

**Synthesis of Biologically Active Thiadiazole Analogs:  
Keys to Unlocking the Mechanisms of Auxin**

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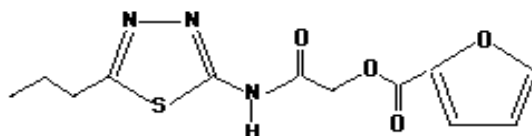
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## Introduction

Biological mechanisms in plants control growth and development. Auxin, specifically indole-3-acetic acid ( $C_{10}H_9NO_2$ ), is the primary plant hormone responsible for stimulating cell development such as growth, division, specialization, and elongation (1). However, the receptor proteins to which auxin binds have not all been identified, and therefore the biological mechanisms that control the expression of auxin are not fully understood. The goal of my study was to synthesize derivatives of an auxin inhibitor that will be used to achieve a better understanding of how auxin stimulates cell development. Plants treated with these derivatives will be examined to determine chemical groups that are responsible for the inhibition of specific auxin-mediated phenotypic traits, using a process known as structure-activity relationship testing. This process ultimately could identify receptor proteins that auxin binds to in order to stimulate plant growth.

A 2004 study by Armstrong *et al.* identified four molecules that inhibit auxin in *Arabidopsis* plants, commonly used for genetics testing (2). These molecules include a furyl acrylate ester of a thiadiazole heterocycle (Figure 1), which specifically inhibits expressions of auxin, such as root hair length or proteolysis. The furyl acrylate ester is composed of a propyl thiadiazole ring, a derivative of glycolic acid, and a derivative of furyl chloride. The study by Armstrong *et al.* is important because synthesizing derivatives of the furyl acrylate ester is the initial step toward identifying the targeted protein of auxin that the furyl acrylate ester binds to.

**Figure 1: Furyl acrylate ester (IUPAC name is 2-oxo-2-(4-propylcyclopenta-1,3-dienylamino)ethylfuran-2-carboxylate)**



Previous research in 2005 by Miller *et al.* involved the synthesis of derivatives of the furoyl acrylate ester using acylation, a substitution reaction in which a nucleophile bonds with a carbon atom of a carbonyl group (2). Based on the Miller study, my first hypothesis was that by varying ends of the furoyl acrylate ester (specifically the propyl thiadiazole ring and the derivative of furoyl chloride) and removing the glycolic acid derivative, an ethyl-amino thiadiazole ring (Figure 2a) could be successfully combined to isosteric acid chlorides (Figures 2b and 2c) using acylation (Figures 3 and 4). I used furoyl chloride for starting material in acylation because a furan ring is present in the furoyl acrylate ester, and would therefore potentially bind to the targeted protein. I used thiophenecarbonyl chloride for starting material in acylation because it is isosteric to furoyl chloride.

