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Design and Synthesis of Novel Benzimidazole Library for the Discovery and

Development of the Next Generation Antibacterial Agents

A Thesis Presented

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Abstract of the Thesis

Design and Synthesis of Novel Benzimidazole Library for the Discovery and Development of the Next Generation Antibacterial Agents

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Tuberculosis, commonly known as "TB," is a contagious infection that is caused by exposure to *Mycobacterium tuberculosis*. Many current anti-TB drugs target bacterial cell wall synthesis, protein synthesis, and fatty acid synthesis. It is important to explore new bacterial targets in fighting the progression of tuberculosis. The bacterial cellular division process as an anti-TB target has not been fully explored and holds great potential in future combinatorial treatment of drug resistant tuberculosis. Since FtsZ (Filamental temperature-sensitive protein Z) is the most essential and abundant protein in bacterial

mitosis, more specifically the coordination of bacterial cytokinesis, it has been targeted as a means to eradicate tuberculosis. The specific targeting of FtsZ for the inhibition of bacterial growth is considered because of its notable similarities with eukaryotic tubulin, while maintaining key differences that can be manipulated for drug development.

Previous research has shown that tubulin polymerization is effectively inhibited by albendazole and thiabendazole. Since FtsZ assembly is the only known prokaryotic mechanism analogous to tubulin polymerization, it was suggested that albendazole and thiobendazole would also inhibit FtsZ at inhibitory concentrations. The compounds interfered and delayed *Mycobacterium tuberculosis* cellular division processes at 16 µg/mL. We have hypothesized that benzimidazoles, the core structure of FtsZ inhibitors, could be developed into broad-spectrum antibacterial agents with novel mechanisms of action. A library of trisubstituted benzimidazoles was synthesized by newly developed polymer-assisted solution phase methods. Several of the benzimidazole compounds exhibited < 0.5 µg/mL MIC_{99} activity in the preliminary screening against Mtb H37RV strain. According to polymerization assays, these compounds arrested Mtb growth by inhibiting FtsZ in dose dependent manner. Further optimization was pursued for diverse 2,5,6- and 2,5,7- trisubstituted benzimidazole compounds in the development of more effective antibacterial agents against tuberculosis.
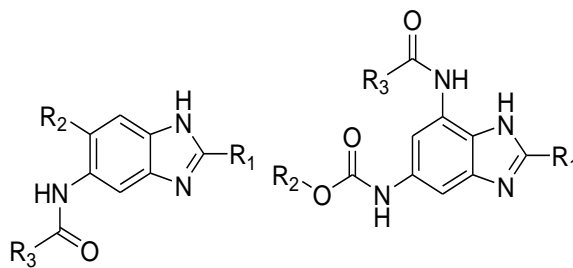
Trisubstituted Benzimidazole

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Chapter 1: Introduction

1.1 The Global Epidemic

In today's considerably advanced society, the global community has witnessed significant medical and scientific breakthroughs that have drastically influenced human existence. Many findings have led to the overall improvement of one's quality of life and the extension of life expectancy. However, numerous health concerns, such as the contraction of infectious diseases, remain as prevalent issues for researchers. Tuberculosis, commonly known as "TB," is a contagious infection that is caused by exposure to *Mycobacterium tuberculosis*, an airborne bacterium.[1] Tuberculosis has devastated many groups of people throughout the world. According to recent reports by the World Health Organization (WHO), "One in three people in the world is infected with dormant TB germs."[3] Approximately two billion people are infected with the microbes that cause tuberculosis and one in ten of those infected will develop active tuberculosis during their lifetime. Without taking appropriate measures to eradicate the disease, many people are at risk of developing drug resistant strains that make treatment difficult and nearly impossible.

A person infected with tuberculosis bacteria is not immediately sick; the body's immune system kills many tuberculosis bacteria that enter the lungs. However, the bacteria that do survive are captured inside macrophages and may remain in a dormant state for many years. The bacteria may leave its dormant state and become active when a person's physical state weakens or "reduce the person's immunity, such as HIV, advancing age, or some medical conditions."[2] When *Mycobacterium tuberculosis* in the

dormant state becomes active, which occurs "about 5 to 10% of infected people,"[1] the bacteria multiply and cause the infected person to become physically ill. Active tuberculosis may cause a wide range of medical problems: prolonged cough, chest pain, fever, chills, night sweats, appetite loss, weight loss, and fatigability. The bacteria mainly target the lungs, but may attack almost any bodily organ when spread through blood, which can be deadly if left untreated (Table 1).[1]

Tuberculosis: A Disease of Many Organs	
Site Infection	*Symptoms of Complications*
Abdominal cavity	Fatigue, swelling, slight tenderness, appendicitis-like pain
Bladder	Painful urination, blood in urine
Bones (mainly children)	Swelling, minimal pain
Brain	Fever, headache, nausea, drowsiness; coma and brain damage if untreated
Pericardium (the membrane around the heart)	Fever, enlarged neck veins, shortness of breath
Joints	Arthritis-like symptoms
Kidneys	Kidney damage, infection around the kidneys
Lymph nodes	Painless, red swelling; may drain pus
Reproductive organs	
Men	Lump in scrotum
Women	Sterility
Spine	Pain, leading to collapsed vertebrae and leg paralysis

Table 1.1: The Effects of Tuberculosis on Bodily Organs

While tuberculosis is in the active state, the infected person has the potential to spread the disease to other people through the air. Transmission may occur when an

infected person in the active state coughs, sneezes, or speaks and another person breathes in the contaminated air, which "can stay in the air for several hours."[1] This extremely simple way of transferring tuberculosis holds great potential for human disaster, particularly for people with impaired immunity, such as AIDS (individuals with the AIDS virus that have been infected with tuberculosis have "50% chance of developing active tuberculosis within 2 months and 5 to 10% chance of developing active disease each year thereafter.)[1] It is believed that "left untreated, each person with active TB disease will infect on average between 10 and 15 people every year."[3]

Tuberculosis remains as a leading killer from a single infectious disease.[4] According to "Global Tuberculosis Control: A short update to the 2009 report" by the World Health Organization, in 2008, there was a global incidence of approximately 9.4 million new TB cases (Figure 1).[5]
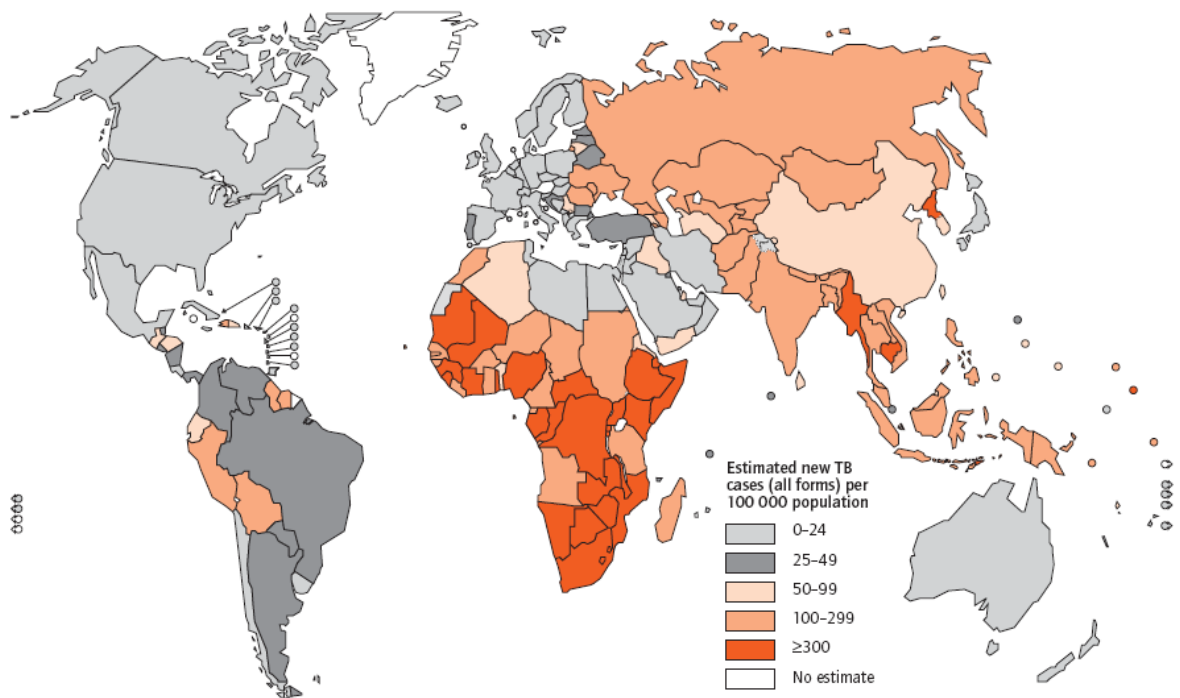
Figure 1.1: Estimated TB Incidence Rates, 2008[5]

Tuberculosis incidence rates maintain at relatively high numbers and "new cases arising each year is still increasing globally."[3] In many instances, tuberculosis persists due to poor chemotherapeutics, inadequate public health resources, reduced immune response due to AIDS, and extreme poverty that led to the eventual emergence of drug resistant strains of Mtb.[1,6] The new drug-resistant strains are very difficult and expensive to treat. When a strain of TB is resistant to two or more "first-line" antibiotics, it is classified as multi-drug resistant TB, or MDR-TB. A combination of second-line drugs is administered to treat MDR-TB. The second-line drugs are less effective than the first-line drugs, and contain more side-effects. Even further, when a strain of TB is resistant to three or more 'second-line' antibiotics, it is classified as extensively drug resistant TB, or XDR-TB. Often, this new stain of TB leaves patients untreatable by currently available anti-TB drugs, and therefore, further investigation of novel drug targets must be pursued to eradicate tuberculosis.

1.2 Current Treatments and Drugs for Tuberculosis

1.2.1 First-line Drugs

The standard treatment of tuberculosis entails two phases: the high intensity phase, and the continuation phase. The high intensity phase is the initial step of treatment. During this phase, a cocktail of rifampicin, isoniazid, ethambutal, and pyrazinamide is administered for two months (Figure 1.2). Then, the continuation phase immediately follows for a six month duration in which rifampicin and isoniazid are given.[7] Typically, two or more drugs with different mechanisms and targets are administered in order to avoid bacterial resistance mechanisms (Figure 1.3).[8] MDR strains are the result of

cumulative mutations that develop from inappropriate patient treatment, so more than one drug is generally given.[8]
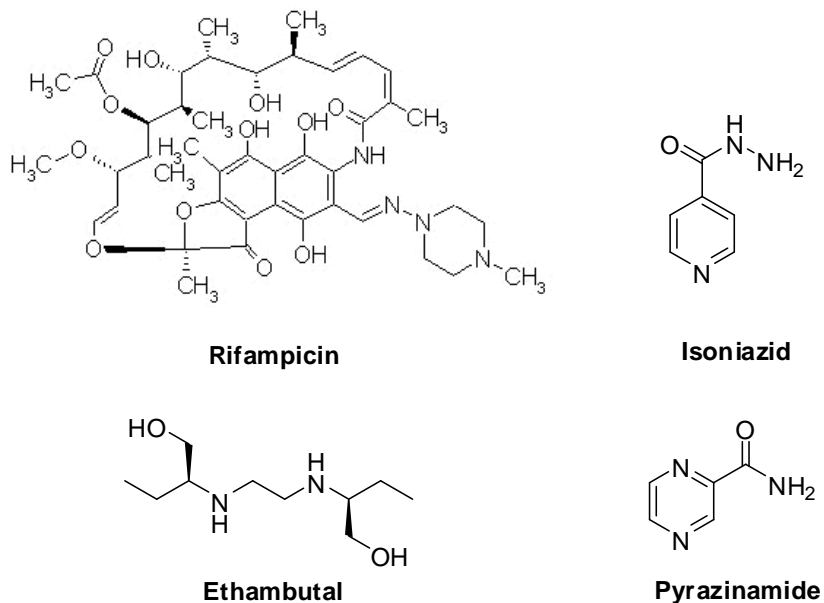
Figure 1.2: Chemical Structures of First-line Anti-TB Drugs

Rifampicin (RMP)[9,10,11,12,20,22]

Rifampicin or rifampin is a semisynthetic derivative of the rifamycins, which is a class of antibiotics that are fermentation products of *Nocardia mediterranei*. It is a broad spectrum agent that has anti-bacterial activity against mycobacteria and other bacteria. The standard dose is 10 mg/ kg of body weight. Rifampin is active at low concentrations (50 % effective dose, ~0.01 μg/mL) against mycobacteria and other gram-positive bacteria. Rifampin specifically inhibits bacterial RNA polymerase, the enzyme responsible for DNA transcription, by forming a stable and tight drug-enzyme complex. The antibiotic binds to the β complex of the core enzyme. The complex sterically blocks elongation of the RNA chain and bacterial growth is prevented.

Isoniazid (INH)[8,13,21,22]

Isoniazid is a narrow spectrum (anti-mycobacterial) agent. It is a prodrug that is activated by enzyme KatG, which maintains activity as catalase and peroxidase. Activated isoniazid causes inhibition of mycolic acid synthesis. Mycolic acid is a long chain fatty acid component of the mycobacterial cell wall. It is believed that two enzymes are targeted by the activated inhibitor: enoyl-acyl carrier reductase and β-ketoacyl-acyl carrier protein synthase. The elongation cycle of fatty acid biosynthesis becomes compromised by isoniazid. Isoniazid has the strongest early bactericidal action, which kills the bacteria, and thus, rendering the patients non-infectious.

Ethambutol (EMB)[14,15,22]

Ethambutol is a narrow spectrum (anti-mycobacterial) agent. It inhibits incorporation of D-arabinose into mycobacterial cell wall arabinogalactan. It is believed that inhibition by ethambutol depletes the sites for transfer of mycolic acid on the 5- position of the D-arabinose on the cell wall. The drug triggers a cascade of changes in the lipid metabolism of mycobacteria that eventually leads to cell death. Ethambutol has inhibitory activity at concentrations of ~3.0 µg/mL.

Pyrazinamide (PZA)[8,16,17,18,19,22]

Pyrazinamide is a narrow spectrum (anti-mycobacterial) agent. It is a nicotinamide analog that is believed to target an enzyme involved in fatty-acid synthesis. Pyrazinamide is a pro-drug that is converted to its active form, pyrazinoic acid (POA), by mycobacterial enzyme nicotinamidase/pyrazinamidase (PZase). First, the antibiotic passes into the

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bacterial cell by passive diffusion, but due to an inefficient efflux system, pyrazinamide builds up in the cytoplasm and becomes converted to its active form. As a result of drug accumulation, POA lowers the intracellular pH to inactivate fatty acid synthase I. Pyrazinamide becomes activated at acidic pH levels. Interestingly, studies have shown that pyrazinamide is especially active against old non-growing bacilli rather than young growing tubercle, which contrasts the function of current anti-TB antibiotics. Pyrazinamide has MIC values of 50-100 mg/L.
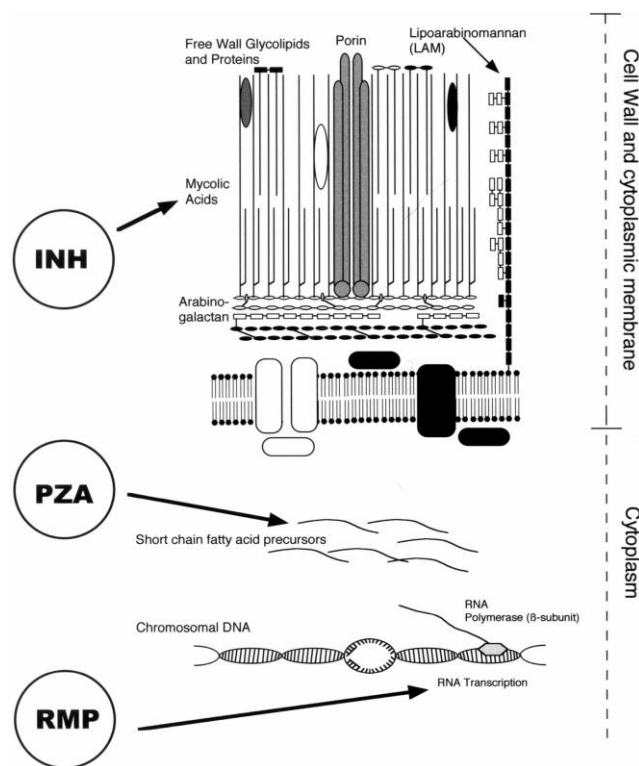
Figure 1.3: Proposed Sites of Action of Isoniazid (INH), Pyrazinamide (PZA), and Rifampin (RMP) on the M. tuberculosis cell[8]

1.2.2 Second-line Drugs

When TB treatment is compromised, multi-drug resistant tuberculosis (MDR-TB) develops. The strain is considered MDR-TB when the bacteria become resistant to two or

more "first-line" antibiotics, at least rifampin and isoniazid. Many studies have shown that a single genetic alteration has not yet been found. Rather, MDR-TB develops by sequential acquisition of mutations at different loci, usually because of inappropriate patient treatment and compliance.[8] The drug resistant mutants are able to replicate and eventually replace the drug sensitive population.[21] Consequently, drug resistant tuberculosis becomes more difficult to treat by standard antibiotics due to their compromised targets and mechanism of action.[20] In order to fight MDR-TB, first-line drugs are combined with second-line drugs. Treatment of MDR-TB involves the consideration of the patient's prior treatment history and adherence, as well as the results of susceptibility testing (Table 1.2).[21] In addition to supplementary medication, MDR-TB requires an extended treatment time of 18 to 24 months.

