SYNTHESIS AND CHARACTERIZATION OF 1,2,4-TRIAZOLO-1,3,4-THIADIAZOLES

By

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ABSTRACT

SYNTHESIS AND CHARACTERIZATION OF 1,2,4-TRIAZOLO-1,3,4-THIADIAZOLES

LOO XIN TONG

In this project, a 1,2,4-triazole and eight 1,2,4-triazolo-1,3,4-thiadiazole derivatives have been successfully synthesized and characterized. On the other hand, 1,2,4-triazolo-1,3,4-thiadiazoles were synthesized through refluxing 1,2,4-triazole and benzoic acid derivatives in the presence of phosphorous oxychloride as the cyclization agent. The 1,2,4-triazolo-1,3,4-thiadiazole derivatives were named as **XT1**, **XT2**, **XT3**, **XT4**, **XT5**, **XT6**, **XT7** and **XT8** for different substituents which were H, 4-Cl, 2-Cl, 2,4-Cl_2, 3-CF_3, 4-CF_3, 4-OCH_3 and 4-NO_2, respectively. The structure of 1,2,4-triazole and 1,2,4-triazolo-1,3,4-thiadiazole derivatives (**XT1-XT8**) were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, DEPT, HMQC and HMBC spectroscopies as well as melting point apparatus. The percentage yield of 1,2,4-triazole obtained was 31 % whereas **XT1-XT8** were synthesized with the percentage yield in the range of 8 – 69 %.

ABSTRAK

SINTESIS DAN KARAKTERISASI 1,2,4-TRIAZOLO-1,3,4-THIADIAZOLES

LOO XIN TONG

Dalam projek ini, lapan derivatif 1,2,4-triazolo-1,3,4-thiadiazol dan 1,2,4-triazol telah disintesiskan dan dikarakterisasikan. Derivatif 1,2,4-triazolo-1,3,4-thiadiazol pula disintesis melalui refluks antara 1,2,4-triazol dengan derivatif asid benzoik. Fosforus oksiklorida digunakan sebagai agen siklisasi dalam reaksi ini. Lapan derivatif 1,2,4-triazolo-1,3,4-thiadiazol ini dinamakan sebagai **XT1**, **XT2**, **XT3**, **XT4**, **XT5**, **XT6**, **XT7** dan **XT8** bagi substituen yang berbeza masing-masing, iaitu H, 4-Cl, 2-Cl, 2,4-Cl_2, 3-CF_3, 4-CF_3, 4-OCH_3 dan 4-NO_2. Struktur 1,2,4-triazol dan derivatif 1,2,4-triazolo-1,3,4-thiadiazol (**XT1-XT8**) dikarakterisasi dengan menggunakan radas takat lebur serta spektroskopi FT-IR, ^{1}H NMR, ^{13}C NMR, DEPT, HMQC dan HMBC. Peratusan hasil 1,2,4-triazol adalah 31 % manakala **XT1-XT8** didapati dalam lingkungan 8 – 69 %.

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Throughout the project, I have received a great amount of assistance and support to bring the project to completion.

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Lastly, my thanks and gratitude to my teammate, Yun Ru, and my other friends who provided stimulating discussions as well as good company during the ups and downs.

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 Universiti Tunku Abdul Rahman or other institutions.

Name: Loo Xin Tong

Date: 29 April 2022

APPROVAL SHEET

This final year project report entitled "**SYNTHESIS AND CHARACTERIZATION OF 1,2,4-TRIAZOLO-1,3,4-THIADIAZOLES**" was prepared by LOO XIN TONG 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

_____ Date: 1/6/2022

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Date: 29 April 2022

PERMISSION SHEET

It is hereby certified that **LOO XIN TONG** (ID No: **19ADB02955**) has completed this final year project report entitled "SYNTHESIS AND CHARACTERIZATION OF 1,2,4-TRIAZOLO-1,3,4-THIADIAZOLES" under the supervision of **ASSOCIATE PROFESSOR 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,

(LOO XIN TONG)

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LIST OF ABBREVIATIONS

^{13}C NMR	Carbon-13 nuclear magnetic resonance
δ_c	Chemical shift of carbon
δ_H	Chemical shift of proton
J	Coupling constant
DNA	Deoxyribonucleic acid
DMSO-d_6	Deuterated dimethyl sulfoxide
DEPT	Distortionless Enhancement by Polarization Transfer
EA	Ethyl acetate
FT-IR	Fourier-Transform Infrared
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Coherence
NMR	Nuclear magnetic resonance
^{1}D NMR	One-dimensional nuclear magnetic resonance
$POCl_3$	Phosphorous oxychloride
KBr	Potassium bromide
^{1}H NMR	Proton nuclear magnetic resonance
R_f	Retention factor
TLC	Thin layer chromatography
^{2}D NMR	Two-dimensional nuclear magnetic resonance
cm^{-1}	Wavenumber

CHAPTER 1

INTRODUCTION

1.1 Triazole

Triazole is a five-membered, heterocyclic compound with the molecular formula of $C_2H_3N_3$. Five-membered heterocyclic compounds containing nitrogen are significant structural fragments for their roles as biologically active compounds, acid-base indicators, corrosion inhibitors, dyes, pesticides and other industrial chemicals. For instance, some triazoles are used to inhibit the formation of fog in photographic emulsions. The name ‘triazole’ was given by the scientist, Bladin, in 1885, and he was the first scientist to describe triazoles’ derivatives. The stability of triazole nucleus is contributed by aromaticity where an aromatic sextet is formed in which each atom connected by double bonds donates one π electron, along with the remaining two electrons from a nitrogen atom. In addition, resonance also contributes to the stability of triazole nucleus, represented by tautomeric forms, where interconversion happens in terms of hydrogen atom being relocated within the compound. Triazole exists in the form of two isomers, which are 1,2,3-triazole and 1,2,4-triazole (Shneine and Alaraji, 2016). The structures of triazoles are shown in Figure 1.1 and Figure 1.2, respectively.

