SYNTHESIS, CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF 1,2,4-TRIAZOLE AND ITS SCHIFF BASE DERIVATIVES

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By

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ABSTRACT

1,2,4-Triazole and its Schiff base derivatives are found to exhibit diverse biological activities which could contribute to medicinal field as novel drugs. The synthesized novel 1,2,4-triazole and its Schiff base compounds were characterized for their antioxidant potential. The 1,2,4-triazole was synthesized by fusion reaction between indole-3-acetic acid and thiocarbohydrazide, while the Schiff bases were synthesized from the condensation reaction between 1,2,4-triazole and a series of benzaldehyde derivatives. The compounds were characterized using modern IR and NMR techniques for structure elucidation.

The antioxidant activity of the synthesized compounds was determined using DPPH assay with BHT as standard antioxidant. 1,2,4-Triazole and its Schiff bases showed moderate antioxidant activities with IC_{50} ranged from 40.3 to 176.4 ppm, except for **SBs 6, 7, 8, and 14**, which showed IC_{50} more than 200 ppm which exceeded the concentration range prepared for the DPPH assay.

ABSTRAK

1,2,4-Triazol dan derivatif basa Schiffnya didapati mempamerkan pelbagai aktiviti biologi yang boleh menyumbang kepada bidang perubatan sebagai ubat baru. 1,2,4-Triazol novel yang disintesis dan sebatian basa Schiffnya telah dicirikan untuk potensi antioksidannya. 1,2,4-Triazol telah disintesis melalui reaksi pelakuran antara indol-3-asid asetik dan thiokarbohidrazid, manakala basa Schiff disintesis daripada tindak balas pemeluwapan antara 1,2,4-triazol dan satu siri terbitan benzaldehid. Sebatian telah dicirikan menggunakan teknik IR dan NMR moden untuk pencirian struktur.

Aktiviti antioksidan bagi sebatian tersintesis ditentukan menggunakan ujian DPPH dengan BHT sebagai antioksidan standard. 1,2,4-Triazole dan basa Schiffnya menunjukkan aktiviti antioksidan sederhana dengan IC_{50} berjulat antara 40.3 hingga 176.4 ppm, kecuali untuk **SB 6, 7, 8, dan 14**, yang menunjukkan IC_{50} lebih daripada 200 ppm yang melebihi julat kepekatan yang disediakan untuk ujian DPPH.

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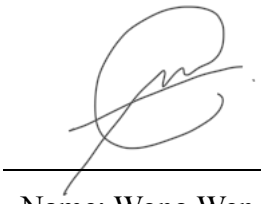
Last but not least, I would like to thank my loving family member and friends for their encouragement and supports, which lead to the completion of this project.

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.



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APPROVAL SHEET

This final year project report entitled "**SYNTHESIS, CHARACTERIZATIONADN ANTIOXIDANT ACTIVITY OF 1,2,4-TRIAZOLE AND ITS SCHIFF BASE DERIVATIVES**" was prepared by WONG WEN LI and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Hons) Chemistry at Universiti Tunku Abdul Rahman.

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Date : 21st September 2023

PERMISSION SHEET

It is hereby certified that WONG WEN LI (ID No: 19ADB05655) has completed this final year project report entitled "SYNTHESIS, CHARACTERIZATIONADN ANTIOXIDANT ACTIVITY OF 1,2,4-TRIAZOLE AND ITS SCHIFF BASE DERIVATIVE" 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,

(WONG WEN LI)

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LIST OF ABBREVATIONS

BHT	Butylated Hydroxytoluene
ABTS	2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid
^{13}C	Carbon-13
℃	Celsius
δ	Chemical shift
J	Coupling constant
D_2O	Deuterated water
DMSO	Dimethyl sulfoxide
DMA	Dimethylacetamide
DMF	Dimethylformamide
DEPT	Distortionless Enhancement by Polarization Transfer
FRAP	Ferric Reducing Antioxidant Power
FT-IR	Fourier-Transform Infrared
g	gram
Hz	Hertz
HMQC	Heteronuclear Multiple Quantum Correlation
HMBC	hetetonuclear multiple bond correlation
IC_{50}	Inhibitory concentration for 50% reduction
mg	miligram
mL	mililitre
mM	milimolar
DIPEA	*N*,*N*-Diisopropylethylamine
nm	nanometer
NBS	*N*-bromosuccinimide
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser Effect

DNPH	O-(2,4-dinitrophenyl)hydroxyamine
1D	One-dimensional
ORAC	Oxygen Radical Absorption Capacity
ppm	Part per million
%	Percentage
^{1}H	Proton
ROS	Reactive Species of Oxygen
R_f	Retention factor
SB	Schiff base
TLC	Thin-Layer Chromatography
TRAP	Total Peroxyl Radical Trapping Antioxidant Parameter
TB	Tuberculosis
2D	Two-dimensional
UV	Ultraviolet
cm^{-1}	Wavenumber
DPPH	2,2-diphenyl-2-picrylhydrazyl

CHAPTER 1

INTRODUCTION

1.1 Introduction of Schiff Base

The term Schiff base is derived from the name of the discoverer, who was a German chemist named Hugo Schiff. He was the first to define the compound produced from the condensation reaction or nucleophilic addition reaction between primary amines and carbonyl compounds in 1864. (Schiff, 1864) Schiff base is characterized by the presence of carbon-nitrogen double bond, which the nitrogen atom is substituted with functional groups such as alkyl or aryl groups but not hydrogen atom. It is represented by the general formula of R R'C=N R'' (where R''≠H). (Raczuk et al., 2022)

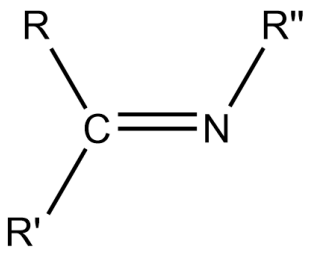


Figure 1.1: Characteristic Structure Fragment of Schiff Bases.

Carbonyl compounds are mainly classified into aldehydes and ketones. Aldehydes are more reactive towards primary amine to form Schiff bases compared to that of ketones. The reactions between ketones and primary amines necessitate certain conditions such as appropriate pH range, catalyst,

choice of solvent that is preferentially forming azeotrope mixture with water, and suitable reaction temperature to initiate the formation of Schiff bases. (Subasi, 2022) This is due to aldehydes have less steric hindrance than ketones. Furthermore, Schiff bases with aryl or aromatic substituents are claimed to be more stable as compared to those with aliphatic alkyl substituent. Aliphatic Schiff bases are labile and readily undergo polymerization, while aromatic Schiff bases are stabilized by their efficient conjugation. (Meena et al., 2023)

1.1.1 Classifications of Schiff Bases

Schiff bases can be further classified based on the functional groups present in the overall structure. Some common types of Schiff bases based on the functional group include hydrazides, hydrazones, and oxime.

1.1.1.1 Hydrazide

Hydrazides are a group of compounds derived from monosubstituted hydrazine. They have a significant structure fragment of a nitrogen bridge (-NH-NH-), with a carbonyl or sulfonyl group attached directly to one of the nitrogen atoms. The general structure of hydrazide moiety is as shown below. This nitrogen bridge is similar to the structure found in amides (peptides), which suggests that hydrazides could mimic peptides.

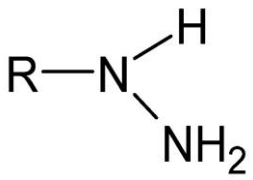


Figure 1.2: Characteristic Structure Fragment of Hydrazide.

Because of the close arrangement of oxygen and nitrogen atoms with lone electron pairs, there's a possibility for the double bond between the carbon and nitrogen atoms to move, resulting in the creation of a hydroxyl group through the oxygen in the carbonyl group.

These hydrazides have limited flexibility because they cannot easily rotate around the C=N bond. This limitation leads to tautomerization, forming iminol forms of hydrazides, which can exist in different geometric shapes known as *E* or *Z* isomers. (Gutowsky and Holm, 1956)

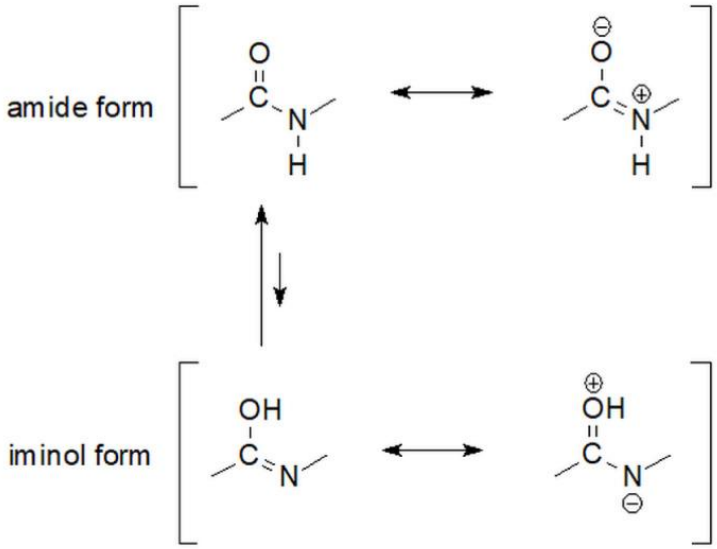


Figure 1.3: Amide-iminol Tautomers of Carbonyl Hydrazides. (Takahashi and Kirikoshi, 2014)

1.1.1.2 Hydrazones

Hydrazones are a group of compounds derived from hydrazides. The characteristic moiety is of the presence of an imine bond (C=N), which could take part in imine-enamine tautomerization due to the presence of α-hydrogen atom. (**Figure 1.5**)

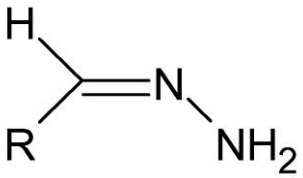


Figure 1.4: Basic Structure of Hydrazones, R can be alkyl or aryl group.

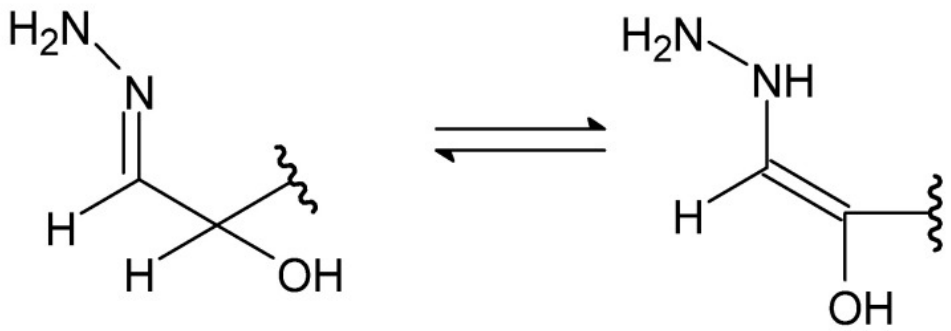


Figure 1.5: Imine–enamine Tautomerization of Hydrazones. (Raczuk et al., 2022)

The N-N bond can be reduced to amino group (-NH_2) or the hydrazone molecule can undergo reductive acylation to form hydrazide. (Perdicchia et al., 2003) The C=N bond in hydrazones is reactive and prone to nucleophilic attack. It can be altered through processes like hydrolysis, oxidation, or reduction, and it can easily return to a carbonyl group (C=O). It can also react with organometallic compounds through nucleophilic addition. (Licandro and Perdicchia, 2004)

1.1.1.3 Oxime

Oximes are characterized by the functional group of R=N-OH, if R group is an alkyl group, the C=N also characterize Schiff base. (**Figure 1.6**) Schiff base with oxime group can be prepared via Friedel–Crafts acylation between phenyl ring and acyl halide, followed by reaction with alkyl nitrite in presence of hydrogen chloride gas. An example of synthetic route of oxime Schiff bases is provided in the **Figure 1.7**. (Dede et al., 2007)

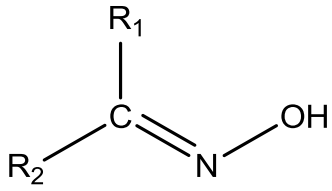


Figure 1.6: General Structure of Oxime Schiff Base.

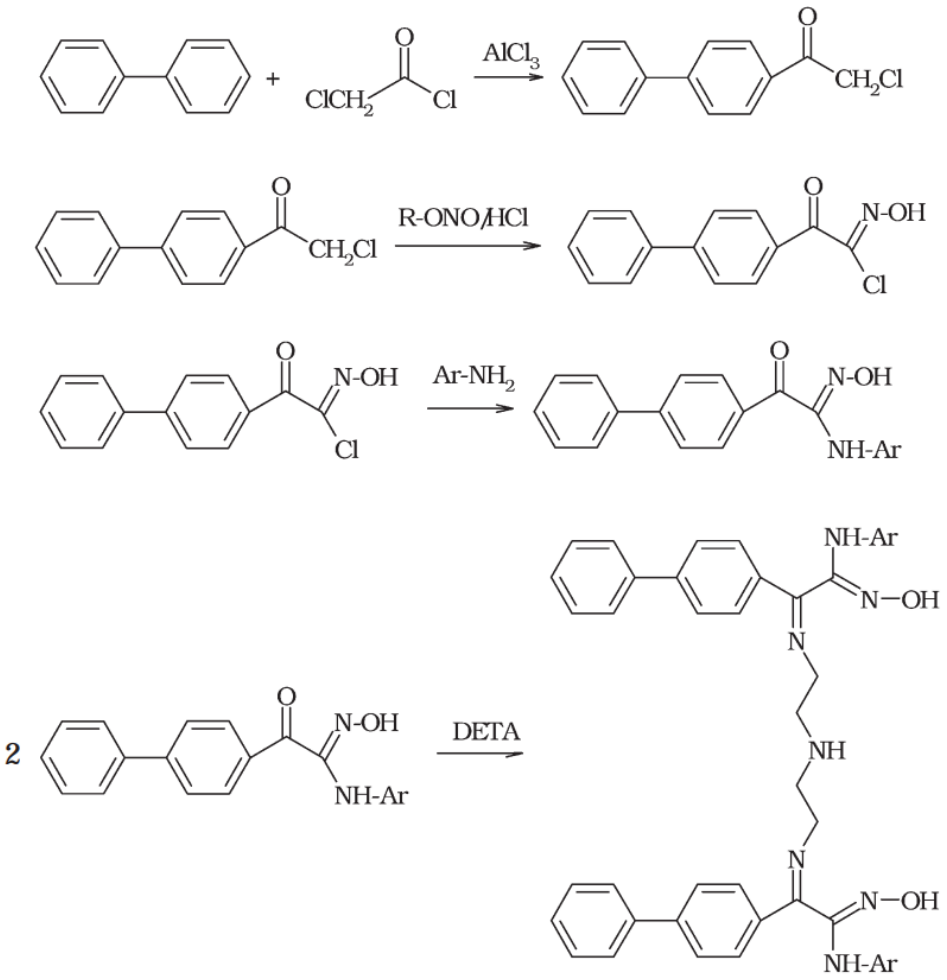


Figure 1.7: Synthesis of Oxime Schiff Base with Functional Group -C=N-OH.

1.1.2 Synthesis of Schiff Bases

In general, Schiff bases are produced from condensation reaction between carbonyl group and primary amine. The condensation reaction consists of two major steps, which involve the addition reaction and elimination reaction. In this case, it is nucleophilic addition, followed by dehydration, where amine is added to carbonyl followed by elimination of water molecules. As it is a reversible reaction, the removal of water molecule may shift the equilibrium to favour the formation of Schiff base, according to Le Chatelier's principle.

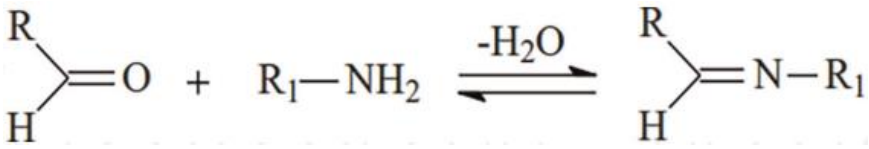


Figure 1.8: General Reaction for Schiff Base Formation.

The reaction mechanism starts with the formation of carbinolamine intermediate from condensation of the carbonyl group with the primary amine, followed by deprotonation and dehydration of the intermediate to form Schiff Base. (**Figure 1.9**) The formation of Schiff base depends on the pH of the reaction system. At low pH, amine will form salt, making free amine molecules less available for the addition reaction. (**Figure 1.10**) This will decrease the rate of addition step, which will make this step the rate-determining step for the reaction.

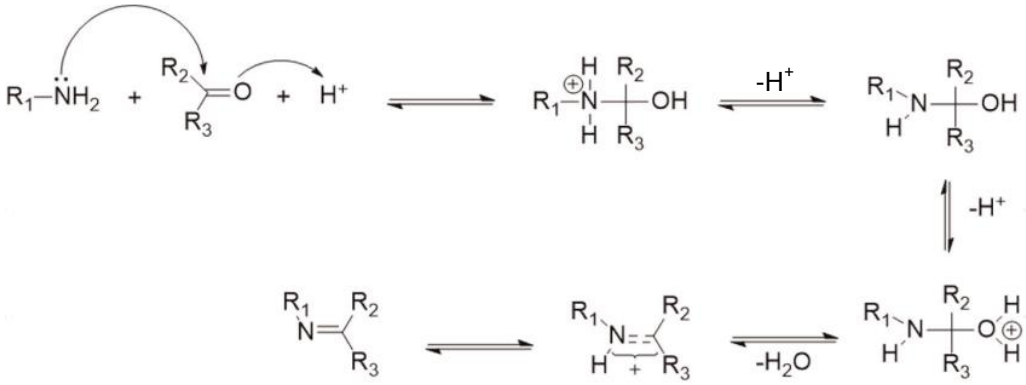


Figure 1.9: Mechanism of Formation of Schiff Base via Condensation Reaction.

