESI):** $C_{16}H_{17}N_4O_2S[M+H]^+$: calcd.: 329.1072; found: 329.1055.

2-Chloro-*N*-phenylpropanamide



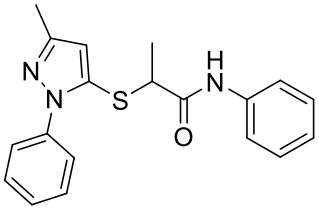
Aniline (100 mg, 1.07 mmol)was used in this reaction and followed the general procedure along with literature precedence to isolate the amide intermediate needed to be carried over for the next step.[43]

***N*-phenyl-2-((1-phenyl-1*H*-tetrazol-5-yl)thio)propanamide (2c)**



2-Chloro-*N*-phenylpropanamide intermediate (40.0 mg, 0.217 mmol) was used in this reaction.The crude mixture of **2c** was purified with DCM/Hexane (1:3) recrystallization solvents to afford **2c** as a white solid (23.0 mg, 47%): mp 123-125˚C. **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 9.48 (s, 1H), 7.61 (d, *J* = 1.3 Hz, 1H), 7.61 – 7.52 (m, 6H), 7.35 – 7.28 (m, 2H), 7.15 – 7.06 (m, 1H), 4.68 (q, *J* = 7.3 Hz, 1H), 1.71 (d, *J* = 7.2 Hz, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 168.21, 154.52, 137.86, 133.16, 130.59, 130.01, 129.02, 124.49, 123.76, 119.73, 77.28, 77.03, 76.78, 45.08, 16.61. **HRMS (ESI):** $C_{16}H_{15}N_5OSNa$ $[M+Na]^+$: calcd.: 348.0900; found: 348.0914.

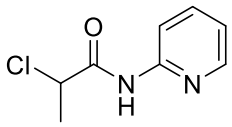
2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-phenylpropanamide (4c)



2-Chloro-*N*-phenylpropanamide intermediate (40.0 mg, 0.217 mmol) was used in this reaction.The crude mixture of **4c** was purified with DCM/Hexane (1:3) recrystallization solvents to afford **4c** as a white solid (26.0 mg,31%): mp 132-134˚C. **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 7.82 (s, 1H), 7.52 – 7.46 (m, 2H), 7.43 (ddd, *J* = 7.9, 6.9, 1.4 Hz, 2H), 7.43 – 7.33 (m, 1H), 7.36 – 7.26 (m, 4H), 7.11 (tt, *J* = 6.9, 1.6 Hz, 1H), 6.36 (s, 1H), 3.58 (q, *J* = 7.3 Hz, 1H), 2.29 (s, 3H), 2.29 (s, 1H), 1.47 (d, *J* = 7.2 Hz, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 168.79, 149.99,

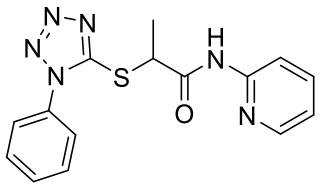
139.14, 137.22, 131.78, 128.98, 128.08, 125.47, 124.64, 119.71, 112.76, 77.27, 77.02, 76.77, 49.18, 17.77, 13.65. **HRMS (ESI):** $C_{19}H_{20}N_3OS$ $[M+H]^+$: calcd.: 338.1327; found: 338.1306.

2-chloro-*N*-2-pyridinyl- propanamide



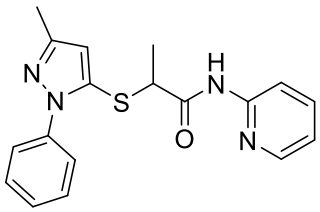
2-aminopyridine (100 mg, 1.06 mmol) was used in this reaction and followed the general procedure along with literature precedence to isolate the amide intermediate needed to be carried over for the next step.[44]

2-((1-phenyl-1*H*-tetrazol-5-yl)thio)-*N*-(pyridin-2-yl)propanamide (2d)



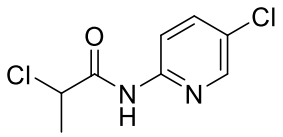
2-chloro-*N*-2-pyridinyl- propanamide intermediate (30.0 mg, 0.192 mmol) was used in this reaction.The crude mixture of **2d** was purified with DCM/Hexane (1:3) recrystallization solvents to afford **2d** as a white solid (26.0 mg, 46%): mp 141-143˚C. **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 9.57 (s, 1H), 8.32 (ddd, *J* = 4.9, 2.0, 0.9 Hz, 1H), 8.16 (dd, *J* = 8.3, 1.2 Hz, 1H), 7.69 (ddd, *J* = 8.4, 7.4, 1.9 Hz, 1H), 7.62 – 7.51 (m, 5H), 7.05 (ddd, *J* = 7.4, 4.9, 1.0 Hz, 1H), 4.76 (q, *J* = 7.3 Hz, 1H), 1.74 (d, *J* = 7.3 Hz, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 168.81, 153.71, 151.06, 148.21, 138.24, 133.28, 130.47, 129.98, 123.76, 120.17, 114.18, 77.28, 77.02, 76.77, 45.81, 17.16. **HRMS (ESI):** $C_{15}H_{14}N_6OSNa$ $[M+Na]^+$: calcd.: 349.0848; found: 349.0832.

2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-(pyridine-2-yl)propanamide (4d)



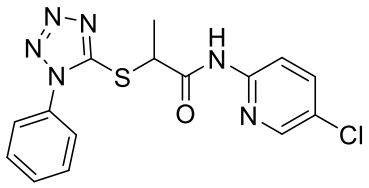
2-chloro-*N*-2-pyridinyl- propanamide intermediate (30.0 mg, 0.192 mmol) was used in this reaction. The crude mixture of **4d** was purified with DCM/Hexane (1:3) recrystallization solvents to afford **4d** as a white solid (38.6 mg, 45%): mp 130-132˚C. **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 8.42 (s, 1H), 8.26 (ddd, *J* = 4.9, 2.0, 0.9 Hz, 1H), 8.10 – 8.04 (m, 1H), 7.68 (ddd, *J* = 8.7, 7.2, 2.0 Hz, 1H), 7.51 – 7.45 (m, 2H), 7.42 – 7.35 (m, 2H), 7.34 – 7.27 (m, 1H), 7.05 (ddd, *J* = 7.3, 4.9, 1.0 Hz, 1H), 6.36 (s, 1H), 3.54 (q, *J* = 7.2 Hz, 1H), 2.28 (s, 3H), 1.44 (d, *J* = 7.2 Hz, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 169.44, 150.80, 149.77, 147.89, 139.14, 138.29, 131.24, 128.86, 127.92, 125.51, 120.09, 113.83, 113.24, 77.28, 77.03, 76.77, 48.73, 17.49, 13.65. **HRMS (ESI):** $C_{18}H_{19}N_4OS$ $[M+H]^+$: calcd.: 339.1280; found: 339.1289.

2-chloro-*N*-(5-chloropyridin-2-yl)propanamide



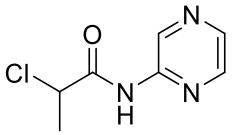
2-amino-5-chloropyridine (100 mg, 0.777 mmol) was used in this reaction and followed the general procedure along with literature precedence to isolate the amide intermediate needed to be carried over for the next step.[45]

***N*-(5-chloropyridin-2-yl)-2-((1-phenyl-1*H*-tetrazol-5-yl)thio)propanamide (2e)**



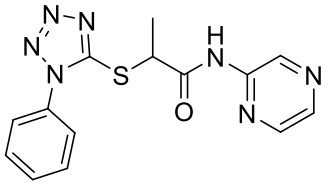
2-chloro-*N*-(5-chloropyridin-2-yl)propanamide intermédiate (30.0 mg, 0.136 mmol) was used in this reaction.The crude mixture of **2e** was purified with DCM/Hexane (1:3) recrystallization solvents to afford **2e** as a white solid (20.0 mg, 41%): mp 116-117˚C. **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 9.73 (s, 1H), 8.26 (dd, *J* = 2.6, 0.8 Hz, 1H), 8.18 – 8.12 (m, 1H), 7.65 (dd, *J* = 8.9, 2.6 Hz, 1H), 7.62 – 7.52 (m, 5H), 4.73 (q, *J* = 7.3 Hz, 1H), 1.72 (d, *J* = 7.3 Hz, 3H).**^{13}C NMR** (126 MHz, $CDCl_3$) δ 168.82, 153.76, 149.39, 146.86, 137.82, 133.22, 130.55, 130.01, 127.16, 123.72, 114.80, 77.28, 77.02, 76.77, 45.42, 16.86. **HRMS (ESI):** $C_{15}H_{14}ClN_6OS$ $[M+H]^+$: calcd.: 361.0638; found: 361.0639.

2-chloro-*N*-(pyrazin-2-yl)propanamide



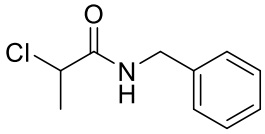
Aminopyrazine (100 mg, 1.05 mmol)was used in this reaction and followed the general procedure along with literature precedence to isolate the amide intermediate needed to be carried over for the next step.[39]

2-((1-phenyl-1*H*-tetrazol-5-yl)thio)-*N*-(pyrazin-2-yl)propanamide (2f)



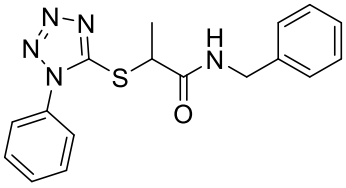
2-chloro-*N*-(pyrazin-2-yl)propanamide intermediate (30.0 mg, 0.161 mmol) was used in this reaction.The crude mixture of **2f** was purified with DCM/Hexane (1:3) recrystallization solvents to afford **2f** as a white solid (32%): mp 137-139˚C. **1H NMR** (500 MHz, Chloroform-d) δ 9.94 (s, 1H), 9.50 (d, J = 1.6 Hz, 1H), 8.35 (d, J = 2.6 Hz, 1H), 8.29 (dd, J = 2.6, 1.6 Hz, 1H), 7.57 (tq, J = 6.0, 3.3, 2.6 Hz, 5H), 4.77 (q, J = 7.3 Hz, 1H), 1.74 (d, J = 7.3 Hz, 3H), 1.33 – 1.23 (m, 1H).**13C NMR** (126 MHz, CDCl3) δ 168.89, 153.86, 148.00, 142.42, 142.23, 141.03, 140.57, 137.07, 136.78, 133.15, 130.63, 130.05, 123.70, 77.29, 77.03, 76.78, 45.02, 34.67, 31.59, 29.06, 25.28, 22.66, 22.31, 16.71, 14.13, 11.44. **HRMS (ESI):** $C_{14}H_{14}N_7OS$ $[M+H]^+$: calcd.: 328.0981; found: 328.0977.

2-Chloro-*N*-(phenylmethyl)propanamide



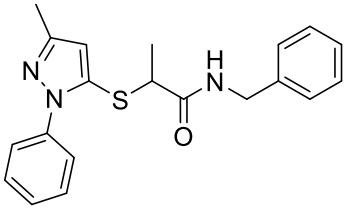
Benzylamine (100 mg, 0.933 mmol) was used in this reaction and followed the general procedure along with literature precedence to isolate the amide intermediate needed to be carried over for the next step.[46]

***N*-benzyl-2-((1-phenyl-1*H*-tetrazol-5-yl)thio)propanamide (2g)**



2-Chloro-*N*-(phenylmethyl)propanamide intermediate (30.0 mg, 0.151 mmol) was used in this reaction.The crude mixture of **2g** was purified with DCM/Hexane (1:3) recrystallization solvents to afford **2g** as a white solid (22.0 mg, 43%): mp 74-76˚C. **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 7.61 – 7.52 (m, 3H), 7.55 – 7.48 (m, 2H), 7.39 (d, *J* = 6.6 Hz, 1H), 7.33 – 7.18 (m, 5H), 4.57 (q, *J* = 7.3 Hz, 1H), 4.45 (d, *J* = 5.8 Hz, 2H), 1.66 (d, *J* = 7.3 Hz, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 170.23, 154.07, 137.77, 133.24, 130.48, 129.95, 128.68, 127.61, 127.50, 123.79, 77.28, 77.02, 76.77, 44.68, 43.92, 16.99. **HRMS (ESI):** $C_{17}H_{17}N_5OSNa$ $[M+H]^+$: calcd.: 362.1052; found: 362.1067.

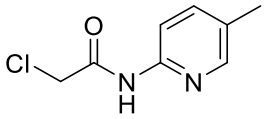
***N*-benzyl-2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)propanamide (4e)**



2-Chloro-*N*-(phenylmethyl)propanamide intermediate (30.0 mg, 0.151 mmol) was used in this reaction.The crude mixture of **4e** was purified with DCM/Hexane (1:3) recrystallization solvents to afford **4e** as a white solid (23.96 mg, 37%): mp 97-99˚C. **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 7.47 – 7.37 (m, 4H), 7.40 – 7.32 (m, 1H), 7.31 (dddd, *J* = 11.0, 6.9, 4.3, 2.3 Hz, 3H), 7.14 – 7.08 (m, 2H), 6.38 (t, *J* = 5.6 Hz, 1H), 6.21 (s, 1H), 4.33 (dd, *J* = 14.8, 6.0 Hz, 1H), 4.27 (dd, *J* =

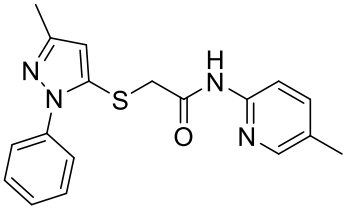
14.7, 5.7 Hz, 1H), 3.54 (q, *J* = 7.3 Hz, 1H), 2.29 (s, 3H), 1.43 (d, *J* = 7.3 Hz, 3H), 1.32 – 1.22 (m, 0H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 170.62, 149.83, 139.18, 137.71, 132.46, 128.94, 128.74, 127.96, 127.69, 127.65, 125.25, 112.04, 77.28, 77.02, 76.77, 48.17, 43.76, 22.34, 17.95, 13.64. **HRMS (ESI):** $C_{20}H_{22}N_3OS$ $[M+H]^+$: calcd.: 352.1484; found: 352.1485.

2-Chloro-*N*-(5-methyl-2-pyridinyl)acetamide



2-amino-5-picoline (100 mg, 0.924 mmol)was used in this reaction and followed the general procedure along with literature precedence to isolate the amide intermediate needed to be carried over for the next step.[47]

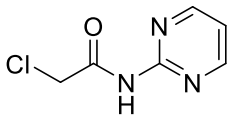
2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-(5-methylpyridin-2-yl)acetamide (3e)



2-Chloro-*N*-(5-methyl-2-pyridinyl)acetamide intermediate (30.0 mg, 0.161 mmol) was used in this reaction.The crude mixture of **3e** was purified by column chromatography using ethyl acetate/hexane (30/70) to afford **3e** as a white solid (22.44 mg, 41%): mp 114-115˚C. **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 8.71 (s, 1H), 8.09 (d, *J* = 2.3 Hz, 1H), 8.01 (d, *J* = 8.4 Hz, 1H), 7.56 – 7.48 (m, 3H), 7.43 (dd, *J* = 8.7, 7.0 Hz, 2H), 7.39 – 7.32 (m, 1H), 6.32 (s, 1H), 3.49 (s, 2H), 2.29 (d, *J* = 12.5 Hz, 6H), 1.26 (d, *J* = 3.9 Hz, 1H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 165.61, 149.96, 148.50, 147.83, 139.10, 138.91, 132.93, 129.73, 129.03, 128.08, 125.24, 113.39,

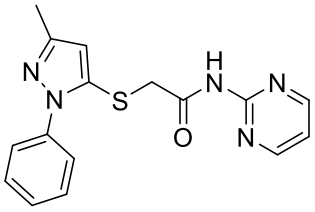
111.00, 77.28, 77.03, 76.77, 39.97, 29.70, 17.85, 13.66, 1.02. **HRMS (ESI):** $C_{18}H_{19}N_4OS$ $[M+H]^+$: calcd.: 339.1280; found: 339.1271.

2-Chloro-*N*-2-pyrimidinylacetamide



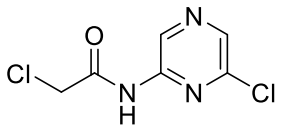
2-aminopyrimidine (100 mg, 1.07 mmol) was used in this reaction and followed the general procedure along with literature precedence to isolate the amide intermediate needed to be carried over for the next step.[48]

2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-(pyrimidin-2-yl)acetamide (3g)



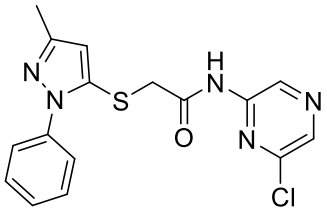
2-Chloro-*N*-2-pyrimidinylacetamide intermediate (30.0 mg, 0.194 mmol) was used in this reaction. The crude mixture of **3g** was purified with DCM/Hexane (1:3) recrystallization solvents to afford **3g** as a white solid (46.22 mg, 81%): mp 159-161˚C. **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 9.34 (s, 1H), 8.54 (d, *J* = 4.9 Hz, 2H), 7.49 – 7.43 (m, 2H), 7.37 – 7.30 (m, 2H), 7.30 – 7.23 (m, 1H), 6.95 (t, *J* = 4.9 Hz, 1H), 6.31 (s, 1H), 3.88 (s, 2H), 2.21 (s, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 158.32, 156.99, 149.75, 139.30, 128.88, 127.84, 125.25, 116.51, 111.62, 77.29, 77.03, 76.78, 40.65, 13.66. **HRMS (ESI):** $C_{16}H_{16}N_5OS$ $[M+H]^+$: calcd.: 326.1076; found: 326.1072.

2-Chloro-*N*-(6-chloro-2-pyrazinyl)acetamide



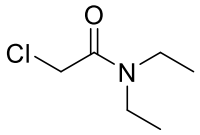
2-amino-6-chloropyrazine (100 mg, 0.772 mmol) was used in this reaction and followed the general procedure along with literature precedence to isolate the amide intermediate needed to be carried over for the next step.[49]

***N*-(6-chloropyrazin-2-yl)-2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)acetamide (3i)**



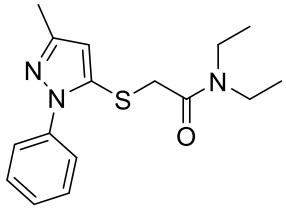
2-Chloro-*N*-(6-chloro-2-pyrazinyl)acetamide (30.0 mg, 0.146 mmol) intermediate was used in this reaction. The crude mixture of **3i** was purified by column chromatography using ethyl acetate/hexane (30/70) to afford **3i** as an oil (46%): mp oil. **1H NMR** (500 MHz, Chloroform-d) δ 9.31 (s, 2H), 8.57 (s, 2H), 8.37 (d, J = 0.6 Hz, 2H), 7.52 – 7.46 (m, 4H), 7.45 – 7.38 (m, 4H), 7.38 – 7.31 (m, 2H), 6.35 (s, 2H), 3.50 (s, 4H), 2.30 (s, 6H), 1.28 (dd, J = 11.3, 2.2 Hz, 1H), 1.28 (s, 1H), 1.26 (s, 1H), 0.91 – 0.82 (m, 1H).**13C NMR** (126 MHz, CDCl3) δ 165.95, 150.15, 146.34, 146.20, 139.80, 138.89, 133.80, 131.93, 129.10, 128.19, 125.34, 111.89, 77.28, 77.03, 76.78, 40.18, 31.59, 29.70, 22.66, 14.13, 13.65. **HRMS (ESI):** $C_{16}H_{15}ClN_5OS$ $[M+H]^+$: calcd.: 360.0686; found: 360.0689.

Chloro-*N*,*N*-diethylacetamide



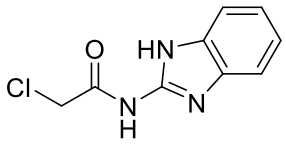
Dietheylamine (100 mg, 0.683 mmol) was used in this reaction and followed the general procedure along with literature precedence to isolate the amide intermediate needed to be carried over for the next step.[50]

***N*,N-diethyl-2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)acetamide (3j)**



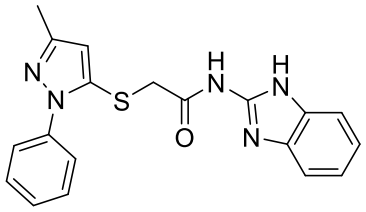
The Chloro-*N*,*N*-diethylacetamide intermediate (30.0 mg, 0.147 mmol) was used in this reaction. The crude mixture of **3j** was purified after workup to afford **3j** as a clear oil (18.20 mg, 30%): mp oil. **1H NMR** (500 MHz, Chloroform-d) δ 7.61 – 7.55 (m, 2H), 7.48 – 7.41 (m, 2H), 7.45 – 7.32 (m, 1H), 7.26 (s, 1H), 6.34 (d, J = 1.5 Hz, 1H), 3.50 (d, J = 1.6 Hz, 2H), 3.32 (qd, J = 7.2, 1.6 Hz, 2H), 3.15 (qd, J = 7.2, 1.5 Hz, 2H), 2.32 (d, J = 1.6 Hz, 3H), 1.25 (s, 0H), 1.08 (td, J = 7.1, 1.3 Hz, 6H). (126 MHz, CDCl3) δ 166.34, 149.60, 139.46, 133.93, 128.89, 128.70, 127.76, 125.63, 125.15, 111.98, 77.28, 77.02, 76.77, 42.42, 40.51, 38.20, 29.70, 14.26, 13.68, 12.89. **HRMS (ESI):** $C_{16}H_{22}N_3OS$ $[M+H]^+$: calcd.: 304.1484; found: 303.1477.

2-(Chloroacetamido)benzimidazole



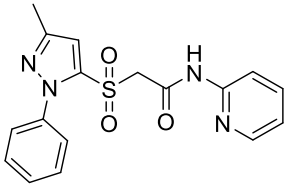
2-aminobenzimidazole (100 mg, 0.751 mmol) was used in this reaction and followed the general procedure along with literature precedence to isolate the amide intermediate needed to be carried over for the next step.[51]

***N*-(1*H*-benzo[d]imidazol-2-yl)-2-((3-methyl-1-phenyl-1H-pyrazol-5-yl)thio)acetamide (3k)**



2-(Chloroacetamido)benzimidazole intermediate (30.0 mg, 0.143 mmol) was used in this reaction. The crude mixture of **3k** was purified with DCM/Hexane (1:3) recrystallization solvents to afford **3k** as a white solid (26.83 mg, 52%): mp 174-176˚C. **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 11.83 (s, 2H), 7.46 (dd, *J* = 7.7, 1.6 Hz, 2H), 7.40 (dt, *J* = 7.5, 3.7 Hz, 2H), 7.33 – 7.19 (m, 5H), 6.27 (s, 1H), 3.62 (s, 2H), 2.24 (s, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 168.80, 149.71, 147.51, 139.11, 132.36, 128.76, 127.76, 125.10, 122.81, 112.54, 77.28, 77.02, 76.77, 39.86, 29.70, 13.62. **HRMS (ESI):** $C_{19}H_{18}N_5OS$ $[M+H]^+$: calcd.: 364.1232; found: 364.1223.

Preparation of 2-((3-methyl-1-phenyl-1H-pyrazol-5-yl)sulfonyl)-N-(pyridin-2-yl)acetamide (5a)



Procedure:

To a tall clear 4 dram vial, equipped with a magnetic stir rod/flea bar, 2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-(pyridine-2-yl)acetamide (50.0 mg, 0.163 mmol) (1.0 eq) was added in the

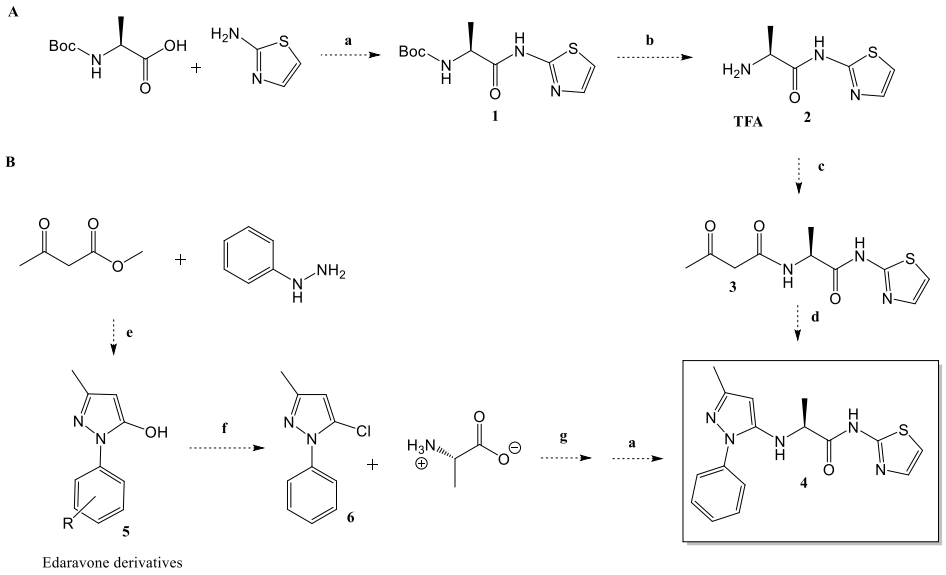
presence of urea-hydrogen peroxide (5.0 eq) and pthalic anhydride (5.0eq) suspended in ethyl acetate (0.3M). The reaction mixture was allowed to stir for 8 hours at ambient room temperature. After 8 hours, HPLC analysis showed complete consumption of starting material resulting in direct conversion to the sulfone species. The reaction mixture was subjected to an aqueous workup where the organic layer was digested with a solution of 10% sodium sulfite (2mL) as well as a solution of aqueous 10% sodium carbonate (2mL). The organic layer was condensed into a yellow solid which was suspended in the anti-solvent diethyl ether where heat was introduced expelling the impurities. After filtration, the resultant pure sulfone was isolated as a white solid[52] (30.08 mg, 52%): mp 148-150°C. **^{1}H NMR** (500 MHz, Chloroform-d) δ 10.83 (s, 1H), 8.28 – 8.21 (m, 2H), 7.65 – 7.57 (m, 2H), 7.51 – 7.41 (m, 3H), 7.31 (ddd, J = 8.6, 7.7, 1.5 Hz, 1H), 7.04 (ddd, J = 7.6, 6.4, 1.9 Hz, 1H), 6.92 (s, 1H), 4.15 (s, 2H), 2.36 (s, 3H). **^{13}C NMR** (126 MHz, CDCl3) δ 159.14, 148.87, 143.49, 139.29, 138.30, 137.42, 129.81, 129.07, 127.89, 127.19, 119.77, 115.39, 114.23, 77.28, 77.02, 76.77, 62.22, 13.46.

CHAPTER THREE

PROPOSED *N*-LINKED SERIES OF INHIBITORS OF OF *N*-SUCCINYL-L,L-DIAMINOPIMELIC ACID DESSUCINYLASE

Proposed Scheme

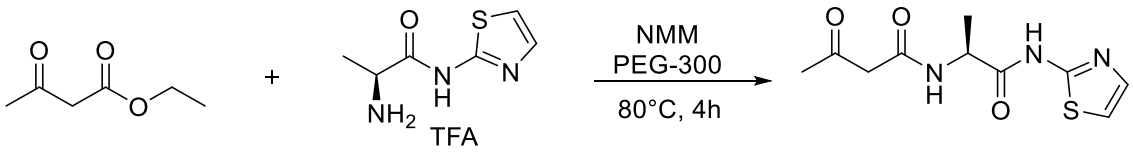
Encouraged by the docking results, the synthesis of pyrazole analog **4** was designed following reported methods starting from the commercially available Boc-protected L-alanine amino acid was allowed to react in the presence of EDCI, *N*-methylmorpholine, and 2-aminothiazole as the amino derivative. The carboxylic acid reacted with the amine through base-mediated coupling to give the L-alanine amide analog **1** which was isolated and purified through column chromatography and characterized by HPLC and NMR.



Scheme 5. Attempted Synthetic routes toward preparation of pyrazole analog 4. Reagents and conditions: (a) EDCI.HCl, HOBt, NMM, DMF, 50 ºC, overnight; (b) CF_3COOH, CH_2Cl_2, rt, 2 h;

(c) ethyl acetoacetate, NMM, PEG-300, 80º C, 4 h; (d) phenylhydrazine; (e) phenylhydrazine, ethyl acetoacetate, acetic acid, 118 ºC, 4 h; (f) edaravone, $POCl_3$, 120 ºC, 12 h; (g) L-alanine, K_2CO_3, DMSO, 80 ºC, 6 h

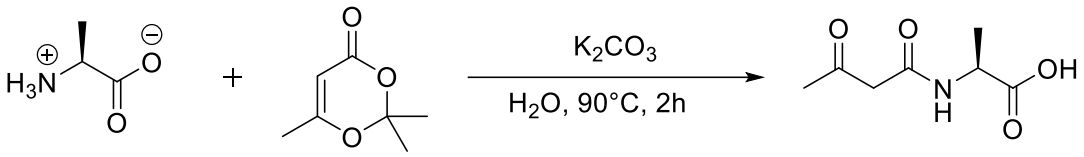
During the course of this work, we deviated from routinely using EDCI and transitioned to the superior coupling reagent propylphosphonic anhydride (T3P).[53] T3P gave better yields with higher purities without the need for column chromatography. Following this reaction, we removed the Boc-protecting group using TFA to give the L-alanine amide derivative **3** which was characterized by HPLC and NMR.



Scheme 6. Nucleophilic acyl substitution of B-keto ester with L-amide derivative under basic conditions.

According to a published procedure, aliphatic amines have been reacted with β-keto esters in the presence of PEG-300 under basic conditions to yield a resultant β-ketoamide. [54] As part of our synthetic design, ethyl acetoacetate was allowed to react with the L-alanine amide derivative with NMM in PEG-300. The reactions that I attempted included conditions varying in temperature with 60 ºC insufficient for the reaction rate while temperatures above 80 ºC yielded hydrolysis of the 2-aminothiazole amide without formation of the β-ketoamide. The results of temperatures above 80 ºC include the decomposition of the amide and possible loss of chirality.

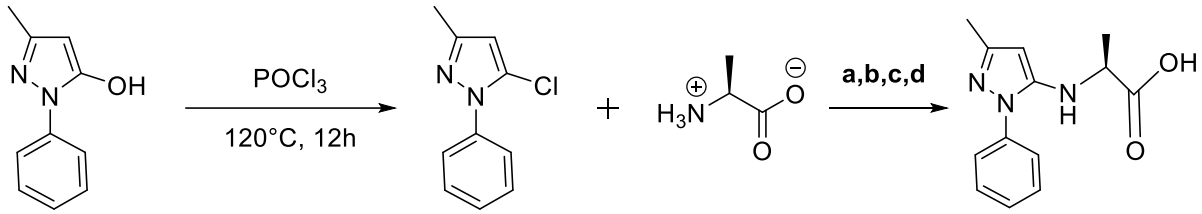
Monitoring the reaction by HPLC, it was observed that a multitude of new peaks corresponding to byproducts did not include the desired compound.



Scheme 7. Synthesis of L- alanine b-betoamide from L-alanine and TMD.

Our approach to forming the pyrazole ring involved the condensation of phenylhydrazine with β-ketoesters and β-ketoamides. Our synthesis, starting with the commercially available amino acids being readily converted to their β-ketoamide derivatives which should undergo cyclization with phenylhydrazine, or a substituted hydrazine, to form the pyrazole ring, enabling point derivatizations in driving SAR. L-Alanine was utilized in exploring and optimizing reaction conditions to make the pyrazole derivative. The synthesis of the β-ketoamide analogs of the amino acids is achieved by reacting L-alanine and with the diketene-acetone adduct, 2,2,6-trimethyl-4H-1,3-dioxin-4-one (TMD). TMD is a stable derivative of diketene which can be pyrolyzed at temperatures above 90ºC providing the acetyldiketene and acetone through a pseudo-retro Diels Alder reaction.[55] In our reactions with TMD we followed the general procedure from Garden, who did not employ amino acids but explored aliphatic amines. L-Alanine β-ketoamide was synthesized by reacting L-alanine with TMD and K_2CO_3 in water and isolated 59% yield (Scheme 3). [56] In parallel, with the successful synthesis of L-alanine β-ketoamide we investigated a few selected reaction conditions to access the 5-aminopyrazole from

β-ketoamide-L-phenylalanine that was explored by our research member Thahani Habeeb. All of the condensation reaction conditions investigated in synthesizing L-phenylalanine pyrazole analog were not successful due to the hydrolysis of the β-ketoamide and were monitored by HPLC. The desired product was not identified by NMR.

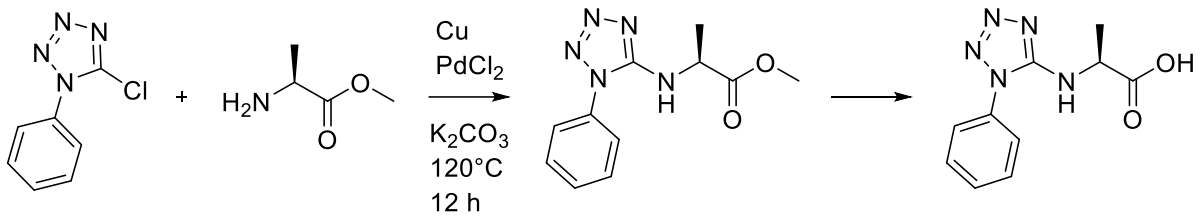


Scheme 8: S_NAr reaction of edaravone with $POCl_3$ to yield 5-chloro-1-phenyl-3-methyl-pyrazole and attempted synthesis of 5-aminopyrazole. Reagents and conditions: (a) cesium carbonate, DMSO, 80 °C, 2h; (b) TEA, DCM, rt, 2h; (c) sodium bicarbonate, ethanol, 80 °C, 48h; (d) cesium carbonate, CuI, DMSO, 80 °C, 48h.

Moving forward, we investigated several selected reaction conditions to access the 5-aminopyrazole starting with the commercially available compound Edaravone and reacting it with phosphorous oxychloride to yield a 5-chloropyrazole.[57, 58] This was accomplished by reacting the enol form[59] of edaravone with phosphorous oxychloride in a S_NAr reaction to give the 5-chloro-1-phenyl-3-methyl-pyrazole.

With 5-chloro-1-phenyl-3-methyl-pyrazole in hand additional S_NAr conditions based on of a limited number of published methods to couple 5-chlororpyrazoles with a primary amine. [60-62] The primary amine in our case would be L-alanine or other amino acids. To the best of our knowledge there are no reported methods for synthesizing substituted amino-pyrazole derivatives from L-amino acids. The reaction conditions consisted of the 5-chloropyrazole in the presence of

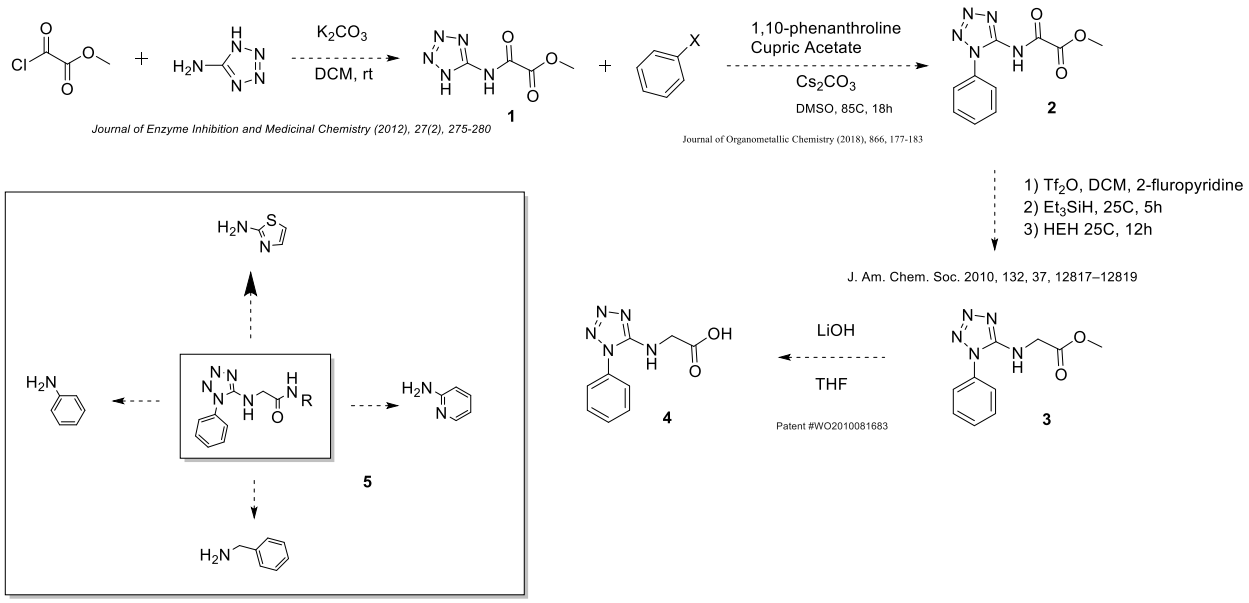
L-alanine in polar aprotic solvents and base. These reactions conditions were monitored by HPLC and showed no consumption of starting material.



Scheme 9. Cross-coupling to access N-linked tetrazole analogs.

Additionally, we investigated a synthetic route derived from a patent procedure.[63] This reaction incorporates the cross-coupling between the commercially available 5-chloro-1H-tetrazole in the presence of methyl ester protected L-alanine, copper, potassium carbonate and palladium chloride at 120°C for 24hrs. Through the course of monitoring the reaction over four days the monitoring resulted in no consumption of starting materials. Additional screenings of conditions were explored with varying lower temperatures as well as differentiating from potassium carbonate to the softer cesium carbonate; however, the narrative was universally continuous in that monitoring resulted in no consumption of starting materials.

Conclusion



Scheme 10. Proposed synthetic route to access N-linked tetrazoles.

A new proposed synthetic route begins by reacting methyl chlorooxoacetate in the presence of 5-amino-1H-tetrazole to produce the respective tetrazole **intermediate 1**. In regards to methyl chlorooxoacetate's ability to partake in a nucleophilic acyl substitution reaction, there are literature sources referencing methyl chlorooxoacetate as a coupling potential. The reported conditions mirror that of acyl substitutions that have been successfully synthesized for the S-linked series. Once the tetrazole amide intermediate **1** is in hand, **1** will undergo a Buchwald-Hartwig reaction using literature conditions coupling the aryl halide to the NH position on the tetrazole. The literature source showcases the success of the cross coupling of the 5-amino-1H-tetrazole, there are no procedures for the tetrazole amide moiety, if this step is problematic, then

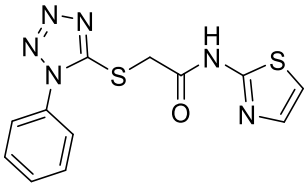
we can cross couple 5-amino-1H-tetrazole with an aryl halide prior to reacting it with the acid chloride. The resulting phenyl-tetrazole amide species would yield **intermediate 2** which would then have the amide bond reduced. The literature source for this step highlights how selective the conditions are for amide reductions in the presence of esters. From **3** to **4** the methyl ester transformation to carboxylic acid is from a patent procedure. Once the carboxylic acid intermediate is isolated this procedure demonstrates reacting non-nucleophilic amino heterocycles utilizing the BOP coupling reagent. Alternatively, the phosphonium salt derivative, PyBOP is far superior given its reactivity and quenching ability at larger scales. This synthesis would allow us to scale up **intermediate 4**, from there we can branch out and react at smaller scales with a variety of amino heterocycles.