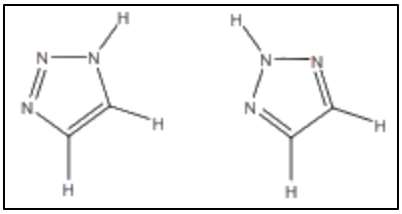


Figure 1.1: Tautomeric forms of 1,2,3-triazole. From left to right, 1*H*-1,2,3-triazole and 2*H*-1,2,3-triazole (Shneine and Alaraji, 2016).

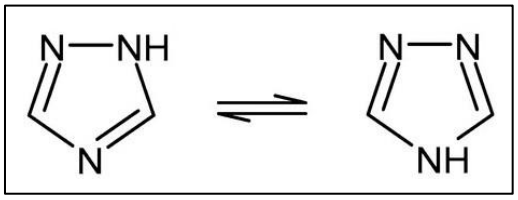


Figure 1.2: Tautomeric forms of 1,2,4-triazole. From left to right, 1*H*-1,2,4-triazole and 4*H*-1,2,4-triazole (MDPI AG, 2022).

Novel drugs of triazole have been discovered and developed through the utilization of bioisosteric replacement technique. Since then, they have been attracting attentions for medicinal chemistry research due to their prominent biological activities detected (Kumari et al., 2021). Bioisosteric replacement is a technique utilized by medicinal chemists when a lead compound in the drug under development fails to exhibit the desired metabolism and pharmacokinetic properties. These constraints are addressed by the technique while still retaining the potency of the lead compound. When bioisosteres are used and structural changes are introduced to the lead compound, alterations can be done to the

compound in terms of dipole, electronic distribution, lipophilicity, polarity, polarizability, pK_a, size and shape, retaining the engagement of potent target at the same time (Dick and Cocklin, 2020).

Triazoles have been comprehensively studied as hydrophobic linkers and peptide bond isosteres. The lone pairs of electrons from both nitrogen atoms in the triazole which are like pyridine behave as hydrogen bond acceptors, imitating the role of oxygen atom in an amide. On the other hand, strong dipole moment exhibited by the CH bond of triazole enables it to act as a donor of hydrogen bond, comparable to the NH bond of amide. These characteristics allow the engagement of hydrogen bonds by the triazole followed by taking part in π interactions (Sainas and Lolli, 2021).

Some marketed medicines containing triazole moiety include anastrazole, litrozole, vorozole, fluconazole, itraconazole, myclobutanil, paclobutrazole, posaconazole, tebuconazole, rizatriptan and ribavirin (Kumari et al., 2021). Table 1.1 shows the marketed medicines with their chemical structures and respective functions.

Table 1.1: Marketed medicines containing triazole moiety.

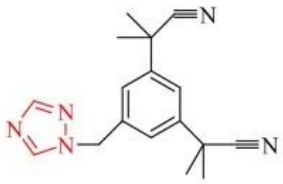
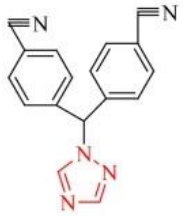
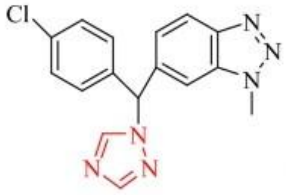
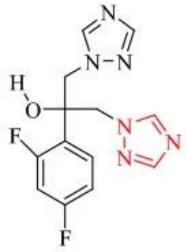
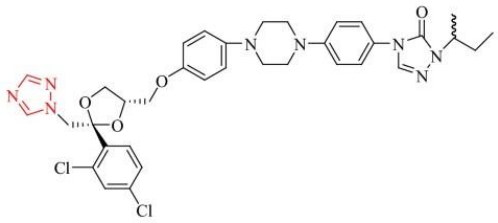
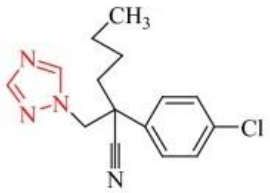
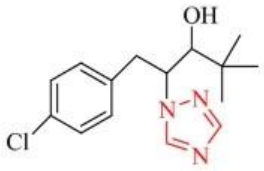
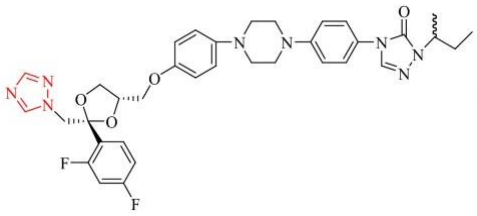
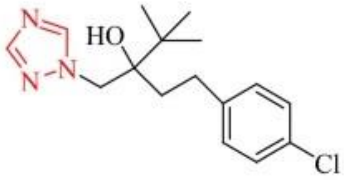
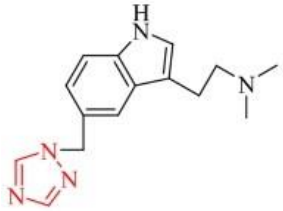
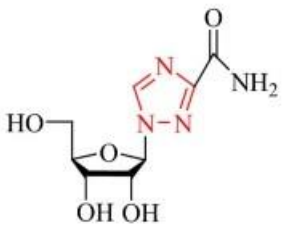
Medicine	Structure	Biological Activity
Anastrazole		Anticancer
Litrozole		
Vorozole		
Fluconazole		Antifungal
Itraconazole		

Table 1.1: Continued.

