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

By

TEH CHOR CHIN

A project submitted to the Department of Chemical Science,

Faculty of Science,

Universiti Tunku Abdul Rahman,

In partial fulfillment of the requirement for the degree of

Bachelor of Science (Hons) Chemistry

September 2016

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ABSTRACT

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Interest has been shown in the synthesis of 1,2,4-triazole Schiff bases due to its wide range of application especially biological activities such as anticancer, antitubercular, antifungal, antibacterial and antioxidant. In this project, 3-[(1H-indol-3-yl)-propyl]-4-amino-1H-1,2,4-triazole-5(4H)-thione was pre-synthesized to act as a starting material for the synthesis of Schiff bases. There are five new Schiff base of 1,2,4-triazole derivatives synthesized: 3-[(1H-indol-3-yl)-propyl]-4-(2,4-dichlorobenzylideneamino)-1H-1,2,4-triazole-5(4H)-thione **(SB 7)**, 3-[(1H-indol-3-yl)-propyl]-4-(3,4-dichlorobenzylideneamino)-1H-1,2,4-triazole-5(4H)-thione **(SB 8)**, 3-[(1H-indol-3-yl)-propyl]-4-(4-methoxybenzylideneamino)-1H-1,2,4-triazole-5(4H)-thione **(SB 6)**, 3-[(1H-indol-3-yl)-propyl]-4-(4-fluorobenzylideneamino)-1H-1,2,4-triazole-5(4H)-thione **(SB 3)** and 3-[(1H-indol-3-yl)-propyl]-4-(2-fluorobenzylideneamino)-1H-1,2,4-triazole-5(4H)-thione **(SB 11)**. Among all 1,2,4-triazole Schiff bases, **SB 6** has the highest percentage yield of 63%. The percentage yield is then decreases in an order of **SB 8**, **SB 11**, **SB 3** and **SB 7** with the value of 49%, 47%, 44% and 33% respectively. The structures of each Schiff bases were characterized and elucidated through a series of instrumental analysis including ^{1}H NMR, ^{13}C NMR, DEPT, HMQC, HMBC and melting point. The antioxidant activity of Schiff bases was determined via DPPH assay using

BHA as standard. **SB 7** was determined to have the highest antioxidant activity with the IC_{50} value of 74.7 $\pm$ 6.43 among the Schiff bases synthesized followed by **SB 6** (77.7 $\pm$ 2.52), **SB 3** (80.0 $\pm$ 1.00), **SB 11** (81.0 $\pm$ 1.73) and lastly **SB 8** (121.0 $\pm$ 1.73).

ABSTRAK

SINTHESIS, PENCIRIAN DAN AKTIVITI ANTIOKSIDAN

1,2,4-TRIAZOLE SCHIFF BASE

Teh Chor Chin

Tumpuan telah diberikan dalam sintesis 1,2,4-triazole Schiff base kerana ia didapati mempunyai pelbagai aplikasi khususnya aktiviti-aktiviti biologi seperti antikanser, antitubercular , antikulat , antibakteria dan antioksidan. Dalam projek ini, , 3-[(1H-indol-3-yl)-propyl]-4-amino-1H-1,2,4-triazole-5(4H)-thione telah pra-disintesis untuk digunakan sebagai bahan permulaan dalam sintesis Schiff base. Lima Schiff base baru bagi 1,2,4-triazole telah disintesis: 3-[(1H-indol-3-yl)-propyl]-4-(2,4-dichlorobenzylideneamino)-1H-1,2,4-triazole-5(4H)-thione **(SB 7)**, 3-[(1H-indol-3-yl)-propyl]-4-(3,4-dichlorobenzylideneamino)-1H-1,2,4-triazole-5(4H)-thione **(SB 8)**, 3-[(1H-indol-3-yl)-propyl]-4-(4-methoxybenzylideneamino)-1H-1,2,4-triazole-5(4H)-thione **(SB 6)**, 3-[(1H-indol-3-yl)-propyl]-4-(4-fluorobenzylideneamino)-1H-1,2,4-triazole-5(4H)-thione **(SB 3)** dan 3-[(1H-indol-3-yl)-propyl]-4-(2-fluorobenzylideneamino)-1H-1,2,4-triazole-5(4H)-thione **(SB 11)**. Antara semua 1,2,4-triazole Schiff base, SB 6 mempunyai hasil peratusan tertinggi, iaitu 63%. Hasil peratusan Schiff base seterusnya berkurang dalam suatu susunan dari **SB 8**, **SB 11**, **SB 3** dan **SB 11** dengan nilai 49%, 47%, 44% dan 33% masing-masing. Struktur bagi setiap Schiff base telah dikenalpasti dan dijelaskan dengan satu siri analisis instrumental termasuk ^{1}H NMR, ^{13}C NMR, DEPT, HMQC, HMBC dan melting point. Aktiviti antioksidan Schiff base

telah dinilaikan dengan kaedah DPPH dan BHA telah digunakan sebagai standard. **SB 7** didapati mengandungi aktiviti antioksidan paling tinggi dengan nilai IC_{50} 74.7 ± 64.3 antara semua Schiff base yang telah disintesis, diikuti dengan **SB 6** (77.7 ± 2.52), **SB 3** (80.0 ± 1.00), **SB 11** (81.0 ± 1.73) dan terakhirnya **SB 8** (121.0 ± 1.73).

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Last but not least, I would like to thank my family and friends for giving me support and encouragement towards the success of this project.

DECLARATION

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

Name : TEH CHOR CHIN

Date :

APPROVAL SHEET

This thesis report entitled **"SYNTHESIS, CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF 1,2,4-TRIAZOLE SCHIFF BASE"** was prepared by TEH CHOR CHIN and submitted as partial fulfillment of the requirements for the degree of Bachelor of Science (HONS) Chemistry at Universiti Tunku Abdul Rahman.

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Date: _____

PERMISSION SHEET

It is hereby certified that **TEH CHOR CHIN** (ID No. **13ADB08160**) has completed this final year project entitled **"SYNTHESIS, CHARACTERIZATION AND ANTIOXIDANT ACTIVITI OF 1,2,4-TRIAZOLE SCHIFF BASE"** supervised by Dr. Sim Kooi Mow from the Department of Chemical Science, Faculty of Science.

I hereby give permission to my supervisor to write and prepare manuscripts of these research findings for publishing in any form, if I do not prepare it within six (6) months from this date, provided that my name is included as one of the authors for this article. The arrangement of the name depends on my supervisor.

Yours truly,

_____

(TEH CHOR CHIN)

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LIST OF SYMBOLS/ABBREVIATIONS

δ	chemical shift
ppm	part per million
mmol	millimol
nm	wavelength
g mol^{-1}	molecular weight
Hz	Hertz
J	coupling constant
SB	Schiff base
IC_{50}	effective concentration for 50% reduction of activity
R_f,	Retention factor
NMR	Nuclear Magnetic Resonance
DEPT	Distortionless Enhancement by Polarization Transfer
HMQC	Heteronuclear Multiple Quantum Correlation
HMBC	Heteronuclear Multiple Bond
LCMS	Liquid Chromatography-Mass Spectroscopy
FT-IR	Fourier Translational Infrared Spectroscopy
TLC	Thin Layer Chromatography
DPPH	2,2-diphenyl-2-picrylhydrazyl hydrate

CHAPTER 1

INTRODUCTION

1.1 Schiff Base

Schiff base was initially discovered by a German-born chemist named Hugo Schiff. In 1864, he discovered the condensation reaction using aldehyde and aromatic amines and studied the product of the reaction. The product formed is been called as Schiff base. Schiff base is a nitrogen analogue of an aldehyde or ketone in which the carbonyl (C=O) group is replaced by an azomethine group with a general formula RHC=N-R_1, where R and R_1 are alkyl, aryl, cyclo alkyl or heterocyclic group (Ashraf *et al*., 2011).

According to Dr. Xavier A. *et al.* (2014), Schiff base of ligands with aldehydes are formed readily compared to that with ketone. Apart from that, Schiff bases that contain aryl substituents substantially more stable and more readily synthesized, while those which contain alkyl substituents are relatively unstable Schiff bases of aromatic aldehydes are more stable due to its effective conjugation whereas those with aliphatic aldehydes are relatively unstable and readily polymerized.

1.1.1 Synthesis of Schiff Bases

Generally, Schiff bases are the product of condensation reaction between ketones or aldehydes with primary amines. This reaction can take place under acid or base catalysis, or upon heating. A water molecule is eliminated to form an imine in the reaction.

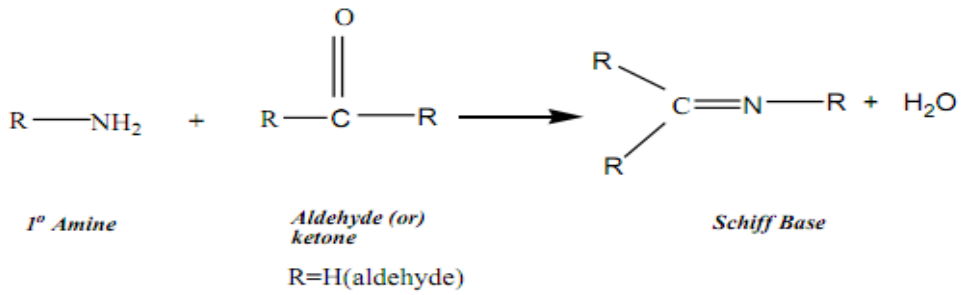


Figure 1.1: General equation of Schiff base formation.

Schiff base is synthesized by a sequence of two reactions which are addition reaction, followed by elimination reaction, in this case, nucleophilic addition followed by dehydration. The amine, which acts as the nucleophile, reacts with the aldehyde or ketone by attacking the carbonyl carbon to give an unstable intermediate compound called carbinolamine.

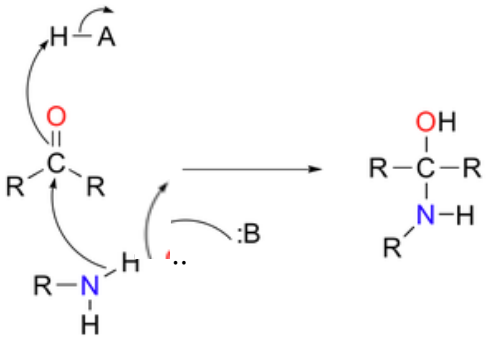


Figure 1.2: Formation of carbinolamine

The nitrogen is then deprotonated, and the electrons from this N-H bond leads to removal of oxygen from the carbon and hence a new C=N double bond (an imine) is formed and a water molecule is eliminated. According to Le Chatelier's principle, removal of water favours the forward reaction to the right.

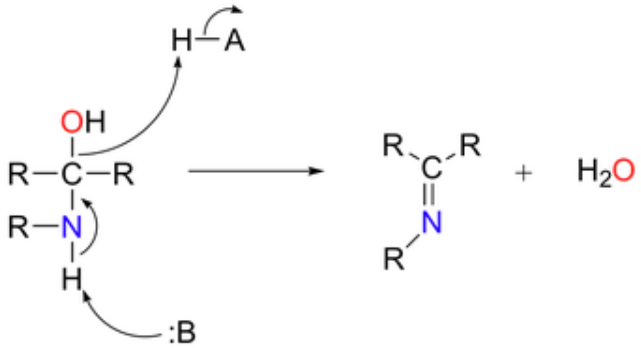


Figure 1.3: Formation of Schiff base from carbinolamine

This reaction can be conducted via acid-catalyzed or base-catalyzed pathways. Since the carbinolamine is an alcohol, it undergoes an acid-catalyzed dehydration rather than base-catalyzed reaction.

General Mechanism:

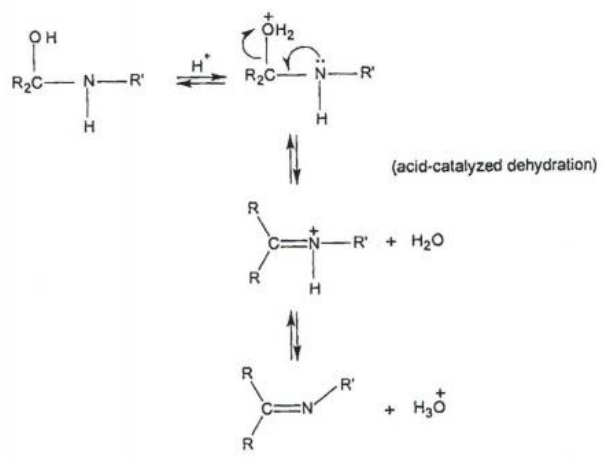


Figure 1.4: Mechanism of acid-catalyzed dehydration

However, the reaction of forming Schiff base via aldehyde or ketone is reversible. Hence, conversion of imine back to their aldehydes or ketones and amines can be carried out by hydrolysis by acid or base (Xavier *et al*., 2014).

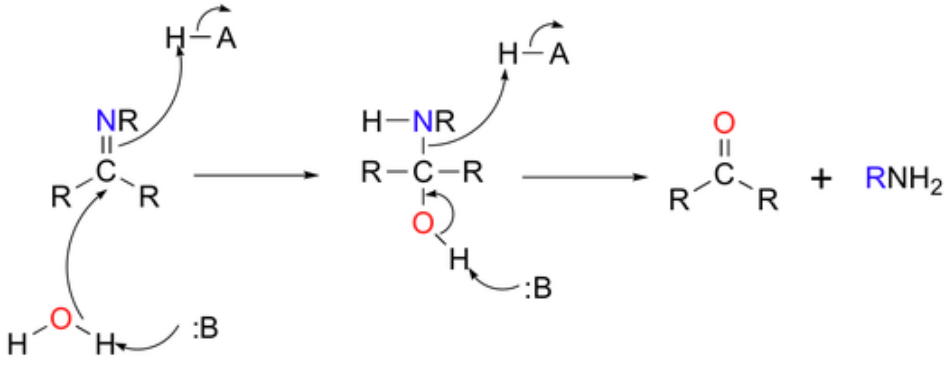


Figure 1.5: Hydrolysis of Schiff base back to aldehyde/ketone

1.1.2 Acid- / Base-catalyzed Schiff Base Formation

Typically the dehydration of the carbinolamine is the rate-determining step of Schiff base formation and the pH of the reaction medium does influence the whole process. Dehydration of carbinolamine can be carried out under base-catalyzed pathway. This reaction is somehow similar to the E2 elimination of alkyl halides except that it is not a concerted reaction. It occurs through two steps by forming an anionic intermediate.

Apart from that, dehydration can also be catalyzed by acid. The acid catalyst protonates the carbinolamine intermediate and converts the hydroxyl group into water molecule as water is a good leaving group. However, the acid concentration cannot be too high because amine is a basic compound. Highly

acidic medium will protonate the amine nucleophile and caused it to become non-nucleophilic. This will lead to the shifting of equilibrium of reaction to the left and eventually terminate the carbinolamine formation. Thus, a reaction medium with mild acidic pH is optimum to carry out the Schiff base synthesis (Xavier *et al*., 2014).

1.1.3 Application of Schiff Base

Based on the research by Patil *et al.* (2012), Schiff bases have been reported in their biological properties such as antibacterial, antifungal, anticancer, antitumor, antimicrobial, insecticidal, anti-inflammatory, and antiviral activities. These have drawn a considerable attention from many researches. The azomethine linkage in the Schiff bases is the one that contributes to the biological activities (Calisir *et al.*, 2010).

In organic chemistry, acylation of Schiff bases by acyl cyanides, acid chlorides and acid anhydrides have led to an addition of the acylation agent to the imine group. This reaction gives positive results in natural product synthesis. Schiff bases are said to be important intermediates in enzymatic reactions of an amino and a carbonyl group of the substrate whereby the enzyme is usually that of a lysine residue. Several studies also show that condensation of salicylaldehyde with different heterocyclic compounds yields derivatives with potent antibacterial and antifungal activity (Xavier *et al*., 2014).

Besides that, Schiff bases also act as important chelating agents which offer great versatility in ligand system. This is because the imine nitrogen which is typically derived from alkyl diamines and aromatic aldehydes is basic and it exhibits π-acceptor properties (Nworie *et al.*, 2016).

Their metal complexes have been widely studied due to the anticancer and herbicidal application. Schiff bases derived from 4-dimethylamine benzaldehyde exhibit significant antibacterial activity and are used as antibodies and anti-inflammatory agents in medicines (Ashraf *et al*., 2011). According to Harvey and Clifford (1950), bis-imine Schiff bases are stable metal complexing agents when they are used with one or more donor atoms to form macrocycles. They are also good in stabilizing metals with high oxidation states.

1.2 1,2,4-Triazole

Triazole is an isomeric chemical compounds with molecular formula $C_2H_3N_3$. Triazole is a basic aromatic heterocyclic compound which consists of a 5-membered ring of two carbon atoms and three nitrogen atoms. Bladin is the one who first named the compound triazole and described its derivatives in early 1885. There are two types of triazole, namely 1,3,4-triazole and 1,2,4-triazole. The structure of 1,2,4-triazole is shown Figure 1.6.

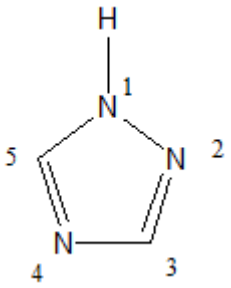


Figure 1.6: Structure of 1,2,4-Triazole

The contribution of one π electron from each atom joined by double bonds and the remaining two electrons from a nitrogen atom forms an aromatic sextet, which give rise to its stability due to the resonance effect.

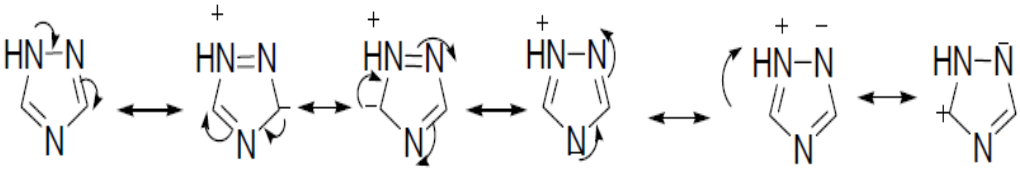


Figure 1.7: Resonance effect of 1,2,4-Triazole

1,2,4-Triazole exhibit two tautomeric forms namely [1H]-1,2,4-triazole and [4H]-1,2,4-triazole (Belapure, 2012).

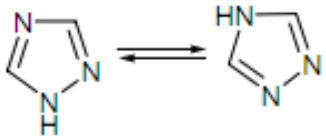
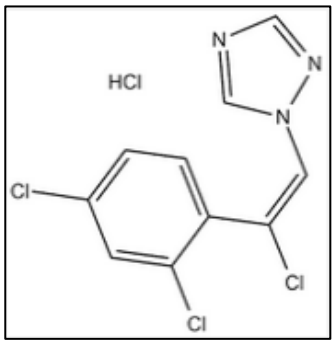


Figure 1.8: Structure of [1H]-1,2,4-triazole and [4H]-1,2,4-triazole

1,2,4-Triazole and their derivatives constitute an important class of organic compounds with diverse agricultural, industrial and biological activities (Zamani *et al.*, 2002), including anti-inflammatory (Mullican *et al.*, 1993), antimicrobial (Guzeldemirci and Kucukbasmac, 2010), anticonvulsant (Kelley *et al.*, 1995) and antifungal (Heubach *et al.*, 1979).

Compounds bearing with 1,2,4-triazole ring are sometimes known as drugs. Vorozole, Letrozole and anatrozole are the examples of non-steroidal drugs (Serdar *et al.*, 2007). Loreclezole, on the other hand, is one the example of anticonvulsant drugs (Bekircan *et al.*, 2006). Fluconazole and Itraconazole are examples of antifungal drugs that can be found in the market (Belapure, 2012). A synthesized compound, 4-[(4-methoxyphenyl)methylenamino]-3-phenyl-5-(4-tolyl)-4H-1,2,4-triazole showed marginal antimicrobial activity towards *Staphylacocus aureus* (Sedar *et al.*, 2007). According to Bayer *et al.* (1971), the antifungal and antimicrobial activities can be enhanced if the triazole exists as a salt or a metallic complex. Apart from that, 1,2,4-triazole is also used in agricultural industry as herbicide and pesticide.



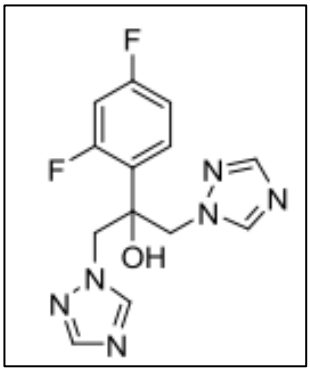
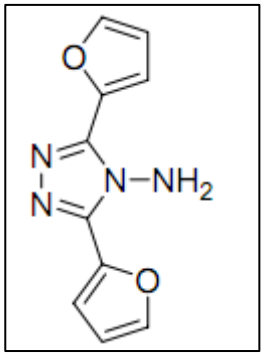


Figure 1.9 a **Figure 1.9 b**

Figure 1.9: Structure of Loreclezole (1.9 a) and Fluconazole (1.9 b) respectively.



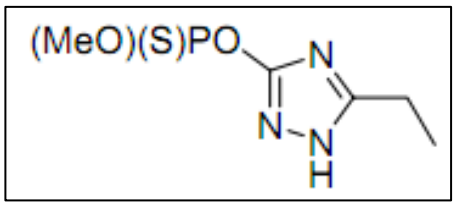


Figure 1.10 a **Figure 1.10 b**

Figure 1.10: Structure of Herbicidal (1.10 a) and Pesticidal (1.10 b) respectively.

1.3 Synthesis of 1,2,4-Triazole Schiff Base

In recent years, the chemistry of 1,2,4-triazole and their fused heterocyclic derivatives has received considerable attention owing to their synthetic and effective biological importance. Some Schiff bases bearing aryl groups or heterocyclic residues also possess excellent biological activities. Thus, 1,2,4-triazole is used to synthesize 1,2,4-triazole Schiff bases by condensation reaction with various benzaldehydes. All the synthesized derivatives are characterized using instrumental analysis such as:

1. Nuclear Magnetic Resonance (^{1}H NMR, ^{13}C NMR),
2. Heteronuclear Multiple Bond Correlation (HMBC),
3. Heteronuclear Multiple Quantum Correlation (HMQC),
4. Distortionless Enhancement by Polarization Transfer (DEPT) NMR,
5. Fourier Translational Infrared Spectroscopy (FT-IR) and
6. Liquid Chromatography-Mass Spectroscopy (LCMS)

1.3.1 Applications of 1,2,4-Triazole Schiff Base

An enormous number of 1,2,4-triazole-containing ring system have been incorporated into a wide variety of therapeutically interesting drugs as they are found to possess considerable biological activities. It is widely used as analgesic, antiviral, antifungal, anticancer, antitubercular, anti-inflammatory and antimicrobial activity (Ye *et al.*, 2007). According to Mobinikhaledi *et al.* (2010), Schiff bases bearing 1,2,4-triazole are reported to be used as drug with significant biological activity. The applications of 1,2,4-triazole Schiff base are discussed below:

1.3.1.1 Antimicrobial activity

Newly synthesized Schiff bases of 4-Amino-5-sulfanyl-4H-1,2,4-triazol-3-yl with various substituted benzaldehydes shown in Figure 1.11 showed their antimicrobial activities against various microorganisms including Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtillis*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and fungus (*Candida albicans*) (Mange *et al.*, 2010).

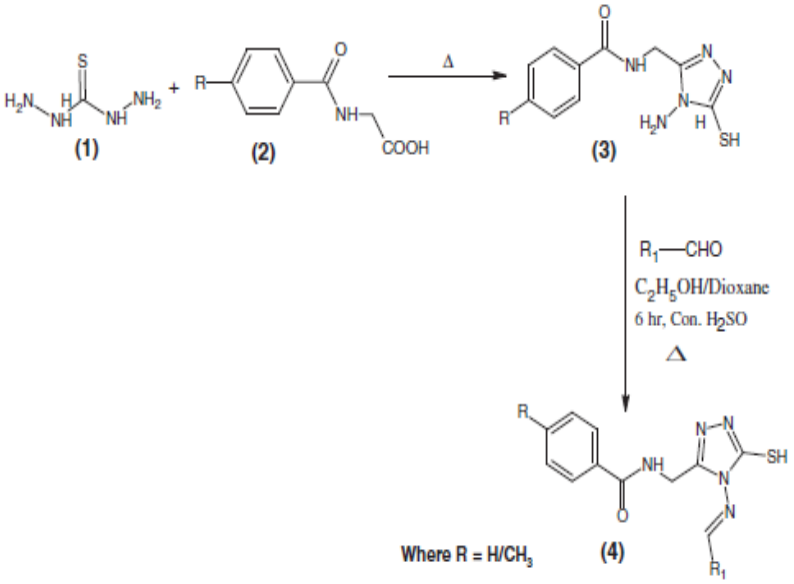


Figure 1.11: Synthesis of 1,2,4-triazole Schiff base by Mange *et al.*

1.3.1.2 Anti-inflammatory activity

A series of 5-aryl-3 alkylthio-1,2,4-triazoles and corresponding sulfones were synthesized and characterized by Tozkoparan *et al* (2007). The anti-inflammatory-analgesic activity of the compounds was evaluated using mice. Several of the compounds showed significant analgesic-anti-inflammatory activity. Alkylsulfones derivatives were found to be much more potent analgesic-anti-inflammatory agents than alkylthio analogs (Tozkoparan *et al.*, 2007).

Figure 1.12: Synthesis of 1,2,4-triazole Schiff base by Tozkoparan *et al.*

1.3.1.3 Anticancer activity

According to Li *et al.* (2012), a new type of chiral 1,2,4-triazole Schiff bases bearing γ-substituted butenolide moiety have been synthesized via tandem Michael addition-elimination reaction. This reaction is conducted under phase-transfer catalysis conditions. The compounds were biologically screened for anticancer activities and the results obtained shows that they possess good anticancer activities towards HeLa (Li *et al.*, 2012).

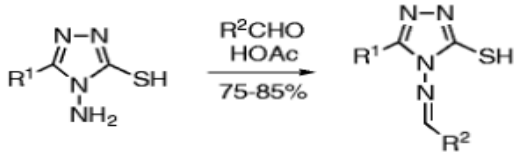


Figure 1.13: Synthesis of 1,2,4-triazole Schiff base by Li *et al.*

1.3.1.4 Antifungal activity

A novel series of Schiff bases based on 4-(benzylideneamino)-5-phenyl-4H-1,2,4-triazole-3-thiol scaffold was synthesized and were evaluated for antifungal activity. Thiocarbohydrazide were fused with substituted benzoic acid and subsequently treated with substituted benzaldehydes to synthesize the Schiff base compounds. The newly synthesized derivatives showed positive antifungal activity against fungal species such as *Microsporum gypseum* but not *Candida albicans* nor *Aspergillus niger* (Gupta and Jain, 2015).

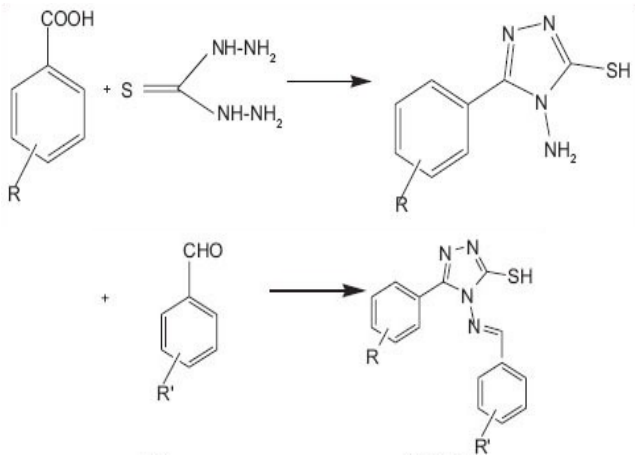


Figure 1.14: Synthesis of 1,2,4-triazole Schiff base by Gupta and Jain.

1.4 Antioxidant Activity

An antioxidant can be defined as a molecule that inhibits the oxidation of other molecules. Though oxygen is an essential component for living, it is a highly reactive atom that is capable of becoming part of potentially damaging molecules, known as "free radicals" (Mandal, 2013). These free radicals are responsible for causing a large number of diseases including cancer, cardiovascular disease, Alzheimer's disease, neural disorders, Parkinson's disease and ageing. Antioxidants are used to ceased or delay the oxidation, as they are capable of stabilizing or deactivating the free radicals, as well as preventing the onset of degenerative diseases (Nur Alam, Bristi and Rafiquzzaman., 2013).

Antioxidant can be obtained either naturally or synthetically. Natural antioxidant such as quercetin and ascorbic acid can be obtained from fruits, vegetables and plants. The synthetic antioxidants available in the market include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroquinone (TBHQ).

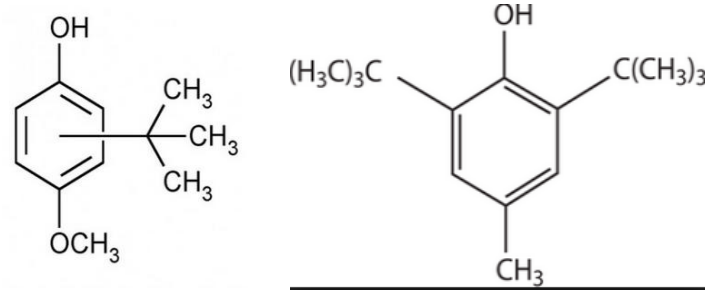


Figure 1.15 a **Figure1.15 b**

Figure 1.15: Structure of BHA (1.15 a) and BHT (1.15 b) respectively

There are several method used for the evaluation of antioxidant activity, mainly divided into two categories, *in vitro* and *in vivo*. *In vivo* are the studies in which the effects of various biological entities are tested on living things, usually animals and plants while *in vitro* are those being done in a laboratory environment using glass wares and apparatus such as test tubes and petri dish. The examples of in vitro method are 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity, hydroxyl radical scavenging activity and hydrogen peroxide scavenging assay. On the other hand, examples for in vivo method are ferric reducing ability of plasma, superoxide dismutase (SOD) method and lipid peroxidation (LPO) assay. However, the most commonly used method for antioxidant activity is DPPH assay as this method is rapid, simple and inexpensive among other free radical scavenging method (Nur Alam, Bristi and Rafiquzzaman., 2013).

Antioxidants are widely used in industries. It is used as stabilizers in fuel and lubricants to prevent oxidation. At the same time, it is also used in gasoline to prevent polymerization that will lead to the formation of engine-fouling residue (Boozer *et al.*, 1955). Apart from that, antioxidants are also added in polymer manufacturing such as rubbers, plastics and adhesives. This is to prevent the oxidative degradation than will cause a loss of strength and flexibility in the materials.

1.4.1 DPPH Assay

Stable free radical DPPH was first discovered by Goldschmidt and Renn in 1922. Its stability is due to the delocalization of non-bonding electron over molecule.

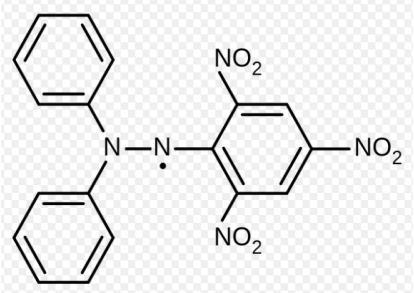


Figure 1.16: Structure of DPPH

This disabled DPPH to form dimer. The free radical of DPPH with an odd electron is in deep violet colour. This makes it visible and has a maximum absorption at ~517 nm. DPPH assay is based on the reduction of DPPH. The antioxidant compounds will pair off the free radical of DPPH by donating a hydrogen atom. The DPPH is then reduced to become DPPH-H and the reduced DPPH is decolourized and turned yellow (Warrier, Nambier and Raman, 1994).

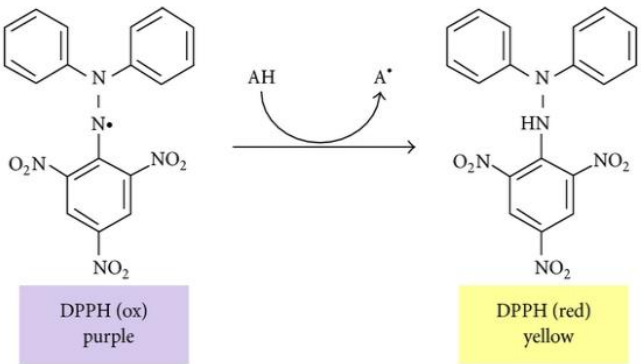


Figure 1.17: Reduction of DPPH

1.5 Objectives of the Researches

1. To synthesis a series of 4-amino-3-(substituted)-5-mercapto-1,2,4-triazole Schiff Base
2. To characterize the structure of pure 1,2,4-triazole Schiff Base and its derivative compounds using spectroscopic techniques
3. To study the antioxidant activity of each synthesized 1,2,4-triazole Schiff base.