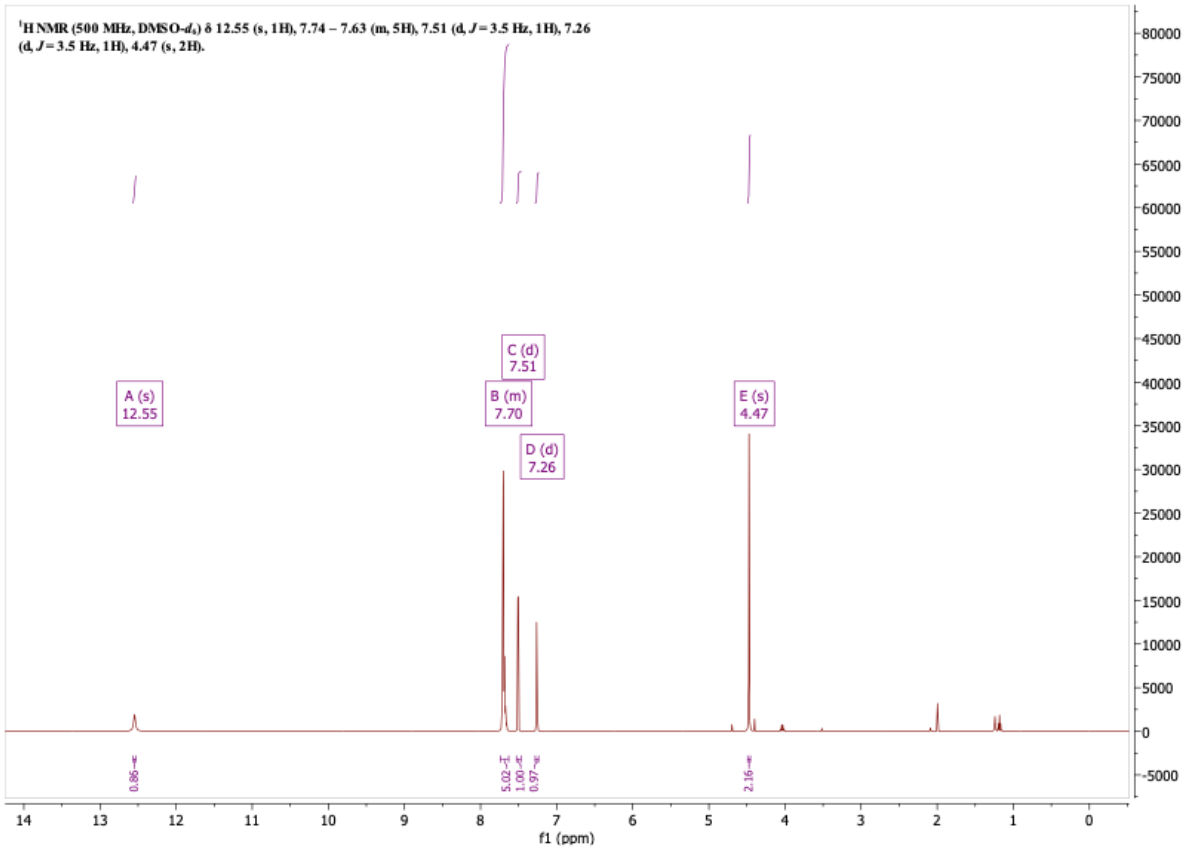
APPENDIX A

SUPPLEMENTAL DATA FOR CHAPTER TWO

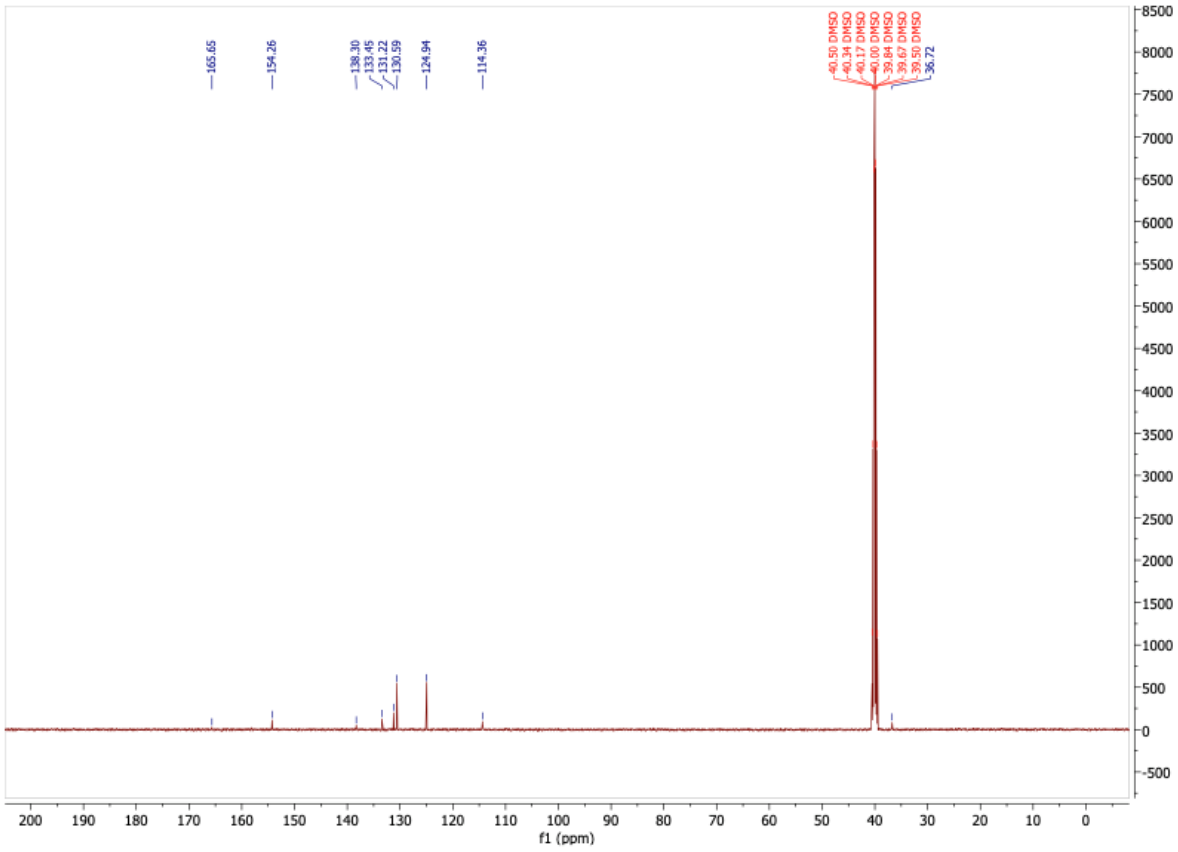
HRMS AND NMR SPECTRA OF COMPOUNDS REPORTED IN CHAPTER TWO

2-((1-phenyl-1*H*-tetrazol-5-yl)thio)-*N*-(thiazol-2-yl)acetamide (1a)





^{1}H spectrum of 2-((1-phenyl-1*H*-tetrazol-5-yl)thio)-*N*-(thiazol-2-yl)acetamide (1a)



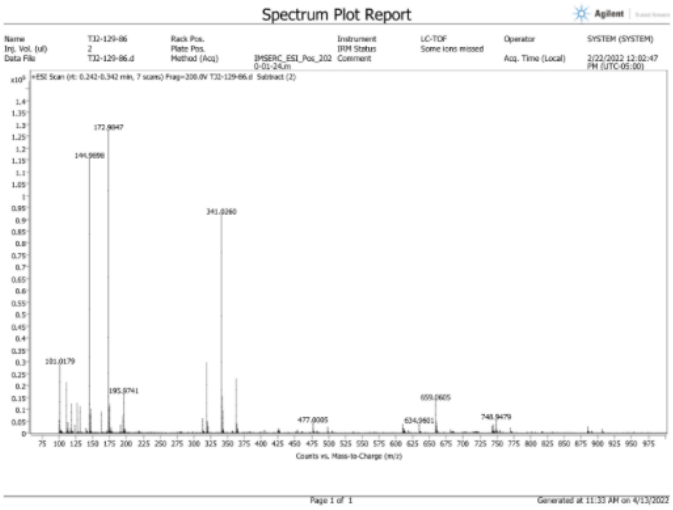
^{13}C spectrum of 2-((1-phenyl-1*H*-tetrazol-5-yl)thio)-*N*-(thiazol-2-yl)acetamide (1a)

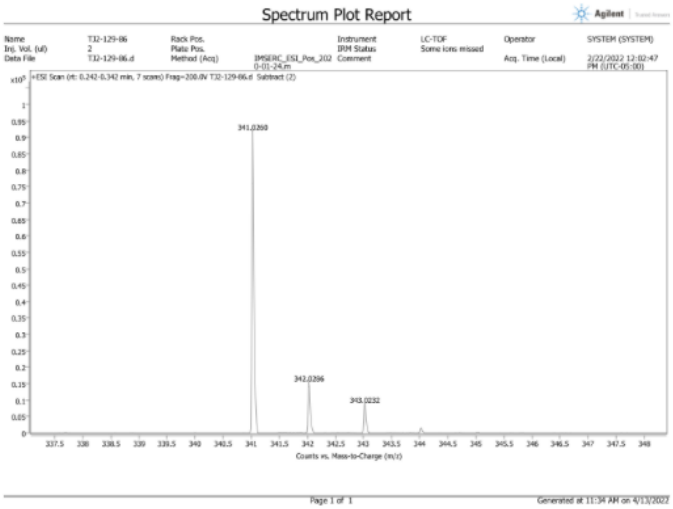
Spectrum Identification Results: + Scan (rt: 0.242-0.342 min) Sub (2) (TJ2-129-86.d)

Best	ID Source	Formula	Species	m/z	Score	Diff (ppm)	Diff (mDa)	Score (MFG)
	MFG	**C12 H10 N6 O S2**	**(M+Na)+**	**341.0260**	**96.05**	**3.42**	**1.09**	**96.05**

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M+Na)+	341.026	99.47	92.57	96.05	96.05	98.9	92463.7	C12 H10 N6 Na O

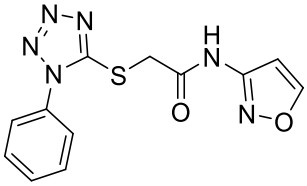
Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z	Diff (ppm)
92605.5	77.55	100	341.025	1	92463.7	100	77.43	341.026	3.01
15653.2	13.11	16.9	342.0272	1.5	16014.1	17.32	13.41	342.0286	4.31
9725.4	8.14	10.5	343.022	1.2	9337.8	10.1	7.82	343.0232	3.39
1429.8	1.2	1.54	344.0236	0.3	1598.3	1.73	1.34	344.024	0.94

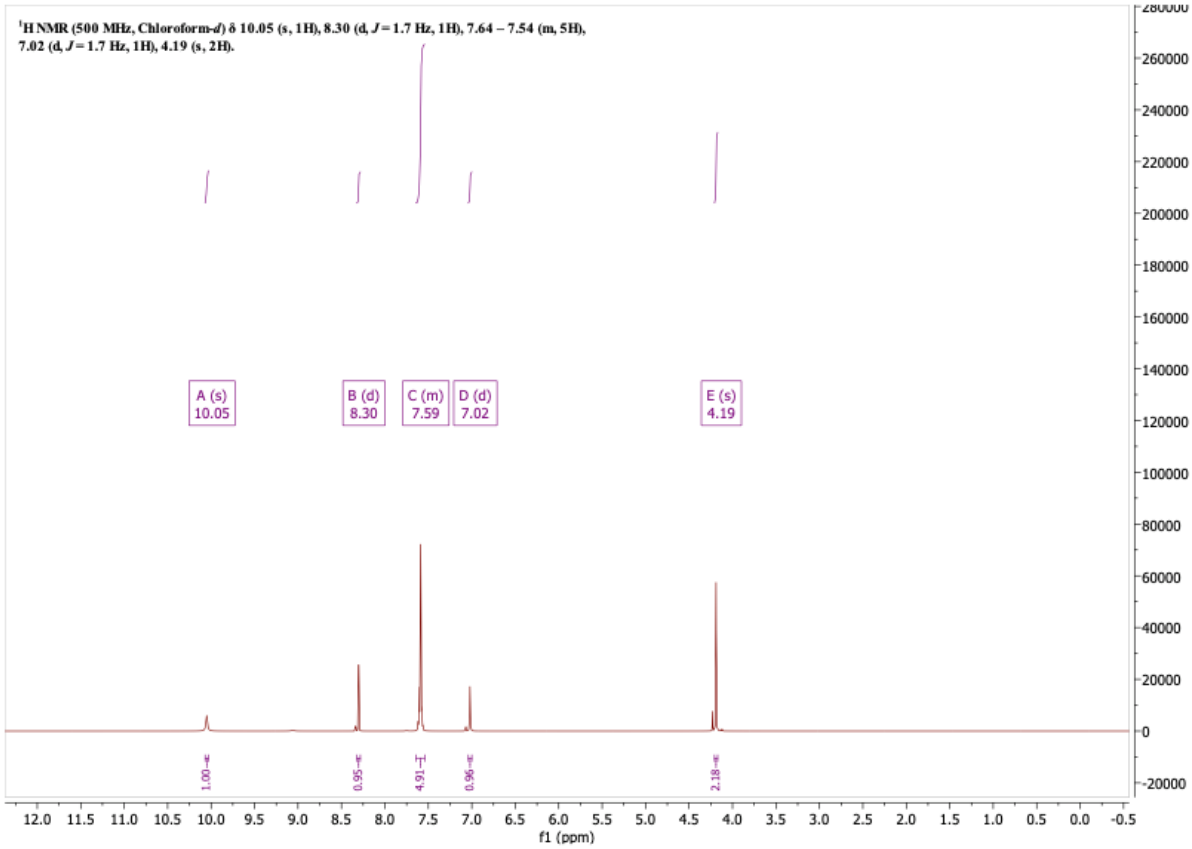




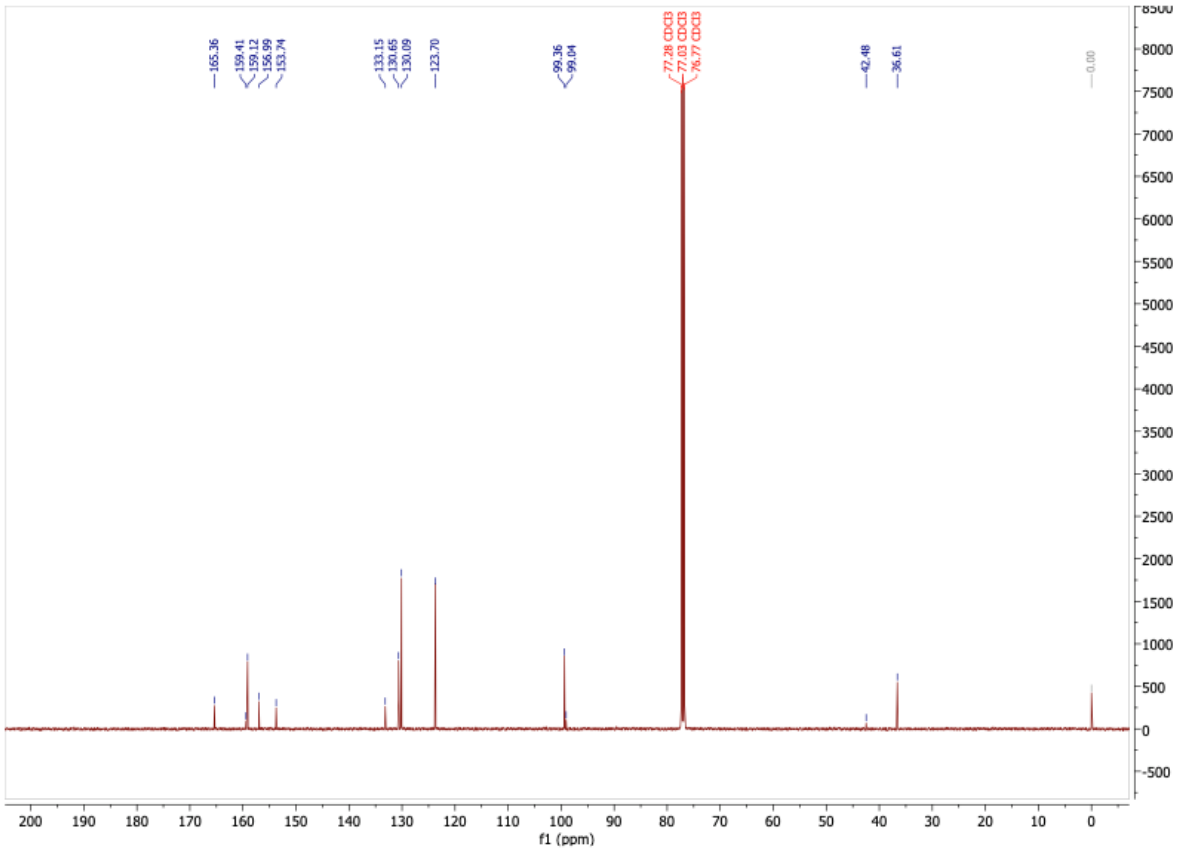
HRMS data of 2-((1-phenyl-1*H*-tetrazol-5-yl)thio)-*N*-(thiazol-2-yl)acetamide (1a)

_N_-(isoxazol-3-yl)-2-((1-phenyl-1_H_-tetrazol-5-yl)thio)acetamide (1b)





^{1}H spectrum of _N_-(isoxazol-3-yl)-2-((1-phenyl-1_H_-tetrazol-5-yl)thio)acetamide (1b)



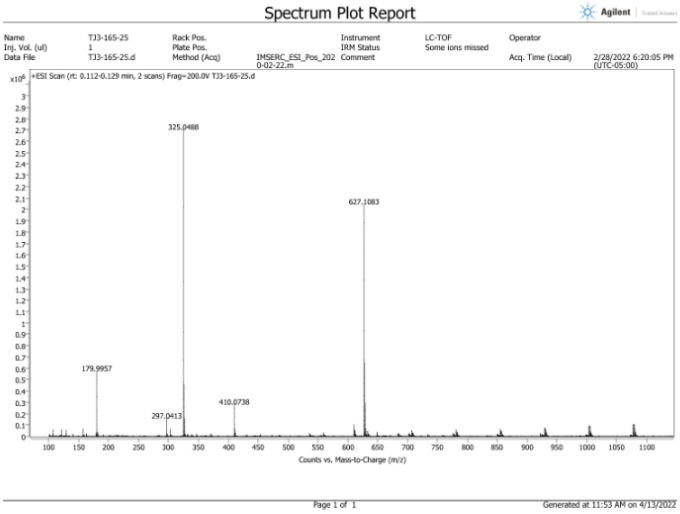
^{13}C spectrum of *N*-(isoxazol-3-yl)-2-((1-phenyl-1*H*-tetrazol-5-yl)thio)acetamide (1b)

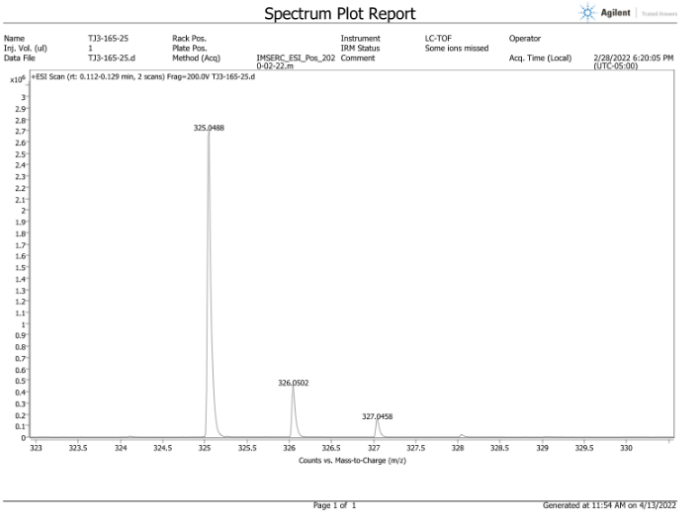
Spectrum Identification Results: + Scan (rt: 0.112-0.129 min) (TJ3-165-25.d)

Best	ID Source	Formula	Species	m/z	Score	Diff (ppm)	Diff (mDa)	Score (MFG)
	MFG	**C12 H10 N6 O2 S**	**(M+Na)+**	**325.0488**	**96.5**	**2.6**	**0.79**	**96.5**

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M+Na)+	325.0488	98.94	95.86	96.5	96.5	94.85	2765375.5	C12 H10 N6 Na O

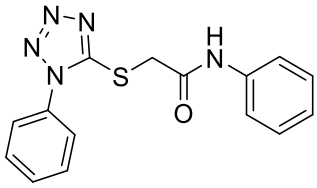
Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z	Diff (ppm)
2762807.9	81.79	100	325.0478	1	2765375.5	100	81.87	325.0488	2.94
446237.9	13.21	16.15	326.0501	0.1	453653.1	16.4	13.43	326.0502	0.2
168868.2	5	6.11	327.0459	-0.1	158885.4	5.75	4.7	327.0458	-0.38

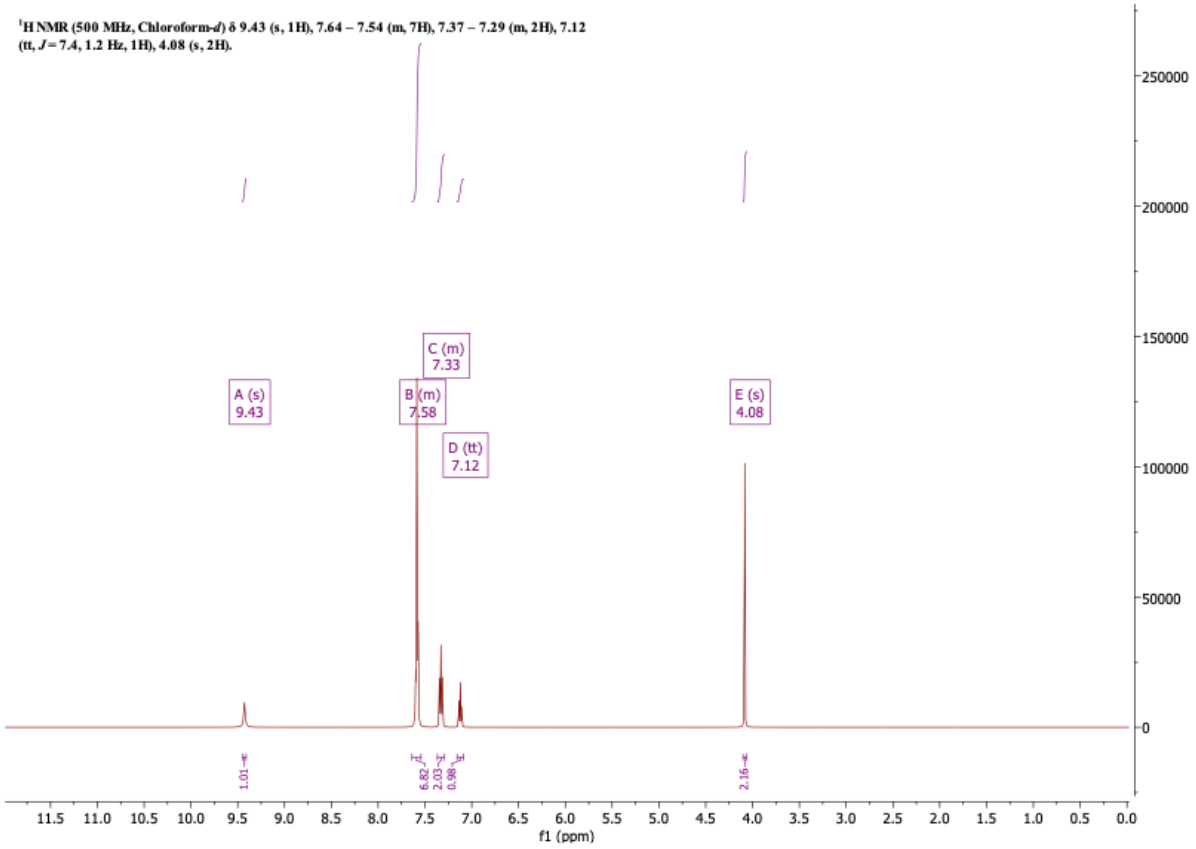




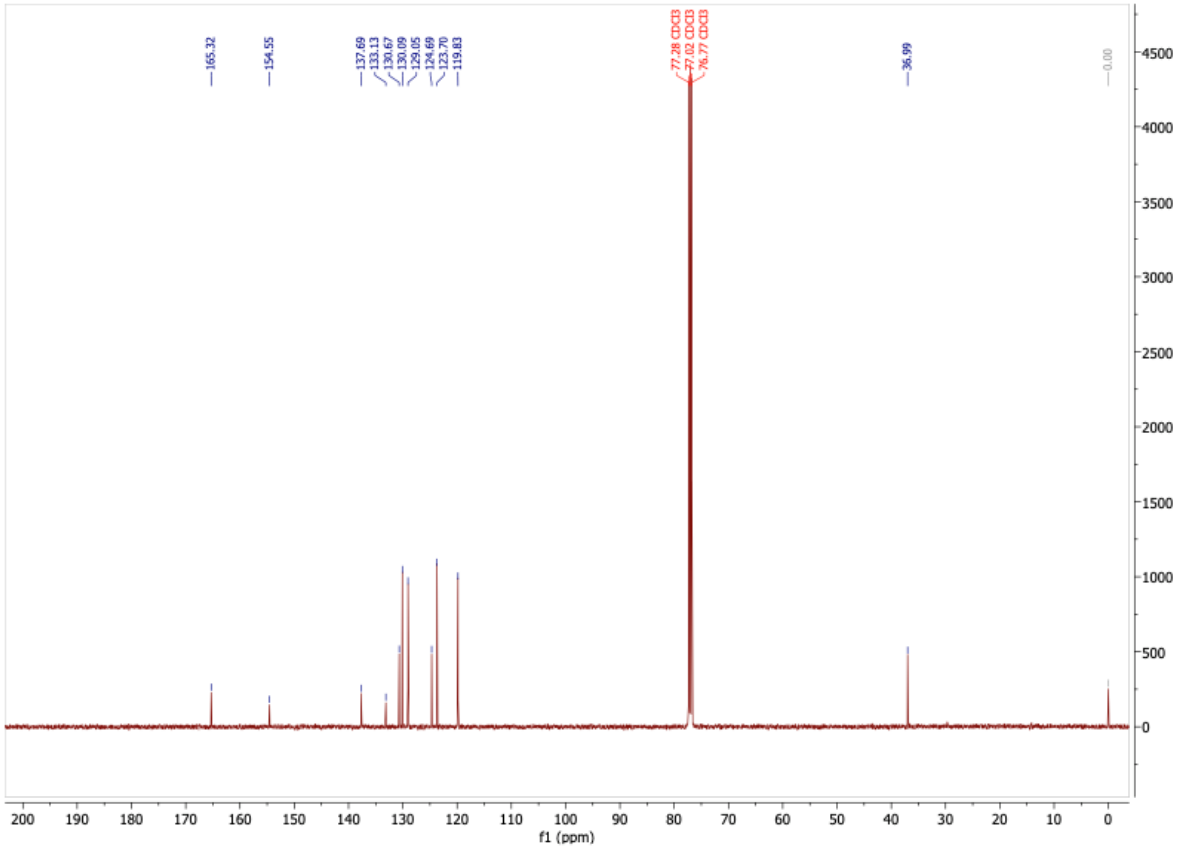
HRMS data of *N*-(isoxazol-3-yl)-2-((1-phenyl-1*H*-tetrazol-5-yl)thio)acetamide (1b)

***N*-phenyl-2-((1-phenyl-1*H*-tetrazol-5-yl)thio)acetamide(1c)**





¹H spectrum of *N*-phenyl-2-((1-phenyl-1*H*-tetrazol-5-yl)thio)acetamide(1c)



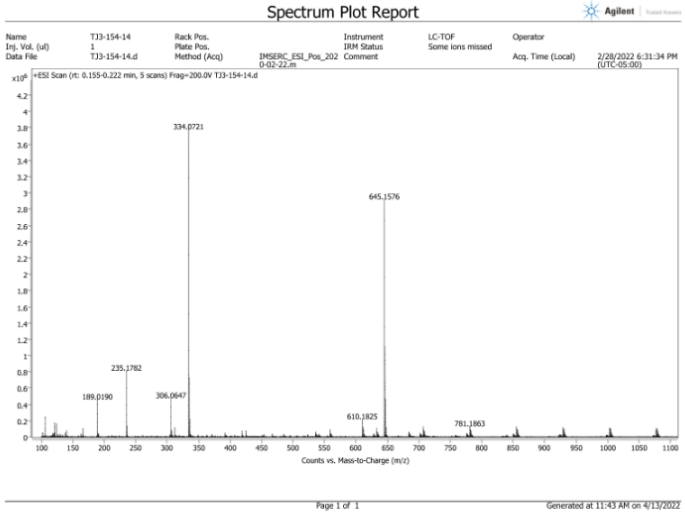
^{13}C spectrum of *N*-phenyl-2-((1-phenyl-1*H*-tetrazol-5-yl)thio)acetamide(1c)

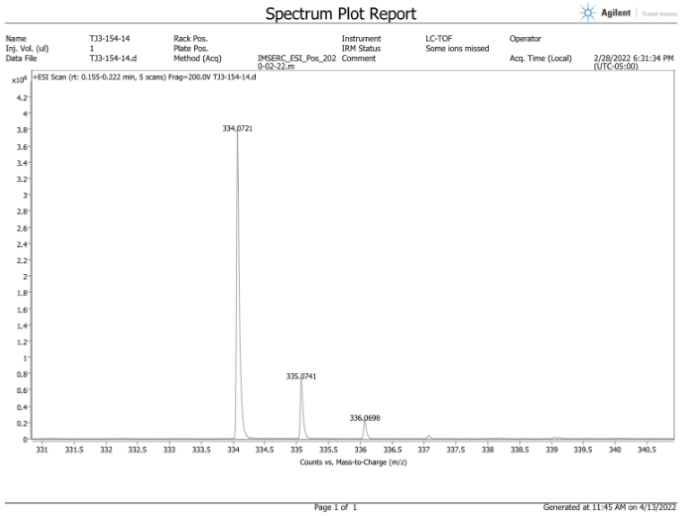
Spectrum Identification Results: + Scan (rt: 0.155-0.222 min) (TJ3-154-14.d)

Best	ID Source	Formula	Species	m/z	Score	Diff (ppm)	Diff (mDa)	Score (MFG)
	MFG	**C15 H13 N5 O S**	**(M+Na)+**	**334.0721**	**93.42**	**-4.34**	**-1.35**	**93.42**

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M+Na)+	334.0721	98.4	88.53	93.42	93.42	97.23	3785105.7	C15 H13 N5 Na O

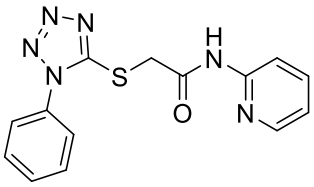
Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z	Diff (ppm)
3789277.3	79.73	100	334.0733	-1.2	3785105.7	100	79.64	334.0721	-3.62
721001.2	15.17	19.03	335.0759	-1.8	740003.8	19.55	15.57	335.0741	-5.46
242349.4	5.1	6.4	336.0719	-2.1	227518.4	6.01	4.79	336.0698	-6.33

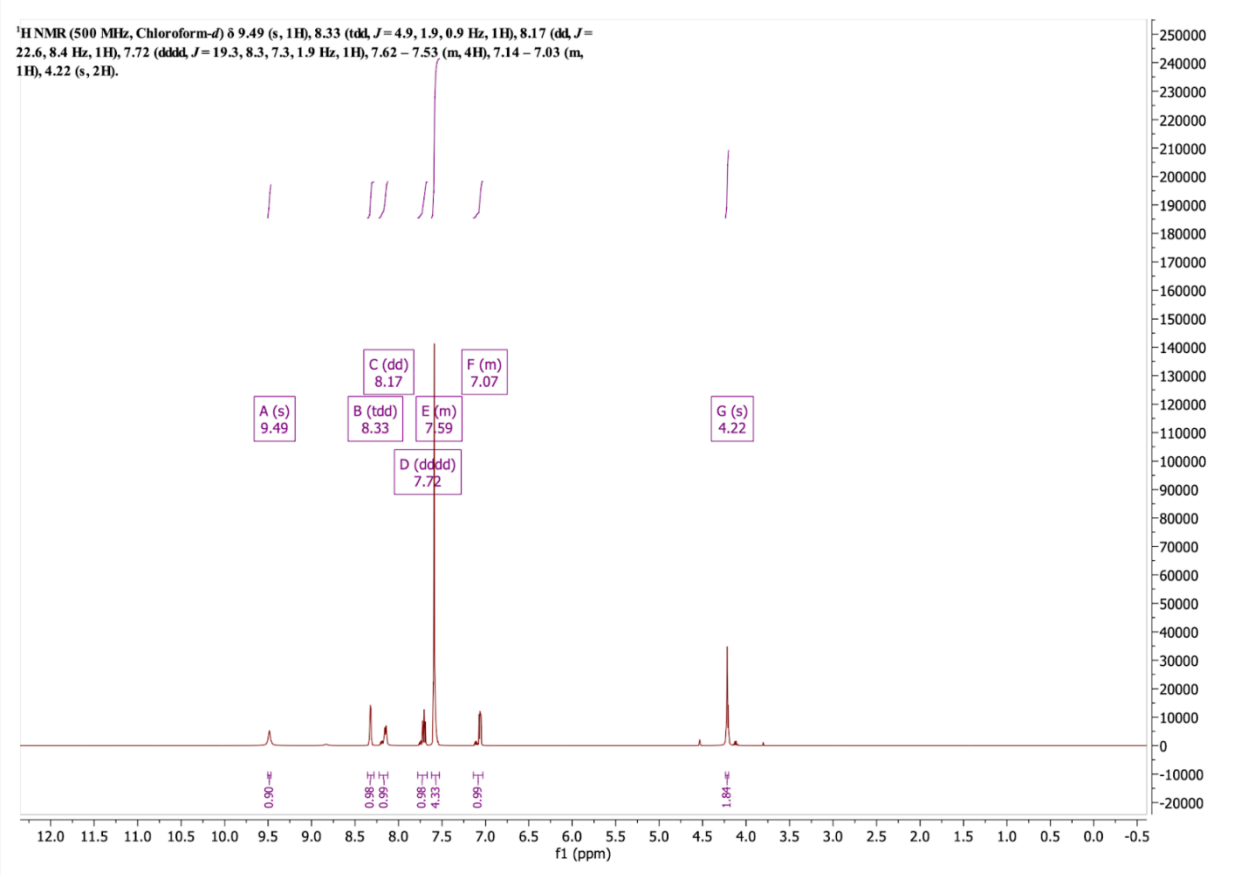




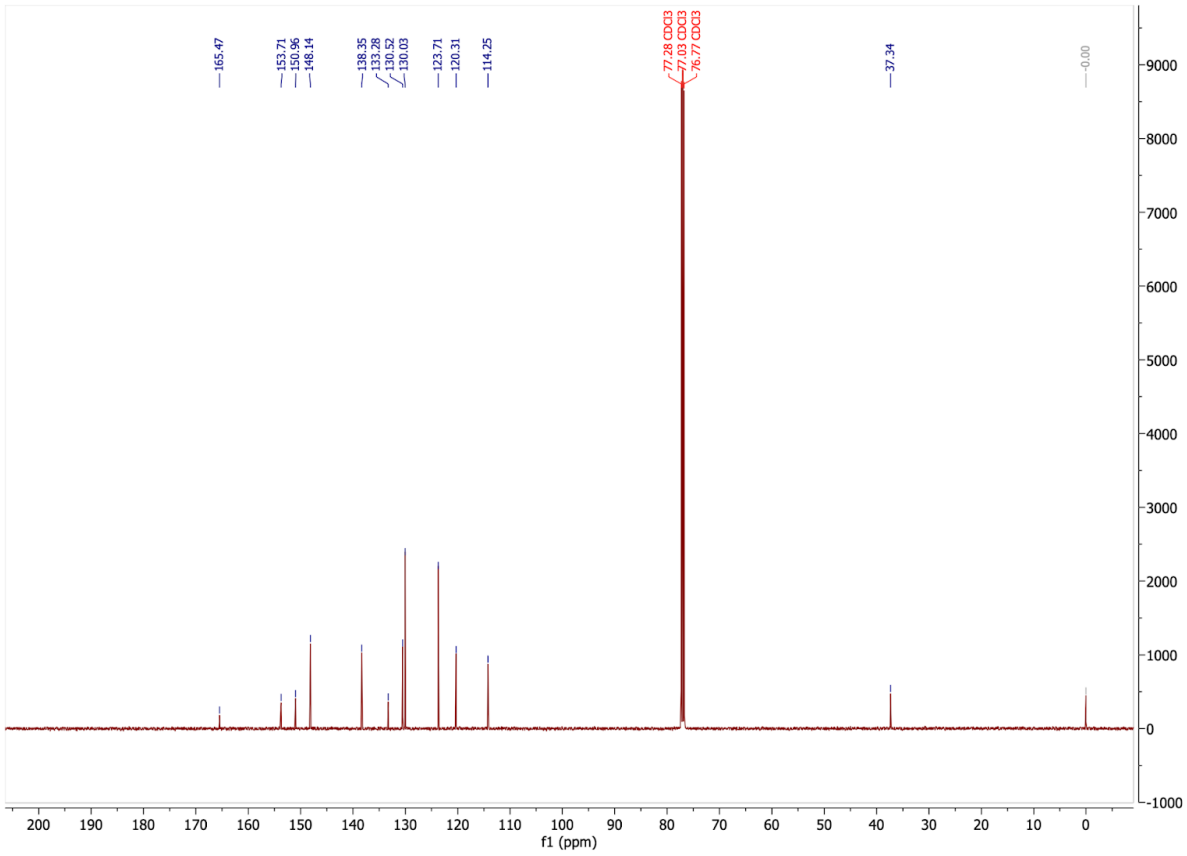
HRMS data of *N*-phenyl-2-((1-phenyl-1*H*-tetrazol-5-yl)thio)acetamide(1c)

2-((1-phenyl-1H-tetrazol-5-yl)thio)-*N*-(pyridine-2-yl)acetamide (1d)





[1]H spectrum of 2-((1-phenyl-1H-tetrazol-5-yl)thio)-*N*-(pyridine-2-yl)acetamide (1d)



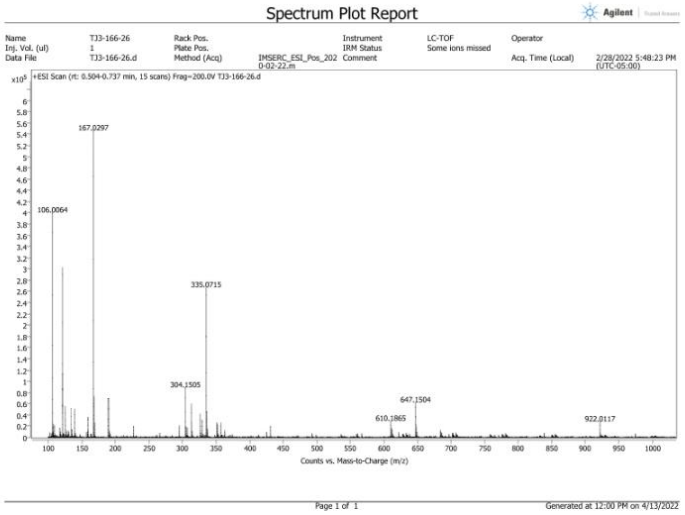
^{13}C spectrum of 2-((1-phenyl-1H-tetrazol-5-yl)thio)-*N*-(pyridine-2-yl)acetamide (1d)

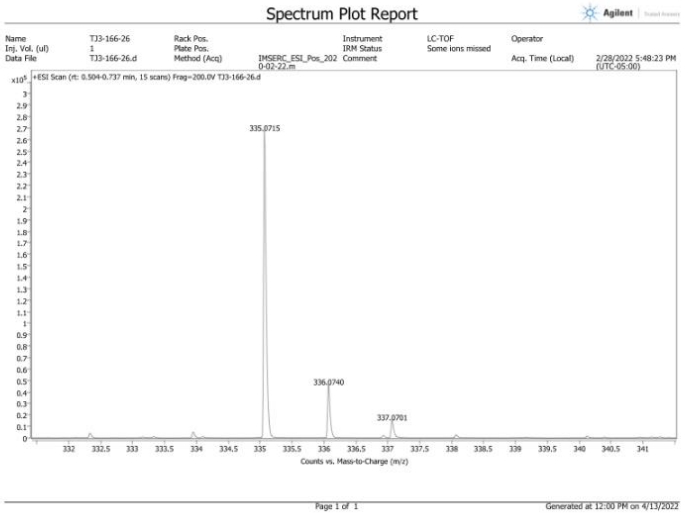
Spectrum Identification Results: + Scan (rt: 0.504-0.737 min) (TJ3-166-26.d)

Best	ID Source	Formula	Species	m/z	Score	Diff (ppm)	Diff (mDa)	Score (MFG)
☑	**MFG**	**C14 H12 N6 O S**	**(M+Na)+**	**335.0715**	**78.37**	**9.52**	**2.97**	**78.37**

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M+Na)+	335.0715	98.41	55.56	78.37	78.37	99.94	276619.9	C14 H12 N6 Na O

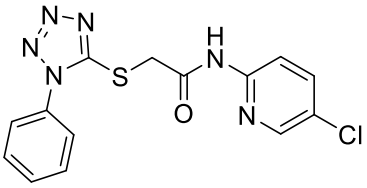
Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z	Diff (ppm)
273177	80.28	100	335.0686	3	276619.9	100	81.29	335.0715	8.82
49990.5	14.69	18.3	336.071	3	48258	17.45	14.18	336.074	8.89
17114.6	5.03	6.27	337.0669	3.2	15404.1	5.57	4.53	337.0701	9.53

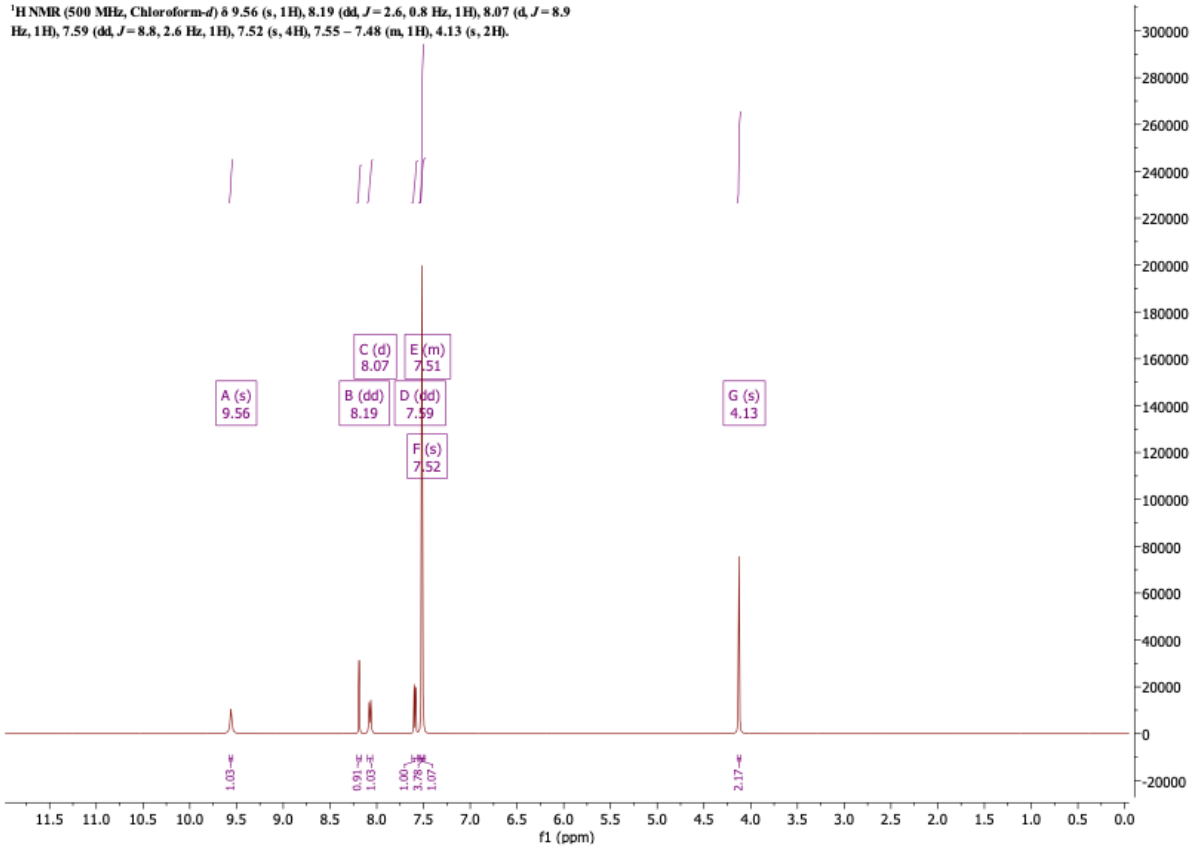




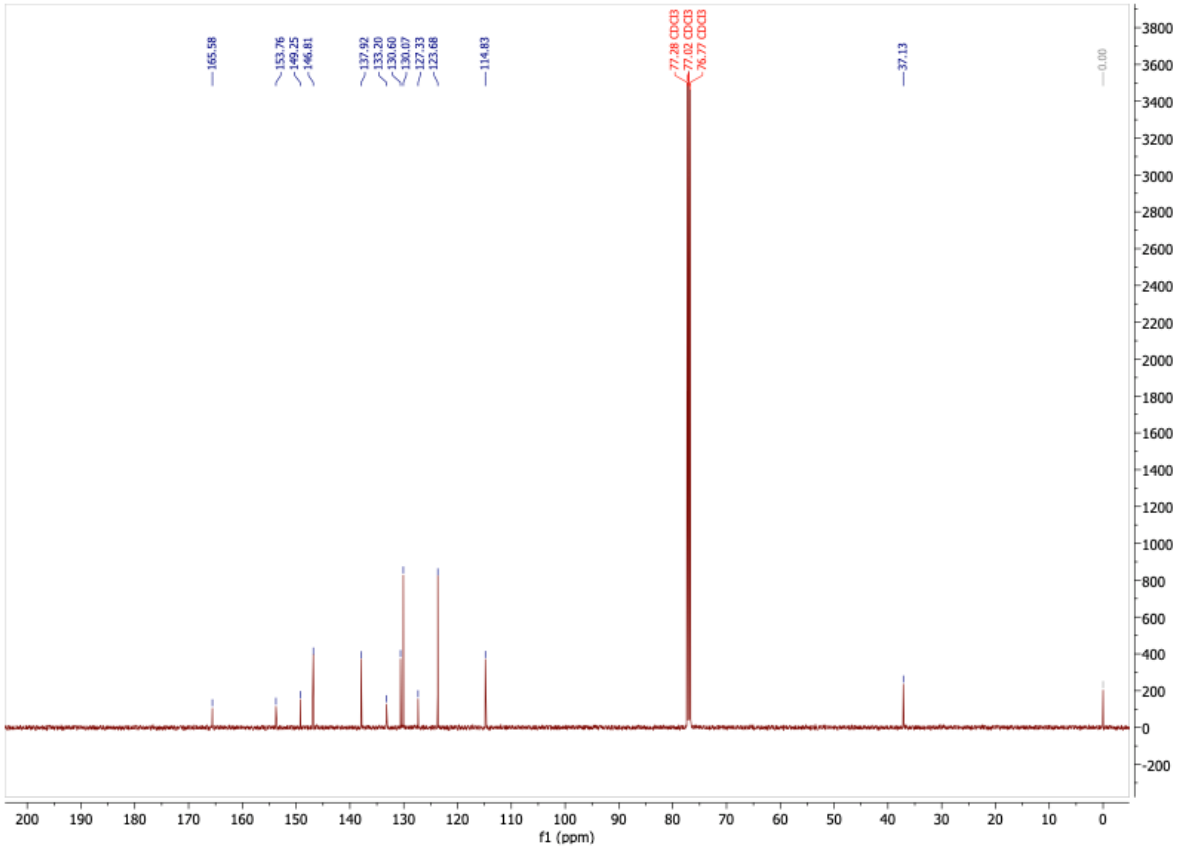
HRMS data of 2-((1-phenyl-1H-tetrazol-5-yl)thio)-*N*-(pyridine-2-yl)acetamide (1d)

_N_-(5-chloropyridin-2-yl)-2-((1-phenyl-1_H_-tetrazol-5-yl)thio)acetamide (1e)





[1]H spectrum of _N_-(5-chloropyridin-2-yl)-2-((1-phenyl-1_H_-tetrazol-5-yl)thio)acetamide (1e)



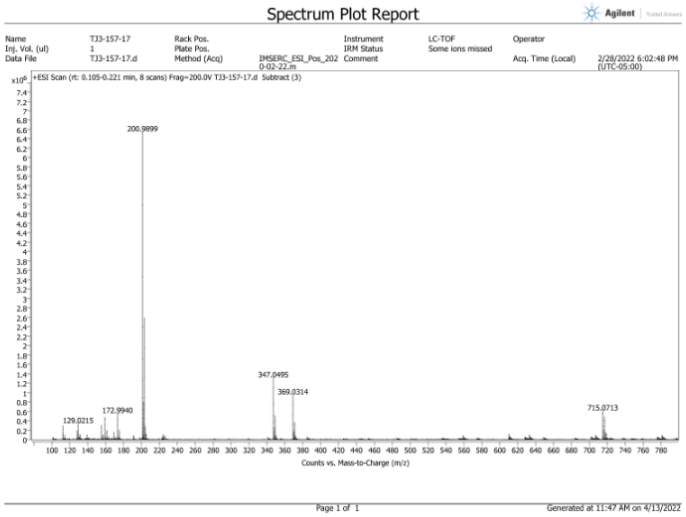
^{13}C spectrum of *N*-(5-chloropyridin-2-yl)-2-((1-phenyl-1*H*-tetrazol-5-yl)thio)acetamide (1e)

