DIARYL BIOLOGICALLY ACTIVE COMPOUNDS: POTENTIAL TREATMENTS FOR PARASITIC DISEASES AND CANCER AND NOVEL SYNTHETIC METHODS

A Thesis By

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Abstract:
Diaryl compounds have inhibitory activity towards multiple parasites and forms of cancer. In this study, new compounds have been synthesized to investigate their inhibitory activities towards Chagas Disease, which is caused by the parasite *Trypanosoma cruzi* and has infected millions of people in Latin America, causing health complications and in many cases death. Additionally, these compounds have been studied for AKT inhibition to inhibit the growth of cancerous cells. In this research, aryl substitutions, as well as the structural core, have been studied, producing compounds that are able to successfully inhibit the AKT pathway and *T. cruzi* epimastigote growth. In addition, the formation of these compounds was studied through the development of a flow reaction for the synthesis of 2-arylidene cycloalkanones, a key intermediate in the synthesis of asymmetric diarylidene cycloalkanones which exhibited strong inhibition towards AKT and *T. cruzi*. With this novel reaction, 2-arylidene cycloalkanones can be synthesized effectively. Additionally, this reaction was validated over 48 hours, and the scope was explored with a small library of starting materials. Through this new reaction, researchers can more efficiently synthesize 2-arylidene cycloalkanones to generate promising compounds for future investigations.

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ACKNOWLEDGMENTS

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CHAPTER 1

INTRODUCTION TO DIARYL BIOLOGICALLY ACTIVE COMPOUNDS

Researchers have discovered compounds composed of two linked aryl rings with the potential for the treatment of many diseases, including multiple parasitic diseases, cancer, viral infections, HIV, and diabetes[1-3]. Additionally, these compounds have been observed to inhibit a wide range of enzymes, controlling cellular mechanisms such as cell growth, proliferation, and survival, inflammation, protein synthesis, and cell death.[1, 4, 5] The chemical scaffold is relatively simple, consisting of two aryl groups linked by a variety of functional groups. Both the aryl substitutions, as well as the linker can be varied to produce a library of analogues.[6] In this work, the synthesis and activity of three types of diaryl biologically active compounds, diarylidene cycloalkanones, triazoles, and amides or sulfonamides, have been explored as potential therapeutic treatments for a plethora of diseases, with an emphasis on antiparasitic and anticancer activities. In addition, new synthetic methods have been developed to aid in the synthesis and evaluation of these compounds.

Diaryl Dienones

The diaryl dienones are a class of compounds that has a broad field of activity and enzyme interaction in pharmacological studies.[1] Notably, it has been shown that these compounds have strong anti-cancer properties due to their affinity towards proteins in the ubiquitin proteosome system, as well as unique antiparasitic activity, antiviral activity, and anti-inflammatory properties.[1, 7, 8] Most commonly, these compounds are formed through aldol condensations between a ketone and an aromatic aldehyde.[8, 9] This forms an α,β-unsaturated ketone that can be conjugated with one or both aryls to form large, complex aromatic networks.[10] Additionally, the central linker can be comprised of longer alkyl chains, saturated or unsaturated, but more commonly is composed of a cycloalkanone due to the biological activity of this group, as shown in Figure 1.[11] This central ketone can also be modified to affect biological activity through the use of heteroatoms or substituents which ideally would also improve specificity and reduce side effects.[1, 6] Monoaryl dienones have also been synthesized, such as in a study of 6-(nitrobenzylidene)cycloalkanones by Das et al., but it is generally

observed that the diarylidene cycloalkanones have superior biological activity.[12] However, these compounds, also known as 2-arylidene cycloalkanones, can be useful in the synthesis of asymmetric diarylidene cycloalkanones which have uniquely beneficial properties due to the ability to fine tune each aryl independently.[9]

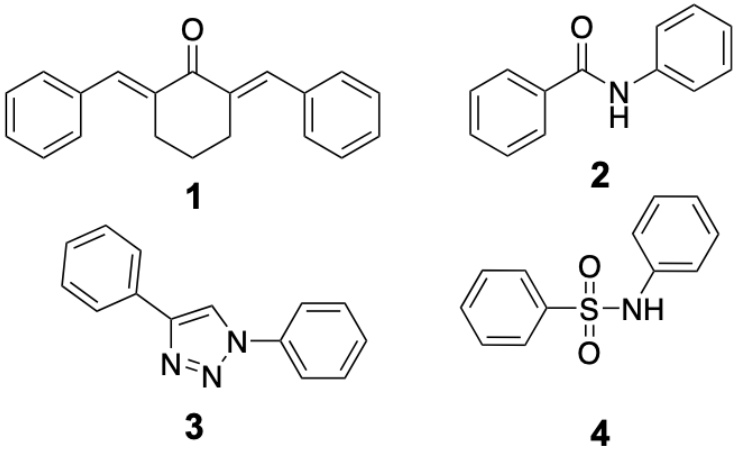


Figure 1. General structures for biologically active diaryl compounds of interest. In numerical order, diarylidene cycloalkanones, diaryl amides, diaryl 1,2,3-triazoles, and diaryl sulfonamides.

Diaryl Triazoles

Another structure of interest in this research has been the diaryl triazole (Compound **3**, Figure 1). These compounds have been studied extensively due to the synthetic ease with which they can be made and their similarity to functional groups found in biological systems, which ultimately leads to potential improvements in bioavailability and biological activity.[13] Generally triazoles are formed through click reactions, which are widely used in synthetic chemistry to couple two compounds together with good yields, specificity, and relative simplicity.[13] A prime example of this is seen in the formation of 1,2,3 triazoles, where an alkyne and azide can be joined to form an additional heteroaromatic ring under easily accessible conditions, allowing syntheses of additional analogues.[6] Furthermore, reaction conditions can be used to control the substitution location, allowing for more compounds to be synthesized.[13, 14] Triazoles are also bioisosteres for amides, esters, carboxylic acids, olefins, and many heteroaromatic rings.[13] The biocompatibility paired with synthetic

accessibility has resulted in numerous triazoles observed to have antifungal and antibacterial activity, cancer inhibition, HIV protease inhibition, antituberculosis effects, and parasitic inhibition.[14, 15]

Diaryl Amides and Sulfonamides

Another linker for diaryl systems is through amides and sulfonamides (Compounds **2** and **4**, Figure 1). These compounds can be formed relatively easily by coupling an amine to either a sulfonyl chloride or a carboxylic acid. Amides have a long-standing history in drug development and are one of the most common functional groups in biology, as this bond is contained in every amino acid, which is the building block of all proteins.[10] These functional groups are biologically compatible and have the benefits of hydrogen bond donors and acceptors, which further improves their solubility and by extension their bioavailability.[6] This makes it comparatively easy for this functional group to form strong associative bonds with other compounds. Sulfonamides, while easy to synthesize, are rarely found in nature.[16] However, they have been used extensively as antibacterial agents, with additional usage as an anticonvulsant; additionally, these compounds have been studied extensively in pharmacology.[16-17] To date, sulfonamide containing compounds have been found to have biological activity for many applications such as cancer inhibition, antiparasitic treatments, and matrix metalloproteinase inhibitors which have demonstrated potential to treat diseases including cancer, diabetes, multiple sclerosis, and arthritis.[18-21]

CHAPTER 2

RESEARCH ON DIARYL BIOLOGICALLY ACTIVE COMPOUNDS FOR THE TREATMENT OF PARASITIC DISEASES

Diaryl inhibitors have been studied extensively for the treatment of parasitic diseases and have shown promising inhibitory activity towards the *Trypanosomatida* order.[22-24] This group of organisms is exclusively parasitic and is notably responsible for African trypanosomiasis, better known as sleeping sickness, American trypanosomiasis, commonly known as Chagas disease, and Leishmaniasis.[25] However, American trypanosomiasis is unique among these because, unlike Leishmaniasis and African trypanosomiasis, it does not have any reliable treatment for adults after a few weeks post-infection, making it one of the more prevalent focuses of antiparasitic inhibitor investigations (although as seen in many of the following studies, researchers are often simultaneously searching for inhibition of several parasites, and these compounds can be seen to have biological activity in these capacities).[9, 23, 24, 26] This, in addition to the prevalence of this disease, has lead the World Health Organization to label Chagas disease as a Neglected Tropical Disease.[27] The CDC estimates that approximately 8 million people are affected by Chagas disease, with approximately 300,000 of those people living in the United States.[28] This disease is caused by the parasite *Trypanosoma cruzi*, which is found in the feces of triatomine bugs, also known as “kissing bugs”.[29] The disease is spread when the triatomine takes a blood meal, and then defecates in the wound, spreading the parasite.[27] Additionally, the disease can be transmitted through blood transfusion and organ transplants from an infected human and congenitally from mother to child.[29] This parasite goes through two phases of its lifecycle; one in the triatomite bug, and one in the human hosts.[27] If this parasite is left unchecked in the human host, it can result in severe cardiomyopathy that often proves fatal.[28]

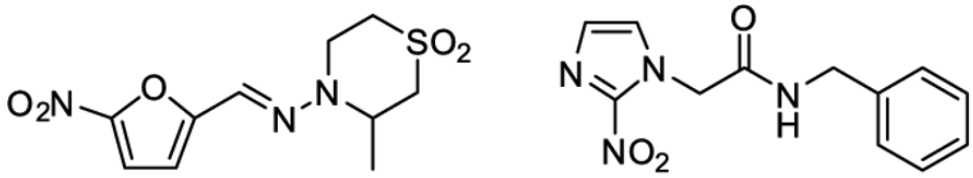
Infection

When humans first become infected, they enter the acute phase of the disease where patients suffer minor symptoms and the amastigotes multiply inside cardiac and other cells and transform into trypomastigotes.[27] Treatment for Chagas disease is the most effective in this phase if it is identified;

however, it is very difficult to treat the disease at this point, as it is rare for the mild symptoms of the acute phase to be diagnosed in time, which has led to preventive Chagas disease screenings.[30] After the first few weeks, the disease then progresses from its acute phase into its chronic phase where more severe symptoms generally occur.[27] Unfortunately, once in the chronic phase, Chagas Disease is usually incurable with the medications currently available, which poses many potential problems for those seeking treatment.[23, 31] Additionally, the currently available treatments have a severe side effect profile; in this phase, the only option is symptom management.[23, 32]

Current Treatment Options

Currently there are two effective treatments for Chagas disease: Benznidazole and Nifurtimox (Figure 2), neither of which is approved for use in adults in the United States, partially due to limited clinical trial data.[33] Furthermore, there is a significant concern about the drugs' toxicity, especially in children and the elderly.[33] Most significantly, these drugs are 60-90% effective in treating Chagas disease in the acute phase but essentially non-effective in the more prevalent chronic phase where the parasitic amastigotes can lie dormant in muscle tissues, protecting them from currently available treatments.[34] Given the low effectiveness of current drugs, undesired side effects, and the impact and widespread nature of this disease, there is a large need to develop alternative compounds to effectively treat Chagas disease, especially for the chronic phase of infection.



Nifurtimox
5

Benznidazole
6

Figure 2. Structures of the two currently available treatments for Chagas Disease.[35]

Diaryl Inhibitor Studies

Symmetrical Diarylidene Cycloalkanones

The lack of sufficient treatment options has led to the application of many new compounds, including diaryl biologically active compounds, to help treat trypanosomatidal infections, with an emphasis on treatments for Chagas disease. In a study by Braga et al., cyclopentanone and cyclohexanone were reacted with many aromatic aldehydes via aldol condensations to produce symmetric diarlyidene cycloalkanones containing heavily conjugated aromatic systems.[8] These compounds were then tested to determine their effectiveness at inhibiting Trypanothione Reductase (TR) and Glutathione Reductase (GR), which are both metabolically necessary enzymes for trypanosomatids and are verified therapeutic targets for the treatment of Chagas disease and Leishmaniasis.[31, 36] However, due to the low selectivity of these compounds towards the above enzymes, the compounds were subsequently studied for Cruzain inhibition. Cruzain, also known as Cruzipain, is a Cysteine protease of *T. cruzi* and a putative therapeutic target, as inhibition of this protease blocks amastigote and epimastigote proliferation, metacyclogenesis, and can increase the survival rate of *T. cruzi* infected animals *in-vivo*.[8, 36, 37] From this study, it was determined that cyclohexanone-based compounds substituted with quinoline, benzene, and pyridine were able to effectively inhibit Cruzain with 30-60% inhibition at 100 μM.[8] This laid the groundwork for the use of diarlyidene cycloalkanones as potential targets for the treatment of Trypanosomatidal infections.

Variable Chain Length Diarylidene Alkenones

Following the work by Braga et al., Aguilera et al. proceeded to study the central linker of diarlyidene ketones to determine if there was an alternative ketone with which to connect the two aryls for the concomitant inhibition of Triosephosphate Isomerase and Cruzipain, both of which are validated enzymatic targets for inhibition of *T. cruzi*.[11] The compounds were tested against *T. cruzi* epimastigotes. In this study, furans, thiophenes, and benzene rings were added to symmetric ketones with one to three conjugated alkenes in between the ketone and aryl. Additionally, five to seven membered cyclic ketones were tested in this study to see what effect the additional rigidity and

hydrophobicity would provide. The research found that a cyclohexanone core, furan aryls, and two conjugated double bonds gave the best inhibition toward *T. cruzi* epimastigotes and both enzymes.[11] Unfortunately, this compound had a low selectivity index of 17, but was also observed to improve *in-vivo* survival among mice infected with *T. cruzi*.[11] The team also studied the docking of these compounds with Triosephosphate Isomerase and observed that the alkyl ring could interact with a critical loop of the enzyme, potentially strengthening enzymatic inhibition.[11]

Asymmetric Diarylidene Cycloalkanones

While Aguilera et al. worked to modify the aryl groups and ketone linker, Din et al. took another approach wherein they chose to synthesize compounds with two different aryls connected by a cycloalkanone core, creating asymmetric diarlyidene cycloalkanones (Figure 3).[9] While symmetric compounds with this general structure had been determined to have biological activity by Das et al., this study was able to show that the asymmetric analogues had antiparasitic activity towards *T. cruzi* epimastigotes and trypomastigotes, as well as *L. amazonesis* promastigotes through the determination of their IC_{50} and EC_{50} values compared to Benznidazole.[9, 12] Din et al. had initially tested a series of symmetric diarylidene cycloalkanones and found that none of the compounds were able to inhibit any of the three parasite types more effectively than Benznidazole.[9] However once adjusting to the asymmetric motif, they found multiple compounds to have superior inhibition compared to benznidazole, with these compounds being almost exclusively substituted at varied positions with methoxy functional groups.[9] The most effective compound was **16** (Figure 3), which was seen to have an IC_{50} of 5.2 μM towards *T. cruzi* epimastigotes, an EC_{50} of 27.4 μM towards *T. cruzi* trypomastigotes, and an IC_{50} of 8.9 μM towards *L. amazonesis* promastigotes, while showing an CC_{50} of 401.2 μM towards monkey kidney Vero cells (Table 1), suggesting that this compound has high selectivity for parasites but low cytotoxicity towards mammalian cells, indicating that there could be a sufficient therapeutic window for treatment.[9] This study explored 21 different compounds and established a promising structural motif that had been relatively unexplored thus far.

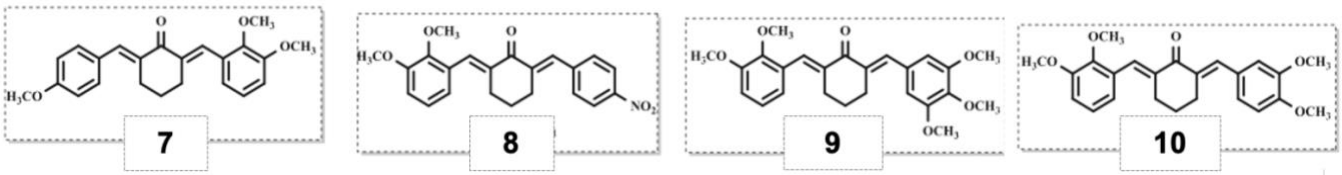


Figure 3. Study of inhibitory activity of asymmetric diarylidene cycloalkanones towards *T. cruzi* epimatigotes, trypomastigotes, and *L. amazonesis* promastigotes.[9]

Table 1. Parasitic inhibition data for promising asymmetric diarylidene cyclohexanones against three types of parasites, with structures in Figure 3.[9]

	T. cruzi Epimastigote	*T. cruzi* Trypomastigote	*L. amazonesis* Promastigote	Vero cells			
Compounds	IC_{50}(μM)	EC_{50}(μM)	IC_{50}(μM)	CC_{50} (μM)	$SI_{e\text{-}v}$[a]	$SI_{t\text{-}v}$[b]	$SI_{p\text{-}v}$[c]
7	5.2 ± 0.8	27.4 ± 3.6	8.9 ± 0.7	401.2 ± 8.8	77.2	14.6	45.1
8	33.3 ± 4.7	27.7 ± 3.8	3.9 ± 0.5	<10 ± 0.0	No Data		
9	3.0 ± 0.0	30.2 ± 1.8	2.6 ± 0.4	29.3 ± 4.8	9.8	1.0	11.3
10	28.5 ± 3.3	23.8 ± 5.4	4.5 ± 1.1	36.1 ± 1.6	1.3	1.5	8.0
Benznidazole (**6**)	6.5±0.7	34.5 ± 7.6	No Data				

Amide and Sulfonamide Containing Diaryl Biologically Active Compounds

Another structure that has shown promise are diaryl amides. In one study by Álvarez et al., diaryl amides with a thiazole connected at the amide core were synthesized based on a previously discovered parasitic inhibitor to create *T. cruzi* inhibitors that were both more effective at treating Chagas Disease while reducing mutagenic and clastogenic effects.[38] This was done by testing the compounds against *T. cruzi* amastigotes, trypomastigotes, and epimastigotes for inhibition, Vero cells for selectivity and toxicity, *Salmonella enterica* for mutagenicity in the Ames test, and *in-vivo* against infected mice while measuring antibody response and parasite count.[38] This study found the best compound, **12** contained the original diaryl thiazole **11**, with an added amide in the center and a piperazine linkage to a propenyl benzene (Figure 4).[38] This increased the distance between the functional groups and added some rigidity to the core, while also breaking the aromatic network via the piperazine. This new compound was found to have an amastigote EC_{50} value of 0.72 μM. vs 3.3 μM for Benznidazole and 0.42 μM for Nifurtimox, and while the inhibition for Nifurtimox was greater,

the new compound showed a superior selectivity index of 433 vs 300 and 200 for Benznidazole and Nifurtimox respectively, based on the cytotoxicity toward Vero cells.[38] This compound also demonstrated strong inhibition towards the epimastigotes and trypomastigotes.[38] In the Ames testing, they found that this new compound was not mutagenic, unlike Benznidazole and Nifurtimox.[38] Based on these positive results they moved toward *in-vivo* testing and found that the compound was able to treat parasitic infections in mice at half the dose of current treatments, and tissue damage was observed to be less than in mice with or without Benznidazole treatment.[38] These *in-vivo* studies also showed this compound was not genotoxic and had a significantly higher LD_{50} than Benznidazole, while allowing for the same level of antibody production.[38] This suggests that these compounds could allow for a modest increase in inhibitory activity while reducing side effects or widening the therapeutic window for Chagas Disease treatments.

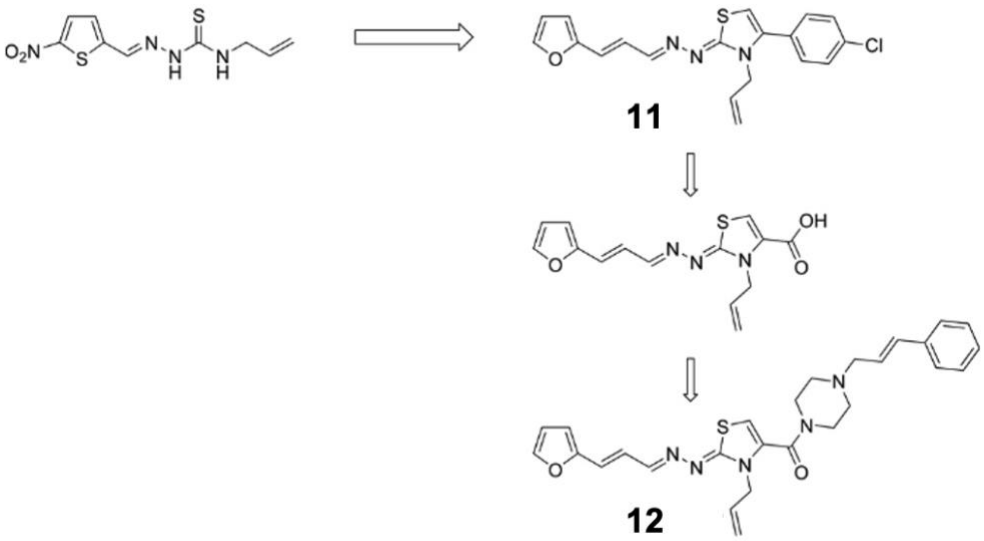
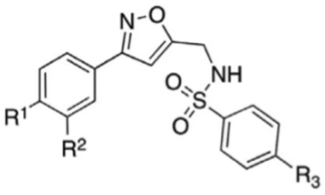


Figure 4. General synthetic pathway and strategy for a study focusing on thiazole-containing diaryl amides for the treatment of Chagas Disease.[38]

In another study, both diaryl amides and sulfonamides were synthesized, with one of the aryls being a 3-nitrotriazole and the other aryl being the main experimental variable.[39] These compounds were tested for activity towards *T. cruzi* amastigotes*, Leishmania donovani*, and *Trypanosoma brucei*. While there were compounds that inhibited the latter two partially, nearly all of the 36 compounds

synthesized were able to provide strong inhibitory activity towards *T. cruzi*, having 2-56 fold activity when compared to Benznidazole, while also showing low cytotoxicity in L6 mouse skeletal muscle cells.[39] Similarly to the last study, the compounds that were the most active had the amide or sulfonamide connected to one aryl, but were physically spaced and not connected to the nitrotriazole ring.[39] It was observed that both the amides and sulfonamides had promising activity, and compounds were evaluated *in-vivo* to further differentiate between them.[39]

Diaryl isoxazolyl-sulfonamides have found promise in the treatment of parasitic diseases, as well as *Herpes Simplex Virus*. In a study by de Rosa et al. 20 of these compounds were synthesized and screened for inhibition against *T. cruzi* amastigotes, *L. amazonesis*, and a subunit of DNA polymerase for human herpesvirus 6A.[26] Compared toward amphotericin B, a current Leishmania treatment, and the antiviral Acyclovir, none of the compounds were close in growth inhibition or viral replication inhibition, although a few of the compounds showed potential activities in these areas with low levels of inhibition.[26] However, **8** and **16** from Figure 5 showed promising activity towards *T. cruzi*, with over 85% growth inhibition at a 50 μM dosing with a selectivity index of approximately 30.[26] Additionally, as in previous studies, it was observed that Nitro substitutions were able to significantly increase biological activity, as the top two compounds contained this substitution.

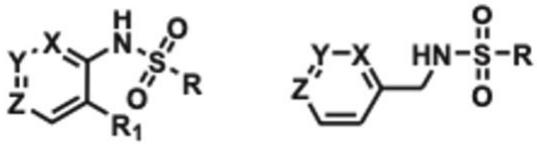


Compound 13: R^1=R^2=H R^3=F
Compound 14: R^1=R^2=OCH_2O R^3=NO_2

Figure 5. Compounds synthesized for the study of biological activity of isoxazolyl-sulfonamides for antiparasitic and antiviral development.[26]

Sulfonamides were a central theme in an antiparasitic SAR study conducted by Peres et al. where the team synthesized diaryl sulfonamides that were either directly connected to both aryls or spaced by a methylene group (**15** and **16** respectively, as seen in Figure 6).[19] They varied the “R”

aryls with methoxy and alkyl substituted benzene and naphthalene systems. While these compounds were not able to reduce infection as effectively as Benznidazole, a few hits were found that showed similar levels of infection reduction, and some conclusions about structural activity were made.[19] Compound **15**, which had a pyridine with the nitrogen in the Y position, gave the best inhibition.[19] For **16**, having a nitrogen in the Y and Z positions showed moderate activity.[19] The systems that displayed the best inhibition had a para-methoxy group relative to the connected R group, indicating that this substitution may interfere with active site binding or has another activity related to this site. Overall, the presence of inhibition in these studies shows that there is potential for these amide and sulfonamide-based diaryl systems in antiparasitic development.



Compound 15 **Compound 16**

Figure 6. SAR study by Peres et al. of diaryl sulfonamides.[19]

1,2,3-Triazole Containing Diaryl Biologically Active Compounds

"Click" reactions have been hugely popular for decades due to their ability to couple reagents easily, selectively, and quickly.[13, 14] One of these click reactions is the alkyne azide Huisgen cycloaddition, where an alkyne and azide react to form a 1,2,3-triazole ring after exposure to heat.[13, 14, 40] This reaction is useful for two reasons, the first being that it is a simple and clean method to couple two somewhat uncommon functional groups; the second is that the 1,2,3-triazole ring that is formed is a heteroaromatic ring, which has unique biological properties, similarly to other heteroaromatic systems.[13, 14] This is similar to the commercial antiparasitic Benznidazole, which has a heteroaromatic nitroimidazole ring (Figure 7). For these reasons, this class of chemistry has garnered interest in several studies in pharmacological research.[13, 14]

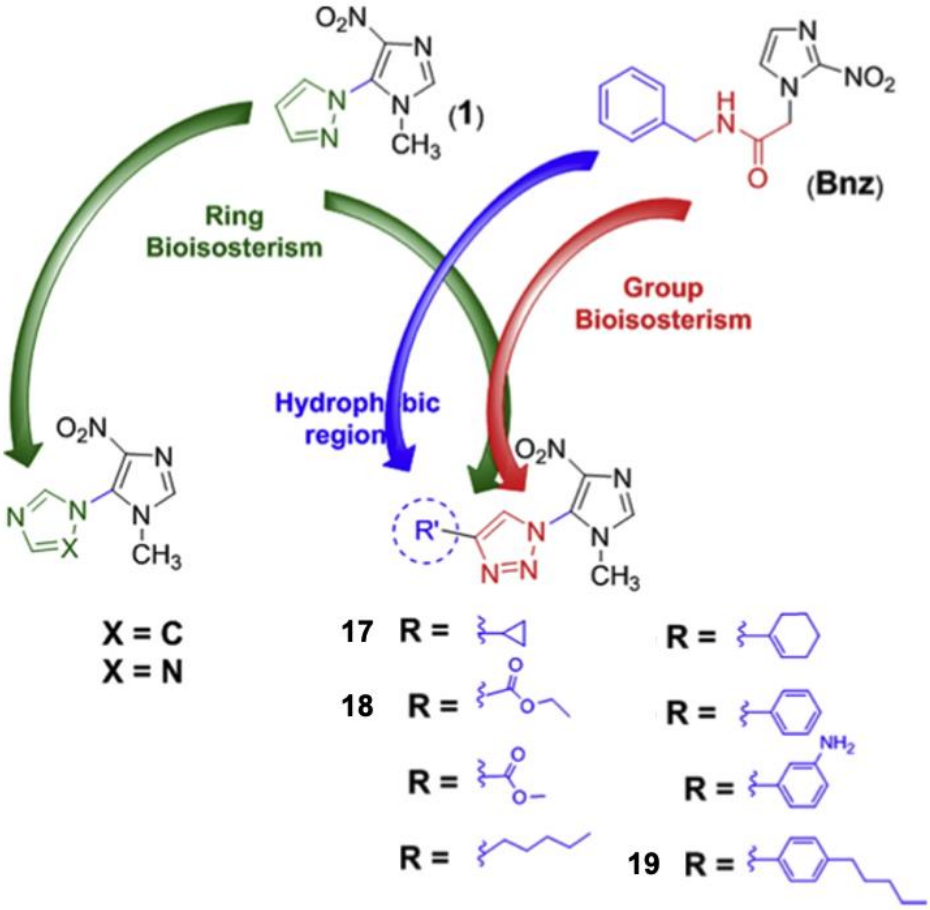


Figure 7. Strategy and compounds formed by do Vale Chaves et al. to understand and improve the inhibitive qualities of benznidazole-like compounds towards *Trypanosoma*.[8]

Many researchers have synthesized 1,2,3-triazole compounds with parasitic inhibition.[8, 26, 41] One such study focused on the synthesis of nitroimidazole derivatives for the evaluation of their trypanocidal, cytotoxic, and genotoxic capabilities. In this study, do Vale Chaves et al. selected these nitroimidazole derivatives for their similarity to Benznidazole, then used click chemistry to connect the ring to a hydrophobic region to study how this hydrophobic area affects trypanosomal inhibition.[15] The triazole ring was selected by do Vale Chaves et al. because it is a bioisostere of the amide group that is also present in Benznidazole.[13, 15] Two compounds with similar bioisoterism to 1,2,3-triazoles were evaluated, shown in green in Figure 7, but no promising activity was found with the pyrazole or 1,2,4-triazole rings.[15] However in the initial screening, **17**-**19** were selected due to their moderate activity towards *T. cruzi* trypomastigotes.[15] These compounds were then evaluated for their activity toward *T. cruzi* amastigotes, and cytotoxicity and genotoxicity to determine their therapeutic potential.

Ultimately, **17** was determined to be the safest compound, while also having the best inhibition for trypomastigotes.[15] This compound was ultimately less selective and effective than Benznidazole, indicating the need for more research into this chemical space that shows antiparasitic activity but is so far unoptimized for this application.[15]

Another study in this structural category was conducted by Faria et al., wherein the team synthesized 92 1,2,3-triazole based diarylidene compounds to test for inhibitory activity toward two strains of *T. cruzi* epimastigotes.[41] Out of these compounds, the team found six that exhibited strong inhibition, as seen in Figure 8 and Table 2. These compounds were tested for selectivity and cytotoxicity toward mouse J774.G8 cells and peritoneal macrophages. Of particular note was **22**, which has strong inhibition of *T. cruzi* with EC_{50} values of less than 0.3 μM compared to 7.85 μM and 19.86 μM for Benznidazole.[41] This compound had lower cytotoxicity than Benznidazole, as observed by the CC_{50} values.[41] While the other compounds were not as promising, this significant jump in activity and therapeutic potential shows that this class of compounds contains significant promise in the synthesis of antiparasitic agents for the treatment of Chagas Disease.

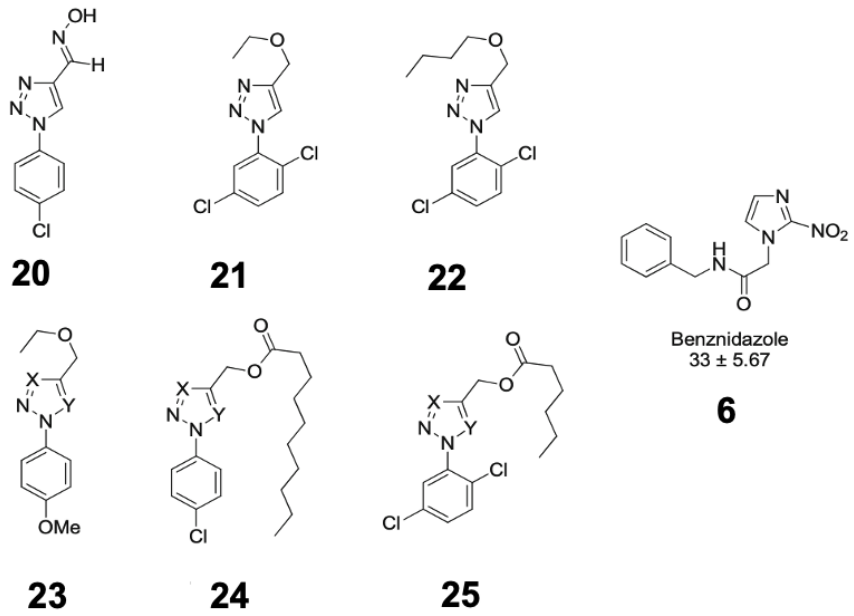


Figure 8. Promising 1,2,3-trizaole diaryl compounds for *T. cruzi* inhibition.[41]

Table 2. Inhibitory activity and toxicity of compounds from Figure 8.[41]

Compounds	Y Strain MTT EC_{50} (μM)	DM28-C strain MTT EC_{50} (μM)	Peritoneal Macrophages LDH CC_{50} (mM)	J774.G8 LDH CC_{50} (mM)
20	6.11	11.39	0.165	0.153
21	0.209	0.246	0.529	0.45
22	0.185	0.3	0.03	0.02
23	7.28	7.96	0.254	0.358
24	103.8	104	0.017	0.008
25	24.08	88.72	0.087	0.024
Benznidazole (**6**)	7.85	19.86	0.037	0.036

From these studies, asymmetric diarylidene cycloalkanones proved to be a promising structure to investigate, as the symmetric compounds have extensive history, but the asymmetric analogues lack a more systematic study.[9] Additionally, amides and sulfonamides have been reported to have biological activity with the potential to interact strongly with hydrogen bond donors and acceptors while maintaining free rotation, unlike the arylidene cycloalkanones, making them an attractive complement for study.[26, 38] Lastly, we were interested in the 1,2,3-triazoles as these compounds can mimic many functional groups that are already present in the body and would provide an aromatic functional group which can provide unique interactions.[13, 15, 41] These four types of compounds would then be connected to aromatic rings that have been found to have biological activity, including furans, pyridines, and benzene rings, including substitutions such as ethers, nitro groups, and halogens.[9, 26, 38, 41]

CHAPTER 3

RESEARCH ON DIARYL BIOLOGICALLY ACTIVE COMPOUNDS FOR THE TREATMENT OF CANCER

In many ways, cancer is a disease that needs no introduction due to its impact and prevalence in every corner of society. In the U.S. alone it was estimated that 1.8 million people would get cancer in 2020 with over 600,000 of those people dying from the disease.[42] Additionally, it has been estimated that 39.5% of people will be diagnosed with cancer at some point in their lifetime.[42] Given the enormous impact of cancer, it is no surprise that researchers have been trying to find a treatment for this disease.

AKT as a Therapeutic Target

One target that has been heavily studied for the treatment of cancer has been the AKT kinase, which is an enzyme that is downstream from phosphatidylinositol 3-kinase (PI3K), and involved in numerous cellular pathways responsible for regulating several cellular activities including cell growth, survival, metabolism, and proliferation (Figure 9).[43, 44] It was observed that AKT is overexpressed in human carcinomas and can be correlated with cancer advancement.[45, 46] Moreover, several upstream kinases in this phosphorylation chain such as PI3K, tumor suppressor PTEN, NF1, and LBK1 have been linked to tumorigenesis, in addition to numerous downstream kinases.[4] The AKT kinase has attracted considerable attention from oncological researchers because, through a kinase cascade, AKT has been observed as a key factor of tumor aggressiveness for a broad range of cancers which, given the many processes tied to AKT regulation, seen in Figure 9, also results in a large swath of biological consequences.[43, 47]

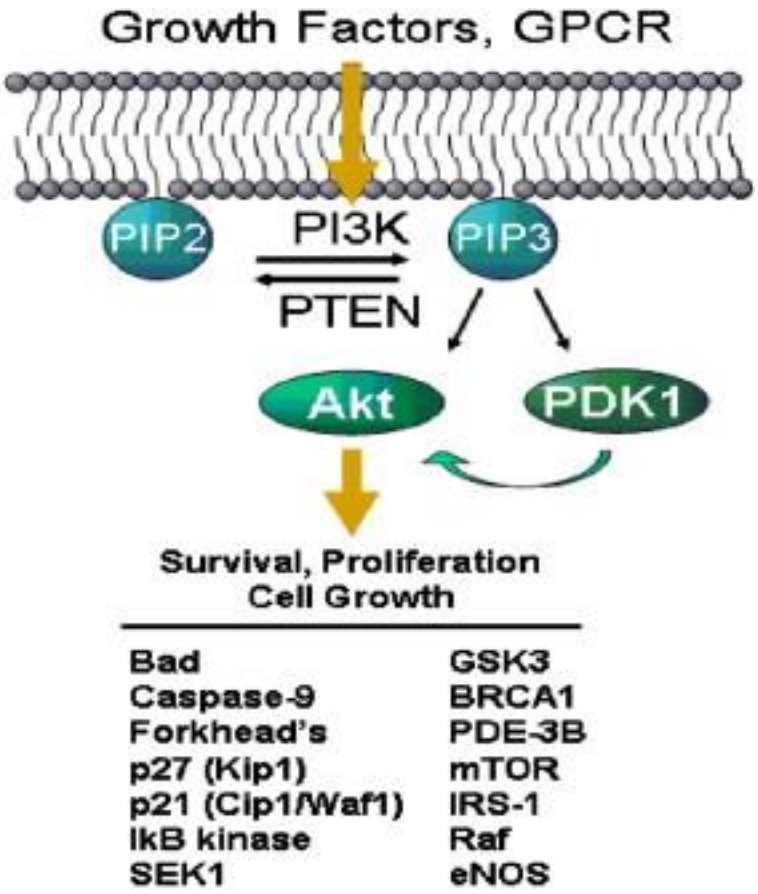


Figure 9. AKT pathway including key regulators, PTEN, GPCRs and growth factors, and PI3K.[48]

Rationale For Targeting AKT

Because AKT controls cell growth, survival, and proliferation, its overexpression in cancer cells contributes to tumor survival and the cell's resistance to apoptosis when faced with cellular stressors.[47] Additionally, PTEN, a known tumor suppressor and upstream kinase that regulates AKT, is inactivated in many cancer lines, as seen in Figure 10; however when reintroduced, PTEN has been observed to restore regulation of AKT in lymphocyte cells, leading to cellular apoptosis while also increasing susceptibility to apoptosis from other mechanisms.[44] This would simultaneously lead to the death of cancer cells while increasing other factors leading to cell cancer apoptosis, making this pathway an enticing strategy for synergy with other cancer treatments.[49] In fact, AKT inhibition has been shown to be effective at amplifying the effectiveness of other cancer therapies as well.[3, 50]

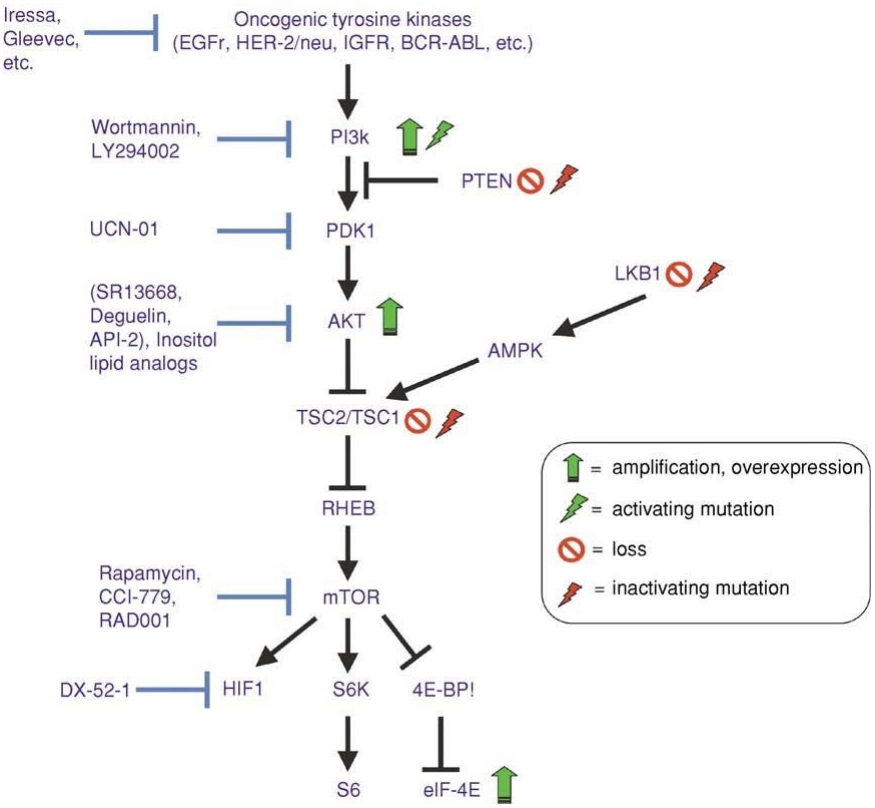


Figure 10. Alterations of AKT expression in human cancer with molecular alterations listed on the right.[47]

To further complicate the problem, the presence of three forms of AKT have been identified in human cells: AKT1, AKT2, and AKT3.[4, 43, 50] While all three forms are generally similar and commonly observed in humans, overexpression of one or more of these variants can be seen in many different forms of cancer.[45, 46] Depending on the type of cancer being studied, researchers have either targeted specific variants of AKT or searched for panAKT inhibitors.[3, 5, 45, 46, 51] This is because inhibition of AKT is only desired where it is overexpressed in relation to cancer, due to the many interactions AKT has with cellular function that could cause undesired side effects.[4, 5] However, there are many studies where panAKT inhibition has shown promising results *in-vivo* as well, as multiple forms of AKT can be overexpressed in some varieties of cancer.[3, 5, 51] For these reasons, researchers in the past decade have been isolating and testing specific isoforms of AKT, or have intentionally targeted pan-AKT inhibitors.[43, 45, 46]

AKT Diaryl Inhibitors

Indazole-Pyridine Inhibitors

One study that has promising results has been Luo et al.'s study of Indazole-Pyridine based AKT inhibitors. In this pathway, Luo's team initially discovered a lead via high throughput screening.[3] This compound was a relatively weak AKT inhibitor with a K_i of 5 μM, but served as a useful starting point for their SAR study.[3] From here, they were able to improve the activity of this compound 30,000 fold when they added an indole ring for a second distal aryl moiety, eliminated the rotational ability of the pyridine, and converted the pyridine to a dihydropyridazine to add hydrogen bonding capabilities to create **26** (Figure 11).[3] To then make this compound orally available, Luo's team swapped the indole for a phenyl to afford **27**, which was orally available at cost of AKT inhibition.[3]

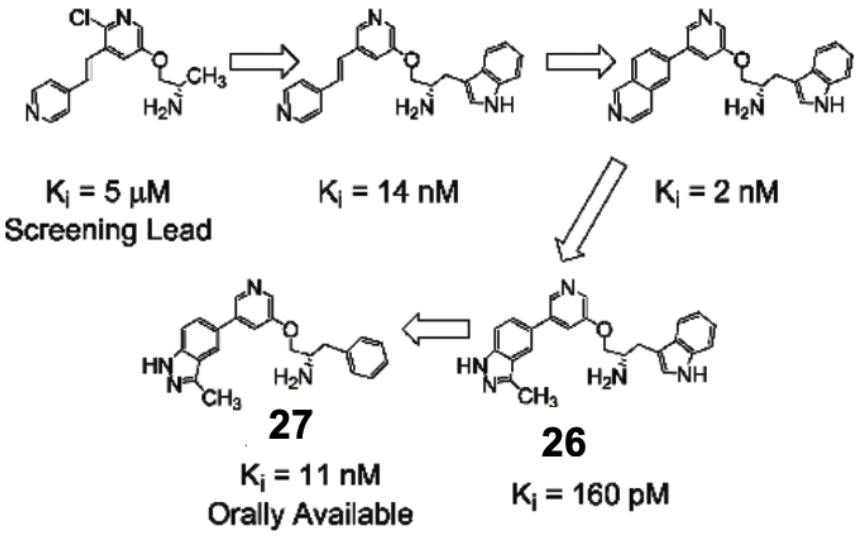


Figure 11. Development Pathway in Luo et al.'s study of indazole-pyridine Inhibitors which produced two promising compounds with different bioavailabilities.[3]

These two compounds were then tested for *in-vitro* and *in-vivo* activity, as well as selectivity for AKT inhibition. These compounds were tested for inhibition against three AKT enzymes, as well as common enzymes to test for selectivity. It was found that **26** inhibited all three AKT enzymes vs. other kinases, with **27** showing the same trend but with generally less specificity and selectivity.[3] Additionally, the phosphorylation of downstream products GSK3a/b, FOXO3, TSC2, and mTOR was observed, further corroborating AKT inhibition.[3] *In-vivo*, it was found that both of these compounds

performed similarly when tested against 3T3 murine fibroblasts in soft agar and mouse tumors.[3] The compounds were also tested in a MiaPaCa-2 human pancreatic cell xenograft model where they were able to significantly slow tumor growth.[3] Additionally, the two compounds' inhibition was tested when combined with paclitaxel, an antimitotic, and rapamycin, a PI3K pathway inhibitor via mTOR.[44] These studies showed that combining AKT inhibitors with established chemotherapeutics can synergistically induce apoptosis more effectively than any of the therapeutics on their own.[3]

Oxindole-Pyridine Based AKT Inhibitors

Another class of diaryl AKT inhibitors was found by Zhu et al., by linking an oxindole ring to an amino propoxy indole through a pyridine backbone (Figure 12).[52] In this study, **28** was reacted with a variety of alkyl halides to form **29** and separately with a variety of aldehydes to form **30**-**46**, as seen in Figure 12.