Drug	Formulation	Average daily dose	Type of antimycobacterial activity	Evaluation in clinical studies
Pyrazinamide	tablet (500 mg)	15–30 mg/g 1000–2000 mg daily	bactericidal at acid pH	+
Ethambutol	tablet (100, 400 mg)	15–30 mg/kg 800–1600 mg daily	bacteriostatic	+
Streptomycin	1 g vial, iv or im injection	20–40 mg/kg (1 g) once daily	bactericidal against exponential phase bacilli	+
Amikacin/kanamycin	500 mg and 1 g vial, iv or im injection	15–30 mg/kg (1 g) once daily	bactericidal against exponential phase bacilli	+
Capreomycin	1 g vial, iv or im injection	15–30 mg/kg (1 g) once daily	bactericidal against exponential phase bacilli	+
p-Aminosalicylic acid	granules (4 g packets); tablet 500 mg	8–12 g/day in two or three doses	bacteriostatic	+
Cycloserine	capsule (250 mg)	10–15 mg/kg usually 500–750 mg in two doses	bacteriostatic	+
Ethionamide/ prothionamide	tablet (250 mg)	15–20 mg/kg usually 500–750 mg/day in single daily or two divided doses	bactericidal	+
Ciprofloxacin	tablet (500 and 750 mg)	500–1000 mg daily	weakly bactericidal	+
Ofloxacin	tablet (400 mg)	400–800 daily	weakly bactericidal	+
Levofloxacin	tablet (500 mg)	500–1000 daily	bactericidal	+
Gatifloxacin	tablet (400 mg)	400 mg daily	bactericidal	−
Moxifloxacin	tablet (400 mg)	400 mg daily	bactericidal	−
Co-amoxiclav	tablet (1000/250 mg)	3000/750 mg in three doses	bactericidal against exponential phase bacilli	+/−
Clarithromycin	tablet (500 mg)	1000 mg	bacteriostatic	−
Linezolid	tablet and vial (600 mg)	1200 mg in two doses	bacteriostatic	−

Table 1.2: Second-line and Several First-line Anti-TB Drugs[21]

Streptomycin[7,20,21,22]

Streptomycin, a broad spectrum antibacterial agent, is a member of the aminoglycoside-aminocyclitol group. It has activity against gram-positive and gram-negative bacteria, and mycobacteria. It is believed that streptomycin binds irreversibly to a ribosomal subunit, which prevents initiation of protein synthesis and interferes with ongoing translation.

Aminoglycosides[7,21]

The aminoglycosides group includes amikacin and kanamycin, which are injectable second-line drugs. Aminoglycosides bind to the small bacterial ribosomal subunit, which compromises the translation process. It reduces the frequency of mRNA reading and in turn, protein synthesis. Aminoglycosides are specifically bacteriocidal against rapidly dividing mycobacteria. The drug fails to be effective against bacteria in the stationary phase.

Polypeptides[7,20,21]

The class of polypeptides includes capreomycin, viomycin, and several other drugs. Capreomycin is a second-line injectable drug. It is a narrow spectrum antibiotic that is produced by *Streptomyces capreolus*. The mechanism of action for capreomycin remains uncertain. However, it is believed that careomycin targets a ribosomal subunit to interfere with translation, and thus, protein synthesis. Viomycin is also known to inhibit the translation process.

p-Aminosalicylic acid (PAS)[7,22]

p-Aminosalicylic acid is a narrow spectrum, bacteriostatic anti-TB agent. Though the mechanism of *p*-aminosalicylic acid is unclear, some inconclusive evidence suggests that the antibiotic competitively blocks the conversion of para-aminobenzoic acid into folic acid. Alternatively, some studies suggest that PAS interferes with the biosynthesis of iron-chelating mycobactins. As a result, mycobacteria become iron deficient and unable to sustain microbial growth.

Thioamides[21,22]

The thioamides group includes ethionamide and prothionamide. Thioamides are bactericidal. It is believed that ethionamide's mechanism of action is similar to isoniazid; it includes a pro-drug activation step and the inhibition of mycolic acid synthesis.

Cycloserine[20,21,22]

D-cycloserine is a broad spectrum agent, which shows activity against a wide range of bacteria, including mycobacteria. It has been used to treat urinary tract infections, but it is mainly used as a second-line agent in combination therapy against MDR-TB. It is believed that D-cycloserine inhibits peptidoglycan synthesis. D-cycloserine, a structural analog of amino acid D-alanine, competitively inhibits D-alanine racemase and D-alanyl-D-alanine synthetase. The drug exhibits its bacteriostatic effect by competitively blocking two steps bacterial cell wall synthesis. Consequently, peptidoglycan in the cell wall is unable to form, thus compromising the structural integrity of the mycobacterial cell wall.

Fluoroquinolones[7,20,21,22]

Fluoroquinolones are considered broad spectrum agents, which show activity against a wide range of bacteria, including mycobacteria. The drugs are particularly effective because they are distributed broadly throughout the body, and also, within cells. Fluoroquinolones are synthetic derivatives of nalidixic acid and this class includes several drugs, such as ciprofloxacin, levofloxacin, ofloxacin, moxifloxacin, and gatifloxacin. It is believed that the antibiotic interacts with DNA gyrase (topoisomerase II) and DNA topoisomerase IV. Topoisomerase IV is not present in *M. tuberculosis*. DNA gyrase catalyzes the unwinding of double stranded DNA, which introduces negative supercoils to DNA. By inhibiting supercoiling, DNA topology dependent processes, such as chromosomal replication and transcription, are compromised. Fluoroquinolones also affect DNA topoisomerase IV, which unlinks DNA strands after chromosomal replication for daughter cell formation. Inhibition of this process interrupts bacterial cellular division. Fluoroquinolones possess the potential to penetrate macrophages and exert their anti-bacterial properties.

1.3 FtsZ: A Novel TB Drug Target

Many current anti-TB drugs target bacterial cell wall synthesis, protein synthesis, and fatty acid synthesis. It is seemingly important to explore new bacterial targets in fighting the progression of tuberculosis. The bacterial cellular division process as an anti-TB target has not been fully explored and holds great potential in future combinatorial treatment of drug resistant tuberculosis. When a host body is contracted with an infectious disease, the foreign agent multiplies in exponential numbers. Cellular growth is

dictated by the efficiency of its cellular division, i.e. mitosis. For bacterial mitosis, FtsZ (Filamental temperature-sensitive protein Z) is the most essential and abundant protein in the dividing process.[23] More importantly, FtsZ acts early in bacterial cellular division (Figure 1.4).[24]
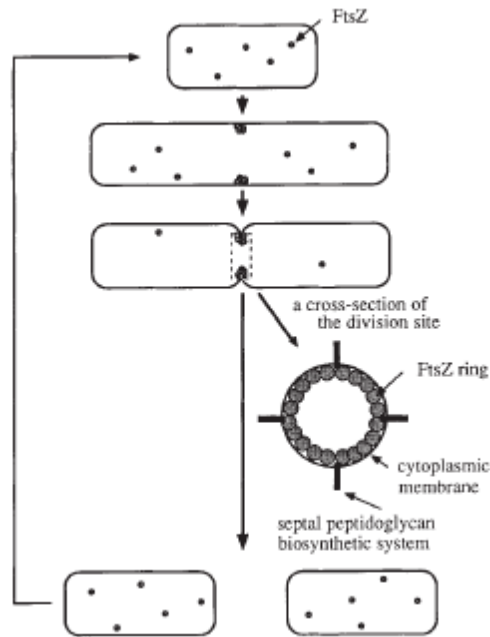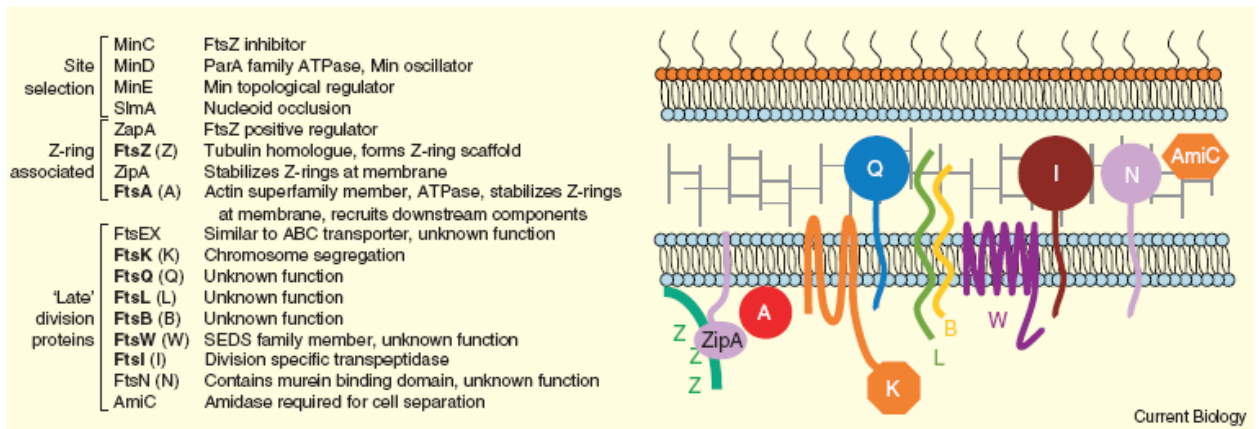

Figure 1.4: Role of FtsZ in Bacterial Cell Division[24]

At the site of future cellular division, FtsZ self-assembles into a ring-like structure on the cytoplasmic surface of the inner membrane.[24] FtsZ polymerizes in the presence of GTP through initiation at a single site mid-cell and appears to grow bidirectionally to form a highly dynamic helical structure.[23,25,26,27,28] The ring-like structure, also known as the Z-ring, is used as a scaffold for crucial cell division components.[25,29] FtsZ interacts with septal-specific peptidoglycan biosynthetic machinery[24] as well as other mitosis dividing proteins, such as FtsA, ZipA, and ZapA,[23] to form a large cell division complex or

divisome (Figure 1.5).[25] The key features, specifically linked to cellular division, of the complex consists of cytoplasmic, inner membrane and periplasmic components.

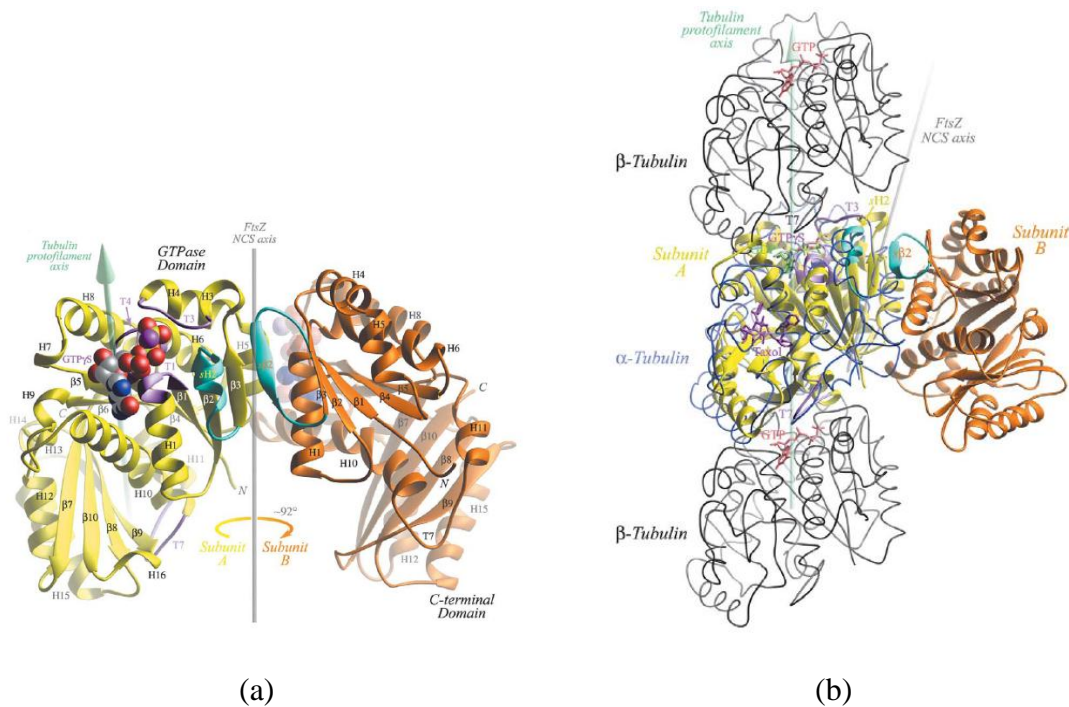**Figure 1.5**: Cell Division Proteins in Bacterial Cellular Division[25]

The Z-ring is extremely dynamic; it continuously remodels itself before and during constriction by its interaction with associated cell division proteins.[23,25,30] However, the driving force of bacterial cell division is still unclear. There are two leading hypotheses: polymer curvature due to GTP hydrolysis, and "purse string" model driven by depolymerization.[25] Several studies have shown that the size of the Z-ring decreases in diameter as septation occurs.[24] Furthermore, depolymerization of the Z-ring leads to constriction and the formation of septum. Eventually, the Z-ring disperses and two daughter cells are formed (Figure 1.6).[23,25]
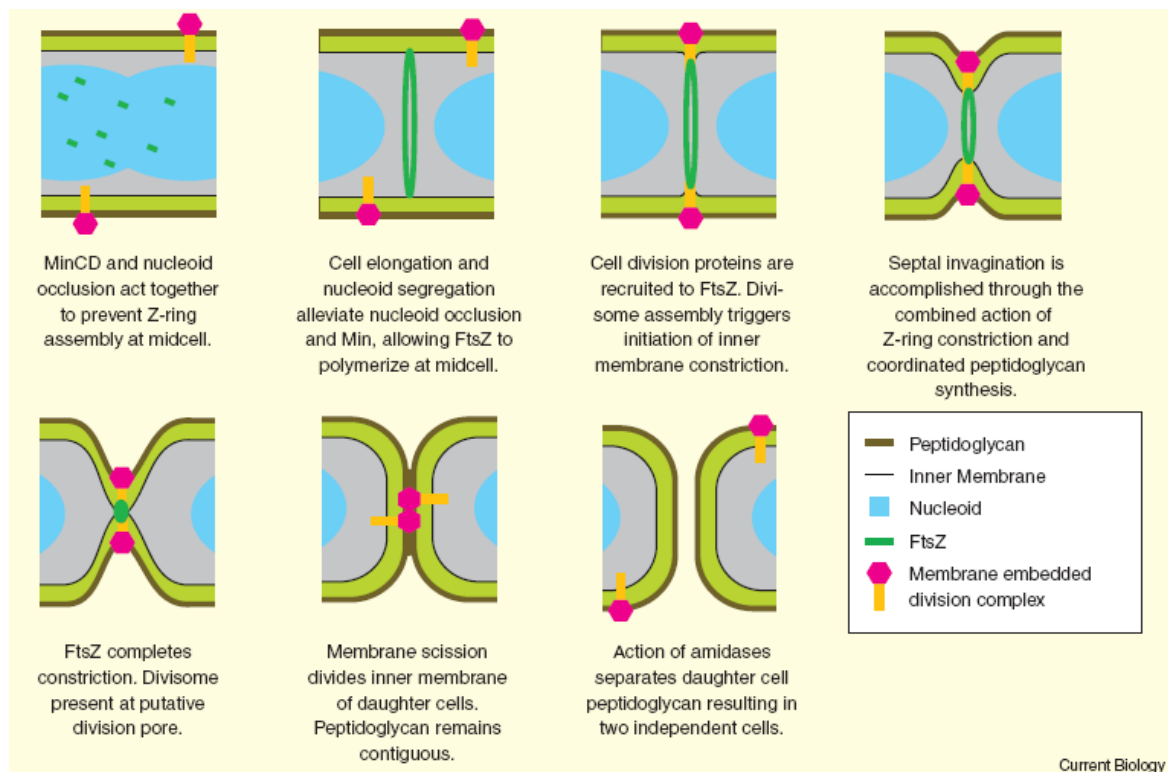
Figure 1.6: Overview of Bacterial Cell Division[25]

The specific targeting of FtsZ for the inhibition of bacterial growth is considered because of its notable similarities with eukaryotic tubulin, while maintaining key differences that can be manipulated for drug development. Tubulin and FtsZ polymerize in a GTP dependent manner.[25] Also, the assembly and disassembly of tubulin polymers and FtsZ polymers are greatly influenced by its associated proteins. More importantly, FtsZ shares a common amino acid sequence, GGGTGTG, to the eukaryotic tubulin signature motif (found in all α, β, and γ- tubulins), (G/A)GGTGSG.[23,31,32,33] In many cases, protein homologs of eukaryotes and prokaryotes indicate shared functionality.[31] In addition to amino acid sequence similarity, FtsZ and tubulin share a common fold, comprised of two domains linked by an α-helix,[34] which is involved in protofilament formation. Despite the similarities between the two proteins, FtsZ and tubulin share a limited sequence

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homology of 10- 18 %, which can be utilized to develop a non-cytotoxic anti-bacterial agent.[23] The two proteins maintain different shapes, as dictated by their subunits. According to crystallographic studies, MtbFtz subunits are associated laterally, unlike tubulin protofilaments, which are associated longitudinally (Figure 1.6).[26]
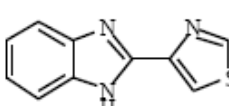

<center>(a)   (b)</center>
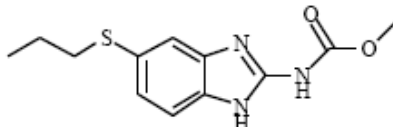
Figure 1.7: Crystal Structures of MtbFtsZ Dimer and Tubulin Protofilaments[26]

(a) MtbFtsZ dimer viewed from inside; (b) αβ-tubulin protofilaments

Since FtsZ has been shown to be a crucial element in bacterial cell division, FtsZ is seemingly an important anti-tuberculosis target that holds great potential for effective treatment. It has structural and functional homology, while maintain limited sequence homology (less than 20 % identity). Known inhibitors of tubulin can be explored for effectiveness against FtsZ, without compromising eukaryotic cells.