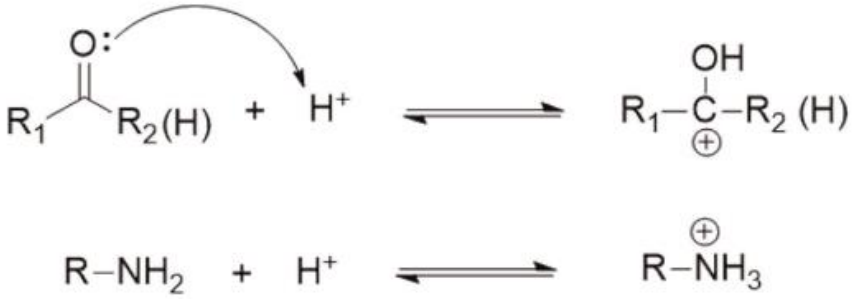


Figure 1.10: Effect of Acidic Medium on Both Reactants, the Carbonyl Group and Amine.

However, at high pH, the presence of hydroxide ions affects the rate of elimination steps. Generally, a relatively lower pH is still preferential for the formation of Schiff base, which is around pHs 3 to 4, this pH range is suitable to facilitate the initiation of nucleophilic addition reaction and elimination reaction at its optimal rate. (Subasi, 2022)

1.1.3 Application of Schiff Bases

Schiff bases can be used as precursors in organic synthesis, which involve four major groups of reactions. The four groups include addition of organometallic reagent to C=N bond to form asymmetric C-C bond, Diels-Alder reaction to produce nitrogen-containing heterocycle, chiral salen metal complex (metal complex with tetradentate Schiff base ligand) being used in asymmetric synthesis, and Staudinger reaction with ketene to produce β-lactams. (Subasi, 2022)

Apart from taking part in organic synthesis, Schiff bases also exhibit wide range of biological activities including antimicrobial, antitubercular (Aboul-Fadl et al., 2003), anticancer (Miri et al., 2013), anticonvulsant, antioxidant (Wei et al., 2006), anthelmintic, anti-inflammatory, analgesic (Sondhi et al., 2006), antiglycation, and antidepressant activities.

In term of antimicrobial activity, research suggested novel Schiff bases derived from isatin was characterized for *in vitro* antibacterial activity and it was assessed by minimum inhibitory concentration (MIC) to compare the activity with standard antimicrobial drugs. Some of the Schiff bases were claimed to be more active than the standard drugs against certain microbes, concluding that the substituted electron donating group is effective in enhancing antimicrobial activity. (Kajal et al., 2013)

Tuberculosis (TB) is an illness caused by the bacterium *Mycobacterium tuberculosis*. While it primarily impacts the lungs, it can also affect various parts of the body. The infection can exist in two states: active or latent, and about 10% of latent infections can become active. This disease spreads through tiny droplets released when an infected person talks, coughs, or sneezes. It is a very popular disease, where statistics show about quarter of the global population is estimated to experience TB infection. (Padda and Reddy, 2023) Therefore, discovery of antitubercular activity on novel compounds is vital to the human community. It is proven that Schiff bases of isonicotinic acid hydrazide exhibit excellent antitubercular activity by enzymatic deactivation process. (Kajal et al., 2013)

1.2 Introduction of 1,2,4-triazole

1,2,4-Triazole is a five-membered heterocycle compound consists of 2 carbon and 3 nitrogen atoms. It possesses molecular formula of $C_2H_3N_3$, which is one of the two isomeric forms of triazoles, with 1,2,3-triazole as the other isomer. 1,2,4-triazole is stabilized by its aromaticity, as aromatic sextet is formed by donation of one π electron from each atom connected by double bonds. Apart from that, the lone pair electrons from nitrogen atom also contributed to its stability. Furthermore, tautomerization can take place in 1,2,4-triazole, which enable the structure to be stabilized by resonance. (Shneine and Alaraji, 2013) There are two possible tautomeric forms, which are 1*H*-1,2,4-triazole and 4*H*-1,2,4-triazole. Studies have suggested that 1*H*-1,2,4-triazole is relatively more stable than the other tautomer. (G A Pinto et al., 2007) The basic structure of 1,2,4-triazole is shown in the figure below.

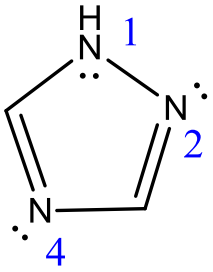


Figure 1.11: Basic Structure of 1*H*-1,2,4-Triazole.

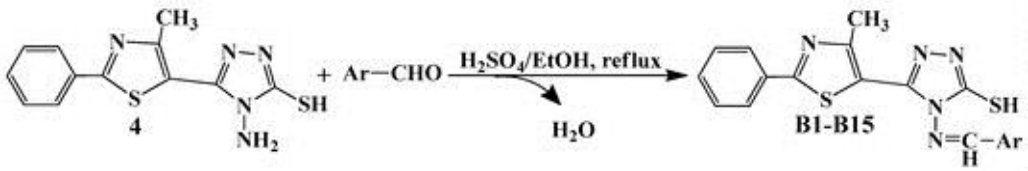
1.3 Schiff Bases Derived from 1,2,4-Triazole

Triazole Schiff base derivatives have shown significant applications in industry, agriculture, and medicine due to their pharmaceutical properties. They serve roles as fungicides, cancer-fighting drugs, materials for pharmaceuticals, polymer protectors, and UV absorbers. With the basic Schiff base structure (-C=N-), they can also act as ligand, to bind with trace metal ions in living organisms, offering various biological benefits and impacting pharmacology. These compounds are versatile and find applications in medicine, materials, and more, including as antibacterial agents, pesticides, and plant growth regulators in healthcare and farming. Recent research has focused on these derivatives, with patents describing new syntheses and novel applications. Attentions have been drawn to recognize the importance of heterocycles derived from 1,2,4-triazole. (Jiang et al., 2020)

1.3.1 Application of 1,2,4-Triazole Schiff Bases

Schiff bases derived from 1,2,4-triazole have broad range of pharmacological properties such as antifungal, anticancer, anticonvulsant, and antibacterial activities. Some of the biological applications of 1,2,4-triazole Schiff base derivatives are discussed below.

A series of novel thiazolyl-triazole Schiff bases were assessed for their antifungal activity toward *Candida* species. Nitro-substituted thiazolyl-triazole Schiff bases shows a significant antifungal activity, which its inhibitory activity is better than the standard antifungal drugs used in the study, which are Fluconazole and Ketoconazole. (Stana et al., 2016)



B1) Ar=2,4-diCl-phenyl
B2) Ar=2,6-diCl-phenyl
B3) Ar=2,3-diCl-phenyl
B4) Ar=3-Cl-phenyl
B5) Ar=4-Br-phenyl
B6) Ar=4-F-phenyl
B7) Ar=2-OH-phenyl
B8) Ar=3-OH-phenyl
B9) Ar=4-OH-phenyl
B10) Ar=3-NO_2-phenyl
B11) Ar=4-NO_2-phenyl
B12) Ar=2-OCH_3-phenyl
B13) Ar=3-OCH_3-phenyl
B14) Ar=2-thiophene
B15) Ar=4-$(CH_3)_2$N-phenyl

Figure 1.12: Synthesis of Thiazolyl-triazole Schiff bases from Thiazolyl-triazole. (Stana et al., 2016)

In another study, a novel 4-amino-1,2,4-triazole Schiff base derivative was synthesized and characterized for their anticancer activity. The synthetic route of the compound is as shown below.

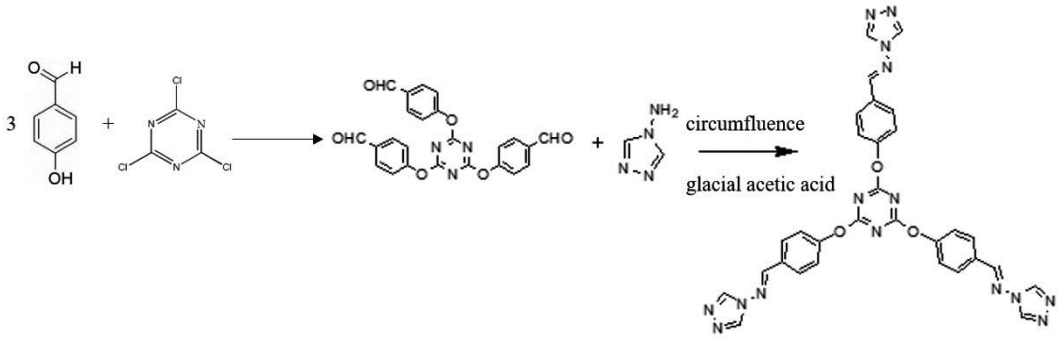


Figure 1.13: Synthetic Route of 4-amino-1,2,4-triazole Schiff Base Derivative.

The study shows that this compound exhibits inhibition effect on the growth of cancer cells, A549 and Bel7402 cells, as the half-maximal inhibitory concentration was determined using 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay. Although there are no suitable Schiff base derivatives in clinical use that can be used as positive control drugs, it is undeniably that this compound exhibits anticancer activity, thus could play an important biological role in medical industry. (Jiang et al., 2020)

1.4 Objectives of this Study

1. To synthesize a series of 1,2,4-triazole Schiff base derivatives.
2. To characterize the structure of pure 1,2,4-triazole and its Schiff base derivatives using FT-IR and NMR spectroscopic techniques.
3. To study the antioxidant activity of synthesized 1,2,4-triazole and its Schiff bases using DPPH assay.

CHAPTER 2

LITERATURE REVIEW

2.1 Synthesis of Indole-3-Acetic Acid

Indole-3-acetic acid is a common precursor for biologically active compounds. Indole-3-acetate moiety is often found in biologically active compounds, some examples are shown in **Figure 2.1**.

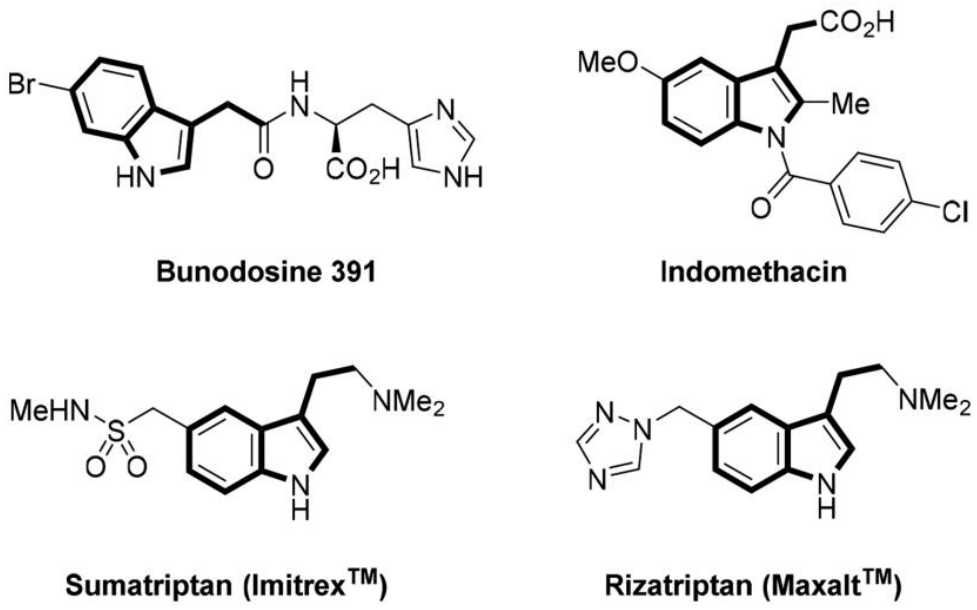


Figure 2.1: Some Bioactive Molecules with Highlighted Indole-3-Acetate Scaffold. (Chen et al., 2018)

Compounds bearing indole ring or benzopyrrole have potential in contributing to medical industry, as most of them are biologically active, which are proven effective to be antidepressant (Hamid et al., 2017), antiviral (Xue et al., 2014), anticancer (Zhuang et al., 2013), anti-inflammatory and analgesic. (Sarva et

al., 2016) Therefore, in this study, 1,2,4-triazole bearing indole ring moiety is to be synthesized and characterized.

There are some possible pathways for the synthesis of indole-3-acetic acid summarized in **Figure 2.2**. Among the synthesis methods, Fischer indole synthesis is the most common method. Besides the conventional methods, there are also some novel synthetic routes reported in the recent studies. (Chen et al., 2018)

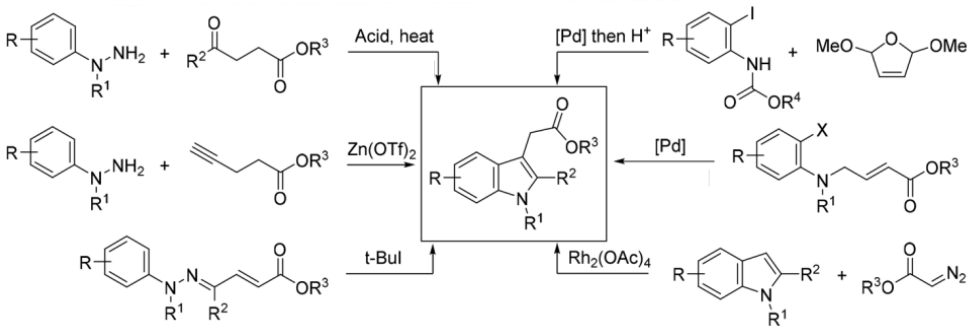


Figure 2.2: Synthetic Routes for the Preparation of Indole-3-Acetic Acid Derivatives.

2.1.1 Synthesis of Substituted Indole-3-Acetic Acid Through Fischer Indole Synthesis

Bullock and Hand (1956) proposed a synthesis using substituted phenylhydrazine hydrochloride and ethyl levulinate as starting materials, in presence of sodium acetate, acetic acid and water. This reaction produced substituted phenylhydrazone intermediate. The intermediate was refluxed with ethanol as solvent and concentrated sulphuric acid as catalyst under nitrogen atmosphere for 2 hours. Then, the reaction mixture was washed with water and sodium bicarbonate solution to remove excess acid to obtain substituted indole-3-acetate. In the last step, the substituted indole-3-acetate was saponified with ethanolic potassium hydroxide, followed by acidification dilute hydrochloric acid.

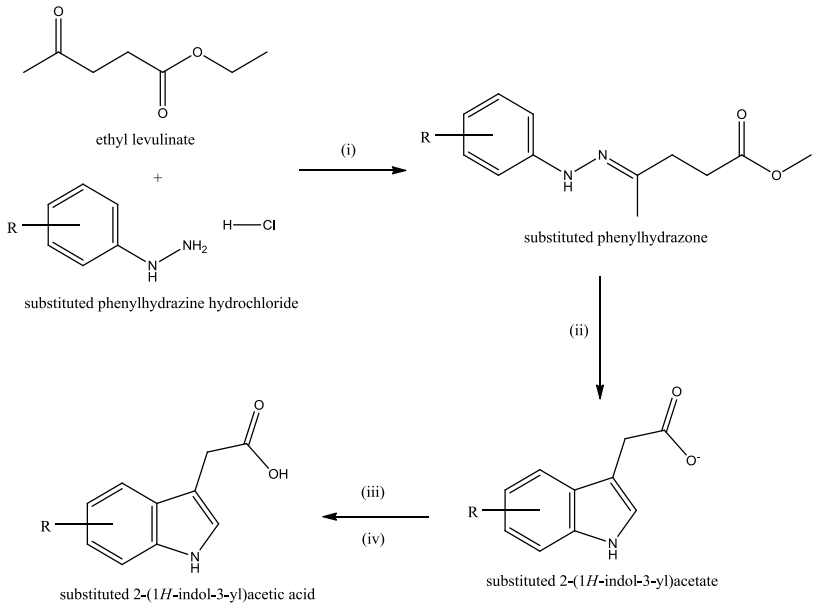


Figure 2.3: Synthetic Route for Substituted Indole-3-Acetic Acid. Reagent and Condition: (i) NaOAc and HOAc; (ii) EtOH and H_2SO_4, reflux 2 hrs under N_2; (iii) KOH, reflux 2 hrs; (iv) HCl. R = 2-methyl, 4-methyl, 2,4-dimethyl, and 4-chloro.

2.1.2 One-Pot Synthesis of Indole-3-acetic Acid

Chen et al. (2018) proposed one-pot synthesis to counter limitation of Fischer synthesis on selectivity. They reported a palladium-catalyzed cascade Tsuji−Trost Reaction and Heck Coupling to synthesize indole-3-acetic acid. (**Figure 2.4**) N-Ts *o*-iodoaniline and ethyl (*E*)-4- acetoxybut-2-enoate were used as the starting materials. The reaction was mediated by palladium (II) complex as metal catalyst, tri(*o*-tolyl)-phosphine as ligand, *N*,*N*-diisopropylethylamine (DIPEA) as base, and dimethylacetamide (DMA) as solvent.

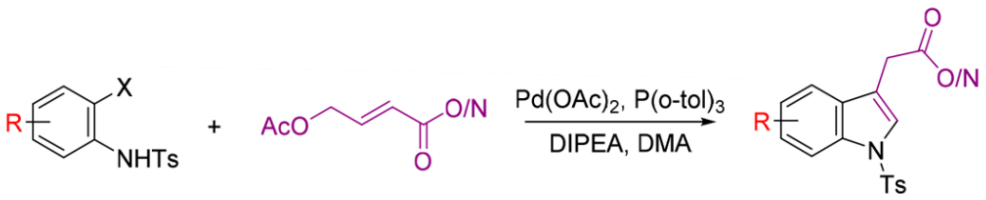


Figure 2.4: Palladium-catalyzed Synthesis of Indole-3-Acetic Acid.