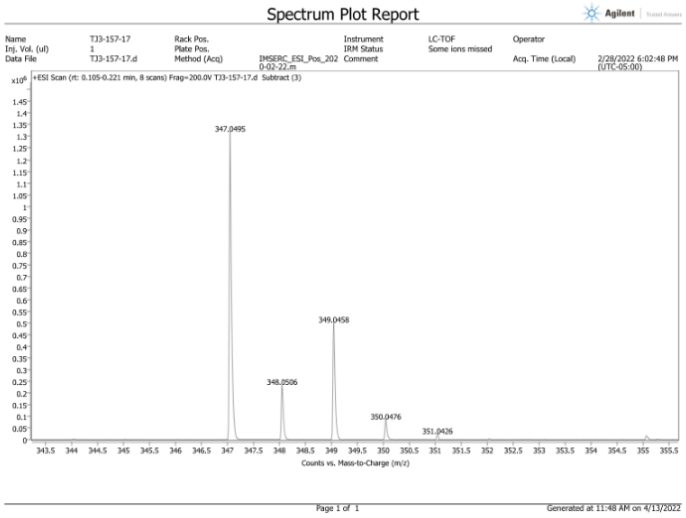
Spectrum Identification Results: + Scan (rt: 0.105-0.221 min) Sub (3) (TJ3-157-17.d)

Best	ID Source	Formula	Species	m/z	Score	Diff (ppm)	Diff (mDa)	Score (MFG)
	MFG	**C14 H11 Cl N6 O S**	**(M+H)+**	**347.0495**	**91.51**	**4.09**	**1.42**	**91.51**

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M+H)+	347.0495	99.89	87.9	91.51	91.51	88.69	1320784.5	C14 H12 Cl N6 O

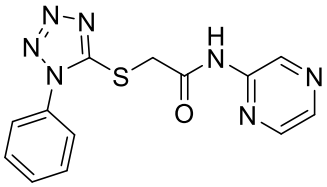
Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z	Diff (ppm)
1314346.8	60.45	100	347.0476	1.9	1320784.5	100	60.75	347.0495	5.45
240521	11.06	18.3	348.0501	0.5	239610	18.14	11.02	348.0506	1.44
502879.6	23.13	38.26	349.0449	0.9	502508.9	38.05	23.11	349.0458	2.5
88883.2	4.09	6.76	350.0471	0.4	86194.6	6.53	3.96	350.0476	1.27
27543.2	1.27	2.1	351.0433	-0.7	25075.9	1.9	1.15	351.0426	-1.97

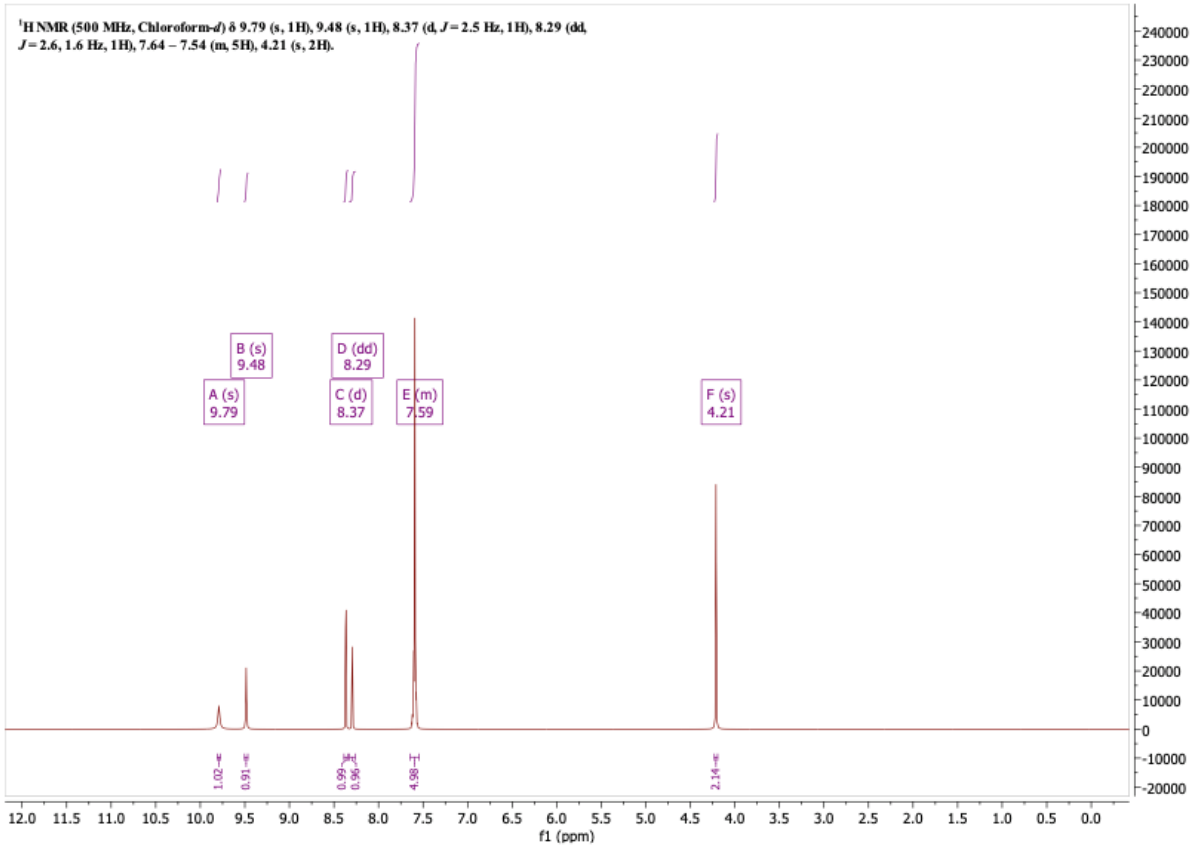




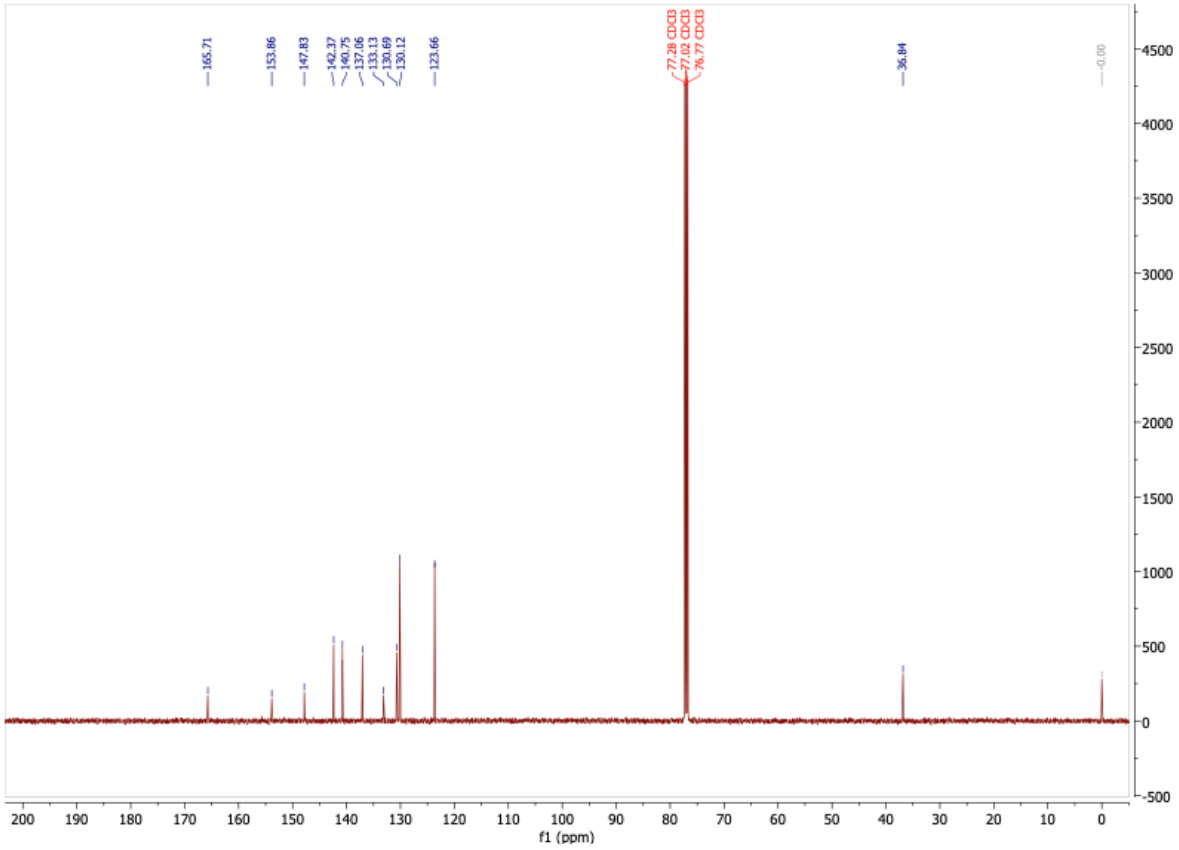
HRMS data of *N*-(5-chloropyridin-2-yl)-2-((1-phenyl-1*H*-tetrazol-5-yl)thio)acetamide (1e)

2-((1-phenyl-1*H*-tetrazol-5-yl)thio)-*N*-(pyrazin-2-yl)acetamide (1f)





¹H spectrum of 2-((1-phenyl-1*H*-tetrazol-5-yl)thio)-*N*-(pyrazin-2-yl)acetamide (1f)



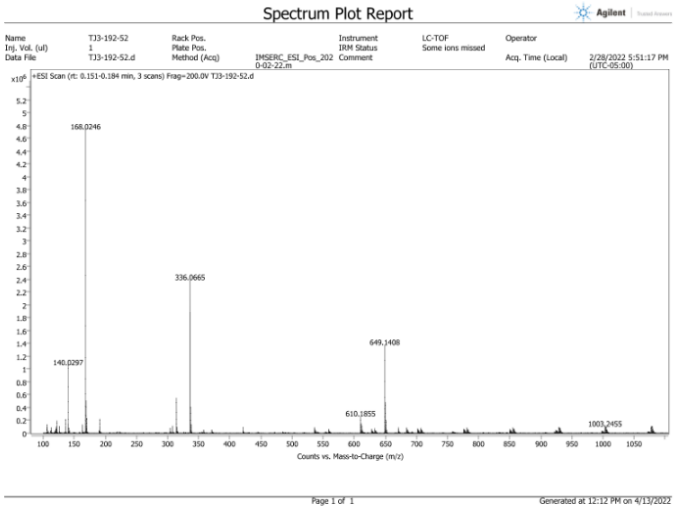
^{13}C spectrum of 2-((1-phenyl-1*H*-tetrazol-5-yl)thio)-*N*-(pyrazin-2-yl)acetamide (1f)

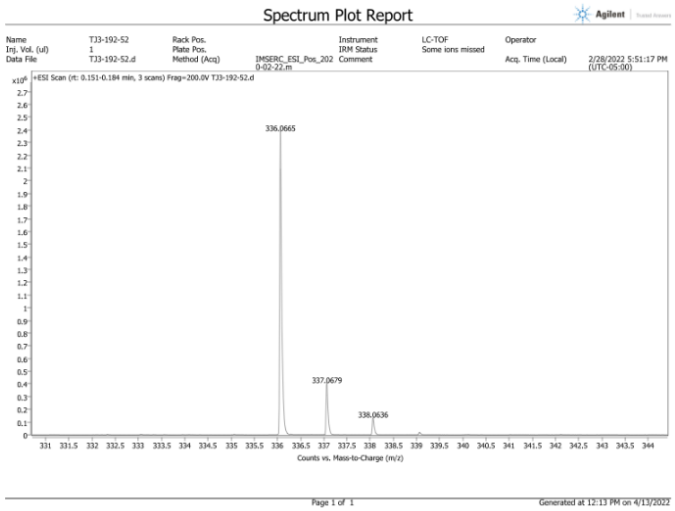
Spectrum Identification Results: + Scan (rt: 0.151-0.184 min) (TJ3-192-52.d)

Best	ID Source	Formula	Species	m/z	Score	Diff (ppm)	Diff (mDa)	Score (MFG)
	MFG	**C13 H11 N7 O S**	**(M+Na)+**	**336.0665**	**81.66**	**8.16**	**2.55**	**81.66**

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M+Na)+	336.0665	99	64.85	81.66	81.66	94.49	2397614.5	C13 H11 N7 Na O

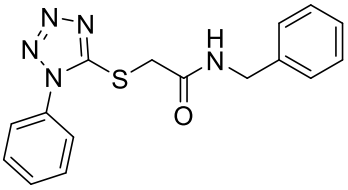
Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z	Diff (ppm)
2384192.7	80.83	100	336.0638	2.7	2397614.5	100	81.29	336.0665	8.15
418948.4	14.2	17.57	337.0661	1.8	415098.2	17.31	14.07	337.0679	5.31
146381.7	4.96	6.14	338.0619	1.7	136810.1	5.71	4.64	338.0636	4.91

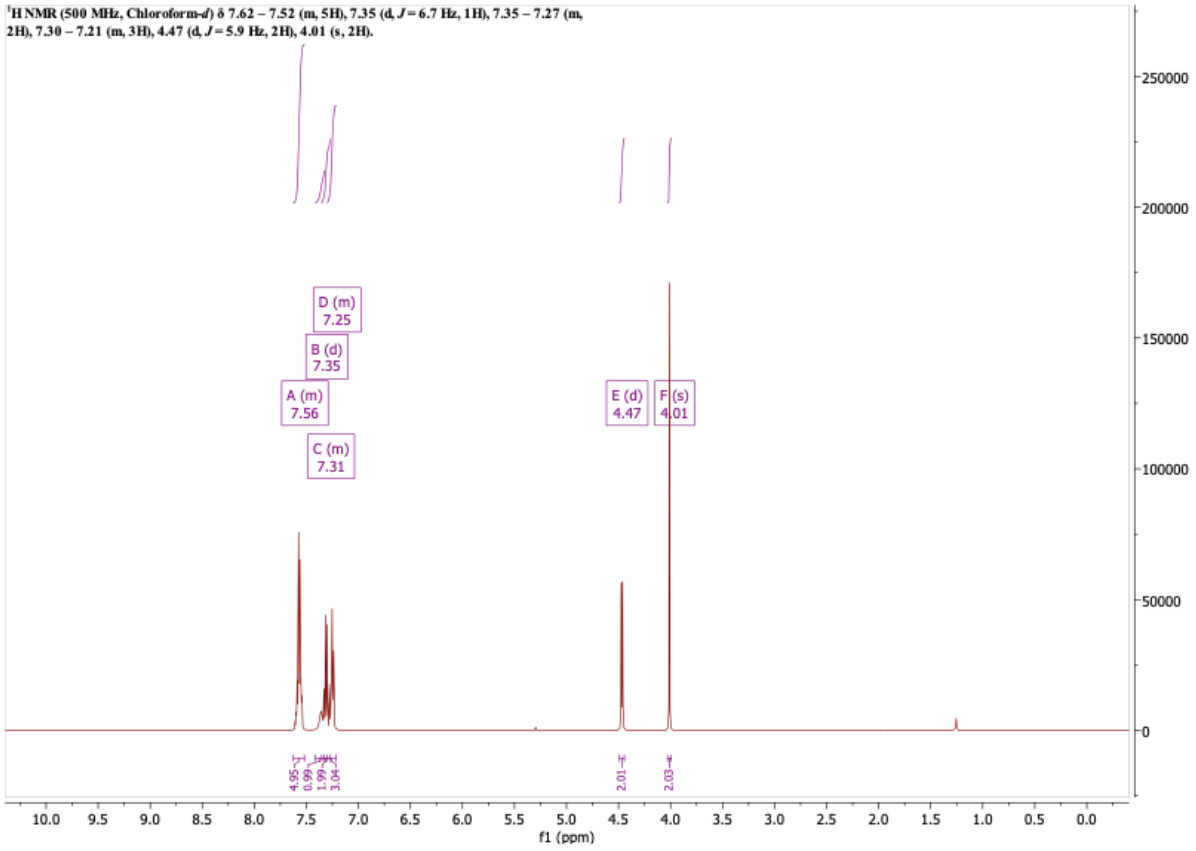




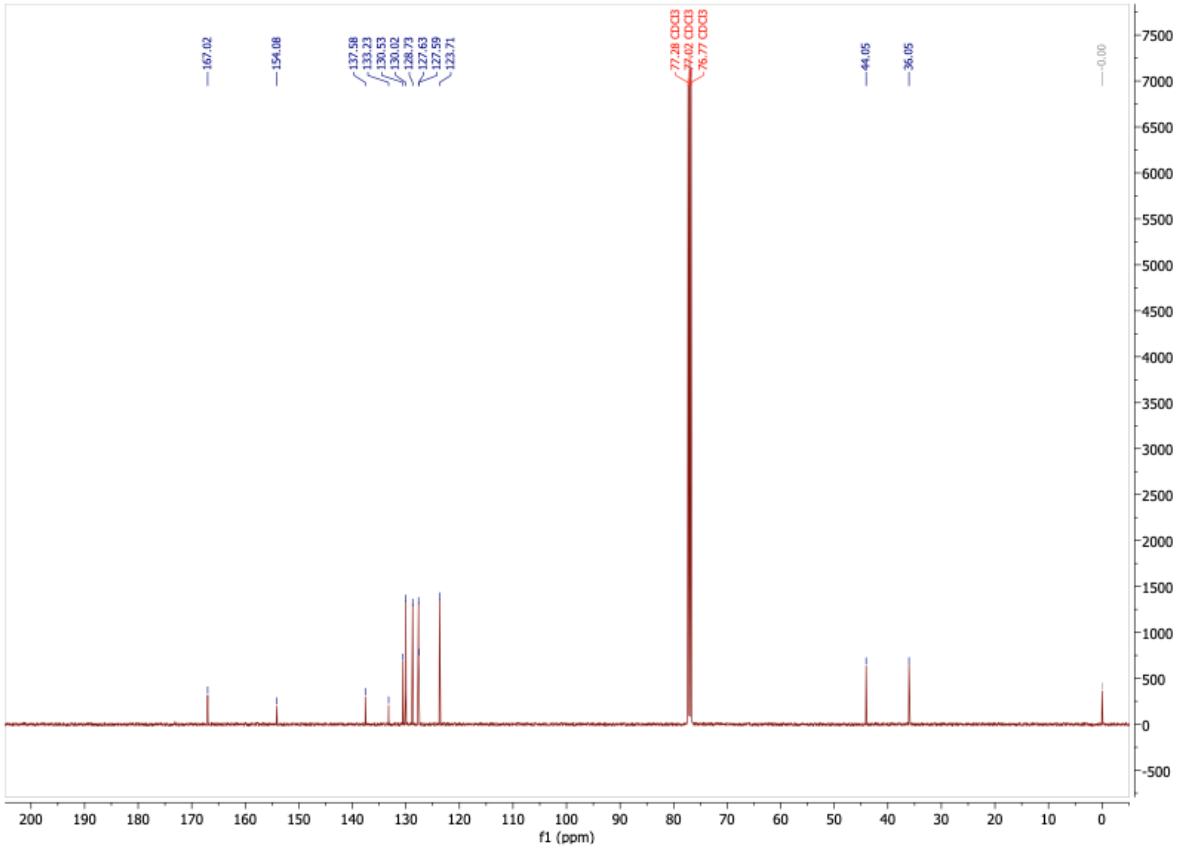
HRMS data of 2-((1-phenyl-1*H*-tetrazol-5-yl)thio)-*N*-(pyrazin-2-yl)acetamide (1f)

***N*-benzyl-2-((1-phenyl-1*H*-tetrazol-5-yl)thio)acetamide (1g)**





^{1}H spectrum of *N*-benzyl-2-((1-phenyl-1*H*-tetrazol-5-yl)thio)acetamide (1g)



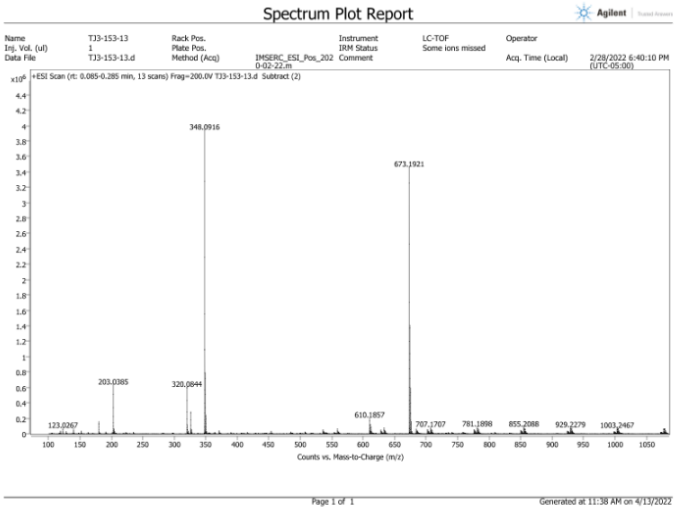
^{13}C spectrum of *N*-benzyl-2-((1-phenyl-1*H*-tetrazol-5-yl)thio)acetamide (1g)

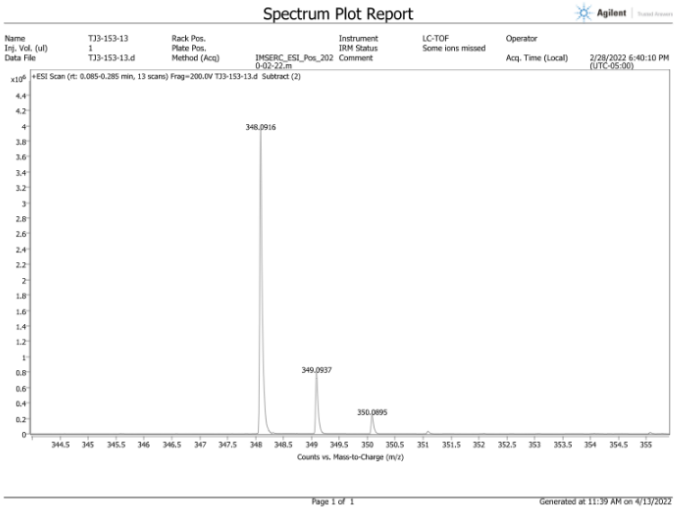
Spectrum Identification Results: + Scan (rt: 0.085-0.285 min) Sub (2) (TJ3-153-13.d)

Best	ID Source	Formula	Species	m/z	Score	Diff (ppm)	Diff (mDa)	Score (MFG)
☑	**MFG**	**C16 H15 N5 O S**	**(M+Na)+**	**348.0916**	**83.4**	**7.69**	**2.5**	**83.4**

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M+Na)+	348.0916	98.64	66.97	83.4	83.4	97.96	3946693.5	C16 H15 N5 Na O

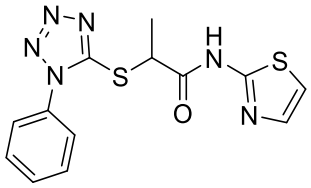
Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z	Diff (ppm)
3941028.4	78.9	100	348.089	2.6	3946693.5	100	79.02	348.0916	7.54
793407.2	15.88	20.13	349.0916	2.1	800958.9	20.29	16.04	349.0937	6.08
260347.6	5.21	6.61	350.0878	1.7	247130.8	6.26	4.95	350.0895	4.94

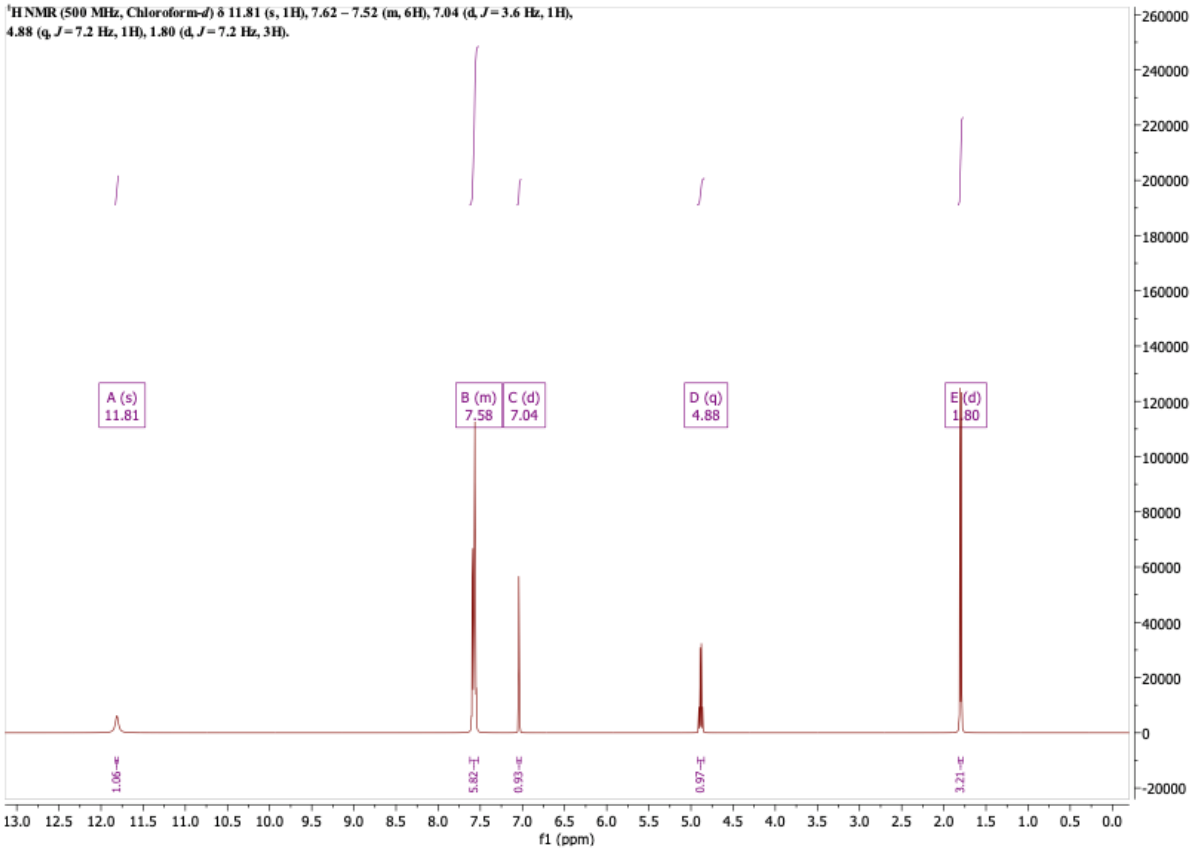




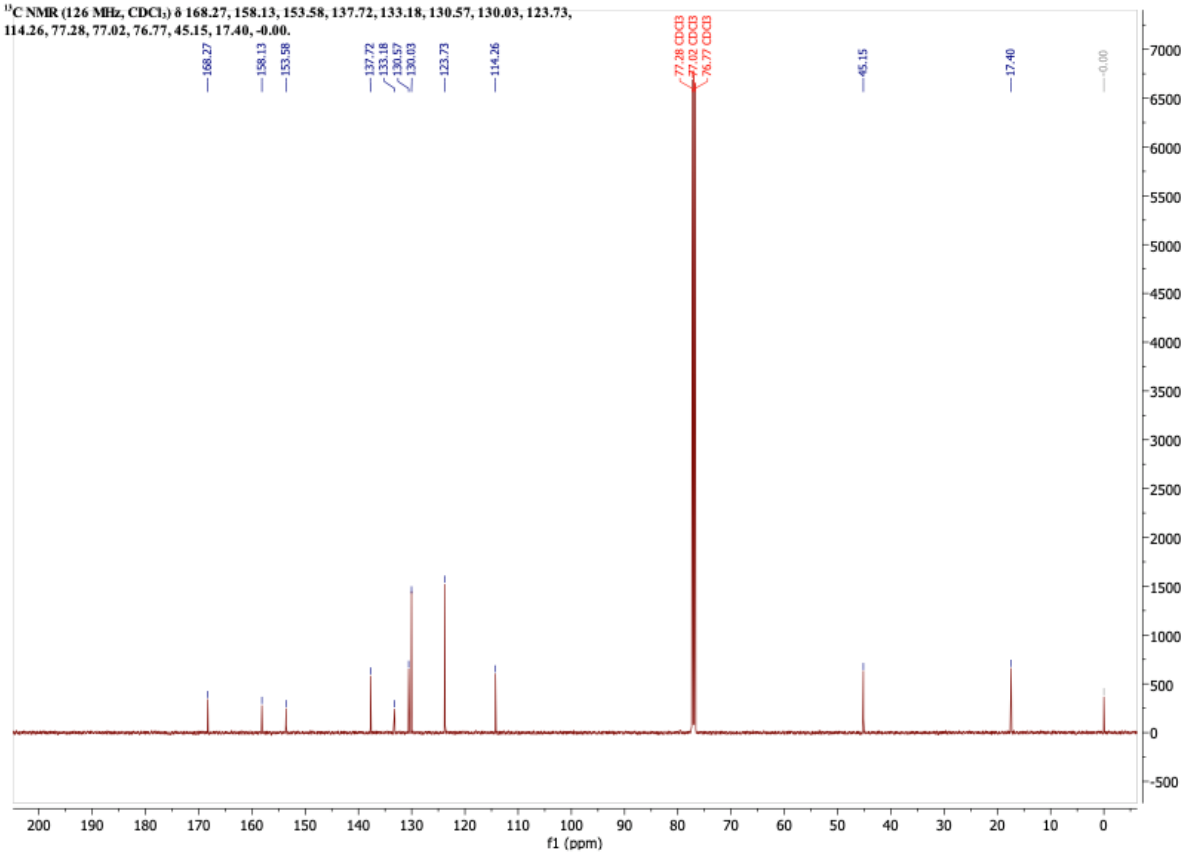
HRMS data of *N*-benzyl-2-((1-phenyl-1*H*-tetrazol-5-yl)thio)acetamide (1g)

2-((1-phenyl-1*H*-tetrazol-5-yl)thio)-*N*-(thiazol-2-yl)propanamide (2a)





[1]H spectrum of 2-((1-phenyl-1*H*-tetrazol-5-yl)thio)-*N*-(thiazol-2-yl)propanamide (2a)



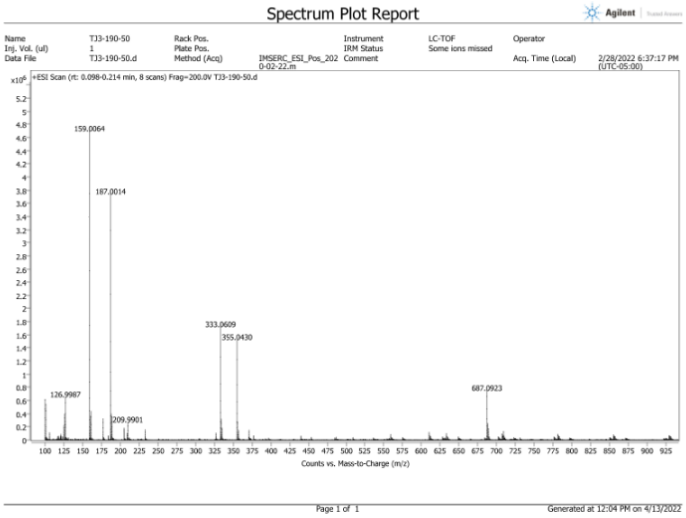
^{13}C spectrum of 2-((1-phenyl-1*H*-tetrazol-5-yl)thio)-*N*-(thiazol-2-yl)propanamide (2a)

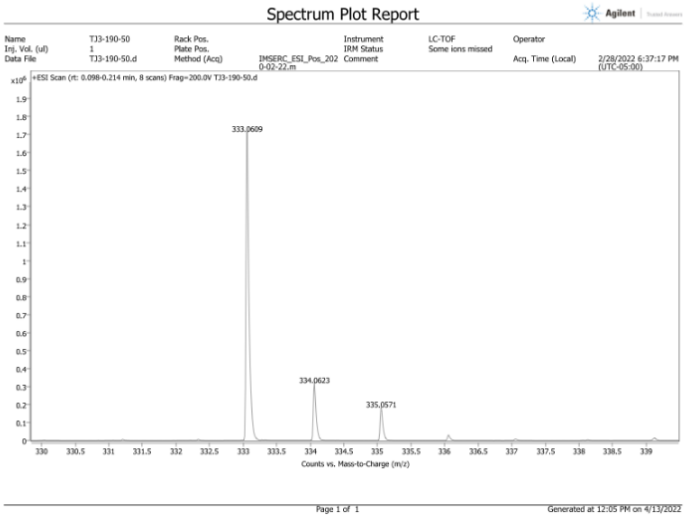
Spectrum Identification Results: + Scan (rt: 0.098-0.214 min) (TJ3-190-50.d)

Best	ID Source	Formula	Species	m/z	Score	Diff (ppm)	Diff (mDa)	Score (MFG)
☑	**MFG**	**C13 H12 N6 O S2**	**(M+H)+**	**333.0609**	**86.71**	**6.14**	**2.04**	**86.71**

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M+H)+	333.0609	99.81	75.73	86.71	86.71	92.96	1769131.9	C13 H13 N6 O S2

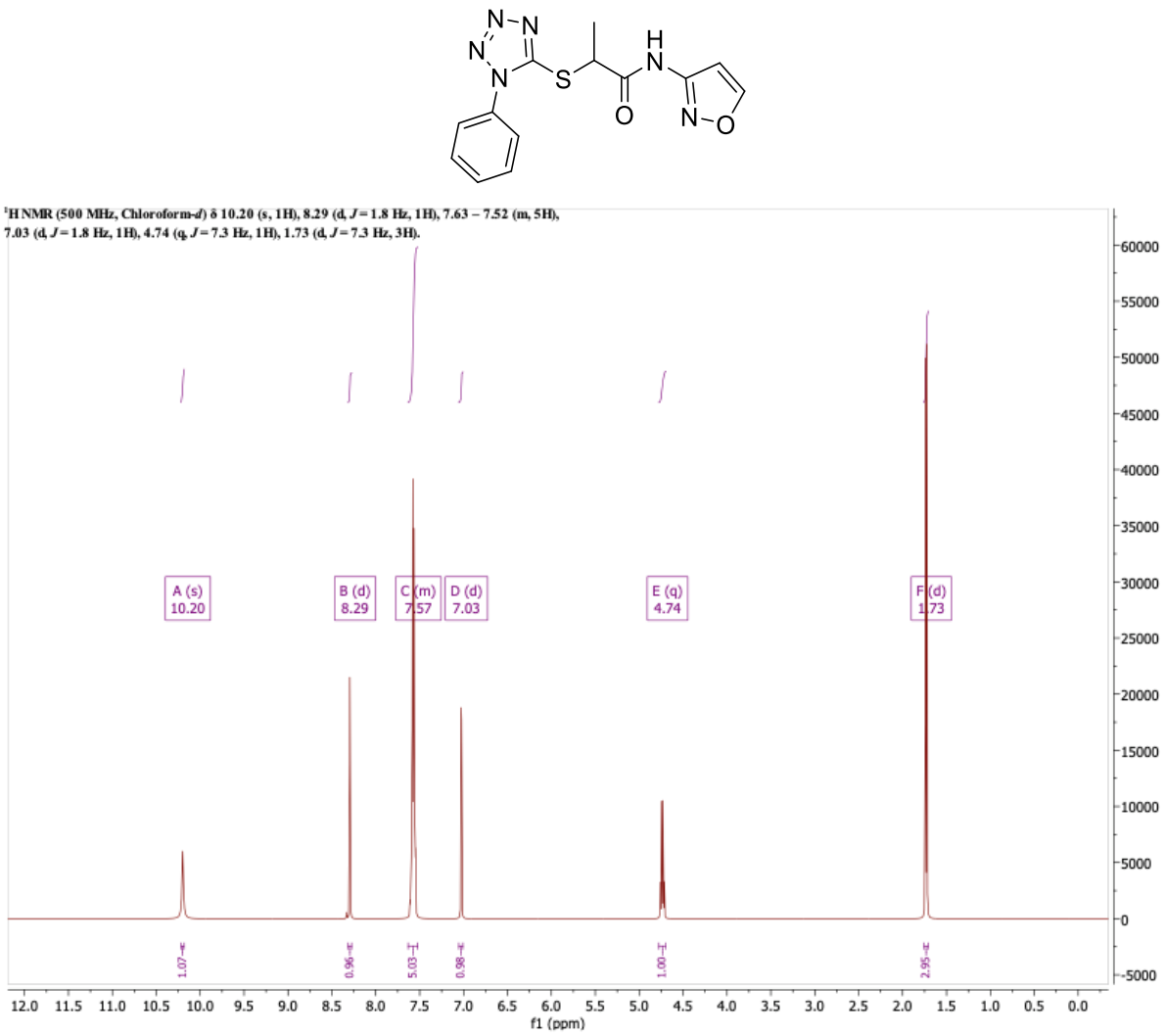
Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z	Diff (ppm)
1767569.4	76.7	100	333.0587	2.3	1769131.9	100	76.77	333.0609	6.8
318501	13.82	18.02	334.0609	1.3	320319.4	18.11	13.9	334.0623	4
188970.3	8.2	10.69	335.0559	1.2	185692.2	10.5	8.06	335.0571	3.62
29363.9	1.27	1.66	336.0575	1.3	29261	1.65	1.27	336.0588	3.91



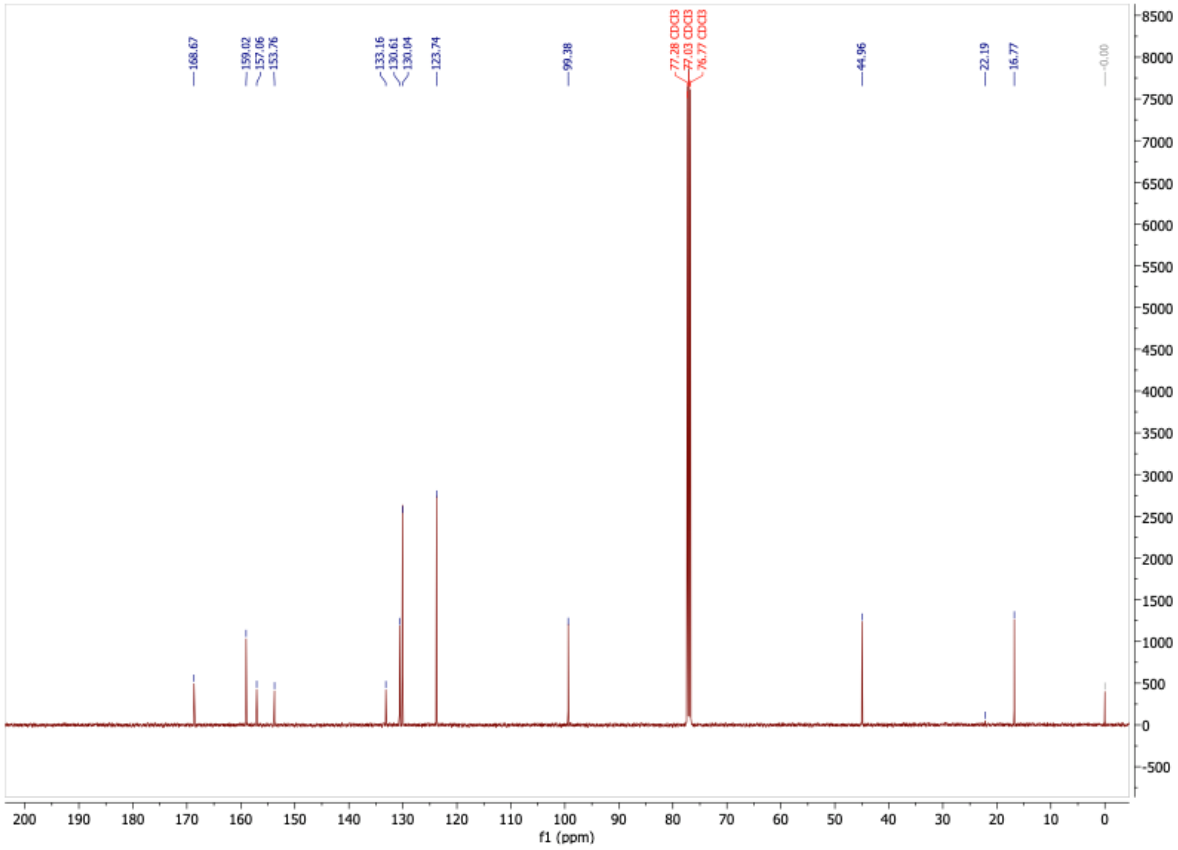


HRMS data of 2-((1-phenyl-1*H*-tetrazol-5-yl)thio)-*N*-(thiazol-2-yl)propanamide (2a)

_N_-(isoxazol-3-yl)-2-((1-phenyl-1_H_-tetrazol-5-yl)thio)propanamide (2b)



[1]H spectrum of _N_-(isoxazol-3-yl)-2-((1-phenyl-1_H_-tetrazol-5-yl)thio)propanamide (2b)



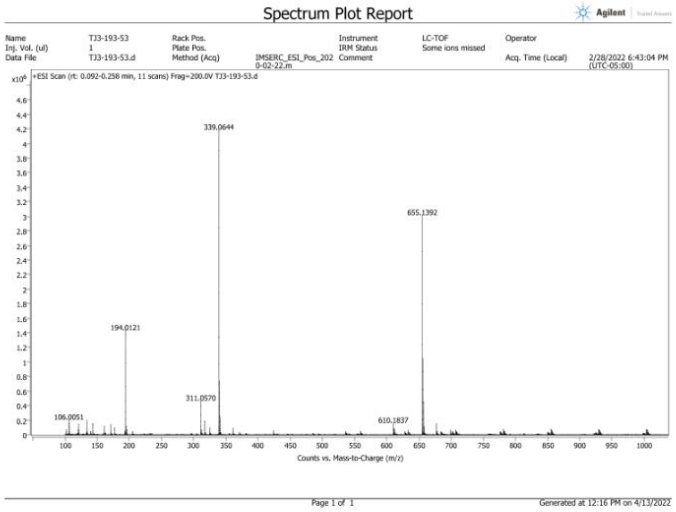
^{13}C spectrum of *N*-(isoxazol-3-yl)-2-((1-phenyl-1*H*-tetrazol-5-yl)thio)propanamide (2b)

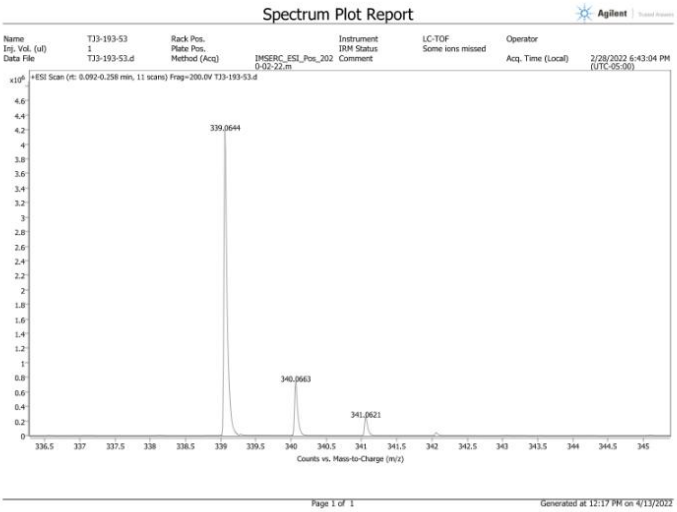
Spectrum Identification Results: + Scan (rt: 0.092-0.258 min) (TJ3-193-53.d)

Best	ID Source	Formula	Species	m/z	Score	Diff (ppm)	Diff (mDa)	Score (MFG)
☑	**MFG**	**C13 H12 N6 O2 S**	**(M+Na)+**	**339.0644**	**96.96**	**2.7**	**0.85**	**96.96**

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M+Na)+	339.0644	99.03	95.32	96.96	96.96	97.74	4278326.8	C13 H12 N6 Na O

