SYNTHESIS AND CHARACTERIZATION OF 1,2,4-TRIAZOLO-1,3,4-THIADIAZOLE DERIVATIVES

By

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A project report submitted to the Department of Chemical Science

Faculty of Science

Universiti Tunku Abdul Rahman

in partial fulfilment of the requirements for the degree of

Bachelor of Science (Hons) Chemistry

May 2022

ABSTRACT

SYNTHESIS AND CHARACTERIZATION OF 1,2,4-TRIAZOLO-1,3,4-THIADIAZOLE DERIVATIVES

CHENG YUN RU

In this project, a 1,2,4-triazole and a series of 1,2,4-triazolo-1,3,4-thiadiazole derivatives have been successfully synthesized. A total of eight 1,2,4-triazolo-1,3,4-thiadiazole derivatives **(YR1**-**YR8)** with different R group substituents (R = H, 2-F, 4-F, 2,4-F_2, 4-Br, 4-CH_3, 4-$N(CH_3)_2$ and 4-NO_2) were synthesized from 1,2,4-triazole and benzoic acid derivatives via condensation reaction in the presence of phosphoryl chloride ($POCl_3$) as cyclization agent. The percentage yield of 1,2,4-triazole was 31 % whereas the percentage yield of the synthesized 1,2,4-triazolo-1,3,4-thiadiazole derivatives **(YR1**-**YR8)** was in the range of 7 – 84 %. The structures of 1,2,4-triazole and a series of 1,2,4-triazolo-1,3,4-thiadiazole derivatives **(YR1**-**YR8)** were characterized and elucidated by using FTIR, ^{1}H NMR, ^{13}C NMR, DEPT, HMQC, HMBC and melting point apparatus.

ABSTRAK

SINTESIS DAN PENCIRIAN BAGI TERBITAN 1,2,4-TRIAZOLO-1,3,4-THIADIAZOL

CHENG YUN RU

Dalam kajian ini, satu 1,2,4-triazol dan satu siri terbitan 1,2,4-triazolo-1,3,4-thiadiazol telah berjaya disintesis. Sebanyak lapan terbitan 1,2,4-triazolo-1,3,4-thiadiazol **(YR1-YR8)** dengan substituen kumpulan R yang berbeza (R = H, 2-F, 4-F, 2,4-F_2, 4-Br, 4-CH_3, 4-$N(CH_3)_2$ and 4-NO_2) telah disintesis daripada 1,2,4-triazol dan pelbagai asid benzoik melalui tindak balas kondensasi dengan menggunakan fosforil klorida ($POCl_3$) sebagai agen kitaran. Hasil peratusan bagi 1,2,4-triazol adalah sebanyak 31 % manakala hasil peratusan bagi terbitan 1,2,4-triazolo-1,3,4-thiadiazol **(YR1-YR8)** adalah antara 7 % hingga 84 %. Struktur 1,2,4-triazol dan satu siri terbitan 1,2,4-triazolo-1,3,4-thiadiazol **(YR1-YR8)** telah dicirikan dan dikenalpasti dengan menggunakan FTIR, ^{1}H NMR, ^{13}C NMR, DEPT, HMQC, HMBC and radas takat lebur.

ACKNOWLEDGEMENT

The completion of this final year project would not be possible without the support from supervisors, friends and family. First and foremost, I would like to express my deepest appreciation to my final year project supervisor, Dr. Sim Kooi Mow for his guidance, advice, patience and encouragement throughout the duration of this project.

Besides, I would like to extend my sincere thanks to Mr. Leong Thung Lim as well as other lab officers for their kind assistance and sharing helpful information, skills and knowledge throughout this project which is not learnt in lectures.

Next, I would like to thank my final year project teammate, Loo Xin Tong as well as other friends for their support, companionship and encouragement throughout the duration of this project. Without them, this project would not be much meaningful and successful.

Last but not least, I would like to thank my beloved parents for their love and encouragement especially in my last semester of studies. I would not be able to enjoy many opportunities without them.

DECLARATION

I hereby declare that this final year project report is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

Name: CHENG YUN RU

Date: 29 April 2022

APPROVAL SHEET

This final year project report entitled "**SYNTHESIS AND CHARACTERIZATION OF 1,2,4-TRIAZOLO-1,3,4-THIADIAZOLE DERIVATIVES"** was prepared by CHENG YUN RU and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Hons) Chemistry at Universiti Tunku Abdul Rahman.

Approved by:

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KM SIM

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FACULTY OF SCIENCE

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PERMISSION SHEET

It is hereby certified that **CHENG YUN RU** (ID No: **18ADB05427**) has completed this final year project report entitled "SYNTHESIS AND CHARACTERIZATION OF 1,2,4-TRIAZOLO-1,3,4-THIADIAZOLE DERIVATIVES" under the supervision of DR. SIM KOOI MOW from the Department of Chemical Science, Faculty of Science.

I hereby give permission to the University to upload the softcopy of my final year project report in pdf format into the UTAR Institutional Repository, which may be made accessible to the UTAR community and public.

Yours truly,

yunru

(CHENG YUN RU)

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LIST OF ABBREVIATIONS

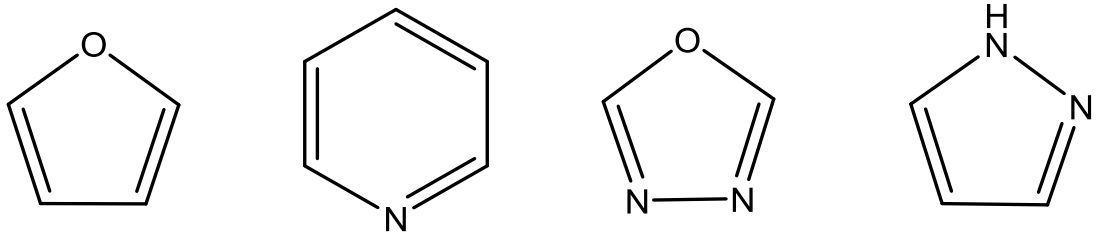
CAN	Ceric ammonium nitrate
δ	Chemical shift
J	Coupling constant
$CDCl_3$	Deuterated chloroform
DMSO-d_6	Deuterated dimethyl sulfoxide
DEPT	Distortionless Enhancement by Polarization Transfer
EA	Ethyl acetate
FTIR	Fourier Transform Infrared
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Coherence
LR	Lawesson's reagent
MW	Microwave
1D-NMR	One-Dimensional Nuclear Magnetic Resonance
$POCl_3$	Phosphoryl chloride
PEG	Polyethylene glycol
R_f	Retention factor
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
2D-NMR	Two-Dimensional Nuclear Magnetic Resonance

CHAPTER 1

INTRODUCTION

1.1 Heterocyclic Chemistry

Heterocyclic chemistry is a major division of organic chemistry whereby approximately 70 % of organic compounds are heterocyclic compounds. Heterocyclic compounds are cyclic compounds with at least one different atom other than carbon as part of the ring system. For instance, sulphur, oxygen and nitrogen are the common heteroatoms. In 1818, the first heterocyclic compound was isolated by Luigi Brugnatelli which was alloxan (Kaur, et al., 2018). Heterocyclic compounds either natural or synthetic are found to be pharmacologically and biologically active which are widely used as antioxidants, copolymer dyes, agrochemicals and pharmaceutical products and other applications (Ji Ram, et al., 2019). Some examples of heterocyclic compounds are furan, pyridine, oxadiazole, pyrazole and others as shown in Figure 1.1.

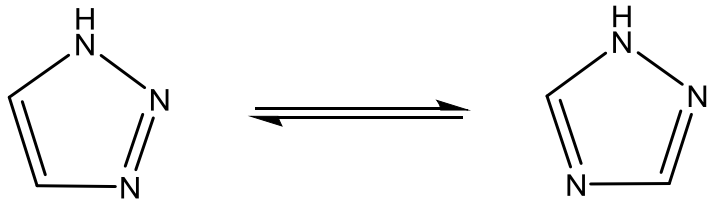


Furan Pyridine Oxadiazole Pyrazole

Figure 1.1: Examples of heterocyclic compounds.

1.2 Triazole

Triazole, which is also known as pyrrodiazoles, is a nitrogen-containing aromatic heterocyclic compound comprising two carbon atoms and three nitrogen atoms with two double bonds in a five-membered unsaturated ring. It has one pyrrole type and two pyridine type nitrogen atoms. The name triazole was given by a scientist named Bladin who described its derivatives in early 1885 (Kaur, et al., 2018). It has chemical formula of $C_2H_3N_3$. It exists in two isomeric forms correspond to the positions of nitrogen atom in the ring which are 1,2,4-triazole and 1,2,3-triazole as shown in Figure 1.2 (Kashyap and Silakari, 2018).



