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Antibacterial Activity, Structure-Activity Relationships, and Scale-Up Reaction of 1,3,4-Oxadiazoles

Olivia Marie Smith
smith21@otterbein.edu

ANTIBACTERIAL ACTIVITY, STRUCTURE-ACTIVITY RELATIONSHIPS, AND SCALE-UP REACTION OF 1,3,4-OXADIAZOLES

By

Olivia Marie Smith

Department of Chemistry

Otterbein University

Westerville, Ohio 43081

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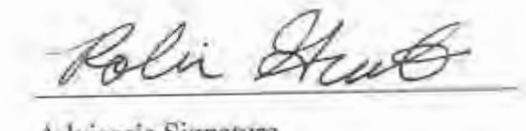
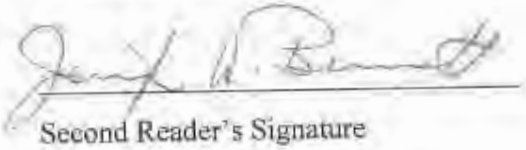
Robin Grote, Ph.D.
Honors Thesis Advisor



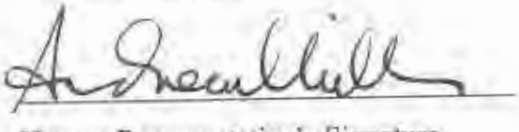
Advisor's Signature

Jennifer Bennett, Ph.D.
Second Reader



Second Reader's Signature

Andrew Mills, Ph.D.
Honors Representative



Honors Representative's Signature

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Abstract

Oxadiazoles are compounds in the field of organic chemistry that have been gathering interest in the medicinal chemistry and microbiology communities for their biological properties, which range from anti-inflammatory agents, to chemotherapy drugs, to antibiotics. The synthesis of oxadiazoles can be difficult due to the expensive and complex nature of the techniques used as well as the volatile reagents and elevated temperatures that are often required in organic synthesis. The Grote lab has recently developed a new method for the synthesis of 1,3,4-oxadiazoles under mild conditions. The goals of this thesis were thus twofold: to develop a viable scale-up procedure for this novel reaction and to determine the antibacterial properties of some of the simple oxadiazoles made in the Grote lab. To determine whether these oxadiazoles possessed any viable antibacterial properties, the Kirby-Bauer disc method was utilized with *E. coli*, *B. megaterium*, and *M. luteus* as the bacterial representatives.

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Introduction

Oxadiazoles have recently received increased attention in the scientific community for their uses in a variety of medicinal compounds. The oxadiazole base structure (Figure 1) is a heterocyclic structure containing one oxygen, two nitrogen, and two carbon atoms, with room for functional groups to attach to the carbons to enable the formation of various derivatives. These functional groups, or R groups, can range in size and complexity from a single atom to a structure larger than the original oxadiazole itself. They can also be placed on either side of the symmetric oxadiazole.

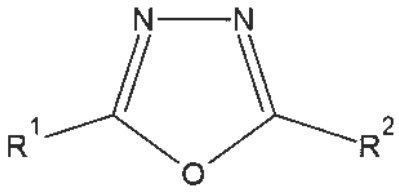

Figure 1. Oxadiazole unit structure.

The substituents on oxadiazole derivatives can provide the compound with a range of chemical properties that are useful in pharmaceuticals, ranging from anti-fungal to anti-cancer behavior.[1] Oftentimes, groups on the oxadiazole are phenyl rings with further substituent modification occurring at the para position to the branched attachment. These can vary from single halogens to more complicated multi-ring structures, but the complexity of the substituent does not seem to have a large influence on biological activity.[8]

Some of the uses of oxadiazoles include cough suppressants (Oxalamine), antimicrobial agents (furamizole), chemotherapy (Zibotentan), and the inhibition of various biological pathways (Butalamine) (Figure 2).[1,2]

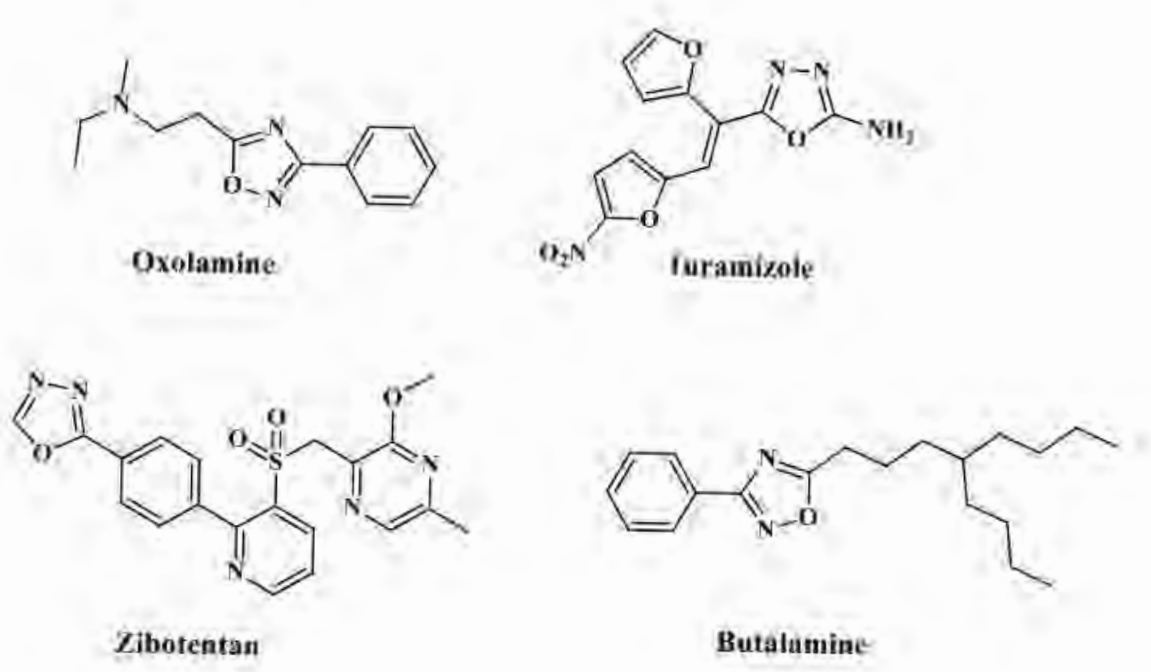

Figure 2. Common commercially available drugs with an oxadiazole scaffold.[1]

The use of oxadiazoles as antibiotics is of significant interest. Common health issues such as bacterial infections are often treated by over-the-counter antibiotics. However, the misuse and overuse of these types of medications can lead to negative side effects like antibiotic resistance.[3,4]