1.4 Drug Development

The Southern Research Institute (SRI) performed Mtb inhibition studies with known tubulin inhibitors.[35-37] Several pyridopyrazine and pteridine compounds were found to have anti-TB activity. Also, Slayden *et al* carried out Mtb inhibition studies with albendazole and thiabendazole.[23] Since FtsZ assembly is the only known prokaryotic mechanism analogous to tubulin polymerization, it was suggested that albendazole and thiobendazole would also inhibit FtsZ at micromolar concentrations.[38,39,40] Thiabendazole and albendazole were found to interfere and delay *Mycobacterium tuberculosis* cellular division processes at 16 μg/mL (figure 1.7).[23,38] The results indicate that thiabendazole and albendazole interfere with Mtb cell division processes at inhibitory concentrations.[23]

Compound	MIC$_{99}$ (H37Rv)
Albendazole	16 μg/mL (61 μM)
Thiabendazole	16 μg/mL (80 μM)

Table 1.3: Polymerization Inhibitors for FtsZ[36]

In addition to cell viability, inactivation of FtsZ polymers results in bacterial cell elongation.[23] Fluroescence-emission image studies have shown that inhibition results in an increase of cell size to $6.2 \pm 1.4 - 9.6 \pm 1.9$ μm (wild-type cells are rod-shaped and are 2.3 ± 0.97 μm long). The elongated cells appear filament-like (figure 1.8).[38,39]
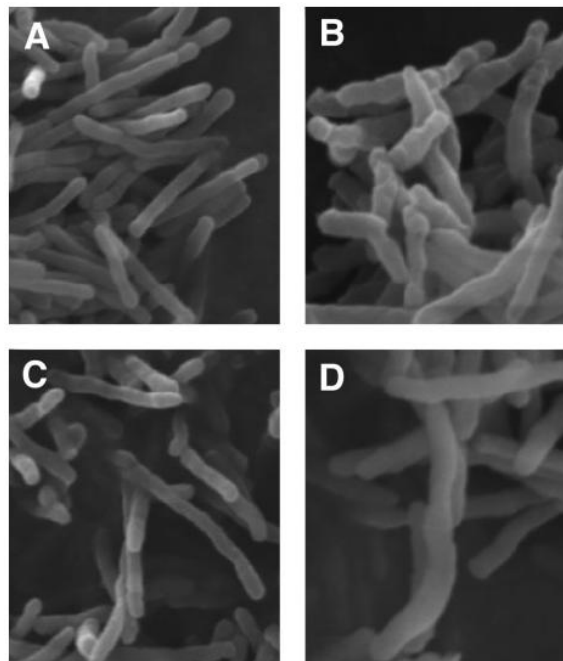
Figure 1.8: Mtb w/ Albendazole and Thiabendazole[38]
(a) Inhibition of FtsZ with 60 µM albendazole for 3 days; (b) Inhibition of FtsZ with 60 µM albendazole for 5 days; (c) Inhibition of FtsZ with 80 µM thiabendazole for 3 days; (d) Inhibition of FtsZ with 80 µM thiabendazole for 5 days

The core structure of pyridopyrazine, pteridine, thiabendazole and albendazole, benzimidazole, was used as a starting point to create a diverse library that would interfere with FtsZ. Extensive literature searches have shown that tribsubstituted benzimidazoles have not been tested for anti-tuberculosis activity. A library of trisubstituted benzimidazole compounds was synthesized by the Ojima lab using novel polymer-assisted solution phase methods. Optimization of the bacterial inhibitor was performed by altering substituents on active positions on benzimidazole (Figure 1.9).
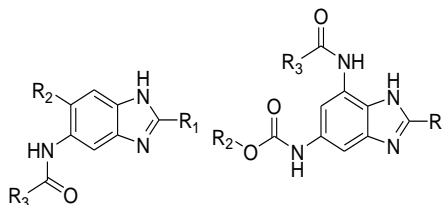
Figure 1.9: Trisubstituted Benzimidazole

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A library of 349 compounds were synthesized and screened against H37Rv (drug sensitive Mtb strain) for its antibacterial activities. In preliminary screening, 26 compounds exhibited moderate MIC_{99} activity, of which 9 compounds (Figure 1.10) exhibited $MIC_{99} \leq 6$ mg/mL.[42] The 9 active compounds were resynthesized and sent to Dr. Richard Slayden's laboratory at Colorado State University for confirmation of MIC_{99} values and they tested positive for antibacterial activity.
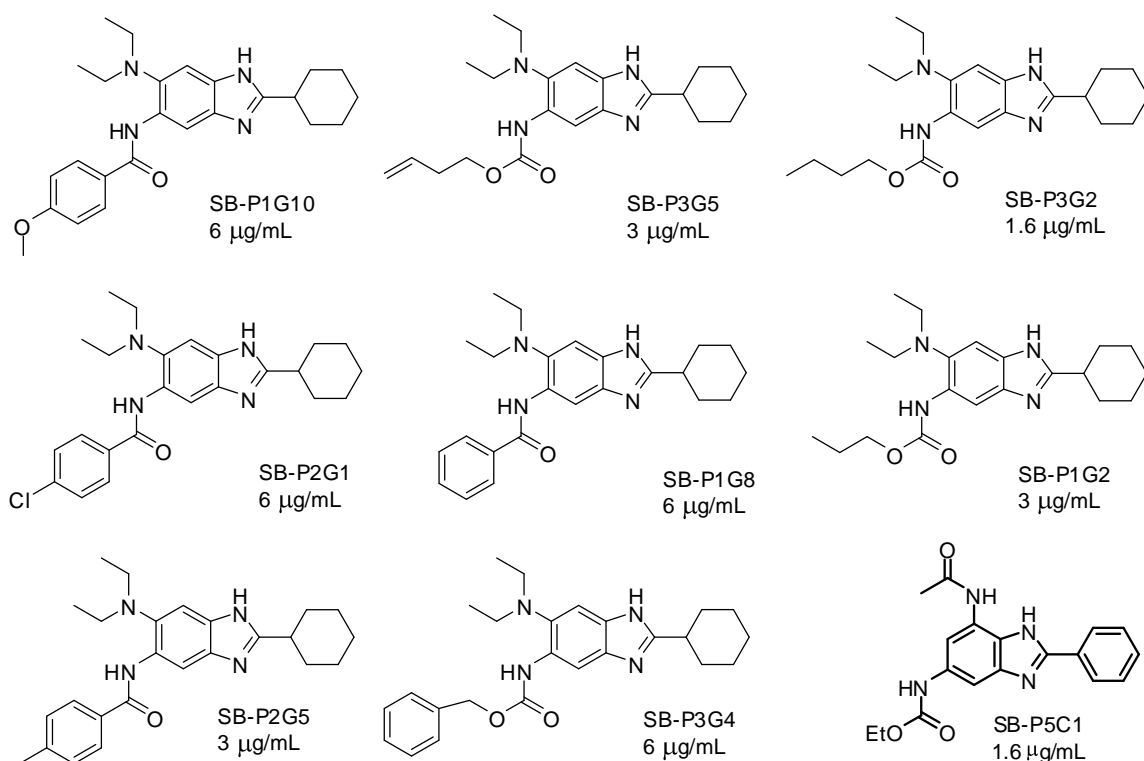
Figure 1.10: Nine Most Active Benzimidazole Compounds

1.5. Light Scattering Studies

To confirm that these 8 compounds indeed inhibit bacterial growth by interacting with FtsZ assembly, light scattering experiments were conducted on FtsZ (de)polymerization by using trisubstituted benzimidazoles SB-P3G5, SB-P3G2, SB-P1G2, and SB-P1G8 (Figure 1.11).[42] It was proven that the degree of FtsZ

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polymerization inhibition corresponded with the varying concentration amounts of benzimidazole. The Ojima lab found that SB-P3G5, at 10-20 μM concentration, had 50% inhibition and polymerization was completely halted at 40 μM concentration; SB-P1G2, at 20 μM concentration, had 50% inhibition and no significant inhibition at 10 μM concentration; and SB-P1G8 and SB-P3G2, at 10 μM, have 50% inhibition and polymerization was completely shut down by 20 μM. Thus, these novel trisubstituted benzimidazole compounds inhibit FtsZ polymerization, which, in turn, modulate Mtb growth.
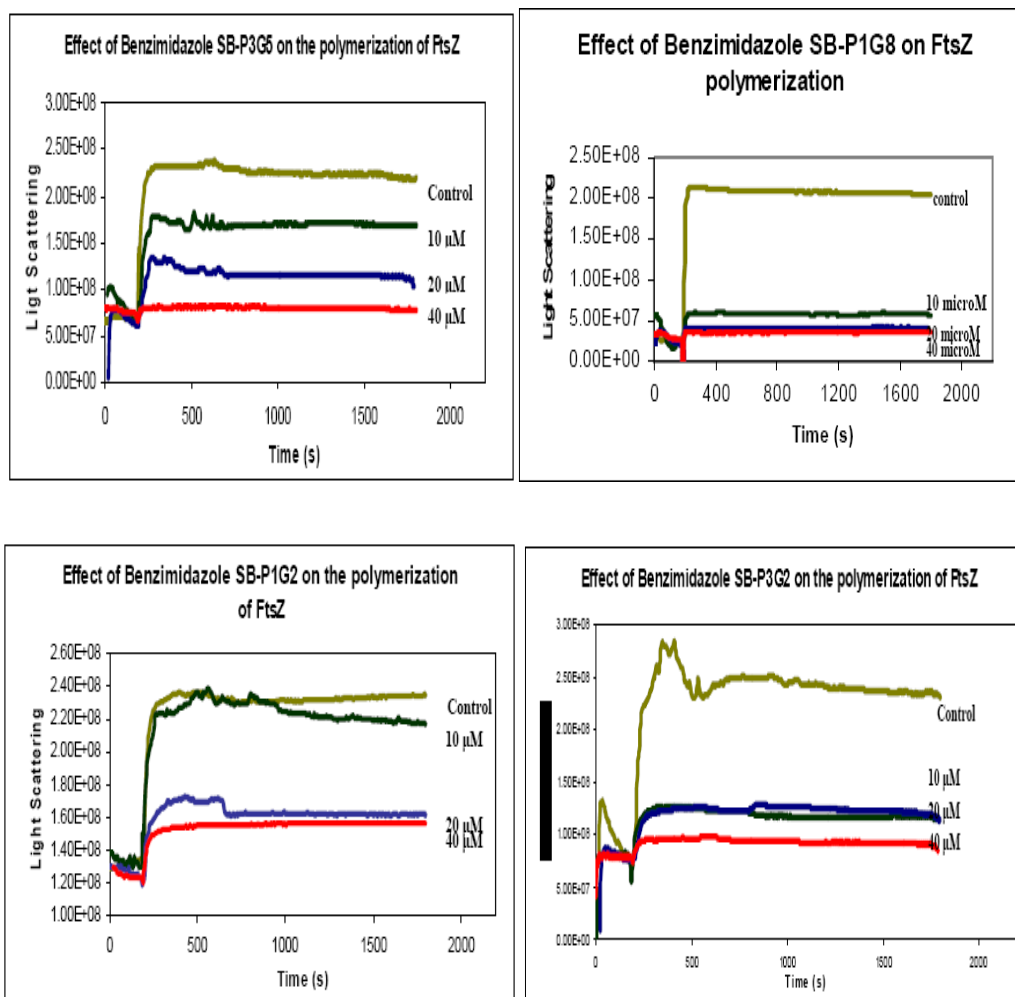
Figure 1.11: Effect of Benzimidazoles on FtsZ Polymerization

1.6 Development of Novel Benzimidazoles

Despite achieving significant inhibitory activity, further optimization of trisubstituted benzimidazoles is desired. The SAR study shows that the diethylamino group at the 6 position and the cyclohexyl group at the 2 position are important for inhibitory activity. The synthesis of more diverse 2,5,6- trisubstituted benzimidazole compounds and 2,5,7-trisubstituted benzimidazole compounds, as well as, the resynthesis of active compounds for confirmation of MIC_{99} values are later described in subsequent chapters.

Chapter 2: Results and Discussion

2.1 Synthesis of Novel 2,5,6-Benzimidazoles

The 2,5,6-trisubstituted benzimidazoles were synthesized as outlined in Scheme **2.1**. The aromatic nucleophilic substitution of commercially available 2,4-dinitro-5-fluoroaniline with N-Boc-piperazine afforded compound 1.1. The acylation of 1.1 with cyclohexylcarbonyl chloride generated acylated intermediate 1.2. Tin (II) chloride mediated reduction and cyclization of intermediate 1.2 afforded intermediate 1.3. The intermediate 1.3 was subjected to acylation conditions to generate novel benzimidazole libraries (**Scheme 2.1**).
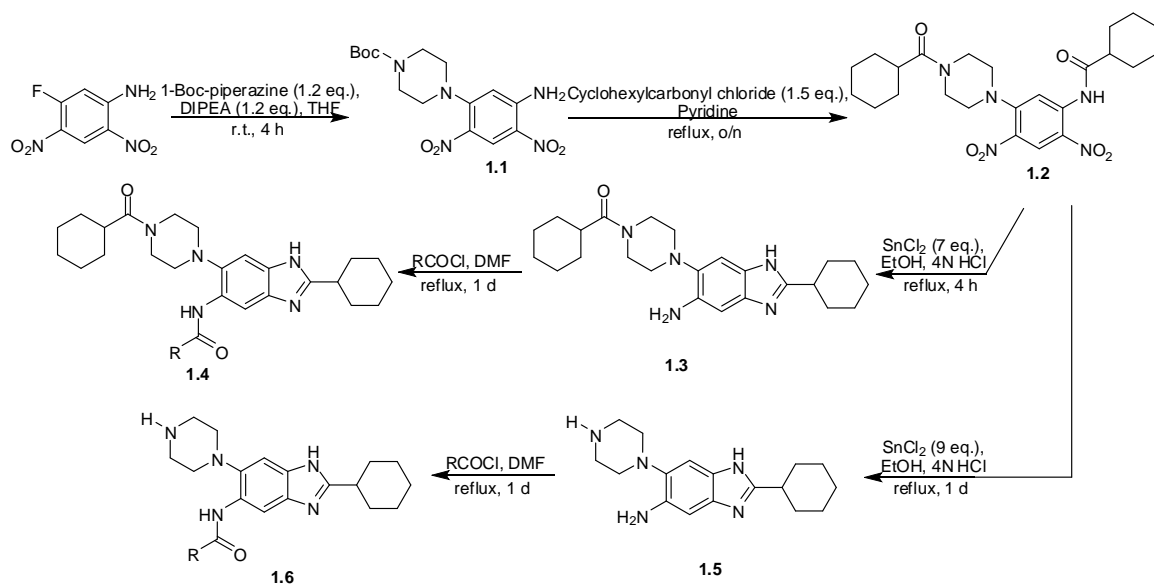
Scheme 2.1: Novel 2,5,6-Benzimidazoles

2.2 Resynthesis of Active 2,5,6-Benzimidazoles

The 2,5,6- trisubstituted benzimidazoles were synthesized as outlined in **Scheme 2.2**. The aromatic nucleophilic substitution of commercially available 2,4-dinitro-5-fluoroaniline with piperidine afforded compound **2.1**. The acylation of **2.1** with the respective substituted acid chloride generated acylated intermediate **2.2**. Tin (II) chloride mediated reduction and cyclization of intermediate **2.2** afforded intermediate **2.3**. The intermediate **2.3** was subjected to acylation conditions to generate benzimidazole libraries of hit compounds (**Scheme 2.2**).

21
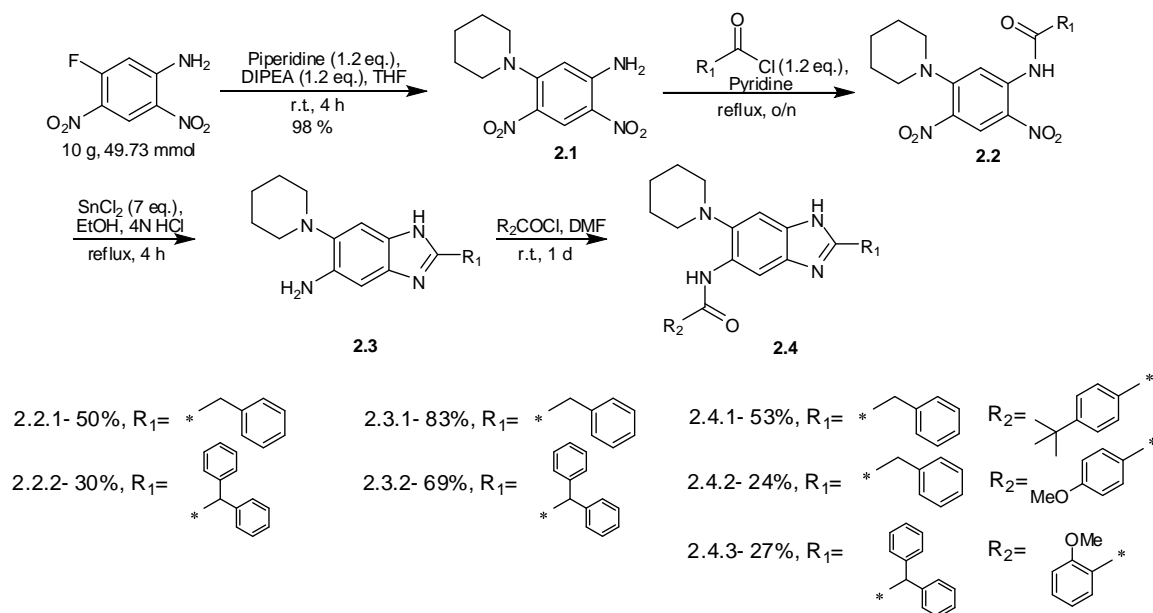
Scheme 2.2: Active 2,5,6-Benzimidazoles

2.3 Synthesis of Novel 2,5,7-Benzimidazoles

The 2,5,7- trisubstituted benzimidazoles were synthesized as outlined in **Scheme 2.3**. Commercially available 5-amino-2,4-dinitrobenzamide was hydrolyzed in presence of HCl to obtain 4-amino-3,5-dinitrobenzoic acid. The acid was converted to the acyl chloride intermediate **3.1**, followed by treatment with sodium azide to afford acyl azide which then, underwent Curtius Rearrangement to form corresponding isocyanate. The isocyanate was subsequently treated with various alcohols to obtain compound **3.2** as a bright red solid. Reduction of intermediate **3.2** followed by treatment with bisulfate salts of aldehyde afforded compound **3.3**. Final acylation of free aromatic amine generated a library of novel benzimidazoles **3.4** (**Scheme 2.3**).
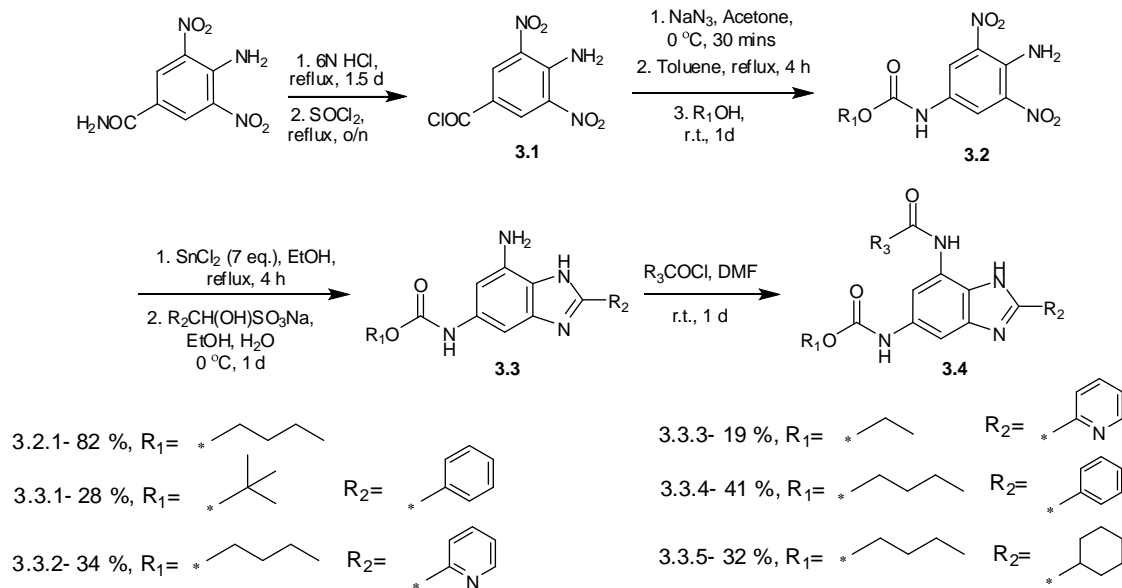
Scheme 2.3: Novel 2,5,7-Benzimidazoles