These compounds were then screened for AKT1 inhibition in an enzyme assay and a mouse xenograph model utilizing MiaPaCa-2 cells, which are human pancreatic cancer cells. Promising probes were then screened for kinase selectivity and cytotoxicity. Ultimately, the most selective AKT inhibitor in this series was **43** with an IC_{50} of 0.17 nM for AKT1 and 100-fold selectivity with AKT2 and AKT3.[52] **28** also showed promising selectivity with an IC_{50} of 3.2 nM for AKT1 and similar selectivity to AKT1.[52] Other compounds also showed strong AKT1 inhibition but were either not specific for AKT1 or showed significant cytotoxicity. Both of these compounds also maintained their activity in the mouse xenograft model, measured by AKT IC_{50} values and inhibition of tumor growth, suggesting that these compounds have a sufficient therapeutic window to be utilized.[52] These results from the general structure of **46** showed that the substitutions of **28** and **29** only maintained activity with one conformation of oxazoline ring attachment or a previously studied quinoline substitution. This resulted in the second series based on **28** to create **30**-**45**, which gave a range of inhibitory activities as R_2 was varied.[52] The more promising substitutions from this series were exclusively heteroaromatic rings, which could be related to the conjugated aryl system in which they were connected.[10]

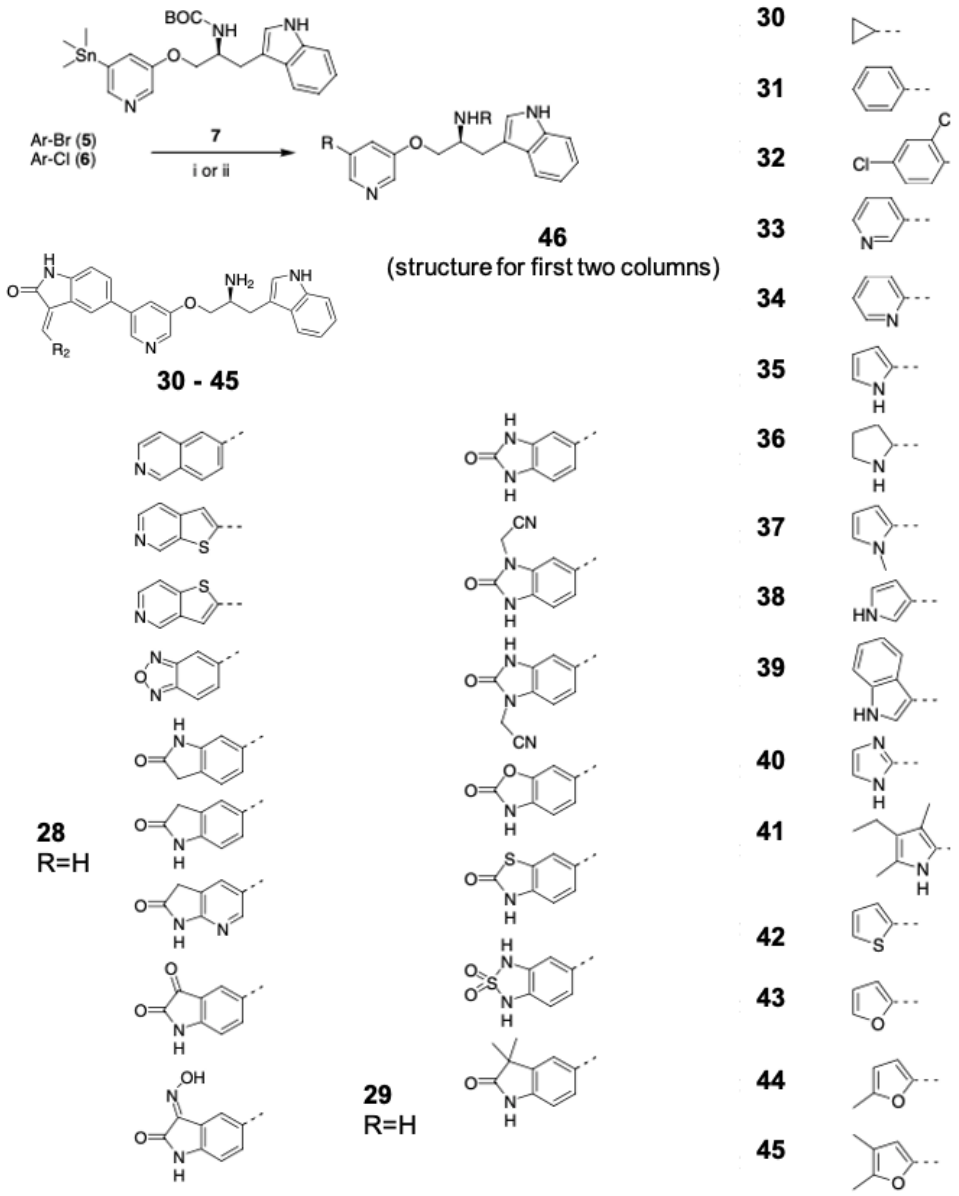


Figure 12. Scheme for Oxazoline-pyridine based AKT inhibitor SAR.[52]

Diarylidene Cycloalkanone AKT Inhibitors

Similarly to the parasitic inhibitors in Chapter 2, diarylidene cycloalkanones have also found application in cancer treatments through their inhibitory qualities toward AKT1. In the research of Jo et al., numerous compounds were screened to identify compounds that inhibited AKT1 by inactivating its binding domain while also ubiquitinating AKT1, which changes the way AKT1 is regulated in the cell.[50, 51] In their screening Jo et al. discovered 12 compounds that were able to prevent AKT1 from moving to the membrane, where it can be phosphorylated and participate in its kinase cascade, and induced collection of AKT1 to a subcellular location in the pericentrosomal region which is responsible

for degrading and recycling cellular proteins.[50, 53] These compounds were then tested against HEK 293 cells that were subject to a mutation in PH domain that ultimately causes AKT1 enrichment at the cellular membrane, which has been identified in human cancers as a marker for AKT overexpression.[43, 50] After confirming the AKT1 enrichment, these cells were treated with the compounds of interest and it was found that seven were able to have 50% or more inhibition of AKT vs. control.[50] Additionally, Jo et al. studied the effects of these compounds when combined with PI3K inhibitors on cancer cell proliferation and death in HeLa cancer cells. The team found that when their AKT inhibitors were mixed with PI3K inhibitors LY294002 and wortmannin, 10-30% of the cells entered mitosis, compared to 45% without.[50] This confirmed the growth inhibitory properties of these compounds. Additionally, they observed that these compounds were able to prevent reactivation of AKT, which often happens after PI3K inhibition in an attempt to restore cellular equilibrium.[50] Lastly, the team tested the efficacy of these compounds in a mouse xenograft tumor model similar to the last study and found that these compounds were able to significantly inhibit tumor growth, confirming the activity of these compounds *in-vivo*.[50]

As seen in Figure 13, many different types of compounds were found to have inhibitory activity toward AKT. However, the most effective compound with strong inhibition *in-vitro* and *in-vivo* was **47**. This compound showed strong efficacy over **48**, giving further evidence that the central linker of these diarlyidene cycloalkanone systems is crucial for proper AKT inhibition.[50] Additionally, many of these aromatic rings contained amides and heteroaromatic nitrogen atoms, suggesting that these groups are important for biological activity and specificity. Lastly, many of these systems have a conjugated diarlyidene system, suggesting that this may be an important factor in the inhibitory activity of these compounds. Regardless, it is clear that the diarylidene class of compounds has significant biological activity for the potential treatment of diseases.

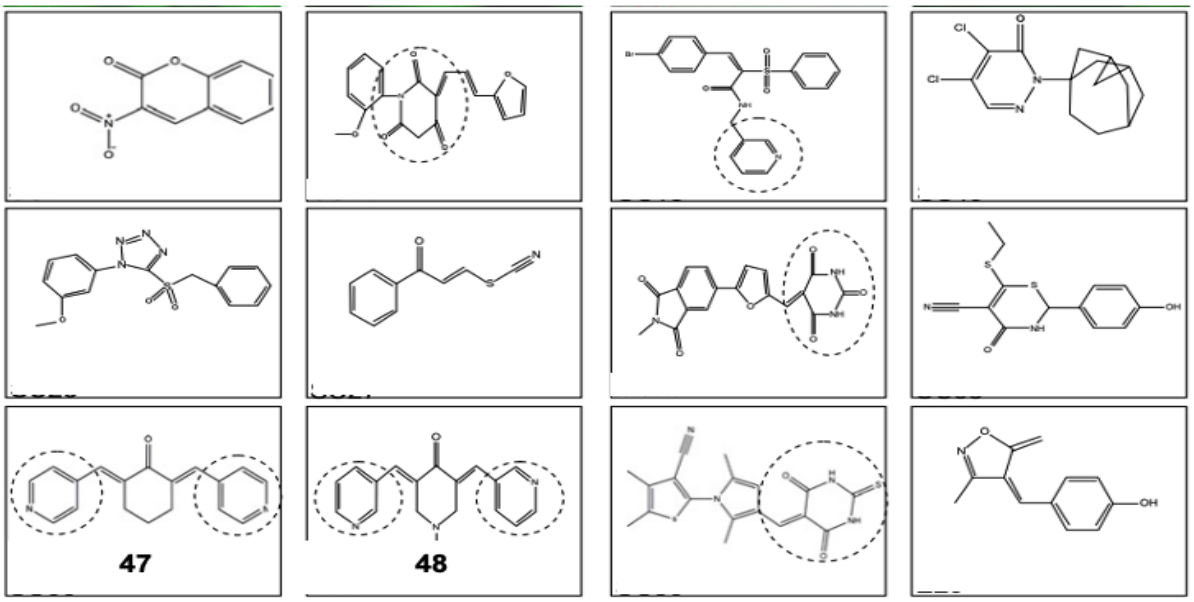


Figure 13. A group of 12 compounds found to have AKT inhibition by Jo. et al.[50]

CHAPTER 4

UTILIZING FLOW CHEMISTRY FOR THE DEVELOPMENT OF DIARYL BIOLOGICALLY ACTIVE COMPOUNDS

When deciding which diaryl inhibitors to evaluate, we noticed that there were many promising candidates with an asymmetric diarylidene cycloalkanone structure. These compounds are generally synthesized by combining the 2-arylidene cycloalkanone intermediate with an aromatic aldehyde to obtain the asymmetric analogue.[9, 12] And while many methods have been reported to synthesize the 2-arylidene cycloalkanone intermediate, these compounds have been difficult to synthesize in good yield and purity due to the formation of the symmetric diarylidene cycloalkanone side product, which negatively impacts the yields and is generally difficult to purify from the desired 2-arylidene cycloalkanone.[9, 12] In addition, there are no reported flow reactor methods in the literature for the synthesis of the target molecule. Because of this, we decided to utilize flow chemistry to apply more precise control over these reactions, allowing for a more efficient synthesis of 2-arylidene cycloalkanones.

Flow Chemistry

In the pharmaceutical industry, synthetic chemists have spent decades trying to improve reaction efficiency, time, and scalability.[40] This resulted in several new methodologies to improve the efficiency with which a new target molecule is made and scaled.[6, 36, 40, 54] A promising method to synthesize molecules is through the use of flow chemistry, as the use of continuous processing is able to provide innumerable benefits that have been detailed in the literature, including unparalleled control of reaction variables and condition, improved safety, easier handling of materials, excellent reproducibility, and unmatched scalability.[55] Flow chemistry at its simplest form involves the use of a flow reactor to conduct chemical reactions or processes. Generally this involves the use of peristaltic pumps to continuously push reagents through some form of reaction catalyst or treatment, temperature differentials, substrate bound catalysts, pressure, purification processes, or UV light to give the desired products.[54, 55] These variables can be combined for a wide assortment of methods, allowing for reactions that can be performed in a matter of minutes and providing new reactions that

could not be possible without this extensive condition control.[40] Additionally, flow systems allow for scalable green chemical reactions, with simple scalability for safe and continuous commercial production.[40, 54, 56]

Flow reactors have also gained considerable attention in the field of pharmaceutica because of the speed in which reactions can be conducted.[40, 54] Typically, when researchers begin a large-scale project to develop a new therapeutic treatment, they will test hundreds or thousands of compounds to determine what types of structural groups show a desired activity for a given target.[40, 43] Then, once these general chemical spaces have been identified, many compounds will be synthesized to determine what smaller structural effects will affect the activity in a desired or undesired fashion.[23, 40] Flow chemistry is extremely appealing in this application because it allows for a large number of compounds to be successively synthesized by simply swapping the reagent feed. This makes it simple to test for a variety of substitutions in a given chemical scope, or to try a large variety of reaction conditions on a reaction by changing one of the variables available in a given system, such as reaction temperature or speed. Additionally, because reagents are being reacted continuously, the time scale for reactions is much smaller.[55] Often, what would normally take days can be done in less than an hour, with continuous output to get a desired yield.[9. 57] Many flow reactors include purification processes as well, further providing time-saving benefits to the pharmaceutical development process.[55]

Aldol Condensations in Continuous Flow

Given their utility, long-standing history, and simplicity, aldol condensations have been studied extensively in the field of flow chemistry.[40, 57] This reaction has been widely utilized, as it is a relatively straightforward method that uses accessible and economical reagents to form carbon-carbon bonds from an aldehyde, ketone, and base.[58] This is appealing because, traditionally, carbon-carbon bonds have been somewhat difficult to synthesize.[58] However, despite being studied extensively, these reactions have faced challenges with catalyst durability and waste generation due to the traditional strong base needed for this reaction.[57] While solid-acid catalysts have provided less problems in this

field, solid-base catalysts have proven problematic due to their limited stability, water sensitivity, and required harsh conditions.[57] As an alternative, Laroche et al. were able to establish an aldol condensation reaction which utilized a basic anion-exchange resin catalyst. These resins have been touted for their environmental sensitivity and water tolerance, but until recently were limited in their use in this capacity.[54, 57]

Using the Amberlyst A26 anion exchange resin, Laroche et al. were able to establish a new aldol condensation method utilizing temperature, flow rate, and solvent control to connect aromatic aldehydes with cyclic ketones containing an additional aromatic functional group.[57] Their study initially began by screening a variety of basic catalysts and solvents, while monitoring the yield of the desired α,β-unsaturated product and the undesired β-hyroxyl product. To run these reactions, a solution containing 0.1 M α-tetralone and 0.105 M benzaldehyde was flowed through a column containing varied anion exchange resins at a rate of 0.15 mL/min at a temperature of 25°C. Out of the ten catalysts that were studied, only two were able to produce the desired α,β-unsaturated product, with one of those creating a ~50:50 yield of both products.[57] Based on this result, Laroche et al. picked the Amberlyst A26 resin, as it did not produce significant byproducts.[57] Following this, reaction temperature and solvent were screened, and it was found that toluene, THF, and ethanol at room temperature were able to maintain similar performance to the initial screening, while DMF gave a mixture of products, although a reaction temperature of 40°C was able to selectively form the β-hydroxyl product which could be useful for other reactions.[57] These solvents were then tested for catalyst longevity, as it has been observed that these catalysts will lose effectiveness over time.[54, 55] Ultimately, a system of 9:1 toluene:ethanol was determined to have the best lifespan, with consistent yields of more than 80% over 120 hours in the model system.[57] Increasing the ethanol level, using 100% toluene, or blending with THF resulted in a decrease in catalyst activity within one to three days, indicating that unsuitable solvent blends were slowly deactivating, instead of regenerating, the catalyst.[57]

This reaction was then tested with various aldehydes and ketones, coupled with α-tetralone and benzaldehyde respectively, to determine the substrate scope of this reaction. From this, Laroche et al. discovered that aromatic aldehydes containing electron withdrawing substituents were able to create high yields of the desired α,β-unsaturated ketone, while electron donating substituents appeared to have a slightly negative effect on the yield overall.[57] Additionally, the reaction was found to be successful with a handful of heteroaromatics, including 2-furyl and 2-thiophene.[57] When testing the scope of ketones in this system, it was found that yields were able to be maintained above 80% and increased toward quantitative yields in some cases, although in many instances the reaction temperature had to be increased to either 40°C or 60°C to maintain activity.[57] It was also found that when the temperature for cyclic ketones was increased, the small amounts of side products were either decreased or eliminated, which is the opposite trend of acyclic ketones.[57] This reaction was then used in a two-step flow synthesis of donepezil, an Alzheimer's treatment, where the target compounds was synthesized with a 92-95% yield and an ability to produce 7.7 g per day, using a 0.1 M solution flowing at 0.15 mL/min.[57]

The Need for A Method to Synthesize 2-arylidene Cycloalkanones

More recently, there has been a great interest in asymmetrical diarylidene cycloalkanones, which demonstrated promising biological activity in a few initial studies and could increase the diversity of compound libraries, potentially leading to molecules with increased activity and binding specificity for a desired therapeutic target.[9, 12] These compounds are generally formed by first reacting a cyclic ketone with an aromatic aldehyde to form a 2-arylidene cycloalkanone intermediate.[9, 12] Following this, the 2-arylidene cycloalkanone is reacted with another aromatic aldehyde to afford the final asymmetric product.[12, 59, 60]

While many methods have been reported to synthesize 2-arylidene cycloalkanones from aromatic aldehydes and cyclic ketones via traditional batch aldol condensations, there are many challenges to forming the 2-arylidene cycloalkanone intermediate with current syntheses.[2, 8, 9, 11, 12] The largest obstacle when using a ketone with two available α-carbons is preventing the formation of

diarylidene cycloalkanone side products when creating the desired monoarylidene cycloalkanone product. Ultimately, this results in poor yields and product mixtures which need to be purified, which is generally very difficult due to the similar molecular properties of the side products and desired products. Flow chemistry is one way to potentially improve these syntheses because it allows one to exert precise control over the conditions of a reaction system, while providing the additional benefit of continuous product formation.[40] Reaction variables such as temperature, heat transfer, mixing, pressure, and production rate can be controlled by a flow reactor, allowing for specific and consistent reaction conditions, increased safety, and unparalleled scalability.[40] Essentially, flow chemistry provides a unique reaction capability by meticulously controlling the flow, heat, and concentrations of the reagents, ideally resulting in a set of reaction conditions that will cleanly and efficiently form the desired 2-arylidene cycloalkanones with various aromatic aldehydes and cycloalkanones.[54] When trying to find a solution for this problem using flow chemistry, it was found that the previously reported aldol condensations used ketones that contained only one available α-carbon, likely to avoid this very issue.[2, 8, 9, 11] Because of this, a new flow reaction would be valuable in the field of flow chemistry and allow the production of biologically useful molecules.

Proposed Work

In this research, numerous diaryl compounds have been synthesized to study their potential as biologically active compounds. These compounds were tested for two general applications: as antiparasitic agents towards the treatment of Chagas disease, and as AKT inhibitors in search of potential cancer treatments. Additionally, this work has focused on new methods for the creation of diaryl compounds in an attempt to improve the syntheses of diaryl compounds so that researchers may quickly and easily synthesize these compounds for study, testing, and manufacturing.

CHAPTER 5

DIARYL BIOLOGICALLY ACTIVE COMPOUNDS AS ANTIPARASITIC AGENTS FOR THE TREATMENT OF CHAGAS DISEASE

Researchers have already detailed the antiparasitic activities of symmetric diarylidene cycloalkanones. While these compounds showed some initial promise toward the treatment of Chagas disease, Din et al. demonstrated that asymmetric diarylidene had more biological activity with further unstudied potential.[9]

Inhibition Assays

Based off the promising results from Din et al., the compounds indicated in Figure 14 were synthesized to further explore the activity of asymmetric diarylidene cycloalkanones, as well as symmetric arylidene cycloalkanones with heterocyclic aromatic structures. These were synthesized via aldol condensations with either cyclohexanone or cyclopentanone. In the case of the symmetric arylidene cycloalkanones, synthesis was achieved in one step using General Procedure A, while the asymmetric compounds were formed through 2-arylidene cycloalkanone intermediates via General Procedure B and C for cyclopentanone and cyclohexanone respectively. Following this the 2-arylidene cycloalkanones were reacted with one equivalent of aldehyde to afford the asymmetric products by following General Procedure A (Figure 14). In the case of compounds containing a 4-pyridine moiety, General Procedure D was followed to afford the final product, as the 4-pyridine carboxaldehyde would result in a complex mixture of products when following the other general procedures.

Inhibitory activity against *T. cruzi* epimastigotes was measured through fluorescence for 60 μM samples of these compounds. Experiments were ran in triplicate, taking measurements every 24 hours for four days. It was determined that the most active compounds in this experiment were **47**, **51**, **52**, and **54**. All but one of these compounds contains a 2,3-dimethoxy moiety, indicating that this group may be improving binding site affinity or some other factor related to bioavailability. The symmetrical analog containing 4-pyridine (**47**) showed promising inhibitory activity. Combining the 2,3-dimethoxy and the 4-pyridine gave asymmetric **52**, which displayed the best activity out of the

series with 42% inhibition, compared to 55% inhibition of growth for Benznidazole, the only FDA approved treatment for Chagas Disease. Additionally, it was observed that the furyl functional group as well as the benzoyl substitution showed negative effects on the inhibition of *T. cruzi* epimastigotes.

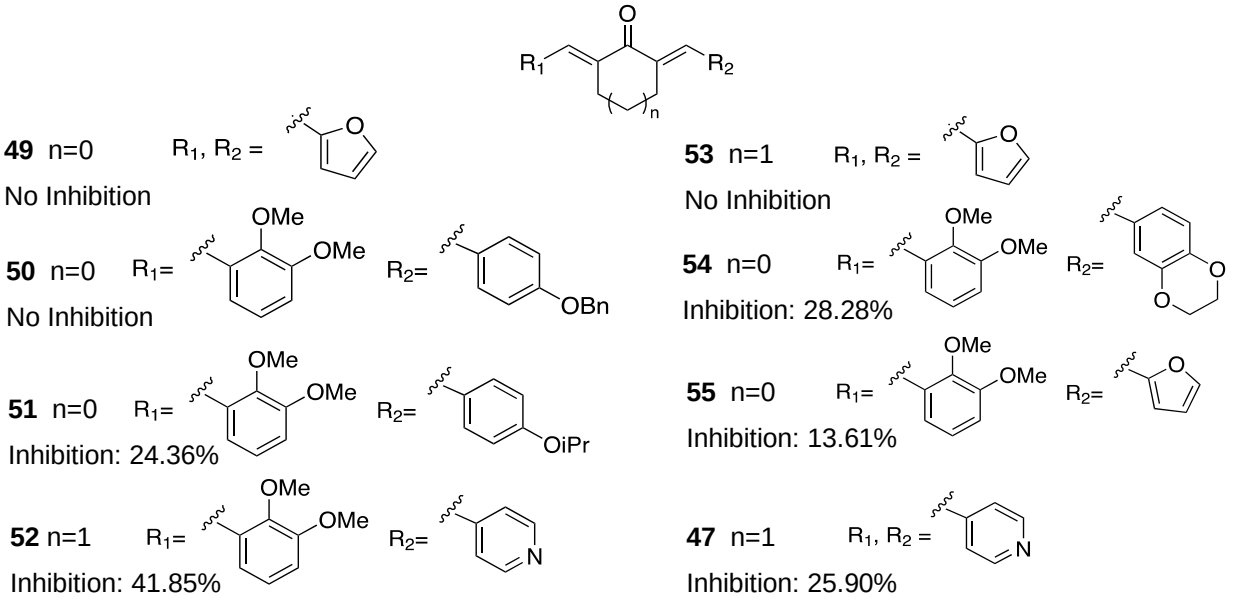
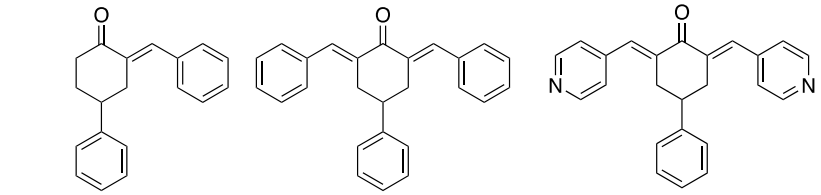


Figure 14. Initial inhibitors synthesized for the investigation of asymmetric diarylidene cycloalkanone inhibitors (60 μM) for the treatment of Chagas Disease.

The Salzameda lab had previously observed that **56**-**58** had promising activity towards the inhibition of *T. cruzi* epimastigotes with EC_{50} values as low as 4.40 μM when measured in triplicate (Figure 15). These compounds all shared a phenyl moiety opposite the ketone, indicating that this functional group can improve binding and inhibition. To study the impact of phenyl and 4-pyridine functional groups, the compounds in Figure 16 were synthesized in the same manner as those from Figure 14. **57** was tested for its selectivity towards *T. cruzi* epimastigotes vs. human HEK 293 cells, and it was found that **57** had a Selectivity Index (SI) of 14, which is roughly 10 times lower than Benznidazole, indicating this compound is more toxic than desired. Due to this, **66** was synthesized to see if a modified structure could maintain activity while reducing the toxicity profile. Additionally, compounds **47** and **52** were retested as controls and were determined to maintain their activity, with **52** showing a significant increase in inhibition relative to **47**, further exemplifying the potential of this

scaffold for future study. Additionally multiple new compounds with improved inhibitory activity were identified, including **59**, **60**, and **66**. **59** was particularly interesting as it showed that the methoxy benzene substitutions resulted in inhibitory activity for **52**. It is possible **52** and **59** interact with different target proteins as the targets of these studies is currently unknown. Further complicating this, **60**, which has three methoxy substitutions, had superior activity compared to **52** and **59**. **66**, which is in many ways structurally similar to **59**, also showed promising inhibitory activity, suggesting that the location and linking of the phenyl substitution could be flexible.



56 EC_{50} = 58.35 μM

57 EC_{50} = 35.32 μM

58 EC_{50} = 4.40 μM

Figure 15. Previously tested arylidene cycloalkanones that exhibited potential towards the treatment of Chagas Disease, but were limited by cytotoxicity.

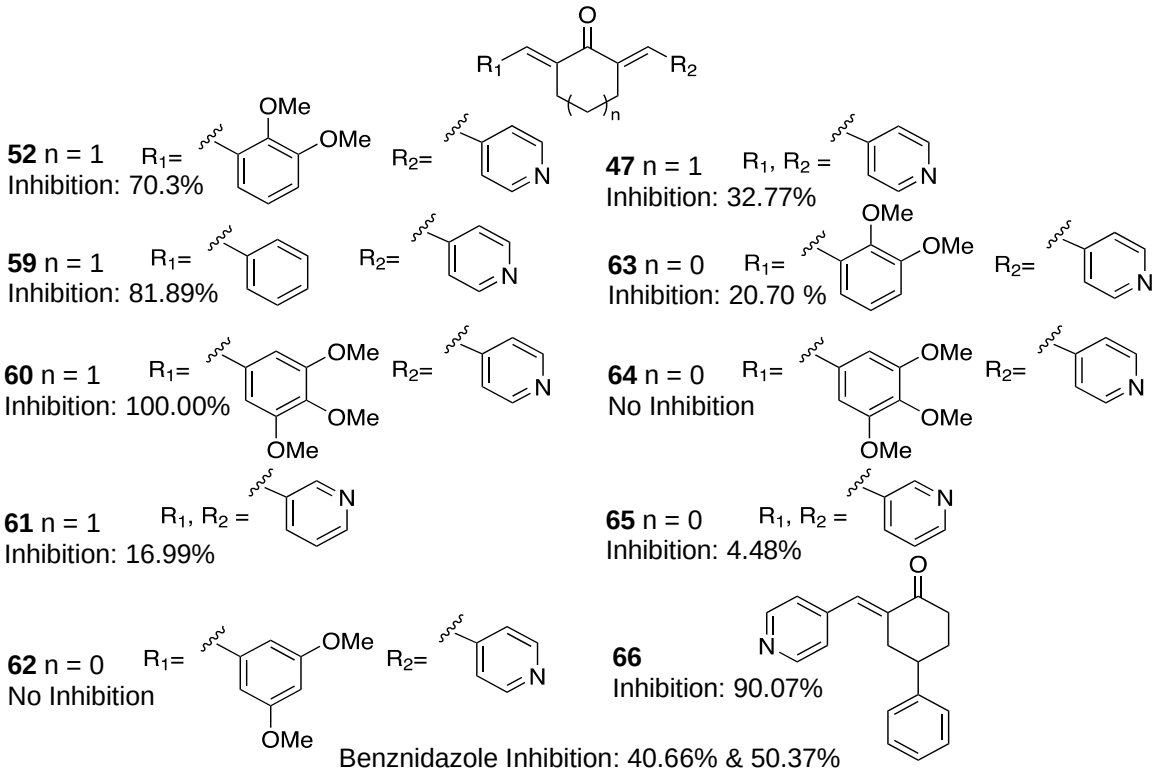


Figure 16. The second round of compounds tested for *T. cruzi* inhibition, with a focus on the 4-pyridine functional group at inhibitor concentrations of 60 μM.

Additional scaffolds were evaluated to determine the importance of the cycloalkanone core for inhibition of *T.* cruzi. Out of the six compounds synthesized, only **71** showed activity with an inhibition of 47.76 % at a 60 μM concentration (Figure 17). This was surprising as methoxy benzene substitutions displayed the best activities in the previous schemes; however comparing **71** to **68**, it was clear that the bromo benzene of **71** has superior inhibitory activity compared to the dimethoxy benzene in **68**.

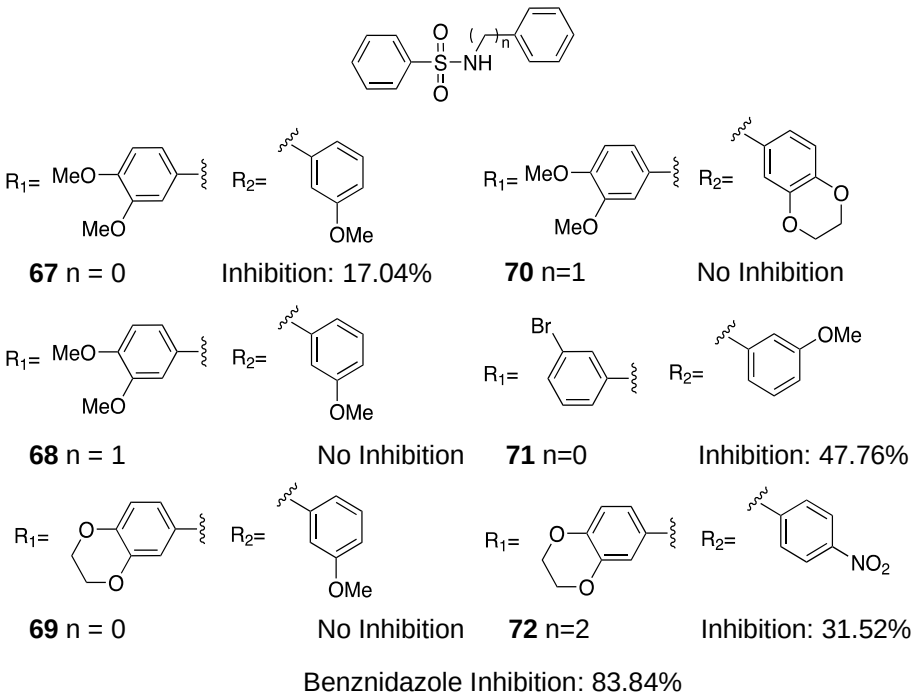


Figure 17. A third series of compounds, focusing on diaryl sulfonamides, which was tested for *T. cruzi* epimastigote inhibition at 50 μM.

Conclusion

Through these studies, multiple new compounds were discovered to have promising inhibition toward T. cruzi epimastigotes growth, with four compounds, **52**, **59**, **60**, and **66**, showing better inhibition at 60 μM than Benznidazole at 100 μM. The general structural conclusions from this study are seen in Figure 18. These compounds shared the same 4-pyridine functional group, indicating that this functional group has a very strong affinity for its active site. Additionally, these compounds had a cyclohexanone-based core. **63** and **64** shared the same substitutions as **52** and **60**, but had a cyclopentanone-based core. Compounds **59** and **66** contained phenyl substitutions that differed in

their linkage and location but gave very similar inhibition values. This indicates that the phenyl substitution provided inhibitory interactions, but the location of the phenyl functional group is at least somewhat flexible. **52** and **60** also shared similarities in their structure as they had multiple methoxy substitutions on the phenyl ring in the 2,3 and 3,4,5 positions respectively, which can act as hydrogen bond acceptors and donate electron density into the aromatic systems of these compounds. Interestingly, compound **62** contained a 3,5-dimethoxy substitution, but exhibited no inhibition at all. This suggests that the methoxy groups have highly specific interactions in their active site which can greatly increase inhibition. However, **62** also shows that if these methoxy groups are in an unfavorable position they can impede inhibitory activity entirely, which could be due to interfering steric interactions or hydrogen bonding with the methoxy groups.

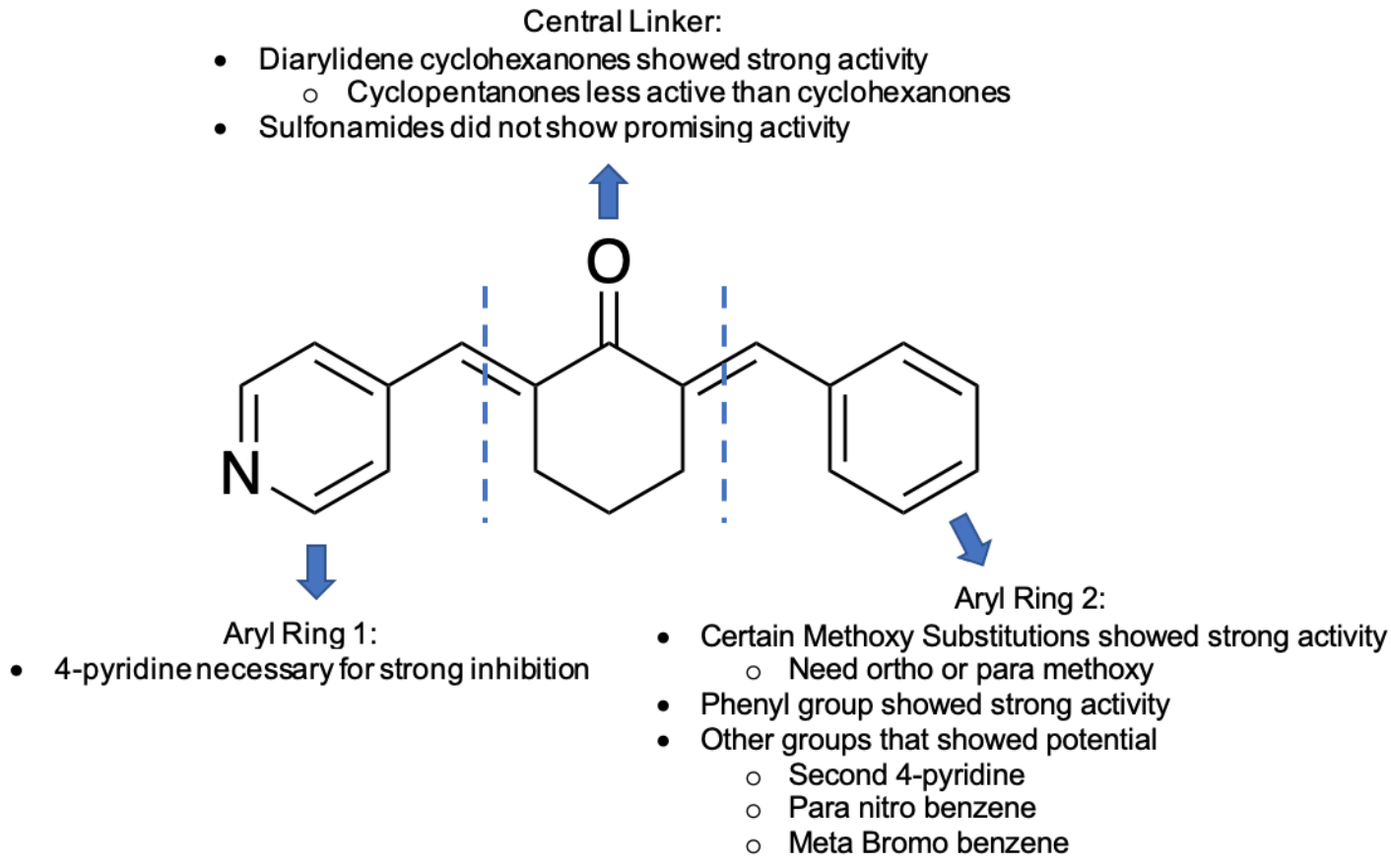


Figure 18. General structural conclusions from SAR study for *T. cruzi* inhibition.

The sulfonamide series in Figure 17 also proved interesting, as two compounds, **71** and **72**, showed potential leads for inhibitory activity. While this linker generally did not give the strong

inhibition relative to Benznidazole, the aryl substitutions provided insight about what functional groups may prove fruitful in future series. As was shown in the diarylidene cycloalkanone compounds, methoxy substituents show great potential for inhibitory activity. Compound **71** also contained a 3-bromo phenyl substitution, indicating that this functional group could provide promise for future series and would be worth additional study. Moreover, compound **72** showed that there is potential in the nitro substitution, as it showed improved inhibition when compared to compound **54**, which contained the other aryl substitution, benzodioxene, with a known inhibitory functional group, 2,3-dimethoxy benzene, but did not show very promising activity. Furthermore, nitro aryl substitutions have shown promise for diaryl inhibitors in the literature, further supporting investigation into this functional group.

CHAPTER 6

DIARYL BIOLOGICALLY ACTIVE COMPOUNDS AS POTENTIAL AKT INHIBITORS FOR THE TREATMENT OF CANCER

Inhibition Screenings

After synthesizing **47** as a potential *T. cruzi* inhibitor, our lab discovered that this compound was of interest to Dr. Bhandari's lab at California State University Long Beach where researchers are studying AKT inhibitors as potential cancer treatments. Because of this, and the many similar compounds we had synthesized, the lab supplied further diarylidene cycloalkanones to Dr. Bhandari's lab to determine their potential for AKT inhibition (Figure 19). For this screening, Western blot analyses was performed to determine the presence of phosphorylated AKT, with differentiation between the two phosphorylation sites: Threonine 308 and Serine 473. Additionally, total AKT (tAKT) was measured in some instances to determine if compounds were inhibiting AKT phosphorylation or the synthesis of AKT itself, as inhibiting the synthesis of AKT would indicate that the cell is reducing overall protein synthesis which is an entirely different mechanism of action that is also under investigation.[61] Additionally, upstream and downstream kinases, Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH) and Activating Transcription Factor 4 (ATF4) respectively, were observed to determine if AKT was being inhibited directly, or if a regulator of AKT was being affected. ATF4 is generally present in low levels under normal conditions.[62] However, ATF4 functions as an ER stress marker as it is expressed in response to ER stress to regulate the cell and is downstream from the PI3K/AKT pathway.[62] This allows one to observe if the inhibitors are acting on an enzyme downstream from AKT. GAPDH is a glycolytic enzyme that is activated by phosphorylated AKT and has been correlated with deregulation in some forms of cancer, as well as tumor progression and cellular death.[63] While the total function of this enzyme is not fully understood, it has been selected as a potential therapeutic target for the treatment of cancer, which is related to AKT function.[63] Tunimyacin (Tun) was also used as an ER stress inducer to stimulate ATF4 synthesis as a positive control through an unfolded protein response that can induce cellular apoptosis.[64] In these assays, inhibition activity was observed by the formation of two bands at the AKT-pT308 location and the

decrease in the amount of AKT-S437 as observed in the AKT-pS473 band, which indicate the modification of AKT and the inhibition of its formation respectively. If these indicators were not seen, it was assumed that AKT was not inhibited in this study.

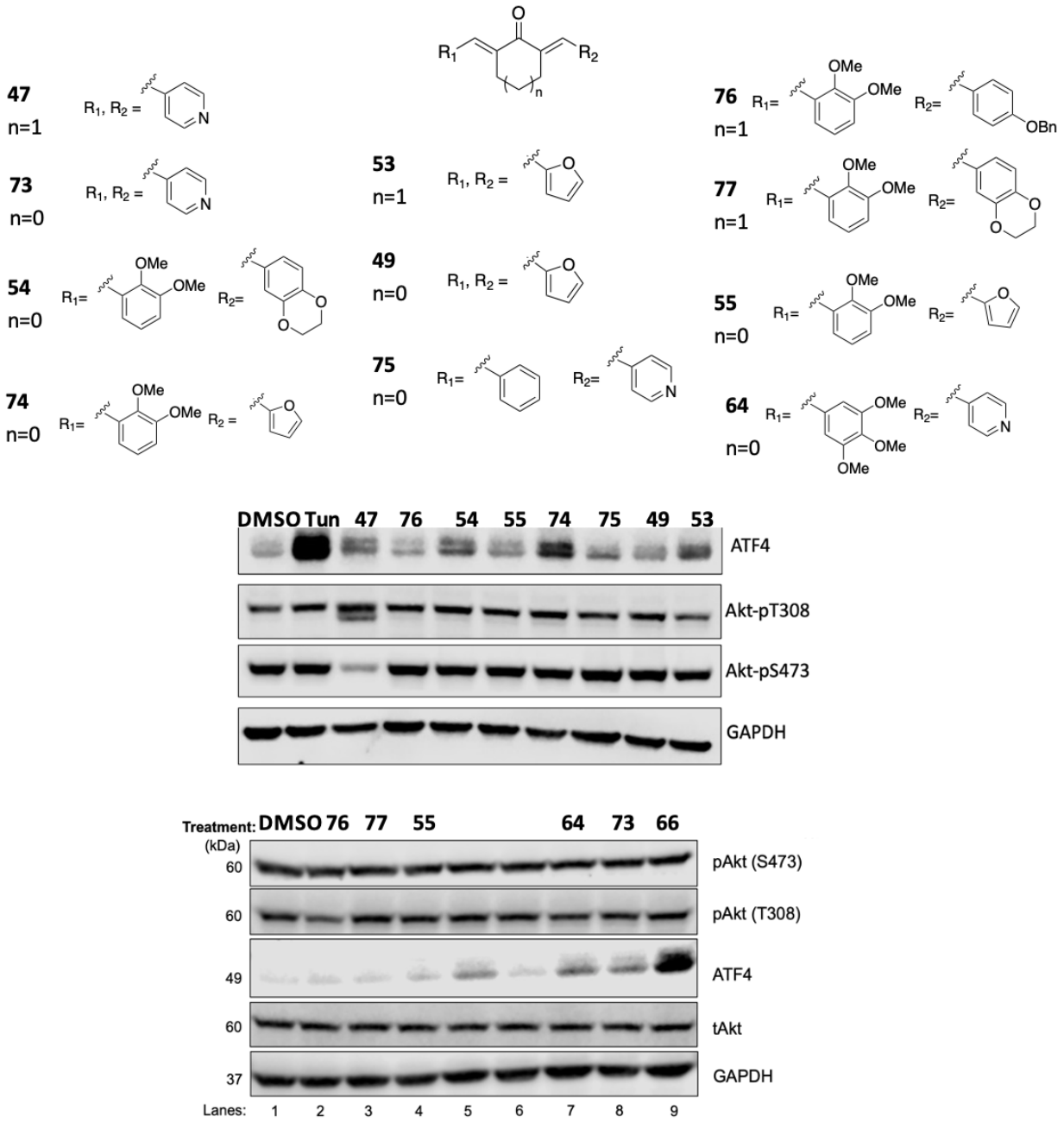


Figure 19. An initial series of diarylidene cycloalkanones provided to the Bhandari Lab to test for AKT inhibition potential.

In the first screening done by Dr. Bhandari's lab, none of the synthesized compounds showed promising activity, with **47** being the only compound to display activity. Because this compound is substituted with two 4-pyridine functional groups, the next series was focused on compounds

containing this functional group. In this series, the 4-pyridine group was maintained on one side of each compound, while the other side was varied with methoxy substitutions (Figure 20). **52** and **60** were found to have strong AKT inhibition. These compounds are very similar, differing only in methoxy substitutions. **63** and **64** also gave inhibition of AKT at levels closer to **47**. Notably, **63** and **64** vary from **52** and **60** in their linker alone, with the first two containing a cyclopentanone while the latter were comprised from cyclohexanone. This suggests that six membered cycloalkanones are likely superior in this application compared to five membered cycloalkanones. Additionally, **61** and **65** were synthesized with a 3-pyridine substitution instead of a 4-pyridine substitution and resulted in complete activity loss in this screening. **78** was also evaluated to determine the effect of adding an alkyl group to **66**, and this was observed to have no activity nor the ATF4 expression of **66**.