Antibiotic resistance especially is becoming a large problem for the modern world, as simple infections can develop into years-long health issues and bacteria are thus getting more deadly by retaining and spreading their resistance genes. As with every other living organism, bacteria are constantly fighting to survive, which means developing resistance to potential destructive compounds in antibiotics. However, humans want to survive as well, fighting against the mildly inconvenient to lethal effects from bacterial infections. This phenomenon has locked humans and bacteria in a struggle to outcompete the other that has lasted for as long as they have coexisted. The pharmaceutical industry has also slowed its efforts to find new and improved

antibiotics due to decreased profits. Clearly, if this problem is not addressed, then many people will suffer and die from bacterial infections that can no longer be treated.[5] The key to overcoming the problems of drug overuse through a public health focus is to educate physicians and the general public about the associated dangers. The key to overcoming this problem from the chemical synthesis route is to then produce new compounds that do not have the same unintentional side effects and hazards. Oxadiazole modifications are showing promise at helping to reduce and reverse antibiotic resistance by assisting other antibiotics through blocking efflux pumps and working as non-β-lactam inhibitors of cell wall biosynthesis.[6,7]

Many antibiotics commercially available today are β-lactam inhibitors, and a flooding of the market and thus the population with these antibiotics has led to an increased development of bacterial resistance to β-lactam inhibitors. As oxadiazoles are a potential non- β-lactam inhibitor, they also therefore have the potential to reduce the use of β-lactam inhibitors as well as the subsequent bacterial resistance. Efflux pumps are a common technique used by bacteria to pump out the antibiotic from its system, preventing the antibiotic from doing any damage. Other antibiotics that would normally be ineffective due to these efflux pumps may be made effective again by the oxadiazoles blocking these pumps, allowing the antibiotic to damage the bacteria and treat the infection. Further research into oxadiazoles as both antibiotics and antibiotic assistants may help open the doors to decreasing antibiotic resistance and create or restore avenues of treating infections.

Scale-Up

Synthesis of 1,3,4-oxadiazoles is often difficult to achieve, requiring highly volatile and toxic chemical reagents that are used in multi-step syntheses, such as sulfuric acid and transition

metals, as well as the need for elevated temperatures in these reactions and the use of complex, expensive, and lengthy techniques such as microwave irradiation and nanoparticles.[9-16]

A straightforward procedure for the synthesis of 1,3,4-oxadiazoles has been achieved by the Grote lab (Figure 3) with a novel two-step, one-pot cyclodehydration that reduces both the use of elevated temperatures and toxic, expensive, highly corrosive reagents. Cyclodehydration reactions describe the process of a condensation reaction that forms a ring.

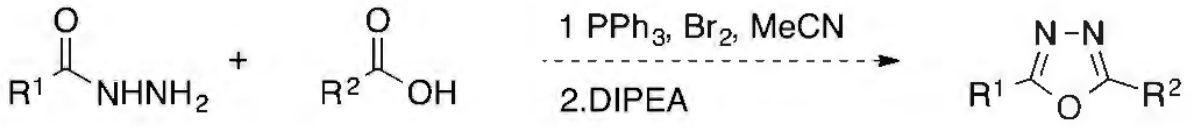

Figure 3. General reaction scheme for Grote synthesis of 1,3,4-oxadiazoles.[17]

The procedural success of this simple, elegant reaction scheme is due to the use of triphenylphosphine dibromide as the key cyclodehydration reagent, which is shelf-stable, easily produced and acquired, and works under standard lab conditions. Triphenylphosphine is a common reagent in organic chemistry synthesis reactions due to its directed reactivity with carbon-based molecules and its ability to form and break both single and double bonds. Combining triphenylphosphine and bromine in the presence of acetonitrile under a nitrogen atmosphere at 0°C will quickly produce triphenylphosphine dibromide to be used in situ. The scale-up for this reaction is promising as the key reagent of triphenylphosphine dibromide has been published in a large-scale formation reaction already.[17,18] Both triphenylphosphine dibromide and an intermediate reactant benzoyl hydrazine (Figure 4) can be made in an undergraduate lab from common, easily handled chemicals, and the ease of production makes this synthesis a prime target for a scale-up reaction.[19]

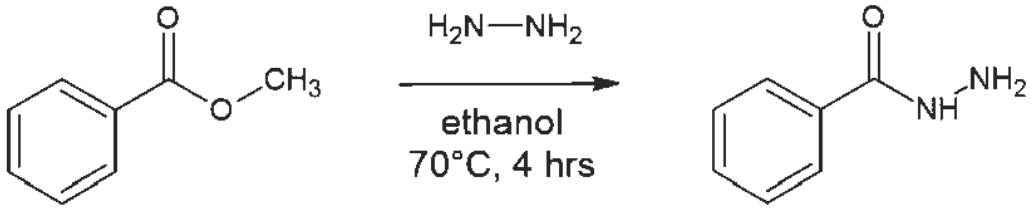

Figure 4. Benzoyl hydrazine synthesis scheme.

This project is a continuation of novel oxadiazole study under Dr. Grote, to further the scientific community's knowledge about the formation of these substances, their medicinal uses, and the viability of these compounds as mass-produced treatments. The previously synthesized oxadiazoles in the Grote lab were studied and tested for their yield based on the electronegativity of the R group. It was found that electron-withdrawing groups tend to stabilize transition state reactions and increase yield of the final product. Some of the oxadiazoles synthesized had R groups of chlorine, bromine, fluorine, hydrogen, and dimethyl amine. A proposed mechanism for the formation of the oxadiazole by the triphenylphosphine dibromide cyclodehydration involves the formation of 1,2-dibenzyoylhydrazine as a key intermediate.[20,21]

This experiment focused on scaling-up the Grote lab two-step one-pot synthesis of the previously made oxadiazoles from the current 0.1 gram to 1 gram and eventually 10 grams. The procedure for a scale-up reaction follows the same general procedure as the original but in much larger numbers in hopes of gaining a larger yield of compound. There are parameters that must be considered when scaling-up a reaction, such as increased time for heating and cooling, using larger and different types of glassware, and using alternative purification methods such as recrystallization. These parameters can make scale-up reactions difficult, but the large amount of yield of potentially medicinally viable compound is worth it. There are also risks associated with

scale-up reactions due to the nature of using large quantities of reagents in large apparatuses, as well the other issues of greater potential for forming larger amounts of side products and the amounts of time and energy needed for these reactions.[22]

Scale-up methods for other medicinally significant compounds also pose unique challenges, as common manufacturing processes such as spray drying make scale-up reactions difficult as they are harder to control in large quantities and quality control thus decreases.[23] The emergence of nanoparticles as a superior drug delivery method contains inherent scale-up challenges as well, again due to the narrowing window of control over quality as production moves towards mass scale as the manufacturing process often requires extreme temperatures, expensive equipment, and overall complexity of synthesis.[24]

In contrast, the novel Grote synthesis for 1,3,4-oxadiazoles is simple and safe enough to be conducted in an undergraduate research lab, and the scale-up should be similar in terms of ease of production. The harshest chemical used is dichloromethane, the trickiest technique is using an inert nitrogen atmosphere, and the largest apparatus is a rotavap. Scaling-up production for this efficient synthesis method from milligrams to multigram quantities would then provide large amounts of compound for further studies. These large-scale compound studies would provide valuable insight into the compounds' ability to treat ailments as well as the viability of mass production of these oxadiazoles for said treatments.[25]

Virtually every pharmaceutical, industrial chemical, cosmetic, and many other chemical-based products are made using scale-up techniques to meet high demand, and the viability of a scale-up reaction of a chemical is thus very useful in determining its mass production potential as well as opening the path to more tests to discover all possible uses of the chemical. This project describes the scale up attempt of this procedure to obtain multigram quantities from a single

reaction, which would demonstrate the synthetic utility of this method and its ability to produce large quantities of reagents and products. Preliminary trials show promise of a viable scale-up using the streamlined Grote lab procedure. The specific oxadiazole derivative considered for scale-up was 2-(4-chlorophenyl)-5-phenyl-1,3,4-oxadiazole.