Figure 2: Starting material used in this study

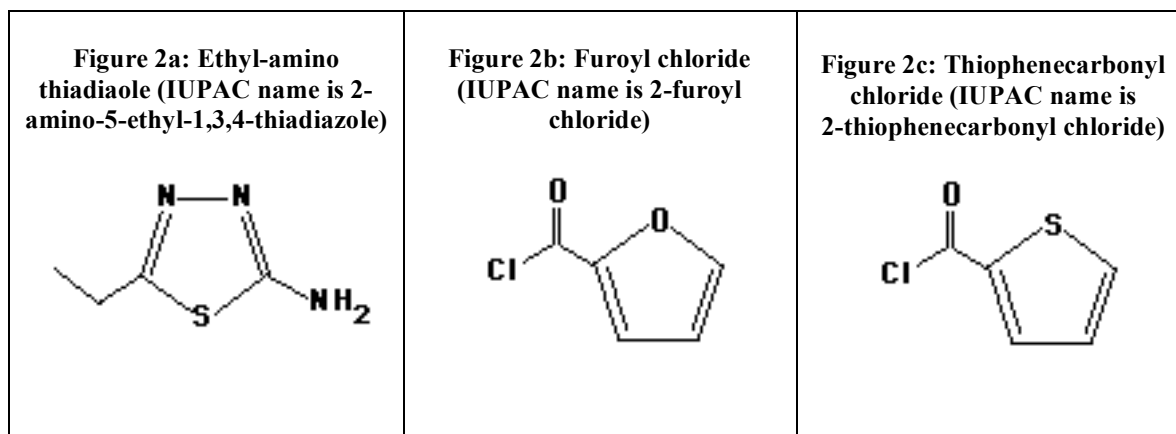
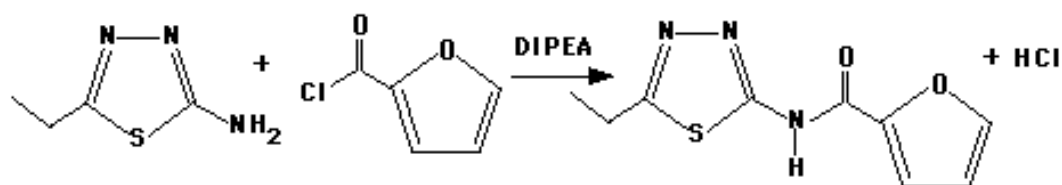
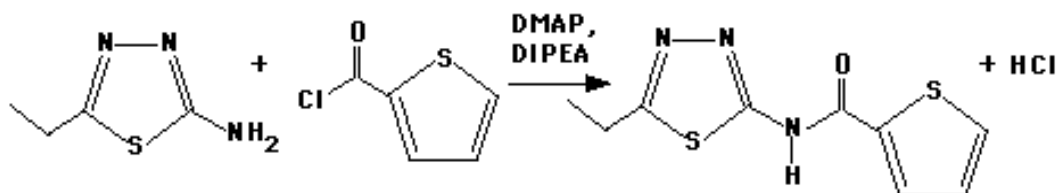


Figure 3: Synthesis of furoyl chloride derivative (IUPAC name is *N*-(5-ethyl-1,3,4-thiadiazol-1-yl)furan-2-carboxamide) through acylation



**Figure 4: Synthesis of thiophenecarbonyl chloride derivative (IUPAC name is *N*-(5-ethyl-1,3,4-thiadiazol-2-yl)thiophene-2-carboxamide) through acylation**



**Ethyl-amino thiadiazole + thiophenecarbonyl chloride → thiophenecarbonyl chloride derivative**

The syntheses for my study involved the formation of amide bonds through acylation reactions. The formation of these amide bonds depended on a substitution reaction by the nucleophilic amino group with the electrophilic acid chloride carbonyl with loss of chloride. Based on this principle of nucleophilic attack, my second hypothesis was that that amide bonds would form between the ethyl-amino thiadiazole and the acid chlorides, yielding a furoyl chloride derivative (Figure 3), or a thiophenecarbonyl chloride derivative (Figure 4), and *N,N*-diisopropylethylamine hydrochloride as a by-product.

## Methodology

*Acylation Reaction:* A round-bottom flask, labeled Flask 1, and a pear-shaped flask, labeled Flask 2, were heated in an oven at 105 °C for 60 minutes. Then, the flasks were cooled to room temperature. In a fume-hood, ethyl-amino thiadiazole (100 mg, 0.77 mmol) was massed and placed in Flask 1. Both flasks were capped with a septum and purged with argon for five minutes. Dichloromethane (7 mL) was added to Flask 1 with stirring. *N,N*-diisopropylethylamine (0.27 mL, 1.55 mmol) was added to Flask 1, and dichloromethane (2 mL) and acid chloride (1.16 mmol) were added to Flask 2. The solution in Flask 2 was added drop-wise to Flask 1 over a 15-minute period. Flask 2 was rinsed with dichloromethane and the solution was transferred to Flask 1. For the synthesis of the thiophenecarbonyl chloride derivative only, 4-dimethylaminopyridine (DMAP,

18.40 mg, 0.16 mmol) was added to Flask 1. The reaction was run overnight; then, silica gel thin-layer chromatography (TLC) was run on the reaction mixture, using 95:5 (by volume) dichloromethane:methanol as eluent.

