SYNTHESES OF NEW BUTYLATED HYDROXYTOLUENE ANALOGUES AND THEIR POTENTIAL APPLICATION AS ANTIOXIDANT FOR SYNTHETIC LUBRICANT OIL

AMIT RANJAN NATH

INSTITUTE FOR ADVANCED STUDIES
UNIVERSITY OF MALAYA
KUALA LUMPUR

2019

SYNTHESES OF NEW BUTYLATED HYDROXYTOLUENE ANALOGUES AND THEIR POTENTIAL APPLICATION AS ANTIOXIDANT FOR SYNTHETIC LUBRICANT OIL

AMIT RANJAN NATH

THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

INSTITUTE FOR ADVANCED STUDIES
UNIVERSITY OF MALAYA
KUALA LUMPUR

2019

UNIVERSITY OF MALAYA

ORIGINAL LITERARY WORK DECLARATION

Name of Candidate: **Amit Ranjan Nath**

Matric No: **HHC150003**

Name of Degree: **Doctor of Philosophy**

Title of Project Paper/Research Report/Dissertation/Thesis ("this Work"):

SYNTHESES OF NEW BUTYLATED HYDROXYTOLUENE ANALOGUES AND THEIR POTENTIAL APPLICATION AS ANTIOXIDANT FOR SYNTHETIC LUBRICANT OIL

Field of Study: **Chemistry**

I do solemnly and sincerely declare that:

(1) I am the sole author/writer of this Work;
(2) This Work is original;
(3) Any use of any work in which copyright exists was done by way of fair dealing and for permitted purposes and any excerpt or extract from, or reference to or reproduction of any copyright work has been disclosed expressly and sufficiently and the title of the Work and its authorship have been acknowledged in this Work;
(4) I do not have any actual knowledge nor do I ought reasonably to know that the making of this work constitutes an infringement of any copyright work;
(5) I hereby assign all and every rights in the copyright to this Work to the University of Malaya ("UM"), who henceforth shall be owner of the copyright in this Work and that any reproduction or use in any form or by any means whatsoever is prohibited without the written consent of UM having been first had and obtained;
(6) I am fully aware that if in the course of making this Work I have infringed any copyright whether intentionally or otherwise, I may be subject to legal action or any other action as may be determined by UM.

Candidate's Signature Date:

Subscribed and solemnly declared before,

Witness's Signature Date:

Name:

Designation:

SYNTHESES OF NEW BUTYLATED HYDROXYTOLUENE ANALOGUES AND THEIR POTENTIAL APPLICATION AS ANTIOXIDANT FOR SYNTHETIC LUBRICANT OIL

ABSTRACT

Antioxidants are familiarly involved in the prevention of cellular damages as well as oxidative damages of chemical products caused by free radicals. Therefore, discovering the efficient multifunctional antioxidants is the continuous research to prevent the oxidation related problems effectively such as oxidative stress and pre-mature oxidation. In search of new multifunctional or multipotent antioxidants, four multifunctional antioxidant structures were prepared by assembling multiple antioxidant functions in a single structure. In this study, N^1-(3,5-di-tert-butyl-4-hydroxybenzylidene)-N^4-(substituted phenyl)-semicarbazone (5.7a-5.7j) and N^1-(3,5-di-tert-butyl-2-hydroxybenzylidene)-N^4-(substituted phenyl)-semicarbazone (5.10a-5.10j) were two envisioned multifunctional antioxidant structures, butylated hydroxyphenyl (BHP) incorporated semicarbazones, which were prepared from thiolated substituted semicarbazides bearing butylated hydroxyphenyl (5.5a-j) upon heating in the acidic ethanol in the presence of 3,5-di-tert-butyl-4-hydroxybenzaldehyde and 3,5-di-tert-butyl-2-hydroxybenzaldehyde respectively. The compounds 5.5a-j were also the designated multifunctional antioxidant structure which is the combination of multiple antioxidant functions such as BHA, thioether and aromatic secondary amine. Series 1 (5.7a-5.7j) and series 2 (5.10a-5.10j) were carried out DPPH assay to investigate the antioxidants properties. Series 1 compounds (5.7a-5.7j) were found as an efficient free radical scavenger in DPPH assay in comparison with standard BHT and Series 2 compounds. The increasing order of IC_{50} were found for Series 1 as follow as 5.7a >5.7b >5.7h >5.7e >5.7c >5.7g >5.7d >5.7i > BHT >5.7j >5.7f. Incorporation of 3,5-di-tert-butyl-4-

hydroxyphenyl to semicarbazones (compounds 5.7a-e, 5.7g-i) increased the antioxidant activity notably. It was concluded that hydrogen bond in Series 2 structure might be responsible for the lower antioxidant properties. Based on the solubility in synthetic lubricant oil trimethylolpropane trioleate (TMPTO) and obtained DPPH results, synthesized compounds were carried out several oxidation tests to evaluate the oxidation stability. The compounds (3,5-di-tert-butyl-4-hydroxy-benzylsulfanyl)-acetic acid methyl ester (6.1) showed promising oxidative stability to TMPTO oil in DSC, RBOT and thermogravimetric analysis in comparison with commercial antioxidants BHT and Irganox 1076. To obtain those above-designated compounds, a new reaction protocol for the preparation of semicarbazones became necessary to develop due to all the contemporary methods suffer from several limitations. Therefore, in this study, an efficient and robust reaction protocol was developed which provides a variety of antioxidant structures based on BHT moiety including the designated structures and also able to overcome the existing limitations. A variety of semicarbazones (28 compounds 4.7a-4.7g, 4.7a_1-4.7g_1, 4.7a_2-4.7g_2, 4.7a_3-4.7g_3) were initially prepared to validate the reaction protocol as proof of versatility, functional group compatibility and higher product yields of this method. This reaction can also help to get distinct substitution derivatives of semicarbazides forming a more carbon-nitrogen bond which will allow further research.

Keywords: Antioxidants, Semicarbazones, BHT, Oxidation stability, and Lubricant oil.

SINTESIS ANALOG HIDROKSITOLUENA TERBUTIL BARU DAN APLIKASI POTENSINYA SEBAGAI ANTIOKSIDAN UNTUK MINYAK PELINCIR SINTETIK

ABSTRAK

Antioksidan biasanya terlibat dalam pencegahan kerosakan selular serta kerosakan oksidatif produk kimia yang disebabkan oleh radikal bebas. Jadi, penemuan antioksidan pelbagai fungsi yang efisien adalah penyelidikan yang berterusan untuk mencegah masalah berkaitan pengoksidaan dengan berkesan misalnya, tekanan oksidatif dan pengoksidaan pra-matang. Dalam mencari antioksidan pelbagai fungsi atau multiplikasi baru, empat struktur antioksidan pelbagai fungsi telah disediakan dengan menghimpunkan pelbagai fungsi antioksidan dalam satu struktur. Dalam kajian ini. N^1-(3,5-di-tert-butil-4-hidroksibenzilidena)-N^4-(fenil tersubstitusi)-semikarbazon (5.7a-5.7j) dan N^1-(3,5-di-tert-butil-2-hidroksibenzilidena)-N^4-(fenil tersubstitusi)-semicarbazon (5.10a-5.10j) adalah dua struktur antioksidan pelbagai fungsi dari hidroksifenil terbutil (BHP) yang digabungkan dengan semikarbazon, yang disediakan daripada semikarbazida diganti berlapis yang mengandungi hidroksifenil terbutil (5.5a-j) etanol berasid masing-masing dengan kehadiran 3,5-di-tert-butil-4-hidroksibenzaldehida dan 3,5-di-tert-butil-2-hidroksibenzaldehida. Komponen 5.5a-j juga merupakan struktur antioksidan pelbagai fungsi yang merupakan gabungan pelbagai jenis antioksidan seperti BHA, thioeter dan amina aromatik sekunder. Siri 1 (5.7a-5.7j) dan Siri 2 (5.10a-5.10j) telah dijalankan untuk menilai sifat-sifat antioksidan. Sebatian Siri 1 (5.7a-5.7j) didapati sebagai pemulung radikal bebas yang cekap dalam ujian DPPH daripada piawai BHT dan sebatian Siri 2. Nilai IC50 yang semakin meningkat telah dijumpai untuk Siri 1 seperti berikut 5.7a> 5.7b> 5.7h> 5.7e> 5.7c> 5.7g> 5.7d> 5.7i> BHT> 5.7j> 5.7f. Gabungan 3,5-di-tert-butil-4-hidroksifenil kepada semikarbazon (sebatian 5.7a-e, 5.7g-i) meningkatkan aktiviti antioksidan. Disimpulkan bahawa ikatan hidrogen dalam struktur Siri 2 mungkin

bertanggungjawab terhadap sifat-sifat antioksidan yang lebih rendah. Berdasarkan kepada kelarutan di dalam minyak pelincir sintetik trimethylolpropane trioleate dan keputusan DPPH yang diperolehi, beberapa ujian pengoksidaan telah dilakukan ke atas sebatian yang disintesis untuk menilai kestabilan pengoksidaan. Sebatian (3,5-di-tert-butil-4-hidroksi-benzilsulfanil) -metil ester asid asetik (6.1) telah menunjukkan kestabilan oksidatif yang dijangka kepada minyak TMPTO dalam analisis DSC, RBOT dan termogravimetrik berbanding dengan antioksidan komersil BHT dan Irganox 1076. Untuk mendapatkan sebatian seperti di atas, protokol reaksi baru untuk penyediaan semikarbazon menjadi perlu untuk dibangunkan disebabkan semua kaedah kontemporari mengalami beberapa kekangan. Oleh itu, dalam kajian ini, protokol tindak balas yang cekap dan mantap telah dibangunkan yang menyediakan pelbagai struktur antioksidan berasaskan BHT termasuk struktur yang ditetapkan dan juga dapat mengatasi kekangan sedia ada. Pelbagai semikarbazon (28 sebatian 4.7a-4.7g, 4.7a_1-4.7g_1, 4.7a_2-4.7g_2, 4.7a_3-4.7g_3) disediakan pada awalnya untuk mengesahkan protokol reaksi sebagai bukti serba boleh, keserasian kumpulan berfungsi dan hasil produk yang lebih tinggi daripada kaedah ini. Tindak balas ini juga dapat membantu dalam menghasilkan penggantian terbitan yang berbeza daripada semikarbazida untuk membentuk ikatan karbon-nitrogen yang lebih besar agar dapat digunakan dalam penyelidikan ini dengan lebih lanjut.

Keywords: Antioksidan, Semicarbazones, BHT, Kestabilan oksidasi, dan minyak pelincir.

ACKNOWLEDGMENTS

I dedicated my thesis to the most important person in my life, my beloved parents Manik Ranjan Nath and Konica Rani Nath.

It was impossible to write this thesis without the great help and support of the kind people around me, to only some of whom it is possible to give particular mention here. For those people, who are their names may not appear here, their efforts and help will still be in my mind forever.

With a deep sense of gratitude and respect, I would like to thank my supervisors **Late Professor Dr. Sharifah Bee Abd Hamid, Professor Dr. Mohd Rafie Bin Johan and Dr. Wageeh Abdulhadi Yehye Dabdawb** for their generous support, outstanding guidance, encouragement, advice and wisdom throughout this Ph.D. journey. I would also thank Prof. Dr. Md. Mahbubul Matin for his inspiring support to continue my research career.

I would like to thank my younger sister Trishna, younger brother Aviram along with my best companion Santanu Baura, and also Sheikh Md. Rasel, Nibedita Mallik, Deen Mohammad, Sharif, Mahbub and Anjon for their continuous mental support. I was also happy to have my senior fellows in NANOCAT, especially Ibrahim Khalil, Dr. Ziaul Karim, Dr. Asing, Dr. Subrata Saha.

I would also like to thank all NANOCAT staffs and friends, specially Mohd Hafiz Ahmad, Syuhada Abas, Syabilah Sazeli, Shahrooz Rahmati will be in my mind forever for their cordial and continuous support to carry out my work smoothly.

Sincerely

Amit Ranjan Nath

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SCHEMES

LIST OF SYMBOLS AND ABBREVIATIONS

ADPA	:	Alkylated diphenylamine
AcOH	:	Acetic acid
ALA	:	Alpha-linolenic acid
BDE	:	Bond dissociation energy (enthalpy change)
BHA	:	Butylated hydroxyanisole
BHP	:	Butylated hydroxyphenyl
BHT	:	Butylated hydroxytoluene
CoAH	:	Co-antioxidant
COX	:	Cyclooxygenase enzyme
DG	:	Dodecyl gallate
DPPH	:	Radical 2,2-diphenyl-1-picrylhydrazyl
DNA	:	Deoxyribonucleic acid
DHA	:	Docosahexaenoic acid
EPA	:	Eicosapentaenoic acid
EtOH	:	Ethanol
EP	:	Extreme-pressure
FDA	:	Food and drug administration
G^+	:	Gram-positive
G^-	:	Gram-negative
GABA	:	Gamma-aminobutyric acid
HALS	:	Hindered-amine-light stabilizers
HPB	:	Hydrophobic interaction
IC_{50}	:	Half maximal inhibitory concentration
MeOH	:	Methanol

MDTI	:	N-methyl-3-methyl-6,7-dihydroxy-1,2.3,4 tetrahydroisoquinoline-3-carboxylic acid
MPAO	:	Multipotent antioxidants
NMR	:	Nuclear magnetic resonance
NSAID	:	Nonsteroidal anti-inflammatory drugs
NADPH	:	Nicotinamide adenine dinucleotide phosphate H
OG	:	Octyl gallate
PG	:	Propyl gallate
PTSA	:	P-Toluenesulfonic acid
ROS	:	Reactive oxygen species
RNS	:	Reactive nitrogen species
RT	:	Room temperature
SAR	:	Structure-activity relationship
TBHQ	:	Tertiary-butylhydroquinone
TBMP	:	2-tert-butyl-4-methylphenol
UV	:	Ultra-violet
ZDDP	:	Zinc dialkyldithiophosphates
α-TOH	:	α-Tocopherol

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.1 Background of the study

Development of environmental-friendly lubricant from renewable resources has drawn a great attention for the last few years to reduce dependency on under earthed fossil-based resources due to the risen ecological concerns and growing preventive protocols (Alias et al., 2009; Erhan & Asadauskas, 2000; Wu et al., 2013). Since dependency on fossil-based resources is no longer reasonable in view of viable, eco-friendly and socio-economic purposes and moreover, the reserve of fossil-based resources is markedly reducing with time. Hence, producing lubricant from renewable resources and increasing the performance level to replace the mineral lubricant oil have been extensively studied for recent years. Synthetic lubricant oils or vegetable oils have gained a great consideration because it derived from cheaper renewable resources and their better lubricant properties than the mineral oils. Unfortunately, due to the lower oxidative and thermal stability it has failed to get wide acceptance. This is because the production of free radical in lubricant oil causes premature oxidative degradation that is responsible for product damage, discoloration and reduced lifetime. Apart from industrial products, free radicals have detrimental effects on the human body. Imbalance of free radicals and antioxidants in the human body causes oxidative stress that is responsible for many chronic diseases. Thus, investigation of multifunctional or multipotent antioxidants which can effectively scavenge the free radicals as well as improve the oxidative stability of synthetic ester-based lubricant oil is the outcome of this research work.

1.2 Problem Statement and Research Scope

Oxidative stress is one of the main causes of etiology and expansion of major human deteriorating diseases such as cancer, alzheimer, parkinson, insomnia, hypertension, heart failure inflammation and others. The imbalance of production of free radical (reactive

oxygen species) and ability of the body to counteract the free radical through by natural system suffers oxidative stress (Finkel & Holbrook, 2000). On the other hand, the oxidative breakdown or premature oxidation of the engine oil caused by free radicals creates sludge and deposits, deteriorates the viscosity characteristics of the oil, and produces acidic bodies that corrode engine parts. The pre-mature oxidation or auto-oxidation process can be stopped or delayed by introducing antioxidant into the products. Antioxidant is a molecule that can inhibit the oxidation process by three different ways such as scavenging free radicals, decomposing peroxides radicals and deactivating metal ions which are prone to produce free radicals (Nath & Yehye, 2018).

To combat the effects of oxidation, engine oils are formulated with an array of antioxidants including, zinc dithiophosphates (ZDDP). The use of ZDDP in engine oils is declining due to the regulative environmental policy. Further, conventional Alkylated diphenylamine (ADPA) antioxidants insufficiently stabilize the lubricants. Butylated hydroxytoluene (BHT) is one of the most commonly used phenolic antioxidants in petroleum products (Rudnick, 2017). Unfortunately, it has classified as a high human health priority by causing environmental pollution. In addition, prevention of cellular oxidative stress become a paramount priority to decline the risk of several chronic diseases. Thus, development of multipotent or multifunctional antioxidants is the now continuous research in search of a more efficient and effective molecule which can act strongly against oxidative problems (e.g. oxidative stress or oxidative stability).

However, another problem was encountered in this study during the preparation of envisioned multipotent antioxidants, which was several limitations of contemporary reaction methods for the preparation of semicarbazones. One of the main problems of these methods is functional group incompatibility. For example, semicarbazones bearing ester, acetyl group could not be prepared by the existing reported procedure. In addition,

product yields were lower in the general semicarbazones preparation method (Nath & Yehye, 2018). Therefore, it became important to discover the efficient and robust reaction protocol for the preparation of semicarbazones in search of new multifunctional or multipotent antioxidants.

1.3 Objectives

The main research focuses on this study are to synthesize the multifunctional or multipotent antioxidants based on BHT analogous and to assess antioxidant activity of synthesized compounds by DPPH assay as well as investigate the oxidative or thermal stability in synthetic ester-based lubricant. Therefore, the objectives of this study are:

1. To design and synthesize of high efficiency multipotent and multifunctional antioxidants based on the BHT moiety.
2. To evaluate the antioxidant activity of the synthesized designated compounds by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay.
3. To evaluate the oxidation stability of ester-based lube oil according to standard oxidative and thermal test.

The novelty of this study is to establish a new and robust reaction method for the synthesis of semicarbazones which offer better functional group compatibility, chemoselectivity and greater product yields. And synthesize the novel and efficient multipotent and multifunctional antioxidants which are anticipated to obtain better antioxidant activity and functionality.

1.4 Overview of chapters

Chapter 1: This chapter introduces the background of this research, problem statements, importance of this study, research objectives and outline of this thesis along with a brief discussion on the correlation of all chapters.

Chapter 2: An extensive literature review has been performed on this study that is included in Chapter 2. It describes free radical and its detrimental effects on the human body and industrial products. It also includes the several class of antioxidants to counteract the free radicals and its applications. For example, a brief description about the endogenous antioxidants that form inside the human body, exogenous natural antioxidants that are not only essential for human health but also industrial products such food, foodstuffs, oil, rubber and others.

Chapter 3: This chapter discusses multipotent or multifunctional antioxidants and their importance. Multipotent or multifunctional antioxidant structures were designated in this study based on rational-design strategy.

Chapter 4: Since exiting reported methods for the semicarbazone preparation have many limitations and it was difficult to synthesize all the designated antioxidant structure by following the contemporary reaction procedures. So, it became necessary to develop a new reaction protocol for the preparation of semicarbazones which would offer functional compatibility, chemo-selectivity and greater products yields. In response to this necessity, an efficient and robust reaction protocol was revealed in this chapter for the synthesis of semicarbazone derivatives which has overcome all previously reported method's limitations. A wide variety of semicarbazones were synthesized by this reaction protocol using several reactive functional groups to investigate the method's versatility.

Another important aspect of this method is that all the designated antioxidant structures were obtained in one reaction protocol.

Chapter 5: In Chapter 5, designated semicarbazone compounds were synthesized by following the established reaction protocol described in chapter four. Two series of semicarbazone derivatives containing butylated hydroxytoluene (BHT) were synthesized and investigated their free radical scavenging properties by DPPH assay. Comparison study along with the role of the position of hydroxyl group on the antioxidant activity was done in this chapter. Chapter 5 also includes the oil oxidation stability tests which were carried out with some compounds blending with synthetic ester-based lubricant oil (trimethylolpropane trioleate, TMPTO).

Chapter 6: Two important compounds were chosen among the synthesized compounds for detailed evaluation of oxidation stability test blending with trimethylolpropane trioleate (TMPTO) as synthetic lubricant oil and were compared with two commercial antioxidants namely, butylated hydroxytoluene (BHT) and Irganox 1076. Two kinds of oil oxidation test such as rotary bomb oxidation test (RBOT) and differential scanning calorimeter (DSC) were described in this chapter as ASTM (American Society for Testing and Materials) procedure. Thermogravimetric analysis (TGA) was also included here for the investigation of thermal decomposition.

Chapter 7: This chapter wraps up the thesis with some concluding remarkable the research findings. Also, the future scopes of this research study have been concluded in this section.

CHAPTER 2: LITERATURE REVIEW

There are enormous applications of antioxidants in our daily life products including from pharmaceuticals, food and foodstuffs to lubricant oil. To balance the amounts of free radicals in tolerance limit, different kinds of enzymatic endogenous antioxidants are produced inside the body to counteract the excessive amounts of free radicals. While body immune system is unable to neutralize the excess free radicals, then it becomes necessary to provide natural or synthetic exogenous antioxidants as a supplement inside the body to balance the free radicals. Moreover, industrial chemical products such as food and foodstuffs suffer from oxidative damages because of auto-oxidation and aerobic oxygens. So, introducing antioxidants into the food and foodstuffs in tolerance limit is essential to protect the food from oxidation. Again, several kinds of antioxidants are applied to petrochemical products such as lubricant oils because antioxidants prolong the lubricant lifetime by the increase of the oxidative and thermal stability. In this chapter, free radicals and different kinds of antioxidants and their applications on industrial products (e.g. food, foodstuffs and lubricant oil) are discussed.

2.1 Free radical and its sources

Free radicals are very well-known substance in the biological system and industrial products. Generally, a molecule or substance with an odd number of electrons in its outermost shell is known as free radical. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) act as free radical. ROS can be found in different forms such as hydroxyls ($HO^{\cdot}$), superoxide ($O_2^{\cdot -}$), peroxyl ($ROO^{\cdot}$), alkoxyl ($RO^{\cdot}$) and nitric oxides ($NO^{\cdot}$) and these are directly responsible for the oxidation in biological system and several industrial products such as foodstuffs, rubber, pharmaceuticals, oil and plastics (Lee et al., 2004). Generally, free radicals are produced in the human body either from endogenous or exogenous sources. Endogenous ROS/RNS are generated from aerobic

respiration, immune cell stimulation, inflammation, infection, cancer and aging. Exogenous free radicals result from air and water pollution, cigarette smoke, alcohol, heavy or transition metals (Cd, Hg, Pb, Fe, As), specific drugs (cyclosporine, tacrolimus, gentamycin, bleomycin), ultra-violet light and ionizing radiation (Valko et al., 2005; Valko et al., 2006; Willcox et al., 2004). Free radicals can be either beneficial or harmful to the human body based on the amount of production inside the body. At average concentrations, free radicals are important in all living organisms and have biological advantages. For instant, phagocytes (neutrophils, macrophages, monocytes) discharge free radicals to abolish the dangerous pathogenic microbes as part of the body's self-defense system (Droge, 2002; Is & Woodside, 2001). The importance of free radical production by the natural immune system can be understood by granulomatous disease. The patients with this disease have abnormal membrane-bound NADPH oxidase system which is unable to release the superoxide anion radical ($O_2^{\cdot -}$), thereby the patients suffer from multiple and persistent infections (Droge, 2002; Valko et al., 2007). It also plays an important role as a signal substance in the different cellular system and contributes to the heart for being able to provide more blood in a stress-filled situation (Andersson et al., 2011; Halliwell, 2007; Is & Woodside, 2001). On the other hand, excessive free radical production causes oxidative stress to the human body. Extreme free radicals can damage the cell membrane, and lipoprotein by lipid peroxidation process. The structure of proteins can be changed by the free radical attack which causes the loss of enzyme activity (Frei, 1994). Free radical can also alter the DNA structure which occurs mutation. Though, the body has its own defense mechanism to counteract the excessive free radicals by DNA repair enzyme and/or producing antioxidants. If the natural immune system is unable to destroy the excessive free radical formed by endogenous and exogenous sources, then the body suffers from oxidative stress. It can cause a variety of chronic and deteriorating diseases as well as the aging process and some severe pathologies. Many

researchers have reported that several diseases such as inflammatory disease (Chatterjee et al., 1996), cancers disease (Sangeetha et al., 1990), degenerative disease (Kar et al., 2010) and chronic diseases (Khalil & Khodr, 2001) are the results of oxidative stress which is caused by excessive free radicals. Most of the diseases were outlined in Figure 2.1 caused by oxidative stress.

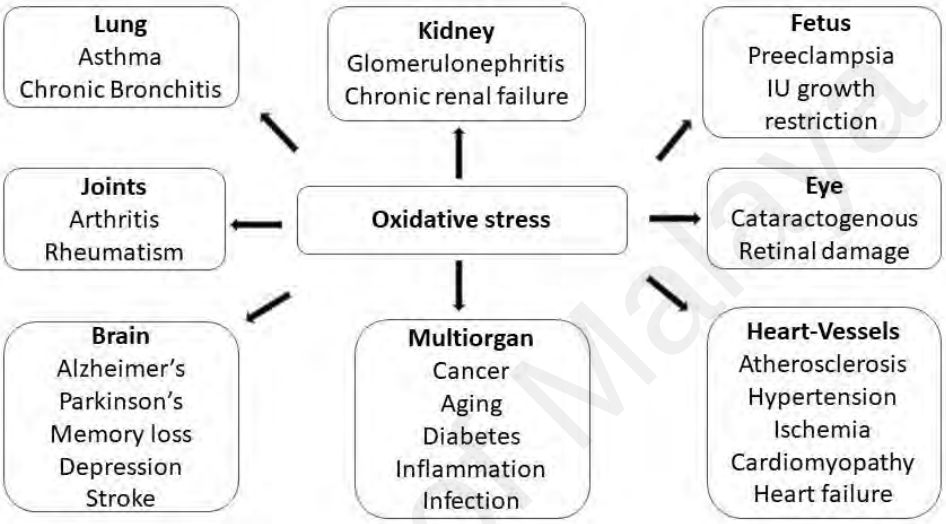


Figure 2.1: Several diseases in the human body caused by oxidative stress.

2.2 Antioxidant and its classification

The body has several defense mechanisms to counteract the invading free radicals by releasing antioxidants, either endogenous antioxidants (naturally occurred) or exogenous antioxidants, come from different foods. The main activity of antioxidants is to counteract the excess free radicals in order to protect the cell (lipoprotein, protein, DNA) from damages and mutations caused by free radicals, eventually contribute to keep away the human body from the several diseases (Pham-Huy et al., 2008). Antioxidants can be classified into two groups: endogenous antioxidants and exogenous antioxidants, while there are two types of endogenous antioxidants namely: enzymatic endogenous antioxidants and non-enzymatic endogenous antioxidants. Enzymatic endogenous

antioxidants are released from the body to counteract the excessive free radicals naturally. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GRx) are some of the examples of enzymatic endogenous antioxidants (Genestra, 2007; Halliwell, 2007; Is & Woodside, 2001). Non-enzymatic endogenous antioxidants are generated by metabolism in the body such as lipoid acid, glutathione, coenzyme Q10, melatonin, uric acid, bilirubin, metal-chelating proteins, transferrin and others.

Exogenous antioxidants are those which are supplied into the body through supplements or diet as that antioxidants cannot be generated in the body naturally. These antioxidants are divided into groups: natural or nutrient antioxidants and synthetic antioxidants.

2.3 Exogeneous Natural Antioxidants

Nutrient or natural antioxidants occur in the plant and their extracts, are usually phenolic and polyphenolic compounds. The nutrient antioxidant deficiency is one of the reasons for the several chronic and degenerative pathologies. Each nutrient has its own properties and antioxidant functionality based on its structure. For example, taking vegetables and fruits enriched with vitamins A, E, and C can reduce the chances of many diseases such as inflammatory processes (Sandoval et al., 2002), cardiovascular (Nivas et al., 2010), cancer (Yi et al., 2005), diabetes (Ghosal, 2005), and hypertension (Schroeder & Adams, 1941). The main reason for the protective properties of these vitamins is attributed to their structure along with minerals such as potassium, zinc, and selenium. Most of these vitamins are phenolic compounds which can act as free radical scavengers, antimutagens and metal chelators (Osawa, 1992).

2.3.1 Vitamin E

Vitamin E is a lipophilic high potency antioxidant. Only α-tocopherol (α-TOH) is one of most bioactive structure among the eight stereoisomers of vitamin E. It can release protons to scavenge the free radicals by converting itself into α-tocopherol radical (α-$TO^{\cdot}$) as seen in Figure 2.2. α-tocopherol radical (α-$TO^{\cdot}$) is more stable radical due to the resonance inside the benzene ring (Fuller, 2004). It has been reported that Vitamin E has excellent preventive properties of breast, prostate and colon cancers, some cardiovascular diseases, arthritis and certain neurological disorders (Thendral et al., 2015). Vegetable oils, nuts, fruits, eggs, whole grains, cereals and meets are the main of sources of vitamin E (Willcox et al., 2004).

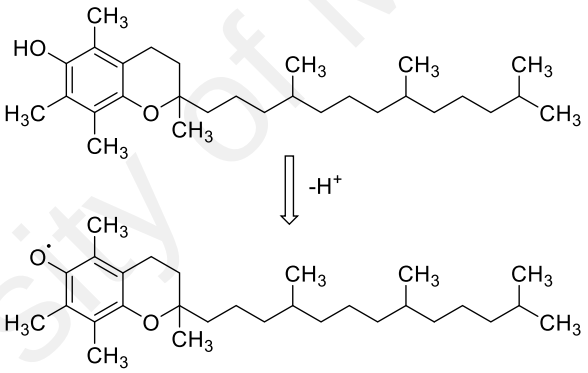


Figure 2.2: Conversion of α-tocopherol (α-TOH) into α-tocopherol radical (α-$TO^{\cdot}$) by donating a proton.

2.3.2 Vitamin C

Vitamin C is a lipophobic or hydrophilic compounds and also familiar as ascorbic acid. It is important elements for the carnitine, collagen, and neurotransmitters biosynthesis and also acts as antioxidant, anti-carcinogenic, anti-atherogenic, and immunomodulator (Li & Schellhorn, 2007). Ascorbic acid can donate one or two protons by forming ascorbate free radical and dehydroascorbic acid respectively as shown in Figure 2.3 (May,

1999). Ascorbic acid shows excellent synergistic effect with vitamin E to counteract the free radicals and also regenerates the reduced form of vitamin E (Pham-Huy et al., 2008). Many researchers reported that vitamin C is good at reducing the rate of stomach cancer, and also at preventing lung and colorectal cancer (Naidu, 2003).

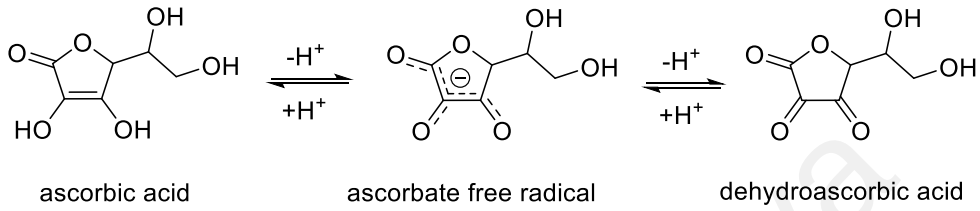


Figure 2.3: Oxidation state of vitamin C or ascorbic acid.

2.3.3 Beta-carotene

Beta-carotene is a fat-soluble provitamin, a member of carotenoid. Oxidation cleavage of beta-carotene according to Figure 2.4 provides active retinol which is familiar as vitamin A (Pham-Huy et al., 2008). Retinol is an excellent antioxidant and is the strong quencher of oxygen singlet. Foods containing a high amount of beta-carotene reduce the chance of lung and prostate cancer (Pham-Huy et al., 2008). Many fruits vegetables (carrots, green plants, squash, spinach), grains, and oil are enriched with beta-carotene (Willcox et al., 2004).

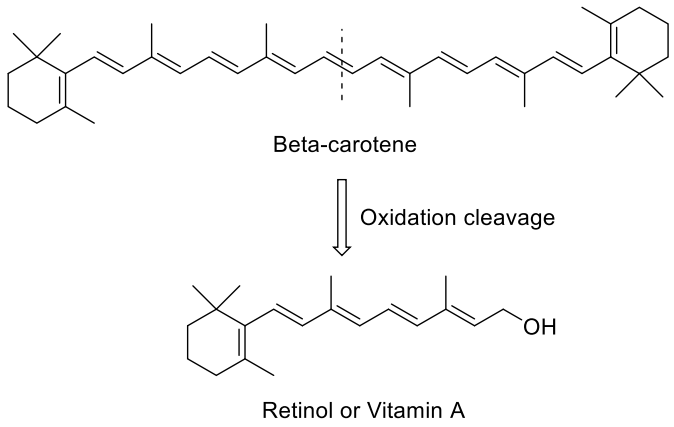


Figure 2.4: Formation of vitamin A (retinol) from Beta-carotene.

2.3.4 Flavonoids

Flavonoids are a group of polyphenolic compounds with excellent antioxidant properties. Based on the chemical structure, flavonoids are divided into many classes such as flavanols, flavanones, flavones, isoflavones, catechins, anthocyanins, proanthocyanidins and around 4000 more flavonoids compounds were reported (Miller, 1996). Few of flavonoids compounds such as quercetin, kaempferol, myricetin, apigenin, and luteolin are shown in Figure 2.5. Due to its potent antioxidant activity, it brings great benevolent effects on human health. They are being used to prevent or reduce of chances of several chronic or degenerative diseases such as cancer, arthritis, memory loss, stroke, Alzheimer's, inflammation, cardiovascular diseases and others (Hanneken, Lin, Johnson, & Maher, 2006). Mainly, flavonoids compounds are found naturally in vegetables, fruits, beverage, tea and wine (Hanneken et al., 2006; Kühnau, 1976).

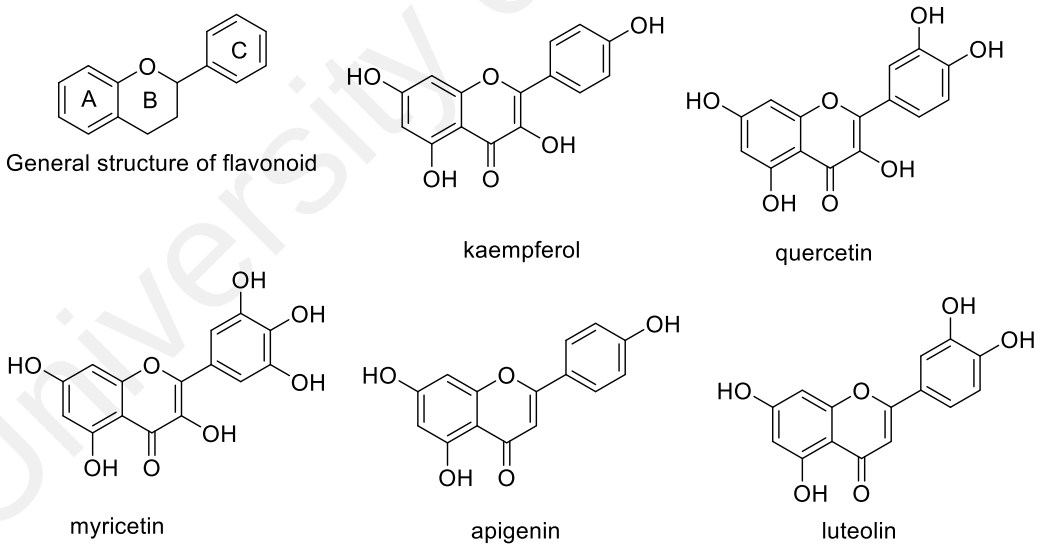


Figure 2.5: Some of the flavonoid compounds.

2.3.5 Lycopene

Lycopene is another member of carotenoid which is familiar for its antioxidant and antiproliferative properties (Seren et al., 2008). Researchers have found the relationship

between high ingestion of lycopene and reduced risk of prostate cancer, though all studies did not show the consistent results (Seren et al., 2008). Different kinds of dishes of tomatoes such tomatoes juice and tomato sauce are the major sources of lycopene which are more bioavailable than that in raw tomatoes (Donaldson, 2004).

2.3.6 Poly saturated phenolic acids

Some poly saturated phenolic acids are also familiar for their antioxidant activity such as Omega-3 and Omega-6 fatty acids. A proper balance of Omega-3 and Omega-6 fatty acids is very important in the good diet because both the substances act synergistically to keep the body healthy (Logan, 2004; Pham-Huy et al., 2008). Omega-6 should be 2-4 times more than Omega-3s in an appropriate balance of a healthy diet. Both the substances are good at preventing many chronic diseases such as cancer, arthritis, depression, psoriasis etc. Among the three major types of Omega-3 fatty acid: eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and alpha-linolenic acid (ALA), the former two are found largely in sea fish such as salmon, tuna, halibut while nut oils and flaxseed are the sources of ALA. Omega-6 fatty acids occur naturally in vegetable oils, nuts, cereals, eggs and poultry (Donaldson, 2004).