Antibacterial Activity

The improved synthesis of oxadiazoles returns to the initial problem of finding new compounds with antibacterial activities while seeking to avoid overuse and the development of antibiotic resistant diseases. Selected oxadiazoles previously synthesized by other members of the Grote lab were used in initial antibacterial testing to determine the compounds' biological activity related to the various substituent groups. The commercially available oxadiazoles used to treat a variety of ailments are often more complex than the oxadiazoles made in the undergraduate Grote lab. This project is also a preliminary study into the structure-activity relationships between the core oxadiazole structure, the possible functional groups, and the specific antibacterial activity observed.

A simple test for biological activity utilizes the Kirby-Bauer method[26], which involves plating a lawn of bacteria on a nutrient agar plate, and placing discs inoculated with the compounds to be tested on the bacterial lawn. After a period of incubation, the inhibition zone of bacterial growth around the disc is measured and compared with a control antibiotic to determine initial antibiotic properties. In general, a larger inhibition zone indicates a greater ability to decrease the growth of and/or destroy bacterial colonies by the compound being studied, which implies greater antibiotic behavior and thus an improved likelihood of the compound being a candidate for an effective and eventually commercially available antibiotic. Of course, diseases can be caused by a number of factors, and disk diffusion susceptibility tests can be used with a

variety of substances besides bacteria, such as fungi and viruses, to test antifungal and antiviral behavior, respectively.[27,28]

However, bacteria and antibiotic resistance are the central concern of this research project, and therefore bacteria will be used in the tests to determine antibiotic behavior of the previously synthesized oxadiazoles. Bacteria have a variety of properties that make the development of both broad- and narrow-spectrum antibiotics difficult. This research project began the initial oxadiazole antibiotic study by comparing its effectiveness against both Gram-negative and Gram-positive bacteria. Gram-positive and -negative designations indicate the structure of the bacterial cell wall, with Gram-positive bacteria having many thick layers of peptidoglycan as their cell wall, while Gram-negative bacteria have a thin peptidoglycan cell wall that is surrounded by a lipopolysaccharide outer membrane. This difference in cell wall structure provides different properties to the bacteria, such as their reaction to different types of antibacterial agents due to their mechanism of action of infiltrating and bursting a cell wall.[29]

The cell envelope, or the combination of the plasma membrane that encases every cell and the cell wall that is unique to prokaryotes for its ability to house single-celled life, is the main barrier between the contents of a single-celled organism and the outside environment. This protective barrier of the peptidoglycan cell wall prevents substances such as antibiotics from simply passing into the main part of the cell and doing fatal damage to its vital processes. Generally, ‘Gram-negative bacteria are more resistant to antibiotics than are their Gram-positive cousins’ (Silhavy et al.) which seems to arise from the lipopolysaccharide and teichoic acid coatings intertwined with the Gram-negative outer membrane, a feature that is absent in Gram-positive bacteria.

As mentioned previously, oxadiazoles have been found to interrupt the biosynthesis of the bacterial cell wall as one of its main forms of antibiotic activity. The Kirby-Bauer method for determining the antimicrobial activity of a compound was thus used to test the biologically antagonistic behavior of the previously synthesized oxadiazoles against both Gram-negative and Gram-positive bacteria to determine the preliminary antibiotic activity from a simple oxadiazole derivative synthesized in the Grote lab. For this purpose, *Escherichia coli*, *Micrococcus luteus*, and *Bacillus megaterium* were chosen to represent the two factions of bacteria.

E. coli bacteria are Gram-negative and can be pathogenic, as *E. coli* O157:H7 is a strain responsible for causing severe irritation in the gastrointestinal tract that, if untreated, could possibly lead to death. Although the rate of infection with *E. coli* O157:H7 is lower than some other common bacterial pathogens, hospitalizations and deaths occur at higher rates, demonstrating the severity of this pathogen. It is a common threat in the Western world due to improper food cleaning, handling, and preparation.[30] *M. luteus* is Gram-positive and, although generally non-pathogenic, is associated with a variety of diseases such as meningitis, endocarditis, and catheter-related infections due its opportunistic behavior as a pathogen.[31] *B. megaterium* is another Gram-positive bacteria that forms spores and is commonly found in soil, with abilities to promote plant growth and discourage the growth of plant pathogens. Even when humans are not directly involved, effects of bacterial interactions can still be felt in fields such as agriculture, since humans heavily depend on crops for food sources and infected crops can cause famine and disease.[32]

There were five different oxadiazole derivatives used in the Kirby-Bauer antibiotic tests, with R groups of H, NMe_2, F, Cl, and Br (Figure 5).

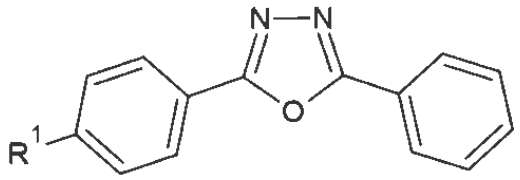

Figure 5. Oxadiazole derivative unit structure.

The results of these compounds' Kirby-Bauer tests were compared with a positive control of tetracycline, which has known antibiotic behavior. Tetracycline is a common, broad-spectrum antibiotic that has been used to treat infections for decades. However, its broad method of action that made it ideal for neutralizing a variety of bacteria and its subsequent frequent use has lead to the development of tetracycline-specific resistance.[33] Thus, as it is widely available, widely used, and has an increasing number of bacteria resistant to its attacks, it is a great candidate for comparison of antibiotic behavior against both Gram-positive and Gram-negative bacteria.

Results and Discussion

Scale-Up

There were two trials conducted for the synthesis of intermediate reagent benzoyl hydrazine (Table 1). The mass recovery from trial one was 0.47 g, and from trial two it was 0.19 g. The percent yield, with methyl benzoate as the limiting factor at 0.022 mol, was 15.72% for trial one and 6.35% for trial two. The scale-up experiment was successful in creating intermediates in the lab and physically executing the procedure. Even though the percent yield was low, the possibility of creating the intermediate in the lab is reassuring that this procedure can be improved and easily conducted in the undergraduate laboratory.

Table 1. Mass Recovery and Yield of Benzoyl Hydrazine Synthesis

	Mass Recovery	Percent Yield
Trial 1	0.47g	15.72%
Trial 2	0.19g	6.35%

The scale-up synthesis for the oxadiazole derivative 2-(4-chlorophenyl)-5-phenyl-1,3,4-oxadiazole was only partially completed, as the final product was unable to be isolated before the completion of this thesis. A solubility screen was conducted on the end compound of the oxadiazole synthesis to determine the route for recrystallization as the standard purification method, which would simplify the procedure as compared to a technique such as column chromatography.