Scheme 2.3 has been slightly modified to improve product yield. Previously, the reduction and cyclization steps were carried out under Pd conditions (see **Figure 2.1**). Ever since the conditions were changed to a one pot system, typical yields have been improved from 18-30 % up to 83%.
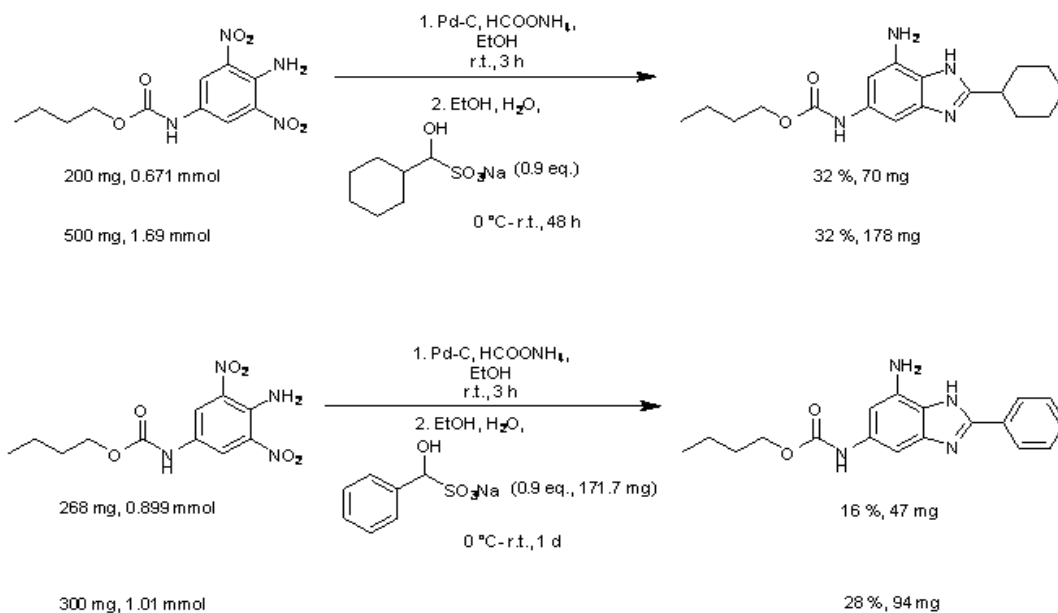
Figure 2.1: Previous Reduction and Cyclization Conditions for 2,5,7- Benzimidazoles

2.4 Synthesis of Bisulfite Salts

The bisulfite salts for 2,5,7- trisubstituted benzimidazoles were synthesized as outlined in

Scheme 2.4. The respective aldehydes are treated with sodium bisulfite to form salts for

2,5,7-benzimidazole formation (**Scheme 2.4**).
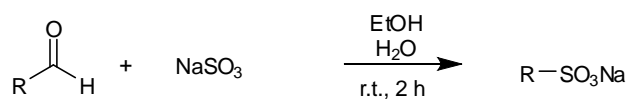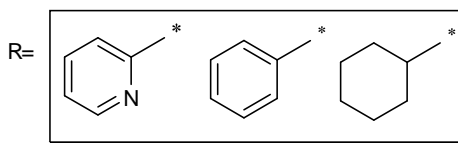
Scheme 2.4: Bisulfite Salts for 2,5,7- Benzimidazoles

2.5 Synthesis of Trisubstituted Benzimidazole Library with Variation at C6 or C7
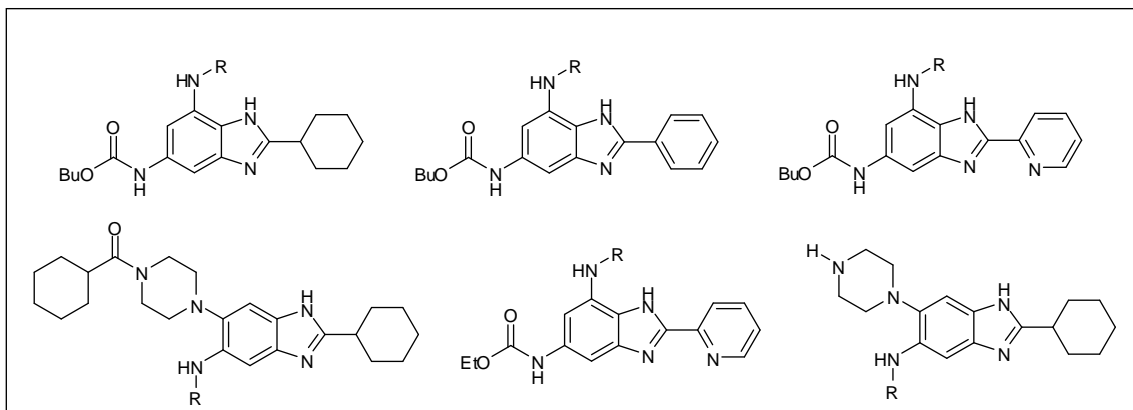
Figure 2.2: Trisubstituted Benzimidazole Intermediates for Library Synthesis

Six final trisubstiuted benzimidazoles were used for library synthesis (**Figure 2.2**). The

final intermediates (0.005 mM) were dissolved in dichloromethane and transferred into

ninety-six well plates. Twelve different acylating agents, isocyanates, and thiocyanates

(1.1 eq.) were dissolved in dichloromethane and added to the individual wells (**Figure 2.3**).
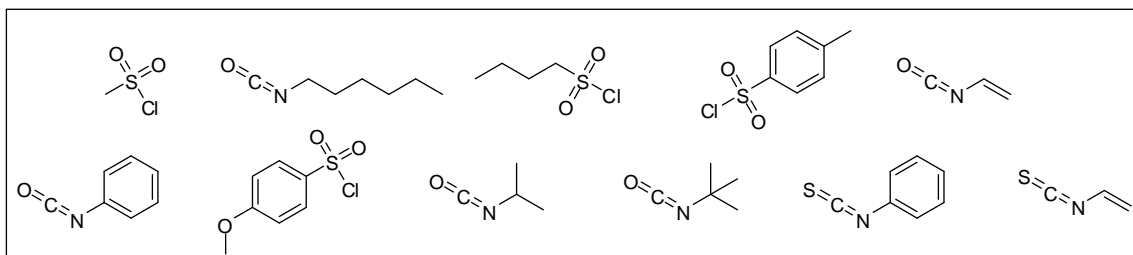
Figure 2.3: Reagents Used in Library Synthesis

The plates were slowly shaken for a day. Aminomethylated resins (10 eq.) were added and shaken for a day to remove excess reagent. Filtering of the resins with dichloromethane produces a library of tribsustituted benzimidazoles (**Scheme 2.5**).
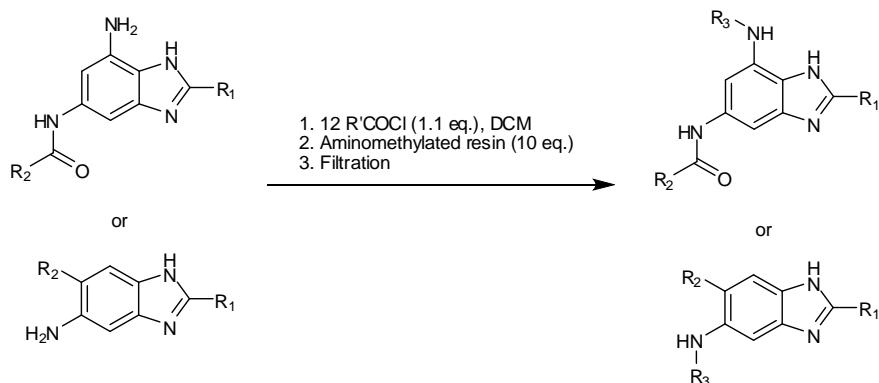
Scheme 2.5: Library Synthesis of Trisubstituted Benzimidazoles

Materials: All chemicals were obtained from commercial sources (VWR, Sigma and Aldrich). Tetrahydrofuran and dichloromethane were dried by passing through drying columns on the Innovative Technologies, Inc. solvent purification system. NMR spectra were recorded on a Varian 300, 400, or 500 NMR spectrometer. The ^{1}H and ^{13}C spectra were calibrated using residual solvent peak as the internal standard ($CDCl_3$: 7.26/77.00ppm). Melting points were measured on a Thomas Hoover Capillary melting point apparatus and are uncorrected. TLC was performed on Merck DC-alufolien with Kieselgel 60F-254 and column chromatography was carried out on silica gel 60 (Merck; 230-400 mesh ASTM) or aluminum oxide (Alfa Aesar; 60 mesh). Mass to charge values were measured by flow injection analysis on an Agilent Technologies LC/MSD VL. High resolution mass spectra were obtained from the Mass Spectrometry Laboratory, University of Illinois at Urbana-Champaign, Urbana, IL.

4-*tert*-Butyl 4-(5-amino-2,4-dinitrophenyl)piperazine-1-carboxylate (1.1):
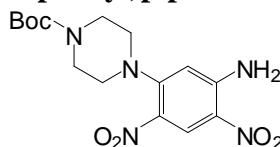
A solution of 4-*tert*-butyl 1-piperazine carboxylate (3.3 g, 18 mmol) in 85 mL of THF was added dropwise to a magnetically stirred solution of 2,4-dinitro-5-fluoroaniline (3.0 g, 15 mmol) and *N*, *N*-diisopropylethylamine (3.1 mL, 18 mmol) in 100 mL of THF. The reaction mixture was stirred at room temperature for 4 h. The solvent was evaporated on a rotary evaporator. The residue was washed with water and dichloromethane three times. The organic layers were dried with magnesium sulfate, filtered, and concentrated to afford the product *tert*-butyl compound **1.1** as a yellow solid (5.018 g, 91.6 %): mp 199-200 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (s, 9 H), 3.09 (t, *J* = 4.8 Hz, 4 H), 3.61 (t, *J* = 5 Hz, 4 H), 6.16 (s, 1 H), 8.91 (s, 1 H); LRMS (ESI) *m/z* cald. 367, found 368.1 (M+1). All data were found to be in agreement with literature values.[42]

N-[5-(4-Cyclohexanecarbonylpiperazin-1-yl)-2,4-dinitrophenyl]cyclohexane carboxamide (1.2):
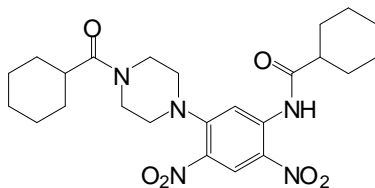
To a solution of **1.1** (5.0 g, 14 mmol) in 15 mL of pyridine, cyclohexanecarbonyl chloride (2.99 g, 20.4 mmol) was added and magnetically stirred. The mixture was microwaved at 100 °C for 50 min. The reaction mixture was diluted with dichloromethane and washed with water three times. The organic layers were dried with magnesium sulfate, filtered, and concentrated. After workup, the residue contained the product **1.2**. The mixture was purified by flash chromatography on silica gel (15- 25% ethyl acetate/hexane) to give compound **1.2** as yellow crystals: mp 123-124 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.2525

(m, 11 H), 1.554 (s, 9 H), 3.309 (m, 4 H), 3.764 (m, 4 H), 8.693 (s, 1 H), 8.927 (s, 1 H), 10.937 (s, 1 H); LRMS (ESI) *m/z* cald. 487, found 488.2 (M+1).

## 6-(4-Cyclohexanecarbonylpiperazin-1-yl)-2-cyclohexyl-1H-1,3-benzodiazol-5-amine (1.3):
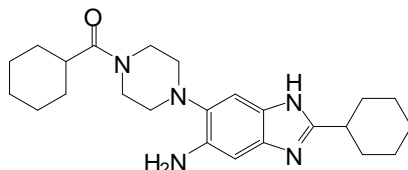
A solution of **1.2** (1.0 g, 2.1 mmol), tin(II) chloride (2.8 g, 15 mmol), and 2N HCl (8 mL) in 42 mL of EtOH was magnetically stirred and refluxed for 4 h. The reaction mixture was cooled and quenched with 30 % NaOH until ~ pH 10. Tin(II) chloride precipitated in solution upon addition of 30 % NaOH. The solution was decanted from the tin(II) chloride solid layer. The solution was washed with water and ethyl acetate three times. The organic layers were dried with magnesium sulfate, filtered, and concentrated. The residue was purified by flash chromatography on aluminum oxide using MeOH/DCM (1:20) as eluent to afford compound **1.3** as a tan solid (586 mg, 70 %): mp 157-160 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.213- 1.826 (m, 20 H), 2.064 (m, 4 H), 2.836 (m, 4 H), 5.290 (s, 2 H), 6.855 (s, 1 H), 7.170 (s, 1 H); ^{13}C NMR (400 MHz, CDCl$_3$) δ 25.605, 25.911, 29.324, 31.762, 38.433, 40.214, 42.305, 46.081, 51.741, 52.367, 135.482, 137.545, 158.379, 174.762; LRMS (ESI) *m/z* cald. 409, found 410.2 (M+1); HRMS (FAB) *m/z* calcd for C$_{24}$H$_{36}$N$_5$OH+: 410.2919, Found: 410.2920 (Δ= -0.2 ppm).

5-Amino-2-cyclohexyl-6-(piperazin-1-yl)-1H-1,3-benzodiazole (1.5):
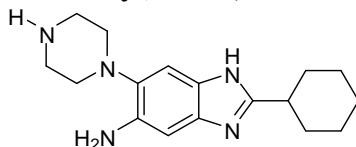
A solution of **1.2** (920 mg, 1.92 mmol), tin (II) chloride (3.28 g, 17.3 mmol), and 4N HCl (16 mL) in 34 mL of EtOH was magnetically stirred and refluxed for 1 day. The reaction mixture was cooled and quenched with 30 % NaOH until ~ pH 10. Tin(II) chloride precipitated in solution upon addition of 30 % NaOH. The solution was decanted from the tin(II) chloride solid layer. The solution was washed with water and ethyl acetate three times. The organic layers were dried with magnesium sulfate, filtered, and concentrated. The residue was purified by flash chromatography on aluminum oxide using MeOH/DCM (1:20) as eluent to afford compound **1.5** as a tan solid (124 mg, 21 %): mp 168-170 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 2.767- 2.875 (m, 9 H), 3.026 (t, 5 H, *J*= 5.1 Hz), 3.300 (m, 2 H), 3.594 (q, 2 H, *J*= 7.2 Hz), 6.891 (s, 1 H), 7.157 (s, 1 H); ^{13}C NMR (500 MHz, CD$_3$OD) δ 26.699, 27.023, 27.347, 27.961, 32.745, 33.065, 33.313, 39.656, 40.171, 47.651, 47.926, 52.575, 53.353, 54.158, 99.877, 100.979, 107.410, 108.097, 133.939, 136.125, 138.463, 138.497, 138.703, 139.561, 139.657, 139.687, 159.438; LRMS (ESI) *m/z* cald. 409, found 410.2 (M+1); HRMS (FAB) *m/z* calcd for C$_{17}$H$_{26}$N$_5$H+: 300.2182, Found: 300.2188 (Δ= -2.0 ppm).