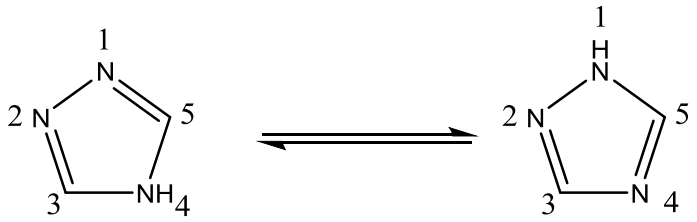
1,2,3-triazole 1,2,4-triazole

Figure 1.2: Two isomers of triazole.

Besides, it appears in white to pale yellow crystalline solid with melting point of 120 ℃ which readily soluble in polar solvents such as alcohol and water. It shows a variety of biological activities such as antioxidant, antimalarial, antitumor, antifungal and other activities. These activities have encouraged researchers to develop various kinds of triazole derivatives with significant biological activities (Kashyap and Silakari, 2018). Among the two isomers, 1,2,4-triazole has a wider range of pharmacological and biological activities (Kaur, et al., 2018).

1.2.1 1,2,4-triazole

1,2,4-triazole is a π-excessive nitrogen-containing aromatic heterocyclic compound which consists of three nitrogen atoms present at the positions 1, 2 and 4 of the ring structure. All atoms in the structure are sp^2 hybridized with 6π electrons delocalized around the ring which leads to its aromatic characteristics. Besides, it can be called as s-triazole which refers to symmetrical. 1,2,4-triazole can undergo tautomerization into two different structures which are 4*H*-1,2,4-triazole and 1*H*-1,2,4-triazole as shown in Figure 1.3 (Ji Ram, et al., 2019). Much research has been revealed that 4H-1,2,4-triazole is less stable than 1*H*-1,2,4-triazole due to high conformation energy (Shneine and Alaraji, 2016).



4*H*-1,2,4-triazole 1*H*-1,2,4-triazole

Figure 1.3: Two tautomeric forms of 1,2,4-triazole.

The parent ring of 1*H*-1,2,4-triazole can be synthesized by the reaction between formamide and hydrazine which is obtained from hydrolysis of 1,2-di(butan-2-ylidene) hydrazine with at 170 ℃ as shown in Figure 1.4. Since there are electronegative nitrogen atoms adjacent to both carbon atoms in 1,2,4-triazole, they are electron deficient and their electron density is low which undergo nucleophilic substitution readily (Ji Ram, et al., 2019).

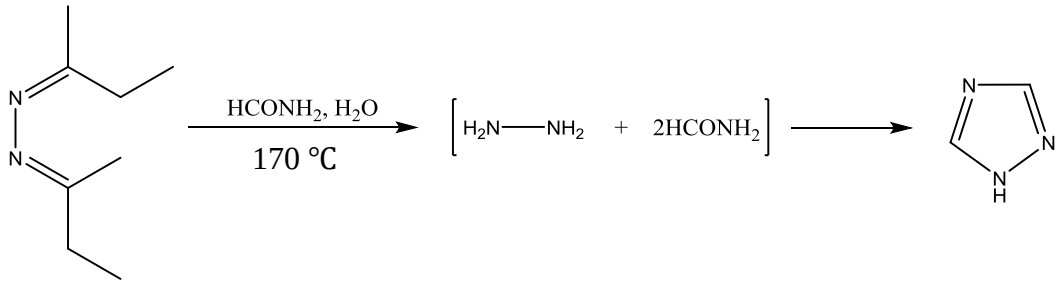


Figure 1.4: Synthesis of 1*H*-1,2,4-triazole.

1,2,4-Triazole compounds can be synthesized using several methods. One of them is known as Pellizzari synthesis whereby it involves the reaction between amide and hydrazide to form an intermediate which is acyl amidrazone followed by intramolecular cyclization to yield 1,2,4-triazole. Figure 1.5 illustrates the reaction of Pellizzari synthesis of 1,2,4-triazole (Ji Ram, et al., 2019).

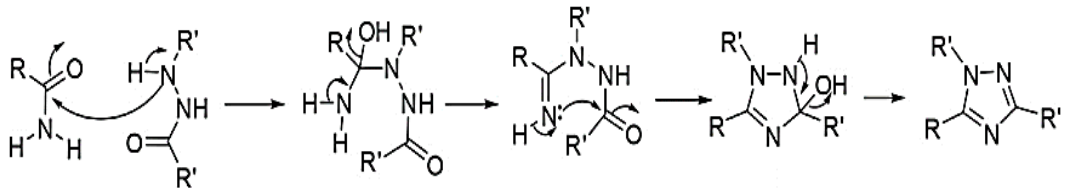
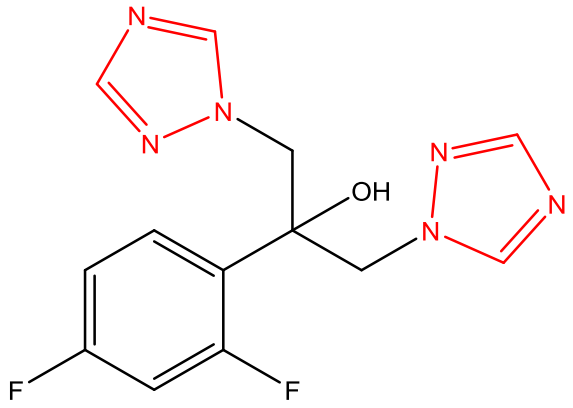
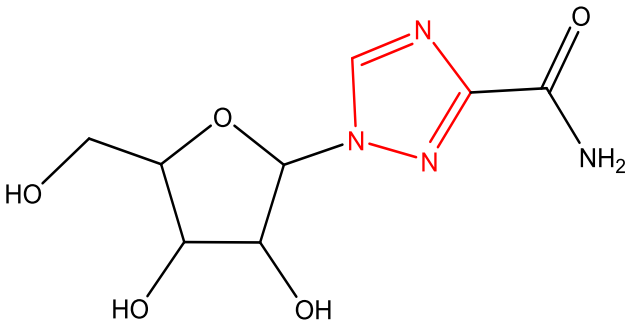
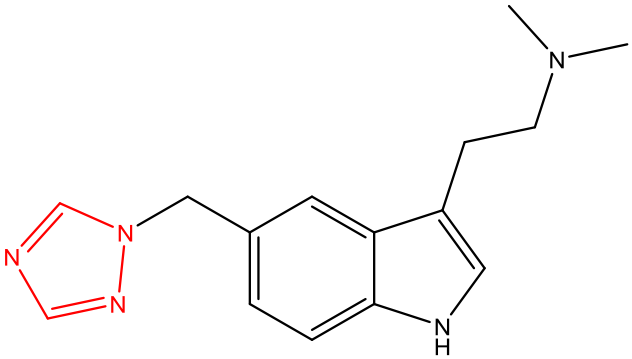
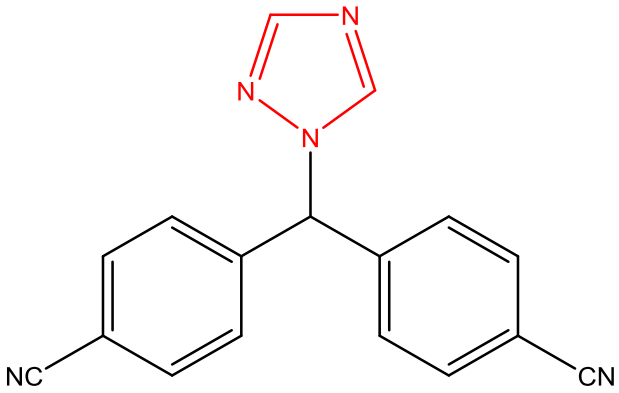
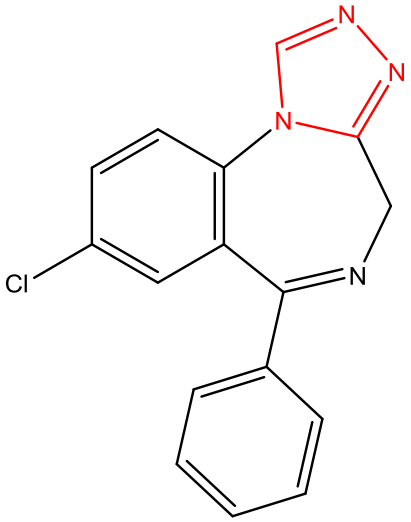


Figure 1.5: Pellizzari synthesis of 1,2,4-triazole compounds.