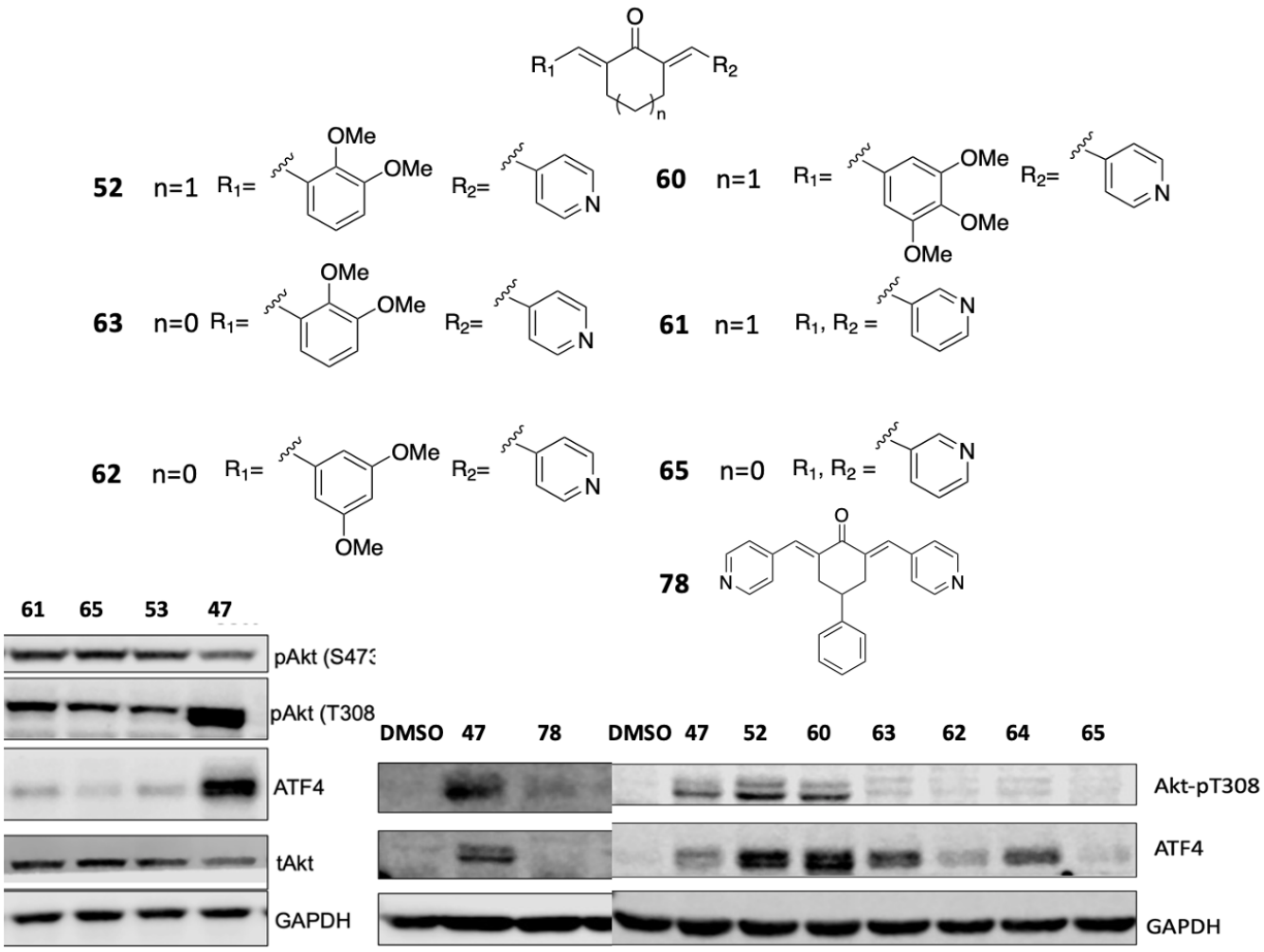


Figure 20. A second series of compounds which were tested for AKT inhibition, focusing on the 4-pyridine functional group.

To test the effect of the central diarylidene linker, different core structures were synthesized, with an emphasis on methoxy aryl structures. While one of these compounds elicited activity with *T. cruzi* inhibition, none of these compounds showed any activity towards AKT inhibition (Figure 21). This lack of activity suggests that having a cycloalkanone as the central linker is likely important for the AKT inhibition of **47**, **52**, and **60**.

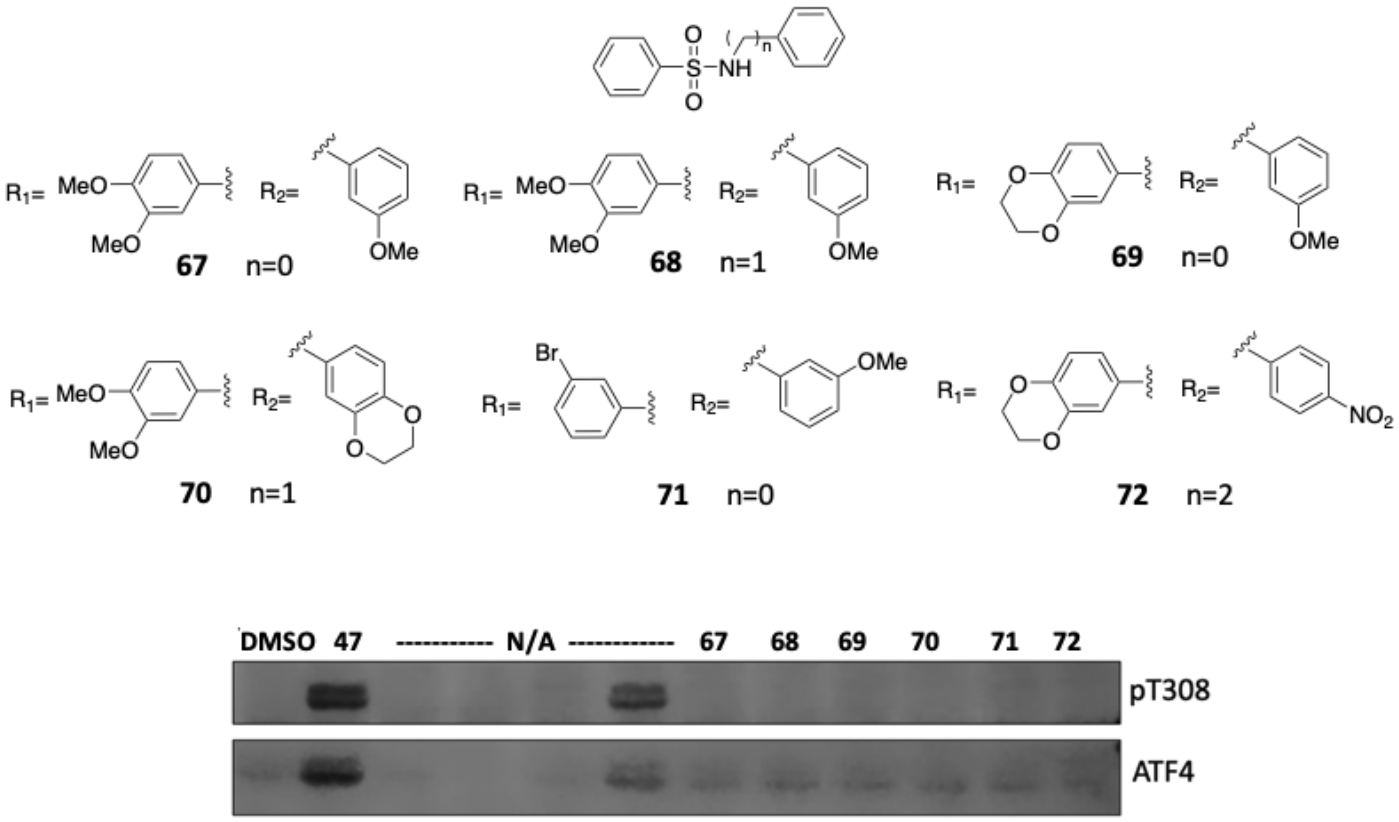


Figure 21. A series of compounds tested for AKT inhibition which focused on the diaryl sulfonamide structural motif.

Conclusion

In this work, multiple compounds were discovered to have inhibitory activity toward AKT, with compounds **47, 52**, and **60** showing the most promise. Interestingly, these compounds (except for **47)** also showed very promising inhibition of *T. cruzi*, further exemplifying the potential biological activity of these diaryl compounds and possibly providing future insight to the mechanism of these compounds. These compounds all had a 4-pyridine functional group, exemplifying the biological activity of this group, and the four compounds that contained a different aryl group were either the

methoxy substituted phenyl rings or phenyl rings without any substitution (Figure 22). Similarly to the parasitic assays, this would suggest some sort of hydrogen bond acceptation or steric interaction is playing a large role in the binding of this compound to AKT. Compound **47** however has a significantly different aryl group, which likely means its binding interactions are different. This could be due to specific interactions near the T308 phosphorylation site or less activity potential in general; however at this time the mechanism is not well enough understood to speculate further.

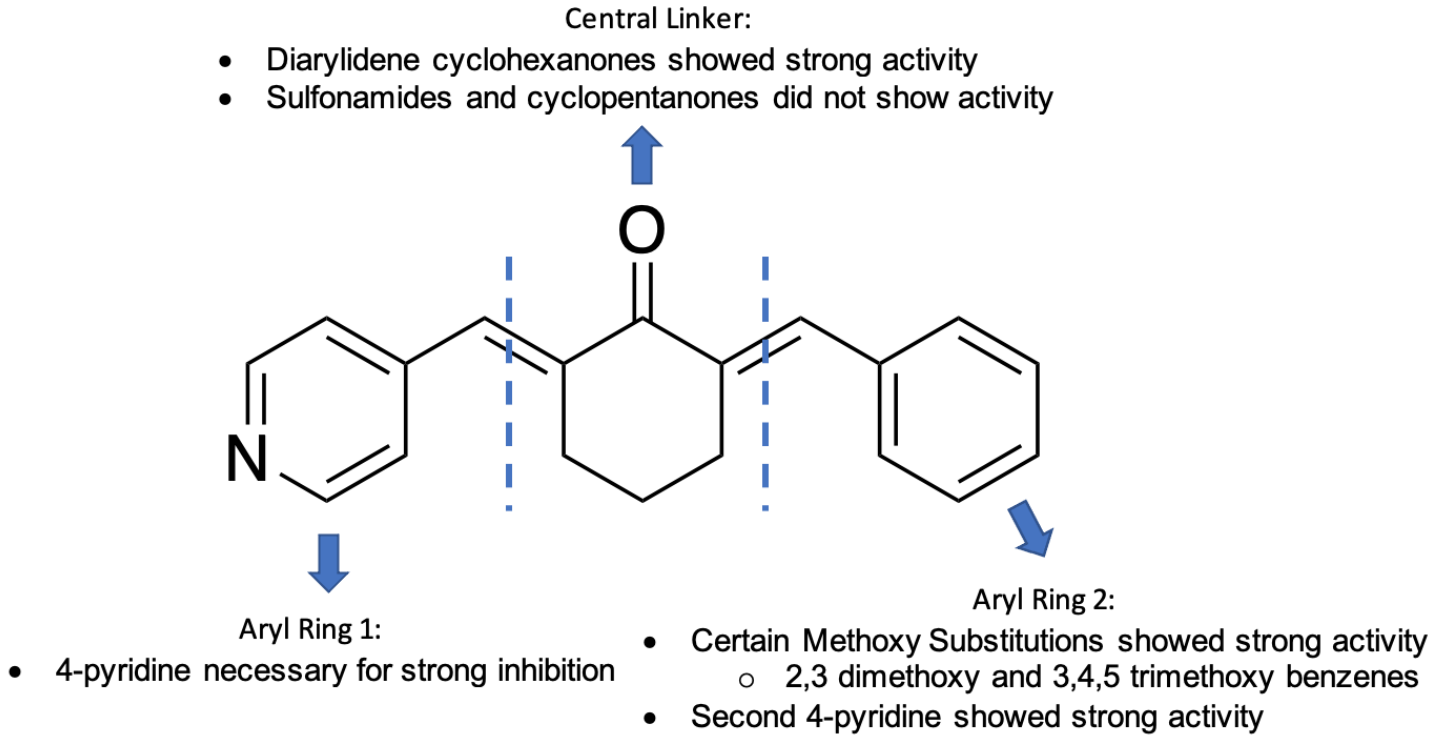


Figure 22. SAR general conclusions by structural component for AKT Inhibition.

In additional similarity to the study of parasitic inhibitors, the cyclohexanone-based systems always outperformed their cyclopentanone counterparts. However, the cyclopentanone based compounds only partially lost inhibitory activity towards AKT versus the total loss seen in the parasitic assays, as can be seen with **63**, **64**, and **73**. Surprisingly, **78** did not show activity despite its structural similarity to **47**. It seems that the addition of an ethyl group to the core cyclohexanone provided some sort of steric or hydrophobic resistance to the compound. This, combined with the low activity of the cyclopentanone series, would suggest that the bottom of the cyclohexanone ring is able to selectively fit into a binding site that is unavailable to the smaller or larger hydrocarbon

cycloalkanones that were tested here. Compounds **61** and **65** also interestingly did not show activity, suggesting that the 3-pyridine moiety is unable to provide the same important interactions to these compounds. This could either be due to binding at the nitrogen site, or the difference in electron donation to the aromatic system which would change the localization of electron density and could therefore affect binding interactions. Unfortunately, the diarylidene sulfonamide series did not show any activity, making it likely that the sulfonamide linkage is unable to bind in the same active site as the diarylidene cycloalkanone compounds.

Future Work

Two of the compounds that showed promising inhibition for *T.* cruzi epimastigotes, **52** and **60**, also gave promising AKT inhibition. For the next round of inhibitor investigations there are currently two series being developed. The first focuses on diaryl amides, which have potential applications in parasitic inhibition and the development of cancer treatments. The amides have been synthesized, purified, and characterized (Figure 23). These compounds have a variety of chain lengths, with aryl substitutions that have proven to have promising biological activity in these applications, namely methoxy groups, nitro groups, and halogens (Figures 7, 12, 19).

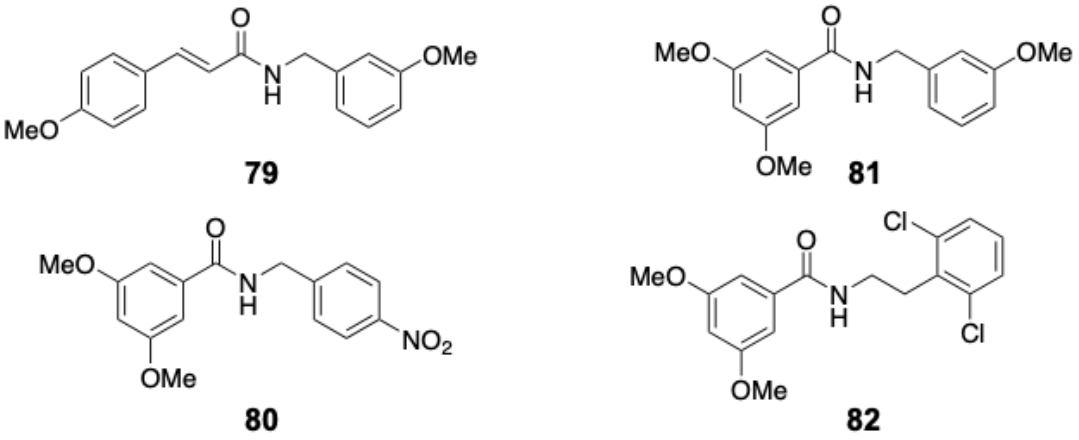


Figure 23. A series of amides synthesized for investigations into AKT and *T. cruzi* inhibition.

A second series of interest would be the synthesis of triazole linked diaryl compounds. These have demonstrated inhibition for both AKT and *T. cruzi* in the literature and could be more analogous

to the arylidene cycloalkanone series, as the 1,2,3-triazole forms a rigid central linker with delocalized electrons.[8, 26, 42] Multiple compounds have been synthesized in this effort, and procedures have been established to create azides and alkynes out of readily available starting materials. The synthesized benzyl methyl azides are shown, which were readily synthesized from benzyl methyl halides (Figure 24). Additionally, an ethynyl benzene was synthesized from an iodobenzene through a trimethyl silyl intermediate. These compounds can then be reacted in a matter of minutes to create diaryl triazoles. This was shown as alkyne **83** was reacted with azide **84** to form triazole **85** in good yield (Figure 24).

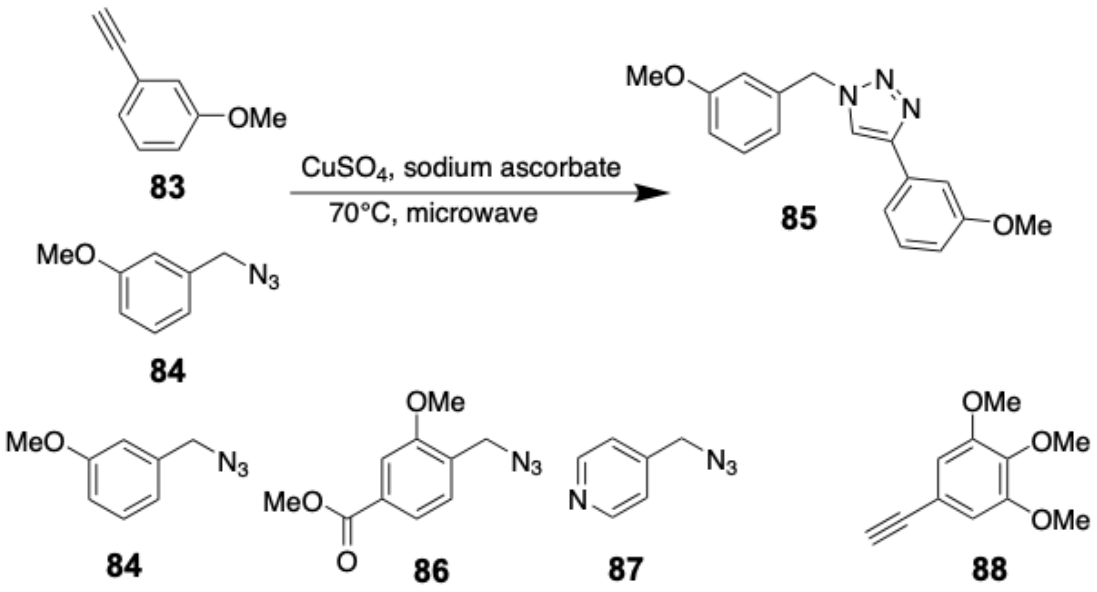


Figure 24. The structural framework and synthetic precursors for a series of diaryl inhibitors linked by a triazole.

Another area of synthetic interest would the diarlyidene phenyl cyclohexanones, such as **66**. These compounds demonstrated activity in the parasitic inhibition series and were claimed to have interesting biological activity by the Bhandari lab; however they also carry some concerns with cytotoxicity which could negate any potential in their current form. However, this may potentially be remedied by modifying the phenyl cyclohexanone, which is extremely hydrophobic and has a negative impact on the bioavailability of this compound. Additionally, it would provide an additional chemical handle for study in the asymmetric diarylidene cycloalkanone series, which proved promising in both applications tested in this work. By adding and optimizing an additional group, one

could potentially make significant strides in specificity and biocompatibility, especially since the compound is relatively small. Groups that would be of particular interest here would be phenyl groups with methoxy, bromo, or nitro substitutions as well as various pyridines, as these functional groups showed promising biological activity for inhibition.

So far, the mechanisms of these compounds are unknown in the applications we have tested. Because of this it would also be useful to track compounds with significant biological interest to determine where they are acting within cells so that their mechanism may be better understood, which would in turn help the design of compounds for a specific target. This would be accomplished through the addition of a fluorescent tag to a compound showing promising activity, then imaging cells after exposure to this compound. This could be done by adding groups at the γ position relative to the cyclohexanone, similarly to **66**. Unfortunately these compounds are small, so fluorescence tags could potentially impact the biological activity compared to the parent compound. However, if successful, this information would be novel and highly useful in future research.

CHAPTER 7

DEVELOPMENT OF A FLOW REACTION FOR THE SYNTHESIS OF 2-ARYLIDENE CYCLOALKANONES

Batch Conditions

Generally, aldol condensation reactions operate with a 1:1 molar ratio of ketone to aldehyde in a polar protic solvent with a catalytic base.[58] However, these conditions are problematic for cycloalkanones with two available α-carbons, as the reaction generally results in a ~2:1 mixture of arylidene cycloalkanone and diarylidene cycloalkanone, as well as residual unreacted cycloalkanone. The purification of the product mixture is difficult and the residual cycloalkanone causes undesired side products if the crude product is carried on to the second aldol condensation. Because of these difficulties, we have explored multiple methods to produce the arylidene cycloalkanone in acceptable purity and yields. The best method from our lab for batch synthesis of 2-arylidene cyclohexanones was adapted from Matsomoto et al. and involved reacting a cyclohexanone and aldehyde in a 3:1 ratio in 0.2 M NaOH over three days.[59] The batch syntheses with a cyclopentanone core were adapted from Wang et al. and used a 5:6 ratio of aldehyde to cyclopentanone in 1:1 diethyl ether and 1 M NaOH over 3 days.[60] These reaction methods produce the desired products with various aromatic aldehydes in good yields (Figure 25).

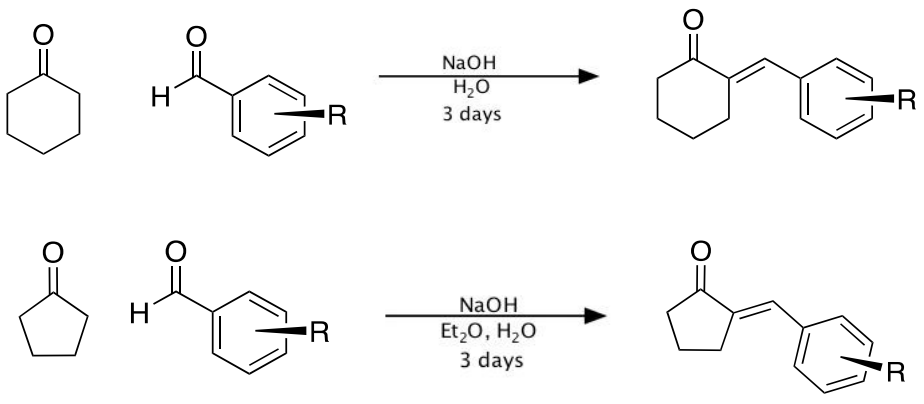


Figure 25. Conditions for batch syntheses of 2-arylidene cycloalkanones from cyclohexanone and cyclopentanone.

The batch methods rely on the solubility differences of the starting materials and products. The reagents were soluble in the aqueous basic layer, but the 2-arylidene cycloalkanones either precipitate or move into the organic layer once formed. This hinders the formation of the unwanted symmetrical diarylidene cycloalkanone species, as the 2-arylidene compound is no longer in solution with the aldehyde and base reagents. This method produces very little diarylidene cycloalkanone but requires a large amount of reagents in the case of the cyclohexanone reaction, as the yields are poor. Additionally, the long reaction time is not ideal for synthesizing a library of analogs and cannot be significantly shortened, as the reaction is extremely slow as monitored by TLC. However, the highlight of this reaction is the high purity of the arylidene cycloalkanone product, which can be further purified by recrystallization. The few products that do not readily precipitate can generally be purified by column chromatography.

Proposed Work

Relying on variable solubility proved to be very successful for the batch reaction, however this method is not ideal for the synthesis of large compound libraries. Robust aldol condensations similar to this application have been detailed by Laroche et al. using flow chemistry.[57] These reactions utilize a single mixed reagent feed in a 9:1 toluene:ethanol solvent system, which flows through a temperature controlled column of polystyrene ammonium hydroxide resin (Amberlyst® A26). This reaction proved to have high yields of 60-95%, with a lifetime of over 120 hours for the reaction between α-tetralone and benzaldehyde.[57] However, said flow reaction has only been reported for α-tetralone. Therefore a need exists for the optimized flow reaction between cycloalkanones containing two α-carbons and aldehydes. As such, we have evaluated the parameters of the flow system, namely the reactants' concentration, column temperature, and flow rate, so that 2-arylidene cycloalkanones can be obtained in good yields and purity in a rapid manner.

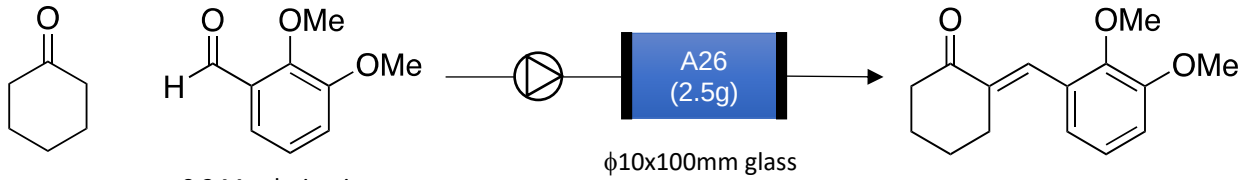
Development of a Method for the Flow Synthesis of 2-Arylidene Cycloalkanones

Initially, a system was designed to determine reaction temperature and flow rates that can be applied to a variety of cycloalkanones and aromatic aldehydes. 2,3-dimethoxybenzaldehyde and

cyclohexanone were selected as the model reactants for our flow chemistry optimization. This is because asymmetric arylidenes containing 2,3-dimethoxy benzaldehyde and other methoxy substituted benzaldehydes are biologically relevant, as they showed strong inhibitory activity towards *T. cruzi* and AKT in our previous studies.[9] The relative reagent and product levels were monitored by GC/MS as the conditions were varied. This was done by first identifying any peaks present through their MS spectra, then integrating the peaks to quantify the approximate amounts of each compound.

From a preliminary trial run, shown in Table 3, it was determined that a rate of 0.1 mL/min at 45°C was able to produce a 2:7 mixture of 2-arylidene cyclohexanones to diarylidene cyclohexanones. While the desired product was present, it was not pure. In an attempt to improve purity, the temperature was varied from 30°C to 60°C, with flow rates from 0.033 to 0.3 mL/min. These flow rates were chosen because LeChatelier's principle would suggest that the formation of the diarylidene cyclohexanones would be promoted by higher reaction temperatures and lower flow rates, as the kinetics of the reaction increase with increased heat and the increased contact time with the catalyst, caused by a lower rate of flow. This initial study determined general reaction conditions that favored the 2-arylidene cyclohexanone at a ratio of ~4:1 versus the diarylidene cyclohexanone. It appeared that the reactions at 45°C and 60°C were able to selectively produce the desired product at 0.1 mL/min and 0.3 mL/ min respectively. When the samples were run below these rates, it resulted in the formation of diarylidene cyclohexanone. Conversely, when the reactions were run at higher rates, the reaction yield drastically decreased. Additionally, it was observed by thin layer chromatography that if lower temperatures were used, no reaction would occur, evidenced by the continued presence of aldehyde and no new detectable products.

Table 3. Determination of optimal flow rate and temperature for a model system of 2-arylidene cycloalkanone production.



0.2 M solution in
9:1 Toluene:Ethanol

ϕ10x100mm glass

Temperature	Flow Rate (mL/min)	Monoarylidene Cyclohexanone Produced	Diarylidene Cyclohexanone Produced	Unreacted Aldehyde	Ratio of Other Compounds
30°C	0.033 0.066 0.1	undetected	present	undetected	many small peaks present
45°C	0.1	18.5%	64.5%	17.0%	
	0.033	37.8%	8.7%	53.5%	
	0.066	36.2%	28.0%	17.4%	18.3%
60°C	0.1	35.6%	62.4%	2.0%	
	0.2	53.3%	30.5%	16.2%	
	0.3	26.6%	72.5%	0.9%	

The concentrations of the reactants were evaluated with the optimized temperature and flow rates of 45°C at 0.1 mL/min and 60°C at 0.3 mL/min (Table 4). The reaction of cyclohexanone and 2,3-dimethoxybenzaldehyde was performed at a variety of concentrations to determine if this effected the ratio of arylidene cycloalkanone to diarylidene cycloalkanone produced. The results shown in Table 4 indicate that a 0.2 M solution gives the highest yield of 2-arylidene cycloalkanone. Concentrations higher than 0.2 M caused an increase in the formation of undesired diarylidene cycloalkanone. Additionally, it was observed that if the reagent solution concentration was less than 0.1 M, no reaction would occur.

Table 4. Determination of ideal concentration for model system, using established temperatures and flow rates from Table 3.

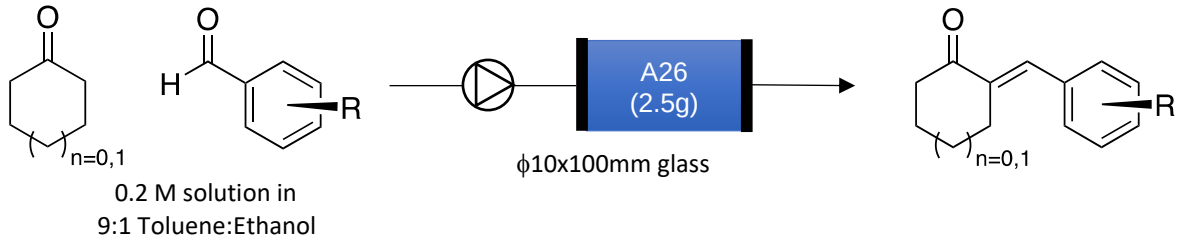
Concentration	Temperature	Flow Rate (mL/min)	Monoarylidene Cyclohexanone Produced	Diarylidene Cyclohexanone Produced	Unreacted Aldehyde
0.05 M	45°C	0.1	0.4%	0%	99.6%
	60°C	0.3	25.0%	0%	75.0%
0.1 M	45°C	0.1	17.2%	0%	82.8%
	60°C	0.3	29.5%	2.3%	68.2%
0.2 M	45°C	0.1	19.0%	trace	81.0%
	60°C	0.3	35.2%	1.4%	62.4%
0.3 M	45°C	0.1	13.4%	trace	86.6%
	60°C	0.3	31.1%	0.6%	66.3%

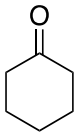
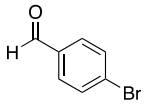
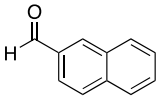
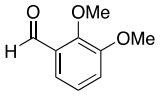
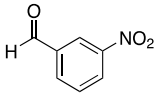
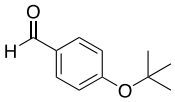
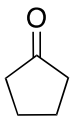
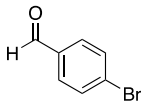
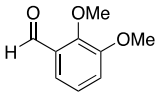
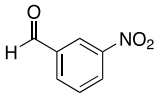
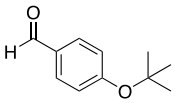
Reaction Validation with Varied Reagents

With these optimized conditions we evaluated the scope of the flow reaction with different aromatic aldehydes and cyclohexanones. Additionally, the desired products from these experiments were isolated and calibration curves were generated from the GC/MS data to determine the yields of the 2-arylidene cycloalkanones (Table 5). The reaction conditions were varied from 30 to 75°C with rates of 0.1 to 1.5 mL/min to identify the optimal reaction conditions or determine if the reaction was not viable. This resulted in yields from 5.8% to 27.5% with Space-Time Yields (STYs) from 92 to 580 grams per liter hour. These significant yields with modest STY values suggest that this reaction can be used to produce compounds both in a lab environment and at larger scales. Generally, mild electron withdrawing groups or electron donating groups reacted similarly to the model system, with ratios of 14:1 arylidene cyclohexanone to diarylidene cyclohexanone obtained in all cases. However the nitro substitution, which is electron withdrawing, significantly impacted the yields for this reaction. Unfortunately increasing the reaction rate in this instance created undesirable diarylidene side products before a similar yield to the model system could be reached. This was also true with benzaldehyde, however the yields with this product were much lower, making this flow reaction unsuitable for some reagents without modification. Furaldehyde was also tested, but significant

byproducts were observed for all conditions evaluated. It is also worth noting that 3-pyridine carboxaldehyde and 4-pyridine carboxaldehyde were evaluated in this flow reaction, but solubility issues resulted in precipitation during the flow reaction. This illustrates the current limitation of this method, namely the use of heterocyclic aldehydes in the reaction, without major modification to the solvent system.

Table 5. Validation of reaction system with variable ketones and aldehydes, along with their optimized reaction conditions and yields.



Ketone	Aldehyde	Temperature	Flow Rate (mL/min)	% Yield 2-Arylidene Cyclohexanone	STY (g/L·hr)
		30°C	0.5	19.4%	336
		75°C	0.5	25.8%	409
		60°C	0.3	15.5%	424
		30°C	0.5	5.8%	92.4
		60°C	0.2	13.6%	580
		45°C	0.3	9.9%	279
		30°C	0.7	14.3%	160
		45°C	0.3	6.7%	157
		60°C	0.4	27.5%	447

All the successful aldehydes were also examined with cyclopentanone to determine the viability of this reaction scheme with other cycloalkanones. As shown in Table 5, the reactivity trends between the cyclohexanone system and cyclopentanone system were similar, giving similar yields and STY values; however the reaction rates likely increased with cyclopentanone compared to reactions with cyclohexanone, as evidenced by the lower optimized temperatures and higher flow rates compared to the cyclohexanone reactions. Because of this it can be concluded that cyclopentanone is a suitable starting material for this flow method, indicating that multiple cycloalkanones can be used without major modifications.

Reaction Lifetime Validation

In the work detailed by Laroche et al., multiple systems were identified to have limited lifetimes, due to the deterioration of catalytic resin.[57] This could be especially concerning for the work detailed here, as even a small deterioration of the resin could result in different reactivity rates, producing less of the desired 2-arylidene cycloalkanone product and creating more side products or other contaminants, which could be observed at lower temperatures and lower flow rates in the aldehyde studies (Tables 3 and 4). Because of this, we decided to confirm the viability of the system over 48 hours. The model system of cyclohexanone and 4-bromobenzaldehyde was chosen, as the results of the 4-bromobenzaldehyde were generally representative of the flow method. As seen in Figure 26, running the reaction for two days, and collecting fractions every 6 or 12 hours, it was determined that this reaction is viable up to 48 hours, as the reactivity is consistent throughout, and 2-arylidene cyclohexanone formation is constant.

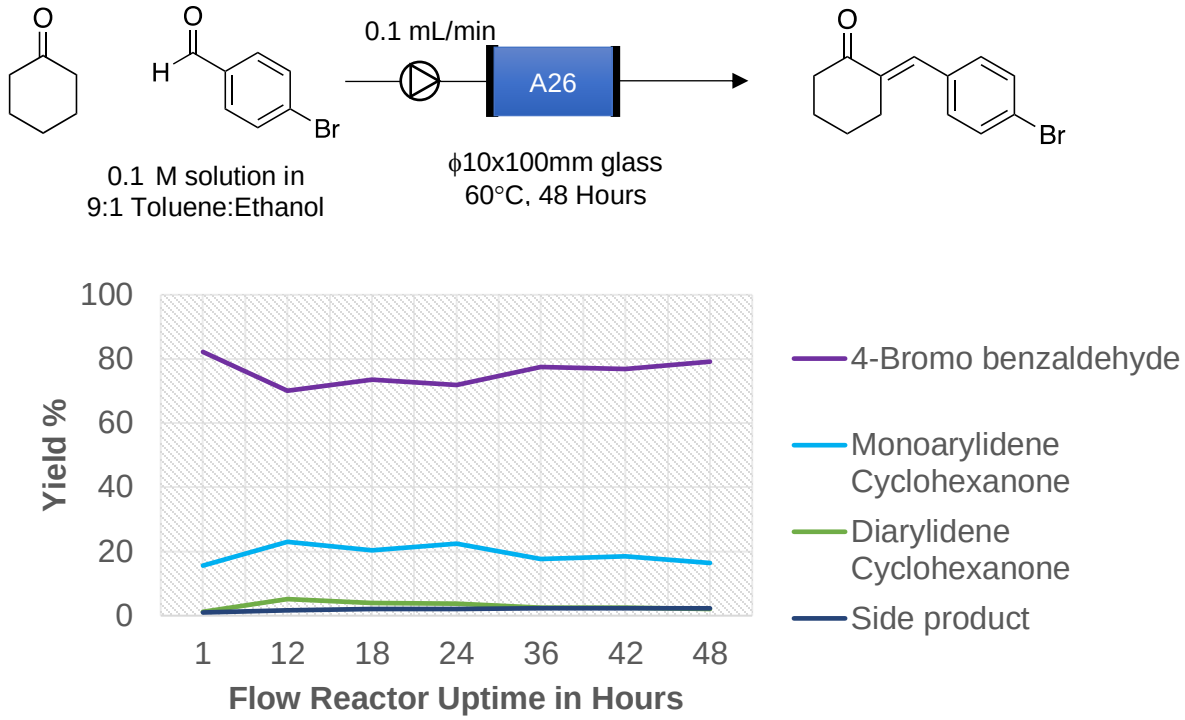


Figure 26. Validation of catalyst regeneration over time.

Conclusion

In this work, a flow reaction was developed to synthesize 2-arylidene cycloalkanones in good purity with an A26 resin column utilizing temperatures from 30 to 75°C and flow rates of 0.2 to 0.7 mL/min. This reaction was validated with multiple cycloalkanones and benzaldehyde derivatives and was shown to have yields of 5.8 to 27.5% and STY values of 92.4 to 580 (g/L·hr). These values were determined via GC/MS through the creation of standard calibration curves. Additionally, the reaction was validated over 48 hours and observed to have a consistent yield over this time frame, suggesting that the catalyst is successfully regenerated during this time frame. This reaction can be used to create large compound libraries for the study of 2-arylidene cycloalkanones, as well as compounds that use these as an intermediate such as asymmetric diarylidene cycloalkanones, which have demonstrated promising biological activity in this work.

Future Work

While this reaction can synthesize many of the 2-arylidene compounds needed for our studies, this reaction could be expanded to utilize heteroaromatic aldehydes, with emphasis on pyridine

carboxaldehydes. When initially testing these compounds, solubility issues were observed which prevented their initial consideration for this reaction. However given the promising observations from their applications in AKT and *T. cruzi* inhibition, it could be worthwhile to expand the scope of this reaction. Furthermore, testing new reaction conditions to try and improve yields and selectivity could be beneficial, as the reaction yields have room to be improved. In this work, yield was optimized by increasing the reaction rate until side products were formed, as the side products are difficult to remove through purification. This ultimately limited the yield of this reaction. However, compounds have been reported in the literature that can selectively form 2-arylidene cycloalkanones over symmetric diarylidene cycloalkanones.[9] Compounds like this could be adapted to flow chemistry and used to improve the yields of this reaction while maintaining the desirable selectivity.

APPENDIX A

GENERAL PROCEDURES

General Procedure A: General Aldol Condensation Procedure for Symmetric Diarylidene Cycloalkanones and Asymmetric Diarylidene Cycloalkanones from 2-Arylidene Cycloalkanones

A ketone or 2-arylidene cycloalkanone (2 mmol) was dissolved in a 10 mL round bottom flask with 2 mL of 100% ethanol and 1.5 mL of 0.2 M NaOH in ethanol. Following this, the selected aldehyde was added (2 mmol if using ketone, or 1 mmol if using 2-arylidene cycloalkanone). The reaction was then stirred overnight and monitored by TLC. Products were extracted with ethyl acetate, washed with deionized water, then dried via rotary evaporation.

General Procedure B: Selective Aldol Condensation for 2-Arylidene Cyclopentanone Synthesis

An aldehyde (5 mmol) and cyclopentanone (6 mmol) were added to a round bottom flask with a mixture of 5 mL of diethyl ether and 5 mL of 1 M NaOH. This solution was then stirred for three days, products were extracted with ethyl acetate, washed with deionized water, then dried via rotary evaporation before being brought to the next step without precipitation.

General Procedure C. Selective Aldol Condensation for 2-Arylidene Cyclohexanone Synthesis

A cyclohexanone (2 mmol) and aldehyde (1 mmol) were added to a round bottom flask containing 10 mL of 0.2 M NaOH in water. This mixture was then stirred for three days and quenched with 2 mL 1.0 M HCl. The product was extracted with ethyl acetate and brine, dried with $MgSO_4$, and verified by TLC and GCMS. The organic layer was then rotovaped and brought to the next step in the synthesis without further purification or isolation.

General Procedure D: Symmetric and Asymmetric Diarylidene Cycloalkanone Synthesis Using 4-Pyridine Carboxaldehyde

For asymmetric diarylidene cycloalkanones, a 2-arylidene cycloalkanone was synthesized using General Procedure B or C. For symmetric diarylidene cycloalkanones a cycloalkanone is used. The starting material (1 mmol) is dissolved in 1.5 mL EtOH and treated with a 10 mol % solution of LiOH in H_2O and allowed to stir for 10 minutes. Following this, 4-pyridine carboxaldehyde was added

to the solution (1 mmol for asymmetric compounds and 2 mmol for symmetric compounds). The reaction was then stirred for 30 minutes and monitored by TLC until aldehyde was consumed. After the reaction, products were precipitated with cold deionized water and thoroughly washed with water. Symmetric dimers were purified through recrystallization in EtOH, while asymmetric products were purified through column chromatography.

General Procedure F: Sulfonamide Couplings

A round bottom flask was charged with an amine (0.5 mmol) and 10 mL of DCM. The flask was then chilled to 0°C before adding the desired sulfonyl chloride (0.5 mmol). The flask was then allowed to return to room temperature before adding diisopropylethylamine (1.5 mmol). The reaction mixture was allowed to stir two hours to overnight before quenching with deionized water. The aqueous mixture was then extracted three times with DCM, followed by a triple wash of the organic layer with 1 M HCl. The resulting organic solution was then dried with $MgSO_4$ and rotary evaporation and purified via flash chromatography.

General Procedure G: Amide Couplings

A carboxylic acid (1 mmol) and an amine (1 mmol) were added to a round bottom flask and dissolved in 5 mL of anhydrous DMF under inert gas. Then EDC (0.75 mmol), HOBT (0.6 mmol), and DIPEA (0.75 mmol) were added to the flask. The reaction mixture was then allowed to stir for four hours and reaction progress was monitored via TLC. Reaction was then quenched with 20 mL of deionized water and extracted three times with ethyl acetate. Following this, the organic solution was washed three times with 1 M HCl, followed by 1 M Na_2CO_3. The organic layer was then dried with brine and $MgSO_4$. Product was purified via flash chromatography to afford the final product.

General Procedure H: Azide Synthesis

Under inert gas, a bromo, chloro, or iodo methyl benzene (3 mmol) was charged to a round bottom flask with 7.5 mL of anhydrous DMF and sodium azide (6 mmol). This was then allowed to stir overnight, and reaction progress was confirmed via TLC. Reaction was then quenched with ~15 mL of deionized water. Product was extracted three times with ethyl acetate, washed three times with

deionized water, then dried with brine followed by $MgSO_4$. Products were purified via flash chromatography with 5-20% Ethyl Acetate in Hexane and confirmed via NMR.

General Procedure I: Alkyne Synthesis

An Iodobenzene (1 mmol) was dissolved in triethylamine (20 mmol) and stirred for 10 minutes under inert gas in a round bottom flask. Following this, the flask was charged with CuI and $PdCl_2PPh_3$ (0.056 mmol each). Following this, Trimethylsilyl acetylene (1.2 mmol) was added to the flask and the reaction mixture was allowed to stir for four hours at room temperature while monitoring reaction progress via TLC. Once the reaction was completed 2 mL of a 1 M KOH in MeOH solution was added dropwise to the flask; the reaction mix was then allowed to stir for 15 minutes. Following this, the methanol was removed through rotary evaporation and products were extracted with ethyl acetate three times followed by deionized water three times. Sample was then dried with $MgSO_4$, purified with flash chromatography, and verified via NMR.

General Procedure J: 1,2,3 Triazole Click reaction

A solution containing azidomethyl benzene (1 mmol), phenyl acetylene (2 mmol), and sodium ascorbate (0.25 mmol) in 2 mL of DMF was prepared in a microwave tube. A separate solution of $CuSO_4$ (0.5mmol) in 0.5 mL of deionized water was prepared, then added to the microwave tube just before sealing and irradiating the sample. Sample was irradiated for 10 minutes at 70°C and reaction progress was verified via TLC. Following reaction completion, the sample was diluted with 15 mL of brine, then extracted with EtOAc three times. Sample was then dried with $MgSO_4$ and purified with flash chromatography.

General Procedure K: Preparation of A26 Resin for Flow System

2.5 g of A26 resin was weighed into a Buchner funnel and rinsed three times with 10mL of deionized water. Then the resin was washed with three 10 mL portions of Ethanol. Following this, the resin is washed with three 10 mL portions of 9:1 Toluene:Ethanol. The resin is then transferred to the column using 9:1 Toluene:Ethanol to help transfer the resin into the column. This column is then

inserted into the flow reactor and the column is allowed to equilibrate for 10 min at the desired temperature while flowing 9:1 Toluene:Ethanol through the system at 0.1 mL/min.

General Procedure L: Preparation of A26 Resin for Flow System

Prepare flow reactor column according to General Procedure K. Following this, the desired aldehyde (3.18 mM) and cycloalkanone (3.0mM) are added to 15 mL of a 9:1 Toluene:Ethanol solution, making sure the reagents fully dissolve. This solution is then fed into the flow reactor, which has been equilibrated according to General Procedure A, at a 0.05-1.5 mL/min ratio. The reactor is allowed to run so that at least 8mL of reaction solution is allowed to pass into the system. After the required volume has passed through the column, a 0.5 mL fraction is collected from the flow system and analyzed via GCMS.

General Workup for Solid Products

Solid dimers were washed with cold water in a Büchner funnel, then dissolved in ethanol. The product was then recrystallized in ethanol and water, or ethanol and ethyl acetate in rare cases. The crystalline solid was then filtered with a Büchner funnel and washed with deionized water. Product was verified by ^{1}H and ^{13}C NMR.

General Workup for Liquid Products

Liquid dimers were purified using Combi flash column chromatography in a 5-20% gradient of ethyl acetate and hexane. The sample was dry loaded into silica. Fractions were collected for each UV-detected peak and TLC was taken for each of the fractions. The correct fractions were then collected and rotovaped to obtain the pure liquid product. Product was verified by ^{1}H and ^{13}C NMR.

***T. cruzi* Epimastigote Assay**

For this assay, a reporter strain of *T. cruzi* epimastigotes with the tdTomato gene was used so that the parasites would fluoresce for quantifiable inhibition measurements. A 96 well plate was incubated with $5x10^{6}$ cells per well, then inoculated with 60 μM of a compound of interest to test for its inhibition. In this experiment, compounds were dissolved in DMSO and a blank control of DMSO was used to control for this. 100 μM Benznidazole was tested as a positive control. Fluorescence was

measured from 540-580 nm every 24 hours for five days with a SpectraMax M3. All measurements were taken in triplicate then averaged to improve accuracy. Percent inhibition calculations were measured by applying a best fit curve to the fluorescence measurements, then taking these slopes and dividing them by the slope of the DMSO control. For dose response curves, a similar methodology was taken, using multiple trials where the dosages of the compound were varied between the trial. The concentrations were then plotted against the fluorescence data to determine which concentration gives 50% inhibition, resulting in the EC_{50}.

AKT Inhibition Western Blot

For this testing HeLa cells (for Figures 20 and 21) or HEK 293 cells (Figures 19 and 20) were exposed to 14.5 μM samples of a potential inhibitor and treated for two hours.

The cell lysates were then run with standard western blot techniques and stained for imaging.