2.2 Synthesis of 1,2,4-Triazole

According to Eicher et al. (2013), 1,2,4-triazole fragment has yet to be found in nature, but there are many synthetic routes reported in the past studies, which can be traced back in 1885, when Bladin first reported the synthesis of 1,2,4-triazole from the reaction between formylhydrazine and formamide. (Bladin, 1885) The discovery is significant to science fields due to its importance in showing high levels of biological activities. The substitution on the 1,2,4-triazole fragment decides the nature of its biological activities. Some of the literatures will be discussed in the following sections.

2.2.1 Synthesis of Unsubstituted 1,2,4-Triazole

Sekiya and Ishikawa (1958) proposed a synthetic route of unsubstituted 1*H*-1,2,4-triazole from condensation reaction of hydrazine sulphate and formamide. Hydrazine sulphate and formamide were heated at 140 □ for 5 hours to produce formohydrazide intermediate, subsequently formation of *N*'-formylformohydrazide intermediate through condensation reaction. In the last step, *N*'-formylformohydrazide undergo condensation with formamide to form 1*H*-1,2,4-triazole, with reported yield of 60 %.

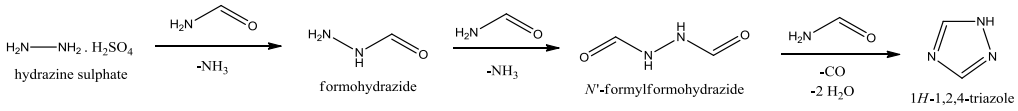


Figure 2.5: Synthetic Route of 1*H*-1,2,4-Triazole Proposed by Sekiya and Ishikawa (1958).

Ainsworth and Jones (1955) proposed a synthetic route which they were able to improve the yield of 1,2,4-triazole. Hydrazine hydrate and formamide were warmed on steam bath for 2 hours. This reaction gave *N*,*N*'-diformylhydrazine (or *N*'-formylformohydrazide) and evolved large quantity of ammonia gas, which is similar to the synthesis proposed by Sekiya and Ishikawa. As 1,2,4-triazole can be produced from either formamide or ammonia. This study suggested if the mixture was to be heated slowly, ammonia will be distilled off and lead to diformylhydrazine being the only material left in the system. Hence, they proposed to heat the synthesized *N*,*N*'-diformylhydrazine with excess ammonia in autoclave at 200 □ for 24 hours. This modified synthetic route improved the yield of 1,2,4-triazole to 70–80 %.

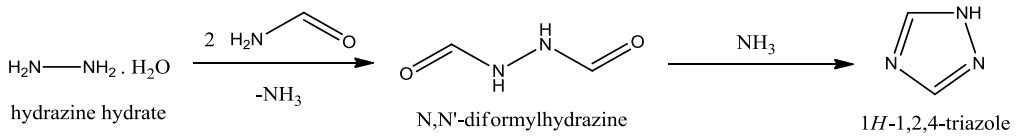


Figure 2.6: Synthetic Route of 1*H*-1,2,4-Triazole Proposed by Ainsworth and Jones (1955).

There is also an alternative method to synthesize 1,2,4-triazole. Nagata et al. (1999) used ketazines instead of hydrazine as starting material. This use of ketazines, especially for acetone azine or methyl ketone ketazine, is preferable as they can be sourced from the industrial production of hydrazine. Ketazine is converted to hydrazine through endothermic hydrolysis, by removing the ketone byproduct. The hydrazine obtained in situ can then react with formamide at 170 □ for 8 hours, to give 1,2,4-triazole.

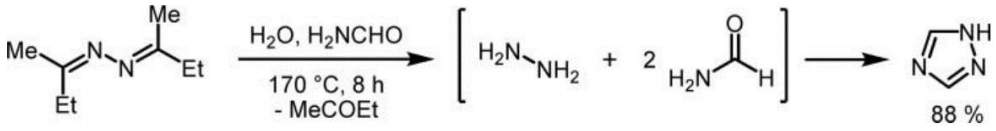


Figure 2.7: Synthetic Route of 1*H*-1,2,4-Triazole Proposed by Nagata et al. (1999).

2.2.2 Synthesis of Amino-substituted Triazoles

The general reaction to synthesize Schiff base is through condensation reaction between primary amine and active carbonyl compounds. Therefore, amino group on triazoles is needed for Schiff base formation, and hence 1,2,4-triazole Schiff base derivatives can be produced. Several methods can be adopted to synthesize amino-substituted triazole, of which some of them are discussed here.

2.2.2.1 From Hydrazines

Yin et al. (2009) proposed a one-pot synthesis of 1,2,4-triazole through the reaction between hydrazine and *N*-cyanoimidate obtained from oxidative amidation of aldehydes. They used various aromatic aldehydes and cyanamide as nitrogen source to synthesize nonamidated product, *N*-cyanoimidate, of which sodium tert-butoxide is used as the base, *N*-bromosuccinimide (NBS) as the oxidant, and methanol as solvent. The synthesis started with reacting aldehyde, cyanamide, and base at room temperature for 30 minutes, followed by addition of oxidant at 50 □ for 12 hours. Then, the synthesized *N*-cyanoimidate was refluxed with phenylhydrazine in methanol for 4 hours to produce 1,2,4-triazole with percent yield of 74-92 %.

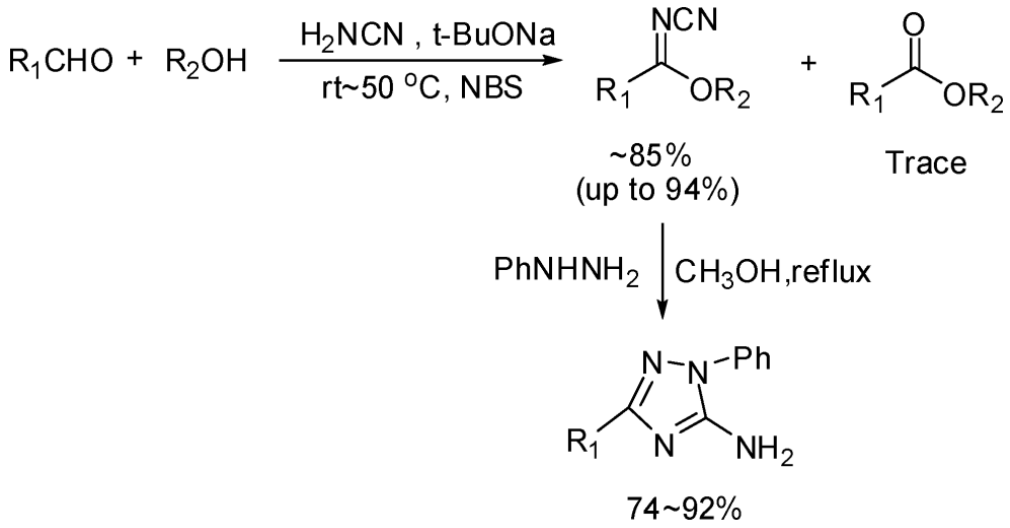


Figure 2.8: One-pot Synthesis of 1,3,5-trisubstituted 1,2,4-Triazole Proposed by Yin et al. (2009).

2.2.2.2 From Thiosemicarbazones

Goswami et al. (1984) proposed a synthetic route by reacting acylhydrazine with carbon disulphide and potassium hydroxide to give potassium salt of thiosemicarbazide. Acylhydrazine was refluxed with carbon disulphide and potassium hydroxide in absolute ethanol for 10 hours to produce the thiosemicarbazone intermediate. Then, the intermediate was treated with hydrazine hydrate by refluxing for 1 hour, followed by acidification to give 1,2,4-triazole.

Figure 2.9: Synthetic Route of 1,2,4-Triazole from Thiosemicarbazone.

2.2.2.3 From Thiocarbohydrazide

Kaplancikli et al. (2008) proposed a reaction between indole-3-acetic acid and thiocarbohydrazide to synthesize 1,2,4-triazole bearing indole ring. Equimolar of thiocarbohydrazide and 1*H*-indole-3-acetic acid was heated in oil bath at 160-170 □ for 2 hours to undergo fusion reaction. The 1,2,4-triazole synthesized can exist in two tautomer forms as shown in **Figure 2.10**.

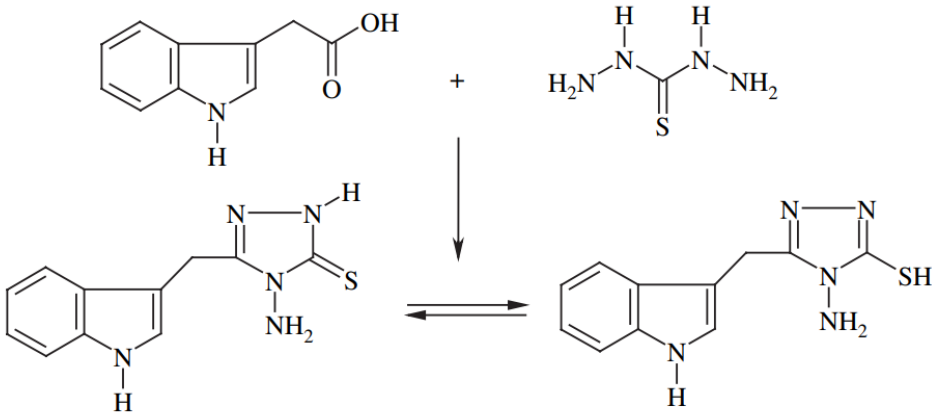


Figure 2.10: Synthetic Route of Indole Bearing 1,2,4-Triazole from Thiocarbohydrazide.

Jin et al. (2007) proposed a synthesis pathway using thiocarbohydrazide and *D*(-)galactono-1,4-lactone as the starting materials. Equimolar of thiocarbohydrazide was added into pyridine solution of *D*(-)galactono-1,4-lactone, subsequently small amount of water was added into the mixture. The reaction mixture was refluxed for 6 hours to produce 4-amino-3-(*D*-galactopentitol-1-yl)-5-mercapto-1,2,4-triazole.

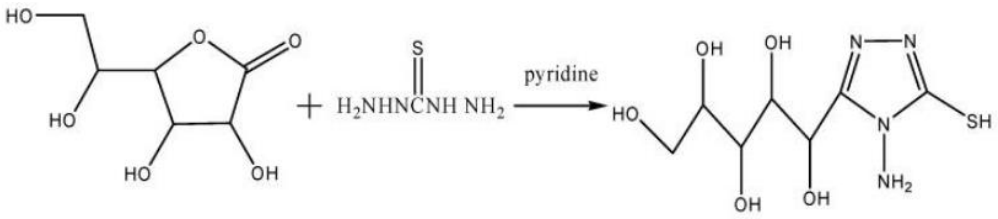


Figure 2.11: Synthetic Route of 4-amino-3-(*D*-galactopentitol-1-yl)-5-mercapto-1,2,4-triazole Proposed by Jin et al. (2007).

2.3 Synthesis of 1,2,4-Triazole Schiff Base Derivatives

Different Schiff bases derived from 1,2,4-triazole have been synthesized and reported in past research. Most of the synthesis are carried out based on the basic principle of Schiff base formation proposed by the discoverer, which is through condensation reaction between primary amine and active carbonyl compounds. Characterization for structure elucidation of the synthesized 1,2,4-triazole Schiff base derivatives can be done using laboratory instruments including Nuclear Magnetic Resonance (NMR) spectrometer and Infrared (IR) spectrophotometer. Besides, applications of the Schiff bases in different fields are also studied to further expand its potential to its fullest. Some synthesis pathways for 1,2,4-triazole Schiff base derivatives are discussed in the following sections.

2.3.1 Synthesis of Schiff Base from Unsubstituted 1,2,4-Triazole

1*H*-1,2,4-triazole was treated with potassium methoxide in methanol to undergo deprotonation. Then, the deprotonated 1,2,4-triazole was heated with O-(2,4-dinitrophenyl)hydroxyamine (DNPH) in dimethylformamide (DMF) at 100 □ for 24 hours, to undergo direct electrophilic amination of the triazolide anion. After acidic workup, the residue was dissolved in methanol prior to addition of benzaldehyde. A relatively low percent yield of 45 % was obtained due to the reaction is uncatalyzed, the condensation reaction is reversible which can revert the formation of Schiff base. (Laus and Klötzer, 1989)

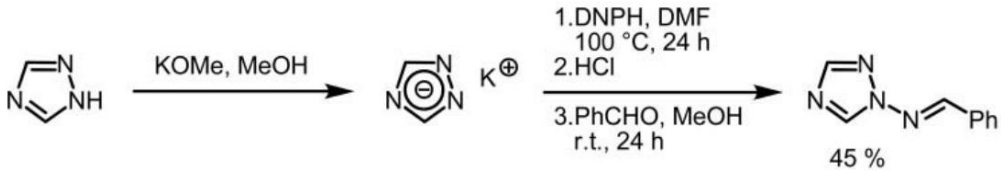


Figure 2.12: Synthetic Route of Schiff Base Derived from Unsubstituted 1,2,4-Triazole. (Holm and Straub, 2011)

2.3.2 Acid-catalyzed Synthesis

Equimolar of 4-amino-5-pentadecyl-4*H*-1,2,4-triazole-3-thiol was mixed with different substituted aldehydes including aliphatic and aromatic aldehydes in ethanol. A few drops of sulphuric acid were added into the reaction mixture prior to reflux for an appropriate duration. The reflux duration was determined by monitoring the reaction progress with thin-layer chromatography. (Kumari et al., 2021)

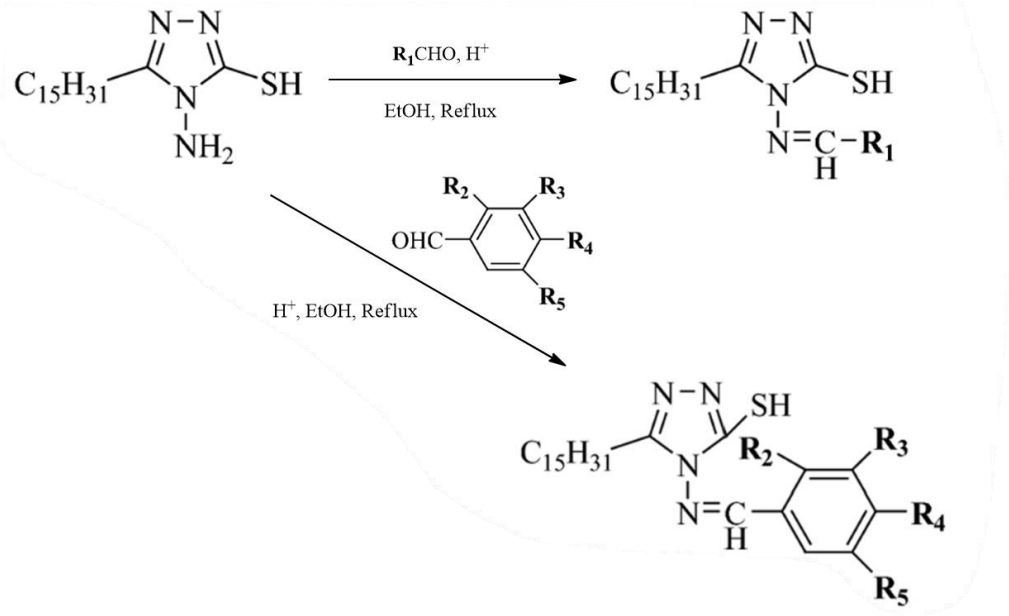


Figure 2.13: Synthetic Route of Schiff Base Proposed by Kumari et al. (2021).

Mixture of ethanol solution of 4-amino-5-(4-methyl-2-phenylthiazol-5-yl)-4*H*-1,2,4-triazole-3-thiol and aromatic or heteroaromatic aldehyde was treated with 2–3 drops of concentrated sulphuric acid as catalyst. The reaction mixture was refluxed for 6 hours. The obtained precipitate was filtered hot and washed with absolute ethanol, and then it was purified by recrystallization from hot DMSO. A series of 4-(substituted benzylideneamino)-5-(4-methyl-2-phenylthiazol-5-yl)-4*H*-1,2,4-triazole-3-thiol was synthesized and characterized by NMR and IR analyses. (Stana et al., 2016)

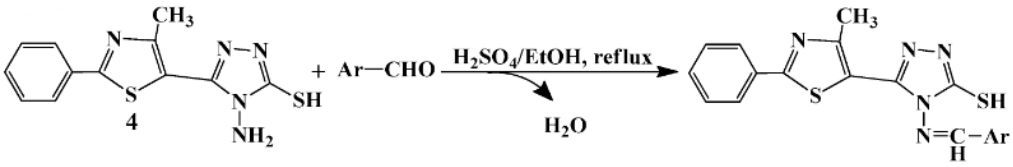


Figure 2.14: Synthetic Route of Schiff Base Proposed by Stana et al. (2016).

Kaplancikli et al. (2008) proposed a synthetic route for Schiff bases derived from 1,2,4-triazole bearing indole ring. Ethanol suspension of arylaldehyde was heated with equimolar of triazole until a clear solution was obtained. Then, a few drops of concentrated sulfuric acid were added as catalyst, and the reaction mixture was refluxed on water bath for 3 hours. The precipitate was then recrystallized from hot ethanol. The products were characterized by IR and NMR analyses to confirm the formation of 5-[(1*H*-Indol-3-yl)methyl]-4-arylideneamino3-mercapto-1,2,4-triazoles. The reported yield for this synthesis ranged from 60–75 %.