CHAPTER 2

LITERATURE REVIEW

2.1 Synthesis of 1,2,4-triazole Schiff Base

Various 1,2,4-triazole Schiff bases have been synthesized through condensation reaction of carbonyl compound (aldehyde / ketone) and primary amine compound. Characterization of the 1,2,4-triazole Schiff bases synthesized is carried out using several laboratory equipment such as Nuclear Magnetic Resonance (NMR) spectrometer. The application of 1,2,4-triazole Schiff bases in various industries is also being studied.

For instance, Sharma, *et al.* (2009) has synthesized Schiff base of 1,2,4-triazole by refluxing 4-(4-amino-5-mercapto-4H-[1,2,4]triazol-3-yl)-benzoic acid and equimolar amount of 10 various substituted aromatic aldehyde in the presence of 3 to 4 drops of sulphuric acid as catalyst. The completion of reaction was monitored using TLC. The solid precipitate formed was filtered, washed with cold water and recrystallized with hot ethanol. The resulting Schiff base was characterized using FT-IR, NMR spectroscopy and mass spectroscopy (Sharma *et al.*, 2009).

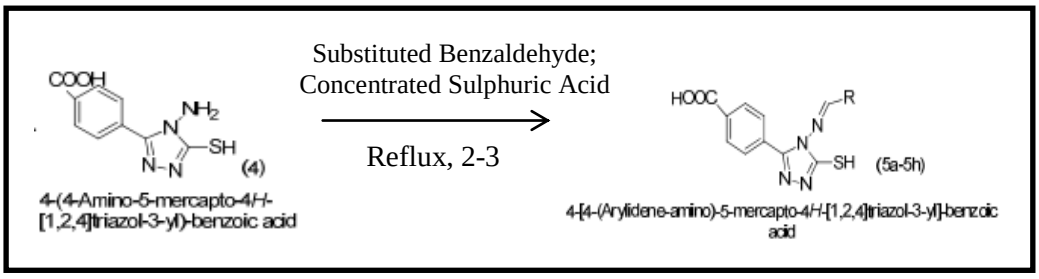


Figure 2.1: Synthesis of Schiff Base by Sharma *et al.*

2.2 Method of Synthesis of 1,2,4-triazole Schiff Base

Schiff base of 1,2,4-triazole can be synthesized via several methods with corresponding advantages and limitations. The synthesis of 1,2,4-triazole Schiff base is mainly categories into two types, which are classical method and conventional method.

2.2.1 Classical Method

Classical method of 1,2,4-triazole Schiff base synthesis involved condensation reaction of primary amine and carbonyl group (aldehyde / ketone), followed by removal of water molecules to form imine group. The elimination of water molecules can be done through molecular sieve, dehydrating agent or azeotropic distillation. The amine group, which acts as a nucleophile, attacked the carbonyl carbon (electrophile) to give an intermediate during the condensation. Schiff base is formed upon dehydration of water molecules from the condensation process. The rate of formation of Schiff base is dependent on the rate of dehydration of the intermediate (Patil *et al.*, 2012; Yang *et al.*, 2002).

In the research of Ye *et al.* (2007), 3-substituted-4-amino-1,2,4-triazole 5-thione was refluxed with appropriate substituted benzaldehyde for 4 hours. Unfortunately, the result was unexpectedly low. Activated molecular sieves (4Å) were then added into the reaction mixture to remove water molecules. As a result, a higher yield of product was achieved and was then purified through filtration and recrystallization from hot ethanol to obtain pure 1,2,4-triazole Schiff base.

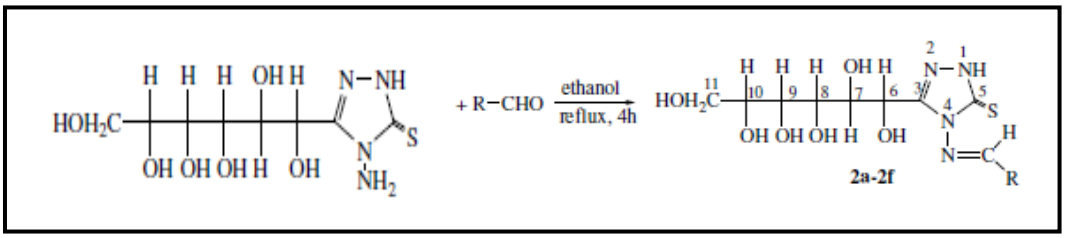


Figure 2.2: Synthesis of Schiff Base by Ye et al.

2.2.2 Conventional Method

Since the classical method of 1,2,4-triazole Schiff base synthesis results in low yield, conventional method of synthesis is introduced. Synthesis of Schiff base using this method can be divided into acid-catalyzed reaction or base-catalyzed reaction. The reaction of condensation between 1,2,4-triazole and aldehyde involved nucleophillic attack by imine group towards carbonyl group of aldehyde. This process gives an intermediate, namely carbinolamine. Acid is more preferable compare to base as the intermediate is an alcohol. The acid helps in deprotonating nitrogen from amine. Apart from that, it also serves as dehydrating agent by protonating the hydroxyl group to make it a better leaving

group. This contributes in solving the difficulties of removal of water as in classical method (Xavier *et al.*, 2014).

2.2.2.1 Acid-Catalyzed Reaction

Mixture of *N*-[(4-amino-5-sulfanyl-4H-1,2,4-triazol-3-yl)methyl]-4-substituted-benzamide and substituted benzaldehyde was treated with 2-3 drops of concentrated sulfuric acid as catalyst in ethanol-dioxane mixture. The reaction mixture was refluxed for 6 hours. After the completion of reaction, the mixture is left to cool in room temperature and the solid precipitate formed was filtered using suction filtration, washed with cold ethanol and recrystallized using hot ethanol. The Schiff base yielded from this reaction is characterized and elucidated using FTIR spectrophotometry, NMR spectroscopy and LC-MS. As a result, the Schiff base is identified as *N*-[(4-{[(*E*)-substituted] amino}-5-sulfanyl-4H-1,2,4-triazol-3-yl)methyl]-4-substituted-benzamide (Mange *et al.*, 2011).

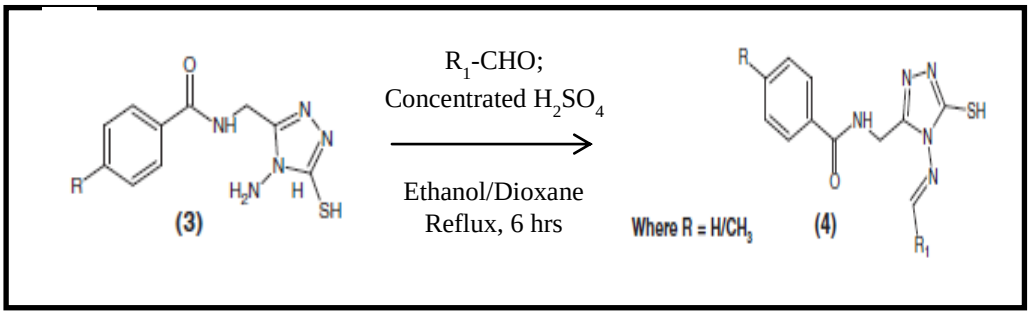


Figure 2.3: Synthesis of Schiff base by Mange *et al.*

In the research of Kaplanckli *et al.*, (2008), Schiff base of 1,2,4-triazole was synthesized by refluxing 4-amino-3 mercapto-5-[(1H-indol-3-yl)methyl]-1,2,4-triazole with arylaldehyde in the presence of concentrated sulfuric acid in ethanol. The structure of resulting Schiff base was elucidated using NMR, IR, mass spectral and elemental analysis. From the spectral analysis, the structure of Schiff base determined is shown in Figure 2.4, namely (5-[(1H-indol-3-yl)methyl]-4-arylideneamino-3-mercapto-1,2,4-triazole (Kaplanckli *et al.*, 2008).

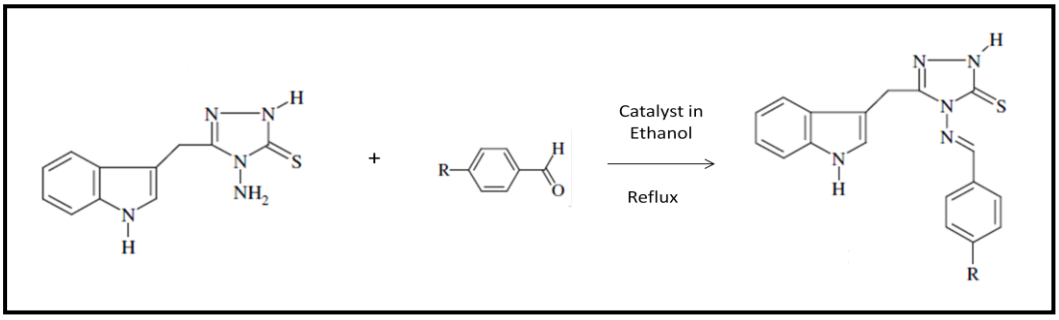


Figure 2.4: Synthesis of Schiff Base by Kaplanckli *et al.*

According to the research of Li *et al.* (2012), the 1,2,4-triazole Sciff base was synthesized via condensation reaction between 4-amino-5-substituted-4H-1,2,4-triazol-3-thiols and aromatic aldehydes. The reaction mixture is treated with glacial acetic acid. The structure of Schiff bases were determined using IR, NMR and Mass Spectroscopy.

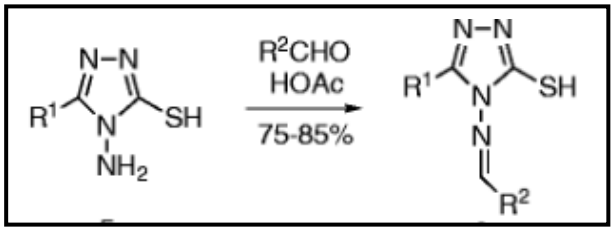


Figure 2.5: Synthesis of Schiff Base by Li *et al.*

2.2.2.2 Base-Catalyzed Reaction

According to the research of Zamani *et al.* (2002), Schiff base of 1,2,4-triazole were synthesized using base-catalyzed reaction by refluxing various solid thiosemicarbazides with sodium hydroxide solution. The mixture was left to cool and filtered after reacted completely. The filtrate was neutralized using hydrochloric acid and the precipitate was filtered and recrystallized with hot ethanol for purification. The 1,2,4-triazole Schiff base produced from this reaction is 2,4-Dihydro-4-(4-methylphenyl)-5-(isomeric pyridyl)-3H-1,2,4-triazole. The reaction pathway is shown in Figure 2.6 (Zamani *et al.*, 2002).

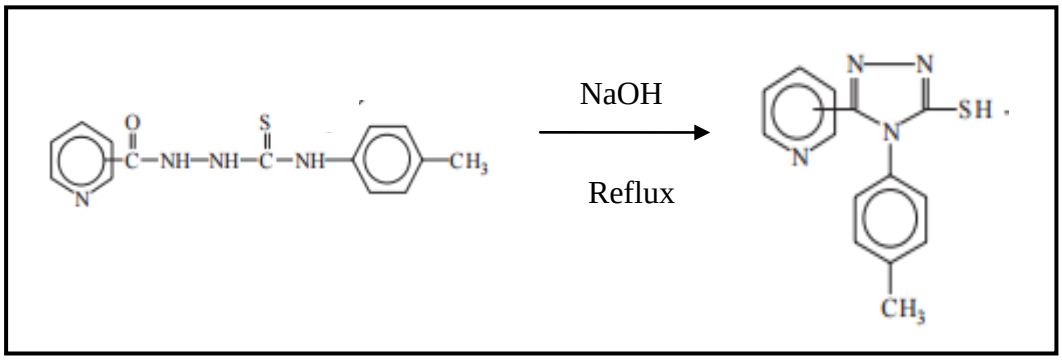


Figure 2.6: Synthesis of Schiff base by Zamani *et al.*

In order to increase the rate of reaction, improve product yield and shorten the reaction duration, conventional method are slightly modified. Microwave irradiation-solvent free method is introduced instead of refluxing for hours.

2.2.2.3 Microwave Irradiation-Solvent Free Method

Microwave activation has become very popular and useful technology in organic chemistry. reaction using microwave irradiation give rise to rate enhancemnet, better yield, ease of manipulation, good conversion, high selectivity and shorter reaction duration. The solvent free microwave synthesis has contributes to greener method as it leads to higher atom economy and environment friendly apart from reducing the hazardous wastes and risk of explosions.

Mixture of 2-methyl-3-[4-amino-5-mercapto-4H-[1,2,4]triazol-3-yl]-1-indole and several substituted benzaldehydes of interest was treated with hydrochloric acid under microwave irradiation for 2-3 minutes. This reaction results in 2-methyl-3-[4-arylideneamino-5-mercapto-4H-[1,2,4]triazol-3-yl]-1-indole derivatives (Gomha and Riyadh, 2011).

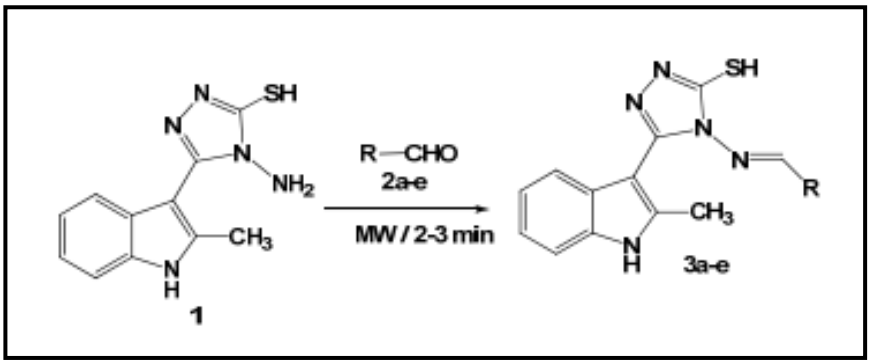


Figure 2.7: Synthesis of Schiff Base by Gomha and Riyadh

According to Karaali *et al.* (2013) research, 1,2,4-triazole and 9H-fluorene-2-carbaldehyde were refluxed for 4 hours in dry ethanol. That same starting material were subjected to microwave activation for 10 minutes with pressure control. From the results obtained, the duration reaction using microwave irradiation was shorter compared to that of using conventional method. Besides, the yield of 1,2,4-triazole Schiff base, 5-methyl/ethyl-4-[(9H-fluoren-3-ylmethylidene)amino]-2,4-dihydro-3H-1,2,4-triazol-3-one, obtained using microwave irradiation was higher compared to that using cnoventional method. The rate of reaction using microwave irradiation is once again proven higher than conventional method (Karaali *et al.*, 2013).

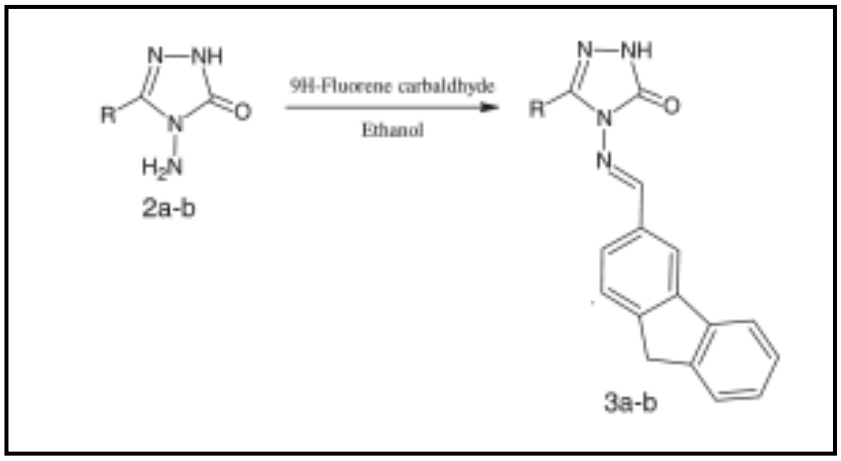


Figure 2.8: Synthesis of Schiff Base by Karaali *et al.*

2.3 Antioxidant Activity

There are a large number of method to evaluate an antioxidant activity including DPPH assay, Folin Ciocaltau (FC) method, Lipid Peroxidation (LPO) assay and Ferric Redusing Antioxidant Power (FRAP) assay . However, DPPH assay is the most common technique used to determine the antioxidant activity of a compound due to its simpleness, convenience, rapidness and inexpensive (Nur Alam, Bristi and Rafiquzzaman., 2013).

The usage of DPPH assay is reviewed. Based on the researches, DPPH has been found to be a stable free radicals due to its delocalization of spare electron over the molecule. The delocalization of electron give rise to the violet colour of free radical DPPH, as the consequence, it is only visible and gives maximum absorption at 517 nm.

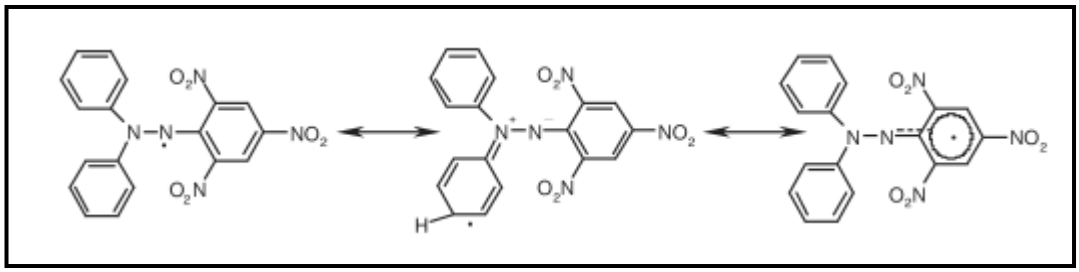


Figure 2.9: Delocalization of electron over DPPH

The stable free radical is also known as good hydrogen abstractor as itself undergoes reduction and being paired off with the hydrogen donated by

antioxidant compounds to produce DPPH-H. The reduction of DPPH led to decolourization of violet colour into yellow colour (Ionita, 2003).

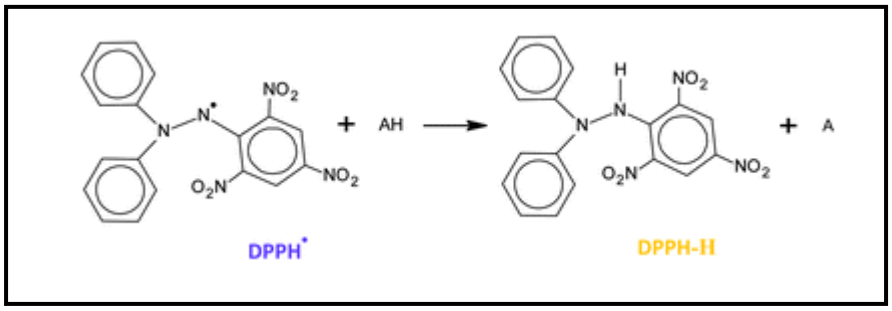


Figure 2.10: Reduction of DPPH into DPPH-H

The absorbance reading of mixture of sample and DPPH solution is obtained after 30 minutes. It is used to calculate the percentage radical scavenging, whereby the percentage value is used to calibrate a graph against concentration. IC_{50} obtained from the graph determined the effective concentration of substrate (antioxidant compound) that inhibit the DPPH activity by 50%.

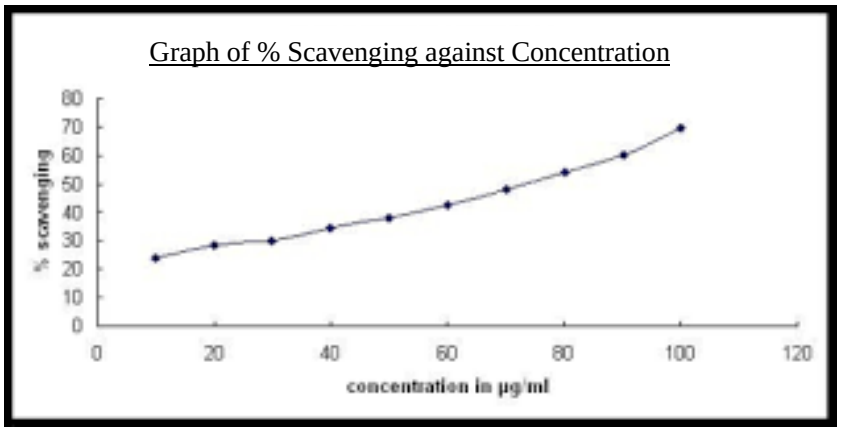


Figure 2.11: Graph of Percentage Radical Scavenging Against Concentration

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 Chemical Used

Table 3.11: Chemical Used in Synthesis of Schiff Base

Chemical Name Molecular Formula	Molecular Weight, g mol^{-1}	Manufacturer Country	State
Indole-3-butyric acid	241.33	Next Gene Science, Malaysia	Solid
Thiocarbohydrazide	106.15	ACROS ORGANIC, Belgium	Solid
2,4-dichlorobenzaldehyde $C_7H_4Cl_2O$	175.02	MERCK, Germany	Solid
3,4-dichlorobenzaldehyde $C_7H_4Cl_2O$	175.01	ACROS ORGANIC, Belgium	Solid
4-methoxybenzaldehyde $C_8H_8O_2$	136.15	Fischer Scientific, UK	Liquid
4-fluorobenzaldehyde C_7H_5FO	124.11	MERCK, Germany	Liquid
2-fluorobenzaldehyde C_7H_5FO	124.11	Fischer Scientific, UK	Liquid
(+)-Tartaric Acid $C_4H_6O_6$	150.09	Fischer Scientific, UK	Solid
Absolute Ethanol C_2H_6O	46.07	Fischer Scientific, UK	Liquid

Table 3.12: Chemical Used in Thin Layer Chromatography

Chemical name Molecular Formula	Molecular Weight, g mol^{-1}	Manufacturer Country	State
Ethyl Acetate $C_4H_8O_2$	88.11	Rinting Scientific, Malaysia	Liquid
Hexane C_6H_{14}	86.18	Rinting Scientific, Malaysia	Liquid
Chloroform	46.07	Rinting Scientific, Malaysia	Liquid
Acetone C_3H_6O	58.08	Rinting Scientific, Malaysia	Liquid

Table 3.13: Chemical Used in Recrystallization

Chemical name Molecular Formula	Molecular Weight, g mol^{-1}	Manufacturer Country	State
Ethanol C_2H_6O	46.07	Rinting Scientific, Malaysia	Liquid

Table 3.14: Chemical Used in Characterization

Chemical name Molecular Formula	Molecular Weight, g mol^{-1}	Manufacturer Country	State
Chloroform-d $CDCl_3$	120.38	ARMAR, Switzerland	Liquid
Dimethyl sulfoxide-d_6 C_2D_6OS	84.17	MERCK, Germany	Liquid

Table 3.15: Chemical Used in Antioxidant Assay

Chemical name Molecular Formula	Molecular Weight, g mol^{-1}	Manufacturer Country	State
2,2-Diphenyl-1-picrylhydrazyl (DPPH) $C_{18}H_{12}N_5O_6$	394.32	SIGMA, Germany	Solid
Methanol CH_4O	32.05	SYNERLAB	Liquid

3.2 Instrument Used

Table 3.21: Instrument Used in the Project

Instrument	Manufacturer	Model
Nuclear Magnetic Resonance Spectrometer	Jeol	ECX-400
Single beam UV Spectrophotometer	Thermo Scientific	GENESYS 10S UV-VIS Spectrophotometer
Melting Point Apparatus	Stuart	SMP 10
Sonicator	Elmasonic	S 100 H

3.3 Methodology

3.3.1 Synthesis of 1,2,4-Triazole

Synthesis of 1,2,4-triazole is done by refluxing 25 mmol of indol-3-butyric acid (5.08 g) with equimolar of thiocarbohydrazide (2.65 g) in 250 mL round bottom flask. Small amount of anti-bumping granules and a magnetic stirring bar are added into the reaction mixture. The reaction mixture is refluxed in an oil bath at 140 ℃ for 7 hours. The completion of reaction is monitored using TLC.

The reaction is said completed when the spot of crude product on TLC plate is at different level with the two starting materials. Once the reaction is completed, the reaction mixture is left to cool at room temperature and washed with sodium bicarbonate solution. This is to remove excess indole-3-butyric acid in the mixture. Sodium bicarbonate solution is prepared by dissolving solid sodium bicarbonate in sufficient distilled water. If the solid is unable to dissolve completely or the solution appears to be cloudy, it is then filtered through filter paper to obtain a clear solution. The clear filtrate (sodium bicarbonate solution) is then added into the round bottom flask containing crude product of 1,2,4-triazole.

The mixture is sonicated with heat to dissolve the crude product. As the solid dissolved, the mixture is left aside to settle down. The mixture appeared to be two immiscible layers after settle down, in which one is organic layer (upper

layer) and another is aqueous layer (lower layer). The organic layer is extracted and transferred to a clean conical flask. Sodium bicarbonate solution is added once again and sonicated to get rid of possible trace amount of starting material in the organic layer.

The mixture subjected to suction filtration, washing with cold ethanol and recrystallization using hot ethanol for purification. TLC is done before and after recrystallization to determine the purity of product. The percentage yield of pure 1,2,4-triazole is calculated. In the meantime, the structure of recrystallized pure product is characterized and elucidated using ^{1}H NMR, ^{13}C NMR, DEPT, HMQC, HMBC and melting point apparatus.

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

3.3.2 Synthesis of 1,2,4-triazole Schiff Base

1,2,4-triazole Schiff base is synthesized by refluxing the pre-synthesized 1,2,4-triazole, namely 3-[(1H-indol-3-yl)-propyl]-4-amino-1H-1,2,4-triazole-5(4H)-thione, with several benzaldehydes listed in Table 3.31.

Table 3.31: Amount of Different Benzaldehyde Used in the Synthesis

Benzaldehyde	**Molecular Weight, g mol^{-1}**	**Amount used for synthesis, (4.0 mmol)**
2,4-dichlorobenzaldehyde	175.02	0.70 g
3,4-dichlorobenzaldehyde	175.01	0.70 g
4-methoxybenzaldehyde	136.15	0.486 mL
4-fluorobenzaldehyde	124.11	0.421 mL
2-fluorobenzaldehyde	124.11	0.421 mL

Mass of reagent needed = Number of mole (mol) × Molecular weight (g/mol)

Volume of reagent needed = mass of reagent needed (g) / Density of reagent (g/mL)

4.0 mmol of starting material, 1,2,4-triazole (1.10 g), is added with 4.0 mmol of benzaldehyde in a 50 mL round bottom flask. An optimum amount of catalyst, (+)-tartaric acid (0.25 g), is added into the reagent mixture, followed by 15 mL of absolute ethanol, to act as the solvent. A few anti-bumping granules are added into the mixture to prevent sudden boiling and at the same time, a magnetic stirring bar is added to stir the mixture evenly and continuously. The mixture in round bottom flask is subjected to reflux in an oil

bath at 90 ℃. The reaction process is monitored using Thin Layer Chromatography.

Once the reaction is completed, the mixture is left to cool to room temperature. A small amount of ice is added into the mixture and stirred to melt in order to aid the process of precipitation. The crude product is then filtered using vacuum suction and washed using cold distilled water. It is then purified by recrystallization using hot ethanol. Lastly, the product is washed with cold ethanol to get rid of impurities residue.

The percentage yield product is calculated using the formula below. Purity of the product is determined using TLC method. Meanwhile, the structure of recrystallized pure product is characterized using NMR spectrometer and melting point apparatus.

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

3.3.3 Recrystallization

The crude product is purified by recrystallization after filtration. Hot ethanol is prepared by heating on a hot plate. A sufficient amount of hot ethanol is added into the crude product to dissolve it. The solution undergoes hot filtration through cotton to get rid of the insoluble impurities such as dust, filter paper residue and others. During hot filtration, the glassware used is preheated to prevent the solution from crystallize in room temperature. This will cause the product to retain on the cotton and filter funnel and eventually cause loss of product and results in reduced percentage yield. A clean and pre-rinsed beaker is used to collect the filtrate. A minimum amount of hot ethanol is added to preheat the beaker. The vapour from solvent will keep the glassware warm. The solution has to remain hot during the filtration process so that no crystallization occurs that the product will retained on the cotton. Small amount of hot ethanol is used to rinse the glass rod and the beaker that initially used to contain the crude product.

After the hot filtration, the clear filtrate is remained heated on the hotplate to concentrate it to a minimum amount. The saturated solution is then cooled to room temperature and left aside for days to allow crystallization. An aluminium foil with a few holes is used to cover the beaker to prevent dust and impurities and at the same time, allow ethanol to vapourize. If the crystallization does not spontaneously, scratching the inner wall of beaker using glass rod just below the surface of the solvent would produce the seed of crystallization and this will aid the crystallization to occur. However, if the

crystallization does not occur after few days, the saturated solution has to undergo concentration or recrystallization again. Next, the solution is immersed in an ice bath to enhance the crystallization process.

TLC technique is performed before and after the recrystallization process. This is to compare the purity of Schiff base product. If there is only one spot observed from the TLC plate, then the product is assumed to be pure. However, the precision and accuracy of purity of product are not secured as TLC technique might not be able to detect small impurities presence. Further characterization and examination using instrumental analysis is required to determine the structure and purity of the compounds.

3.4 Characterization

3.4.1 Melting Point Apparatus

Stuart SMP10 melting point apparatus is used to determine the melting point of the Schiff base compounds. Through the melting point, a brief idea of the compound purity can be determined. A pure compound will give a narrow range of melting point whereas those with less purity will have larger range of melting point. This is because presence of impurities in compound will cause the melting point to deviate and results in wider melting point range.

The range of melting point is determined by recording the temperature at which the compound started to melt to the temperature at which the sample is melted completely.

The Schiff base compound is grinded finely and filled into an open capillary tube. The capillary tube is then inserted into the melting point apparatus with a temperature set around 200℃. The melting process is observed through a magnifying scope. The determination of melting point is repeated at least twice to obtain a precise and constant melting point range.

3.4.2 Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectroscopy is a research technique that determined the physical and chemical properties of atoms or molecules via exploiting the magnetic properties of certain atomic nuclei. The most commonly studied nuclei are carbon and hydrogen. NMR spectroscopy can be used to determine the content and purity of a compound as well as its molecular structure.

In this project, the model of NMR spectrometer used to characterize the Schiff base compounds is JEOL ECX-400 NMR Spectrometer. The irradiation frequency of proton NMR is ~400 MHz while the irradiation frequency of carbon-13 NMR is ~ 100 MHz. apart from ^{1}H NMR and ^{13}C NMR, Distortionless Enhancement by Polarization Transfer (DEPT), Heteronuclear Multiple Quantum Coherence (HMQC) and Heteronuclear Multiple Bond Coherence (HMBC) are obtained from NMR spectrometer. By analyzing and elucidating these spectra, the structure of compounds can be characterized.

To prepare NMR sample, 10 mg of each derivative sample inside a sample vial is dissolved in minimum amount of DMSO-d_6 and diluted with small amount of deuterated chloroform. The sample is then subjected to sonicate to ensure dissolve completely. The dissolved sample is then transferred to a NMR tube and filled up to around 4 cm height using a clean dropper to prevent contamination. The NMR tube is then labelled and ready to be analyzed. Tetramethylsilane (TMS) is used as an internal standard for the analysis.

3.4.3 Thin Layer Chromatography (TLC)

TLC technique is a chromatography technique used to separate non-volatile mixture. However, in this project, TLC is used to monitor the completion of reaction and to determine the purity of the product by observing the number of spot (compounds) present on the TLC plate. The TLC used is an aluminium foil coated with silica gel. Linear lines are drawn at 1 cm from the edge of the TLC plate to indicate the solvent front and base line. The outline of the TLC plate is shown in Figure 3.1. A minimum amount of reaction mixture removed from refluxing system is diluted with small amount of chloroform and acetone. The diluted mixture is then spotted on the base line using a capillary tube. The starting materials, 1,2,4-triazole and respective benzaldehydes, are dissolved in acetone and chloroform separately and spotted on the same TLC plate with the product to act as a reference. The spot would be at least 0.5 cm away from each other. The spotted TLC plate is examined under UV light to ensure that the amount of spots applied is adequate.