2.4 Synthetic Antioxidants and their applications

Due to the instability of natural antioxidants, several synthetic antioxidants have been utilized to protect the cell and industrials goods from oxidation damages. It was found that many synthetic antioxidants have shown better antioxidant activity than the natural antioxidants which can be easily produced in the laboratory (Makahleh et al., 2015). Synthetic antioxidants have been widely used in the food industries, pharmaceuticals, rubber and oil industries. Synthetic antioxidants can be classified into two groups: primary antioxidant and secondary antioxidants based on their mode of action. Primary

antioxidants are also known as free radical scavengers while secondary antioxidants are called peroxide decomposer.

2.4.1 Synthetic antioxidants in food and edible oil industries

Nutritional quality of food, its flavor, consumer acceptability and toxicity of food products can be influenced by the oxidation of its ingredients (Alamed et al., 2009). Generally, auto-oxidation is one of the main reasons for the deterioration or rancidity of fat, oil or related food which initiated by free radicals (Sowbhagya & Bhattacharya, 1976). These free radicals may arise either from the air oxygen or degradation of carbon-based compounds which is dissolved in the aqueous or lipid phase of the food. The auto-oxidation produces hydroperoxides and decomposition of hydroperoxides produces more free radicals (Figure 2.6). Initially, the concentration of free radicals is low, and the rate of oxidation is also slow. Decomposition of peroxides increases the concentration of free radicals as well as oxidation rate. The time of slow oxidation state is called induction period or shelf life, which can be extended by introducing antioxidants into food products (Bradley & Min, 1992; Pokorny, 2007).

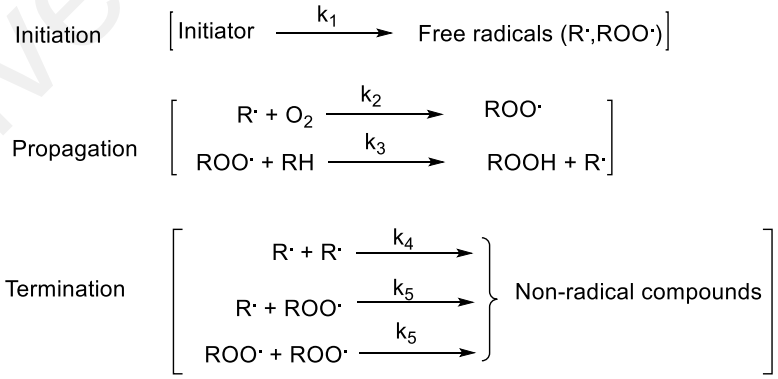


Figure 2.6: Auto-oxidation reaction in food products.

History of utilizing synthetic antioxidants in food industries is over 50 years old. There are extensive applications of synthetic antioxidants in food industries and they are mostly

phenolic antioxidants. Although, many phenolic compounds have been reported as strong antioxidants, only a few of them are used for food stabilization due to the strict health safety regulations. For example, food industries have started using butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA) as antioxidant or stabilizer since late 1950s and TBHQ got approval to be used in food in Europe in 2004, but not yet in Japan (Makahleh et al., 2015). Most commonly used synthetic antioxidants in food industries are butylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA), tertiary-butylhydroquinone (TBHQ), propyl gallate (PG), octyl gallate (OG) and dodecyl gallate (DG) (Figure 2.7) (Makahleh et al., 2015). Since *p*-substituted phenolic compounds exert low toxicity, most of the synthetic phenolic antioxidants are *p*-substituted while phenolic antioxidants found in nature are o-substituted compounds (Pospisil & Klemchuk, 1989). So, the structures of synthetic phenolic antioxidants are altered by inserting various substituents such as alkyls to improve the antioxidant properties because increasing the solubility in fats or oil and minimize the toxicity (Hudson, 2012).

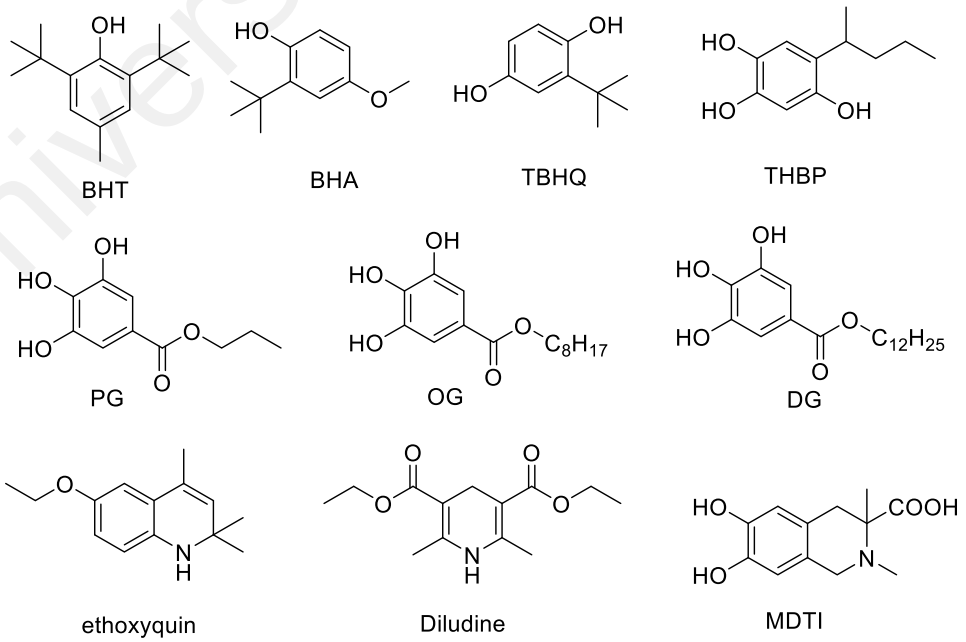


Figure 2.7: Structure of most common synthetic antioxidants in food products.

Butylated hydroxyanisole (BHA) is a phenolic antioxidant which contains one tertiary butyl and methoxy group in its structure. It is white waxy flakes, and the combination of two isomers, 3-tertiary-butyl-4-hydroxyanisole (90%) and 2-tertiary-butyl-4-hydroxyanisole (10%). It is a widely used synthetic food antioxidant which is very soluble in fats and oils but water insoluble and a more efficient in preventing deterioration of flavor and color of essential oils (Shahidi & Ambigaipalan, 2015). It is widely applied in the fats and oil products; meat and meat products; mixed food, dry foods etc. BHA provides excellent synergistic effect in combination with BHT, TBHQ or PG (Shahidi et al., 1992).

Butylated hydroxytoluene (BHT) is a commercially available white crystalline hindered phenolic antioxidant. It was found less effective than BHA in food products due to the presence of two tertiary butyl group which provide obstruction of releasing proton by steric hindrance effect (Nanditha & Prabhasankar, 2008). It was reported that BHT is better at delaying oxidation of animal fats than vegetable oils (Yen & Duh, 1994). BHT is found more efficient at rubber and petroleum industries than food products, though it is widely used in food packaging because of its volatile nature.

Tertiary-butylhydroquinone (TBHQ) is a di-phenolic commercially available antioxidant. It is highly efficient for delaying oxidation deterioration of vegetable oil, edible fats and meat products because it does not affect the color, flavor, and odor of food products even in presence of iron (Kashanian & Dolatabadi, 2009). It is used either alone or in combination with butylated hydroxyanisole and butylated hydroxytoluene at a maximum usage level of 200 ppm depending on the fat content and vegetable oil to get better synergistic results (Shahidi, 2004).

Propyl gallate (PG) is a commercially available white crystalline tri-phenolic antioxidant. It is an esterification product of gallic acid and propyl alcohol. It is soluble

in fat and effective antioxidant for edible fats, oil, bakery products, as well as lubricant oil (Zurita et al., 2007). PG causes discoloration in food products as it forms blue colored complexes with iron, so it is used with citric acid as a chelator to avoid the effect of the pro-oxidative iron and copper catalysts (Shahidi & Naczk, 2004). Good synergistic results of PG were found while using with BHA and BHT (Buck, 1984).

Table 2.1: Chemical properties of commonly used synthetic phenolic antioxidant in food products (André et al., 2010; Makahleh et al., 2015; Shahidi & Ambigaipalan, 2015).

Antioxidants Name	Chemical properties					
	Melting point (°C)	Boiling point (°C)	Density (gm/cm^3) at 20°C	Synergism	Solubility	
					Water	Ethanol and fat
BHA	50-52	268	-	BHT and Gallates	Insoluble	Soluble
BHT	69-70	265	0.89	BHA	Insoluble	Soluble
PG	146-148	181 ± 20.8	1.36 ± 0.06	BHA	Soluble	Soluble
DG	95-98	-	-	BHA	Soluble	Soluble
TBHQ	126-128	291 ± 20	1.09 ± 0.06	-	Soluble	Soluble

Among nitrogen bearing heterocyclic compounds, only ethoxyquin (6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline) and the structurally related N-methyl-3-methyl-6,7-dihydroxy-1,2.3,4-tetrahydroisoquinoline-3-carboxylic acid (MDTI) were reported to be used in vegetable and fish products (Kouřimská et al., 1993; Thorisson et al., 1992). Diludine (2,6-dimethyl-3,5-diethoxycarbonyl-1,4-dihydropyridine) was reported as a stabilizer of carotene and some pharmaceuticals. It also showed a synergistic effect with α-tocopherol in the autoxidation of methyl oleate (Kouřimská et al., 1993).

Only antioxidants ability of any synthesized compounds is not considered to be used in food products, since its adverse effects on human health are the most important factors to get approval as food antioxidants (Hilton, 1989). For example, it was reported that injection of the high level of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) in foodstuffs were responsible for liver damage and carcinogenesis (Biparva et al., 2012).

It was also stated that amongst the several food additives the synthetic phenolic antioxidants such as BHA, TBHQ, PG and 2-tert-butyl-4-methylphenol (TBMP) have the ability to form molecular complexes with a nucleic acid and lead to alter the double helical structure of DNA (Dolatabadi & Kashanian, 2010). The maximum usage level of synthetic phenolic antioxidants in food products is firmly controlled by governments due to their adverse health effects.

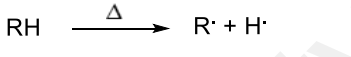
2.4.2 Antioxidants in the lubricant oil

Thermo-oxidation stability of synthetic lubricant is now pre-requisite to get world-wide approval to reduce the earthed fossil-based lubricant. Maintenance cost of different machinery parts and detrimental effects on the environment are significantly involved with the stability of lubricant oils (Rohrbach et al., 2005). So, modern lubricants should be more naturally benevolent and have an extended performance level and most reduced life-cycle-cost (LCC) than traditional lubricants. Synthetic or vegetable lubricant oils are a feasible and sustainable source of environmentally friendly oil which derived from renewable resources. As of late, it has been gaining an ever-increasing amount of consideration because of its better lubricant properties and renewable raw materials.

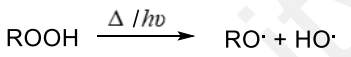
2.4.2.1 Degradation of lubricant oil

It is important to understand the mechanism of oxidation of hydrocarbon or synthetic based oil before the discussion about the roles of several antioxidants in lubricant oil. Lubricant or polymer oxidation is started by the experience of hydrocarbons or other peroxide molecules with oxygen and heat. Formation of free radicals triggers the oxidation reaction of lubricant oil and it is called initiation of free radical chain reaction. Free radicals can be formed in lubricant oil in many ways. For example, homolytic cleavage of hydrocarbon molecule occurred at high temperature and produces free radicals (eqs. 2.1-2.2). It is practically impossible to manufacture synthetic oil or polymer without any traces of hydroperoxides. The hydroperoxide bond is comparatively weak and cleaves homolytically by the absorption of UV light to yield radicals (eq.2.3).

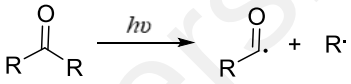
Free radical produced by high temperature and absorption of UV light



(2.1)



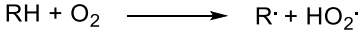
(2.2)



(2.3)

Again, at high temperature, some oxidatively sensitive substrates can directly associate with oxygen to produce free radicals according to eq. 2.4.

Free radical produced by the reaction with oxygen



(2.4)

In addition, a trace of transition metal ions influences the rate of oxidation of polymer or oil products greatly by catalyzing the decomposition of peroxide molecule to free radicals. These metal ions can actively decompose peroxide radicals in both their

lower and higher oxidation state. Among them the metal ions such as copper, iron, cobalt, manganese ions can undergo electron transfer reactions are most efficient peroxide decomposer (Salem et al., 2000).

Free radical produced by metal activator

$$ROOH + M^{n} \longrightarrow ROO^{\cdot} + M^{(n-1)} + H^{+} \quad (2.5)$$

$$ROOH + M^{(n-1)} \longrightarrow RO^{\cdot} + M^{(n-2)} + OH^{-} \quad (2.6)$$

Though the rate of free radical formation reactions is very slow, but free radical-mediated reactions are so fast, and it forms enormous amounts of free radicals by repeating the reaction eqs. 2.7-2.8 many times until it forms a non-radical molecule.

Chain propagation reaction

$$R^{\cdot} + O_2 \longrightarrow ROO^{\cdot} \quad (2.7)$$

$$ROO^{\cdot} + RH \longrightarrow ROOH + R^{\cdot} \quad (2.8)$$

The termination step can be active in attenuation or final stage of the oxidation process if no more radicals are formed in the initial stage. In chain termination reaction, all the free radicals produced in the initial and chain propagation steps would combine together to form nonradicals molecules which cause corrosion, sludge, deposit formation, unexpected increase in the viscosity and molecular weight, other properties, and eventually damage the machinery parts (Soleimani et al., 2018).

Termination reaction

$$2R^{\cdot} \longrightarrow R\text{-}R \quad (2.9)$$

$$R^{\cdot} + ROO^{\cdot} \longrightarrow ROOR \quad (2.10)$$

$$2ROO^{\cdot} \longrightarrow \text{Nonradical molecules} \quad (2.11)$$

2.4.2.2 Classification of lubricant antioxidants

Antioxidants, a class of compounds, can control the oxidation process in the oil and consequently, prevent oil from pre-mature oxidative failure and thickening. The natural antioxidants used in the mineral oil are known as polycycloaromatics, triglycerides, sulfur and nitrogen containing heterocyclic compounds, while in biological systems these are familiar as tocopherol, flavonoids, zeaxanthin, lutein, lycopene and others. (Mortier et al., 2010). Natural antioxidants were found inefficient to inhibit the oxidative degradation of the lubricant and polymer under severe working condition. Thus, several kinds of synthetic antioxidants were introduced as per industrial lubricant demands. Generally, antioxidants for lubricant or polymer compounds can be classified into three major groups: Primary antioxidants (free radical scavengers); secondary antioxidants (peroxide decomposer) and metal deactivators (Dresel, 2007).

(a) ***Primary antioxidants***

Primary antioxidants are also known as free radical scavengers or hydrogen donating antioxidants. This class of antioxidants inhibits the alkyl or alkyl peroxide radical by donating a hydrogen atom to interrupt the free radical chain mechanism of auto-oxidation reaction. Hindered phenol and secondary aromatic amine are well known free radical scavengers.

I. Hindered phenolic antioxidants

Hindered phenol, a simple mono-phenolic antioxidant, protect the oil from pre-mature oxidative degradation by inhibiting the free radicals in the initial stage. It has a very complex mechanism of inhibiting the oxidation reaction showing in Figure 2.8 with an example of 2,6-di-tert-butyl-p-cresol (1) (Pospíšil, 1988). 2,6-di-tert-butyl-p-cresol

(BHT) converts itself into many oxidation products (2, 3, 4, 5, and so on) such as quinone derivatives during the inhibition of auto-oxidation reaction.

The different substituents on the phenolic ring significantly affect the antioxidants of phenolic antioxidants. For example, the antioxidant activity of phenolic compounds increases dramatically if the hydroxyl group is sterically hindered by at least one bulky alkyl group in the ortho position. Thus, all the commercial phenolic antioxidants have been synthesized in this same manner (Nixon et al., 1956; Wasson & Smith, 1953). This is because steric hindrance of phenolic antioxidant provides greater stability of phenoxy radical shown in Figure 2.8 (resonance of phenoxy radical).

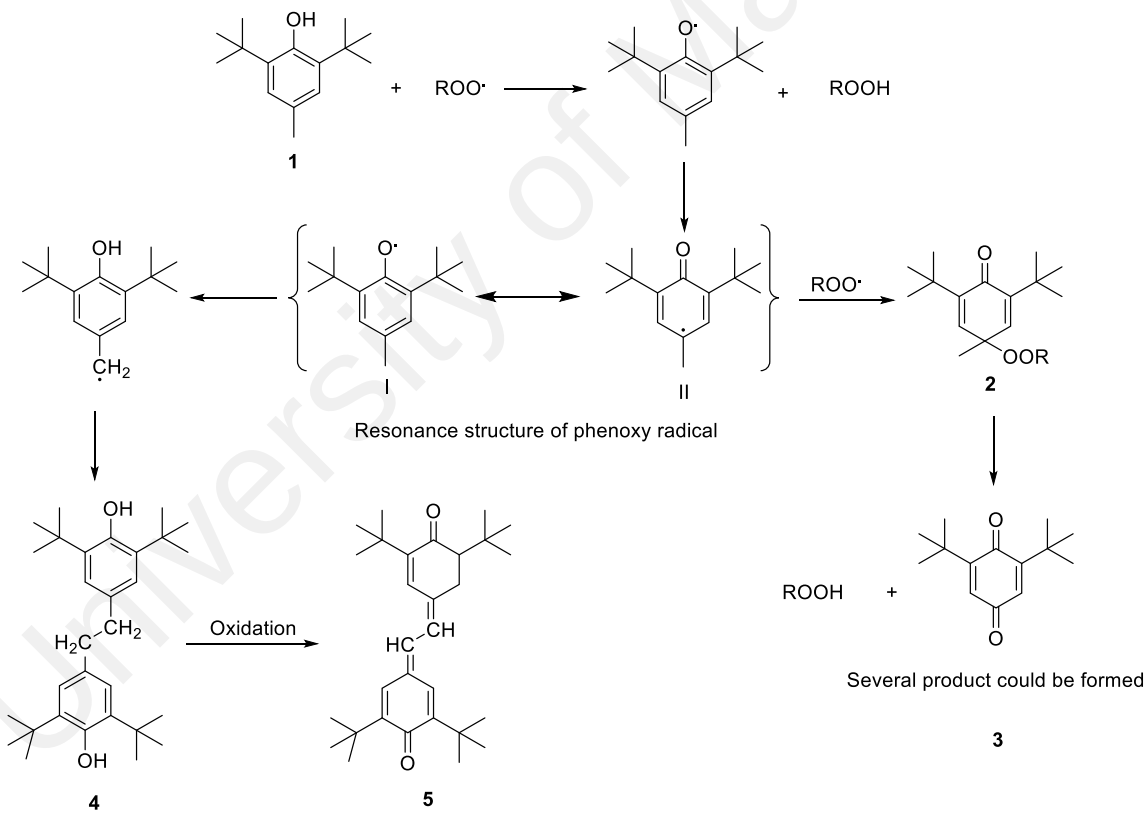


Figure 2.8: Chemical transformation of 2,6-di-tery-butyl-p-cresol during oxidation. (Pospíšil, 1988)

Moreover, para-substituted on the phenolic ring plays an important role such as replacing the methyl group in the 2,6-di-tert-butyl-p-cresol with tertiary butyl group decreases the effectiveness of antioxidant activity of BHT. Because tertiary butyl group

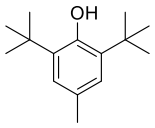
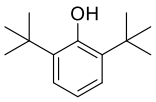
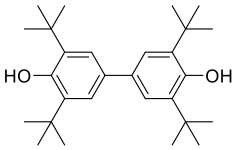
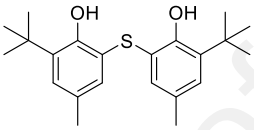
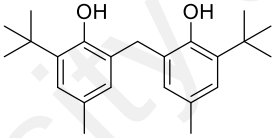
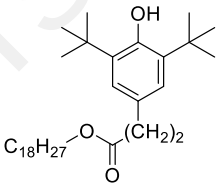
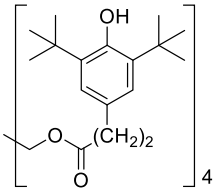
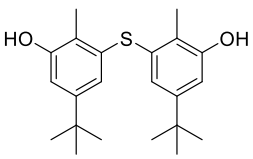
in the para position 2,6-di-tert-butyl-p-cresol preclude the formation of antioxidant compounds (4) that is formed by dimerization of radical (I) shown in Figure 2.8. Strong electron-withdrawing groups such as cyano, carboxyl and others. on the para position of phenolic ring decrease the antioxidant activity as these groups reduce the ability of phenol to donate proton to alkoxy and peroxyl radicals of the substrate (Jones et al., 1952).

The solubility of hindered phenol into the substrates is another important factor on its antioxidant activity. Ester groups are better at dissolving itself into lubricant or polymer products than the alkyl groups. Different alkyl ester (methyl, hexyl, dodecyl, octadecyl) of 3,5-di-tert-butyl-4-hydroxyhydrocinnamic acid were studied into polypropylene at 140°C to investigate oxidation inhibition ability. Octadecyl ester of 3,5-di-tert-butyl-4-hydroxyhydrocinnamic acid was found most effective among all the ester compounds. This difference of antioxidant activity for different ester into polypropylene might be attributed to the differences of solubility of them into the substrate (Scott, 2012).

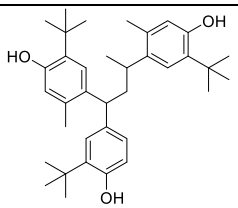
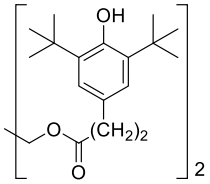
The volatility of hindered phenol has a significant effect on the activity of antioxidants. Incorporation of long aliphatic chain into antioxidants would decrease the volatility and increase the solubility in the lubricant oil. That long aliphatic chain improves the oxidation stability of antioxidants along with the increasing molecular weight. Commercially available di, tri, and poly phenolic antioxidants are the combination of low molecular weight antioxidant with low volatility (Mark, 2013).

Some commercially available hindered phenolic antioxidants used in lubricant oil and polymer products are listed below in Table 2.3 with structure and physical properties.

Table 2.2: Commercial phenolic antioxidant with physical properties**.**

CAS no.	Structure	Physical state	Melting temp (°C)
128-37-0		white to yellow powder	69 - 73
128-39-2		white solid	34 - 37
118-82-1		yellow powder	154
90-66-4		Cream to slightly pink powder	83 - 85
119-47-1		Pale cream to white powder	120 - 132
2082-79-3		White Crystalline powder	49 - 54
6683-19-8		White Crystalline granular	110 - 125
96-69-5		White to off-white powder	160

Continued Table 2.3

CAS no.	Structure	Physical state	Melting temp (°C)
1843-03-4		White Powder	180
35074-77-2		Crystalline, white granules or crystalline powder	105

II. Secondary aromatic amine

Secondary aromatic amines are highly effective to inhibit peroxide radical. This class of antioxidants is more efficient than phenolic antioxidants for the oxidative inhibition of easily oxidized organic materials. Secondary aromatic amine transformed into the different molecule (quinine imines) during oxidation reaction as shown in Figure 2.9 (Sedlar et al., 1982). This type of antioxidants derived from p-phenylenediamine, diphenylamine and they are generally substituted by the different aliphatic chain and aromatic ring to increase their solubility and reduce volatility into the substrates.

Besides secondary aromatic amine, hindered amines are also reported as extremely efficient in protecting polyolefins from photooxidative degradation (Dexter et al., 2002; Thomas et al., 2000). They are also known as hindered-amine-light stabilizers (HALS) and mostly derived from 2,2,6,6-tetramethylpiperidine.

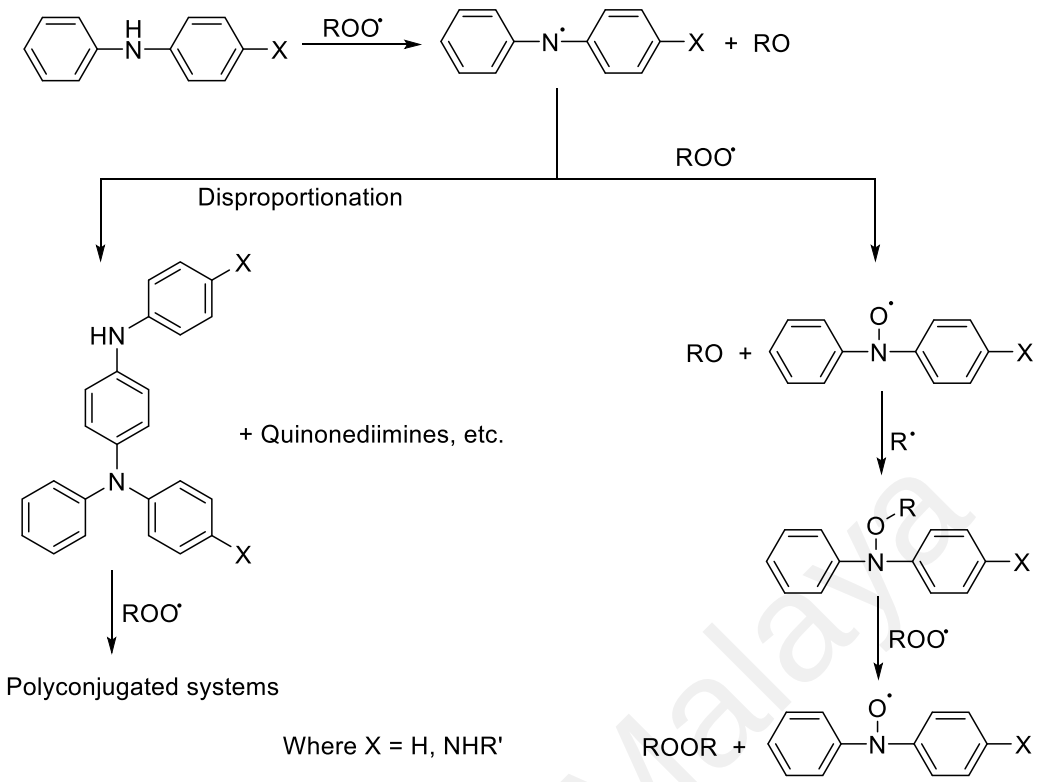
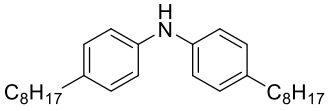
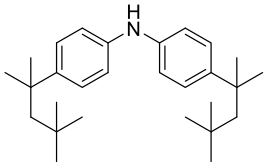
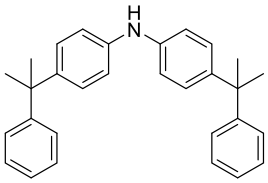
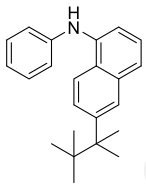
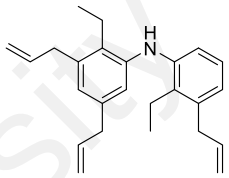
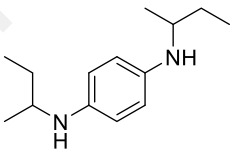
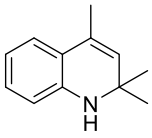


Figure 2.9: Oxidation transformation of aromatic amine (Haidasz et al., 2014; Sedlar et al., 1982).

Hydroxylamines are also powerful free radical scavengers on an equivalent weight basis in contrast with phenolic antioxidants. They act as a sequential source of hydrogen and have the ability to decompose hydroperoxide. They are very effective at low temperature and not useful for long-term thermal stability (Mare & Coppinger, 1963).

Table 2.3: Some commercially used amino antioxidants with physical properties.

CAS number	Structure	Physical properties	Melting point (°C)
101-67-7		brown flakes	96 - 97
15721-78-5		Off White powder	-
10081-67-1		White to off-white solid	98 - 102
68259-36-9		Yellow to brown powder	> 75
68608-77-5		Liquid	-
101-96-2		Amber to red or dark reddish black liquid.	-
147-47-7		Dark brown oil	-

(b) ***Secondary antioxidant***

Homolytic breakdown of peroxide and hydroperoxide accelerates the rate of oxidation chain reaction. Secondary antioxidants convert the peroxide and hydroperoxide into non-

radical molecules and thus, they are also known peroxide decomposers. Generally, organosulfur and organophosphate compounds are excellent peroxide decomposers and familiar as secondary antioxidants. Extensive researches have been done to develop the highly potential antioxidants over the years (Rudnick, 2017). Following oil-soluble organometallics are the major class of antioxidants:

- Sulfur compounds
- Sulfur–nitrogen compounds
- Phosphorus compounds
- Sulfur–phosphorus compounds
- Organocopper compounds
- Boron compounds
- Other organometallic compounds

I. Sulfur and sulfur-nitrogen compounds

Organosulfur compounds are popular in lubricant compositions for their excellent antioxidant properties, extreme-pressure (EP) and antiwear properties. Many researchers have revealed the relationship between the structure of organosulfur compounds and their antioxidant, extreme-pressure and antiwear properties (Bala et al., 1996; Becker & Knorr, 1996; Qiu et al., 2006). Aromatic sulfides are one of the classes of sulfur additives that are utilized as oxidation and corrosion inhibitors like dibenzyl sulfide and di-xylyl disulfide belong to this class of additives (Rudnick, 2017). Sulfur-containing aliphatic ester was found as excellent antioxidants additives in lubricant oil. For example, di-alkyl ester of thiodipropionic acid is a potential hydroperoxide decomposer. A molecule of di-alkyl ester of thiodipropionic acid can decompose at least 20 mol of peroxide at elevated temperature (Dexter et al., 2002; Hawkins, 1984). The reactions of di-alkyl ester of

thiodipropionic acid during the decomposition of hydroperoxide are shown in Figure 2.10 (Dexter et al., 2002).

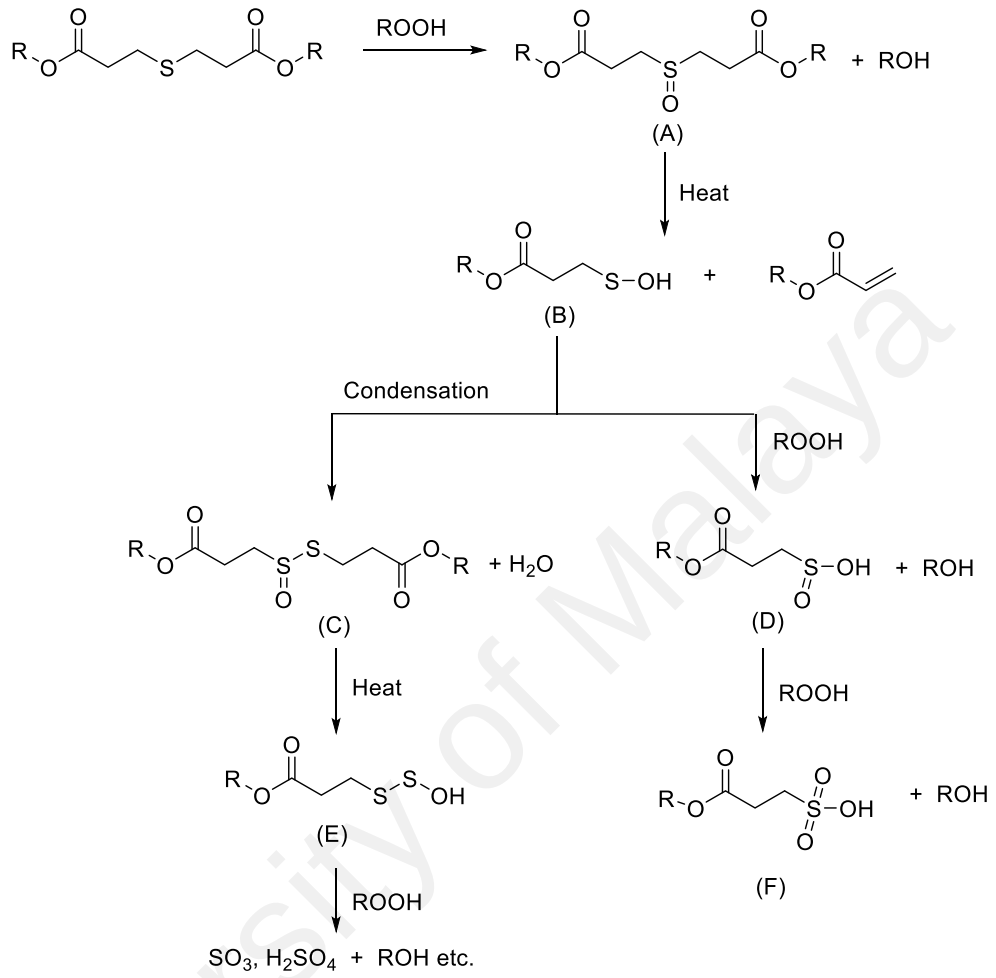


Figure 2.10: Hydroperoxide decomposition by di-alkyl thiodipropionic acid (Dexter et al., 2002; Ye & King III, 2003)

di-alkyl ester of thiodipropionic acid would transform into sulfoxide (A) by converting the hydroperoxide into alcohol as shown in Figure 2.10. This sulfoxide molecule (A) would be transformed into sulfenic acid (B) and alkene by heat. This sulfenic acid would undergo either condensation reaction to give condensation product (C) or reaction with hydroperoxide again to give sulfinic acid (D). At high temperature, this condensation product (C) would be converted into product (E) which can react with hydroperoxide again and give the acidic product. On the other hand, sulfinic acid (D) would convert to

sulfonic acid (F) by the reaction with hydroperoxide. The reaction products such as sulfonic acid, sulfur trioxide, sulfur dioxide and sulfuric acid are also capable of decomposing hydroperoxide to non-radical molecules. It was found that one equivalent of sulfur dioxide enabled to decompose approximately 20,000 equivalents of cumene hydroperoxide (Bridgewater & Sexton, 1978). However, this class of sulfur containing di-alkyl ester exhibits excellent oxidation stability at high temperature, though they are rather weak antioxidants at low temperature.

Sulfur-nitrogen bearing antioxidants such as thiocarbamate compounds are one of the main class of additives possessing antioxidants, antiwear and anticorrosion properties. It is well known that di-thiocarbamate combined with a metal such as zinc, copper, bismuth, and molybdenum show admirable antioxidant and antiwear properties in lubricant oil. One of the commercially important metal di-thiocarbamate is molybdenum di-thiocarbamate. It is an ashless antioxidant and widely utilized in engine crankcase lubricating oil because of their outstanding good oxidative stability, thermal resistance and anticorrosion properties (DeVries & King, 1981).

Thiadiazole derivatives are found as multifunctional additives because of their ashless antiwear, extreme pressure and antioxidant properties and belong to sulfur-nitrogen bearing additives. For example, a monomeric 2-alkylesterthio-5-mercapto-1,3,4-thiadiazole were reported to prolong the oxidative inhibition of engine oils in thin-film oxidation conditions (Yao, 2006). Thiadiazole derivatives are also used in combination with alkylated diphenylamine and organomolybdenum compounds to get better synergistic effect (Karol et al., 2004). Alkylated phenothiazines are also sulfur-nitrogen bearing antioxidants, which have been applied in the aviation fluids and engine oils to stabilize ester-based lubricant (Rudnick, 2017; Schumacher et al., 1991).

II. Phosphorous and phosphorous-sulfur compounds

Elemental phosphorous, like elemental sulfur was used as an oxidation inhibitor in the early 1900s (Rudnick, 2017). Since it is not soluble in oil, extensive research has been done to develop oil-soluble organophosphate compounds. Naturally derived phosphorus compounds: lecithin have been used as antioxidants, and many patents have been filed on these compounds describing their usage either alone or in combination with other antioxidants (Colclough et al., 1996; Holzinger, 1964). However, synthetic phosphite esters show good oxidation stability in lubricant oil. Many patents have described the oxidation stability of alkyl and aryl phosphites such as tributyl phosphites and triphenyl phosphites in lubricant base oil (Moran et al., 1936; Moran & Kozacik, 1939).

The antioxidants containing both sulfur-phosphorous shows effective oxidative stability in lubricant base oil than those antioxidants containing either sulfur or phosphorous. Metal dialkyldithiophosphates have been widely used antioxidants in lubricant oil as a class of sulfur-phosphorous additives. Zinc dialkyldithiophosphates (ZDDPs) are well-known multifunctional additives in this class of sulfur-phosphorous additives used in lubricant composition since they possess antioxidants, antiwear and extreme pressure properties together (Bec et al., 1999; Habeeb et al., 1987; Roper & Bell, 1995). As ZDDPs decompose into volatile phosphorous species which are capable of coating and clogging the supporting composition in catalytic media. To overcome this problem, researchers have tried to reduce the phosphorous amount in preparing zinc dialkyldithiophosphates so that it can produce lesser amounts of volatile phosphorous (Selby et al., 2005; Sheets et al., 2010).