In addition, 1,2,4-triazole and its derivatives exhibit multiple pharmacological activities such as antifungal, antiviral, antimigraine, anxiolytic and other properties. Table 1.1 shows the examples of 1,2,4-triazole containing drugs in clinical use with their chemical structures and functions (Kaur, et al., 2018).

Table 1.1: Examples of 1,2,4-triazole containing drugs in clinical use.

Drug name	Chemical structure	Function
Fluconazole		Antifungal
Ribavirin		Antiviral
Rizatriptan		Antimigraine

Letrozole		Anticancer (Breast cancer)
Estazolam		Anxiolytic

1.3 Thiadiazole

Thiadiazole is a subcategory of azole compounds whereby the name thiadiazole originated from the Hantzsch–Widman nomenclature (Sahu, et al., 2021). It is a ubiquitous aromatic heterocyclic compound containing two pyridine type nitrogen atoms, two carbon atoms and a sulphur atom with two double bonds as a part of the five-membered unsaturated ring. It has the chemical formula of $C_2H_2N_2S$. It exists in four isomeric forms correspond to the positions of nitrogen in the ring which are 1,2,4-thiadiazole, 1,2,3-thiadiazole, 1,3,4-thiadiazole and 1,2,5-

thiadiazole shown in Figure 1.6. The ring stability of thiadiazole depends on the substituents that are attached to it and the position of the heteroatoms (Ji Ram, et al., 2019).

1,2,4-thiadiazole 1,2,3-thiadiazole 1,3,4-thiadiazole 1,2,5-thiadiazole

Figure 1.6: Four isomers of thiadiazole.

Thiadiazole exhibits a wide range of applications in organic synthesis, pharmaceutical and biological fields which attract researchers to develop new synthesis methods. Besides, it is also used as metal chelating agents, oxidation inhibitors, dyes and other applications. Among four isomers of thiadiazoles, 1,3,4-thiadiazole has been reviewed more than other isomers (Kumar, et al., 2013).

1.3.1 1,3,4-thiadiazole

In 1882, 1,3,4-thiadiazole was first synthesized by Emil Fischer and the characteristics of the ring system was developed by Kuhn and Freud in 1890 (Sahu, et al., 2021). It is an aromatic heterocyclic compound which consists of one sulphur atom and two nitrogen atoms present at the positions 1, 3 and 4 of the ring structure respectively as shown in Figure 1.7. Since nitrogen and sulphur are electron withdrawing groups, the carbon atoms at position 2 and 5 in the ring are

electron deficient. Hence, they are susceptible to nucleophilic attack but inert towards electrophilic substitution (Hu, et al., 2014).

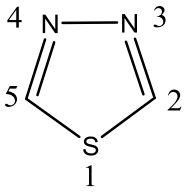


Figure 1.7: Numbering system of 1,3,4-thiadiazole.

In addition, the sulphur with its electron withdrawing ability gives rise to high aromaticity with weak base properties of 1,3,4-thiadiazole. In aqueous acid solution, it is stable but in aqueous base solution, the ring will be cleaved due to low stability. Generally, it can be prepared through sulphuration of diacylhydrazines followed by cyclization as illustrating in Figure 1.8 (Hu, et al., 2014).

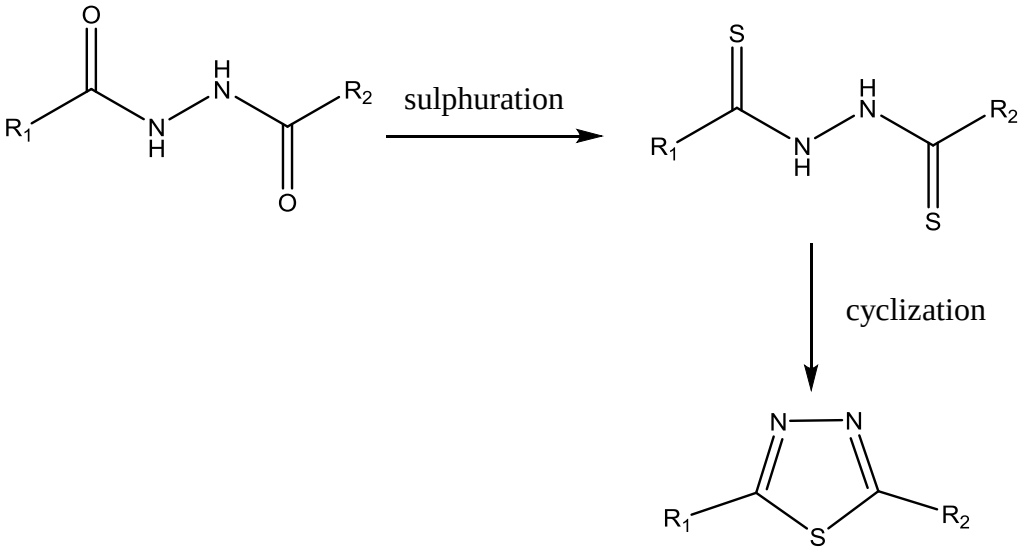
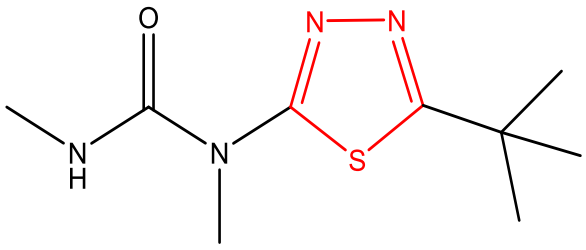
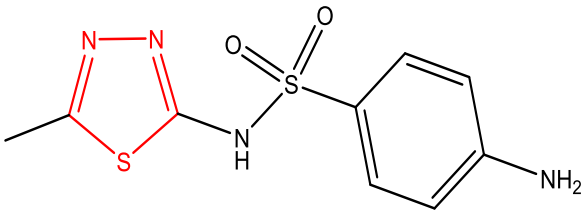
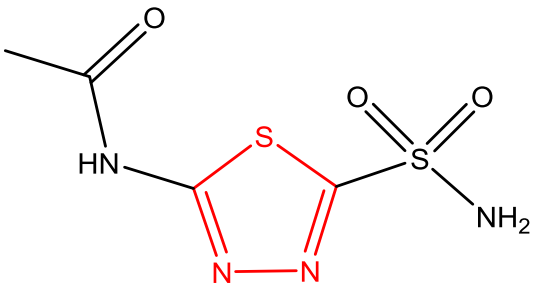
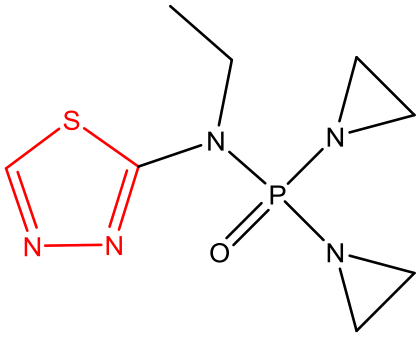
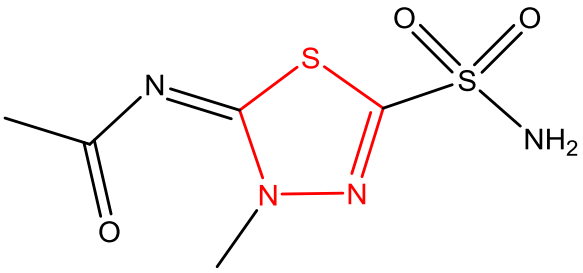


Figure 1.8: Synthesis of 1,3,4-thiadiazole from diacylhydrazines.

Besides, 1,3,4-thiadiazole and its derivatives have a lot of biological activities such as antibiotic, antioxidant, anti-inflammatory, antimicrobial, antidepressant and other activities. It is also applied agrochemical field such as fungicides, herbicides, insecticides and bactericides. Furthermore, it is applied in the electrochemistry and optics field as it has the characteristics of electron deficient which acts as a good electron acceptor with better chemical and thermal stability. Table 1.2 shows the examples of 1,3,4-thiadiazole containing compounds used in pharmaceutical and agrochemical field (Hu, et al., 2014).

Table 1.2: Examples of 1,3,4-thiadiazole containing compounds used in pharmaceutical and agrochemical field.