*Aqueous Rinses and Drying of Reaction Mixture:* The product from the acylation reaction was rinsed three times with 10 mL of dH<sub>2</sub>O, and the resulting mixture was stirred vigorously for five minutes. The mixture was poured into a separatory funnel, rinsing with 3 mL of dH<sub>2</sub>O, then rinsing twice with 3 mL of dichloromethane. The organic layer was drained into an erlenmeyer flask. The organic layer was returned to the separatory funnel, and extracted twice with 10 mL of dH<sub>2</sub>O, three times with 10 mL of 10% hydrochloric acid, and once with 10 mL of saturated sodium chloride. The organic layer was then transferred to a clean erlenmeyer flask. The original erlenmeyer flask used to collect the organic layer was then rinsed into this clean erlenmeyer flask with dichloromethane. Two scoops of magnesium sulfate were added to the product mixture, and the product mixture was dried for 30 minutes to remove any H<sub>2</sub>O present. The product was filtered into a tared flask, and then rotovaped. The product was analyzed with <sup>1</sup>H nuclear magnetic resonance spectroscopy (<sup>1</sup>H NMR).

*Purification of Crude Product:* Flash chromatography was run using 95:5 (by volume) dichloromethane:methanol as eluent. Fractions containing the product were collected and rotovaped. TLC was run using a 95:5 (by volume) dichloromethane:methanol eluent to confirm purity. A 10-mg sample of the product was collected, and <sup>1</sup>H NMR and <sup>13</sup>C NMR were run. A 1-2-mg sample was collected and an infrared (IR) spectroscopy test was run. All NMR and IR spectra were analyzed to characterize the product and confirm purity.

## Results

Synthesis of the furoyl chloride derivative yielded an off-white solid. TLC of the crude product mixture showed a high concentration of ethyl-amino thiadiazole starting material in the reaction mixture, the presence of unidentified intermediates, and the presence of the desired furoyl chloride derivative. After flash chromatography was run, TLC showed the presence of only the furoyl chloride derivative.

The  $^1\text{H}$  NMR spectrum (Figure 5; all NMR and IR spectra are in the Appendix) showed triplet peaks at 1.42, 1.45, and 1.47 ppm, and quartet peaks at 3.06, 3.08, 3.11, and 3.13 ppm, representing the five protons of the ethyl group attached to the thiadiazole ring. Doublet of doublet peaks at 6.61, 6.62, 6.63, 7.52, 7.53, 7.63, and 7.64 ppm represent the three unique protons of the furan ring.

The  $^{13}\text{C}$  NMR spectrum (Figure 6) showed peaks at 13.86, 23.51, 76.58, 77.20, 77.42, 112.66, 117.70, 145.50, 146.18, 155.64, 159.39, and 166.65 ppm, indicating the presence of nine carbon atoms in the product.

The IR spectrum (Figure 7) showed a peak at  $1671\text{ cm}^{-1}$ , representing an amide bond, peaks at  $2963$  and  $2926\text{ cm}^{-1}$ , showing  $\text{sp}^3$  hybridization in the product, and peaks at  $3099$  and  $3133\text{ cm}^{-1}$ , indicating  $\text{sp}^2$  hybridization in the product.

Synthesis of the thiophenecarbonyl chloride derivative yielded a white solid. TLC analysis of the reaction mixture initially showed a high concentration of ethyl-amino thiadiazole starting material still present, as well as the presence of unidentified intermediates, and a very low concentration of the thiophenecarbonyl chloride derivative. Subsequently, TLC qualitatively showed the presence of a higher concentration of the desired product along with the presence of starting material and intermediates after

further reaction following the addition of the catalyst DMAP and another half equivalent of acid chloride.

The  $^1\text{H}$  NMR spectrum (Figure 8) showed triplet peaks at 1.45, 1.47, and 1.50 ppm, representing the three identical ethyl protons, and quartet peaks at 3.07, 3.09, 3.12, and 3.14 ppm, representing the two identical ethyl protons. Doublet of doublet peaks at 7.19, 7.20, 7.22, 7.68, 7.70, and 8.43 ppm represent the three unique protons of the thiophene ring.