Researchers have also incorporated molybdenum into sulfur-phosphorous additives such as organomolybdenum phosphorodithioate complexes to have efficient oxidation stability in lubricant base oil. Oil-soluble molybdenum compounds are preferred

multifunctional additives in certain conditions than the other additives because of their antioxidant, antiwear, antifriction and extreme pressure properties. Thus, they have been utilized commercially in engine oil, industrial and automotive lubricant oil, metalworking fluids and grease (Rudnick, 2017). The dialkylphosphorodithioic acid derivatives are now one of the widely used ashless multifunctional additives to avoid the use of metal dithiophosphates that cause toxicity, waste disposal, filter clogging and pollution. In addition, phosphorodithioate ester derivatives incorporated with a hindered phenol are also found to have good antioxidant properties in base oil (Morris, 1956; Rudnick, 2017).

III. Organocopper compounds

The trace of a transition metal such as copper in the lubricant composition was considered as free radicals producer or oxidation promotor. Interestingly, oil-soluble copper salts exert antioxidant properties in lubricant base oil in the optimum range of concentration 100-200 ppm (Colclough, 1993; Klaus et al., 1992). For example, some oil-soluble organo-copper compounds such as copper oleates, copper naphthenates, copper stearates, copper polyisobutylene succinic anhydrides have shown synergistic effect with multi-ring aromatic compounds in reducing deposit formation in base oil at elevated temperature (Rudnick, 2017; Soleimani et al., 2018). In addition, some compounds such as copper dithiocarbamates or thiophosphates, copper naphthenates and oleates have exhibited synergistic with phenothiazines in regulating viscosity index in lubricant base oil (Field, 1998).

IV. Boron antioxidants

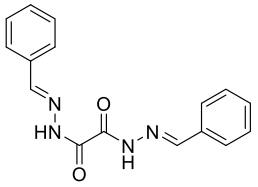
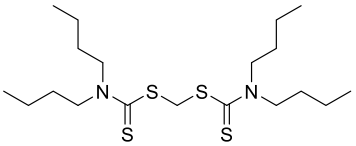
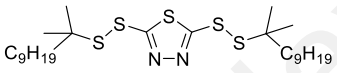
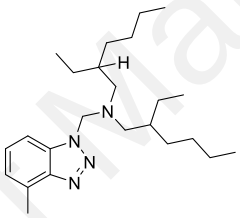
Boron antioxidants have drawn a great interest of additives industries in the results of searching eco-friendly antioxidants to reduce the use of environmentally unfriendly additives. Some boron esters were found to be active oxidative inhibitor along antiwear

and anti-friction properties in either use of individually or in a mixture of other additives (Yao et al., 2002). For example, borated epoxides such as borated 1,2-epoxy-mixed C_{15}-C_{18} alkanes and borated 1,2-Epoxytetradecane were patented as antioxidant and antiwear corrosivity reducers, friction reducers (Horodysky, 1983). A few more examples of boron antioxidants are borated single and mixed alkanediols (Horodysky, 1988), phenol esters of hindered phenyl borates (e. g. di-n-butyl 2,6-Di-tert-butylphenyl borate) (Braid, 1985), and reaction products of boric acid with the condensates of phenols with aromatic or aliphatic aldehydes (Koch, 1993). Many patents were issued describing the other boron antioxidants in lubricant composition (Horodysky & Herd, 1985; Horodysky & Kaminski, 1981, 1984).

(c) ***Metal deactivators***

It is known that metal ions can accelerate the oxidation reaction in lubricant by decomposing the hydroperoxides to free radicals. The ability of metal ions to produce free radicals can be counteracted by introducing metal deactivators (Chalk & Smith, 1957). These metal deactivators are generally chelators or polydentate ligands and form coordination bond with a metal ion which can increase the potential difference between their oxidized and reduced states. Thus, the ability of metal ions of free radical production (according to eqs. 2.5 - 2.6) from peroxides or hydroperoxides can be inhibited. Because metal complexes, which are formed by metal deactivators and metal ions, are unable to interact with hydroperoxides to form free radicals (Dexter et al., 2002). Some commercial metal deactivators for lubricant base oil were listed with structure in Table 2.5.

Table 2.4: Commercial metal deactivators for lubricant oil

CAS number	Structure
6629-10-3	
36878-20-3	
59656-20-1	
94270-86-7	

CHAPTER 3: DESIGNATION OF MULTIPOTENT ANTIOXIDANT AND ITS PREPARATION METHODS

3.1 Multipotent antioxidant

Generally, antioxidants can protect biological and chemical substances from oxidative damage associated with free radical. Free radical oxidation of substances (biological and chemical) occurs through a chain reaction that comprises of three stages (i.e. initiation, propagation and termination) and antioxidants exert their radical scavenging effects through various mechanisms to prevent the oxidation reaction. But the compounds having antioxidant activity along with other pharmacological properties become multipotent antioxidants and also called multifunctional antioxidants as they contain multiple antioxidant functions in their structure. This type of antioxidant compound has drawn a great interest for the treatment of several chronic diseases such as cancer, Alzheimer's disease and so on (Zhang et al., 2006). Rational-design strategy and structure-activity relationship are the primary things needed to take under consideration for the designation of the multipotent antioxidant structure. Multifunctional antioxidants are efficient free radical scavenger by providing the auto-synergistic antioxidant system (Farzaliev et al., 1978). Generally, the multifunctional or multipotent antioxidant is the hybrid structure that can be obtained by either the combination of two or more antioxidant functions or the combination of antioxidant function and pharmacophore group in one structure. For example, thiazolidinone derivatives were combined with butylated hydroxyphenyl (BHP) moiety by Kato *et al.* (Kato et al., 1999) to obtained multipotent antioxidant activity. Likewise, semicarbazones would become potential multipotent antioxidant because they have significant anticonvulsant, anticancer and antimicrobial properties along with the antioxidant activity.

3.2 Multipotent activity of semicarbazones

An extensive research has done on the semicarbazone derivatives for the last 20 years due to its excellent biological properties. The literature uncovered that semicarbazones have risen as a compound with a wide scope of biological activities including anticonvulsant, antitubercular, anticancer and antioxidant activity (Ahsan et al., 2011). Semicarbazones are considered as a class of imine derivatives because its general preparation involves with the formation of imine bond by the reaction of semicarbazides and aldehydes or ketones (Layer, 1963).

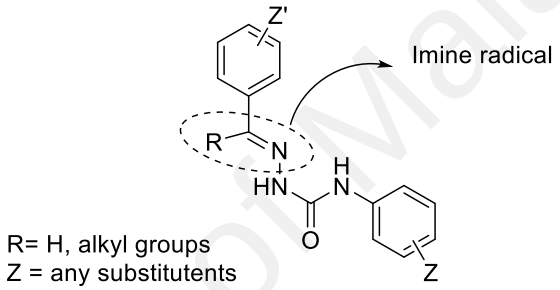


Figure 3.1: General structure of semicarbazones

3.2.1 Importance of semicarbazones

Semicarbazones were found as potential therapeutic agents that exhibit anticonvulsant, anticancer, antimicrobial and antioxidant properties. Generally, it has been expansively studied for its anticonvulsant properties. The reasons for the anticonvulsant activity of semicarbazones are their several smaller functional groups that also exhibit anticonvulsant properties (Jain et al., 2011). The smaller functional group of semicarbazones are shown in Figure 3.2.

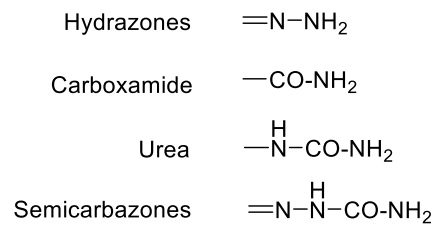


Figure 3.2: Concepts of semicarbazones as anticonvulsant agents.

Some hydrazones derivatives were found as potential anticonvulsant agents in the result of finding effective antiepileptic drugs. For example, Menthone aryl acid hydrazones, some hydrazones of indole-3-carboxaldehyde were reported (Figure 3.3) as a new class of anticonvulsant agents (Jain et al., 2011; Popp, 1984).

Carbamazepine is a member of the carboxamide group, one of the earliest drugs for the diagnosis of grandmal epilepsy. Oxacarbamazepine (Ghaemi et al., 2003) and eslicarbamazepine (Elger et al., 2009) are the modified compounds of carbamazepine have been applied clinically as antiepileptic drugs shown in Figure 3.3.

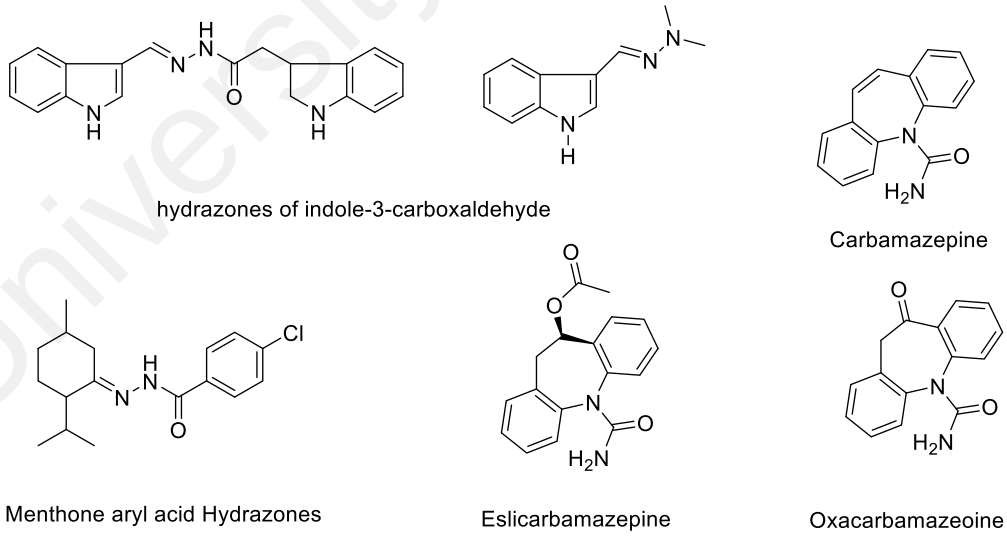


Figure 3.3: Hydrazones and carboxamide derivative as anticonvulsant agents.

Acyclic urea and cyclic ureide derivatives were found as potent anticonvulsant agent and still preferred drugs for the epilepsy treatment. Phenacemide (Coker, 1986),

hydantoin derivatives, phenytoin (Hardman & Limbird, 2001) are the urea derivatives and applied to epilepsy patients in the hospitals. A succinimide derivative of ethosuximide is a commercial drug for the treatment of absence seizures (Patsalos, 2005). Some structures of antiepileptic drugs derived from urea are shown in Figure 3.4.

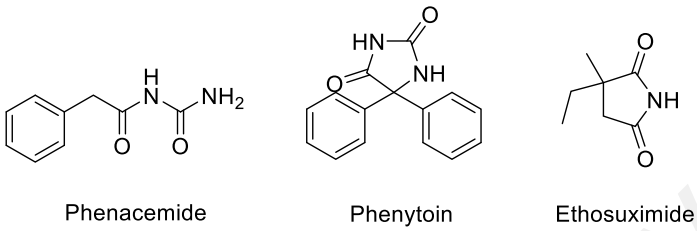
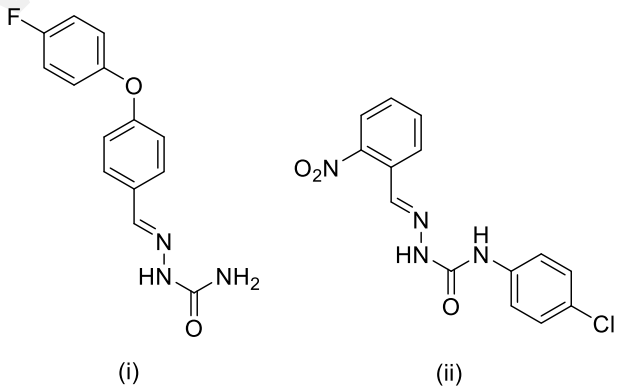


Figure 3.4: Antiepileptic drugs derived from ureides.

Among the above derivatives, semicarbazones have attained an important place as anticonvulsant agents which are orally active and having less side effects and neurotoxicity. 4-(4-Flurophenoxy) benzaldehyde semicarbazone (Dimmock et al., 1999) and *N′*-(4-chlorophenyl)-N^4-(2-nitrobenzaldehyde) semicarbazone (Pandeya et al., 1999) were discovered (Figure 3.5) as leading antiepileptic drugs with lesser side effects and neurotoxicity where former molecule acts as Na channel blocker and later one acts as GABA transaminase inhibitors.



i = 4-(4-Flurophenoxy)-benzaldehyde semicarbazone

ii = *N′*-(4-chlorophenyl)-N^4-(2-nitrobenzaldehyde) semicarbazone

Figure 3.5: Semicarbazones structure of leading antiepileptic drug.

Some semicarbazones derivatives bearing curcumin were examined for the antiproliferative, antioxidant activity and compared with curcumin by Dutta and his group in 2005 (Dutta et al., 2005). Semicarbazone derivatives of curcumin showed enhanced antiproliferative activity in vitro study against estrogen-dependent breast cancer cell line MCF-7 than curcumin.

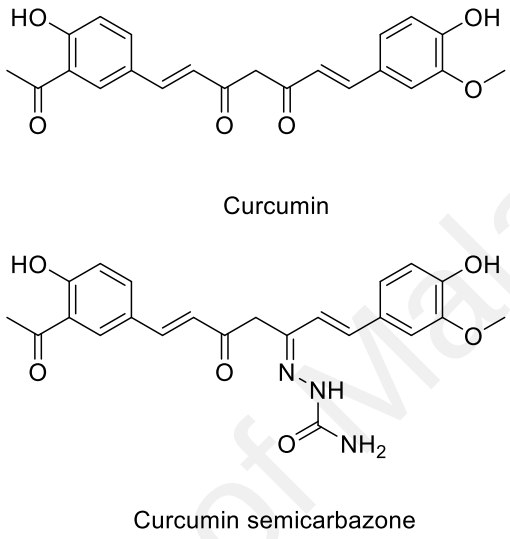


Figure 3.6: Curcumin and curcumin semicarbazones as anticancer agents

Semicarbazone compounds are a neutral ligand, can make coordination bond to metal ions by its azomethine N and carbamoyl O. The several binding modes can be observed if additional coordinating functional group is present in the semicarbazone derivatives. Semicarbazones can be considered as a tridentate ligand that can provide stronger binding ability to metal ions. Semicarbazones and its diversified metal complexes showed important biological properties including anticancer properties (Enyedy et al., 2014). In 2013, Enyedy and his group reported (Enyedy et al., 2014) several metal complexes such as copper(II), vanadium(IV/V), iron(II)/(III) and gallium(III) complexes of salicylaldehyde semicarbazone (SSC, HL) and its 5-bromo derivative (Br-SSC, HL) as anticancer agents for solution speciation. Among these metal complexes gallium (III) salicylaldehyde semicarbazones showed clearly higher stability to physiological pH.

Several copper complexes of substituted semicarbazones and thiosemicarbazones were reported and examined on the prostatic cancer cell line by Venkatachalam *et al.* (Venkatachalam et al., 2016). In vitro anticancer studies of several naphthoquinone semicarbazones and thiosemicarbazones nickel(II) complexes on MCF-7 human breast cancer cells showed that semicarbazone derivatives with nickel(II) complexes are more actively preventing cell proliferation than thiosemicarbazone analogues (Afrasiabi et al., 2005). A series of octahedral ruthenium(II) complexes of phenanthrenequinone semicarbazone and thiosemicarbazone derivatives showed excellent cytotoxic activity against breast cancer cell lines (MCF-7, MDA-MB-468) and skin cancer cell line (A431) (Anitha et al., 2013).

Antimicrobial and antivirus properties of semicarbazone metal complexes were extensively examined for past years. Sriram and co-workers (Sriram et al., 2004) synthesized a series of semicarbazones containing acetamide phenyl and nitrite group and investigated anti-tuberculous properties. Semicarbazones with acetamide phenyl substituents were found to be active against *Mycobacterium tuberculosis* H37Rv, but activity varies with the position of nitrile group on the benzylidene ring. N^1-(4-acetamido phenyl)-N^4-(3-nitro benzylidene) semicarbazone was found more active against *M. tuberculous* than 2-nitro, 4-nitro benzylidene semicarbazones. A series of semicarbazones, obtained by the treatment of 3-methyl-2-benzothiazolinone hydrazone and selected isocyanate or isothiocyanate, was applied on several bacterial strains such as *Bacillus subtilis* (G^+), *Escherichia coli* (G^-), *Pseudomonas aeruginosa* (G^-) and *Staphylococcus aureus* (G^+) and fungal strains such as *Aspergillus flavus* and *Candida albicans*. Most of the semicarbazones synthesized by Yosef et al. (Yosef & Ibrahim, 2016) showed antibacterial and antifungal properties. In 2012, Jafri and his group prepared many semicarbazone derivatives by microwave assisted reaction and investigated antibacterial, antitumor, antioxidant properties (Jafri et al., 2012).

Unfortunately, no significant antibacterial properties were found in all the synthesized compounds, but all semicarbazone derivatives exhibited noteworthy inhibition against tumor development on potato discs induced by AT 10. They also revealed that the semicarbazones derived from 2-nitrobenzaldehyde and acetophenone showed the potent antioxidant activity in DPPH assay. Anti-HIV-1 (HTLV-III_B) and HIV-2 (ROD) properties of 4-aminoacetophenone and (±)-3-Menthone aryl semicarbazone derivatives were investigated by Mishra and coworkers (Mishra et al., 1998; Mishra et al., 2002).

Antioxidant activity of semicarbazone compounds was widely investigated by several researchers. For example, chalconesemicarbazone derivatives as new pharmacophore were synthesized and studied antioxidant activity following reducing power assay by Singhal and coworkers (Singhal et al., 2014).

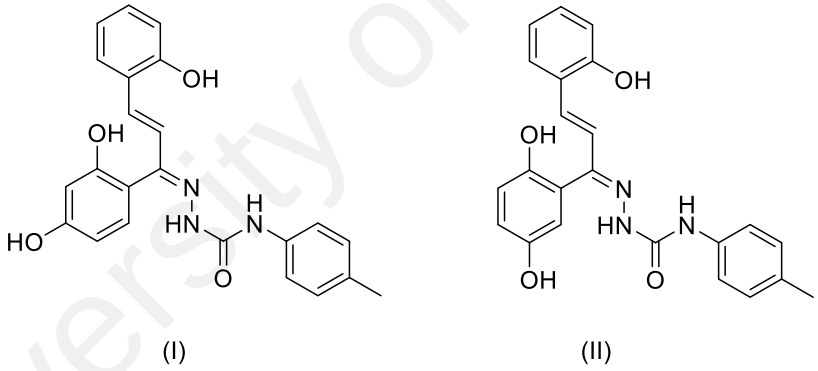


Figure 3.7: Chalconosemicarbazone derivatives as an efficient antioxidant.

1-[1-(2,4-dihydroxyphenyl)-3-(2-hydroxyphenyl) allylidene]-4-(4- methylphenyl) semicarbazone (I) and 1-[1-(2,5-dihydroxyphenyl)-3-(6-hydroxyphenyl) allylidene]-4-(4-methylphenyl) semicarbazone (II) (showed in Figure 3.7) were found to be most efficient antioxidant in power reducing assay. In the DPPH assay, these chalconesemicarbazone derivatives showed good free radical scavenging activity and compound (II) was found most potent antioxidant (Singhal et al., 2011). So, it was found that semicarbazone compounds and its different metal complexes possess excellent

antioxidant properties along with significant biological functions. Incorporation of any antioxidant function into the semicarbazones might increase their antioxidant activity significantly.

3.3 Rational design of multipotent antioxidant

A rational-design strategy is one of the important techniques to develop a way of discovering new multipotent antioxidant structures. The contemporary approach of rational design strategy of multipotent antioxidant is to assemble two or more antioxidant radicals which may contain biological properties using linkers in one structure (Zhang et al., 2006). According to the rational-design strategy, four multipotent antioxidants were designed structures in this study by combining two or more important antioxidant functions in one structure. In first two designated structure showing in Figure 3.8, butylated hydroxyphenyl was incorporated into semicarbazones to obtain better multipotent antioxidant properties. Because hindered phenols are well-known effective free radical scavengers and have been utilizing in the pharmaceuticals, food, foodstuffs and industrials products. Similarly, semicarbazones were also reported to exhibit antioxidant properties along with many important biological properties that were discussed here earlier. Thus, it was anticipated that combination of butylated hydroxyphenyl and semicarbazones would provide the potential antioxidant activity. Two kinds of butylated hydroxyphenyl namely 3,5-di-tert-butyl-4-hydroxyphenyl and 3,5-di-tert-butyl-2-hydroxyphenyl were envisioned to incorporate into semicarbazones, respectively. The purpose of preparing two series of semicarbazone bearing two different butylated hydroxyphenyl is to study the role of the ortho and para hydroxyl group of butylated hydroxyphenyl incorporated semicarbazones on the antioxidant activity.

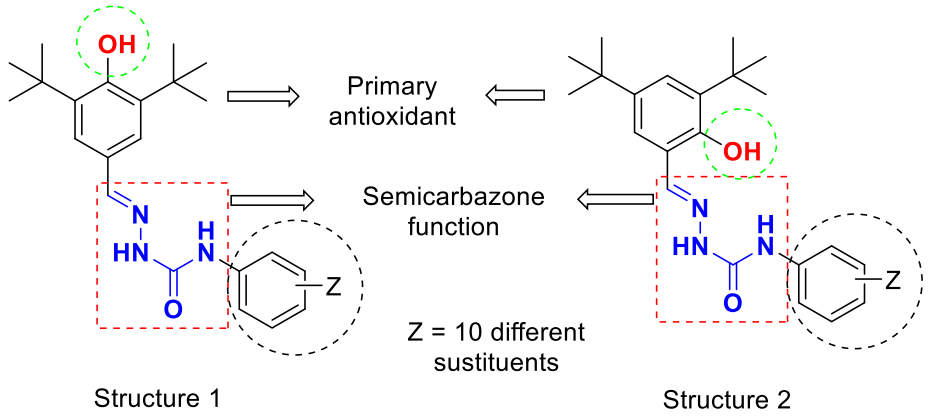


Figure 3.8: Multipotent antioxidant structure 1 and 2 by rational-design strategy.

The other two designated antioxidant structures are shown in Figure 3.9. These following structures were designated by the combination of three important antioxidant functions e.g. Structure 4 is combined of a hindered phenolic, thioether and ester moiety; Structure 3 comp rises of hindered phenolic, thioether and secondary aromatic amine. Where hindered phenol and secondary aromatic amine are called primary antioxidant that act as a free radical scavenger; thioether is excellent peroxide decomposer known as secondary antioxidant and ester moiety plays an important role on the solubility of antioxidant.

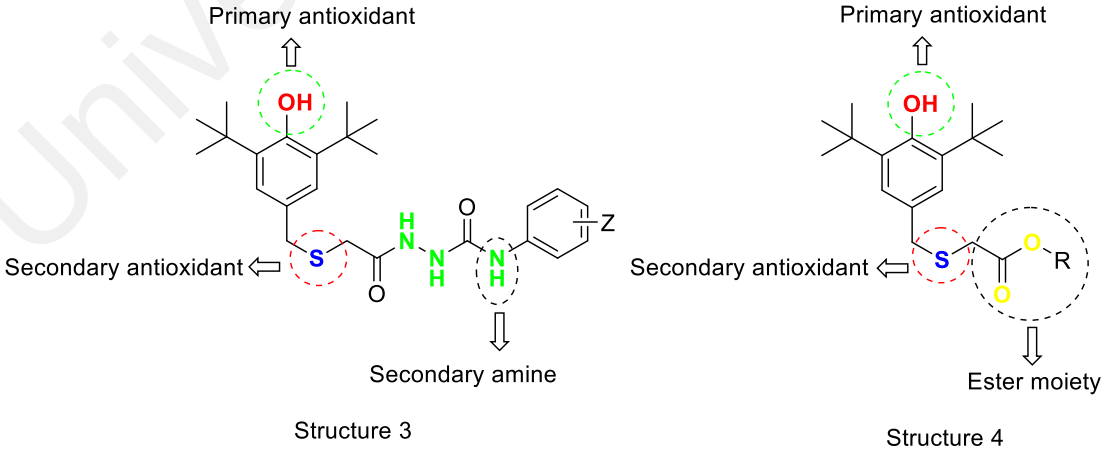


Figure 3.9: Multifunctional antioxidant Structures 3 and 4 by rational design strategy.

3.3.1 Importance of assembled functional radicals

3.3.1.1 Phenolic antioxidant radical

It was found in a recent (Kunchandy & Rao, 1990) study that phenolic antioxidants are the predominant class of multipotent antioxidants and have been widely found in pharmaceutical products. For example, curcumin is well known natural multipotent antioxidant and anti-inflammatory derived from turmeric. It shows good radical scavenging ability (Kunchandy & Rao, 1990), can able to suppress the tumor initiation, promotion and metastasis. In addition, it has been widely utilized for the prevention or treatment of cancer and pharmacologically and it is safe to be used in human application (Aggarwal et al., 2003).

Apart from natural phenolic antioxidants, synthetic hindered phenolic antioxidant has an extensive application across several industrial products including pharmaceutical, food, lubricant, polymer and paint industries (Hertog et al., 1993; Yehye et al., 2015). Hindered phenolic antioxidants are highly effective additives for suppressing the formation of acidic and insoluble products of oil oxidation in engine oil and industrial lubricants. They function as free radical scavengers and provide protection during high-temperature processing operations and also during end use at elevated temperatures (Abraham et al., 2003). Butylated hydroxytoluene (BHT) is one of the most commonly used phenolic antioxidants in petroleum products. Bond dissociation enthalpies of O-H play an important role on the antioxidant activity of phenolic antioxidants. For instance, it reported that presence of CN, NO_2, CHO, COOR, and COOH substituents in para position in BHT decrease the antioxidant activity by increasing the BDE value of the O-H bond, while Cl, Ph, and CH=CH-Ph increase the antioxidant activity lowering the BDE value (Brigati et al., 2002). Again, ionization of values of O-H are important parameters to be effective antioxidant (Klein et al., 2006). It is likely that changes in BDE and IP will

be strongly correlated and indeed this correlation will play an important role in the design of antioxidant.

3.3.1.2 Semicarbazone structure

The importance of semicarbazones has already been discussed earlier in this chapter. In brief, semicarbazones derivatives are a versatile pharmacophore and have been extensively applied for the treatment of epilepsy (Pandeya, 2012). Its metal complexes show anticancer activities against several cancer cell lines are also widely reported (Afrasiabi et al., 2005; Venkatachalam et al., 2016). Researchers also revealed its anti-HIV properties (Mishra et al., 1998), anti-tuberculous (Sriram et al., 2004) and anti-malaria properties (Oliveira et al., 2008). Antioxidant properties of semicarbazones were also reported which may be attributed due to presence of secondary N-H bond and keto-enol tautomerism of semicarbazone function (Dutta et al., 2005).

3.3.1.3 Thioether

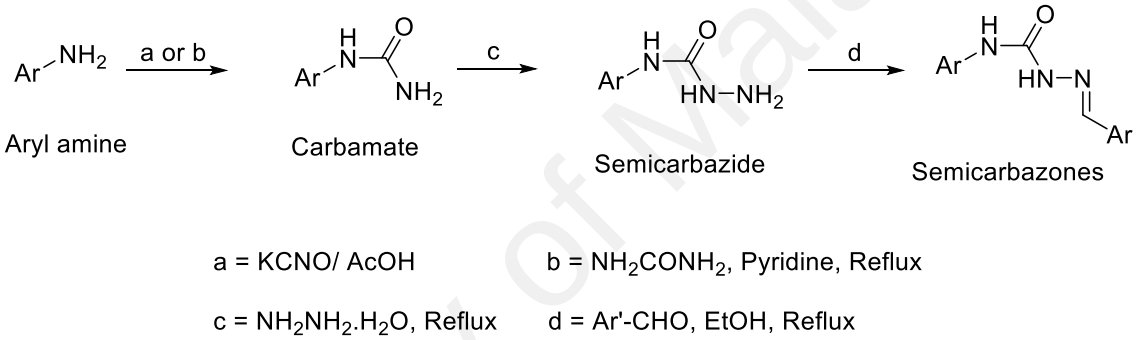
The presence of sulfur as thiol or thioether in biological systems is critical for the upkeep of cellular redox potentials and protein thiol-disulfide ratios, additionally for the immunity of cells from reactive oxygen species. Several naturally occurring thiolated compounds have been recognized that play an essential role in cellular systems such as coenzyme M, trypanothione, mycothiol, ergothioneine and the ovothiols (Hand & Honek, 2005). In addition, several synthetic thiolated compounds such as thiourea (Reid, 1953), propylthiouracil (Reid, 1953), 1,3-dimethyl-2-thiourea (Lewis et al., 1994) and hydroxyphenylurea derivatives (Nakao et al., 2001) were found very active scavengers of reactive oxygen species and free radicals. Generally, thiolated compounds were found such as thioether are classified a secondary antioxidant because of its strong peroxide decomposer properties. Roles of thiolated compounds and its mechanism were elaborately discussed in secondary antioxidant part of Chapter 2.

3.3.2 Importance of multifunctional antioxidants on oxidation stability

One of the important applications of multifunctional antioxidant is in lubricant oil to increase the oxidative stability of the oil. The multifunctional antioxidant can increase the oxidation stability of lubricant oil by offering antioxidant synergism. According to the antioxidant synergistic system in lubricant oil, there are three types of antioxidant synergisms namely, homo synergism; hetero synergism and auto synergism. Synergism antioxidant system can provide an effective solution to the problems where a single antioxidant is inadequate to offer acceptable results. Homo synergism antioxidant describes the combined use of two or more same types of an antioxidant such as a mixture of hindered phenolic antioxidant and secondary aromatic amine which both are the primary antioxidants. The combination of two different types of the antioxidant mixture (e.g. primary and secondary antioxidants) is known as an antioxidant composite that can exhibit hetero antioxidant synergism in lubricant oil. Auto-synergism antioxidant can be obtained by combining two or more different kinds of antioxidant functions in one structure. For example, a structure containing phenolic antioxidant and secondary antioxidant, or peroxide decomposer can exhibit auto synergism effect (Nath & Yehye, 2018; Rudnick, 2017). Our designated multifunctional antioxidant Structures 3 and 4 are the combinations of primary antioxidant such as phenolic and secondary aromatic amine and secondary antioxidant as a thioether (Figure 3.9). Multifunctional antioxidants would able to decrease the use of several antioxidants together which may also provide a negative effect on the synergistic system. The necessity of the use of antioxidants composite in the lubricant oil is to obtain the hetero-synergism which increase the oxidation inhibition time along with thermal stability. Interestingly, due to the combination of several antioxidant functions in multifunctional antioxidant, it plays a vital role on increase the oxidation and thermal stability of lubricant oil (Rudnick, 2017), which has been discussed elaborately in chapter 6.

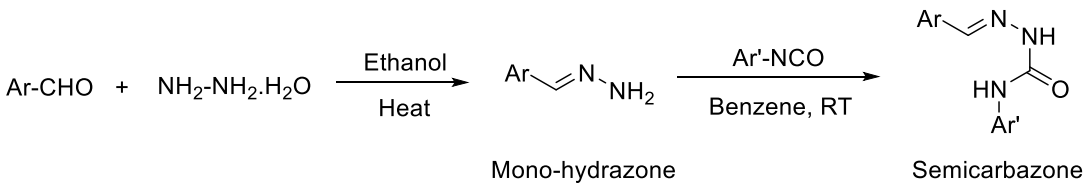
3.4 Semicarbazones preparation methods

It was found in the literature that there are three established methods for the preparation of semicarbazones. The well-known method of preparation of semicarbazones involves the reaction of aryl amine and urea or sodium cyanate in presence of glacial acetic acid to obtain carbamate which was treated with hydrazine to convert into semicarbazides, then the semicarbazides were refluxed with aldehyde to afford semicarbazones as shown in Scheme 3.1 (Asis et al., 1996; Asís et al., 1999; Yogeeswari et al., 2005).



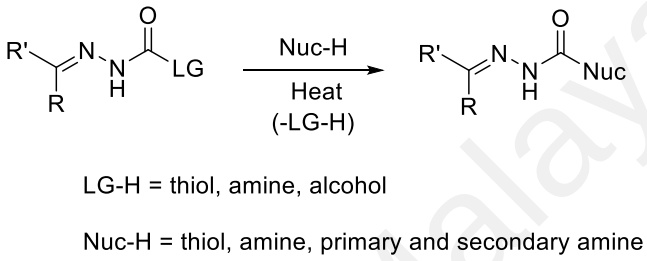
Scheme 3.1: General preparation method of semicarbazones

Due to the difficulties of obtaining some naphthyl semicarbazides, another preparation method of semicarbazones was reported (Asís et al., 1999). The treatment of appropriate mon-hydrazone with aryl isocyanate at room temperature provided the expected semicarbazones by the method (Scheme 3.2). The aldehyde mono-hydrazone were obtained from aldehyde in ethanol upon heating in presence of hydrazine (Thiel & Achmatowicz, 2003).



Scheme 3.2: Preparation of semicarbazones from mono-hydrazone.

Garland and coworkers (Garland et al., 2013) revealed a method for the preparation of complex hydrazones and semicarbazones by using the simple structure of hydrazone and semicarbazones molecule. In this method, semicarbazones can be accessed by the treatment of simple semicarbazone with a variety of leaving the group with a nucleophile such as primary and secondary amine at high temperature as showing in Scheme 3.3.



Scheme 3.3: Semicarbazones preparation via Imino-isocyante intermediate.

3.4.1 Importance of new method

An extensive literature survey on the semicarbazone preparation methods helps us to identify several limitations of existing methods. Functional group incompatibility is one of the major limitations of all the reported methods (Thiel & Achmatowicz, 2003). For example, synthesis of ester or acetyl group containing semicarbazone become difficult by following the widely reported semicarbazone preparation method because ester, acetyl groups are very reactive to the amine group. It became necessary to establish a new and efficient method for the preparation of semicarbazones which can alleviate the limitations of previously reported methods. Thus, in this study, an efficient and facile reaction protocol was established for semicarbazones which has been broadly discussed in Chapter 4. To validate the newly established method, a wide variety of semicarbazones (28 compounds) were synthesized which may provide important biological properties such as anticonvulsant properties along with the antioxidant activity. Because According to

Dimmock pharmacophore model (Dimmock et al., 1996), following structural features of an anticonvulsant should contain as shown in Figure 3.10: (i) aryl binding site responsible for hydrophobic interaction (HPB site); (ii) two-electron donor system (C=N); and most importantly (iii) a hydrogen bonding area as represented by carboxamide -$CONH_2$ function (HBD).

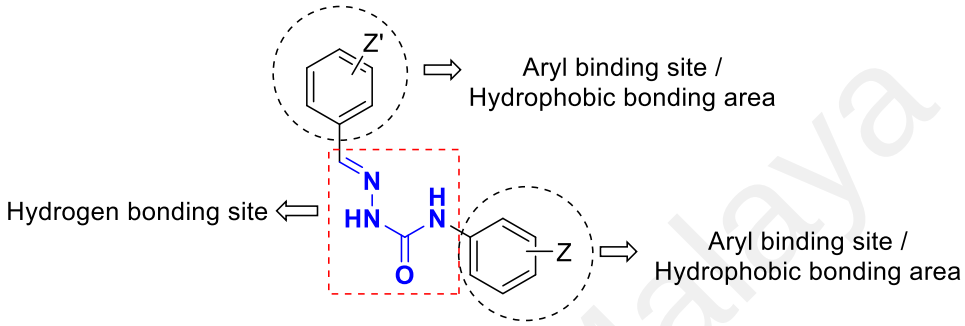


Figure 3.10: Structural features of anticonvulsant agents according to Dimmock pharmacophore model (Dimmock et al., 1996).