Medicine	Structure	Biological Activity
Myclobutanil		Antifungal
Paclobutrazole		
Posaconazole		
Tebuconazole		
Rizatriptan		Antimigrain

Ribavirin		Antiviral

1.1.1 1,2,4-Triazole

Among the two isomeric forms of triazole which are 1,2,3-triazole and 1,2,4-triazole, substitution can be done on positions three, four and five of triazole ring but it is found that the groups attached to the nitrogen atom at the fourth position contribute to the largest change in physicochemical and biological properties. The hydrogen bonding is favored and metabolic degradation is stable. This contributes to increased solubility and favorability in binding bimolecular targets. 1,2,4-triazole has been getting attentions from researchers owning to its ability in terms of biological activities such as anticancer, anticonvulsant, anti-inflammatory, antimicrobial, antimigrain, antioxidant, antiparasytic, anti-urease and antiviral (Kumari et al., 2021). One of the substituted 1,2,4-triazoles is 3-mercapto-1,2,4-triazole, which is being studied in this project. There are two tautomeric forms that exist for this compound, which are shown in Figure 1.3. The thione form has the mobile hydrogen attached to the nitrogen whereas for the thiol form, the sulfur has the attachment of the mobile hydrogen, where thione form exists predominantly (Shneine and Alaraji, 2016).

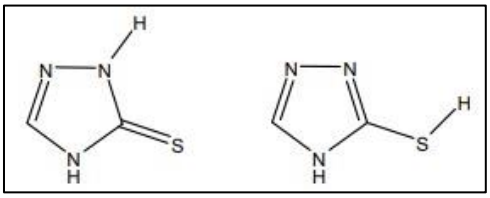


Figure 1.3: From left to right, thione form and thiol form (Shneine and Alaraji, 2016).

1.2 Thiadiazole

Thiadiazole is a five-membered, heterocyclic compound with the molecular formula of $C_2H_2N_2S$. There are four isomeric forms of thiadiazole where the nitrogen atoms are located at different positions for each isomer as shown in Figure 1.4.

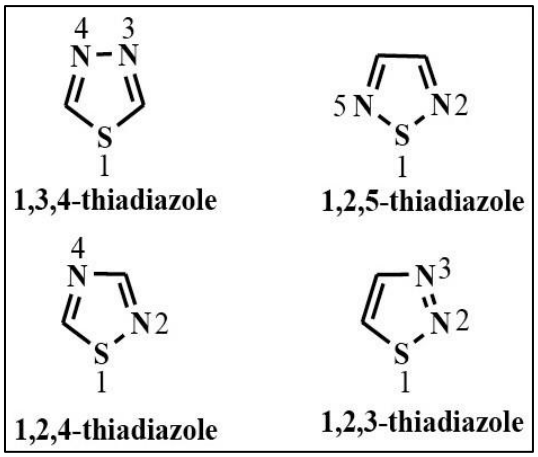
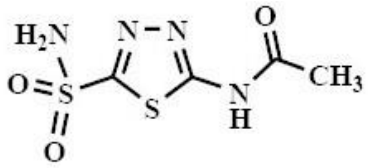
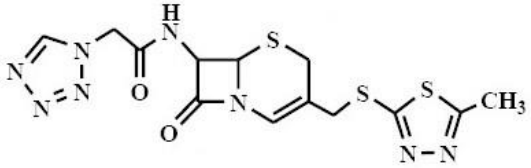
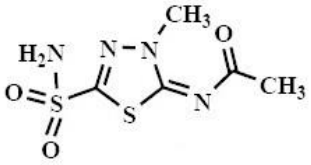
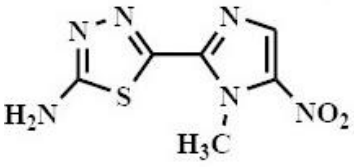
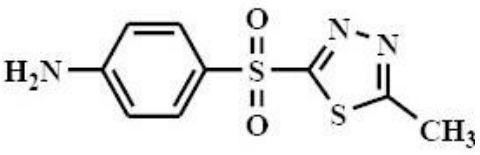


Figure 1.4: Isomers of thiadiazoles (Babalouei and Tahghighi, 2017).

Thiadiazole exhibits remarkable therapeutic potential, making it to be favored by medicinal chemists for years. The mesoionic character of the ring by different regions of positive and negative charges allows it to cross cellular membranes easily and have strong interactions with biological targets. Moreover, it has high liposolubility due to the presence of sulfur atom, contributing to improved

pharmacokinetic properties and the biological activity of compounds bearing the thiadiazole ring. Some marketed drugs containing thiadiazole moiety include acetazolamide, cefazolin, methazol amide, megazol and sulfamethizole (Babalouei and Tahghighi, 2017). Table 1.2 shows the marketed drugs with their chemical structures and respective functions.

Table 1.2: Marketed drugs containing thiadiazole moiety.

Drug	Structure	Biological Activity
Acetazolamide		Carbonic anhydrase inhibitor
Cefazolin		First generation cephalosporin
Methazol amide		Diuretic drug
Megazol		Antiparasitic drug
Sulfamethizole		Antimicrobial

1.2.1 1,3,4-Thiadiazole

The chemistry of 1,3,4-thiadiazole was developed following the discovery and finding of hydrazine and phenylhydrazines during the late 19th century. The first description was made by Fischer in 1882 and then followed by further development by Bush and his coworkers. However, Goerdler et al. first demonstrated the actual nature of the ring system in 1956 (Sharma, Verma, Prajapati and Sharma, 2013). 1,3,4-thiadiazoles are widely used in agricultural applications, including bactericides, fungicides, insecticides, herbicides and pesticides (Yakan, 2020). Published studies have reported that 1,3,4-thiadiazole derivatives show the most promising potential therapeutic activities. Compounds bearing this thiadiazole moiety have been reported to possess the potential of antibacterial, analgesic, anticonvulsant, antifungal, anti-leishmanial, anti-inflammatory, antidepressant, antipsychotic, antituberculosis, as well as anticancer, especially. The heterocyclic ring of 1,3,4-thiadiazole is a bioisostere of a pyrimidine, which is the backbone of the structures of three nucleobases of the DNA. As a result, 1,3,4-thiadiazole molecules that are cytostatic-active can interfere with DNA synthesis and subsequently hinder the replication of human tumor and bacterial cells. This leads to the inhibition of multiplication of bacterial and cancer cells (Janowska et al., 2022).

There are several common methods used to synthesize 1,3,4-thiadiazoles. Synthesis by one-bond formation is carried out through the ring-closing reaction of an acylated thiosemicarbazide, as shown in Figure 1.5.