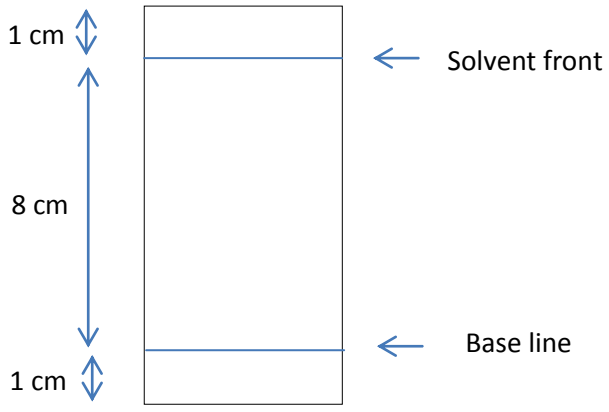


Figure 3.1: Outline of TLC plate

The solvent used is a mixture of ethyl acetate and hexane in a ratio of 1 : 1. 6 mL of each solution is measured and poured into a covered container. The solvent bath prepared must not higher than the base line. The TLC plate is immersed into the solvent bath and left to stand without any disturbance. Once the solvent reached solvent front via capillary action, the TLC plate is removed from the container. It is then observed under UV lamp for the separated spots.

The spots observed from the TLC plate under UV lamp appear to be in one spot (for a pure compound) or maximum two spots (due to excess compounds or impurities). The spots that appear at the same distance from the base line show that the compounds are identical.

Apart from determining the purity of compounds, it is also used to compare the polarity of 1,2,4-triazole Schiff base compounds. The distance of spot of respective derivatives from base line is recorded. Retention factor, R_f, of the compound are calculated using the formula below:

$$\text{Retention factor} = \frac{\text{Distance travelled by sample from base line}}{\text{Distance travelled by solvent front from base line}}$$

3.5 Antioxidant Activity Analysis

2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) assay is used to determine the antioxidant activity of the Schiff base compounds synthesized. GENESYS 10S UV-Vis Spectrophotometer is used to study the amount of the purple colour stable DPPH radical been reduced by a series of sample solution with known concentration. The absorbance is read at wavelength of 517 nm. This is because the deep purple colour of DPPH can only be detected around this wavelength.

500 ppm of sample is initially prepared by dissolving 5 mg of sample in 10 mL of methanol in a 10 mL volumetric flask. A serial dilution is performed to prepare a series of sample with concentration: 200 ppm, 100 ppm, 50 ppm, 25 ppm, 12.5 ppm and 6.25 ppm, from the stock solution by diluting with methanol. 1 mL of sample of each concentration is transferred to a sample vial with cover. This process is performed using a micropipette to enhance the accuracy.

The standard used in antioxidant activity determination is butylated hydroxyanisole (BHA). It acts as a reference to compare the antioxidant activity of each sample against DPPH radical. The preparation of standard solution is same as the preparation of sample solution.

3.84 mg of DPPH is added into a 100 mL volumetric flask and topped up with methanol to prepare 0.1 mM DPPH solution. The DPPH solution is inverted few times and allowed to stand for few minutes for homogenous mixing. 4 mL of DPPH solution is added into the sample series and shook vigorously for homogenous mixing. Since DPPH is a photo-sensitive reagent, the sample solution is covered with aluminium foil and incubated in the dark for 30 minutes. The absorbance of each solution is measured at 517 nm using the single beam UV-Vis spectrophotometer.

A blank solution is prepared by mixing methanol and DPPH solution in a ratio of 1 : 4 to eliminate the absorbance that caused by the solution other than the analyte. The antioxidant activity is repeated twice to obtain a constant value of $IC_{50.}$ IC_{50} is the effective concentration required for 50% reduction of DPPH free radical after 30 minutes. This value is obtained from the graph of percentage radical scavenging against concentration calibrated. The percentage radical scavenging is calculated using the formula below:

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

Where, A_{blank} and A_{sample} are absorbance of blank and sample respectively.

3.6 Calculation

- Mass = Molecular weight (g mol^{-1}) × Number of mole (mol)
  - Used to calculate mass of starting material required for the synthesis.

- Volume = Mass (g) / Density (g mL^{-1})
  - Used to calculate the volume of liquid starting material required for the synthesis.

- Percentage yield = $\frac{\text{Experimental mass of product}}{\text{Theoretical mass of product}} \times 100\%$
  - Used to calculate the percentage of product obtained from the synthesis.

- Retention factor = $\frac{\text{Distance travelled by sample from base line}}{\text{Distance travelled by solvent front from base line}}$
  - Used to calculate the fraction of an analyte in the mobile phase of a chromatographic system.

- Percentage radical scavenging = $\frac{(A_{blank} - A_{sample})}{A_{blank}} \times 100\ \%$
  - Used to determine the effective concentration required to reduce 50% of a specific biological process.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Synthesis of 1,2,4-triazole

Equimolar of indole-3-butyric acid and thiocarbohydrazide are prepared in a round bottom flask. Both reagents are in solid form in the room temperature. A few anti-bumping granules are added to prevent over-boiling and spilling of mixture. Magnetic stirring bar is added into the reaction mixture to ensure even and continuous stirring for homogenous reaction. Fusion only occur when temperature of reaction mixture reach the boiling point of both reagents. Hence, the reaction mixture is refluxed in an oil bath 140 ℃ for 7 hours. Oil bath is chosen instead of using water bath due to several reasons. The boiling point of oil is higher than that of water and the specific heat capacity of oil is greater than that of water. Thus oil bath is preferred over water bath.

Sodium bicarbonate is added into the crude product of 1,2,4-triazole. This step is to remove excess indole-3-butyric acid. The crude product is then filtered using vacuum suction to filter off macro sized impurities and washed with cold ethanol. Recrystallization using hot ethanol is carried out to further purify the product. The hot ethanol is used to dissolve solid product before filtered through cotton wool. The clear filtrate is concentrated to around 50 mL and left to crystallize in room temperature. TLC analysis before and after recrystallization is used to compare and determine the purity of product

obtained. The pure product is then subjected to instrumental analysis for characterization of structure. The synthesis pathway of 1,2,4-triazole via condensation of indole-3-butyric acid and thiocarbohydrazide is shown in Figure 4.1.

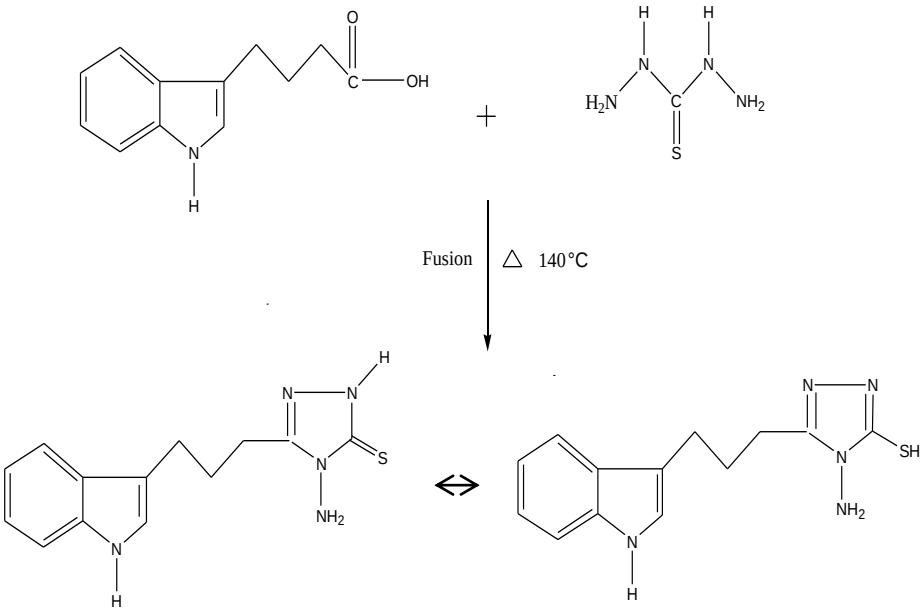


Figure 4.1 Synthesis Pathway of 1,2,4-triazole

4.1.1 Discussion on 3-[(1H-indol-3-yl)-propyl]-4-amino-1H-1,2,4-triazole-5(4H)-thione

1,2,4-triazole synthesized is one of the starting materials used in Schiff base synthesis. Instrumental analysis such as ^{1}H NMR, ^{13}C NMR, DEPT, HMQB and HMBC spectroscopic method were carried out to characterize the compound obtained. The structure and physical properties of 1,2,4-triazole are shown in Figure 4.2 and Table 4.1 respectively.

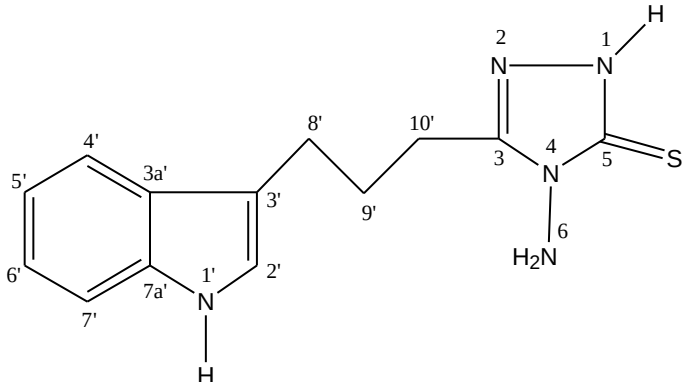


Figure 4.2: Structure of 3-[(1H-indol-3-yl)-propyl]-4-amino-1H-1,2,4-triazole-5(4H)-thione

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

Physical Appearance	Brown solid
Melting Point (°C)	171-173
Percentage yield (%)	54
Molecular Formula	$C_{13}H_{15}N_5S$
Molecular Weight (g mol^{-1})	273.36
R_f value [Ethylacetate : Hexane – 2 : 1]	0.38

From ^{1}H NMR spectrum in Figure 4.3, NH-1 and NH-1’ signals appeared at the downfield region, which is δ 13.32 and δ 10.55 respectively. This high chemical shift is due to the deshielding effect contributed by the aromatic ring. Signal at δ 5.33 belongs to NH-6. This signal is not as deshielded as NH-1 and NH-1’ because it is not directly attached to the 5 membered aromatic ring. The two doublet signals at δ 7.42 and δ 7.25 belongs to the H-4’ and H-7’ from the aromatic ring respectively. Two triplet peaks at δ 6.98 and δ 6.89 belongs to H-6’ and H-5’. However, it can be observed that the intensity of triplet at H-6’ are higher than that of H-5’. Thus, there might be an overlapped signal peak at δ 6.98. Signals at δ2.72 (2H, t), δ 2.64 (2H, t) and δ 2.00 (2H, m) belongs to H-8’, H-10’ and H-9’respectively. These three peaks appear to be least deshielded because they are of aliphatic protons.

^{13}C NMR spectrum of 1,2,4-triazole is shown in Figure 4.5. According to spectrum, signal peaks at δ 166.07 and δ 152.51 belong to C-5 and C-3 respectively. This is because both carbon atoms are attached to electronegative atom such as sulphur (S) atom and nitrogen (N) atom. These electronegative elements tend to attract electron from C-5 and C-3, hence resulting to become deshielded. Signal peaks in the range of δ 110.0 to δ 140.0 belong to the indole carbon. These peaks appear at downfield is due to the delocalizing of electrons and resonance effect in the aromatic rings. Aliphatic carbons (C-8’,C-9’, C-10’) are least deshielded. Hence, the signal peaks appear at δ 26.62, δ 24.60 and δ 24.56. Signal peaks at δ 79.13 and δ 40.03 belong to the solvent peak of chloroform ($CDCl_3$) and deuterated DMSO respectively.

DEPT analysis is used to differentiate carbon atom from methyl, methylene and methane group. Those do not appear at DEPT but show signal peak at ^{13}C NMR indicate the presence of quaternary carbon. In DEPT-135 (Appendix A), there are 3 negative peaks in the range of δ 20.0 to δ 30.0, indicating three CH_2 group in the structure. These three peaks matched the carbons at C-8', C-9' and C-10'.

As for 2D NMR, HMQC shows short range coupling between carbon atom and proton across single bond correlation. The assignment of proton and carbon signals can be done by referring to their direct bonding. Long range coupling between carbon atom and proton across two to three bond correlations is shown in HMBC. In the HMQC spectrum (Appendix B), H-9' is directly bonded to C-9' at δ 26.62, which is a methylene group (CH_2). One bond correlation between carbon and proton in indole aromatic ring gave δ_H 7.42/ δ_C 118.66, δ_H 7.25/ δ_C 111.76, δ_H 7.01/ δ_C 122.62, δ_H 6.98/ δ_C 121.27 and δ_H 6.89/ δ_C 118.58, for H/C at position 4', 7', 2', 6' and 5' respectively. There are 3 signal peaks (δ 13.31, δ 10.55 and δ 5.33) that do not correlate with any carbon atom at ^{13}C of HMQC. This indicates that those are protons bonded to nitrogen atom. According to HMBC spectrum (Appendix C), H-9' peak is has correlation with four carbon peaks at δ 114.12 (C-3'), δ 152.51 (C-3), δ 24.60 (C-8') and δ 24.56 (C-10').

Table 4.2: Summary of ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectral data ($CDCl_3$/DMSO-d_6) of 1,2,4-triazole

Position	^{1}H (δH, ppm)	Remarks	^{13}C (δC, ppm)
1	13.32	1H, s	-
2	-	-	-
3	-	-	152.5
4	-	-	-
5	-	-	166.1
6	5.33	2H, s	-
1’	10.55	1H, s	-
2’	7.01	1H, s	122.6
3’	-	-	114.1
3’a	-	-	127.5
4’	7.42	1H, d, J = 7.96 Hz	118.7
5’	6.89	1H, t, J = 7.96 Hz	118.6
6’	6.98	1H, t, J = 7.96 Hz	121.3
7’	7.25	1H, d, J = 7.96 Hz	111.8
7’a	-		136.8
8’	2.72	2H, t, J = 7.32 Hz	24.6
9’	2.00	2H, q, J = 7.32 Hz	26.6
10’	2.64	2H, t, J = 7.32 Hz	24.6

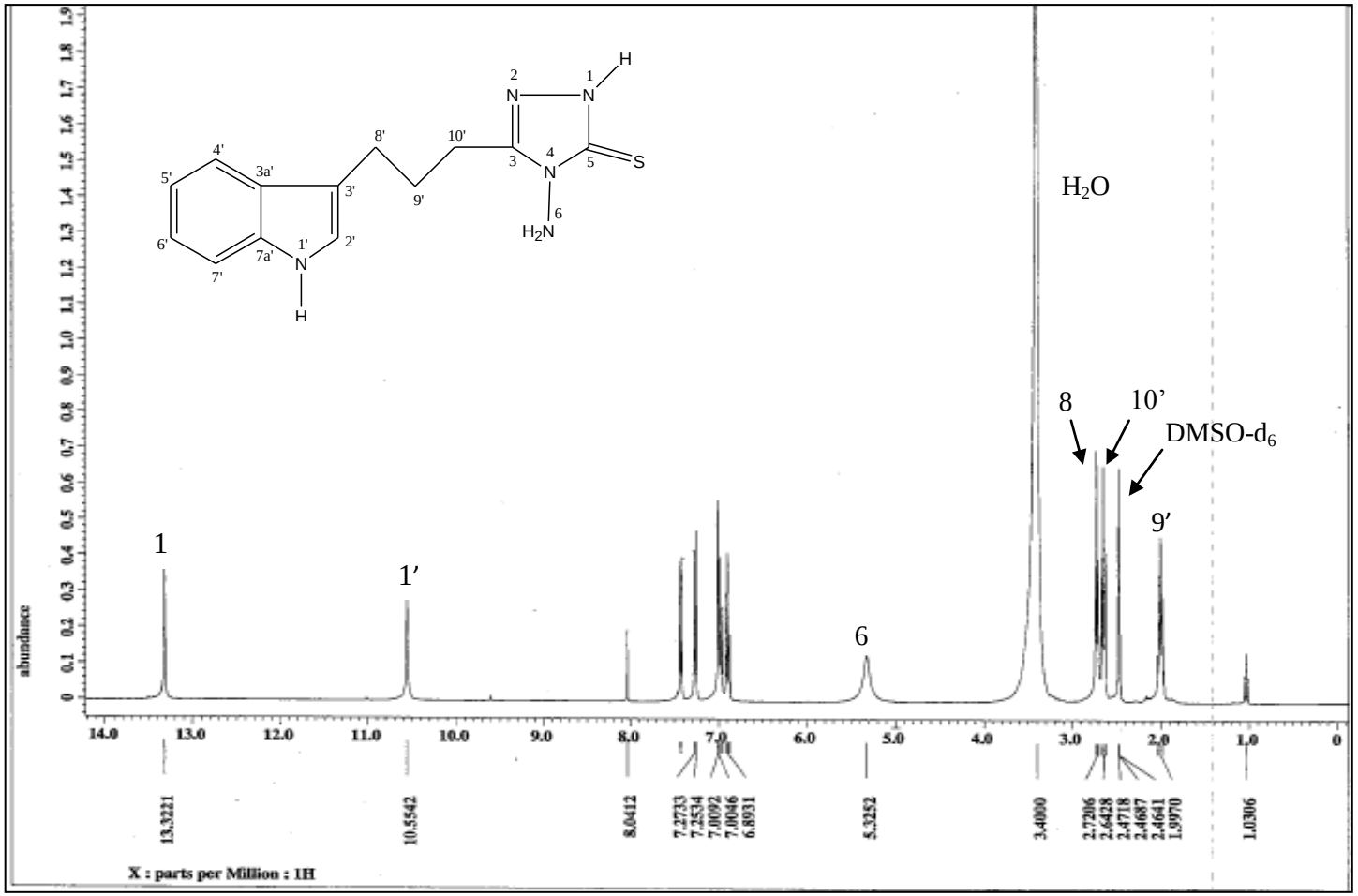


Figure 4.3: ^{1}H NMR Spectrum of 1,2,4-Triazole

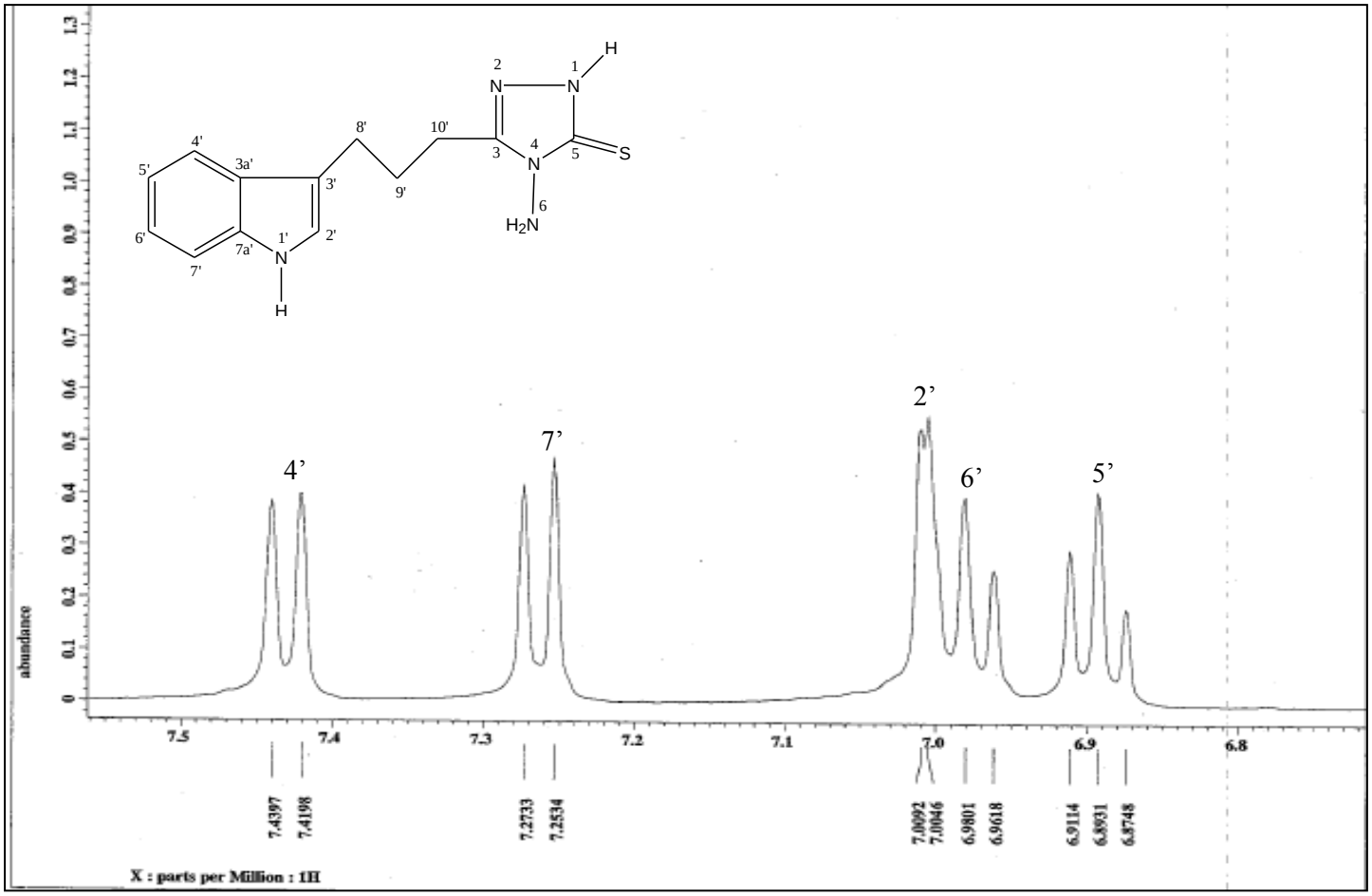


Figure 4.4: ^{1}H NMR Spectrum of 1,2,4-Triazole

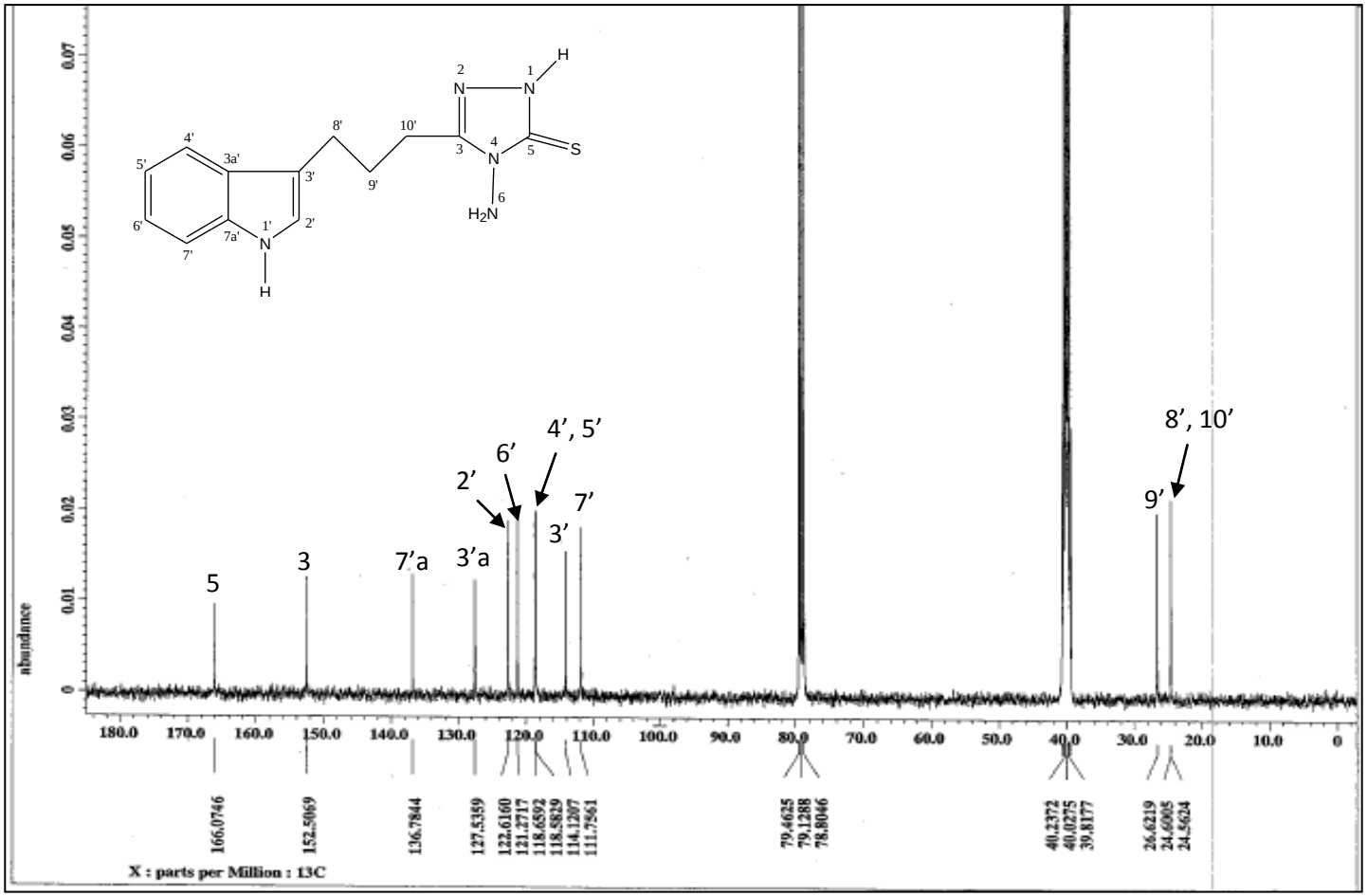


Figure 4.5: ^{13}C NMR Spectrum of 1,2,4-Triazole

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

4.2.1 Discussion on 3-[(1H-indol-3-yl)-propyl]-4-(2,4-dichlorobenzylideneamino)-1H-1,2,4-triazole-5(4H)-thione (SB 7)

Condensation reaction of 3-[(1H-indol-3-yl)-propyl]-4-amino-1H-1,2,4-triazole-5(4H)-thione and 2,4-dichlorobenzaldehyde in ethanol using tartaric acid as catalyst has yield **SB 7**. The structure and physical properties of compound **SB 7** are shown in Figure 4.6 and Table 4.3 respectively.

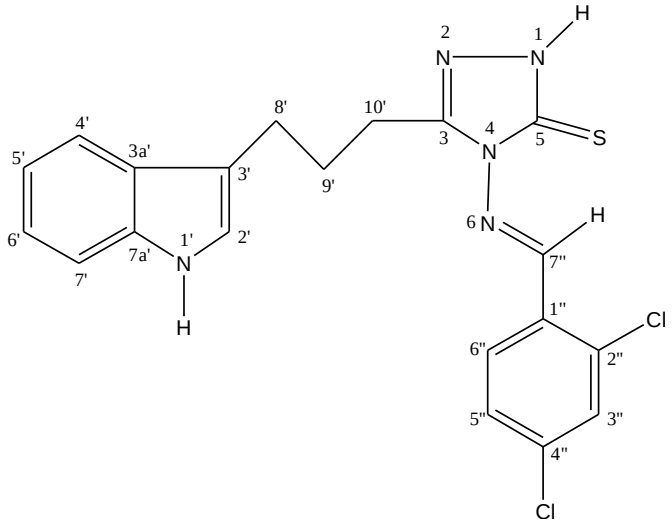


Figure 4.6: Structure of SB 7

Table 4.3: Summary of Physical Properties of SB 7

Physical Appearance	Yellow Solid
Melting Point (℃)	193 – 196
Percentage Yield (%)	33.0
Molecular Formula	$C_{20}H_{17}N_5SCl_2$
Molecular Weight (g mol^{-1})	430.3468
R_f value [Ethylacetate : Hexane – 1 : 1]	0.59

Based on ^{1}H NMR spectrum of **SB 7** in Figure 4.7, NH-1 and NH-1' show signal peaks at downfield, δ 13.58 and δ 10.40 respectively similarly as in 1,2,4-triazole since both proton are from 5 membered aromatic ring. H-7" (10.92) shows strong signal peak at downfield as it belongs to Schiff Base proton. Apart from that, there are a few signal peaks from **SB 7** that are similar as that of 1,2,4-triazole. The signal peaks of benzene ring, H-4' (δ 7.38), H-5' (δ 6.86), H-6' (δ 6.97) and H-7' (δ 7.24), do not have much different as compare to spectrum of 1,2,4-triazole. A singlet peak of H-3" are observed at δ 7.44 while doublet peaks are observed for H-5" and H-6" at δ 7.78 and δ 7.26 respectively. From Figure 4.9, the multiplet peak for H-9' appears at δ 2.03 whereas the overlapped triplet peak of H-8' and H-10' appears at δ 2.76.

By comparing ^{13}C NMR (Figure 4.10) and DEPT (Appendix D), there are 7 quaternary carbons belongs to C-3 (δ 152.19), C-5 (δ 161.82), C-3' (δ 113.98), C-3'a (δ 127.46), C-7'a (δ 136.77), C-2" (δ 138.07) and C-4" (δ 136.49). There are three negative peaks shown in DEPT-135, indicating CH_2 in the structure, C-8' (δ 24.73), C-9' (δ 27.00) and C-10' (δ 24.55). The correlation between proton and carbon atoms in the structure is further confirmed in HMQC and HMBC.

In HMQC (Appendix E), the doublet peaks showing at H-6" (7.26) and H-5" (δ 7.78) are directly correlated to C-6" (δ 128.17) and C-5" (δ 128.55) respectively. The H-3", on the other hand, is correlated to C-3" at δ 129.90. In

HMBC (Appendix F), Schiff base proton (H-7” at δ 10.92) has correlation with C-1” (δ 129.69), C-2” (δ 138.07) and C-6” (128.17).