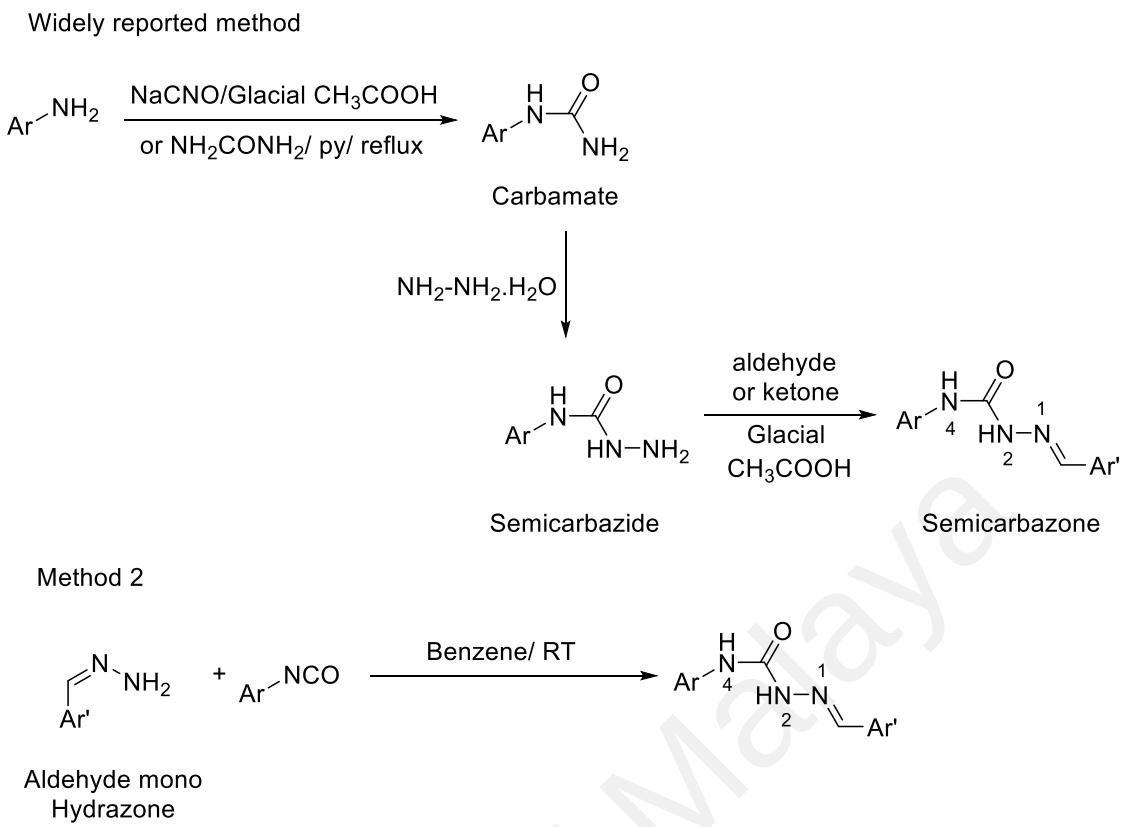
In this study the established reaction protocol for semicarbazones comprises of five steps reaction. An interesting aspect of this method is that the designated multifunctional antioxidant Structures 3 and 4 can be obtained in the second and fourth step of this reaction protocol. A variety of stable molecule containing either butylated hydroxytoluene or active carbon-nitrogen bond can be obtained in every step of this reaction protocol. Since 90% of pharmaceuticals contain a nitrogen atom in their structure, so the development of carbon-nitrogen bond in the molecule becomes important. In this aspect, established reaction protocol in this study (in Chapter 4) would help to make a wide variety of nitrogen-containing molecule which might have the excellent biological properties to reduce the risk of several chronic diseases.

CHAPTER 4: ACID HYDRAZIDE: A POTENTIAL REAGENT FOR SYNTHESIS OF SEMICARBAZONES

4.1 Introduction

The reinforcing of effectual routes to nitrogen-bearing molecules is predominant priority since more than 90% pharmaceutical products have no less than one nitrogen atom in their framework as well as one reaction out of six accomplished in the pharmaceutical industries associated with the development of a carbon-nitrogen bond (Dugger et al., 2005; Roveda et al., 2009). Semicarbazone have earned interest in medicinal and pharmaceutical fields due to broad spectrum biological activities such as anticonvulsant, antitumor and anticancer properties (Dimmock & Baker, 1994; Liu et al., 2014; Mishra et al., 2002; Pandeya, 2012; Sriram et al., 2004; Yogeeswari et al., 2004; Yogeeswari et al., 2005; Yogeeswari et al., 2005; Yogeeswari et al., 2004). Its metal complexes also exhibit significant activity against bacteria, fungi and viruses which extends the versatility of the application (Noblía et al., 2004; Pelosi et al., 2010). Moreover, substituted semicarbazones can be used as a platform chemical to prepare benzotriazepin compounds which are also important class of drug due to their psychostimulant, antidepressant, anorexigenic and antihypertensive properties (Douchez & Lubell, 2015).

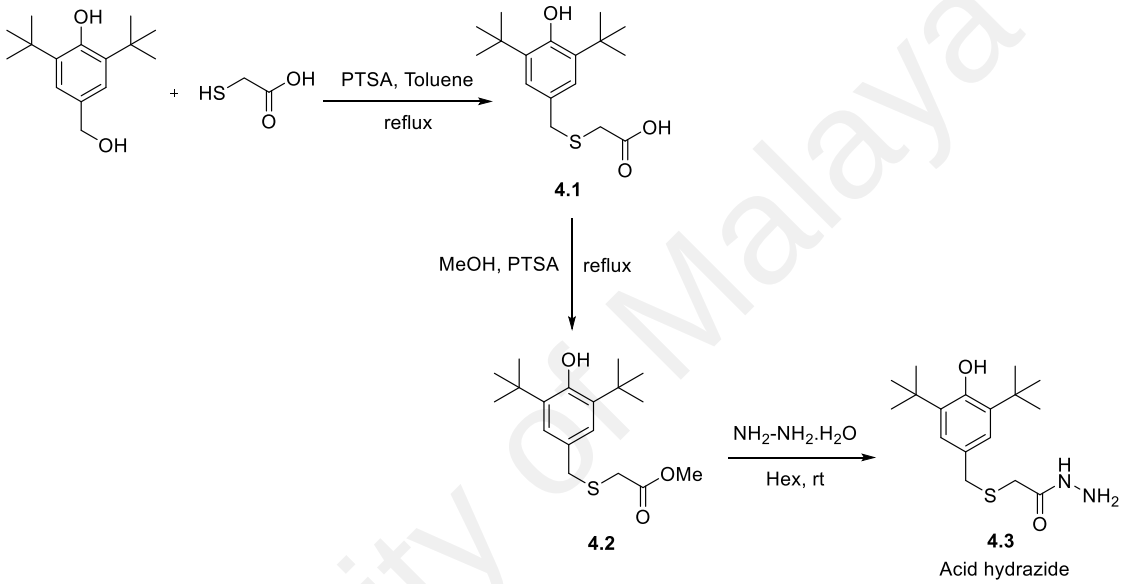
The well-known synthetic route of semicarbazones is a reaction between semicarbazides and aldehyde where semicarbazide compound is obtained by the treatment of carbamate compounds with hydrazine as seen in the Scheme 4.1 (Jafri et al., 2012; Jung et al., 2014; Pandeya et al., 2000; Yogeeswari et al., 2005; Yogeeswari et al., 2005a). Moreover, formation of desired semicarbazides proved challenging and most were commercially unavailable.



Scheme 4.1: Conventional methods for the preparation of semicarbazone (Jafri et al., 2012; Jung et al., 2014; Pandeya et al., 2000; Yogeeswari et al., 2005; Yogeeswari et al., 2005a).

Consequently, Asis *et al.* (Asís et al., 1999) reported another method to prepare semicarbazones involving the reaction between appropriate mono hydrazone and isocyanate. However, this widely reported method (Asís et al., 1999; Sriram et al., 2004; Yogeeswari et al., 2005; Yogeeswari et al., 2005a; Yogeeswari et al., 2004) is limited to some functional groups such as ester or acyl group. That's why semicarbazone bearing these functional group on aromatic ring attached with N-4 (Scheme **4.1**) could not be synthesized easily due to the reactivity of NH_2 of urea, hydrazine and aromatic aldehyde mono-hydrazone towards some active functional groups such as ester, oxadiazole, and other carbonyl compounds (Saravanan et al., 2006). Moreover, the yield of most of the semicarbazones using such synthesis technique was measured poor. Garland and coworkers (Garland et al., 2013) also reported the preparation of semicarbazone and

hydrazone derivatives from common semicarbazones and hydrazones respectively *via* Imino-isocyanates where various amines and anilines were used as nucleophiles with hydrazones. However, this protocol required a simple semicarbazone as the starting of the synthesis technique where N-4 part of semicarbazones was only replaced by amines or anilines as nucleophile via nucleophilic substitution reaction to form a complex semicarbazone derivative.



Scheme 4.2: Preparation of acid hydrazide 4.3.

Therefore, there is a room for designing a robust synthetic route to prepare new semicarbazones, which offer better functional group tolerance as well as chemoselectivity. Hence, it requires extensive research to outline a viable synthesis technique which leads this study to develop a potential reaction route. An effective and facile approach was adopted in this research to prepare diverse semicarbazones containing ester or acetyl group on aromatic ring attached with N-4. Acid hydrazide 4.3 was a pre-requisite precursor which was prepared according to the previously reported method as shown in the Scheme **4.2** (Ariffin et al., 2014).

The newly employed synthesis technique involved the preparation of substituted semicarbazides in order to protect the free hydrazine moiety of acid hydrazides. To the best of our knowledge, this is the first ever reported in our study the reaction protocol for the synthesis of semicarbazones from free hydrazine moiety protected substituted semicarbazide. The functional group which are very reactive to free hydrazine, were used in this reaction because no free hydrazine moiety was present in the substituted semicarbazides. This is one of the main advantages of this reaction over the existing methods. In addition, the by-product of this reaction (3,5-Di-tert-butyl-4-hydroxy-benzylsulfanyl)-acetic acid ethyl ester proved the involvement of the solvent into the reaction. A diverse semicarbazone derivatives were synthesized to validate the reaction protocol by seven different substituted semicarbazides containing reactive functional groups such as acetyl, ester and others.

4.2 Materials and methods

All starting materials were of analytical grade and double-distilled water was used throughout the experiment. The solvents and reagents were supplied by Sigma-Aldrich and Merck, Malaysia and used without further purification. Pre-coated silica gel plates (0.25 mm) was utilized as a part of observing reaction by analytical thin layer chromatography and imagined by UV light. Column chromatography was done on Silica Gel 60 (particle size: 0.040–0.063mm). IR spectra were recorded using FTIR-ATR of the solid samples. Melting points were approximated. 600 MHz and 150 MHz NMR spectrometer were used for ^{1}H NMR and ^{13}C NMR respectively and Tetramethylsilane was used as a reference. Chemical shifts (δ) are accounted for in ppm with respect to the residual solvent peak ($CHCl_3$, $\delta = 7.26$; DMSO-d_6, $\delta = 2.5$ for proton spectra; $^{13}CDCl_3$, $\delta = 77.0$; DMSO-d_6, $\delta = 40$ for carbon spectra). High resolution mass spectra were recorded on a time-of-flight Q-TOF LCMS system.

4.2.1 Synthesis of 2-((3,5-di-tert-butyl-4-hydroxybenzyl) thio) acetohydrazide (4.3):

2-((3,5-di-tert-butyl-4-hydroxybenzyl)thio)acetohydrazide was synthesized according to previously described method.(Ariffin et al., 2014) Compound was solid and stored at ambient temperature.

4.2.2 General procedure of substituted semicarbazides 4.5a-g (Gram scale) A:

1 gm (3.12 mmol) of acid hydrazide was stirred in 20 ml toluene then equimolar aryl isocyanate was introduced. Reaction was monitored by TLC and all the substituted semicarbazides formed white precipitate during the reaction. The solid was filtered and washed several times with toluene. Dichloromethane (CH_2Cl_2) was used for the compounds **4.5c** and **4.5e** since 2,4-di-chlorophhenylisocyanate and 4-acylphenylisocyanate are not soluble in toluene. Compound structure was confirmed by IR, ^{13}C NMR, ^{1}H NMR and HRMS analysis.

2-(2-(3,5-di-*tert*-butyl-4-hydroxybenzylthio)acetyl)-N-4-phenylhydrazinecarboxamide **(4.5a).** Yield: 1.42 g (99%); white solid; mp 128-130^{o}C.^{1}H NMR (600 MHz, DMSO-d_6): $\boldsymbol{\delta}$ 10.10 (s, 1H,), 9.67 (s, 1H), 9.60 (b, 1H), 7.45 (s, 2H), 7.34 (t, J=6 Hz, 1H), 7.17 (t, J=6 Hz, 1H), 7.08 (s, 2H), 6.90 (s, 1H), 3.79 (s, 2H), 3.17 (s, 2H), 1.38 (s, 18H); ^{13}C NMR (150 MHz, DMSO-d_6): $\boldsymbol{\delta}$ 181.4, 169.4, 153.3, 139.6, 139.5, 128.9, 128.6, 126.1, 125.7, 125.5, 36.7, 34.9, 33.4, 30.8; FT-IR (cm^{-1}, ATR) 3619, 3252, 2960, 1605; HRMS (Q-TOF) m/z: $[M+Na]^+$ Calcd for $C_{24}H_{33}O_3N_3SNa^+$ 466.2140; Found 466.2161.

2-(2-(3,5-di-*tert*-butyl-4-hydroxybenzylthio)acetyl)-N-4-(fluorophenyl) hydrazinecarboxamide **(4.5b)**. Yield: 1.41 g (98%); white solid; mp 160-162^{o}C. ^{1}H NMR (600MHz, DMSO-d_6) $\boldsymbol{\delta}$ ppm 9.81 (s, 1H), 8.76 (s, 1H), 8.15 (s, 1H), 7.47 (dd, 2H, J= 6),

7.10 (t, *J*= 6, 2H), 7.07 (s, 2H), 6.89 (s, 1H), 3.78 (s, 2H), 3.12 (s, 2H), 1.37 (s, 18H); ^{13}C NMR (150 MHz, DMSO-d_6): ***δ*** 169.5, 158.6, 157.1, 153.3, 136.4, 139.6, 128.9, 120.7, 115.6, 115.5, 36.5, 34.9, 32.9, 30.8; FT-IR (cm^{-1}, ATR) 3634, 3292, 2955, 1656; HRMS (Q-TOF) m/z: $[M+Na]^+$ Calcd for $C_{24}H_{32}FO_3N_3SNa^+$ 484.2046; Found 484.2051.

2-(2-(3,5-di-*tert*-butyl-4-hydroxybenzylthio)acetyl)-N-(2,4-di-chlorophenyl) hydrazinecarboxamide **(4.5c)**. Yields: 1.56 g (98%); white solid; mp. 106-108°C; ^{1}H NMR (600MHz, DMSO-d6): ***δ*** 10.00 (s, 1H), 8.91 (s, 1H), 8.33 (b, 1H), 8.11 (d, 1H, J= 6 Hz), 7.62 (d, 1H, J= 2.4 Hz), 7.37 (dd, J= 3, 6 Hz, 2H), 7.07 (s, 2H), 6.89 (s, 1H), 3.78 (s, 2H), 3.13 (s, 2H), 1.37 (s, 18H); ^{13}C NMR (150 MHz, DMSO-d6): ***δ*** 169.4, 155.1, 153.3, 139.6, 135.5, 129.1, 128.8, 128.1, 126.8, 125.7, 123.3, 122.6, 36.5, 34.9, 32.6, 30.8; FT-IR (cm^{-1}, ATR) 3644, 3184, 2956, 1591; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{24}H_{31}O_3N_3Cl_2SNa^+$ 534.1355; Found 534.1373.

2-(2-(3,5-di-*tert*-butyl-4-hydroxybenzylthio)acetyl)-N-(3-methylphenyl)hydrazinecarboxamide **(4.5d).** Yield: 1.35 gm (95%); white; solid mp. 106-108oC; ^{1}H NMR (600 MHz, DMSO-d6): ***δ*** 9.80 (s, 1H), 8.63 (s, 1H), 8.10 (s, 1H), 7.29 (s, 1H), 7.25 (d, 1H), 7.15 (t, 1H), 7.08 (s, 2H), 6.88 (s, 1H), 6.79 (d, 1H), 3.78 (s, 2H), 3.13 (s, 2H), 2.26 (s, 3H),1.38 (s, 18H); ^{13}C NMR (150 MHz, DMSO-d6): ***δ*** 169.5, 155.7, 153.3, 139.9, 139.6, 138.3, 128.9, 128.9, 125.7, 123.1, 119.4, 116.1, 36.5, 34.9, 32.9, 30.8, 21.7; FT-IR (cm^{-1}, ATR) 3361.2, 2916.2, 2853.3, 1607.2; HRMS (Q-TOF) m/z: $[M+Na]^+$ Calcd for $C_{25}H_{35}O_3N_3SNa^+$ 480.2291; Found 480.2312.

2-(2-(3,5-di-*tert*-butyl-4-hydroxybenzylthio)acetyl)-N-(4-acetylphenyl)hydrazinecarboxamide **(4.5e).** Yields: 1.36 g (90%); white solid mp. 147-149°C; ^{1}H NMR (600 MHz, DMSO-d6): ***δ*** 9.88 (s, 1H), 9.83 (s, 1H), 9.15 (s, 1H), 8.33 (s, 1H), 7.88 (d, J=9 Hz, 2H), 7.60 (d, J=8.4 Hz, 2H), 7.08 (s, 2H), 6.88 (s, 1H), 2.53 (s, 3H), 1.38 (s, 18H); ^{13}C NMR (150 MHz, DMSO-d6): ***δ*** 196.8, 169.7, 153.3 144.8, 139.7,

131.0, 130.1, 129.9, 128.9, 125.7, 117.9, 36.5, 34.9, 32.9, 30.8, 26.8; FT-IR (cm-1, ATR) 3368, 3092, 2917.5, 2850.3, 1613.2; HRMS (Q-TOF) m/z: $[M+Na]^+$ Calcd for $C_{26}H_{36}O_4N_3SNa^+$ 486.2421; Found 486.2435.

2-(2-(3,5-di-*tert*-butyl-4-hydroxybenzylthio)acetyl)-N-(3-ethoxycarbonylphenyl) hydrazinecarboxamide **(4.5f).** Yield: 1.45 g (90%); white solid; mp 157-159°C; 1H NMR (600MHz, DMSO-d6): ***δ*** 9.83 (s, 1H), 9.02 (s, 1H), 8.23 (s, 1H), 8.12 (s, 1H), 7.75 (d, J=7.8 Hz, 1H), 7.57 (d, J=7.8 Hz, 1H), 7.41 (t, J=7.8 Hz, 1H), 6.87 (s, 1H), 7.08 (s, 2H), 4.32 (q, J=7.2 Hz, 2H), 1.38 (s, 18H), 1.32 (t, J=7.2 Hz, 3H); 13C NMR (150 MHz, DMSO-d6): ***δ*** 169.6, 166.2, 155.7, 153.3, 140.5, 140.4, 139.6, 130.8, 129.6, 129.5, 128.9, 125.7, 123.1, 119.4, 61.2, 36.6, 34.9, 32.9, 30.8, 14.7; FT-IR (cm-1, ATR) 3374.7, 3092.3, 2917.5, 2850.3 1619.9; HRMS (Q-TOF) m/z: [M+Na]+ Calcd for C27H37O5N3SNa+ 538.2346; Found 538.2363.

2-(2-(3,5-di-*tert*-butyl-4-hydroxybenzylthio)acetyl)-N-(1-naphthyl)hydrazinecarboxamide **(4.5g).** Yield: 1.42 g (92%); white solid; mp 123-125°C; ^{1}H NMR (600 MHz, DMSO-d6): ***δ*** 9.96 (s, 1H), 8.79 (s, 1H), 8.43 (s, 1H), 8.06 (d, J=12 Hz, 1H), 7.92 (m, 1H), 7.80 (b, 1H), 7.67 (d, J=6 Hz, 1H), 7.53 (m, 2H), 7.46 (t, J=6 Hz, 1H), 7.08 (s, 2H), 6.90 (s, 1H), 3.80 (s, 2H), 3.16 (s, 2H), 1.36 (s, 18H); ^{13}C NMR (600 MHz, DMSO-d6): ***δ*** 169.6, 156.4, 153.3, 139.7, 134.6, 134.2, 128.9, 128.7, 126.4, 126.2, 126.2, 125.7, 124.2, 122.4, 119.4, 36.6, 34.9, 32.9, 30.8; FT-IR (cm-1, ATR) 3639, 3302, 2961, 1610; HRMS (Q-TOF) m/z 4$[M+H]^+$ Calcd for $C_{28}H_{36}O_3N_3S^+$ 494.2472; Found 494.2473.

4.2.3 General procedure for the synthesis of semicarbazone B:

0.65 mmol of substituted semicarbazides and equimolar quantity of aldehyde were mixed together in approximately 10 ml of ethanol in round bottle flask. Then the reaction

mixture was stirred at 90°C for 4-5 hours in presence catalytic amount of HCl (1.6 mmol) until the disappearance of the starting materials spot in the thin layer chromatography. Then the reaction mixture was allowed to warm to ambient temperature, stirred for around half an hour. In most of the reactions, the target compounds formed solid precipitate during stirring at ambient temperature. The precipitate was filtered and washed with hexane for several times. Then the resultant products were dried and either recrystallized from hexane or column chromatography. On the other hand, the reaction mixture was concentrated whereas no precipitate was found, and residue was purified by column chromatography on silica gel with hexane/EtOAc mixture as the eluent.

(3,5-Di-*tert*-butyl-4-hydroxy-benzylsulfanyl)-acetic acid ethyl ester **(4.8).** Yield: 0.77 g - 0.88 g, (70-80%); brown gummy liquid; Rf. 0.35 (hexane: EtOAc-1:1); 1H NMR (600 MHz, DMSO-d6): ***δ*** 7.03 (s, 1H), 6.92 (s, 1H), 4.10 (q, 2H, J= 7.2 Hz), 3.71 (s, 2H), 3.18 (s, 2H), 1.37 (s, 18H), 1.20 (t, J= 7.2 Hz, 3H); 13C NMR (150 MHz, DMSO-d6): ***δ*** 170.5, 153.4, 139.7, 128.6, 125.7, 61.1, 36.3, 34.9, 32.7, 30.8, 14.5; FTIR (cm^{-1}, ATR): 3564, 2955, 2914, 2873, 1728 HRMS (Q-TOF) m/z: [M+H]+ Calcd for $C_{19}H_{31}O_3S^+$ 338.1916; Found 338.1912.

2-benzylidene-N-phenylhydrazine-1-carboxamide **(4.7a).** Yield: 139.9 mg (90%); white solid; mp 178-180°C; Rf. 0.58 (hexane: EtOAc-2:1); 1H NMR (600 MHz, DMSO-d6): ***δ*** 10.76 (s, 1H), 8.91 (s, 1H), 7.97 (s, 1H), 7.87 (d, J=6.6 Hz, 2H), 7.66 (d, J=7.8 Hz, 2H), 7.44 (t, J=6.6, 7.8 Hz, 2H), 7.40 (t, J=7.2 Hz,1H), 7.30 (t, J=7.8, 8.4 Hz, 1H),7.02 (t, J=7.2 Hz, 1H); 13C NMR (150 MHz, DMSO-d6): ***δ*** 153.5, 141.3, 139.5, 134.9, 129.9, 129.5, 128.9, 127.5, 122.9, 120.4; FT-IR (cm-1, ATR) 3371, 3198, 3094, 2973, 2889, 1680; HRMS (Q-TOF) m/z: [M+H]+ Calcd for C14H14N3O+ 240.1131; Found 240.1149.

2-benzylidene-N-(4-fluorophenyl)hydrazine-1-carboxamide **(4.7b).** Yield: 150.41 mg (90%); white solid; mp 172-174°C; Rf. 0.52 (hexane: EtOAc-2:1); 1H NMR (600 MHz, DMSO-d6): ***δ*** 10.75 (s, 1H), 8.96 (s, 1H), 7.96 (s, 1H), 7.86 (d, J=7.2 Hz, 2H), 7.67 (dd, J=3.6, 5.4 Hz, 2H), 7.44 (t, J=7.2 Hz, 2H), 7.40 (t, J=7.2 Hz, 1H), 7.14 (t, J=9, 8.4 Hz, 2H); 13C NMR (150 MHz, DMSO-d6): ***δ*** 159.1, 157.5, 153.7, 141.3, 135.9, 134.9, 129.9, 129.1, 127.5, 122.3, 115.3; FT-IR (cm-1, ATR) 3377, 3193, 3102, 2958, 2832, 1680; HRMS (Q-TOF) m/z: [M+H]+ Calcd for C14H13FN3O+ 258.1037; Found 258.1052.

2-benzylidene-N-(2,4-dichlorophenyl)hydrazine-1-carboxamide **(4.7c).** Yield: 183.5 mg (92%); white solid; mp 222-223°C; Rf. 0.57 (hexane: EtOAc-2:1); ^{1}H NMR (600 MHz, DMSO-d6): ***δ*** 11.17 (s, 1H), 8.94 (s, 1H), 8.19 (d, J=9 Hz, 1H), 8.01 (s, 1H), 7.73 (d, J=7.2 Hz, 2H), 7.70 (d, J=2.4 Hz, 1H), 7.47 (t, J=7.2 Hz, 2H), 7.45 (d, J=2.4 Hz, 1H), 7.43 (t, J=3 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d6): ***δ*** 152.7, 142.2, 134.9, 134.4, 130.3, 129.4, 129.1, 128.3, 127.4, 127.2, 124.3, 122.7; FT-IR (cm-1, ATR) 3341, 3190, 3093, 2962, 2864, 1689; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{14}H_{12}C_{l2}N_3O^+$ 308.0352; Found 30.0364.

2-benzylidene-N-(m-tolyl)hydrazine-1-carboxamide **(4.7d)**. Yield: 148 mg (90%); white solid; mp. 165-167°C; R_f. 0.54 (hexane: EtOAc-2:1); ^{1}H NMR (600 MHz, DMSO-d_6): ***δ*** 10.73 (s, 1H), 8.80 (s, 1H), 7.96 (s, 1H), 7.85 (d, *J*=7.2 Hz, 2H), 7.48 (s, 1H), 7.47 (d, *J*=6 Hz, 1H), 7.43 (t, *J*=6.6, 7.8 Hz, 2H), 7.40 (t, *J*=7.2 Hz, 1H), 7.18 (t, *J*=7.8 Hz, 1H), 6.84 (d, *J*=7.8 Hz, 1H), 2.30 (s, 3H); ^{13}C NMR (150 MHz DMSO-d_6): ***δ*** 153.5, 141.2, 139.4, 138.1, 134.9, 129.9, 129.1, 128.8, 127.5, 123.7, 120.8, 117.4, 21.6; FT-IR (cm^{-1}, ATR) 3356, 2916, 2853, 1628; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{15}H_{16}N_3O^+$ 254.1288; Found 254.1306.

N-(4-acetylphenyl)-2-benzylidenehydrazine-1-carboxamide **(4.7e)**. Yield: 157 mg (86%); white solid; mp 180-182°C; R_f. 0.55 (hexane: EtOAc-2:1); ^{1}H NMR (400 MHz,

DMSO-d_6): **δ** 10.93 (s, 1H), 9.24 (s, 1H), 7.99 (s, 1H), 7.93 (d, *J*=9 Hz, 2H), 7.88 (d, *J*= 6 Hz, 2H), 7.85 (d, 2H), 7.45 (m, 2H, *J*= 7.8 Hz), 7.42 (t, *J*= 7.2 Hz, 1H), 2.54 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): **δ** 196.9, 153.2, 144.2, 142.0, 134.7, 131.4, 130.1, 129.7, 129.1, 127.6, 119.1, 22.7; FT-IR (cm^{-1}, ATR) 3361, 3200, 3099, 2957, 2857, 1693; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{16}H_{16}N_3O_2^+$ 282.1237; Found 282.1248).

ethyl-3-(2-benzylidenehydrazine-1-carboxamido)benzoate **(4.7f)**. Yield: 161.8 mg (80%); white solid; mp. 126-128°C; R_f. 0.59 (hexane: EtOAc-2:1); ^{1}H NMR (600 MHz, DMSO-d_6): **δ** 9.18(s, 1H), 10.84 (s, 1H), 7.96 (d, 1H), 7.62 (d, 1H), 8.34 (s, 1H), 7.98 (s, 1H), 7.45 (dt, *J*= 1.8, 8.4 Hz, 3H), 7.41 (t, 1H), 7.45 (t, *J*= 7.2 Hz, 2H), 7.88 (d, 2H), 4.33(q, *J*=7.2 Hz, 2H), 1.33(t, *J*=7.2 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): **δ** 193.7, 166.3, 153.8, 153.6, 141.6, 140.1, 134.8, 129.9, 129.1, 127.7, 125.1, 123.6, 120.9, 61.2, 14.70; FT-IR (cm^{-1}, ATR) 3361, 3193, 3058, 2917, 2850, 1633; HRMS (Q-TOF) m/z: $M+H]^+$ Calcd for $C_{17}H_{18}N_3O_3^+$ 312.1343; Found 312.1357.

2-benzylidene-N-(naphthalen-2-yl)hydrazine-1-carboxamide **(4.7g)**. Yield: 169.2 mg (90%); white solid; mp 196-198°C; R_f. 0.43 (hexane: EtOAc-2:1); ^{1}H NMR (400 MHz, DMSO-d_6): **δ** 10.84 (s, 1H), 9.19 (s, 1H), 8.03 (s, 1H), 7.98 (d, *J*=8.4 Hz, 1H), 7.96 (d, *J*=7.8 Hz, 1H), 7.88 (d, *J*=7.2 Hz, 2H), 7.77 (d, *J*=8.4 Hz, 1H), 7.72 (d, *J*=7.2 Hz, 1H), 7.57 (t, *J*=6.6 Hz, 1H), 7.55 (t, *J*=7.2 Hz, 1H), 7.52 (t, *J*=7.8 Hz, 1H), 7.44 (t, *J*=7.2 Hz, 2H), 7.40 (t, *J*=7.2 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6): **δ** ppm: 154.4, 141.2, 135.0, 134.4, 134.2, 129.8, 129.1, 129.1, 128.6, 127.4, 126.4, 126.4, 126.1, 125.3, 122.9, 122.3; FT-IR (cm^{-1}, ATR) 3410, 3199, 3109, 2965, 2868, 1701; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{18}H_{16}N_3O^+$ 290.1288; Found 290.1303.

2-(4-fluorobenzylidene)-N-phenylhydrazine-1-carboxamide **(4.7a$_1$)**. Yield: 153.8 mg (92%); white solid; mp. 160-162°C; R_f. 0.45 (hexane: EtOAc-2:1);^{1}H NMR (600 MHz, DMSO -d_6): **δ** 10.75 (s, 1H), 8.92 (s, 1H), 7.95 (s, 1H), 7.93 (d, *J*=6, 2.4 Hz, 2H), 7.65

(d, *J*=7.8 Hz, 2H), 7.30 (t, *J*=7.8 Hz, 2H), 7.27 (d, *J*=9 Hz, 2H), 7.03 (t, *J*=7.2 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6): ***δ*** 162.4, 153.5, 140.1, 139.5, 131.5, 129.6, 128.9, 122.9, 120.4, 115.9; FT-IR (cm^{-1}, ATR) 3377, 3193, 3102, 2958, 2832, 1680; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{14}H_{13}N_3FO^+$ 258.1037; Found 258.1052.

2-(4-fluorobenzylidene)-N-(4-fluorophenyl)hydrazine-1-carboxamide **(4.7b$_1$)**. Yield: 169.8 mg (95%); white solid; mp 170-172°C; R_f. 0.57 (hexane: EtOAc-2:1); ^{1}H NMR (600 MHz, DMSO-d_6): ***δ*** 10.74 (s, 1H), 8.98 (s, 1H), 7.95 (s, 1H), 7.92 (d, *J*=3, 5.4 Hz, 2H), 7.66 (dd, *J*=1.8, 5.4 Hz, 2H), 7.26 (t, *J*=9 Hz, 2H), 7.14 (t, *J*=9 Hz, 2H); ^{13}C NMR (150 MHz, DMSO-d_6): ***δ*** 162.4, 157.5, 153.7, 140.2, 135.9, 131.5, 129.6, 122.3, 115.9, 115.3; FT-IR (cm^{-1}, ATR) 3368, 2917, 2850, 1633; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{14}H_{11}N_3F_2O^+$ 276.0943; Found 276.0961.

N-(2,4-dichlorophenyl)-2-(4-fluorobenzylidene)hydrazine-1-carboxamide **(4.7c$_1$)**. Yield: 190.2 mg (90%); white solid; mp 235-237°C; R_f. 0.54 (hexane: EtOAc-2:1); ^{1}H NMR (600 MHz, DMSO-d_6): ***δ*** 11.17 (s, 1H), 8.93 (s, 1H), 8.16 (d, 1H), 8.01 (s, 1H), 7.81 (dd, *J*=3, 6 Hz, 2H), 7.70 (d, *J*=2.4 Hz, 1H), 7.43 (dd, *J*=2.4, 6.6Hz, 1H), 7.30 (d, *J*=9 Hz, 2H); ^{13}C NMR (150 MHz, DMSO-d_6): ***δ*** 162.6, 152.7, 141.1, 134.9, 131.1, 129.3, 129.1, 128.3, 127.5, 124.6, 122.9, 116.5; FT-IR (cm^{-1}, ATR) 3341, 3197, 3086, 2962, 2831, 1689; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{14}H_{11}N_3Cl_2FO^+$ 326.0258; Found 326.0263.

2-(4-fluorobenzylidene)-N-(*m*-tolyl)hydrazine-1-carboxamide **(4.7d$_1$)**. Yield: 149.8 mg (85%); white solid; mp 152-154°C; R_f. 0.47 (hexane: EtOAc-2:1); ^{1}H NMR (600 MHz, DMSO-d_6): ***δ*** 10.72 (s, 1H), 8.82 (s, 1H), 7.95 (s, 1H), 7.92 (dd, *J*=3, 6 Hz, 2H), 7.48 (d, *J*=4.2 Hz, 1H), 7.46 (s, 1H), 7.26 (d, *J*=9 Hz, 2H), 7.18 (t, *J*=7.8 Hz, 1H), 6.84 (d, *J*=7.2 Hz, 1H), 2.29 (s, 1H); ^{13}C NMR (600 MHz, DMSO-d_6): ***δ*** 162.4, 153.5, 140.0, 139.4, 138.1, 131.5 129.6, 128.8, 123.7, 120.9, 117.5, 115.9, 21.6; FT-IR (cm^{-1}, ATR)

3365, 2916, 2848, 1628; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{15}H_{15}N_3FO^+$ 272.1194; Found 272.1209.

N-(4-acetylphenyl)-2-(4-fluorobenzylidene)hydrazine-1-carboxamide **(4.7e$_1$)**. Yield: 171 mg (88%), white solid; mp 232-233°C; R_f. 0.55 (hexane: EtOAc-2:1); ^{1}H NMR (600 MHz, DMSO-d_6): ***δ*** 10.93 (s, 1H), 9.26 (s, 1H), 7.98 (s, 1H), 7.95 (d, *J*=5.4 Hz, 2H), 7.92 (d, *J*=8.4 Hz, 2H), 7.86 (d, *J*=9 Hz, 2H), 7.28 (t, *J*=9 Hz, 2H), 2.54, (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): ***δ*** 196.9, 164.1, 162.5, 153.2, 144.3, 140.9, 131.4, 131.3, 129.8, 129.7, 119.1, 116.1, 26.9; FT-IR (cm^{-1}, ATR) 3368, 3200, 3099, 2964, 2857, 1693; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{16}H_{15}N_3FO_2^+$ 300.1143; Found 300.1158.

ethyl-3-(2-(4-fluorobenzylidene)hydrazine-1-carboxamido)benzoate **(4.7f$_1$)**. Yield: 175.4 mg (82%); white solid; mp 142-144°C; R_f. 0.58 (hexane: EtOAc-2:1); ^{1}H NMR (600 MHz, DMSO-d_6): ***δ*** 10.82 (s, 1H), 9.18 (s, 1H), 7.96 (s, 1H), 7.95 (d, 2H,), 7.95 (d, *J*=5.4 Hz, 1H), 7.63 (d, *J*=7.8 Hz, 1H), 7.45 (t, *J*=7.8 Hz, 1H), 7.28 (d, *J*=9 Hz, 2H), 4.33 (q, *J*=7.2 Hz, 2H),1.33 (t, *J*=7.2 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): ***δ*** 166.3, 164.1, 162.4, 153.6, 140.5 131.5, 130.7, 129.8, 129.2, 125.1, 123.6, 120.8, 115.9, 61.2, 14.7; FT-IR (cm^{-1}, ATR) 3374, 3200, 3085, 2964, 1680; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{17}H_{17}N_3FO_3^+$ 330.1248; Found 330.1264.

2-(4-fluorobenzylidene)-N-(naphthalen-2-yl)hydrazine-1-carboxamide **(4.7g$_1$)**. Yield: 179.7 mg (90%); white solid; mp 212-214°C; R_f. 0.39 (hexane: EtOAc-2:1); ^{1}H NMR (600 MHz, DMSO-d_6): ***δ*** 10.85 (s, 1H), 9.23 (s, 1H), 8.01 (s, 1H), 7.99 (d, *J*=7.8 Hz, 1H), 7.97 (m, *J*=4.2 Hz, 1H), 7.95 (d, *J*=6 Hz, 1H), 7.79 (d, *J*=7.8 Hz, 1H), 7.69 (d, *J*=7.2 Hz, 1H), 7.57 (t, *J*=8.4 Hz, 2H), 7.53 (t, *J*=7.8 Hz, 1H), 7.28 (d, *J*=9 Hz, 2H); ^{13}C NMR (600 MHz, DMSO-d_6), ***δ*** 154.5, 139.9, 134.5, 134.2, 131.7, 129.5, 129.2, 128.6, 126.3, 126.1, 125.4, 123.1, 122.71, 116.0; FT-IR (cm^{-1}, ATR) 3320, 3193, 2959, 2862, 1641; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{18}H_{15}N_3FO^+$ 308.1194; Found 308.1208.