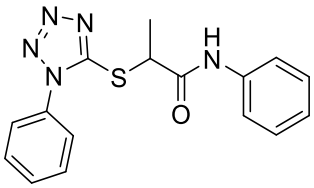
Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z	Diff (ppm)
4275144.3	80.94	100	339.0635	1	4278326.8	100	81	339.0644	2.87
737726.8	13.97	17.26	340.0659	0.4	742530.1	17.36	14.06	340.0663	1.14
268943	5.09	6.29	341.0618	0.2	260957.3	6.1	4.94	341.0621	0.71

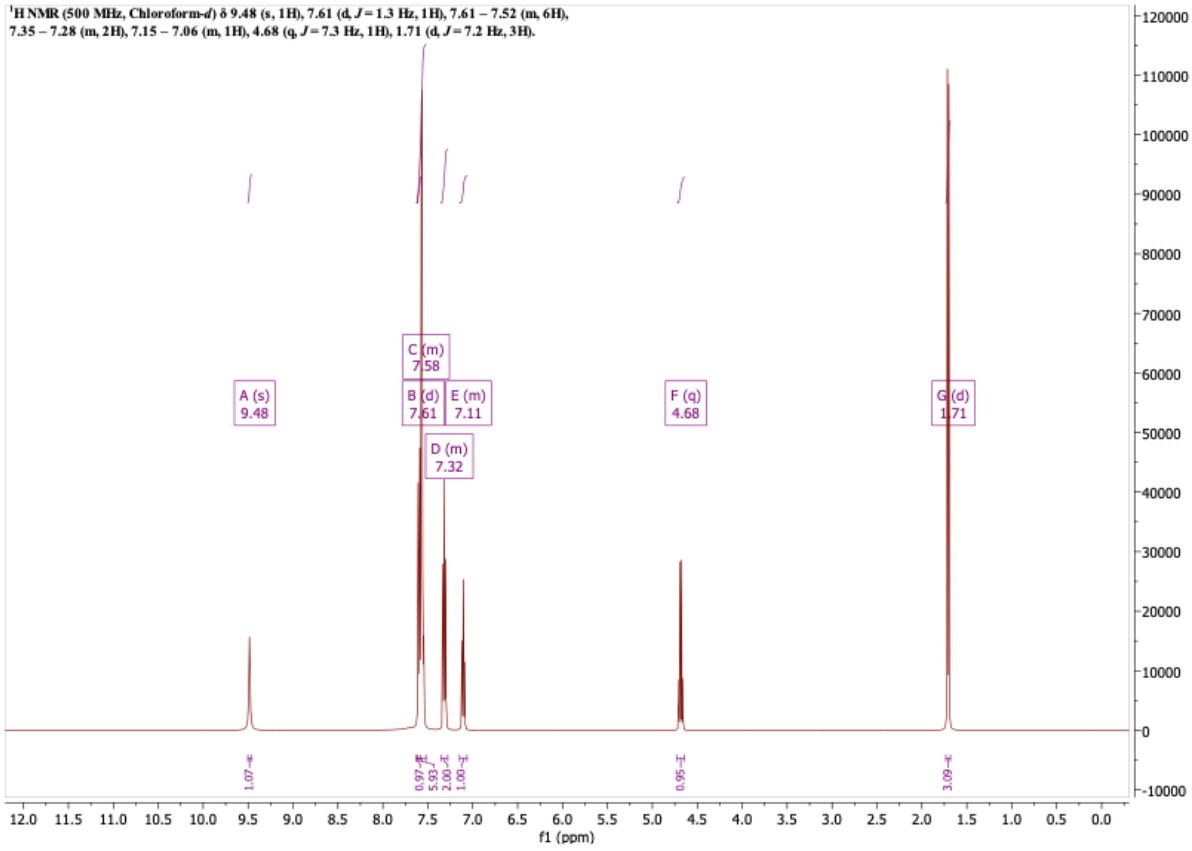




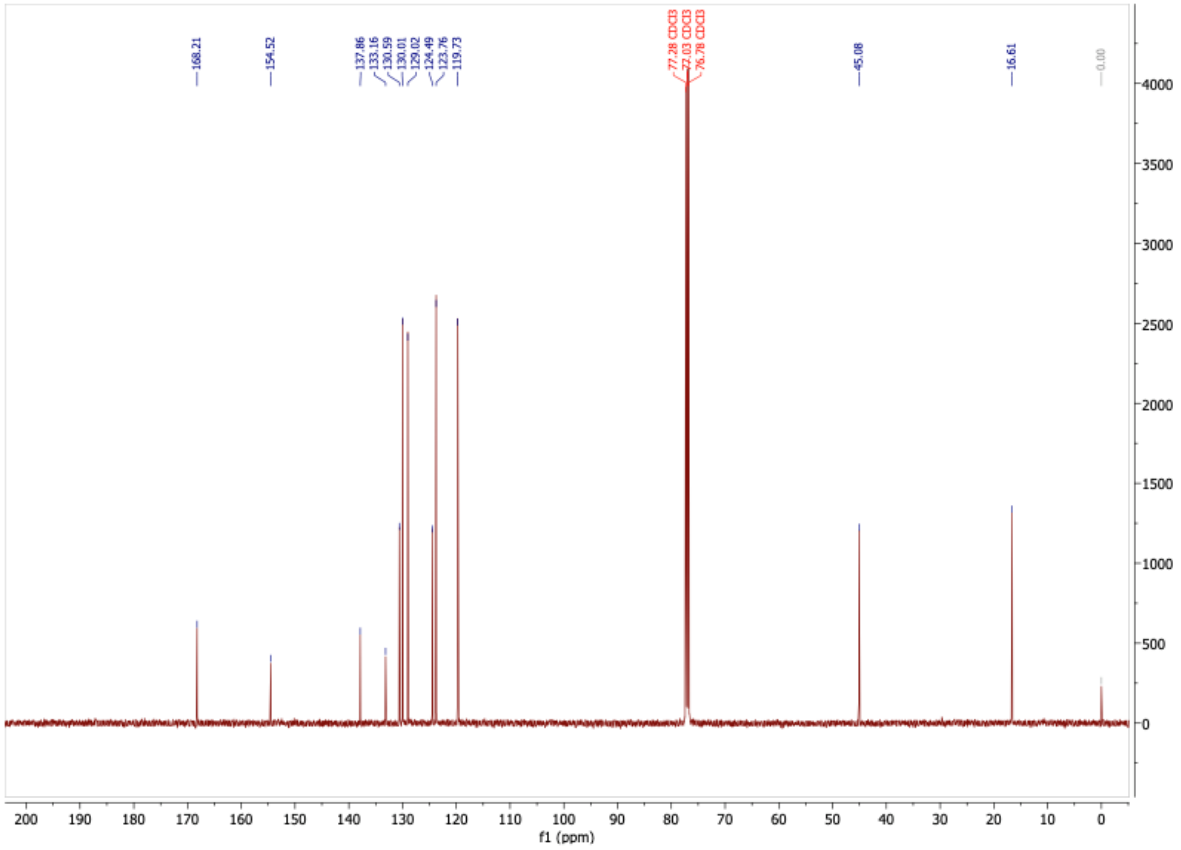
HRMS data of *N*-(isoxazol-3-yl)-2-((1-phenyl-1*H*-tetrazol-5-yl)thio)propanamide (2b)

_N_-phenyl-2-((1-phenyl-1_H_-tetrazol-5-yl)thio)propanamide (2c)





[1]H spectrum of _N_-phenyl-2-((1-phenyl-1_H_-tetrazol-5-yl)thio)propanamide (2c)



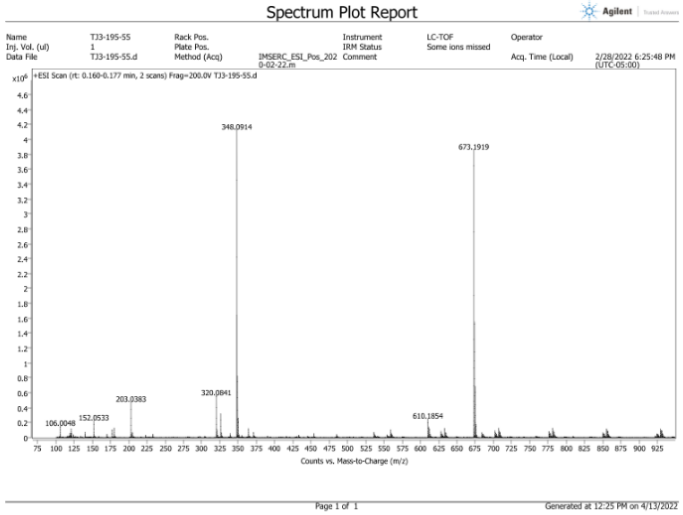
^{13}C spectrum of *N*-phenyl-2-((1-phenyl-1*H*-tetrazol-5-yl)thio)propanamide (2c)

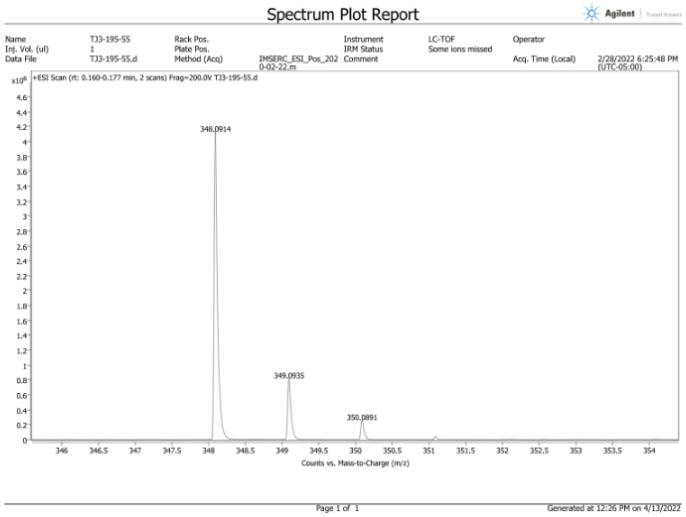
Spectrum Identification Results: + Scan (rt: 0.160-0.177 min) (TJ3-195-55.d)

Best	ID Source	Formula	Species	m/z	Score	Diff (ppm)	Diff (mDa)	Score (MFG)
☑	**MFG**	**C16 H15 N5 O S**	**(M+Na)+**	**348.0914**	**85.18**	**7.12**	**2.31**	**85.18**

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M+Na)+	348.0914	98.79	70.94	85.18	85.18	97.35	4130689.2	C16 H15 N5 Na O

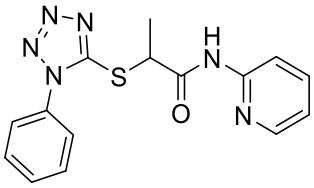
Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z	Diff (ppm)
4123654.5	78.9	100	348.089	2.5	4130689.2	100	79.04	348.0914	7.06
830173.5	15.88	20.13	349.0916	1.9	832255.3	20.15	15.92	349.0935	5.49
272412.1	5.21	6.61	350.0878	1.3	263295.6	6.37	5.04	350.0891	3.82

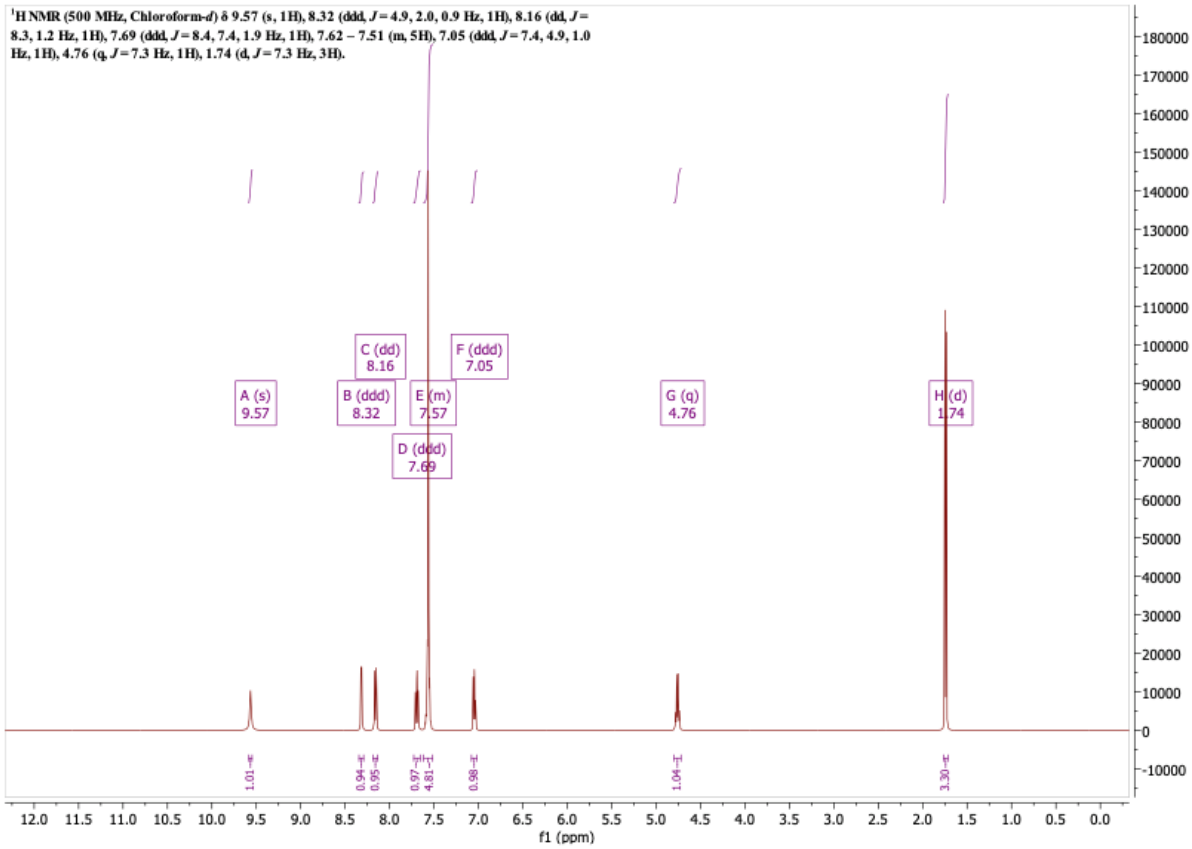




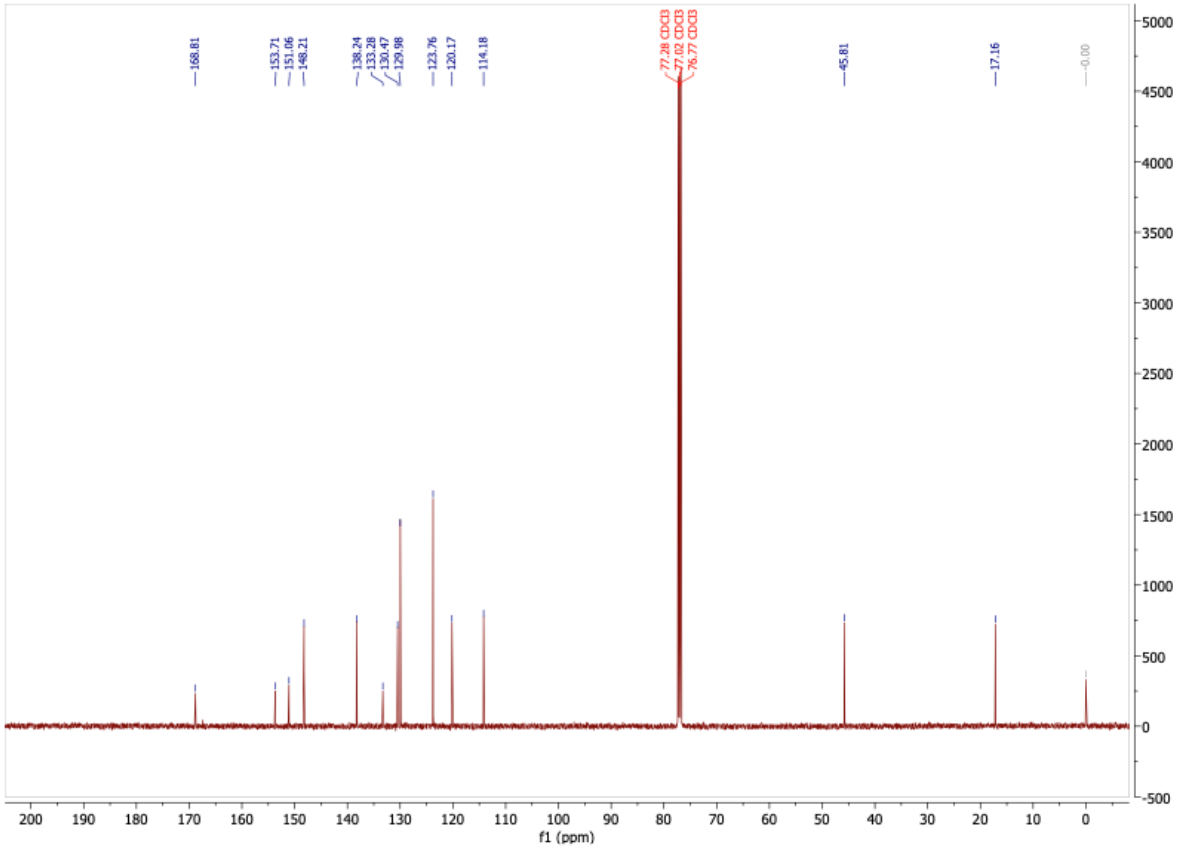
HRMS data of *N*-phenyl-2-((1-phenyl-1*H*-tetrazol-5-yl)thio)propanamide (2c)

2-((1-phenyl-1*H*-tetrazol-5-yl)thio)-*N*-(pyridin-2-yl)propanamide (2d)





[1]H spectrum of 2-((1-phenyl-1*H*-tetrazol-5-yl)thio)-*N*-(pyridin-2-yl)propanamide (2d)



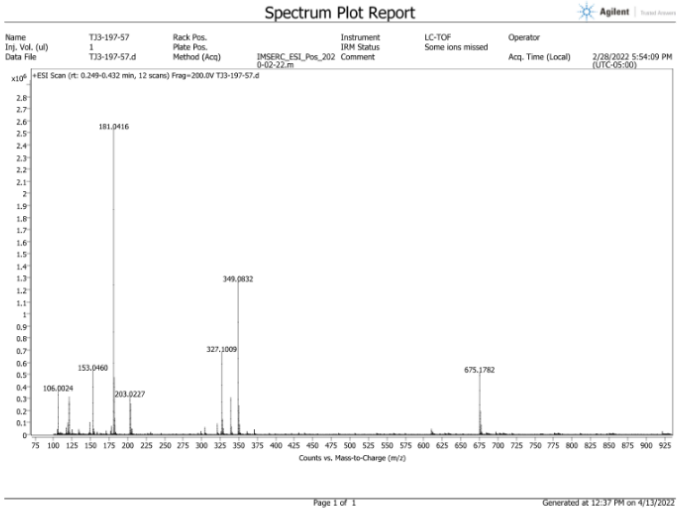
^{13}C spectrum of 2-((1-phenyl-1*H*-tetrazol-5-yl)thio)-*N*-(pyridin-2-yl)propanamide (2d)

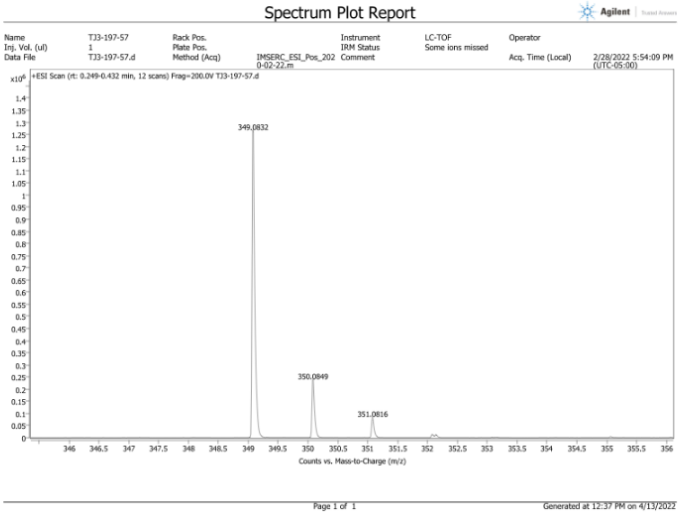
Spectrum Identification Results: + Scan (rt: 0.249-0.432 min) (TJ3-197-57.d)

Best	ID Source	Formula	Species	m/z	Score	Diff (ppm)	Diff (mDa)	Score (MFG)
	MFG	**C15 H14 N6 O S**	**(M+Na)+**	**349.0832**	**95.24**	**-3.45**	**-1.13**	**95.24**

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M+Na)+	349.0832	99.47	92.21	95.24	95.24	96.23	1329590.1	C15 H14 N6 Na O

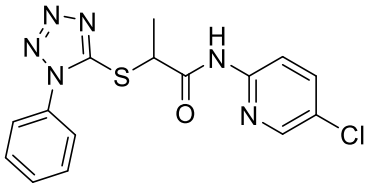
Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z	Diff (ppm)
1321249.1	78.83	100	349.0842	-1	1329590.1	100	79.33	349.0832	-2.83
256378.3	15.3	19.4	350.0867	-1.9	247166.5	18.59	14.75	350.0849	-5.29
85450.5	5.1	6.47	351.0828	-1.2	85865.6	6.46	5.12	351.0816	-3.45
12904.2	0.77	0.98	352.0839	-1	13359.9	1	0.8	352.083	-2.74

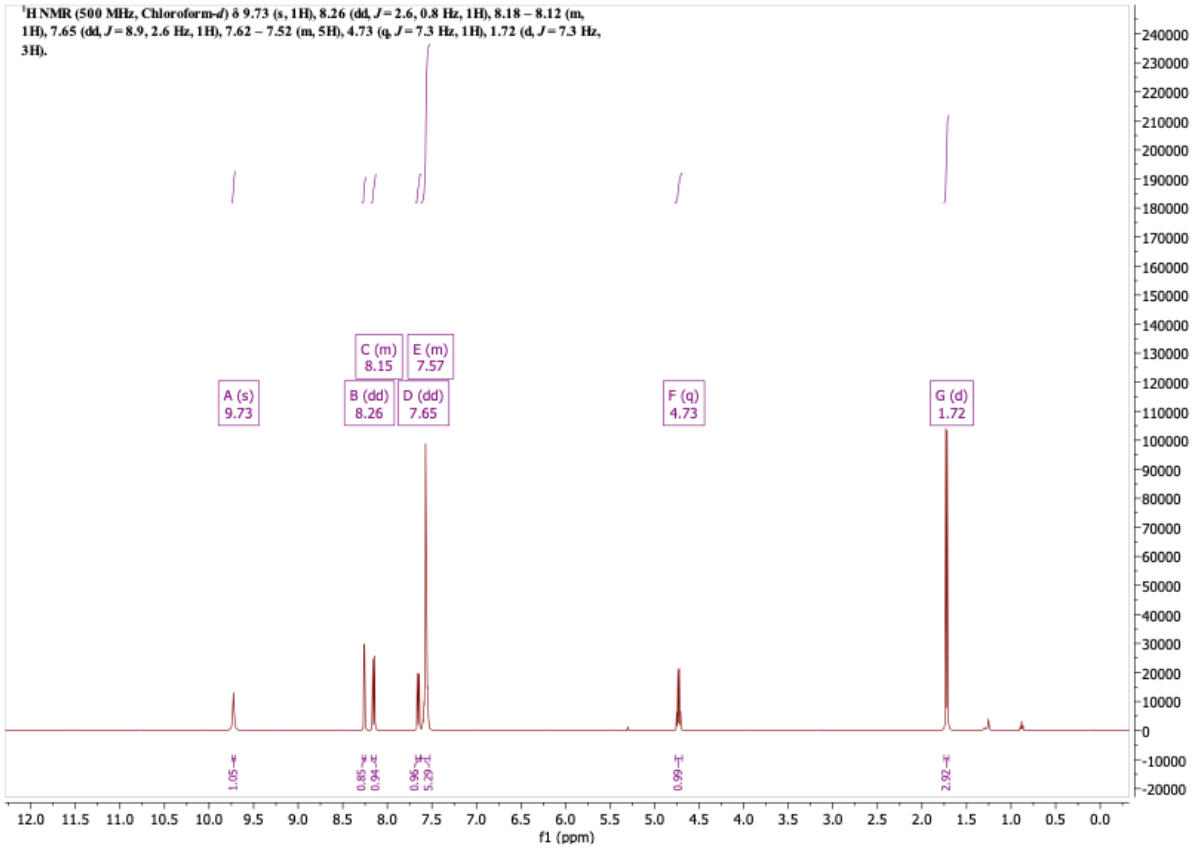




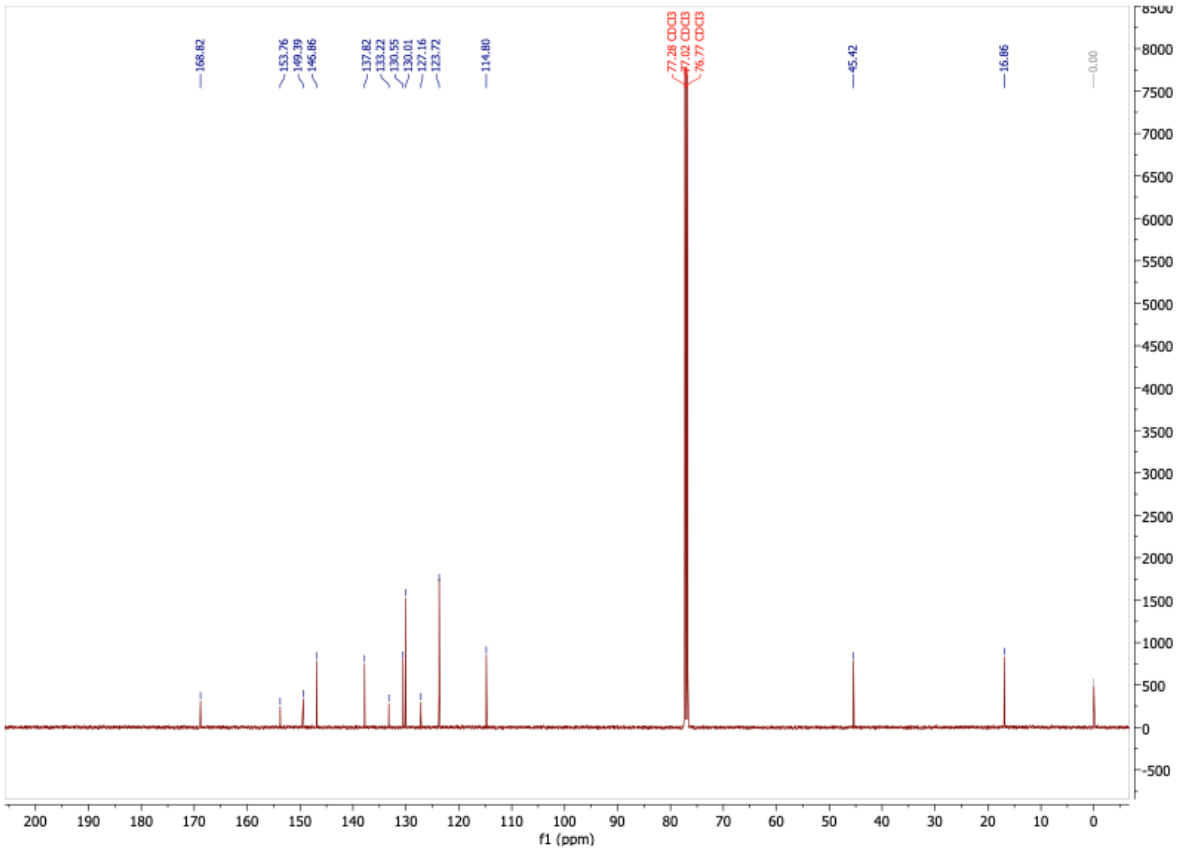
HRMS data of 2-((1-phenyl-1*H*-tetrazol-5-yl)thio)-*N*-(pyridin-2-yl)propanamide (2d)

_N_-(5-chloropyridin-2-yl)-2-((1-phenyl-1_H_-tetrazol-5-yl)thio)propanamide (2e)





¹H spectrum of _N_-(5-chloropyridin-2-yl)-2-((1-phenyl-1_H_-tetrazol-5-yl)thio)propanamide (2e)



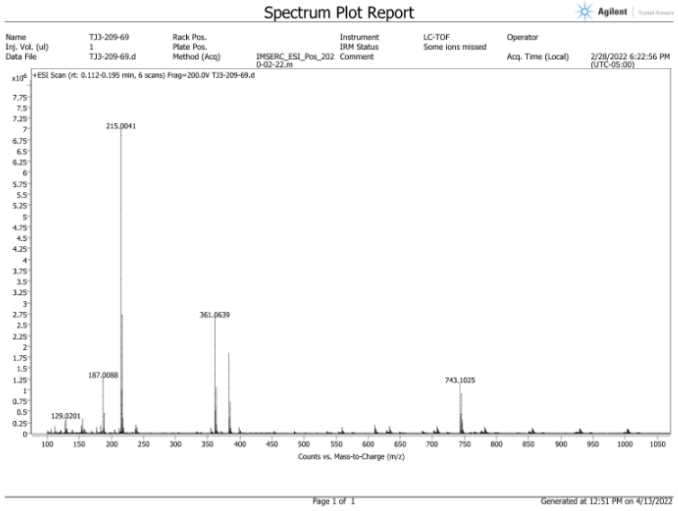
^{13}C spectrum of *N*-(5-chloropyridin-2-yl)-2-((1-phenyl-1*H*-tetrazol-5-yl)thio)propanamide (2e)

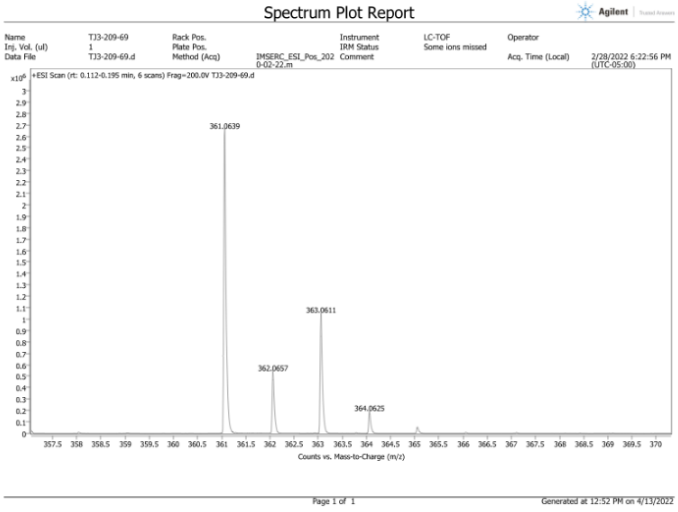
Spectrum Identification Results: + Scan (rt: 0.112-0.195 min) (TJ3-209-69.d)

Best	ID Source	Formula	Species	m/z	Score	Diff (ppm)	Diff (mDa)	Score (MFG)
☑	**MFG**	**C15 H13 Cl N6 O S**	**(M+H)+**	**361.0639**	**98.66**	**1.23**	**0.44**	**98.66**

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M+H)+	361.0639	99.65	98.79	98.66	98.66	97.22	2725171	C15 H14 Cl N6 O

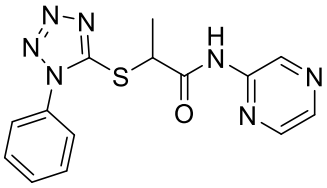
Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z	Diff (ppm)
2739849.3	59.8	100	361.0633	0.6	2725171	100	59.48	361.0639	1.73
531646.9	11.6	19.4	362.0658	-0.1	548050.8	20.11	11.96	362.0657	-0.34
1053833.3	23	38.46	363.0606	0.5	1056090.7	38.75	23.05	363.0611	1.3
196863.8	4.3	7.19	364.0629	-0.4	196676.7	7.22	4.29	364.0625	-1.04
59465	1.3	2.17	365.0592	-0.4	55669.1	2.04	1.22	365.0588	-1.14

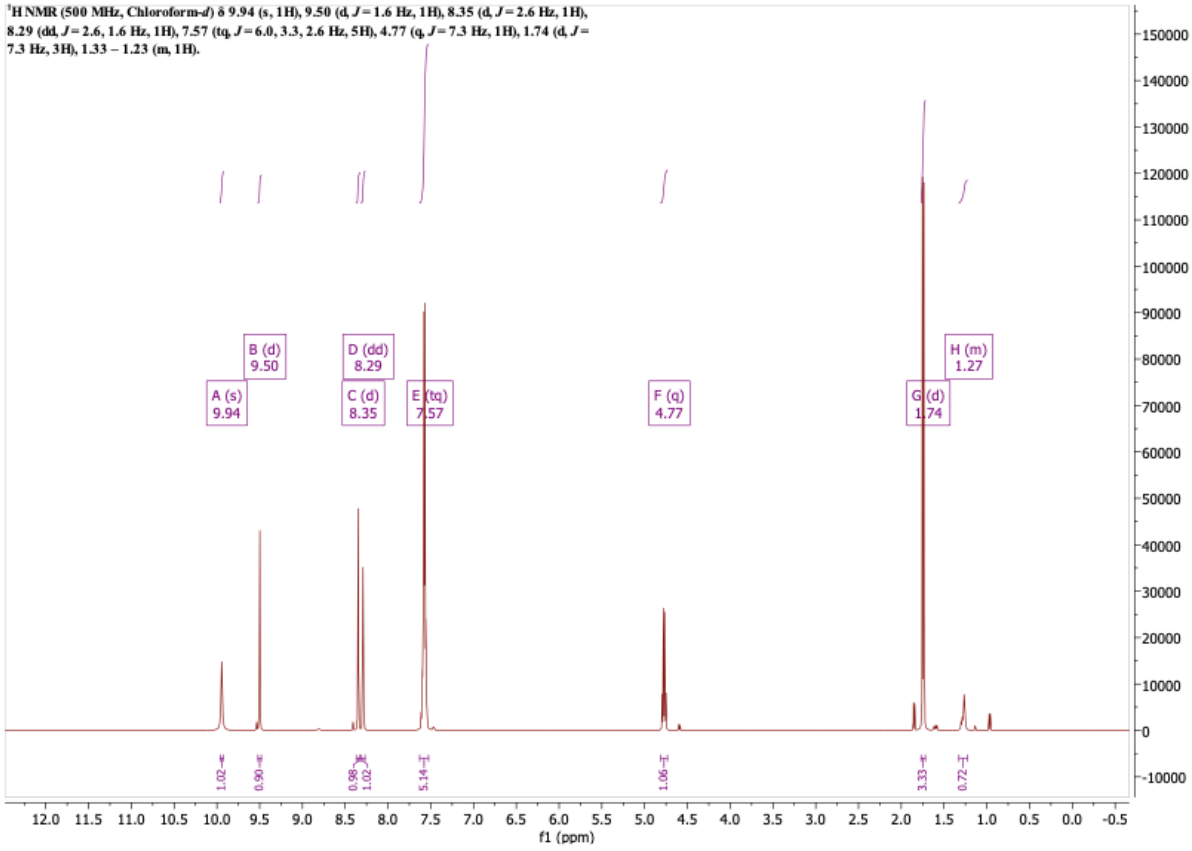




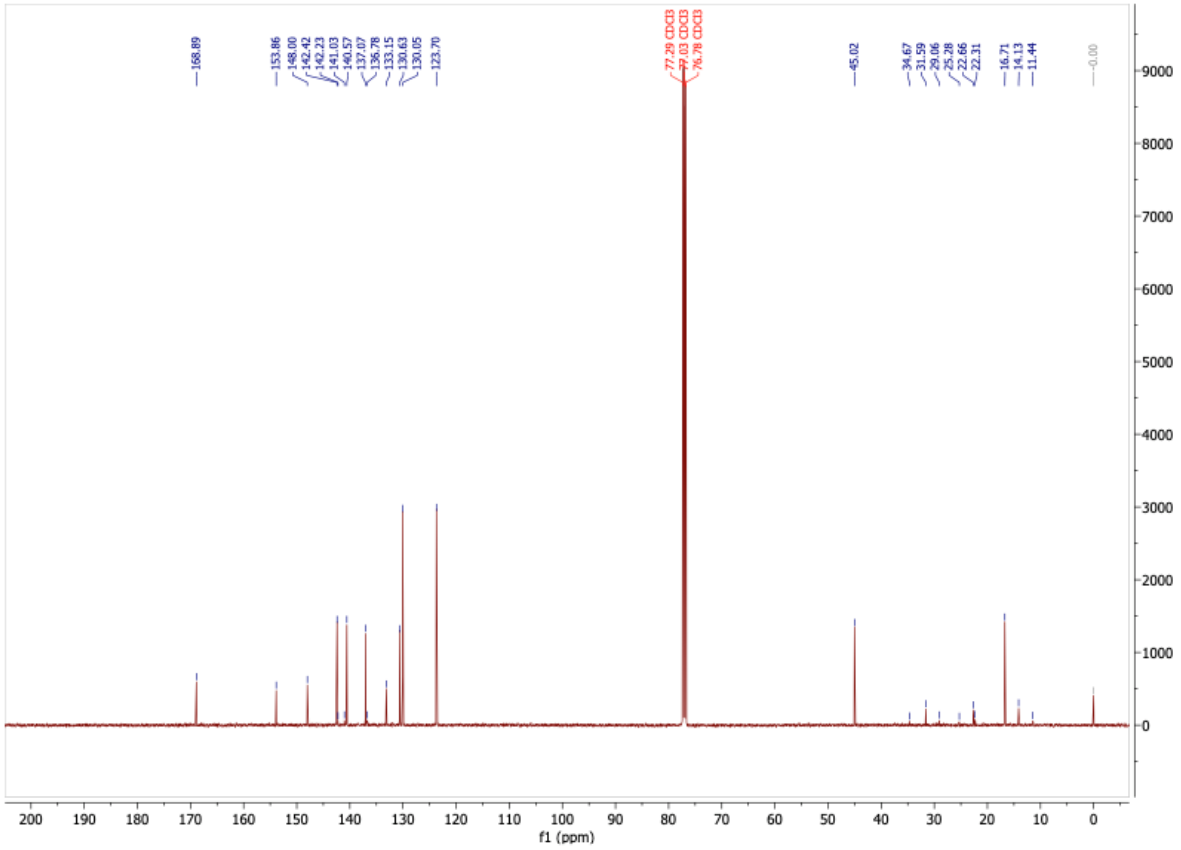
HRMS data of *N*-(5-chloropyridin-2-yl)-2-((1-phenyl-1*H*-tetrazol-5-yl)thio)propanamide (2e)

2-((1-phenyl-1*H*-tetrazol-5-yl)thio)-*N*-(pyrazin-2-yl)propanamide (2f)





¹H spectrum of 2-((1-phenyl-1*H*-tetrazol-5-yl)thio)-*N*-(pyrazin-2-yl)propanamide (2f)



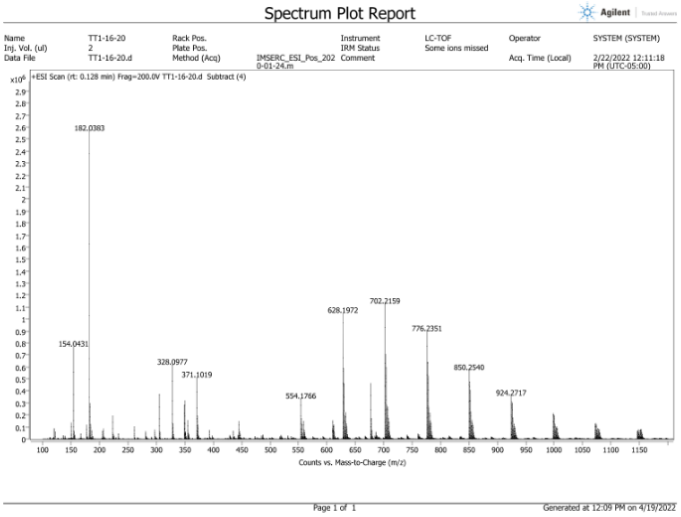
^{13}C spectrum of 2-((1-phenyl-1*H*-tetrazol-5-yl)thio)-*N*-(pyrazin-2-yl)propanamide (2f)

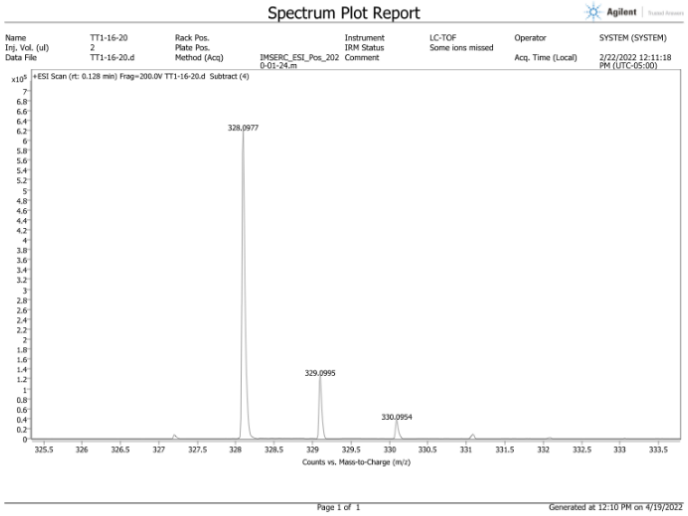
Spectrum Identification Results: + Scan (rt: 0.128 min) Sub (4) - TT1-16-20.d (TT1-16-20.d)

Best	ID Source	Formula	Species	m/z	Score	Diff (ppm)	Diff (mDa)	Score (MFG)
	MFG	**C14 H13 N7 O S**	**(M+H)+**	**328.0977**	**98.68**	**-0.11**	**-0.03**	**98.68**

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M+H)+	328.0977	97.43	99.99	98.68	98.68	97.56	625716.9	C14 H14 N7 O S

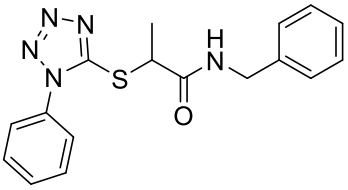
Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z	Diff (ppm)
635218.1	79.39	100	328.0975	0.2	625716.9	100	78.21	328.0977	0.69
118709.5	14.84	18.69	329.0999	-0.4	127619.1	20.4	15.95	329.0995	-1.15
40248.5	5.03	6.34	330.0959	-0.5	38062.2	6.08	4.76	330.0954	-1.52
5918.7	0.74	0.93	331.097	-11.6	8696.6	1.39	1.09	331.0853	-35.12