Table 4.4: Summary of ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectral data ($CDCl_3$/DMSO-d_6) of SB 7

Position	^{1}H (δH, ppm)	Remark	^{13}C (δC, ppm)
1	13.58	1H, s	-
2	-	-	-
3	-	-	152.2
4	-	-	-
5	-	-	161.8
6	-	-	-
1’	10.40	1H, s	-
2’	6.94	1H, s	122.5
3’	-	-	114.0
3’a	-	-	127.5
4’	7.38	1H, d, J = 7.96 Hz	118.5
5’	6.86	1H, t, J = 7.96 Hz	118.6
6’	6.97	1H, t, J = 7.96 Hz	121.3
7’	7.24	1H, d, J = 7.96 Hz	111.7
7’a	-	-	136.8
8’	2.76	2H, t, J = 7.32 Hz	24.7
9’	2.03	2H, q, J = 7.32 Hz	27.0
10’	2.76	2H, t, J = 7.32 Hz	24.6
1’’	-	-	129.7
2’’	-	-	138.1
3’’	7.44	1H, s	129.9
4’’	-	-	136.5
5’’	7.78	1H, d, J = 7.92 Hz	128.5
6’’	7.26	1H, d, J = 7.92 Hz	128.2
7’’	10.97	1H, s	154.3

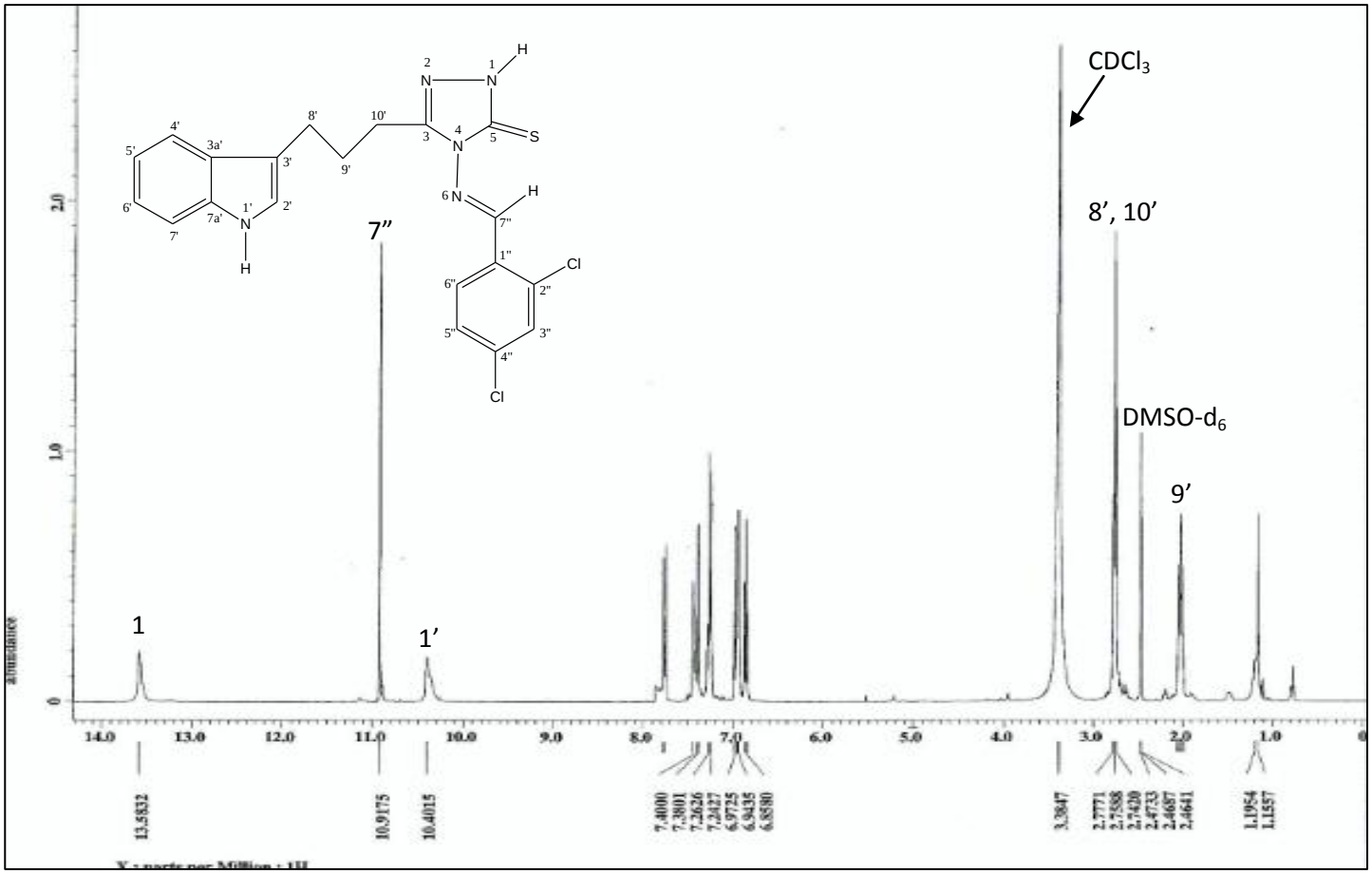


Figure 4.7: ^{1}H NMR Spectrum of SB 7

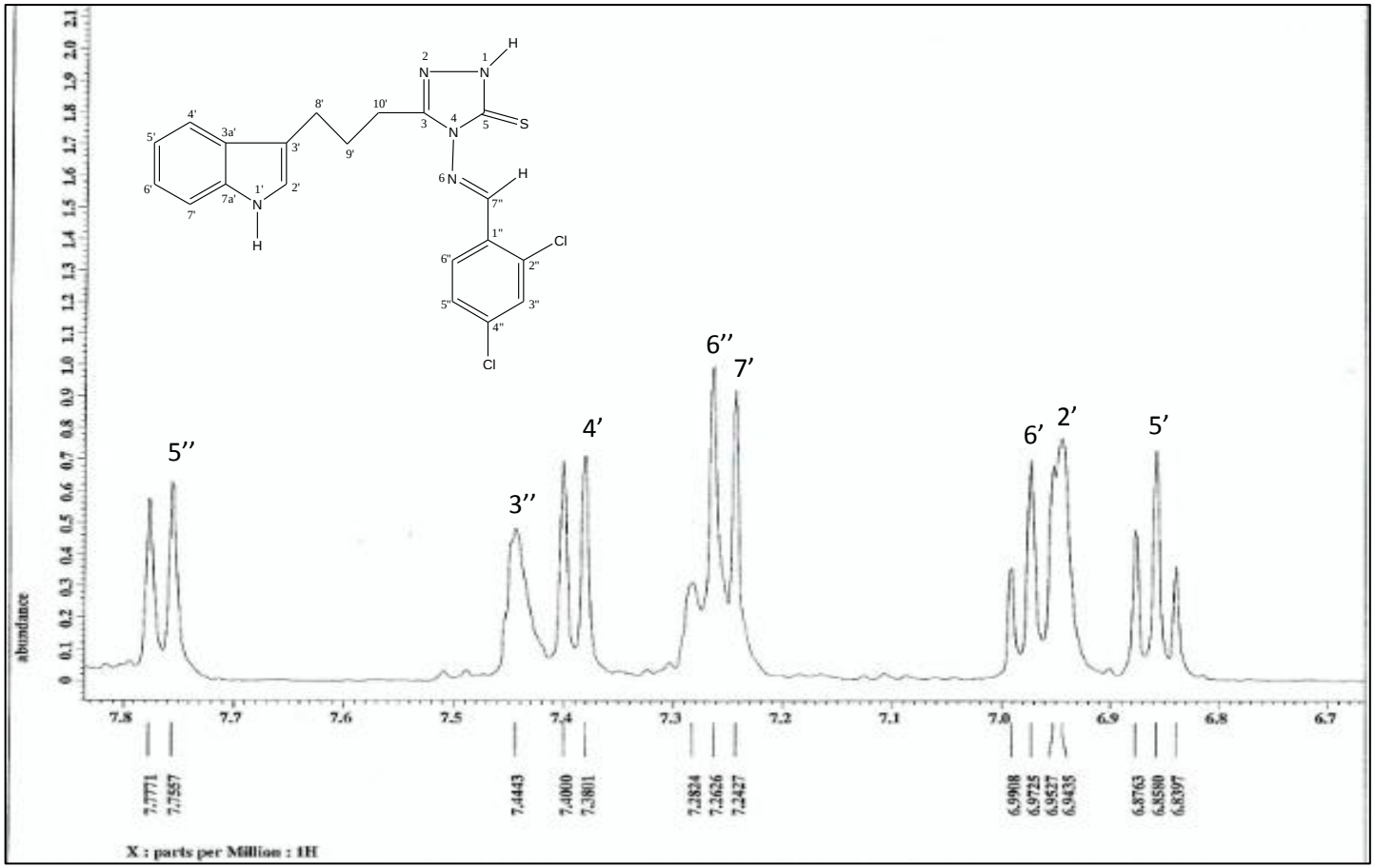


Figure 4.8: ^{1}H NMR Spectrum of SB 7

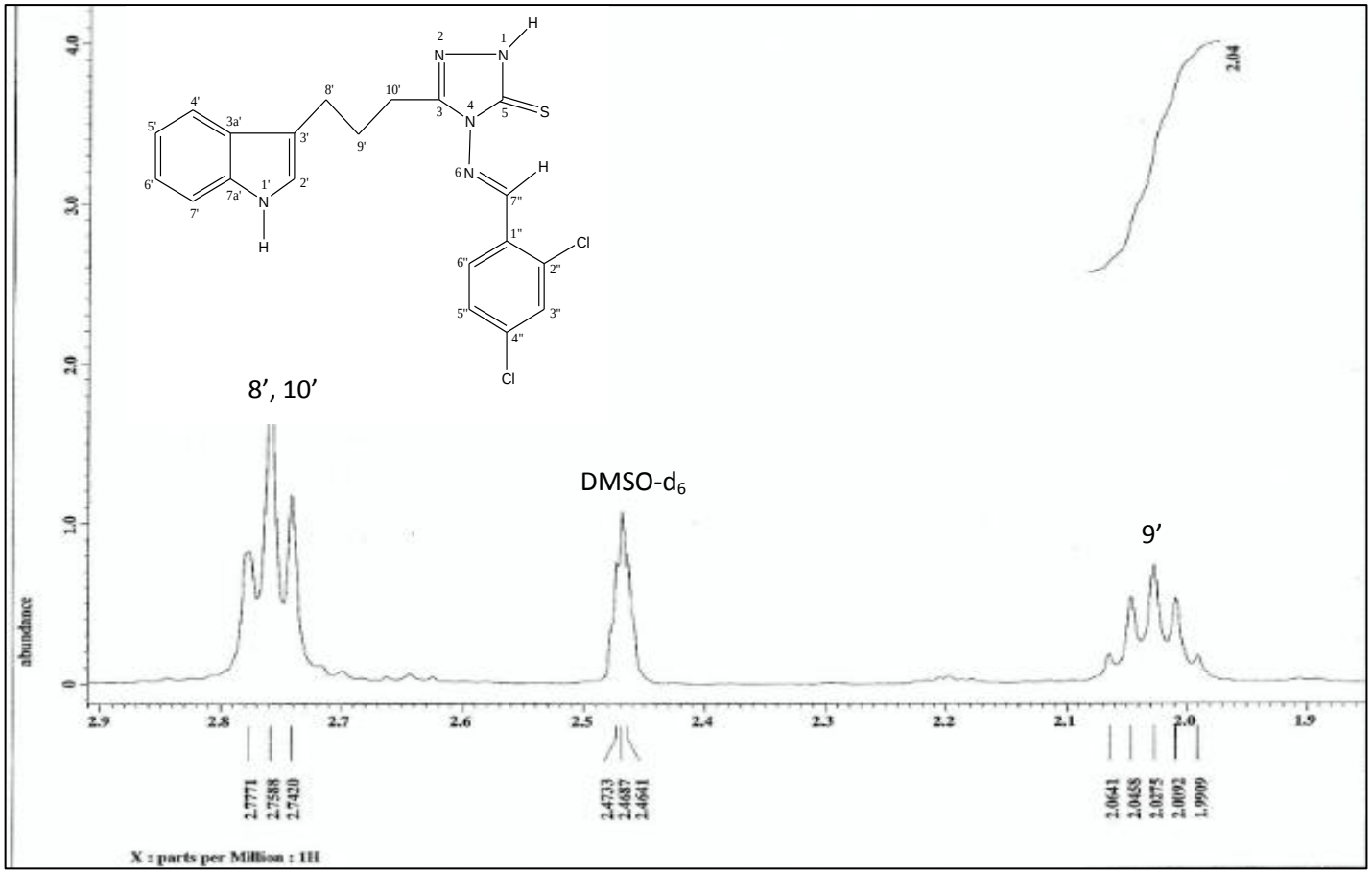


Figure 4.9: ^{1}H NMR Spectrum of SB 7

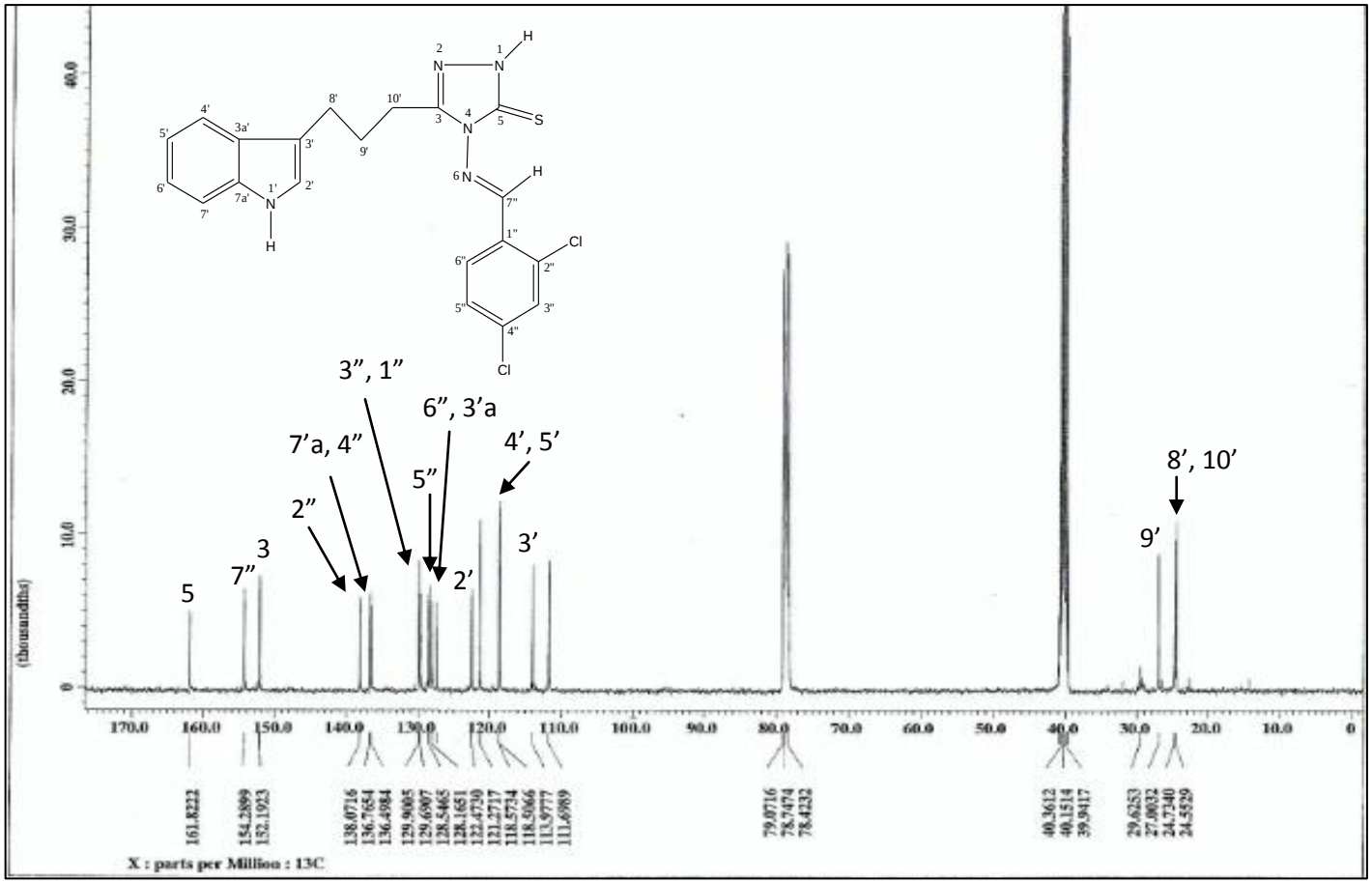


Figure 4.10: ^{13}C NMR Spectrum of SB 7

4.2.2 Discussion on 3-[(1H-indol-3-yl)-propyl]-4-(3,4-dichlorobenzylideneamino)-1H-1,2,4-triazole-5(4H)-thione (SB 8)

Condensation reaction of 3-[(1H-indol-3-yl)-propyl]-4-amino-1H-1,2,4-triazole-5(4H)-thione and 3,4-dichlorobenzaldehyde in ethanol using tartaric acid as catalyst has yield **SB 8**. The structure and physical properties of compound **SB 8** are shown in Figure 4.11 and Table 4.5 respectively.

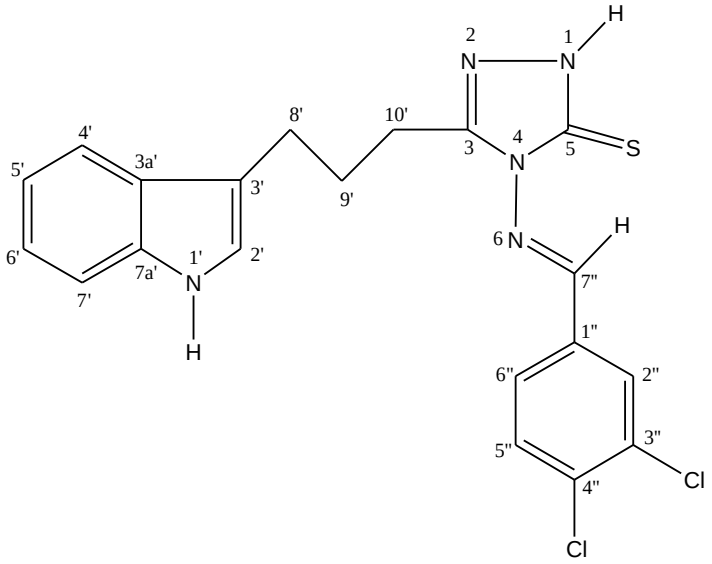


Figure 4.11: Structure of SB 8

Table 4.5: Summary of Physical Properties of SB 8

Physical Appearance	Pale Brown Solid
Melting Point (°C)	165 – 168
Percentage Yield (%)	49
Molecular Formula	$C_{20}H_{17}N_5SCl_2$
Molecular Weight (g mol^{-1})	430.3468
R_f value [Ethylacetate : Hexane – 1 : 1]	0.55

From the ^{1}H NMR spectrum in Figure 4.12**, SB 8** has similar chemical shift compound as **SB 7** at δ 13.66 (H-1), δ 10.56 (H-1'), δ 10.22 (H-7"), δ 7.38 (H-4'), δ 7.25 (H-7'), δ 6.97 (H-6'), δ 6.95 (H-2') and δ 6.85 (H-5'). Apart from that, the multiplet peak for H-9' appears at δ 2.03 and the overlapped triplet peak of H-8' and H-10' appears at δ 2.76, are also similar as for **SB 7**. A singlet peak of H-2" are observed at δ 7.89 while doublet peaks are observed for H-5" and H-6" at δ 7.58 and δ 7.61 respectively. Signal peaks at δ 2.47 and δ 3.38 are determined as DMSO-d_6 and $CDCl_3$ solvent peaks.

By comparing ^{13}C NMR (Figure 4.14) and DEPT (Appendix G), there are 8 quaternary carbons belongs to C-3 (δ 151.92), C-5 (δ 161.88), C-3' (δ 113.97), C-3'a (δ 127.49), C-7'a (δ 136.79), C-1" (δ 135.58), C-3" (δ 132.88) and C-4" (δ 133.36). There are three negative peaks shown in DEPT-135, indicating CH_2 in the structure, C-8' (δ 24.78), C-9' (δ 26.86) and C-10' (δ 24.56). The correlation between proton and carbon atoms in the structure is further confirmed in HMQC and HMBC.

In HMQC (Appendix H), the doublet peaks showing at H-6" (δ 7.61) and H-5" (δ 7.58) are directly correlated to C-6" (δ 128.12) and C-5" (δ 131.56) respectively. The H-2" (δ 7.89), on the other hand, is correlated to C-2" at δ 130.17. In HMBC (Appendix I), H-2" at δ 7.89 has correlation with C-1" (δ 135.58), C-3" (δ 132.88), C-6" (δ 128.12) and C-7" (δ 158.39).

Table 4.6: Summary of ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectral data ($CDCl_3$/DMSO-d_6) of SB 8

Position	^{1}H (δH, ppm)	Remarks	^{13}C (δC, ppm)
1	13.66	1H, s	-
2	-	-	-
3	-	-	151.9
4	-	-	-
5	-	-	161.9
6	-	-	-
1’	10.56	1H, s	-
2’	6.95	1H, s	121.3
3’	-	-	114.0
3’a	-	-	127.5
4’	7.38	1H, d, J = 7.92 Hz	118.6
5’	6.85	1H, t, J = 7.92 Hz	118.6
6’	6.97	1H, t, J = 7.92 Hz	122.6
7’	7.25	1H, d, J = 7.92 Hz	111.8
7’a	-	-	136.8
8’	2.76	2H, t, J = 7.32 Hz	24.8
9’	2.04	2H, q, J = 7.32 Hz	26.9
10’	2.76	2H, t, J = 7.32 Hz	24.6
1’’	-	-	135.6
2’’	7.89	1H, s	130.2
3’’	-	-	132.9
4’’	-	-	133.4
5’’	7.58	1H, d, J = 7.92 Hz	131.6
6’’	7.61	1H, d, J = 7.92 Hz	128.1
7’’	10.22	1H, s	158.4

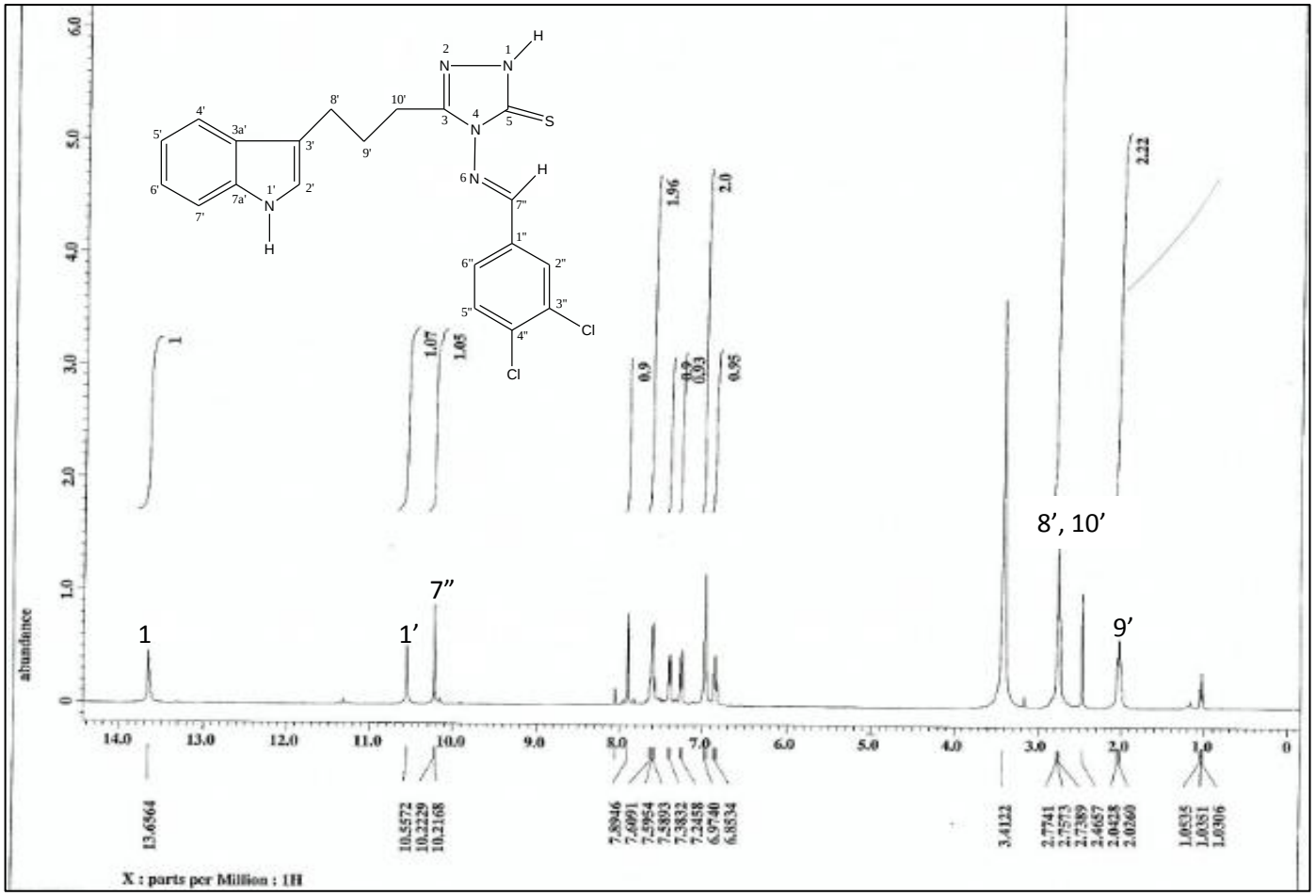


Figure 4.12: ^{1}H NMR Spectrum of SB 8

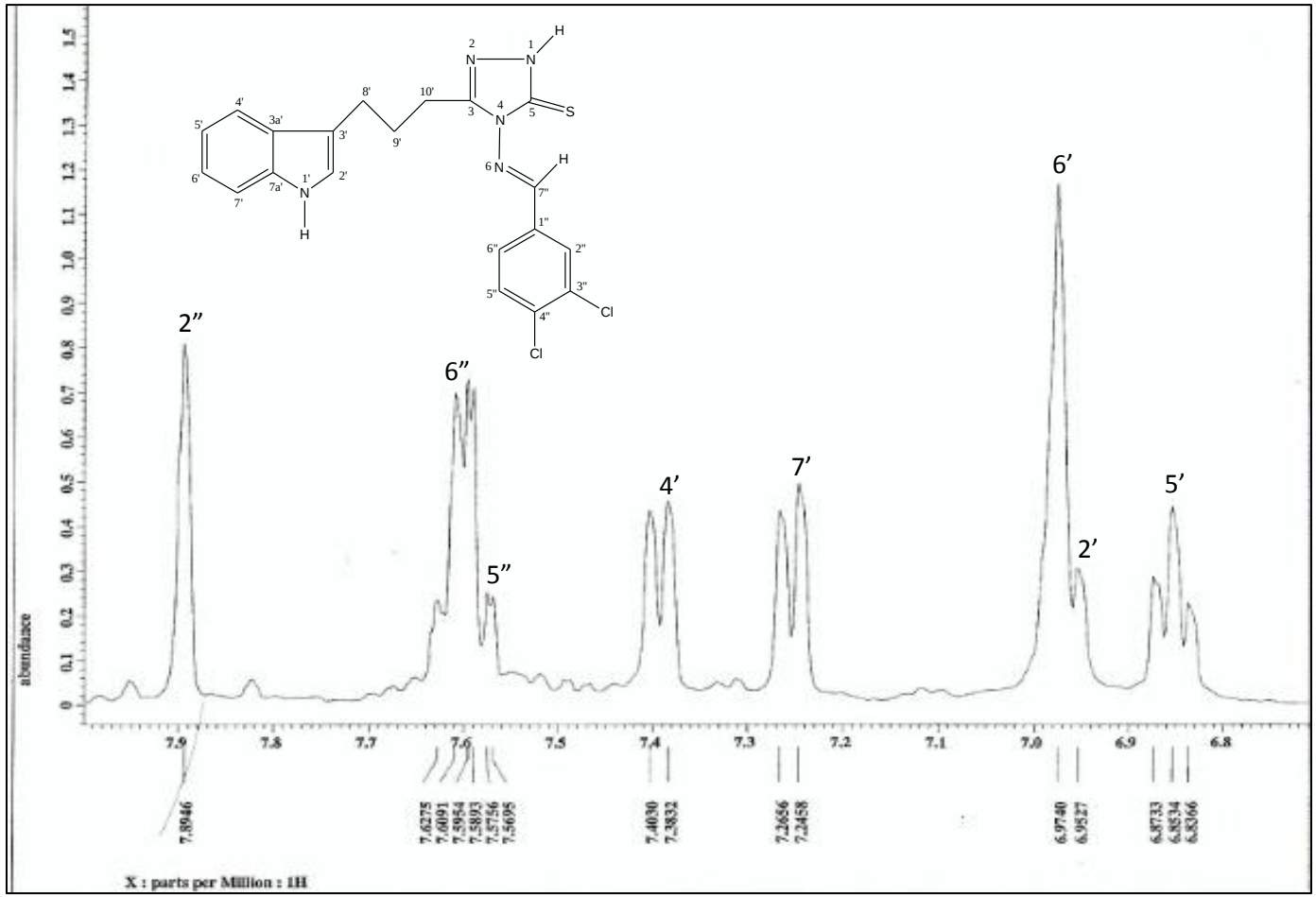


Figure 4.13: ^{1}H NMR Spectrum of SB 8

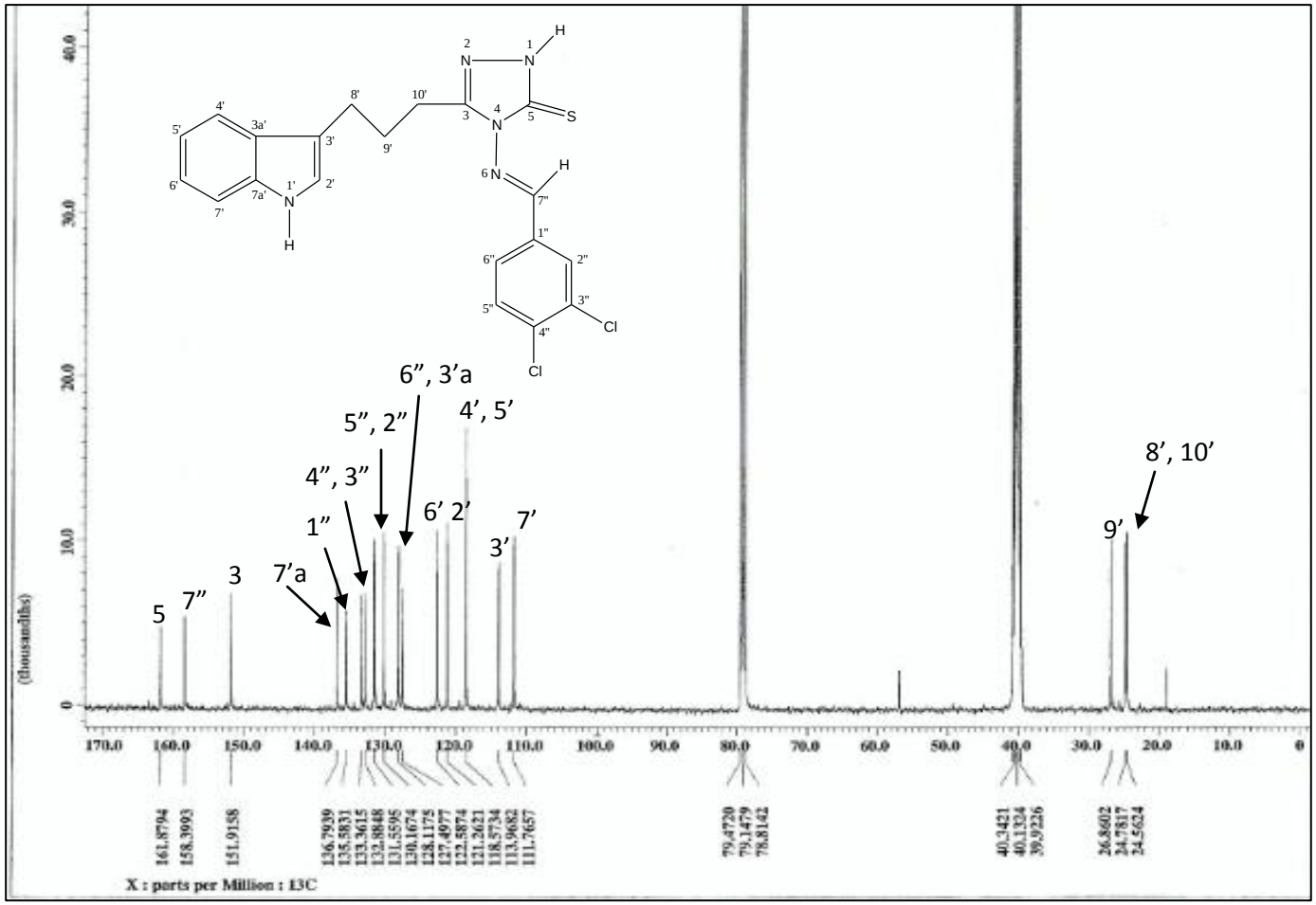


Figure 4.14: ^{13}C NMR Spectrum of SB 8

4.2.3 Discussion on 3-[(1H-indol-3-yl)-propyl]-4-(4-methoxybenzylideneamino)-1H-1,2,4-triazole-5(4H)-thione (SB 6)

Condensation reaction of 3-[(1H-indol-3-yl)-propyl]-4-amino-1H-1,2,4-triazole-5(4H)-thione and 4-methoxybenzaldehyde in ethanol using tartaric acid as catalyst has yield **SB 6**. The structure and physical properties of compound **SB 6** are shown in Figure 4.15 and Table 4.7 respectively.