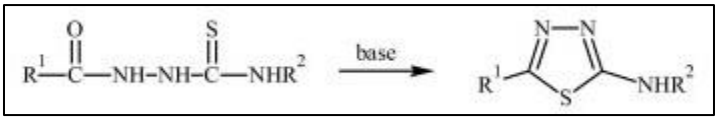


Figure 1.5: One-bond formation of thiadiazole (Othman, Kihel and Amara, 2019).

On the other hand, synthesis by two-bond formation is carried out through 1,3-dipolar cycloaddition as shown in Figure 1.6.

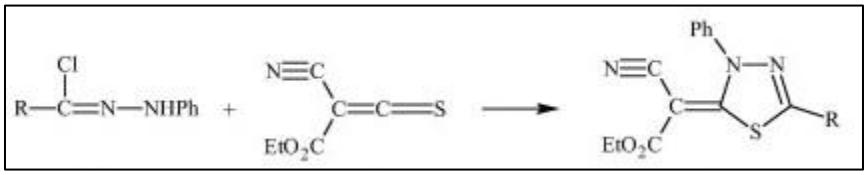


Figure 1.6: Synthesis of mercapto-thiadiazoles by 1,3-dipolar cycloaddition reaction (Othman, Kihel and Amara, 2019).

Meanwhile, simple mercapto-thiadiazoles are synthesized as shown in Figure 1.7.

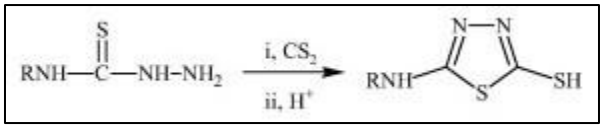


Figure 1.7: Synthesis of mercapto-thiadiazole (Othman, Kihel and Amara, 2019).

Besides, 1,3,4-thiadiazoles can also be synthesized by refluxing 1,3,4-oxadiazoles in ethanolic hydrochloric acid as shown in Figure 1.8 (Othman, Kihel and Amara, 2019).

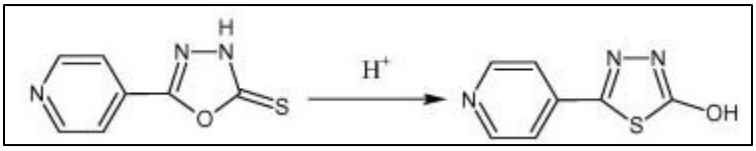


Figure 1.8: Synthesis of 1,3,4-thiadiazole from 1,3,4-oxadiazole (Othman, Kihel and Amara, 2019).

1.3 Objectives

I. To synthesize a 1,2,4-triazole and a series of 1,2,4-triazolo-1,3,4-thiadiazole compounds.

II. To characterize the structure of 1,2,4-triazole and 1,2,4-triazolo-1,3,4-thiadiazoles by using FT-IR, 1D-NMR (^{1}H, ^{13}C and DEPT) and 2D-NMR (HMQC and HMBC).

CHAPTER 2

LITERATURE REVIEW

2.1 Synthesis of 1,2,4-triazole

According to Kaplancıklı, Zitouni, Ozdemir and Revial (2007), thiocarbohydrazide was heated with 1*H*-indole-3-acetic acid to produce 4-amino-3-mercapto-5-[(1*H*-indole-3-yl)methyl]-1,2,4-triazole. 0.1 mol of thiocarbohydrazide and 0.1 mol of 1*H*-indole-3-acetic acid were subjected to heating in an oil bath at 160 ^{o}C to 170 ^{o}C for 2 hours. Hot water was used to disperse the fused mass to obtain the triazole. Recrystallization was then carried out on the product using methanol. The reaction is illustrated in Figure 2.1.

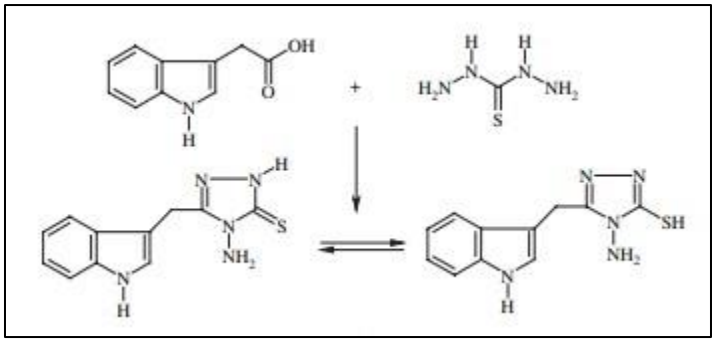


Figure 2.1: Synthesis of 1,2,4-triazole from thiocarbohydrazide and 1*H*-indole-3-acetic acid by Kaplancıklı, Zitouni, Ozdemir and Revial (2007).

Besides, 1,2,4-triazole was synthesized in a similar manner as reported by Nkurunziza and Kalluraya (2018). 5-substituted-4-amino-3-mercapto-1,2,4-triazole was synthesized through cyclocondensing thiocarbohydrazide with various carboxylic acids, including acetic acid, butyric acid, formic acid and propionic acid. The reaction was carried out under reflux condition. The synthesis is illustrated in Figure 2.2.

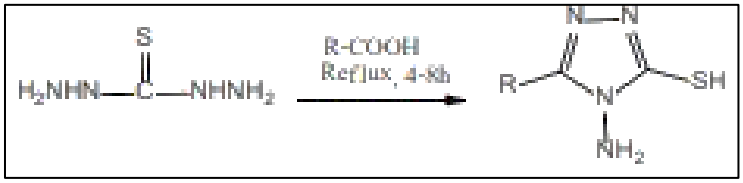


Figure 2.2: Synthesis of 5-substituted-4-amino-3-mercapto-1,2,4-triazole by Nkurunziza and Kalluraya (2018).