The  $^{13}\text{C}$  NMR spectrum (Figure 9) showed peaks at 13.73, 23.68, 76.58, 77.20, 77.43, 128.48, 132.15, 133.54, 136.28, and 159.80 ppm, indicating the presence of nine carbon atoms in the product.

The IR spectrum (Figure 10) showed a peak at  $1647\text{ cm}^{-1}$ , representing an amide bond; a peak at  $2975\text{ cm}^{-1}$ , showing  $\text{sp}^3$  hybridization in the product; and peaks at  $3033\text{ cm}^{-1}$  and  $3069\text{ cm}^{-1}$ , indicating the presence of  $\text{sp}^2$  hybridization in the product.

## Conclusion

Results showed that both the furoyl chloride derivative and the thiophenecarbonyl chloride derivative were successfully synthesized and purified. NMR spectra for both products indicated the desired number and arrangement of hydrogen atoms and carbon atoms, and IR spectra for both products confirmed the presence of the indicated functional groups.

The catalyst DMAP produced greater amounts of the thiophenecarbonyl chloride derivative. The addition of another half equivalent of acid chloride further improved the yields of the thiophenecarbonyl chloride derivative.

In the future, the furoyl chloride and thiophenecarbonyl chloride derivatives can be applied to *Arabidopsis* plants to see if phenotypic changes are exhibited by

*Arabidopsis*, signaling binding with a target protein of auxin to inhibit its expression.

Because the synthesis of derivatives of auxin inhibitors is the foundation for these structure-activity relationship studies, different derivatives of the furyl acrylate ester should also be synthesized to determine chemical groups necessary for the inhibition of auxin.

Once structure-activity relationships of the furyl acrylate ester inhibitor have been determined with the use of derivatives, affinity-based separation techniques such as column chromatography can be used to isolate the protein. The identification of target proteins of auxin will enable molecular examination of how auxin operates in plants. Because auxin plays a major role in plant growth, this knowledge would be useful in applications such as designing herbicides or producing genetically-engineered plants to suit changing environmental conditions (3).

### **Acknowledgments**

Dr. Rebecca C. Hoye and the Minnesota Academy of Science provided me with this research opportunity and supervised my study. Dr. Hoye also provided me with background literature on chemical genetics, organic synthesis, and previous and ongoing research related to my study. Ms. Lois Fruen guided me as I wrote my paper. Matthew Weiss, Sarah Miller, and Johann Bergholz at Macalester College helped me in the laboratory and taught me the techniques necessary to start my research. Finally, the Breck School research class offered constant support and suggestions for the presentation of my work.

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Appendix

Figure 5:  $^1\text{H}$  NMR spectrum of *N*-(5-ethyl-1,3,4-thiadiazol-1-yl)furan-2-carboxamide (furoyl chloride derivative)