Compound	**Chemical structure**	**Function**
Tebutherion		Herbicide
Sulfamethizole		Antibiotic

Acetazolamide		Diuretic
Azetepa		Antineoplastic (Anticancer)
Methazolamide		Carbonic anhydrase inhibitor

1.4 Objectives

1. To synthesize a 1,2,4-triazole and a series of 1,2,4-triazolo-1,3,4-thiadiazole.
2. To characterize the structure of 1,2,4-triazole and 1,2,4-triazolo-1,3,4-thiadiazoles by using FTIR, 1D-NMR (^{1}H, ^{13}C and DEPT), 2D-NMR (HMQC and HMBC) and melting point apparatus.

CHAPTER 2

LITERATURE REVIEW

2.1 Synthesis of 1,2,4-triazole

There are several methods have been reported for the preparation of 1,2,4-triazole which can be generally divided into conventional and green methods. In conventional method, a study done by Kaplancıklı, et al. (2008) whereby 1,2,4-triazole was prepared by conventional heating equimolar of indole-3-acetic acid (0.1 mol) and thiocarbohydrazide (0.1 mol) at 160 – 170 ℃ oil bath for 2 hours. The resultant mixture formed was triturated in hot water to yield crude triazole and purified by recrystallization using methanol. Figure 2.1 shows the synthesis of 1,2,4-triazole from thiocarbohydrazide and indole-3-acetic acid by conventional heating.

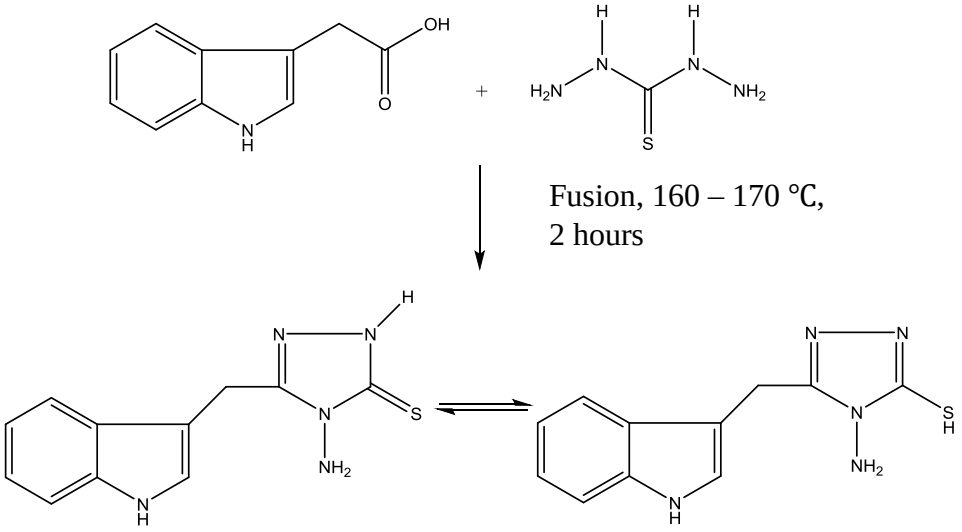


Figure 2.1: Synthesis of 1,2,4-triazole from thiocarbohydrazide and indole-3-acetic acid by conventional heating.

Furthermore, another conventional method of synthesizing 1,2,4-triazole was performed by Lin, et al. (2017). The first step involves the formation of benzoyl hydrazine by reaction of benzoic acid ester with hydrazine hydrate in ethanol. The carbon disulphide was added dropwise into the reaction mixture followed by the addition of potassium hydroxide upon stirring for 12 hours at room temperature to form the intermediate of potassium dithiocarbazinate. It was added with excess hydrazine hydrate and refluxed for three hours to undergo cyclization and yield 1,2,4-triazole. Figure 2.2 shows the multistep synthesis of 1,2,4-triazole by conventional heating.

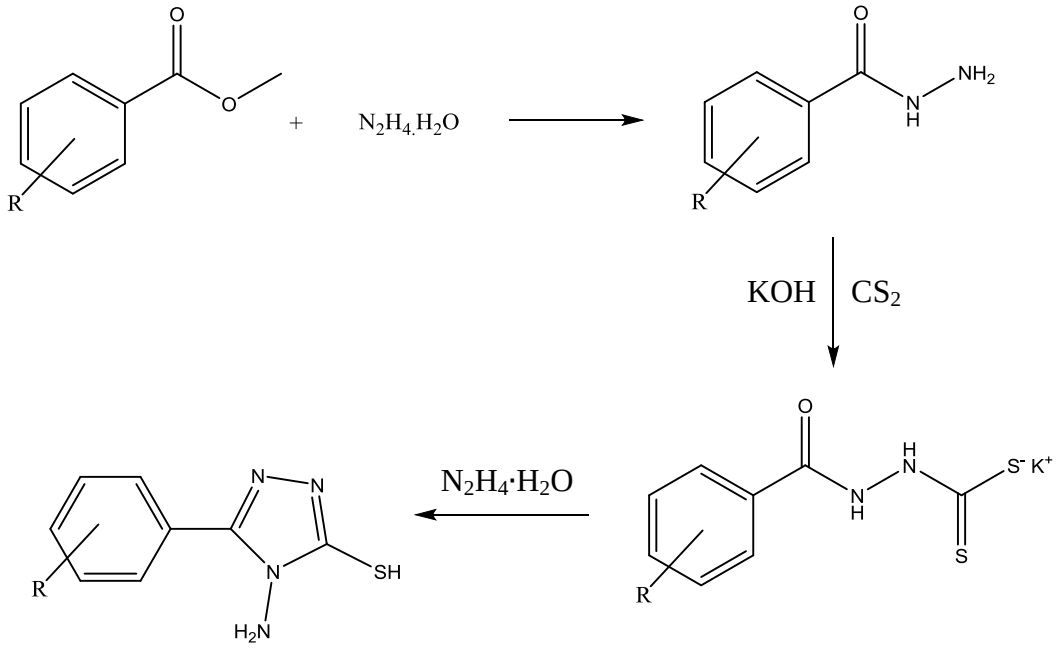


Figure 2.2: Multistep synthesis of 1,2,4-triazole by conventional heating.

In the green method, Shelke, et al. (2015) reported that a simple and environmentally friendly method has been established to synthesize 1,2,4-triazoles via microwave-assisted synthesis. It was first done by irradiating a mixture of hydrazine and formamide with a ratio of 1:2 at 140 ℃ and 230 W for 10 minutes without using a catalyst as well as under solventless conditions. However, a product with low yield was obtained under these conditions. There was no significant increment of yield when increasing the temperature or reaction time to 160 ℃ and 30 minutes respectively. A large improvement in terms of yield was examined by adjusting the ratio of hydrazine to formamide to 1:20 and increasing reaction temperature to 160 ℃. Figure 2.3 shows one-pot synthesis of 1,2,4-triazole via microwave irradiation. Table 2.1 shows the modification of reaction conditions.

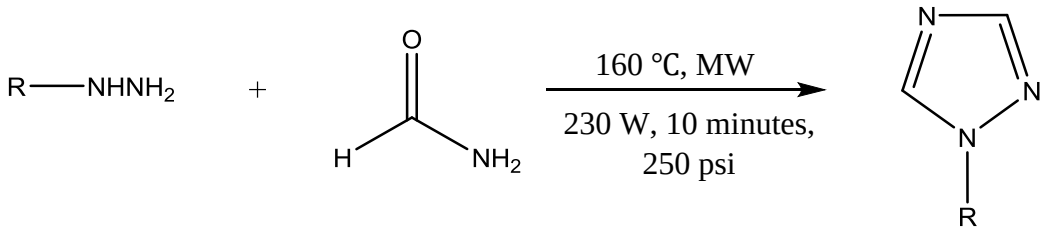


Figure 2.3: One-pot synthesis of 1,2,4-triazole via microwave irradiation.

Table 2.1: Modification of reaction conditions.

Formamide (mmol)	Temperature (℃)	Time (min)	Yield (%)
2.0	140	10	20
2.0	140	30	28
2.0	160	10	24
10.0	160	10	52
15.0	160	10	63
20.0	160	10	74
25.0	160	10	71

In addition, Nakka, et al. (2015) conducted a green and environmentally friendly reaction for the preparation of 1,2,4-triazole through oxidative cyclization using polyethylene glycol (PEG) as green and recyclable reaction medium. They first conducted the reaction of amidrazone and aldehyde by heating in ethanol at 80 ℃ in the presence of ceric ammonium nitrate (2 mol%) as catalyst. However, the desired product was obtained with low yield. Thus, a series of adjustments was done on reaction conditions to improve the yield. Different types of solvent and amount of ceric ammonium nitrate (CAN) were used and finally they found that using PEG as solvent and 5 mol% of CAN gave the best yield. Besides, the reaction time was decreased instead of only increasing the product yield. Figure 2.4 shows the synthesis of 1,2,4-triazole using PEG as a recyclable reaction medium. Table 2.2 shows the adjustment of reaction conditions.