2-(3-methoxybenzylidene)-N-phenylhydrazine-1-carboxamide **(4.7a$_2$)**. Yield: 157.4 mg (90%); white solid; mp 130-132°C; R_f. 0.55 (hexane: EtOAc-2:1); ^{1}H NMR (600 MHz, DMSO-d_6): ***δ*** 10.74 (s, 1H), 8.91 (s, 1H), 7.93 (s, 1H), 7.65 (d, *J*= 7.2 Hz, 2H), 7.45 (s, 1H), 7.35 (d, *J*= 7.8 Hz, 1H), 7.32 (t, *J*= 7.8 Hz, 1H), 7.29 (t, *J*= 7.8 Hz, 2H), 7.01 (t, *J*= 7.2 Hz, 1H), 6.95 (dd, *J*= 1.2, 6.6 Hz, 1H), 3.83 (s, 3H); ^{13}C NMR (150 MHz DMSO-d_6): ***δ*** 160.02, 153.5, 141.2, 139.5, 136.3, 130.2, 128.9, 123.0, 120.4, 115.9, 115.9, 112.1, 55.7; FT-IR (cm^{-1}, ATR) 3371, 3193, 3094, 2963, 2832, 1680; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{15}H_{16}N_3O_2^+$ 270.1237; Found 270.1252.

N-(4-fluorophenyl)-2-(3-methoxybenzylidene)hydrazine-1-carboxamide **(4.7b$_2$)**. Yield: 164.2 mg (88%); white solid; mp. 125-127°C; R_f. 0.41 (hexane: EtOAc-2:1); ^{1}H NMR (600 MHz, DMSO-d_6): ***δ*** 10.76 (s, 1H), 9.00 (s, 1H), 7.93 (s, 1H), 7.67 (dd, *J*= 1.8, 5.4 Hz, 2H), 7.45 (s, 1H), 7.36 (d, *J*= 7.2 Hz, 1H), 7.34 (t, *J*= 7.2 Hz, 1H), 7.14 (t, *J*= 9 Hz, 2H), 6.97 (dd, *J*= 1.2, 6.6 Hz, 1H), 3.82 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): ***δ*** 160.01, 157.6, 153.7, 141.2, 136.3, 135.9, 130.1, 122.4, 120.4, 115.9, 115.3, 112.1, 55.7; FT-IR (cm^{-1}, ATR) 3314, 3240, 2998, 2957, 2836, 1673; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{15}H_{15}N_3FO_2^+$ 288.1143; Found 288.1155.

N-(2,4-dichlorophenyl)-2-(3-methoxybenzylidene)hydrazine-1-carboxamide **(4.7c$_2$)**. Yield: 201.6 mg (92%); white solid; mp 200-201°C; R_f. 0.61 (hexane: EtOAc-2:1); ^{1}H NMR (600 MHz, DMSO-d_6): ***δ*** 10.76 (s, 1H), 9.00 (s, 1H), 8.21 (d,1H, *J*= 9 Hz), 7.98 (s, 1H), 7.70 (s, 1H), 7.44 (dd, *J*= 2.4, 6 Hz, 1H), 7.37 (t, *J*= 7.8 Hz, 1H), 7.33 (s, 1H), 7.28 (d, *J*= 7.8 Hz, 1H), 7.00 (dd, *J*= 2.4, 5.4 Hz, 1H), 3.80 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): ***δ*** 160.0, 152.9, 141.9, 135.9, 134.9, 130.5, 129.1, 128.4, 127.4, 124.2, 122.6, 120.1, 116.6, 111.1, 55.5; FT-IR (cm^{-1}, ATR) 3360, 3203, 3099, 2969, 2831, 1696; HRMS (Q-TOF) m/z $[M+H]^+$ Calcd for $C_{15}H_{14}N_3Cl_2O_2^+$ 33.0458; Found 338.0464.

2-(3-methoxybenzylidene)-N-(*m*-tolyl)hydrazine-1-carboxamide **(4.7d$_2$)**. Yield: 147.2 mg (80%); white solid; mp 142-144°C; R$_f$. 0.50 (hexane: EtOAc-2:1); ^{1}H NMR (600 MHz, DMSO-*d*$_6$): ***δ*** 10.75 (s, 1H), 8.85 (s, 1H), 7.93 (s,1H,), 7.50 (s, 1H), 7.46 (d, *J*= 8.4 Hz, 1H), 7.44 (s, 1H), 7.37 (d, *J*= 7.8 Hz, 1H), 7.34 (t, *J*= 7.8 Hz, 1H), 7.18 (t, *J*= 7.8 Hz, 1H), 6.97 (dd, *J*= 1.2, 6.6 Hz, 1H), 6.85 (d, *J*= 7.8 Hz, 1H), 3.82 (s, 3H), 2.30 (s, 3H); ^{13}C NMR (150 MHz DMSO-*d*$_6$): ***δ*** 160.0, 153.5, 141.1, 139.4, 138.1, 136.3, 130.2, 128.7, 123.7, 120.9, 120.3, 117.6, 115.8, 112.1, 55.7, 21.7; FT-IR (cm^{-1}, ATR) 3371, 3193, 3083, 2952, 2837, 1680; HRMS (Q-TOF) m/z [M+H]$^+$ Calcd for $C_{16}H_{18}N_3O_2^+$ 284.1394; Found 284.1407.

N-(4-acetylphenyl)-2-(3-methoxybenzylidene)hydrazine-1-carboxamide **(4.7e$_2$)**. Yield: 157.3 mg (82%); white solid; mp 129-131°C; R$_f$. 0.52 (hexane: EtOAc-2:1); ^{1}H NMR (600 MHz, DMSO-*d*$_6$): ***δ*** 10.96 (s, 1H), 9.29 (s, 1H), 7.96 (d,1H, *J*=9 Hz), 7.93 (d, *J*=9 Hz, 2H), 7.86 (d, *J*=9 Hz, 2H), 7.48 (s, 1H), 7.39 (d, *J*=7.2 Hz, 1H), 7.36 (t, *J*=7.8 Hz, 1H), 6.99 (dd, *J*=1.2, 6.6 Hz, 1H), 3.83 (s, 3H), 2.53 (s, 3H); ^{13}C NMR (150 MHz, DMSO-*d*$_6$): ***δ*** 196.9, 160.0, 153.3, 144.3, 141.9, 136.1, 131.4, 130.2, 129.7, 120.5, 119.1, 116.0, 112.3, 55.7, 26.9; FT-IR (cm^{-1}, ATR) 3354, 3185, 3082, 2951, 2837, 1693; HRMS (Q-TOF) m/z: [M+H]$^+$ Calcd for $C_{17}H_{18}N_3O_3^+$ 312.1343; Found 312.1355.

ethyl-3-(2-(3-methoxybenzylidene)hydrazine-1-carboxamido)benzoate **(4.7f$_2$)**. Yield: 188.5 mg (85%); white solid; mp 104-106°C; R$_f$. 0.57 (hexane: EtOAc-2:1); ^{1}H NMR (600 MHz, DMSO-*d*$_6$): ***δ*** 10.85 (s, 1H), 9.21 (s, 1H), 8.35 (s, 1H), 7.95 (s, 1H), 7.93 (d, *J*= 1.8Hz, 1H), 7.62 (d, *J*= 7.2 Hz, 1H), 7.48 (s, 1H), 7.45 (t, *J*= 7.8 Hz, 1H), 7.40 (d, *J*= 7.8 Hz, 1H), 7.35 (t, *J*= 7.8 Hz, 1H), 6.98 (dd, *J*=1.8, 6.6 Hz, 1H), 4.33 (q, *J*=7.2 Hz, 2H), 3.83 (s, 3H),1.33 (t, *J*=7.2 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-*d*$_6$): ***δ*** 166.3, 160.0, 153.6, 141.5, 140.0, 136.2, 130.7, 130.1, 129.2, 125.1, 123.6, 120.9, 120.4, 115.8, 112.3,

61.2, 55.7, 14.7; FT-IR (cm^{-1}, ATR) 3394, 3193, 3092, 2978, 1687; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{18}H_{20}N_3O_4^+$ 342.1448; Found 342.1458.

2-(3-methoxybenzylidene)-N-(naphthalen-2-yl)hydrazine-1-carboxamide **(4.7g$_2$)**. Yield: 186.7 mg (90%); white solid; mp. 194-196°C; R_f. 0.57 (hexane: EtOAc-2:1); ^{1}H NMR (600 MHz, DMSO-*d*$_6$): ***δ*** 10.88 (s, 1H), 9.24 (s, 1H), 8.00 (d, *J*=3.6 Hz, 1H), 7.99 (s, 1H), 7.97 (d, *J*=7.2 Hz, 1H), 7.79 (d, *J*=8.4 Hz, 1H), 7.73 (d, *J*=7.2 Hz, 1H), 7.56 (dt, *J*=1.2, 2.4 Hz, 2H), 7.53 (t, *J*=7.2 Hz, 1H), 7.52 (s, 1H), 7.36 (d, *J*=5.4 Hz, 1H), 7.34 (t, *J*=7.8 Hz, 1H), 6.98 (d, *J*=2.4, 6.6Hz, 1H), 3.83 (s, 3H), 2.53 (s, 3H); ^{13}C NMR (150 MHz, DMSO-*d*$_6$): ***δ*** 160.1, 154.4, 141.0, 136.4, 134.4, 134.2, 130.2, 129.1, 128.6, 126.4, 126.3, 126.1, 125.3, 122.3, 120.4, 116.1, 111.4, 55.7; FT-IR (cm^{-1}, ATR) 3380, 3187, 3091, 2959, 2868, 1683; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{19}H_{18}N_3O_2^+$ 320.1394; Found 320.1402.

2-(4-isopropylbenzylidene)-N-phenylhydrazine-1-carboxamide **(4.7a$_3$)**. Yield: 146.2 mg (80%); white solid; mp 140-142°C; R_f. 0.65 (hexane: EtOAc-2:1); ^{1}H NMR (600 MHz, DMSO-*d*$_6$): ***δ*** 10.67 (s, 1H), 8.85 (s, 1H), 7.93 (s, 1H), 7.76 (d, *J*=7.8 Hz, 2H), 7.66 (d, *J*=7.8 Hz, 2H), 7.30 (d, *J*=7.8 Hz, 2H), 7.30 (t, *J*=7.8 Hz, 2H), 7.02 (t, *J*=7.8 Hz, 1H), 2.93 (m, *J*=7.2 Hz, 1H), 1.22 (d, *J*=7.2 Hz, 6H); ^{13}C NMR (150 MHz, DMSO-*d*$_6$): ***δ*** 153.5, 150.4, 141.3, 139.5, 132.6, 128.9, 127.6, 127.0, 122.9, 120.3, 33.8, 24.2; FT-IR (cm^{-1}, ATR) 3377, 3193, 3089, 2952, 2863, 1675; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{17}H_{20}N_3O^+$ 282.1601; Found 282.1615.

2-(4-isopropylbenzylidene)-N-(4-fluorophenyl)hydrazine-1-carboxamide **(4.7b$_3$)**. Yield: 153.6 mg (79%), white solid; mp 114-116°C; R_f. 0.50 (hexane: EtOAc-2:1); ^{1}H NMR (600 MHz, DMSO-*d*$_6$): ***δ*** 10.70 (s, 1H), 8.94 (s, 1H), 7.93 (s, 1H), 7.76 (d, *J*=7.8 Hz, 2H), 7.67 (dd, *J*=4.8 Hz, 2H), 7.30 (d, *J*=7.8 Hz, 2H), 7.14 (t, *J*=9 Hz, 2H), 2.92 (m, *J*=7.2 Hz, 1H), 1.22 (d, *J*=6.6 Hz, 6H); ^{13}C NMR (600 MHz, DMSO-*d*$_6$): ***δ*** 157.4, 153.7,

150.4, 141.4, 135.9, 132.6, 127.6, 127.0, 122.2, 115.3, 33.8, 24.2; FT-IR (cm^{-1}, ATR) 3368, 2917, 2850, 1633; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{17}H_{19}N_3FO^+$ 300.1507; Found 300.1519.

2-(4-isopropylbenzylidene)-N-(2,4-dichlorophenyl)hydrazine-1-carboxamide **(4.7c$_3$)**. Yield: 186 mg (82%); white solid; mp 191-193°C; R_f. 0.55 (hexane: EtOAc-2:1); ^{1}H NMR (600 MHz, DMSO-d_6): ***δ*** 11.11 (s, 1H), 8.94 (s, 1H), 8.24 (d, 1H), 7.98 (s, 1H), 7.70 (d, *J*=2.4 Hz, 1H), 7.64 (d, *J*=7.8 Hz, 2H), 7.42 (dd, *J*=2.4, 6.6 Hz, 1H), 7.33 (d, *J*=8.4 Hz, 2H), 2.92 (m, *J*=6.6 Hz, 1H), 1.21 (d, *J*=6.6 Hz, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): ***δ*** 152.7, 151.0, 142.3, 134.9, 132.1, 129.0, 128.4, 127.4, 127.2, 124.0, 122.3, 33.8, 24.2; FT-IR (cm^{-1}, ATR) 3350, 3193, 3115, 2959, 1689; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{17}H_{18}N_3Cl_2O^+$ 350.0821; Found 350.0831.

2-(4-isopropylbenzylidene)-N-(*m*-tolyl)hydrazine-1-carboxamide **(4.7d$_3$)**. Yield: 157.3 mg (82%); white solid; mp 146-148°C; R_f. 0.68 (hexane: EtOAc-2:1); ^{1}H NMR (600 MHz, DMSO-d_6): ***δ*** 10.67 (s, 1H), 8.77 (s, 1H), 7.92 (s, 1H), 7.75 (d, *J*=7.8 Hz, 2H), 7.49 (s, 1H), 7.46 (d, *J*=8.4 Hz, 1H), 7.29 (d, *J*=8.4 Hz, 2H), 7.17 (t, *J*=7.8 Hz, 1H), 6.83 (d, *J*=7.8 Hz, 1H), 2.90 (m, *J*=7.2 Hz, 1H), 2.30 (s, 3H), 1.22 (d, 6H, *J*=7.2 Hz); ^{13}C NMR (150 MHz, DMSO-d_6): ***δ*** 153.5, 150.4, 141.3, 139.4, 138.1, 132.6, 128.8, 127.5, 127.0, 123.6, 120.7, 117.4, 33.8, 24.2, 21.6; FT-IR (cm^{-1}, ATR) 3377, 3188, 3089, 2958, 2853, 1680; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{18}H_{22}N_3O^+$ 296.1757; Found 296.1770.

N-(4-acetylphenyl)-2-(4-isopropylbenzylidene)hydrazine-1-carboxamide **(4.7e$_3$)**. Yield: 147.1 mg (70%); white solid; mp. 136-138°C; R_f. 0.48 (hexane: EtOAc-2:1); ^{1}H NMR (600 MHz, DMSO-d_6): ***δ*** 10.90 (s, 1H), 9.25(s, 1H), 8.00 (s, 1H), 7.98 (d, *J*=9 Hz, 2H), 7.91 (d, *J*=9 Hz, 1H), 7.83 (d, *J*=8.4 Hz, 1H), 7.37 (d, *J*=8.4 Hz, 2H), 2.98 (m, *J*=6.6 Hz, 1H), 2.59 (s, 3H), 1.28 (d, *J*=7.2 Hz, 6H); ^{13}C NMR (150 MHz DMSO-d_6): ***δ*** 197.0, 153.2, 150.6, 144.3, 140.1, 132.4, 131.4, 129.7), 127.7, 127.0, 119.1, 33.8, 26.8, 24.2;

FT-IR (cm^{-1}, ATR) 3365, 3180, 3018, 2964, 2857, 1673; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{19}H_{22}N_3O_2$ 324.1707; Found 324.1718.

2-(4-isopropylbenzylidene)-N-(3-(pent-1-en-2-yl)phenyl)hydrazine-1-carboxamide **(4.7f3)**. Yield: 172.2 mg (75%); white solid; mp 120°C; R_f. 0.57 (hexane: EtOAc-2:1); ^1H NMR (600 MHz, DMSO-d_6): ***δ*** 10.75 (s, 1H), 9.13 (s, 1H), 8.33 (s, 1H), 7.96 (s, 1H,), 7.95 (s, 1H), 7.78 (d, *J*=8.4 Hz, 2H), 7.62 (d, *J*=7.8 Hz, 1H), 7.44 (t, *J*=7.8 Hz, 1H), 7.30 (d, *J*=8.4 Hz, 2H), 4.33 (q, *J*=7.2 Hz, 2H), 2.92 (m, *J* =6.6 Hz, 1H), 1.33 (t, *J*=7.2 Hz, 3H), 1.22 (d, *J*=7.2 Hz, 6H); ^{13}C NMR (600 MHz, DMSO-d_6): ***δ*** 166.3, 153.6, 150.5, 141.7, 140.1, 132.5, 130.7, 129.2, 127.7, 127.0, 124.9, 123.5, 120.9, 61.2, 33.8, 24.2, 14.7; FT-IR (cm^{-1}, ATR) 3448, 3193, 3092, 2957, 2884, 1664; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{20}H_{24}N_3O_3^+$ 354.1812; Found 354.1824.

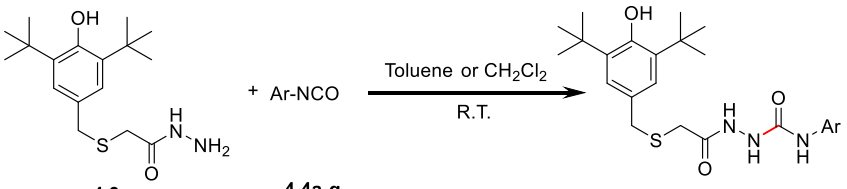
2-(4-isopropylbenzylidene)-N-(naphthalen-2-yl)hydrazine-1-carboxamide **(4.7g3)**. Yield: 183 mg (85%); white solid; mp 171-173°C; R_f. 0.72 (hexane: EtOAc-2:1); ^1H NMR (600 MHz, DMSO-d_6): ***δ*** 10.85 (s, 1H), 9.18 (s, 1H), 8.01 (s, 1H), 8.00 (s, 1H), 7.96 (d, 1H, *J*= 8.4 Hz), 7.77 (d, 2H, *J*= 7.8 Hz), 7.74 (d, 1H, *J*=8.4 Hz), 7.58 (t, *J*= 8.4 Hz, 1H), 7.54 (t, *J*= 7.2 Hz, 1H), 7.51 (t, *J*= 7.8 Hz, 1H), 7.31 (d, *J*= 8.4 Hz, 2H), 2.92 (m, *J*= 6 Hz, 1H), 1.22 (d, *J*= 6 Hz, 6H); ^{13}C NMR (600 MHz, DMSO-d_6): ***δ*** 154.4, 150.4, 141.3, 134.4, 134.2, 132.7, 128.7, 128.6, 127.4, 127.1, 126.4, 126.1, 125.1, 122.8, 121.8, 33.8, 24.2; FT-IR (cm^{-1}, ATR) 3350, 3195, 3110, 2960, 1689; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{21}H_{22}N_3O^+$ 332.1752; Found 332.1773.

4.3 Results and Discussion

Reaction between acid hydrazide and aryl isocyanates is a straightforward reaction at room temperature as reported by many researchers (Drummond & Johnson, 1988; Majumdar et al., 2014). Herein, we carried out this reaction between prepared acid

hydrazide **4.3** and phenyl isocyanate at ambient temperature utilizing three different solvents that is toluene, dichloromethane (DCM) and ethanol separately. Interestingly, this reaction was favored by all these three solvents and the reaction products started to form almost instantly in toluene but in the case of DCM and ethanol it took around 10 and 20 minutes respectively the products solid to be visible. So, most of the reaction was accomplished in toluene but few aryl isocyanates were insoluble in this media and in this case, the reaction was executed utilizing DCM. The results of the reaction were listed in Table **4.1**. The reaction completion period was observed very short (2-5 min) for those substituted semicarbazides where phenyl isocyanate **4.4a**, 4-fluorophenyl isocyanate **4.4b**, 2,4-dichlorophenyl isocyanate **4.4c**, m-tolyl isocyanate **4.4d**, and naphthyl isocyanate **4g** were used, but in the case of 4-acetylphenyl isocyanate **4.4e** and 3-(ethoxycarbonyl)phenyl isocyanate **4.4f,** the reaction completion time was noticeably high (5 hours). This is possibly owing to the nature of the substituents in benzene ring of aryl isocyanates which determines the reactivity of the aryl isocyanates.

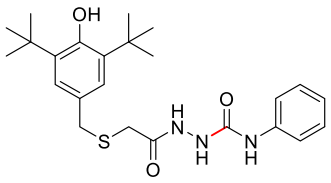
Table 4.1: Evaluation of the reaction of substituted semicarbazides formation. [a-c]



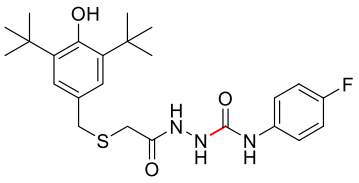
4.3
Acid Hydrazide

4.4a-g

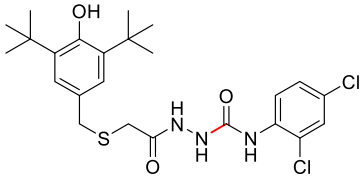
4.5a-g



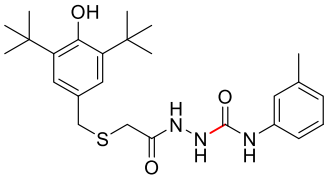
4.5a
2 min, 99% [d]



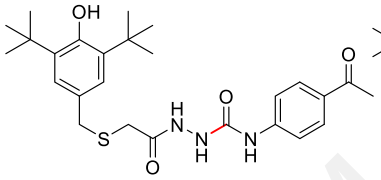
4.5b
2 min, 98% [d]



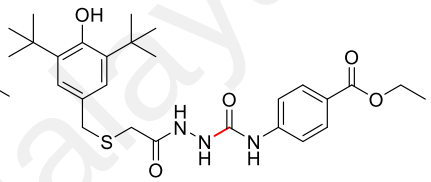
4.5c
2 min, 98% [e]



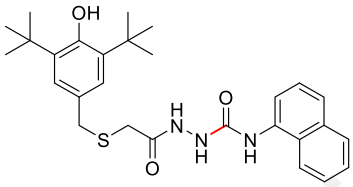
4.5d
5 min, 95% [d]



4.5e
5 hours, 90% [e]



4.5f
5 hours, 90% [d]



4.5g
5 min, 92% [d]

[a] Reaction condition: 3 (3.12 mmol), 4.4a-g (3.12 mmol), room temperature. [b] reaction completion time. [c] isolated yields. [d] toluene was used as solvent. [e] dichloromethane was used as solvent.

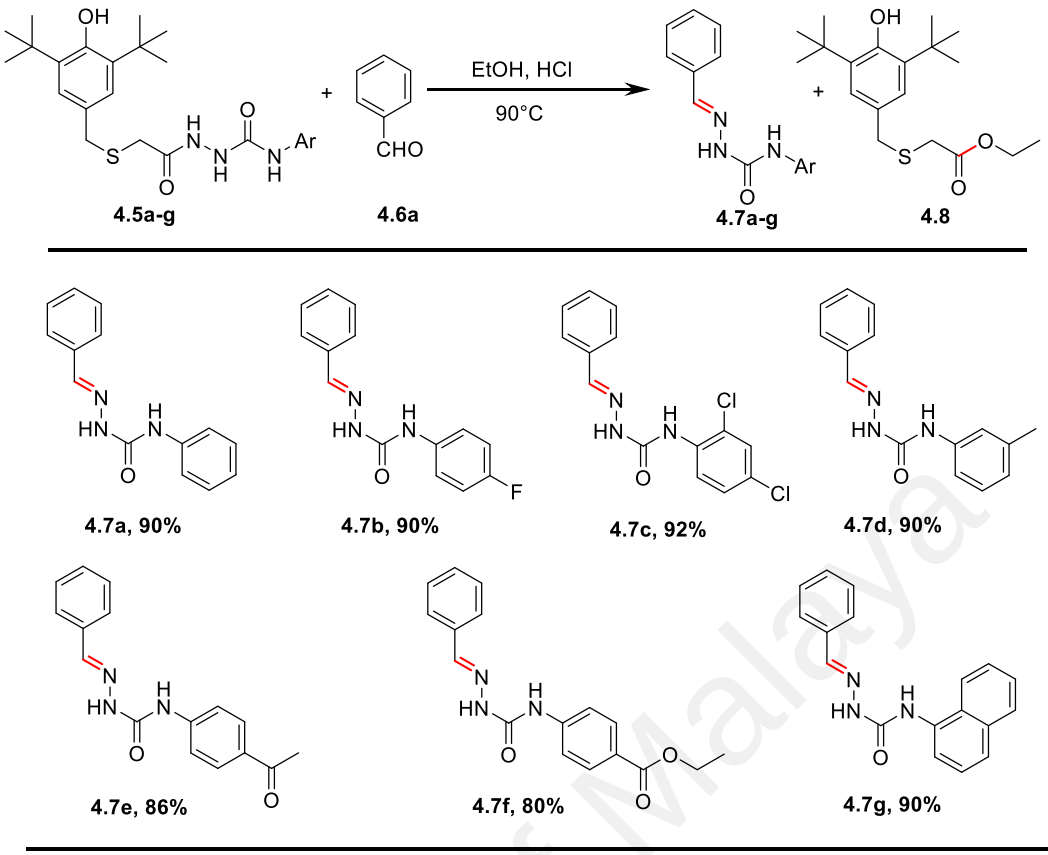
It was observed that the reaction time was markedly short in presence of weakly electron withdrawing body such as –F, Cl (**4.4b, 4.4c**) or even absence of any substituents (**4.4a** and **4g**) in benzene ring or naphthyl ring of the aryl isocyanate. Likewise, for the presence of moderately benzene ring deactivating groups such as $–COCH_3$ and $–COOC_2H_5$, the reaction took approximately 5 hours to complete.

This is probably attributed to the resonance effect of benzene ring bearing acyl or ester group. For further investigation m-tolyl isocyanate was utilized to this reaction to examine

the effect of ring activating group. Interestingly, this reaction also completed in short time (2-5 min). However, the product yields of all the substituted semicarbazide (≥ 90 %) was highly promoted.

After **4.5a-g** compounds in hand, we performed the reaction of substituted semicarbazides and benzaldehyde according to the Table **4.2**. Best turnover was found at around 90°C in the presence of catalytic amount of HCl in solvent ethanol. Physical properties of the synthesized semicarbazones (**4.7a-4.7g)** were outlined in Table **4.2**. According to Table **4.2**, N^1-benzylidene-N^4-phenyl-semicarbazone (**4.7a**) was segregated with 90% yield along with (3,5-Di-tert-butyl-4-hydroxy-benzylsulfanyl)-acetic acid ethyl ester (**4.8)** by this method while Asis *et al.* got 32% yield of this compound (**4.7a**) by their reported method (Asís et al., 1999). The semicarbazones bearing 4-fluoro (**4.7b**); 2,4-di-chloro (**4.7c**) and 3-methyl (**4.7d**) substitution of benzene ring attached with N-4 provided about 90% more yields. Enchantingly, semicarbazones with N^4-(3-ethoxycarbonylphenyl) (**4.7f**) and N^4-(4-acetylphenyl) (**4.7e**) moiety were also synthesized with more than 80% yields.

Table 4.2: Evaluation of N^1-benzylediene-N^4- (substituted phenyl) semicarbazone. [a-b]



[a] Reaction condition: 4.5a-g (0.65 mmol), benzaldehyde 4.6a (0.65 mmol), HCl (1.6 mmol), Ethanol (10 ml), 90°C. [b] isolated yields.

In case of entry **4.7g**, N^1-benzylidene-N^4-naphthalene-semicarbazone was isolated with 90% yields by this reporting method though Asis *et al.* (Asís et al., 1999) reported 20% yields of this product and Azam *et al.* obtained 79% yields by utilizing the broadly reported method (Azam et al., 2010). In the previously reported procedure N^4-phenyl ring of semicarbazones relies upon the selective phenyl amine which was taken as starting materials in the initial step. For instance, starting with ester substituted phenyl amine to get N^4-(alkoxycarbonylphenyl) semicarbazones was extremely troublesome due to the high reactivity of hydrazine towards the ester moiety in the second step (Saravanan et al., 2006).

To examine versatility of this revealing method three different benzaldehyde having electron donating and withdrawing groups were used in this research. Herein, we carried out this reaction with m-anisaldehyde, 4-fluorobenzaldehyde, cuminaldehyde to prepare

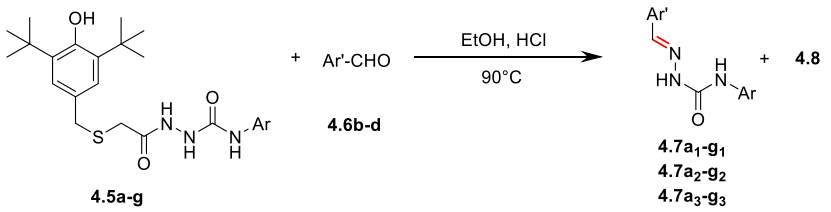
semicarbazones containing different substitution of N^1-benzylidene ring in the same reaction condition and the results were outlined in Table **4.3**.

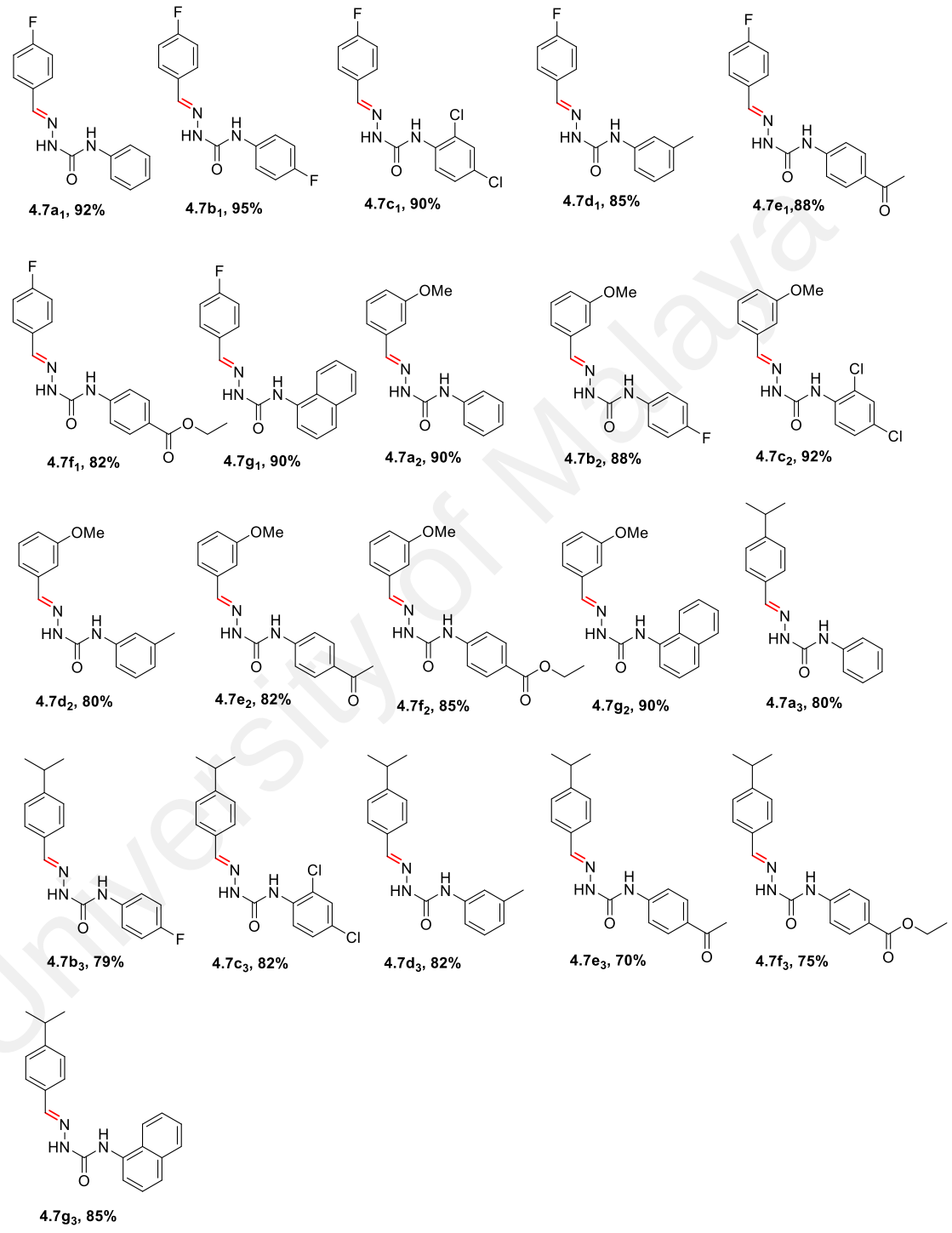
At to begin with, we endeavored to prepare the semicarbazones with N^1-(4-fluorobenzylidene)-ring. Gratifyingly, 4-fluorobenzaldehyde brought expected semicarbazones with better yields reacting with all substituted semicarbazides (**4.5a-g**). Phenyl (**4.7a$_1$**), 4-fluorophenyl (**4.7b$_1$**) and 2,4-dichloro-phenyl (**4.7c$_1$**) semicarbazone with N^1-(4-fluorobenzylidene)-ring were obtained with 92%, 95%, and 90% yields respectively.

Though N^1-(4-fluorobenzylidene)-N^4-naphthyl-semicarbazone (**4.7g$_1$**) was isolated with 80% product yields by earlier reported method (Azam et al., 2010), interestingly yields increased to 90% of this compound (**4.7g$_1$**) by the reaction of 2-(2-(3,5-di-tert-butyl-4-hydroxybenzylthio)acetyl)-N-naphthyhydrazinecarboamide (**4.5g**) with 4-fluorobenzaldehyde.

Then we assembled the semicarbazones containing N^1-(3-methoxybenzylidene)-ring by treating 3-methoxybenzaldehyde with pre-prepared substituted semicarbazides (**4.5a-g**). Phenyl (**4.7a$_2$**); 4-fluoro (**4.7b$_2$**); 2,4-di-chloro (**4.7c$_2$**); and naphthyl (**4.7g$_2$**) semicarbazones were accessed with around 90% product yields and for the rest of that (**4.7d$_2$-4.7f$_2$**) turnover were found more than 80%. As noticed in Table **4.3** product yields of the compounds **4.7e$_3$, 4.7f$_3$** bearing N^1-(4-(2-isopropyl)-benzylidene)-ring was around 70% more where phenyl (**4.7a$_3$**), 4-fluorophenyl (**4.7b$_3$**), 2,4-dichloro (**4.7c$_3$**), m-tolyl (**4.7d$_3$**) and naphthyl (**4.7g$_3$**) semicarbazones were discovered with 80% a greater amount.

Table 4.3: Evaluation of semicarbazones from three different benzaldehydes. [a-b]

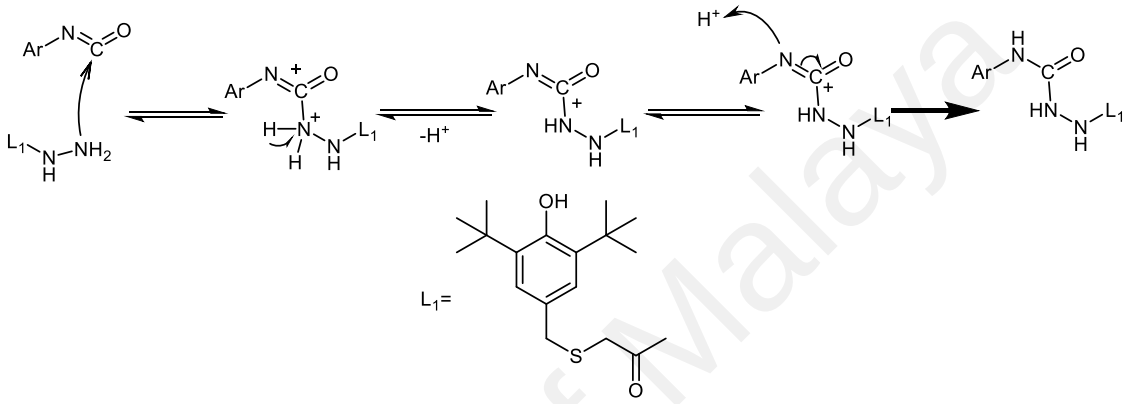




[a] Reaction condition: 5a-g (0.65 mmol), 6b-e (0.65 mmol), HCl (1.6 mmol), Ethanol (10 ml), 90°C. [b] isolated yields. 4.7a1-g1 reaction performed with 4-fluorobenzaldehyde.

4.7a$_2$-g$_2$ reaction performed with m-anisaldehyde. 4.7a$_3$-g$_3$ reaction performed with cuminaldehyde.

The formation of substituted semicarbazide from acid hydrazide and aryl isocyanate by nucleophilic addition mechanism is shown in Scheme **4.3**. Hydrazine moiety of acid hydrazide **4.3** might attack at the carbonyl carbon of isocyanate forming an intermediate having two positive charges.