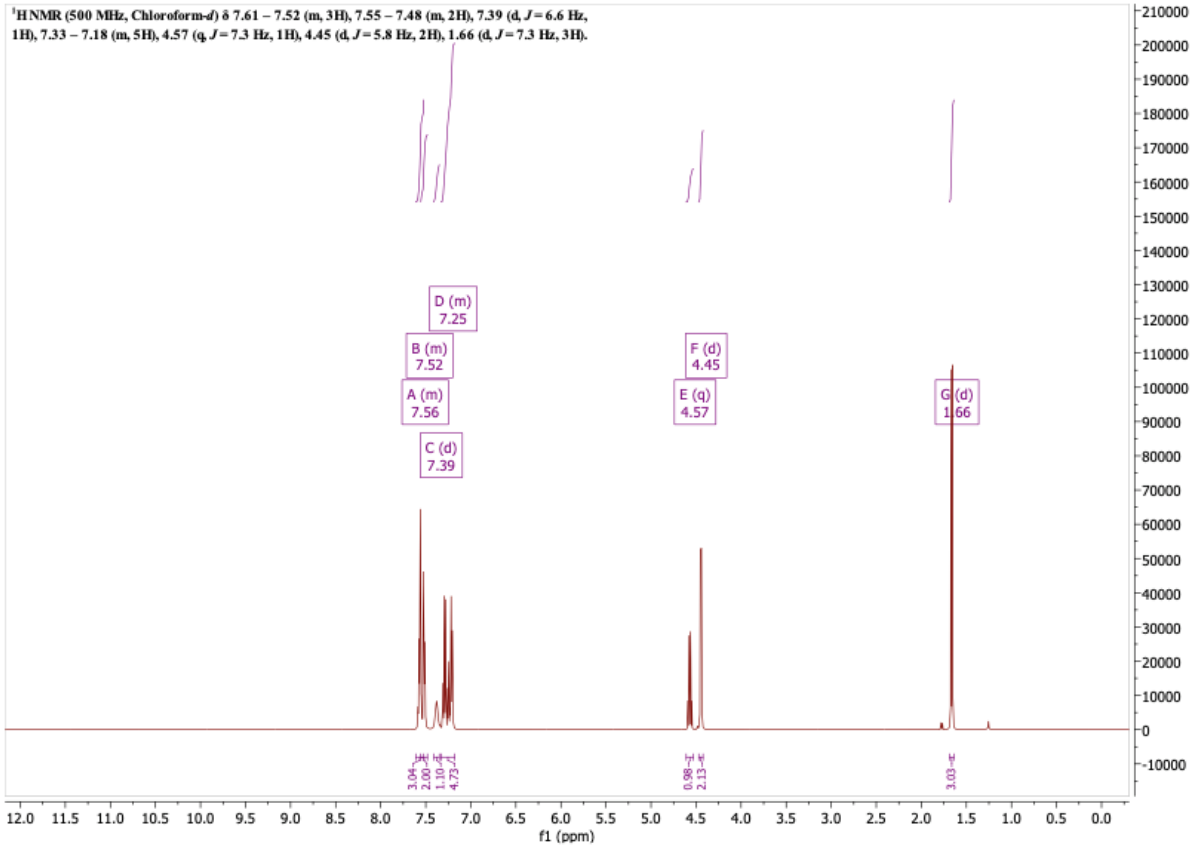




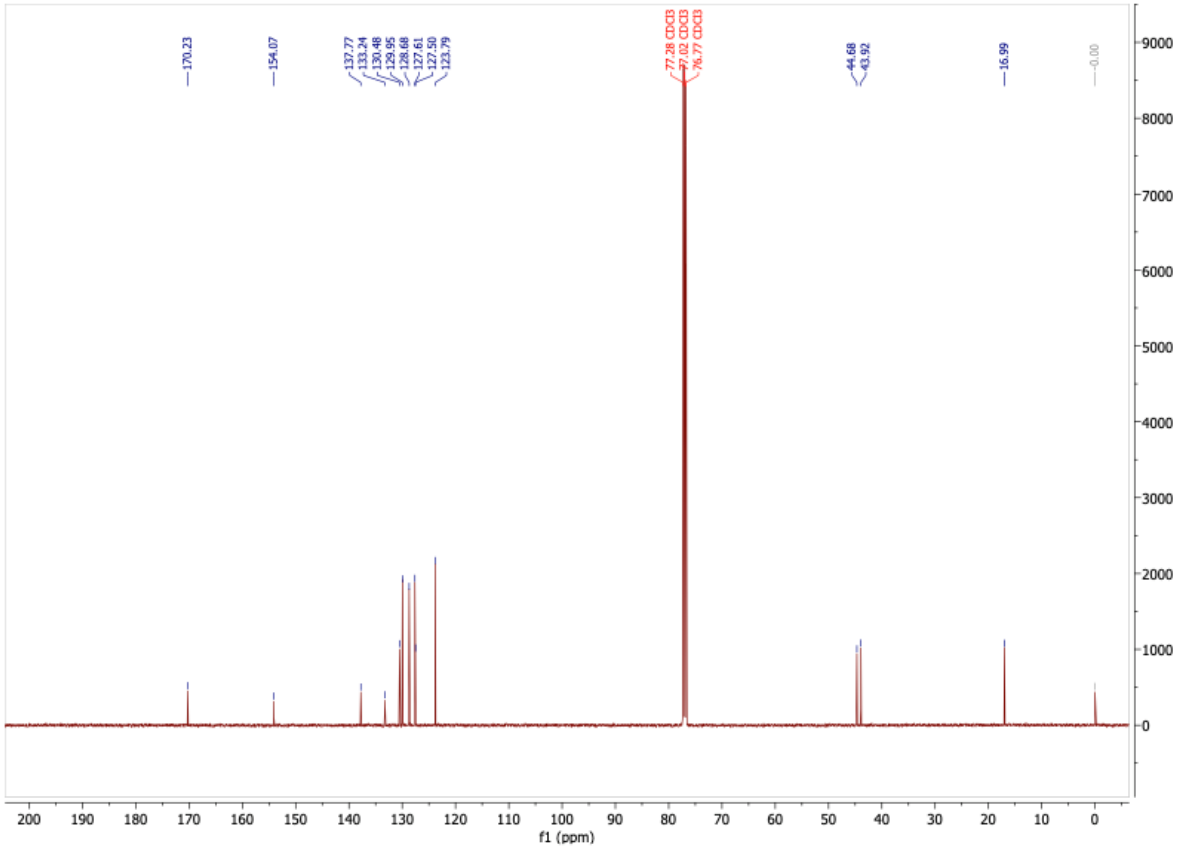
HRMS data of 2-((1-phenyl-1*H*-tetrazol-5-yl)thio)-*N*-(pyrazin-2-yl)propanamide (2f)

_N_-benzyl-2-((1-phenyl-1_H_-tetrazol-5-yl)thio)propanamide (2g)





^{1}H spectrum of *N*-benzyl-2-((1-phenyl-1*H*-tetrazol-5-yl)thio)propanamide (2g)



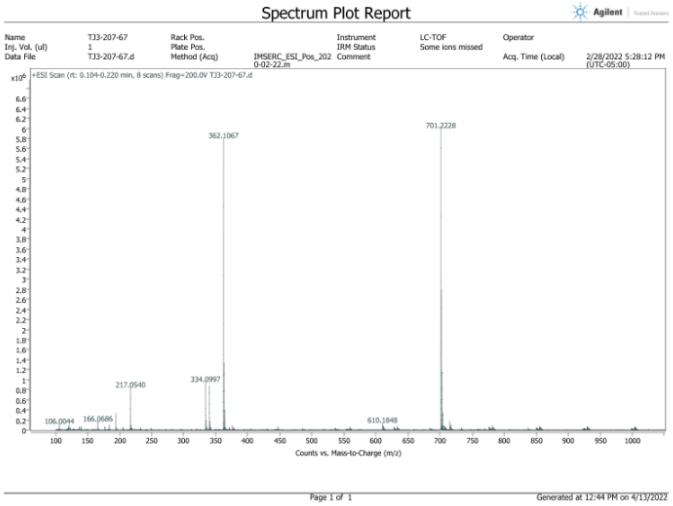
^{13}C spectrum of *N*-benzyl-2-((1-phenyl-1*H*-tetrazol-5-yl)thio)propanamide (2g)

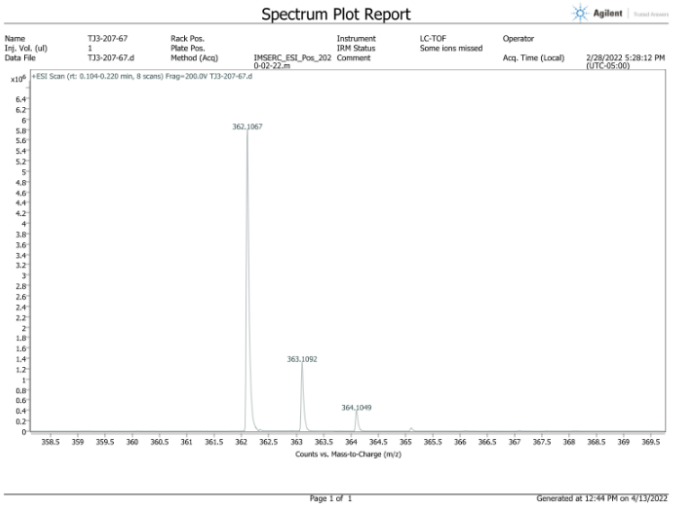
Spectrum Identification Results: + Scan (rt: 0.104-0.220 min) (TJ3-207-67.d)

Best	ID Source	Formula	Species	m/z	Score	Diff (ppm)	Diff (mDa)	Score (MFG)
	MFG	**C17 H17 N5 O S**	**(M+Na)+**	**362.1067**	**88.93**	**5.9**	**2**	**88.93**

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M+Na)+	362.1067	98.56	78.12	88.93	88.93	99.01	5918960.5	C17 H17 N5 Na O

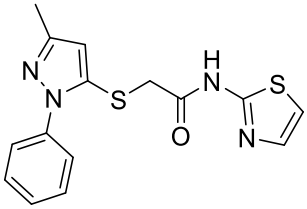
Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z	Diff (ppm)
5979778.9	77.42	100	362.1046	2.1	5918960.5	100	76.63	362.1067	5.77
1269899.3	16.44	21.24	363.1073	1.9	1337973.4	22.6	17.32	363.1092	5.25
408341.6	5.29	6.83	364.1037	1.2	403554.4	6.82	5.22	364.1049	3.29
65554.5	0.85	1.1	365.1047	0.8	63085.9	1.07	0.82	365.1056	2.29

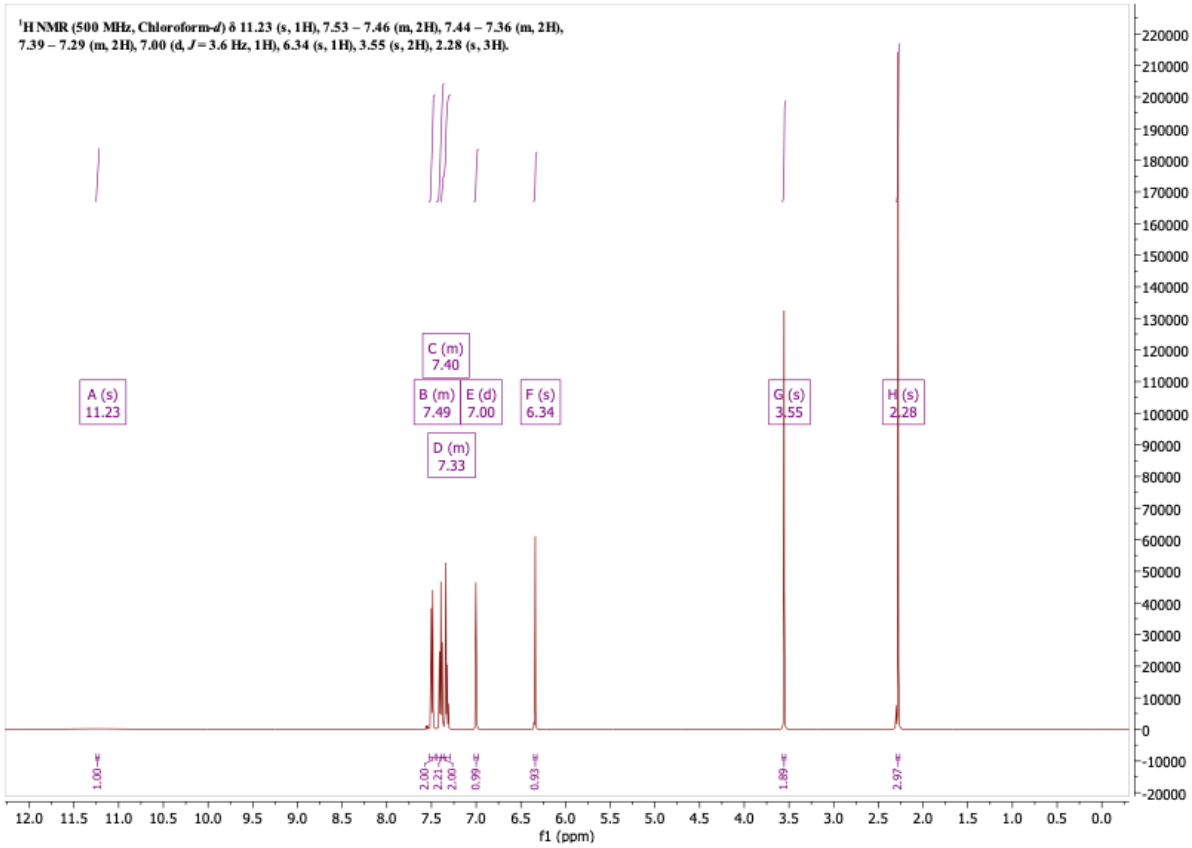




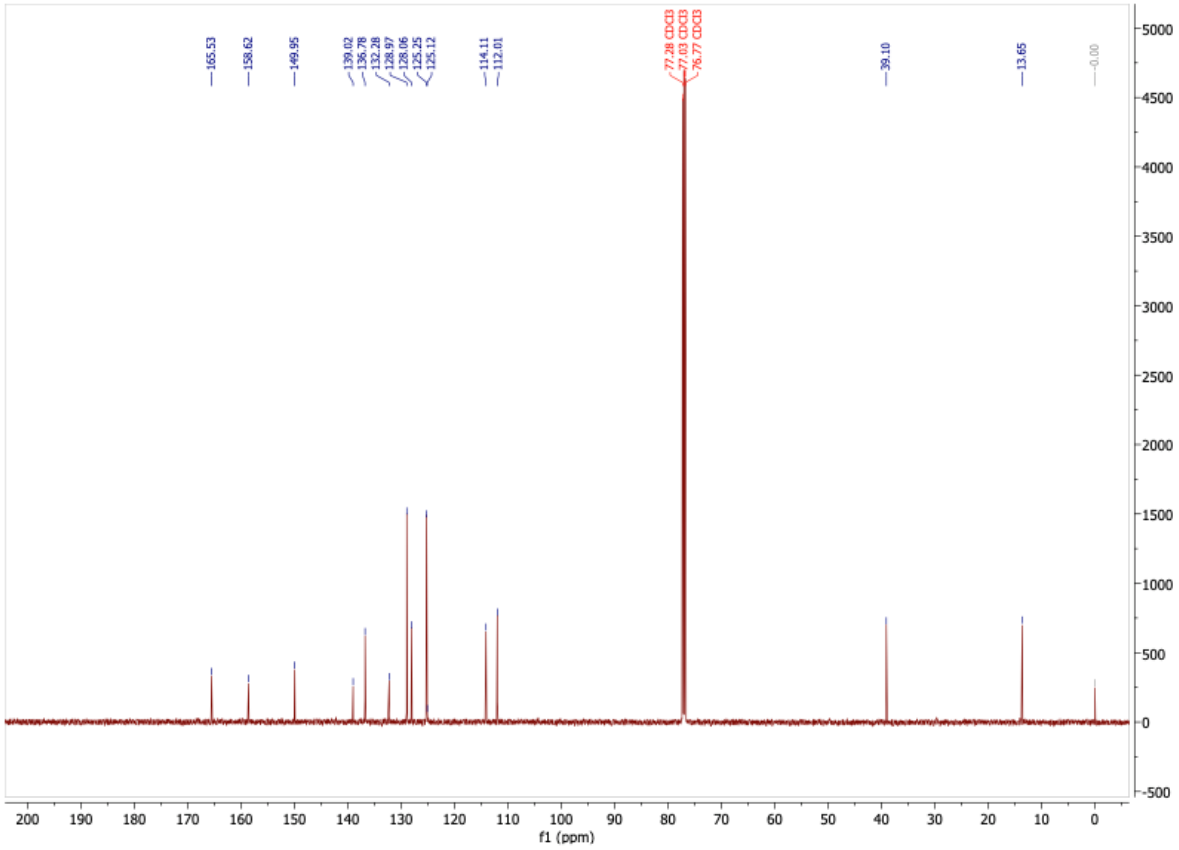
HRMS data of *N*-benzyl-2-((1-phenyl-1*H*-tetrazol-5-yl)thio)propanamide (2g)

2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-(thiazol-2-yl)acetamide (3a)





1H spectrum of 2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-(thiazol-2-yl)acetamide (3a)



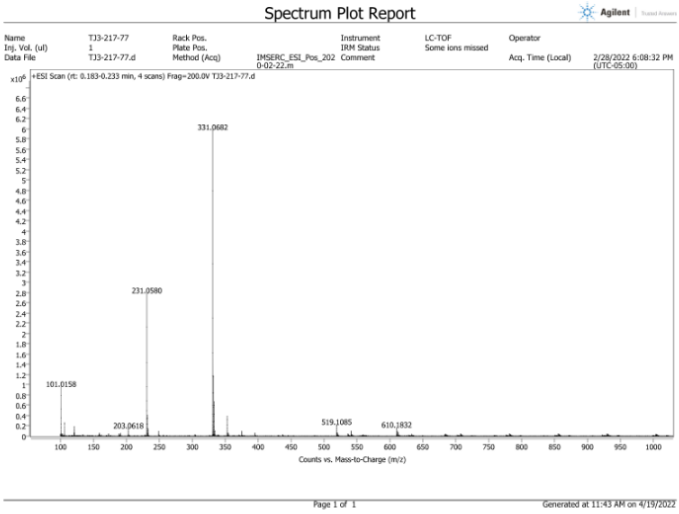
^{13}C spectrum of -((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-(thiazol-2-yl)acetamide (3a)

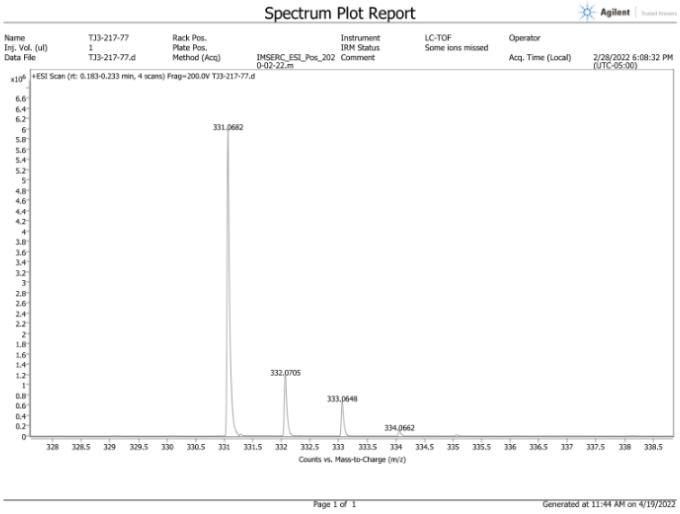
Spectrum Identification Results: + Scan (rt: 0.183-0.233 min) (TJ3-217-77.d)

Best	ID Source	Formula	Species	m/z	Score	Diff (ppm)	Diff (mDa)	Score (MFG)
	MFG	**C15 H14 N4 O S2**	**(M+H)+**	**331.0682**	**99.18**	**-0.46**	**-0.15**	**99.18**

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M+H)+	331.0682	98.99	99.84	99.18	99.18	98.1	5989212.7	C15 H15 N4 O S2

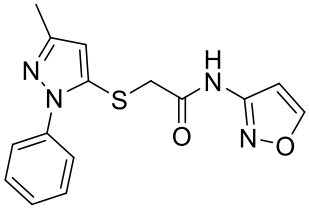
Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z	Diff (ppm)
6054409.3	75.62	100	331.0682	0	5989212.7	100	74.8	331.0682	-0.09
1179074.4	14.73	19.47	332.0708	-0.3	1228655.4	20.51	15.35	332.0705	-0.86
663167.4	8.28	10.95	333.0657	-0.8	677096.8	11.31	8.46	333.0648	-2.49
110001.9	1.37	1.82	334.0674	-1.2	111688	1.86	1.39	334.0662	-3.54

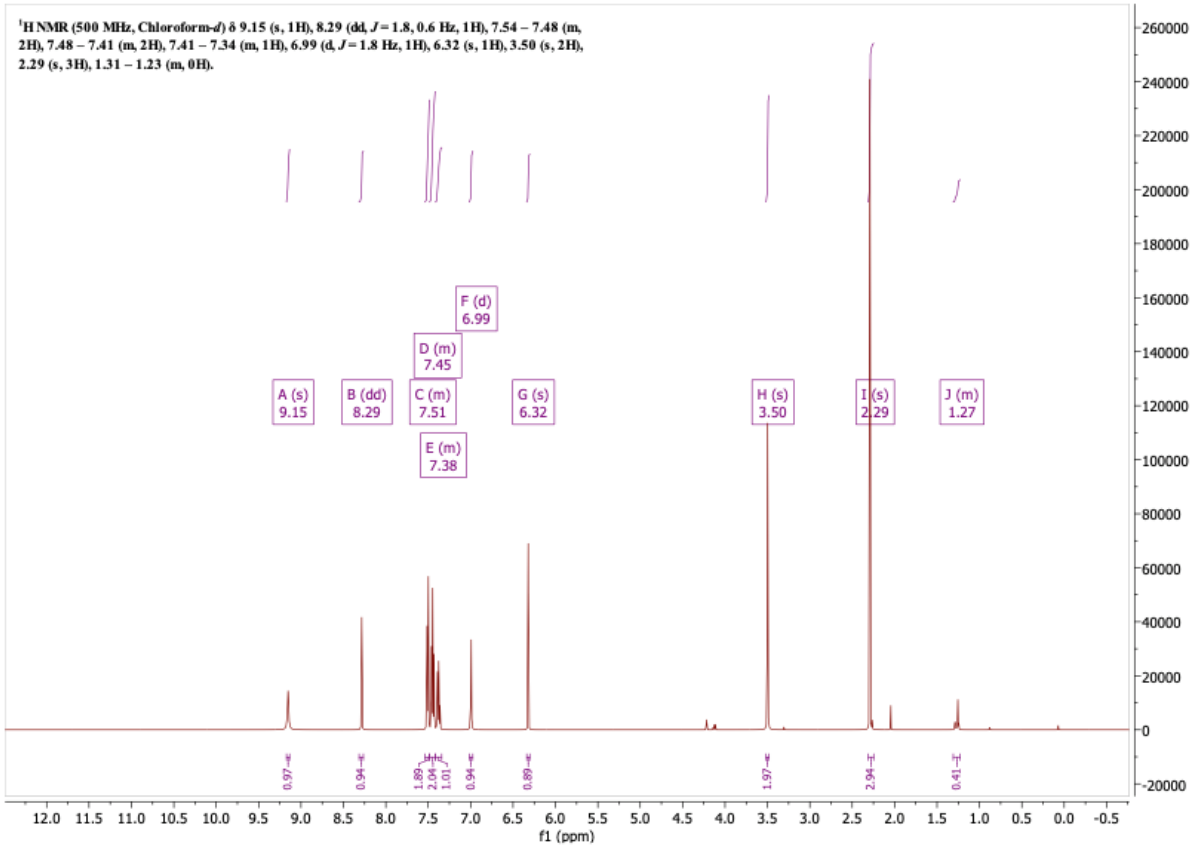




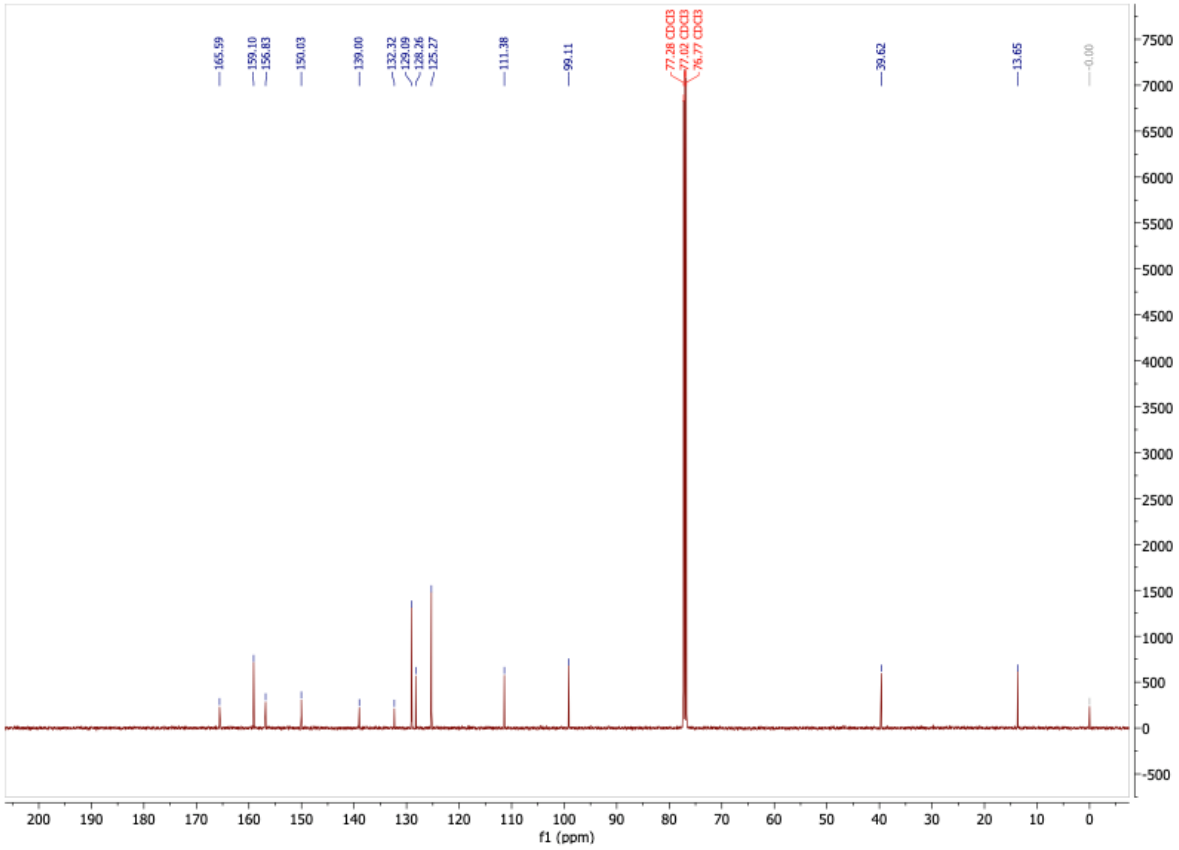
HRMS data of -((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-(thiazol-2-yl)acetamide (3a)

_N_-(isoxazol-3-yl)-2-((3-methyl-1-phenyl-1_H_-pyrazol-5-yl)thio)acetamide (3b)





[1]H spectrum of _N_-(isoxazol-3-yl)-2-((3-methyl-1-phenyl-1_H_-pyrazol-5-yl)thio)acetamide (3b)



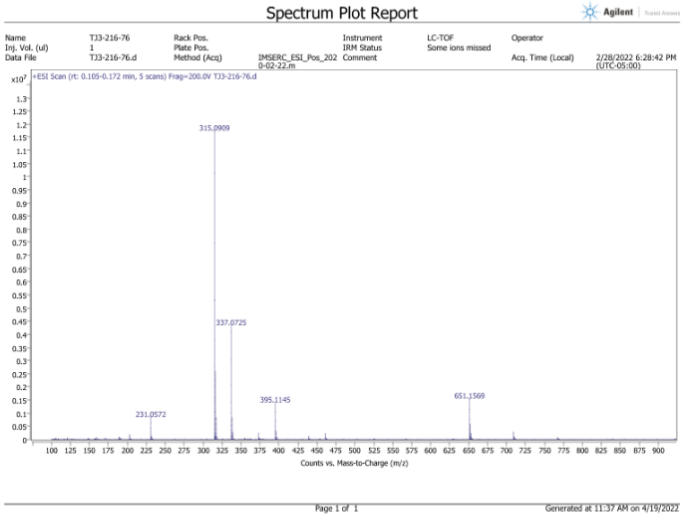
^{13}C spectrum of *N*-(isoxazol-3-yl)-2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)acetamide (3b)

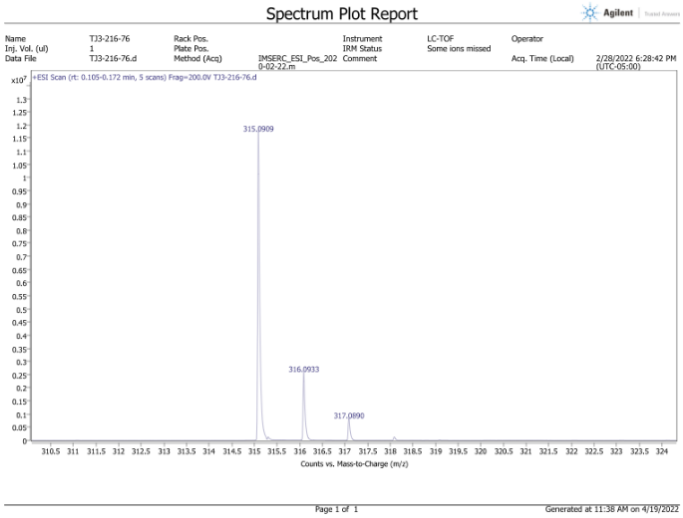
Spectrum Identification Results: + Scan (rt: 0.105-0.172 min) (TJ3-216-76.d)

Best	ID Source	Formula	Species	m/z	Score	Diff (ppm)	Diff (mDa)	Score (MFG)
☑	**MFG**	**C15 H14 N4 O2 S**	**(M+H)+**	**315.0909**	**97.08**	**-0.78**	**-0.25**	**97.08**

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M+H)+	315.0909	91.56	99.57	97.08	97.08	98.71	11766002.2	C15 H15 N4 O2 S

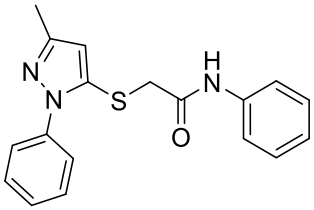
Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z	Diff (ppm)
12163469.4	79.21	100	315.091	-0.1	11766002.2	100	76.63	315.0909	-0.44
2277387.5	14.83	18.72	316.0937	-0.5	2612514.7	22.2	17.01	316.0933	-1.56
796025.4	5.18	6.54	317.0898	-0.8	851414.9	7.24	5.54	317.089	-2.5
118193.2	0.77	0.97	318.0912	-1.6	125143.7	1.06	0.81	318.0897	-4.97

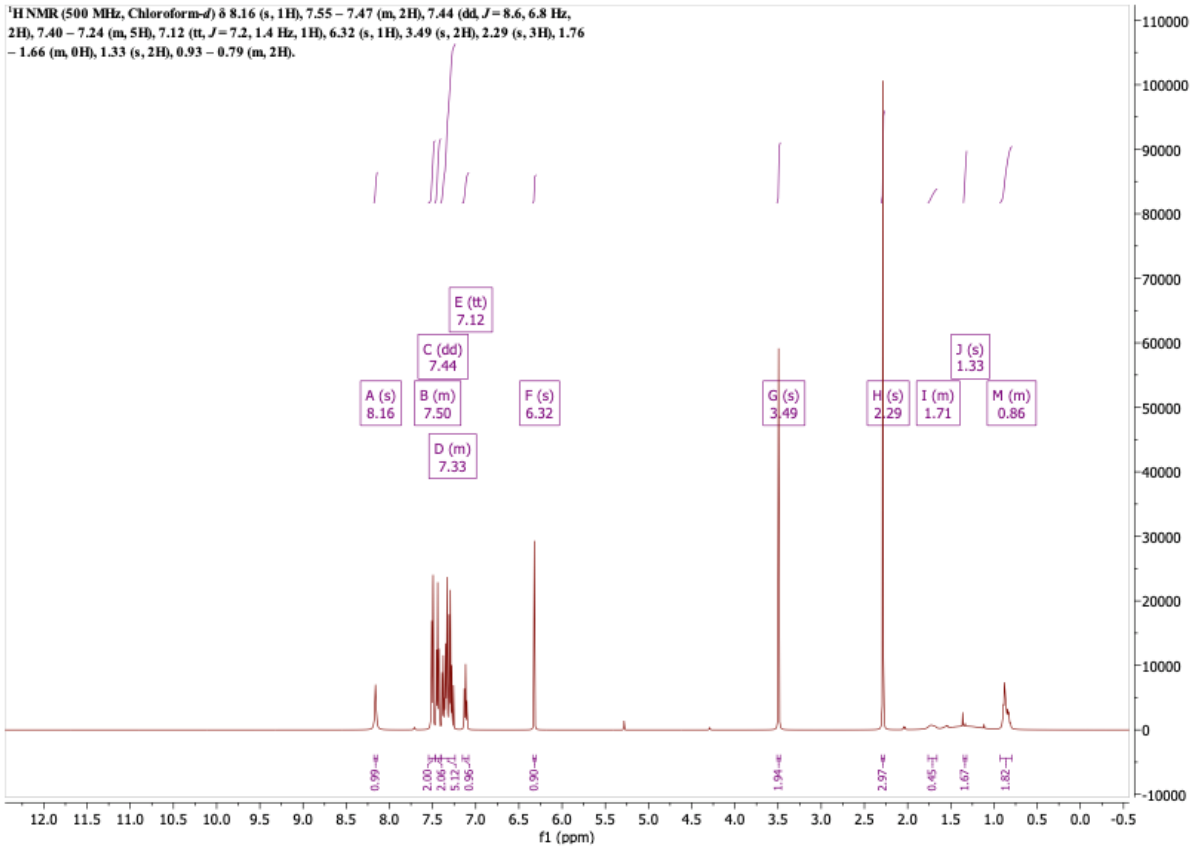




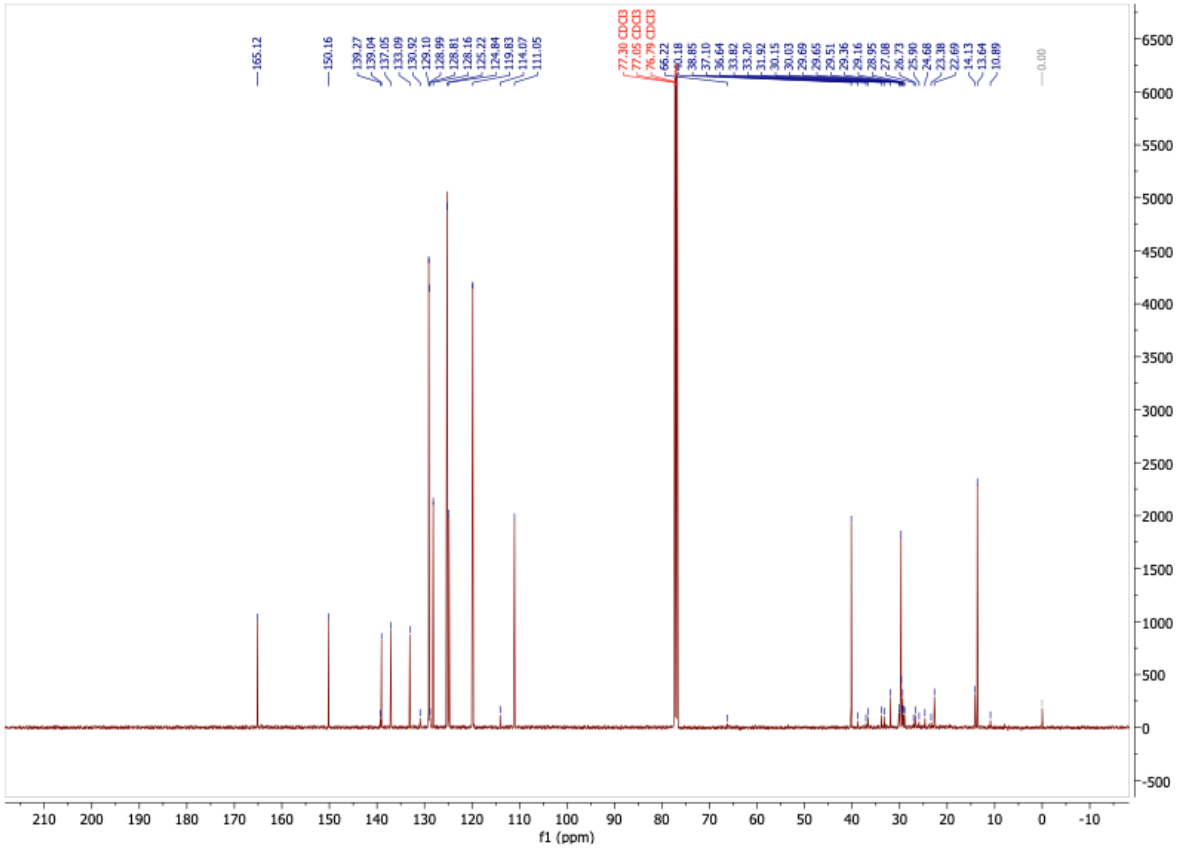
HRMS data of *N*-(isoxazol-3-yl)-2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)acetamide (3b)

2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-phenylacetamide (3c)





[1]H spectrum of 2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-phenylacetamide (3c)



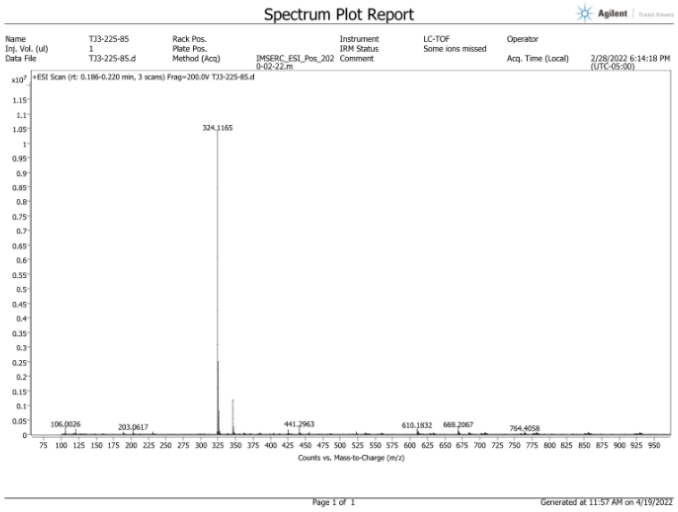
^{13}C spectrum of 2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-phenylacetamide (3c)

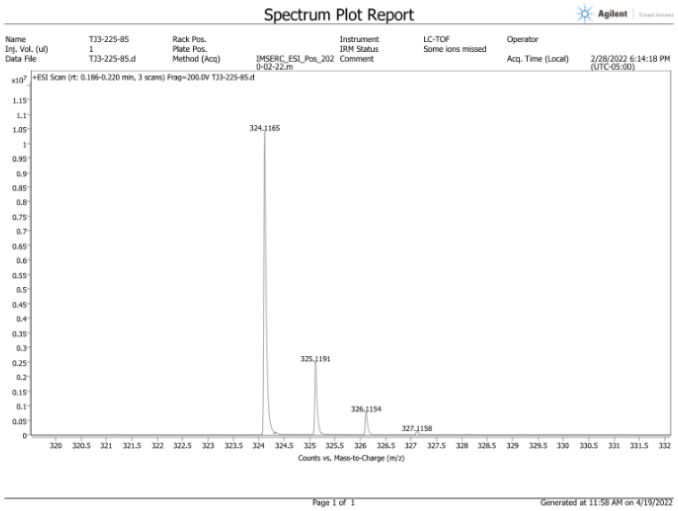
Spectrum Identification Results: + Scan (rt: 0.186-0.220 min) - TJ3-225-85.d (TJ3-225-85.d)

Best	ID Source	Formula	Species	m/z	Score	Diff (ppm)	Diff (abs. ppm)	Diff (mDa)	Score (MFG)
☑	MFG	C18 H17 N3 O S	(M+H)+	324.1165	97.65	-0.4	0.4	-0.13	97.65

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M+H)+	324.1165	92.56	99.88	97.65	97.65	99.27	10841570.1	C18 H18 N3 O S

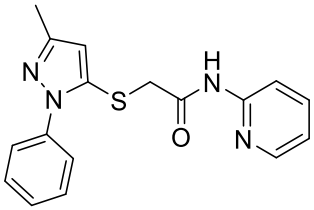
Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z	Diff (ppm)
10841570.1	77.15	100	324.1165	0	10485337.6	100	74.61	324.1165	-0.12
2341667.9	16.66	21.6	325.1194	-0.4	2624535.6	25.03	18.68	325.1191	-1.09
748264.9	5.32	6.9	326.1158	-0.4	813162.9	7.76	5.79	326.1154	-1.19
121449.6	0.86	1.12	327.117	-1.2	129916.3	1.24	0.92	327.1158	-3.56

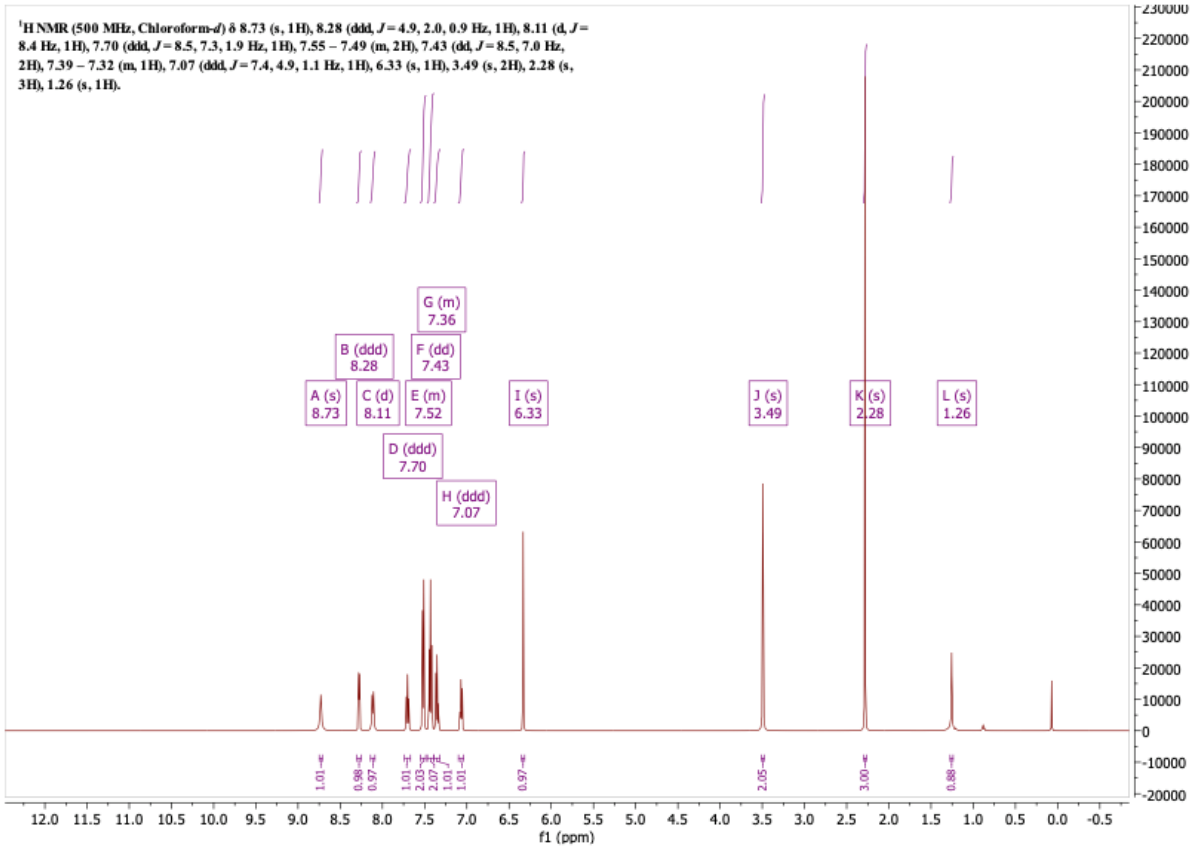




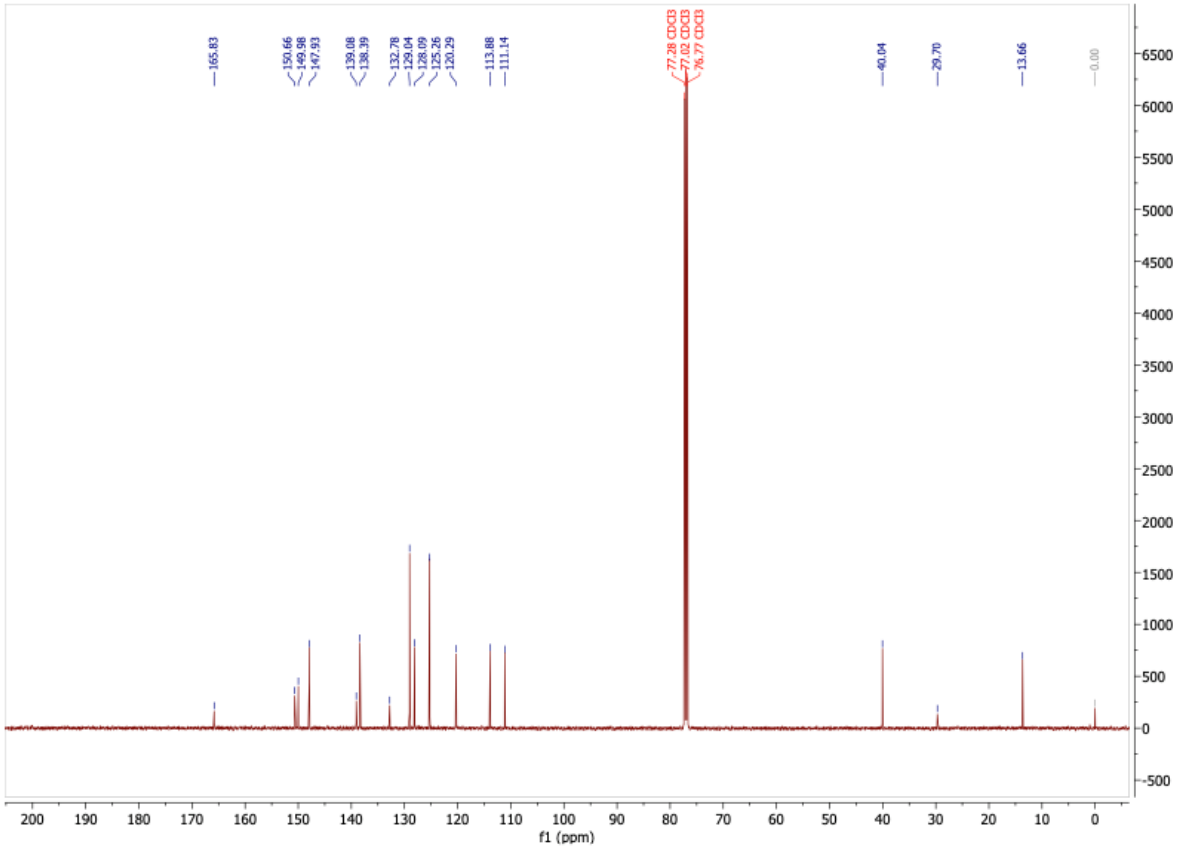
HRMS data of 2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-phenylacetamide (3c)