The results from the solubility screen (Table 2) show that DCM and diethyl ether are good candidates for a recrystallization procedure as purification. Ethanol was able to dissolve the compound but was not chosen as a viable recrystallization solvent due its difficulty in being

removed from the final compound, and hexanes were unable to dissolve the compound in the first place. Ethyl acetate led to an incomplete dissolution of the compound and slowly evaporated from the solution. Diethyl ether created a slow crash of the product, requiring a smaller flask and minimum amount of solvent which still resulted in the creation of triphenylphosphine oxide as a side product. DCM resulted in the product oiling out first before eventually forming crystals that were also impure due to the presence of triphenylphosphine oxide as side product. The solutions and crystals were combined to be placed back on the rotavap but became lodged in the apparatus. Thus, the final mass recovery and ^{1}H NMR were unable to be conducted before the conclusion of this thesis and it cannot be determined whether the scale-up was successful in creating the desired end product on a one gram scale with sufficient purity.

Table 2. Solubility Screen Results of 2-(4-chlorophenyl)-5-phenyl-1,3,4-oxadiazole

	DCM	Diethyl ether	Ethanol	Hexanes	Ethyl acetate
Oxadiazole dissolution	Soluble	Soluble	Soluble	Insoluble	Incomplete dissolution
Result of recrystallization attempt	Oil, then crystals with side product	Crystals with side product	Unable to recrystallize	N/a	Evaporation of solvent

The preliminary ^{1}H NMR and ^{13}C NMR analysis of the crude product from the scale-up synthesis before and after the solubility screen shows a lack of defined peaks. The ^{13}C NMR spectra (Appendix, Figure 13) showed a noisy background with some peaks between 120 and 130 ppm, which could indicate the presence of carbon in both aromatic rings and double bonds.[34] This is reassuring as the oxadiazole should have two phenyl rings, though the reactants and side

product triphenylphosphine also contain aromatic rings. Unfortunately, due to the background noise that could not be reduced, not much else could be interpreted and concluded with confidence. The peaks in the ^{1}H NMR spectra (Appendix, Figures 9-12) ranged from 7.03 to 8.17 ppm for the compound, both before and after the solubility screen and between diethyl ether and DCM, which places the compound solidly in the aromatic region, again reassuring the presence of reactants, side products, and the desired end product, or some combination thereof.[35] Since the peaks could not be differentiated, though, it is unclear about the progression and success of the synthesis in regards to forming the end product as far as is readily known. It is assumed that triphenylphosphine is the side product overshadowing the detection and lowering the purity of the desired end product oxadiazole, as it is a common side product when utilizing triphenylphosphine as a reagent.[36] There are methods to purify products by removing the triphenylphosphine oxide though, which creates more triphenylphosphine and may be a viable purification step in future syntheses.[37]

Antibacterial Activity

After the incubation period, the plates were observed and inhibition zones were measured (Figures 6-8). The results for each trial of the oxadiazole derivatives are shown below (Table 1) for each of the five R groups of H, NMe_2, F, Cl, Br at concentrations of 1 µg/µL, 0.5 µg/µL, and 0.25 µg/µL, as well as the positive and negative controls. There were consistent results of no inhibition from the negative control and steady inhibition from the positive control (Figures 6-8, Table 1), as to be expected. The 30 µg tetracycline positive control displayed an inhibition zone of 1.1 cm for *E. coli*, 1.5 cm for *M. luteus*, and 1.0 cm for *B. megaterium*. Since the positive control did display inhibition ability, it can be concluded that any lack of inhibition from the

oxadiazoles is due to their properties and not any issues with bacterial colony growth or other experimental preparation. There was no significant inhibition of any of the bacterial strains from any of the oxadiazoles tested, so it can be assumed that either these specific oxadiazole derivatives have no significant antibacterial activity against these strains of bacteria, the concentrations of the oxadiazole solutions were too low to exhibit antibiotic activity, or a combination of the two. This lack of inhibition from the oxadiazoles also indicates that these simple core oxadiazole structures may not be the most important characteristic for antibacterial activity, and perhaps different or more complex structures may exhibit activity instead, or these specific derivatives may have activity but against other species of bacteria.

Table 3. Inhibition Zone Area Radii for Each Antibacterial Trial of Selected Oxadiazole Derivatives in cm.

	E. coli trial 1	*E. coli* trial 2	*M. luteus* trial 1	*M. luteus* trial 2	*B. megaterium* trial 1	*B. megaterium* trial 2
H (1 µg/µL)	0.0	0.0	0.0	0.0	0.0	0.0
H (0.5 µg/µL)	0.0	0.0	0.0	0.0	0.0	0.0
H (0.25 µg/µL)	0.0	0.0	0.0	0.0	0.0	0.0
NMe_2 (1 µg/µL)	0.0	0.0	0.0	0.0	0.0	0.0
NMe_2 (0.5 µg/µL)	0.0	0.0	0.0	0.0	0.0	0.0
NMe_2 (0.25 µg/µL)	0.0	0.0	0.0	0.0	0.0	0.0
F (1 µg/µL)	0.0	0.0	0.0	0.0	0.0	0.0
F (0.5 µg/µL)	0.0	0.0	0.0	0.0	0.0	0.0
F (0.25 µg/µL)	0.0	0.0	0.0	0.0	0.0	0.0
Cl (1 µg/µL)	0.0	0.0	0.0	0.0	0.0	0.0
Cl (0.5 µg/µL)	0.0	0.0	0.0	0.0	0.0	0.0
Cl (0.25 µg/µL)	0.0	0.0	0.0	0.0	0.0	0.0
Br (1 µg/µL)	0.0	0.0	0.0	0.0	0.0	0.0
Br (0.5 µg/µL)	0.0	0.0	0.0	0.0	0.0	0.0
Br (0.25 µg/µL)	0.0	0.0	0.0	0.0	0.0	0.0
Tetracycline (30 µg)	1.1	1.1	1.5	1.5	1.0	1.0
DI water	0.0	0.0	0.0	0.0	0.0	0.0