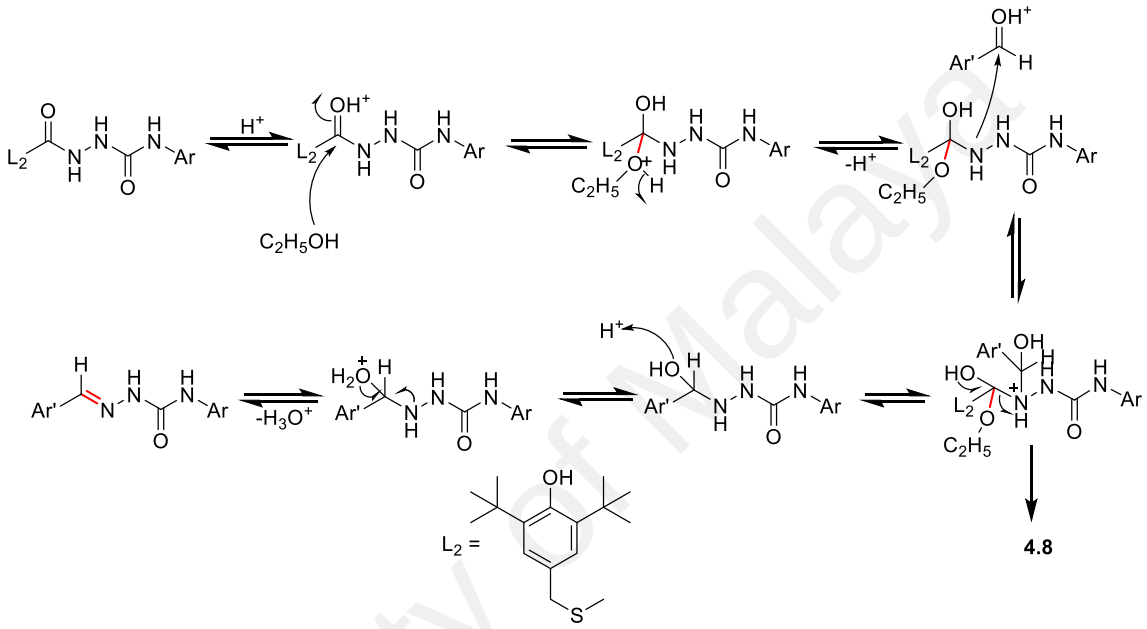


Scheme 4.3: Proposed mechanism for the formation of substituted semicarbazide.

In the subsequent step positively charged nitrogen of hydrazine part released a proton to become stable ion and on the other hand, the nitrogen of aryl isocyanate moiety might be attracted by this roaming proton and formed N-H bond. It could be anticipated from the different reaction completion period that the resonance of aromatic ring of aryl isocyanate bearing different functional group affects the action of nitrogen. Thus, formation of compounds **4.5e** and **4.5f** took longer time than the other substituted semicarbazides.

Since (3,5-di-tert-butyl-4-hydroxy-benzylsulfanyl)-acetic acid ethyl ester (**4.8**) was found as reaction product along with semicarbazones in every reaction, indicates that solvent ethanol participated into the reaction as a substrate as well and this product **4.8** could be used in the preparation of acid hydrazide **4.3** according to the Scheme **4.4**. Therefore, taking this into account a plausible mechanism was drawn for the formation

of semicarbazones in the Scheme 4.4. Protonation and nucleophilic attack of ethanol might be occurred at the same carbonyl carbon of substituted semicarbazides then the neighboring nitrogen might attack the protonated aldehyde resulting the intermediate (**i**). This intermediate (**i**) released the compounds **4.8** and again protonated. At the last step, it formed semicarbazone by giving up hydronium ion.



Scheme 4.4: Proposed mechanism for the semicarbazone formation via substitution reaction.

In widely semicarbazones preparation (Scheme **4.1**) begins with reaction of aniline with the urea in the presence of pyridine or sodium cyanate in glacial acetic acid resulted in the formation of carbamate compound, which is then reacted with hydrazine to give semicarbazide. Condensation of semicarbazide with aromatic aldehyde in the presence of glacial acetic acid provides semicarbazone. Although this method is more popular in the synthesis of semicarbazones, unfortunately the use of aniline and terminal amino group in carbamate greatly limits its ability to prepare diverse semicarbazones. For instant, some certain carbamates bearing ester or acetyl group are very difficult to synthesize because urea or cyanate molecule are more susceptible to react with other reactive functional

group instead of amino group which leads the unwanted product instead of carbamate.(Asís et al., 1999) In addition, formation of semicarbazide in the second step by the reaction of carbamate bearing ester or acetyl group with hydrazine is more problematic because hydrazine is very reactive to carbonyl or ester group. In second method shown in Scheme **4.1**, aromatic aldehyde mono hydrazone, a key molecule in the preparation of semicarbazone obtained from the reaction of aldehyde with hydrazine, which also experiences the similar problems due to the unwanted reactions between hydrazine molecule and other functional groups. Furthermore, the reaction of hydrazone with isocyanate is not a functional group compatible. These above limitations are due to the presence of the terminal amino group which makes both methods limited to offering a wide variety of semicarbazones. Interestingly, the problem has been solved in this work by protecting the terminal hydrazine moiety of acid hydrazide with aryl isocyanates, which led to have the substituted semicarbazides bearing several active functional groups. Then the treatment of substituted semicarbazides with aldehyde in acidic ethanol directed to a substitution reaction which results are the semicarbazones and (3,5-di-tert-butyl-4-hydroxy-benzylsulfanyl)-acetic acid ethyl ester (**4.8**) with greater yields than the existing methods.

4.4 Conclusion

In conclusion, a variety of substituted semicarbazides were accessed easily by a straightforward reaction of acid hydrazide with aryl isocyanate bearing different substituents in order to protect free hydrazine moiety of acid hydrazide. A nucleophilic substitution reaction of substituted semicarbazides with diverse substituted benzaldehyde has been established by which an extensive assortment of semicarbazone compounds were synthesized with notable yield. Since hydrazine moiety is not free in the substituted semicarbazides it can easily be converted into diverse complex semicarbazone

compounds bearing different reactive functional group such as ester and acetyl group. Again, the obtained by-product **4.8** proved the involvement of ethanol into reaction. Furthermore, this by-product **4.8** could also be used in the preparation of acid hydrazide which would be another interesting aspect of this method. therefore, this reaction protocol pointedly offers a simple and robust alternative for the preparation of diverse semicarbazone derivatives resolving the limitations of conventional methods. This reaction can also help to get distinct substitution derivatives of semicarbazides forming more carbon-nitrogen bond which are under development.

CHAPTER 5: SYNTHESIS AND ANTIOXIDANT ACTIVITY EVALUATION OF BUTYLATED HYDROXYPHENYL INCORPORATED SEMICARBAZONES

5.1 Introduction

Oxidative stress is one of the main causes of etiology and expansion of major human deteriorating disease. The imbalance of production of free radical (reactive oxygen species) and the ability of the body to counteract the free radical causes oxidative stress (Rahman et al., 2012). So excessive free radicals exert toxic effects throughout the body especially responsible for cancer and other chronic diseases (Lobo et al., 2010; Pham-Huy et al., 2008). Apart from the oxidative stress in the human body, free radicals cause auto-oxidation and premature oxidation in industrial products such as oils, rubbers, food, foodstuffs and so on (Aguilar et al., 2010; Hovorka & Schöneich, 2001; Shelton, 1959). Premature oxidation is one of a major obstacle to developing the oxidatively and thermally stable synthetic lubricant where eco-friendly synthetic lubricants become a necessity of the modern world.

So, development of eco-friendly synthetic lubricant from sustainable resources has drawn a great interest for the last few years to replace the mineral lubricants because of the risen environmental concerns and preventive protocols (Alias et al., 2009; Erhan & Asadauskas, 2000; Wu et al., 2013). Because reliance on fossil-based resources is no longer pragmatic in perspective of feasible, eco-friendly and financial purposes and in addition, the reserve of fossil-based resources is notably diminishing with time. As a result, Synthetic lubricant oils or vegetable oils have gained a great consideration because it derived from cheaper renewable resources and their better lubricant properties than the mineral oils (Nath et al., 2018). Unfortunately, due to the lower oxidative and thermal stability, it has failed to get wide acceptance. This is because the production of free radical

in lubricant oil causes premature oxidative degradation that is responsible for product damage, discoloration and reduced lifetime.

The pre-mature oxidation or auto-oxidation process and excess free radical production in vivo or in vitro can be stopped or delayed by introducing antioxidant into the substrates. Antioxidants can inhibit the different stages of the oxidation process in industrial products by their different mood of actions or mechanisms such as scavenging free radicals or decomposing peroxides radicals and deactivating metal ions. Thus, these are divided into three main groups based on their mode of action: free radical scavenger or primary antioxidant, the other peroxide decomposer or secondary antioxidant and metal chelator or deactivator (Koleva et al., 2002). For the past few years, researchers have been trying to develop efficient multipotent or multifunctional antioxidant by assembling two or more antioxidant functions in one structure (Nath & Yehye, 2018; Nath et al., 2018). Because high efficient multifunctional antioxidants would be an effective solution to overcome the problems caused by free radical such as oxidative stress in the human body and low oxidative stability of industrial product such as synthetic lubricant oil.

Butylated hydroxytoluene (BHT) is one of the widely used antioxidant and semicarbazones are extensively reported pharmacophore which also possesses excellent antioxidant properties. BHT is well known synthetic primary antioxidant. It has been extensively used in the food industry, pharmaceuticals, rubber and lubricant due to its excellent free radical scavenging capacity (Yehye et al., 2015). It is identified as safe for use in food in low concentration as a food preservative. Thus, it has been utilized in the food industry, food packaging, fish products and others (Stuckey, 1972). BHT is also broadly utilized in combination with other antioxidants such as butylated hydroxyanisole (BHA), propyl gallate and citric acid for the stabilization of oils and high-fat foods

(Mukhopadhyay, 2006). BHT also exhibited good synergism results with other antioxidants in the application of rubber and lubricant oil.

Semicarbazones, are versatile pharmacophore agents, have earned interest in medicinal and pharmaceutical fields due to broad-spectrum biological activities such as anticonvulsant, antitumor, and anticancer properties (Liu et al., 2014; Pandeya, 2012; Yogeeswari et al., 2004; Yogeeswari et al., 2005; Yogeeswari et al., 2005a; Yogeeswari et al., 2004a). Since semicarbazones are also known as tridentate or neutral ligand since they contain multiple donor atoms in their structure. Their metal complexes also exhibit significant activity against bacteria, fungi and viruses which extends the versatility of the application (Pelosi et al., 2010). Moreover, substituted semicarbazones can be used as a platform chemical to prepare benzotriazepin compounds which are also an important class of drug due to their psychostimulant, antidepressant, anorexigenic and antihypertensive properties (Douchez & Lubell, 2015). Some researcher also revealed their antioxidant activity as they have two secondary amino in its structure (Jafri et al., 2012; Singhal et al., 2011). Sabari and coworkers reported some new semicarbazone derivative of curcumin for its antioxidant and antimicrobial properties (Dutta et al., 2005). It is believed that favorable substitution of semicarbazones would affect significantly its free radical scavenging ability. Example like, Chalconesemicarbazones, a pharmacophore model, were found good antioxidant activity (Singhal et al., 2014).

Comparing to mono-functional antioxidants such as hindered phenol and aromatic amine, inhibiting action of multifunctional antioxidants is more complicated and effective because their structures contain multiple antioxidant functions which involved in the termination of free radical chain reaction through different mechanisms (Denisov & Denisova, 2013; Varatharajan & Pushparani, 2017). Multifunctional or multipotent antioxidants have been used widely into pharmaceuticals, rubber, and petroleum because

of their efficacy in terminating free radicals. This type of antioxidants has drawn a tremendous attention for the treatment of several diseases (Zhang et al., 2006). Example like, thiazolidinone derivatives incorporated with butylated hydroxyphenyl (BHP) exhibit antioxidant activity along with Ca^{2+} overload inhibitor and Ca^{2+} channel blocker (Kato et al., 1999).

Therefore, discovering effective multipotent antioxidants is now paramount need to effectively counteract the free radicals to reduce the risk of oxidative stress as well as to give better oxidative stability to synthetic lubricant oil. Herein, we have envisioned to make multipotent semicarbazone incorporated with two types of butylated hydroxyphenyl (BHP) as an efficient antioxidant. The purpose of this study is to develop a group of efficient multipotent semicarbazones which can exhibit excellent antioxidant activity and to study the role of the ortho and para hydroxyl group of butylated hydroxyphenyl incorporated semicarbazones on the antioxidant activity. Antioxidant properties of these synthesized compounds were investigated by DPPH assay and effects of different substituents on the antioxidant activity were also studied. Furthermore, some synthesized compounds were chosen based on easy solubility in trimethylolpropane trioleate as synthetic ester based-lubricant for the evaluation of oxidative stability by differential scanning calorimeter (DSC) and compared with commercial antioxidant BHT.

5.2 Materials and Methods

General remarks. All chemicals and reagents were of analytical grade and supplied by Sigma-Aldrich and Merck, Malaysia and used without further purification. Double-distilled water was used throughout the experiment. Pre-coated silica gel plates (0.25 mm) was utilized for analytical thin layer chromatography to monitor the reaction and imagined by UV light. Column chromatography was done on Silica Gel 60 (particle size:

0.040–0.063mm). IR spectra were recorded using FTIR-ATR of the solid samples. Melting points were approximated. 600 MHz and 150 MHz NMR spectrometer were used for ^{1}H NMR and ^{13}C NMR respectively and Tetramethylsilane was used as a reference. Chemical shifts (δ) were measured for in ppm with respect to the residual solvent peak ($CHCl_3$, δ = 7.26; DMSO-d_6, δ = 2.51 for proton spectra; $^{13}CDCl_3$, δ = 77.0; DMSO-d_6, δ = 40 for carbon spectra). High-resolution mass spectra were performed on a time-of-flight Q-TOF LCMS system.

5.2.1 Synthesis of 2-((3,5-di-*tert*-butyl-4-hydroxybenzyl) thio) acetohydrazide (5.3) and substituted semicarbazides 5.5a-j

2-((3,5-di-tert-butyl-4-hydroxybenzyl)thio)acetohydrazide was synthesized according to a previously described method (Ariffin et al., 2014). Acid hydrazide (1 g, 3.12 mmol) was stirred in toluene (20 mL) then an equimolar amount of aryl isocyanate was introduced, and the reaction was monitored by TLC. All the substituted semicarbazides formed white precipitates during the reaction, which were filtered and washed several times with toluene. Dichloromethane (CH_2Cl_2) was used for compounds **5.5c**, **5.5d** and **5.5i** since 2,4-dichlorophhenylisocyanate, 4-cyanophenylisocyanate and 4-acylphenylisocyanate are not soluble in toluene. Compound structures were confirmed by IR, ^{13}C NMR, ^{1}H NMR and HRMS analyses. We have already reported the following synthesized substituted semicarbazides **5.5a-5.5c, 5.5f, 5.5h, 5.5i,** and **5.5j** with characterization data in the literature (Nath & Yehye, 2018a). Herein, compounds **5.5d**, **5.5e**, **5.5g** and **5.5i** have been reported with characterization data. All the synthesized compounds were solid and stored at ambient temperature.

2-(2-(3,5-Di-*tert*-butyl-4-hydroxybenzylthio)acetyl)-N-(4-cyanophenyl)hydrazinecarboxamide (**5.5d**): Yield: 1.39 g (95%); white solid; mp 145-146°C; ^{1}H NMR (600 MHz, DMSO-d6): ***δ*** 9.89 (s, 1H), 9.27 (s, 1H), 8.43 (s, 1H), 7.72

(d, J=6 Hz, 2H), 7.67 (d, J=12 Hz, 2H), 7.07 (s, 2H), 6.88 (s, 1H), 3.78 (s, 2H), 3.14 (s, 2H), 1.38 (s, 18H). 13C NMR (150 MHz, DMSO-d6): ***δ*** 169.5, 155.2, 153.3, 144.7, 139.6, 133.6, 128.9, 125.7, 119.75, 118.7, 103.9, 36.5, 34.9, 32.9, 30.8. FT-IR (cm^{-1}, ATR) 3589, 3337, 2956, 2234, 1601. HRMS (Q-TOF) m/z: [M+H]+ Calcd for $C_{25}H_{32}O_3N_4S^+$ 468.2273; Found 468.2286.

2-(2-(3,5-Di-*tert*-butyl-4-hydroxybenzylthio)acetyl)-N-(3-methoxyphenyl)hydrazinecarboxamide **(5.5e):** Yield: 1.40 g (95%); white solid; mp 136-137°C; ^{1}H NMR (600 MHz, DMSO-d6): ***δ*** 9.82 (s, 1H), 8.72 (s, 1H), 8.12 (s, 1H), 7.16 (d, J=6 Hz, 2H), 7.08 (s, 2H), 6.99 (d, J=6 Hz, 1H), 6.98 (s, 1H), 6.55(d, J=6 Hz, 1H), 3.79 (s, 2H), 3.71 (s, 3H), 3.13 (s, 2H), 1.38 (s, 18H). 13C NMR (150 MHz, DMSO-d6): ***δ*** 169.5, 160.1, 155.6, 153.3, 141.3, 139.6, 129.9, 129.4, 128.9, 128.7, 125.7, 111.2, 107.8, 104.7, 55.4, 36.6, 34.9, 32.9, 30.8. FT-IR (cm^{-1}, ATR) 3624, 3288, 2911, 1683. HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{25}H_{36}N_3O_4S^+$ 473.2348; Found 473.2391.

2-(2-(3,5-Di-*tert*-butyl-4-hydroxybenzylthio)acetyl)-N-(4-sec-butylphenyl)hydrazinecarboxamide *(***5.5g**): Yield: 1.4 g (90%); white solid; mp 152-154°C; ^{1}H NMR (600 MHz, DMSO-d6): ***δ*** 9.80 (s, 1H), 8.61 (s, 1H), 8.07 (s, 1H), 7.35 (d, J=6 Hz, 2H), 7.10 (d, J=6 Hz, 2H), 7.08 (s, 2H), 6.88 (s, 1H), 3.78 (s, 2H), 3.12 (s, 2H), 2.54 (m, J=6 Hz, 1H), 1.54 (m, J=6 Hz, 2H), 1.37 (s, 18H), 1.16 (d, J=6 Hz, 3H), .76 (t, J=6 Hz, 3H). ^{13}C NMR (150 MHz, DMSO-d6): ***δ*** 169.5, 155.9, 153.3, 139.6, 137.8, 129.4, 128.9, 128.7, 127.5, 125.7, 119.1, 118.8, 36.6, 34.9, 32.9, 30.8, 22.3, 12.5. FT-IR (cm^{-1}, ATR) 3640, 3225, 2964, 2870, 1706, 1606; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{28}H_{42}N_3O_3S^+$ 500.2947; Found 500.2995.

2-(2-(3,5-Di-t*ert*-butyl-4-hydroxybenzylthio)acetyl)-N-(4-ethoxycarbonylphenyl) hydrazinecarboxamide (**5.5i**): Yield: 1.5 g (90%); white solid; mp 228-229°C; 1H NMR (600 MHz, DMSO-d6): ***δ*** 9.88 (s, 1H), 9.16 (s, 1H), 8.33 (s, 1H), 7.87 (d, J=9 Hz, 2H),

7.61 (d, J=8.4 Hz, 2H), 7.10 (s, 2H), 6.89(s, 1H), 4.28 (q, J=7.2 Hz, 2H), 3.78 (s, 2H), 3.13 (s, 2H), 1.37 (s, 18H), 1.31 (t, J=6.6 Hz, 3H). 13C NMR (150 MHz, DMSO-d6): ***δ*** 169.5, 165.9, 153.3, 144.7, 139.6, 130.7, 128.9, 125.7, 123.3, 118.0, 60.8, 36.5, 34.9, 32.9, 30.8, 14.7; FT-IR (cm-1, ATR) 3640, 3225, 2964, 2870, 1706, 1606; HRMS (Q-TOF) m/z: [M+Na]+ Calcd for C27H37N3O5NaS+ 538.2362; Found 538.2352.

5.2.2 General procedure for the synthesis of semicarbazones

0.65 mmol of substituted semicarbazides and an equimolar quantity of aldehyde were taken into round bottle flask with approximately 10 ml of ethanol. Then the mixture was stirred at 90°C for 4-5 hours in the presence of catalytic amount of HCl (1.6 mmol) until the disappearance of the starting materials spot in the thin layer chromatography. Afterward, the reaction mixture was allowed to warm to ambient temperature and stirred for around half an hour. The expected semicarbazones started the formation of solid precipitate during ambient temperature stirring in some of the reactions. The precipitate was filtered and washed with hexane for several times. Then the resultant products were dried and either recrystallized from hexane or column chromatography. However, the reaction in which no precipitates were found, the reaction mixture was concentrated and purified by column chromatography on silica gel with hexane/EtOAc mixture as the eluent.

2-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-N-phenylhydrazine-1-carboxamide **(5.7a)**: compounds 7a was obtained as white solid product. Yields: 92% (219.76 mg); mp. 178-180°C; ^{1}H NMR (600 MHz, DMSO-d_6): ***δ*** 10.48 (s, 1H), 8.77 (s, 1H), 7.89 (s, 1H), 7.62 (d, 2H, *J*=7.8 Hz), 7.48 (s, 2H), 7.29 (t, 2H, *J*= 7.8 Hz), 7.01 (t, 1H, *J*=7.2), 1.43 (s, 18H); ^{13}C NMR (150 MHz, DMSO-d_6): ***δ*** 156.0, 153.4, 142.9, 139.6, 129.0, 126.1, 124.1, 122.8, 119.9, 35.0, 30.7; FTIR (cm^{-1}, ATR): 3631, 3386, 3192, 3116, 2964, 2879, 1679; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{22}H_{30}N_3O_2^+$ 268.23; Found 268.2329.

2-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-N-(4-fluorophenyl)hydrazine-1-carboxamide **(5.7b):** obtained as white solid. Yields: 94% (235.37 mg); mp. 184-185°C; ^{1}H NMR (600 MHz, DMSO-d_6): ***δ*** 10.49(s, 1H), 8.87 (s, 1H), 7.88 (s, 1H), 7.64 (dd, 2H, *J*=4.8 Hz), 7.48 (s, 2H), 7.29 (s, 1H), 7.13 (t, 2H, *J*=9 Hz), 1.42 (s, 18H); ^{13}C NMR (150 MHz, DMSO-d_6): ***δ*** 157.4, 156, 153.7, 142.9, 139.6, 136.1, 126.1, 124.2, 121.9, 115.5, 35.0, 30.8; FTIR (cm^{-1}, ATR): 3337, 3331, 2964, 2879, 1712, 1658; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{22}H_{29}FN_3O_2^+$ 386.22; Found 386.2251.

2-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-N-(2,4-dichlorophenyl)hydrazine-1-carboxamide **(5.7c):** obtained as white solid. Yields: 90% (254.7); mp. 231-232°C; ^{1}H NMR (600 MHz, DMSO-d_6): ***δ*** 11.1(s, 1H), 9.1 (s, 1H), 8.4 (d, 1H, *J*= 9 Hz), 7.97 (s, 1H), 7.75 (d, 2H, *J*=2.4 Hz), 7.54 (s, 2H), 7.48 (dd, 1H, *J*=2.4 Hz), 7.46 (s, 1H), 1.46 (s, 18H); ^{13}C NMR (150 MHz, DMSO-d_6): ***δ*** 156.4, 152.6, 143.3, 139.8, 135.1, 128.9, 128.6, 126.5, 124.7, 123.8, 122.4, 120.6, 35.0, 30.6; FTIR (cm^{-1}, ATR): 3637, 3192, 2964, 2873, 1694; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{22}H_{28}Cl_2N_3O_2^+$ 436.16; Found 436.1556.

N-(4-cyanophenyl)-2-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)hydrazine-1-carboxamide **(5.7d):** obtained as white solid; Yields: 90% (229.61 mg); mp. 216-217°C; ^{1}H NMR (600 MHz, $CDCl_3$): ***δ*** 8.32 (s, 1H), 8.29 (s, 1H), 7.66 (s, 1H), 7.60 (d, 2H, *J* = 9 Hz), 7.56 (d, 2H, *J*= 9 Hz), 7.40 (s, 2H), 5.47 (s, 1H), 1.42 (s, 18H); ^{13}C NMR (150 MHz, $CDCl_3$): ***δ*** 156.3, 152.5, 143.8, 142.3, 136.6, 133.4, 124.4, 118.9, 106.11, 34., 30; FTIR (cm^{-1}, ATR): 3610, 3198, 3104, 2976, 2216, 1697; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{23}H_{28}N_4O_2Na^+$ 415.2110; Found 415.2112.

2-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-N-(3-methoxyphenyl)hydrazine-1-carboxamide **(5.7e):** obtained as white solid. Yields: 95% (245.3 mg); mp. 203-204°C; ^{1}H NMR (600 MHz, DMSO-d_6): ***δ*** 10.48(s, 1H), 8.76 (s, 1H), 7.89 (d, 1H, *J*= 9 Hz), 7.48 (s, 1H), 7.33 (d, 2H, *J*=2.4 Hz), 7.29 (s, 2H), 7.19 (t, 1H, *J*=8.4 Hz), 7.15 (d, 1H, *J*=8.4

Hz), 6.59(d, 1H, *J*=6.6 Hz), 3.74 (s, 1H) 1.43 (s, 18H); ^{13}C NMR (150 MHz, DMSO-d_6): ***δ*** 160.0, 156.1, 153.4, 142.9, 140.9, 139.6, 129.8, 126.1, 124.1, 112.6, 108.1, 105.7, 55.4, 35.0, 30.7; FTIR (cm^{-1}, ATR): 3640, 3431, 3234, 2955, 1676; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{23}H_{32}N_3O_3^+$ 398.21; Found 398.2449.

2-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-N-(m-tolyl)hydrazine-1-carboxamide **(5.7f)**: obtained as white solid. Yields: 90% (223.19 mg); mp. 212-213°C; ^{1}H NMR (600 MHz, DMSO-d_6): ***δ*** 10.49(s, 1H), 8.70 (s, 1H), 7.87 (s, 1H), 7.68 (s, 1H), 7.48 (s, 2H), 7.35 (d, 1H, *J* = 7.2 Hz), 7.31 (s, 1H), 7.18 (t, 1H, *J* = 8.4 Hz), 6.83 (d, 1H, *J* = 7.2 Hz), 2.29 (s, 3H) 1.42 (s, 18H); ^{13}C NMR (150 MHz, DMSO-d_6): ***δ*** 160.4, 156.0, 153.5, 142.8, 139.6, 138.1, 128.9, 126.1, 124.1, 123.5, 120.4, 117.2, 35.0, 30.6, 21.7; FTIR (cm^{-1}, ATR): 3283, 3228, 2986, 2670, 1688, 1618; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{23}H_{32}N_3O_2^+$ 382.25; Found 382.2492.

N-(4-(*sec*-butyl)phenyl)-2-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)hydrazine-1-carboxamide **(5.7g):** obtained as white solid. Yields: 87% (239.37 mg); mp. 150-151°C; ^{1}H NMR (600 MHz, DMSO-d_6): ***δ*** 10.44(s, 1H), 8.68 (s, 1H), 7.88 (s, 1H), 7.69 (s, 1H), 7.50(d, 2H, J= 9 Hz), 7.47 (s, 2H), 7.29 (s, 2H), 7.12 (d, 2H, *J*=8.4 Hz), 2.54 (m, 1H, *J*= 7.2 Hz) 1.53 (m, 2H, J= 7.2 Hz) 1.42 (s, 18H), 1.18 (d, 3H, *J*= 6 Hz), 0.77 (t, 3H, *J*= 7.2 Hz); ^{13}C NMR (150 MHz, DMSO-d_6): ***δ*** 160.6, 156.0, 153.6, 141.6, 139.6, 137.3, 128.7, 127.3, 126.1, 124.1, 120.3, 35.0, 31.1, 30.7, 30.4, 22.4, 12.6; FTIR (cm^{-1}, ATR): 3628, 3107, 2958, 2876, 1688; HRMS (Q-TOF) m/z: $[M+Na]^+$ Calcd for $C_{26}H_{37}N_3NaO_2^+$ 446.28; Found 446.2785.

N-(4-acetylphenyl)-2-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)hydrazine-1-carboxamide **(5.7h)**: obtained as white solid. Yields: 88% (234.25); mp. 180-182°C; ^{1}H NMR (600 MHz, DMSO-d_6): ***δ*** 10.49(s, 1H), 8.87 (s, 1H), 7.88 (s, 1H), 7.64 (dd, 2H, *J*=6 Hz), 7.48 (s, 2H), 7.29 (s, 1H), 7.14 (d, 2H, *J*=6 Hz), 2.54 (s, 3H),1.42 (s, 18H); ^{13}C

NMR (150 MHz, DMSO-d_6): $\boldsymbol{\delta}$ 157.4, 156, 153.7, 142.1, 139.6, 136.1, 126.1, 125.7, 121.9, 115.5, 35.0, 30.8, 29.9; FTIR (cm^{-1}, ATR): 2982, 2882, 2685, 1724, 1670; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{24}H_{32}N_3O_3^+$ 410.2444; Found 410.2451

ethyl-4-(2-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)hydrazine-1-carboxamido)benzoate **(5.7i)**: obtained as white solid. Yields: 85% (242.86 mg); mp. 235-236°C; ^{1}H NMR (600 MHz, $CDCl_3$): $\boldsymbol{\delta}$ 8.39 (s, 1H), 8.29 (s, 1H), 7.98 (d, 2H, *J*= 8.4 Hz), 7.67 (s, 1H), 7.55 (d, 2H, *J*= 9 Hz), 7.41 (s, 2H), 5.45 (s, 1H), 4.31 (q, 2H, *J*= 7.2 Hz), 1.42 (s, 18H), 1.33 (t, 3H, *J*= Hz); ^{13}C NMR (150 MHz, $CDCl_3$): $\boldsymbol{\delta}$ 166.3, 156.1, 152.7, 143.3, 142.3, 136.6, 130.9, 125.0, 124.2, 118.2, 60.8, 34.4, 30.2, 14.4; FTIR (cm^{-1}, ATR): 3431, 3234, 3170, 2955, 2867, 1707, 1676; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{25}H_{34}N_3O_4^+$ 440.25; Found 440.2555.

2-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-N-(naphthalen-2-yl)hydrazine-1-carboxamide **(5.7j)**: obtained as white solid. Yields: 92% (249.7 mg); mp. 220-221°C; ^{1}H NMR (600 MHz, $CDCl_3$): $\boldsymbol{\delta}$ 9.22(s, 1H), 8.91 (s, 1H), 8.18 (d, 1H, *J*= 7.2 Hz), 7.95(d, 1H, *J*= 4.8 Hz),7.83 (d, 1H), 7.76 (), 7.58 (d, 1H, *J*=7.8 Hz), 7.51 (s, 2H), 7.45 (t, 2H, *J*=8.4 Hz), 7.44 (d, 1H, *J*=2.4 Hz), 1.43 (s, 18H); ^{13}C NMR (150 MHz, $CDCl_3$): $\boldsymbol{\delta}$ 155.9, 154.0, 142.6, 136.5, 134.2, 132.9, 128.9, 126.2, 126.1, 125.9, 124.1, 123.9, 120.1, 117.5, 34.4, 30.2; FTIR (cm^{-1}, ATR): 3619, 3389, 3086, 2961, 2867, 1691; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{26}H_{32}N_3O_2^+$ 418.25; Found 418.2473.

2-(3,5-di-*tert*-butyl-2-hydroxybenzylidene)-N-phenylhydrazine-1-carboxamide **(5.10a)**: obtained as white solid. Yields: 95% (226.93 mg); mp. 212-213°C; ^{1}H NMR (600 MHz, $CDCl_3$): $\boldsymbol{\delta}$ 10.07(s, 1H), 9.41 (s, 1H), 8.04 (s, 1H), 7.58 (d, 2H, *J*= 7.8 Hz), 7.46 (s, 1H), 7.42 (s 1H), 7.40 (t, 2H, *J*= 7.8 Hz), 7.17 (t, 1H, *J*= 7.2 Hz), 7.08 (s, 1H), 1.49 (s, 9H) 1.39 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$): $\boldsymbol{\delta}$ 154.2, 152.7, 148.2, 141.7, 137.4, 136.7, 129.1, 126.9, 125.9, 124.2, 120.0, 116.7, 35.1, 34.2, 31.5, 29.5; FTIR (cm^-

^{1}, ATR): 3631, 3386, 3192, 2964, 2879, 1679; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{22}H_{30}N_3O_2^+$ 268.23; Found 268.2329.

2-(3,5-di-*tert*-butyl-2-hydroxybenzylidene)-N-(4-fluorophenyl)hydrazine-1-carboxamide **(5.10b)**: obtained as white solid. Yields: 96% (240.38 mg); mp. 213-214°C; ^{1}H NMR (600 MHz, DMSO-d_6): ***δ*** 11.24 (s, 1H), 10.46 (s, 1H), 9.91 (s, 1H), 8.21 (s, 1H), 7.54 (d, 2H, *J*= 4.8, 4.2 Hz), 7.27 (s, 1H), 7.2 (s 1H), 7.14 (t, 2H, *J*= 9 Hz), 1.41 (s, 9H) 1.28 (s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6): ***δ*** 158.9, 157.4, 154.0, 152.8, 146.8, 140.9, 136.1, 125.8, 125.2, 121.6, 118.2, 115.7, 35.1, 34.4, 31.8, 29.8; FTIR (cm^{-1}, ATR): 3337, 3331, 2964, 2879, 1712, 1658; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{22}H_{29}FN_3O_2^+$ 386.22; Found 386.2230.

2-(3,5-di-*tert*-butyl-2-hydroxybenzylidene)-N-(2,4-dichlorophenyl)hydrazine-1-carboxamide **(5.10c)**: obtained as white solid. Yields: 92% (23.72); mp. 212-213°C; ^{1}H NMR (600 MHz, DMSO-d_6): ***δ*** 9.85 (s, 1H), 8.82 (s, 1H), 8.28 (d, 1H *J*=9 Hz), 8.16 (s, 1H), 7.93 (s, 1H), 7.35 (d, 1H, *J*= 1.8 Hz), 7.34 (d, 1H, *J*= 1.8 Hz), 7.21 (dd, 1H, *J*= 2.4, 6.6 Hz), 6.99 (s, 1H), 1.38 (s, 9H) 1.25 (s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6): ***δ*** 156.9, 154.4, 151.9, 148.4, 141.8, 136.9, 133.4, 128.8, 127.9, 127.2, 125.9, 123.3, 120.9, 116.3, 35.2, 34.2, 31.5, 29.7; FTIR (cm^{-1}, ATR): 3637, 3192, 2964, 2873, 1694; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{22}H_{28}Cl_2N_3O_2^+$ 436.16; Found 436.1549.

N-(4-cyanophenyl)-2-(3,5-di-*tert*-butyl-2-hydroxybenzylidene)hydrazine-1-carboxamide **(5.10d)**: obtained as white solid. Yields: 90% (229.61 mg); mp. 245-246°C; ^{1}H NMR (600 MHz, DMSO-d_6): ***δ*** 11.26 (s, 1H), 10.67 (s, 1H), 9.56 (s, 1H), 8.26 (s, 1H), 7.76 (d, 2H *J*=9 Hz), 7.73 (d, 2H *J*=9 Hz), 7.28 (d, 1H, *J*= 1.8 Hz), 7.22 (d, 1H, *J*= 1.8 Hz), 1.41 (s, 9H) 1.28 (s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6): ***δ*** 154.2, 152.2, 147.8, 144.4, 141.0, 136.1, 133.6, 125.9, 125.5, 119.7, 119.3, 118.0, 104.2, 35.1, 34.4, 31.8,

29.8; FTIR (cm^{-1}, ATR): 3610, 3198, 3104, 2976, 2216, 1697; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{23}H_{29}N_4O_2^+$ 393.51; Found 393.2296.

2-(3,5-di-*tert*-butyl-2-hydroxybenzylidene)-N-(3-methoxyphenyl)hydrazine-1-carboxamide **(5.10e)**: obtained as white solid. Yields: 95% (245.3 mg); mp. 192-194°C; ^{1}H NMR (600 MHz, DMSO-d_6): ***δ*** 11.49 (s, 1H), 10.46 (s, 1H), 9.08 (d, 1H *J*=9 Hz), 8.27 (s, 1H), 7.32 (s, 1H), 7.30 (s, 1H), 7.26 (s, 1H), 7.25 (d, 1H, *J*= 7.2 Hz), 7.08 (d, 1H, , *J*= 7.2 Hz), 6.65 (d, 1H, , *J*= 6 Hz), 3.80 (s, 3H),1.47 (s, 9H) 1.34 (s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6): ***δ*** 160.09, 154.2, 152.2, 146.9, 141.0, 136.03, 129.9, 125.7, 125.2, 118.2, 111.8, 108.2, 105.3, 55.4, 35.1, 34.5, 31.8, 29.7; FTIR (cm^{-1}, ATR): 3640, 3431, 3234, 2955, 1676; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{23}H_{32}N_3O_3^+$ 398.21; Found 398.2446.