2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-(pyridine-2-yl)acetamide (3d)





[1]H spectrum of 2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-(pyridine-2-yl)acetamide (3d)



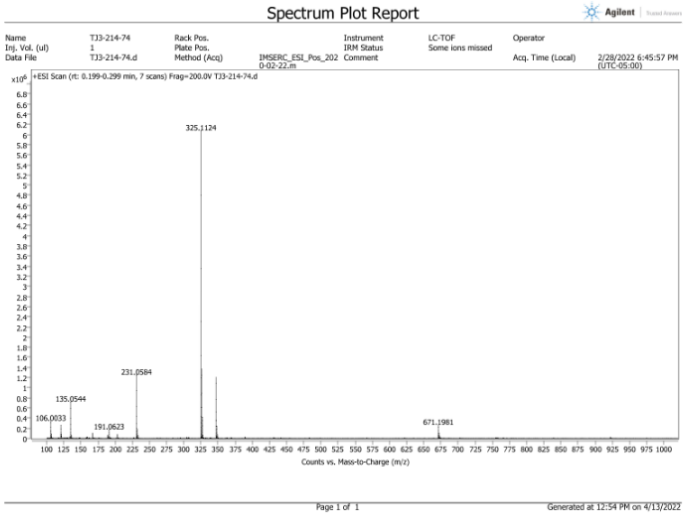
^{13}C spectrum of 2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-(pyridine-2-yl)acetamide (3d)

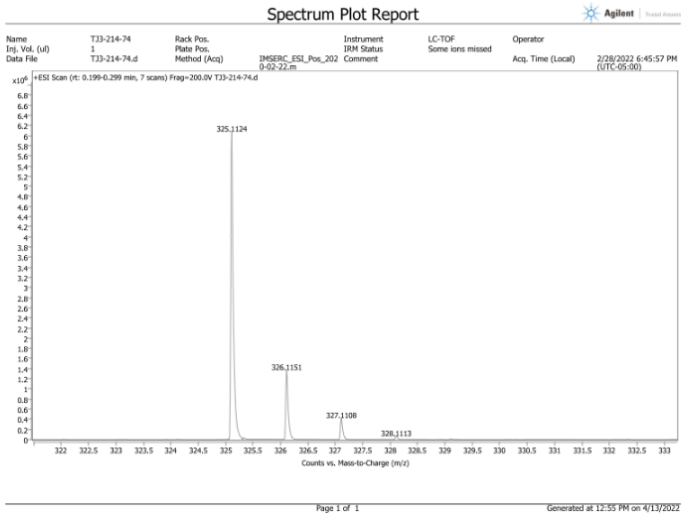
Spectrum Identification Results: + Scan (rt: 0.199-0.299 min) (TJ3-214-74.d)

Best	ID Source	Formula	Species	m/z	Score	Diff (ppm)	Diff (mDa)	Score (MFG)
☑	**MFG**	**C17 H16 N4 O S**	**(M+H)+**	**325.1124**	**98.16**	**1.76**	**0.57**	**98.16**

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M+H)+	325.1124	97.69	97.8	98.16	98.16	99.43	6132921.2	C17 H17 N4 O S

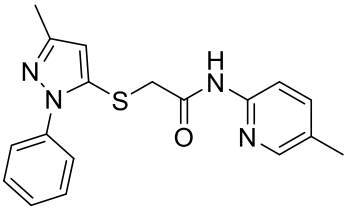
Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z	Diff (ppm)
6226517.6	77.7	100	325.1118	0.6	6132921.2	100	76.53	325.1124	1.94
1299550.8	16.22	20.87	326.1145	0.5	1389865	22.66	17.34	326.1151	1.65
420443	5.25	6.75	327.1108	0	424118.6	6.92	5.29	327.1108	-0.11
66723.4	0.83	1.07	328.112	-0.6	66330	1.08	0.83	328.1113	-1.97

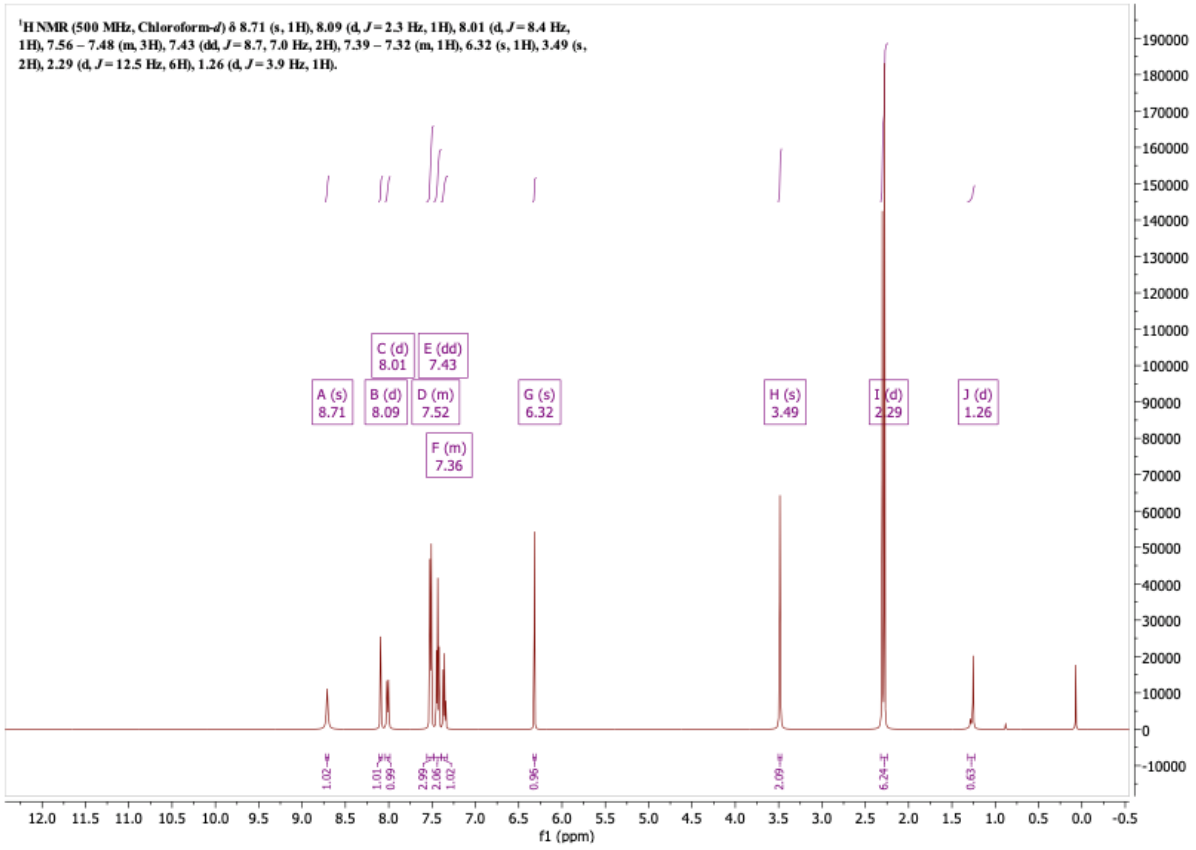




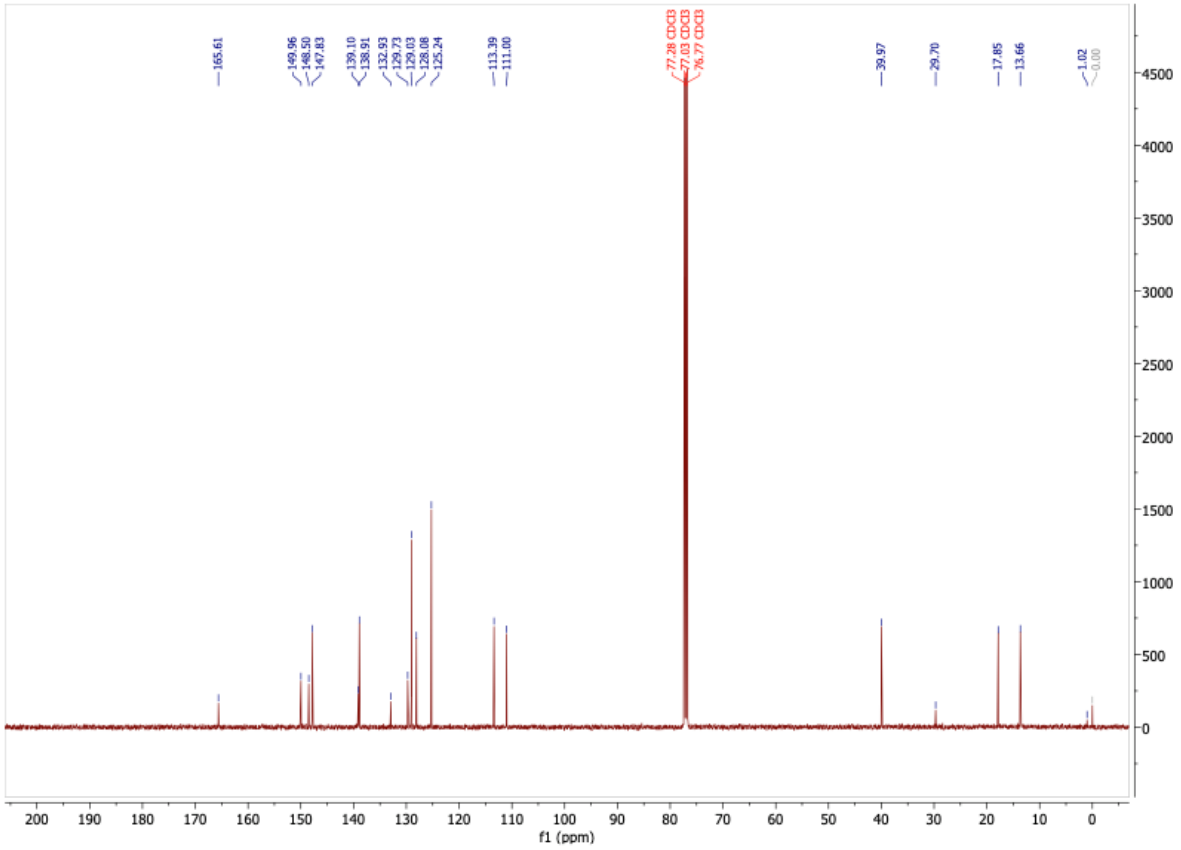
HRMS data of 2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-(pyridine-2-yl)acetamide (3d)

2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-(5-methylpyridin-2-yl)acetamide (3e)





^{1}H spectrum of 2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-(5-methylpyridin-2-yl)acetamide (3e)



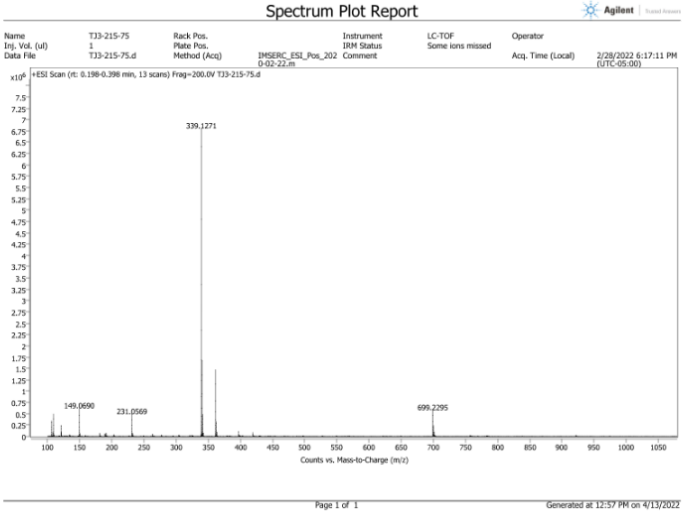
^{13}C spectrum of 2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-(5-methylpyridin-2-yl)acetamide (3e)

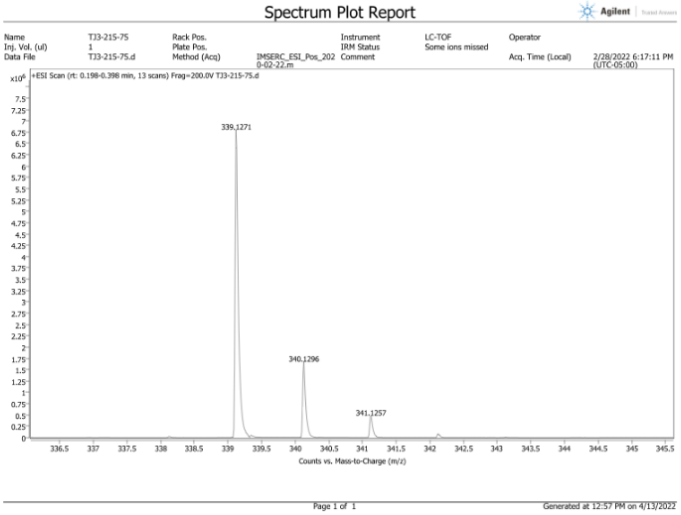
Spectrum Identification Results: + Scan (rt: 0.198-0.398 min) (TJ3-215-75.d)

Best	ID Source	Formula	Species	m/z	Score	Diff (ppm)	Diff (mDa)	Score (MFG)
	MFG	**C18 H18 N4 O S**	**(M+H)+**	**339.1271**	**98.37**	**-1.12**	**-0.38**	**98.37**

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M+H)+	339.1271	96.92	99.06	98.37	98.37	98.72	6966534.9	C18 H19 N4 O S

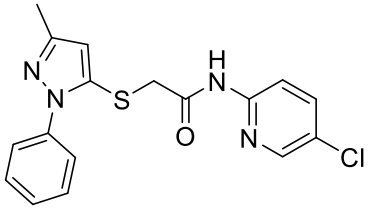
Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z	Diff (ppm)
7084870.6	76.86	100	339.1274	-0.3	6966534.9	100	75.58	339.1271	-0.78
1556957.3	16.89	21.98	340.1302	-0.6	1677315.1	24.08	18.2	340.1296	-1.83
494754	5.37	6.98	341.1267	-1	493824.7	7.09	5.36	341.1257	-2.85
81209.5	0.88	1.15	342.1278	-1.7	80116.7	1.15	0.87	342.1261	-4.83

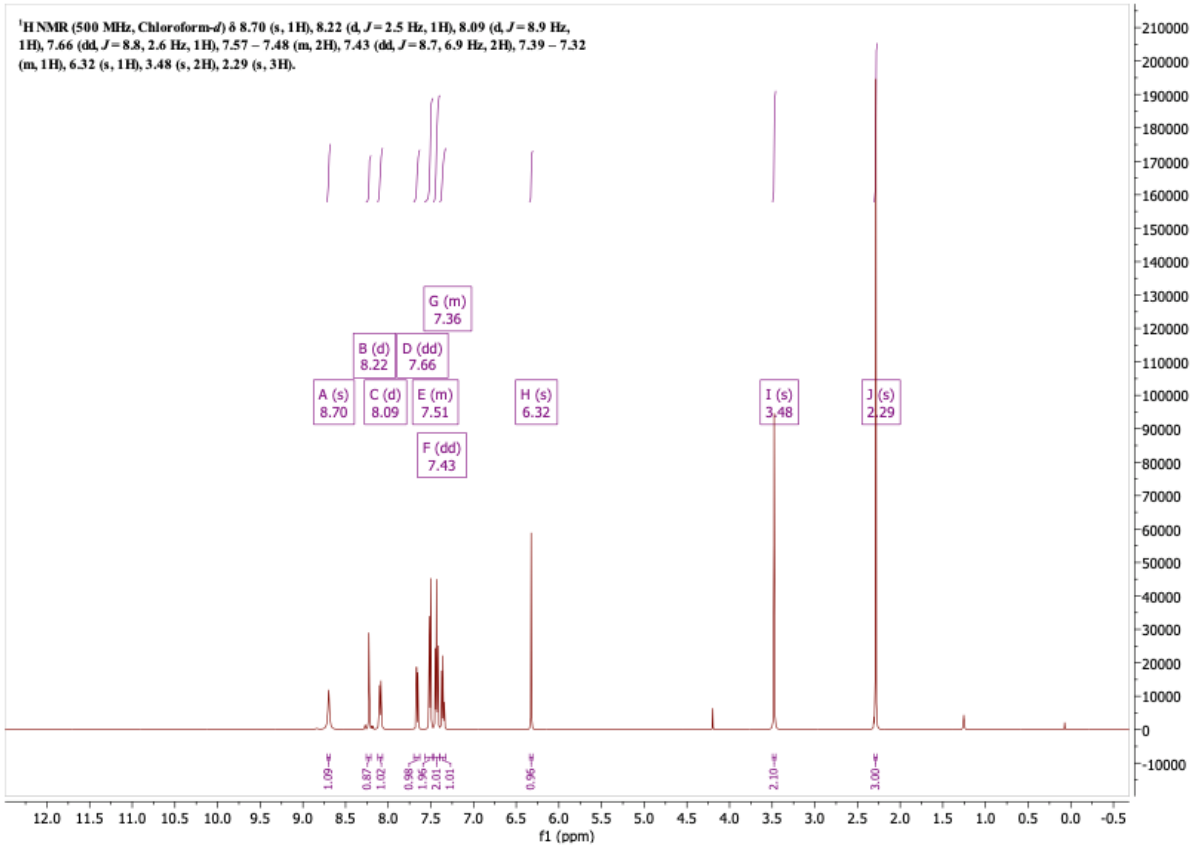




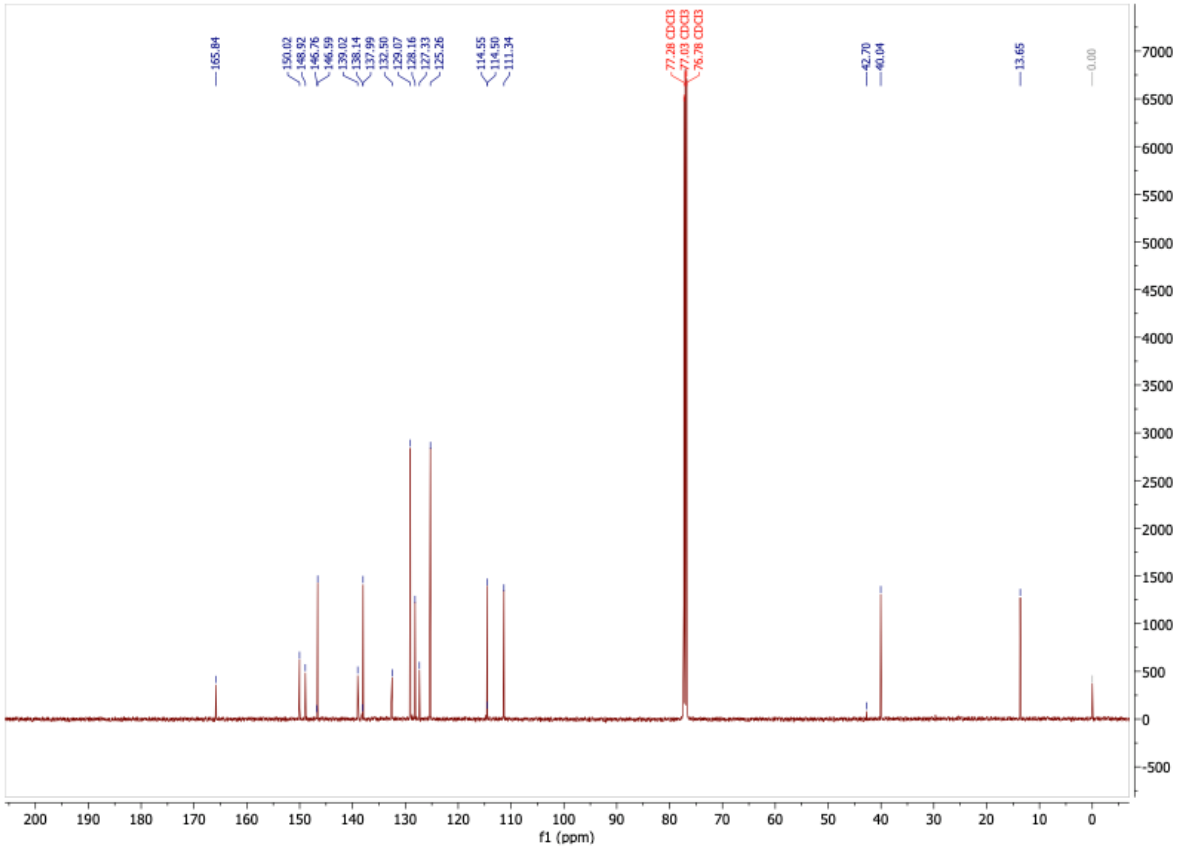
HRMS data of 2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-(5-methylpyridin-2-yl)acetamide (3e)

_N_-(5-chloropyridin-2-yl)-2-((3-methyl-1-phenyl-1_H_-pyrazol-5-yl)thio)acetamide (3f)





¹H spectrum of _N_-(5-chloropyridin-2-yl)-2-((3-methyl-1-phenyl-1_H_-pyrazol-5-yl)thio)acetamide (3f)



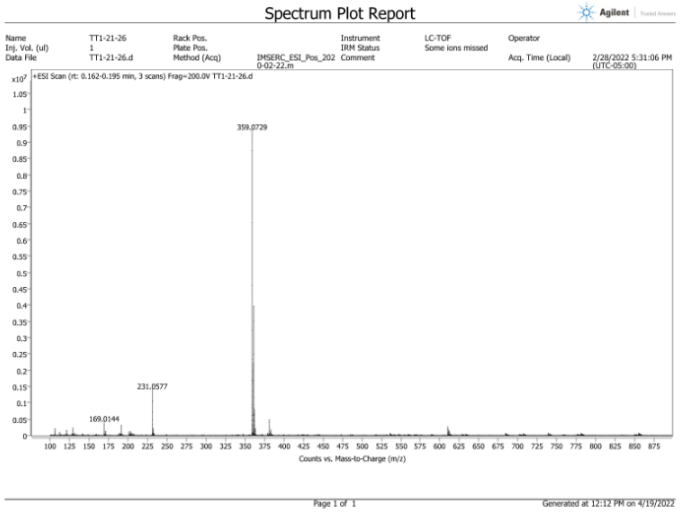
^{13}C spectrum of *N*-(5-chloropyridin-2-yl)-2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)acetamide (3f)

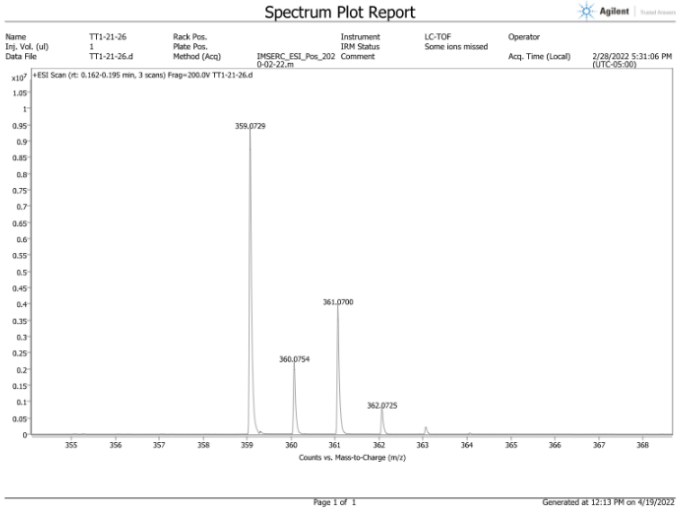
Spectrum Identification Results: + Scan (rt: 0.162-0.195 min) - TT1-21-26.d (TT1-21-26.d)

Best	ID Source	Formula	Species	m/z	Score	Diff (ppm)	Diff (mDa)	Score (MFG)
☑	MFG	C17 H15 Cl N4 O S	(M+H)+	359.0729	98.84	-0.02	-0.01	98.84

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M+H)+	359.0729	96.37	100	98.84	98.84	99.47	9564175.3	C17 H16 Cl N4 O

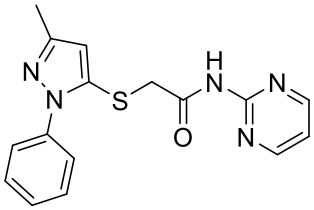
Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z	Diff (ppm)
9927094.5	58.95	100	359.0728	0.1	9564175.3	100	56.8	359.0729	0.33
2070765	12.3	20.86	360.0756	-0.2	2240875.8	23.43	13.31	360.0754	-0.42
3846335.7	22.84	38.75	361.0702	-0.1	3997937.7	41.8	23.74	361.07	-0.41
768859.1	4.57	7.75	362.0727	-0.2	809698.5	8.47	4.81	362.0725	-0.54
225845.4	1.34	2.28	363.0692	-0.8	226212.4	2.37	1.34	363.0684	-2.15

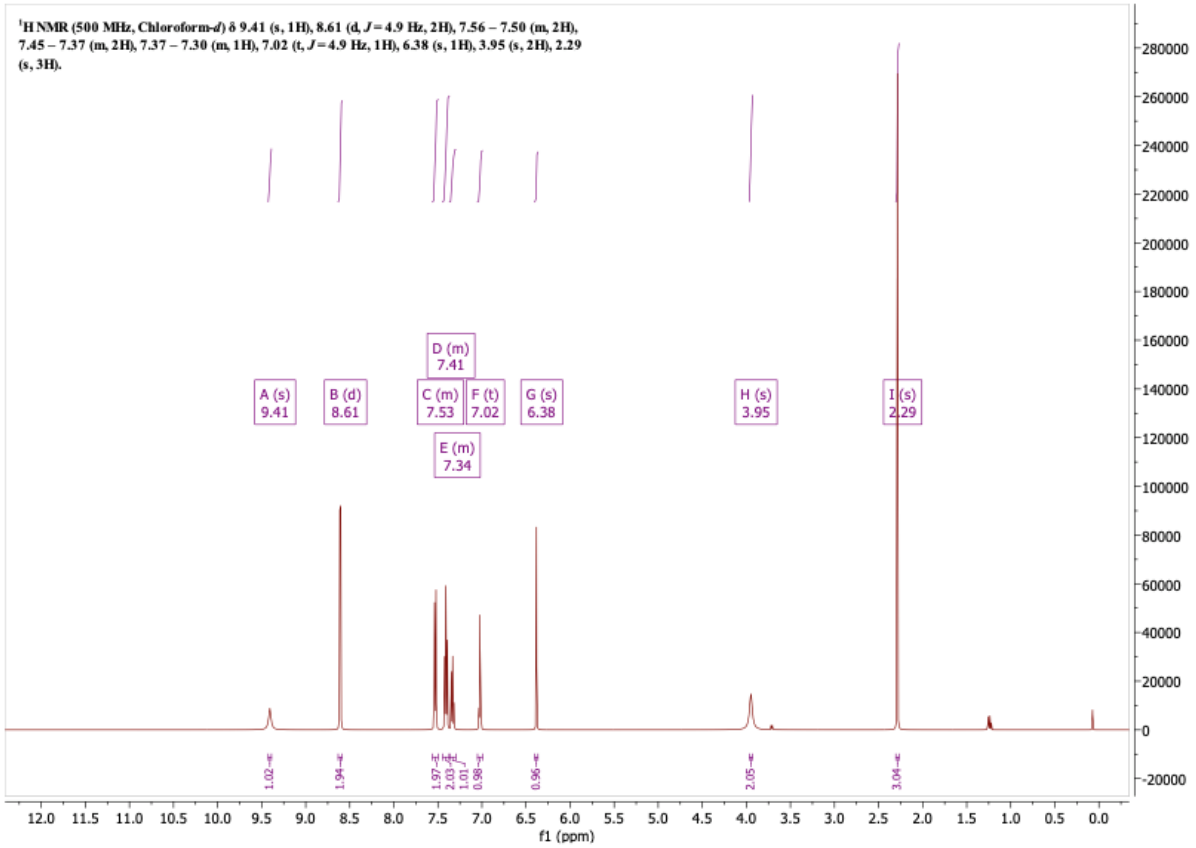




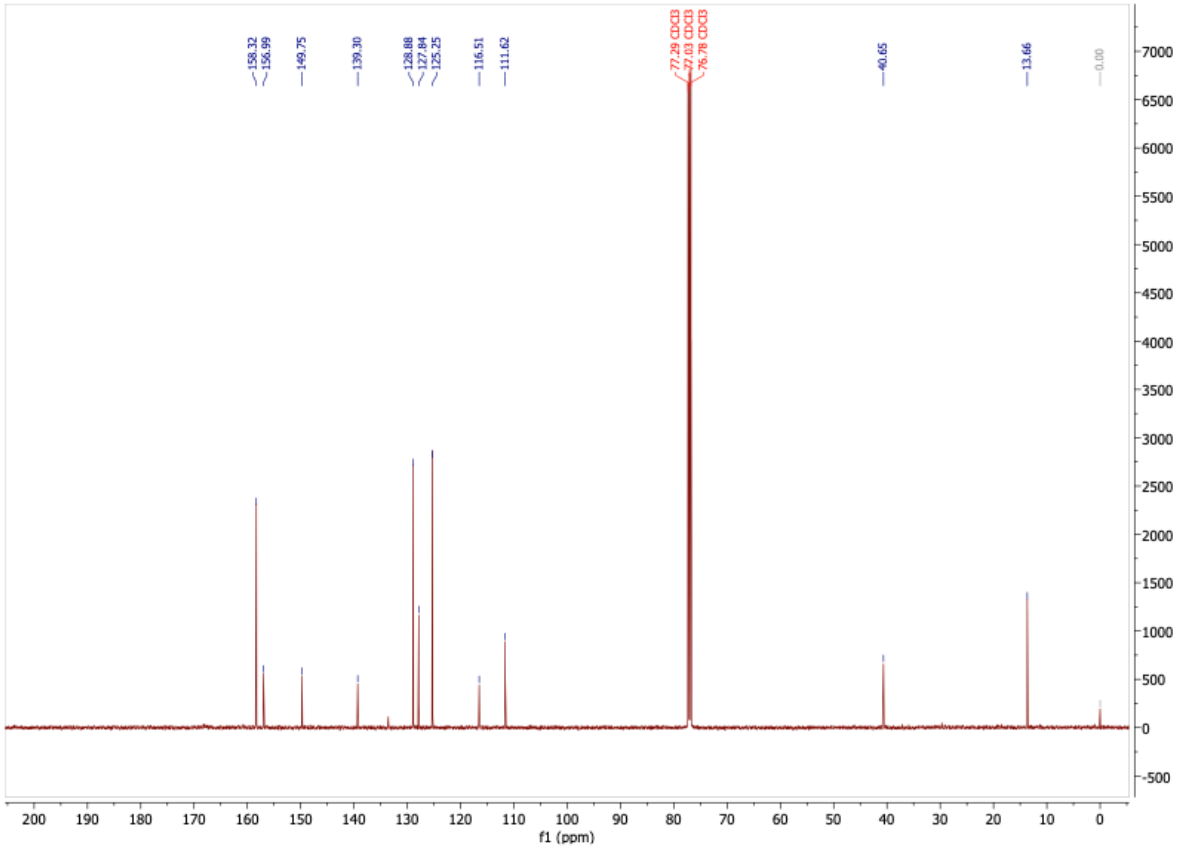
HRMS data of *N*-(5-chloropyridin-2-yl)-2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)acetamide (3f)

2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-(pyrimidin-2-yl)acetamide (3g)





¹H spectrum of 2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-(pyrimidin-2-yl)acetamide (3g)



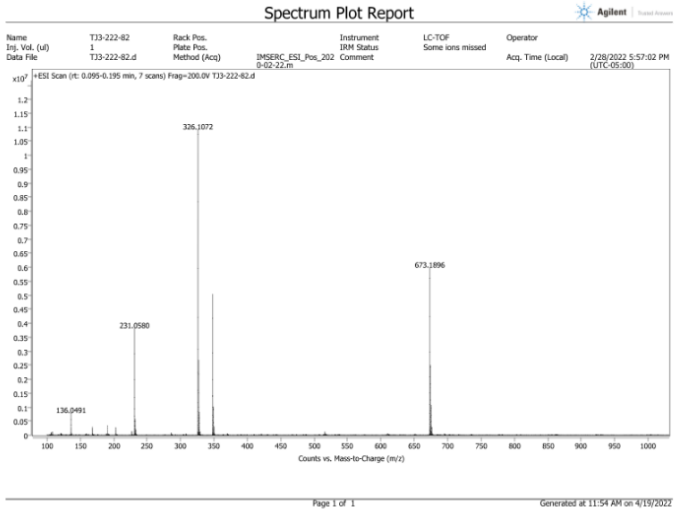
^{13}C spectrum of 2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-(pyrimidin-2-yl)acetamide (3g)

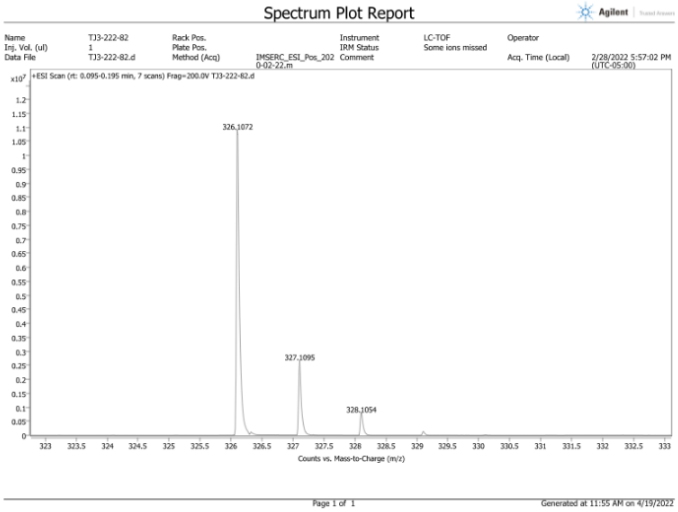
Spectrum Identification Results: + Scan (rt: 0.095-0.195 min) - TJ3-222-82.d (TJ3-222-82.d)

Best	ID Source	Formula	Species	m/z	Score	Diff (ppm)	Diff (mDa)	Score (MFG)
☑	**MFG**	**C16 H15 N5 O S**	**(M+H)+**	**326.1072**	**97.39**	**0.34**	**0.11**	**97.39**

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M+H)+	326.1072	92.19	99.92	97.39	97.39	98.56	11375980.8	C16 H16 N5 O S

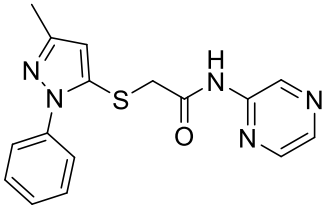
Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z	Diff (ppm)
11764542.7	78.26	100	326.107	0.2	11375980.8	100	75.68	326.1072	0.71
2369788.9	15.76	20.14	327.1097	-0.2	2682191.6	23.58	17.84	327.1095	-0.46
777447.9	5.17	6.61	328.1058	-0.5	841755.2	7.4	5.6	328.1054	-1.38
120470.1	0.8	1.02	329.107	-1.4	132321.9	1.16	0.88	329.1056	-4.12

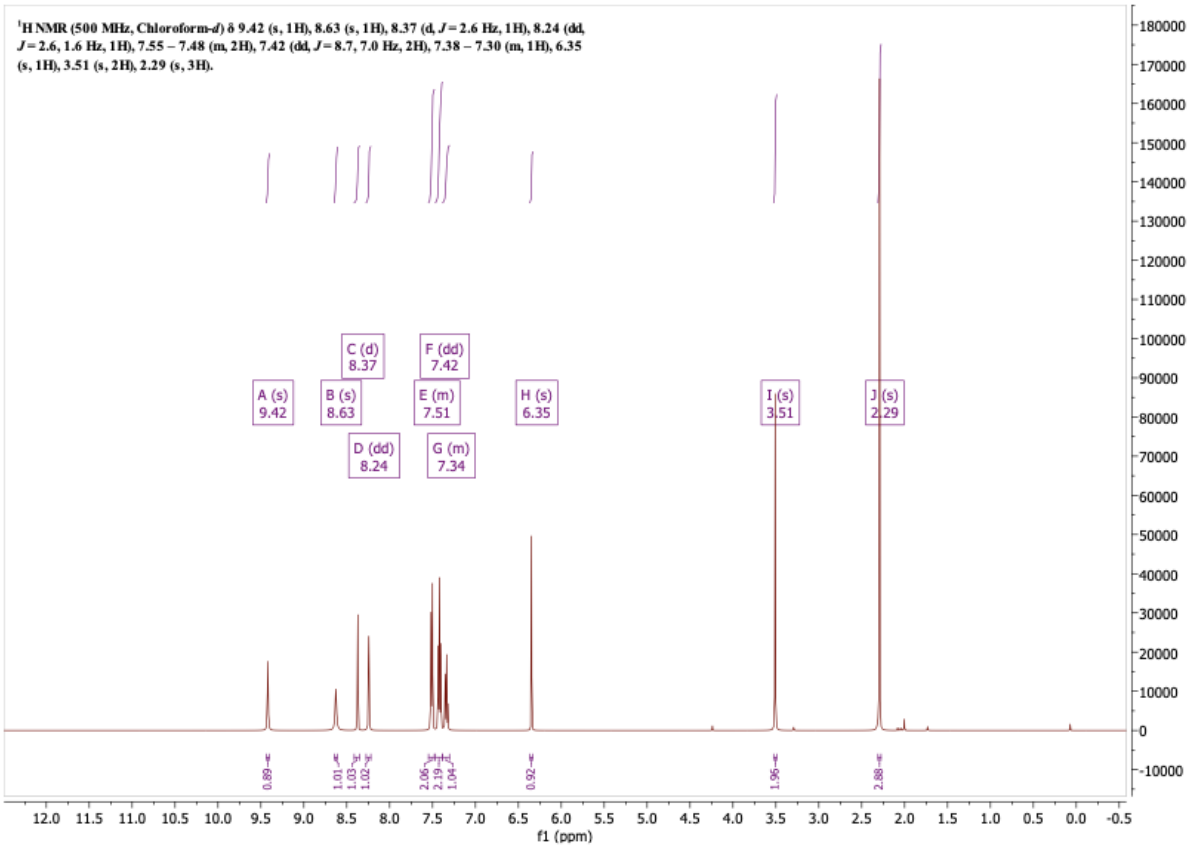




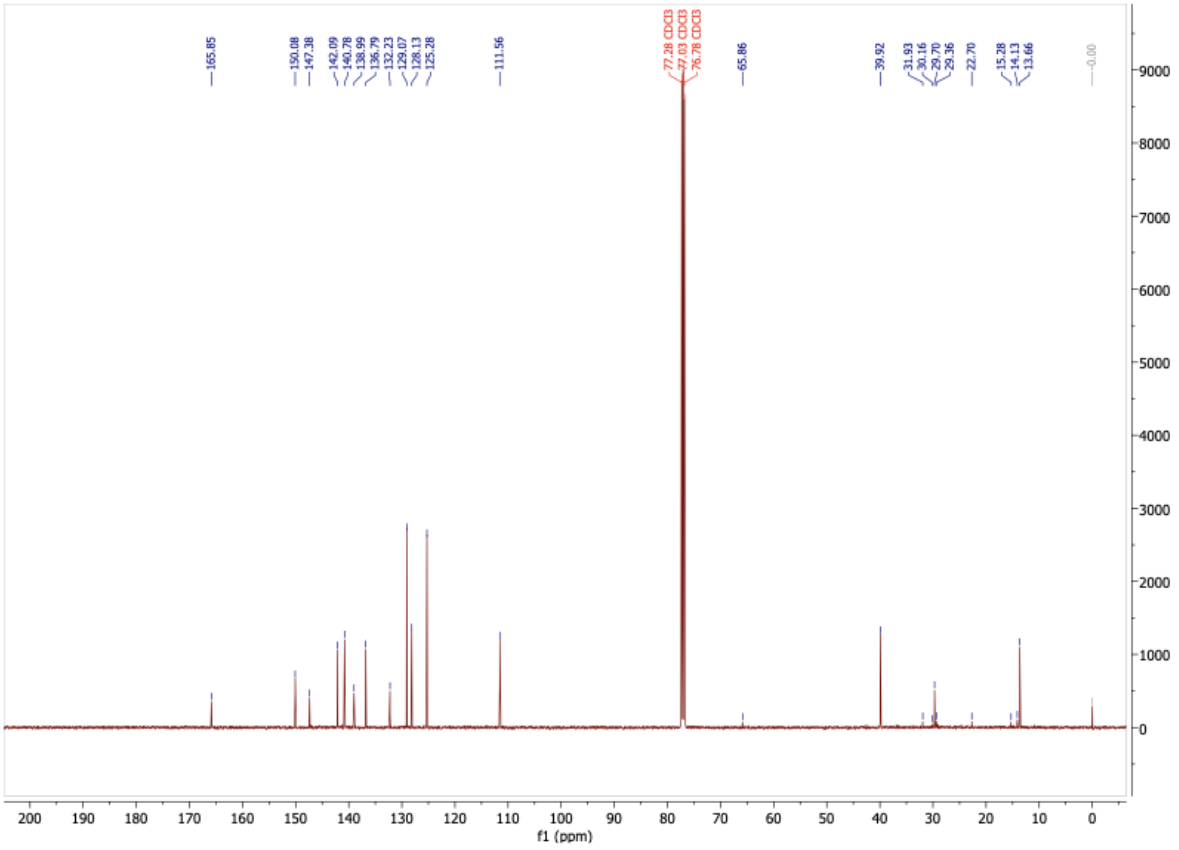
HRMS data of 2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-(pyrimidin-2-yl)acetamide (3g)

2-((3-methyl-1-phenyl-1H-pyrazol-5-yl)thio)-N-(pyrazin-2-yl)acetamide (3h)





[1]H spectrum of 2-((3-methyl-1-phenyl-1H-pyrazol-5-yl)thio)-N-(pyrazin-2-yl)acetamide (3h)



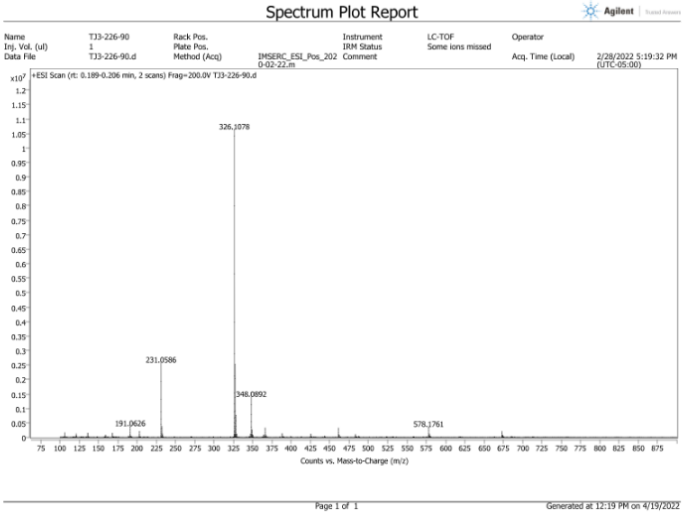
^{13}C spectrum of 2-((3-methyl-1-phenyl-1H-pyrazol-5-yl)thio)-N-(pyrazin-2-yl)acetamide (3h)