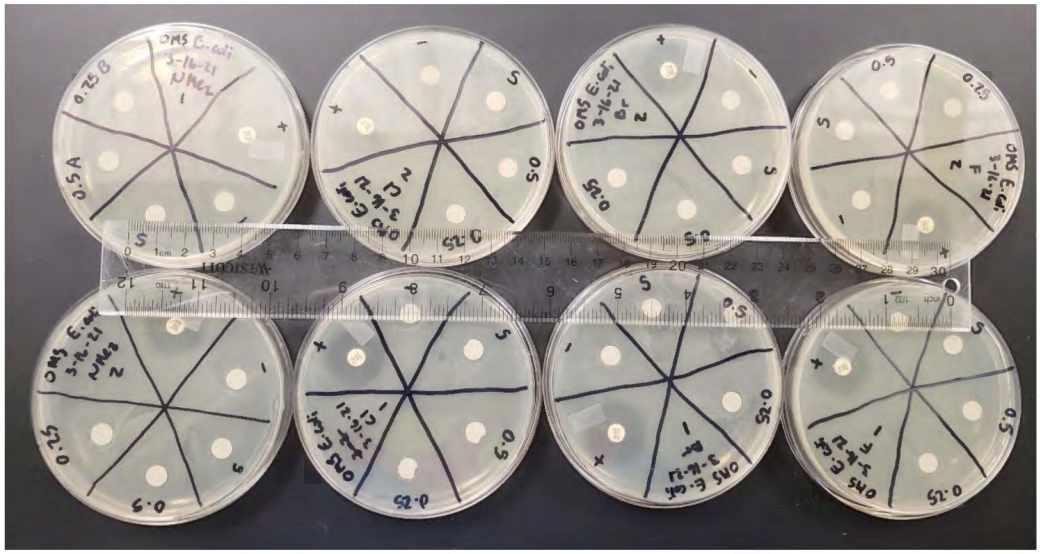

Figure 6. *E. coli* plates after incubation.

Figure 7. *M. luteus* plates after incubation.

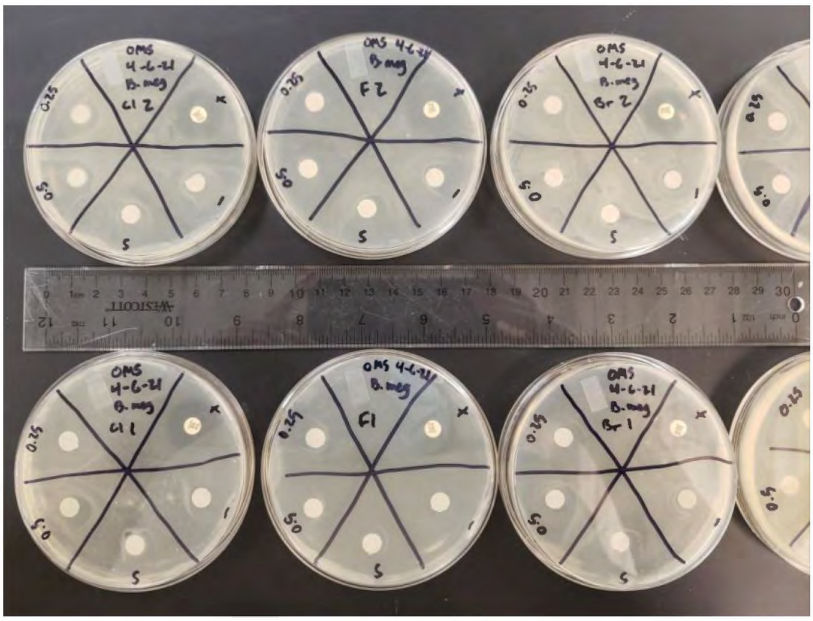

Figure 8. *B. megaterium* plates after incubation.

Materials and Methods

Scale-Up

The scale-up synthesis began with two trials to create one of the key intermediate reactants for the cyclodehydration, benzoyl hydrazine. For the first trial, in a 100 mL round-bottom flask (RBF), 2.77 mL of methyl benzoate, 9 mL of hydrazine monohydrate, and 30 mL of ethanol were combined. The solution was heated and stirred at 70°C and 300 rpm for 4 hours, then run to dryness on the rotavap. The compound was recrystallized from 3 mL ethanol then pressed dry with filter paper. After drying, the product was placed in a new RBF and pumped dry under nitrogen. For the second trial, in a 100 mL round-bottom flask (RBF), 2.77 mL of methyl benzoate, 9 mL of hydrazine monohydrate, and 30 mL of ethanol were again combined. The solution was heated and stirred at 70°C and 300 rpm for 4 hours, then run to dryness on the rotavap as before. Then, the compound was dissolved in 25 mL water and 25 mL dichloromethane (DCM) and placed in a separatory funnel. The organic layer was separated and washed with another 25 mL of water, then dried under approximately 2 g sodium sulfate. The compound was gravity filtered, placed in a new RBF, run to dryness on the rotavap, and pumped dry under nitrogen.

For the cyclodehydration reaction, 5.0082g of triphenylphosphine was placed in a 100 mL RBF and pumped dry under nitrogen. Using a syringe, 20 mL anhydrous acetonitrile was added to the RBF and the flask was placed in an ice bath. Approximately 0.89 mL of bromine was added dropwise, and the solution was stirred for 5 minutes, then 10 more minutes at room temperature. Then, 0.675 g 4-chlorobenzoic acid was added to the solution, which was then stirred for 10 minutes at room temperature, before adding 0.585 g stock benzoyl hydrazide and approximately 9 mL of more anhydrous acetonitrile and stirring for another 10 minutes at room

temperature. The flask was placed in an ice bath and 6 mL of DIPEA was added dropwise. The solution was left to stir at room temperature overnight, then quenched with 25 mL water while still stirring. The solution was transferred to a separatory funnel, where 25 mL water and 50 mL DCM were added. The organic layer was separated, washed with another 50 mL DCM, then washed with 50 mL sodium bicarbonate and dried over sodium sulfate. The compound was gravity filtered into a new, preweighed RBF and run to dryness on the rotavap. A preliminary ^{1}H NMR was run on the crude product, as well as a solubility screen with diethyl ether, ethyl acetate, DCM, ethanol, and hexanes. A ^{13}C NMR was then run on the DCM-recrystallized product, and ^{1}H NMR was run on the DCM- and diethyl ether-recrystallized products.

Antibacterial Activity

A 1 µg/µL amount of oxadiazole in DMSO was prepared for each of the five R groups, in addition to using DI water for negative control, 30 µg tetracycline disks from Hardy Disks for positive control, and dilute oxadiazole solutions at 0.5 µg/µL and 0.25 µg/µL. The 200 microliters of bacteria culture in nutrient broth was pipetted onto Mueller-Hinton agar plates and spread with a sterilized spreader. Autoclaved filter paper disks cut with a hole punch were inoculated by pipetting 15 microliters of solution onto the disk and left to dry overnight. Sterilized tweezers were used to place each disk onto the plate, evenly spread out from other disks to allow for growth of the inhibition zone. This was conducted in duplicate for each 1 µg/µL oxadiazole, as well as the 0.5 µg/µL dilution, 0.25 µg/µL dilution, and controls. Plates were then incubated at 37°C overnight for both *E. coli* and *B. megaterium*, and over the course of one week for *M. luteus*.

Future Directions

Further research and experimentation with the scale-up procedure would likely result in greater yield and higher efficiency, as well as providing plenty of compound for studying synthesis of other oxadiazole derivatives and their biological activity. This could be achieved by conducting further solubility screens, exploring column chromatography as a purification method, and removing the triphenylphosphine oxide side product which may even create more triphenylphosphine, acting as a catalyst and restoring the key reagent. Other oxadiazole derivatives may be used as the target end product, which would adjust the procedure and perhaps result in higher yields due to an improvement in mechanistic efficiency, Any product created would then also be useful as a new oxadiazole to use in antibiotic tests. If the adjustments to these procedures based on mechanistic and/or other chemical properties are successful, this would also be promising as a generally viable scale-up method, with more lab work hopefully leading to a larger scale of production.