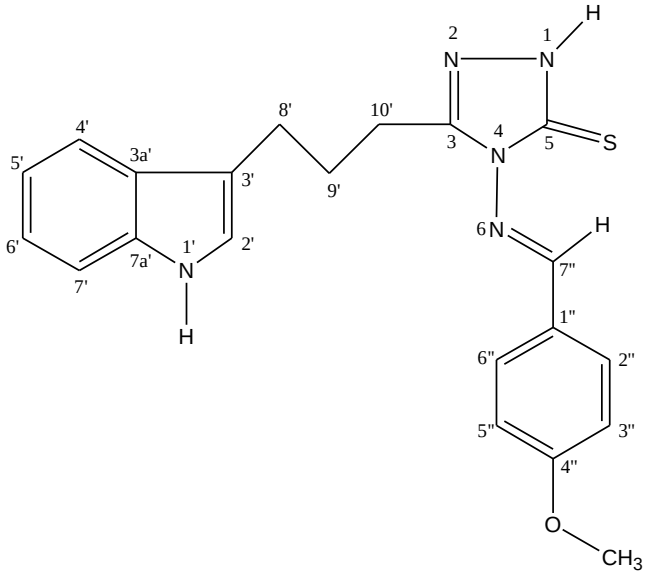


Figure 4.15: Structure of SB 6

Table 4.7: Summary of Physical Properties of SB 6

Physical Appearance	Brown Solid
Melting Point (°C)	186 – 189
Percentage Yield (%)	63
Molecular Formula	$C_{21}H_{21}N_5SO$
Molecular Weight (g mol^{-1})	301.49
R_f value [Ethylacetate : Hexane – 1 : 1]	0.45

According to the ^{1}H NMR spectrum in Figure 4.16, similarly, H-1, H-1' and H-7" shows signal peaks at high field region, δ 13.40, δ 10.19 and δ 9.89. The protons of indole ring have slight chemical shifting as compare to that of 1,2,4-triazole. The peaks appears at δ 6.91 (H-2'), δ 7.39 (H-4'), δ 6.86 (H-5'), δ 6.91 (H-6') and δ 7.24 (H-7'). The overlapping triplet peaks of H-8' and H-10' appears at δ 2.73 and δ 2.72 respectively while multiplet peak of H-9' appear at δ 2.02. As for the aromatic protons of Schiff base, H-2" and H-6" have overlapping peaks at δ 7.62 while the overlapping doublet peaks of H-3" and H-5" appears at δ 6.88. There is a singlet peak with strong intensity at δ 3.77 belongs to H-4" (-OCH_3). The strong intensity is due to the chemically equivalence of the three protons.

By comparing ^{13}C NMR (Figure 4.18) and DEPT (Appendix J), there are 7 quaternary carbons belongs to C-3 (δ 151.82), C-3' (δ 114.11), C-3'a (δ 127.40), C-7'a (δ 136.72), C-1" (δ 125.17) and C-4" (δ 163.03). There are three negative peaks shown in DEPT-135, indicating CH_2 in the structure, C-8' (δ 24.92), C-9' (δ 26.73) and C-10' (δ 24.59). The correlation between proton and carbon atoms in the structure is further confirmed in HMQC and HMBC.

In HMQC (Appendix K), the strong singlet peak of H-4" (-OCH_3) at δ 3.77 shows direct correlation with C-4" (-OCH_3) at δ 55.64. The Schiff base proton (H-7") at δ 9.89 is also directly correlated with C-7" at δ 161.54. In HMBC (Appendix L), H-6" at δ 7.62 has correlation with C-2" (δ 130.54) and C-4" (δ

163.03) while H-5” at δ 6.88 has correlation with C-4” (δ 163.03) and C-3” (δ 114.57).

Table 4.8: Summary of ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectral data ($CDCl_3$/DMSO-d_6) of SB 6

Position	^{1}H (δH, ppm)	Remarks	^{13}C (δC, ppm)
1	13.39	1H, s	-
2	-	-	-
3	-	-	151.8
4	-	-	-
5	-	-	132.0
6	-	-	-
1’	10.19	1H, s	-
2’	6.90	1H, s	122.3
3’	-	-	114.1
3’a	-	-	127.4
4’	7.39	1H, d, J = 7.96 Hz	118.5
5’	6.85	1H, t, J = 7.96 Hz	118.6
6’	6.97	1H, t, J = 7.96 Hz	121.3
7’	7.23	1H, d, J = 7.96 Hz	111.6
7’a	-	-	136.7
8’	2.73	2H, t, J = 7.32Hz	24.9
9’	2.01	2H, q, J = 7.32Hz	26.7
10’	2.71	2H, t, J = 7.32 Hz	24.6
1”	-	-	125.2
2”	7.62	2H, d, J = 8.56 Hz	130.5
3”	6.88	2H, d, J = 8.56 Hz	114.6
4”	-	-	163.0
5”	6.88	2H, d, J = 8.56 Hz	114.6
6”	7.62	2H, d, J = 8.56 Hz	130.5
7”	9.89	1H, s	161.5
4”-OCH_3	3.77	3H, s	55.6

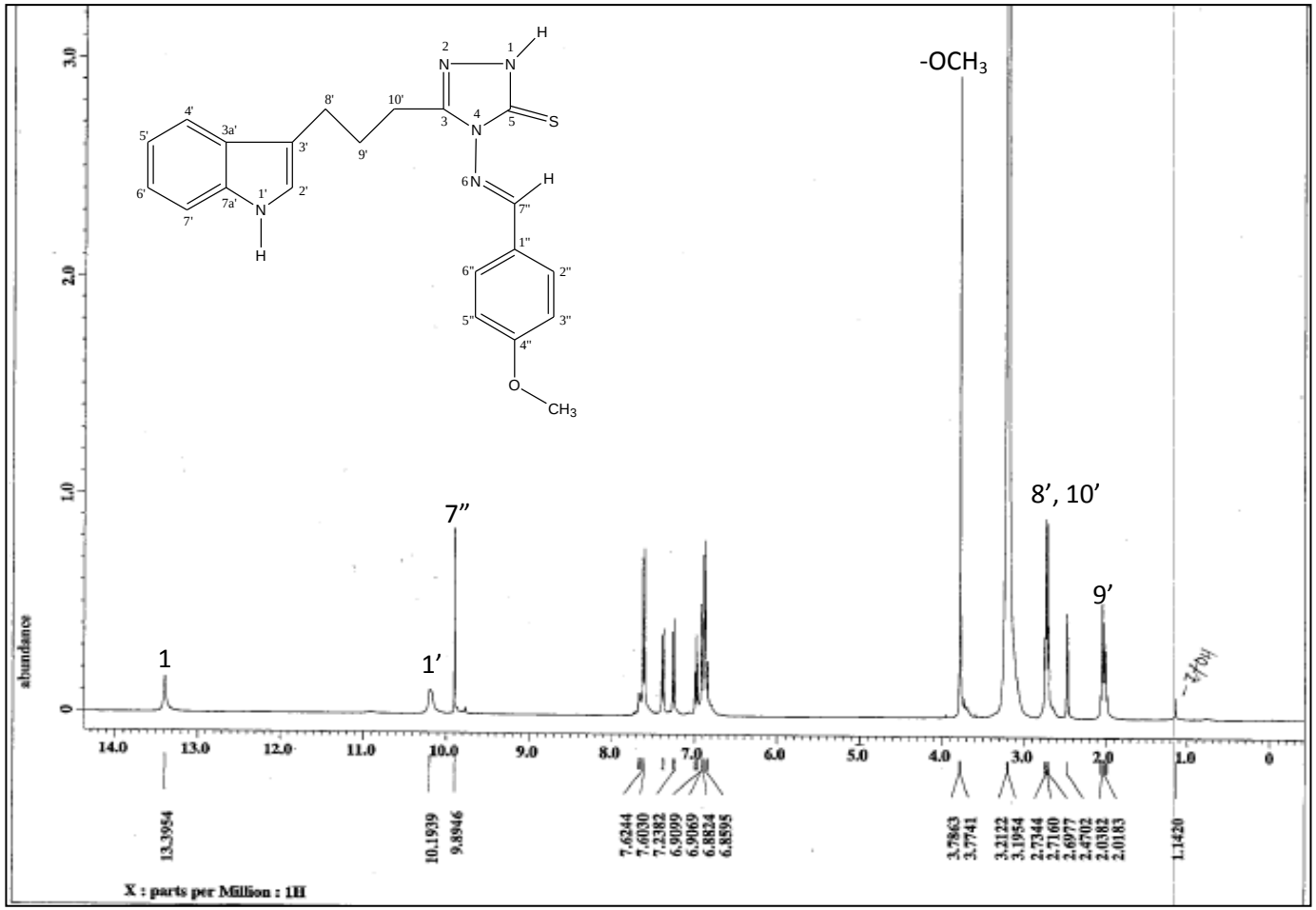


Figure 4.16: ^{1}H NMR Spectrum of SB 6

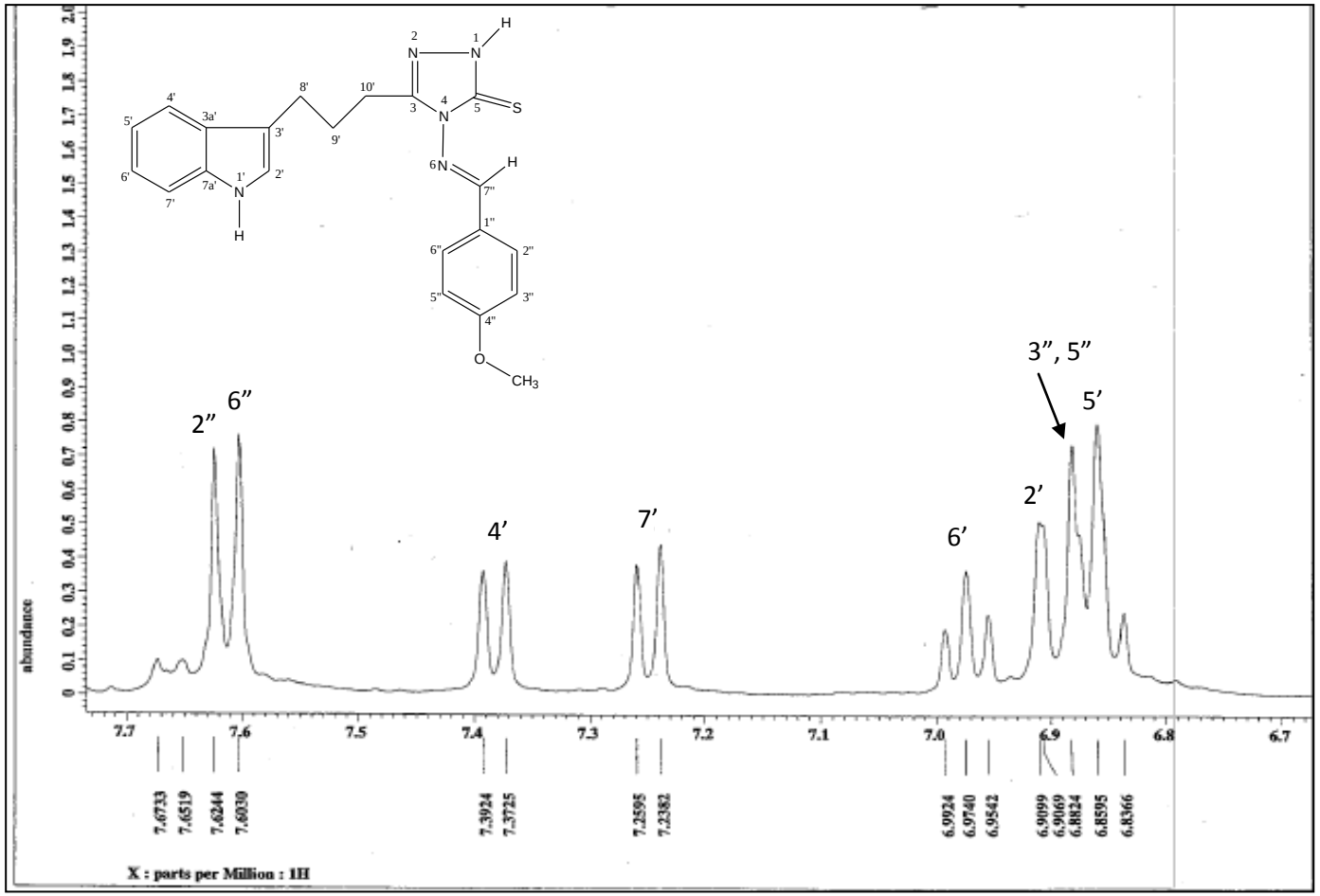


Figure 4.17: ^{1}H NMR Spectrum of SB 6

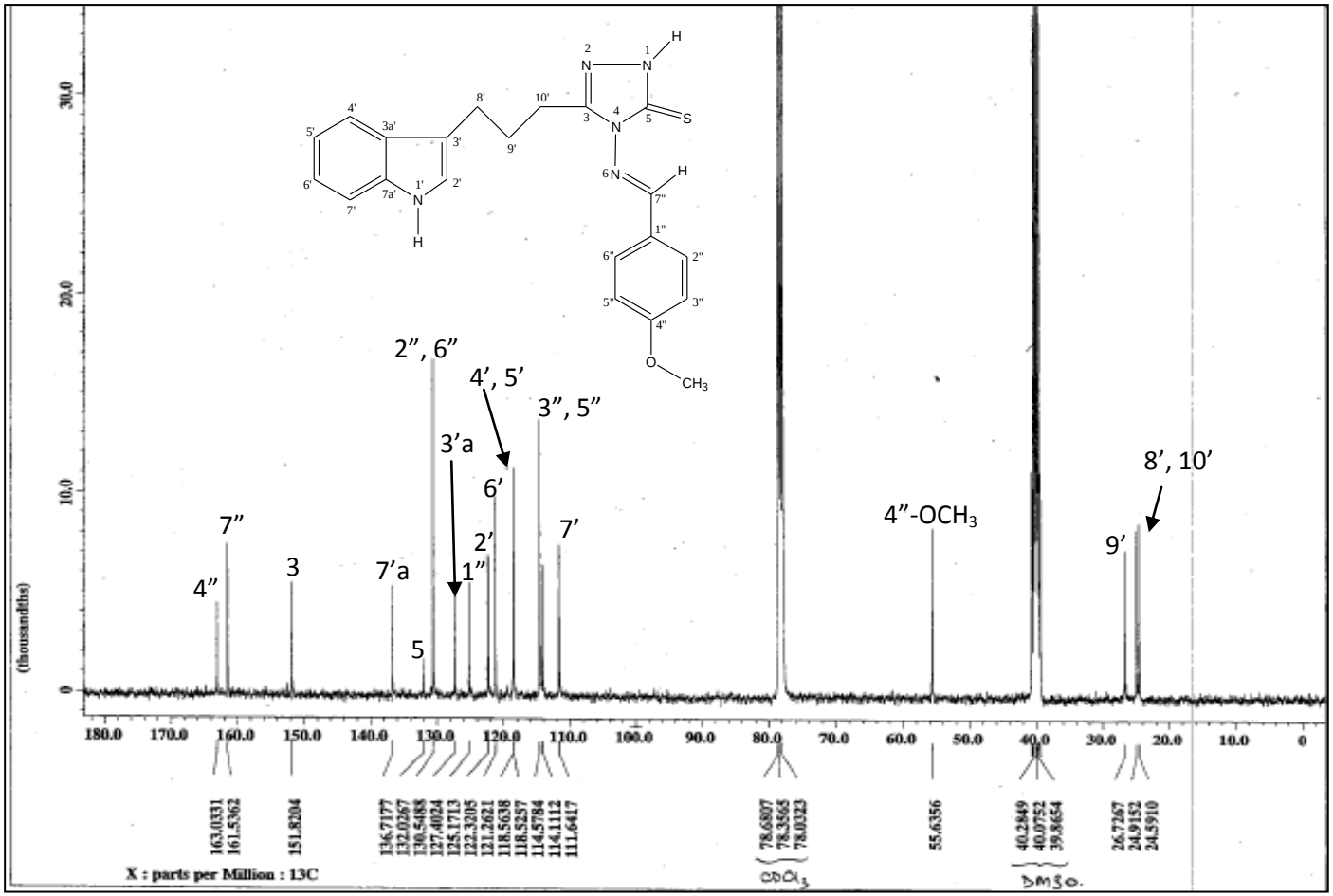


Figure 4.18: ^{13}C NMR Spectrum of SB 6

4.2.4 Discussion on 3-[(1H-indol-3-yl)-propyl]-4-(4-fluorobenzylideneamino)-1H-1,2,4-triazole-5(4H)-thione (SB 3)

Condensation reaction of 3-[(1H-indol-3-yl)-propyl]-4-amino-1H-1,2,4-triazole-5(4H)-thione and 4-fluorobenzaldehyde in ethanol using tartaric acid as catalyst has yield **SB 3**. The structure and physical properties of compound **SB 3** are shown in Figure 4.19 and Table 4.9 respectively.

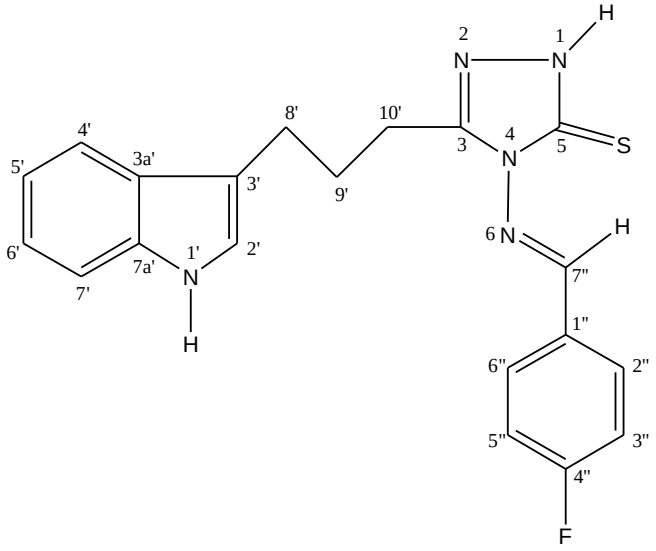


Figure 4.19: Structure of SB 3

Table 4.9: Summary of Physical Properties of SB 3

Physical Appearance	Brown Solid
Melting Point (°C)	186 – 188
Percentage Yield (%)	44
Molecular Formula	$C_{20}H_{18}N_5SF$
Molecular Weight (g mol^{-1})	379.4531
R_f value [Ethylacetate : Hexane – 1 : 1]	0.51

According to ^{1}H NMR in Figure 4.20, similar as other Schiff base compounds, H-1, H-1’ and H-7” appeared as the most deshielded protons, appearing at δ 13.63, δ 10.60 and δ 10.06 respectively. H-8’, H-9’ and H-10’ have the most shielding effect. The triplet peaks of H-8’ and H-10’ show signal peaks at δ 2.76 and δ 2.74 respectively while multiplet peak of H-9’ appear at δ 2.02. The protons of indole ring have slight chemical shifting as compare to that of 1,2,4-triazole. The peaks appears at δ 7.00 (H-2’), δ 7.40 (H-4’), δ 6.86 (H-5’), δ 6.97 (H-6’) and δ 7.26 (H-7’). The doublet peak of H-3” and H-5” overlapped each other and shows signals at δ 7.77. Same case is apply for H-2” and H-6”, their overlapped doublet signal peaks appears at δ 7.00 and δ 6.97 respectively. The overlapping might due to the reason that both protons at that particular position are symmetry to each other.

By comparing ^{13}C NMR (Figure 4.22) and DEPT (Appendix M), there are 7 quaternary carbons belongs to C-3 (δ 151.81), C-5 (δ 161.77), C-3’ (δ 113.98), C-3’a (δ 127.54), C-7’a (δ 136.82), C-1” (δ 129.29) and C-4” (δ 166.30). There are three negative peaks shown in DEPT-135, indicating CH_2 in the structure, C-8’ (δ 24.84), C-9’ (δ 26.90) and C-10’ (δ 24.58). The correlation between proton and carbon atoms in the structure is further confirmed in HMQC and HMBC.

In HMQC (Appendix N), the overlapped doublet peaks of H-3” and H-5” at δ 7.77 shows direct correlation with overlapped doublet peak of C-3” and C-5” at δ 131.15. The Schiff base proton (H-7”) at δ 10.06 is also directly correlated

with C-7" (160.81). In HMBC (**Appendix O**), H-2' at δ 7.00 has correlation with C-3' (δ 113.98), C-3'a (δ 127.54), C-4' (δ 118.59) and C-7' (δ 111.79).

Table 4.10: Summary of ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectral data ($CDCl_3$/DMSO-d_6) of SB 3

Position	^{1}H (δH, ppm)	Remarks	^{13}C (δC, ppm)
1	13.63	1H, s	-
2	-	-	-
3	-	-	151.8
4	-	-	-
5	-	-	161.8
6	-	-	-
1'	10.60	1H, s	-
2'	7.00	1H, s	122.7
3'	-	-	114.0
3'a	-	-	127.5
4'	7.40	1H, d, J = 7.96 Hz	118.6
5'	6.86	1H, t, J = 7.96 Hz	118.6
6'	6.97	1H, t, J = 7.96 Hz	121.3
7'	7.26	1H, d, J = 7.96 Hz	111.8
7'a	-	-	136.8
8'	2.76	2H, t, J = 7.32 Hz	24.8
9'	2.02	2H, q, J = 7.32 Hz	26.9
10'	2.74	2H, t, J = 7.32 Hz	24.6
1''	-	-	129.3
2'',6''	7.76	2H, d, J = 7.92 Hz	131.1
3'',5''	7.20	2H, dd, J = 7.92, 5.48 Hz	116.5
4''	-	-	166.3
7''	10.06	1H, s	160.8

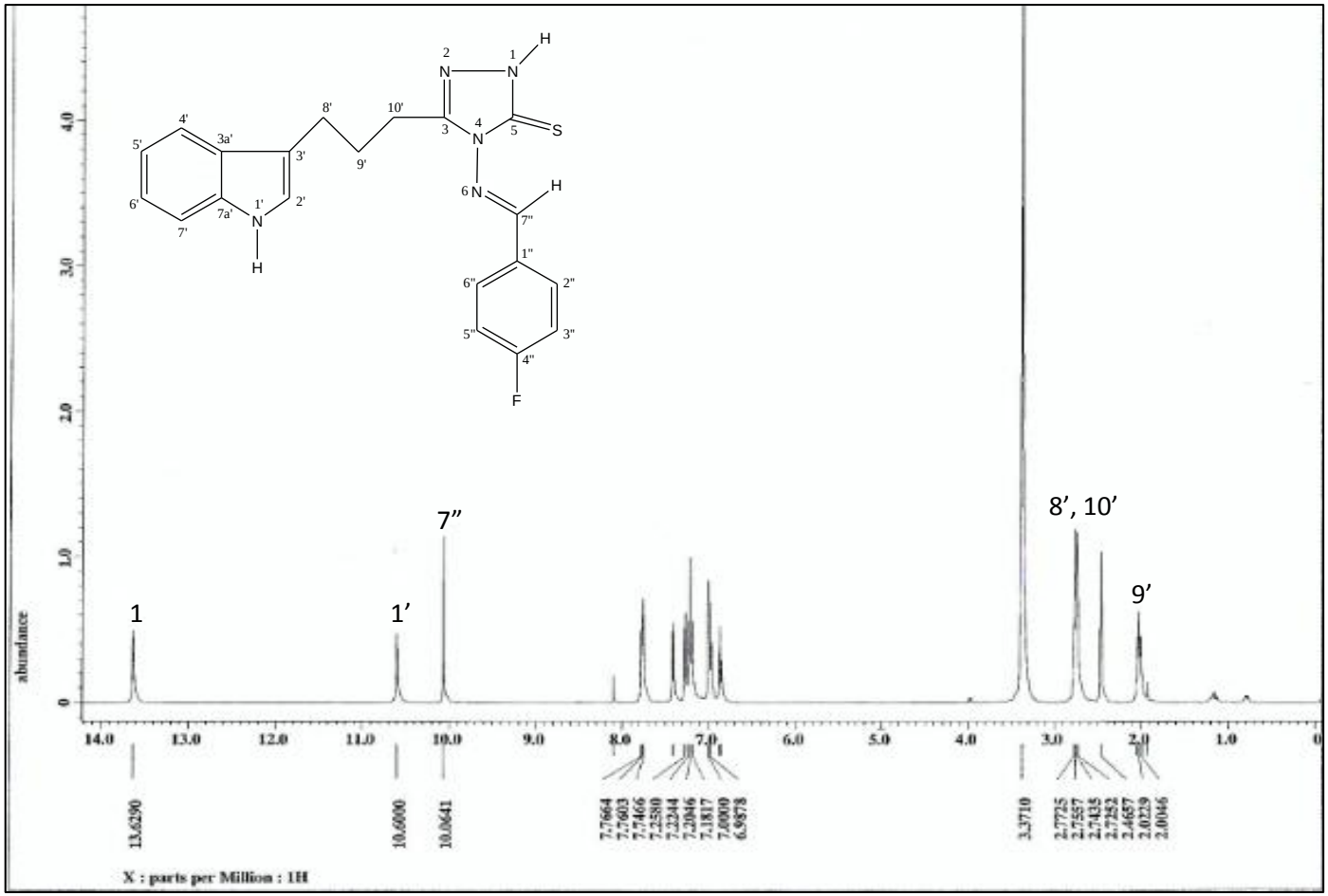


Figure 4.20: ^{1}H NMR Spectrum of SB 3

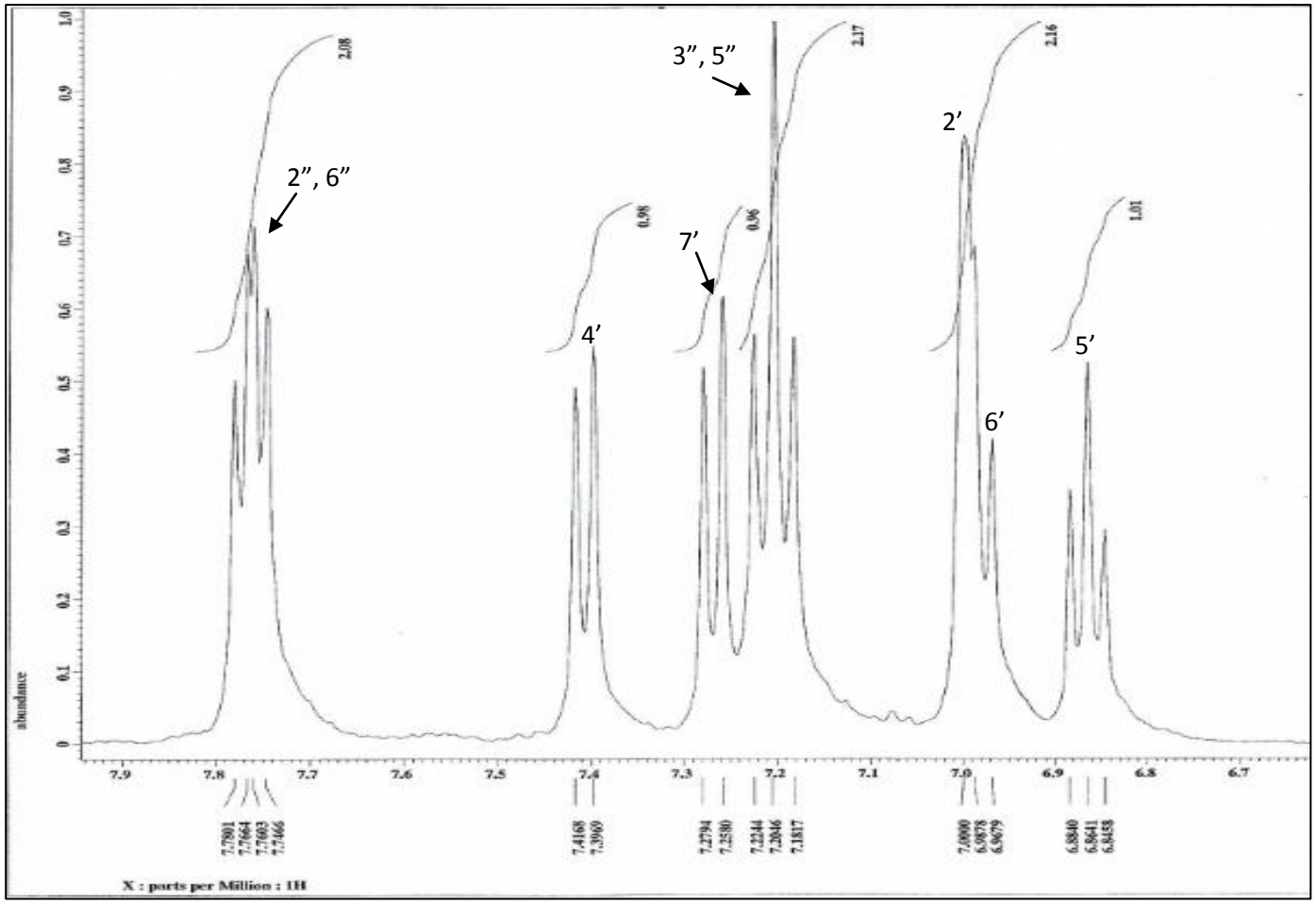


Figure 4.21: ^{1}H NMR Spectrum of SB 3

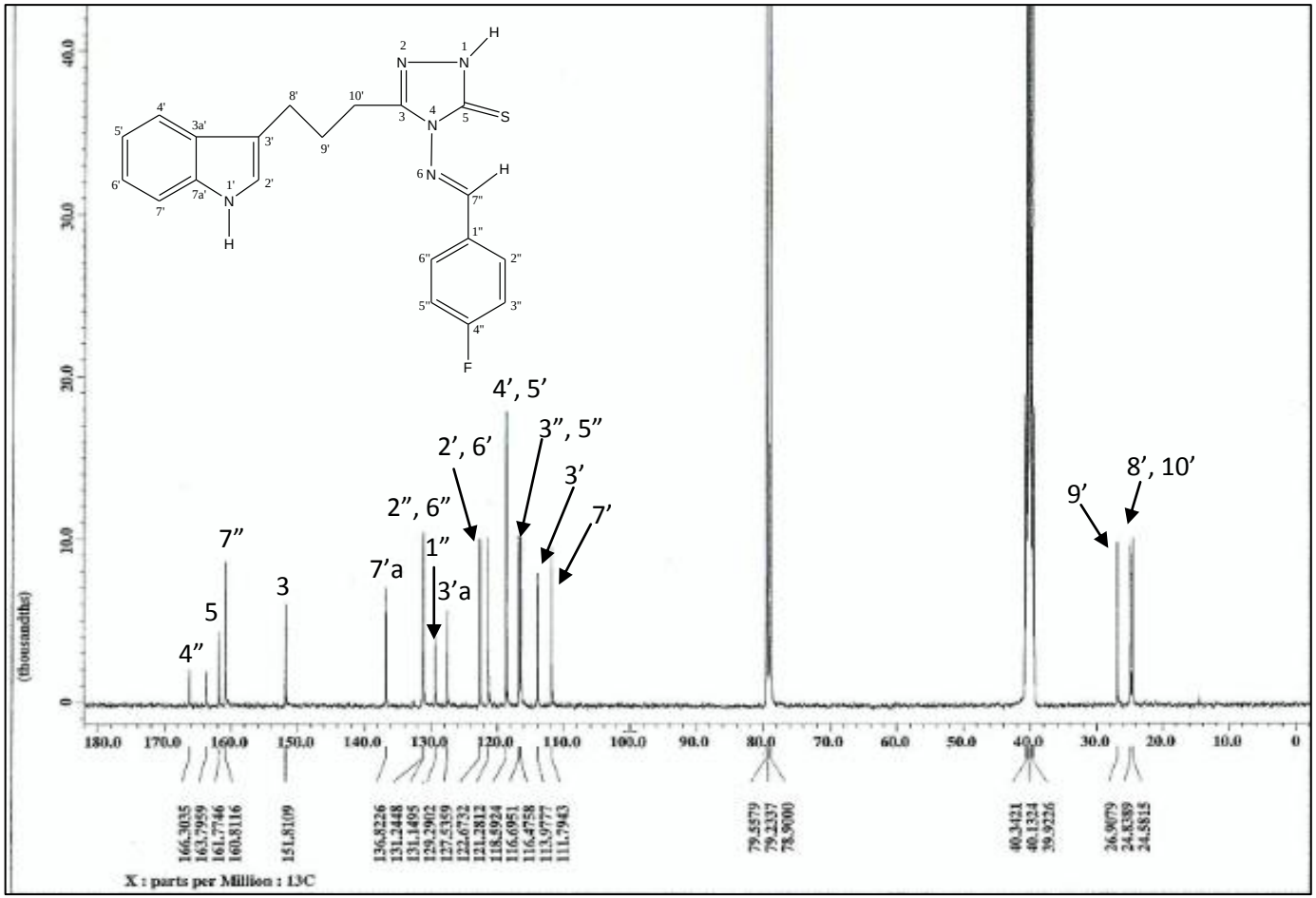


Figure 4.22: ^{13}C NMR Spectrum of SB 3

4.2.5 Discussion on 3-[(1H-indol-3-yl)-propyl]-4-(2-fluorobenzylideneamino)-1H-1,2,4-triazole-5(4H)-thione (SB 11)

Condensation reaction of 3-[(1H-indol-3-yl)-propyl]-4-amino-1H-1,2,4-triazole-5(4H)-thione and 2-fluorobenzaldehyde in ethanol using tartaric acid as catalyst has yield **SB 11**. The structure and physical properties of compound **SB 11** are shown in Figure 4.23 and Table 4.11 respectively.