APPENDIX B

COMPOUND CHARACTERIZATION

Compound 47: *2,6-bis(pyridin-4-ylmethylene)cyclohexan-1-one*

Synthesized with General Procedure C and D. 28% yield. Confirmed by GCMS and NMR.

^{1}H NMR (400 MHz, DMSO) δ 8.10, 7.77, 7.77, 7.77, 7.47, 6.81, 6.80, 3.40, 2.77, 2.77, 2.76, 2.74, 2.74, 1.79, 1.77, 1.76.

^{13}C NMR (101 MHz, DMSO) δ 188.14, 146.10, 144.66, 134.95, 126.94, 122.24, 111.57, 28.14, 21.83.

Compound 49: *2,5-bis(furan-3-ylmethylene) cyclopentane-1-one*

Synthesized with General Procedure A. 74% yield. Confirmed by GCMS and NMR.

^{1}H NMR (400 MHz, Chloroform-d) δ 7.92 (s, 1H), 7.50 (s, 1H), 7.15 (ddt, J = 18.0, 15.9, 4.7 Hz, 3H), 7.00 – 6.88 (m, 2H), 4.35 – 4.25 (m, 4H), 3.89 (d, J = 8.4 Hz, 6H), 3.04 (s, 4H).

^{13}C NMR (101 MHz, Chloroform-d) δ 196.18 , 153.00 , 149.25 , 144.98 , 143.51 , 138.69 , 135.72 , 133.54 , 130.25 , 129.57 , 127.79 , 125.08 , 123.76 , 121.58 , 119.28 , 117.62 , 113.42 , 77.39 , 64.62 , 64.23 , 61.51 , 55.88 , 26.57 .

Compound 50: *2-(4-(benzyloxy)benzylidene)-5-(2,3-dimethoxybenzylidene)cyclopentane-1-one*

Synthesized with General Procedure B and D. 46% yield. Confirmed by GCMS and NMR.

^{1}H NMR (400 MHz, Chloroform-d) δ 7.90 (t, J = 2.9 Hz, 1H), 7.75 (dt, J = 1.6, 0.8 Hz, 1H), 7.53 – 7.43 (m, 2H), 7.21 – 7.05 (m, 2H), 6.96 (dd, J = 8.1, 1.6 Hz, 1H), 6.68 – 6.62 (m, 1H), 3.88 (d, J = 8.2 Hz, 6H), 3.09 – 2.98 (m, 2H), 2.95 – 2.85 (m, 2H), 1.29 – 1.22 (m, 0H).

^{13}C NMR (101 MHz, Chloroform-d) δ 195.72, 152.99, 149.27, 145.33, 143.99, 138.79, 136.55, 130.12, 127.93, 123.87 (d, J = 16.7 Hz), 122.87, 121.54, 113.49, 110.48, 77.41, 61.51, 55.86, 26.15 (d, J = 7.1 Hz).

Compound 51: *2-(2,3-dimethoxybenzylidene)-5-(4-isopropxybenzylidene)cyclopentane-1-one*

Synthesized with General Procedure C and D. 53% yield. Confirmed by GCMS and NMR.

^{1}H NMR (400 MHz, Chloroform-d) δ 7.91 (s, 1H), 7.56 – 7.46 (m, 3H), 7.19 – 7.00 (m, 2H), 6.98 – 6.84 (m, 3H), 4.58 (hept, J = 6.1 Hz, 1H), 3.85 (d, J = 1.6 Hz, 7H), 3.03 – 2.94 (m, 4H), 1.34 (d, J = 6.1 Hz, 6H).

^{13}C NMR (101 MHz, Chloroform-d) δ 196.13 , 159.06 , 152.95 , 149.20 , 138.75 , 138.51 , 134.92 , 133.73 , 132.60 , 130.21 , 128.15 (d, J = 19.1 Hz), 127.48 , 123.80 , 121.54 , 115.83 , 113.40 , 77.53 , 69.94 , 61.45 , 55.83 , 26.56 , 21.98.

Compound 52: *2-(2,3-dimethoxybenzylidene)-6-(pyridin-4-ylmethylene)cyclohexan-1-one*

Synthesized with General Procedure B and D. 14% yield. Confirmed by GCMS and NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68, 8.66, 7.97, 7.67, 7.32, 7.30, 7.28, 7.11, 7.09, 7.07, 6.97, 6.96, 6.95, 6.95, 6.94, 3.91, 3.90, 3.90, 3.89, 3.89, 3.87, 3.85, 3.82, 3.82, 3.82, 3.81, 2.93, 2.93, 2.91, 2.90, 2.90, 2.86, 2.85, 2.84, 2.83, 2.82, 2.42, 1.83, 1.81, 1.80, 1.78, 1.77, 1.64, 1.40, 0.03, 0.02, 0.01.

^{13}C NMR (101 MHz, $CDCl_3$) δ 189.80, 152.89, 149.97, 143.51, 139.88, 136.92, 133.47, 130.09, 124.08, 123.51, 122.07, 113.05, 61.16, 55.88, 28.55, 28.40, 22.91, -0.01.

Compound 53: *2,6-bis(pyridin-3-ylmethylene)cyclohexan-1-one*

Synthesized with General Procedure C and D. 73% yield. Confirmed by GCMS and NMR.

^{1}H NMR (400 MHz, DMSO-d6) δ 8.13 – 8.06 (m, 1H), 7.77 (t, J = 1.8 Hz, 1H), 7.47 (d, J = 2.1 Hz, 1H), 6.83 – 6.76 (m, 1H), 2.76 (dq, J = 6.5, 2.2 Hz, 2H), 1.77 (tt, J = 7.4, 5.6 Hz, 1H).

^{13}C NMR (101 MHz, DMSO-d6) δ 188.14 , 146.09 , 144.66 , 134.95 , 126.94 , 122.24 , 111.57 , 40.57 , 40.36 , 40.15 , 28.14 , 21.83 .

Compound 54: *2-((2,3-dihydrobenzo[1,4]dioxin-6-yl)methylene)-5-(2,3-dimethoxybenzylidene) cyclopentan-1-one*

Synthesized with General Procedure B and D. 64% yield. Confirmed by GCMS and NMR.

^{1}H NMR (400 MHz, Chloroform-d) δ 7.92 (s, 1H), 7.50 (s, 1H), 7.15 (ddt, J = 18.0, 15.9, 4.7 Hz, 3H), 7.00 – 6.88 (m, 2H), 4.35 – 4.25 (m, 4H), 3.89 (d, J = 8.4 Hz, 6H), 3.04 (s, 4H).

^{13}C NMR (101 MHz, Chloroform-d) δ 196.18, 153.00, 149.25, 144.98, 143.51, 138.69, 135.72, 133.54, 130.25, 129.57, 127.79, 125.08, 123.76, 121.58, 119.28, 117.62, 113.42, 77.39, 64.62, 64.23, 61.51, 55.88, 26.57.

Compound 55: *2-(2,3-dimethoxybenzylidene)-5-(furan-2-ylmethylene) cyclopentane-1-one*

Synthesized with General Procedure B and D. 71% yield. Confirmed by GCMS and NMR.

^{1}H NMR (400 MHz, Chloroform-d) δ 7.93 – 7.83 (m, 1H), 7.53 (d, J = 1.9 Hz, 1H), 7.35 – 7.25 (m, 1H), 7.16 – 7.07 (m, 1H), 7.04 (t, J = 8.0 Hz, 1H), 6.93 – 6.85 (m, 1H), 6.63 (d, J = 3.6 Hz, 1H), 6.47 (dd, J = 3.4, 1.8 Hz, 1H), 3.81 (s, 6H), 2.95 (s, 4H).

^{13}C NMR (101 MHz, Chloroform-d) δ 195.64 , 152.90 , 152.63 (d, J = 2.0 Hz), 149.23 , 145.06 , 138.94 , 138.46 , 135.85 , 135.22 , 130.05 , 127.97 , 127.62 , 123.81 , 121.49 , 119.95 , 119.70 , 115.94 (d, J = 6.4 Hz), 113.49 , 112.66 , 77.59 , 76.95 , 61.42 , 55.78 , 26.65 , 26.16 (d, J = 6.4 Hz), 25.75 .

Compound 59: *2-(benzylidene)-6-(pyridin-4-ylmethylene)cyclohexan-1-one*

Synthesized with General Procedure C and D. 6.5% yield. Confirmed by GCMS and NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82, 7.50, 7.48, 7.44, 7.43, 7.41, 7.37, 7.36, 7.28, 7.25, 3.75, 3.73, 3.28, 2.95, 2.19, 1.83, 1.81, 1.27, 1.26, 1.24, 0.02.

^{13}C NMR (101 MHz, $CDCl_3$) δ 190.46, 136.98, 136.20, 135.98, 130.37, 128.59, 128.38, 58.47, 30.92, 28.46, 23.02, 18.39.

Compound 60: *2-(pyridin-4-ylmethylene)-6-(3,4,5-trimethoxybenzyidene)cyclohexan-1-one*

Synthesized with General Procedure C and D. 7.4% yield. Confirmed by GCMS and NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63, 8.62, 7.72, 7.62, 7.27, 7.26, 6.70, 3.87, 3.87, 2.95, 2.88, 1.82, 1.81.

^{13}C NMR (101 MHz, $CDCl_3$) δ 189.45, 153.01, 149.94, 143.39, 139.66, 138.13, 134.83, 133.30, 131.10, 124.03, 108.02, 60.93, 56.20, 28.39, 28.24, 22.70.

Compound 61: *2,6-bis(pyridin-3-ylmethylene)cyclohexan-1-one*

Synthesized with General Procedure A. 54% yield. Confirmed by GCMS and NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66, 8.65, 7.96, 7.66, 7.30, 7.29, 7.28, 7.08, 6.96, 6.94, 3.90, 3.84, 2.91, 2.83, 1.79, 1.77, 0.01.

^{13}C NMR (101 MHz, $CDCl_3$) δ 195.80, 151.65, 149.92, 137.07, 130.66, 123.68, 99.98, 98.46, 30.92, 26.44.

Compound 62: *2-(3,5-dimethoxybenzylidene)-5-(pyridin-4-ylmethylene)cyclopentane-1-one*

Synthesized with General Procedure B and D. 8.7% yield. Confirmed by GCMS and NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71, 8.70, 7.56, 7.47, 7.43, 7.42, 7.30, 7.28, 6.76, 6.54, 3.87, 3.85, 3.15, 2.18, 2.18, 1.70, 1.27, 0.01.

^{13}C NMR (101 MHz, $CDCl_3$) δ 203.80, 160.89, 150.36, 142.82, 141.70, 137.21, 136.87, 135.13, 130.42, 124.06, 108.88, 101.96, 55.45, 30.90, 26.48.

Compound 63: *2-(2,3-dimethoxybenzylidene)-5-(pyridin-4-ylmethylene)cyclopentane-1-one*

Synthesized with General Procedure B and D. 8.7% yield. Confirmed by GCMS and NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.69, 8.68, 7.98, 7.45, 7.41, 7.40, 7.37, 7.28, 7.18, 7.16, 7.13, 7.11, 7.09, 6.99, 6.97, 3.89, 3.88, 3.88, 3.84, 3.09, 2.17, 2.17, 2.17, 1.94, 1.26, -0.00.

^{13}C NMR (101 MHz, $CDCl_3$) δ 195.70, 153.03, 150.31, 149.49, 142.92, 141.95, 137.51, 130.16, 129.78, 129.57, 124.04, 123.82, 121.56, 113.93, 61.55, 55.89, 30.90, 26.60, 26.49.

Compound 64: *2-(3,4,5-trimethoxybenzylidene)-5-(pyridin-4-ylmethylene)cyclopentane-1-one*

Synthesized with General Procedure B and D. 87% yield. Confirmed by GCMS and NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71, 8.70, 7.58, 7.54, 7.48, 7.43, 7.42, 7.38, 7.28, 6.86, 3.96, 3.93, 3.16, 2.18, 1.67, 1.27, 0.01.

^{13}C NMR (101 MHz, $CDCl_3$) δ 197.16, 170.57, 153.32, 150.37, 141.73, 135.46, 130.97, 130.28, 124.67, 124.04, 108.40, 106.98, 99.99, 61.01, 56.23, 54.40, 26.34.

Compound 65: *2,5-bis(pyridin-3-ylmethylene)cyclopentane-1-one*

Synthesized with General Procedure A. 87% yield. Confirmed by GCMS and NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80, 8.55, 8.47, 7.84, 7.65, 7.52, 7.34, 7.24, 3.11.

^{13}C NMR (101 MHz, $CDCl_3$) δ 200.61, 151.60, 149.86, 138.79, 136.99, 130.56, 123.63, 26.38.

Compound 66: *4-phenyl-2-pyridin-4-ylmethylene)cyclohexan-1-one*

Synthesized with General Procedure D. 69% yield. Confirmed by NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61, 8.47, 7.74, 7.35, 7.33, 7.28, 7.27, 7.25, 7.16, 7.12, 7.10, 6.97, 3.84, 3.79, 3.46, 3.25, 3.22, 3.04, 3.02, 2.48, 2.16, 2.02.

^{13}C NMR (101 MHz, $CDCl_3$) δ 188.50, 149.89, 149.43, 144.55, 143.42, 142.98, 138.16, 135.13, 129.24, 128.94, 128.59, 127.28, 126.71, 126.62, 124.10, 50.49, 40.10, 35.60, 30.91.

Compound 67: *3,4-dimethoxy-N-(3-methoxyphenyl)benzenesulfonamide*

Synthesized with General Procedure F. 32% yield. Confirmed by NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45, 7.45, 7.43, 7.43, 7.28, 7.26, 7.26, 6.93, 6.90, 6.68, 6.68, 6.67, 6.66, 6.53, 6.52, 6.51, 6.51, 5.91, 4.72, 4.71, 4.69, 3.94, 3.88, 3.18, 3.16, 3.15, 3.13, 2.68, 2.66, 2.65, 1.26, 0.00.

^{13}C NMR (101 MHz, $CDCl_3$) δ 152.52, 149.18, 147.84, 146.36, 131.33, 121.70, 121.04, 110.42, 109.44, 108.87, 108.31, 101.00, 56.18, 56.15, 44.33, 35.32.

Compound 68: *3,4-dimethoxy-N-(3-methoxybenzyl)benzenesulfonamide*

Synthesized with General Procedure F. 31% yield. Confirmed by NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38, 7.38, 7.34, 7.33, 7.32, 7.31, 7.28, 7.14, 7.12, 7.10, 6.88, 6.86, 6.73, 6.73, 6.72, 6.69, 6.68, 6.68, 6.68, 6.67, 6.66, 6.66, 6.66, 6.64, 6.64, 6.64, 6.64, 6.62, 6.62, 6.62, 6.62, 4.28, 4.28, 4.27, 4.27, 4.26, 4.26, 4.24, 4.24, 4.24, 4.23, 4.23, 4.22, 4.22, 3.77, 3.74, 2.19, 1.91, 0.02.

^{13}C NMR (101 MHz, $CDCl_3$) δ 160.25, 147.68, 143.48, 137.94, 131.39, 130.01, 121.06, 117.71, 116.96, 113.01, 110.68, 106.53, 64.49, 64.08, 55.31, 30.96.

Compound 69: *N-(3-methoxyphenyl)-2,3-dihydrobenzo[1,4]dioxine-6-sulfonamide*

Synthesized with General Procedure F. 26% yield. Confirmed by NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16, 8.16, 8.15, 8.14, 8.14, 8.13, 7.33, 7.32, 7.31, 7.31, 7.30, 7.30, 7.29, 7.28, 7.27, 7.26, 6.95, 6.93, 5.96, 4.47, 4.46, 4.44, 4.35, 4.34, 4.34, 4.33, 4.33, 4.33, 4.30, 4.30, 4.29, 4.29, 4.28, 4.28, 3.31, 3.29, 3.28, 3.26, 2.94, 2.93, 2.91, 2.06, 1.59, 1.30, 1.28, 1.27, 1.26, 0.02, 0.01.

^{13}C NMR (101 MHz, $CDCl_3$) δ 143.59, 129.70, 123.85, 120.76, 117.76, 116.56, 64.53, 64.17, 43.71, 35.93.

Compound 70: *N-((2,3-dihydrobenzo[1,4]bioxin-6-yl)methyl)-3,4-dimethoxybenzenesulfonamide*

Synthesized with General Procedure F. 31% yield. Confirmed by NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09, 8.08, 8.08, 7.95, 7.95, 7.94, 7.94, 7.93, 7.93, 7.92, 7.92, 7.85, 7.84, 7.84, 7.84, 7.83, 7.82, 7.82, 7.82, 7.49, 7.47, 7.45, 7.34, 7.32, 7.30, 7.28, 7.07, 7.07, 7.07, 7.06, 7.05, 7.05, 7.04, 7.04, 6.65, 6.65, 6.64, 6.64, 6.63, 6.63, 6.62, 6.62, 6.57, 6.56, 6.56, 3.76, 2.06, 1.29, 1.28, 0.02.

^{13}C NMR (101 MHz, $CDCl_3$) δ 160.20, 140.88, 137.19, 134.57, 131.39, 130.55, 129.98, 127.21, 123.54, 122.85, 117.00, 116.56, 55.50.

Compound 71: *3-bromo-N-(3-methoxyphenyl)benzenesulfonamide*

Synthesized with General Procedure F. 8.5% yield. Confirmed by NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46, 7.46, 7.44, 7.43, 7.28, 7.25, 7.25, 7.15, 7.13, 7.11, 7.01, 6.87, 6.85, 6.73, 6.72, 6.72, 6.67, 6.66, 6.66, 6.65, 6.64, 6.64, 3.91, 3.81, 3.75, 2.19, 0.01.

^{13}C NMR (101 MHz, $CDCl_3$) δ 160.30, 152.80, 137.89, 130.01, 121.30, 113.59, 110.86, 110.42, 109.68, 107.28, 56.12, 55.32.

Compound 72: *N-(4-nitrophenethyl)-2,3-dihydrobenzo[1,4]dioxine-6-sulfonamide*

Synthesized with General Procedure F. 7.5% yield. Confirmed by NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49, 7.49, 7.47, 7.47, 7.31, 7.31, 7.28, 7.17, 7.15, 7.13, 6.92, 6.89, 6.77, 6.76, 6.76, 6.76, 6.75, 6.74, 6.74, 6.72, 6.71, 6.71, 5.22, 5.20, 5.18, 4.09, 4.09, 4.08, 3.93, 3.86, 3.84, 3.72, 2.04, 1.26, 1.26, 0.01.

^{13}C NMR (101 MHz, $CDCl_3$) δ 159.75, 152.53, 149.12, 137.89, 131.51, 129.62, 121.11, 120.00, 113.43, 113.22, 110.50, 109.60, 56.17, 55.16, 47.19.

Compound 73: *2,5-bis(pyridin-4-ylmethylene)cyclopentane-1-one*

Made with General Procedure B then A. 54% yield. Confirmed by GCMS and NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.73, 8.73, 8.72, 8.72, 7.52, 7.44, 7.44, 7.43, 7.43, 7.28, 3.19, 0.01.

^{13}C NMR (101 MHz, $CDCl_3$) δ 195.36, 150.45, 142.48, 140.80, 131.58, 124.10, 26.38, -0.01.

Compound 74: *2-(2,3-dimethoxybenzylidene)-5-(furan-3-ylmethylene) cyclopentane-1-one*

Made with General Procedure B then A. 45% yield. Verified by NMR.

^{1}H NMR (400 MHz, Chloroform-d) δ 7.91 (t, J = 2.5 Hz, 1H), 7.61 (d, J = 1.7 Hz, 1H), 7.41 (t, J = 2.4 Hz, 1H), 7.26 – 7.07 (m, 2H), 6.97 (dd, J = 8.1, 1.5 Hz, 1H), 6.75 – 6.69 (m, 1H), 6.55 (dd, J = 3.4, 1.8 Hz, 1H), 3.89 (d, J = 9.6 Hz, 6H), 3.14 – 3.00 (m, 4H).

^{13}C NMR (101 MHz, Chloroform-d) δ 195.84 , 153.01 , 152.74 , 149.31 , 145.03 , 139.08 , 135.26 , 130.23 , 127.86 , 123.75 , 121.58 , 120.07 , 116.01 , 113.46 , 112.62 , 61.52 , 55.89 , 26.23 (d, J = 7.7 Hz).

Compound 75: *2-(benzylidene)-5-(pyridin-4-ylmethylene)cyclopentane-1-one*

Made with General Procedure C then A. 7.2% yield. Confirmed by GCMS and NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63, 7.61, 7.60, 7.58, 7.47, 7.46, 7.44, 7.43, 7.41, 7.40, 7.38, 7.31, 7.28, 7.24, 7.20, 7.19, 7.17, 7.09, 7.07, 7.07, 7.07, 7.05, 7.04, 3.13, 3.13, 3.12, 3.11, 2.19, 2.19, 2.18, 0.02, 0.01.

^{13}C NMR (101 MHz, $CDCl_3$) δ 196.31, 158.77, 156.09, 137.44, 135.96, 135.87, 133.65, 133.36, 132.54, 130.72, 129.95, 129.33, 128.76, 124.16, 119.76, 118.30, 76.71, 30.93, 26.49.

Compound 76: *2-(4-(benzyloxy)benzylidene)-6-(2,3-dimethoxybenzylidene)cyclohexan-1-one*

Made with General Procedure C then A. 27% yield. Confirmed by GCMS and NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95, 7.94, 7.80, 7.79, 7.48, 7.47, 7.46, 7.41, 7.39, 7.37, 7.37, 7.28, 7.28, 7.27, 7.19, 7.19, 7.17, 7.15, 7.09, 7.09, 7.07, 7.07, 7.04, 7.03, 7.02, 7.02, 6.95, 6.93, 6.93, 3.91, 3.90, 3.84, 3.84, 3.83, 3.83, 2.96, 2.94, 2.93, 2.83, 2.82, 2.80, 1.85, 1.83, 1.82, 1.81, 1.79, 1.78, 0.02, 0.02, 0.02, 0.01.

^{13}C NMR (101 MHz, $CDCl_3$) δ 190.16, 157.96, 156.34, 152.87, 148.30, 137.50, 136.60, 136.30, 135.20, 132.25, 132.20, 130.79, 129.89, 123.94, 123.46, 122.15, 119.58, 118.09, 112.73, 61.13, 55.87, 28.74, 28.49, 23.16.

Compound 77: *2-(2,3-dihydrobenzo[1,4]dioxin-6-yl)methylene)-6-(2,3-dimethoxybenzylidene) cyclohexan-1-one*

Made with General Procedure C then A. 34% yield. Confirmed by GCMS and NMR.

^{1}H NMR (400 MHz, DMSO) δ 7.76, 7.72, 7.52, 7.49, 7.44, 7.42, 7.38, 7.29, 7.27, 7.22, 7.13, 7.11, 7.07, 7.06, 7.05, 7.03, 7.01, 6.99, 6.97, 6.94, 6.94, 6.93, 6.79, 6.74, 6.69, 4.35, 4.29, 4.27, 4.20, 4.10, 3.90, 3.87, 3.83, 3.82, 3.79, 3.77, 3.72, 3.72, 3.71, 3.71, 3.69, 3.67, 3.65, 3.64, 3.61, 3.52, 3.50, 3.45, 3.43, 3.33, 3.17, 2.86, 2.85, 2.80, 2.78, 2.77, 2.74, 2.51, 2.50, 2.50, 2.50, 2.49, 2.24, 2.09, 2.08, 2.08, 1.92, 1.83, 1.70, 1.68, 1.52, 1.23, 1.14, 1.06, 0.85, -0.01.

^{13}C NMR (101 MHz, DMSO) δ 206.92, 152.95, 148.26, 144.80, 137.48, 136.40, 135.17, 131.67, 131.67, 131.08, 129.77, 124.80, 124.21, 122.11, 119.48, 117.65, 114.22, 64.81, 64.45, 60.97, 56.23, 49.05, 31.13, 28.41, 28.19.

Compound 78: *(2,6)-4-phenyl-2,6-bis(pyridin-4-ylmethylene)cyclohexan-1-one*

Synthesized with General Procedure C and D. 15% yield. Confirmed by LCMS and NMR.

Compound 79: *N-(3-methoxybenzyl)-3-(4-methoxyphenyl)acrylamide*

Made with General Procedure G. 65% yield. Confirmed by NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58, 7.55, 7.34, 7.32, 7.18, 7.16, 7.14, 7.13, 7.12, 6.86, 6.84, 6.82, 6.81, 6.77, 6.75, 6.73, 6.73, 6.46, 6.42, 5.25, 4.44, 4.43, 4.11, 4.09, 4.07, 4.06, 3.74, 3.69, 2.00, 1.25, 1.23, 1.21, 1.18, 1.17.

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.22, 166.58, 160.76, 159.77, 140.52, 140.14, 129.59, 129.34, 127.57, 119.94, 118.53, 114.15, 113.24, 112.76, 60.40, 55.24, 55.09, 53.52, 43.55, 20.99, 14.17.

Compound 80: *3,5-dimethoxy-N-(4nitrobenzyl)benzamide*

Made with General Procedure G. 58% yield. Confirmed by NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20, 8.17, 7.42, 7.40, 7.28, 7.26, 6.82, 6.70, 6.58, 6.22, 3.90, 3.85, 3.82, 3.77, 3.75, 3.73, 3.72, 3.08, 3.07, 3.05, 0.01.

^{13}C NMR (101 MHz, $CDCl_3$) δ 160.95, 146.66, 129.67, 123.89, 107.64, 106.49, 104.80, 103.54, 55.57, 40.82, 35.64.

Compound 81: *3,5-dimethoxy-N-(3-methoxybenzyl)benzamine*

Made with General Procedure G. 85% yield. Confirmed by NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28, 7.24, 7.22, 7.20, 7.00, 6.94, 6.93, 6.90, 6.88, 6.85, 6.85, 6.84, 6.81, 6.80, 6.79, 6.79, 6.78, 6.55, 6.54, 6.54, 5.28, 4.56, 4.54, 4.53, 3.93, 3.80, 3.78, 3.77, 3.77, 3.75, 3.73, 3.70, 3.57, 2.17, 2.16, 2.10, 2.02, 1.25, 0.01.

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.32, 160.82, 159.83, 139.83, 136.53, 129.70, 120.00, 113.40, 112.87, 104.98, 103.63, 55.48, 55.17, 44.00.

Compound 82: *N-(2,6-dichlorophenethyl)-3,5-dimethoxybenzamide*

Made with General Procedure G. 92% yield. Confirmed by NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26, 7.24, 7.08, 7.06, 7.06, 7.04, 6.89, 6.88, 6.71, 6.69, 6.68, 6.53, 6.52, 6.51, 3.79, 3.75, 3.74, 3.73, 3.71, 3.70, 3.68, 3.29, 3.27, 3.25.

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.48, 160.74, 136.71, 135.73, 134.96, 128.35, 128.31, 104.80, 103.69, 55.46, 38.58, 30.86.

Compound 84: *1-(azidomethyl)-3-methoxybenzene*

Made with General Procedure H. 99% yield. Confirmed by NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36, 7.34, 7.32, 6.95, 6.95, 6.95, 6.94, 6.94, 6.94, 6.93, 6.92, 6.91, 4.34, 3.85.

^{13}C NMR (101 MHz, $CDCl_3$) δ 159.99, 136.94, 129.91, 120.42, 113.85, 113.70, 55.24, 54.74.

Compound 85: *1-(3-methoxybenzyl)-4-(3-methoxyphenyl)-1H-1,2,3-triazole*

Made with General Procedure H. 99% yield. Confirmed by NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72, 7.41, 7.40, 7.40, 7.40, 7.31, 7.30, 7.30, 7.29, 7.29, 7.28, 7.25, 7.23, 7.21, 7.21, 7.19, 6.83, 6.83, 6.82, 6.82, 6.81, 6.81, 6.81, 6.80, 6.80, 6.79, 6.79, 6.78, 5.44, 3.76, 3.69, 2.84, 2.79, 2.79.

^{13}C NMR (101 MHz, $CDCl_3$) δ 160.04, 159.99, 147.89, 136.23, 131.87, 130.16, 129.86, 120.15, 120.09, 118.07, 114.14, 114.06, 113.64, 110.73, 55.25, 55.23, 53.99.

Compound 86: *Methyl 4-(azidomethyl)-3-methoxybenzoate*

Made with General Procedure H. 79% yield. Confirmed by NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61, 7.61, 7.59, 7.59, 7.52, 7.52, 7.28, 7.26, 4.35, 3.87, 3.86.

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.52, 157.22, 131.36, 129.35, 129.03, 121.97, 111.08, 55.52, 52.14, 49.70.

Compound 87: *4-(azidomethyl)pyridine*

Made with General Procedure H. 97% yield. Confirmed by NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53, 8.53, 8.52, 8.52, 7.17, 7.17, 7.16, 7.16, 7.16, 4.32.

^{13}C NMR (101 MHz, $CDCl_3$) δ 150.08, 144.43, 122.32, 53.14.

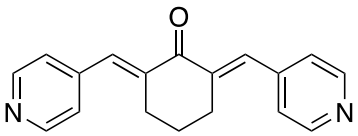
Compound 88: *5-ethynyl-1,2,3-trimethoxy benzene*

Made with General Procedure I. 91.10% yield. Confirmed by GCMS and NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36, 7.36, 7.34, 7.32, 7.32, 6.96, 6.95, 6.95, 6.95, 6.95, 6.94, 6.94, 6.94, 6.93, 6.92, 6.91, 4.34, 3.85.

^{13}C NMR (101 MHz, $CDCl_3$) δ 160.00, 136.96, 129.91, 120.42, 113.85, 113.71, 55.23, 54.73.

Compound 47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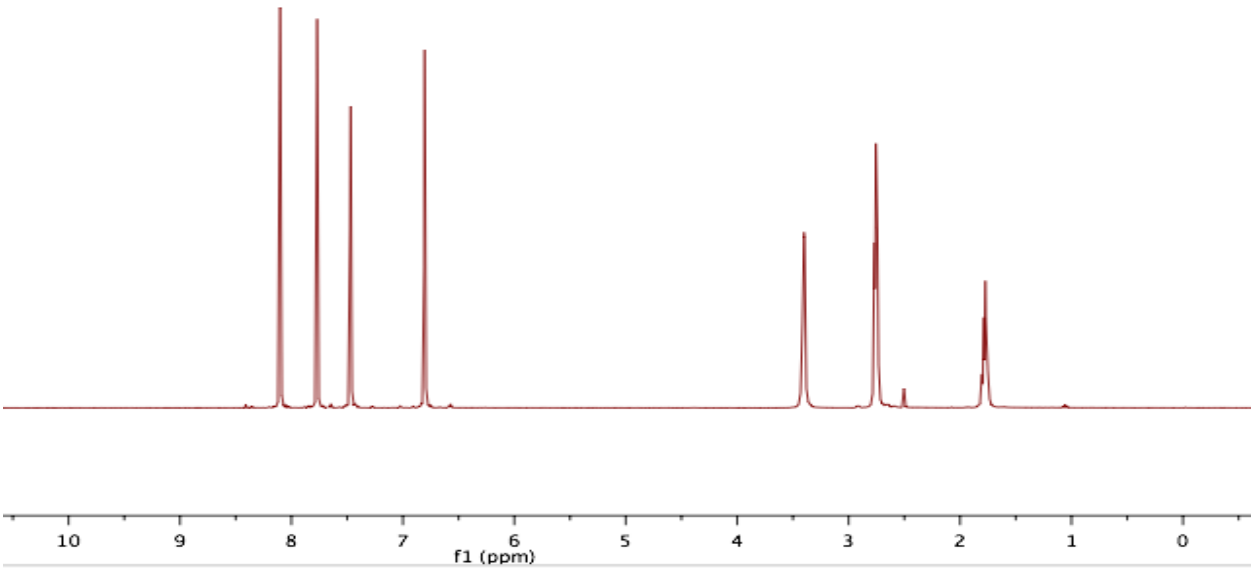
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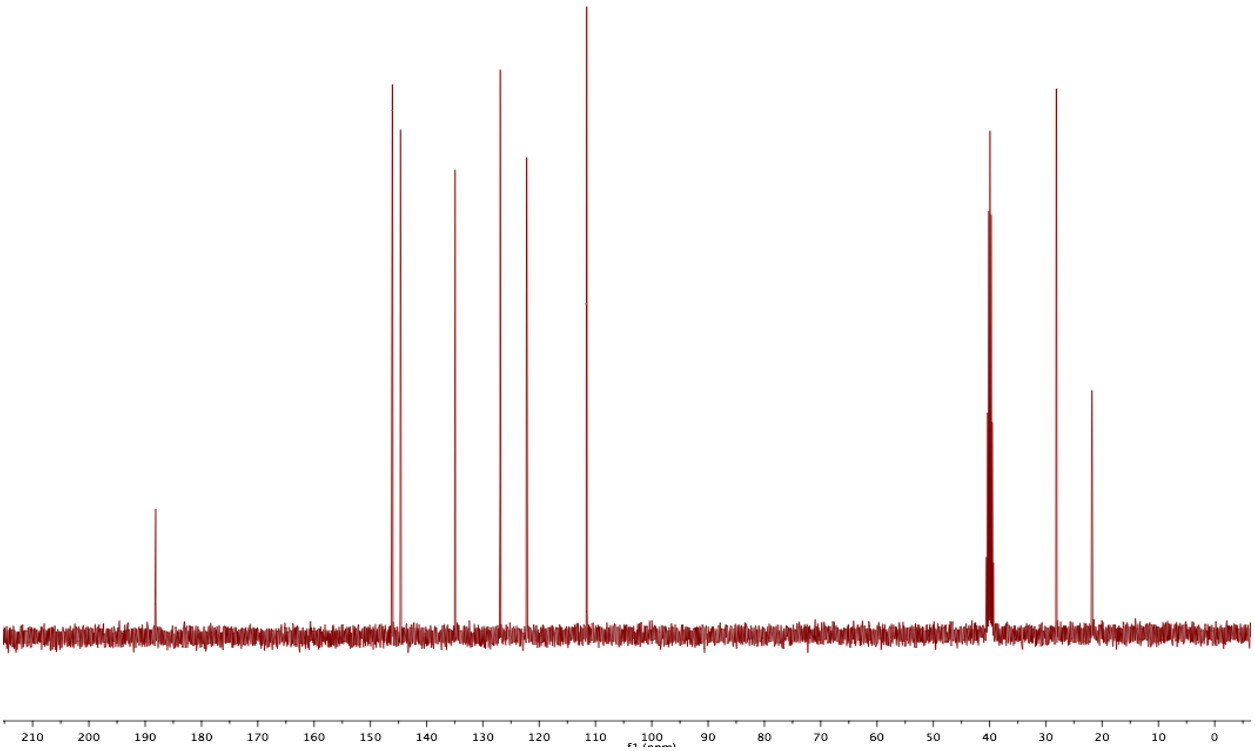
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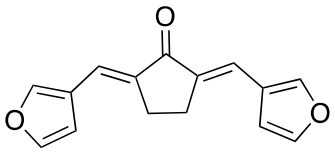
Compound 49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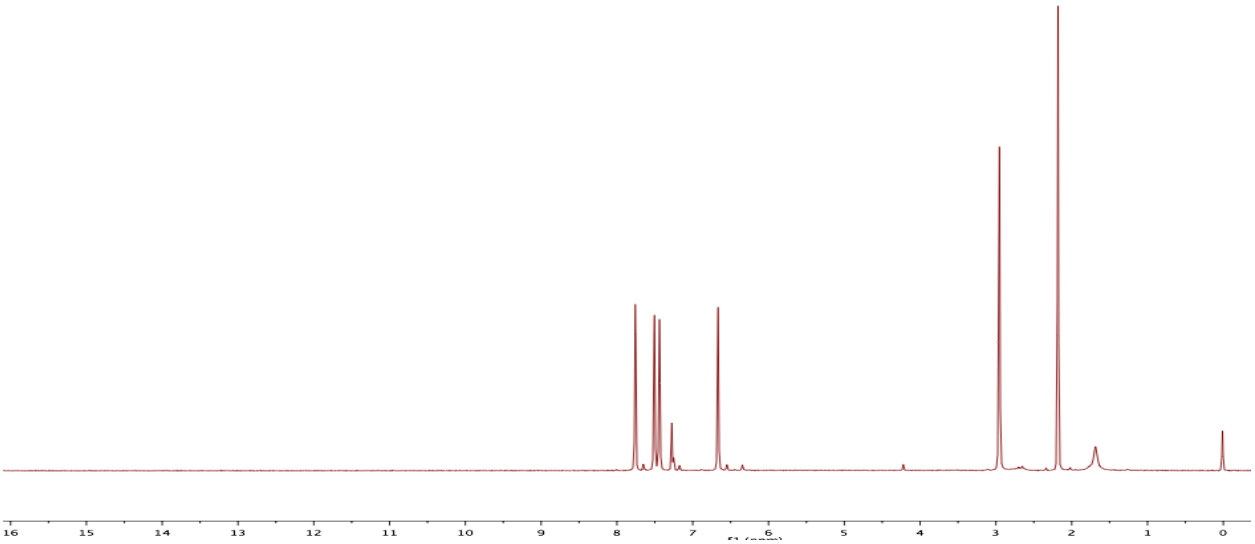
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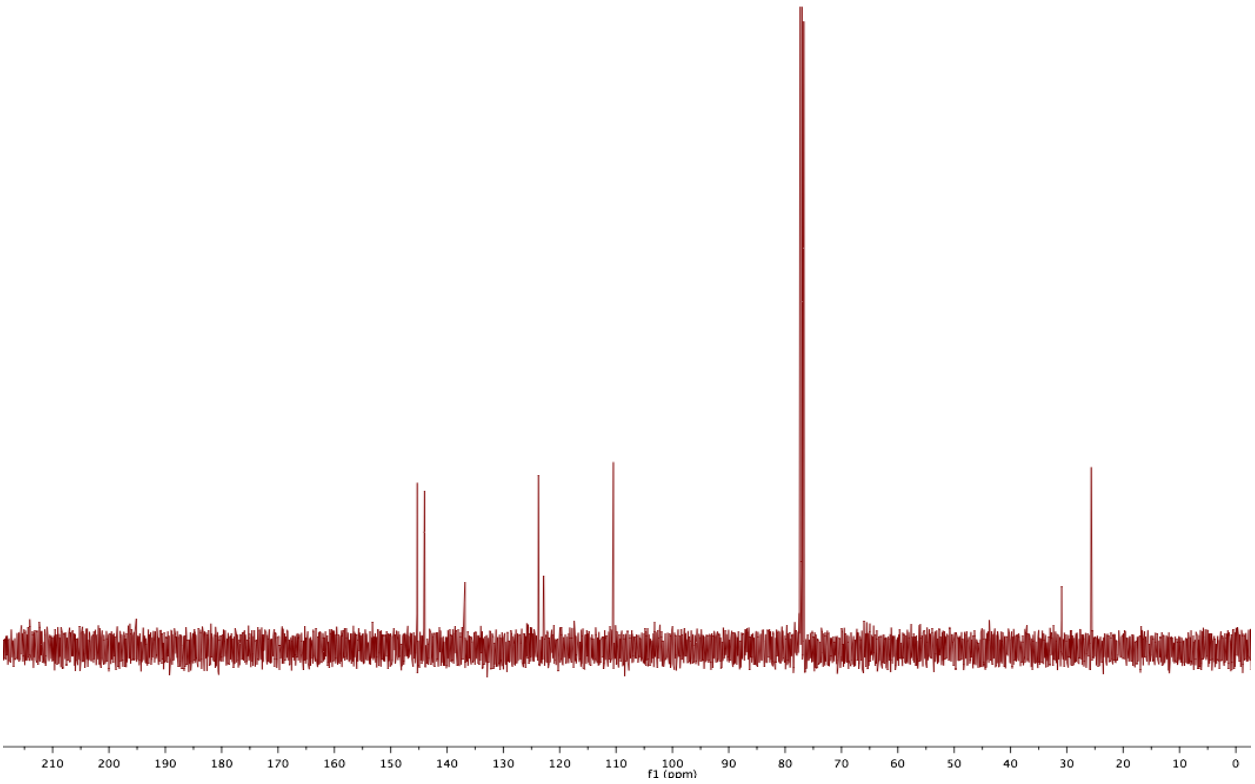
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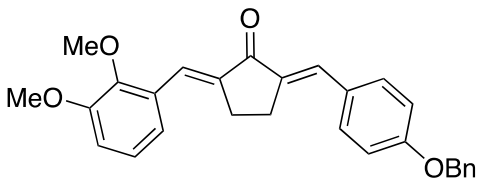
Compound 50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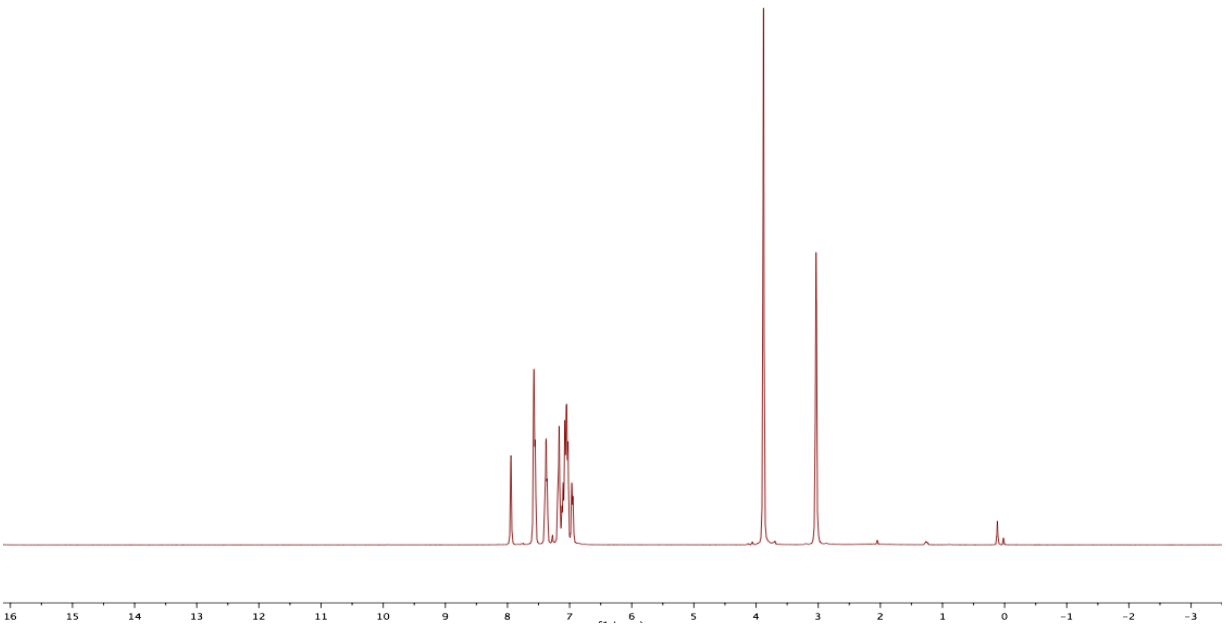
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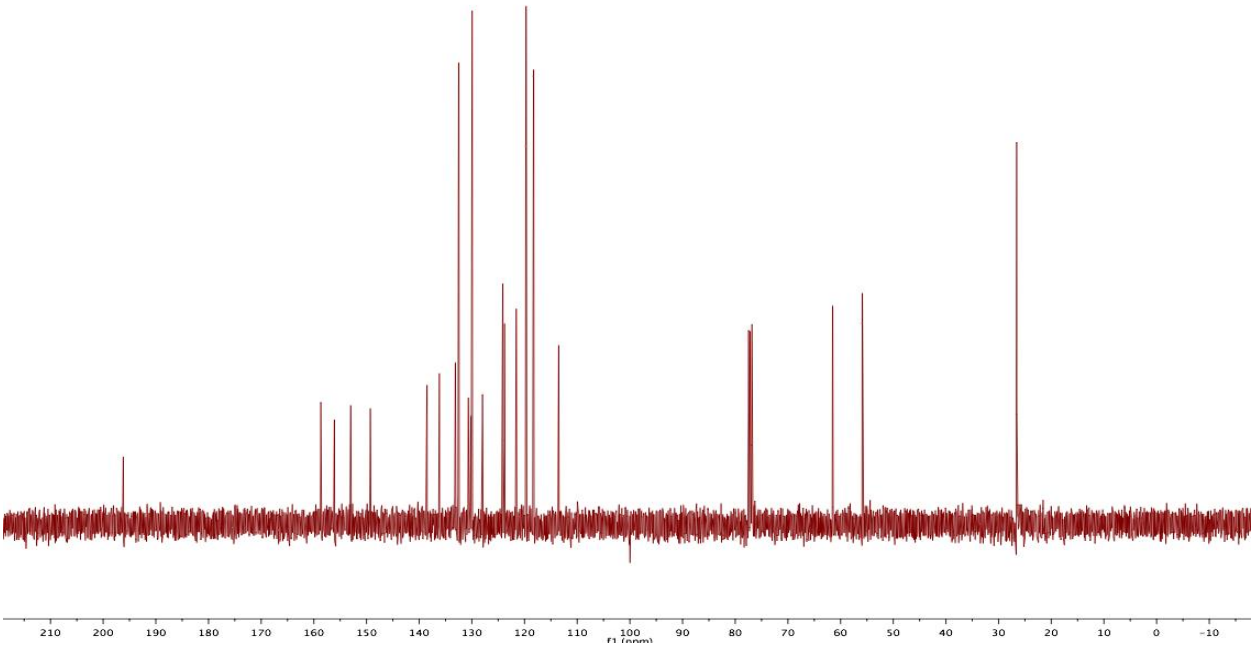
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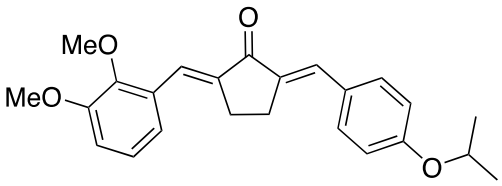
−10