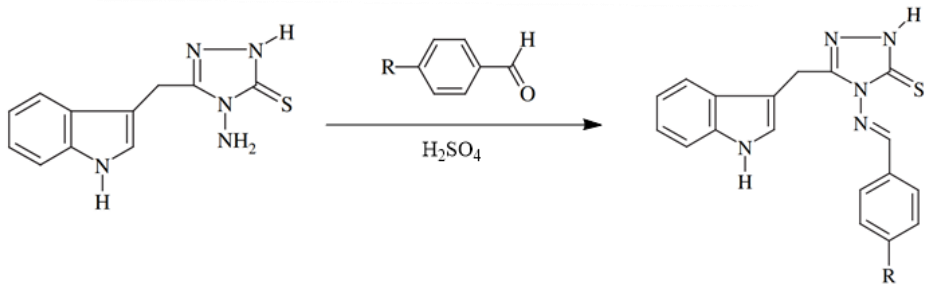


Figure 2.15: Synthetic Route of Schiff Base Proposed by Kaplancikli et al. (2008).

Sameer Al-Rawi and Abed Nashaan (2023) proposed a synthesis method to synthesize symmetrical 1,2,4-triazole derivative. 5,5'-(4-amino-4*H*-1,2,4-triazole-3,5-diyl)bis(benzene-1,2,3-triol) was mixed with equimolar of benzaldehydes followed by addition of 5 drops of glacial acetic acid as acid catalyst. The reaction mixture was refluxed in ethanol for 6 hours. The precipitated solid was filtered, dried, and recrystallized from methanol. The products were characterized with IR and NMR analyses to elucidate the structure, it was identified that the products were 5,5'-(4-(substituted benzylideneamino)-4*H*-1,2,4-triazole-3,5-diyl)bis(benzene-1,2,3-triol)

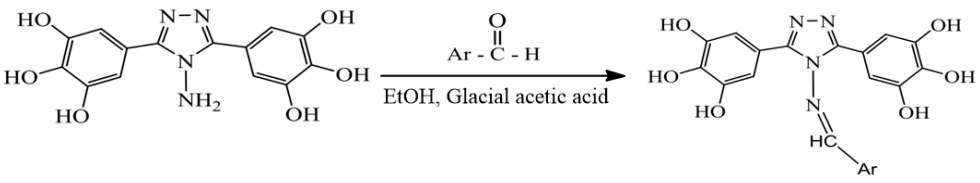


Figure 2.16: Synthetic Route of Schiff Base Proposed by Sameer Al-Rawi and Abed Nashaan (2023)

2.3.3 Microwave-Assisted Synthesis

Microwave-mediated reaction uses a greener approach in term of energy consumption as it normally requires lower reaction temperature than conventional method. Although previously there were studies reported microwave irradiation synthesis of Schiff base, the reactions were carried out with organic solvents and acid catalysis, which is less eco-friendly. Therefore, Mermer and Boulebd (2023) proposed a microwave-irradiated approach with greener solvent system to synthesize 1,2,4-triazole Schiff base. They were able to optimize the solvent system and reaction time to achieve higher yield. The best optimization was using solvent with acetic acid to water in 3:2 ratio, reaction carried out at 200 □ for 25 minutes to obtain percent yield of 89.8 %. NMR and IR characterizations were performed to confirm the formation of (*E*)−3-((1*H*-benzo[*d*][1,2,3]triazol-1-yl)methyl)−4-((substituted benzylidene)amino)−1*H*-1,2,4-triazole-5(4*H*)-thione.

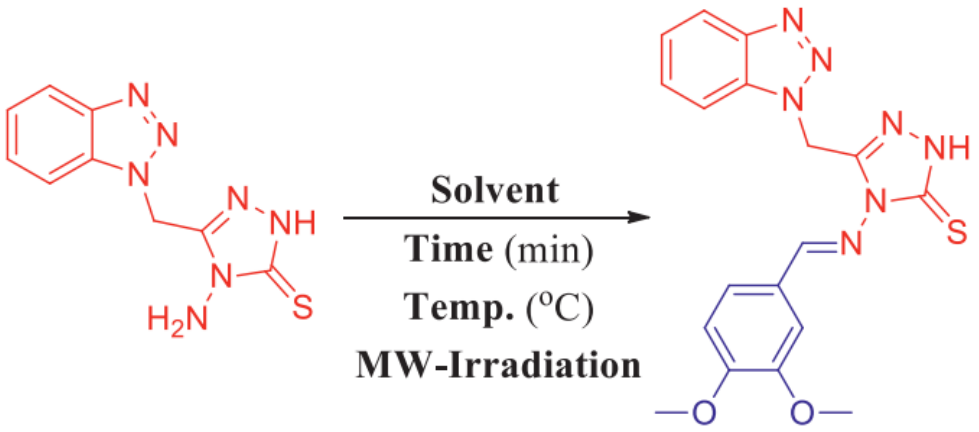


Figure 2.17: Microwave-Assisted Synthesis of Schiff Base Proposed by Mermer and Boulebd (2023).

2.4 Antioxidant Assay

Antioxidants are substances that are able to neutralize free radicals in biological cells, of which the free radicals can damage cells of living organisms. Oxidative stress caused by free radicals can take part in developing common diseases, like diabetes, high blood pressure, preeclampsia, atherosclerosis, acute renal failure, Alzheimer's and Parkinson's. Biological cells are capable at creating reactive species of oxygen (ROS) through metabolising oxygen that are potentially harmful to living organisms. High levels of ROS in biological cells can lead to deficient cell operation, aging, or disease. (Rodrigo, 2009)

There are some common methods that can be used to measure antioxidant activity which include Oxygen Radical Absorption Capacity (ORAC) test, Total Peroxyl Radical Trapping Antioxidant Parameter (TRAP) test, Ferric Reducing Antioxidant Power (FRAP) test, 2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) test, and [2,2-di(4-tert-octylphenyl)-1-picrylhydrazyl] (DPPH) test. Among the tests, DPPH assay is the most commonly used antioxidant assay, due to its simplicity, rapidness, convenience, and economical nature.

DPPH• is a stable radical, found in both solid and liquid states. Its unique low reactivity is mostly influenced by how well the molecule's surrounding parts shield its hydrazyl structure, rather than by extended conjugation. The DPPH

neutralization test works by antioxidants donating electrons to neutralize the DPPH radical. This process changes the colour of DPPH from purple, which is measurable at 517 nm, to pale yellow. The fading of purple colour or lowering of absorbance at 517 nm indicates how active the antioxidant is. Antioxidant activity through the DPPH neutralization method is often reported as IC_{50}, which is the concentration of the antioxidant needed to reduce the initial DPPH concentration by 50%. (Munteanu and Apetrei, 2021) IC_{50} can be obtained from the graph of percentage scavenging against concentration of potential antioxidant.

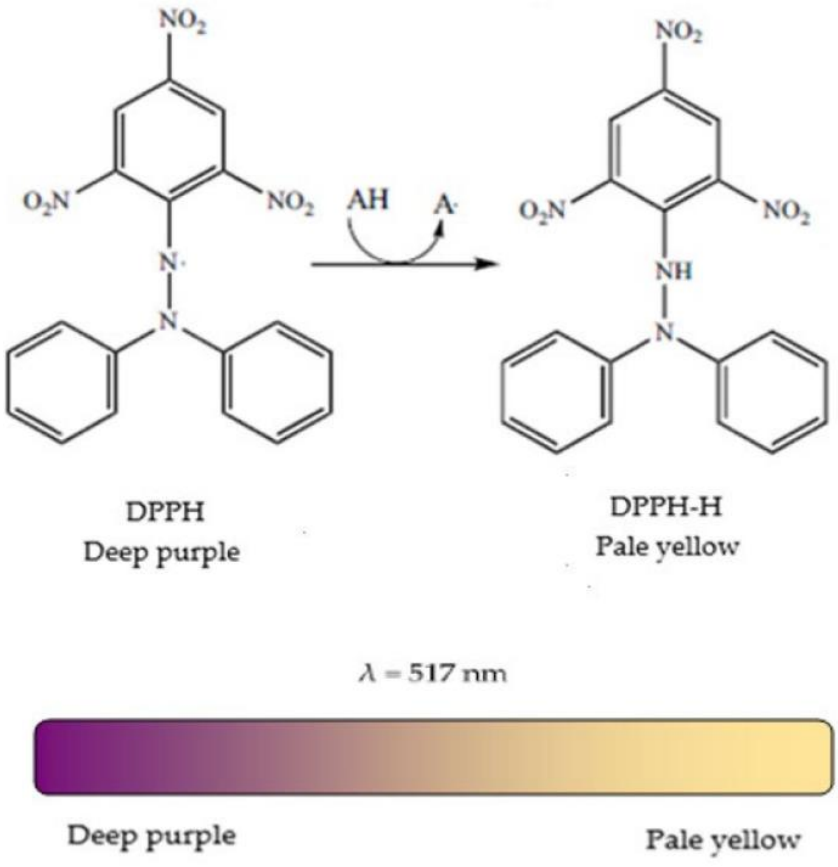


Figure 2.18: Reduction of DPPH by An Antioxidant (AH).

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 Chemicals Used

Table 3.1: Chemicals Used for Synthesis of 1,2,4-Triazole.

Chemical/Solvent	Molecular Weight, g/mol	Manufacturer	Country of Origin
Acetic Acid $C_2H_4O_2$	60.05	Acros Organic	Belgium
Ethyl Acetate $C_4H_8O_2$	88.11	LAB-SCAN	Ireland
Absolute Ethanol C_2H_6O	46.07	Fischer Scientific	Malaysia
Hydrochloric Acid HCl	36.46	Fischer Scientific	Malaysia
Sodium Hydroxide NaOH	40.00	Fischer Scientific	Malaysia
Sulphuric Acid H_2SO_4	98.08	Fischer Scientific	UK
Thiocarbohydrazide CH_6N_4S	106.15	Acros Organic	Belgium
Sodium Bicarbonate $NaHCO_3$	84.01	Fischer Scientific	UK
Sodium Sulphate Na_2SO_4	142.04	Merck	Germany

Table 3.2: Chemicals Used for Synthesis of Schiff Bases.

Chemical	Molecular Weight, g/mol	Manufacturer	Country of Origin
Benzaldehyde C_7H_6O	106.12	Merck	Germany
2,4-dichlorobenzaldehyde $C_7H_4Cl_2O$	175.01	Merck	Germany
3,4-dichlorobenzaldehyde $C_7H_4Cl_2O$	175.01	Acros Organic	Belgium
2-fluorobenzaldehyde C_7H_5FO	124.11	Merck	Germany
4-fluorobenzaldehyde C_7H_5FO	124.11	Merck	Germany
2-chlorobenzaldehyde C_7H_5ClO	140.57	Merck,	Germany
4-chlorobenzaldehyde C_7H_5ClO	140.57	Merck	Germany
2-bromobenzaldehyde C_7H_5BrO	185.02	Acros Organic	Belgium
4-bromobenzaldehyde C_7H_5BrO	185.02	Acros Organic	Belgium
4-methylbenzaldehyde C_8H_8O	120.15	Merck	Germany
2-methoxybenzaldehyde $C_8H_8O_2$	136.15	Merck	Germany
4-methoxybenzaldehyde $C_8H_8O_2$	136.15	Acros Organic	Belgium
2-nitrobenzaldehyde $C_7H_5NO_3$	151.12	Merck	Germany
4-nitrobenzaldehyde $C_7H_5NO_3$	151.12	Merck	Germany
2-hydroxybenzaldehyde $C_7H_6O_2$	122.12	Acros Organic	USA

Table 3.3: Chemicals Used for Thin-Layer Chromatography (TLC)

Solvent	Molecular Weight, g/mol	Manufacturer	Country of Origin
Ethyl Acetate $C_4H_8O_2$	88.11	LAB-SCAN	Ireland
Hexane C_6H_{14}	86.18	Merck	Germany
Chloroform $CHCl_3$	119.38	Acros Organic	Belgium
95% Ethanol C_2H_6O	46.07	Systerm	Malaysia

Table 3.4: Chemical Used for Recrystallization

Solvent	Molecular Weight, g/mol	Manufacturer	Country of Origin
95% Ethanol C_2H_6O	46.07	Systerm	Malaysia

Table 3.5: Chemicals Used for Characterization

Chemical/Solvent	Molecular Weight, g/mol	Manufacturer	Country of Origin
Dimethyl Sulfoxide-d_6 C_2D_6OS	84.17	Fischer Scientific	UK
Deuterated Water D_2O	20.03	Sigma Aldrich	Germany

Table 3.6: Chemicals Used for Antioxidant Assay

Chemical/Solvent	Molecular Weight, g/mol	Manufacturer	Country of Origin
2,2-diphenyl-1-picrylhydrazyl (DPPH) $C_{18}H_{12}N_5O_6$	394.32	Sigma Aldrich	Germany
2,6-di-tert-butyl-4-methylphenol (BHT) $C_{15}H_{24}O$	220.35	Merck	Germany
Methanol CH_4O	32.04	Synerlab	Malaysia

3.2 Instruments Used

Table 3.7: Instruments Used for Characterization

Instrument	Manufacturer	Model
Nuclear Magnetic Resonance (NMR) Spectrometer	JEOL	NM-70010S4L1
Melting Point Apparatus	Stuart	SMP 10
Fourier-Transform Infrared Spectrometer	PerkinElmer	Spectrum EX 1
Double Beam UV-Visible Spectrophotometer	Thermo Scientific	Genesys 180

3.3 Methodology

3.3.1 Synthesis of 1,2,4-Triazole

Synthesis of 1,2,4-triazole bearing an indole ring is started by refluxed in an oil bath for 7 hours, the temperature of the oil bath was monitored and kept at 80 □. The completion of reaction was monitored using TLC technique. Upon completion of reaction, the reaction mixture was cooled down to room temperature, with aid of running tap water. The cooled reaction mixture was poured into a beaker containing crushed ice, the mixture was stirred and extracted thrice with 30 mL of ethyl acetate. The extraction was performed to remove acetic acid from the mixture. Acetic acid dissolved in water, it was being extracted to the aqueous layer, and the aqueous layer was discarded. The organic layer was combined into a clean conical flask, followed by addition of anhydrous sodium sulphate to remove traces of aqueous layer. The dried organic layer was collected in a 250-mL round bottom flask, and it was subjected to solvent evaporation using rotary evaporator. 20 mL of absolute ethanol, 2 mL of concentrated sulphuric acid, a few boiling chips, and a magnetic stirring bar were added into the flask. The reaction mixture was refluxed in an oil bath at 80 □ for 7 hours. The completion of reaction was indicated using TLC technique. The reaction mixture was cooled down to room temperature and mixed with crushed ice. The mixture was stirred and extracted with 30 mL of ethyl acetate thrice. Solvent extraction was performed to remove hydrochloric acid from the organic layer. Then, suitable amount of anhydrous sodium sulphate was added into the combined organic layer to

remove traces of aqueous layer. The dried organic layer was collected in a 250-mL round bottom flask, and it was subjected to solvent evaporation using rotary evaporator. 15 mL of ethanol, saturated ethanolic sodium hydroxide, and a magnetic stirring bar were added into the flask. The mixture was stirred at room temperature for 1.5 hours. The completion of reaction was verified using TLC technique. 20 mL of water was added into the reaction mixture. Then, 6 M sulphuric acid was added while the mixture was cooled in an ice bath, until the mixture is acidic, tested with pH test strips. The acidic mixture was then subjected to solvent extraction with 30 mL of ethyl acetate followed by extraction with 30-mL of water, to remove excess acid, with each extraction performed thrice. The organic layer was collected and dried with suitable amount of anhydrous sodium sulphate. The dried organic layer was collected in a 250-mL round bottom flask and subjected to solvent evaporation to obtain crude indole acetic acid. The crude indole acetic acid was then subjected to purification via recrystallization and cold washing using ethanol.

25 mmol of synthesized indole-3-acetic acid and equimolar of thiocarbohydrazide (2.65 g), along with some boiling chips and a magnetic stirring bar were added into a 250-mL round bottom flask. The solid mixture was heated in an oil bath to 140 □ for 7 hours, to undergo fusion reaction. The completion of reaction was monitored using TLC technique. Then, the reaction mixture was cooled down to room temperature followed by addition of saturated sodium bicarbonate solution. The mixture was stirred and subjected to vacuum filtration, to obtain crude 1,2,4-triazole. The crude

product was subjected to purification via recrystallization and cold washing with ethanol.

Figure 3.1: Synthetic Route of 1,2,4-Triazole and Its Schiff Bases Derivatives.

3.3.2 Synthesis of 1,2,4-Triazole Schiff Bases

2 mmol of synthesized 1,2,4-triazole with equimolar of benzaldehyde derivatives were added into 50-mL round bottom flask, followed by addition 15 mL of absolute ethanol as solvent, a few boiling chips and a magnetic stirring bar. The reaction mixture was refluxed in an oil bath at 80 □ for 8 hours. A series of benzaldehyde derivatives was used, and the amount used is reported in **Table 3.8**. The mass and volume of reagents needed were calculated using formula below:

Mass of Reagent Needed = Number of Mole (mol) × Molecular Weight (g/mol)

Volume of Reagent Needed = $\frac{Mass\ of\ Reagent\ Needed\ (g)}{Density\ of\ Reagent\ (g/mL)}$

The completion of reaction was monitored using TLC technique. Then, the reaction mixture was cooled to room temperature followed by mixing with crushed ice, to aid the precipitation of product. The mixture was stirred and filtered under vacuum condition, to remove water soluble tartaric acid. The crude 1,2,4-triazole Schiff bases were purified via recrystallization and cold washing with ethanol.