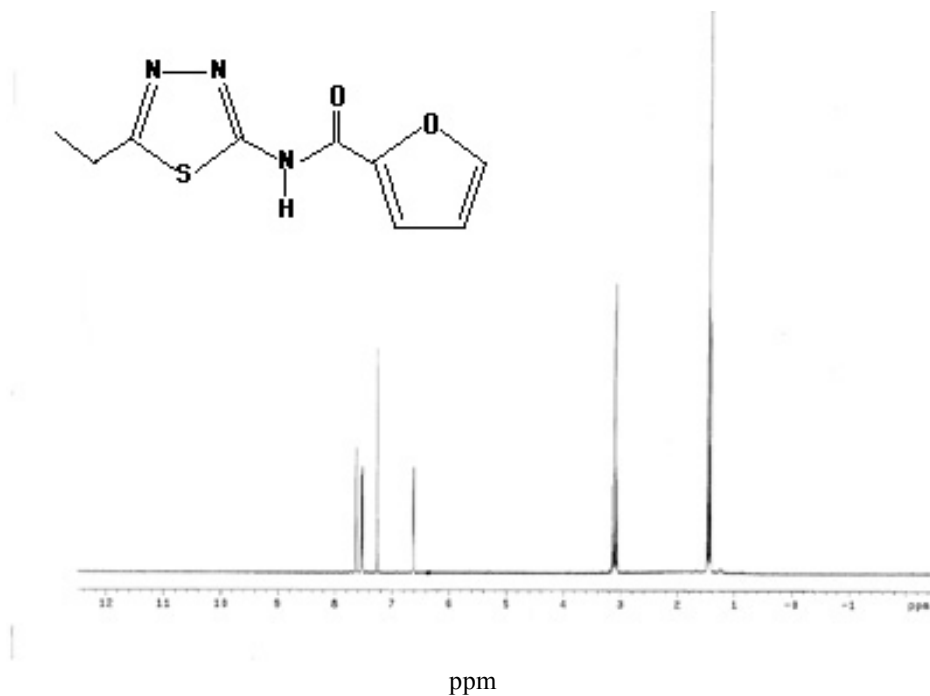


Figure 6:  $^{13}\text{C}$  NMR spectrum of *N*-(5-ethyl-1,3,4-thiadiazol-1-yl)furan-2-carboxamide (furoyl chloride derivative)