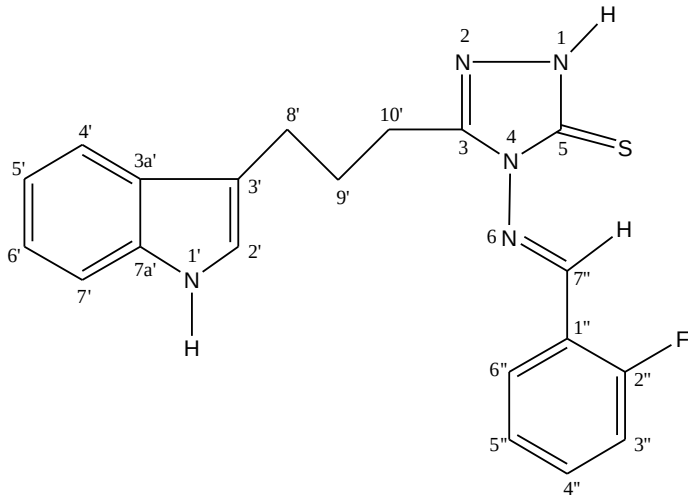


Figure 4.23: Structure of SB 11

Table 4.11: Summary of Physical Properties of SB 11

Physical Appearance	Pale Brown Solid
Melting Point (℃)	188 – 193
Percentage Yield (%)	47
Molecular Formula	$C_{20}H_{18}N_5SF$
Molecular Weight (g mol^{-1})	379.4531
R_f value [Ethylacetate : Hexane – 1 : 1]	0.51

According to ^{1}H NMR in Figure 4.24, similar as other Schiff base compounds, H-1, H-1' and H-7" appeared at the most downfield, which is at δ 13.64, δ 10.54 and δ 10.59 respectively. H-8', H-9' and H-10' have the most shielding effect, showing signal peaks at δ 2.77, δ 2.04 and δ 2.75 respectively. The protons of indole ring have slight chemical shifting as compare to that of 1,2,4-triazole. The peaks appears at δ 6.96 (H-2'), δ 7.42 (H-4'), δ 6.86 (H-5'), δ 6.98 (H-6') and δ 7.25 (H-7'). H-5" shows a triplet signal peak at δ 7.83 whereas H-3" shows a doublet peak at δ 7.22. Although the neighbouring carbon of C-4" (C-5" and C-3") each consists of single proton, H-4" shows a doublet signal peak instead of triplet because the carbon at position 3" and 5" are symmetry to each other.

By comparing ^{13}C NMR **(**Figure 4.26) and DEPT (Appendix P), there are 8 quaternary carbons belongs to C-3 (δ 152.06), C-5 (δ 161.80), C-3' (δ 114.01), C-3'a (δ 127.51), C-7'a (δ 136.81), C-1" (δ 161.01), C-2" (δ 165.55) and C-7" (153.62). There are three negative peaks shown in DEPT-135, indicating CH_2 in the structure, C-8' (δ 24.86), C-9' (δ 26.92) and C-10' (δ 24.63). The correlation between proton and carbon atoms in the structure is further confirmed in HMQC and HMBC.

In HMQC (**Appendix Q**), the doublet peaks showing at H-3" (δ 7.22) and triplet peak at H-5" (δ 7.83) are directly correlated to C-3" (δ 125.28) and C-5" (δ 127.29) respectively. The H-2" (δ 7.89), on the other hand, is correlated to C-2" at δ 130.17. In HMBC (**Appendix R**), H-5" at δ 7.83 has correlation with

C-1” (δ 161.01), C-2” (δ 163.55), C-3” (δ 125.28), C-4” (δ 134.49) and C-7” (δ 153.62).

Table 4.12: Summary of ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectral data ($CDCl_3$/DMSO-d_6) of SB 11

Position	^{1}H (δH, ppm)	Remarks	^{13}C (δC, ppm)
1	13.64	1H, s	-
2	-	-	-
3	-	-	152.1
4	-	-	-
5	-	-	161.8
6	-	-	-
1’	10.54	1H, s	-
2’	6.96	1H, s	122.6
3’	-	-	114.0
3’a	-	-	127.5
4’	7.42	1H, d, J = 7.96 Hz	118.6
5’	6.86	1H, t, J = 7.96 Hz	118.6
6’	6.98	1H, t, J = 7.96 Hz	121.3
7’	7.25	1H, d, J = 7.96 Hz	111.8
7’a	-	-	136.8
8’	2.77	2H, t, J = 7.32 Hz	24.9
9’	2.04	2H, q, J = 7.32 Hz	26.9
10’	2.75	2H, t, J = 7.32 Hz	24.6
1’’	-	-	161.0
2’’	-	-	163.5
3’’	7.22	1H, m	125.3
4’’	7.52	1H, m	134.5
5’’	7.83	1H, t, J = 7.92 Hz	127.3
6’’	7.20	1H, m	116.6
7’’	10.59	1H, s	153.6

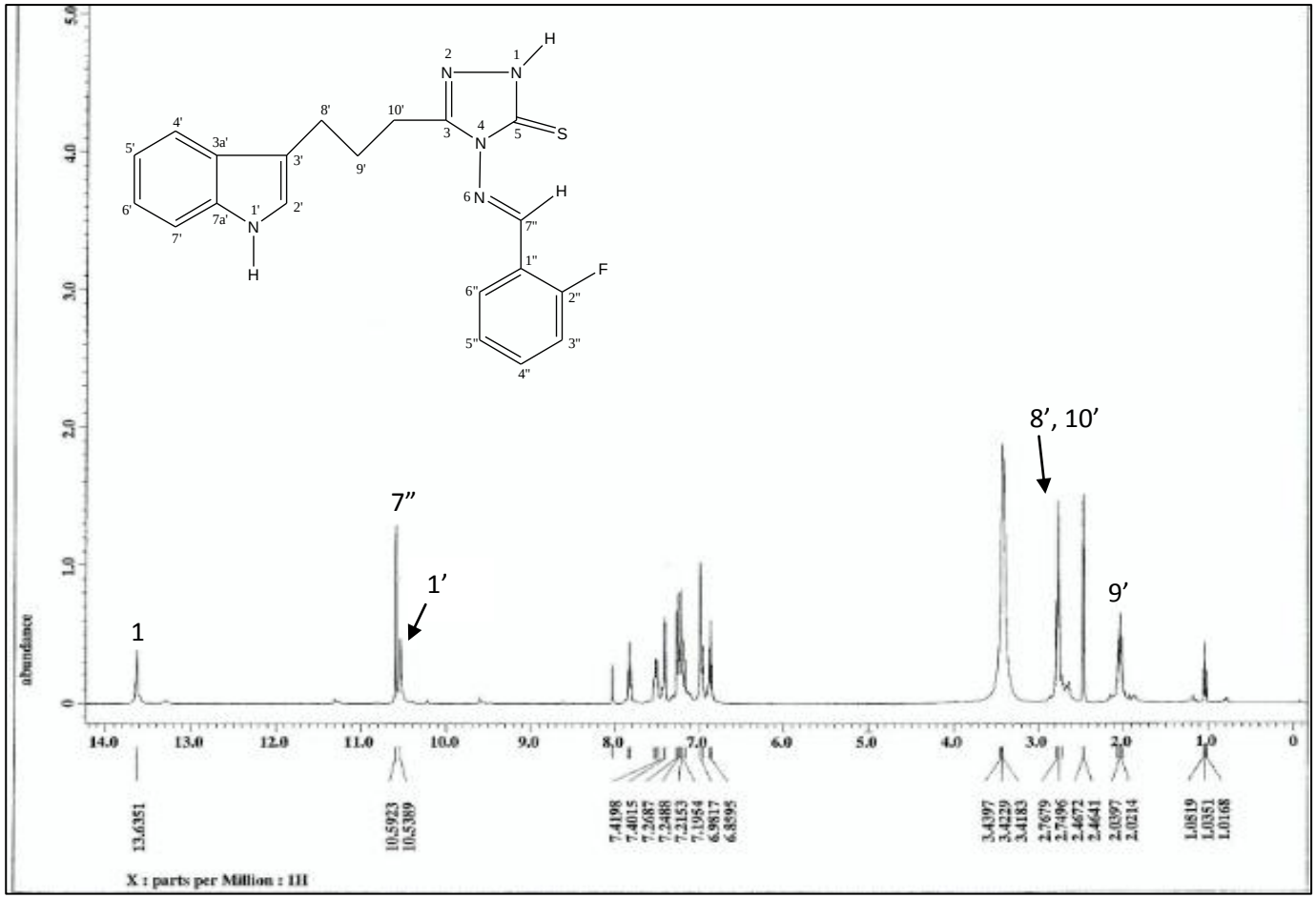


Figure 4.24: ^{1}H NMR Spectrum of SB 11

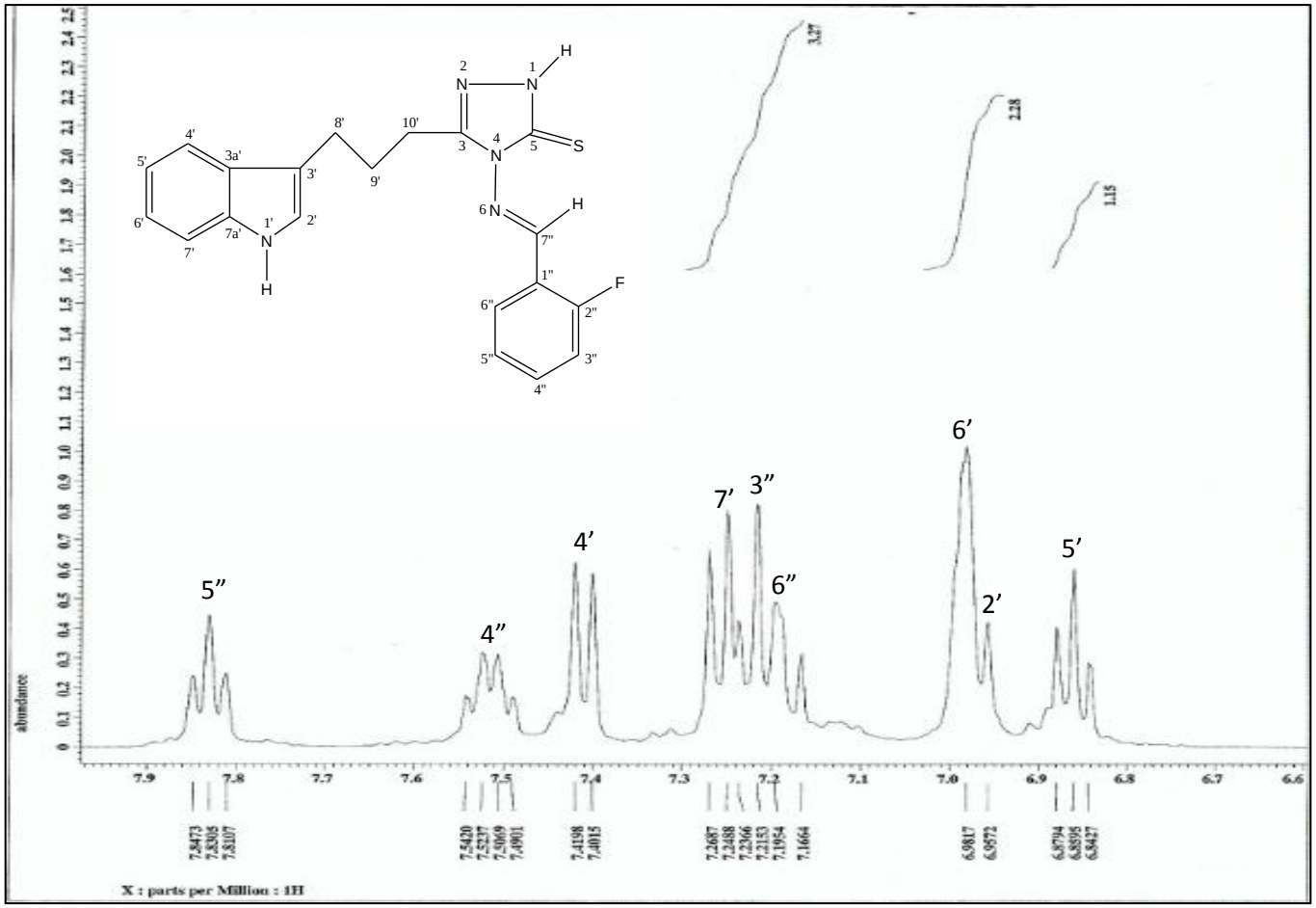


Figure 4.25: ^{1}H NMR Spectrum of SB 11

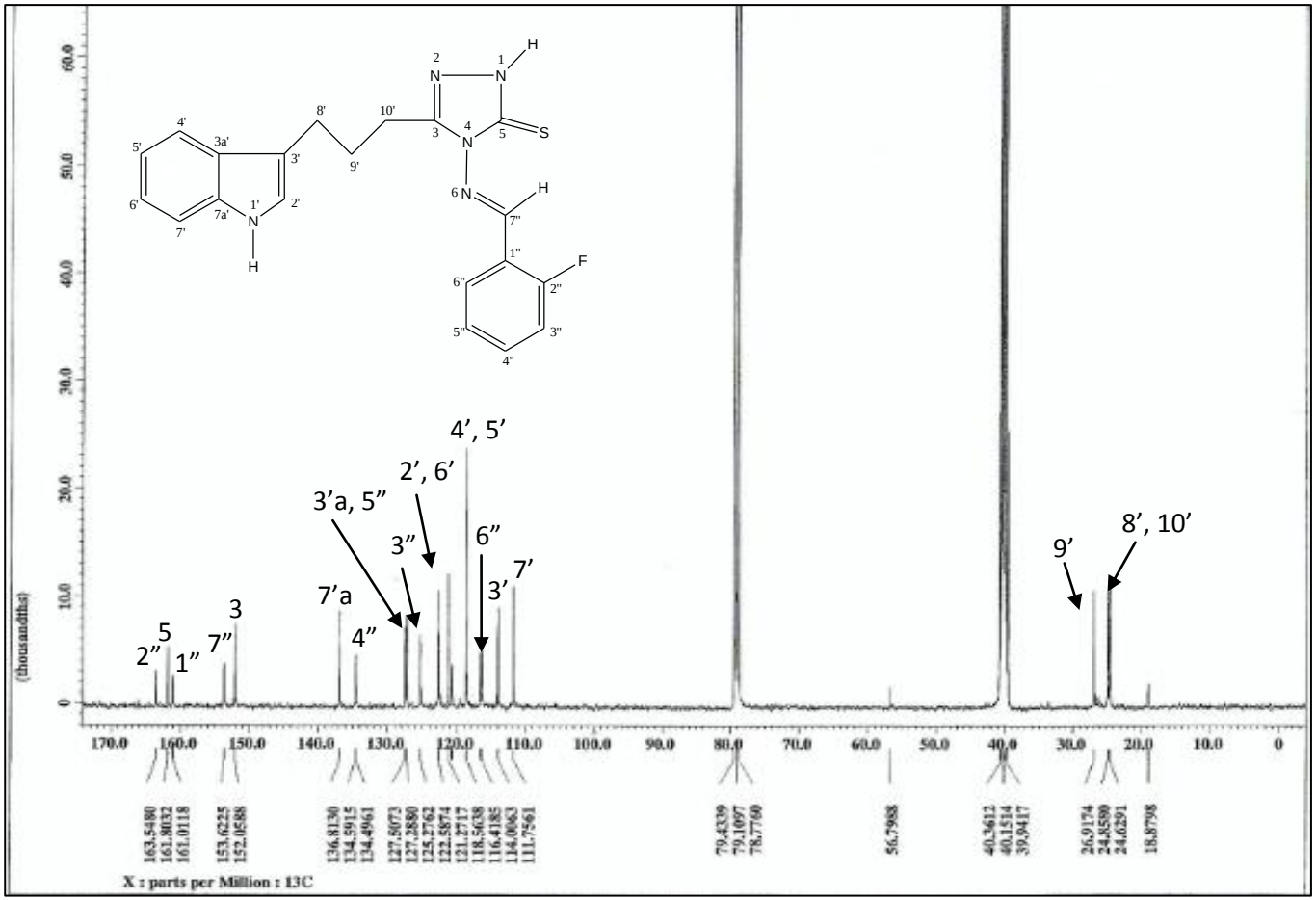


Figure 4.26: ^{13}C NMR Spectrum of SB 11

4.3 Antioxidant Activity

The antioxidant activity of 1,2,4-triazole and its Schiff bases are determined by using DPPH assay. The standard used is butylated hydroxyanisole (BHA) to act as a reference. IC_{50} represents the effective concentration of an inhibitor to reduce a specific biological process by 50%. The effective concentration required to reduce the DPPH free radical after 30 minutes is determined from the calibrated graph of percentage radical scavenging against concentration in Figure 4.27. The IC_{50} and standard deviation of each compound are tabulated in Table 4.13.

Table 4.13: IC_{50} of samples

Compounds	**IC_{50}**
1,2,4-triazole	102.3 ± 2.52
SB 7	74.7 ± 6.43
SB 8	121.0 ± 1.73
SB 6	77.7 ± 2.52
SB 3	80.0 ± 1.00
SB 11	81.0 ± 1.73
Standard BHA	25.0 ± 4.36

By comparing the data in Table 4.13, **SB 7** has the lowest value of IC_{50} followed by **SB 6**, **SB 3**, **SB 11** and lastly **SB 8**. In other words, **SB 7** has the highest antioxidant activity. This is because **SB 7** consists of halogen atoms, in which it induces electron-withdrawing effect and able to delocalize the lone pair electrons and stabilizes the compound via resonance effect. **SB 6** contains methoxy-substituted benzaldehyde. The $–OCH_3$ also enables formation of lone pair electrons and subsequently stabilizes the compound through resonance effect.

Apart from that, by comparing the IC_{50} and structure of each Schiff bases, it can be noticed that substitution at C-2" and C-4" position of Schiff base aromatic ring gives low value of IC_{50}. This can be explained as the antioxidant activity of Schiff base with substituents at ortho- and/or para- position is higher than that in meta-position as it enables the delocalization of electrons and hence inhibiting the radical activity of DPPH.

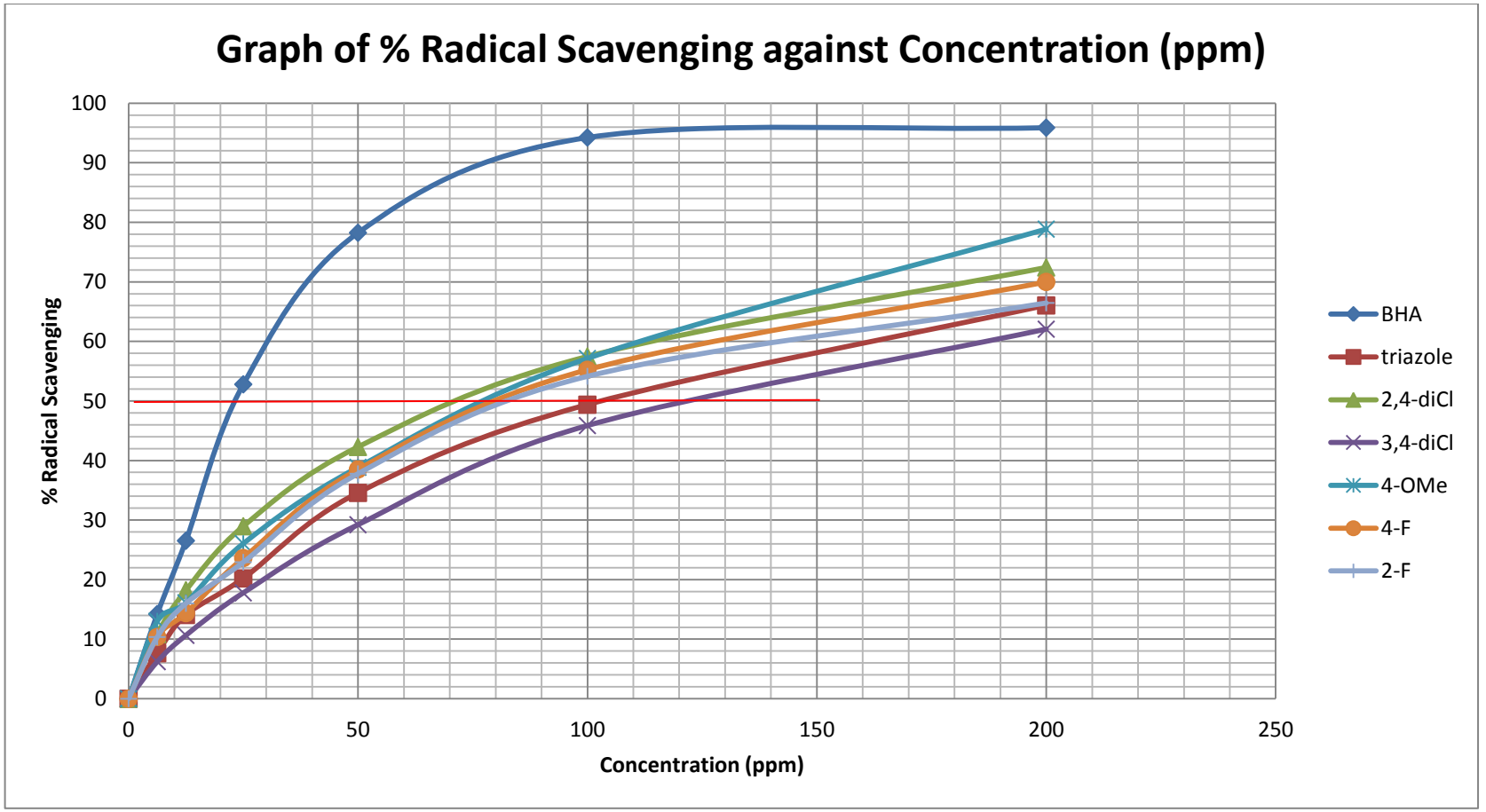


Figure 4.27: Graph of DPPH Percentage Radical Scavenging against Concentration (ppm)

CHAPTER 5

CONCLUSION

5.1 Conclusion

5 Schiff bases of 1,2,4-triazole were successfully synthesized in this project. Those compounds were synthesized by a presynthesized of 1,2,4-triazole, namely 3-[(1H-indol-3-yl)-propyl]-4-amino-1H-1,2,4-triazole-5(4H)-thione. Those Schiff bases are 3-[(1H-indol-3-yl)-propyl]-4-(2,4-dichlorobenzylideneamino)-1H-1,2,4-triazole-5(4H)-thione **(SB 7)**, 3-[(1H-indol-3-yl)-propyl]-4-(3,4-dichlorobenzylideneamino)-1H-1,2,4-triazole-5(4H)-thione **(SB 8)**, 3-[(1H-indol-3-yl)-propyl]-4-(4-methoxybenzylideneamino)-1H-1,2,4-triazole-5(4H)-thione **(SB 6)**, 3-[(1H-indol-3-yl)-propyl]-4-(4-fluorobenzylideneamino)-1H-1,2,4-triazole-5(4H)-thione **(SB 3)** and 3-[(1H-indol-3-yl)-propyl]-4-(2-fluorobenzylideneamino)-1H-1,2,4-triazole-5(4H)-thione **(SB 11)**. The structures of each Schiff bases were characterized and elucidated through a series of instrumental analysis including ^{1}H NMR, ^{13}C NMR, DEPT, HMQC, HMBC and melting point.

The antioxidant activity of Schiff bases was determined via DPPH assay using BHA as standard. Among the Schiff bases of 1,2,4-triazole synthesized, **SB 7** has the highest antioxidant activity with the IC_{50} value of 74.7 ± 6.43. This is due to the halogen substituents at ortho- and para- position of the Schiff base aromatic ring that contribute to resonance effect that stabilized the structure

while inhibiting the radical activity of DPPH. **SB 6, SB 3** and **SB 11** has moderate antioxidant activity with IC_{50} value of 77.7±2.52, 80.0 ± 1.00 and 81.0 ± 1.73 respectively. Last but not least, **SB 8** showed relatively high IC_{50} value of 121.0 ± 1.73. Its antioxidant activity is even weaker than that of the starting material, 1,2,4-triazole. Thus, Schiff base with ortho- and/or para-substituents is said to give higher antioxidant activity.

5.2 Further study

1,2,4-Triazole Schiff bases has been reported to have various biological activities. Further studies can be done on antimicrobial activity, anti-inflammatory activity, anti-cancer activity, anti-tubercular activity and others.

Besides, detail study in the mechanism of 1,2,4-triazole Schiff base scavenging activity on DPPH radical can be made to learn the relationship between the substituents on Schiff base aromatic ring and their position towards antioxidant activity.

REFERENCES

Ashraf, M.A., Mahmood, K. and Wajid, A., 2011. Synthesis, characterization and biological activity of Schiff bases. *International Conference on Chemistry and Chemical Process*, IPCBEE 10, pp. 1-7.

Bayer, H.O., Cook, R.S. and Von, M.W.C., 1971. Fungicidal metal salt complexes of 4-substituted-1,2,4-triazoles. ZA7004373A, 1971.

Bekircan, O. and Bektas, H., 2006. Synthesis of new bis-1,2,4-triazole derivatives. *Molecules*, 11, pp. 469-477.

Belapure, A.S., 2012. Synthesis and Catalytic Applications of 1,2,4-triazoles in oxidative processes. PhD thesis, University of Tennessee.

Boozer, C.E., Hammond, G.S., Hamilton, C.E. and Sen, J.N., 1955. Air oxidation of hydrocarbon. 1II. The stoichiometry and fate of inhibitors in benzene and chlorobenzene. *Journal of American Chemical Society*. 77 (12): pp.3233-3237.

Calisir, M.M., Kocyigit-Kaymakcioglu, B., Ozbek, B. and Otuk, G., 2010. Synthesis and antimicrobial activity of some novel Schiff bases containing 1,2,4-triazole-3-thione. *E-Journal of Chemistry*, 7(1), pp. 458-464.

Gomha,S.M. and Riyadh, S.M., 2011. Synthesis under microwave irradiation of [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles and other diazoles bearing indole moieties and their antimicrobial evaluation. *Molecules*, 16, pp. 8244-8256.

Gupta, D. and Jain, D.K., 2015. Synthesis, antifungal and antibacterial activity of novel 1,2,4-triazole derivatives. *Journal of Advanced Pharmacy Technology Research*, 6(3), pp. 141-146.

Guzeldemirci, N.U. and Kucukbasmac, O., 2010. Synthesis and antimicrobial activity evaluation of new 1,2,4-triazoles and 1,3,4-thiadiazoles bearing imidazo[2,1-*b*] thiazole moiety. *European Journal of Medical Chemistry*, 45 (2010), pp. 1338-1345

Harvey, K.B. and Clifford, C., 2004. Bis(*N,N*-Disalicylethlene diamine-methoxyphenylmethylidene). M 5321J, *Mol. Bank*. pp. 352.

Heubach, G., Sachse, B. and Buerstell, H., 1979. 1,2,4-Triazole derivatives. *German Offen. 2, 826, 760; Chemical Abstracts*, 92, 181200h.

Ionita, P., 2003. Is DPPH stable free radical a good scavenger for oxygen active species? *Chemical Papers*, 59 (1), pp. 11-16.

Karaali, N., Mentese, E.,Yilmaz, F., Usta, A and Kahveci, B., 2013. Microwave-assisted synthesis of some 1H-1,2,4-triazol-3-one derivatives. *South African Journal of Chemistry*, 66, pp. 72-76.

Kaplancikli, Z.A., Turan-Zitouni, G., Ozdemir, A. and Revial, G., 2007. New triazololothiadiazine derivatives as possible antimicrobial agents. *European Journal of Medicinal Chemistry*, 43, pp. 155-159.

Kelley, J.L., Koble,C.S., Davis, R.G., Mc Lean, E.W., Soroko, F.E. and Cooper B.R., 1995. 1-(Fluorobenzyl)-4-amino-1H-1,2,3-triazolo[4,5-c]pyridines: synthesis and anticonvulsant activity. *Journal of Medical Chemistry*, 38, pp. 4131-4134.

Li, X., Li, X.Q., Liu, H.M., Zhou, X.X. and Shao, Z.H., 2012. Synthesis and evaluation of antitumor activities of novel chiral 1,2,4-triazole Schiff bases bearing γ -butenolide moiety. *Organic and Medicinal Chemistry Letters*. [Online]. Available at:

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3490741/

Li, Z., Gu, Z., Yin, K., Zhang,R., Deng, Q., Xiang, J., 2009. Synthesis of substituted-phenyl-1,2,4-triazol-3-thione analogue with modified D-glucopyranosyl residues and their antiproliferative activites. *European Journal of Medicinal Chemistry*, 44, pp. 4716-4720.

Mandal, A., 2013. What are antioxidant? Science and Medicine. [Online]. Available at: http://www.news-medical.net/health/What-are-Antioxidants.aspx

Mange, Y.J., Isloor, A.M., Malladi, S., Isloor, S. and Fun, Hoong-Kun., 2011. Synthesis and antimicrobial activities of some novel 1,2,4-triazole derivatives. *Arabian Journal of Chemistry*, 6, pp.177-181.

Mullican, M.D., Wilson, M.W., Conner, D.T., Kostlan, C.R., Schrier D.J. and Dyer, R.D., 1993. Design of 5-(3,5-di-tert-butyl-4-hydroxyphenyl)-1,3,4-thiadiazoles, 1,3,4-oxadiazoles and 1,2,4-triazoles as orally active nonulcerogenic anti-inflammatory agents. *Journal of Medical Chemistry*, 36, pp.1090-1099.

Nur Alam, M., Bristi, N.J. and Rafiquzzaman, M., 2013. Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal*, 21 (2), pp. 143-152.

Nworie, F.S., Nwabue, F.I, Elom N.I. and Eluu, S.O., 2016. Schiff base and schiff base metal complexes: from syntheses to applications. *Journal of Basic and Applied Research* 2(3), pp. 295-305.

Patil, S., Jadhav, S.D. and Patil, U.P., 2012. Natural acid catalyzed Schiff base under solvent-free condition. *Archieves of Applied Science Research*, 4(2), pp. 1074-1078.

Serdar, M., Gumrukcuoglu, N., Karaoglu, S.A. and Demirbas, N., 2007. Synthesis of some novel 3,5-diaryl-1,2,4-triazole. *Turkish Journal of Chemistry*, 31, pp. 315-326.

Sharma, V., Mehta, D.K., Das, R. and Kaur, P.K., 2016. Synthsis of some novel triazole derivativesas Schiff bases and their antimicrobial evaluation. *International Journal of Pharmacy and Pharmaceutical Science*, 8 (9), pp. 1-4.

Shekhar, T.C. and Anju, G., 2014. Antioxidant activity by DPPH radical scavenging method of *Ageratum conyzoides* Linn. Leaves. *American Journal of Ethnomedicine*, 1,(4), pp. 244-249.

Tozkoparan, B., Kupeli, E., Yesilada, E. and Ertan, M., 2007. Preparation of 5-aryl-3-alkylthio-1,2,4-triazoles and corresponding sulfones with anti-inflammatory-analgesic activity. *Bioorganic & Medicinal Chemistry*, 15 (4), pp. 1808-1814.

Warrier, P.K., Nambier, V.P.K. and Raman Kutty, C., 1994. Indian medicinal plants – A xompendium of 500 species, *Orient Longman Limited, Madras*, Volume I, pp. 95-97.

Xavier A. and Srividhya N., 2014. Synthesis and study of Schiff base ligands. *IOSR Journal of Applied Chemistry*, 7 (11), Ver. I., pp. 06-15.