In addition, 1,2,4-triazole is also reported to be synthesized by a one-pot reaction through heating potassium dithiocarbazinate, hydrazine hydrate and water under reflux. In this study, 0.1 mol of potassium dithiocarbazinate, 0.3 mol of hydrazine hydrate and 5 ml of water were heated under reflux for 8 hours. A clear solution formed following the formation of H_2S. The reaction mixture was then subjected to dilution with 5 ml of cold water, before being acidified with dilute hydrochloric acid to give a white precipitate. Filtration was carried out on the precipitate with washing using water. Lastly, recrystallization was done using 80 % aqueous ethanol to produce 4-amino-5-mercapto-3-pyridyl-1,2,4-triazole. The product was

formed by the cyclization of potassium dithiocarbazinate with hydrazine hydrate (Singh and Singh, 2009). The reaction is illustrated in Figure 2.3.

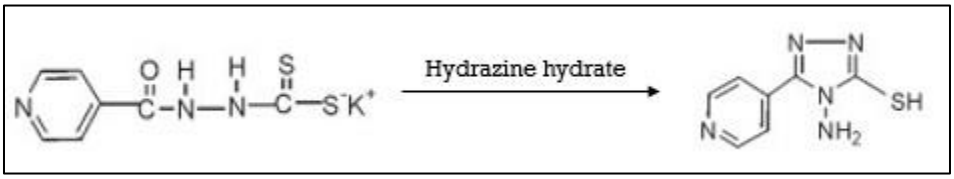


Figure 2.3: Synthesis pathway of 1,2,4-triazole by Singh and Singh (2009).

2.2 Synthesis of 1,3,4-thiadiazole

According to Yakan (2020), 1 mol of *N*-arylthiosemicarbazides and 1 mol of benzoic acid were mixed and cooled in the refrigerator. After adding 3 mol of phosphorous oxychloride into the mixture drop-wise with stirring, reflux was carried out at 90 ^{o}C for 4 hours. Upon completion of the reaction, the mixture was allowed to cool to room temperature and then placed in ice-cold water while stirring continued, followed by neutralization using ammonia. Then, filtration was carried out on the precipitate with washing using water. Lastly, recrystallization was done with a suitable solvent. The synthesis is illustrated in Figure 2.4.

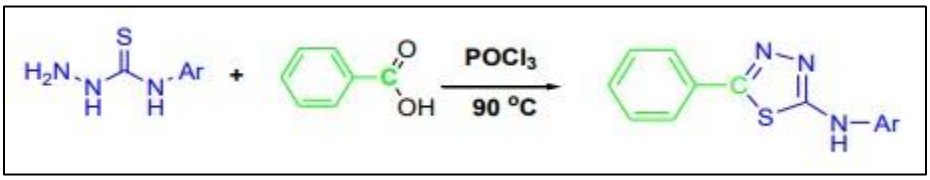


Figure 2.4: Synthesis of 1,3,4-thiadiazole from N-arylthiosemicarbazides and benzoic acid by Yakan (2020).

According to Janowska et al. (2022), 1,3,4-thiadiazoles were synthesized from the reaction between 1,4-disubstituted thiosemicarbazides and concentrated sulfuric acid at room temperature. The yields obtained were in the range of 18 % to 70 %. The reaction is illustrated in Figure 2.5.

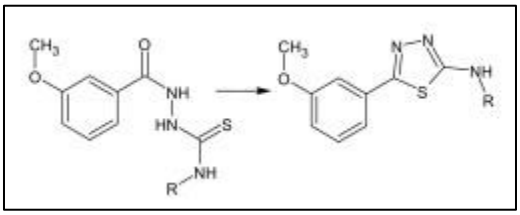


Figure 2.5: Synthesis of 1,3,4-thiadiazole performed by Janowska et al. (2022).

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

The fusion of 1,2,4-triazole and 1,3,4-thiadiazole rings leads to formation of heterocyclic compounds that exhibit substantial range of pharmacological properties (Trafalis et al., 2021). The triazolothiadiazole derivatives were synthesized by condensing triazole and thiadiazole molecules, as reported by Kanaoka in 1956. The resulting compounds have been found to exhibit extensive biological activities, including bactericidal, fungicidal, herbicidal, insecticidal, analgesic, anticancer, anticonvulsant and anti-inflammatory (Lin et al., 2017). For instance, Trafalis et al. (2021) reported that 1,2,4-triazolo-1,3,4-thiadiazoles were found to have good binding to the ATP (adenosine triphosphate) binding site of Akt1 and Akt2, where Akt is known as protein kinase B with three isoforms consisting of Akt1, Akt2 and Akt3. As inhibition of Akt1 and Akt2 phosphorylation is linked to cancer treatment, the low acute toxicity and

anticancer acitivity of 1,2,4-triazolo-1,3,4-thiadiazoles make them promising cancer therapeutic agents.

According to Trafalis et al. (2021), 0.268 mmol of 2-((4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)methyl)-N,N-disubstituted-4,5-dimethoxybenzene sulfonamide and 0.268 mmol of carboxylic acids were added with 0.4 ml of phosphorous oxychloride. The mixture was subjected to heating under reflux for 2 hours with continuous stirring. Upon completion of the reaction, the mixture was allowed to cool to room temperature and then placed in ice, followed by adding aqueous potassium carbonate to adjust the pH to the value of eight. Extraction of the mixture was then carried out with dichloromethane. The drying of the organic extracts was done using sodium sulfate, and then filtration followed. Purification of the residue was done by silica gel column chromatography. The reaction is illustrated in Figure 2.6.