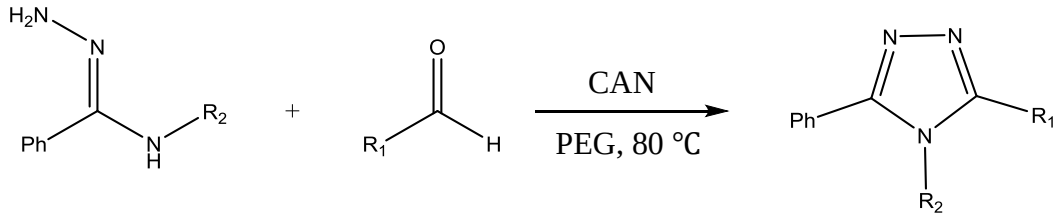


Figure 2.4: Synthesis of 1,2,4-triazole using PEG as a recyclable reaction medium.

Table 2.2: Adjustment of reaction conditions.

Solvent	**CAN (mol%)**	**Time (h)**	**Yield (%)**
Ethanol	2	3	61
1,4-dioxane	2	3	53
Acetonitrile	2	3.5	48
PEG-300	2	2	82
Toluene	2	4	28
PEG-300	5	1	96
PEG-300	10	0.5	71

By comparing conventional and green methods, both green methods give higher yield than conventional methods. Microwave-assisted synthesis enhances the rate of reactions by providing uniform heating of samples which results in high energy efficiency. Besides, it provides higher yield by minimizing side reactions (Shelke, et al., 2015). Next, polyethylene glycol is a green and recyclable solvent. It is also a cheap, non-toxic and water soluble which is easy to remove from the final

product. Ceric ammonium nitrate (CAN) is also cheap, high reactivity and eco-friendly catalyst (Nakka, et al., 2015). As for conventional methods, high temperature is required with longer reaction time but giving low yield. Some reactions need expensive reagents and involve multistep synthesis which leads to formation of side products (Shelke, et al., 2015).

2.2 Synthesis of 1,3,4-thiadiazole

The most common way to synthesize 1,3,4-thiadiazole is through cyclization of diacylhydrazine. Various studies have been done using Lawesson's reagent (LR) or phosphorus sulphide act as thiation agent in different solvents such as dimethylformamide, dichloromethane and others. In conventional method, Kumar, et al. (2010) prepared 1,3,4-thiadiazole from 1.2-diacylhydrazine and Lawesson's reagent to carry out thionation followed by oxidative cyclization in different solvents under reflux at 80 ℃ for 5 hours. Among different types of solvents used such as dioxane, toluene, tetrahydrofuran and xylene, tetrahydrofuran gave the highest yield as compared to other solvents. Figure 2.5 shows the synthesis of 1,3,4-thiadiazole from diacylhydrazine under reflux.

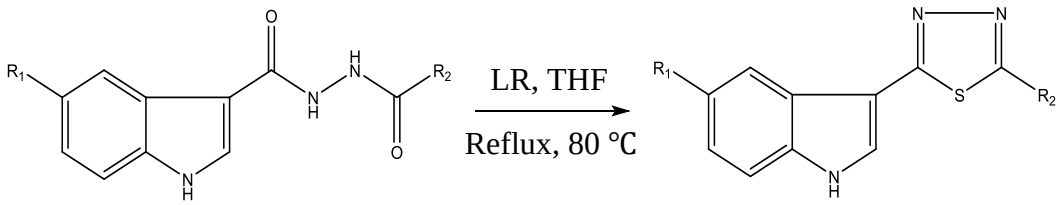


Figure 2.5: Synthesis of 1,3,4-thiadiazole from diacylhydrazine by conventional heating.

However, conventional methods require longer reaction time at elevated temperatures but provide low yield with formation of by-products such as thiation agent and solvents used which are not eco-unfriendly. Hence, microwave assisted synthesis was introduced to overcome these issues which is a simple and greener method. However, the reaction shown in Figure 2.5 was also performed under microwave irradiation and gave a mixture of 1,3,4-thiadizole with by-products. Thus, a series of adjustments in reaction conditions and reagents used can be done to enhance the reactivity of the reaction.

Another synthesis of 1,3,4-thiadiazole was conducted by Kiryanov, et al. (2001) whereby 4-bromobenzohydrazide was underwent *N*-acylation using myristoyl chloride to form unsymmetrical *N*,*N*'-diacylhydrazine. It was then cyclized into 1,3,4-thiadiazole under microwave irradiation in 13 minutes with a yield of 91 %. Figure 2.6 shows the synthesis of 1,3,4-thiadiazole from diacylhydrazine under microwave irradiation.

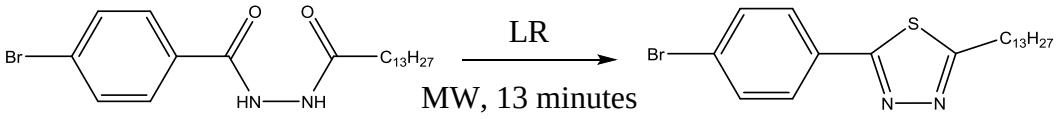


Figure 2.6: Synthesis of 1,3,4-thiadiazole from diacylhydrazine under microwave irradiation.

2.3 Synthesis of 1,2,4-triazolo-1,3,4-thiadiazole

A study done by Raval, et al. (2010) stated that the synthesis of 1,2,4-triazolo-1,3,4-thiadiazole can be done through conventional heating and microwave irradiation. For traditional method, equimolar of various benzaldehyde derivatives (0.01 mol) and 3-mercapto-4-amino-5-[(4-chloro-3-methylphenoxy)methyl]-4H-1,2,4-triazole (0.01 mol) in the presence of *p*-toluenesulphonic acid as catalyst in dry *N*,*N*-dimethylformamide were refluxed for 10-12 hours. After refluxing, the solution was cooled and recrystallized using suitable solvents such as methanol.

Similarly for microwave method, equimolar of 3-mercapto-4-amino-5-[(4-chloro-3-methylphenoxy)methyl]-4*H*-1,2,4-triazole (0.01 mol) and various benzaldehyde derivatives (0.01 mol) in the presence of *p*-toluenesulphonic acid as catalyst in dry *N*,*N*-dimethylformamide were mixed with acidic alumina. It was dried and put inside the alumina bath. The reaction was carried out under microwave irradiation with irradiation power of 350 W for 4-6 minutes and 500 W for 2-3.5 minutes. The reaction mixture was cooled to room temperature. Dry toluene was used to extract the crude product followed by filtration and recrystallization using methanol. Figure 2.7 shows the synthesis of 1,2,4-triazolo-1,3,4-thiadiazole via conventional and microwave methods by Raval, et al. (2010).

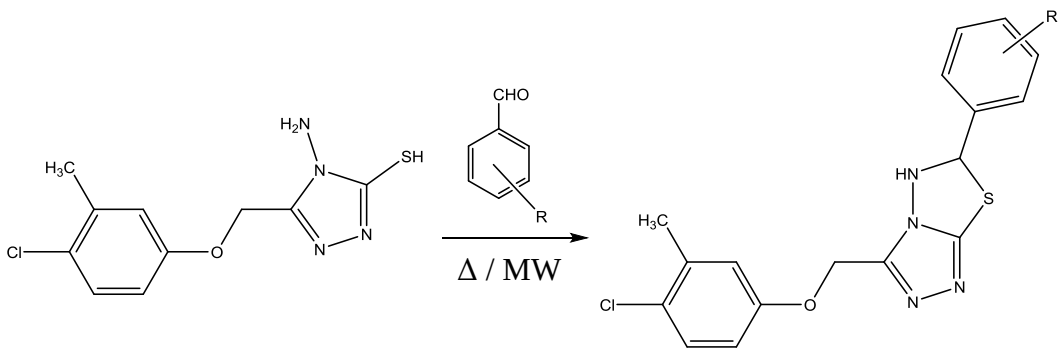


Figure 2.7: Synthesis of 1,2,4-triazolo-1,3,4-thiadiazole via conventional and microwave methods by Raval, et al. (2010).