Future studies on the biological activity of 1,3,4-oxadizoles should focus on structure-activity relationships (SAR) to determine the viability of synthesis and the antibiotic activity, as well as their activity when paired with other antibiotics.[6] Literature research on the microbes that are typically affected by oxadiazole-specific antibiotic activity would be a good place to begin this search. For example, Gram-negative bacteria such as *Shigella flexneri* and *Helicobacter pylori* are more susceptible to the oxadiazoles' ability to stall ribosomes.[8] In addition, some biological activity seems to improve with the replacement of the F moiety as an R group by an F_3C moiety.[8]

Besides adjusting its functional groups, the oxadiazole base itself can also be modified while retaining some antibiotic activity. However, the strength of activity seems to be highly

centered around the addition of an amide group to the overall molecule, and its relative position to the oxadiazole scaffold, which is usually on the α- or β-carbon if it is associated with biological activity. These starting points would greatly help improve the future directions of this project by exploring these specific relationships and possibly developing procedures, such as syntheses and antibiotic tests, and narrowing down the desired end products based on these findings in order to develop improved synthesis efficiency and products with high antibiotic efficacy.

Appendix

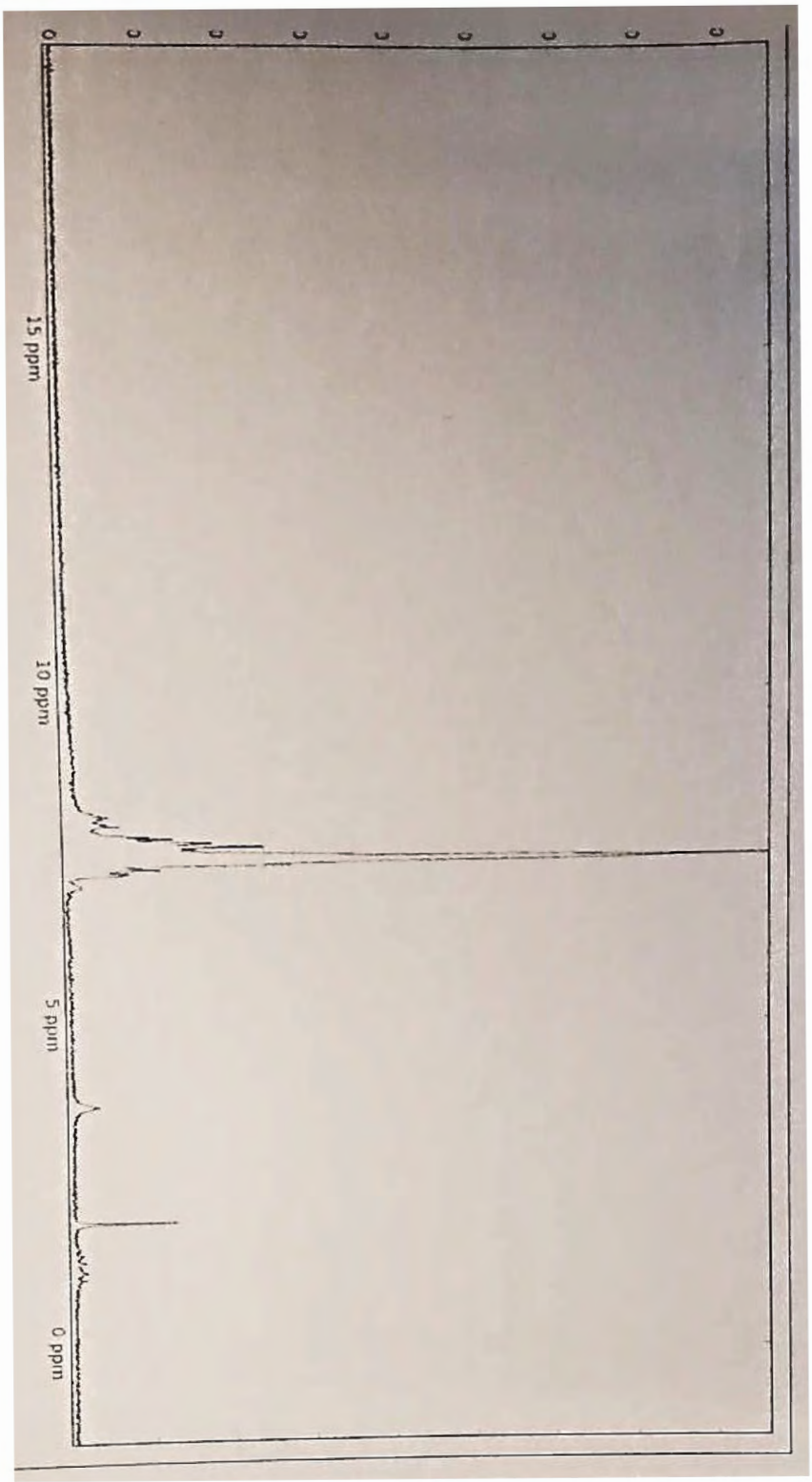

Figure 9. Preliminary ^{1}H NMR spectra of crude oxadiazole derivative.