Table 3.8: A Series of Benzaldehyde Derivatives with Their Amounts Used for the Syntheses

Benzaldehydes	Molecular Weight, g/mol	Amount (2 mmol)
Benzaldehyde	106.12	0.204 mL
2,4-dichlorobenzaldehyde	175.01	0.35 g
3,4-dichlorobenzaldehyde	175.01	0.35 g
2-fluorobenzaldehyde	124.11	0.211 mL
4-fluorobenzaldehyde	124.11	0.215 mL
2-chlorobenzaldehyde	140.57	0.226 mL
4-chlorobenzaldehyde	140.57	0.28 g
2-bromobenzaldehyde	185.02	0.234 mL
4-bromobenzaldehyde	185.02	0.37 g
4-methylbenzaldehyde	120.15	0.236 mL
2-methoxybenzaldehyde	136.15	0.27 g
4-methoxybenzaldehyde	136.15	0.244 mL
2-nitrobenzaldehyde	151.12	0.30 g
4-nitrobenzaldehyde	151.12	0.30 g
2-hydroxybenzaldehyde	122.12	0.211 mL

3.3.3 Purification – Recrystallization

The crude products were purified via recrystallization from hot ethanol. Ethanol was heated on hot plate. Then, minimum amount of hot ethanol was added into the crude product for dissolution. The hot solution was hot filtered through cotton wool to remove insoluble impurities such as filter paper residue and dust from the solution. The glassware used such as glass funnel was pre-heated in oven prior to hot filtration, to minimize loss of product through

crystallization onto glassware at room temperature. Minimum amount of hot ethanol was added into a clean beaker for filtrate collection. The beaker was heated on hot plate, which the ethanol vapour will keep the glassware warm. The solution was heated throughout the filtration to avoid undesired precipitation on glassware.

After filtration, the filtrate was heated to saturate the solution to minimum solvent. The saturated solution was cooled to room temperature and set aside to allow crystallization. If the crystallization does not spontaneously, glass rod was used to scratch the inner wall of beaker to induce crystallization by producing the seed of crystallization. However, if crystallization was not observed after a few days, concentration of the solution will be performed, and eventually recrystallization procedure will be repeated to induce crystallization.

TLC technique was done before and after recrystallization, to compare the purity of products briefly. A single spot was expected to be observed from the TLC plate, which will be assumed to be pure. However, further characterization is needed fundamentally, to determine the structure and purity of product, as TLC technique may not be sensitive enough to detect traces of impurities.

The percentage yield of the compounds can be determined using formula:

$$\text{Percentage yield} = \frac{\text{Actual Mass of Product}}{\text{Theoretical Mass of Product}} \times 100\%$$

3.4 Characterization

3.4.1 Thin-Layer Chromatography (TLC)

TLC technique is a two-dimensional chromatography technique for separation of non-volatile substances. In this project, this technique is mainly used to monitor reaction progress and to verify completion of reaction by looking at the number of spot and comparing the retardation factors, R_f between starting material and product. By using an appropriate solvent system, the number of spots can represent the number of compounds present in the product, therefore a single spot is expected to be observed upon completion of reaction.

Aluminium-backed TLC plate coated with silica gel was used. TLC plate with dimension of 5 cm × 10 cm was cut out and a horizontal line was drawn using pencil at 1 cm from top and bottom of the plate indicating the solvent front and base line. The outline of the TLC plate is as shown in **Figure 3.2**. A minimum amount of reaction mixture was removed from the refluxing system and diluted with chloroform to reduce the polarity of ethanol solvent from the system. Then, the mixture was spotted on the base line of TLC plate using a capillary tube. The respective starting materials were dissolved in ethanol, diluted with chloroform, and spotted onto the same TLC plate to serve as a reference. The spotted TLC plate was examined under short-waved ultraviolet (UV) lamp with wavelength of 254 nm, to ensure the spots are adequate to be observed under UV before development.

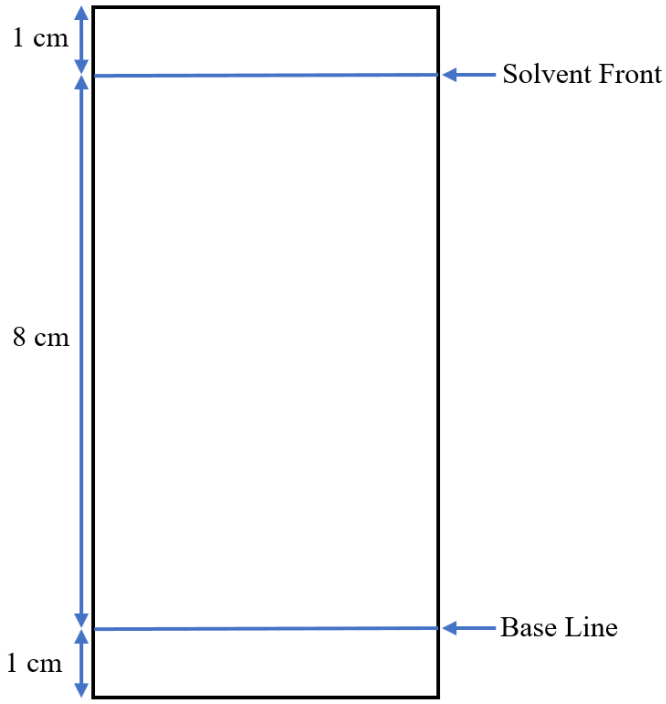


Figure 3.2: Outline of TLC Plate.

The solvent system used for development was a mixture of ethyl acetate and hexane with composition of 1:1. 6 mL of each solvent was measured and added into a covered glass container to serve as the development chamber. The volume of solvent added was chosen based on the height of solvent level in the chamber must not be higher than the base line on TLC plate. A piece of filter paper was immersed into the solvent system to saturate the atmosphere in the chamber. The spotted TLC plate was then carefully placed into the chamber and allowed to stand without disturbance. The TLC plate was removed from the chamber once the solvent front hit the 1-cm line marked from the top of TLC plate. The plate was observed under short-waved UV lamp. The spots observed was marked with pencil.

The observation for one single spot indicate a pure compound, while observation of more than one spot may indicate incomplete reaction as the extra spot is due to starting material, or presence of impurities. The distance travelled by the sample from base line can be determined using a ruler. For the spots that have same travel distance from base line, it is said to have similar polarity and assumed to be the same compound.

As the solvent system is relatively non-polar compared to the silica gel on TLC plate, it serve as a brief polarity study on the compounds. The further the sample spots travelled, the non-polar it is, compared to those travelled in short distance. This is mainly used to study the polarity of 1,2,4-triazole and its Schiff base derivatives. The distance travelled by the sample and solvent were measured and recorded for the calculation of retardation factor, R_f, represented by the formula below:

$$\text{Retardation Factor, } R_f = \frac{\text{Distance Travelled by Sample from Base Line}}{\text{Distance Travelled by Solvent from Base Line (Solvent Front)}}$$

3.4.2 Melting Point Determination

Stuart SMP 10 melting point apparatus was used to determine the melting point of a substance. This characterization technique provide a brief idea on the purity of compound. A pure compound should give a narrow range of melting point, while compound with impurities will have a relatively wider range of melting point. Furthermore, the melting point of an impure compound will appear to be in a lower range compared to pure compound. This is because impurities cause defects in the crystalline lattice structure of the pure compound, which lead to weaker intermolecular interactions, making it easier to be overcame.

Figure 3.3: Stuart SMP 10 Melting Point Apparatus.

1,2,4-Triazole and its Schiff base derivatives appear in crystal form at room temperature, which is readily to be used for melting point determination. An

open capillary tube was filled with the compound of interest, and was tapped a few times on the closed end to ensure the crystal was at the closed end. The capillary tube was inserted into the melting point apparatus with plateau temperature to be set around 200 □. The melting process was observed through a magnifying lens. The temperature at which the compound started to melt to the temperature at which the sample is fully melted were recorded as the melting point range of the compound. Then, the heating was stopped to allow cooling, as to prepare for next measurement.

3.4.3 Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectroscopy is a non-destructive spectroscopic technique that is widely used to study molecular structure, composition, and interactions of molecules at the atomic level. NMR spectroscopy relies on the magnetic properties of certain atomic nuclei, the most common ones would be the nuclei of hydrogen (^{1}H or proton) and carbon-13 (^{13}C), to provide valuable information about chemical compounds.

JEOL NM-70010S4L1 spectrometer was used to study the structures of the synthesized 1,2,4-triazole and its Schiff base derivatives. The irradiation frequency of proton NMR is around 400 MHz while the irradiation frequency of ^{13}C NMR is around 100 MHz. Besides that, Distortionless Enhancement by Polarization Transfer (DEPT), Heteronuclear Multiple Quantum Coherence (HMQC) and Heteronuclear Multiple Bond Coherence (HMBC) experiments

were also studied. The spectra were analyzed to elucidate the structure of the compounds.

For sample preparation, 20 mg of each sample was dissolved in DMSO-d_6 in separate sample vials. The sample solution was sonicated to aid dissolution. The sample solution was then transferred to an NMR tube and the tube was filled up to around 4 cm height using a clean dropper. The NMR tube was then labelled and ready for NMR analysis. An additional analysis involved addition of deuterated water (D_2O) into the sample solution. About 10 % volume of D_2O was added into the NMR tube containing sample solution and was shaken well before sending for proton NMR analysis.

3.4.4 Fourier-Transform Infrared (FT-IR) Spectroscopy

FT-IR spectroscopy is a common spectroscopic technique used to study the vibrational modes of molecules. It gives the general information on which functional groups are present in the chemical compounds.

In this project, PerkinElmer Spectrum EX 1 spectrometer was used for FT-IR analysis. The analysis covered the frequency range from 4000 cm^{-1} to 400 cm^{-1}.The sample was prepared in KBr pellet form, which small amount of sample solid was grinded with suitable amount of potassium bromide, and the solid mixture was pressed into a small pellet.

IR spectra were obtained from the analysis, identification of functional group can be achieved based on the significant bands at particular frequency range.

Figure 3.4: PerkinElmer Spectrum EX 1 Spectrometer.

3.5 Antioxidant Assay

2,2-diphenylpicrylhydrazyl (DPPH) assay was used to study the antioxidant activities of 1,2,4-triazole and its Schiff base derivatives. Butylated hydroxytoluene (BHT) was used as a standard antioxidant for comparison of antioxidant activity.

Genesys 180 Double Beam UV-Visible spectrophotometer was used to study the loss of DPPH absorbance at 517 nm. The maximal absorption of the purple DPPH radical take place at 517 nm. A series of sample solution with known concentration was prepared to study the loss of DPPH.

3.94 mg of DPPH was dissolved in 100 mL of methanol using 100-mL volumetric flask to prepare 0.1 mM of DPPH methanol solution. The DPPH solution was kept in dark and cooled condition, as it is light- and heat-sensitive. Then, 5 mg of each sample was dissolved in 10 mL of methanol using 10-mL volumetric flask, to prepare the stock 500 ppm solution. Serial dilution using methanol was performed to prepare a series of concentration: 200 ppm, 100 ppm, 50 ppm, 25 ppm, 12.5 ppm and 6.25 ppm. 1 mL of sample of each concentration was transferred using micropipette to a sample vial with cover. A blank was also prepared using 1 mL of methanol instead of sample solution. Then, 4 mL of the prepared 0.1 mM of DPPH methanol solution was added into each sample vial and shaken vigorously for mixing purpose. The sample vials were covered in aluminium foil and incubated in dark condition for 30 minutes. The absorbance of each solution was measured at 517 nm, in descending order of concentration of sample solution.

Inhibitory concentration for 50 % reduction (IC_{50}) of DPPH radical can be determined from the graph of percentage radical scavenging against concentration of sample solution. While the percentage radical scavenging can be calculated using the formula below:

$$\text{Percentage radical scavenging} = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100\%$$

; A_{blank} and A_{sample} represent the absorbance of blank and sample, respectively.

3.6 Calculations

i. Mass of Starting Materials

Used to calculate mass of solid starting material required for the synthesis.

Mass = Number of Mole (mol) × Molecular Weight (g/mol)

ii. Volume of Starting Materials

Used to calculate the volume of liquid starting material required for the synthesis.

$$\text{Volume} = \frac{Mass\ of\ Reagent\ Needed\ (g)}{Density\ of\ Reagent\ (g/mL)}$$

iii. Percentage Yield of Product

Used to calculate the percent yield of synthesized product.

$$\text{Percentage yield} = \frac{\text{Actual Mass of Product}}{\text{Theoretical Mass of Product}} \times 100\%$$

iv. Retardation Factor

Used to calculate the ratio of the distance travelled by spot to the distance travelled by solvent.

$$\text{Retardation Factor} = \frac{\text{Distance Travelled by Sample from Base Line}}{\text{Distance Travelled by Solvent from Base Line (Solvent Front)}}$$

v. Percentage Radical Scavenging

Used to determine the percent scavenging of DPPH radical by sample.

$$\text{Percentage radical scavenging} = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100\%$$

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Synthesis of 1,2,4-Triazole

Synthesis of 1,2,4-triazole was carried out using thiocarbohydrazide and indole-3-acetic acid. Equimolar of thiocarbohydrazide and indole-3-acetic acid were heated to 140 □ for 7 hours. The reaction temperature is set based on the boiling point of both reagents, as to allow fusion reaction to take place. Then, sodium bicarbonate solution was introduced to neutralize and remove unreacted indole-3-acetic acid from the crude 1,2,4-triazole. The crude product was filtered, recrystallized, and washed with cold ethanol. The purified product was then subjected to characterization for structure elucidation.

4.1.1 Proposed Mechanism for Synthesis of 1,2,4-Triazole

Formation of 1,2,4-triazole starts with nucleophilic attack of thiocarbohydrazide on the indole-3-acetic acid, followed by proton transfer. Then, dehydration takes place which condition the intermediate to be able to undergo intramolecular condensation between amine and carbonyl group. Proton transfer and dehydration then take place to form the 1,2,4-triazole. (**Figure 4.1**)

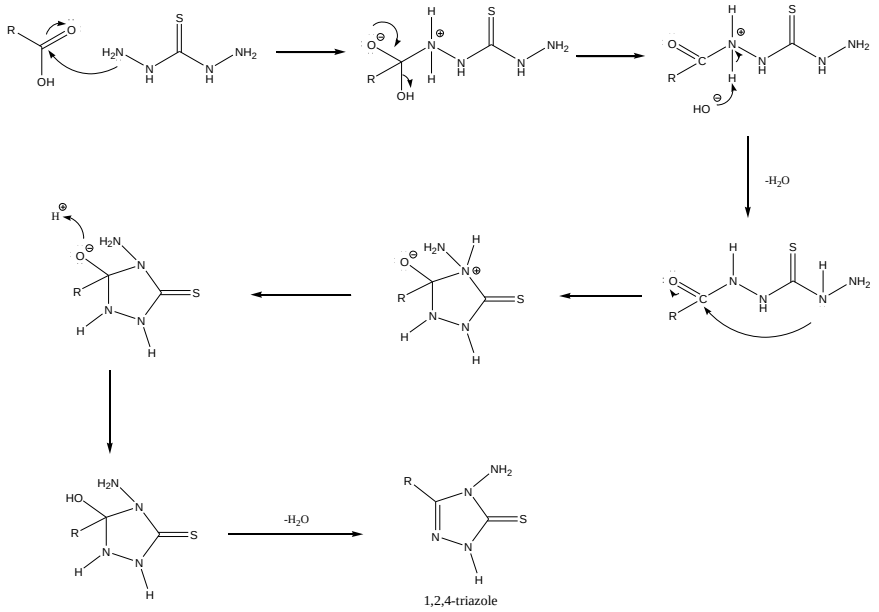


Figure 4.1: Mechanistic Scheme of Formation of 1,2,4-Triazole.

4.1.2 Characterization of 1,2,4-Triazole

The synthesized 1,2,4-triazole is one of the precursors for Schiff base synthesis. The 1,2,4-triazole was characterized by melting point determination, TLC, IR, and NMR analysis, of which the NMR analysis include ^{1}H NMR, ^{13}C, NMR, DEPT, HMQC, and HMBC experiments. The structure of the synthesized 1,2,4-triazole is shown in Error! Reference source not found. and the physical properties are summarized in **Table 4.1**.

Table 4.1: Summary of Physical Properties of 1,2,4-Triazole.

Physical Appearance	Cream White Solid
Melting Point Range (□)	211 – 214
Percentage Yield (%)	45
R_f Value (Hexane:EA=1:1)	0.44

The IR spectrum of 1,2,4-triazole (Error! Reference source not found.) shows absorption band at 3500 –3300 cm^{-1}, due to N–H stretch. The absorption bands at 1630 and 1294 cm^{-1} were assigned to C=C and C=S stretching, respectively. While other absorption bands were observed at 3245 and 3160 cm^{-1} for C=C-H, 2947 and 2928 cm^{-1} for C-H stretching, and 1464 cm^{-1} due to C-H bending.