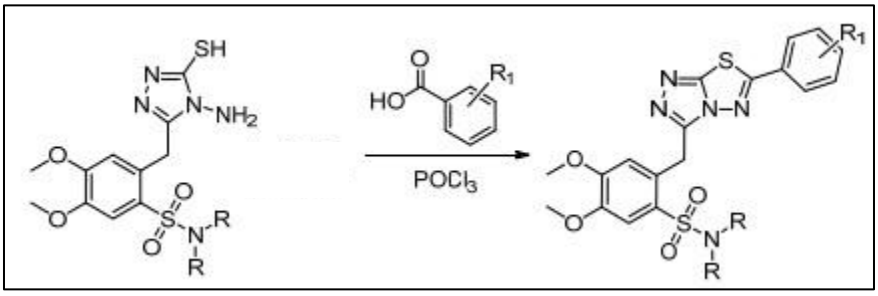


Figure 2.6: Synthesis of 1,2,4-triazolo-1,3,4-thiadiazole derivatives by Trafalis et al. (2021).

On the other hand, Baburajeev et al. (2017) reported the synthesis of 1,2,4-triazolo-1,3,4-thiadiazoles through sulfated ceria catalyzed cyclization reaction. 1 mmol of 4-amino-5-phenyl-4H-1,2,4-triazole-3-thiol and 1 mmol of various carboxylic acids were added with 20 mol % of sulfated ceria in 10 ml of dimethylformamide and 0.1 mmol of phosphorous oxychloride. Then, reflux was carried out for 10 hours. Filtration and washing of the catalyst were done with water. The removal of solvent was carried out under reduced pressure and the concentrated mass was placed in ice, followed by adjusting the pH to the value of eight using potassium carbonate and potassium hydroxide. Then, separation was carried out on the solid obtained by filtration with washing using water. Lastly, recrystallization was done with a suitable solvent. The reaction is illustrated in Figure 2.7.

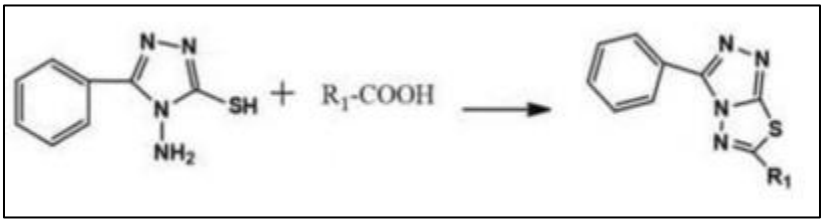


Figure 2.7: Synthesis of 1,2,4-triazolo-1,3,4-thiadiazoles by Baburajeev et al. (2017).

They reported that the efficiency of the catalyst for the cyclization was enhanced by the modification of sulfated ceria with anions such as sulfate ions which led to the formation of excellent acidic catalyst. Completion of most reactions was achieved within 10 hours and the separation of undissolved sulfated ceria was

done by simple filtration. The product resulted in good yield. The plausible mechanism of the synthesis is shown in Figure 2.8.

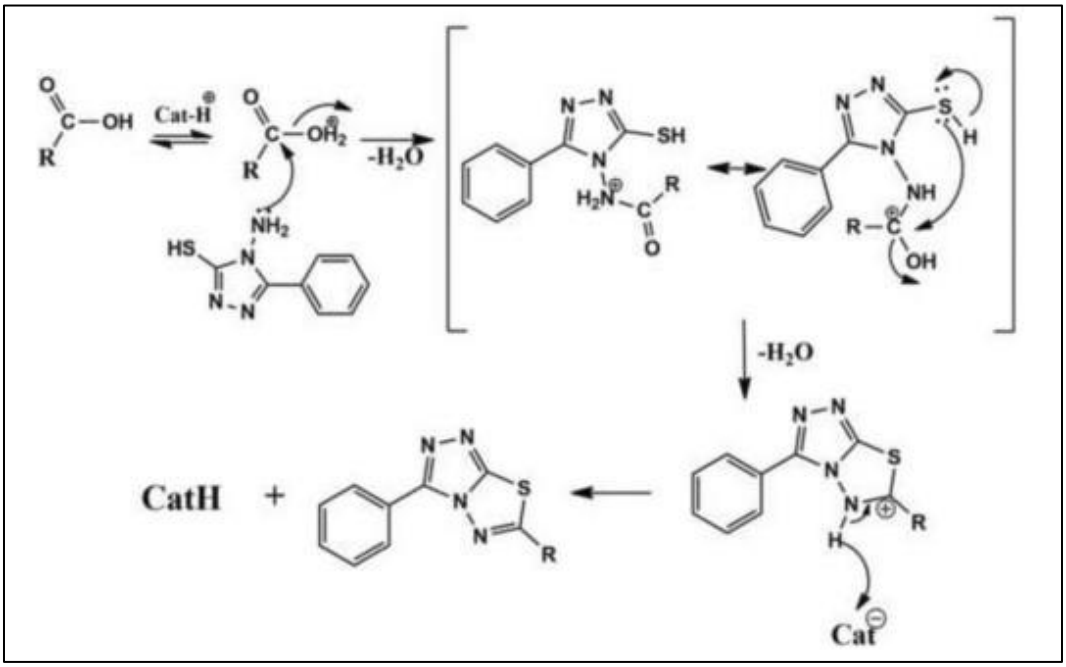


Figure 2.8: Plausible mechanism of cyclization and synthesis of 1,2,4-triazolo-1,3,4-thiadiazoles by Baburajeev et al. (2017).

In the first step, the acid was protonated followed by dehydration. At the same time, an intermediate was formed upon the attacking of the acylium ion that was electron-deficient by the lone pair electrons from nitrogen. The intermediate then underwent neighboring group engagement with nucleophilic sulfur, resulting in the formation of C-S bond after the water molecule was eliminated. In the last step, deprotonation led to the formation of 1,2,4-triazolo-1,3,4-thiadiazole (Baburajeev et al., 2017).

Furthermore, Gomha and Riyadh (2011) reported a microwave-assisted synthesis of 1,2,4-triazolo-1,3,4-thiadiazoles. The condensation of 2-methyl-3-[4-amino-5-mercapto-4*H*-[1,2,4]triazol-3-yl]-1*H*-indole was carried out with various aldehydes in dimethylformamide with the addition of hydrochloric acid in catalytic amount which formed 4-arylideneamino-[1,2,4]triazole Schiff's base derivatives. Then, the 4-arylideneamino[1,2,4]triazole derivatives were brominated in acetic acid with the presence of anhydrous sodium acetate. This led to the formation of 1,2,4-triazolo-1,3,4-thiadiazoles after dehydrobromination from the intermediate. The pathway of synthesis is shown in Figure 2.9.