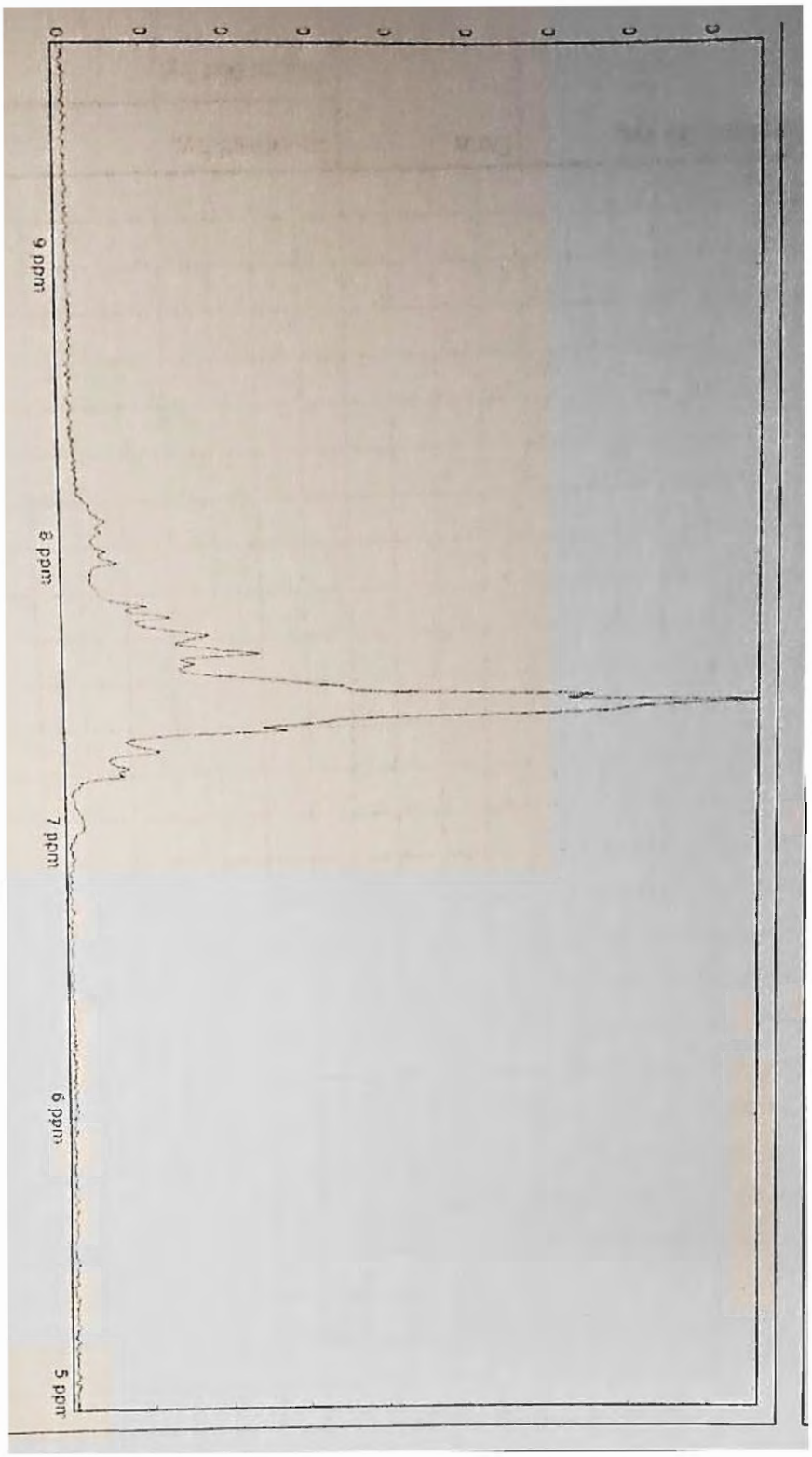

Figure 10. Preliminary ^{1}H NMR spectra of crude oxadiazole derivative, zoomed in for clarity.

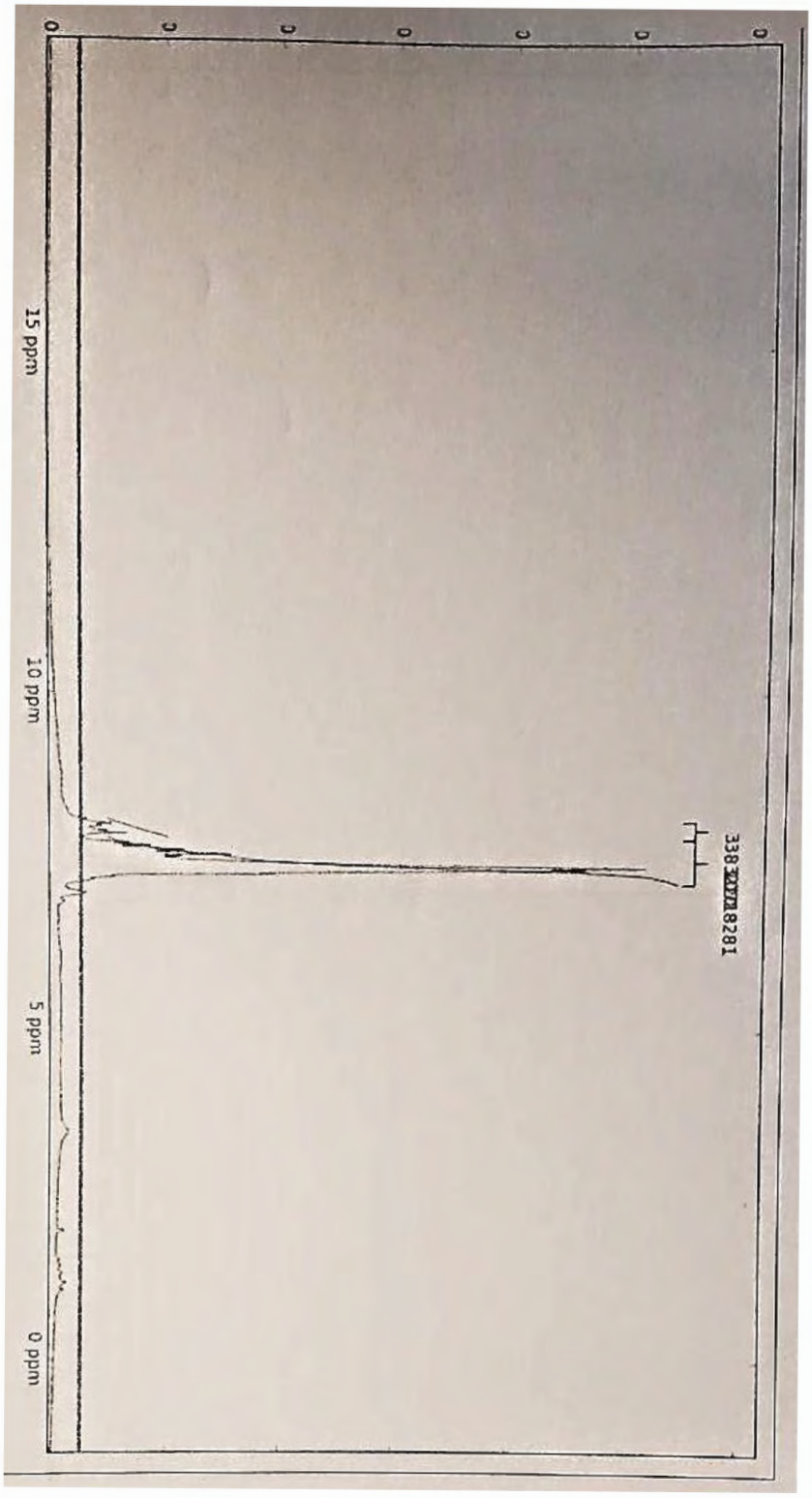

Figure 11. Crude oxadiazole derivative ^{1}H NMR spectra after diethyl ether solubility screen.