To conclude, microwave method showed higher yield than conventional method. The reaction time for microwave irradiation were shorter than conventional heating. Dipole polarization of the molecules is responsible for most of the microwave heating which helps to speed up the reaction. The comparison was made in terms of yield and reaction time for both methods as shown in Table 2.3. Higher yields of compounds were obtained in microwave irradiation as compared to conventional heating. Besides, the yield can be increased when increasing the irradiation power in microwave method (Raval, et al., 2010).

Table 2.3: Comparison of conventional and microwave methods to synthesize 1,2,4-triazolo-1,3,4-thiadiazole.

R	Yield			Reaction time		
	Microwave (%)		Conventional	Microwave (min)		Conventional
	350 W	500 W	(%)	350 W	500W	(hour)
H	75	84	57	4.0	3.0	10
2-Cl	83	90	54	6.0	2.0	12
4-Cl	81	86	59	5.5	2.0	12
2,4-Cl_2	72	82	59	4.5	2.0	10.5
4-NO_2	79	88	51	5.0	3.0	10.5
3-Br	83	92	43	6.0	3.5	12
2-OCH_3	79	88	47	4.5	2.0	12
4-OCH_3	77	83	48	5.0	2.5	12
2-OH	74	83	63	5.0	3.0	11

Similar procedure was done by Ramaprasad, et al. (2012) whereby they used equimolar of 4-amino-5-(5'-fluoro-2'-methoxybiphenyl-3-yl)-4H-1,2,4-triazole-3-thiol (1.58 mmol) and various aromatic carboxylic acid derivatives (1.58 mmol) in the presence of phosphoryl chloride in solventless conditions. The reaction was done in both conventional and microwave method. For conventional method, the reflux was carried out at 110 ℃ for 7 hours instead of 10-12 hours in previous discussion. Besides, additional step was done which was adding sodium carbonate to neutralize the reaction mixture before further purification of product.

For microwave method, the procedures were similar with conventional method with the irradiation power of 70 W at 50 ℃ for 5 minutes which was lower than previous discussion. Similarly, microwave method showed higher yield than conventional method. Figure 2.8 shows the synthesis of 1,2,4-triazolo-1,3,4-thiadiazole via conventional and microwave methods by Ramaprasad, et al. (2012).

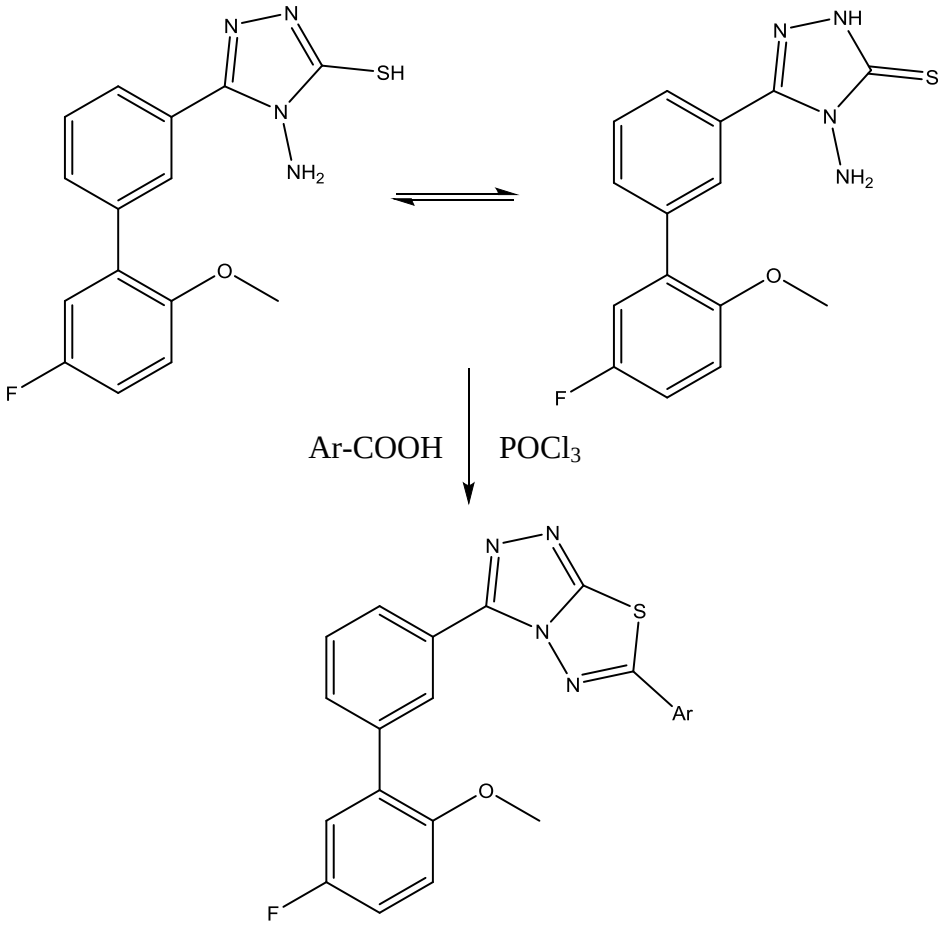


Figure 2.8: Synthesis of 1,2,4-triazolo-1,3,4-thiadiazole via conventional and microwave methods by Ramaprasad, et al. (2012).

In addition, another synthesis of 1,2,4-triazolo-1,3,4-thiadiazole conducted by Lin, et al. (2017) by adding various benzoic acid derivatives (4.5 mmol) dropwise to a solution of 4-amino-5-substituted-4H-1,2,4-triazole-3-thiols (3 mmol) in the presence of phosphoryl chloride (25 mL). The reaction mixture was refluxed for 3 hours with continuous stirring. Then, it was poured into ice water to enhance the formation of precipitates and sodium hydroxide solution was added to neutralize the pH of the mixture. The precipitates were filtered and washed with ethanol. The crude products were recrystallized from absolute ethanol and allowed to dry and yield 3,6-disubstituted-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles. The yields of the product range between 32 % to 59 %. Figure 2.9 shows the synthesis of 1,2,4-triazolo-1,3,4-thiadiazole under reflux.

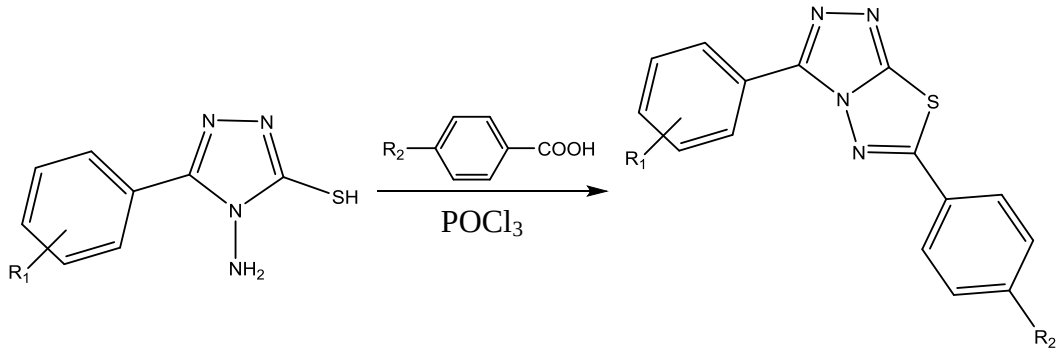


Figure 2.9: Synthesis of 1,2,4-triazolo-1,3,4-thiadiazole under reflux.

Besides, some biological activities such as antifungal and antibacterial activities were tested on synthesized 3,6-disubstituted-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles. Based on the results, all derivatives showed certain level of antifungal and antibacterial activities. Specifically, the compounds substituted

with fluorophenyl (R_1) and nitrophenyl (R_2) at 3,6-positions in the triazolothiadiazole rings showed significant antifungal and antibacterial activities. Hence, some structural modifications can be done to enhance their biological activities.

2.4 Phosphoryl chloride as cyclization agent

Phosphoryl chloride is a colourless to pale yellow liquid with chemical formula of $POCl_3$. Recently, it has been used as cyclization agent in synthesis of various heterocyclic compounds including 1,3,4-oxadiazole, 1,3,4-thiadiazole, 1,2,4-triazolo-1,3,4-thiadiazole, isoquinoline and others (Mahdi, et al., 2020). For instance, Bischler-Napieralski synthesis of 1-benzoyl dihydroisoquinoline was conducted by Shankar, et al. (2012) whereby 2-oxo-*N*-phenethylacetamide underwent cyclodehydration reaction with $POCl_3$ at 80 ℃ for 12-14 hours. An isomeric mixture of 1-benzoyl dihydroisoquinoline was isolated with 70 % yield after purification by column chromatography. Figure 2.10 shows the Bischler-Napieralski synthesis of 1-benzoyl dihydroisoquinoline in the presence of $POCl_3$.