Compound 51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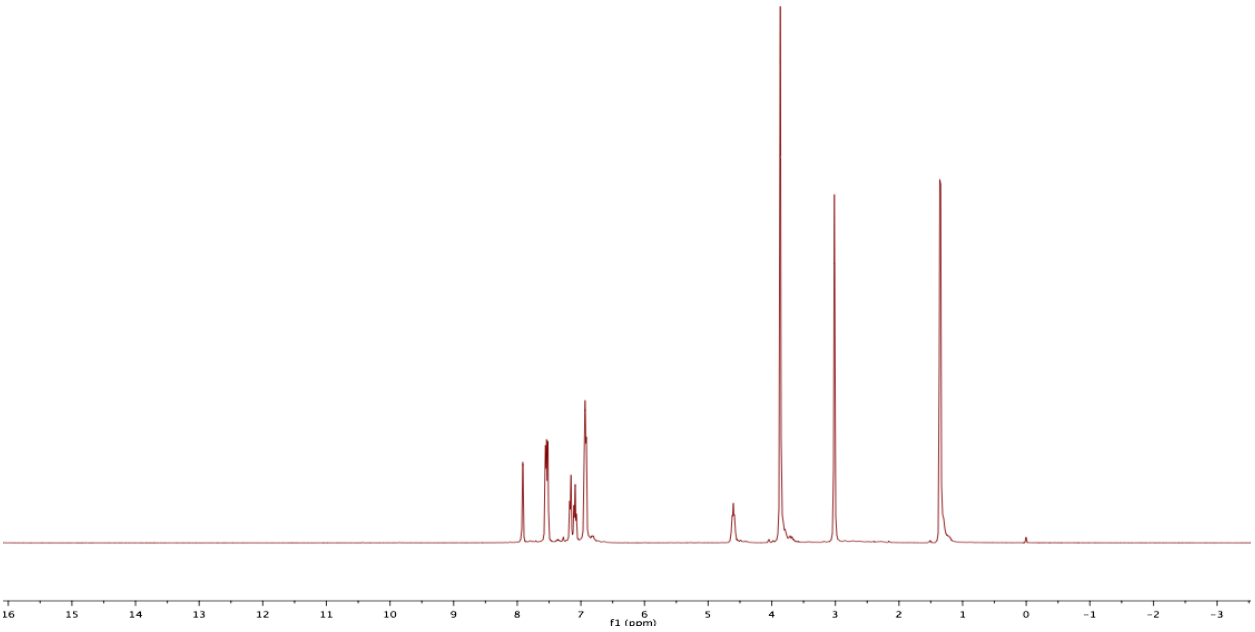
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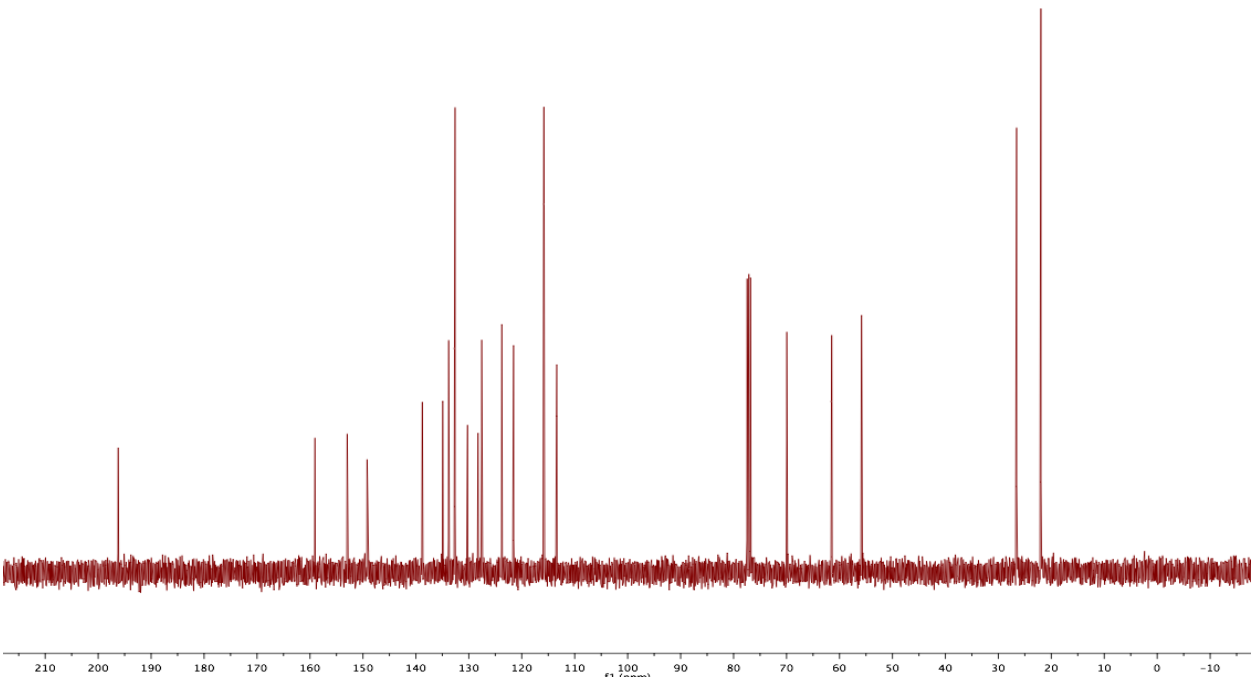
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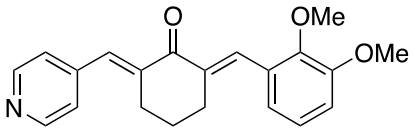
Compound 52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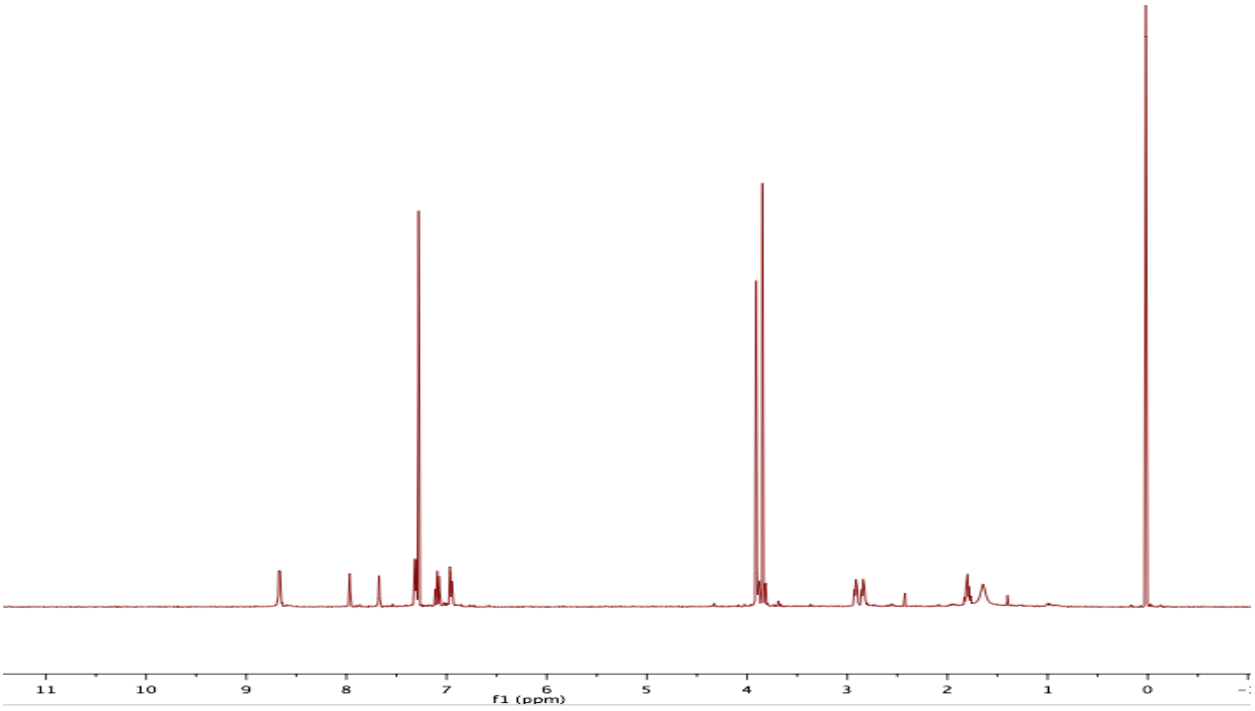
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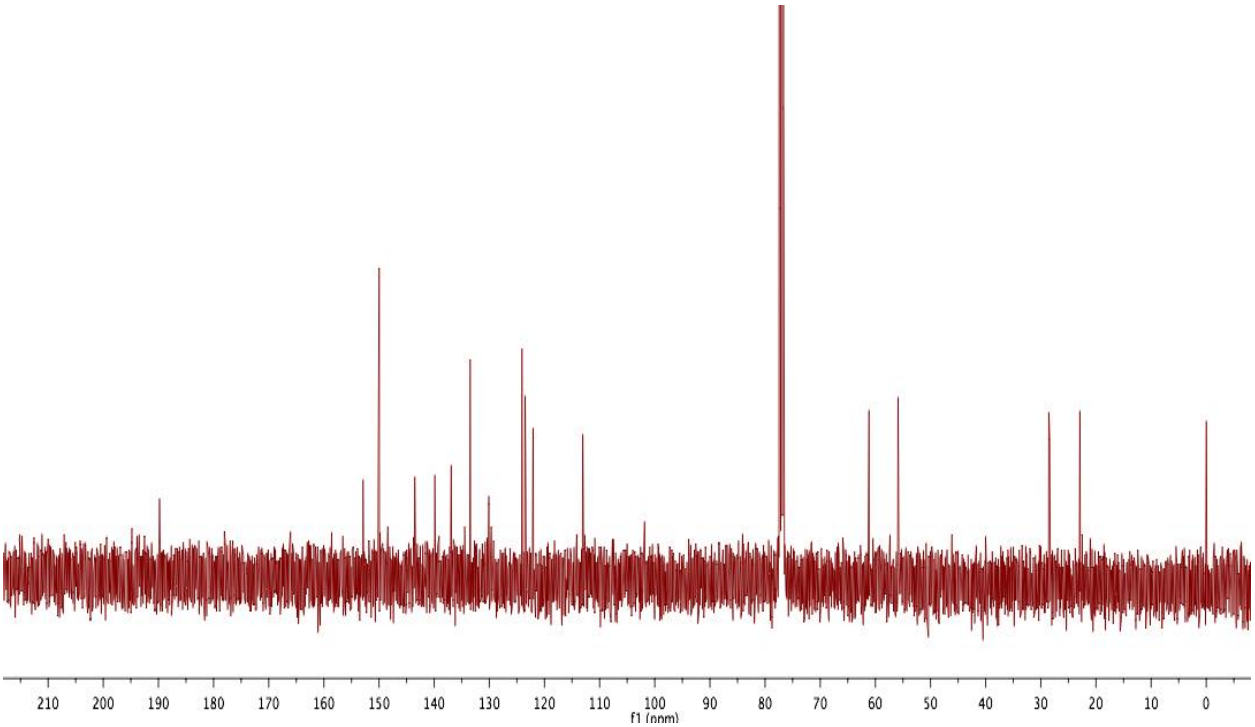
f1 (ppm)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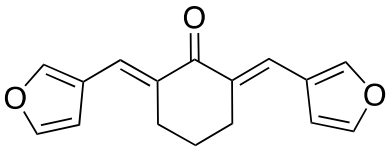
f1 (ppm)

Compound 53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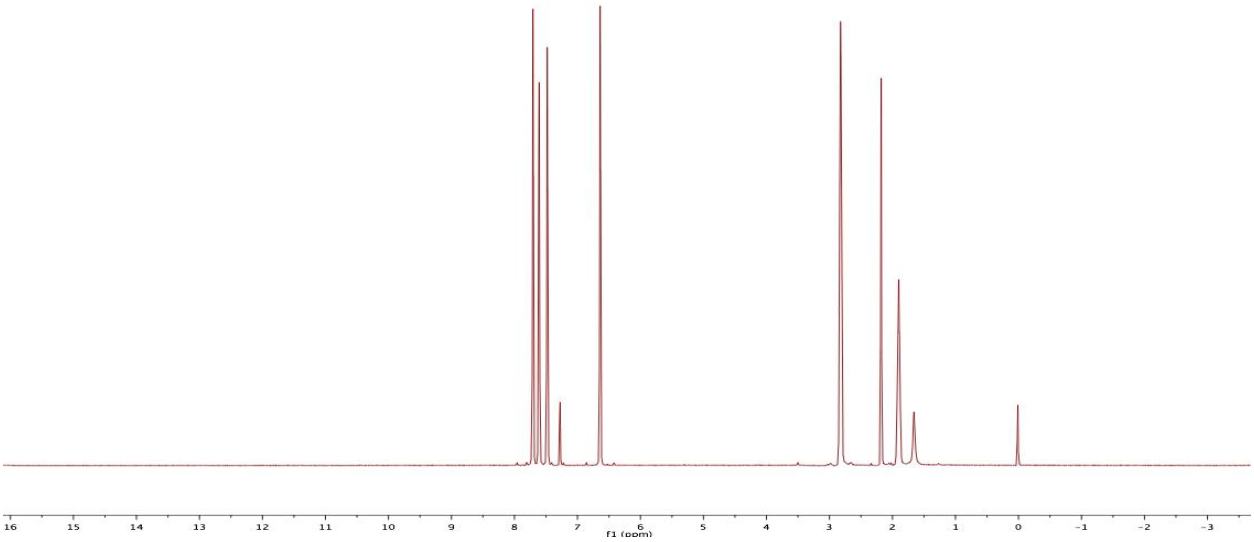
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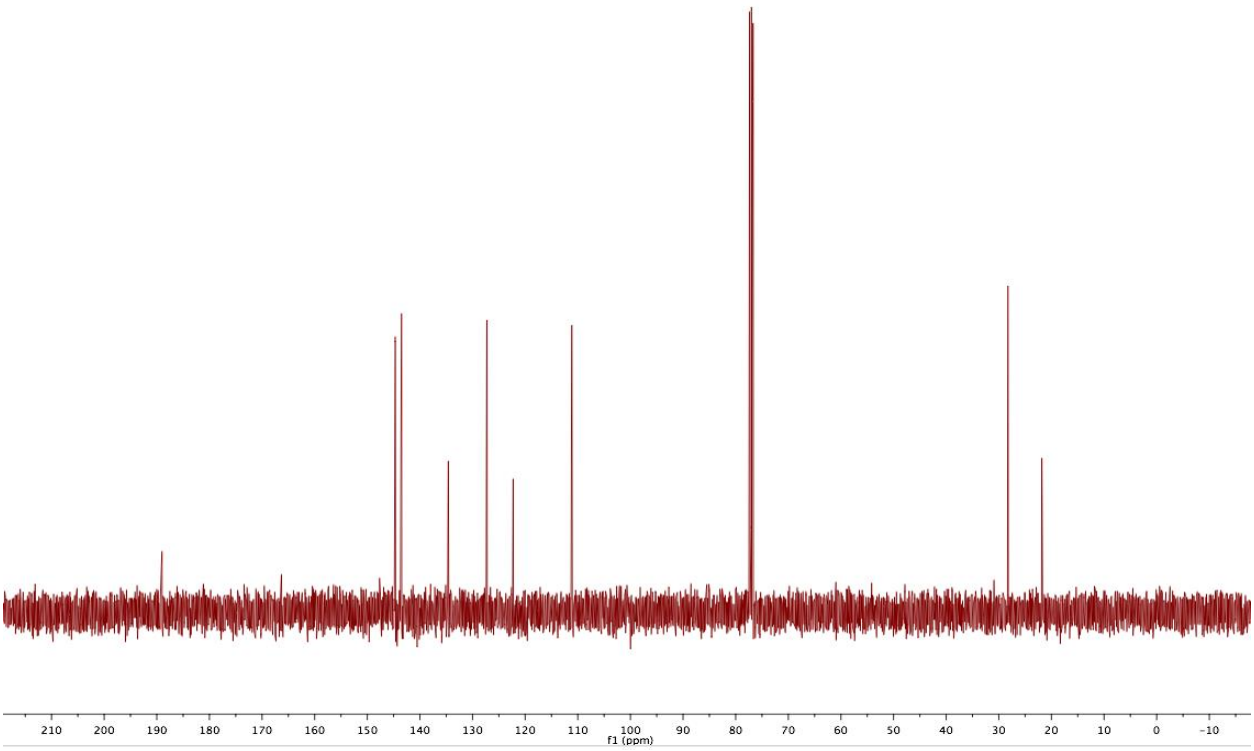
−3
f1 (ppm)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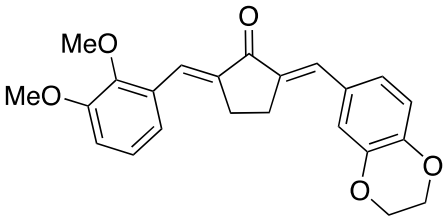
−10
f1 (ppm)

Compound 54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MeO
O
O
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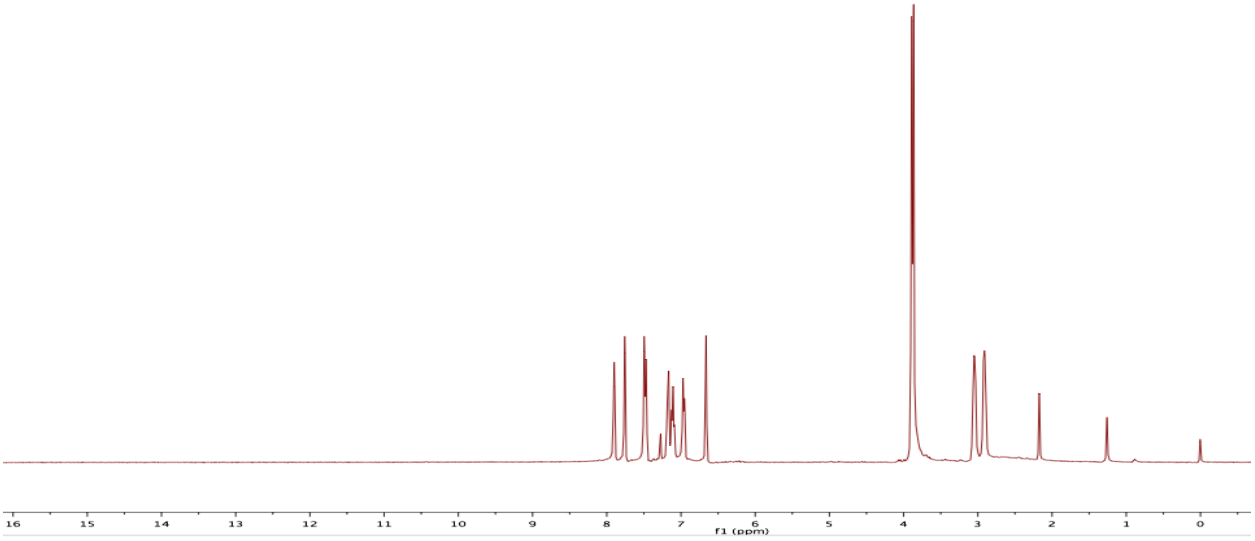
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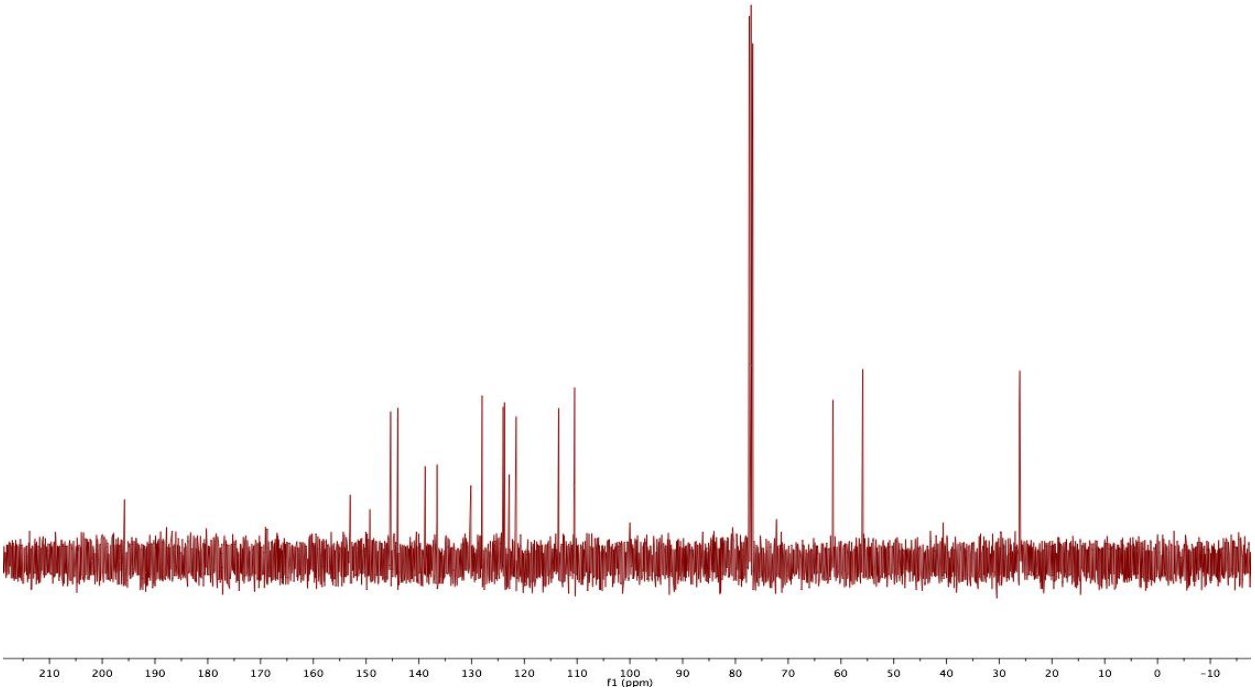
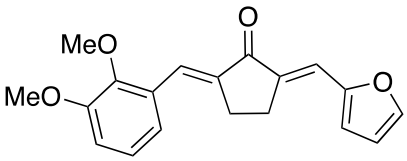
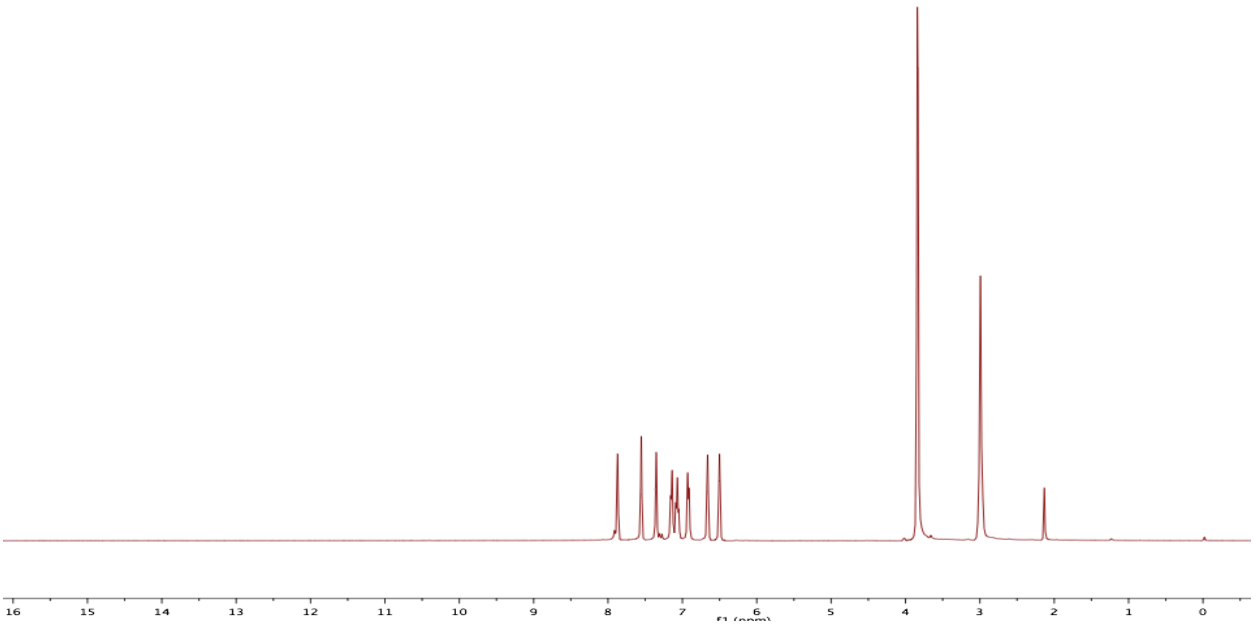
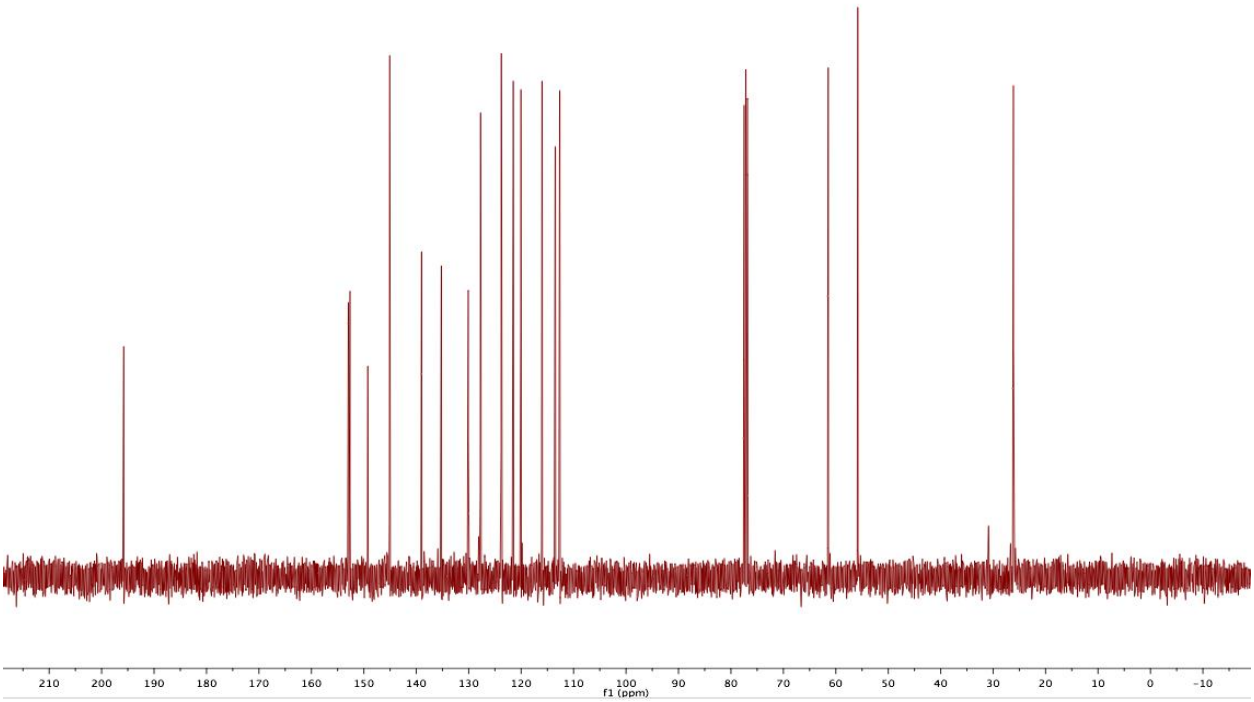
f1 (ppm)


210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

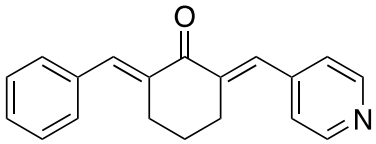
Compound 55

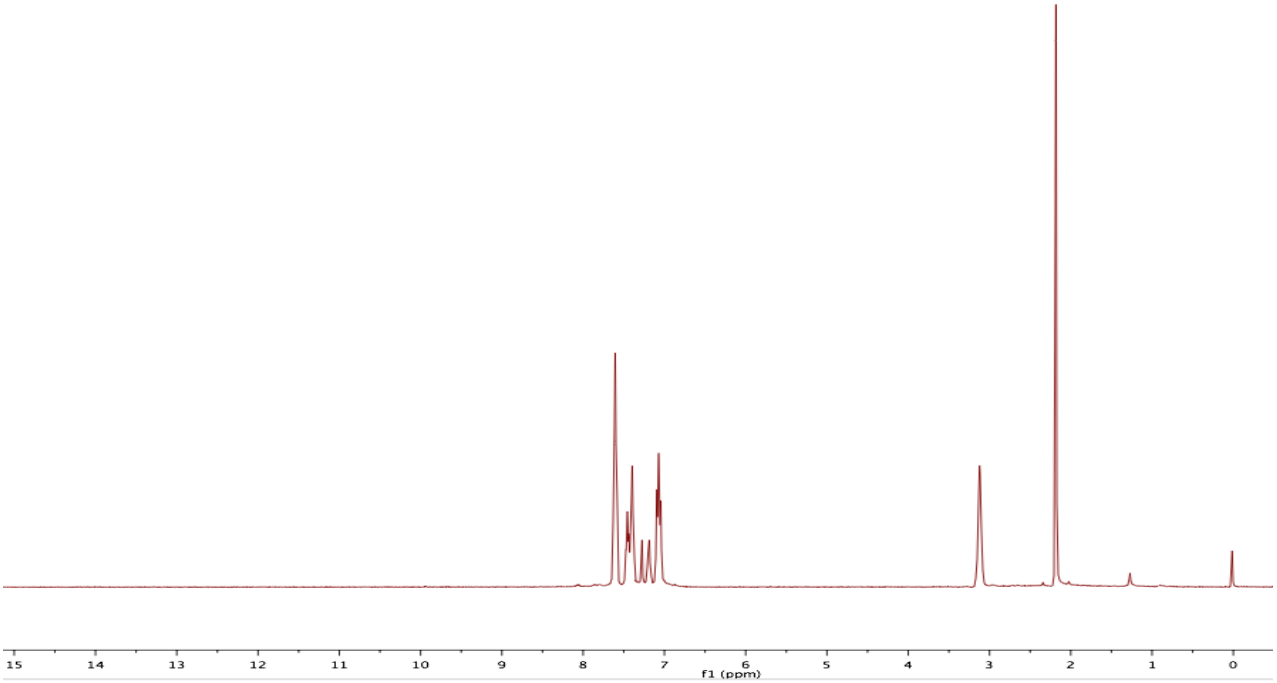

MeO
MeO
O
O

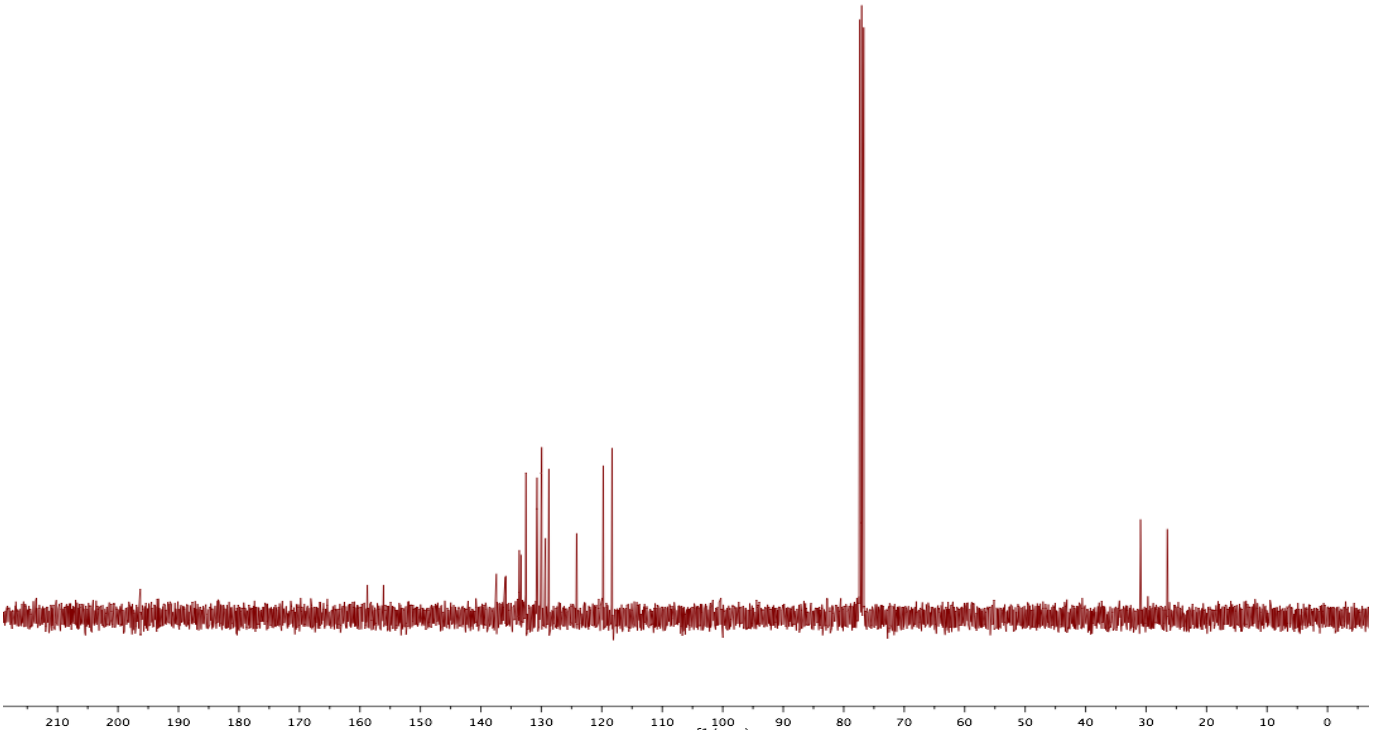

16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0


210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

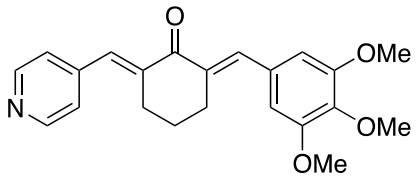
Compound 59

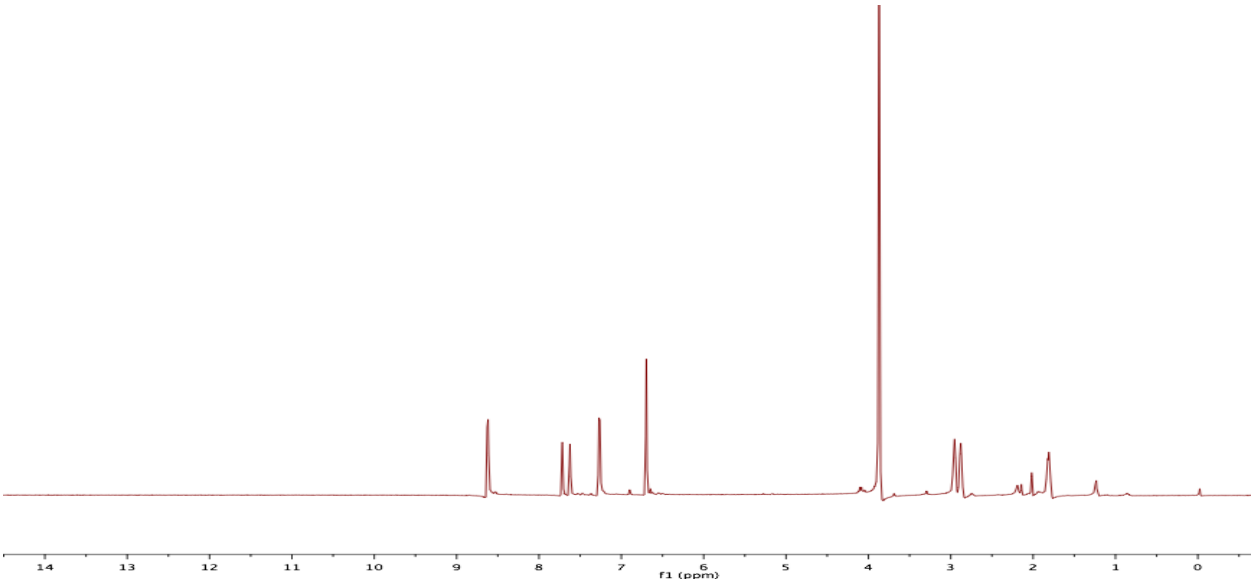

O
N

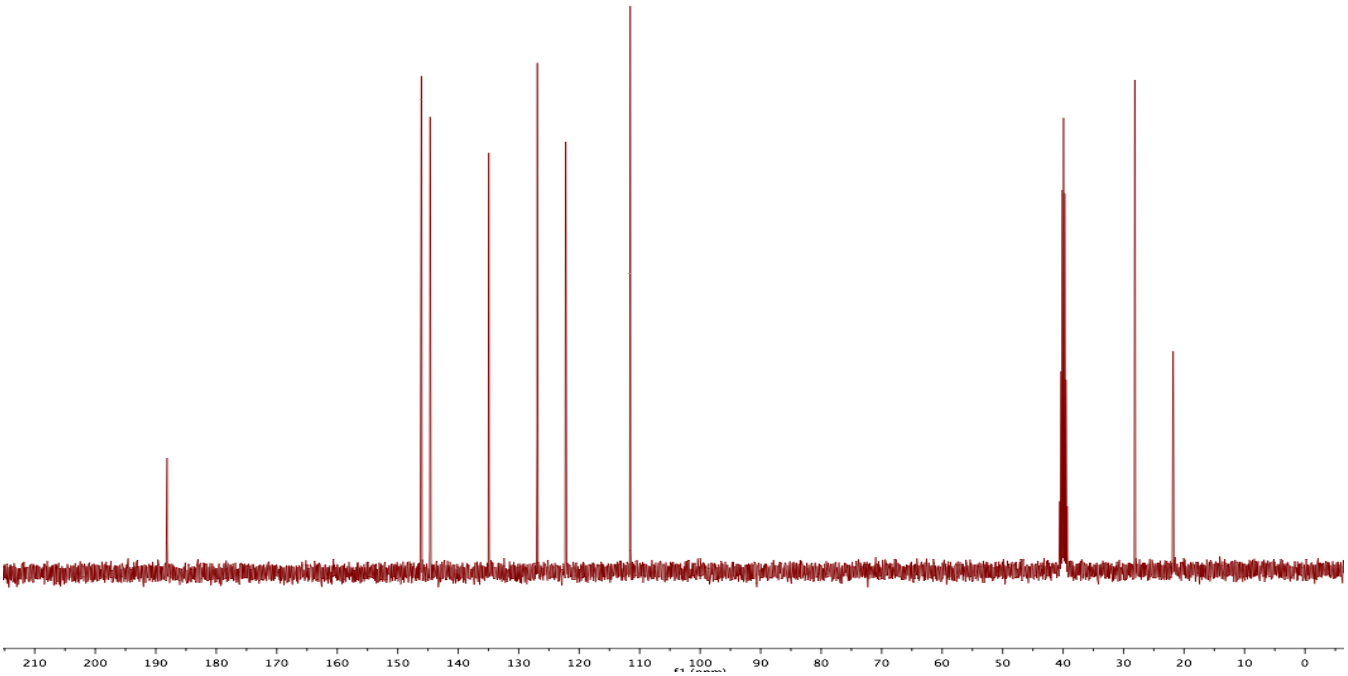

15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)


210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

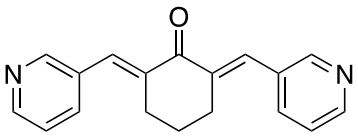
Compound 60

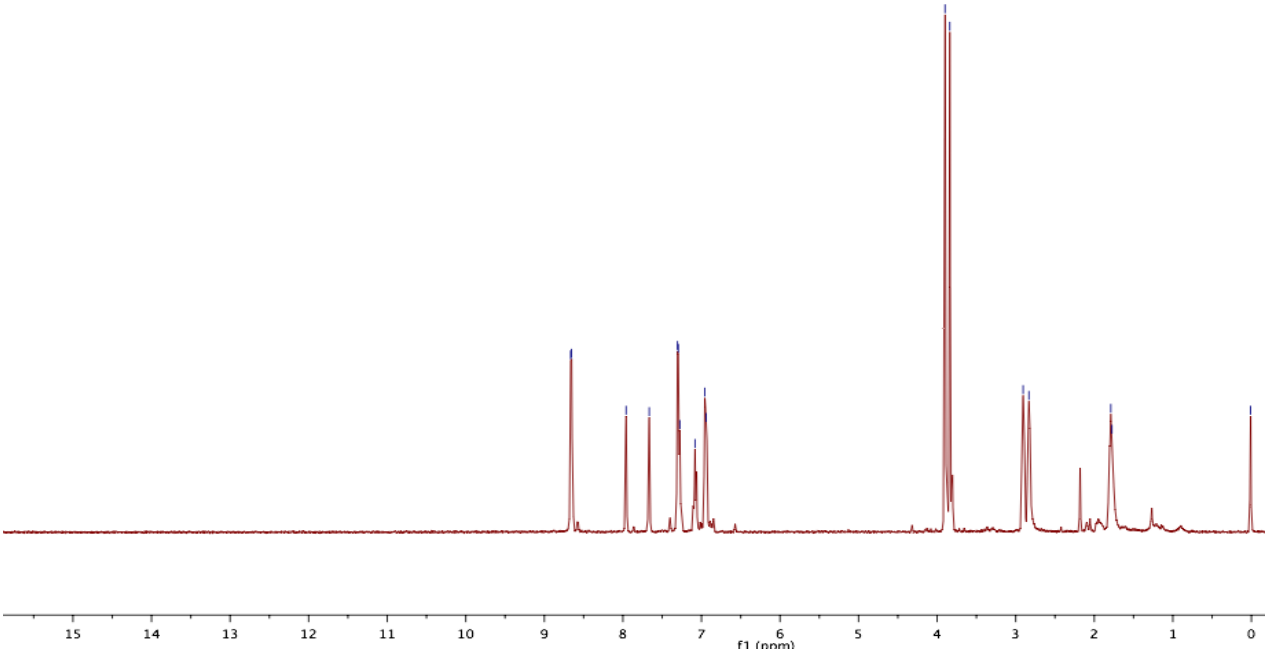

O
N
OMe
OMe
OMe

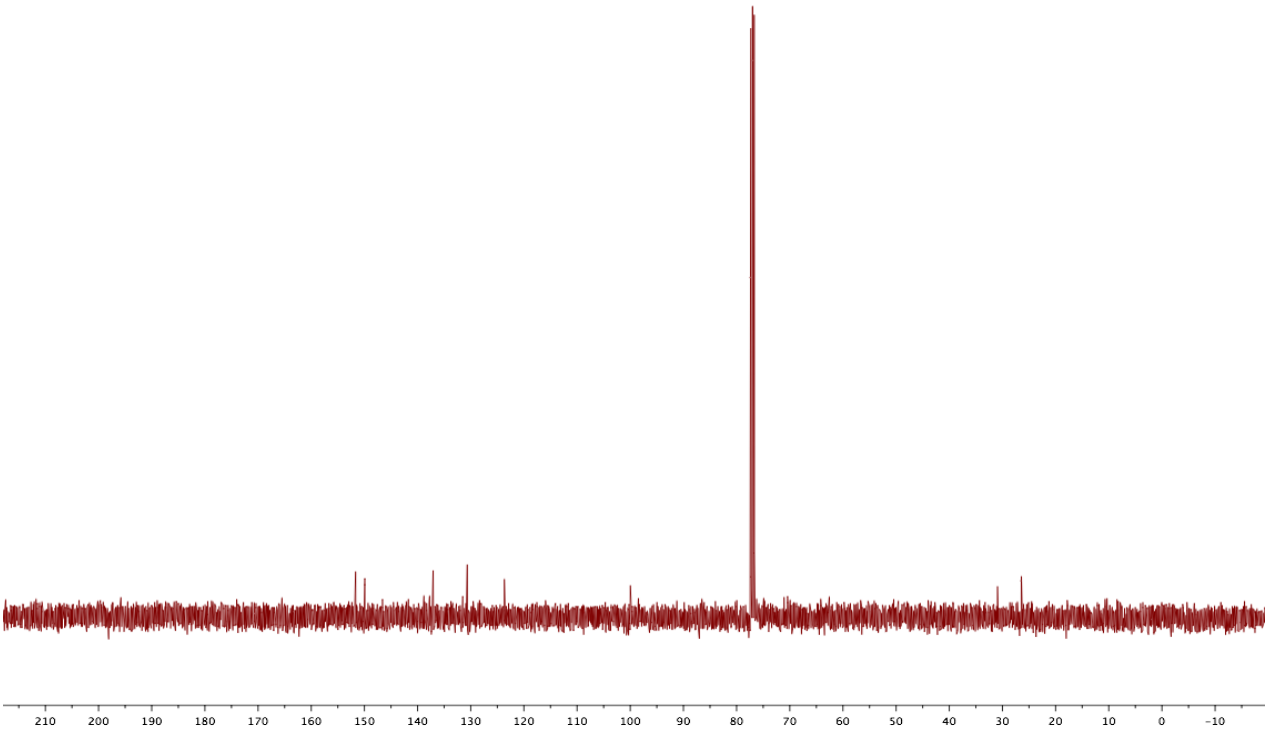

14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)