2-(3,5-di-*tert*-butyl-2-hydroxybenzylidene)-N-(m-tolyl)hydrazine-1-carboxamide ***(5.10f)***: obtained as white solid. Yields: 90% (223.19 mg); mp. 215-216°C; ^{1}H NMR (600 MHz, DMSO-d_6): ***δ*** 11.40 (s, 1H), 10.41 (s, 1H), 8.95 (s, 1H), 8.21 (s, 1H), 7.37 (s, 1H), 7.31 (d, 1H, *J*=8.4 Hz), 7.27 (d, 1H, *J*=2.4 Hz), 7.20 (d, 1H, *J*=1.8Hz), 7.18 (t, 1H, , *J*= 7.8 Hz), 6.84 (d, 1H, , *J*= 7.2 Hz), 2.29 (s, 3H),1.41 (s, 9H) 1.28 (s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6): ***δ*** 154.1, 152.5, 146.7, 140.9, 139.7, 138.3, 136.0, 129.0, 125.7, 125.2, 123.5, 120.1, 118.2, 116.8, 35.1, 34.4, 31.8, 29.8, 21.7; FTIR (cm^{-1}, ATR): 3283, 3228, 2986, 2670, 1688, 1618; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{23}H_{32}N_3O_2^+$ 382.25; Found 382.2492.

N-(4-(*sec*-butyl)phenyl)-2-(3,5-di-*tert*-butyl-2-hydroxybenzylidene)hydrazine-1-carboxamide **(5.10g)**: obtained as white solid. Yields: 87% (239.37 mg); mp. 208-209°C; ^{1}H NMR (600 MHz, $CDCl_3$): ***δ*** 9.95 (s, 1H), 8.78 (s, 1H), 7.91 (s, 1H), 7.36 (d, 2H, *J*=8.4 Hz), 7.32 (s, 1H), 7.27 (s, 1H), 7.12 (d, 2H, *J*=8.4 Hz), 6.99 (s, 1H), 2.53 (m, 1H, , *J*= 7.2 Hz), 1.52 (t, 2H, , *J*= 7.2 Hz), 1.39 (s, 9H) 1.24 (s, 9H), 1.17 (d, 3H, *J*=7.2 Hz), 0.77 (t,

3H, *J*= 7.2 Hz); ^{13}C NMR (150 MHz, $CDCl_3$): ***δ*** 154.2, 152.8, 147.8, 143.8, 141.7, 136.7, 134.9, 127.7, 126.8, 125.9, 120.7, 116.6, 41.2, 35.1, 34.2, 31.5, 31.2, 29.5, 21.9, 12.2; FTIR (cm^{-1}, ATR): 3628, 3107, 2958, 2876, 1688; HRMS (Q-TOF) m/z: $[M+Na]^+$ Calcd for $C_{26}H_{37}N_3NaO_2^+$ 446.28; Found 446.2785.

N-(4-acetylphenyl)-2-(3,5-di-*tert*-butyl-2-hydroxybenzylidene)hydrazine-1-carboxamide (**5.10h**): obtained as white solid. Yields: 88% (242.86 mg); mp. 207-208°C; ^{1}H NMR (600 MHz, DMSO-d_6): ***δ*** 9.80 (s, 1H), 8.68 (s, 1H), 7.93 (d, 1H, J= 3 Hz), 7.91 (s, 1H), 7.60 (s, 1H), 7.58 (s, 1H), 7.56 (s, 1H), 7.35 (s, 1H, J= 2.4 Hz), 7.00(d, 1H, J= 2.4 Hz), 2.52 (s, 3H), 1.40 (s, 9H) 1.25 (s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6): ***δ*** 196.9, 165.3, 156.9, 154.2, 148.6, 142.0, 136.8, 132.7, 129.9, 127.3, 126.1, 118.9, 116.4, 35.2, 34.3, 31.4, 29.5, 26.4; FTIR (cm^{-1}, ATR): 2982, 2882, 2685, 1724, 1670; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{24}H_{32}N_3O_3^+$ 410.24; Found 410.2451.

ethyl-4-(2-(3,5-di-*tert*-butyl-2-hydroxybenzylidene)hydrazine-1-carboxamido)benzoate **(5.10i)**: obtained as white solid. Yields: 90% (257.143 mg); mp. 227-228°C; ^{1}H NMR (600 MHz, DMSO-d_6): ***δ*** 9.95 (s, 1H), 9.33 (s, 1H), 8.09 (d, 2H, *J*= 12 Hz), 8.06 (s, 1H), 7.67 (d, 2H, *J*= 8.4 Hz), 7.44 (s, 1H), 7.09 (s, 1H), 4.41 (q, 2H, *J*= 6 Hz), 1.49 (s, 9H), 1.43 (t, 3H, *J*= 6 Hz), 1.25 (s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6): ***δ*** 166.2, 154.2, 152.4, 148.8, 141.9, 141.6, 136.8, 130.9, 127.2, 126.1, 125.8, 118.9, 116.5, 60.9, 35.2, 34.2, 31.5, 29.5, 14.4; FTIR (cm^{-1}, ATR): 3431, 3234, 3170, 2955, 2867, 1707, 1676; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{25}H_{34}N_3O_4^+$ 440.25; Found 440.2555.

2-(3,5-di-*tert*-butyl-2-hydroxybenzylidene)-N-(naphthalen-2-yl)hydrazine-1-carboxamide **(5.10j)**: obtained as white solid. Yields: 95% (257.84 mg); mp. 205-206°C; ^{1}H NMR (600 MHz, DMSO-d_6): ***δ*** 10.25 (s, 1H), 9.62 (s, 1H), 7.95 (s, 1H), 7.92 (d, 2H, *J* = 6.6 Hz), 7.85 (s, 1H), 7.82 (d, 1H, *J*= 7.8 Hz), 7.50 (d, 1H, *J*= 8.4), 7.46 (d, 1H, *J*=

4.2), 7.44 (d, 1H, *J*= 4.8), 7.32 (d, 1H, *J*= 2.4 Hz), 6.95 (d, 1H, *J*= 2.4 Hz), 1.34 (s, 9H), 1.24 (s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6): **δ** 165.4, 154.4, 153.8, 148.3, 141.7, 136.8, 134.3, 128.8, 126.8, 126.6, 126.2, 125.9, 125.8, 120.8, 120.5, 116.7, 35.2, 34.2, 31.5, 29.5; FTIR (cm^{-1}, ATR): 3619, 3389, 3086, 2961, 2867, 1691; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{26}H_{32}N_3O_2^+$ 418.25; Found 418.2490.

5.2.3 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay

DPPH radical scavenging assay was carried out according to the literature (Blois, 1958; O. P. Sharma & Bhat, 2009) with some modifications. Various concentrations with range of 60, 40, 20, 10 µM/mL for samples DPPH solution (50µL, 0.203 mM in MeOH) was added. Around 1 mg of synthesized compounds were dissolved in MeOH (5.0 mL, 100%) as a stock solution. This stock solution was diluted to a range of final extraction concentrations 60, 40, 20, and 10 µM. A negative control with the same DPPH concentration in MeOH without sample was used. Each assay was carried out in triplicates. The mixture was then incubated in dark for 60 min at room temperature. Absorbance at 517 nm for each sample was measured. BHT was used as positive control. The free radical scavenging activity of the compounds will be calculated as a percentage of radical inhibition by using the formula:

$$\text{Percentage of Inhibition (\%)} = [(Ac - As)/Ac] \times 100, \quad 5.1$$

where, As = Absorbance of the compounds/ positive control, and

Ac = Absorbance of control (MeOH solution)

The required concentration was determined to achieve 50 % inhibition (IC_{50}) of DPPH radical and the percentage of DPPH inhibition for each compound will be plotted against extract concentration.

5.2.4 Differential scanning calorimetry (DSC)

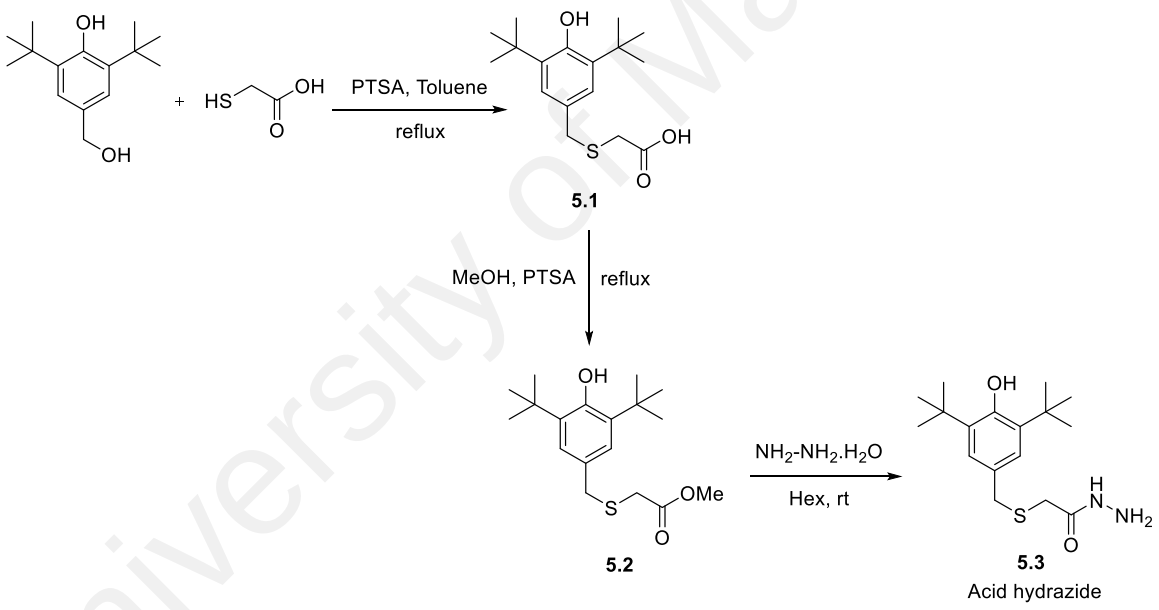
Temperature ramping DSC: Differential scanning calorimetry was conducted for the evaluation of oxidation induction time and oxidation onset temperature by utilizing Perkin Elmer DSC 4000. For the assessment of OIT, 5 mg of sample oil was placed in an open aluminum pan under 2 bar pressures of nitrogen. Metal indium was used for the temperature calibration at 20°C heating rate. The sample was heated in a nitrogen atmosphere until reached the isothermal temperature 150°C then switched to oxygen gas at 20 mL/min rate.

Programmed temperature DSC: This experiment was also carried out by the same instrument. 3mg of oil sample was placed in an open aluminum pan for the maximum interaction between oil and reactant gas in order to avoid gas diffusions limitations. Temperature scanning rate of 20 ◦C/min was kept in the temperature ramping experiments and 99.99% pure oxygen flow was maintained at 20 ml/min.

5.3 Results and Discussion

5.3.1 Synthesis of BHA incorporated semicarbazones

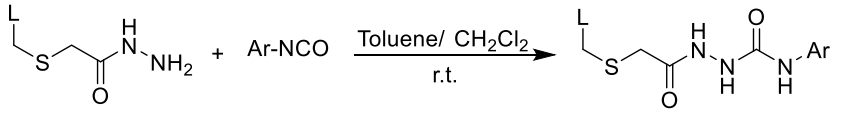
Recently, we disclosed a robust reaction protocol for the synthesis of semicarbazones to skip the limitations of previously reported methods such as functional group incompatibility and lower yields and ohters. This reaction protocol involved with the synthesis of acid hydrazide that was reacted with aryl isocyanate to obtain substituted semicarbazides, it was then treated with benzaldehyde to get expected semicarbazones (Nath & Yehye, 2018). Acid hydrazide was prepared by following the Scheme 5.1 stated in the literature (Ariffin et al., 2014; Nath & Yehye, 2018).



Scheme 5.1: Preparation of Acid Hydrazide (**5.3**)

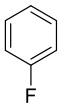
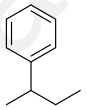
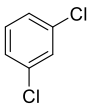
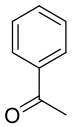
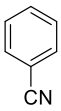
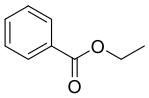
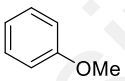
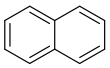
Afterward, 10 substituted semicarbazides were prepared by the reaction of a pre-prepared acid hydrazide and 10 different aryl isocyanate at room temperature using two different solvents; toluene and dichloromethane based on the solubility of the reactants. All the substituted semicarbazides (**5.4a-j**) were obtained with notable yields but at different reaction completion period. The results are summarised in Table 5.1.

Table 5.1: Evaluation of the reaction of substituted semicarbazides formation 5.5a-j.[a-c]



5.3 **5.4a-j** **5.5a-j**

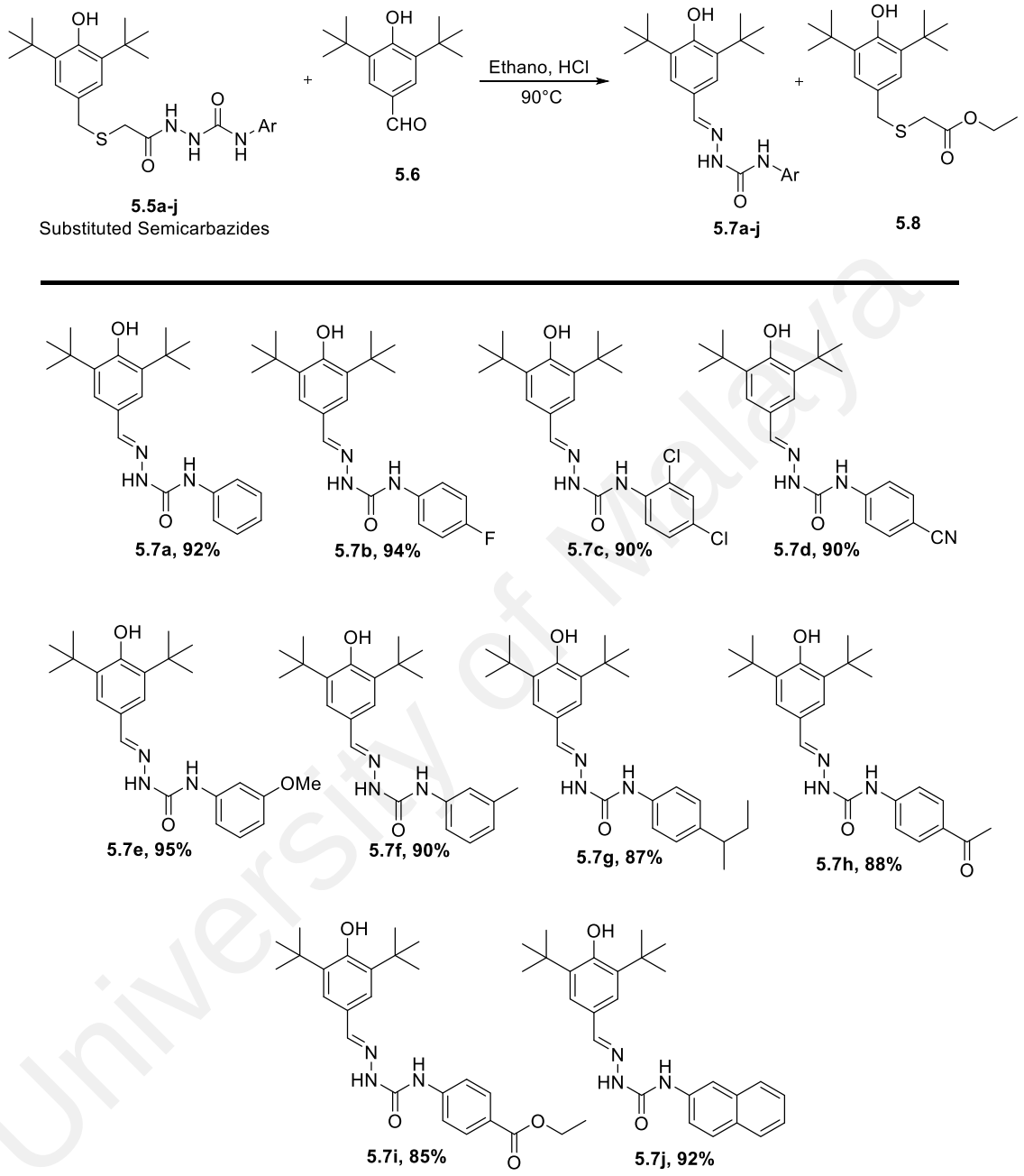
Substituted Semicarbazides

Substituted semicarbazide	Ar	Yields %	Substituted Semicarbazide	Ar	Yields %
5.5a		99[b]	**5.5f**		90[b]
5.5b		98[b]	**5.5g**		90[b]
5.5c		98[c]	**5.5h**		95[b]
5.5d		95[c]	**5.5i**		90[c]
5.5e		95[b]	**5.5j**		90[b]

[a] Reagents and conditions: 3 (3.12 mmol), 5.4a–g (3.12 mmol), room temperature. [b] toluene was used as a solvent; [c] Dichloromethane was used as a solvent.

Then the synthesized substituted semicarbazides were taken into the reaction with 3,5-di-tert-butyl-4-hydroxy benzaldehyde in presence of acidic ethanol at 90°C. and the results were outlined in Table 5.2. 2-(3,5-di-*tert*-butyl-4-hydroxy benzylidene)-N¬-(substituted phenyl)hydrazine-1-carboxamide (**5.7a-j**) were obtained successfully with notable yields. Semicarbazones containing 4-fluoro phenyl **5.7b**, 2,4-di-chloro phenyl **5.7c**, 4-cyano phenyl **5.7d,** 3-methoxy phenyl **5.7e**, m-tolyl **5.7f**, naphthalene **5.7j** attached at N-4 were isolated with around 90% more product yields. Again, acetyl **5.7h**, ethyl ester **5.7i** substituted phenyl semicarbazones provided more than 80% yields.

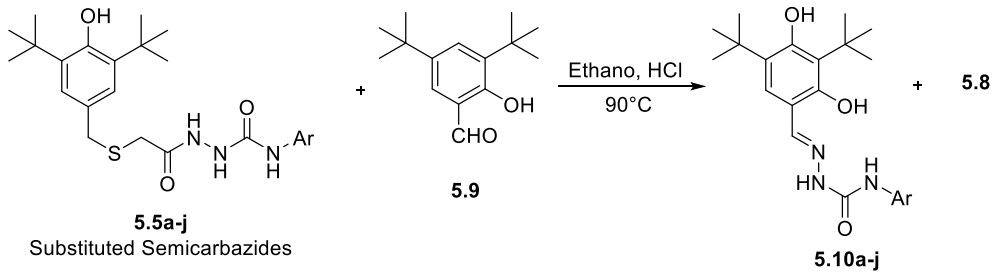
Table 5.2: Evaluation of 2-(3,5-di-*tert*-butyl-4-hydroxy benzylidene)-N-(substituted phenyl)hydrazine-1-carboxamide.[a-b]

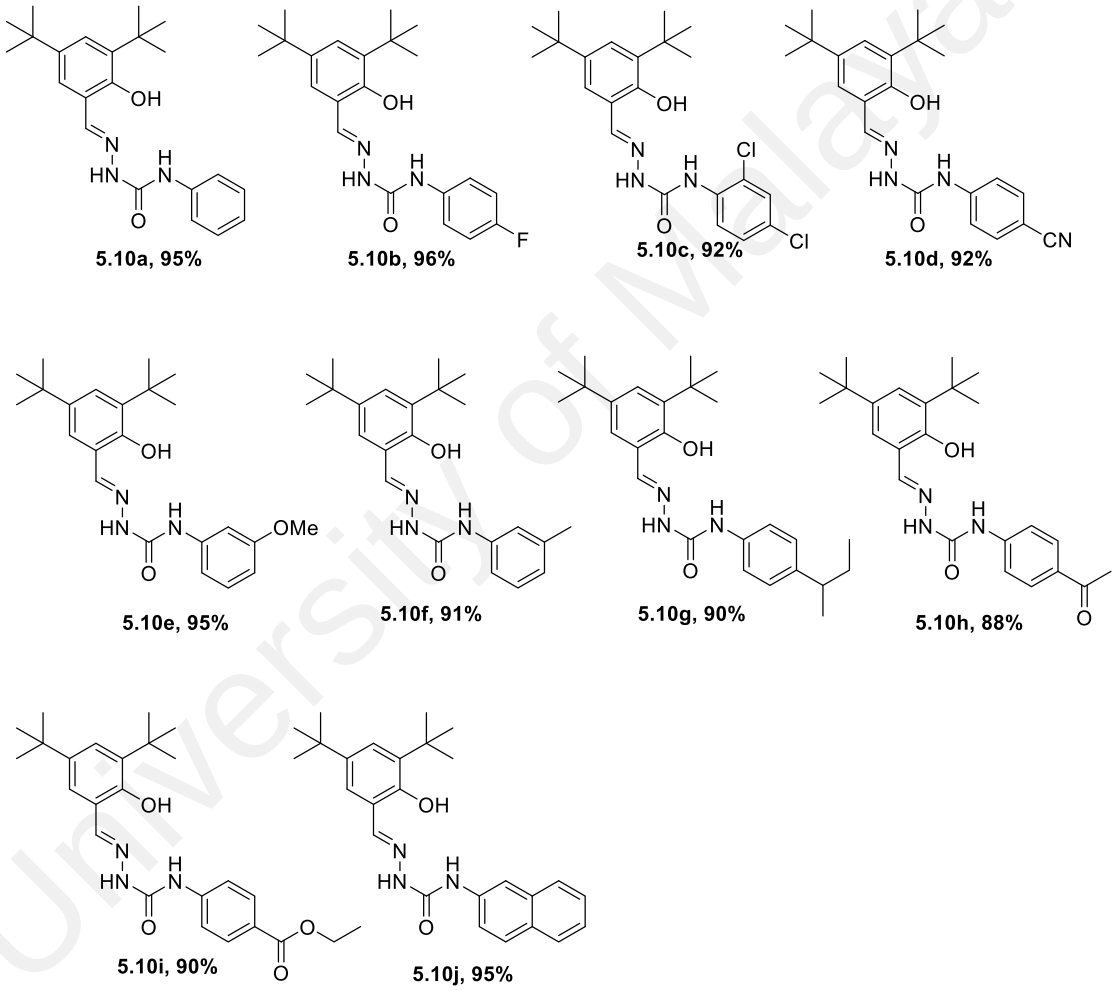


[a] Reagents and conditions: **5.5a–g** (0.65 mmol), **6** (0.65 mmol), HCl (1.6mmol), ethanol (10 mL), 90°C; [b] Isolated yield.

After obtaining the substituted semicarbazones (**5.7a-j**) containing 3,5-di-tert-butyl-4-hydroxyphenyl, we prepared another series of same substituted semicarbazones attaching with 3,5-di-tert-butyl-2-hydroxyphenyl to investigate antioxidant activity of both series.

Table 5.3: Evaluation of 2-(3,5-di-*tert*-butyl-2-hydroxy benzylidene)-N-(substituted phenyl)hydrazine-1-carboxamide. [a-b]





[a] Reagents and conditions: **5.5a–g** (0.65 mmol), **5.9** (0.65 mmol), HCl (1.6mmol), ethanol (10 mL), 90°C; [b] Isolated yield.

Thus, pre-prepared substituted semicarbazides (**5.5a-j**) were reacted with 3,5-di-tert-butyl-2-hydroxy benzaldehyde **5.9** upon heating with acidic ethanol. Interestingly, 3,5-

di-tert-butyl-2-hydroxy benzaldehyde also went into reaction with all the synthesized substituted semicarbazides (**5.5a-j**) and also provided the noticeable yields, the results were shown in Table 5.3. All 2-(3,5-di-*tert*-butyl-2-hydroxy benzylidene)-N-(substituted phenyl)hydrazine-1-carboxamide (**5.10a-j**) were obtained with around 90% more products yields.

5.3.2 Antioxidant activity evaluation by DPPH assay

The free radical scavenging ability of series 2-(3,5-di-*tert*-butyl-4-hydroxy benzylidene)-N-(substituted phenyl)hydrazine-1-carboxamide (**5.7a-j**) and 2-(3,5-di-*tert*-butyl-2-hydroxy benzylidene)-N-(substituted phenyl)hydrazine-1-carboxamide (**5.10a-j**) were evaluated by a non-enzymatic method DPPH assay. Reduction of DPPH by antioxidant causes the color changes from purple to yellow and it is also observed by the decreasing absorption at 517 nm in the spectrophotometer. The free radical scavenging properties of the synthesized semicarbazones were investigated by the interaction between experimental compounds and 0.2 mM 1,1-diphenyl-2-picryl-hydrazine (DPPH) for 30 mins. The antioxidant activities of the series 1 of 2-(3,5-di-*tert*-butyl-4-hydroxy benzylidene)-N-(substituted phenyl)hydrazine-1-carboxamide (**5.7a-j**) obtained from DPPH assay were summarized in Figure 5.1.

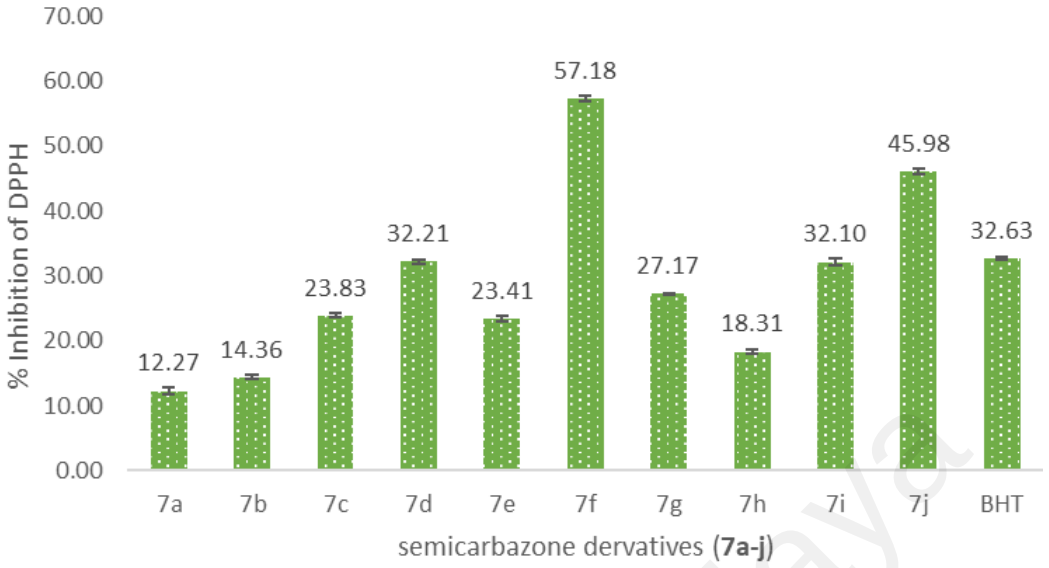


Figure 5.1: Antioxidant activity of synthesized compounds (5.7a-j) by DPPH assay.

Although all the compounds (**5.7a-j**) possessed the reasonable free radical scavenging properties, most of them exhibited promising oxidation inhibition in comparison with standard BHT. The lower value of IC_{50} of compounds indicates the better antioxidant activity. Compounds (**5.7a-e**, **5.7g**, **5.7h**) showed the lower IC_{50} values than standard BHT. The increasing order of IC_{50} can be made from results in Figure 5.1 as 5.7a > 5.7b > 5.7h > 5.7e > 5.7c > 5.7g > 5.7d > 5.7i > BHT > 5.7j > 5.7f.

The series of 2-(3,5-di-*tert*-butyl-2-hydroxy benzylidene)-N-(substituted phenyl)hydrazine-1-carboxamide (**5.10a-j**) were also conducted with DPPH assay and results were shown in Figure 5.2. Free radical scavenging properties of these compounds can be ordered from the obtained results as follows: 5.10e > 5.10i > 5.10h > BHT > 5.10b > 5.10g > 5.10c > 5.10d > 5.10a > 5.10f > 5.10j. In this series, 3-methoxyphenyl semicarbazones, 3-ethoxy carbonyl phenyl semicarbazone and 4- acetyl phenyl semicarbazones showed better radical scavenging properties than standard BHT, though the rest of the compounds exhibited antioxidant properties.

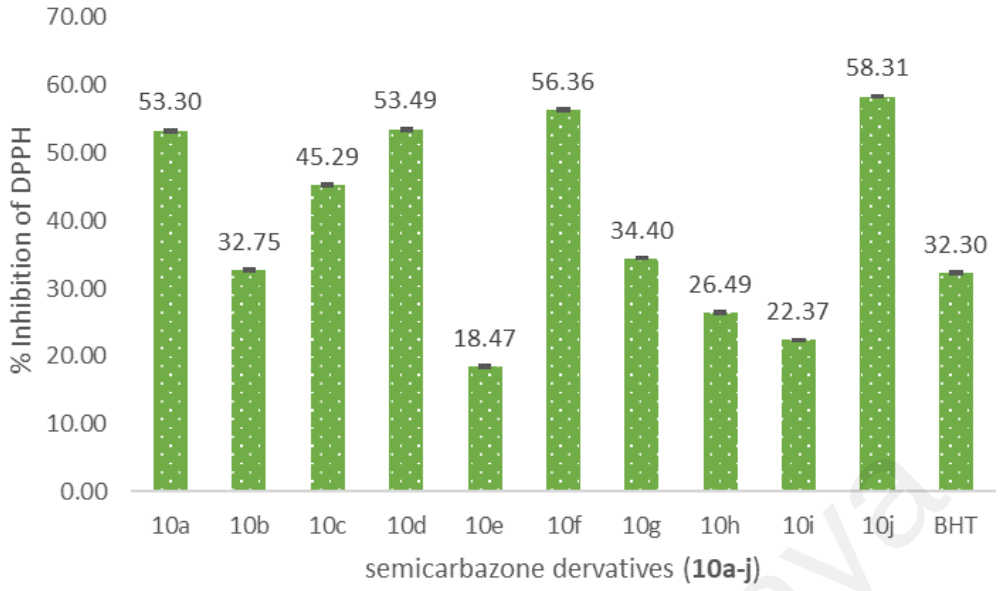


Figure 5.2: Antioxidant activity of synthesized semicarbazones (5.10a-j) by DPPH assay.

Generally, it is observed in DPPH assay results (Figure 5.1 and Figure 5.2) that 2-(3,5-di-t*ert*-butyl-2-hydroxy benzylidene)-N-(substituted phenyl)hydrazine-1-carboxamide offer the better antioxidant activity than 2-(3,5-di-*tert*-butyl-2-hydroxy benzylidene)-N-(substituted phenyl)hydrazine-1-carboxamide. This may primarily be attributed to the position of hydroxyl radical (-OH) on the benzylidene ring at N-1 of semicarbazones and the substituents effects of the N-4 ring.

Higher stability of phenoxy radical and lower BDE values of O-H bonds are the prerequisite of a hindered phenol to be antioxidant. It is well-known that antioxidant activity of butylated hydroxytoluene (BHT) varies with the values of bond dissociation enthalpies (BDE) of O-H bond and stability of phenoxy radical. The BDE values of O-H in butylated hydroxytoluene depends on the OH group position on the benzene ring and its substituents (Yehye et al., 2015). And, the stability of phenoxy radical depends on the steric hindrance. Therefore, in butylated hydroxytoluene, the sterically hindered hydroxyl group is obtained by flanking two bulky groups like tertiary butyl groups to attain more

stable phenoxy radical. Thus, the phenoxy radical, formed by donating a proton to reduce free radical, is not affected by any electrophiles readily.

In the case of semicarbazones, keto-enol tautomerism might play an important role on their antioxidant properties. Hydroxyl group of enol form of semicarbazones and/ or secondary aromatic amine might involve in the H-atom transfer mechanism (or single electron transfer) to reduce the free radicals.

In the structure of first Series 1 (**5.7a-j**), the para hydroxyl group of butylated hydroxyphenyl (BHP) is flanked by two tertiary butyl groups, so the antioxidant activity of butylated hydroxyphenyl may remain same as BHT due to the favorable position (ortho) of O-H group but may be affected by the substituents. Consequently, phenyl semicarbazones bearing BHP **5.7a** showed promising antioxidant activity (IC_{50}12.27µM or 4.51 µg/L) in DPPH assay in comparison with BHT (IC_{50} 32.63 µM). The oxidation inhibition of phenyl semicarbazone without any substituents (N^1-benzylediene-N^4-phenyl-semicarbazones) was reported 33.29 µg/L (IC_{50} value) in 0.1 mM DPPH concentration by Jafri *et al* (2012) (Jafri et al., 2012). While phenyl semicarbazones incorporated with BHP (**5.7a**) showed significant antioxidant activity (IC_{50} 4.51 µg/L) in 0.2 mM DPPH concentration in this study. This is may due to the combination of two antioxidant functions in one structure that able to exhibit the antioxidant synergistic effect. But.this antioxidant activity varies with the presence of different substituents at N-4 ring of semicarbazones. BHA incorporated semicarbazones containing 4-fluoro **5.7b**, 4-acetyl **5.7h**, 3-methoxy **5.7e**, 4-chloro **5.7c**, 4-Cyano **5.7d**, 4-ester **5.7i** and 4-sec-butyl **5.7g** at N-4 benzene ring of semicarbazone exhibited lower IC_{50} values than BHT, but 3-methyl **5.7f** and naphthyl **5.7j** substituted semicarbazones showed higher IC_{50} values.

In the structure of the second series, the ortho hydroxyl group of BHP moiety is not flanked by two tertiary butyl groups in 3,5-di-tert-butyl-2-hydroxy benzylidene ring, this

may increase the BDE values O-H groups resulting the lower antioxidant activity of the compounds of this series in comparison with series 1. BDE values of O-H groups of this series might be affected by the hydrogen bond which would occur between the hydrogen of OH group and nitrogen of imine group. Due to the formation of a hydrogen bond between H and N, it becomes difficult to abstract the proton from -OH group because of high BDE values. It can be displayed by Figure 5.3 that describes the probable formation of hydrogen bond in series 2 structure (**5.10a-j**).

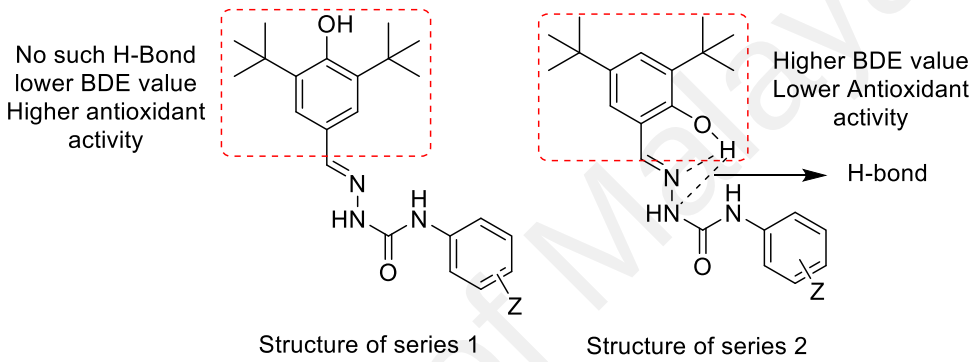


Figure 5.3: Probable structure for the formation of hydrogen bond in series 2.

This hydrogen bond might decrease the normal antioxidant activity of butylated hydroxyphenyl which results into the lower antioxidant activity of Series 2 compounds. This series may not be able to provide any synergistic effect between BHP and semicarbazones functional group because of the formation of H-bond. Thus, the antioxidant activitiy of Series 2 might be obtained only from semicarbazones functions which has been described earlier. Interestingly, 3-methoxyphenyl **5.10e**, 4-ethyl carbonyl phenyl **5.10i** and 4-acetyl phenyl **5.10h** semicarbazones incorporated with 3,5-di-tert-butyl-2-hydroxyphenyl showed better antioxidant activity than standard BHT in DPPH assay. However, it can be concluded from the above discussion that semicarbazone bearing 3,5-di-tert-butyl-4-hydroxyphenyl can be effective antioxidant compounds than the semicarbazones incorporated with 3,5-di-tert-butyl-2-hydroxyphenyl.

5.3.3 Oxidation stability Assessment of TMPTO

After the evaluation of antioxidant properties of the series 1 and series 2 compounds, we proceeded to investigate the oxidation stability of some synthesized compounds by blending in trimethylolpropane trioleate as ester-based lubricant oil. Since all the compounds were not soluble in the trimethylolpropane trioleate (TMPTO), so only a few compounds which were easily soluble in TMPTO, were taken to carry out the lubricant oil oxidation stability test. Compounds **5.7a**, **5.7b**, **5.7c**, **5.7e**, **5.7g** and **5.10a** were easily soluble in TMPTO and carried out two types of differential scanning calorimeter test namely: temperature ramping DSC and programmed temperature DSC at two isothermal temperature 150°C and 125°C. The samples were blended in TMPTO with very less amount (0.25 wt.%) and conducted temperature ramping DSC with a constant scanning rate of 20°C/min to assess their oxidation decomposition temperature. The obtained results from temperature ramping DSC analysis were outlined in Figure 5.4.