From ^{1}H NMR spectrum (

Figure 4.2), 1-NH and 1'-NH signals were observed at the downfield region, which are located at δ 13.40 and δ 10.81, respectively. This is due to the protons are deshielded by the electronegative nitrogen atom and aromatic rings, predominantly deshielding effect or anisotropic effect is contributed by aromatic rings. While the signal for 6-NH was observed at δ 5.50, it is not as affected by the deshielding effect as it is not attached to the aromatic ring directly. The peak assignment for amine protons is further confirmed by addition of deuterated water. Amine protons (-NH) can undergo acidic proton transfer with D_2O, which the hydrogen atoms on the amine group will be replaced by deuterium atoms. The newly formed N-D group will not be detected in ^{1}H NMR (**Figure 4.3**). When the ^{1}H NMR spectra before and after addition of deuterated water are compared, the peaks absent in the spectrum after addition are identified as amine protons.

The doublet signals were assigned to aromatic H-4' and H-7', respectively. While the triplet signals belong to aromatic H-5' and H-6', respectively. For singlet signals observed at δ 3.99 were assigned to H-8', they appear at the upfield region because they are of aliphatic protons, not affected by the deshielding effect by aromatic ring. To eliminate the ambiguity for the assignment of aromatic protons of indole ring, as there are 2 doublet and 2 triplet signals observed in the ^{1}H NMR spectrum, one-dimensional (1D) Nuclear Overhauser Effect (NOE) experiment can be used. NOE experiment estimates proton-to-proton distances though space interaction. A peak of interest can be selected manually to be subjected to irradiation or excitation. Transfer of energy from the irradiated proton can take place to the protons that

are close in space, which is normally less than 5 Å. In the spectrum (Error! Reference source not found.), 1'-NH peak at δ 10.81 was irradiated, as it is appearing as negative peak, and enhancement of peaks were observed. Another NOE experiment was carried out to irradiate signal at δ 7.20, of which it also enhance the 1'-NH peak. (**Figure 4.4**).

In the ^{13}C NMR spectrum of 1,2,4-triazole (**Figure 4.5**), there are two signals observed at the downfield region, δ 166.0 and δ 152.3, were assigned to C-5 and C-3 respectively because the carbon atoms are bonded with electronegative nuclei such as sulphur and nitrogen atoms. These nuclei tend to withdraw electrons towards themselves and thereby deshielding the neighbouring atoms. Signals observed in the range of δ 140.0 to δ 100.0 belong to the indole carbons. They are in relatively downfield region due to the aromaticity of indole ring. Whereas the signals at δ 20.4 are assigned to C-8'. As they are aliphatic carbon, which experience less deshielding effect, therefore appear in the upfield region.

Distortionless Enhancement by Polarization Transfer (DEPT) analysis is used to distinguish different types of carbons like methyl ($-CH_3$), methylene ($-CH_2$), and methine groups. (-CH) Only DEPT-135 analysis was performed, which gives negative peaks for methylene group, and positive peaks for methyl and methine groups. When comparing DEPT spectrum (**Appendix A**) with ^{13}C NMR spectrum, the disappearance of peaks in DEPT spectrum indicates those peaks are of quaternary carbons. A negative peak at δ 20.4 was observed, which indicates the signal belongs to methylene carbon, which can be directly

assigned to C-8' as it is the only methylene carbon present in the structure of 1,2,4-triazole.

While for two-dimensional (2D) NMR analysis, Heteronuclear Multiple Quantum Correlation (HMQC) data were used to reveal short-range coupling between carbon and hydrogen atoms across single bond correlation. The assignment of peaks is supported by referring to the direct bonding of C-H shown in HMQC spectrum. (**Appendix B**)

Heteronuclear Multiple Bond Correlation (HMBC) analysis is another 2D-NMR technique which indicate the long-range coupling of carbon and hydrogen atoms through two- and three-bond correlations. In HMBC spectrum, (**Appendix C**) 6-NH at δ 5.50 showed three-bond coupling (^3J) with C-3. Besides, ^3J coupling was observed for 1'-NH with C-3' and C-3a'. The summary of NMR spectral data of 1,2,4-triazole is tabulated in **Table 4.2.**

Table 4.2: Summary of NMR Spectral Data of 1,2,4-Triazole.

Position	^{1}H δ (ppm) {Multiplicity}	^{13}C δ (ppm)	HMBC	
			^{2}J	^{3}J
1	13.39 {1H, *s*}	-	-	-
3	-	152.3	-	-
5	-	166.0	-	-
6	5.50 {2H, *s*}	-	-	C-3
1’	10.8 {1H, *s*}	-	-	C-3’
2’	-	133.8	-	-
3’	-	103.6	-	-
4’	7.39 {1H, *d*}	118.2	C-5’	
5’	6.93 {1H, *t*}	120.7	C-4’	-
6’	6.86 {1H, *t*}	118.9	C-7’	-
7’	7.20 {1H, *d*}	110.9	C-6’	
8’	3.99 {2H, *s*}	20.4	C-3, C-3’	

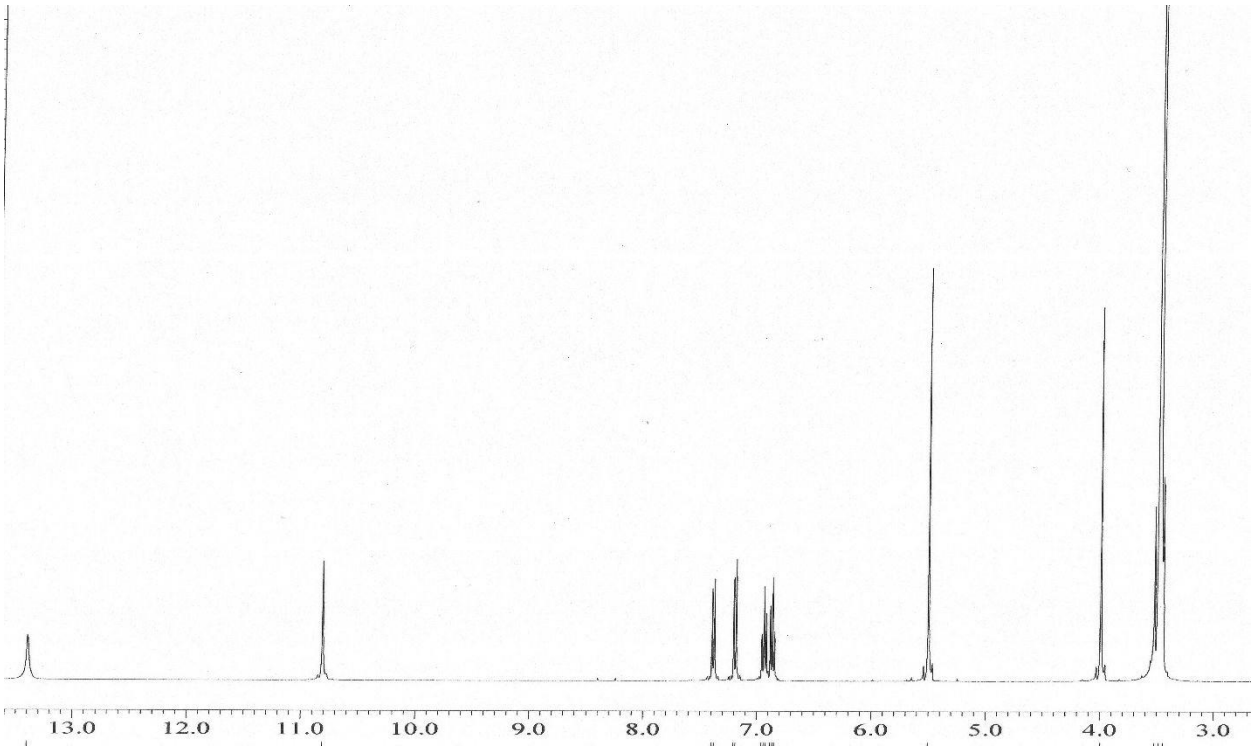


Figure 4.2: ^{1}H NMR spectrum of 1,2,4-Triazole. (400 MHz, Solvent: DMSO-d_6)

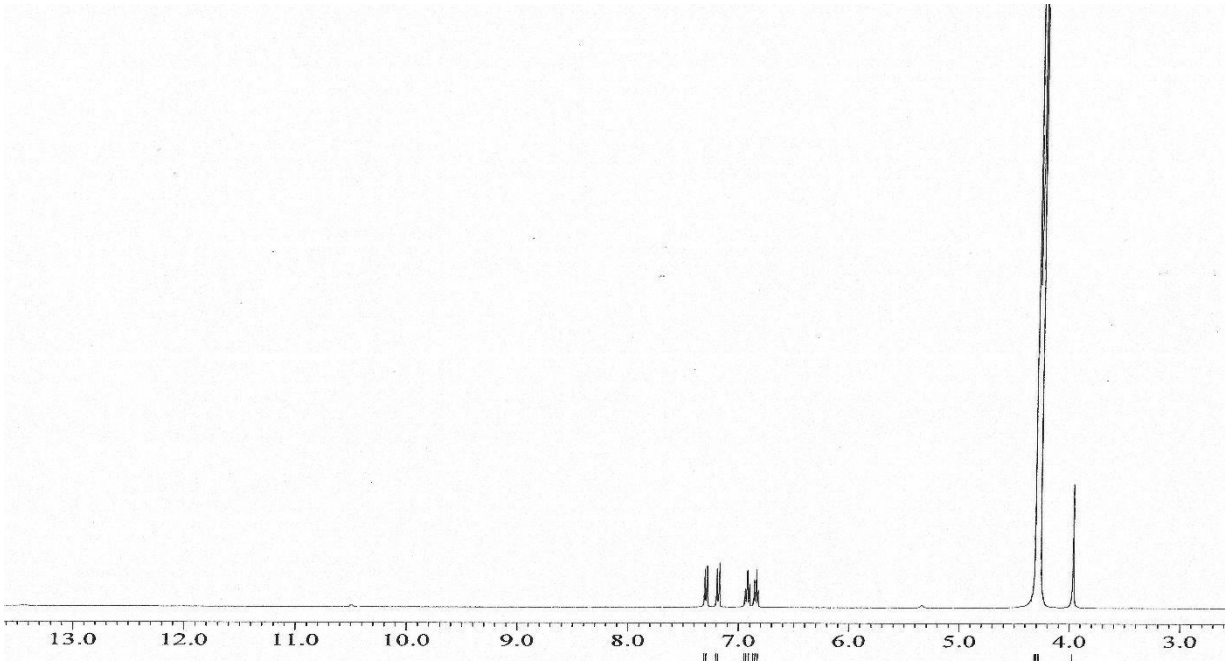


Figure 4.3: ^{1}H NMR Spectrum of 1,2,4-Triazole After Addition of D_2O. (400 MHz, Solvent: DMSO-d_6)

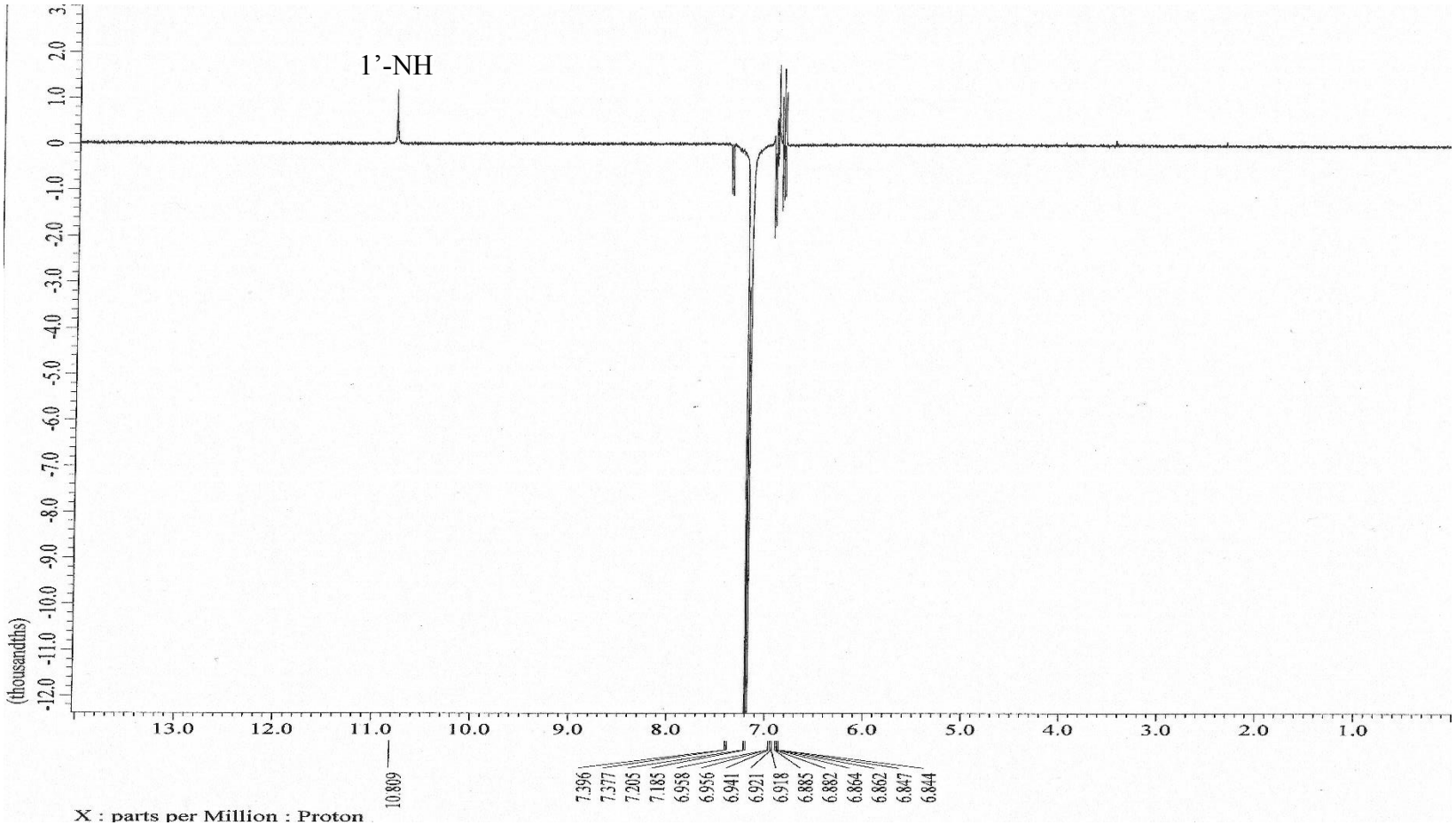


Figure 4.4: NOE Spectrum of 1,2,4-Triazole. (Irradiation at δ 7.20)

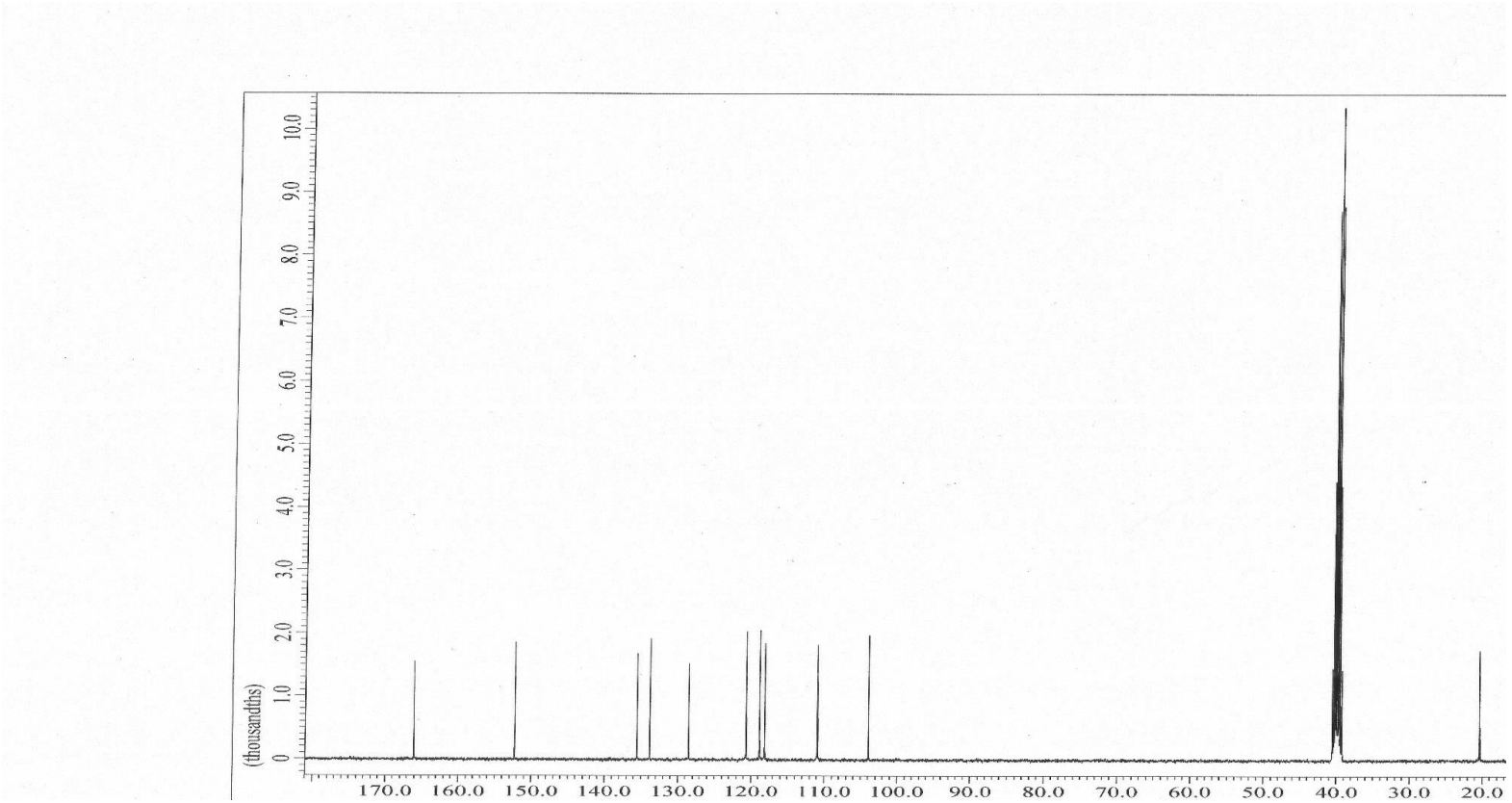


Figure 4.5: ^{13}C Spectrum of 1,2,4-Triazole. (100 MHz, Solvent: DMSO-d_6)

4.2 Synthesis of 1,2,4-Triazole Schiff Base Derivatives

Fifteen new Schiff bases derived from 1,2,4-triazole were synthesized through acid-catalyzed condensation reaction between 1,2,4-triazole and a series of benzaldehyde derivatives. 1,2,4-triazole and benzaldehyde derivatives were refluxed at 80 □ for 8 hours and absolute ethanol as solvent. Upon completion of reaction, the reaction mixture was mixed with ice, stirred and subjected to vacuum filtration. The crude Schiff bases were recrystallized and washed with cold ethanol. The structure of the synthesized Schiff bases (**SBs 1-15**) is depicted in **Figure 4.6**.