210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

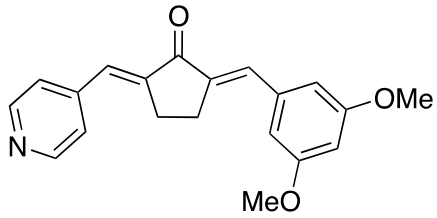
Compound 61 181/95

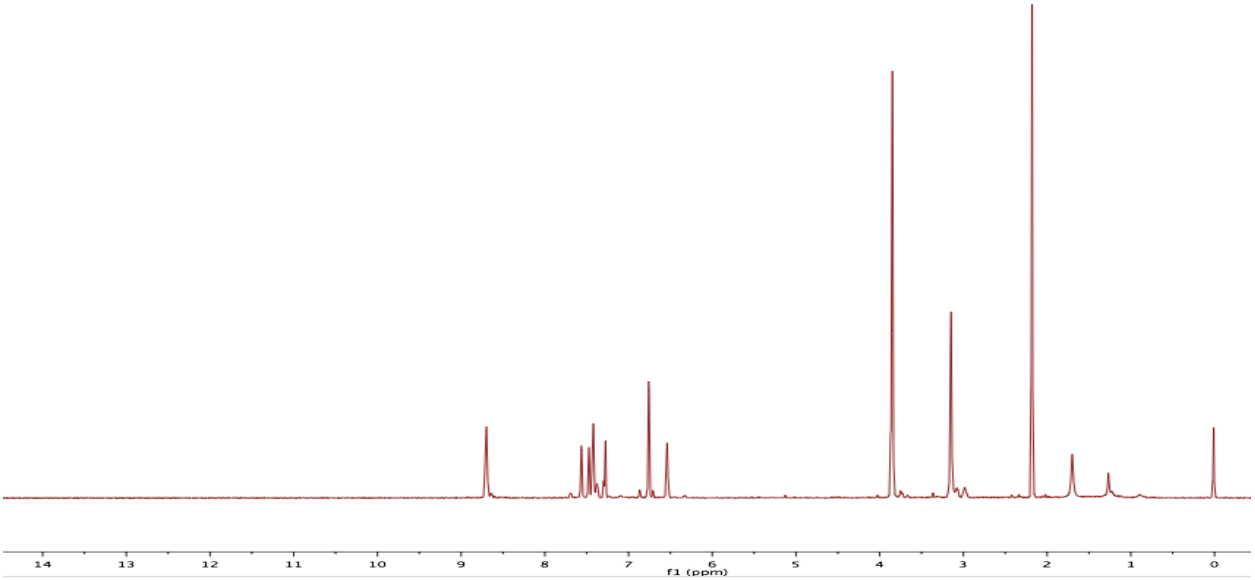


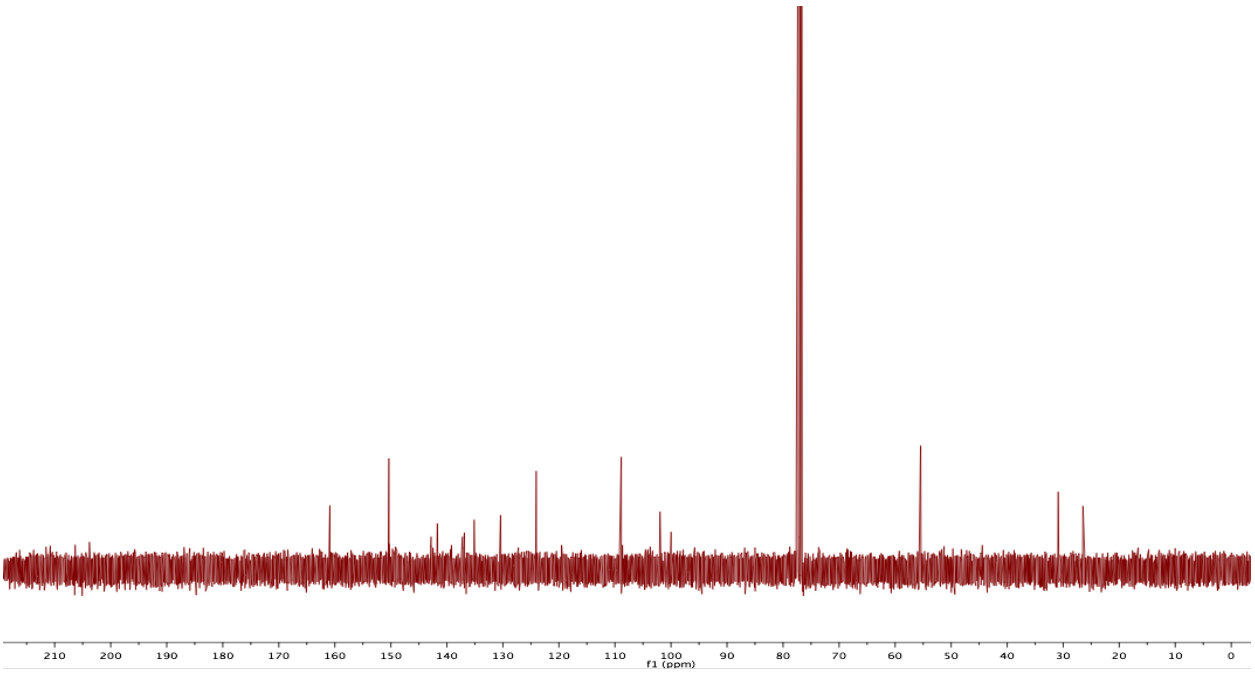




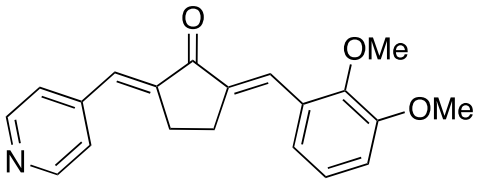
Compound 62

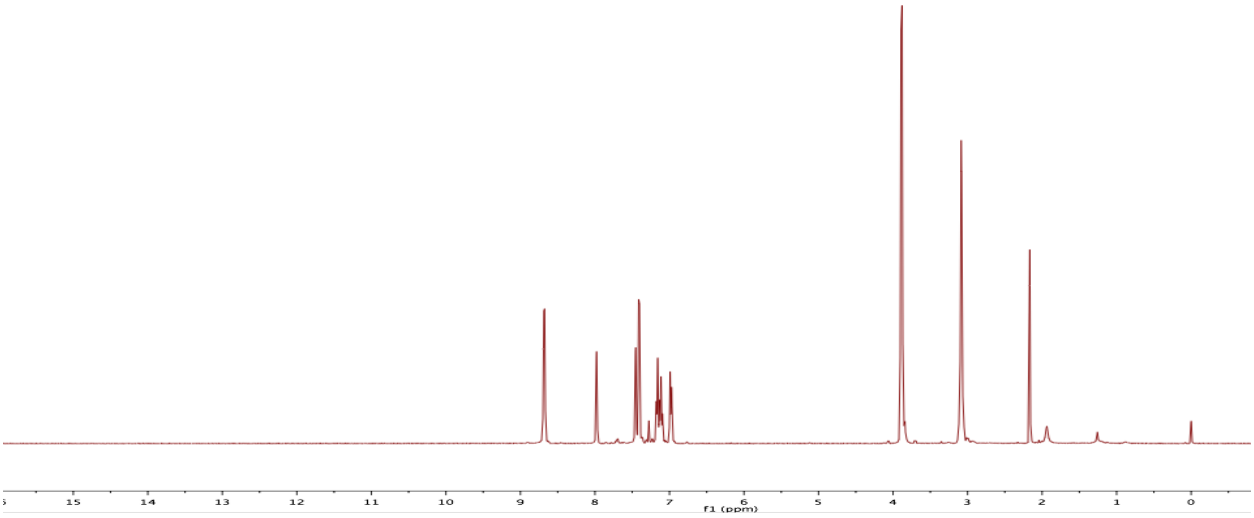

O
OMe
N
MeO

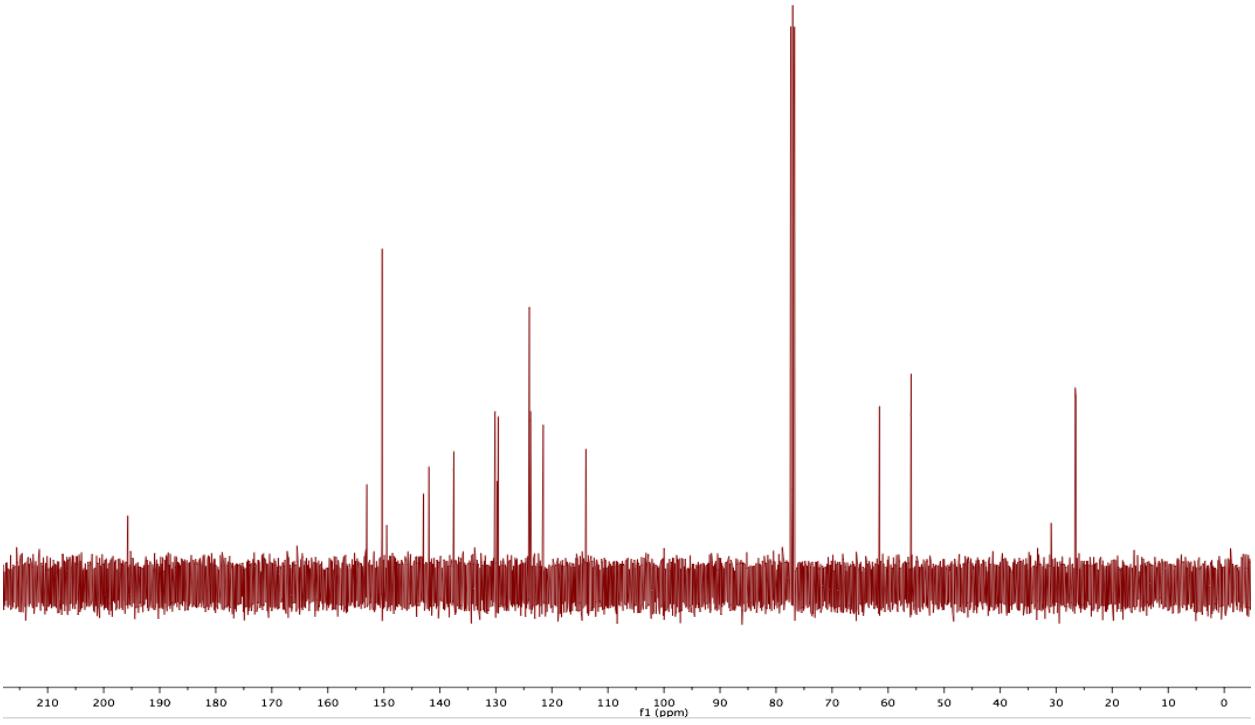

14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)


210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

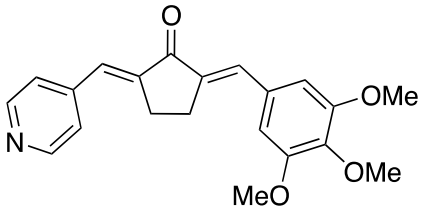
Compound 63

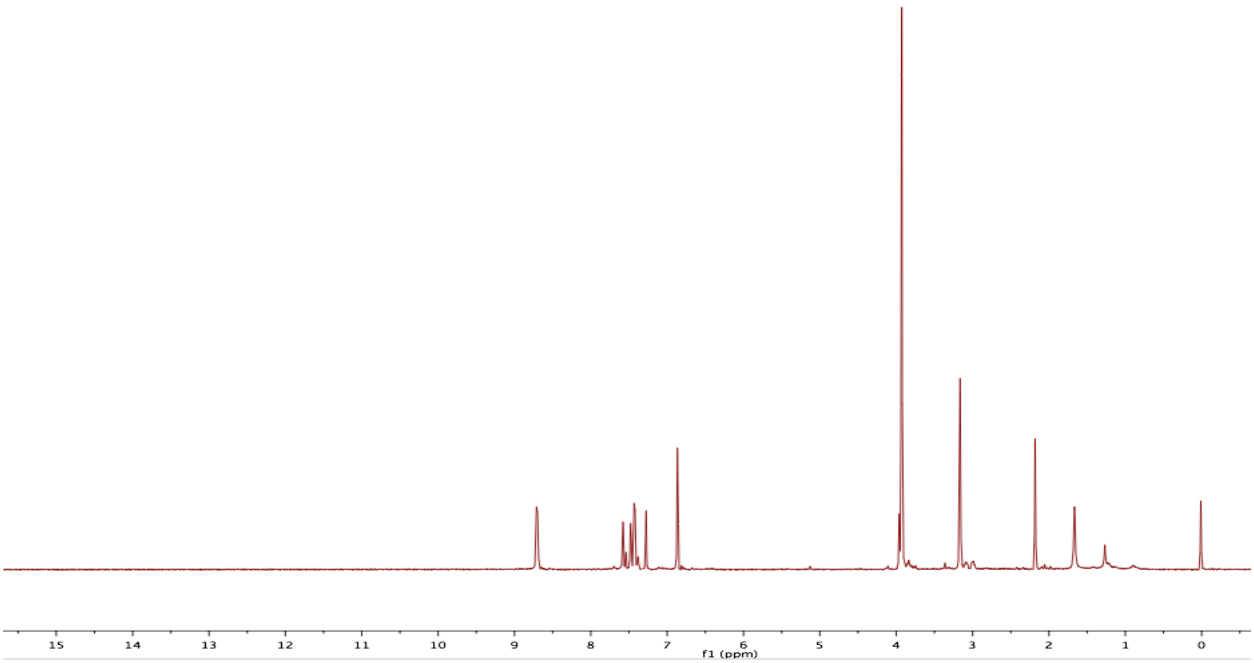

O
OMe
OMe
N

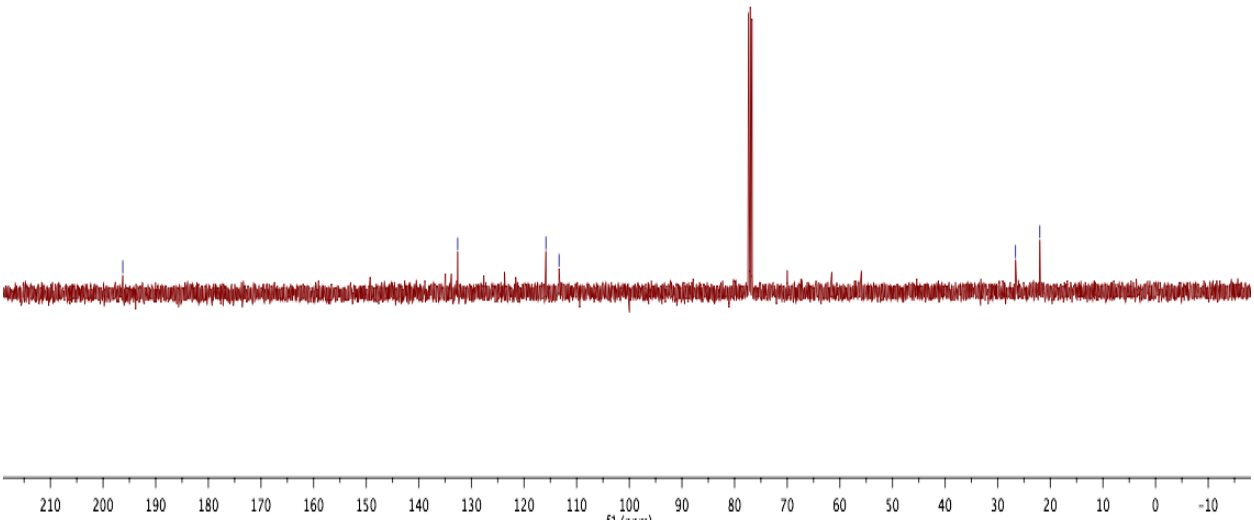

15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)


210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

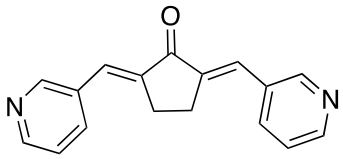
Compound 64

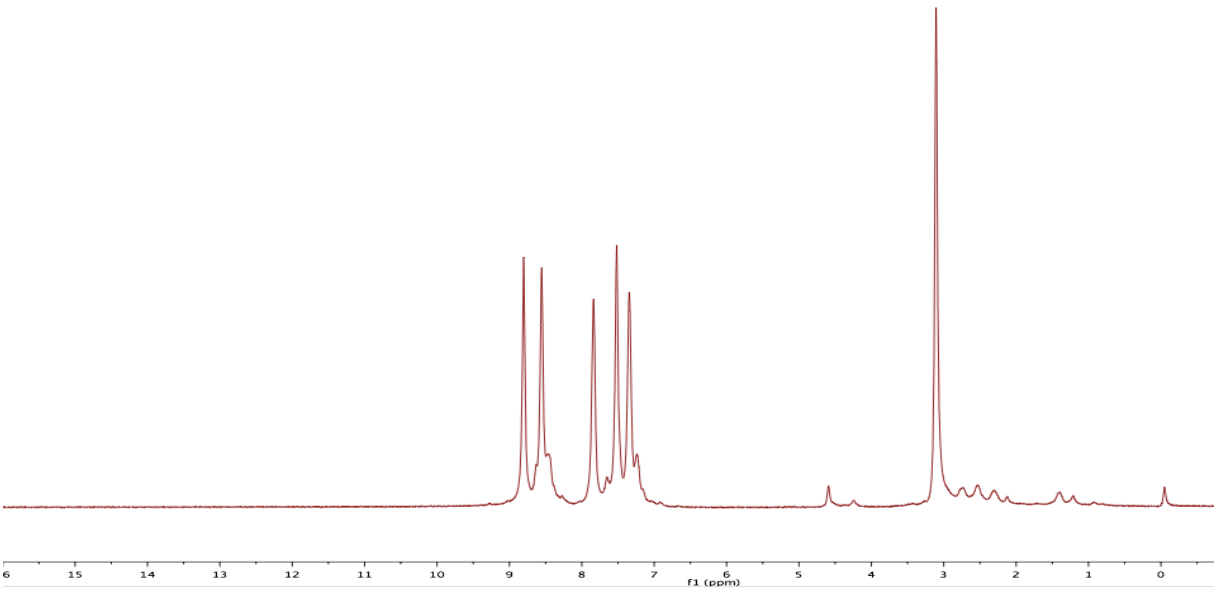

O
N
OMe
OMe
MeO

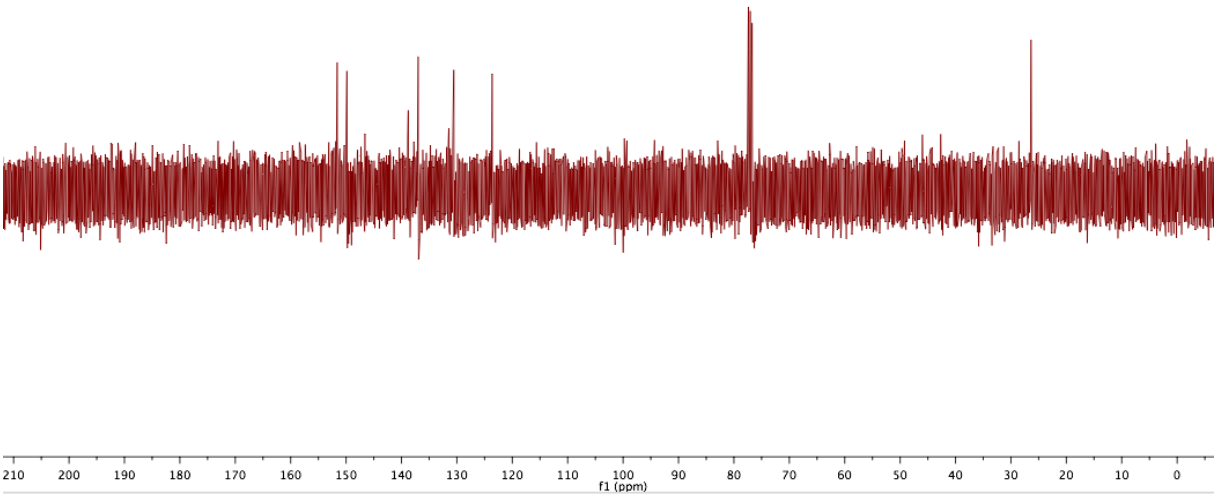

15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)


210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10

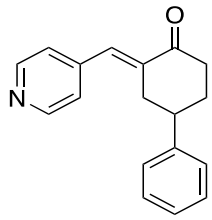
Compound 65

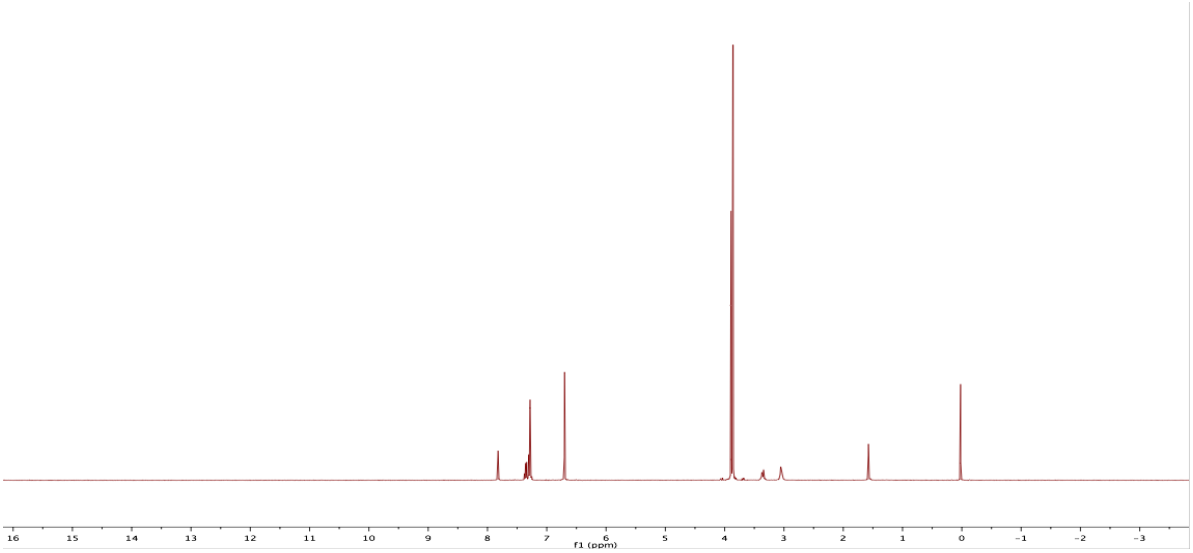

O
N
N

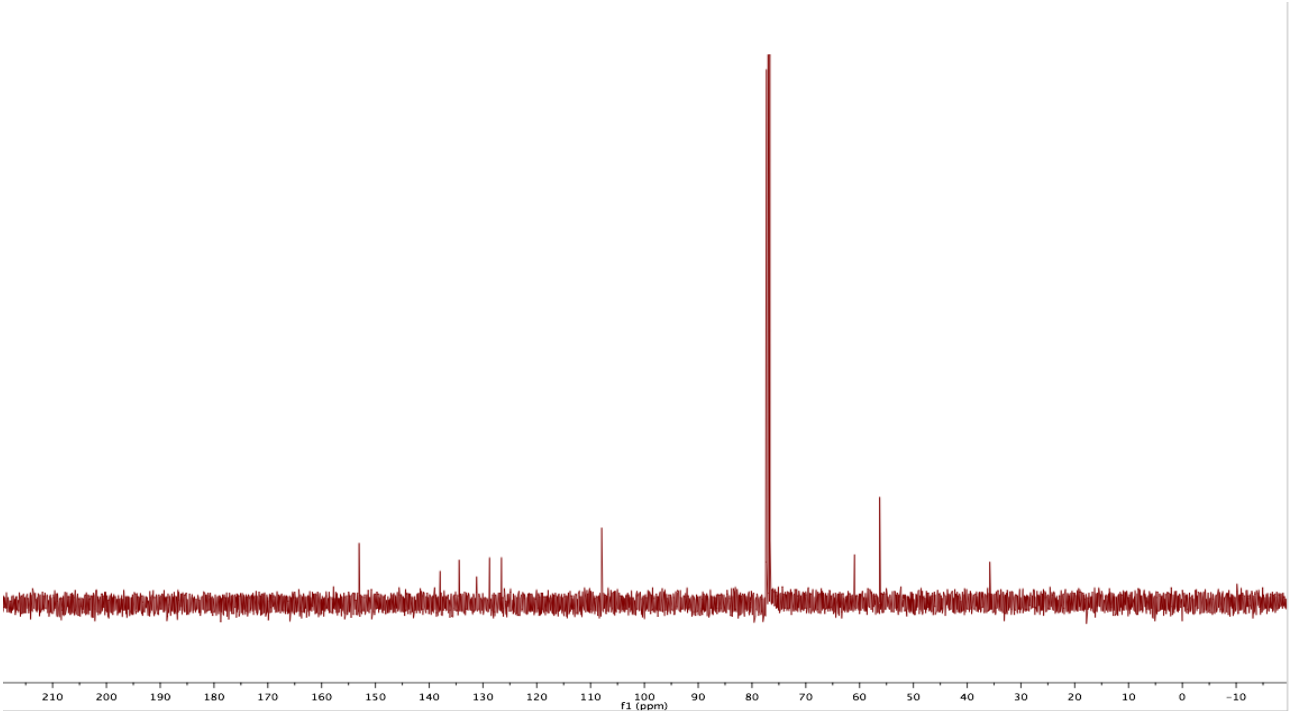

6
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)


210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

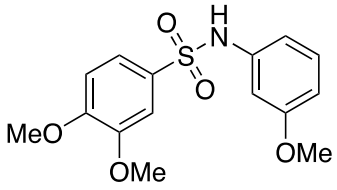
Compound 66

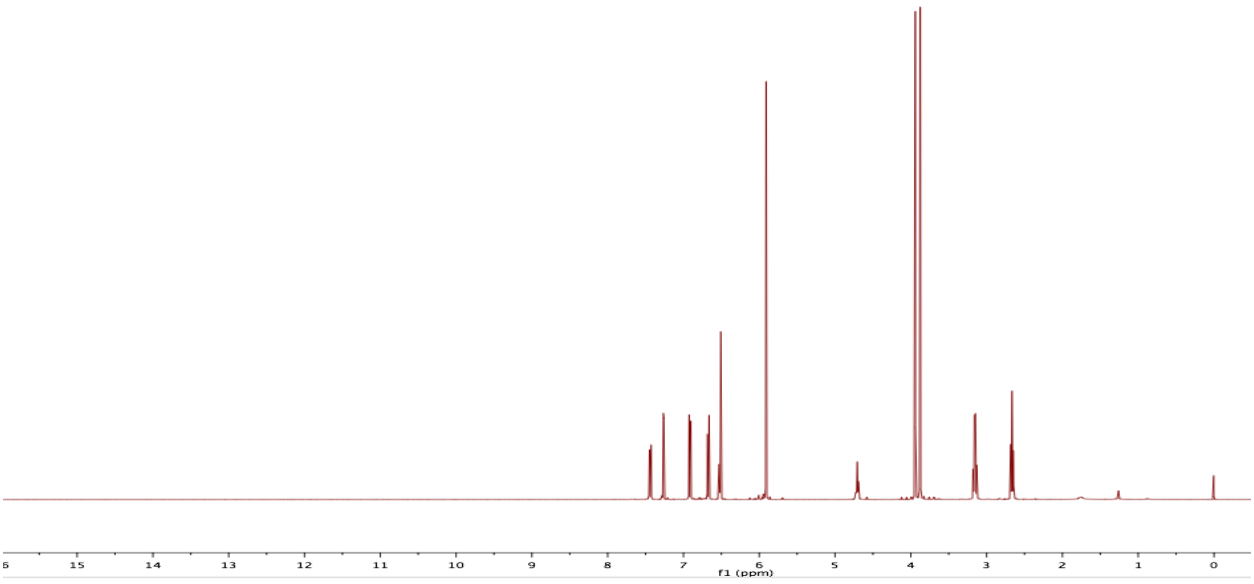

O
N

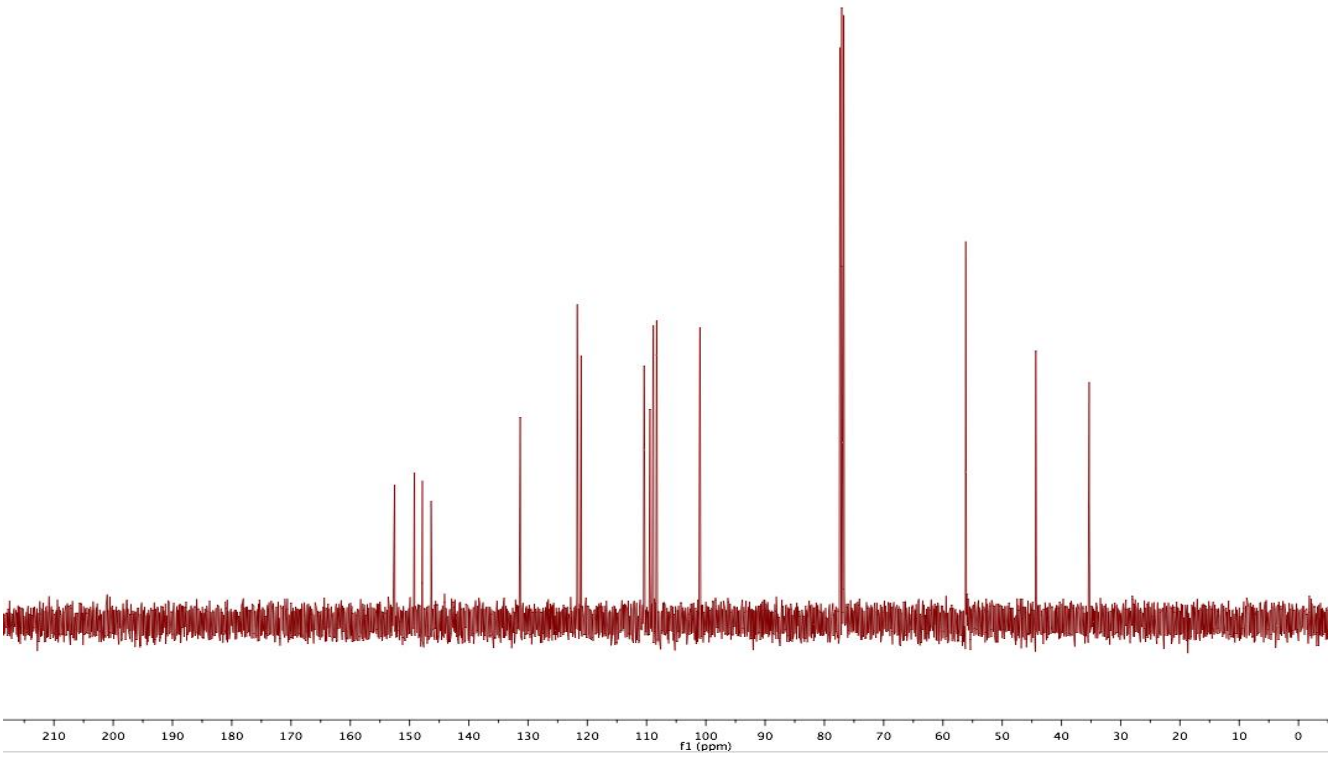

16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2 −3
f1 (ppm)


210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)

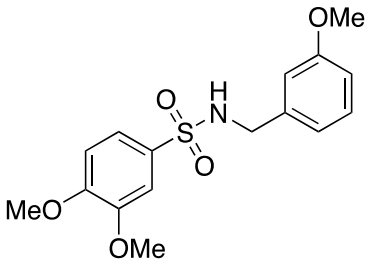
Compound 67

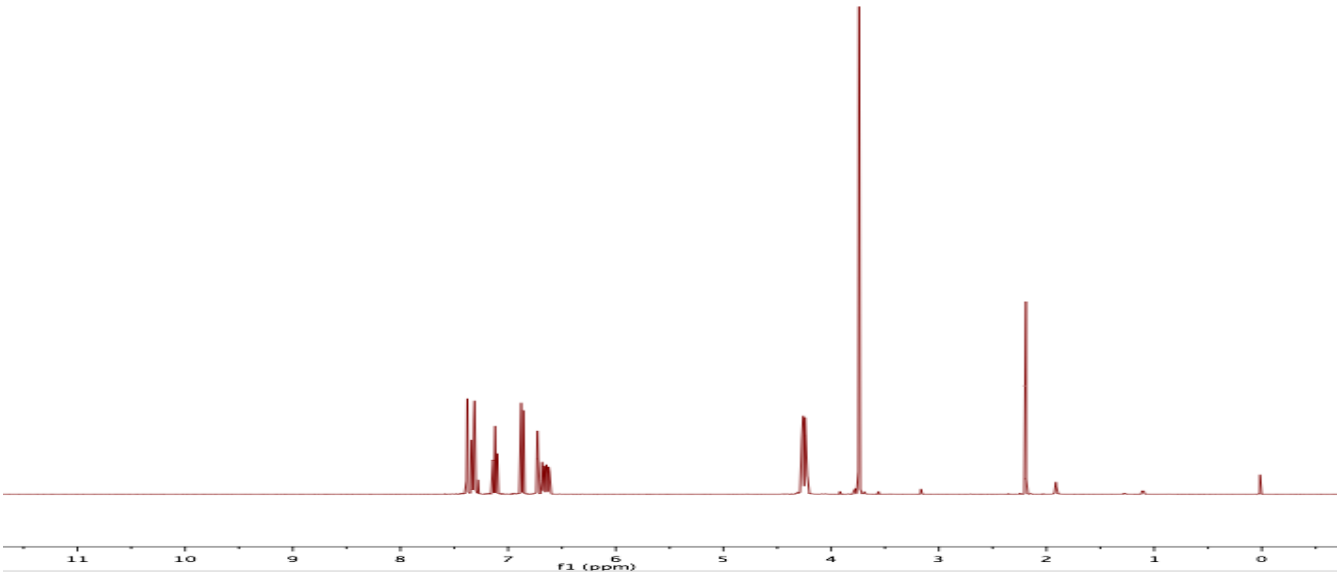


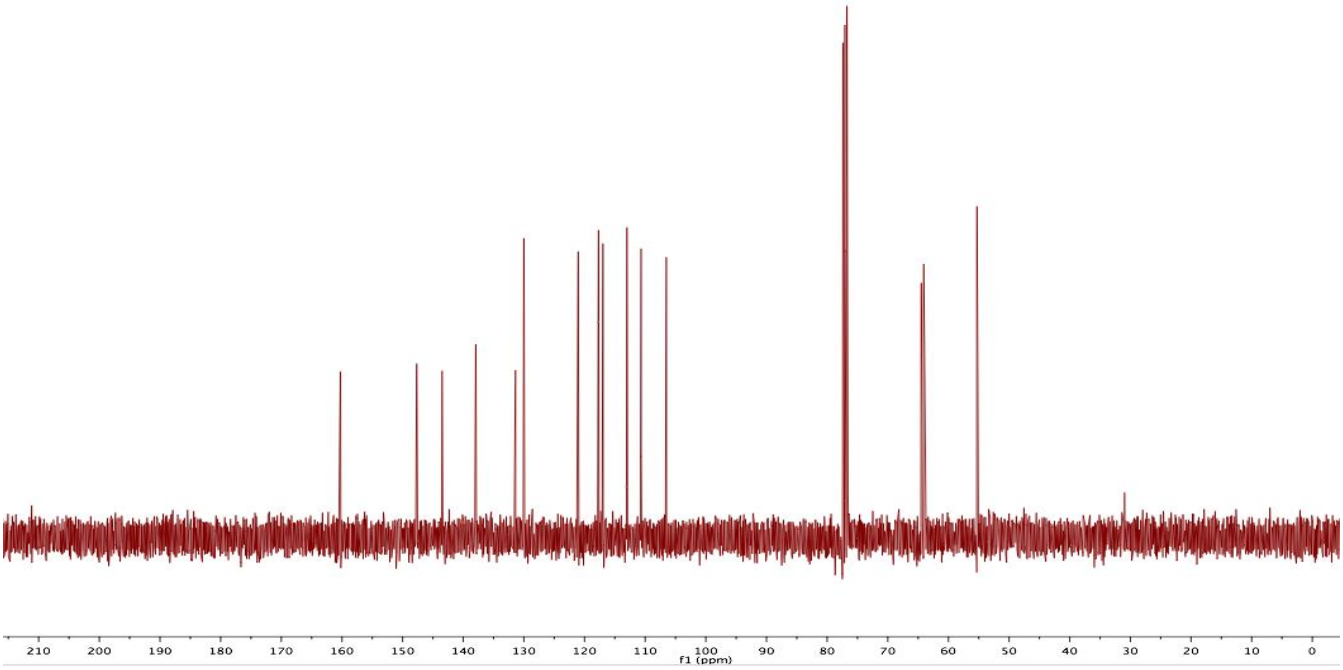




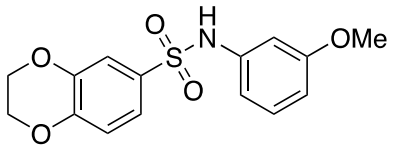
Compound 68

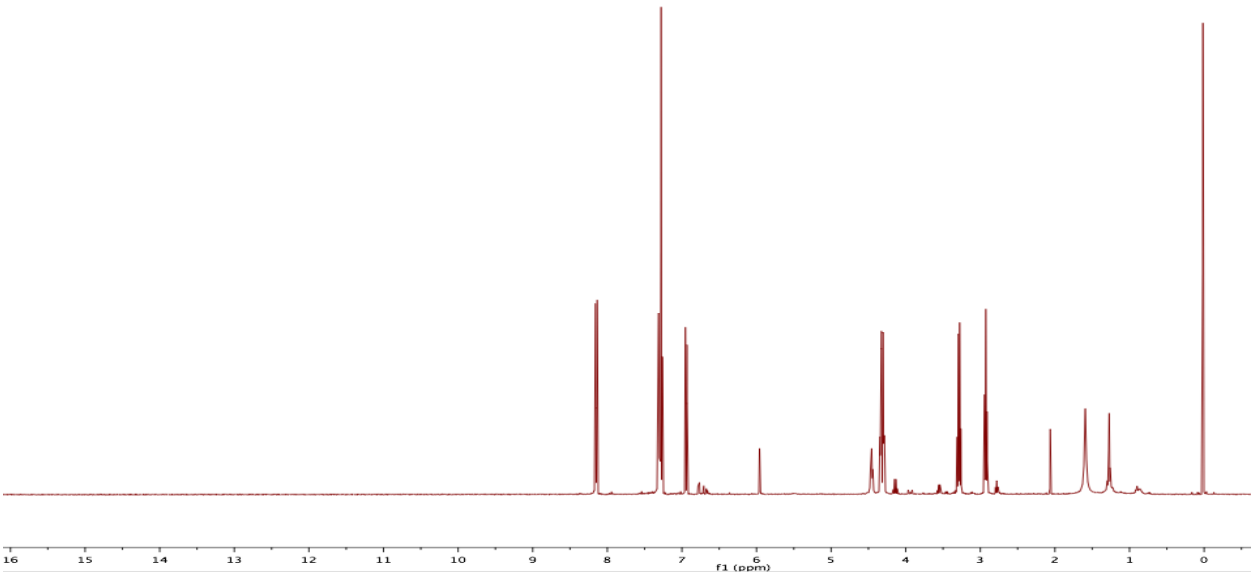

OMe
H
N
O
S
O
MeO
OMe

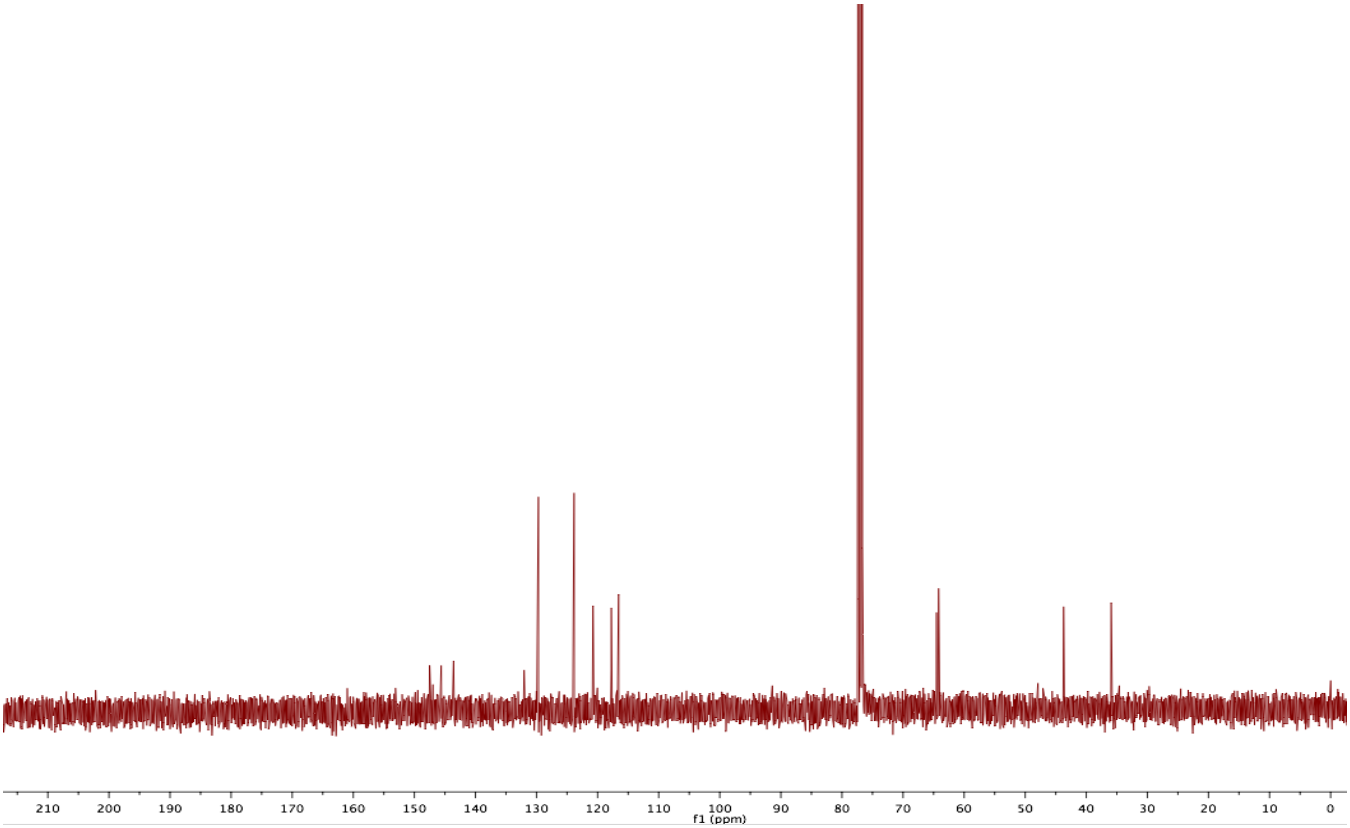

11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)