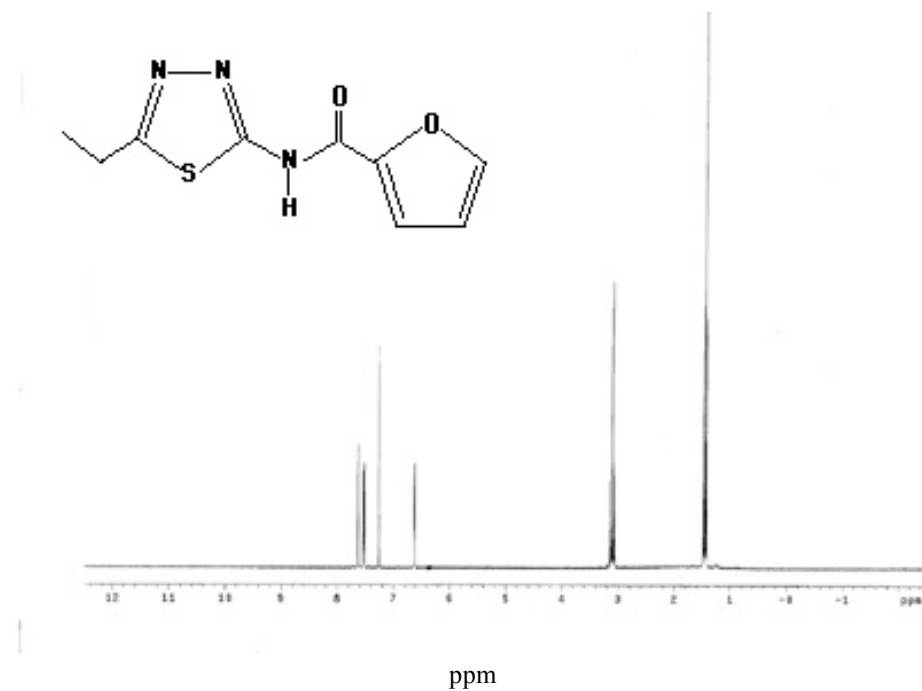
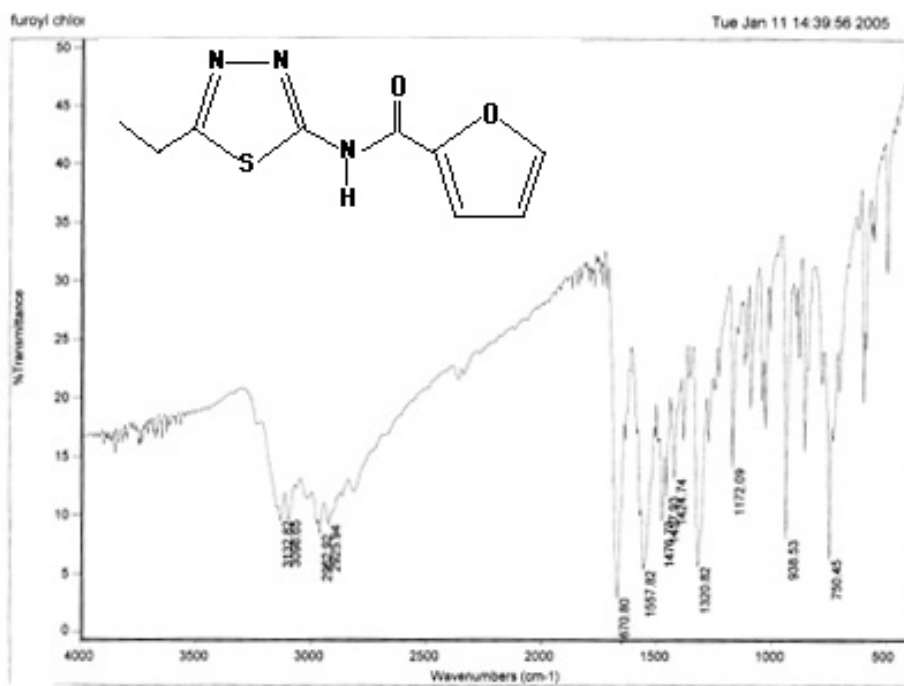
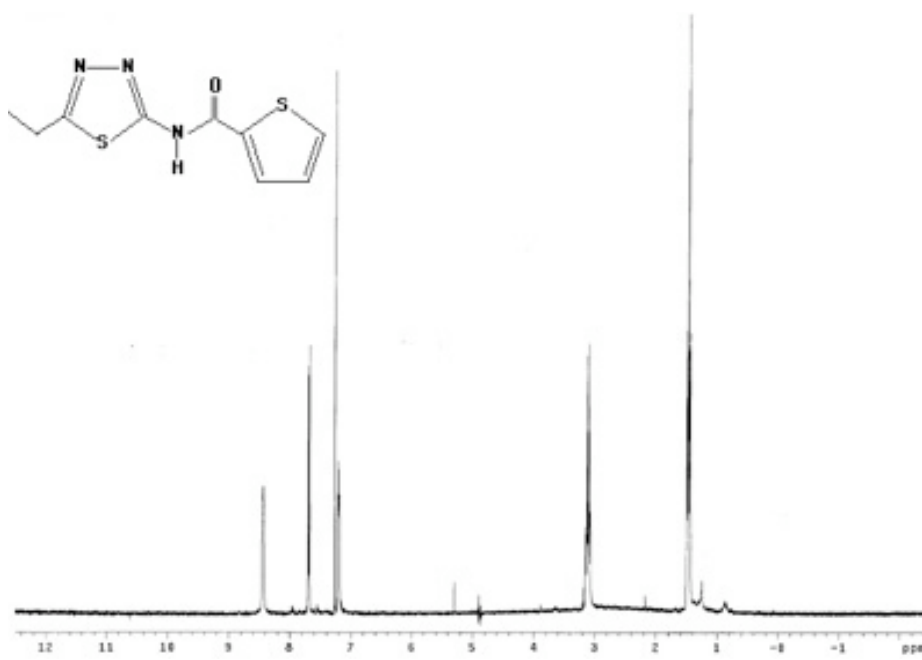


Figure 7: IR spectrum of *N*-(5-ethyl-1,3,4-thiadiazol-1-yl)furan-2-carboxamide (furoyl chloride derivative)