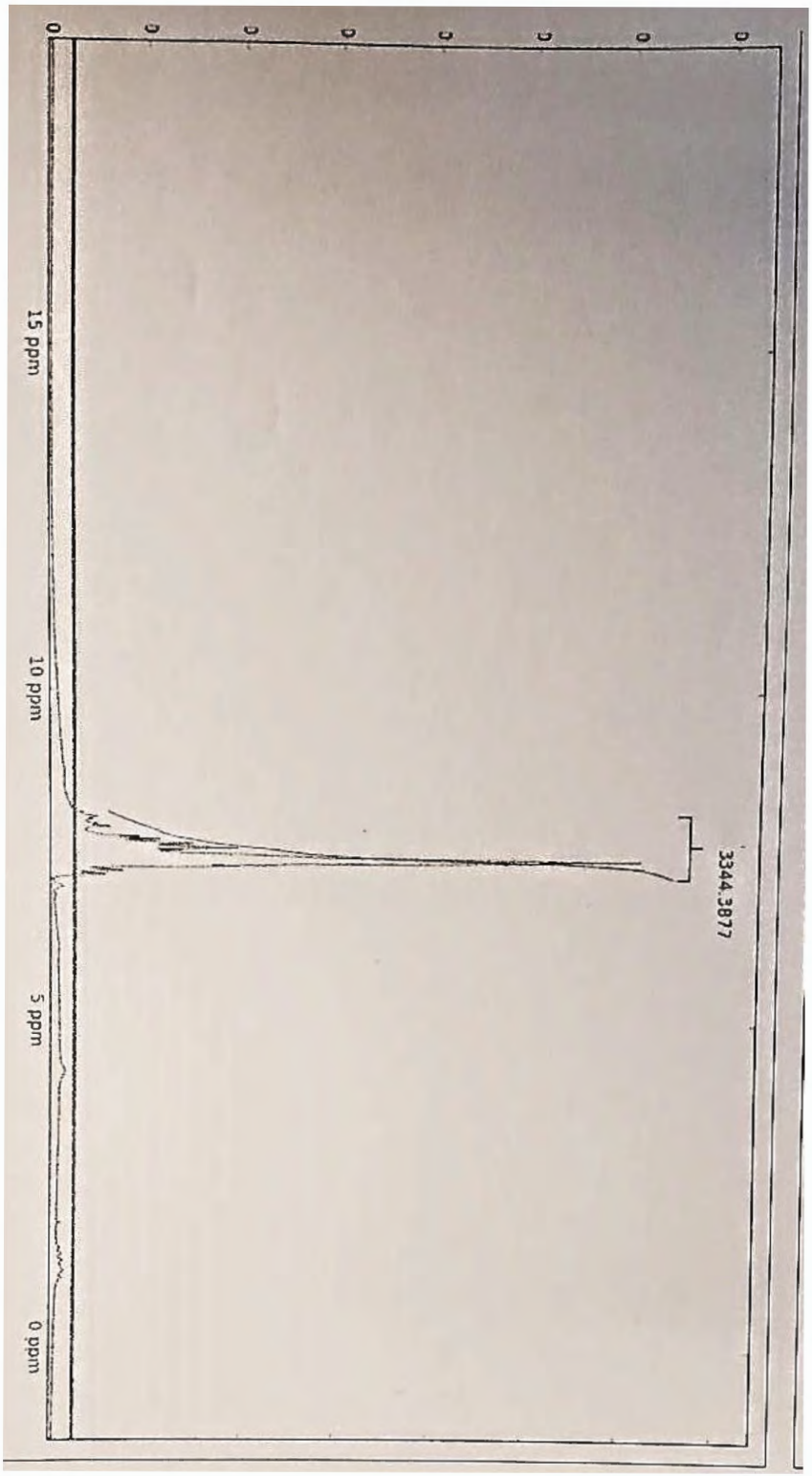

Figure 12. Crude oxadiazole derivative ^{1}H NMR spectra after DCM solubility screen.

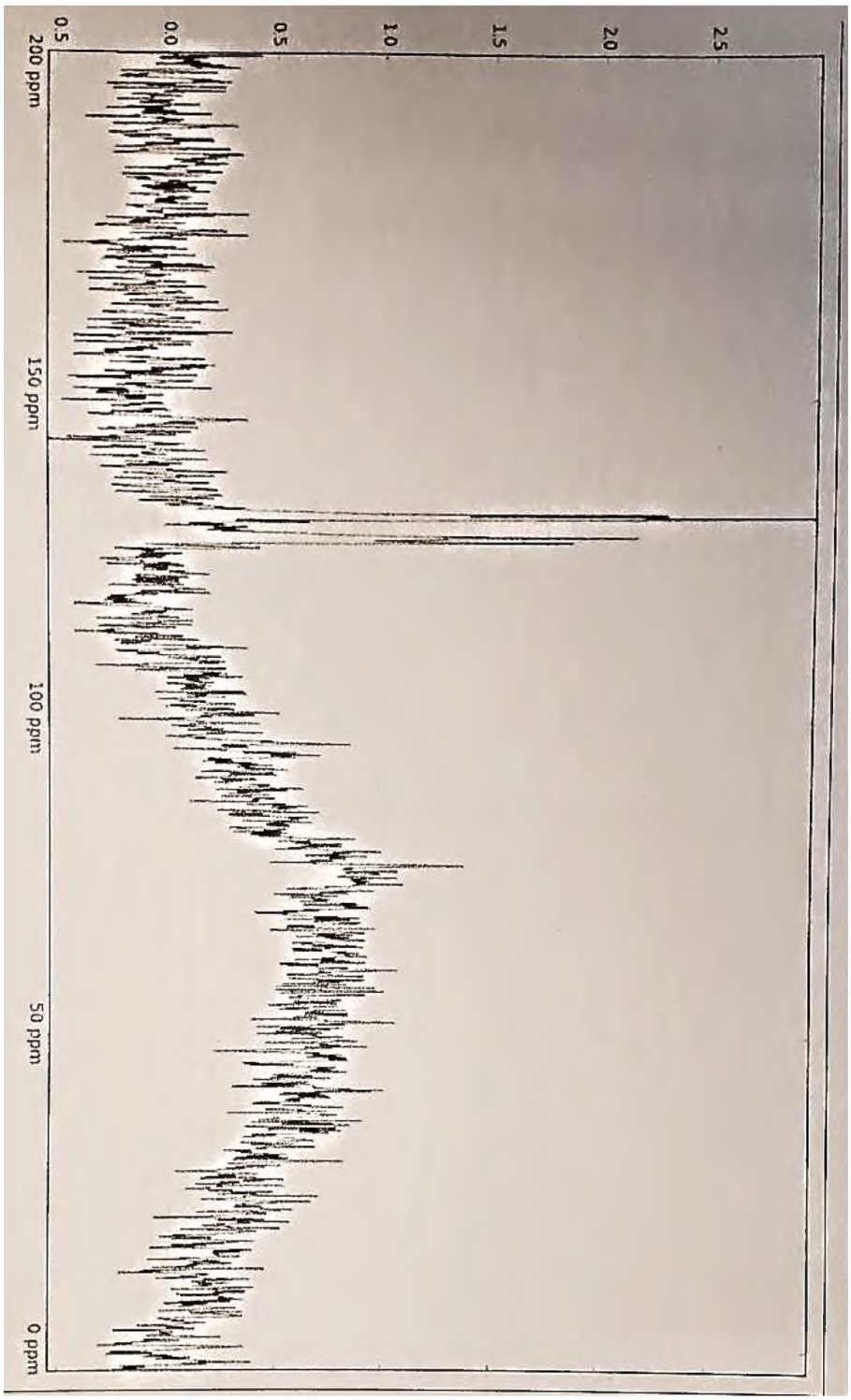

Figure 13. Crude oxadiazole derivative C^{14} NMR spectra after DCM solubility screen.

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