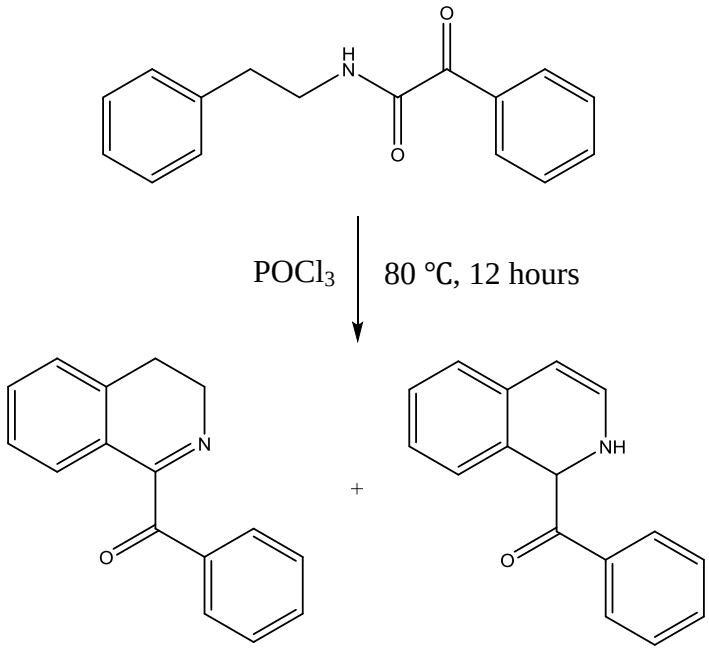


Figure 2.10: Bischler-Napieralski synthesis of 1-benzoyl dihydroisoquinoline in the presence of $POCl_3$.

In addition, a study conducted by Yakan (2020) show that phosphoryl chloride act as cyclization agent in synthesis of the 1,3,4-thiadiazole compounds whereby equimolar of *N*-arylthiosemicarbazides and benzoic acid derivatives were cooled in the refrigerator and phosphoryl chloride was added dropwise by stirring. The reaction was then refluxed at 90 ℃ for 4 hours. Figure 2.11 shows the synthesis of 1,3,4-thiadiazole compounds in the presence of $POCl_3$.

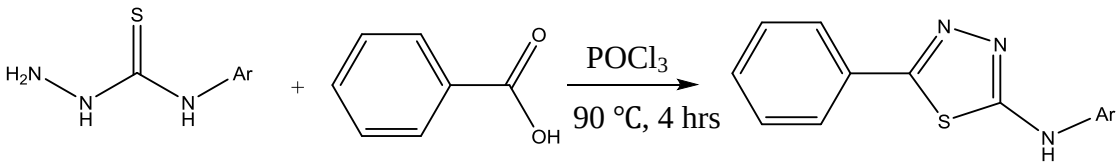


Figure 2.11: Synthesis of 1,3,4-thiadiazole compounds in the presence of $POCl_3$.

Furthermore, Verma, et al. (2017) revealed that various studies have been done in synthesis of 1,3,4-oxadiazole by researchers using $POCl_3$ as cyclization agent and they concluded that it is the most conventional method. Some reactions for the synthesis of 1,3,4-oxadiazole in the presence of $POCl_3$ done by researchers were shown in Figure 2.12.

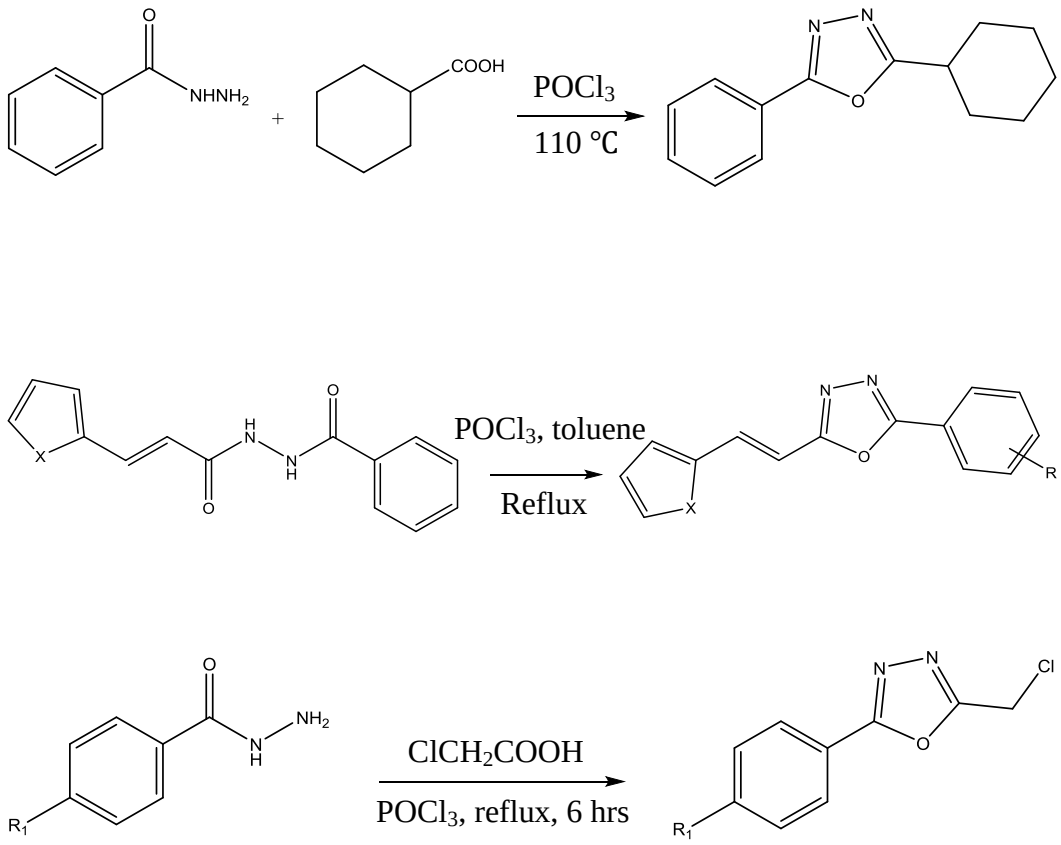


Figure 2.12: Reactions for the synthesis of 1,3,4-oxadiazole in the presence of $POCl_3$.

CHAPTER 3

MATERIALS AND METHODS

3.1 Chemicals used

Table 3.1 shows the list of chemicals used in the synthesis of 1,2,4-triazole.

Table 3.1: List of chemicals used in the synthesis of 1,2,4-triazole.

Chemical	Molecular weight, g/mol	Manufacturer	Country
Thiocarbohydrazide	106.15	Acros Organics	Belgium
Sodium bicarbonate	84.01	Fisher Scientific	UK

Table 3.2 shows the list of chemicals used in the synthesis of 1,2,4-triazolo-1,3,4-thiadiazole.

Table 3.2: List of chemicals used in the synthesis of 1,2,4-triazolo-1,3,4-thiadiazole.

Chemical	Molecular weight, g/mol	Manufacturer	Country
Benzoic acid	122.12	Merck	Germany
2-fluorobenzoic acid	140.11	Acros Organics	Belgium
4-fluorobenzoic acid	140.11	Merck	Germany
2,4-difluorobenzoic acid	158.11	Thermo Fisher Scientific	UK
4-bromobenzoic acid	201.02	Merck	Germany
4-methylbenzoic acid	136.15	Merck	Germany
4-dimethylaminobenzoic acid	165.19	Acros Organics	Belgium
4-nitrobenzoic acid	167.12	Merck	Germany
Phosphoryl chloride	153.33	Merck	Germany
Sodium bicarbonate	84.01	Fisher Scientific	UK

Table 3.3 shows the list of chemicals used in Thin Layer Chromatography (TLC).

Table 3.3: List of chemicals used in Thin Layer Chromatography (TLC).

Chemical	Molecular weight, g/mol	Manufacturer	Country
95 % ethanol	46.07	Bumi Pharma	Malaysia
Chloroform	119.38	R&M Chemicals	UK
Ethyl acetate	88.11	Lab-Scan	Ireland
Hexane	86.18	Merck	Germany

Table 3.4 shows the list of chemicals used in recrystallization.

Table 3.4: List of chemicals used in recrystallization.

Chemical	Molecular weight, g/mol	Manufacturer	Country
95 % ethanol	46.07	Bumi Pharma	Malaysia

Table 3.5 shows the list of chemicals used in Fourier Transform Infrared Spectrophotometry (FTIR).