SB	R_1	R_2	R_3
1	H	H	H
2	Cl	H	Cl
3	H	Cl	Cl
4	F	H	H
5	H	H	F
6	Cl	H	H
7	H	H	Cl
8	Br	H	H
9	H	H	Br
10	H	H	CH_3
11	OCH_3	H	H
12	H	H	OCH_3
13	NO_2	H	H
14	H	H	NO_2
15	OH	H	H

Figure 4.6: Structures of the 1,2,4-Triazole Schiff bases. (SBs 1-15)

4.2.1 Proposed Mechanism for Synthesis of 1,2,4-Triazole Schiff Base Derivatives

The condensation reaction between 1,2,4-triazole and benzaldehyde derivative begins with nucleophilic addition of the amino group of 1,2,4-triazole onto the carbonyl group of aromatic aldehydes, to afford a tetrahedral intermediate. The intermediate undergo proton transfer to give carbinolamine, followed by protonation of the carbinolamine which is facilitated by the acid catalyst. The protonation condition the hydroxyl group into a better leaving group, $-OH_2^+$, which can be eliminated as water molecule. Then, deprotonation take place to produce Schiff bases derived from 1,2,4-triazole and re-form the acid catalyst.

4.2.2 Characterization of 1,2,4-Triazole Schiff Bases

Condensation reaction between 1,2,4-triazole and a series of benzaldehyde derivatives was carried out to yield **SB 1** to **SB 15**. The summary of physical properties of the synthesized 1,2,4-triazole Schiff bases is tabulated in **Table 4.3** and **Table 4.4**, reporting the physical appearance, melting point range, percentage yield, and R_f value of the compounds, in which the R_f value was obtained using solvent composition of hexane to ethyl acetate in ratio of 1:1.

Table 4.3: Summary of Physical Properties of SBs 1 – 8.

SB	1 (H)	2 (2,4-Cl_2)	3 (3,4-Cl_2)	4 (2-F)	5 (4-F)	6 (2-Cl)	7 (4-Cl)	8 (2-Br)
Physical Appearance	Cream white solid	Pale brown solid	Pale brown solid	Pale brown solid	Pale brown solid	Pale brown solid	Pale brown solid	Pale brown solid
Percentage Yield (%)	45	27	55	33	58	71	69	66
R_f Value (Hexane:EA=1:1)	0.63	0.62	0.61	0.63	0.59	0.63	0.63	0.58

Table 4.4: Summary of Physical Properties of SBs 9 – 15.

SB	9 (4-Br)	10 (4-CH_3)	11 (2-OCH_3)	12 (4-OCH_3)	13 (2-NO_2)	14 (4-NO_2)	15 (2-OH)
Physical Appearance	Pale brown solid	Brown solid	Dark brown solid	Brown solid	Pale orange solid	Orange solid	Pale brown solid
Percentage Yield (%)	91	43	28	40	76	88	43
R_f Value (Hexane:EA=1:1)	0.60	0.61	0.50	0.54	0.54	0.63	0.53

4.2.3 FT-IR Characterization of 1,2,4-Triazole Schiff Bases

Major absorption bands for the synthesized Schiff bases were observed in their expected range. While most of the IR vibrational bands observed in the spectrum of 1,2,4-triazole such as N-H, C=C-H, C-H, C=S stretching, and C-H and N-H bending, are still present in the IR spectra of its Schiff bases, the emergence of new C=N band at 1599-1580 cm^{-1} indicates the formation of Schiff base. However, there are some compounds do not show vibrational band around this region, which include **SB 6**, **SB 10**, **SB 13**, and **SB 14**, this is because the C=N band is obscured by other absorption band, which are C-H bending and N-O stretching. It is also noticeable that **SB 11** do not display C=C stretching, which is obscured by the strong C=N band. Furthermore, **SB 15** with hydroxyl substituent does not show O-H band as it is overlapped with N-H stretching that is due to the amine proton on indole moiety. The IR spectra are provided in **Appendices D – R**, whereas the summary of characteristic bands from IR spectra of **SBs 1 – 15** are tabulated in **Table 4.5** and

Table 4.6.

Table 4.5: Summary of Assignment of Characteristic FT-IR Bands for SBs 1 – 8.

SB / υ (cm^{-1})	1 (H)	2 (2,4-Cl_2)	3 (3,4-Cl_2)	4 (2-F)	5 (4-F)	6 (2-Cl)	7 (4-Cl)	8 (2-Br)
N-H_{str}	3400-3301	3410-3353	3324	3416	3398-3326	3316	3462-3247	3321
C=C-H	3100-3050	3086-3051	3096-3054	3107-3055	3102-3056	3053-3032	3159-3055	3053
C-H_{str}	2930-2759	2934-2763	2943-2742	2945-2757	2944-2778	2909-2885	2926	2913-2854
C=C	1604	1638-1618	1623-1607	1611	1636-1613	1636-1603	1684	1619
C=N	1572	1583	1580	1585	1602	1589	1593	1586
C-H_{bend}	1461	1461	1463	1462	1463	1458	1463	1459
C=S	1281	1273	1287	1283	1279	1274	1282	1273
N-H_{bend}	741	740	741	762-737	738	755	740	755

Table 4.6: Summary of Assignment of Characteristic FT-IR Bands for SBs 9 - 15.

SB / υ (cm^{-1})	9 (4-Br)	10 (4-CH_3)	11 (2-OCH_3)	12 (4-OCH_3)	13 (2-NO_2)	14 (4-NO_2)	15 (2-OH)
N-H_{str}	3436-3317	3379-3318	3423	3401	3429	3396-3326	3396-3342
C=C-H	3098-3055	3048	3105-3050	3145-3045	3098-3052	3104-3056	3101-3058
C-H_{str}	2946-2781	2921-2854	2941-2753	2935-2749	2937-2756	2945-2779	2944-2781
C=C	1636-1611	1638-1623	-	1646-1636	1636-1605	1617	1621
C=N	1582	1602	1592	1601	1565	1577	1603
C-H_{bend}	1464	1456	1461	1460	1462	1463	1463
C=S	1279	1274	1253	1259	1283	1278	1288
N-H_{bend}	737	742	741	745	738	739	741
C-N	-	-	-	-	1342	1345	-

4.2.4 NMR Characterization of 1,2,4-Triazole Schiff Bases

The synthesized Schiff bases derived from 1,2,4-triazole were characterized using 1D-NMR experiments such as ^{1}H NMR and ^{13}C NMR experiments as well as 2D-NMR experiments like HMQC and HMBC experiments.

Most of the peaks were assigned similarly as compared to 1,2,4-triazole, except for additional peaks from aromatic ring derived from benzaldehyde derivatives. Besides, by comparing ^{1}H NMR spectra of the Schiff bases with 1,2,4-triazole, the 6-NH that appear at δ 5.50 in 1,2,4-triazole was not observed in the spectra of Schiff bases, which is an indication of formation of Schiff base through reaction between 6-NH amino group and carbonyl group of benzaldehyde derivatives. Besides, the appearance of imine (H-7" and C-7") peaks in Schiff bases spectra also indicate the formation of Schiff base.

The NMR characterization of **SB 1** is discussed in detail in the following section, while other Schiff base compounds will be grouped into Schiff bases with 2"-, 4"-, and di-substituted at the aromatic ring derived from benzaldehyde derivatives.

4.2.4.1 Discussion and Structure Elucidation of SB 1

Similar as 1,2,4-triazole, signals of 1-NH and 1'-NH appear at downfield region due to the deshielding of aromatic triazole and indole moieties. In the ^{1}H spectrum (**Figure 4.7**) new peak at δ 9.64 is assigned to H-7", it is in the downfield region due to the deshielding of Schiff base or imine functional group. The signal peaks for aromatic protons (H-4', H-5',H-6', and H-7') of indole moiety do not deviate much as compared to 1,2,4-triazole, which only deviate by 0.01 – 0.03 ppm. In the aromatic proton region, there are two new peaks at δ 7.85 and δ 7.51. H-2"and H-6" are of equivalent protons which give signal of 2H, and it appeared as a doublet signal as it coupled with the neighbouring H-3"or H-5". Similarly, H-3" and H-5" are equivalent protons, producing 2H integration, and show as triplet signal as it coupled with the neighbouring H-2" or H-6", and H-4".

The ^{13}C NMR spectrum (**Figure 4.8**) was interpreted with the supporting data from DEPT, HMQC, and HMBC experiments. The carbon peaks were assigned similar to 1,2,4-triazole. The new peaks were assigned to C-7", C-3"&5", and C-2"&6", respectively. HMBC spectrum supported the assignment of Schiff base carbon (C-7") with H-7" correlated ^{2}J coupled with C-1", and it was checked with HMQC spectrum to verify the assignment of C-7". The peak assignment for C-3"&5" and C-2"&6" were also based on HMBC spectrum, as it reveal H-2"&6" ^{3}J coupled with C-4" and C-7". The NMR spectral data for **SB 1** are summarized in **Table 4.7**.

Table 4.7: Summary of NMR Spectral Data of SB 1.

Position	^{1}H δ (ppm) {Multiplicity}	^{13}C δ (ppm)	HMBC	
			^{2}J	^{3}J
1	13.39 {1H, *s*}	-	-	-
3	-	152.3	-	-
5	-	166.0	-	-
6	5.50 {2H, *s*}	-	-	C-3
1’	10.8 {1H, *s*}	-	-	C-3’
2’	-	133.8	-	-
3’	-	103.6	-	-
4’	7.39 {1H, *d*}	118.2	C-5’	
5’	6.93 {1H, *t*}	120.7	C-4’	-
6’	6.86 {1H, *t*}	118.9	C-7’	-
7’	7.20 {1H, *d*}	110.9	C-6’	
8’	3.99 {2H, *s*}	20.4	C-3, C-3’	

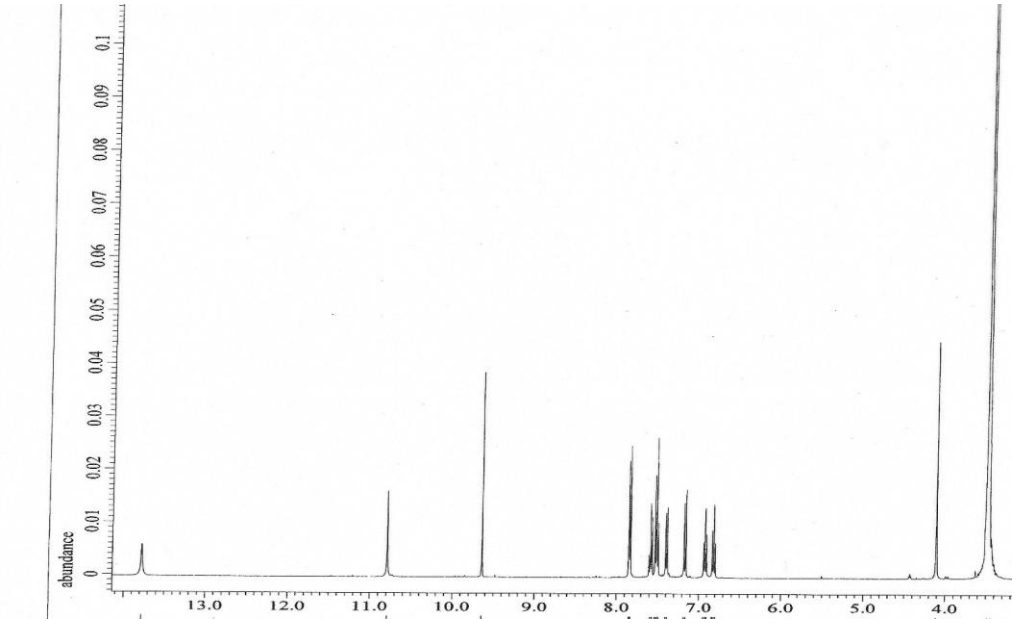


Figure 4.7: ^{1}H NMR Spectrum of SB 1. (400 MHz, Solvent: DMSO-d_6)

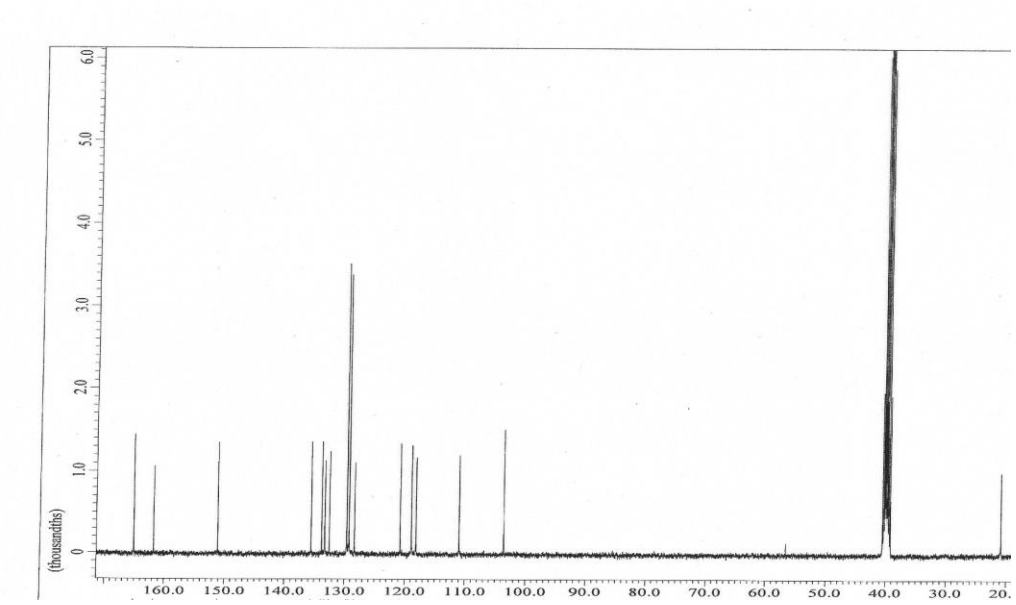


Figure 4.8: ^{13}C Spectrum of SB 1. (100 MHz, Solvent: DMSO-d_6)

4.2.4.2 Discussion and Structure Elucidation of *para*-Substituted Schiff Bases (SB 5, SB 7, SB 9, SB 10, SB 12, and SB 14

SB	R
5	F
7	Cl
9	Br
10	CH_3
12	OCH_3
14	NO_2

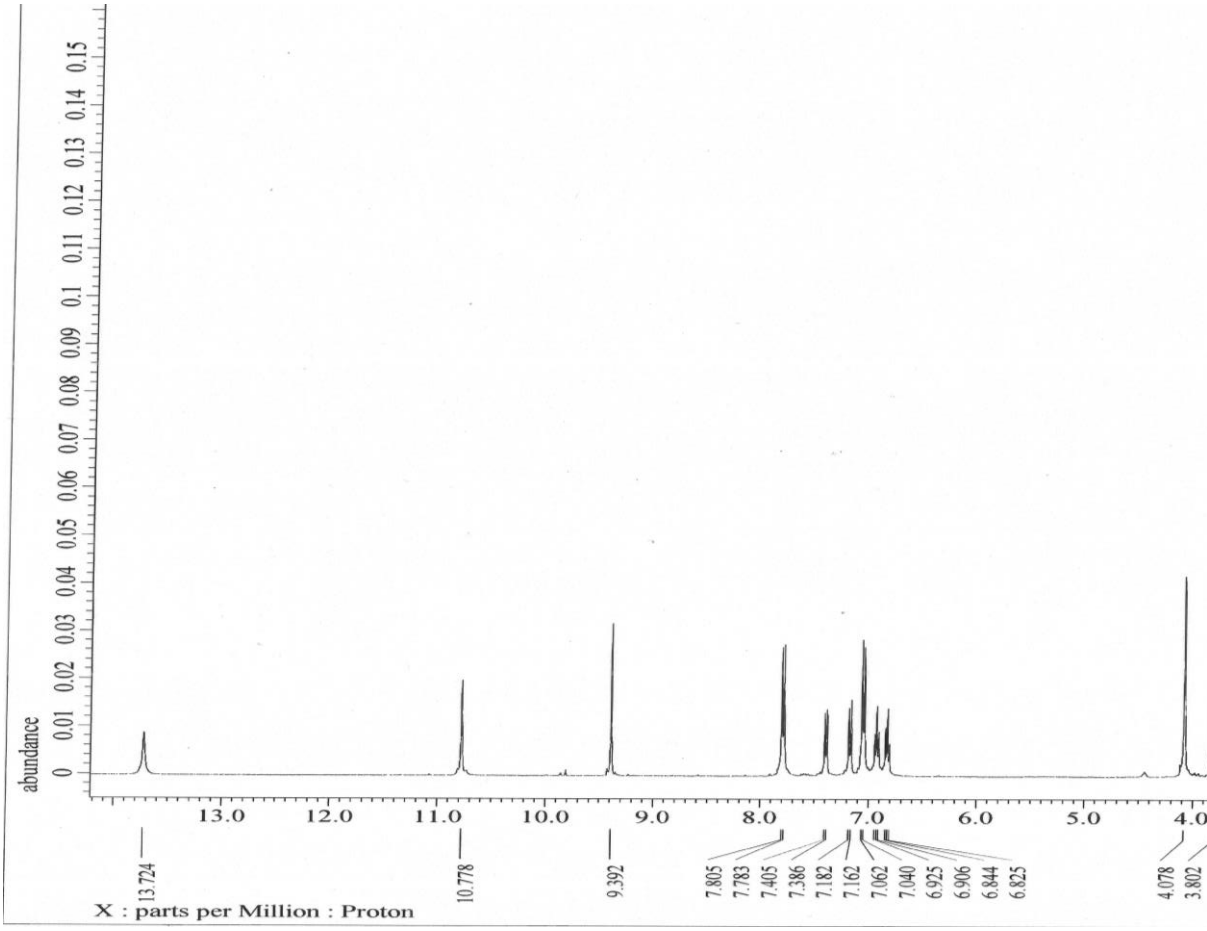
Figure 4.9: Structures of SB 5, SB 7, SB 9, SB 10, SB 12, and SB 14.