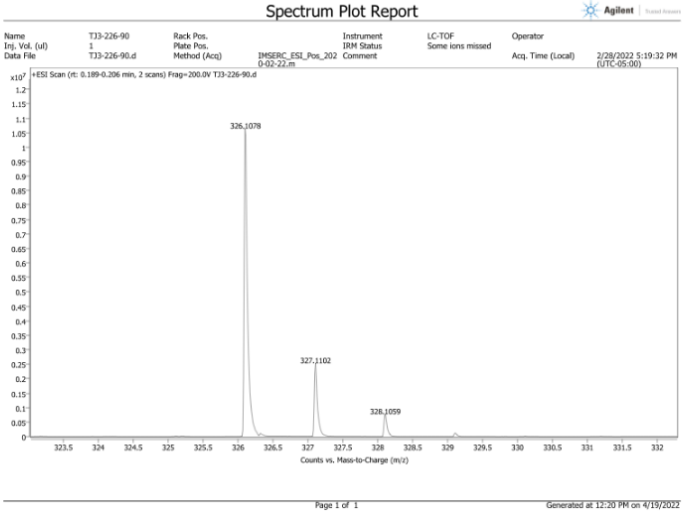
Spectrum Identification Results: + Scan (rt: 0.189-0.206 min) - TJ3-226-90.d (TJ3-226-90.d)

Best	ID Source	Formula	Species	m/z	Score	Diff (ppm)	Diff (mDa)	Score (MFG)
	MFG	**C16 H15 N5 O S**	**(M+H)+**	**326.1078**	**96.58**	**2.19**	**0.71**	**96.58**

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M+H)+	326.1078	94.77	96.59	96.58	96.58	98.75	11062634.1	C16 H16 N5 O S

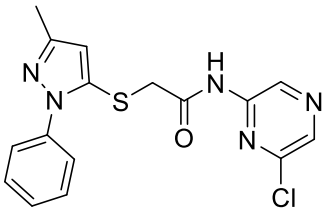
Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z	Diff (ppm)
11359885.2	78.26	100	326.107	0.8	11062634.1	100	76.21	326.1078	2.53
2288276.8	15.76	20.14	327.1097	0.5	2534269.3	22.91	17.46	327.1102	1.54
750706.5	5.17	6.61	328.1058	0.1	794948.3	7.19	5.48	328.1059	0.27
116326.3	0.8	1.02	329.107	-1	123343.1	1.11	0.85	329.106	-3.03

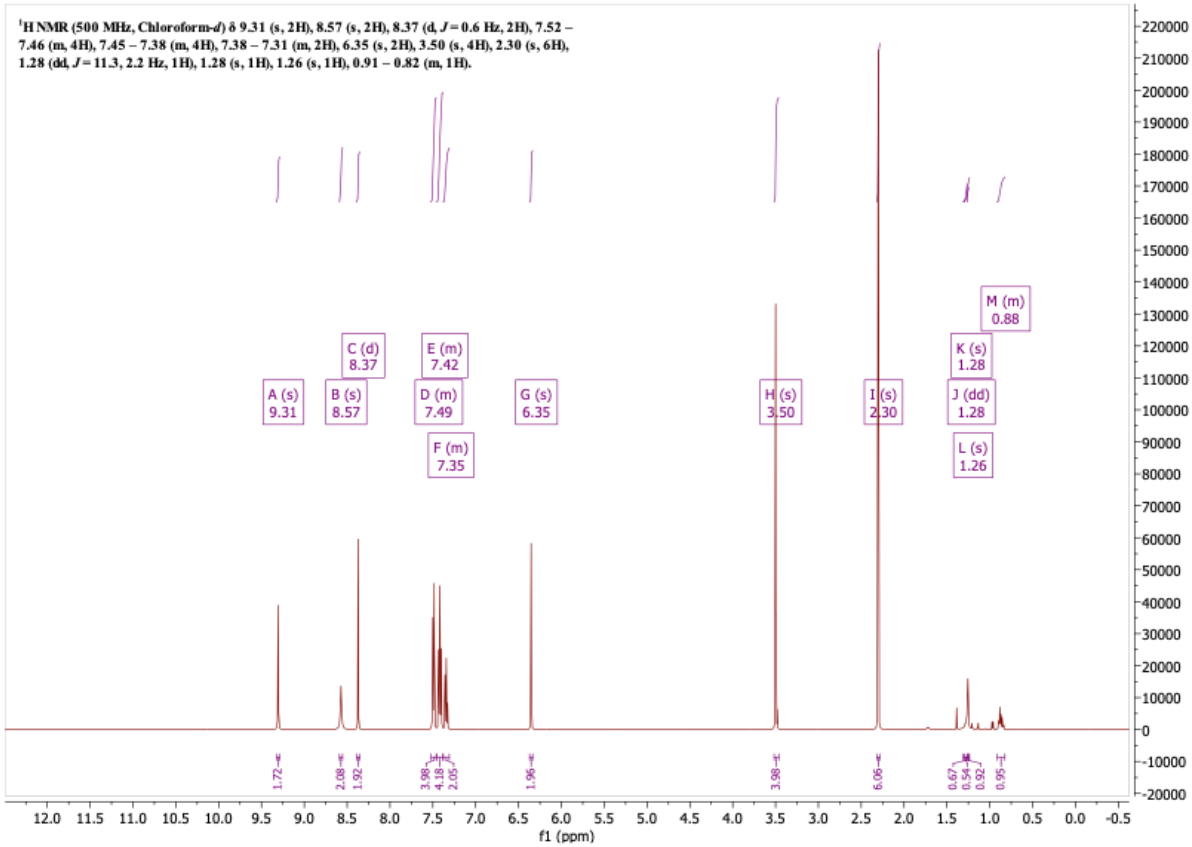




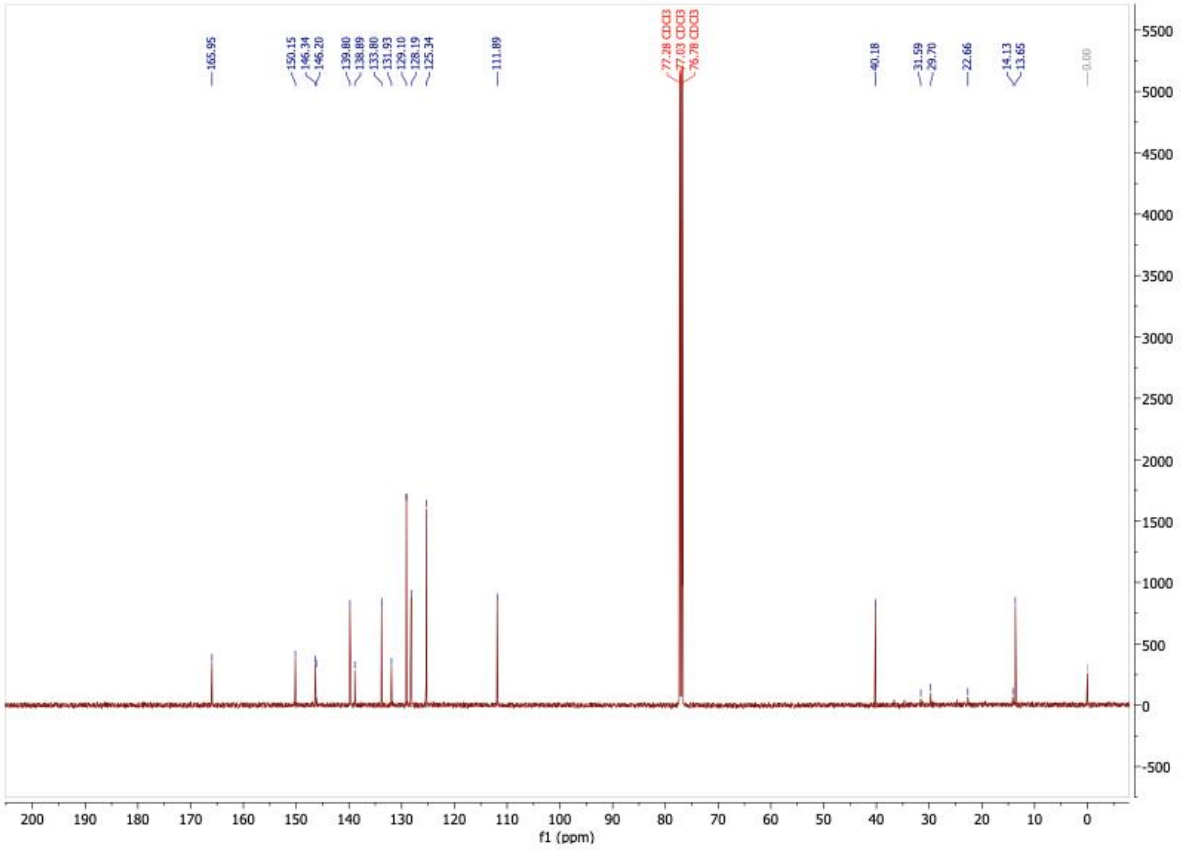
HRMS data of 2-((3-methyl-1-phenyl-1H-pyrazol-5-yl)thio)-N-(pyrazin-2-yl)acetamide (3h)

***N*-(6-chloropyrazin-2-yl)-2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)acetamide (3i)**





[1]H spectrum of *N*-(6-chloropyrazin-2-yl)-2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)acetamide (3i)



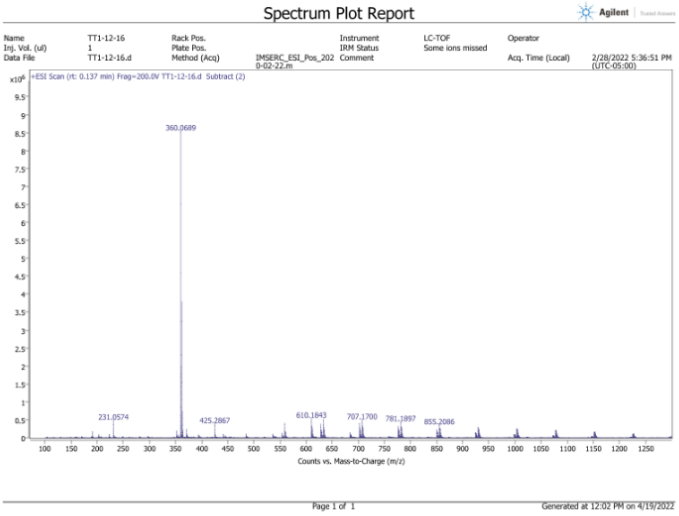
^{13}C spectrum of *N*-(6-chloropyrazin-2-yl)-2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)acetamide (3i)

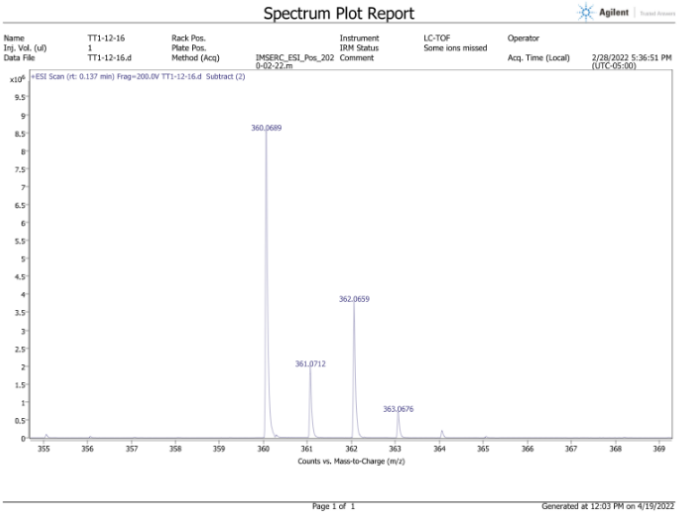
Spectrum Identification Results: + Scan (rt: 0.137 min) Sub (2) - TT1-12-16.d (TT1-12-16.d)

Best	ID Source	Formula	Species	m/z	Score	Diff (ppm)	Diff (mDa)	Score (MFG)
☑	**MFG**	**C16 H14 Cl N5 O S**	**(M+H)+**	**360.0689**	**97.34**	**1.8**	**0.64**	**97.34**

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M+H)+	360.0689	95.49	97.45	97.34	97.34	99.34	8950168.5	C16 H15 Cl N5 O

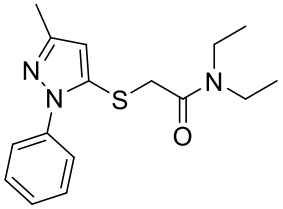
Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z	Diff (ppm)
9334587.5	59.38	100	360.068	0.8	8950168.5	100	56.93	360.0689	2.29
1879237.7	11.95	20.13	361.0707	0.5	2009981.9	22.46	12.79	361.0712	1.44
3603324.4	22.92	38.6	362.0654	0.5	3799873.2	42.46	24.17	362.0659	1.45
696792.8	4.43	7.46	363.0678	-0.2	755650.8	8.44	4.81	363.0676	-0.52
207385.9	1.32	2.22	364.0642	-0.6	205653.8	2.3	1.31	364.0636	-1.64

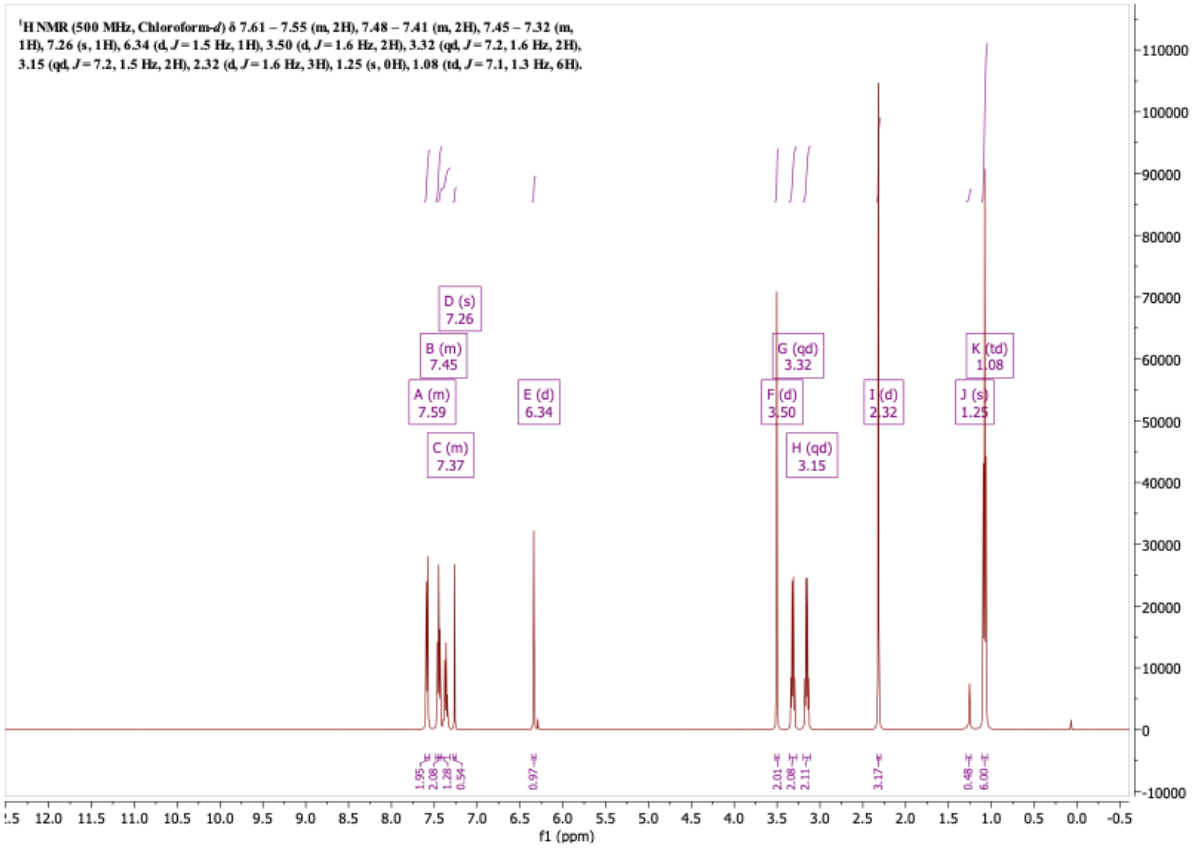




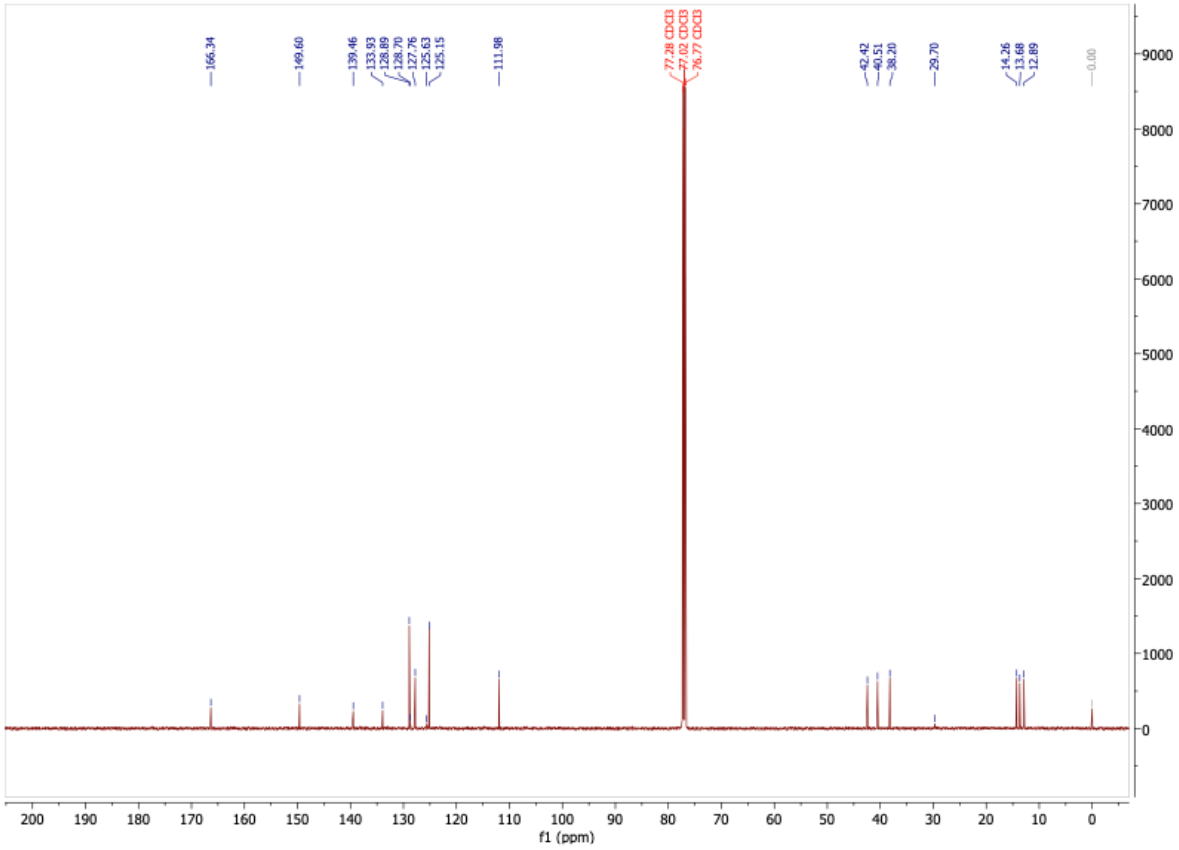
HRMS data of *N*-(6-chloropyrazin-2-yl)-2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)acetamide (3i)

_N_,N-diethyl-2-((3-methyl-1-phenyl-1_H_-pyrazol-5-yl)thio)acetamide (3j)





[1]H spectrum of _N_,N-diethyl-2-((3-methyl-1-phenyl-1_H_-pyrazol-5-yl)thio)acetamide (3j)



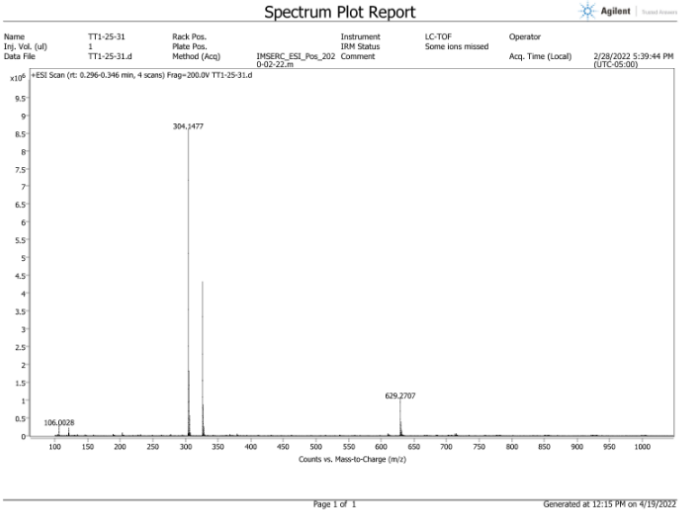
^{13}C spectrum of *N*,N-diethyl-2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)acetamide (3j)

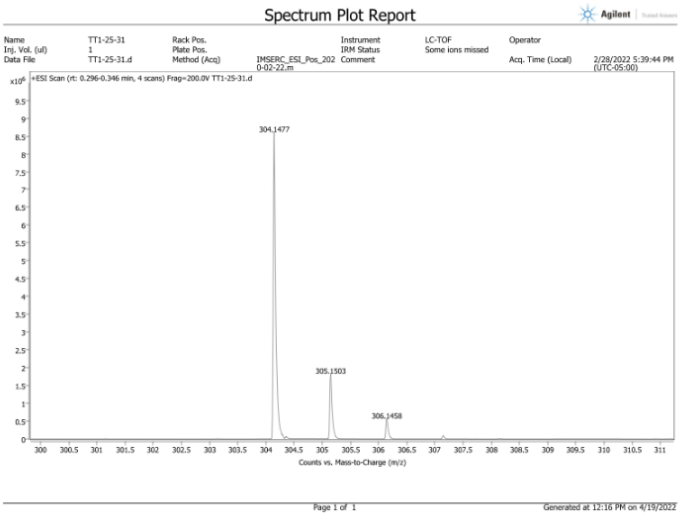
Spectrum Identification Results: + Scan (rt: 0.296-0.346 min) - TT1-25-31.d (TT1-25-31.d)

Best	ID Source	Formula	Species	m/z	Score	Diff (ppm)	Diff (mDa)	Score (MFG)
☑	**MFG**	**C16 H21 N3 O S**	**(M+H)+**	**304.1477**	**98.82**	**-0.78**	**-0.24**	**98.82**

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M+H)+	304.1477	97.39	99.59	98.82	98.82	99	8610679.5	C16 H22 N3 O S

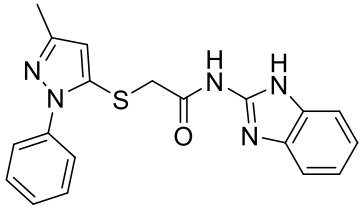
Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z	Diff (ppm)
8755245.4	78.78	100	304.1478	-0.1	8610679.5	100	77.48	304.1477	-0.48
1705682.3	15.35	19.48	305.1507	-0.4	1839212	21.36	16.55	305.1503	-1.3
567220.7	5.1	6.48	306.1466	-0.9	576302.4	6.69	5.19	306.1458	-2.86
85886.9	0.77	0.98	307.148	-1.5	87841.5	1.02	0.79	307.1465	-4.77

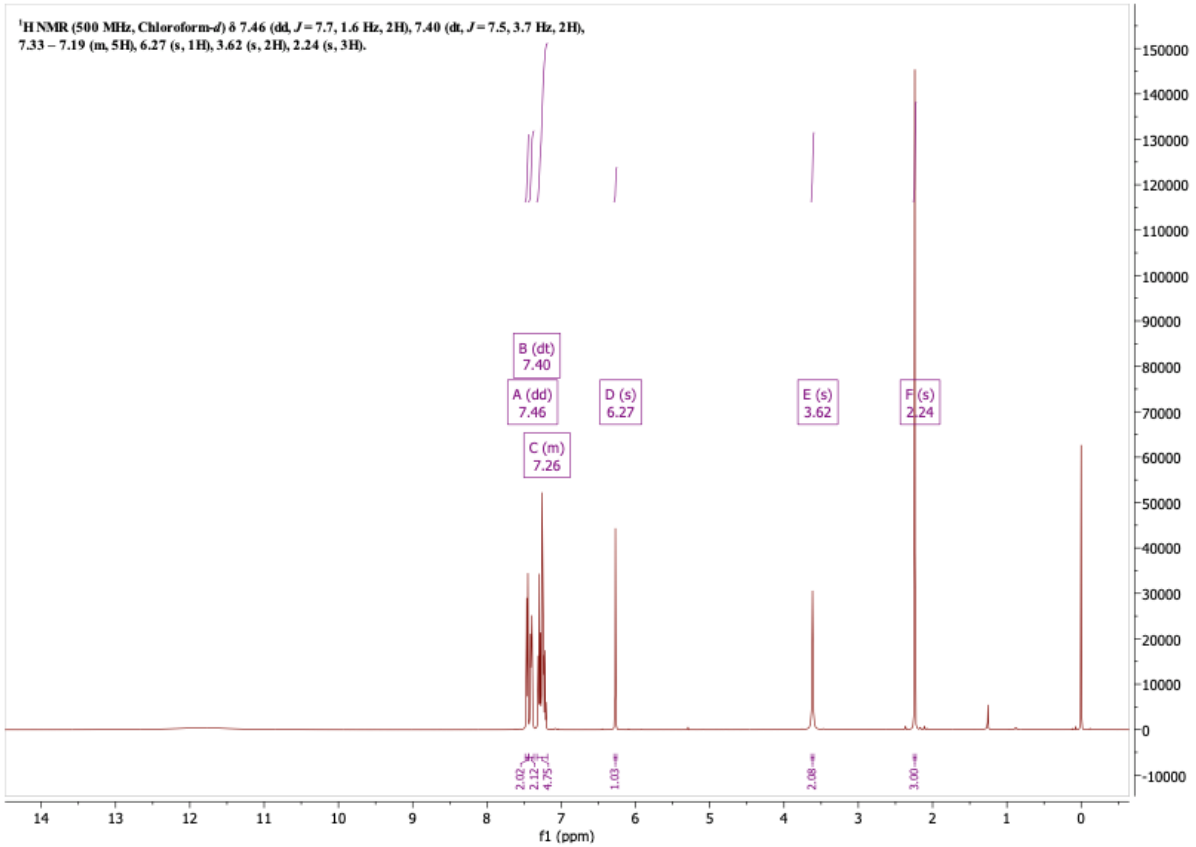




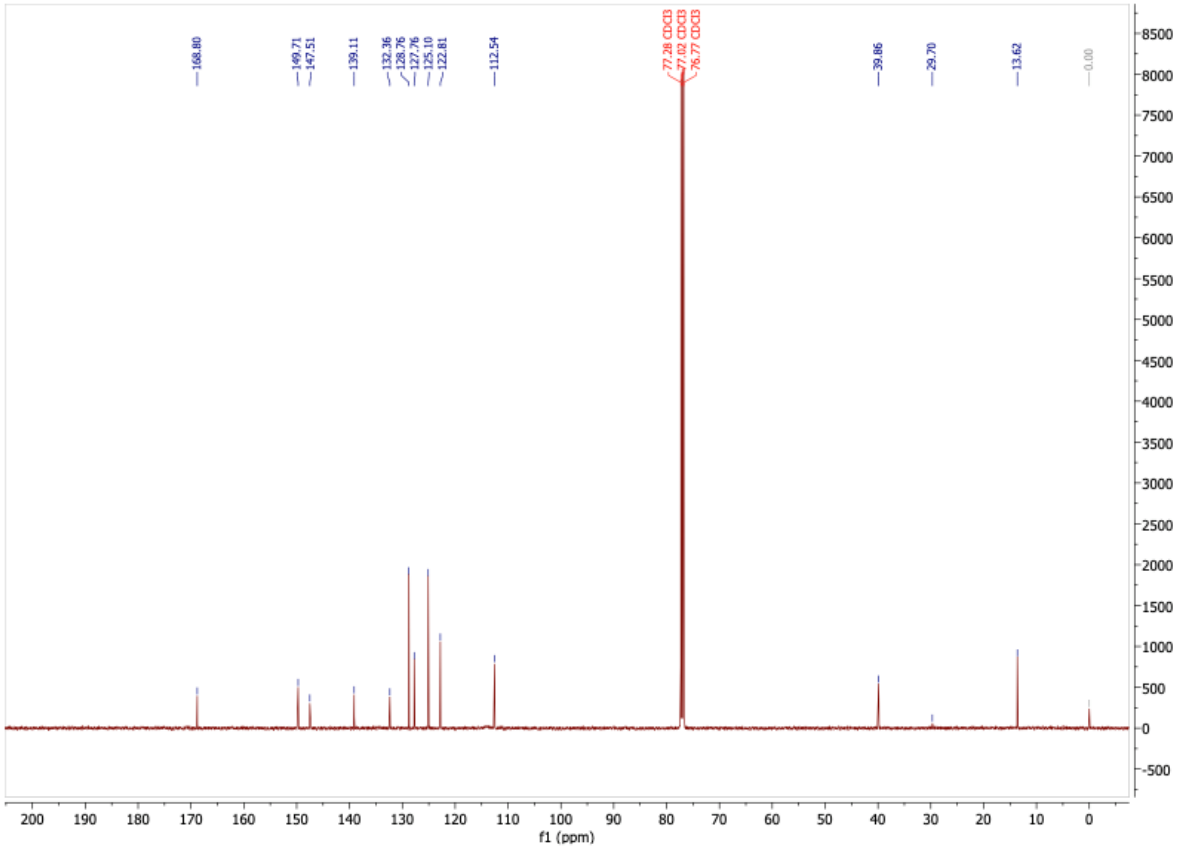
HRMS data of *N*,N-diethyl-2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)acetamide (3j)

_N_-(1_H_-benzo[d]imidazol-2-yl)-2-((3-methyl-1-phenyl-1H-pyrazol-5-yl)thio)acetamide (3k)





[1]H spectrum of _N_-(1_H_-benzo[d]imidazol-2-yl)-2-((3-methyl-1-phenyl-1H-pyrazol-5-yl)thio)acetamide (3k)



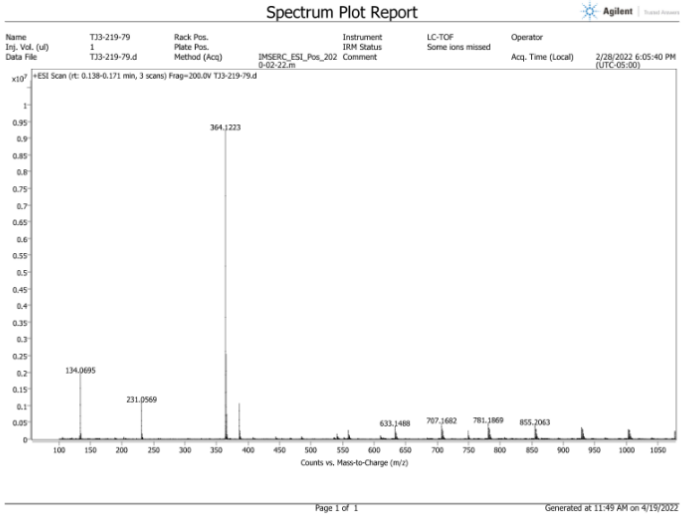
^{13}C spectrum of *N*-(1*H*-benzo[d]imidazol-2-yl)-2-((3-methyl-1-phenyl-1H-pyrazol-5-yl)thio)acetamide (3k)

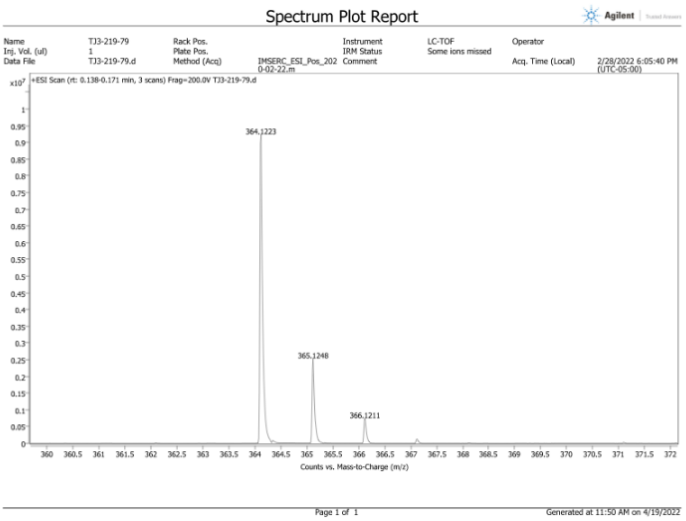
Spectrum Identification Results: + Scan (rt: 0.138-0.171 min) - TJ3-219-79.d (TJ3-219-79.d)

Best	ID Source	Formula	Species	m/z	Score	Diff (ppm)	Diff (mDa)	Score (MFG)
☑	**MFG**	**C19 H17 N5 O S**	**(M+H)+**	**364.1223**	**96.86**	**-1.23**	**-0.45**	**96.86**

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M+H)+	364.1223	92.2	98.78	96.86	96.86	98.64	9405303.1	C19 H18 N5 O S

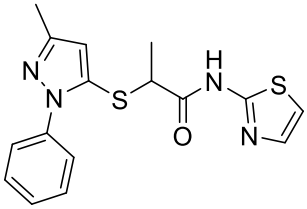
Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z	Diff (ppm)
9733731.4	75.78	100	364.1227	-0.3	9405303.1	100	73.22	364.1223	-0.89
2278784	17.74	23.41	365.1254	-0.6	2544216.7	27.05	19.81	365.1248	-1.78
710803.2	5.53	7.3	366.1222	-1.1	767887	8.16	5.98	366.1211	-2.94
121409.6	0.95	1.25	367.1231	-1.8	127321.3	1.35	0.99	367.1212	-5

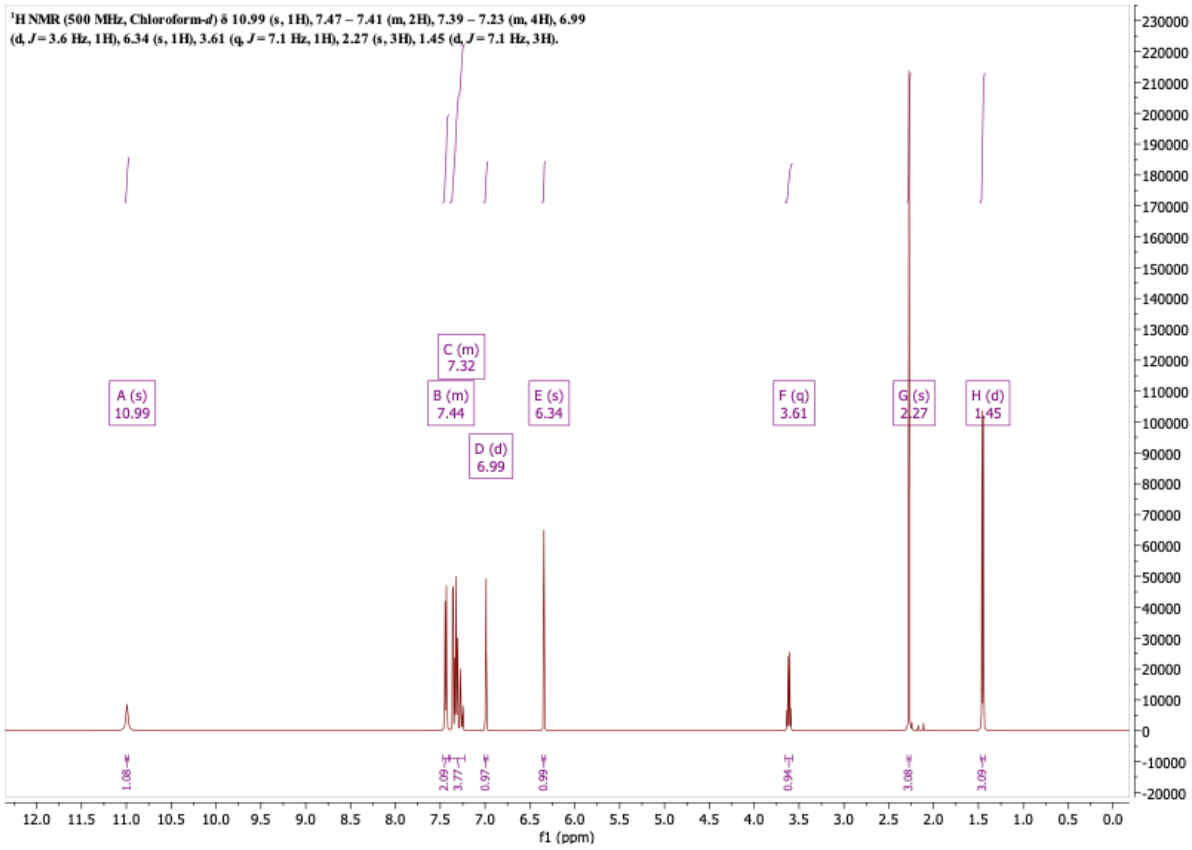




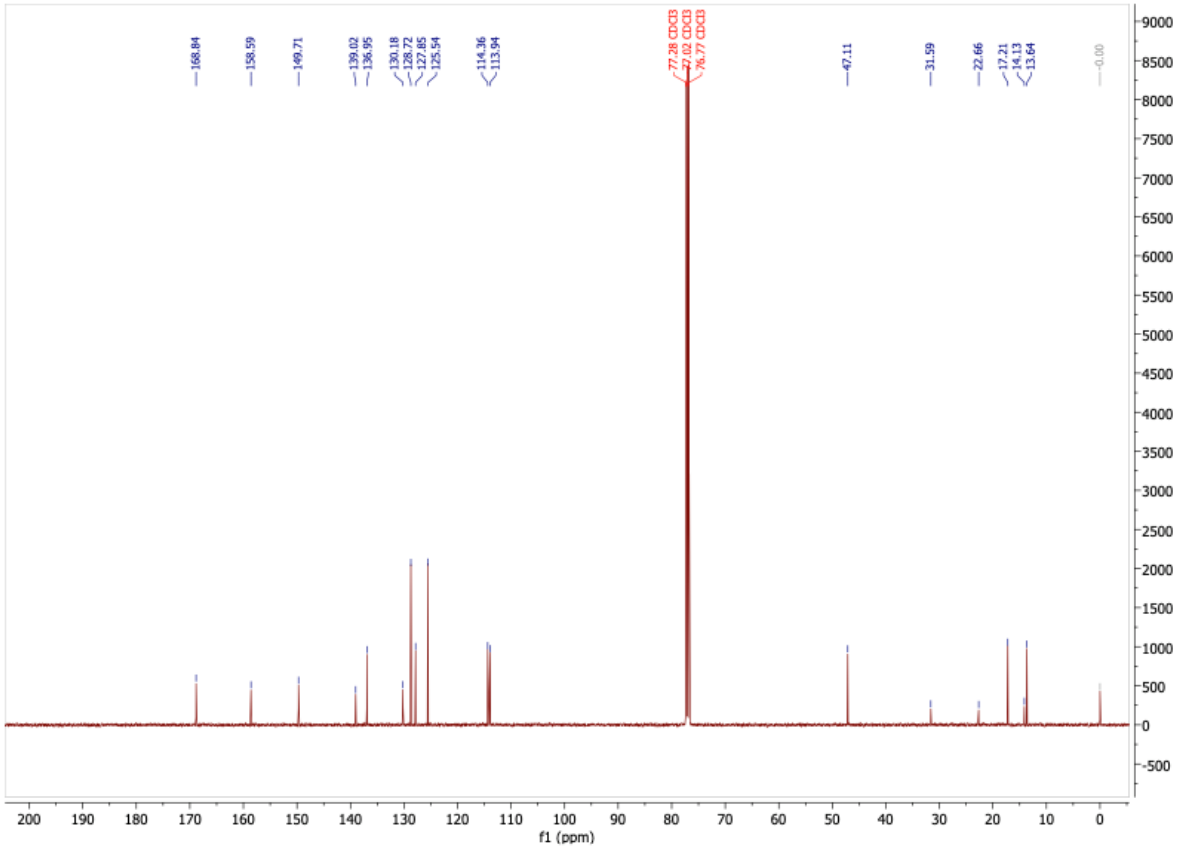
HRMS data of *N*-(1*H*-benzo[d]imidazol-2-yl)-2-((3-methyl-1-phenyl-1H-pyrazol-5-yl)thio)acetamide (3k)

2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-(thiazol-2-yl)propanamide (4a)





[1]H spectrum of 2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-(thiazol-2-yl)propanamide (4a)



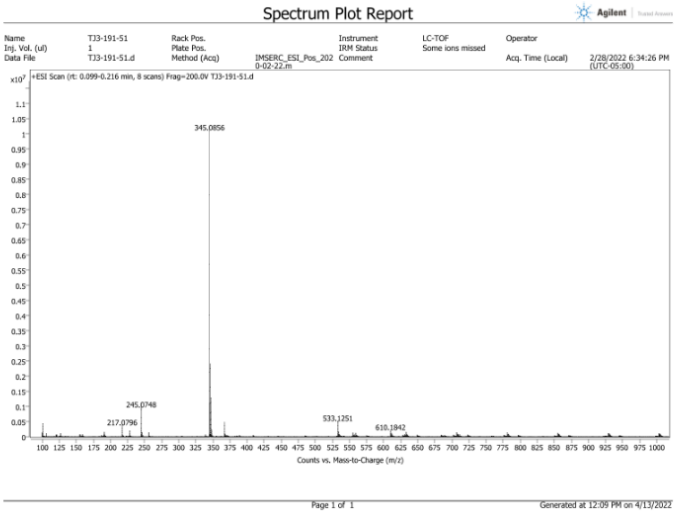
^{13}C spectrum of 2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-(thiazol-2-yl)propanamide (4a)

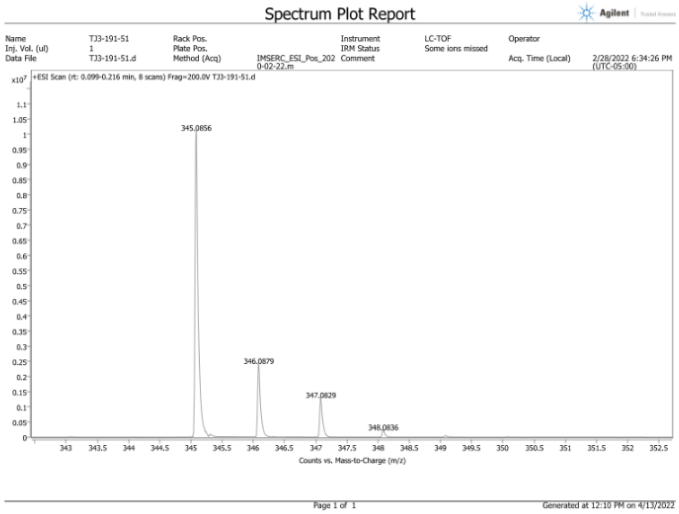
Spectrum Identification Results: + Scan (rt: 0.099-0.216 min) (TJ3-191-51.d)

Best	ID Source	Formula	Species	m/z	Score	Diff (ppm)	Diff (mDa)	Score (MFG)
☑	**MFG**	**C16 H16 N4 O S2**	**(M+H)+**	**345.0856**	**89.84**	**4.83**	**1.66**	**89.84**

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M+H)+	345.0856	92.32	83.63	89.84	89.84	99.27	10276547.9	C16 H17 N4 O S2

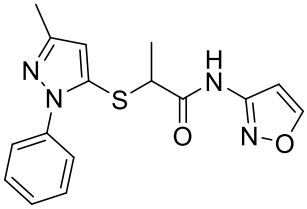
Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z	Diff (ppm)
10678765.7	74.8	100	345.0838	1.8	10276547.9	100	71.98	345.0856	5.12
2197605.9	15.39	20.58	346.0865	1.5	2468489.8	24.02	17.29	346.0879	4.2
1192692.5	8.35	11.17	347.0815	1.5	1297581	12.63	9.09	347.0829	4.23
206946.7	1.45	1.94	348.0832	0.4	233392.1	2.27	1.63	348.0836	1.12