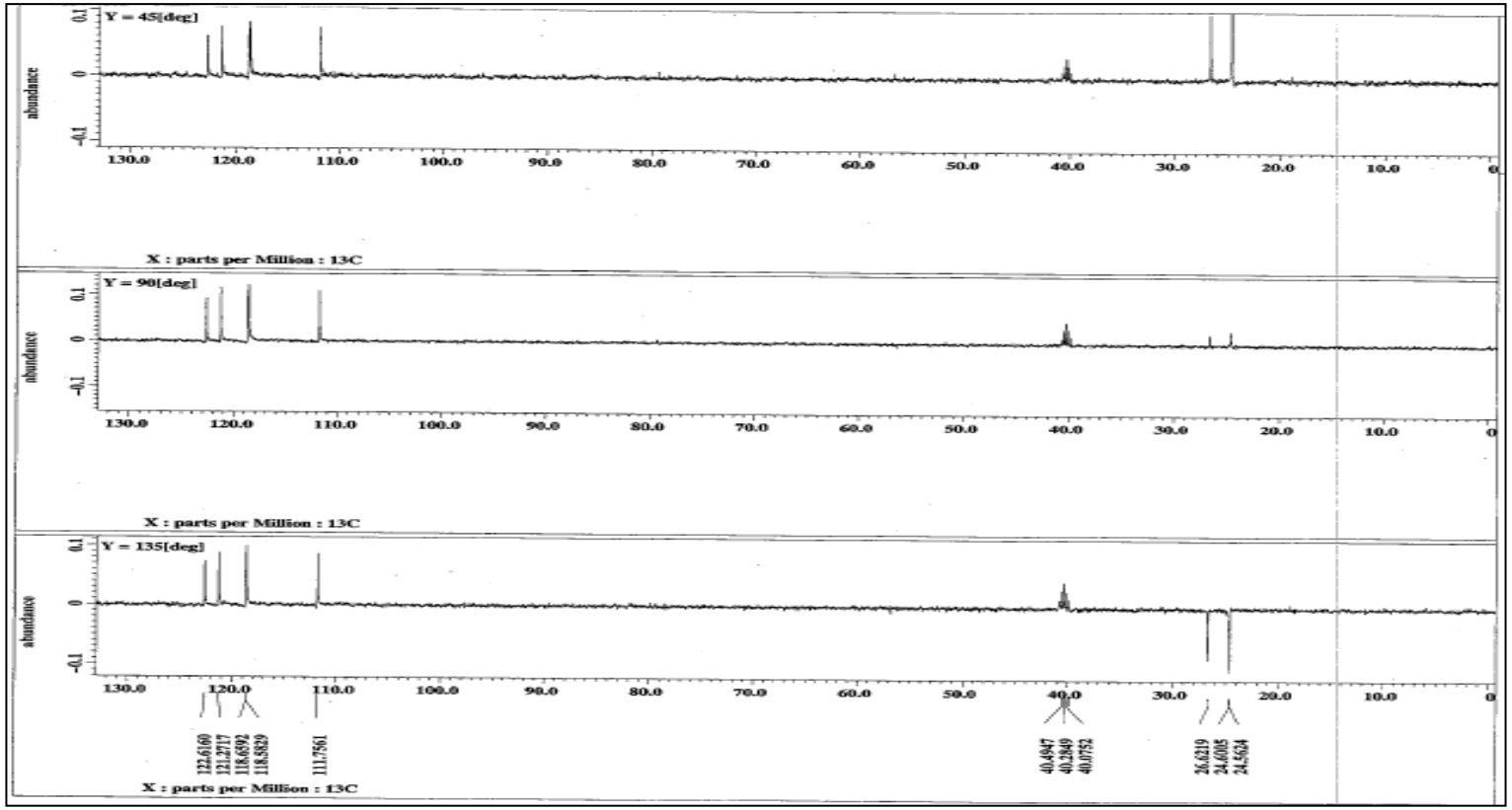
Yang, H.J., Sun, W.H., Li, Z.L. and Ma, Z., 2002. The rapid synthesis of Schiff-base without solvent under microwave irradiation. *Chinese Chemical Letters,* 13 (1), pp. 3-6.

Ye, X.X., Chen, Z.F., Zhang, A.J. and Zhang L.X., 2007. Synthesis and biological evaluation of some novel Schiff's bases from 1,2,4-triazole. *Molecules*, 12, pp. 1202-1209.

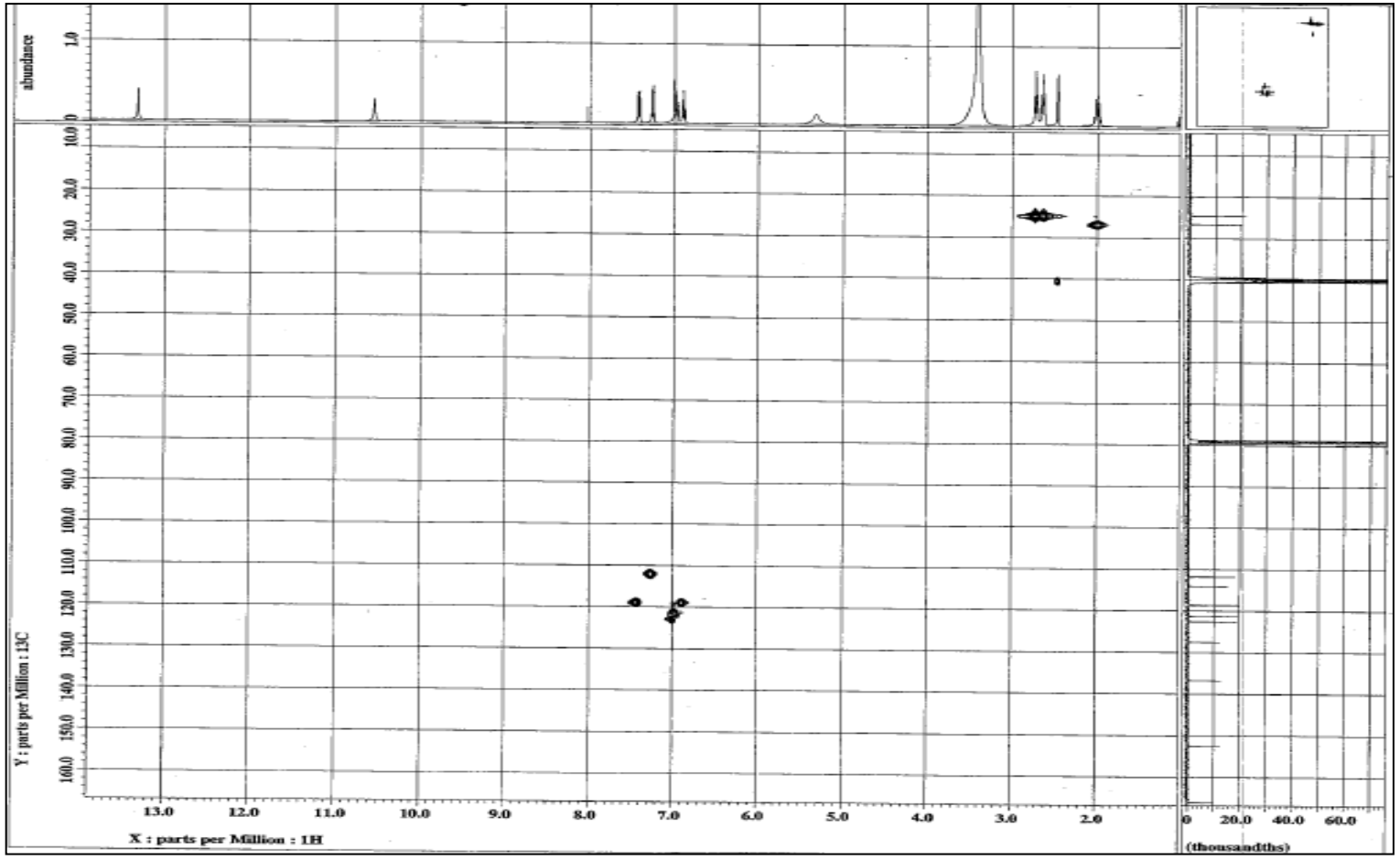
Zamani, K., Faghihi, K., Sangi, M.R. and Zolgharnein, J., 2002. Synthesis of some new substituted 1,2,4-triazole and 1,3,4-thiadazole and their derivatives. *Turkish Journal of Chemistry*, 27, pp. 119-125.

APPENDICES

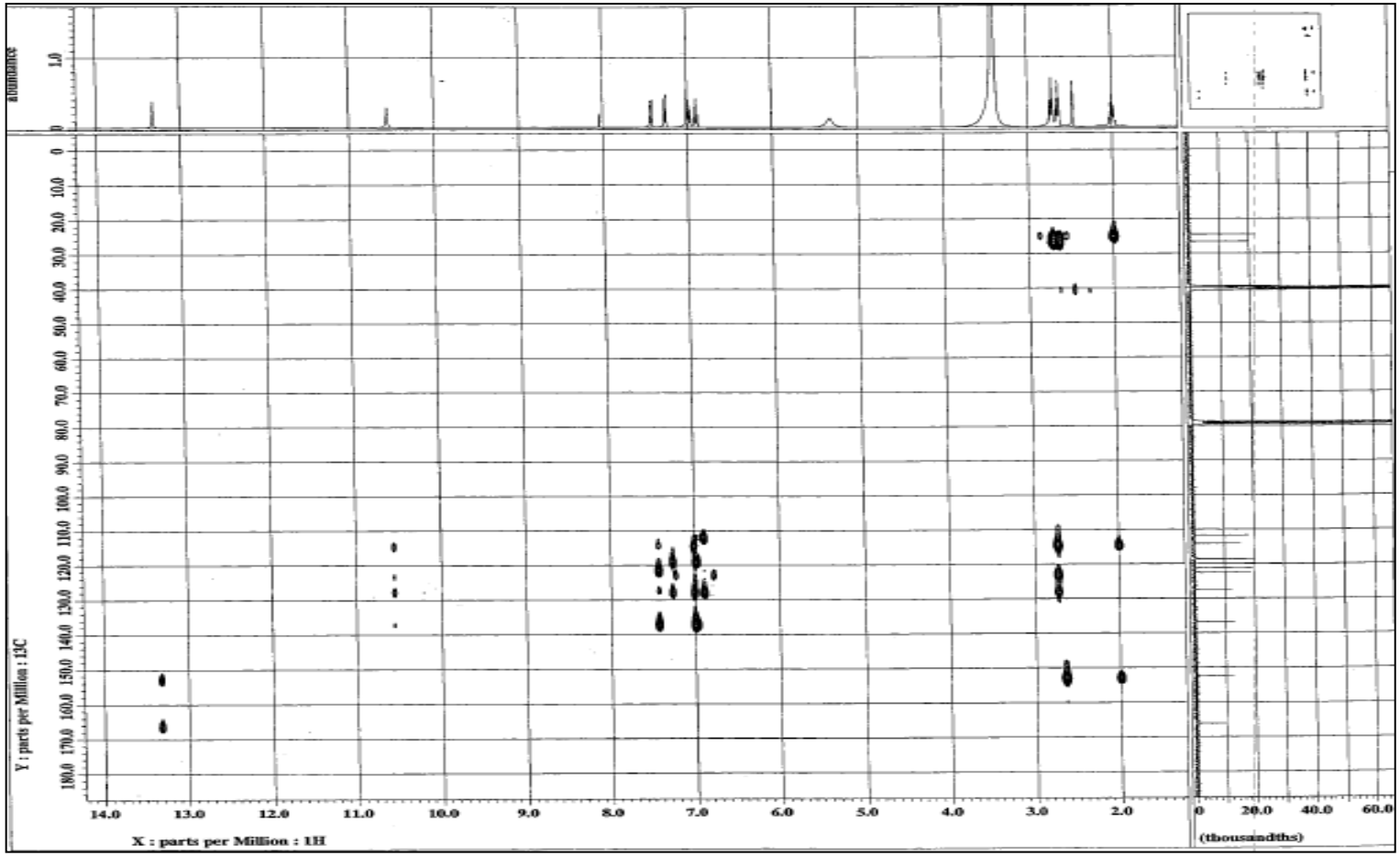
Appendix A: DEPT Spectrum of 1,2,4-Triazole



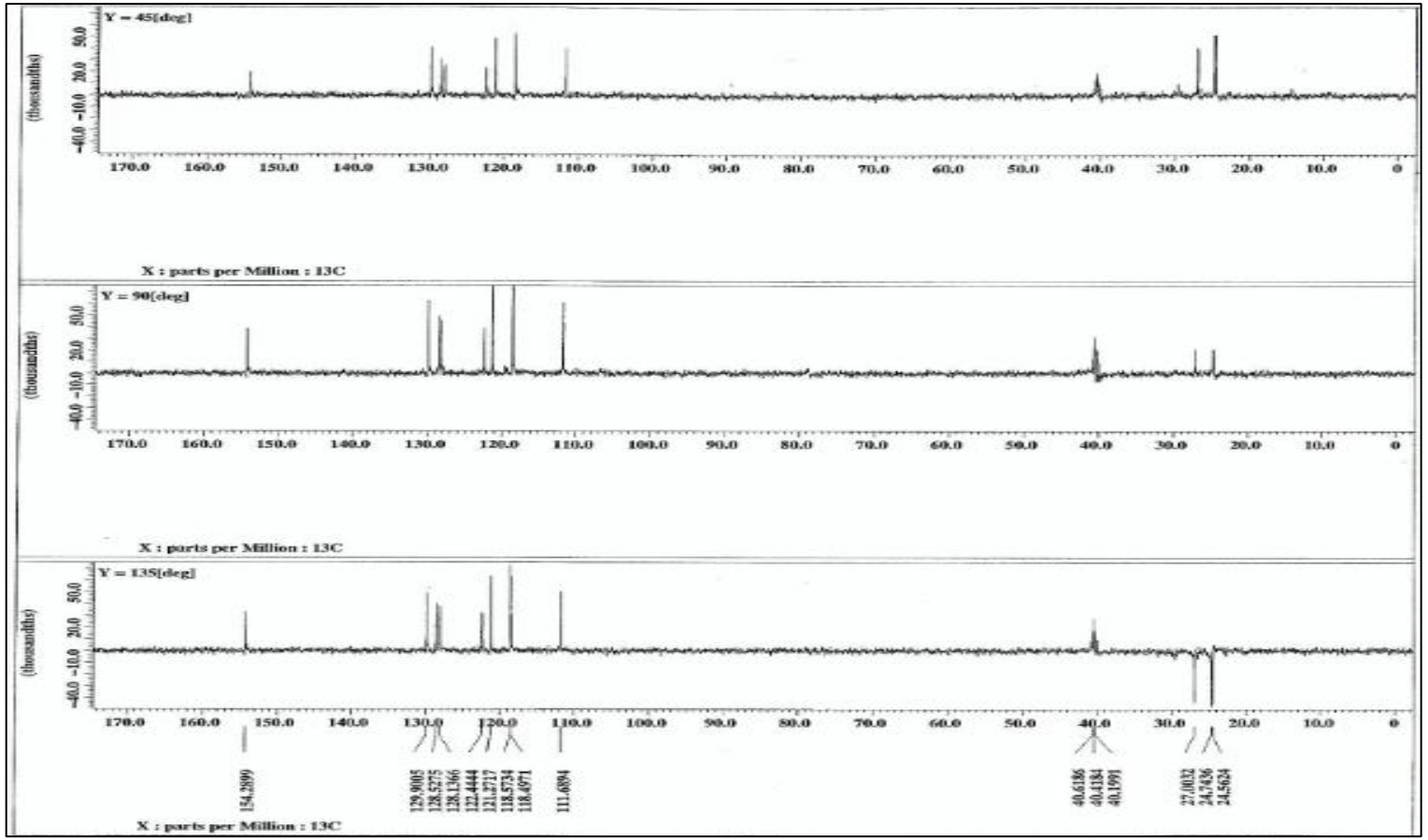
Appendix B: HMQC Spectrum of 1,2,4-Triazole



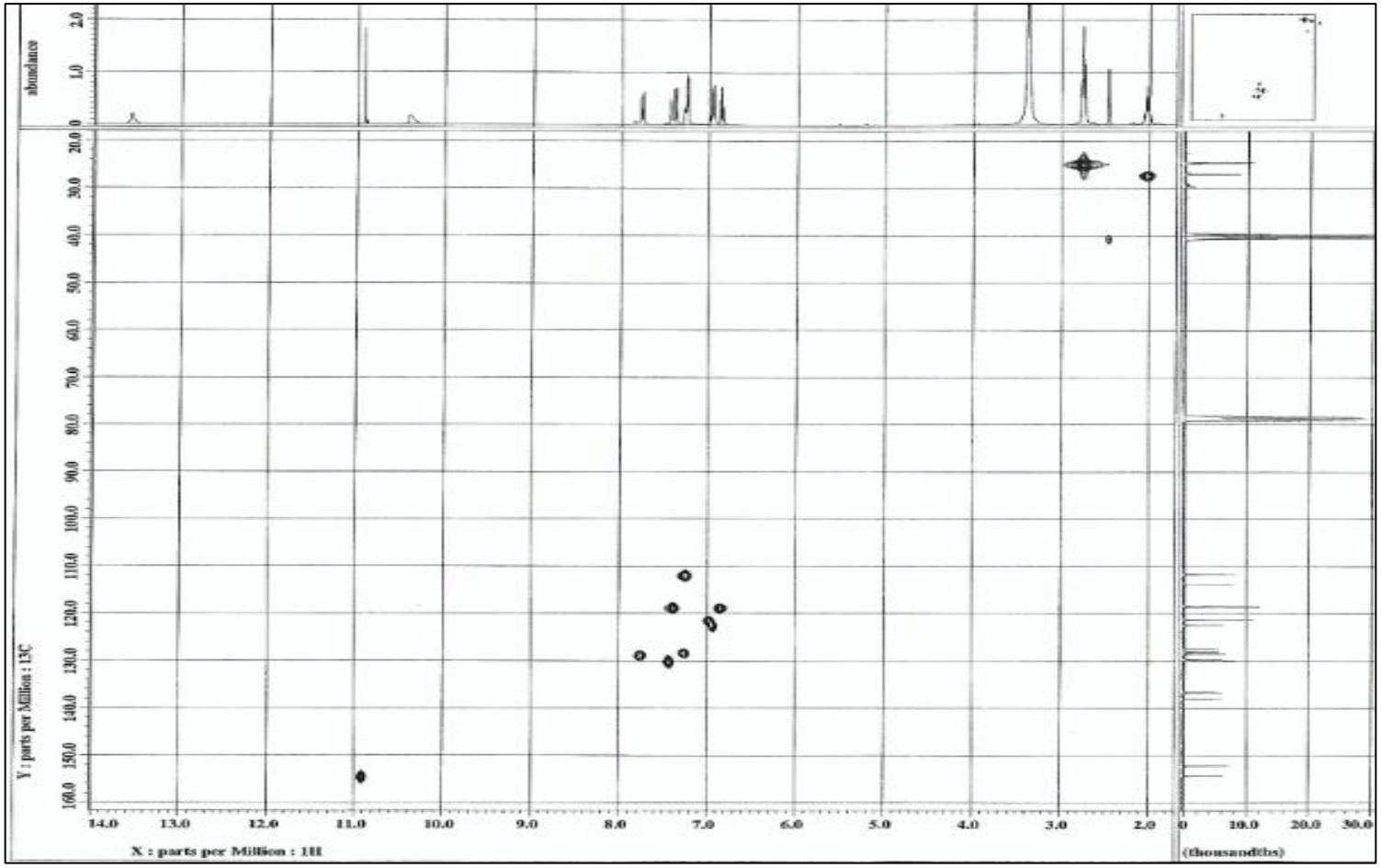
Appendix C: HMBC Spectrum of 1,2,4-Triazole