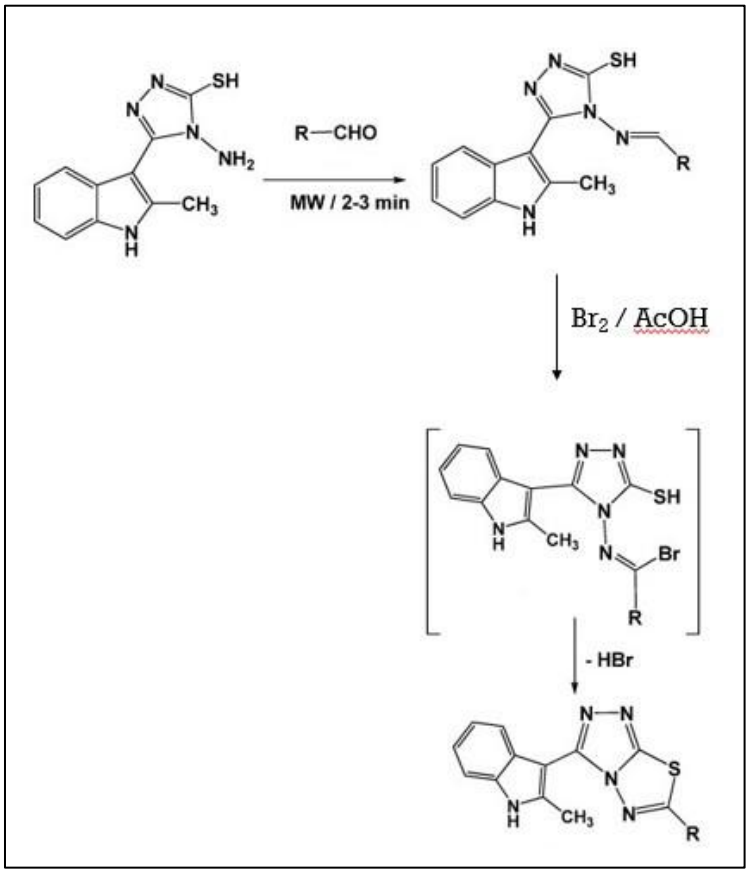


Figure 2.9: Microwave-assisted synthesis of 1,2,4-triazolo-1,3,4-thiadiazoles by Gomha and Riyadh (2011).

Besides, according to Lin et al. (2017), 3 mmol of 4-amino-5-substituted-4H-1,2,4-triazole-3-thiols was added with 25 ml of phosphorous oxychloride. Then, 4.5 mmol of substituted benzoic acid was added dropwise. The reaction mixture was subjected to refluxing for 3 hours with stirring. Upon completion of the reaction, the mixture was placed in ice water followed by adding sodium hydroxide solution to adjust the pH to a value of eight. The precipitates formed were filtered and washed with ethanol for three times. Lastly, recrystallization was done using absolute ethanol followed by dying to obtain 1,2,4-triazolo-1,3,4-thiadiazoles. The reaction is illustrated in Figure 2.10.

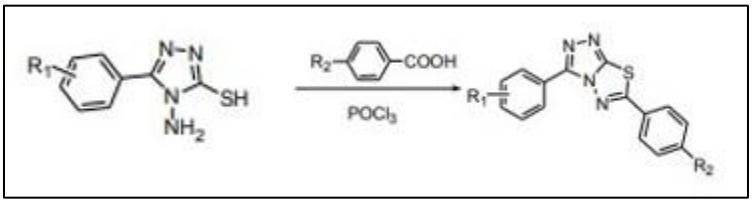


Figure 2.10: Synthesis of 1,2,4-triazolo-1,3,4-thiadiazoles by Lin et al. (2017).

Next, Sarafroz et al. (2019) reported the synthesis of 1,2,4-triazolo-1,3,4-thiadiazoles by condensing equimolar of 1,2,4-triazole with different substituted or unsubstituted aryl acids for 5 to 6 hours in the presence of 10 ml of phosphorous oxychloride. Then, the reaction mixture was stirred in ice-cold water for 4 to 5 hours before being left overnight. The precipitate obtained was separated and neutralized using aqueous alkali. Lastly, dehydration was done followed by recrystallization using ethanol. The reaction is illustrated in Figure 2.11.

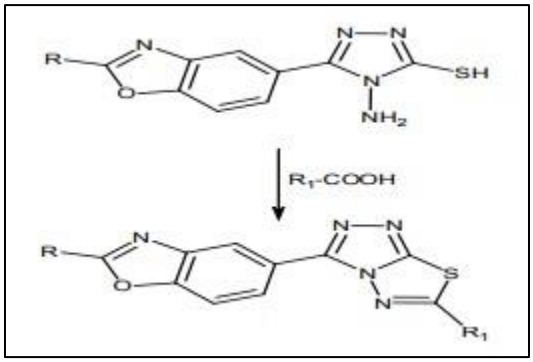


Figure 2.11: Synthesis of 1,2,4-triazolo-1,3,4-thiadiazoles by Sarafroz et al. (2019).

CHAPTER 3

MATERIALS AND METHODS

3.1 List of chemicals

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

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

Chemical	Manufacturer	Country
Thiocarbohydrazide	Acros Organics	Belgium
Sodium bicarbonate	Fischer Scientific	UK
95 % ethanol	Bumi Pharma	Malaysia

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

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

Chemical	**Manufacturer**	**Country**
Benzoic acid	Merck	Germany
4-chlorobenzoic acid	Merck	Germany
2-chlorobenzoic acid	Merck	China
2,4-dichlorobenzoic acid	SRL	India
3-(trifluoromethyl)benzoic acid	Alfa Aesar	Great Britain
4-(trifluoromethyl)benzoic acid	Acros Organics	China
4-methoxybenzoic acid	Acros Organics	Great Britain
4-nitrobenzoic acid	Merck	Germany
Phosphorous oxychloride	Merck	Germany
Sodium bicarbonate	Fischer Scientific	UK
95 % ethanol	Bumi Pharma	Malaysia

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

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

Chemical	**Manufacturer**	**Country**
Ethyl acetate	LAB-SCAN	Ireland
n-hexane	Merck	Germany

Table 3.4 shows list of chemicals used in Nuclear Magnetic Resonance (NMR).