The signal peaks were assigned relative to **SB 1**, with distinctive disappearance of signals for H-4", as the carbon at position 4" is substituted as illustrated in **Figure 4.9**. Generally, the trend for aromatic carbons to be deshielded by electron withdrawing groups and shielded by electron donating group is also observed for characterization of *para*-Substituted Schiff bases. As it is a substitution at *para*-position, the aromatic benzene ring retains its symmetry, which the carbon or proton at positions 2" and 6" as well as 3" and 5" are of equivalency, similar to **SB 1**.

Among the Schiff bases, **SB 5** which fluorine is substituted at C-4" position shows coupling with neighbouring carbon and proton, where H-2"&6" and H-3"&5" experience two- and three-bonds coupling with fluorine. Besides, the signal of H-4' overlapped with H- H-3"&5" and form multiplets.

The peak assignment is supported by DEPT, HMQC, and HMBC experiments, in which the spectra are given in the **Appendix** section. The summary of

spectral data for **SB 5**, **SB 7**, **SB 9**, **SB 10**, **SB 12**, and **SB 14** are provided in Error! Reference source not found. – Error! Reference source not found.. The ^{1}H and ^{13}C NMR spectra are displayed in Error! Reference source not found. – Error! Reference source not found.**.**


abundance
0 0.01 0.02 0.03 0.04 0.05 0.06 0.07 0.08 0.09 0.1 0.11 0.12 0.13 0.14 0.15
13.0 12.0 11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0
13.724
10.778
9.392
7.805
7.783
7.405
7.386
7.182
7.162
7.062
7.040
6.925
6.906
6.844
6.825
4.078
3.802
X : parts per Million : Proton

4.2.4.2 Discussion and Structure Elucidation of di-Substituted Schiff Bases (SB 2 and SB 3)

SB	R_1	R_2	R_3
2	Cl	H	Cl
3	H	Cl	Cl

Figure 4.10: Structures of SB 2 and SB 3.

As the Schiff bases are disubstituted at the aromatic side chain, with **SB 2** has chlorine atoms substituted at C-2" and C-4" position and **SB 3** has chlorine substituent at C-3" and C-4" position, as illustrated in **Figure 4.10**, the correspondent substituted aromatic carbon will not have proton signal. Besides, the aromatic ring is not symmetrical anymore with substituent at such positions. Similar to **SB 8**, the proton signal of 1-NH for **SB 2** was not observed at the expected chemical shift, which is because of the active amine proton exchanges, which the signal has been treated as noise and corrected.

The peak assignment is supported by DEPT, HMQC, and HMBC data, in which the spectra are given in the **Appendix** section. The summary of spectral data for **SB 2** and **SB 3** are provided in Error! Reference source not found. and Error! Reference source not found.. The ^{1}H and ^{13}C NMR spectra are displayed in Error! Reference source not found. – Error! Reference source not found..

4.3 Antioxidant Activity of 1,2,4-Triazole and its Schiff Bases

1,2,4-Triazole and a series of its Schiff base derivatives have been synthesized and characterized for their potential as antioxidant. The antioxidant activities of the compounds were determined by deploying DPPH assay. Butylated hydroxytoluene (BHT) was used as a positive control to the assay. Methanol solutions of the synthesized 1,2,4-triazole and its Schiff bases were prepared in concentrations of 6.25, 12.5, 25.0, 50.0, 100.0, and 200.0 ppm by serial dilution method. 0.1 mM of methanolic DPPH solution was prepared and mixed with each concentration of compound solutions in 4:1 ratio. The mixture was incubated in dark condition for 30 minutes prior to absorbance measurement at 517 nm. The inhibitory concentration to reduce DPPH free radicals after 30 minutes by 50 %, IC_{50} is determined from the calibrated graph of percentage radical scavenging versus concentration of compound solution. The IC_{50} values of each compound were determined and tabulated in Error! Reference source not found..

IC_{50} value is inversely related to the radical scavenging ability of the compounds, which indicate the lower the IC_{50} value, the more capable the compound to scavenge or stabilize free radicals. Among the tested compounds, 1,2,4-triazole has the highest antioxidant activity, holding the lowest IC_{50} of 40.3 ppm. While **SB 12** has the highest antioxidant activity among the Schiff bases, having IC_{50} of 69.9 ppm, followed by **SB 3**, **SB 9**, **SB 13**, **SB 1**, **SB 5**, **SB 4**, **SB 10**, **SB 15**, **SB 2**, and **SB 11**. While for the IC_{50} of **SB 6**, **SB 7**, **SB 8**, and **SB 14** are above 200 ppm.

It is suggested that 1,2,4-triazole stabilizes the free radical by donating electrons through the nitrogen on indole ring as well as triazole ring. Both indole and triazole rings are aromatic, in which electron delocalization can take place and stabilize the compound through resonance effect. It has higher antioxidant activity as compared with its Schiff bases can be justified by the nitrogen atom on indole ring is less hindered by aromatic ring that the Schiff bases have, which introduce a better interaction with DPPH radicals. Among the Schiff bases, **SB 12** with methoxy group substituted at *para*-position of the aromatic ring has the highest antioxidant activity. This indicates that the methoxy substituent enables the formation of lone pair electrons and reduce the radicals, which is subsequently stabilized by resonance effect take place on the aromatic ring derived from the aromatic aldehyde.

CHAPTER 5 CONCLUSION

5.1 Conclusion

A 1,2,4-triazole, and a series of 15 new Schiff base derivatives have been successfully synthesized in this project. The synthesized Schiff bases are labelled as **SB 1** to **SB 15**. The structures of the compounds were characterized and elucidated using FT-IR, ^{1}H NMR, NOE, ^{13}C NMR, DEPT, HMQC, and HMBC experiments.

The antioxidant activity of the synthesized compounds was studied using DPPH assay. The inhibitory concentration for 50% reduction of DPPH radical by the compounds was determined. Most of the Schiff bases have moderate antioxidant activity as compared to the standard BHT, expect for **SB 6**, **SB 7**, **SB 8**, and **SB 14**, in which the IC_{50} exceeded the concentration range (0 to 200 ppm) prepared for the assay, thereby undetermined. Based on the results, it is suggested that the aromatic indole and triazole moieties allow electron delocalization through resonance effect which stabilized the compound. Besides, it is also suggested that the methoxy group at C-4" position (**SB 12**) is able to form lone pair electrons and stabilize the compound through resonance of the aromatic benzene ring.

5.2 Future Studies

Several factors to improve product yield can be studied, such as solvent interactions, pH of the medium, reaction temperature, choice of catalyst, and amount of catalyst used. Furthermore, 1,2,4-triazole and its Schiff bases have been reported to exhibit various biological activities apart from antioxidant activity. Studies on other activities such as antiviral, antibacterial, antimalarial, antifungal, and anticancer activities can be extended to explore the potential of the synthesized 1,2,4-triazole and its Schiff base derivatives. Besides, various 1,2,4-triazole Schiff bases with different substituents can be synthesized using different benzaldehyde derivatives to study the effect of substituents on antioxidant activities.

REFERENCES

Aboul-Fadl, T., Mohammed, F.A.H. and Hassan, E.A.S., 2003. Synthesis, antitubercular activity and pharmacokinetic studies of some Schiff bases derived from 1- alkylisatin and isonicotinic acid hydrazide (INH). *Archives of Pharmacal Research*, 26(10), pp.778–784.

Ainsworth, C. and Jones, R.G., 1955. Isomeric and Nuclear-substituted β-Aminoethyl-1,2,4-triazoles. *Journal of the American Chemical Society*, 77(3), pp.621–624.

Bladin, J.A., 1885. Ueber von Dicyanphenylhydrazin abgeleitete Verbindungen. *Berichte der deutschen chemischen Gesellschaft*, 18(1), pp.1544–1551.

Bullock, M.W. and Hand, J.J., 1956. Syntheses of Some Substituted Indole-3-Acetic Acids. *Journal of the American Chemical Society*, 78(22), pp.5852–5854.

Chen, D. et al., 2018. One-Pot Synthesis of Indole-3-acetic Acid Derivatives through the Cascade Tsuji-Trost Reaction and Heck Coupling. *Journal of Organic Chemistry*, 83(12), pp.6805–6814.

Dede, B., Karipcin, F. and Cengiz, M., 2007. Novel Schiff Bases with Oxime Groups and Their Homo- and Heteronuclear Copper(II) Complexes. *Collection of Czechoslovak Chemical Communications*, 72(10), pp.1383–1397.

Eicher, T., Hauptmann, S. and Speicher, A., 2013. *The Chemistry of Heterocycles Structures, Reactions, Synthesis, and Applications.* 3rd ed., Weinheim, Germany: John Wiley & Sons, Incorporated.

Pinto, D. C. G. A., Santos, C. M. M. and Silva, A.M. S., 2007. Trivandrum-695 023, Kerala, India Recent Research Developments in Heterocyclic Chemistry. *Research Signpost*, 37(2).

Goswami, B.N., Kataky, J.C.S. and Baruah, J.N., 1984. Synthesis and antibacterial activity of 1-(2,4-dichlorobenzoyl)-4-substituted thiosemicarbazides, 1,2,4-triazoles and their methyl derivatives. *Journal of Heterocyclic Chemistry*, 21(4), pp.1225–1229.

Gutowsky, H.S. and Holm, C.H., 1956. Rate Processes and Nuclear Magnetic Resonance Spectra. II. Hindered Internal Rotation of Amides. *The Journal of Chemical Physics*, 25(6), pp.1228–1234.

Hamid, H.A., Ramli, A.N.M. and Yusoff, M.M., 2017. Indole Alkaloids from Plants as Potential Leads for Antidepressant Drugs: A Mini Review. *Frontiers in Pharmacology*, 8(FEB).

Holm, S.C. and Straub, B.F., 2011. Synthesis of N-Substituted 1,2,4-Triazoles. A Review. *http://dx.doi.org/10.1080/00304948.2011.593999*, 43(4), pp.319–347.

Jiang, G.W. et al., 2020. Preparation and antitumor effects of 4-amino-1,2,4-triazole Schiff base derivative. *Journal of International Medical Research*, 48(2).

Jin, J.Y. et al., 2007. Synthesis and Biological Activity of Some Novel Derivatives of 4-Amino-3-(D-galactopentitol-1-yl)-5-mercapto-1,2,4-triazole. *Molecules : A Journal of Synthetic Chemistry and Natural Product Chemistry*, 12(8), p.1596.

Kajal, A. et al., 2013. Schiff Bases: A Versatile Pharmacophore. *Journal of Catalysts*, 2013, pp.1–14.

Kaplancikli, Z.A., Turan-Zitouni, G., Özdemir, A. and Revial, G., 2008. New triazole and triazolothiadiazine derivatives as possible antimicrobial agents. *European Journal of Medicinal Chemistry*, 43(1), pp.155–159. Available at: https://pubmed.ncbi.nlm.nih.gov/17499887/

Kumari, M. et al., 2021. Synthesis and biological evaluation of heterocyclic 1,2,4-triazole scaffolds as promising pharmacological agents. *BMC Chemistry*, 15(1), pp.1–16.

Laus, G. and Klötzer, W., 1989. Synthesis of 1-Amino-1*H*-1,2,4-triazoles. *Synthesis (Germany)*, 1989(4), pp.269–272.

Licandro, E. and Perdicchia, D., 2004. N-Acylhydrazines: Future Perspectives Offered by New Syntheses and Chemistry. *European Journal of Organic Chemistry*, 2004(4), pp.665–675.

Meena, R. et al., 2023. Schiff Bases and Their Metal Complexes: Synthesis, Structural Characteristics and Applications. *Schiff Base in Organic, Inorganic and Physical Chemistry*, pp.1–22.

Mermer, A. and Boulebd, H., 2023. An eco-friendly method for the synthesis of 1,2,4-triazole-Schiff base derivatives in aqueous medium and DFT calculations. *Journal of Molecular Structure*, 1271, pp.134102–134111.

Miri, R., Razzaghi-Asl, N. and Mohammadi, M.K., 2013. QM study and conformational analysis of an isatin Schiff base as a potential cytotoxic agent. *Journal of Molecular Modeling*, 19(2), pp.727–735.

Munteanu, I.G. and Apetrei, C., 2021. Analytical Methods Used in Determining Antioxidant Activity: A Review. *International Journal of Molecular Sciences*, 22(7), pp.3380–3410.

Nagata, N., Nishizawa, C. and Kurai, T., 1999. *Method of Producing 1,2,4-Triazole*, 6002015

Padda, I.S. and Reddy, K.M., 2023. Antitubercular Medications. *StatPearls*.

Perdicchia, D. et al., 2003. A new 'one-pot' synthesis of hydrazides by reduction of hydrazones. *Tetrahedron*, 59(39), pp.7733–7742.

Raczuk, E., Dmochowska, B., Samaszko-Fiertek, J. and Madaj, J., 2022. Different Schiff Bases—Structure, Importance and Classification. *Molecules*, 27(3), pp.1–24.

Rodrigo, Ramon., 2009. *Oxidative Stress and Antioxidants : Their Role in Human disease* 1st ed., New York, United States: Nova Biomedical Books.

Sameer Al-Rawi, M. and Abed Nashaan, F., 2023. Design, Synthesis, and Biological Activity of New Thiazolidine-4-One Derived from Symmetrical 4-Amino-1,2,4-Triazole. *Chemical Methodologies*, 7(2), pp.106–111.

Sarva, S. et al., 2016. Synthesis, antibacterial and anti-inflammatory activity of bis(indolyl)methanes. *Chinese Chemical Letters*, 27(1), pp.16–20.

Schiff, H., 1864. Mittheilungen aus dem Universitätslaboratorium in Pisa: Eine neue Reihe organischer Basen. *Justus Liebigs Annalen der Chemie*, 131(1), pp.118–119.

Sekiya, M. and Ishikawa, S., 1958. Reactions of Formamide. III. The Reactions of Formamide with Phenylhydrazones and Azine. *YAKUGAKU ZASSHI*, 78(5), pp.549–550.

Shneine, J.K. and Alaraji, Y.H., 2013. Chemistry of 1, 2, 4-Triazole: A Review Article . *International Journal of Science and Research*, 5(3), pp.1411–1419.

Sondhi, S.M. et al., 2006. Synthesis, anti-inflammatory, analgesic and kinase (CDK-1, CDK-5 and GSK-3) inhibition activity evaluation of benzimidazole/benzoxazole derivatives and some Schiff's bases. *Bioorganic & Medicinal Chemistry*, 14(11), pp.3758–3765.

Stana, A. et al., 2016. New Thiazolyl-triazole Schiff Bases: Synthesis and Evaluation of the Anti-Candida Potential. *Molecules 2016*, 21(11), pp.1595–1614.

Subasi, N.T., 2022. Overview of Schiff Bases. *Schiff Base in Organic, Inorganic and Physical Chemistry*.

Takahashi, O. and Kirikoshi, R., 2014. Intramolecular cyclization of aspartic acid residues assisted by three water molecules: a density functional theory study. *Computational Science & Discovery*, 7(1), pp.1–13.

Wei, D., Li, N., Lu, G. and Yao, K., 2006. Synthesis, catalytic and biological activity of novel dinuclear copper complex with Schiff base. *Science in China, Series B: Chemistry*, 49(3), pp.225–229.

Xue, S. et al., 2014. Synthesis and antiviral activity of some novel indole-2-carboxylate derivatives. *Acta Pharmaceutica Sinica B*, 4(4), pp.313–321.

Yin, P. et al., 2009. Highly efficient cyanoimidation of aldehydes. *Organic Letters*, 11(23), pp.5482–5485.

Zhuang, S.H. et al., 2013. Synthesis and anticancer activity of 2,4-disubstituted furo[3,2-b]indole derivatives. *European Journal of Medicinal Chemistry*, 66, pp.466–479.