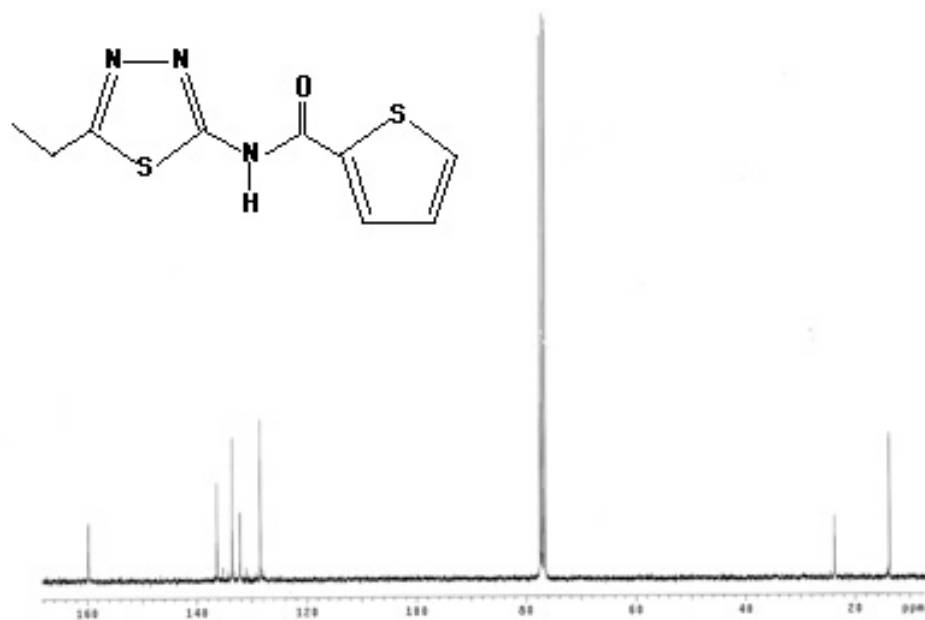
cm<sup>-1</sup>

Figure 8: <sup>1</sup>H NMR spectrum of *N*-(5-ethyl-1,3,4-thiadiazol-1-yl)thiophene-2-carboxamide (thiophene-carbonyl chloride derivative)



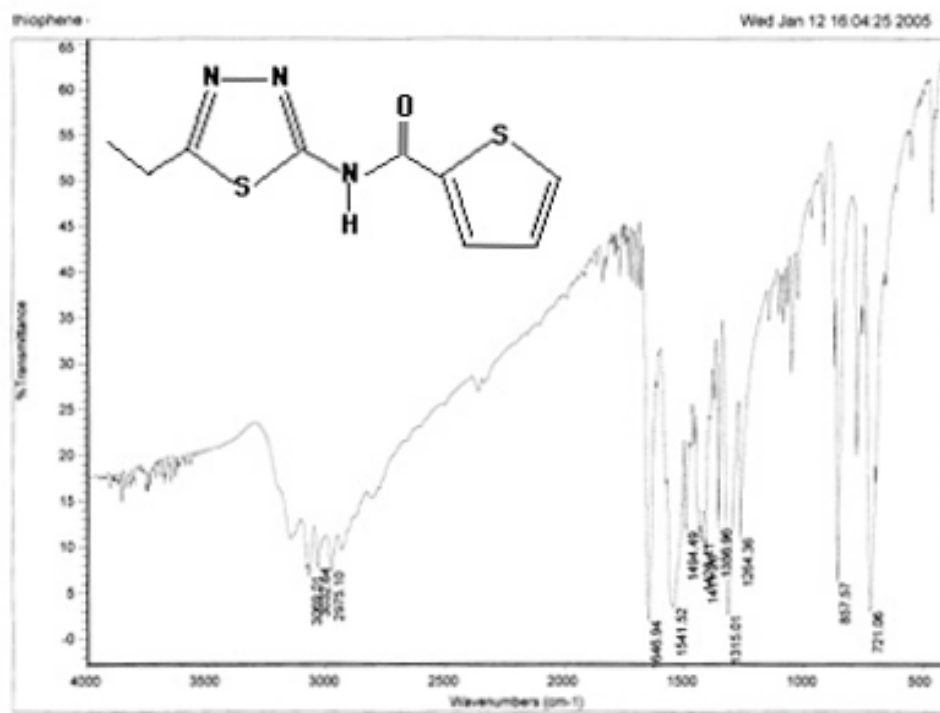
ppm

Figure 9:  $^{13}\text{C}$  NMR spectrum of *N*-(5-ethyl-1,3,4-thiadiazol-1-yl)thiophene-2-carboxamide (thiophene-carbonyl chloride derivative)



ppm

Figure 10: IR spectrum of *N*-(5-ethyl-1,3,4-thiadiazol-1-yl)thiophene-2-carboxamide



$\text{cm}^{-1}$