Table 3.4: List of chemicals used in Nuclear Magnetic Resonance (NMR).

Chemical	**Manufacturer**	**Country**
DMSO-d_6	Fischer Scientific	UK
Deuterated chloroform	Merck	Germany

3.2 List of equipment

Table 3.5 shows the list of equipment used in this study.

Table 3.5: List of equipment used in this study.

Equipment	**Model**
FT-IR Spectrophotometer	Perkin Elmer, 2000-FTIR Spectrophotometer (Spectrum RX1)
Melting Point Apparatus	Stuart SMP10 melting point apparatus
NMR Spectrophotometer	JEOL, FT-NMR Spectrometer JNM-ECX 400

3.4 Characterization of products

3.4.1 Fourier-Transform Infrared (FT-IR) Spectroscopy

FT-IR works in such a way where molecules are excited to a state of higher energy after the absorption of infrared radiation. The energies associated with these vibrations are quantized. The absorption is selective where only specific frequencies of infrared radiation are absorbed by a molecule. During the process of absorption, the absorbed frequencies of infrared radiation will match the natural vibrational frequencies of the sample molecule. The amplitude of the vibrational motions of the bonds in the molecule is increased by the energy absorbed. The natural frequency of vibration is unique and different for each type of bond. Therefore, there will not be two molecules of different structure having an infrared absorption pattern that is exactly the same. This allows the determination of structural information about a molecule.

The analysis was performed at the frequency of 4000 cm^{-1} to 400 cm^{-1}. First of all, small amount of sample and potassium bromide (KBr) were ground into fine powder. Then, the die insert was inserted into the holder plate and placed on the lower die. The sample with KBr was poured into the center of the die insert. After connecting the upper die, the whole assembly was then inserted into the press to press the sample into a pellet. After completing the press, the upper die and lower die were removed and the sample pellet was retrieved. The pellet was then inserted into the instrument for the analysis which produced the IR spectrum.

3.4.2 Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR works by the principle of spin property of the nucleus of an atom where a magnetic field is created by the rotating charge. During the analysis, the sample is subjected to a strong magnetic field in the instrument. This causes the nuclear magnet to line up with the magnetic field or against the magnetic field. These two possible states result in an energy difference associated with the energy of radio frequency known as resonant frequency. Each proton in a molecule has a unique and different resonant frequency. This allows the determination of chemical environment in the immediate vicinity of a particular hydrogen atom, such as nearby double bonds and atoms as well as the types of bonding.

There are two common experiments of NMR, namely ^{1}H NMR and ^{13}C NMR. ^{1}H NMR determines the number and types of hydrogen atoms present in a molecule while ^{13}C NMR determines the number and types of carbon atoms present in a molecule. During the analysis, some of the nuclei in the aligned state get excited to the opposed state upon the striking of a strong pulse of radio frequency energy. When the nuclei relax to the aligned state, they release radio frequency energy at the exact resonant frequency of the relaxing nucleus. For ^{1}H NMR, one specific frequency is detected for each proton in the molecule. The frequencies are mixed and then subjected to Fourier transform. This results in the NMR spectrum which is a graph of frequency against intensity that depicts the different frequencies as peaks in the graph (Jacobsen, 2017).

In the laboratory, 10 mg of sample was dissolved by deuterated chloroform and dimethyl sulfoxide solvent in a sample vial. It was then subjected to the NMR tube to a 4-cm-height. Deuterated chloroform was used as solvent to prevent the appearance of interfering signals from protons present in the solvent itself.

3.5 Calculations

I. Determination of mass of starting materials needed prior to the synthesis:

Mass = number of mole (mol) x molar mass (g/mol)

II. Determination of the retention factor (R_f) from TLC analysis:

$$R_f = \frac{Distance\ traveled\ by\ sample\ (cm)}{Distance\ traveled\ by\ solvent\ (cm)}$$

III. Determination of percentage yield for the synthesized product:

$$\text{Percentage yield} = \frac{Actual\ yield\ (g)}{Theoretical\ yield\ (g)}\ x\ 100\ \%$$

CHAPTER 5

CONCLUSION

5.1 Conclusion

In conclusion, a 1,2,4-triazole and eight 1,2,4-triazolo-1,3,4-thiadiazoles (**XT1, XT2, XT3, XT4, XT5, XT6, XT7 and XT8**) were successfully synthesized. Compounds **XT1-XT8** were synthesized with the percentage yield in the range of 8 – 69 %. The structures of 1,2,4-triazole and 1,2,4-triazolo-1,3,4-thiadiazoles were characterized by instrumental analyses including FT-IR spectroscopy and NMR spectroscopy consisting of ^{1}H NMR, ^{13}C NMR, DEPT, HMQC and HMBC. The melting points obtained for compounds **XT1-XT8** were in the range of 152 – 237 ^{o}C.

5.2 Further studies

As studies reported that 1,2,4-triazolo-1,3,4-thiadiazoles exhibit strong pharmacological properties, further studies can be done on the properties such as antibacterial, anticancer, antimicrobial, antimigrain and antioxidant activities. Besides, 1,2,4-triazolo-1,3,4-thiadiazoles can also be synthesized with some structural modifications by using benzoic acids with different substituents. Furthermore, green synthesis of 1,2,4-triazolo-1,3,4-thiadiazoles can be studied where more green methods can be utilized during the synthesis such as using microwave irradiation to increase the energy efficiency of the reaction.

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