Table 3.5: List of chemicals used in Fourier Transform Infrared Spectrophotometry (FTIR).

Chemical	Molecular weight, g/mol	Manufacturer	Country
Potassium bromide	119.00	Fisher Scientific	UK

Table 3.6 shows the list of chemicals used in Nuclear Magnetic Resonance (NMR).

Table 3.6: List of chemicals used in Nuclear Magnetic Resonance (NMR).

Chemical	Molecular weight, g/mol	Manufacturer	Country
Chloroform-*d*	120.38	Merck	Germany
Dimethyl sulfoxide-d_6	84.17	Fisher Scientific	UK

3.2 Instruments used

Table 3.7 shows the list of instruments used in the study.

Table 3.7: List of instruments used in the study.

Instrument	Model
FTIR Spectrophotometer	Perkin Elmer, 2000-FTIR Spectrophotometer (Spectrum RX 1)
NMR Spectrometer	JEOL, FT-NMR Spectrometer JNM-ECX 400
Melting point apparatus	Stuart SMP10 Melting Point Apparatus

3.3.3 Purification of products through recrystallization

The crude 1,2,4-triazole and 1,2,4-triazolo-1,3,4-thiadiazoles were purified by recrystallization using hot 95% ethanol after vacuum filtration. Firstly, ethanol was added with boiling chips and heated to boil. An appropriate amount of hot ethanol and boiling chips were used to dissolve the crude product. The solution underwent a quick hot filtration through cotton wool in preheated glass funnel to remove undissolved impurities. Preheated glassware was used to prevent the crystal formation on the wall of the glassware which results in lower percentage yield. Then, the filtrate was continued to boil until saturated. After that, it was left aside by covering loosely with aluminium foil with holes to evaporate at room temperature to have crystallization. The reformed product was rinsed with cold ethanol for several times and the solution was removed by sucking out to obtain pure product. It was dried in the oven, collected in sample vial and weighed. The purity of the product was checked using thin layer chromatography (TLC) followed by characterization using Fourier Transform Infrared (FTIR) spectrophotometer and Nuclear Magnetic Resonance (NMR) spectrometer.

3.4 Characterization of products

3.4.1 Thin Layer Chromatography (TLC)

Thin layer chromatography is a chromatographic technique used to separate, identify and to determine the purity individual compounds present in the mixture. In this project, it was used to determine the purity of products by comparing the spots before and after recrystallization. It was conducted on a sheet of aluminium

foil coated with thin layer of silica gel which acted as stationary phase. It depends on the interaction between compounds and both stationary and mobile phase (Saurabh, 2021).

Firstly, small amount of sample was dissolved with appropriate amount of ethanol and chloroform. The sample solution was spotted on the sketched TLC plate side by side with the starting materials used by using capillary tube to have better comparison. The TLC chamber was set up by using a mixture of hexane and ethyl acetate with ratio of (1:1) which acted as mobile phase. Then, the TLC plate was put inside the chamber. It was removed when the solvent reached at the solvent front and visualised under ultraviolet (UV) lamp. The spots observed were marked and their retention factor (Rf) were calculated using the following formula (Saurabh, 2021).

$$\mathrm{Rf} = \frac{\text{Distance travelled by sample from baseline (cm)}}{\text{Distance travelled by solvent front from baseline (cm)}}$$

The lower the Rf value, the higher the polarity of the compound whereby it has stronger affinity towards the stationary phase.

3.4.2 Melting Point Apparatus

The melting point of synthesized compounds were measured using melting point apparatus. It is an easy and fast method to verify the purity of the compounds. As stated by Brittain (2009), the melting point of a compound is the temperature at which some parts of solid start to melt until the whole solid melts completely. A high purity compound has narrow melting point range which usually between 1-

2 ℃. However, the presence of small quantity of impurities will alter the melting point of the compound and broaden the melting point range.

The melting point measurements began with gently tapping the open end of the capillary tube into the powdered solid sample for several times to fill the capillary tube with sample. The tube was inverted and tap gently on the table with the aid of glass funnel to let the crystals fall to the closed end of the capillary tube. Then, it was inserted into the available compartment. The plateau temperature was set closer to expected melting point. The melting process was observed and the melting point was measured when the sample began to melt until it was fully melted. The measurements were done twice for a more precise melting point range.

3.4.3 Fourier Transform Infrared (FTIR) Spectroscopy Analysis

Fourier Transform Infrared (FTIR) spectroscopy is an analytical technique which is used to determine structural information by identifying the types of functional groups present in the sample and producing an infrared absorption spectrum. The principle behind FTIR spectroscopy is that when infrared radiation passes through the sample, some of the radiation is absorbed by the sample while some of them is transmitted. The radiation that passes through the sample is recorded. As different compounds with different structures absorb certain frequencies of infrared radiation, the spectra are unique and act as fingerprint to distinguish among the molecules (Merck, 2022).

To conduct FTIR analysis, a very small amount of sample was ground and mixed with anhydrous potassium bromide into powder by using pestle and mortar. It was transferred into the centre of the Die insert. It was then pressed under required pressure. The sample turned into pellet form with potassium bromide under high pressure. Lastly, the pellet formed was transferred into sample holder and ready for analysis. The analysis was conducted at the frequency of 4000 cm^{-1} to 400 cm^{-1}.

3.4.4 Nuclear Magnetic Resonance (NMR) Spectroscopy Analysis

Nuclear Magnetic Resonance (NMR) spectroscopy is a non-destructive analytical technique which involves magnetic field to identify the structure and purity of the compounds. Many nuclei have been studied but the most commonly used are hydrogen and carbon nuclei. There are several NMR analyses were used to determine the structure of compounds which are ^{1}H NMR, ^{13}C NMR, DEPT, HMQC and HMBC.

The principle involved in NMR spectroscopy is that all nuclei are electrically charged and most of them have spin. When the applied external magnetic field is absorbed by atom nuclei, there is an increase in absorption energy from low to high energy level. The energy absorption takes place is same with the radio frequency. As the spins return to low level, the energy emitted is also at the same

frequency. Therefore, the signal which matches with the energy transfer is measured which generates the NMR spectrum (Aryal, 2022).

To conduct NMR analysis, as for 1,2,4-triazole, about 10 mg of sample was dissolved with a mixture of deuterated chloroform ($CDCl_3$) and dimethyl sulfoxide (DMSO-d_6) solvent with ratio of (1:1). As for 1,2,4-triazolo-1,3,4-thiadiazoles, only deuterated dimethyl sulfoxide was used to dissolve the sample. The dissolved sample was transferred to the NMR tube with a clean and dry dropper until the height of 4 cm and capped to avoid evaporation of solvent. Deuterated solvents were used in NMR analysis to ensure that they will not interfere with the analysis of the compounds.

3.5 Calculations

I. Determination of the mass of starting materials required in the synthesis:

Mass = number of mole (mol) × molecular weight (g/mol)

II. Determination of the product percentage yield:

$$\text{Percentage yield} = \frac{\text{Experimental mass of product (g)}}{\text{Theoretical mass of product (g)}} \times 100\ \%$$

III. Determination of the retention factor (Rf) value of the product from TLC:

$$\text{Rf} = \frac{\text{Distance travelled by sample from baseline (cm)}}{\text{Distance travelled by solvent front from baseline (cm)}}$$

CHAPTER 5

CONCLUSION

5.1 Conclusion

In conclusion, a 1,2,4-triazole and a series of 1,2,4-triazolo-1,3,4-thiadiazole derivatives **YR1**-**YR8** were synthesized in this project. The structure of 1,2,4-triazole and 1,2,4-triazolo-1,3,4-thiadiazoles derivatives were characterized and elucidated by using FTIR, ^{1}H NMR, ^{13}C NMR, DEPT, HMQC, HMBC and melting point apparatus. The percentage yield of 1,2,4-triazole was 31 % and whereas the percentage yield of 1,2,4-triazolo-1,3,4-thiadiazole derivatives was in the range of 7 – 84 %.

5.2 Further study

Various studies and research have reported that 1,2,4-triazolo-1,3,4-thiadiazole possess a wide range of biological and pharmacological activities. Therefore, further study on antioxidant, antifungal, antibacterial and anticancer activities can be conducted on the synthesized 1,2,4-triazolo-1,3,4-thiadiazoles. Furthermore, the synthesis of 1,2,4-triazole and 1,2,4-triazolo-1,3,4-thiadiazole derivatives can be conducted by microwave irradiation or using green solvent such as polyethylene glycol to employ green synthesis.

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