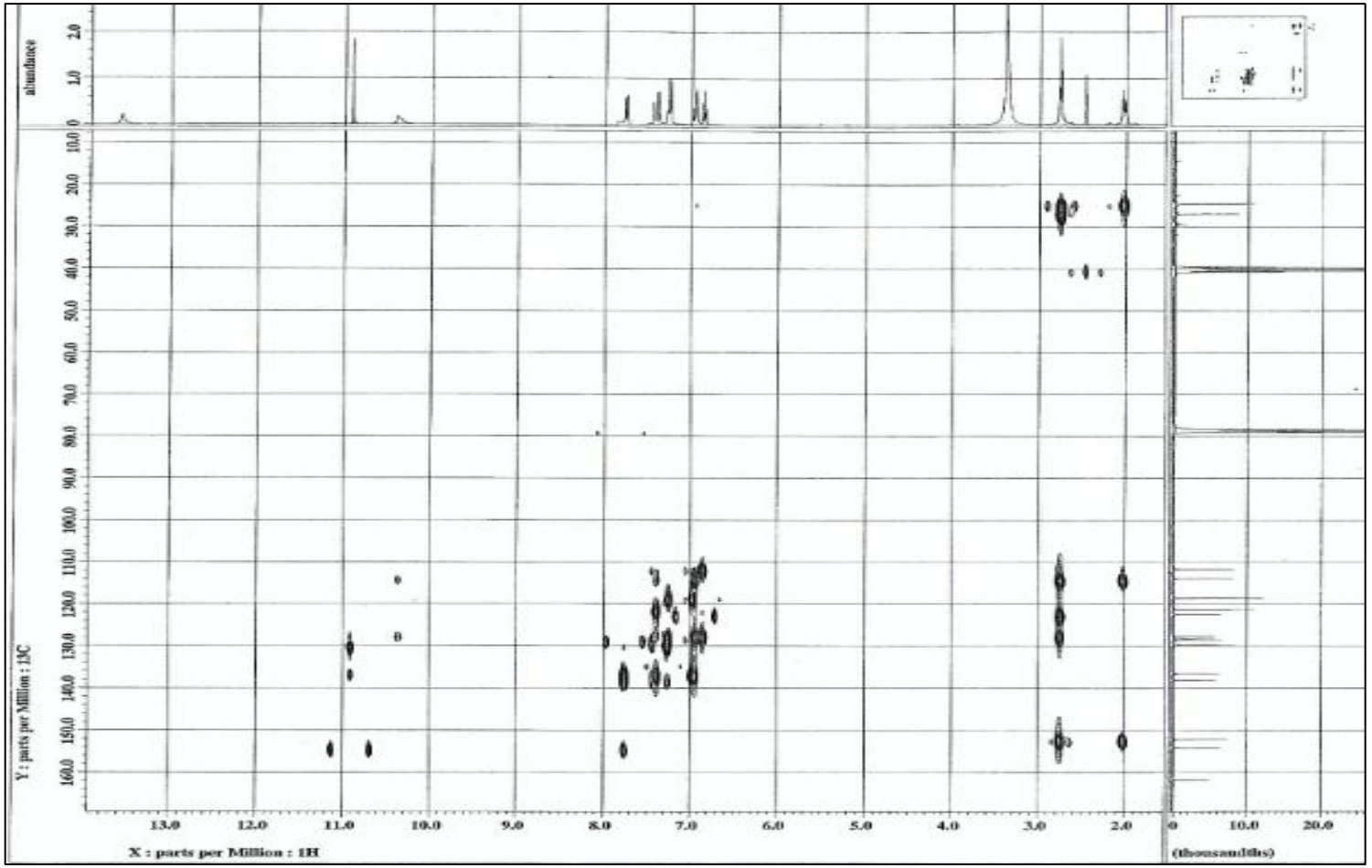
Appendix D: DEPT Spectrum of SB 7



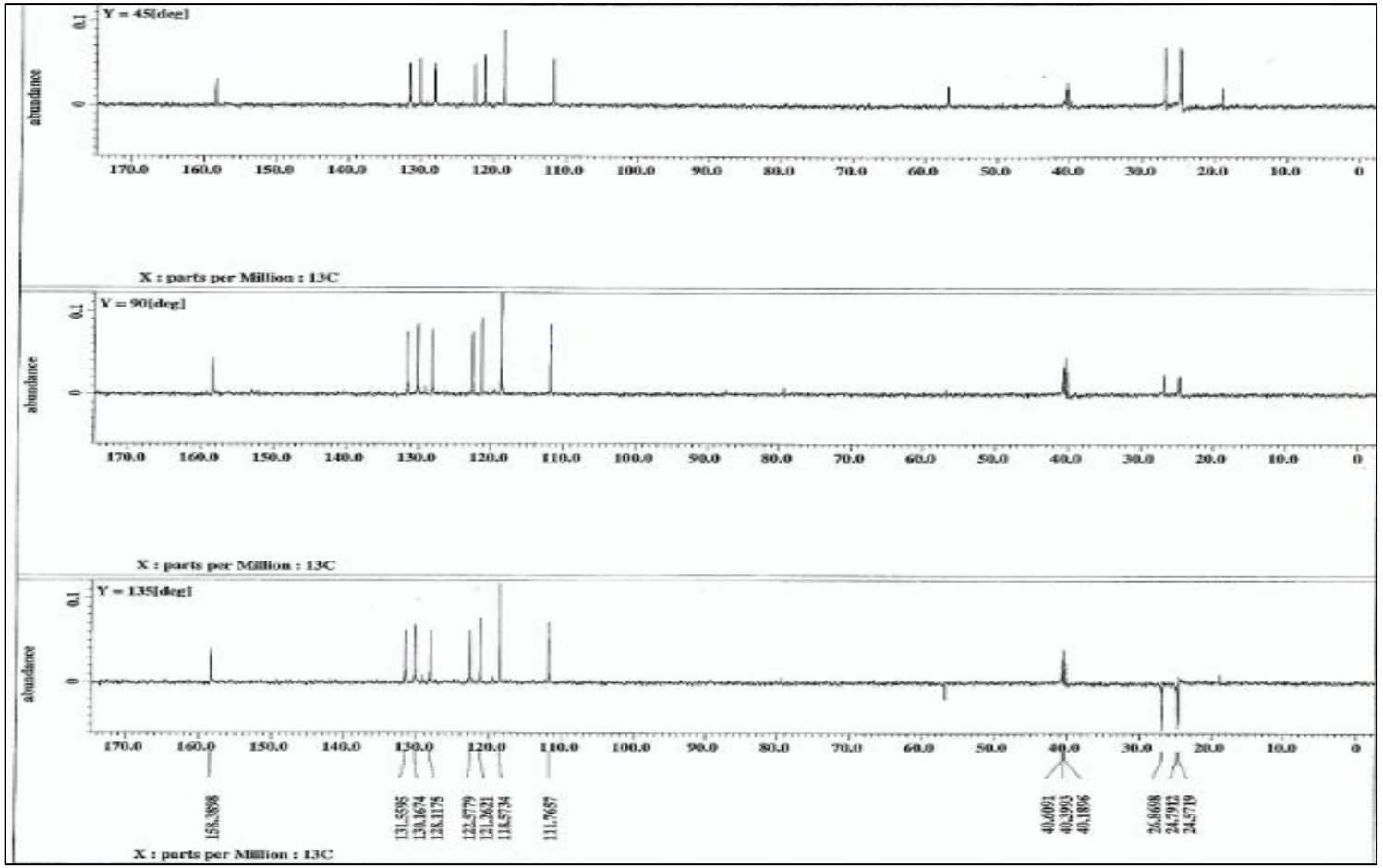
Appendix E: HMQC Spectrum of SB 7



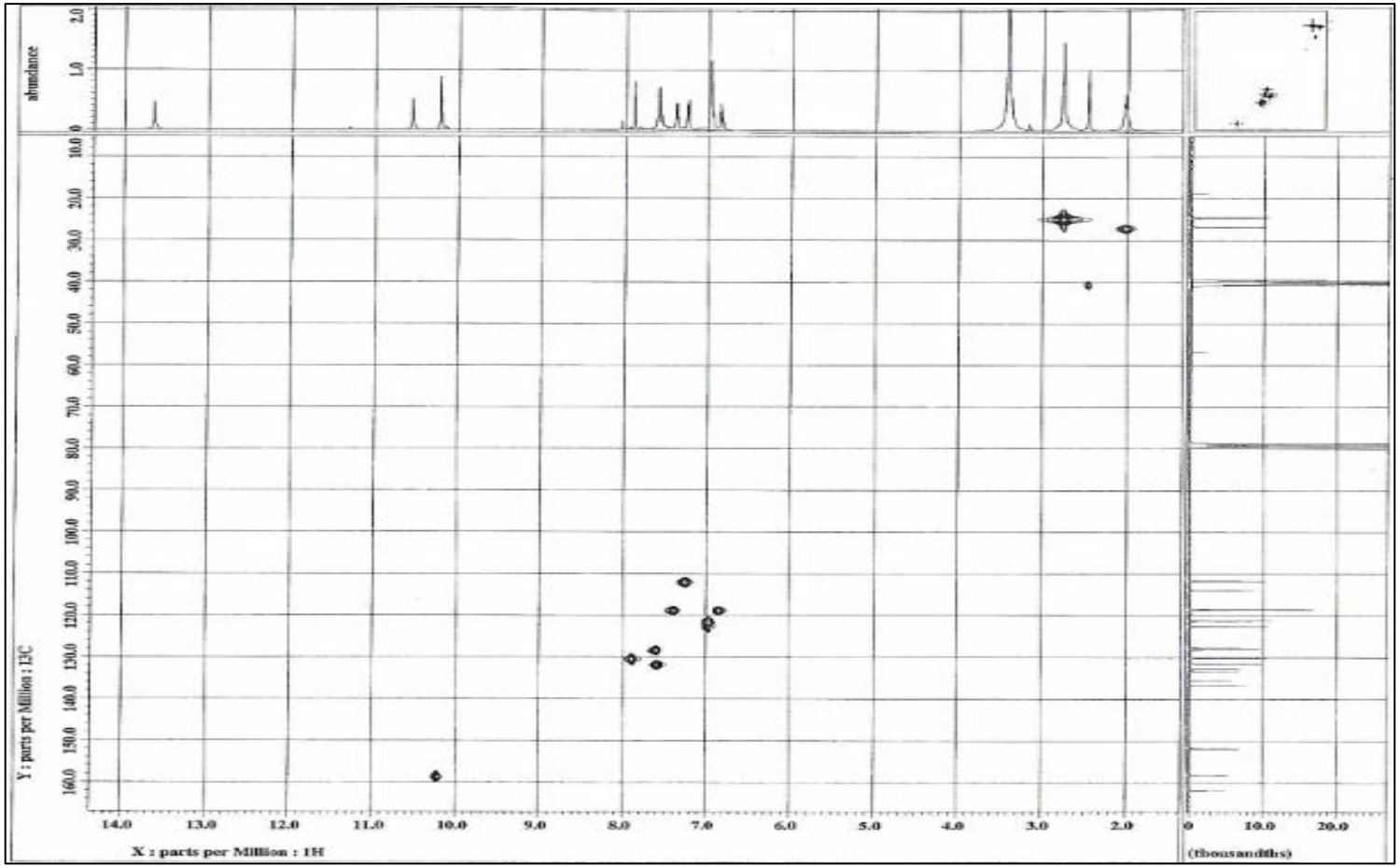
Appendix F: HMBC Spectrum of SB 7



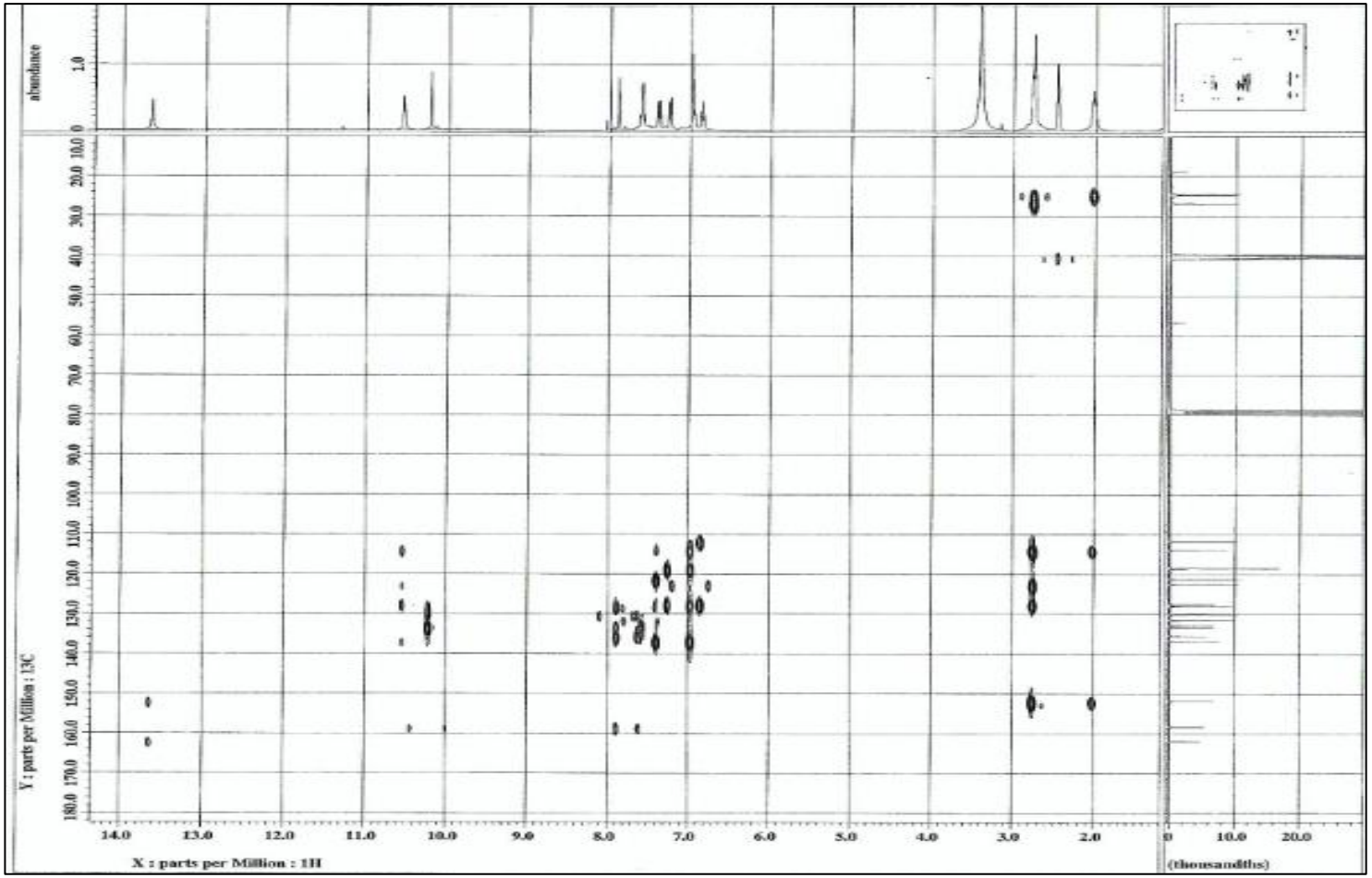
Appendix G: DEPT Spectrum of SB 8



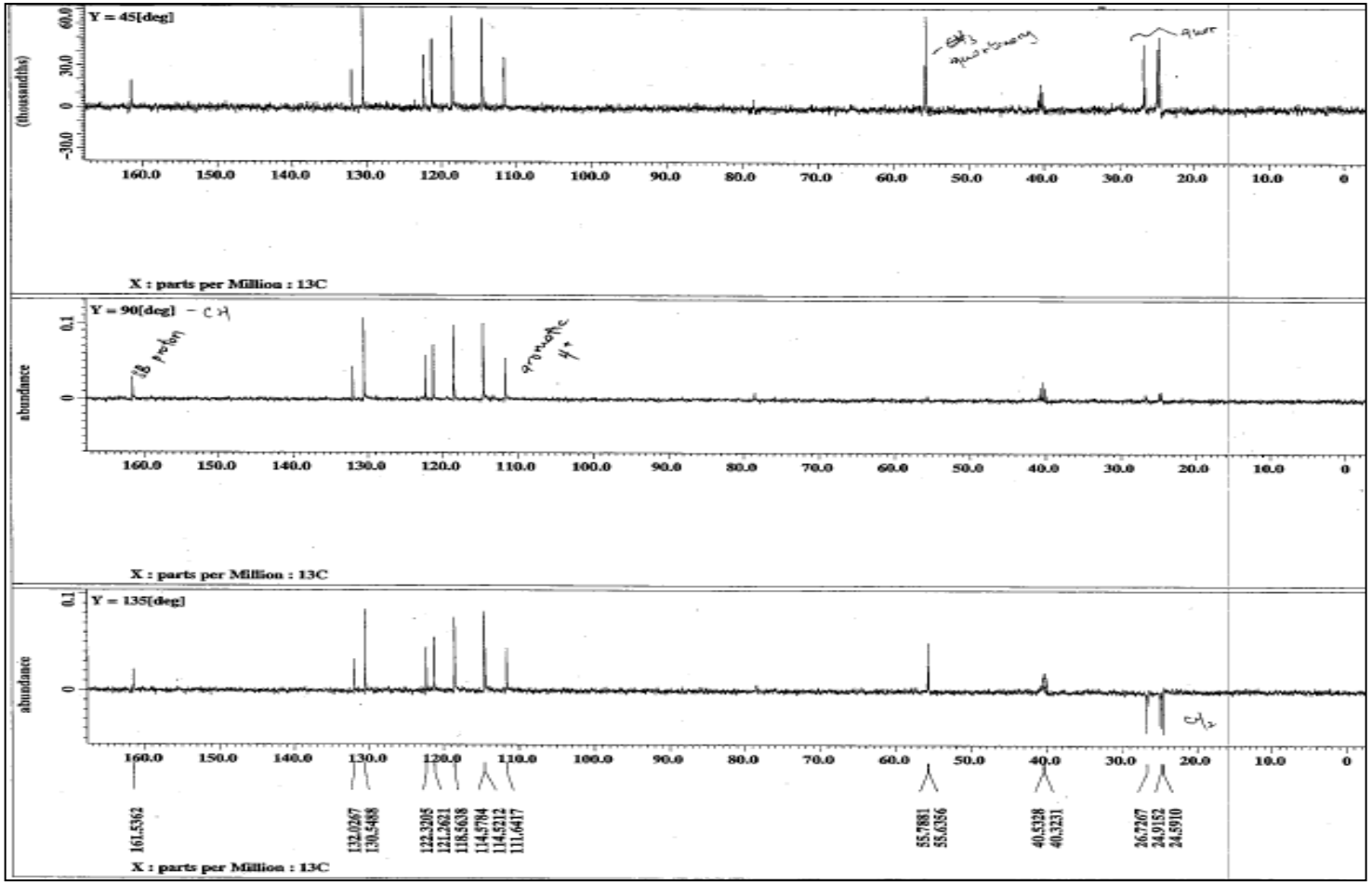
Appendix H: HMQC Spectrum of SB 8



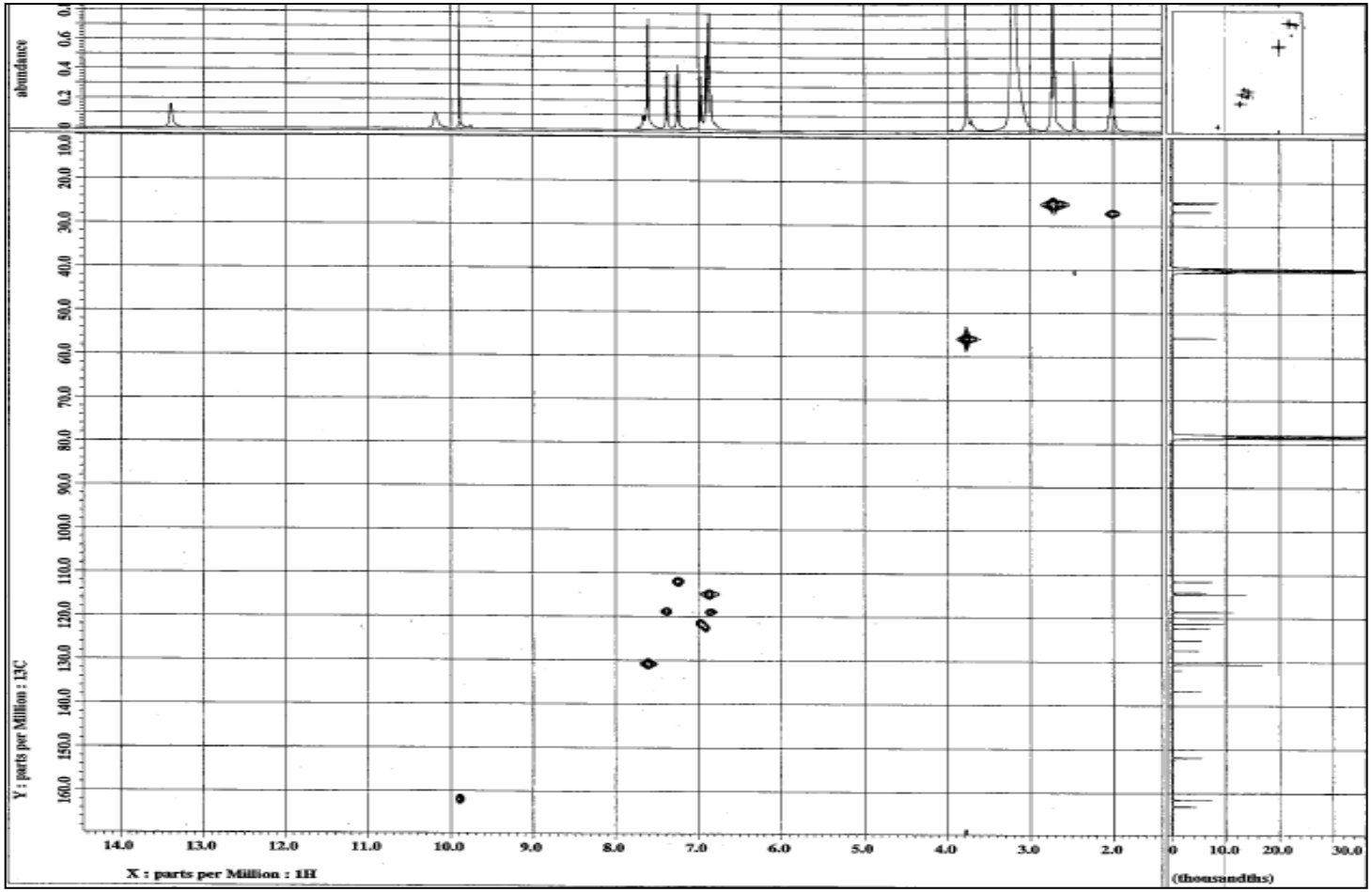
Appendix I: HMBC Spectrum of SB 8



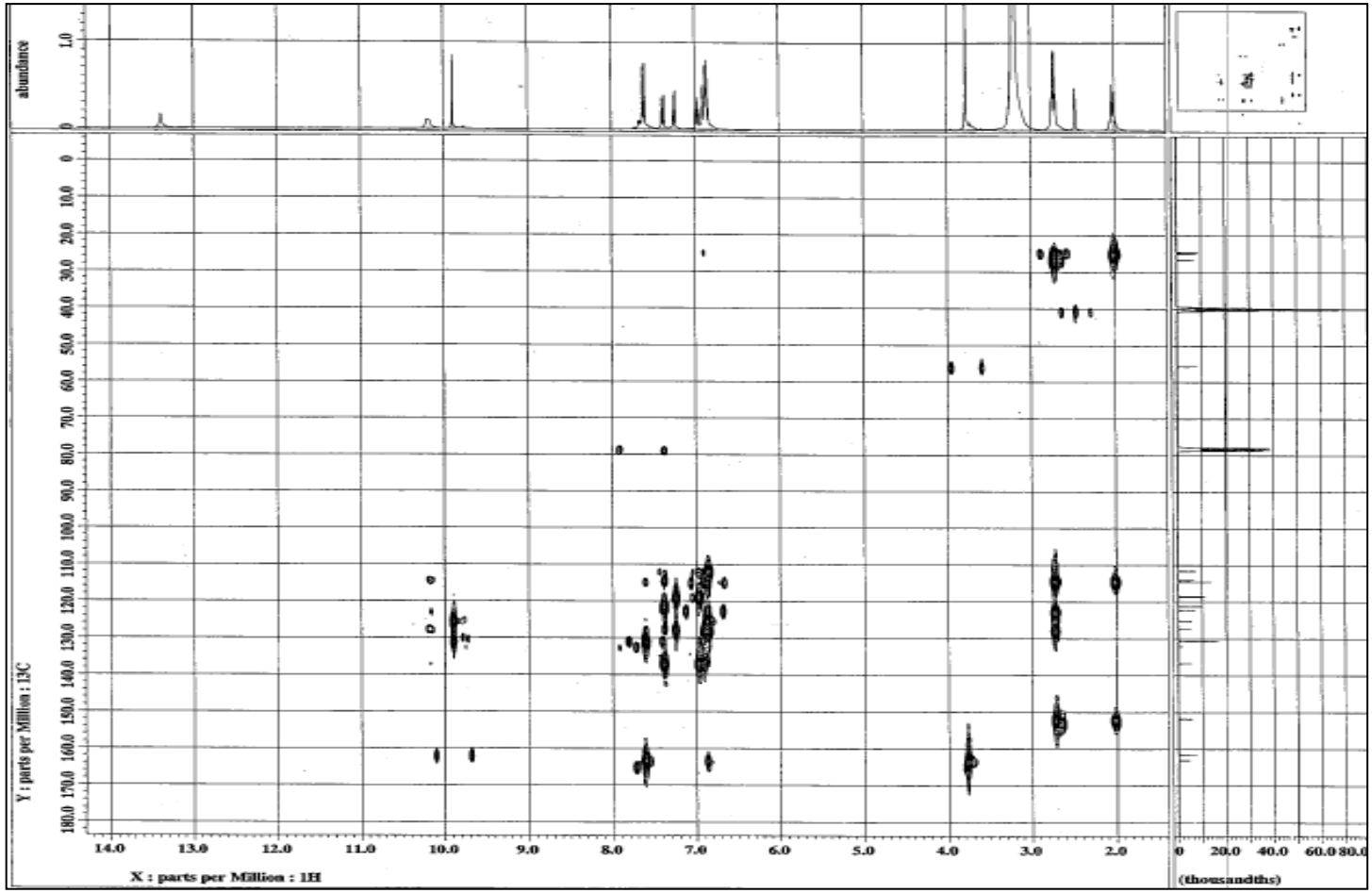
Appendix J: DEPT Spectrum of SB 6



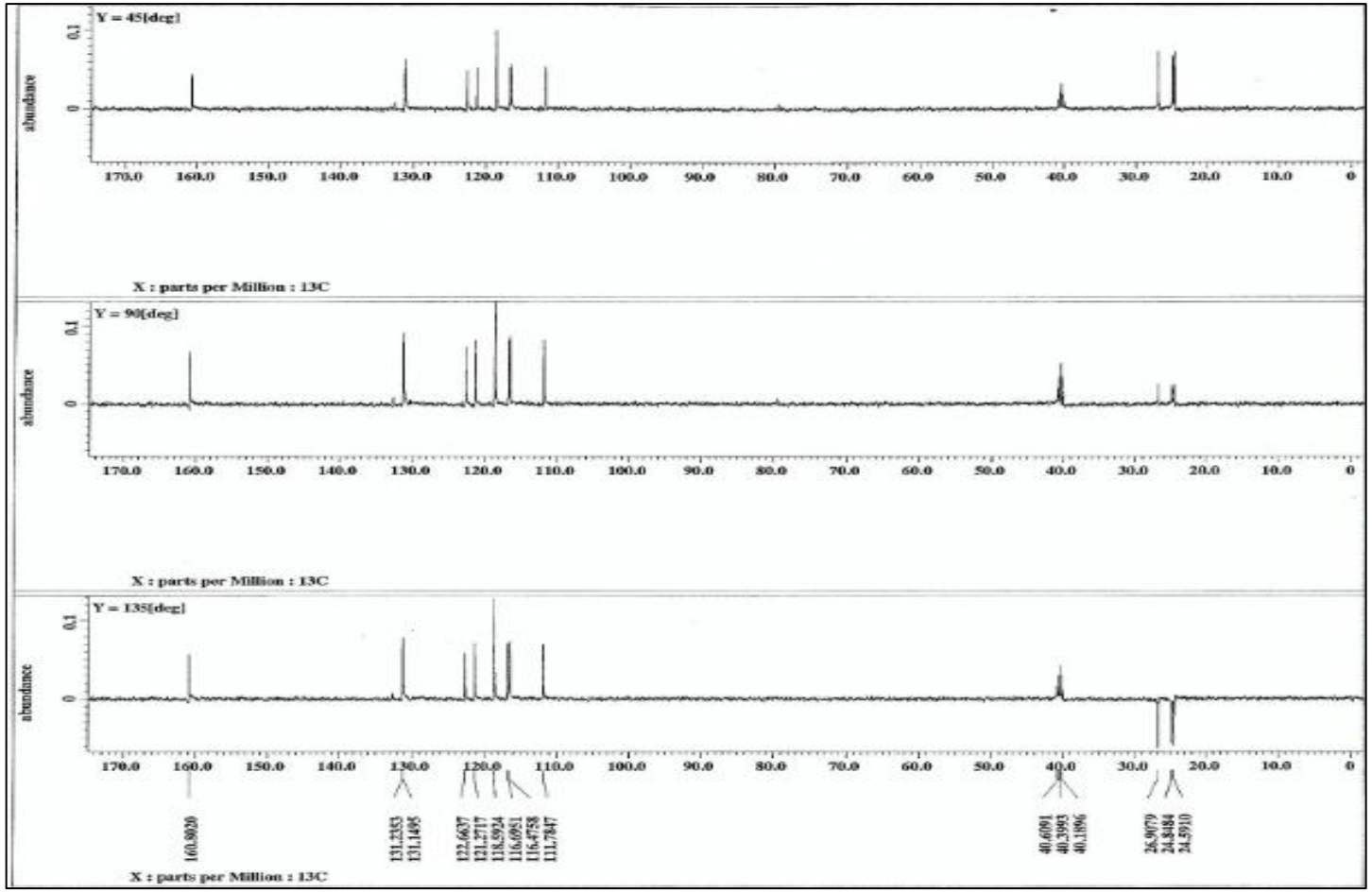
Appendix K: HMQC Spectrum of SB 6



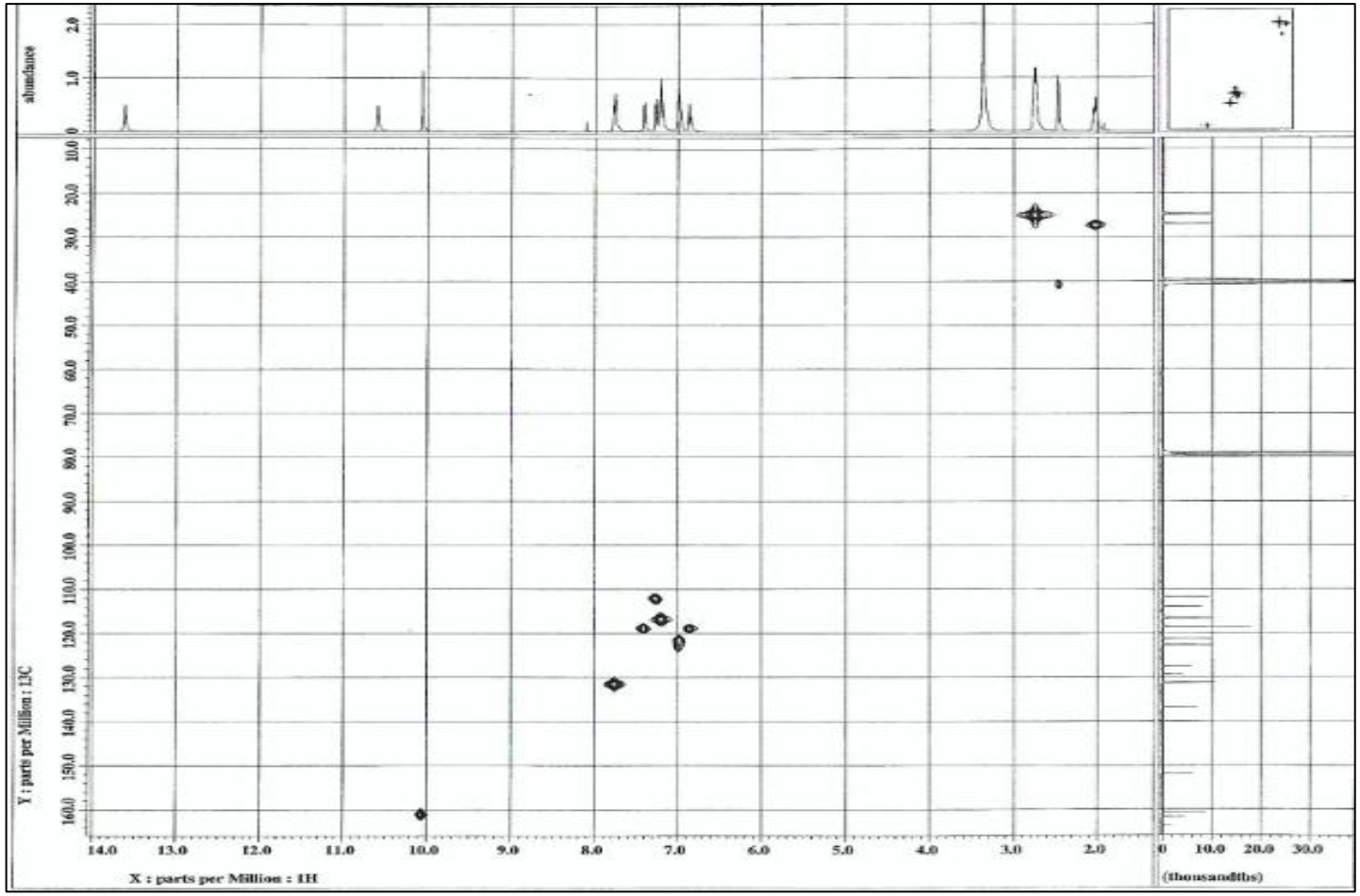
Appendix L: HMBC Spectrum of SB 6



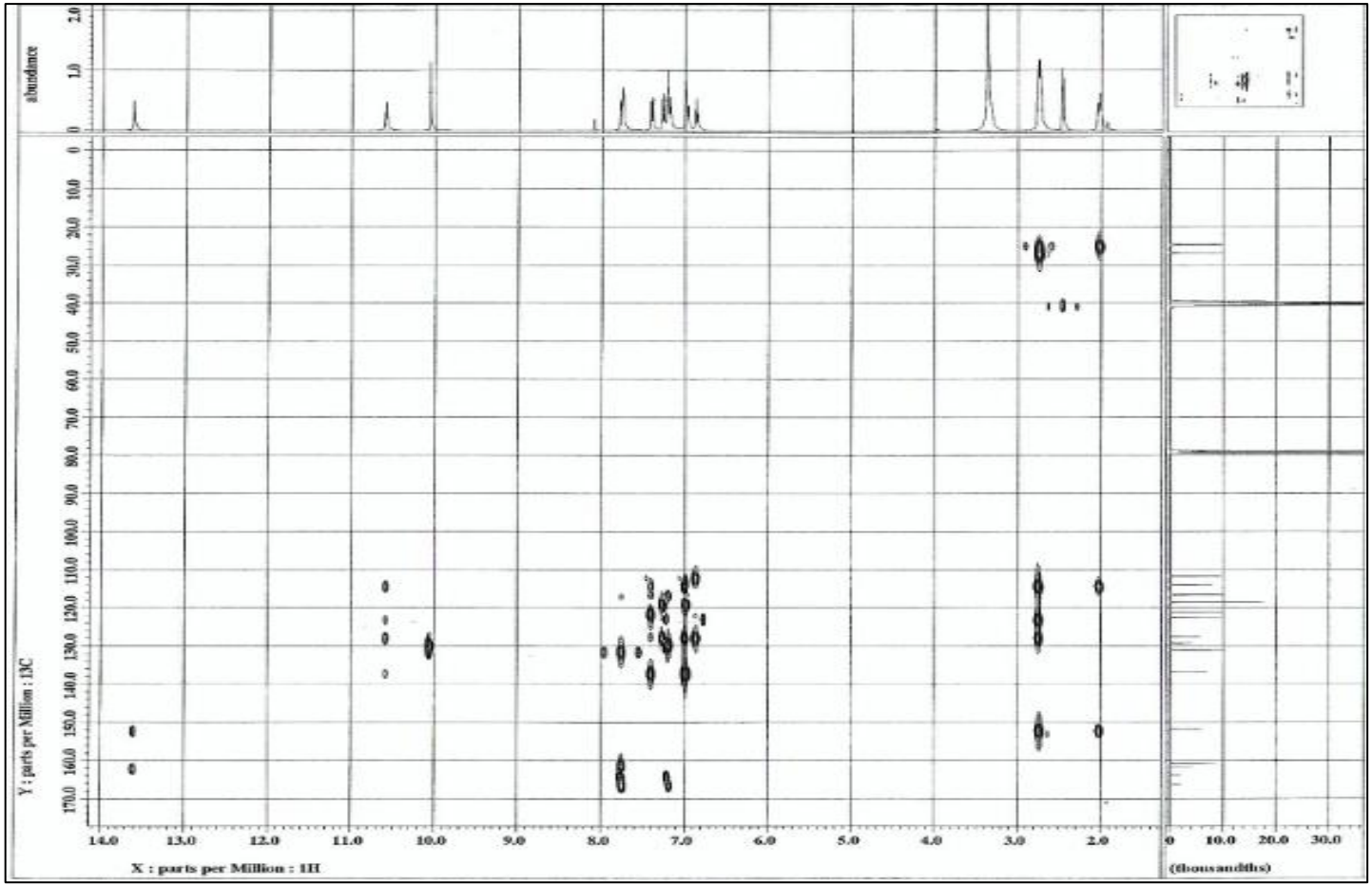
Appendix M: DEPT Spectrum of SB 3



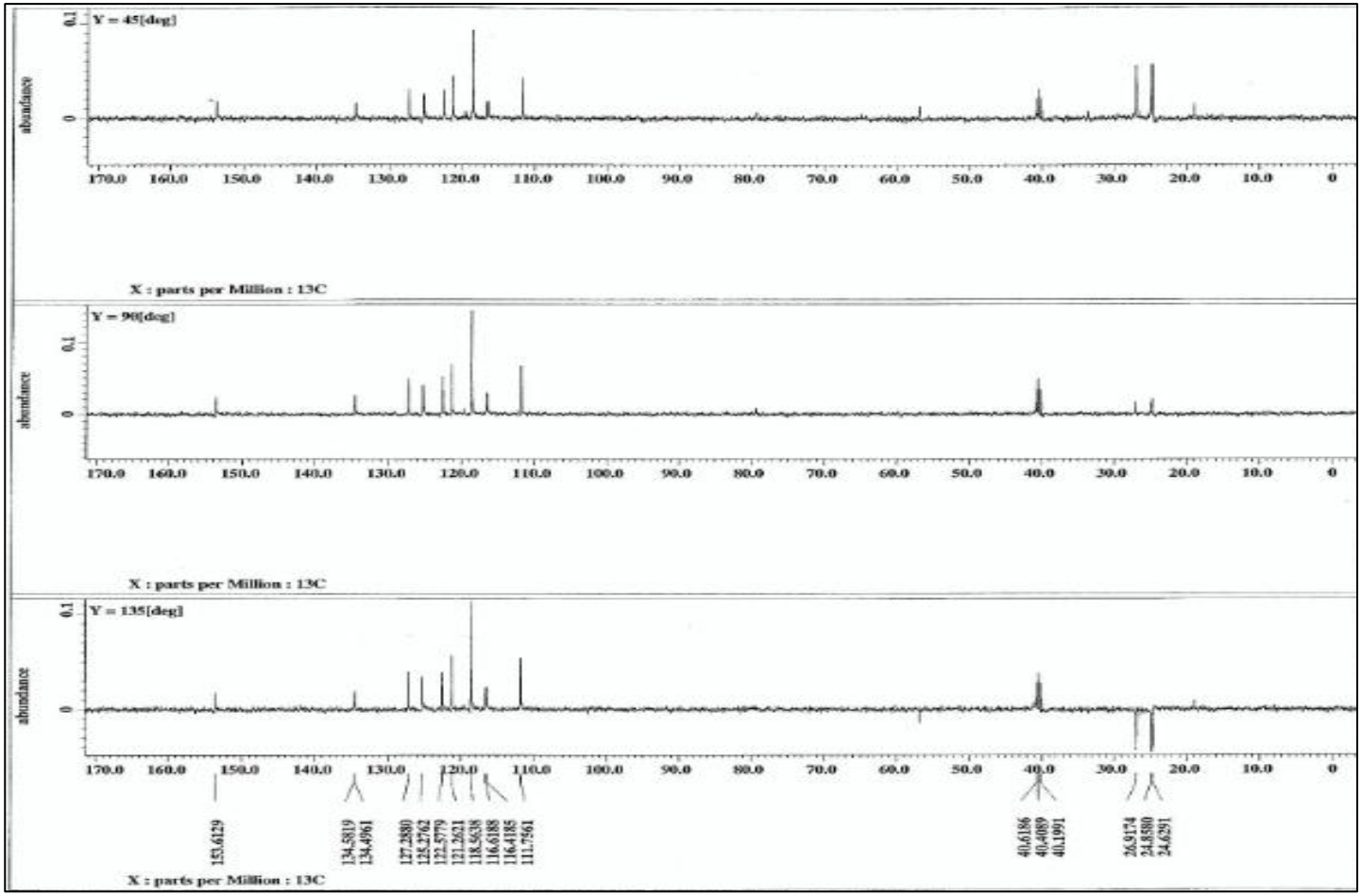
Appendix N: HMQC Spectrum of SB 3



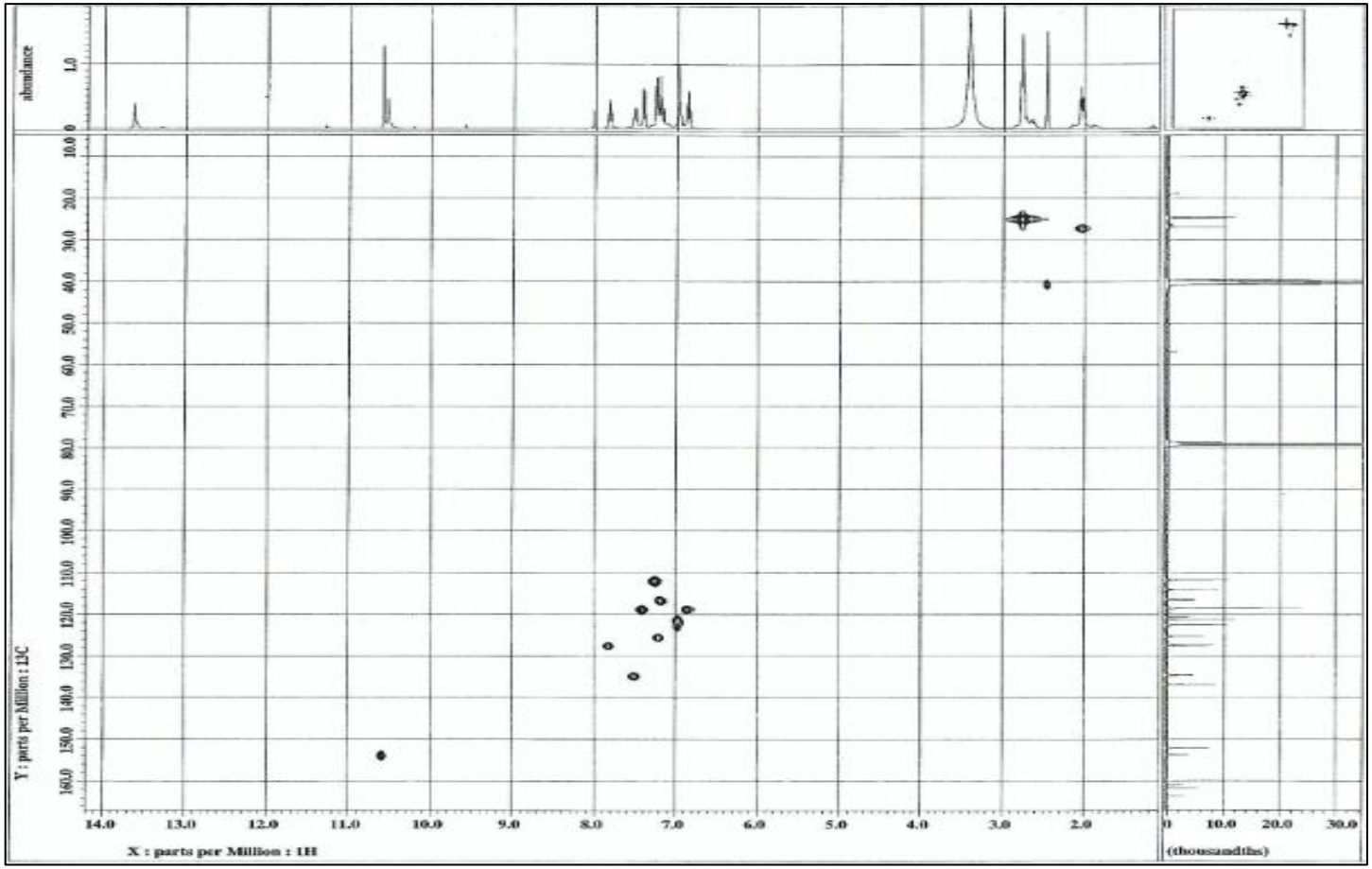
Appendix O: HMBC Spectrum of SB 3



Appendix P: DEPT Spectrum of SB 11



Appendix Q: HMQC Spectrum of SB 11



Appendix R: HMBC Spectrum of SB 11