2,4-Dinitro-5-(piperidin-1-yl)aniline (2.1):
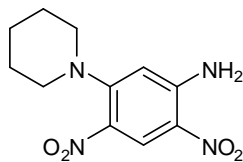
A solution of piperidine (2.95 mL, 29.8 mmol) in 50 mL of THF was added dropwise to a magnetically stirred solution of 2,4-dinitro-5-fluoroaniline (5.0 g, 25 mmol) and *N*, *N*-diisopropylethylamine (5.2 mL, 30 mmol) in 150 mL of THF. The reaction mixture was stirred at room temperature for 1 day. The solvent was evaporated on a rotary evaporator. The residue was washed with water and dichloromethane three times. The organic layers were dried with magnesium sulfate, filtered, and concentrated to afford the product 2,4-dinitro-5-(piperidin-1-yl)aniline **2.1** as a yellow solid (6.5 g, 98 %): mp 137-139 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.628-1.732 (m, 6 H), 3.092 (t, 4 H, *J*= 4.8 Hz), 6.122 (s, 1 H), 6.420 (s, 2 H), 8.832 (s, 1 H); LRMS (ESI) *m/z* cald. 266, found 267.0 (M+1). All data were found to be in agreement with literature values.[43]

_N_-[2,4-Dinitro-5-(piperidin-1-yl)phenyl]-2-phenylacetamide (2.2.1):
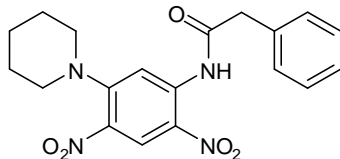
To a solution of **2.1** (4.0 g, 15 mmol) in 12 mL of pyridine, phenylacetyl chloride (3.49 g, 22.56 mmol) was added and magnetically stirred. The mixture was refluxed for 1 day. The reaction mixture was diluted with dichloromethane and washed with water three times. The organic layers were dried with magnesium sulfate, filtered, and concentrated. The residue was purified by flash chromatography using ethyl acetate/hexanes (1:1) as eluent to afford compound **2.2.1** as a yellow solid (2.9 g, 50 %): mp 131 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.702 (s, 6 H), 3.245 (d, 4 H, *J*= 5.1 Hz), 3.834 (s, 2 H), 7.395 (m, 5 H), 8.585 (s, 1 H), 8.792 (s, 1 H), 10.814 (s, 1 H); LRMS (ESI) *m/z* cald. 384, found 385.1 (M+1). All data were found to be in agreement with literature values.[43]

_N_-[2,4-dinitro-5-(piperidin-1-yl)phenyl]-2,2-diphenylacetamide (2.2.2):
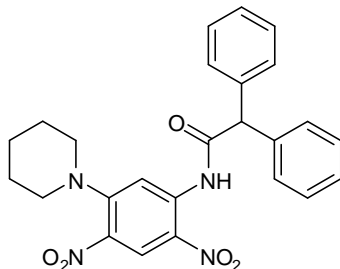
To a solution of **2.1** (2.0 g, 7.5 mmol) in 18 mL of pyridine, diphenylacetyl chloride (4.33 g, 28.8 mmol) was added and magnetically stirred. The mixture was refluxed for 2 days. The reaction mixture was diluted with dichloromethane and washed with water three times. The organic layers were dried with magnesium sulfate, filtered, and concentrated. The residue was purified by flash chromatography on silica gel using ethyl acetate/hexanes (1:1) as eluent to afford compound **2.2.2** as a yellow solid (1.054 g, 30

%): mp 116- 119 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.692 (s, 6 H), 3.2465 (d, 4 H, J= 5.7 Hz), 5.169 (s, 1 H), 7.280-7.417 (m, 10 H), 8.664 (s, 1 H), 8.793 (s, 1 H), 10.996 (s, 1 H); LRMS (ESI) m/z cald. 460, found 462.1 (M+1). All data were found to be in agreement with literature values.[43]

**5-Amino-2-benzyl-6-(piperidin-1-yl)-1H-1,3-benzodiazole (2.3.1):**
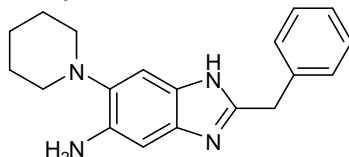
A solution of **2.2.1** (500 mg, 1.3 mmol), tin (II) chloride (1.73 g, 9.10 mmol), and 4N HCl (8 mL) in 17 mL of EtOH was magnetically stirred and refluxed for 4 h. The reaction mixture was cooled and quenched with 30 % NaOH until ~ pH 10. Tin(II) chloride precipitated in solution upon addition of 30 % NaOH. The solution was decanted from the tin(II) chloride solid layer. The solution was washed with water and ethyl acetate three times. The organic layers were dried with magnesium sulfate, filtered, and concentrated. The residue was purified by flash chromatography on aluminium oxide using ethyl acetate as eluent to afford compound **2.3.1** as a brown solid (331 mg, 83 %): mp 191- 193°C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.302-1.748 (m, 6 H), 2.803 (s, 4 H), 4.172 (m, 2 H), 6.740 (s, 1 H), 7.217 (m, 6 H); LRMS (ESI) m/z cald. 306, found 307.1 (M+1). All data were found to be in agreement with literature values.[43]

5-Amino-2-(diphenylmethyl)-6-(piperidin-1-yl)-1H-1,3-benzodiazole (2.3.2):
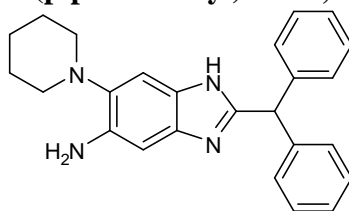
A solution of **2.2.2** (870 mg, 1.89 mmol), tin(II) chloride (2.5 g, 13.2 mmol), and 4N HCl (13 mL) in 27 mL of EtOH was magnetically stirred and refluxed for 4 hours. The reaction mixture was cooled and quenched with 30 % NaOH until ~ pH 10. Tin(II) chloride precipitated in solution upon addition of 30 % NaOH. The solution was decanted from the tin (II) chloride solid layer. The solution was washed with water and ethyl acetate three times. The organic layers were dried with magnesium sulfate, filtered, and concentrated. The residue was purified by flash chromatography on aluminium oxide using ethyl acetate as eluent to afford compound **2.3.2** as a brown solid (496 mg, 69 %): mp 125- 127°C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.259- 1.711 (m, 6 H), 2.782 (s, 4 H), 5.719 (s, 1 H), 6.698 (s, 2 H), 7.168- 7.320 (m, 12 H); LRMS (ESI) m/z cald. 382, found 383.2 (M+1). All data were found to be in agreement with literature values.[43]

_N_-[2-Benzyl-6-(piperidin-1-yl)-1H-1,3-benzodiazol-5-yl]-4-_tert_-butylbenzamide (2.4.1):
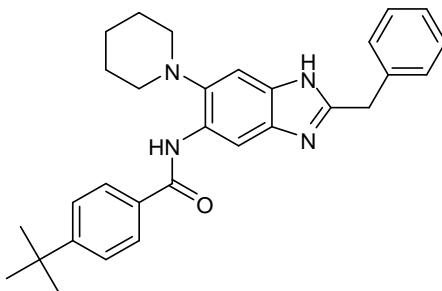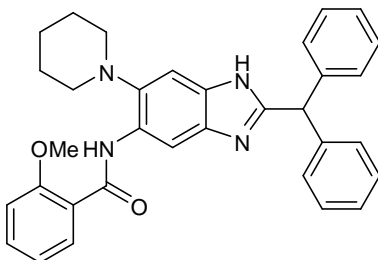

To a solution of **2.3.1** (100 mg, 0.326 mmol) in DCM (6 mL), 4-_tert_-butylbenzoyl chloride (1 eq., 0.059 mL) was added and magnetically stirred at room temperature for 1 day. The solvent was evaporated on a rotary evaporator. The residue was purified by flash chromatography on silica gel using ethyl acetate/hexanes (1:1) as eluent to afford compound **2.4.1** as a tan solid (82 mg, 53 %): mp >200 °C; [^1]H NMR (500 MHz, CDCl$_3$) δ 1.1175 (s, 9 H), 1.676 (d, 6 H, _J_= 69 Hz), 2.771 (s, 4 H), 4.319 (s, 4 H), 7.091-7.849 (m, 10 H), 8.897 (s, 1 H), 9.877 (s, 1 H); [^13]C NMR (500 MHz, CDCl$_3$) δ 23.759, 27.101, 29.633, 31.075, 33.726, 34.966, 54.358, 76.744, 77.000, 77.256, 103.125, 108.320, 125.870, 126.690, 127.189, 128.761, 128.761, 128.925, 130.431, 131.053, 131.946, 134.906, 141.039, 152.631, 155.621, 164.986; LRMS (ESI) _m/z_ cald. 466, found 467.3 (M+1). All data were found to be in agreement with literature values.[43]

_N_-[2-Benzyl-6-(piperidin-1-yl)-1H-1,3-benzodiazol-5-yl]-4-methoxybenzamide (2.4.2):
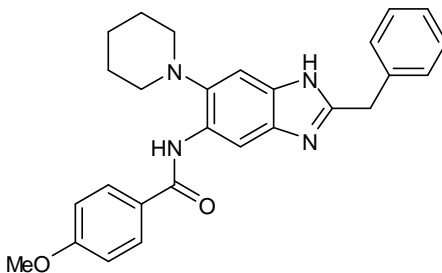
To a solution of **2.3.1** (100 mg, 0.326 mmol) in DCM (6 mL), 4-methoxybenzoyl chloride (1 eq., 0.0545 mL) was added and magnetically stirred at room temperature for 1 day. The solvent was evaporated on a rotary evaporator. The residue was purified by flash chromatography on silica gel using ethyl acetate/hexanes (1:1) as eluent to afford compound **2.4.2** as a tan solid (34 mg, 24 %): mp >200 °C; [^1]H NMR (300 MHz, CDCl$_3$) δ 1.234- 2.171 (m, 6 H), 2.851 (s, 4 H), 3.880 (d, 5 H, _J_= 91.2 Hz), 6.976- 7.512 (m, 8 H), 7.932 (m, 3 H), 8.785 (s, 1 H), 9.935 (s, 1 H); LRMS (ESI) _m/z_ cald. 440, found 441.2 (M+1). All data were found to be in agreement with literature values.[43]

N-[2-(Diphenylmethyl)-6-(piperidin-1-yl)-1H-1,3-benzodiazol-5-yl]-2-methoxybenzamide (2.4.3):

To a solution of **2.3.2** (100 mg, 0.261 mmol) in DCM (6 mL), 2-methoxybenzoyl chloride (1 eq., 0.035 mL) was added and magnetically stirred at room temperature for 1 day. The solvent was evaporated on a rotary evaporator. The residue was purified by flash chromatography on silica gel using ethyl acetate/hexanes (1:1) as eluent to afford compound 2.4.3 as a tan solid (36 mg, 27 %,): mp 153- 156 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.738 (m, 9 H), 2.871 (s, 4 H), 4.135 (d, 3 H, J= 12.3), 5.875 (s, 1 H), 7.112-7.359 (m, 12 H), 8.273 (d, 2 H, J= 6.6 Hz); 10.789 (s, 1 H); LRMS (ESI) m/z cald. 516, found 517.3 (M+1). All data were found to be in agreement with literature values.[43]

4-Amino-3,5-dinitrobenzoyl chloride (3.1):
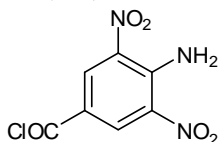
A solution of 5-amino-2,4-dinitrobenzamide (2 g, 8.84 mmol) in 50 mL of 6N HCl was magnetically stirred and refluxed for 1.5 days. The solvent was decanted and the benzoic acid product was concentrated in *vacuo*. The crude product was dissolved in 150 mL of SOCl$_2$, and magnetically stirred and refluxed for 1 day. The reaction mixture was cooled and concentrated by micro distillation. The product was further concentrated in *vacuo* to afford acid chloride **3.1** as a yellow solid in quantitative yield.

n-Butyl N-(4-amino-3,5-dinitrophenyl)carbamate (3.2.1):
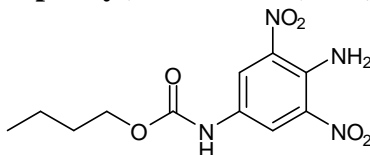
A solution of NaN$_3$ (0.66 g, 10.2 mmol) in 12 mL of ice cold water was added dropwise to a magnetically stirred solution of-amino-3,5-dinitrobenzoyl chloride **3.1** (1 g, 4.08 mmol) in 40 mL of acetone. The reaction mixture was stirred at 0 °C for an hour. The residue was washed with water, saturated sodium chloride, and dichloromethane three times. The organic layers were dried with magnesium sulfate, filtered, and concentrated in *vacuo* to afford the azide intermediate. The crude product (0.967 g) was dissolved in 25 mL of toluene, and magnetically stirred and refluxed for 4 h. The reaction mixture was cooled to room temperature to give the isocyanate intermediate. 25 mL of n-butanol was added and magnetically stirred at room temperature for 1 day. The mixture was purified by recystallization from hexanes to give butyl carbamate product **3.2.1** as a bright red solid (1.052 g, 82.5 %): mp 155- 156 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 0.958 (m, 3 H),

1.380- 1.716 (m, 4 H), 4.203 (t, 2, J= 6.6 Hz), 6.686 (s, 1 H), 8.32 (s, 1 H), 8.646 (s, 2 H); LRMS (ESI) m/z cald. 298, found 299.1 (M+1); HRMS (FAB) m/z calcd for $C_{11}H_{15}N_4O_6H+$: 299.0982, Found: 299.0992 (Δ= -3.3 ppm).

tert-Butyl *N*-(7-amino-2-phenyl-1H-1,3-benzodiazol-5-yl)carbamate (3.3.1):
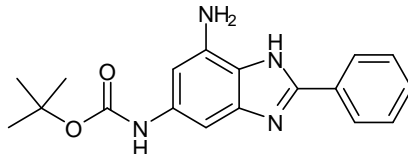
A solution of *tert*-butyl *N*-(4-amino-3,5-dinitrophenyl)carbamate (283 mg, 0.95 mmol), and tin(II) chloride (1.32 g, 6.65 mmol), in 20 mL of EtOH was magnetically stirred and refluxed for 4 h. The reaction mixture was cooled in an ice bath. A solution of sodium phenyl(sulfonatooxy)methanol (375 mg, 0.855 mmol) in 11 mL of H_2O was added to the reaction flask in a dropwise manner. The reaction was magnetically stirred at 0 °C to room temperature for 2 days. The solution was quenched with 30 % NaOH until ~ pH 10. Tin(II) chloride precipitated in solution upon addition of 30 % NaOH. The solution was decanted from the tin(II) chloride solid layer. The solution was washed with water and ethyl acetate three times. The organic layers were dried with magnesium sulfate, filtered, and concentrated. The residue was purified by flash chromatography on aluminium oxide using ethyl acetate as eluent to afford compound **3.3.1** as a brown solid (75 mg, 28 %): mp 224-226 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.47 (s, 9 H), 6.48 (s, 1 H), 7.10 (s, 1 H), 7.31 (m, 5 H), 8.22 (s, 1 H); LRMS (ESI) m/z cald. 324, found 325.1 (M+1).

Butyl N-[7-amino-2-(pyridin-2-yl)-1H-1,3-benzodiazol-5-yl]carbamate (3.3.2):
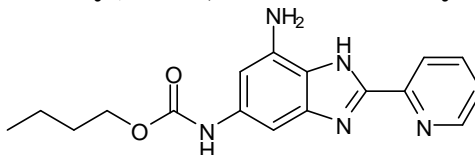
A solution of **3.2.1** (500 mg, 1.6 mmol), and tin(II) chloride (2.855 g, 14.4 mmol), in 30 mL of EtOH was magnetically stirred and refluxed for 4.5 h. The reaction mixture was cooled in an ice bath. A solution of sodium pyridin-2-yl(sulfonatooxy)methanol (183 mg, 1.44 mmol) in 18 mL of H_2O was added to the reaction flask in a dropwise manner. The reaction was magnetically stirred at 0 °C to room temperature for 2 days. The solution was quenched with 30 % NaOH until ~ pH 10. Tin(II) chloride precipitated in solution upon addition of 30 % NaOH. The solution was decanted from the tin(II) chloride solid layer. The solution was washed with water and ethyl acetate three times. The organic layers were dried with magnesium sulfate, filtered, and concentrated. The residue was purified by flash chromatography on aluminum oxide using MeOH/DCM (1:20) as eluent to afford compound **3.3.2** as a brown solid (183 mg, 34 %): mp 95- 97 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.923 (m, 9 H), 1.383 (m, 2 H), 1.625 (m, 2 H), 4.142 (t, 2 H, J= 6.6 Hz), 6.452 (s, 1 H), 6.856 (m, 1 H), 7.079 (m, 1 H), 7.461 (m, 1 H), 7.745 (t, 1 H, J= 7.5 Hz), 8.336 (q, 1 H, J= 8.1 Hz), 8.532 (m, 1 H), 10.727 (s, 1 H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 14.230, 20.222, 20.294, 32.336, 32.413, 32.371, 65.792, 121.920, 122.328, 123.850, 124.960, 125.097, 125.384, 138.398, 138.451, 138.863, 139.039, 141.068, 149.166, 149.772, 149.959, 150.013, 150.825, 151.218, 156.516, 158.595; LRMS (ESI) m/z cald.

325, found 326.1 (M+1); HRMS (FAB) *m/z* calcd for $C_{17}H_{20}N_5O_2H+$: 326.1610, Found: 326.1617 (Δ= -2.1 ppm).

Ethyl *N*-[7-amino-2-(pyridin-2-yl)-1H-1,3-benzodiazol-5-yl]carbamate (3.3.3):
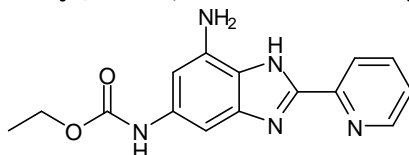
A solution of ethyl *N*-(4-amino-3,5-dinitrophenyl)carbamate (500 mg, 1.98 mmol), and tin(II) chloride (2.623 g, 13.83 mmol), in 25 mL of EtOH was magnetically stirred and refluxed for 1 day. The reaction mixture was cooled in an ice bath. A solution of sodium pyridin-2-yl(sulfonatooxy)methanol (459 mg, 2.17 mmol) in 15 mL of H_2O was added to the reaction flask in a dropwise manner. The reaction was magnetically stirred at 0 °C to room temperature for 2 days. The solution was quenched with 30 % NaOH until ~ pH 10. Tin(II) chloride precipitated in solution upon addition of 30 % NaOH. The solution was decanted from the tin(II) chloride solid layer. The solution was washed with water and ethyl acetate three times. The organic layers were dried with magnesium sulfate, filtered, and concentrated. The residue was purified by flash chromatography on aluminum oxide using MeOH/DCM (1:20) as eluent to afford compound **3.3.3** as a brown solid (105 mg, 19 %): mp 112- 114 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.351 (m, 3 H), 4.297 (q, 2 H, *J*= 7.2 Hz), 6.498 (d, 1 H, *J*= 1.8Hz), 6.708 (s, 1 H), 7.223 (m, 1 H), 7.384 (m, 1 H), 7.888 (m, 1 H), 8.446 (d, 1 H, *J*= 7.8 Hz), 8.662 (d, 1 H, *J*= 4.2 Hz); ^{13}C NMR (500 MHz, CD$_3$OD) δ 14.253, 15.065, 15.153, 61.901, 100.312, 101.464, 121.264, 121.878, 121.897, 122.530, 125.151, 125.589, 137.448, 137.715, 138.314, 138.497, 138.520, 139.008, 139.367, 139.478, 149.158, 149.696, 149.967, 150.474, 150.737, 150.829, 151.596, 156.406; LRMS (ESI) *m/z* cald. 297, found 298.0 (M+1); HRMS (FAB) *m/z* calcd for $C_{15}H_{16}N_5O_2H+$: 298.1293, Found: 298.1304 (Δ= -3.7 ppm).

n-Butyl *N*-(7-amino-2-phenyl-1H-1,3-benzodiazol-5-yl)carbamate (3.3.4):
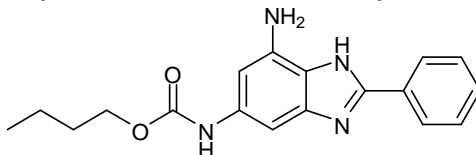
A solution of **3.2.1** (1.0 g, 3.4 mmol), and tin(II) chloride (4.65 g, 23.5 mmol), in 70 mL of EtOH was magnetically stirred and refluxed for 4.5 h. The reaction mixture was cooled in an ice bath. A solution of sodium phenyl(sulfinatooxy)methanol (643 mg, 3.02 mmol) in 30 mL of H_2O was added to the reaction flask in a dropwise manner. The reaction was magnetically stirred at 0 °C to room temperature for 1 day. The solution was quenched with 30 % NaOH until pH 10. Tin(II) chloride precipitated in solution upon addition of 30 % NaOH. The solution was decanted from the tin(II) chloride solid layer. The solution was washed with water and ethyl acetate three times. The organic layers were dried with magnesium sulfate, filtered, and concentrated. The residue was purified by flash chromatography on aluminium oxide using MeOH/DCM (1:20) as eluent to afford compound **3.3.4** as a brown solid (447 mg, 41%): mp 186-187 °C; ^{1}H NMR (300 MHz, CD$_3$OD) δ 0.978 (t, 3 H, *J*= 7.2), 1.473 (m, 2 H), 1.678 (m, 2 H), 4.130 (t, 2 H, *J* = 6.6), 6.30 (s, 2 H), 7.186 (s, 1 H), 7.497 (m, 5 H), 8.011 (m, 2 H); ^{13}C NMR (500 MHz,

CDCl$_3$) δ15.347, 21.431, 33.560, 128.580, 131.353, 132.062, 132.588, 138.390, 140.324, 152.499, 157.740; LRMS (ESI) *m/z* cald. 324, found 325.1 (M+1); HRMS (FAB) *m/z* calcd for C$_{18}$H$_{21}$N$_4$O$_2$H+: 325.1673, Found: 325.1665 (Δ= 2.5 ppm).