210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

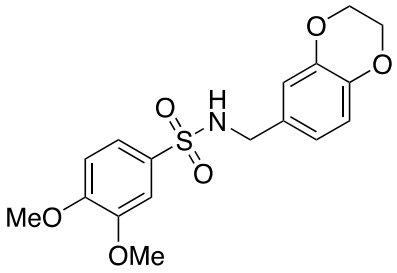
Compound 69

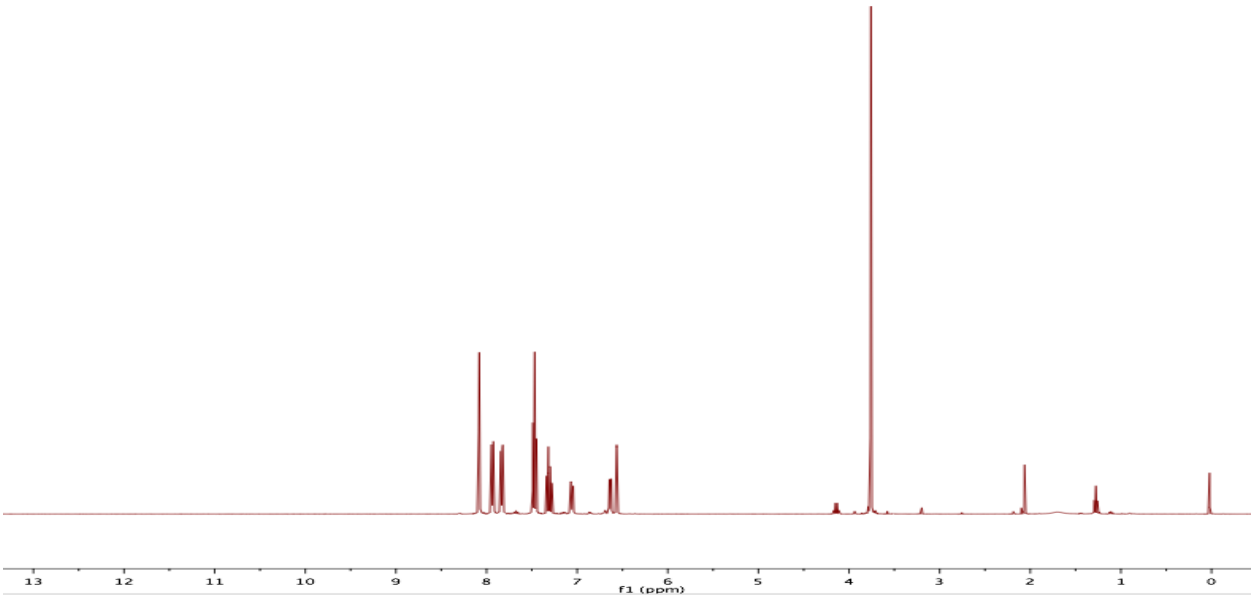

O
H
N
S
O
O
O
O
OMe

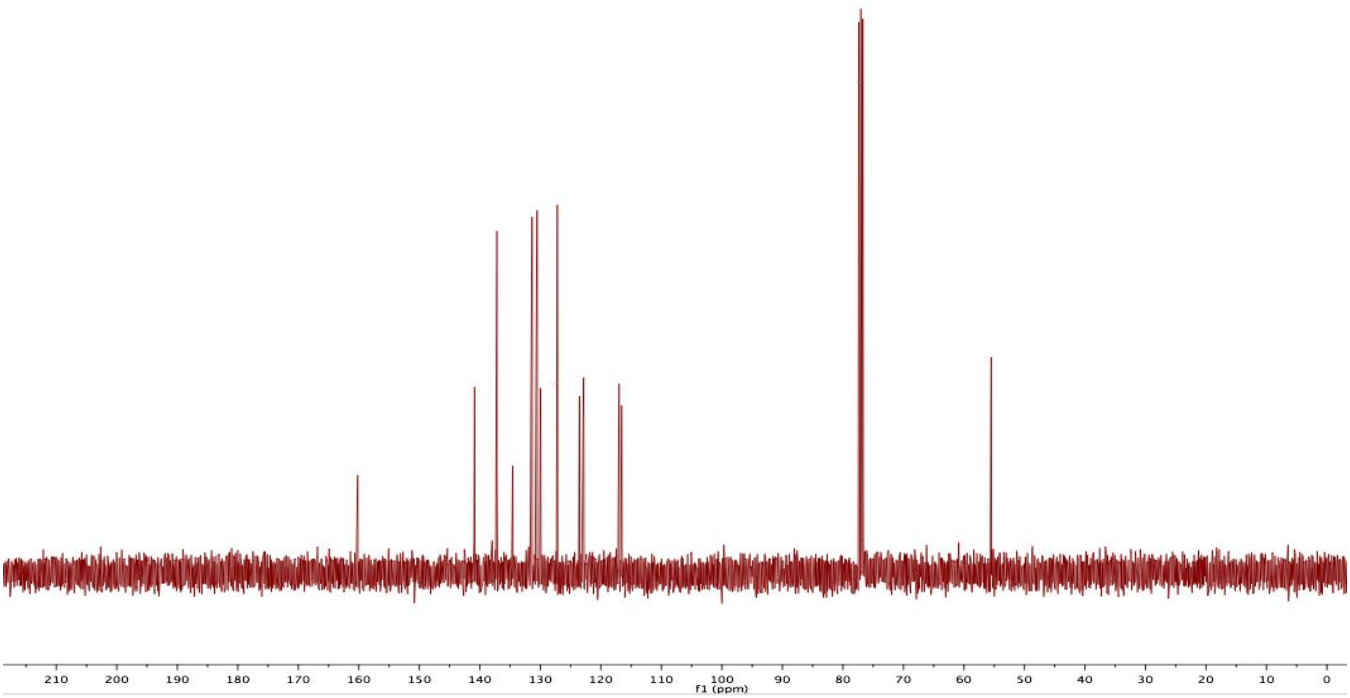

16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)


210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

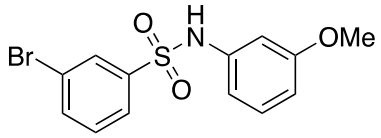
Compound 70

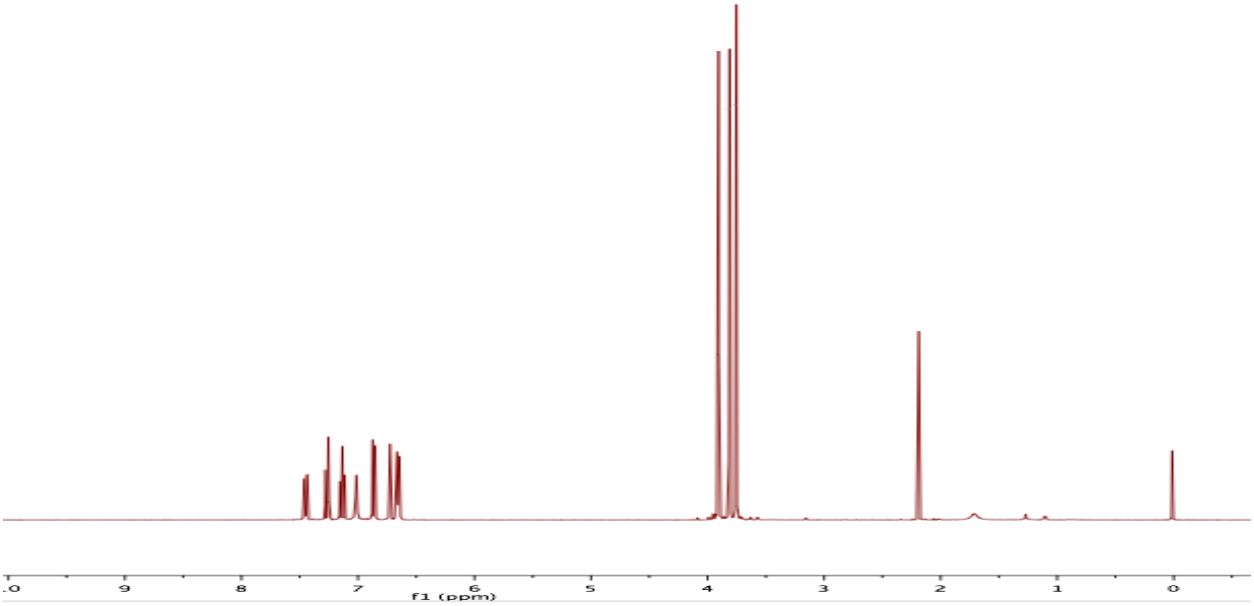

O
O
H
N
O
S
O
MeO
OMe

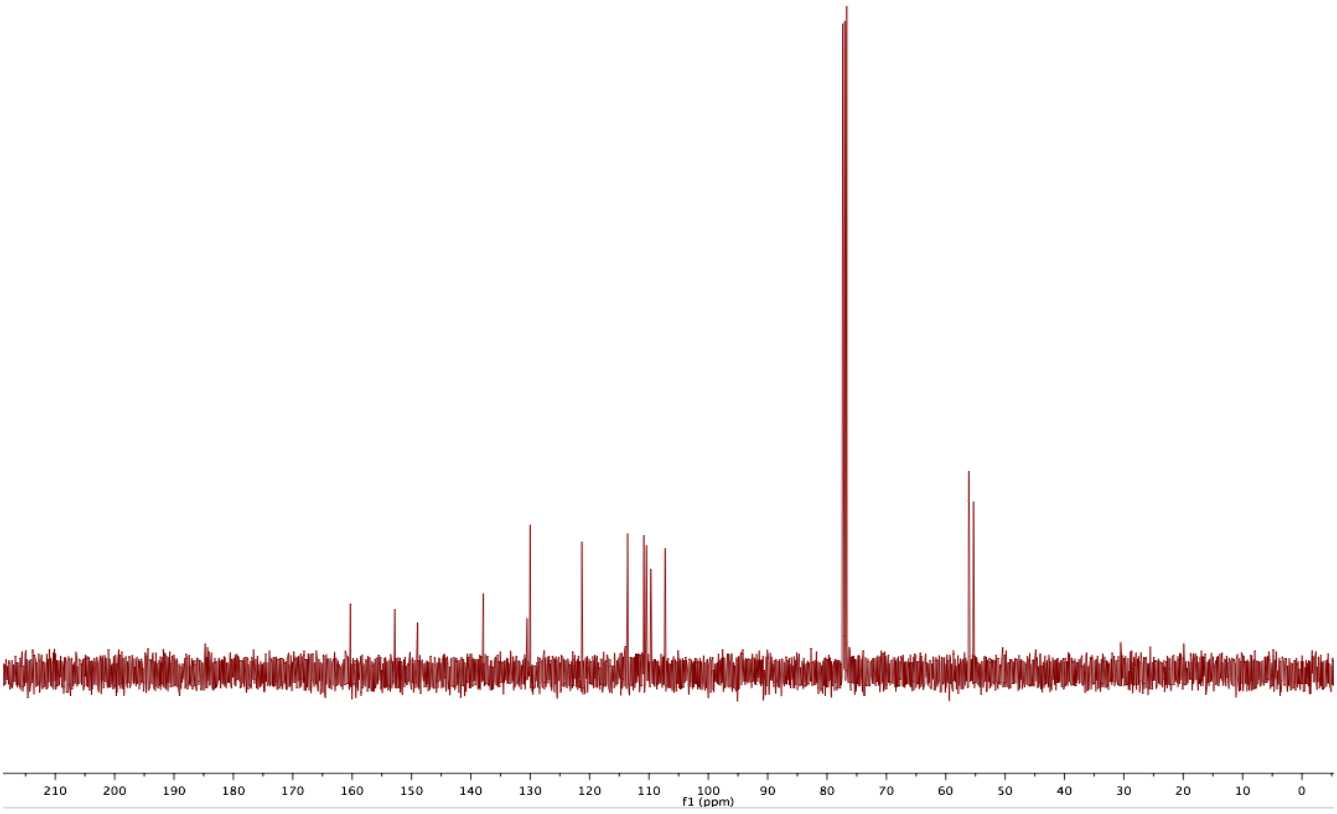

13
12
11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)


210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

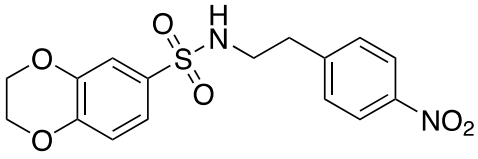
Compound 71

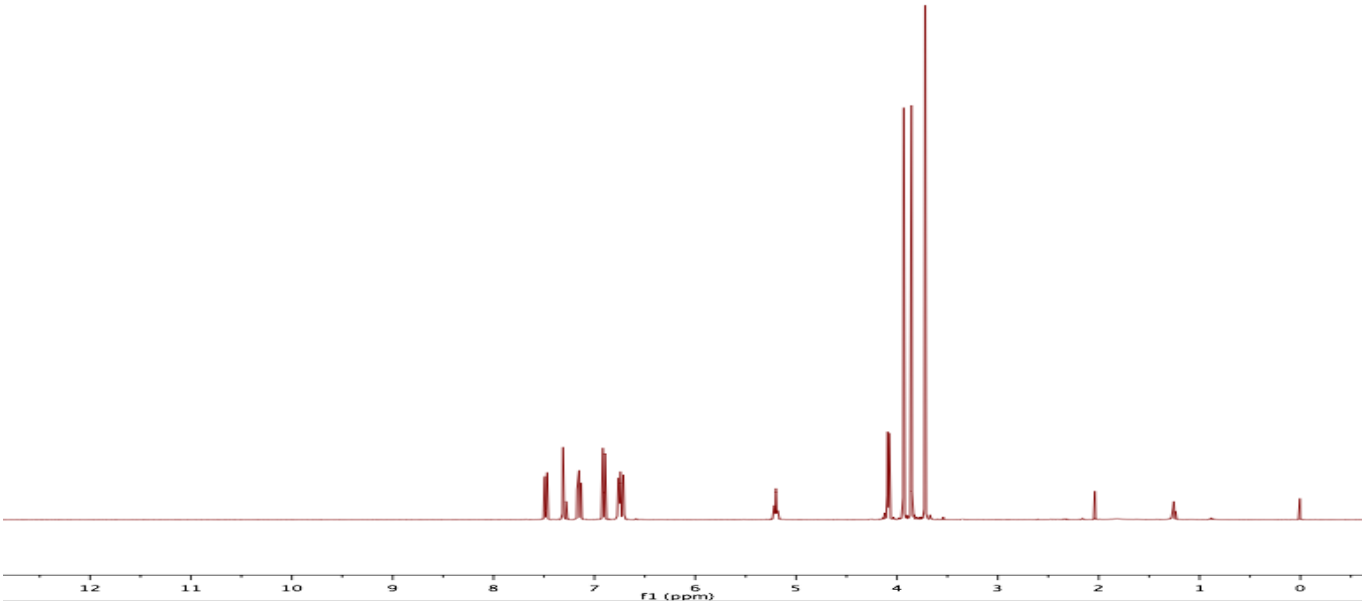


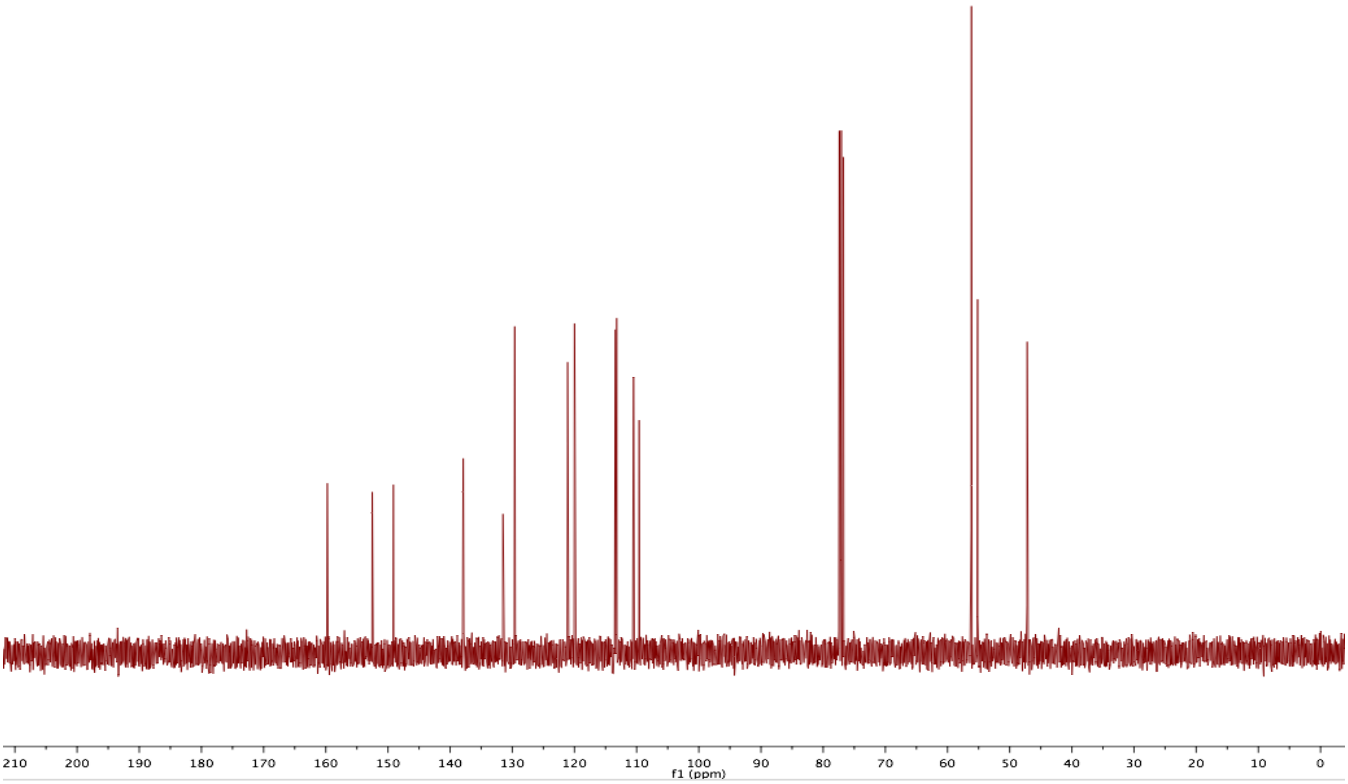




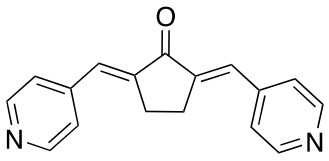
Compound 72

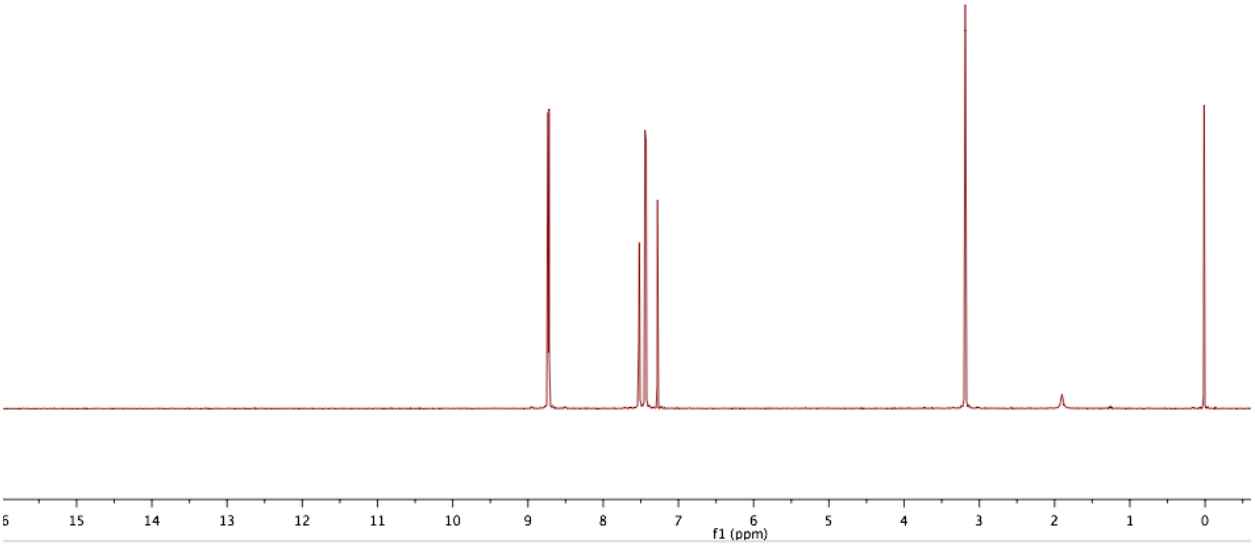

O
H
N
S
O
O
O
O
NO2

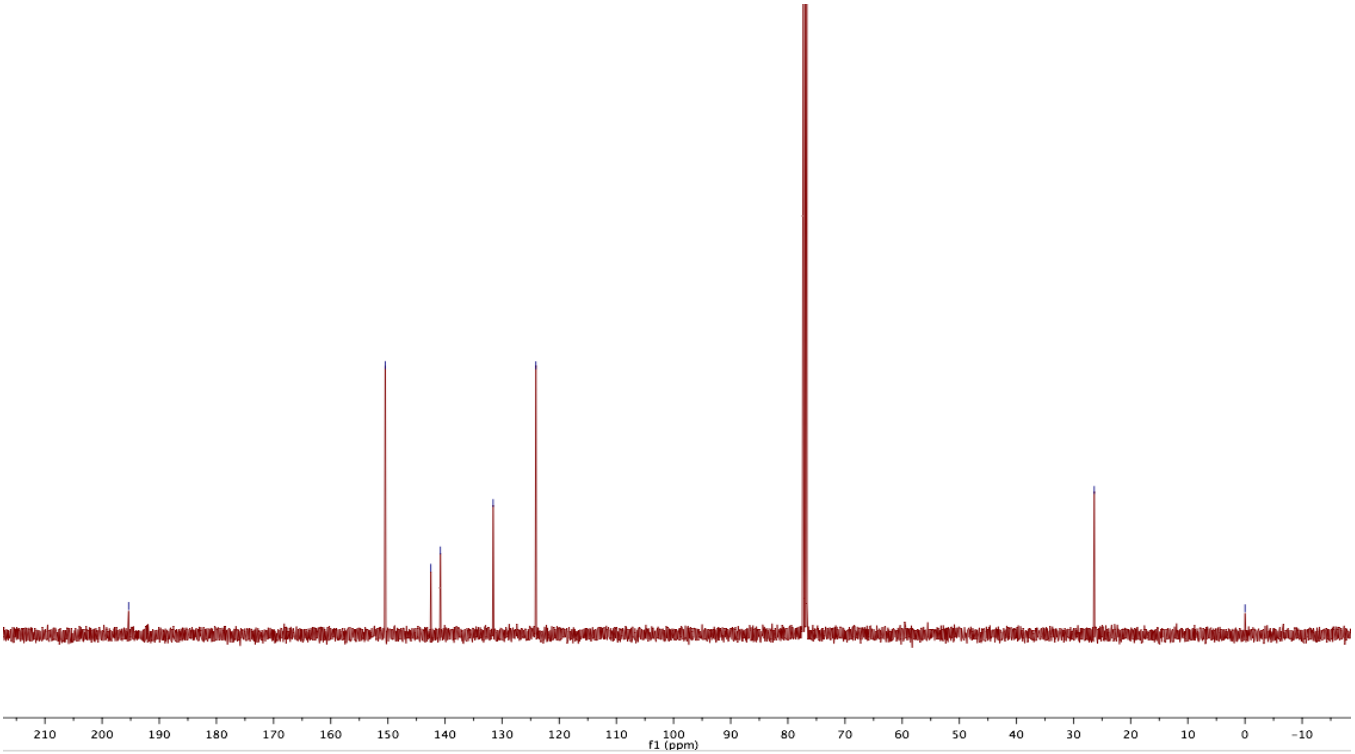

12
11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)


210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

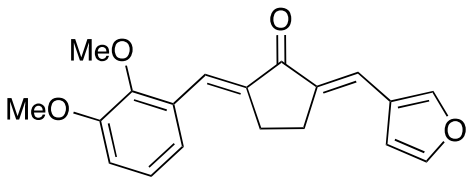
Compound 73

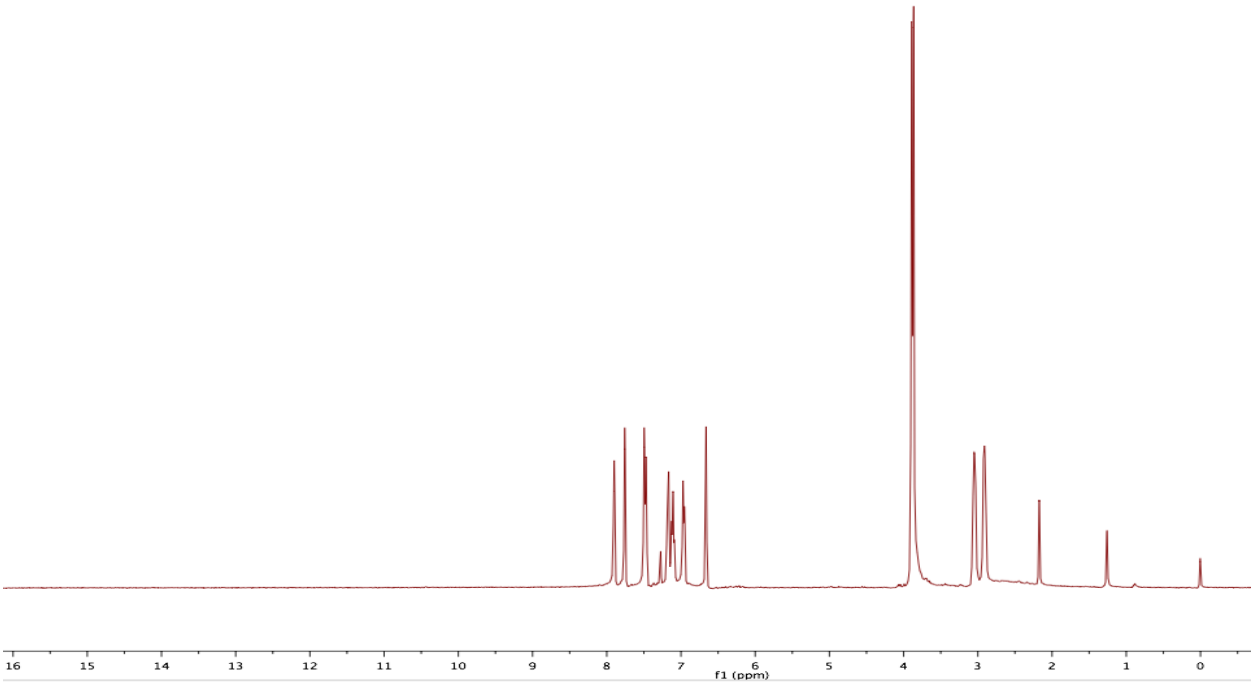

O
N
N

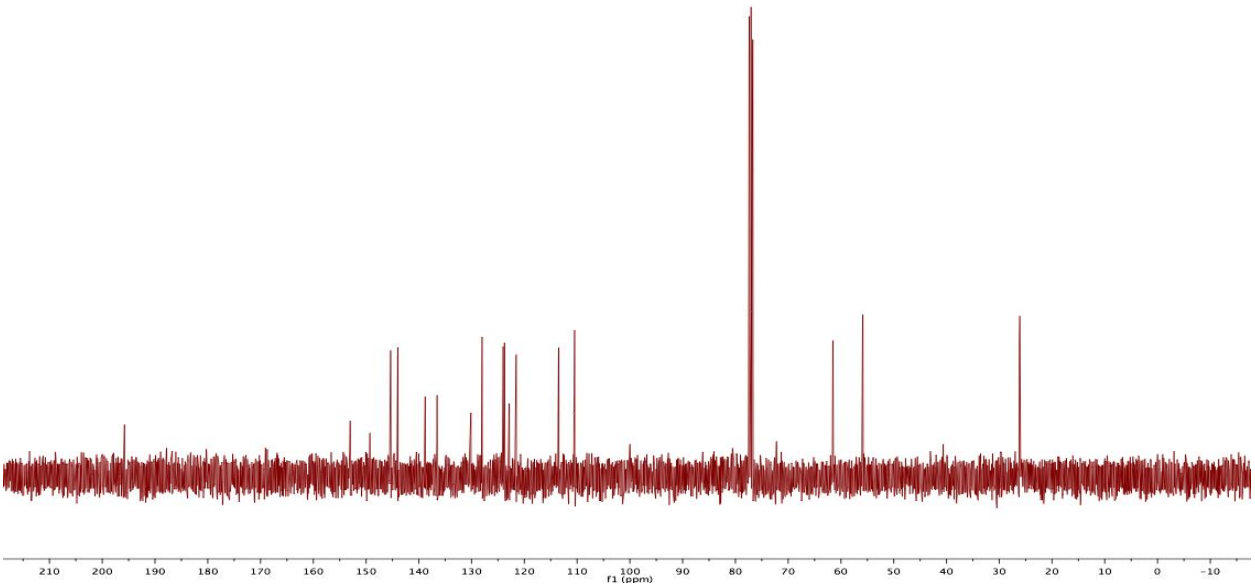

15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)


210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

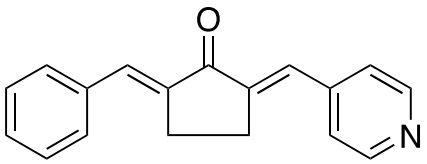
Compound 74

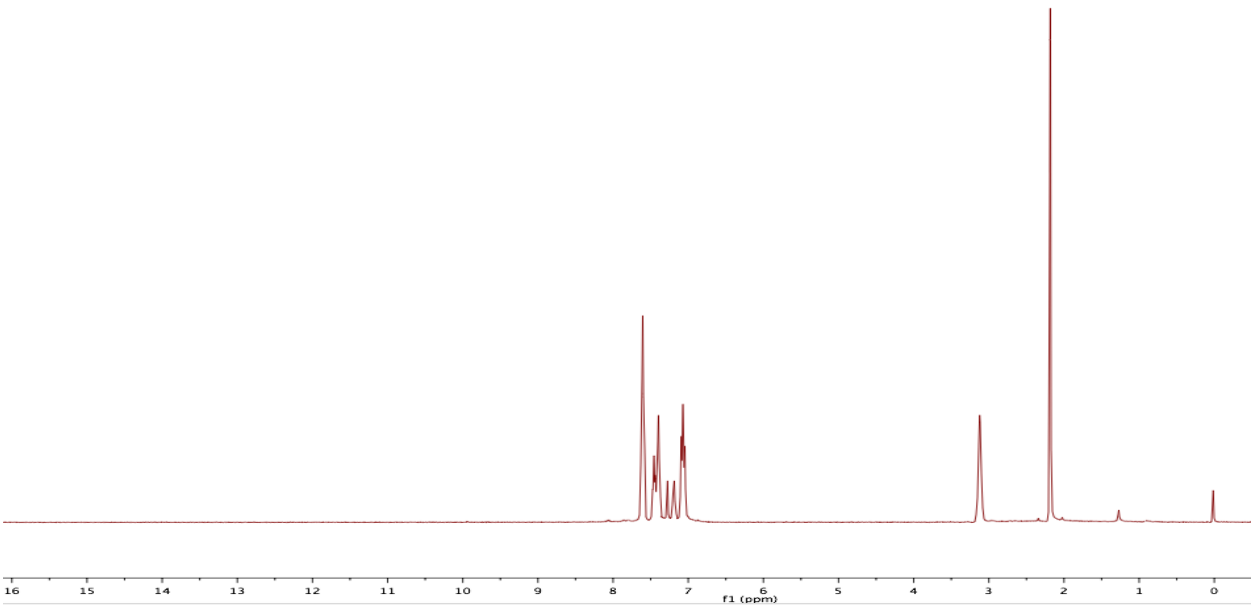

MeO
MeO
O
O

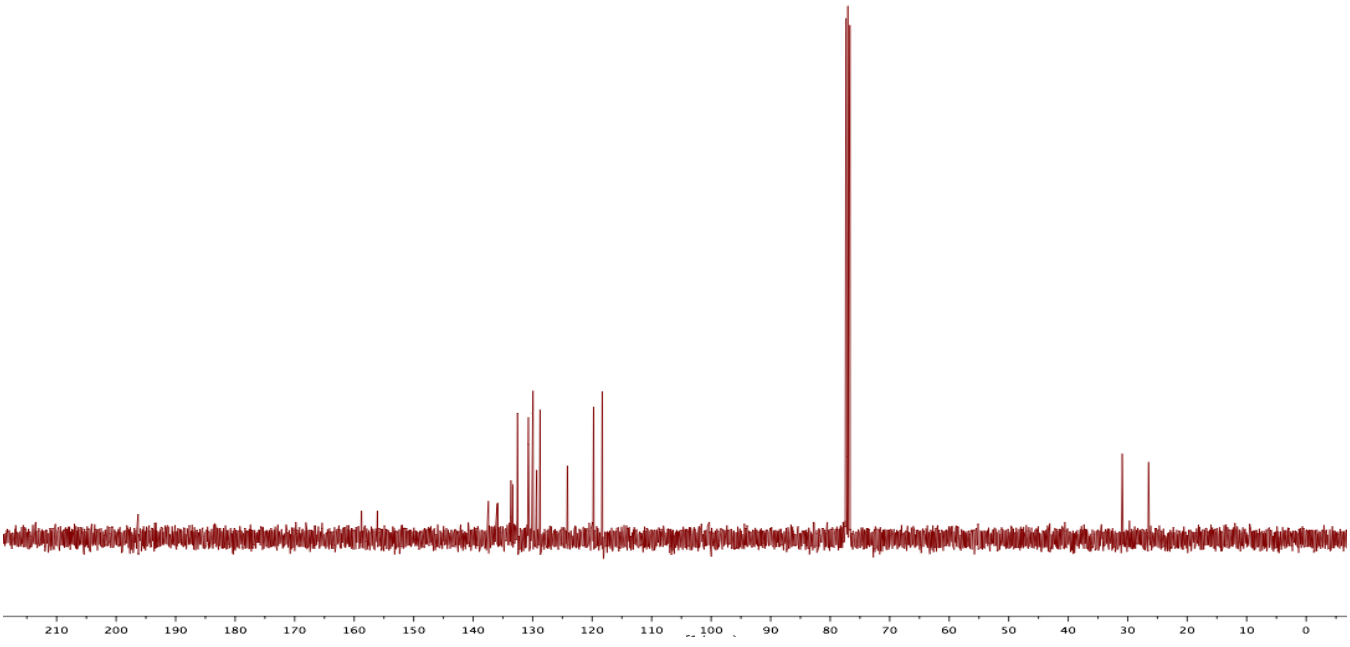

16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0
f1 (ppm)


210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

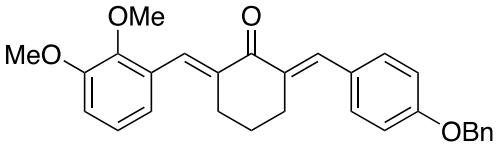
Compound 75

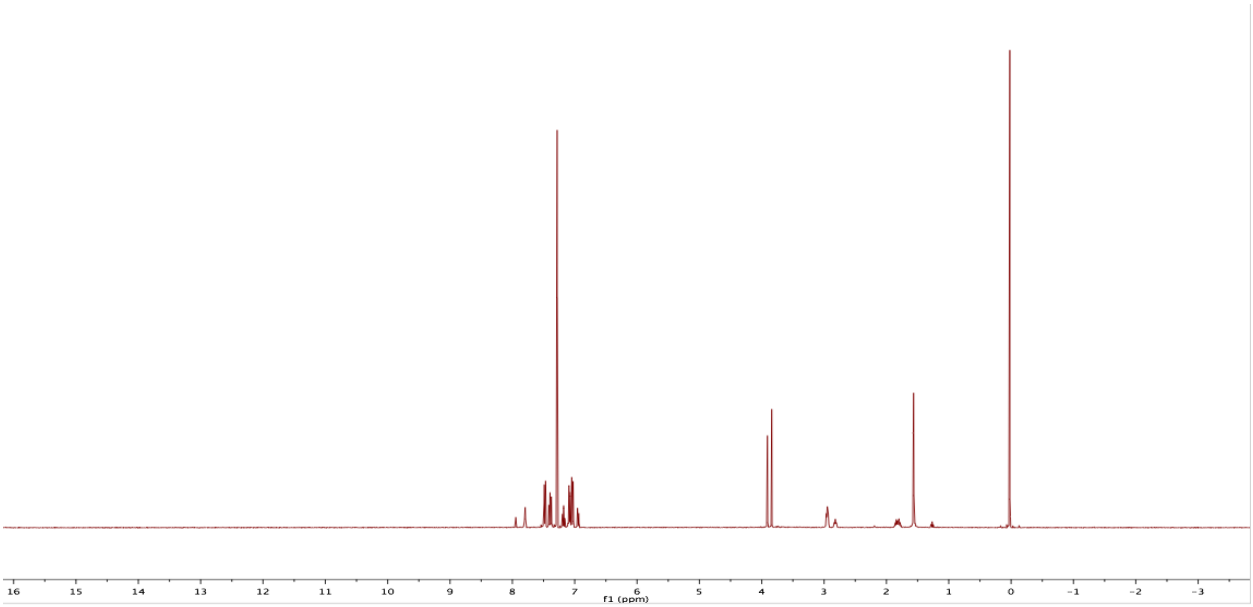

O
N

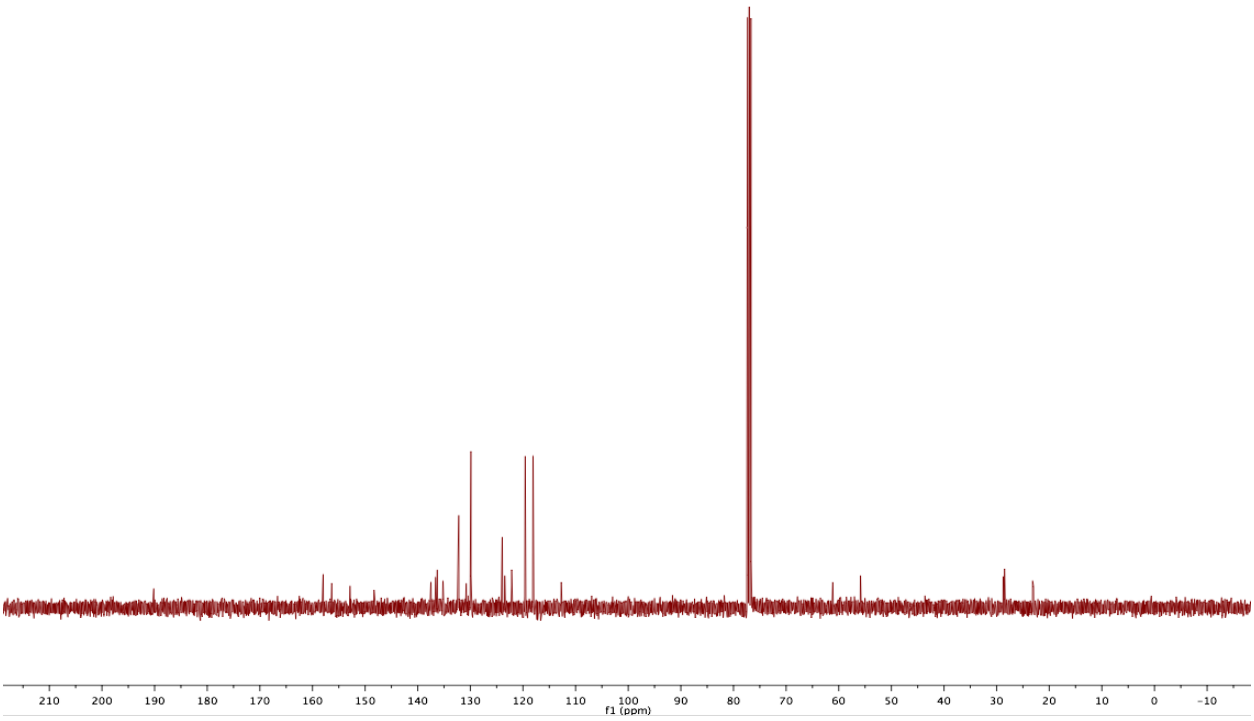

16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)


210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

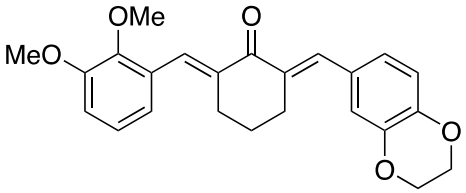
Compound 76

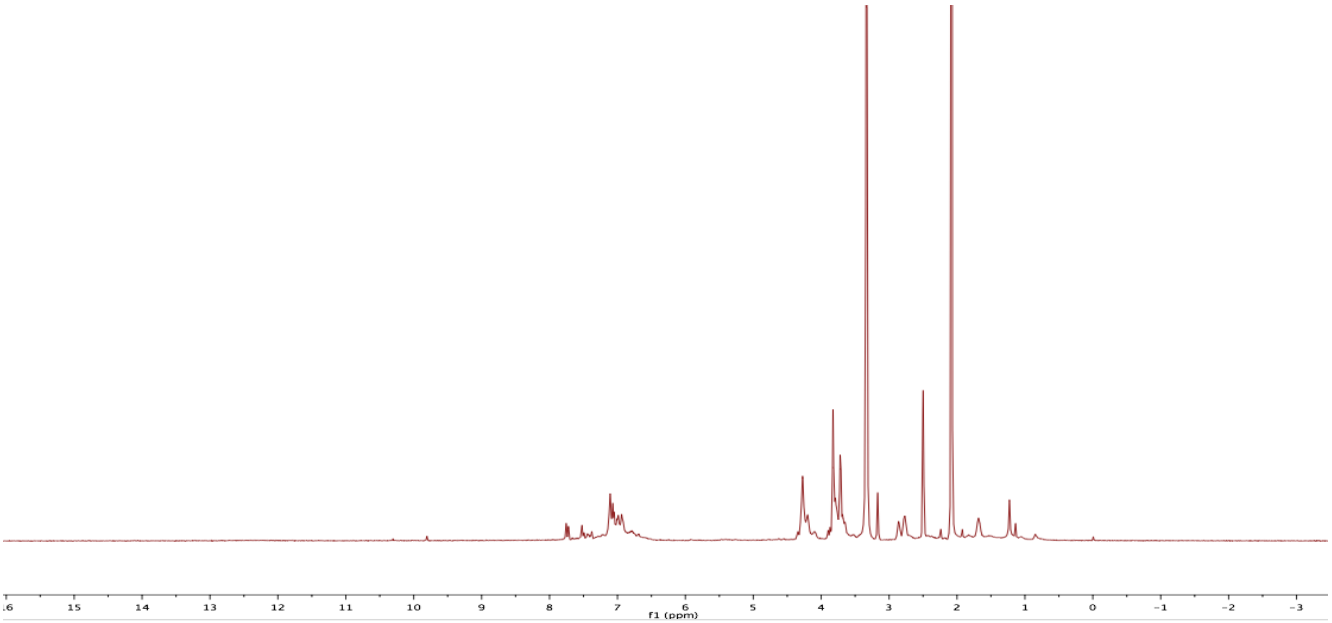

OMe
O
MeO
OBn

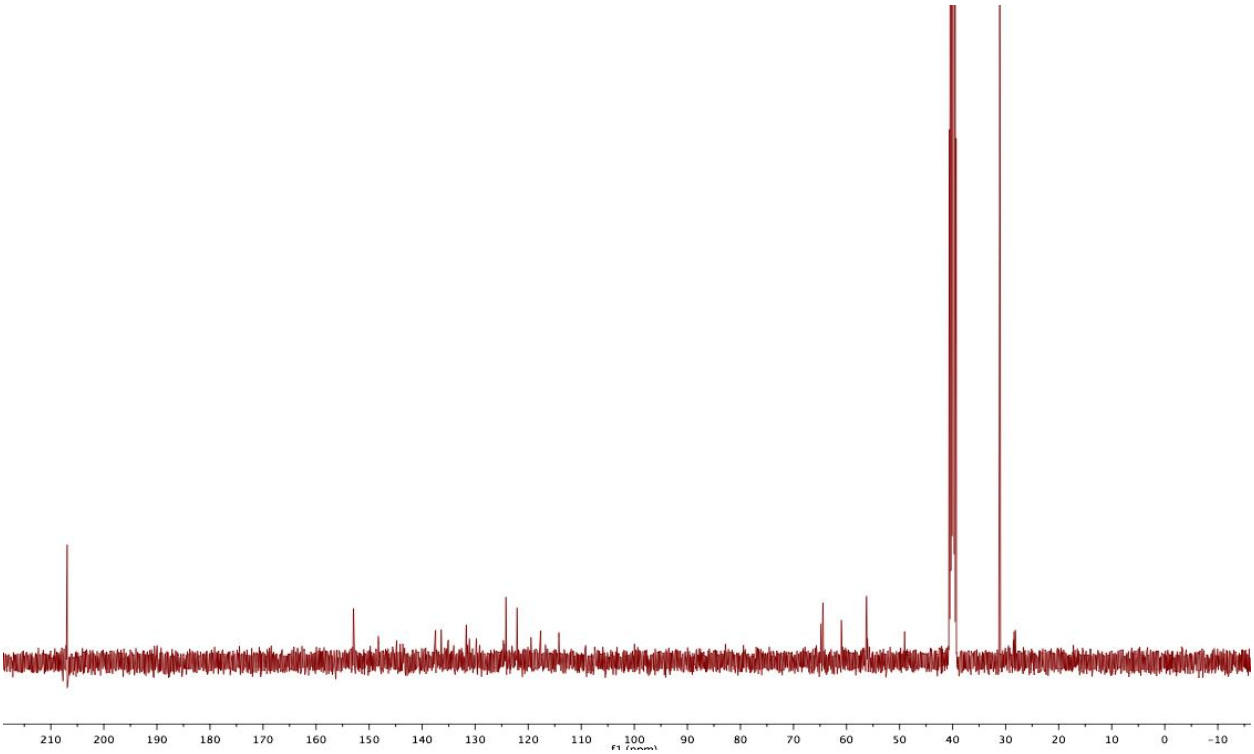

16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3
f1 (ppm)