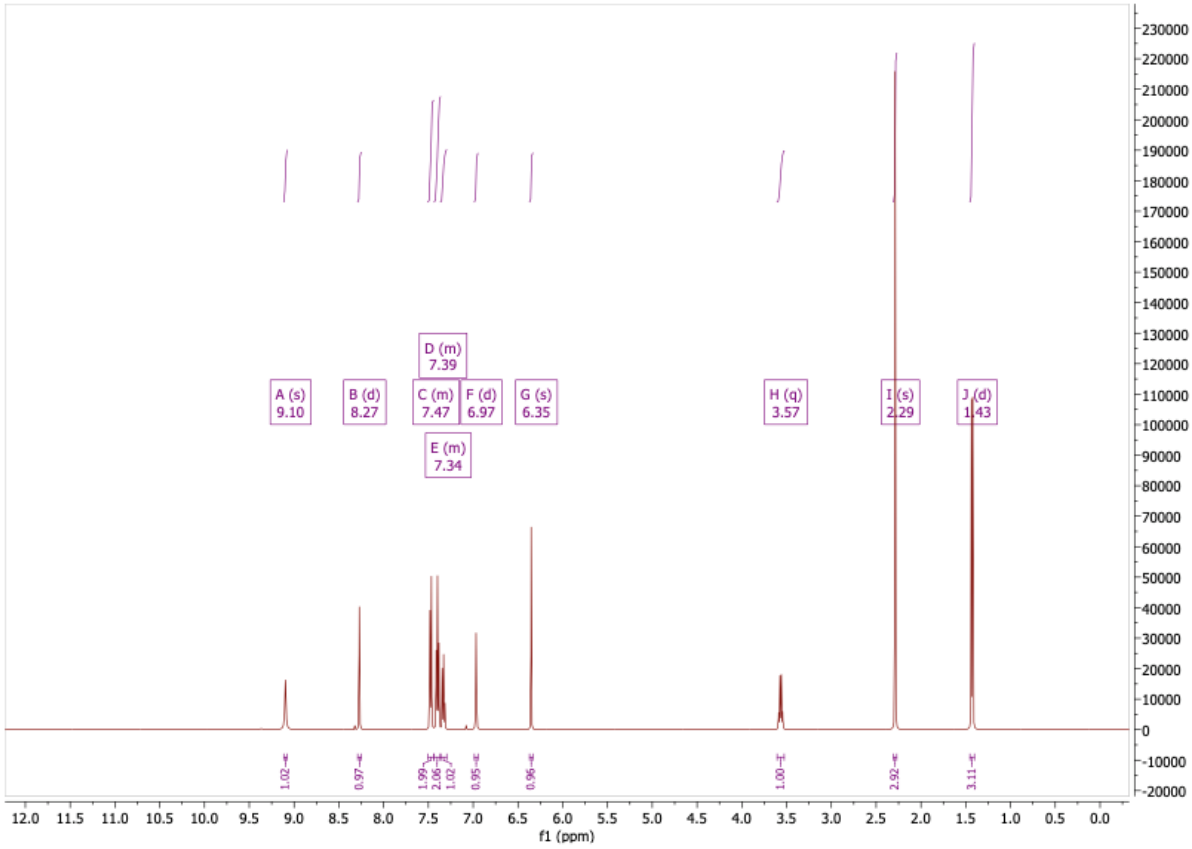




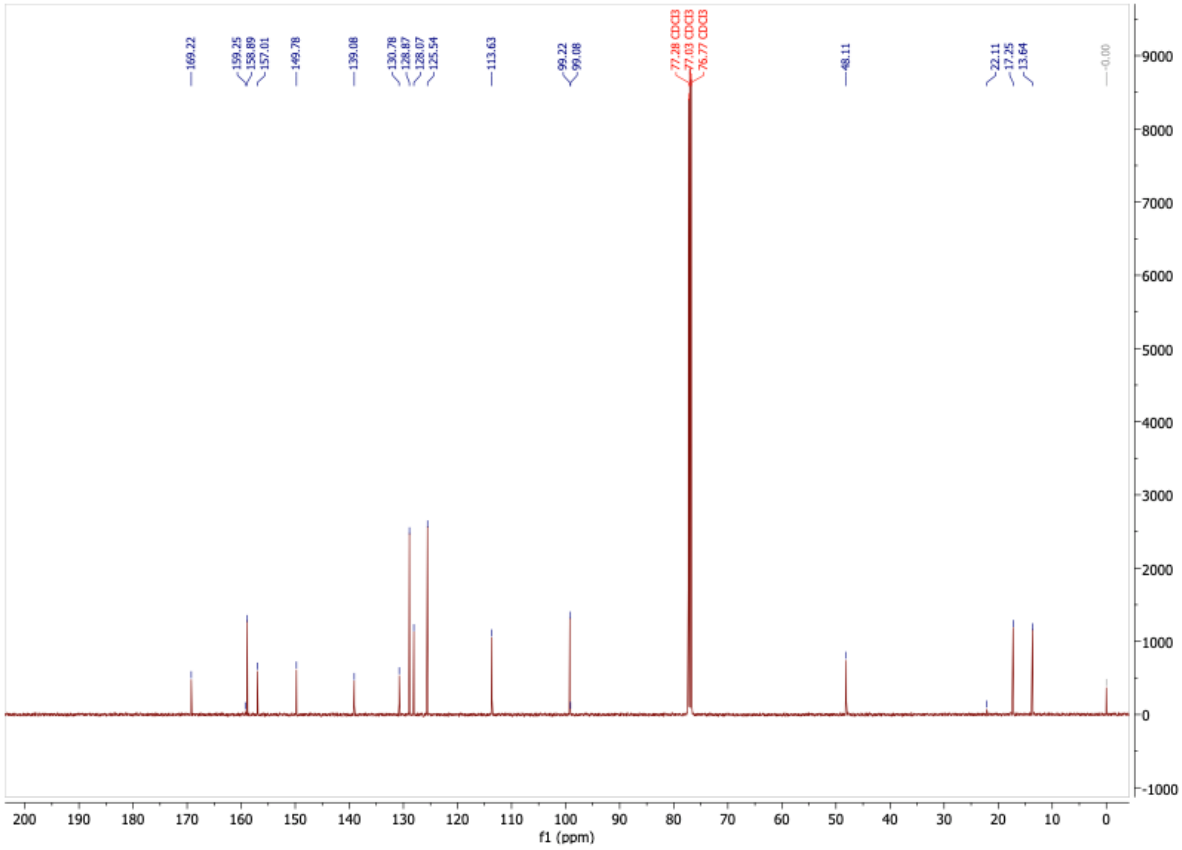
HRMS data of 2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-(thiazol-2-yl)propanamide (4a)

_N_-(isoxazol-3-yl)-2-((3-methyl-1-phenyl-1_H_-pyrazol-5-yl)thio)propanamide (4b)





[1]H spectrum of _N_-(isoxazol-3-yl)-2-((3-methyl-1-phenyl-1_H_-pyrazol-5-yl)thio)propanamide (4b)



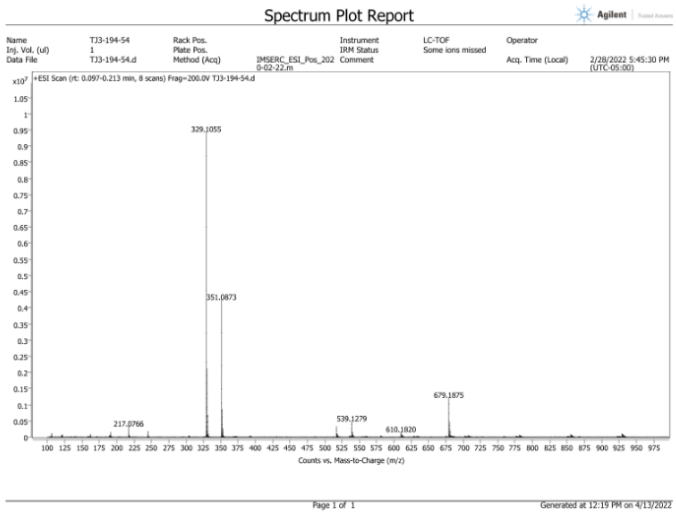
^{13}C spectrum of *N*-(isoxazol-3-yl)-2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)propanamide (4b)

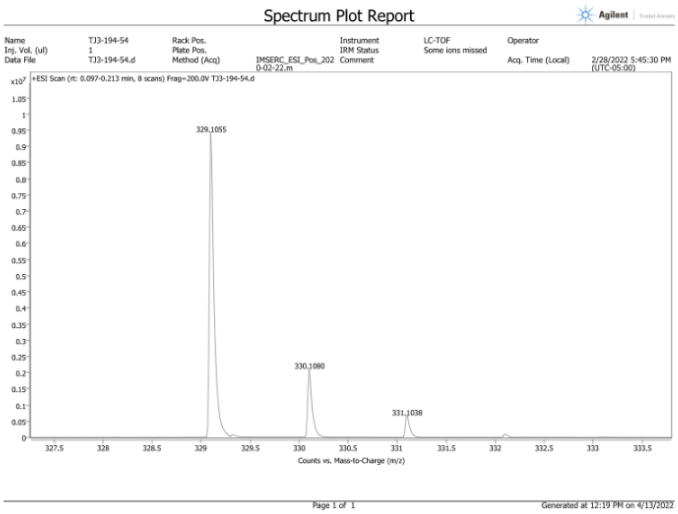
Spectrum Identification Results: + Scan (rt: 0.097-0.213 min) (TJ3-194-54.d)

Best	ID Source	Formula	Species	m/z	Score	Diff (ppm)	Diff (mDa)	Score (MFG)
☑	**MFG**	**C16 H16 N4 O2 S**	**(M+H)+**	**329.1055**	**93.64**	**-3.79**	**-1.24**	**93.64**

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M+H)+	329.1055	95.24	90.06	93.64	93.64	98.88	9598134.3	C16 H17 N4 O2 S

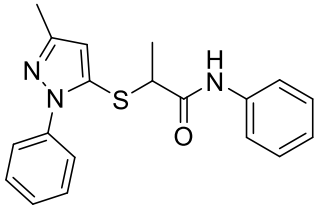
Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z	Diff (ppm)
9831764.9	78.36	100	329.1067	-1.1	9598134.3	100	76.49	329.1055	-3.47
1949417.6	15.54	19.83	330.1094	-1.4	2152993	22.43	17.16	330.108	-4.38
663787.3	5.29	6.75	331.1057	-1.8	693154.1	7.22	5.52	331.1038	-5.54
102647.7	0.82	1.04	332.107	-2.5	103336	1.08	0.82	332.1046	-7.43

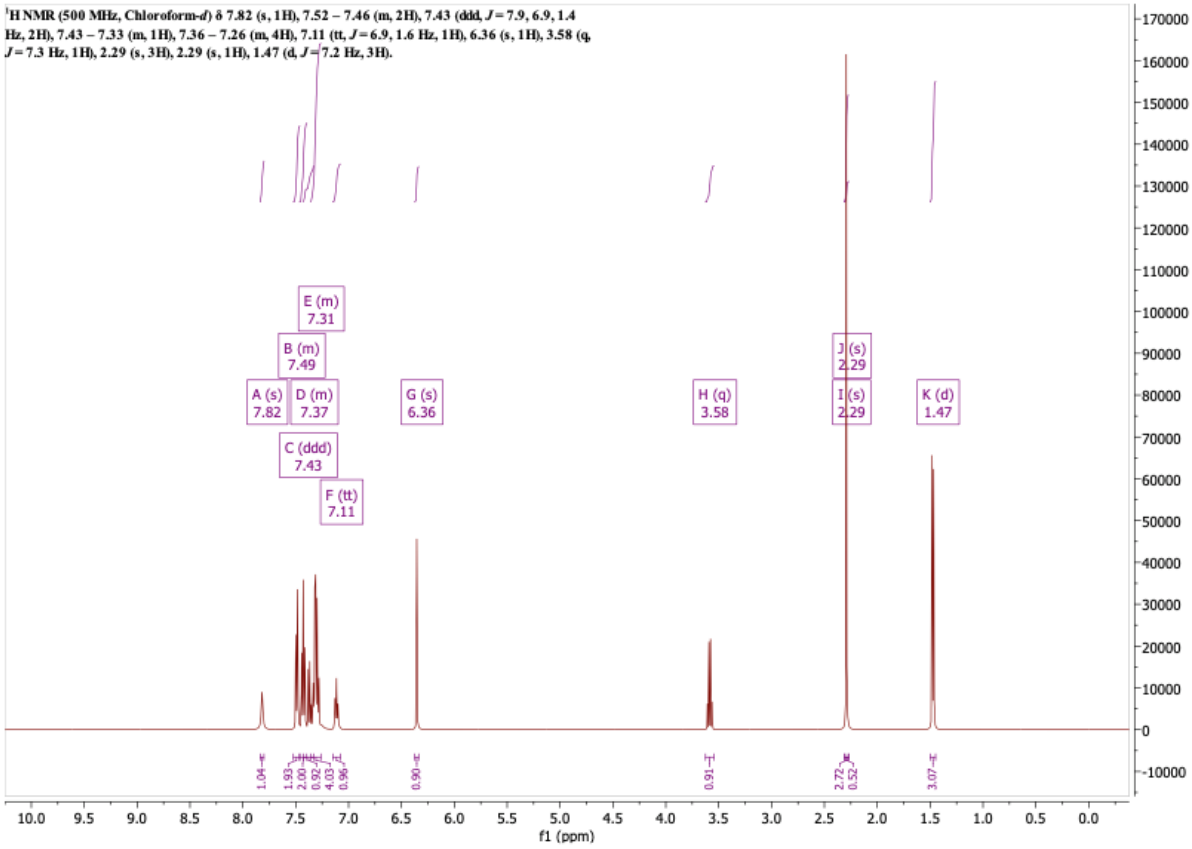




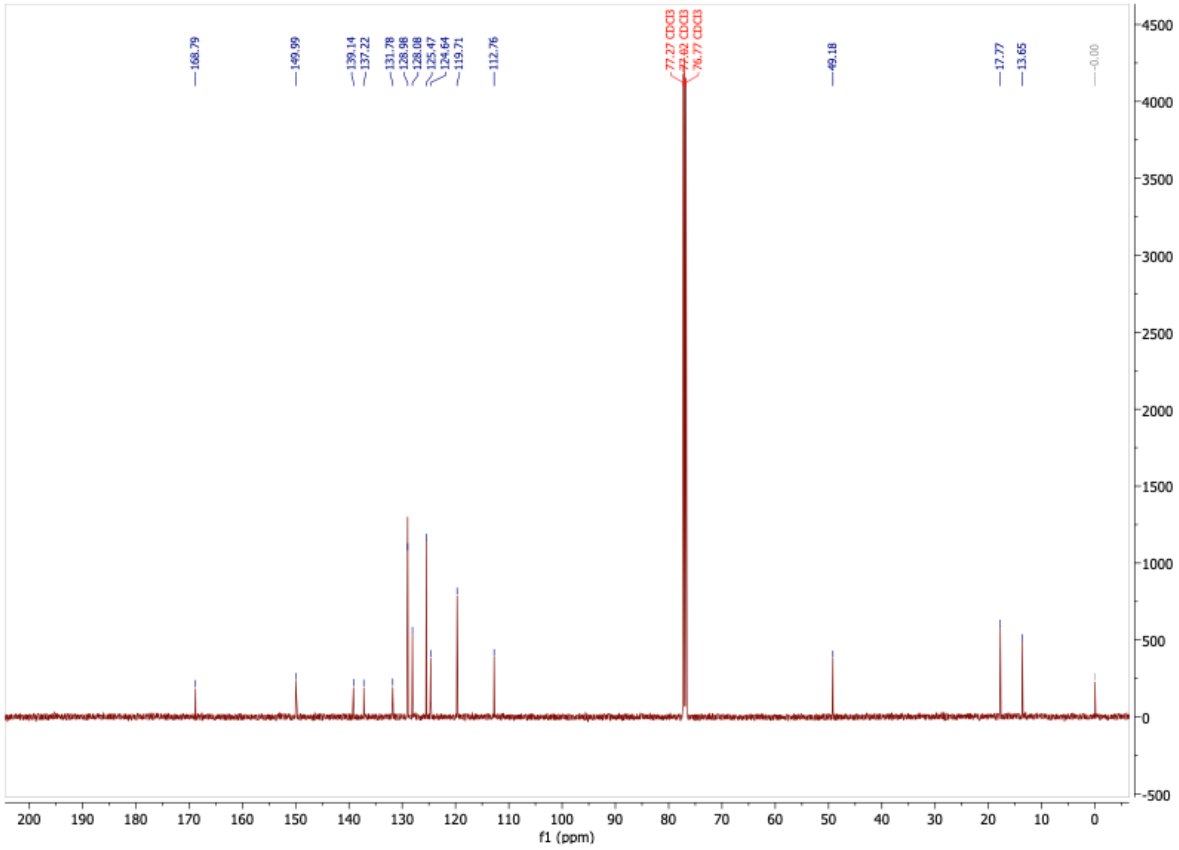
HRMS data of *N*-(isoxazol-3-yl)-2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)propanamide (4b)

2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-phenylpropanamide (4c)





[1]H spectrum of 2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-phenylpropanamide (4c)



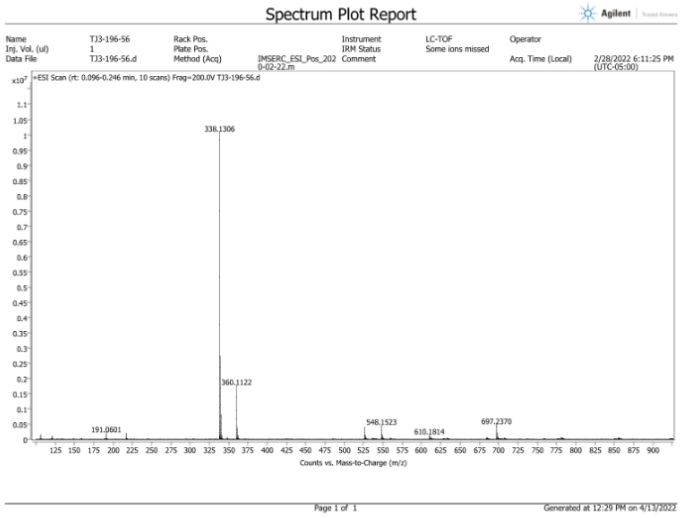
^{13}C spectrum of 2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-phenylpropanamide (4c)

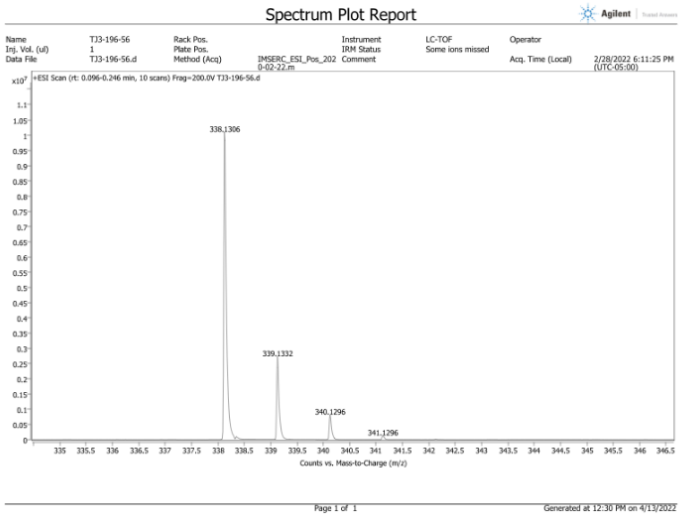
Spectrum Identification Results: + Scan (rt: 0.096-0.246 min) (TJ3-196-56.d)

Best	ID Source	Formula	Species	m/z	Score	Diff (ppm)	Diff (mDa)	Score (MFG)
☑	**MFG**	**C19 H19 N3 O S**	**(M+H)+**	**338.1306**	**89.65**	**-4.89**	**-1.65**	**89.65**

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M+H)+	338.1306	92.31	83.56	89.65	89.65	98.65	10452599.7	C19 H20 N3 O S

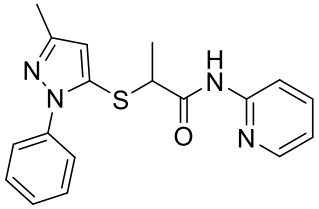
Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z	Diff (ppm)
10804745.5	76.31	100	338.1322	-1.5	10452599.7	100	73.83	338.1306	-4.5
2453060.7	17.33	22.7	339.1351	-1.9	2745858.7	26.27	19.39	339.1332	-5.71
771527.9	5.45	7.14	340.1317	-2.1	822674.4	7.87	5.81	340.1296	-6.07
129280	0.91	1.2	341.1328	-3.2	137481.4	1.32	0.97	341.1296	-9.31

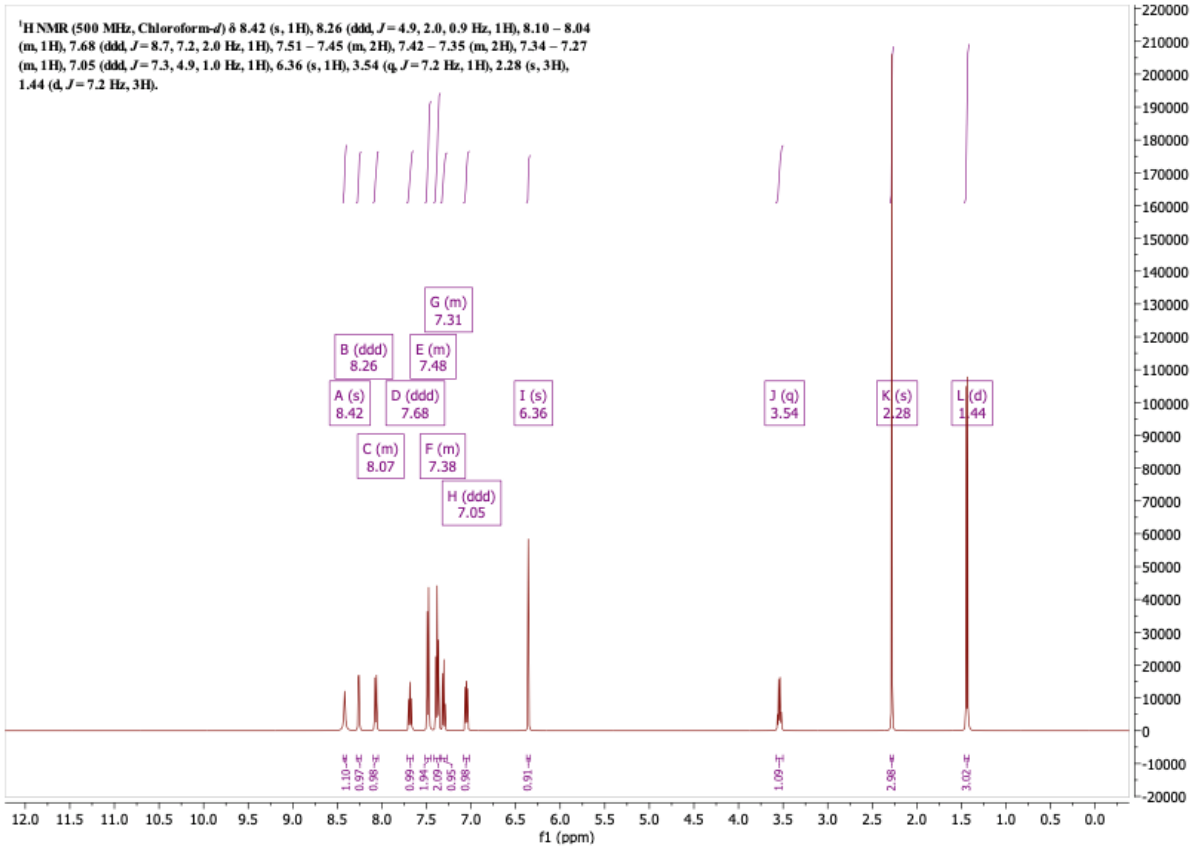




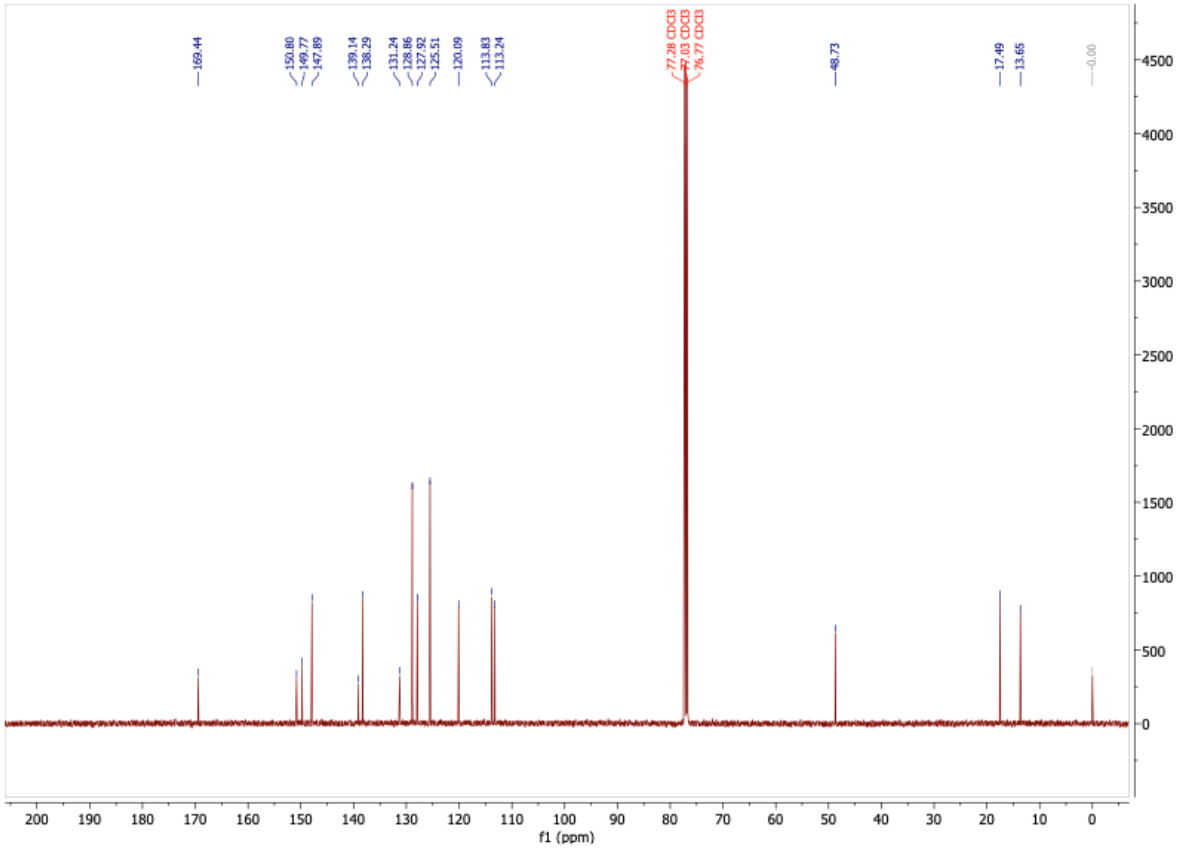
HRMS data of 2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-phenylpropanamide (4c)

2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-(pyridine-2-yl)propanamide (4d)





[1]H spectrum of 2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-(pyridine-2-yl)propanamide (4d)



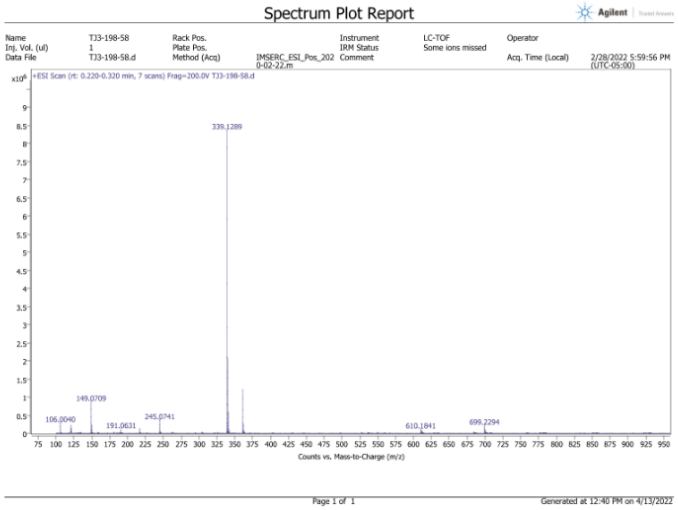
^{13}C spectrum of 2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-(pyridine-2-yl)propanamide (4d)

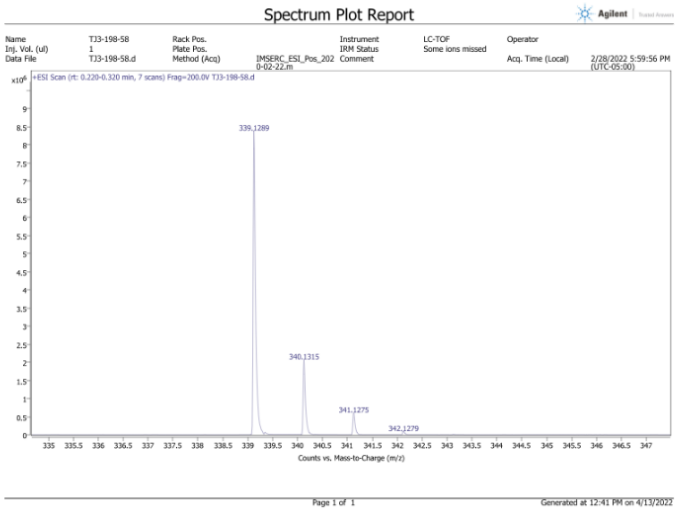
Spectrum Identification Results: + Scan (rt: 0.220-0.320 min) (TJ3-198-58.d)

Best	ID Source	Formula	Species	m/z	Score	Diff (ppm)	Diff (mDa)	Score (MFG)
☑	MFG	C18 H18 N4 O S	(M+H)+	339.1289	92.78	4.23	1.43	92.78

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M+H)+	339.1289	96.54	87.44	92.78	92.78	98.97	8587940.8	C18 H19 N4 O S

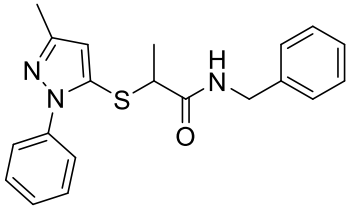
Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z	Diff (ppm)
8755587.1	76.86	100	339.1274	1.5	8587940.8	100	75.39	339.1289	4.51
1924110.7	16.89	21.98	340.1302	1.3	2081878.3	24.24	18.28	340.1315	3.7
611424.2	5.37	6.98	341.1267	0.8	621880.6	7.24	5.46	341.1275	2.37
100359.9	0.88	1.15	342.1278	0.1	99782.3	1.16	0.88	342.1279	0.41

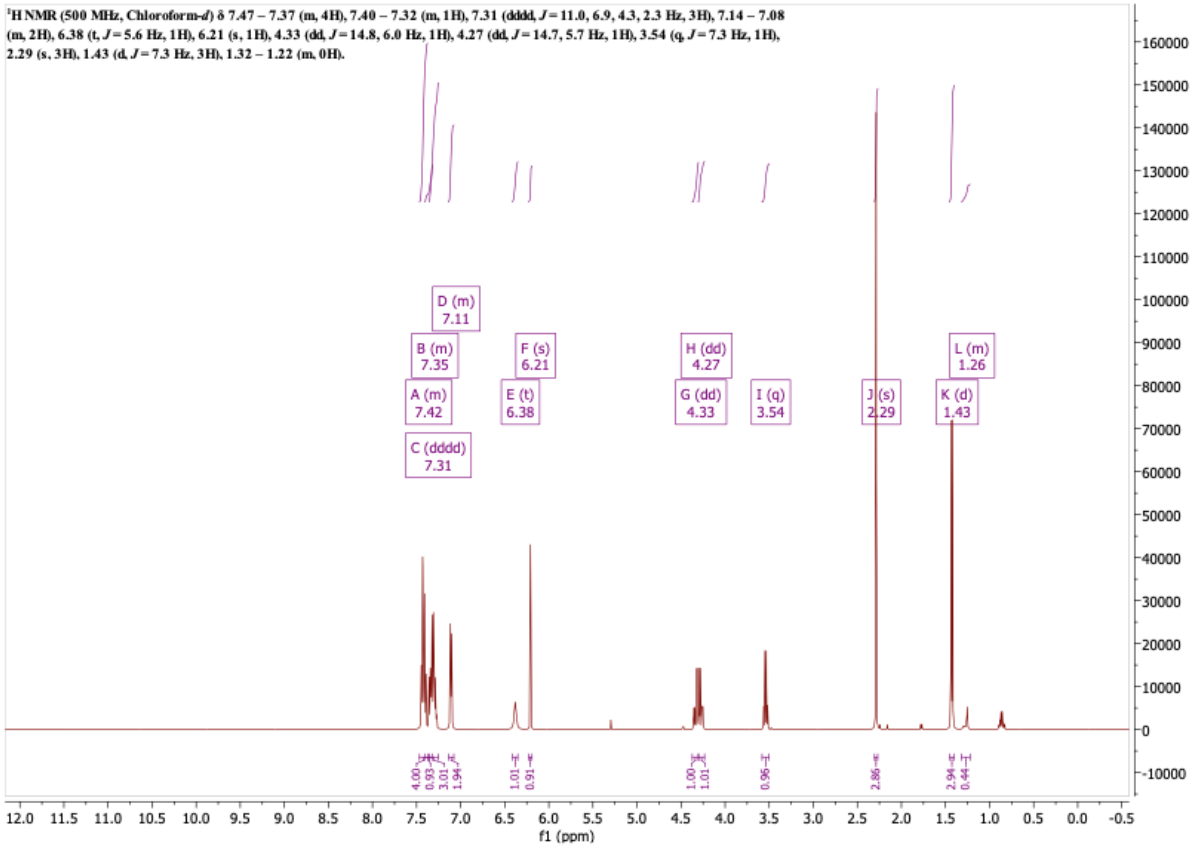




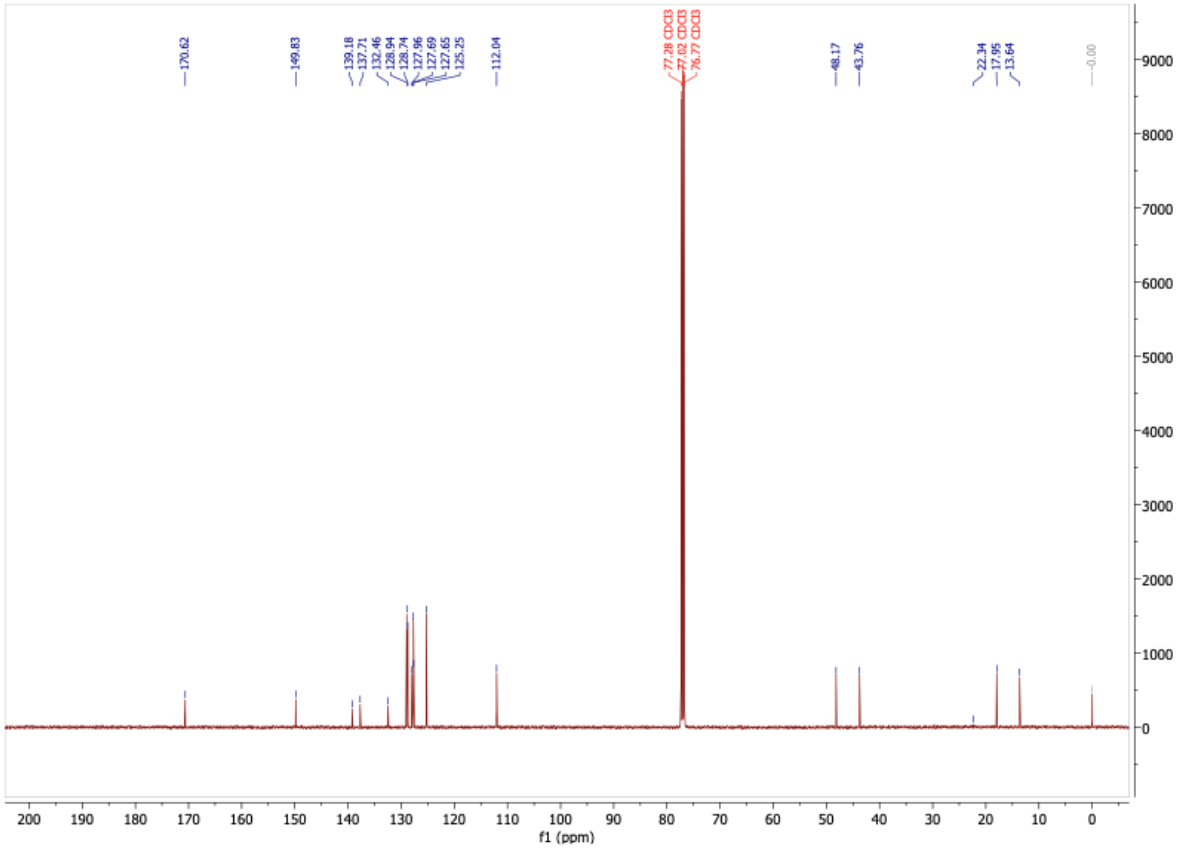
HRMS data of 2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)-*N*-(pyridine-2-yl)propanamide (4d)

_N_-benzyl-2-((3-methyl-1-phenyl-1_H_-pyrazol-5-yl)thio)propanamide (4e)





[1]H spectrum of _N_-benzyl-2-((3-methyl-1-phenyl-1_H_-pyrazol-5-yl)thio)propanamide (4e)



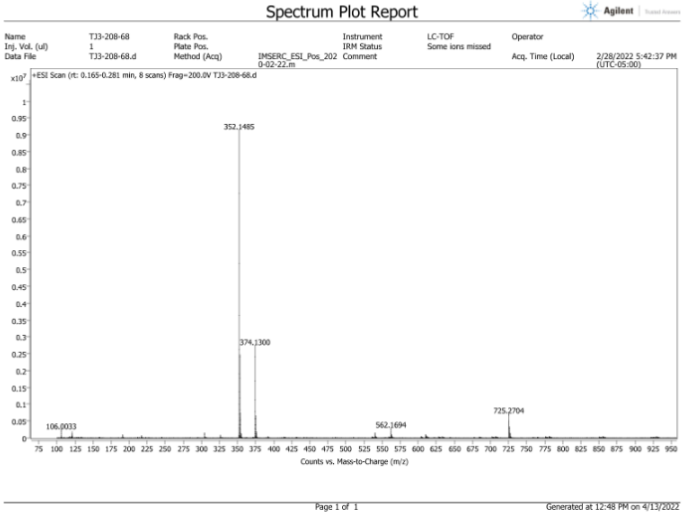
^{13}C spectrum of *N*-benzyl-2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)propanamide (4e)

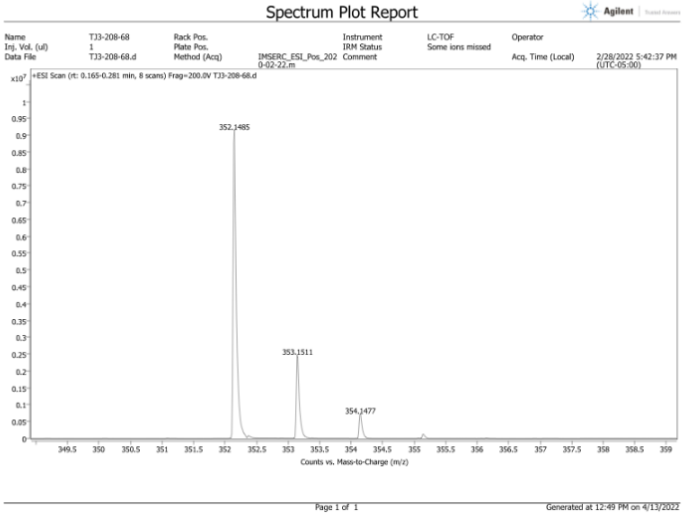
Spectrum Identification Results: + Scan (rt: 0.165-0.281 min) (TJ3-208-68.d)

Best	ID Source	Formula	Species	m/z	Score	Diff (ppm)	Diff (mDa)	Score (MFG)
	MFG	C20 H21 N3 O S	(M+H)+	352.1485	96.23	1.62	0.57	96.23

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M+H)+	352.1485	91.17	97.97	96.23	96.23	98.81	9276369.4	C20 H22 N3 O S

Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z	Diff (ppm)
9616003	75.49	100	352.1478	0.7	9276369.4	100	72.82	352.1485	1.97
2289390	17.97	23.81	353.1508	0.3	2572114.2	27.73	20.19	353.1511	0.93
710783	5.58	7.39	354.1476	0.1	762466.3	8.22	5.99	354.1477	0.34
122646.5	0.96	1.28	355.1485	-0.9	127872.6	1.38	1	355.1476	-2.65

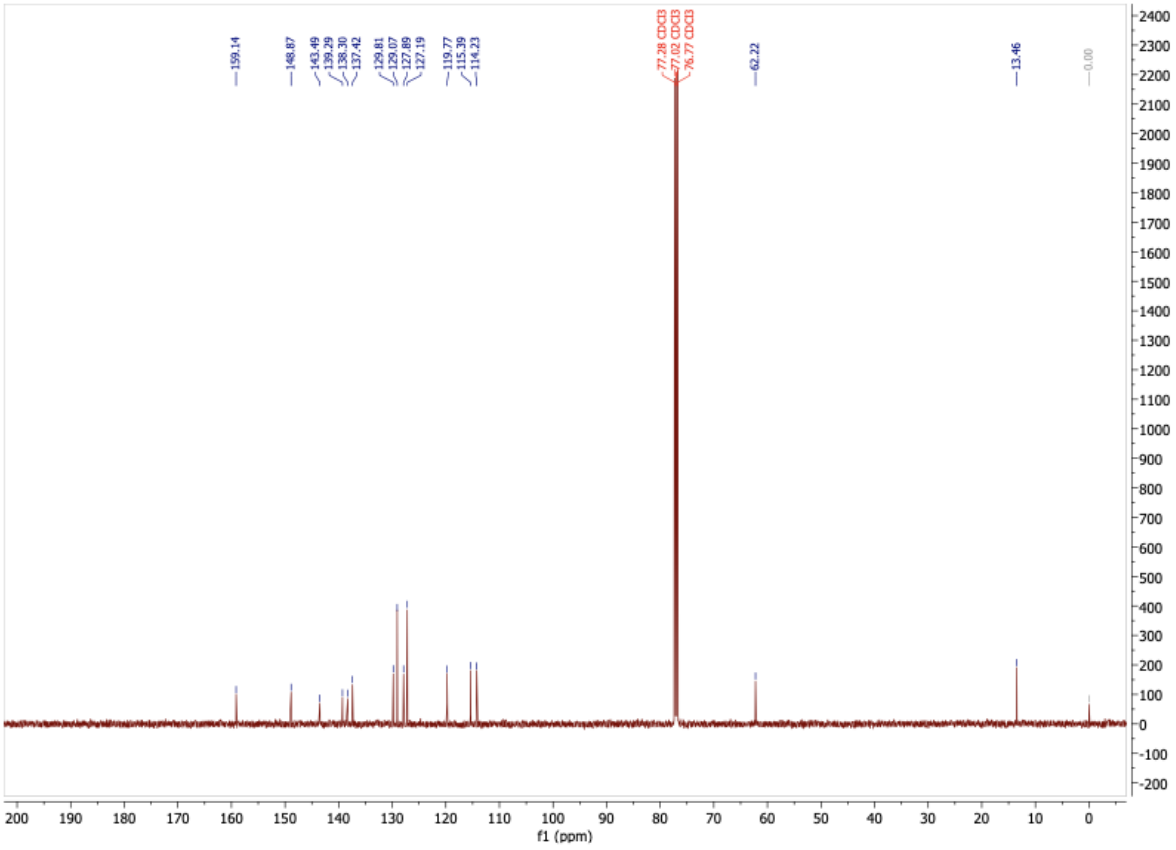




HRMS dataof *N*-benzyl-2-((3-methyl-1-phenyl-1*H*-pyrazol-5-yl)thio)propanamide (4e)

2-((3-methyl-1-phenyl-1H-pyrazol-5-yl)sulfonyl)-N-(pyridin-2-yl)acetamide (5a)

^{1}H spectrum of 2-((3-methyl-1-phenyl-1H-pyrazol-5-yl)sulfonyl)-N-(pyridin-2-yl)acetamide(5a)



^{13}C spectrum of 2-((3-methyl-1-phenyl-1H-pyrazol-5-yl)sulfonyl)-N-(pyridin-2-yl)acetamide(5a)

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VITA

Thomas DiPuma was born in Lima, Peru. He attended undergrad here at Loyola where he earned his Bachelor's degree in biochemistry. He pursued a thesis-based Master's degree under Dr. Daniel Becker where his research focused on drug discovery by synthesis of a series of small molecule inhibitors.

While attending graduate school, he was awarded a research fellowship over the summer and has spent multiple years as a teaching assistant. He was also awarded a departmental scholarship. All of these experiences and opportunities have inspired his interest in teaching, which he will be looking into while continuing onward with his next step.