### *n*-Butyl *N*-(7-amino-2-cyclohexyl-1H-1,3-benzodiazol-5-yl)carbamate (3.3.5):
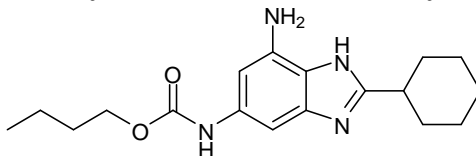
A solution of **3.2.1** (200 mg, 0.671 mmol), Pd-C (74 mg), ammonium formate (1.1 g) in 30 mL of EtOH was magnetically stirred and refluxed for 2 h. The reaction mixture turned from red to dark brown. The catalyst and excess ammonium formate were filtered through celite with 40 mL of ethanol to get a solution of fully reduced butyl carbamate in ethanol. 43 mL of water was added to the reaction mixture and was magnetically stirred at room temperature. The bisulfate salt (132 mg, 0.604 mmol) in 4 mL of water was added to the reaction mixture in a dropwise manner. The reaction was stirred for 2 days. The solvent was evaporated on a rotary evaporator. The residue was purified by flash chromatography on aluminum oxide using ethyl acetate/hexanes (2:1) as eluent to afford compound **3.3.5** as a brown solid (70 mg, 32 %): mp 180.5-181.5 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 0.918-2.045 (m, 17 H), 2.75 (m, 1 H), 4.147 (t, 2 H, *J* = 6.6 Hz), 6.35 (s, 2 H), 6.75 (s, 1 H), 7.05 (s, 1 H); LRMS (ESI) *m/z* cald. 298, found 299.1 (M+1); HRMS (FAB) *m/z* calcd for C$_{18}$H$_{26}$N$_4$O$_2$H+: 331.2127, Found: 331.2134 (Δ= -2.1 ppm).

Sodium pyridin-2-yl(hydroxy)methyl sulfinate (4.1.1):
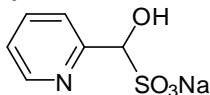
A solution of sodium bisulfite (1.9 g, 18.67 mmol) in 12 mL of water was added dropwise to a magnetically stirred solution of 2-pyridine-carboxaldehyde (2 g, 18.67 mmol) in 60 mL of EtOH for 2 hours. Precipitation occurred in solution and the product was filtered with ethanol. The product **4.1.1** was afforded as a pale yellow solid in quantitative yield: ^{1}H NMR (300 MHz, D$_2$O) δ 5.420 (s, 1 H), 7.301 (m, 1 H), 7.537 (d, 1 H, *J*= 7.8 Hz), 7.762 (m, 1 H), 8.349 (m, 1 H).

Sodium phenyl(hydroxy)methyl sulfinate (4.1.2):
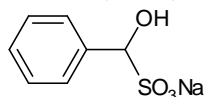
A solution of sodium bisulfite (1.96 g, 18.6 mmol) in 12 mL of water was added dropwise to a magnetically stirred solution of benzaldehyde (2.0 g, 19 mmol) in 60 mL of EtOH for 2 hours. Precipitation occurred in solution and the product was filtered with ethanol. The product **4.1.2** was afforded as a white crystal solid (97 %, 3.85 g). All data were found to be in agreement with literature values.

Sodium cyclohexyl(hydroxy)methyl sulfinate (4.1.3):
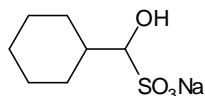
A solution of sodium bisulfite (1.96 g, 18.6 mmol) in 14 mL of water was added dropwise to a magnetically stirred solution of cyclohexane carboxaldehyde (2.0 g, 18 mmol) in 70 mL of EtOH for 2 hours. Precipitation occurred in solution and the product was filtered with ethanol. The product **4.1.3** was afforded as a white crystal solid (80 %, 3.05 g).

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N-[5-(4-cyclohexanecarbonylpiperazin-1-yl)-2,4-dinitrophenyl]cyclohexane carboxamide (1.2):

^{1}H-NMR:

5-Amino-6-(4-cyclohexanecarbonylpiperazin-1-yl)-2-cyclohexyl-1H-1,3-benzodiazole (1.3):

¹H-NMR:

5-Amino-6-(4-cyclohexanecarbonylpiperazin-1-yl)-2-cyclohexyl-1H-1,3-benzodiazole (1.3):

^{13}C-NMR:

CS-109

Data Collected on:
  inv500-inova500
Archive directory:
  /export/home/wberger/vnmrsys/data
Sample directory:

File: CARBON

Pulse Sequence: s2pul
Solvent: CDCl3

Temp. 25.0 C / 298.1 K
User: 1-14-87

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.300 sec
Width 31421.8 Hz
328 repetitions
OBSERVE C13, 125.6986743 MHz
DECOUPLE H1, 499.8973075 MHz
  Power 45 dB
  continuously on
  WALTZ-16 modulated
DATA PROCESSING
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FT size 131072
Total time 3 hr, 50 min
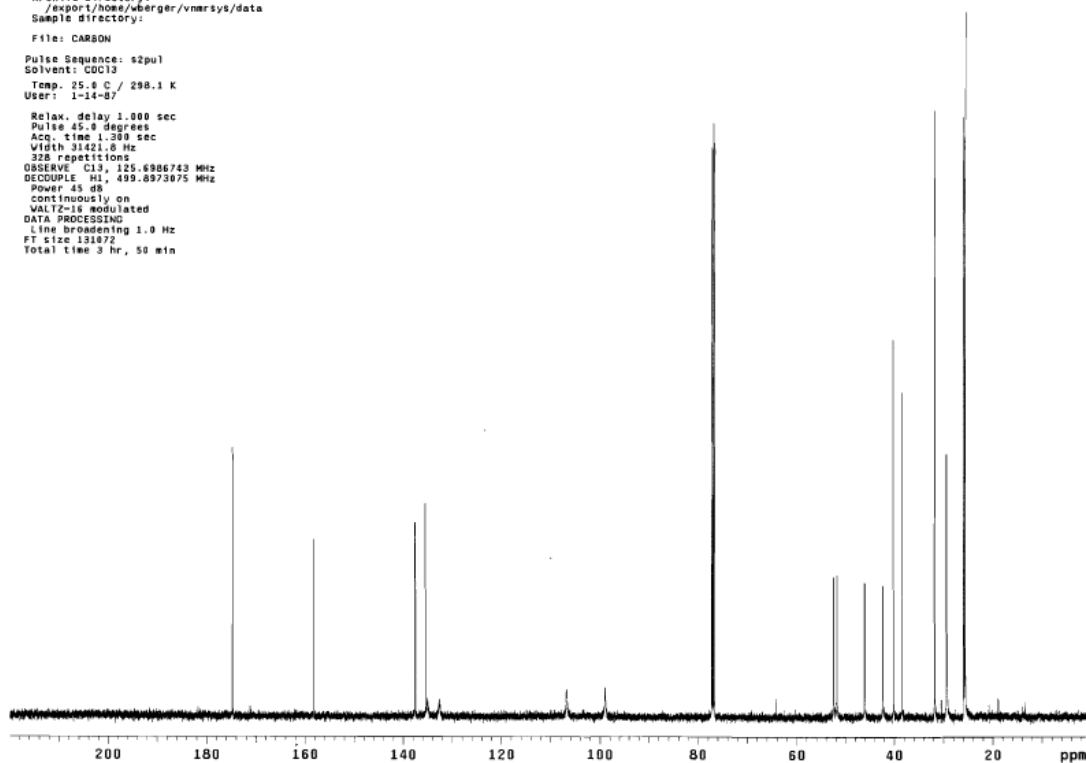
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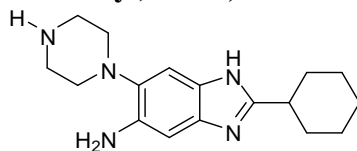
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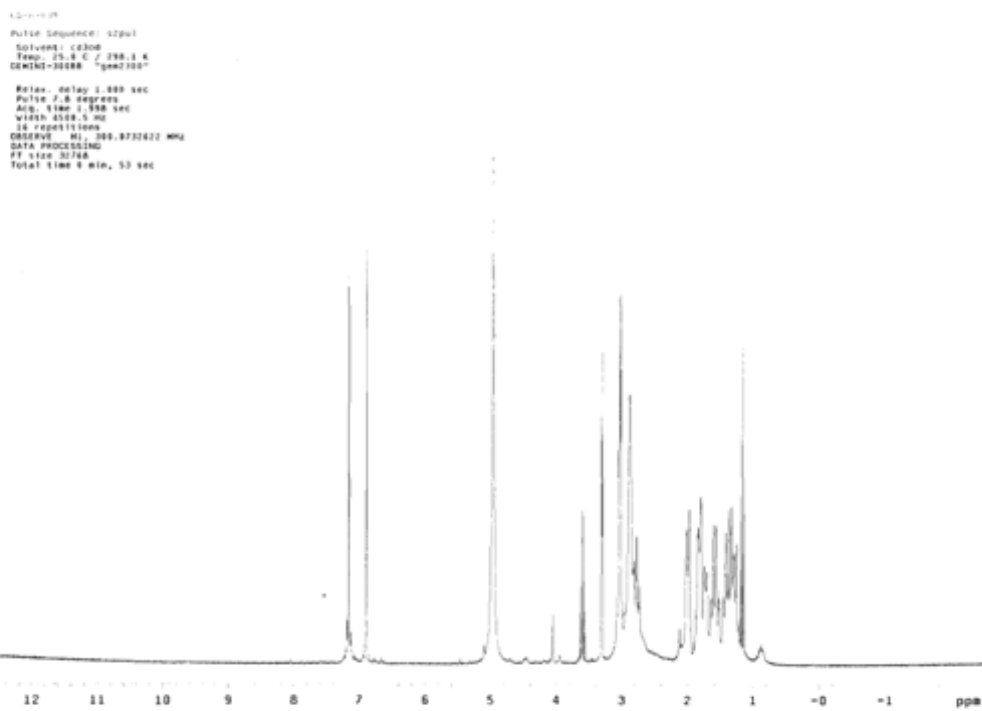
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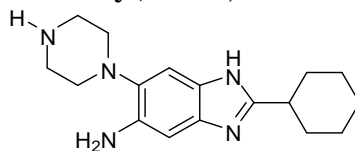
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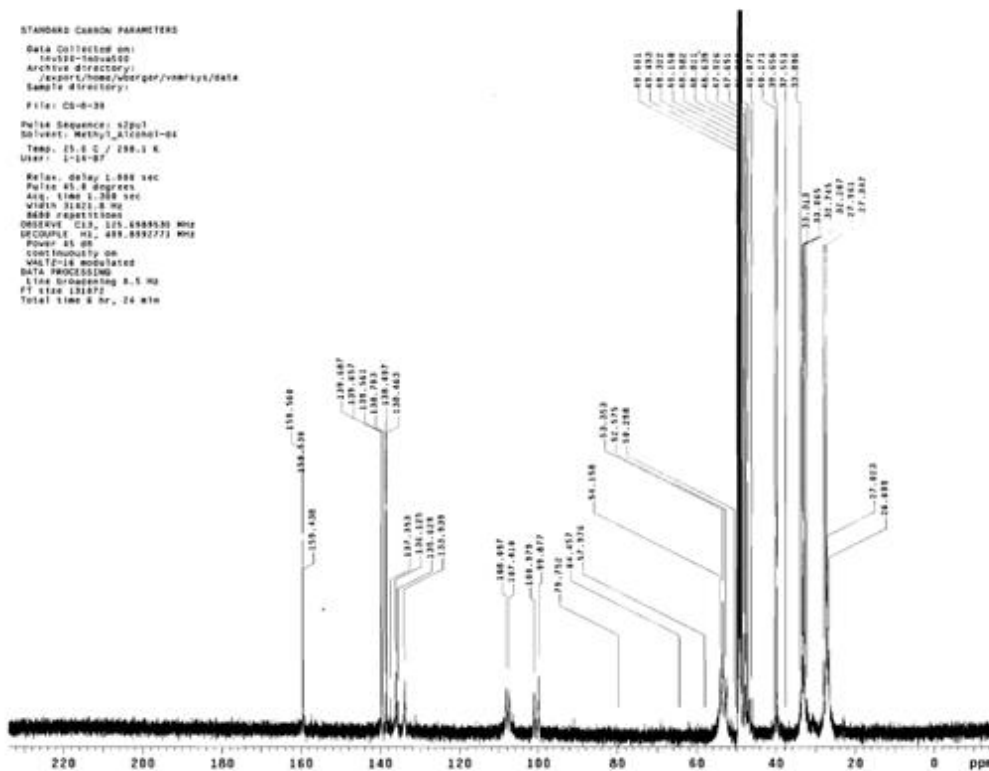
Appendix 2: Spectra for Active 2,5,6- Benzimidazoles

2,4-Dinitro-5-(piperidin-1-yl)aniline (2.1):
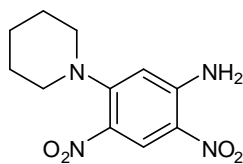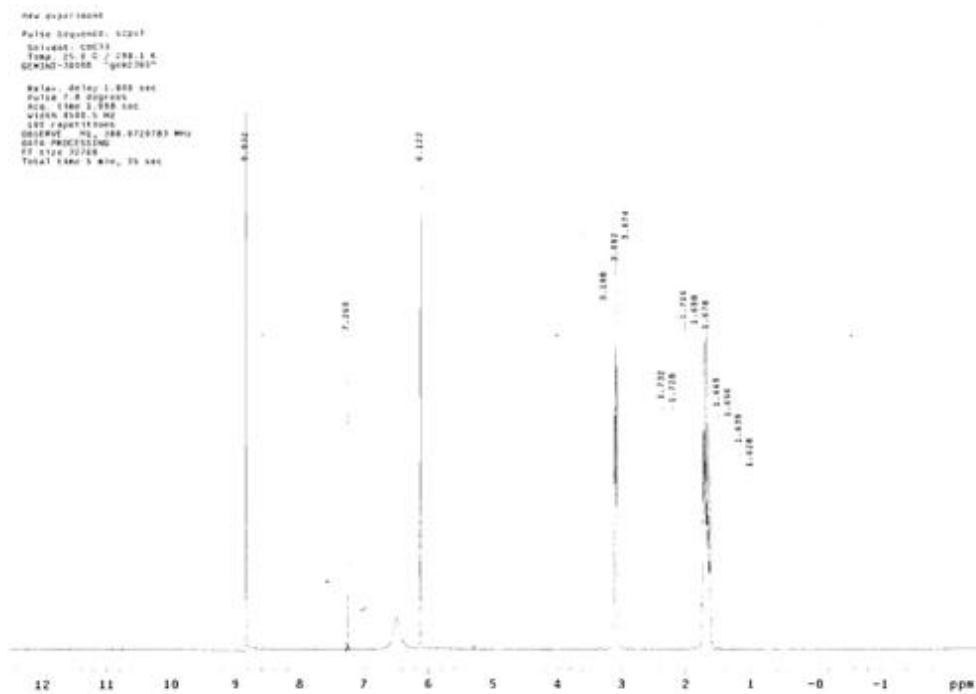
^{1}H-NMR:

_N_-[2,4-dinitro-5-(piperidin-1-yl)phenyl]-2-phenylacetamide (2.2.1):

^{1}H-NMR:

CS-93

Pulse Sequence: s2pul

Solvent: COCl3
Temp. 25.0 C / 298.1 K
GEMINI-300BB "gen2300"

Relax. delay 1.000 sec
Pulse 7.8 degrees
Acq. time 1.998 sec
Width 4500.5 Hz
16 repetitions
OBSERVE   H1, 300.0720783 MHz
DATA PROCESSING
Line broadening 0.5 Hz
FT size 32768
Total time 0 min, 0 sec

12   11   10   9   8   7   6   5   4   3   2   1   -0   -1   ppm

1.05   1.02   2.01   6.28 1.21
        1.00   5.13   4.13   1.63

N-[2,4-dinitro-5-(piperidin-1-yl)phenyl]-2,2-diphenylacetamide (2.2.2):

¹H-NMR:

5-Amino-2-benzyl-6-(piperidin-1-yl)-1H-1,3-benzodiazole (2.3.1):
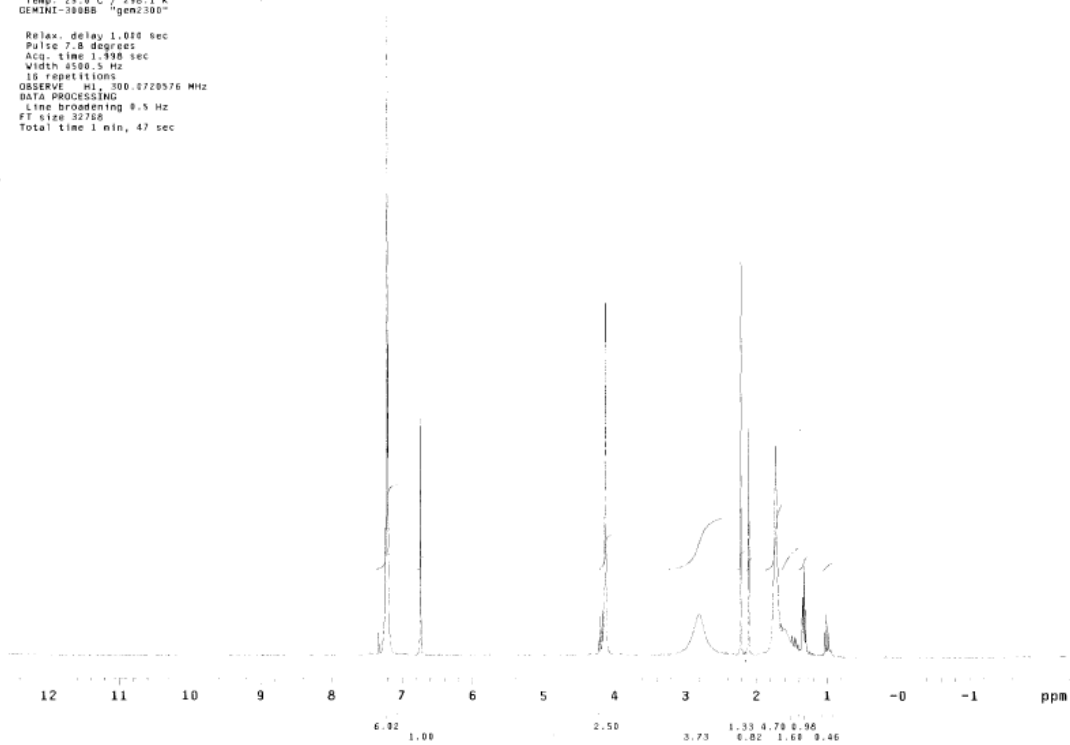
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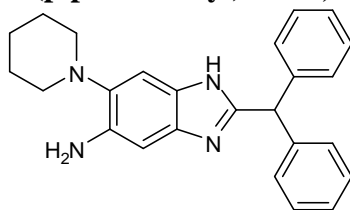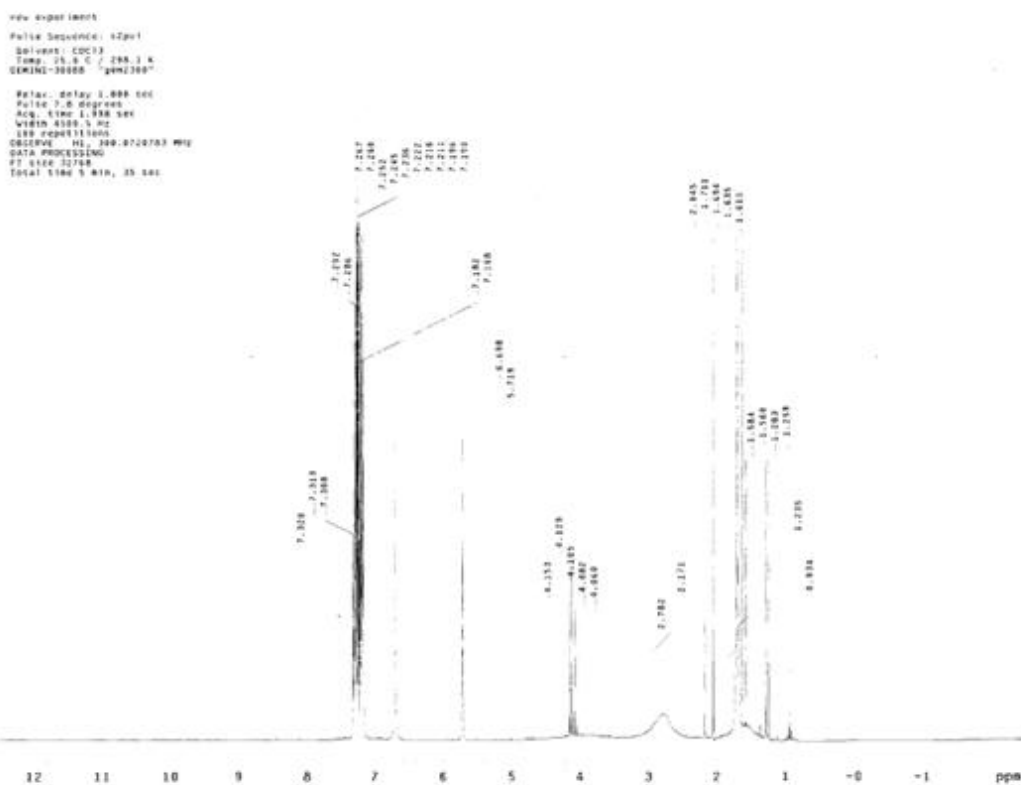
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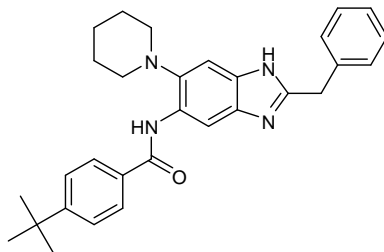
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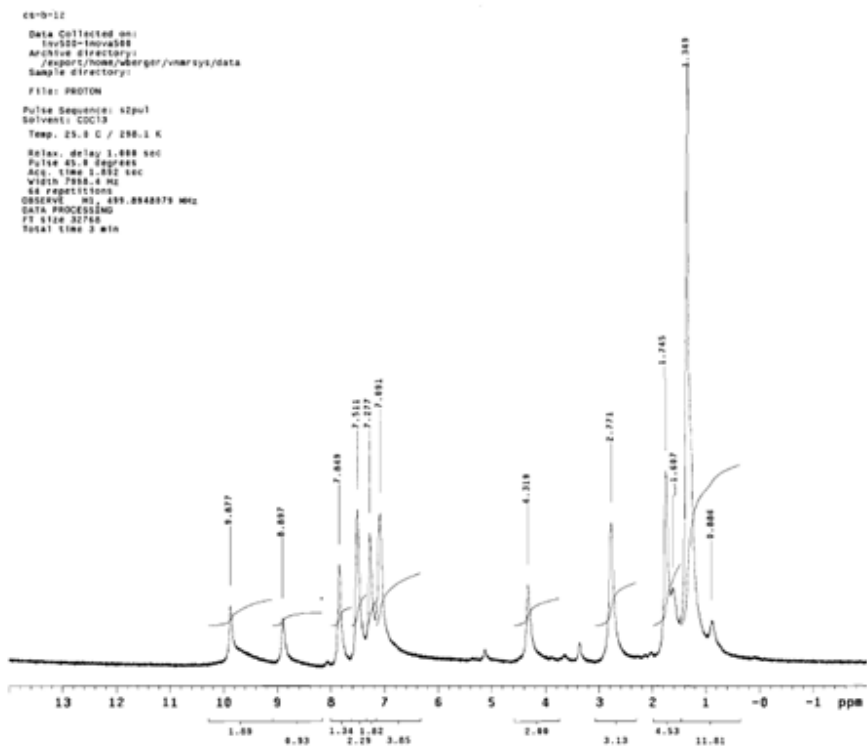
***N*-[2-benzyl-6-(piperidin-1-yl)-1H-1,3-benzodiazol-5-yl]-4-*tert*-butylbenzamide (2.4.1):**
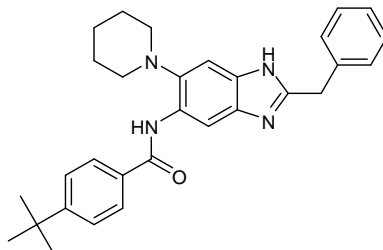
¹³C-NMR:
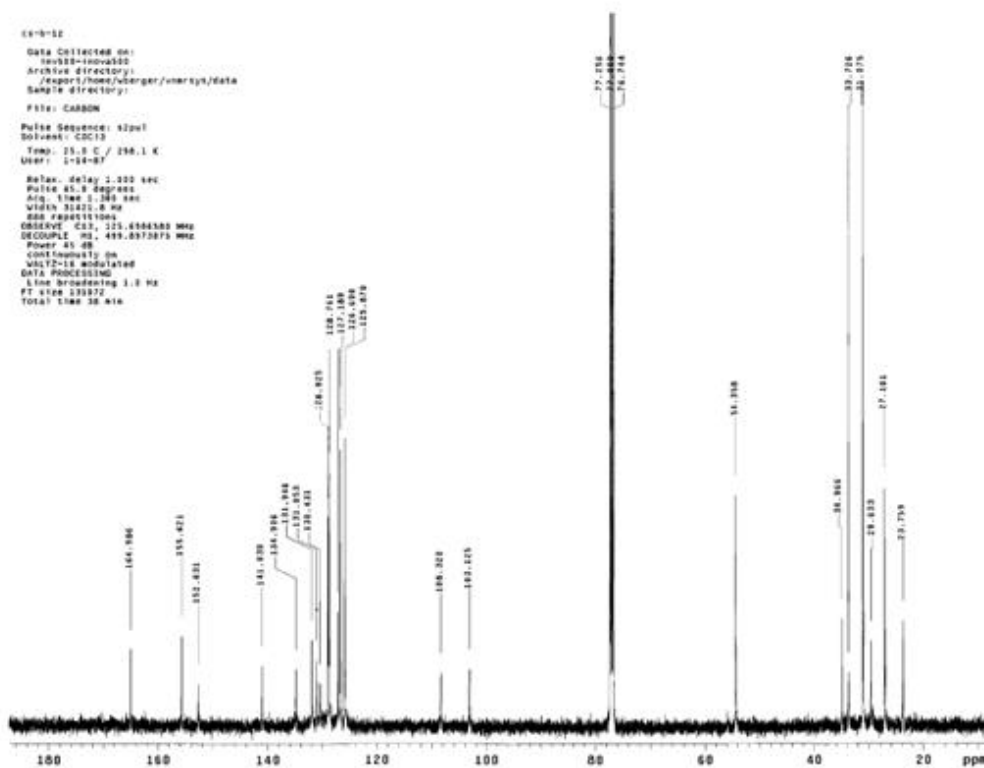
N-[2-benzyl-6-(piperidin-1-yl)-1H-1,3-benzodiazol-5-yl]-4-methoxybenzamide (2.4.2):
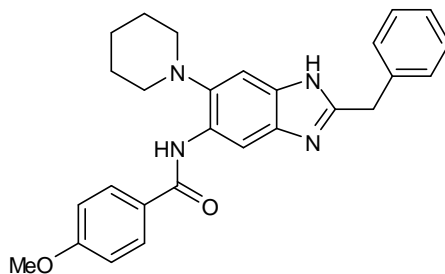
¹H-NMR:
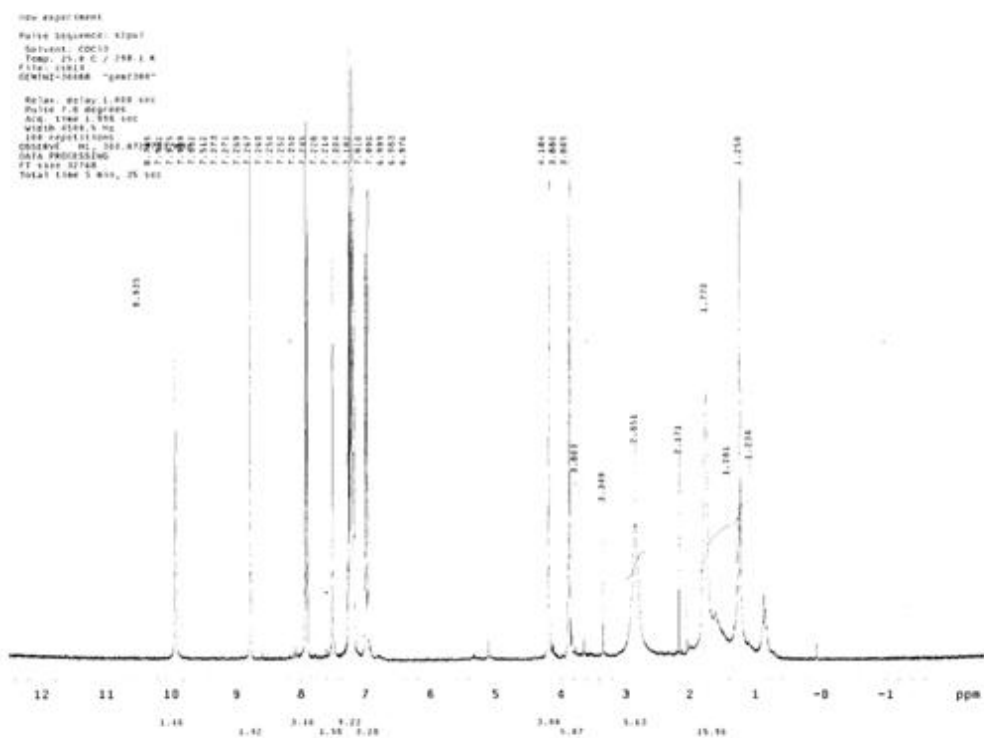
***N*-[2-(diphenylmethyl)-6-(piperidin-1-yl)-1H-1,3-benzodiazol-5-yl]-2-methoxybenzamide (2.4.3):**

¹H-NMR:
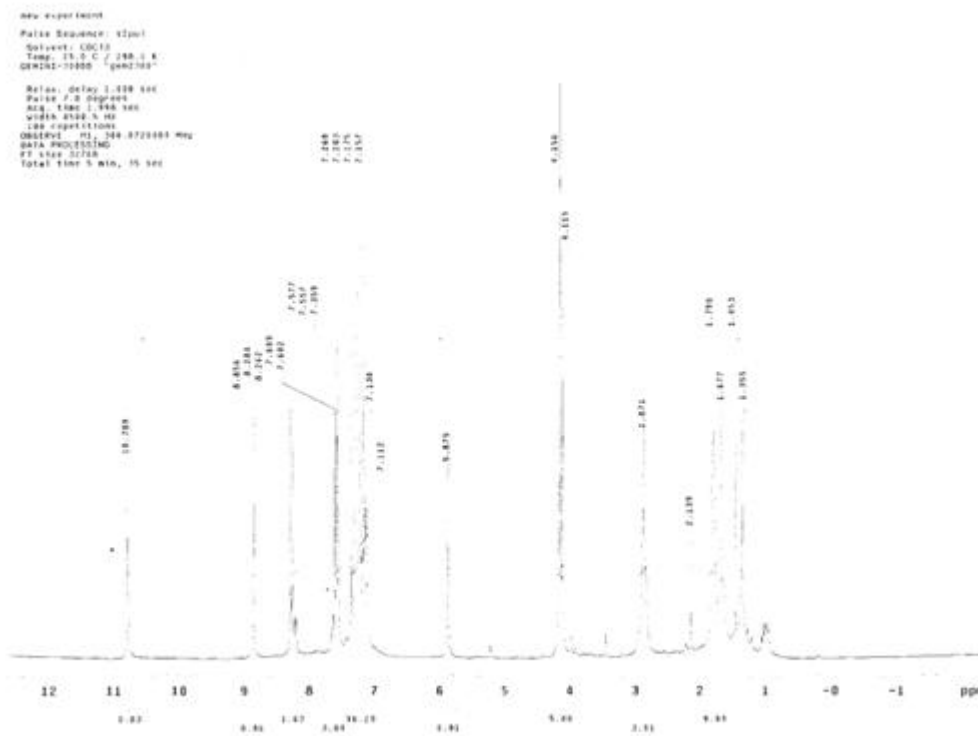
Appendix 3: Spectra for Novel 2,5,7- Benzimidazoles

Butyl N-(4-amino-3,5-dinitrophenyl)carbamate (3.2.1):
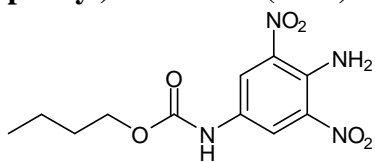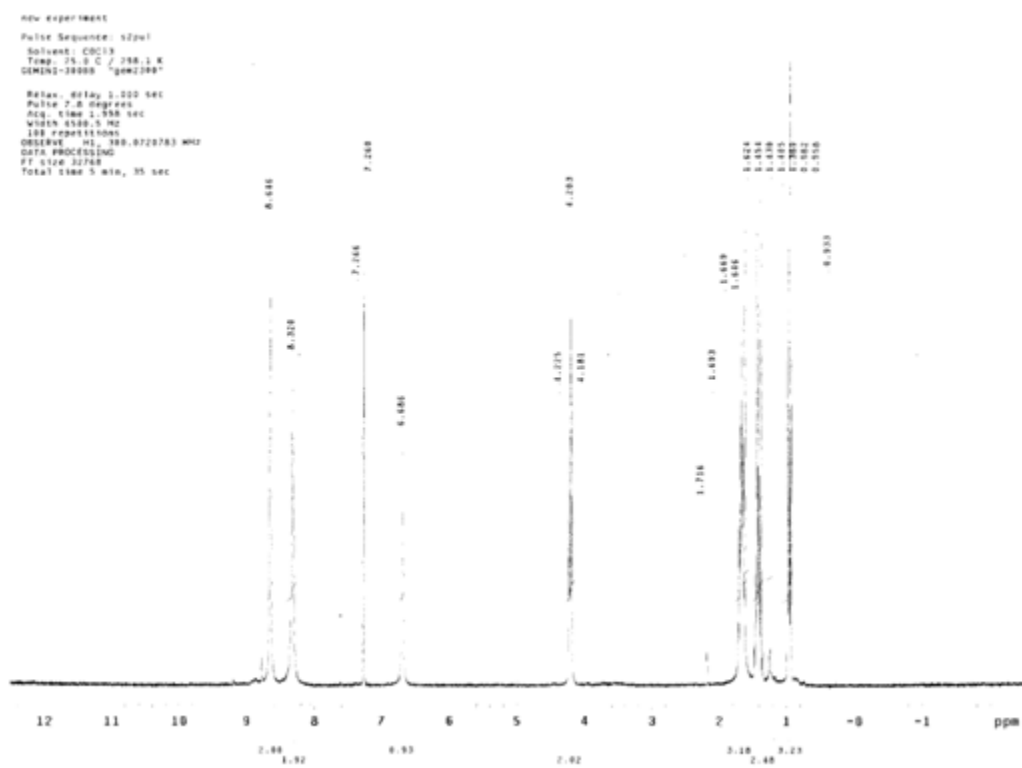
[superscript]1[/superscript]H-NMR:

_n_-Butyl _N_-[7-amino-2-(pyridin-2-yl)-1H-1,3-benzodiazol-5-yl]carbamate (3.3.2):

[chemical structure]

¹H-NMR:

[NMR spectrum]
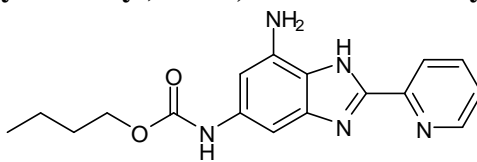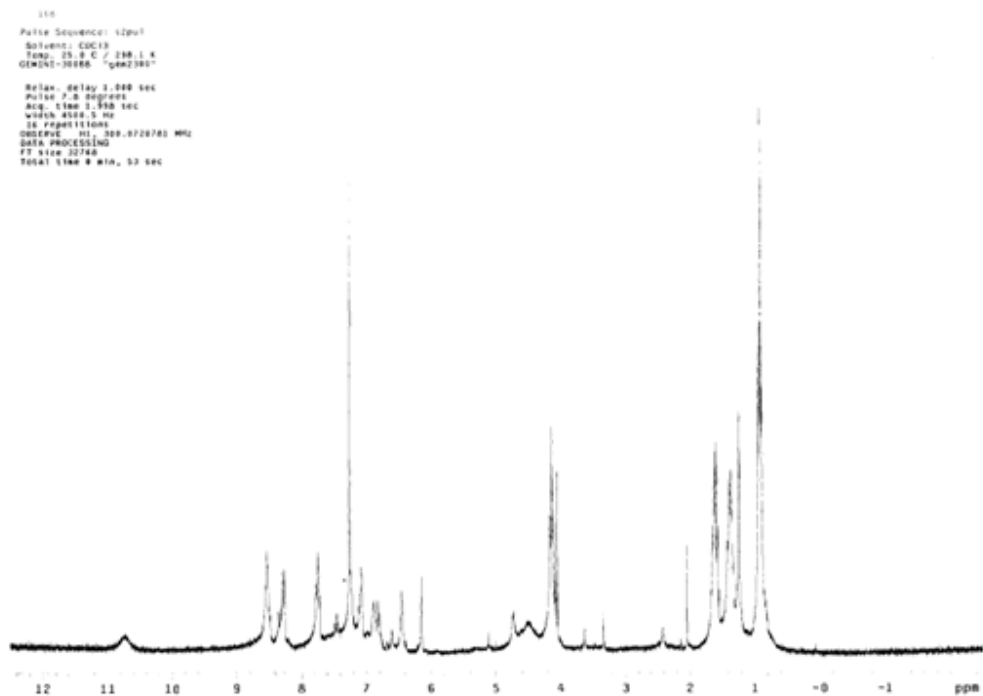
_n_-Butyl _N_-[7-amino-2-(pyridin-2-yl)-1H-1,3-benzodiazol-5-yl]carbamate (3.3.2):
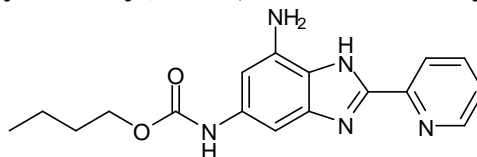
¹³C-NMR:
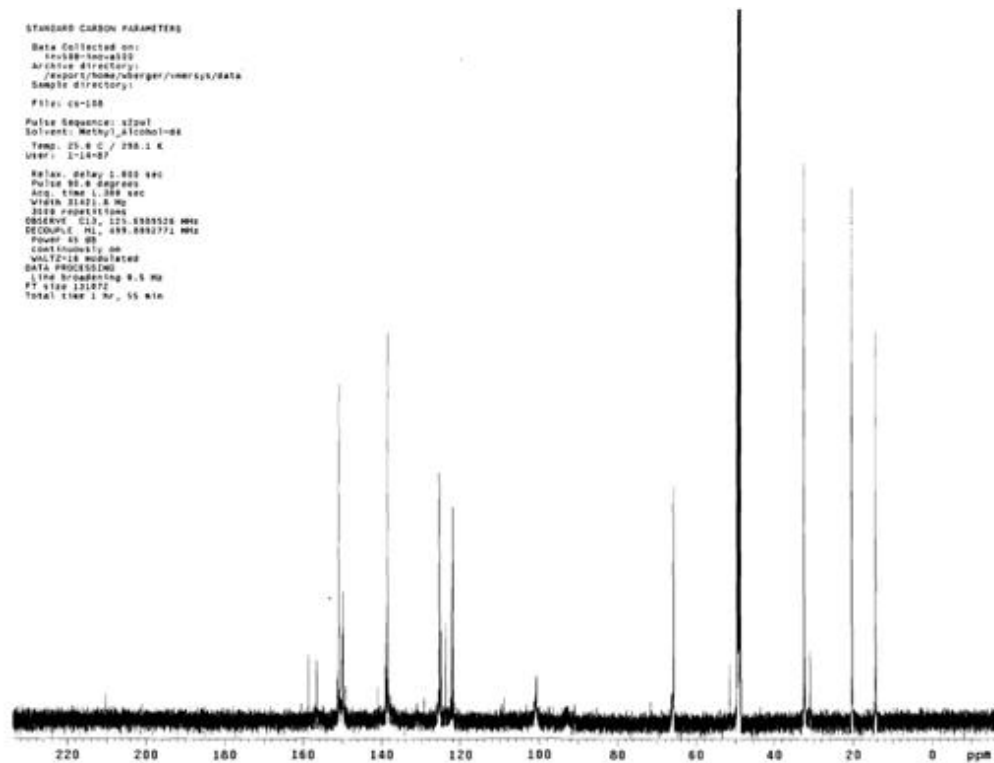
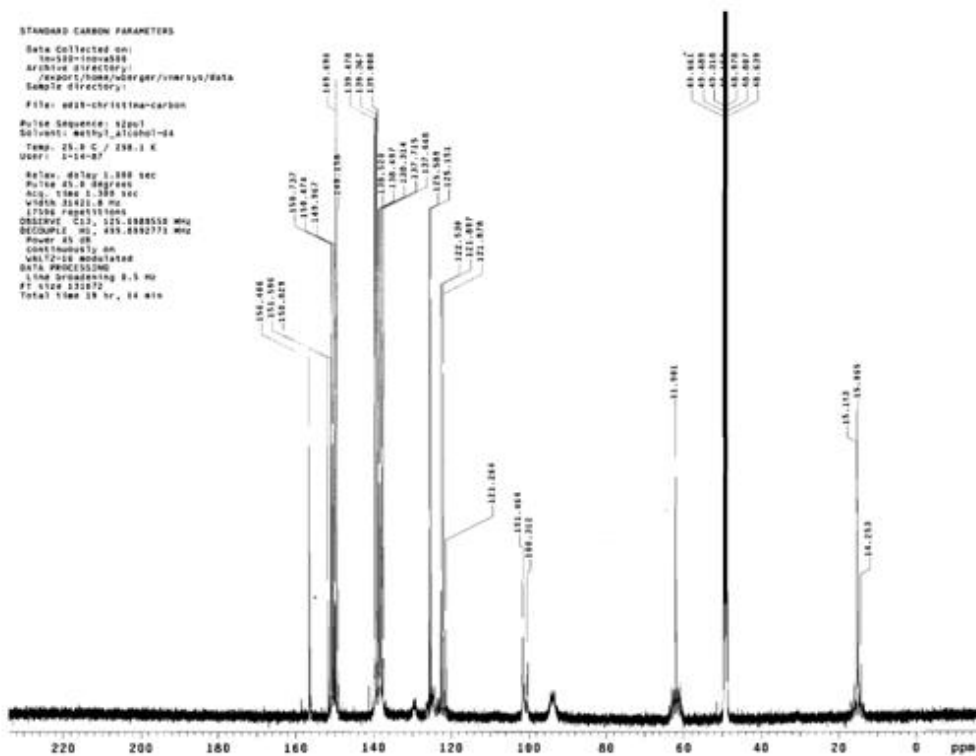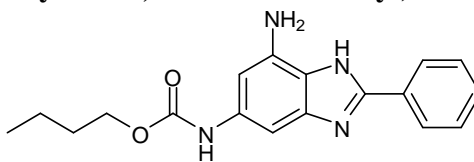**Ethyl *N*-[7-amino-2-(pyridin-2-yl)-1H-1,3-benzodiazol-5-yl]carbamate (3.3.3):**
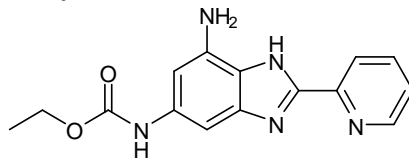
¹H-NMR:
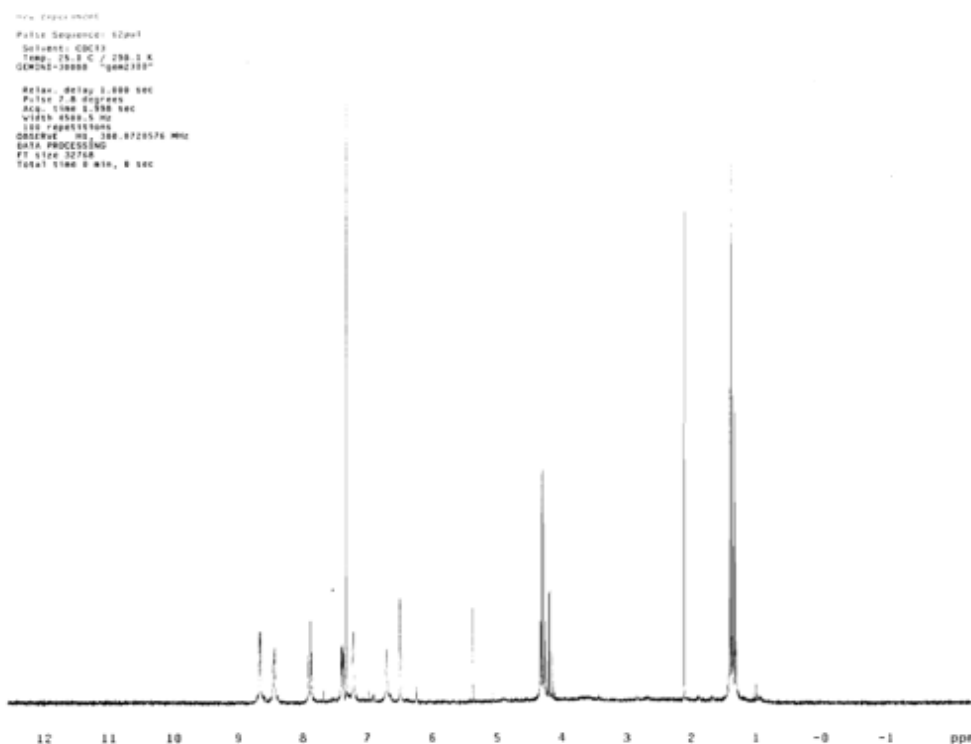
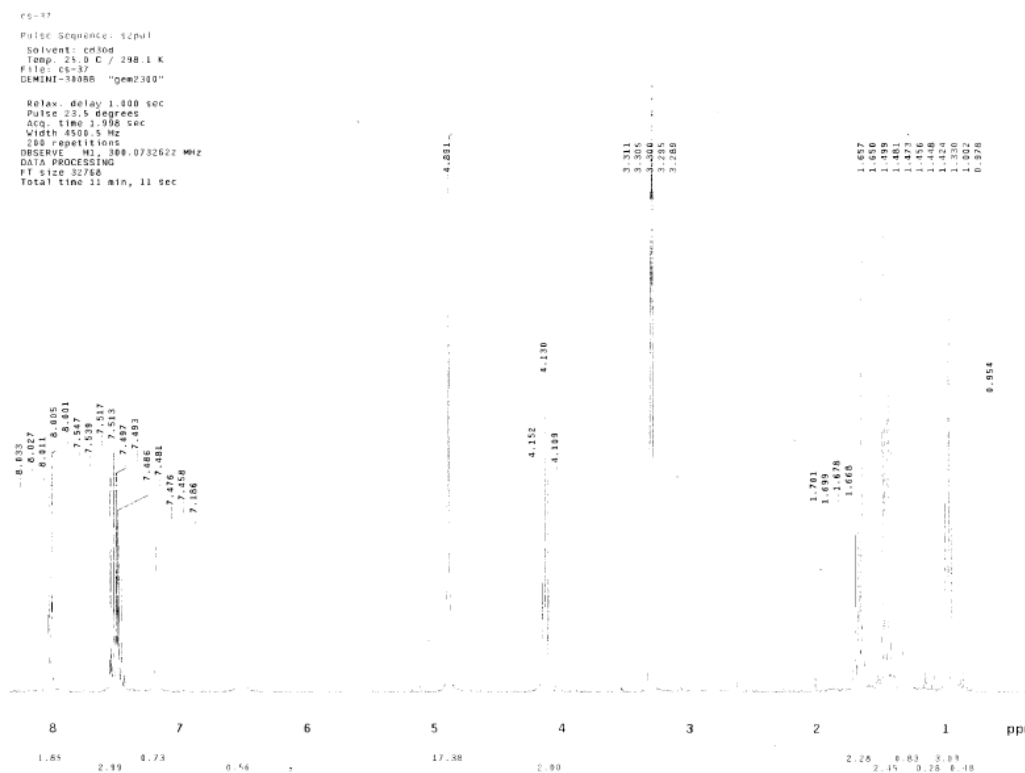**Ethyl *N*-[7-amino-2-(pyridin-2-yl)-1H-1,3-benzodiazol-5-yl]carbamate (3.3.3):**
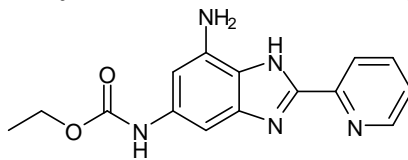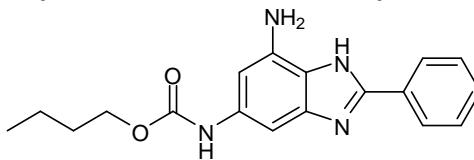

¹³C-NMR:

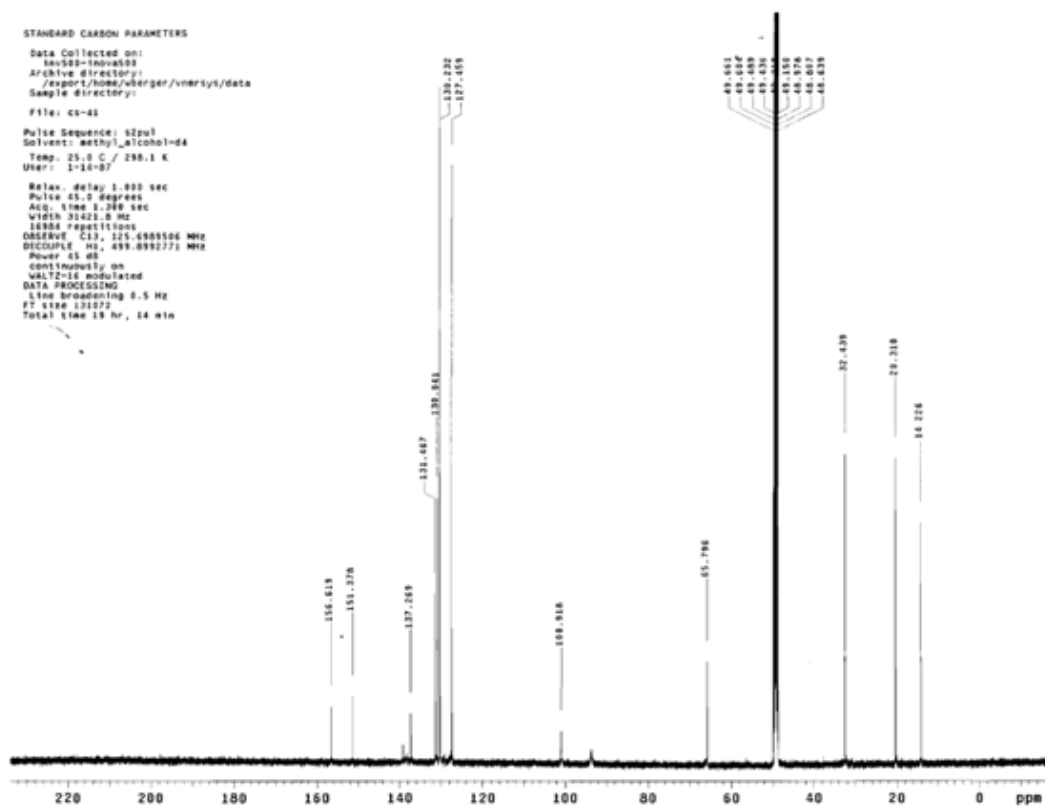***n*-Butyl N-(7-amino-2-phenyl-1H-1,3-benzodiazol-5-yl)carbamate (3.3.4):**

¹H-NMR:

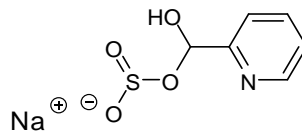**_n_-Butyl N-(7-amino-2-phenyl-1H-1,3-benzodiazol-5-yl)carbamate (3.3.4):**

^{13}C-NMR:

_n_-Butyl _N_-(7-amino-2-cyclohexyl-1H-1,3-benzodiazol-5-yl)carbamate (3.3.5):

¹H-NMR:

Sodium pyridin-2-yl(hydroxy)methyl sulfinate (4.1.1):

^{1}H-NMR:

CS-05

Pulse Sequence: s2pul

Solvent: D2O
Temp. 25.0 C / 298.1 K
GEMINI-300BB "gem2300"

Relax. delay 1.000 sec
Pulse 7.8 degrees
Acq. time 1.998 sec
Width 4500.5 Hz
16 repetitions
OBSERVE   H1, 300.0728539 MHz
DATA PROCESSING
Line broadening 0.5 Hz
FT size 32768
Total time 0 min, 53 sec