210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

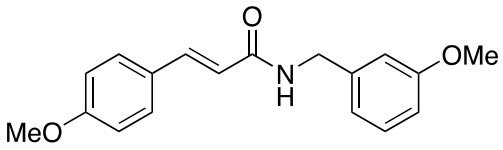
Compound 77

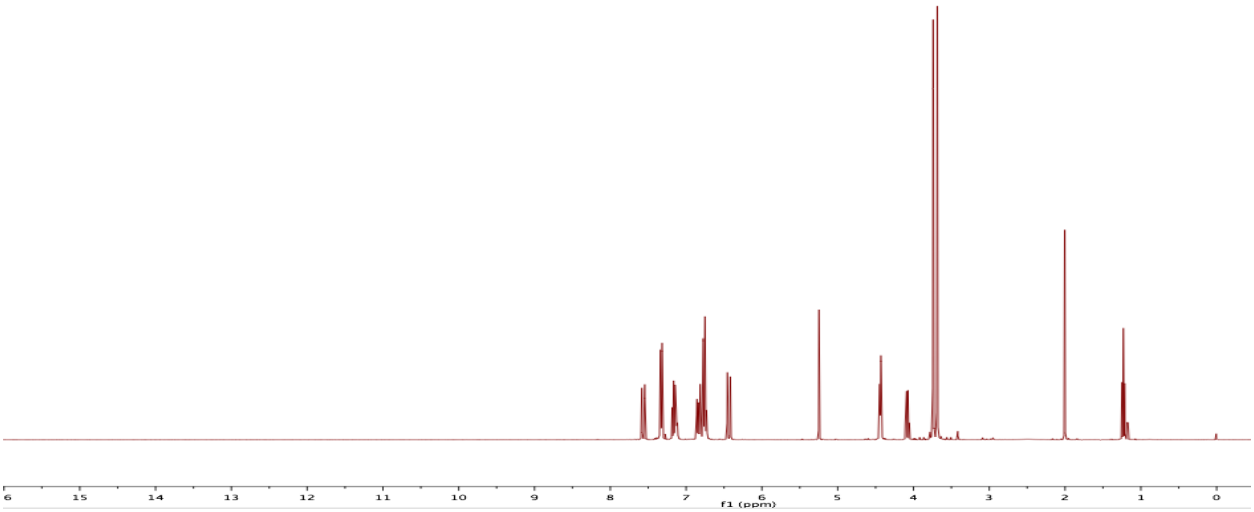


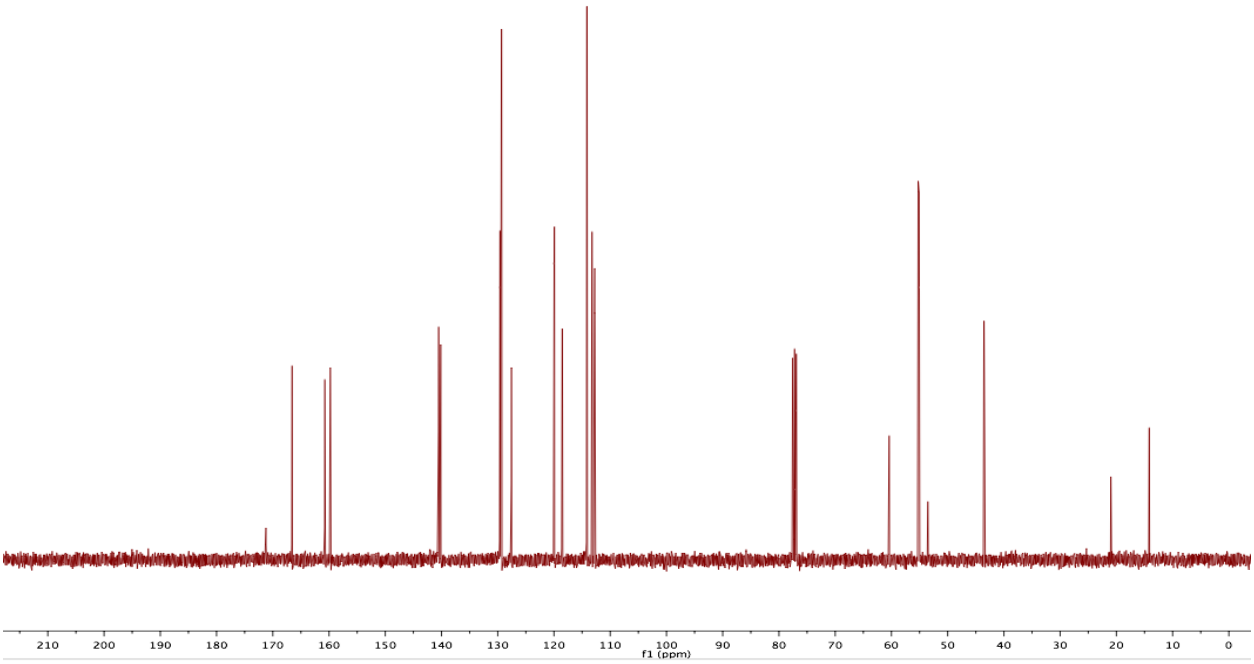




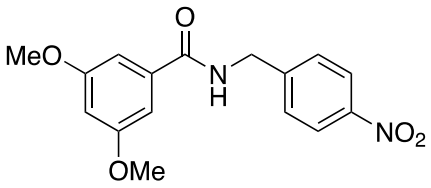
Compound 79

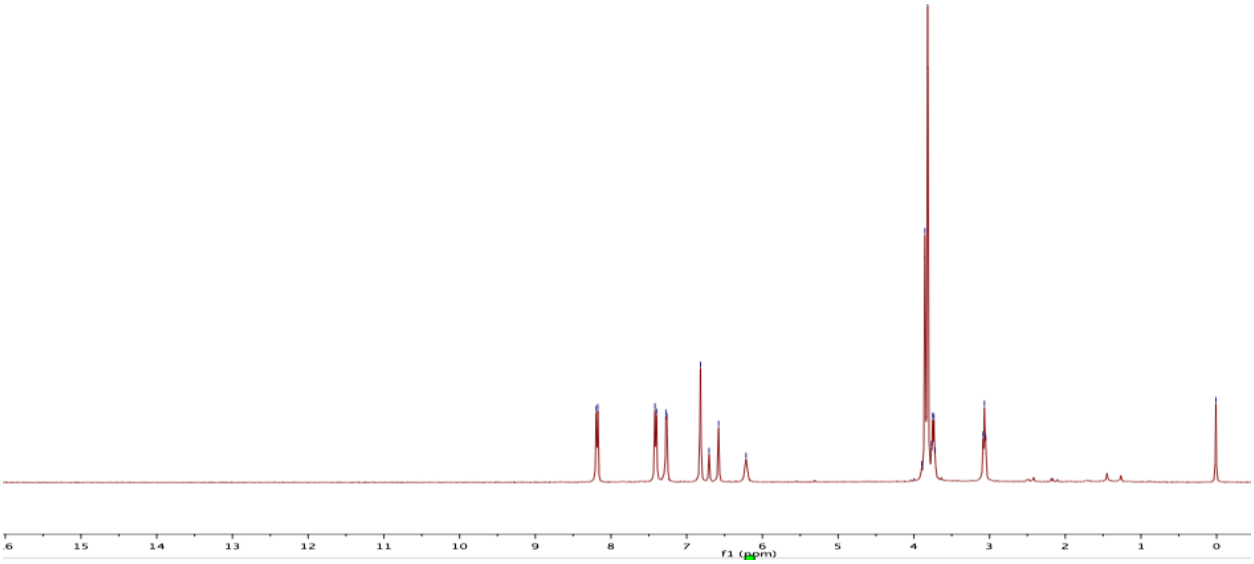


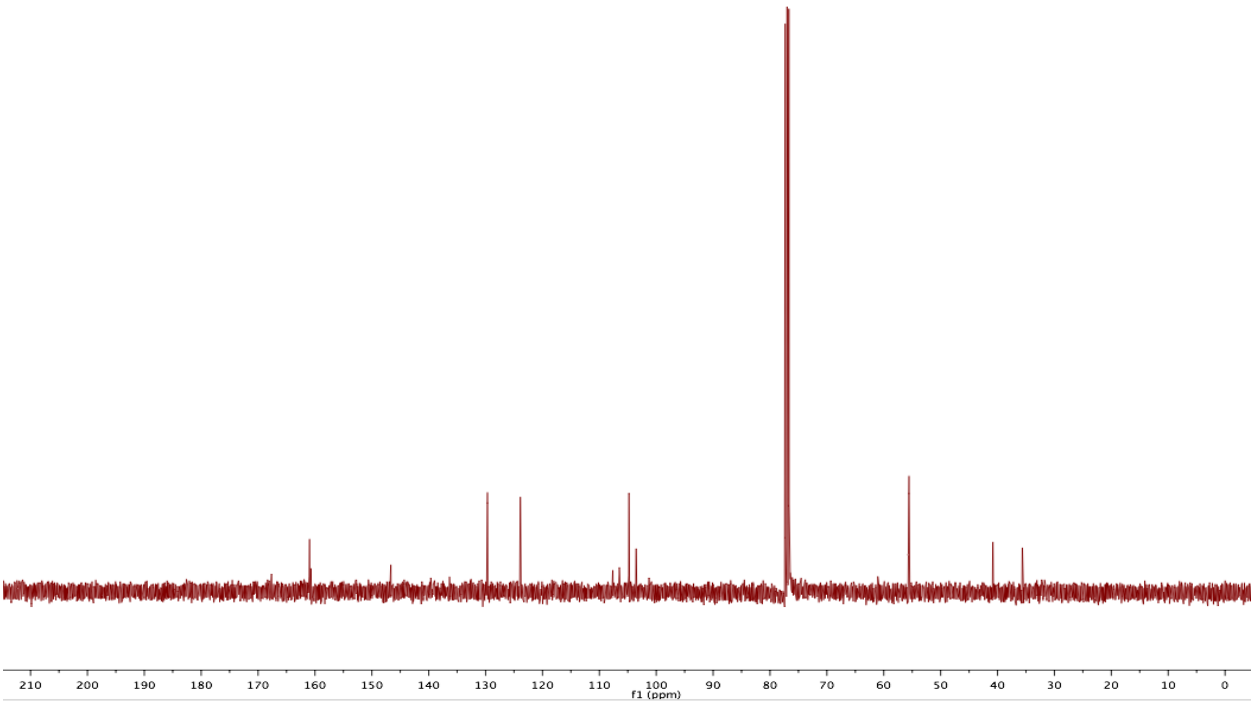




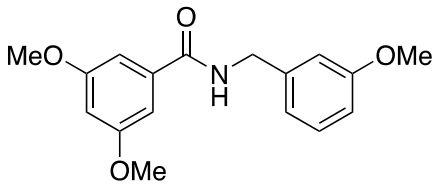
Compound 80

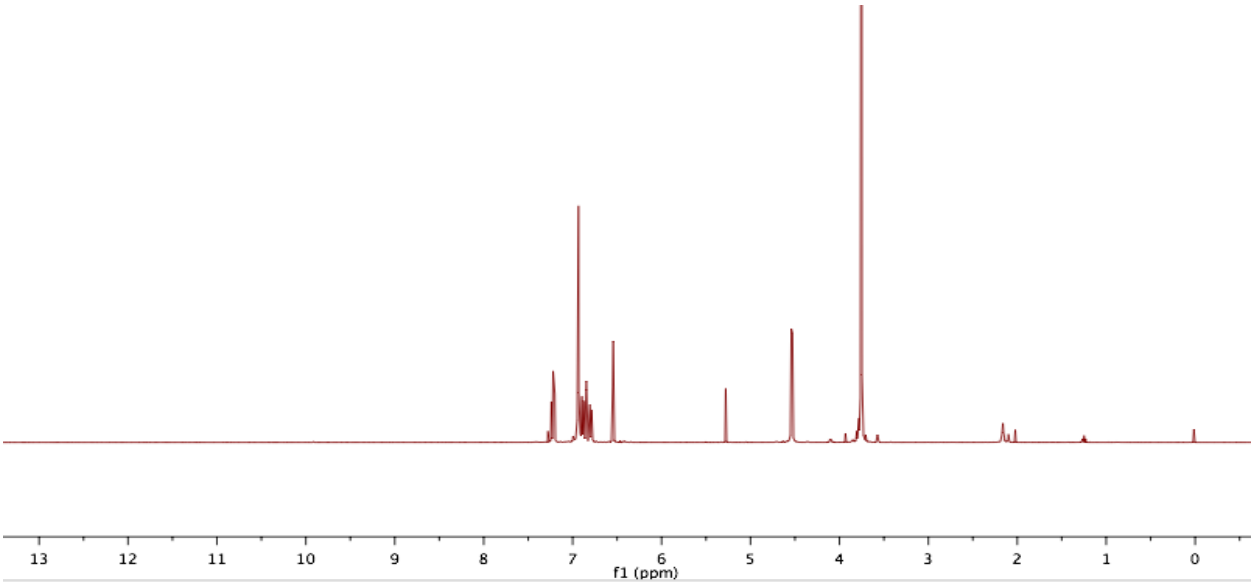


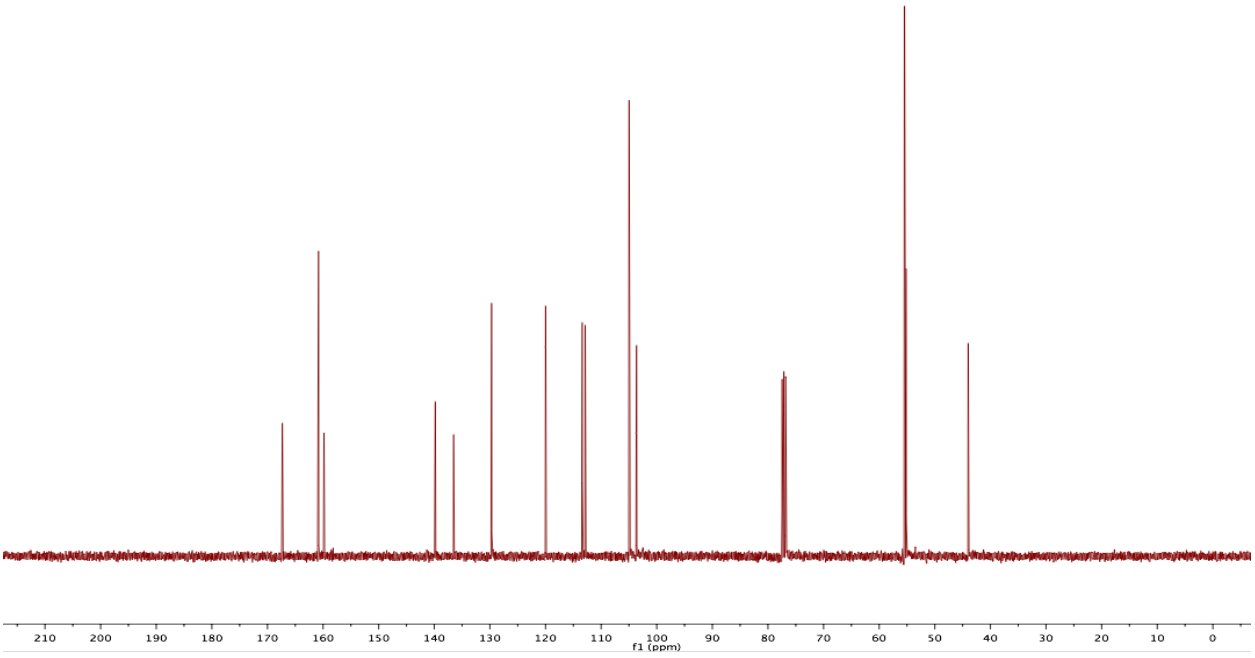




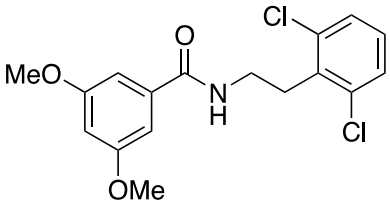
Compound 81

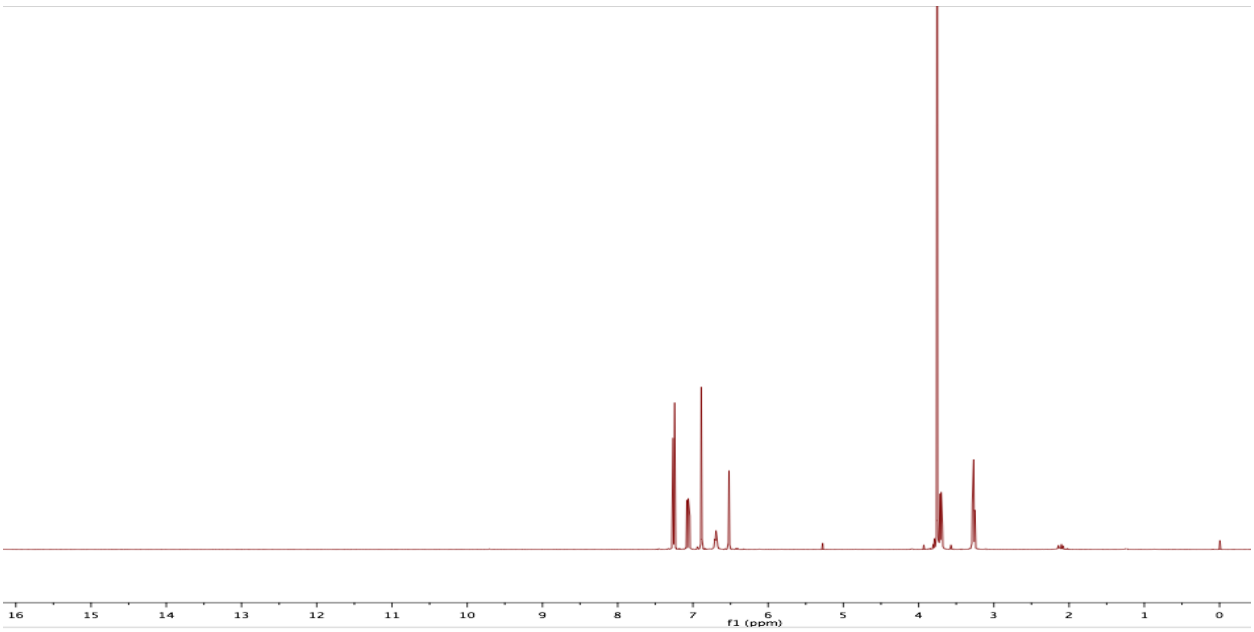

O
MeO
N
H
OMe
OMe

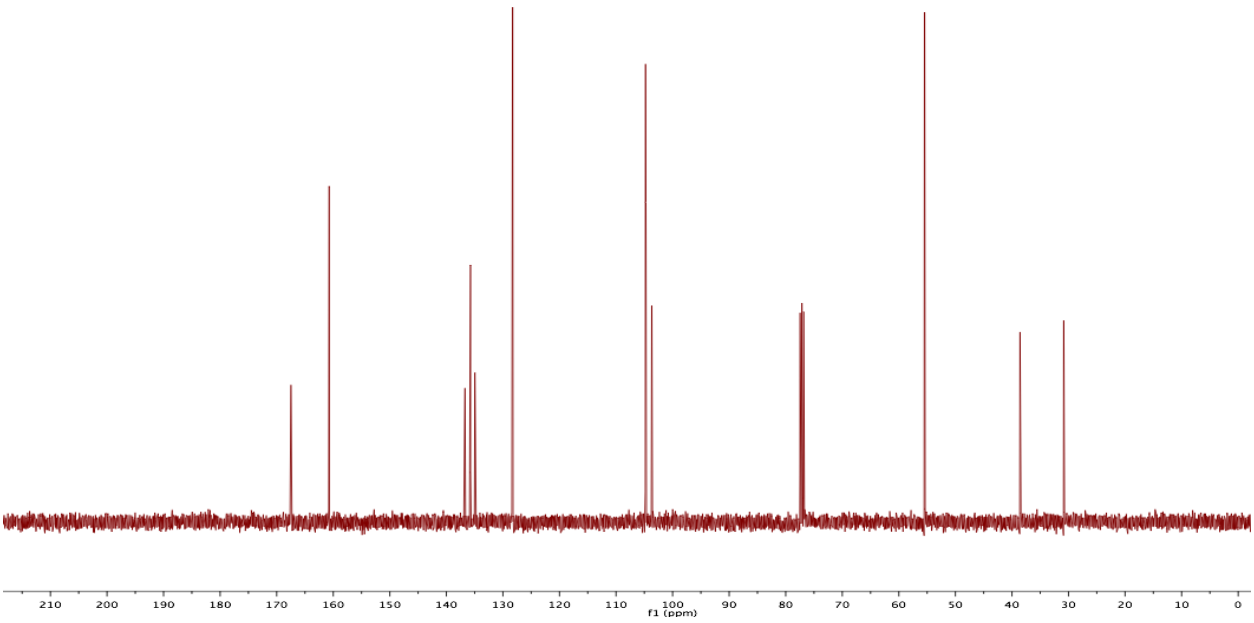

13
12
11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)


210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

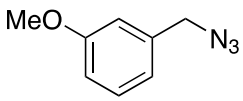
Compound 82

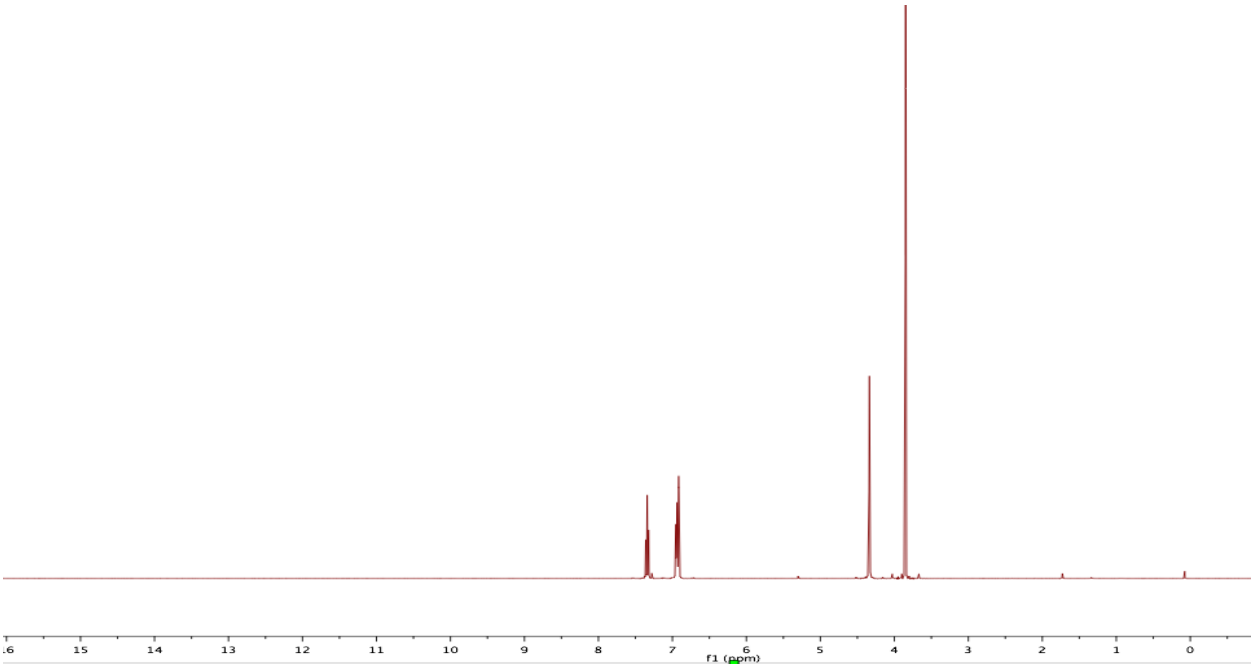

Cl
O
MeO
N
H
Cl
OMe

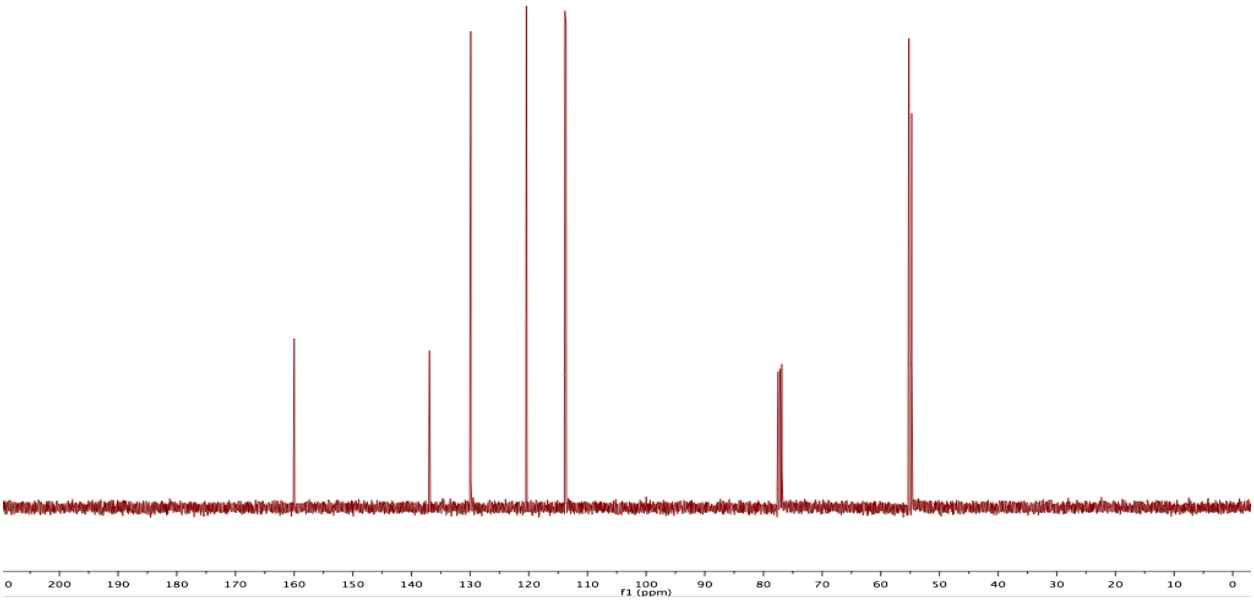

16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)


210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

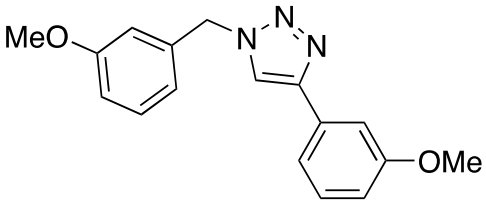
Compound 84

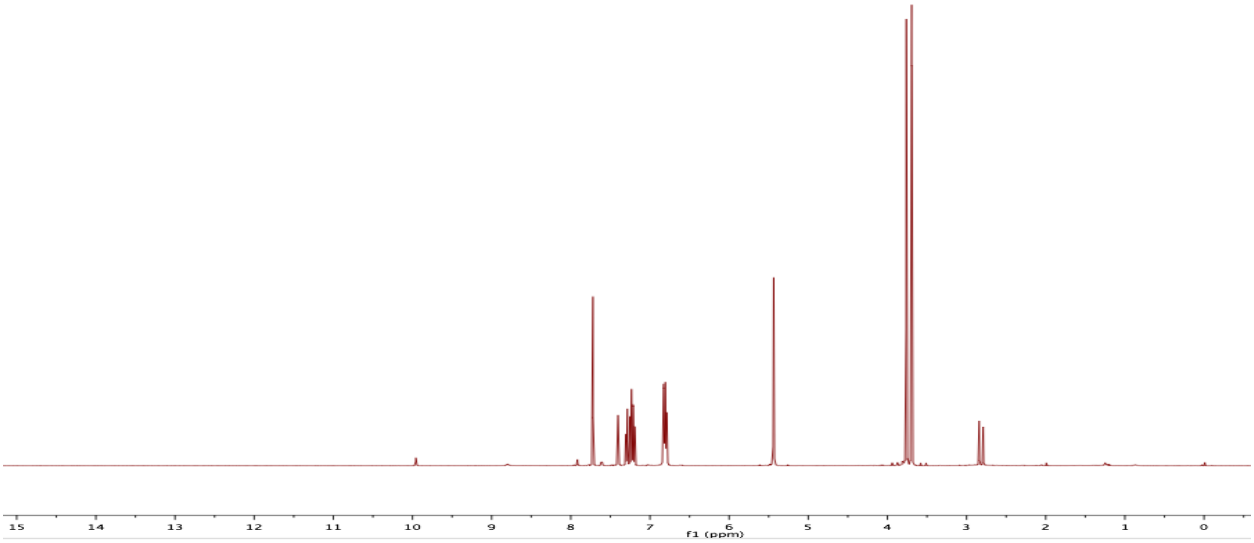


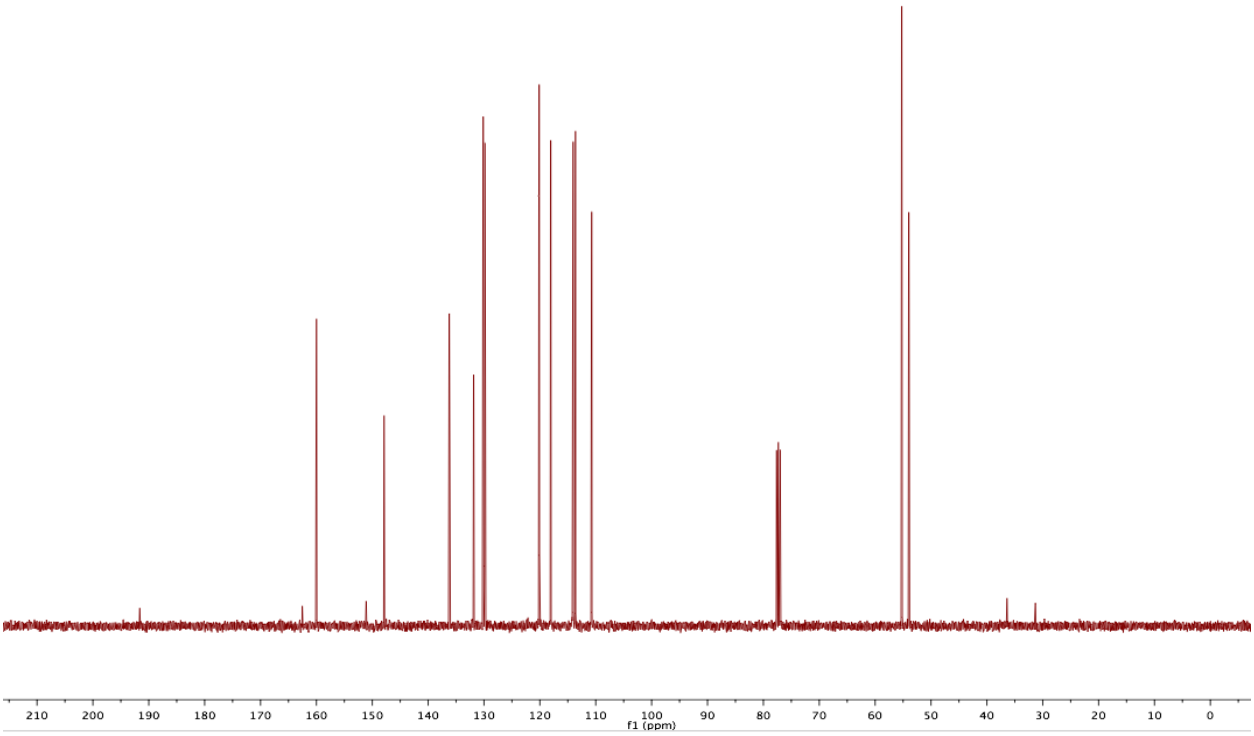




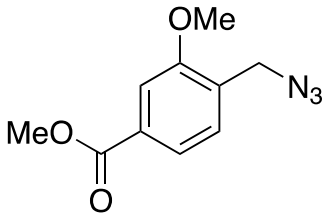
Compound 85

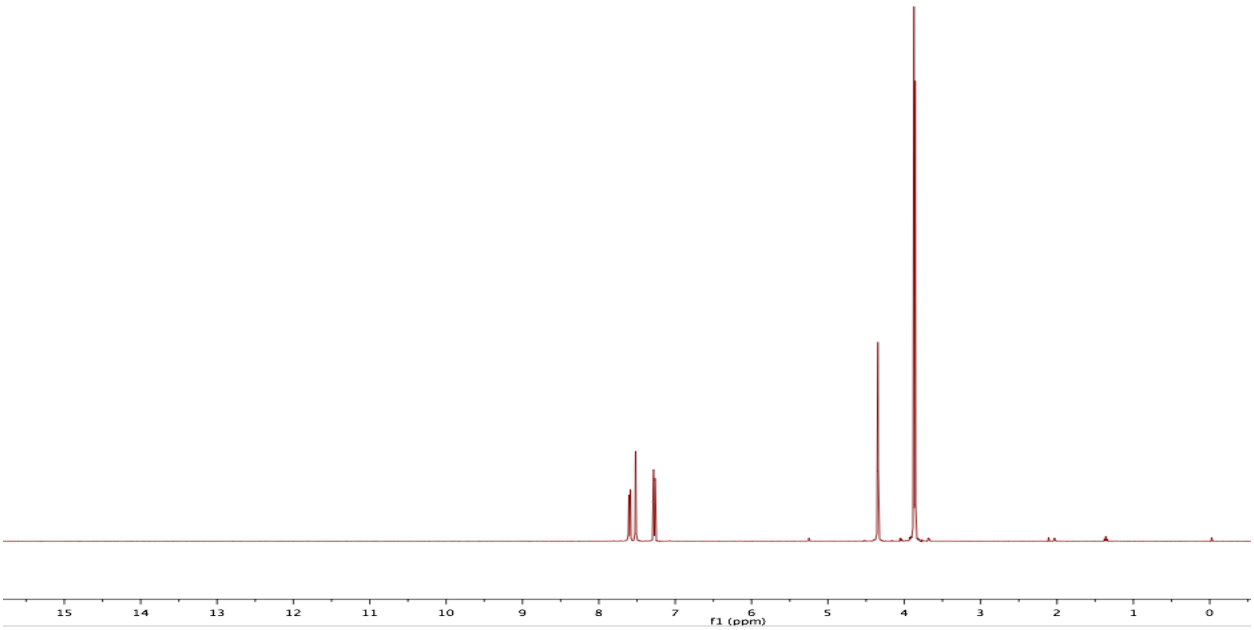

MeO
N
N
N
OMe

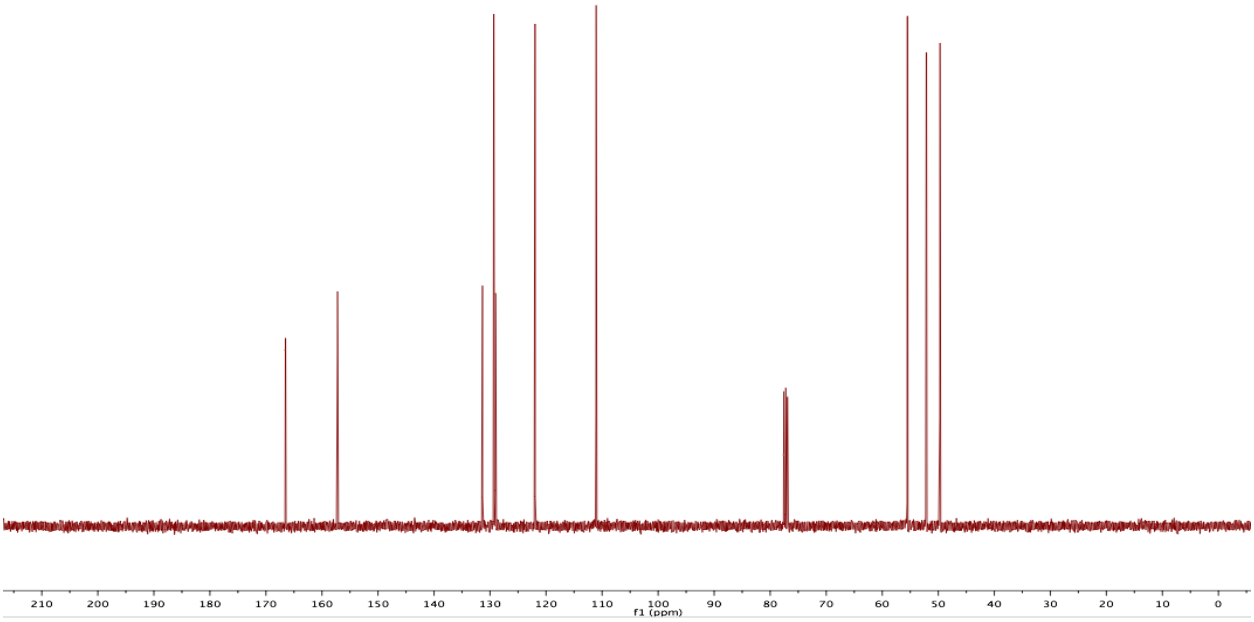

15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)


210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

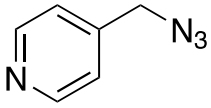
Compound 86

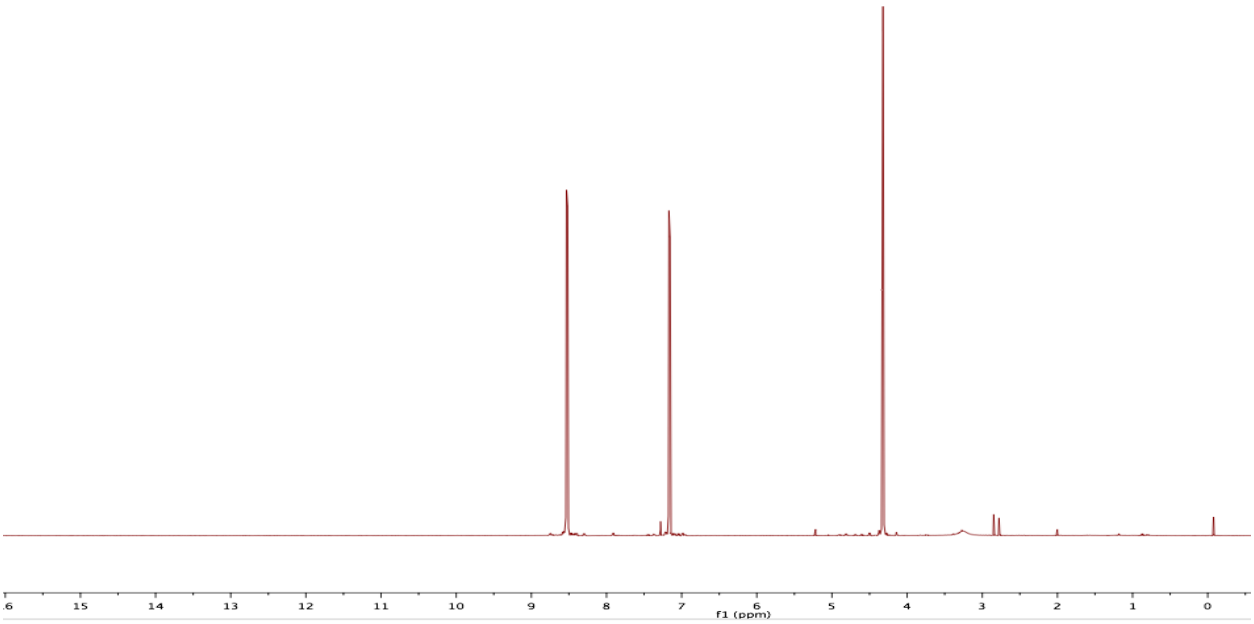

OMe
N3
MeO
O

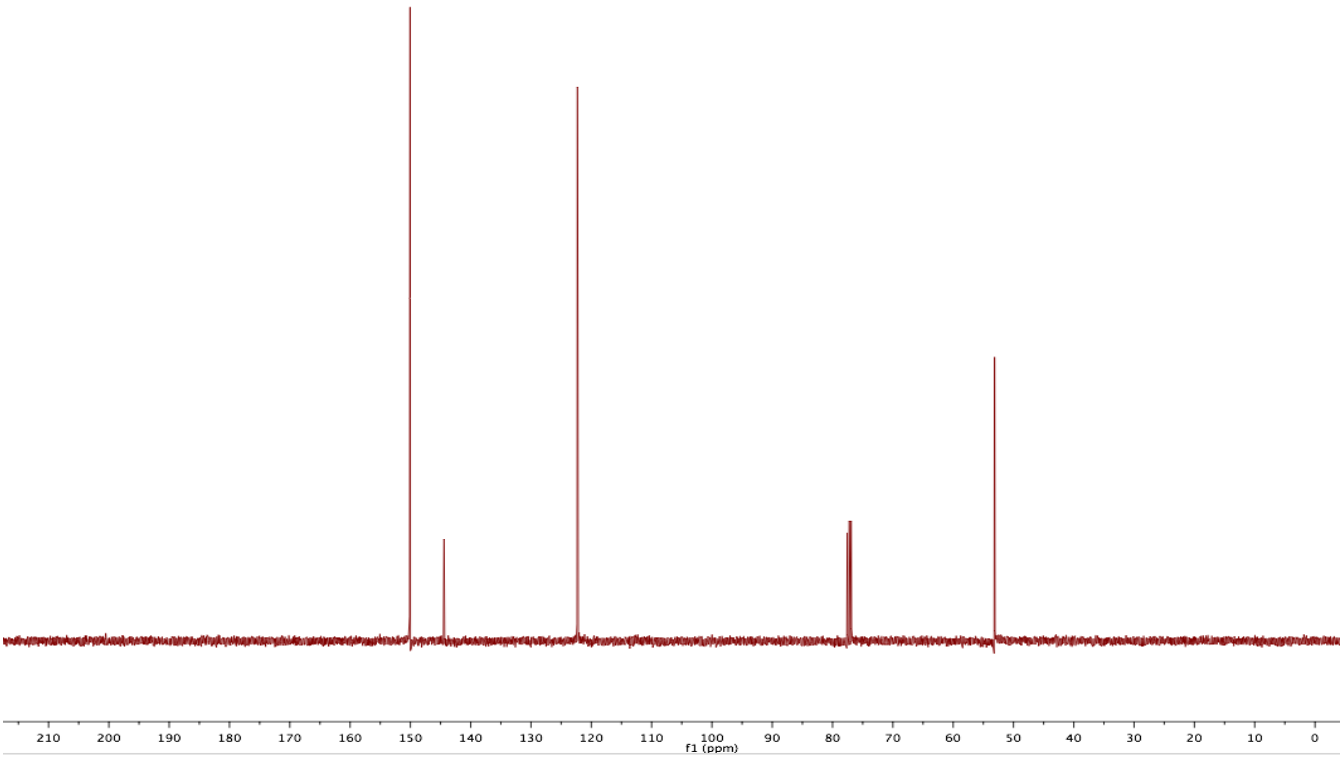

15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0
f1 (ppm)


210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

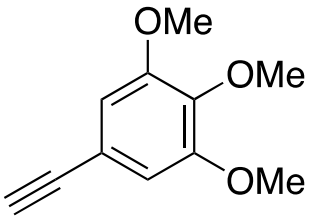
Compound 87

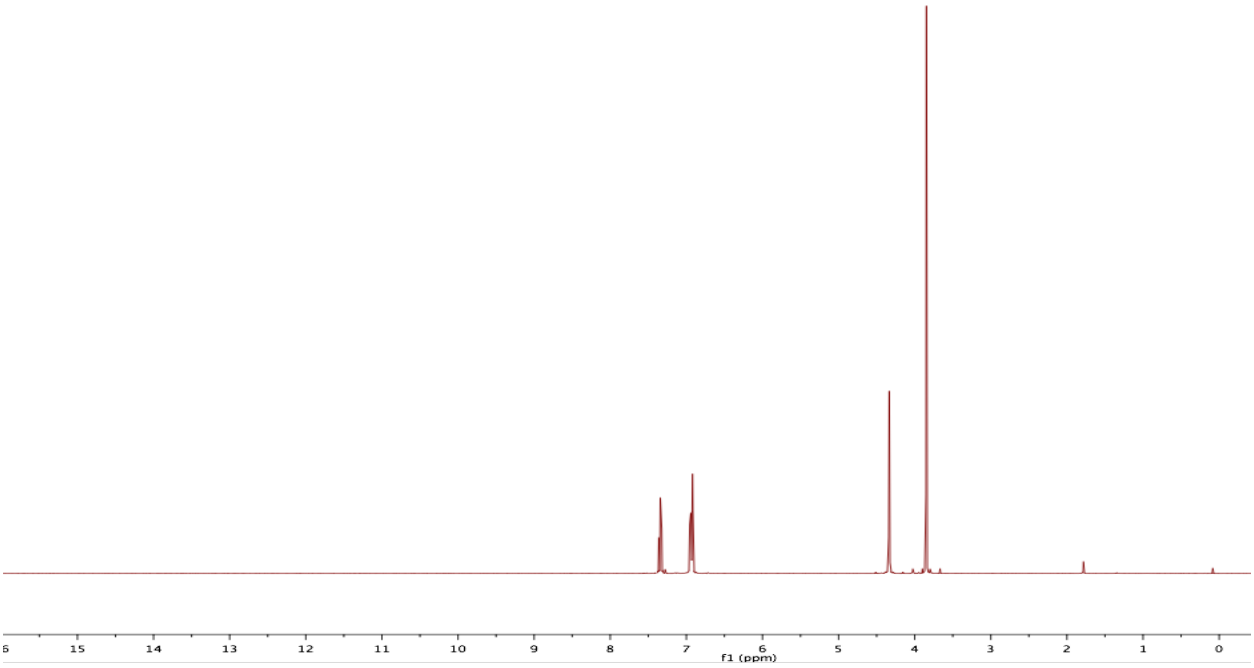


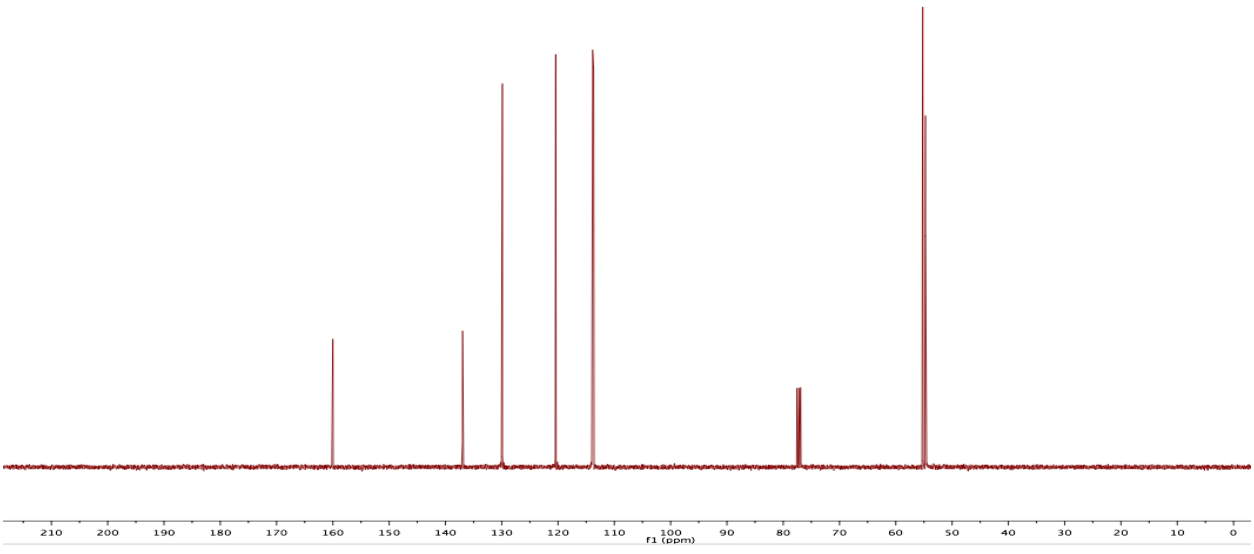




Compound 88







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