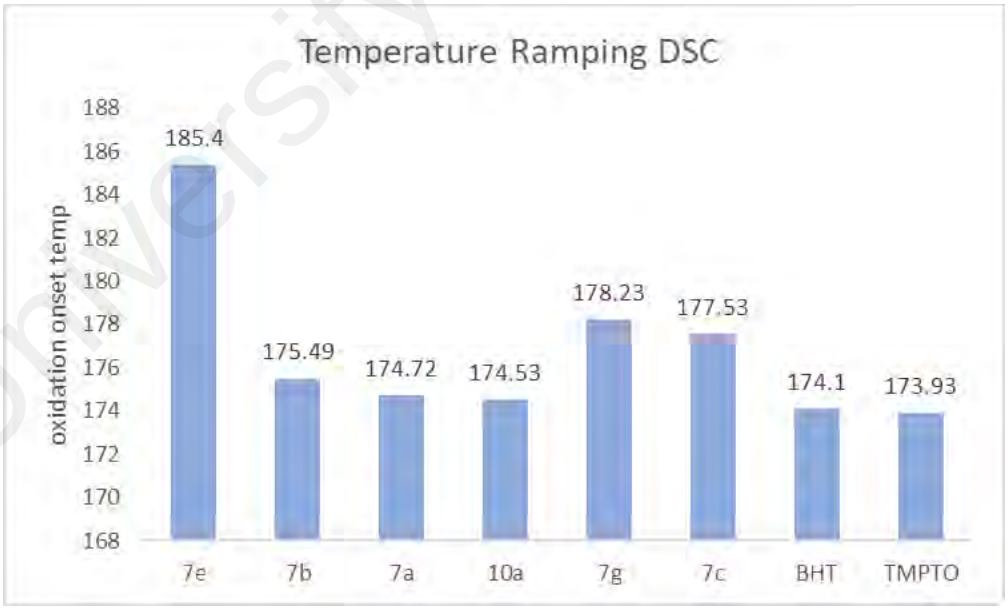


Figure 5.4: Temperature ramping DSC analysis for the TMPTO with 0.25 wt.% antioxidants

From Figure 5.4, Base oil TMPTO exhibited the oxidation onset temperature (OOT) at 173.93°C, OOT of TMPTO incorporated with 0.25% of BHT, compounds **5.7a** and **5.10a** did not show any significant difference. N^1-(3,5-di-tert-butyl-4-hydroxy benzylidene)-N^4-(3-methoxyphenyl)-semicarbazones **5.7e** showed higher OOT at 185.4°C.

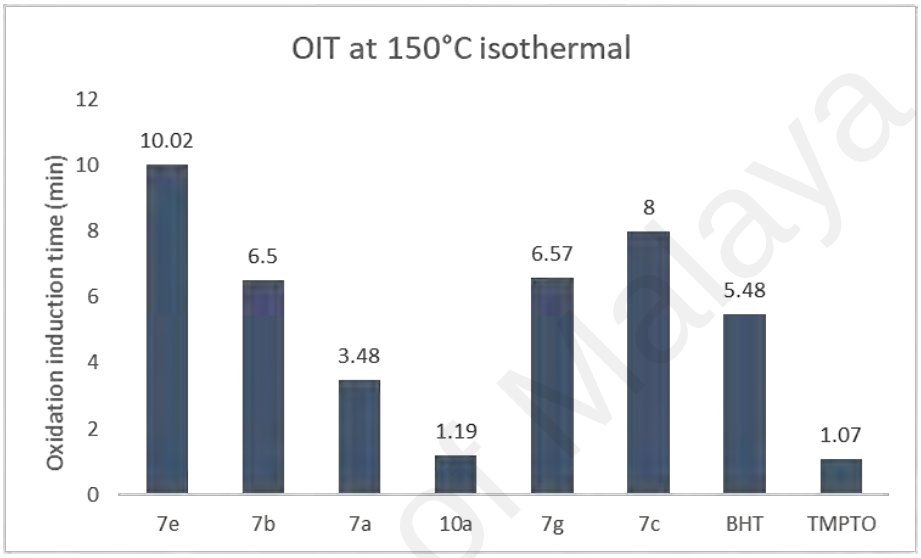


Figure 5.5: Oxidation induction time of TMPTO incorporated with 0.25% experimental sample at 150°C isothermal.

Afterward, all experimental samples were carried out for programmed temperature DSC at 150°C isothermal temperature for the evaluation of oxidation induction time. OIT of the experiments samples were outlined in Figure 5.5. Oxidation induction time (OIT) of TMPTO base oil was observed 1.07 min, incorporation of 0.25 wt% of compounds 7a and 9a didn't show any significant increase of OIT. But TMPTO blended with 0.25 wt% of compound **5.7e** and **5.7c** showed better OIT values (10.02 min and 8 min respectively) in comparison with BHT (5.48 min). 4-fluorophenyl **5.7b** and 4-sec-butyl-phenyl **5.7g** semicarbazones incorporated with BHT showed almost identical OIT values (6.5 and 6.57 min respectively).

This experiment was again carried out at 125°C isothermal temperature with the same blended TMPTO oil to observe the change of OIT. And the obtained results were displayed in Table 5.4. It was found that 0.25 wt.% 7e and 7c increased oxidation induction time of TMPTO 40.48 min and 41.42 min respectively at 125°C isothermal, while Blank TMPTO showed 2.25 min OIT.

Table 5.4: Oxidation induction time of TMPTO incorporated with 0.25% experimental sample at 125°C isothermal.

Compounds No.	0.25 wt.% **7a**	0.25 wt.% **7b**	0.25 wt.% **7c**	0.25 wt.% **7e**	0.25 wt.% **7g**	0.25 wt.% **10a**	0.25 wt.% BHT	TMPTO
OIT Min	8.26	-	40.48	41.42	-	7.42	15.2	2.25

'-' no OIT was observed for the 7c and 7g

It was found in the DSC results that, oxidation stability of TMPTO increased significantly in the combination of compounds **5.7e** and **5.7c** in comparison with BHT. It was reported that the compounds having two or more antioxidant functions in one structure can be able to exert auto-synergism (Nath & Yehye, 2018; Nath et al., 2018). Higher oxidation resistance of these compounds may be attributed to the effect of auto-synergism. Due to the presence of two antioxidant functions (BHA and semicarbazone function) in the one structure, the synthesized semicarbazone may be able to exhibit auto-synergistic effect to protect the oil from pre-mature oxidation. But the OIT results of compounds **7a** obtained by programmed temperature DSC didn't show any significant effect in the oxidation stability of lubricant oil. So, no antioxidant synergism were observed in the oil oxidation stability test for the compound 7a, though it showed the highest antioxidant activity in non enzymatic free radical scaveger test DPPH assay (Figure 5.1) by auto-synersigm. It was found in the literature that that the combination of

primary antioxidants (phenolic antioxidant and secondary aromatic amino antioxidant) exhibits negative synergistic effect in the oxidation of lubricant oil (Rudnick, 2017). Unfortunately, both butylated hydroxyphenyl (hindered phenol) and semicarbazone function (probably aromatic secondary amine) in structure of synthesized semicarbazones act as primary antioxidant. This may be a probable reason for the lower oxidation stability of the synthesized compounds in oil. Interestingly, OIT results of compounds 7e, 7c, 7b, and 7g shows better oxidation stability in comparison with BHT.

Generally, semicarbazones were reported as neutral tridentate ligand due to the presence of azomethine N and carbamoyl O, which can provide the stronger binding ability to metal ions. So several binding modes can be observed if additional coordinating functional group is present in the semicarbazone derivatives (Casas et al., 2000; Enyedy et al., 2014). Thus the metal ions can easily be deactivated by appropriate semicarbazones through the formation of several coordination bond between donor atoms and metal ions. Deactivating metal ions in the lubricant oil is prerequisite to acclerate the oxidation stability since metal ions are very susceptible to convert the acid and hydroperoxide into free radicals. Therefore, metal deactivators or chelators are one of the important antioxidant class in lubricant additives (Dresel, 2007). Generally, metal deactivators or chelators deactivated the metal ions in lubricant oil which are responsible for accelerating oxidation reaction by the production of free radicals (Waynick, 2001). Therefore all chelating metal deactivators contain multiple donor atoms which are able to deactivate the metal ions by the formation of complex compounds. Thus semicarbazones can act as a metal deactivator because of having multiple donor atoms. Due to the presence of additional donor atoms as substituents at N-4 ring of semicarbazones, several binding modes of semicarbazones might be observed and would be effective metal deactivator. Compounds 7e and 7c were found better oxidative stability in TMPTO in DSC experiment and this may due to the presence of 3-methoxy (7e) and 2,4-di-chlorine (7c)

substituents at N-4 ring of semicarbazones. Thus, methoxy and chlorine groups can act as donor atom and their favorable position at N-4 ring of semicarbazone structure would enhance the metal deactivator properties by the increase the metal binding mode (O'Keefe et al., 2014; Takao et al., 2007) which can be shown by Figure 5.6.

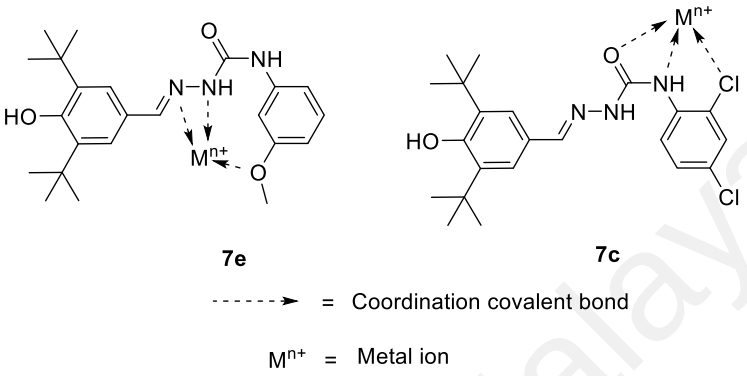


Figure 5.6: Metal binding mode of compound 5.7e and 5.7c.

Therefore, incorporation of butylated hydroxyphenyl into semicarbazones provided significant free radical sccavenging properties shown in DPPH results. Though it was not observed in differential scanning calorimeter test (oxidation stability) of lubricant oil. However presence of electron withdrawing substituents or donor atom at N-4 ring of semicarbazones may increase the metal binding mode which would help to efficiently deactivate the metal ions in the lubricant oil. Moreover, insertion of suitable functional groups at the appropriate position of the N-4 ring of semicarbazones help to obtain several metal binding modes which are very useful to prepare a variety of metal complexes with important chemical properties.

5.4 Conclusion

In conclusion, two series of semicarbazones bearing 3,5-di-tert-butyl-4-hydroxyphenyl (**5.7a-j**) and 3,5-di-tert-butyl-2-hydroxyphenyl (**5.9a-j**) were accessed easily by the reaction of substituted semicarbazides with 3,5-di-tert-butyl-4-hydroxybenzaldehyde and 3,5-di-tert-butyl-2-hydroxybenzaldehyde respectively.

Antioxidant evaluation test DPPH assay revealed that Series 1 (**5.7a-j**) exhibited promising free radical scavenging properties than Series 2 (**5.10a-j**). It was discussed that better antioxidant activity of Series 1 was found for the synergistic effect of butylated hydroxyphenyl and semicarbazones functions, while the formation of hydrogen bond lowers antioxidant activity of Series 2 (**5.10a-j**). Compounds **5.7a**, **5.7b**, **5.7c**, **5.7e**, **5.7g**, **5.10a** and **BHT** were separately blended with trimethylolpropane trioleate in 0.25 wt.% to carry out the oxidation stability test by differential scanning calorimeter. Compounds **5.7e**, **5.7b**, **5.7c** showed better oxidative stability of synthetic lubricant oil. The probable reason was concluded that incorporation of electron withdrawing group (3-methoxy and 2,4-di-chloro) at the N-4 position of semicarbazones (Series 1) make efficient metal deactivator or chelator since they can act as polydentate ligand because of having multiple donor atom in their structure. It was anticipated that the synthesized compounds in this study would provide significant pharmacological properties which are under investigation since semicarbazone derivatives possess important pharmacophore properties as anticonvulsant, anticancer etc.

CHAPTER 6: ESTER OF THIOLATED BUTYLATED HYDROXYTOLUENE: POTENTIAL ANTIOXIDANT FOR SYNTHETIC LUBRICANT OIL

6.1 Introduction

Rising ecological concerns and growing restrictive protocols have stimulated the improvement of environmental-friendly lubricants throughout the world for the last few years (Alias et al., 2009; Erhan & Asadauskas, 2000; Wu et al., 2013). The future lubricants should be more sustainable and have an extended performance level with most reduced life-cycle-cost (LCC) than the traditional lubricants. Vegetable oils are feasible and sustainable source of environmentally friendly oil which derived from the synthetic polyol esters. As of late, it has been gaining an ever-increasing amount of consideration because of its cheaper and renewable raw materials. The synthetic polyol ester-based lubricants are the result of the reaction of fatty acid derived from naturally occurred oils or fats and neopentyl glycol (NPG), trimethylolpropane (TMP) and pentaerythritol (PE) which are mostly petrochemical derivatives. These ester-based lubricants have extensive applications rather than mineral lubricants due to its improved viscosity-temperature performance, low-temperature flow properties and high biodegradability (Gryglewicz et al., 2003; Nagendramma, 2011; Padmaja et al., 2012). One of the widely utilized ester-based lubricant is trimethylolpropane trioleate (TMPTO) which is synthesized by the reaction of trimethylolpropane, a petrochemical derivative with naturally derived oleic acid (Alias et al., 2009; Kiriliauskaitė et al., 2011). It has excellent lubricity, high viscosity index, better oxidative resistance and low-temperature properties in contrast with vegetable oil and could be used as a green lubricant (Rajendiran et al., 2016). TMPTO can be employed as ecofriendly hydraulic oil and water yacht engine oil. It is also widely applied in cold rolling of a steel plate, steel drawing oil and other metal-working fluids (Kiriliauskaitė et al., 2011; Qiao et al., 2017).

Although TMPTO shows better lubricant properties comparing to mineral oil, unfortunately it has failed to get wide approval as lubricant due to its poor oxidation stability. One of the main reasons of its low stability is due to the presence of unsaturated bonds in the oleic acid part of TMPTO molecule, which are readily affected by the radical attack and consequently suffer oxidative degradation resulting in the different acid molecule (Alias et al., 2009; Kiriliauskaitė et al., 2011; Sharma et al., 2007). Thus, oxidation causes the formation of acidic products, insoluble compounds, sludge and increases the acidity and viscosity of lubricant which actively affect machinery parts during its industrial life -service (Korcek et al., 1986; Quinchia et al., 2011). Therefore, high efficiency multi-potent antioxidant must be used in the lubricant composition in order to avoid the early oxidation degradation. because it can prolong oxidative retardation time as per industrial application requirement by protecting the oil from premature oxidation (Duangkaewmanee & Petsom, 2011; Quinchia et al., 2011). Researchers are trying to develop more oxidative stable ester-based lubricant oil adopting many ways such as designing new multipotent antioxidant structure; preparing a composite of commercial antioxidant and nanoparticles; introducing ecofriendly multifunctional additives and others (Huang et al., 2018).

Antioxidants protect the oil from auto-oxidation in different ways based on their structure and mode of action. Generally, there are two types of antioxidant; one is known as free radical scavenger or primary antioxidant and the other one is secondary antioxidant or peroxide decomposer. Hindered phenols and arylamines are free radical scavenger as they can remove the free radical like alkoxy or alkyl per-oxy radical by donating hydrogen atom inside the oil. On the other hand, organosulfur compounds are peroxide decomposer since they can help to form peroxide compounds from different peroxide radical. Primary antioxidants or free radical scavenger react with free radical to form stable compounds and inhibit the chain propagation of oxidation reaction whereas the main role of secondary

antioxidants is to decompose the hydroperoxide as hydroperoxide decomposition forms a higher number of free radical that lead the propagation and branch oxidation process (Rudnick, 2009). A variety of antioxidants have been utilizing to extend the oxidation retardation time of lubricant oil (Dunn, 2005; Karavalakis & Stournas, 2010; Liang et al., 2006; Mousavi et al., 2006; Somayaji & Aswath, 2009).

It was observed that the effect of using two or more antioxidants together brings greater oxidation stability than that of any individual antioxidants which is familiar as antioxidant synergism (Rawat et al., 2015). Synergistic antioxidant systems offer effective solutions to problems where an individual antioxidant is insufficient to offer acceptable results (Rudnick, 2017). The combination of different antioxidants in certain ratio can result in a good synergistic effect where oxidation stability is better than the individually used antioxidant (Guzman et al., 2009; Duangkaewmanee & Petsom, 2011; Fox & Stachowiak, 2007; Hanneken et al., 2006; Hu et al., 2007; Hu, Wei, et al., 2007; Hu et al., 2006; Liang et al., 2006; Qiu et al., 2006; Saravanan et al., 2006; Valko et al., 2007; Zeng et al., 2007). Organosulfur compounds are one of the important additives in the lubricant compositions because of their antioxidant, antiwear and extreme pressure properties. These have been usually used with the hindered phenol or aromatic amine to get better synergistic results. For instant, combination of phenols and sulfides or aromatic phosphates exhibits better synergism at higher temperature (heterosynergism) while the mixture of phenols and aromatic amine show a little negative effects (Homosynergism) (Fox & Stachowiak, 2007). As oxidation resistance ability of sulfides depends on their molecular structure, several kinds of organic sulfides such as different chain length of alkyl substitution, cyclic substitution and polysulfides functionality of sulfides were examined in the lubricant composition for their excellent oxidative resistance (Anderson, 1969; Qiu et al., 2006). Example like, Polychlorinated diphenyl sulfides (PCDPSs), sulfur analogues of polychlorinated diphenyl ethers (PCDEs) have been used in the gas turbine oil for their

potential thermal-resistant (Chen et al., 2018; Sutton et al., 2016). In spite of these potential antioxidant activities, presence of sulfur in the engine oil poison the catalytic effect and reduce the emission life system by fouling catalytic exhaust converters (Barnes et al., 2001; Hakan, 2001; Yamaguchi et al., 1999), Therefore, according to the modern environmental, economic and technical requirement the lowest amount of sulfur containing additive should be used in the advance engine oil formulations (David, 2002; Dugger et al., 2005; Hu, et al., 2007; Hu, Wei, et al., 2007; Valko et al., 2007; Zeng et al., 2007). However, absence of organosulfur compounds in the additive packages would decrease the oxidation resistance of oil, so it becomes important to study on different synergistic antioxidant systems to reduce the amount of sulfur molecule in additive package (Hu et al., 2007; Hu, Wei, et al., 2007; Valko et al., 2007; Zeng et al., 2007).

Therefore, the demand of multifunctional additives is high in order to cut down the number of such additives containing more sulfur atom because an individual multifunctional additive can exhibit several properties together such as antioxidant, antiwear, friction modifier and extreme pressure resistance (Singh et al., 2014; Singhal et al., 2014). Several multifunctional lubricant additives were reported in past years such as ecofriendly dimercaptothiadiazole derivatives (Chen et al., 2012); condensation products of different amines with di(alkylphenyl)phosphorodithioic acid (Swami et al., 2003); and rapseed oil methyl ester as multifunctional fuel additives (Hancsók et al., 2008). Some heterocyclic derivatives (such as benthiazole, thiazole, cyclotriphosphazene and triazine etc.) were also reported as excellent multifunctional lubricant additives for their extreme pressure resistance, antiwear, anticorrosion, antioxidant properties and high thermal stabilities (Aguilar et al., 2010; Azam et al., 2010; Brigati et al., 2002; Dolatabadi & Kashanian, 2010; Droge, 2002; He et al., 2002; Huang et al., 2004; Kar et al., 2010; Karavalakis & Stournas, 2010; Koleva et al., 2002; Li et al., 2010; Mishra et al., 2002; Mortier et al., 2010; Pelosi et al., 2010; Sandoval et al., 2002; Sheets et al., 2010;

Xiangqiong et al., 2005; Xu et al., 2000; Yao et al., 2002; Zeng et al., 2007; Zhang et al., 1999).

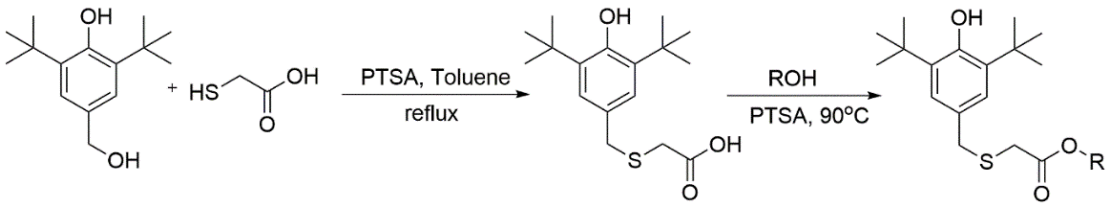
Likely multifunctional additives, multifunctional antioxidants would play vital role in the lubricant composition to avoid multiple antioxidant using for homo-synergistic or hetero-synergistic effect. Because multifunctional antioxidants contain two different antioxidant functional group that exhibit radical scavenging and peroxide decomposing functions in their structure which can offer auto-synergistic antioxidant system (Rudnick, 2017). We envisioned to assemble two types of antioxidants function in one structure in order to get better auto-synergistic antioxidant effect in synthetic lube oil. In this study, we are reporting for the first time two potential multifunctional antioxidants for synthetic ester based lubricant oil which exhibit greater oxidation stability than commercial antioxidant BHT and Irganox 1076. The two reporting compounds- 3,5-di-tert-butyl-4-hydroxy-benzylsulfanyl)-acetic acid methyl ester (6.1) and (3,5-di-tert-butyl-4-hydroxy-benzylsulfanyl)-acetic acid ethyl ester (6.2) were engineered by the combination of three important antioxidant functions: BHT, thioether and ester moiety. The oxidation stability and thermal stability were monitored by using DSC, RBOT and thermogravimetric analysis. The purpose of this study is to develop the efficient multifunctional antioxidant that can provide the more oxidative and thermal stable synthetic ester based lubricant oil by offering strong auto-synergistic effect.

6.2 Materials and Method

6.2.1 Synthesis of Ester of thiolated BHT

(3,5-Di-tert-butyl-4-hydroxy-benzylsulfanyl)-acetic acid methyl ester **6.1** and (3,5-Di-tert-butyl-4-hydroxy-benzylsulfanyl)-acetic acid ethyl ester **6.2** were prepared in the 2nd step of semicarbazones preparation method stated in Chapter four and five (Scheme 6.1). In brief, BHT alcohol was refluxed with thioglycolic acid in the presence of catalytic

amount of P-toluenesulfonic acid (PTSA) to obtain 2-((3-(tert-butyl)-4-hydroxy-5-isopropylbenzyl) thio) acetic acid. It was further refluxed with methanol and ethanol to obtain **6.1** and **6.2** respectively.



Scheme 6.1: Preparation of Ester of thiolated BHT. R= CH3, 6.1; R= CH2CH3, 6.2.

6.2.2 Rotary Bomb Oxidation test ASTM D2272

Rotary bomb oxidation test (RBOT) was carried out according to ASTM D 2272. A covered glass vessel containing 50 g of TMPTO base oil and TMPTO base oil with antioxidant, 5 mL of water and copper coil catalyst was placed in a stainless-steel vessel equipped with highly pure oxygen gas to a gauge pressure of 620 kPa (90 psi). Then the bomb was placed in an oil bath at a constant temperature of 150°C which rotated axially at an angle of 30° from the horizontal with 100 rpm. The pressure in the bomb become recorded with time, and the oxidation induction time (OIT) of RBOT is the time at which the maximum pressure of the bomb has dropped by 175.1 kPa (25.4 psi). Depletion of antioxidants causes the fast pressure drop due to the consumption of higher quantities of oxygen and it coincides to the time while oxidation rate of the oil inside the vessel is at its maximum.

6.2.3 Differential scanning calorimetry (DSC)

Differential scanning calorimetry was conducted for the evaluation of oxidation induction time and oxidation onset temperature by utilizing Perkin Elmer DSC 4000. For the assessment of OIT, 5 mg of sample oil was placed in an open aluminum pan under 2

bar pressures of nitrogen. Metal indium was used for the temperature calibration at 20°C heating rate. The sample was heated in nitrogen atmosphere until reached the isothermal temperature 150°C then switched to oxygen gas at 20 mL/min rate.

Programmed temperature DSC: This experiment was also carried out by the same instrument. 3mg of oil sample was placed in an open aluminum pan for the maximum interaction between oil and reactant gas in order to avoid gas diffusions limitations. Temperature scanning rate of 20°C/min was kept in the temperature ramping experiments and 99.99% pure oxygen flow was maintained at 20 ml/min

6.2.4 Thermogravimetric analysis (TGA)

Conventional TGA: The thermal stability of TMPTO in oxygen was inspected by examining the incipient oxidation temperature (IOT) by the way of thermogravimetric analysis (TGA) over a temperature limit from ambient to approximately 600°C, at scanning rate of 10°C/min using a thermal analyzer.

Isothermal TGA: Isothermal thermogravimetric analysis (TGA) was carried out using same TGA instrument in two different environments: oxygen or nitrogen with a flow rate of 50 mL/min. Isothermal TGA was accomplished in order to investigate the weight loss of oil sample caused by oxidation degradation. In this experiment, 5-10 mg of oil sample was heated to 250°C with a scanning rate of 20 °C/min and kept at 200 °C isothermally for 2 hours. The remaining weight of the sample during the heating time was determined.

6.3 Result and Discussion

6.3.1 Structure confirmation

The structure of (3,5-di-tert-butyl-4-hydroxy-benzylsulfanyl)-acetic acid methyl ester **6.1** was confirmed by Arifin and coworkers (Ariffin et al., 2014). Herein, **6.2** structure was confirmed by ^{1}H NMR, ^{13}C NMR and High-resolution Q-TOF mass analysis. 600 MHz and 150 MHz NMR spectrometer were used for ^{1}H NMR and ^{13}C NMR respectively and Tetramethylsilane was used as an interanl reference. Chemical shifts (δ) were accounted for in ppm with respect to the residual solvent peak (DMSO-d_6, δ = 2.5 for proton spectra; DMSO-d_6, δ = 40 for carbon spectra). Spectral peaks of ^{1}H NMR, ^{13}C NMR and obtained mass are given below. ^{1}H NMR (600 MHz, DMSO-d_6): $\boldsymbol{\delta}$ 7.03 (s, 1H), 6.92 (s, 1H), 4.10 (q, 2H, J= 7.2 Hz), 3.71 (s, 2H), 3.18 (s, 2H), 1.37 (s, 18H), 1.20 (t, 3H, J= 7.2 Hz); ^{13}C NMR (150 MHz, DMSO-d_6): $\boldsymbol{\delta}$ 170.5, 153.4, 139.7, 128.6, 125.7, 61.1, 36.3, 34.9, 32.7, 30.8, 14.5; HRMS (Q-TOF) m/z: $[M+H]^+$ Calcd for $C_{19}H_{31}O_3S^+$ 338.1916; Found 338.1912.

As seen in the ^{1}H NMR spectrum (Figure 6.1), singlet peak (indicated by 7) at 1.37 ppm with 18H represented the two tert-butyl group on the benzene ring. A triplet (1) with 3H and quartet (2) with 2H signal appeared at 1.20 ppm and 4.10 ppm respectively indicating the 5 hydrogens of the ethyl group. Two -CH_2 group (3 and 4) beside sulfur (seen in the structure in Figure 6.1) appeared as a singlet at 3.71 ppm and 3.18 ppm respectively. Hydroxyl group (-OH) and two hydrogens in benzene ring were observed at 6.92 and 7.03 ppm. These all peaks in ^{1}H NMR confirmed the structure of **6.2**.

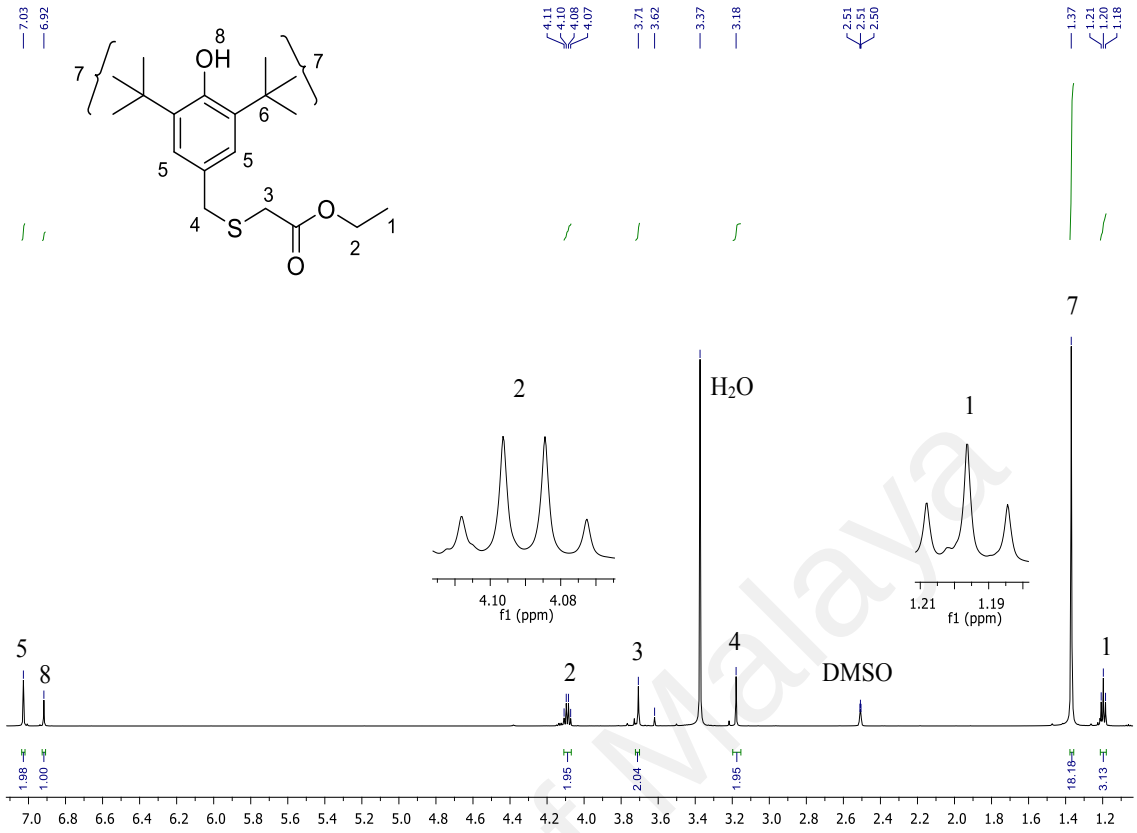


Figure 6.1: Confirmation of 6.2 structure by ^{1}H NMR.

6.3.2 Oxidation stability test

Differential Scanning Calorimetry (DSC), Rotary Bomb Oxidation Test (RBOT) and thermogravimetric analysis were performed in order to investigate the oxidation stability of trimethylolpropane trioleate (TMPTO) as synthetic ester-based lubricant oil with our synthesized compounds and commercial antioxidants. At the beginning, DSC was utilized for the determination of Oxidation induction time (OIT) and Oxidation Onset temperature (OOT) where OIT was measured at 150°C isothermal condition. OIT results of all the compounds were outlined in Figure 6.2. Three different weight percentage- 0.5 wt.%, 1.5 wt.%, 3 wt.% of experimental compounds were used to evaluate the oxidation stability of TMPTO (synthetic lubricant oil).

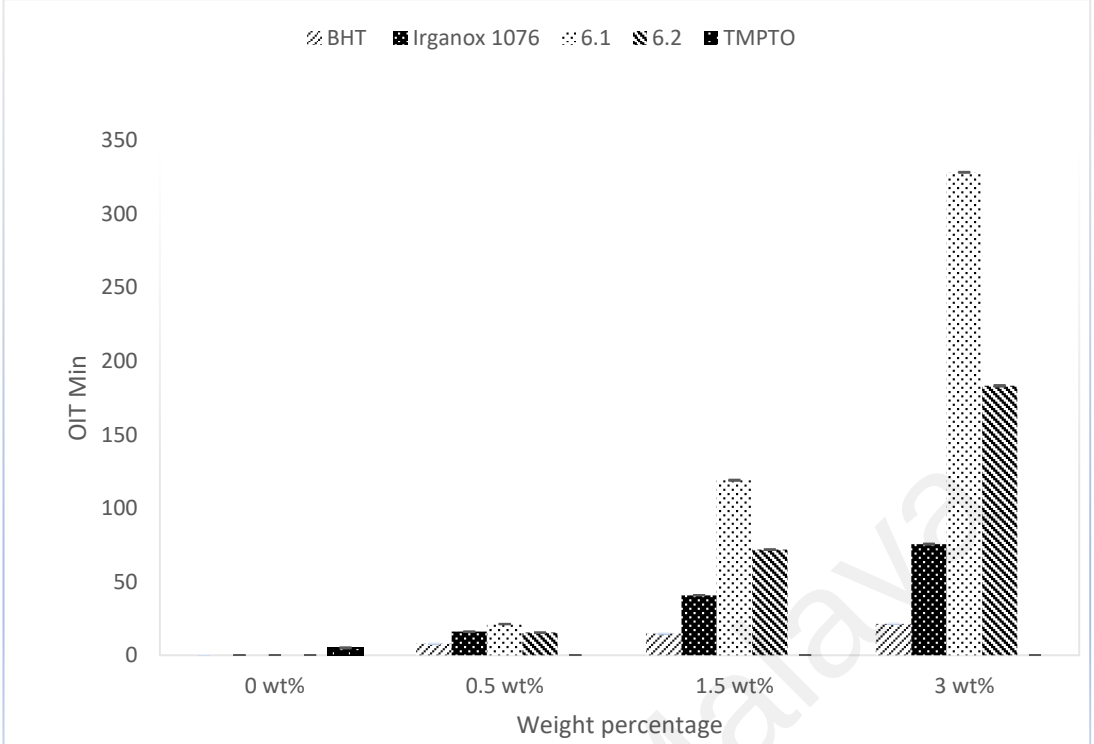


Figure 6.2: Oxidation Induction Time of three different wt.% obtained by DSC at 150°C isothermal temperature.

As seen in Figure 6.2, base oil TMPTO showed 5 min Oxidation induction time but it increased to 21.2 min and 15.39 min with the combination of 0.5 wt.% of methyl ester of BHT (6.1) and ethyl ester of BHT (6.2) respectively. Again, incorporation of 1.5 wt.% of antioxidants, TMPTO showed highest OIT 119.02 min for 6.1 and lowest OIT 14.83 for BHT. In addition, at 3 wt.% of experimental compounds, TMPTO with 6.1 exhibited 15 times stronger oxidation stability than BHT, 4.34 times better than Irganox 1076 and 1.8 times higher than 6.2.

Then temperature ramping experiment with a constant scanning rate of 20°C/min was carried out with the same experimental samples to evaluate their oxidation onset temperature showing in Figure 6.3. Oxidation onset temperature (OOT) for TMPTO base oil was observed 180.51°C. Interestingly, incorporation of 1.5 wt.% of experimental antioxidants, OOT of TMPTO increased by 30°C for synthesized methyl ester of thiolated BHT (6.1) than TMPTO base oil while it was increased by 20°C and 18°C for synthesized

ethyl ester (6.2) and Irganox 1076 respectively. TMPTO showed its highest OOT at 217.26°C with 3 wt.% 6.1 and lowest OOT was observed almost identical to TMPTO base oil with 0.5 wt.% BHT.

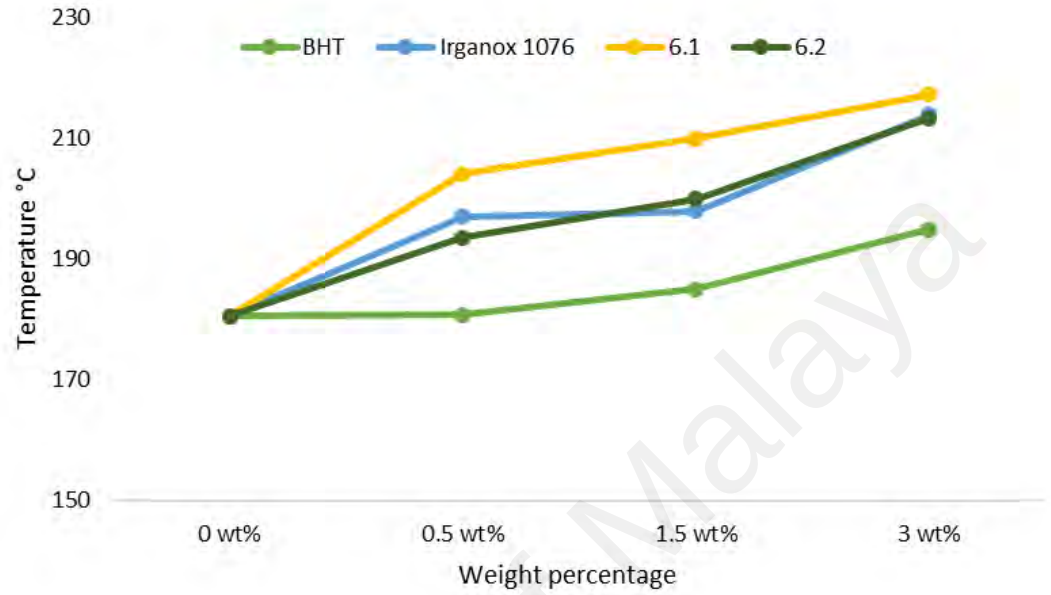


Figure 6.3: Oxidation Onset Temperature (OOT) of three different wt.% obtained by DSC.

After evaluating the OIT and OOT result obtained by DSC, it was clear that **6.1** showed better oxidation stability to synthetic lubricant oil in comparison with all the tested compounds. So, bulk oil oxidation test such as RBOT experiment was carried out with BHT, Irganox 1076 and **6.1** of three different weight percentage like before and the results were outlined in Figure 6.4.

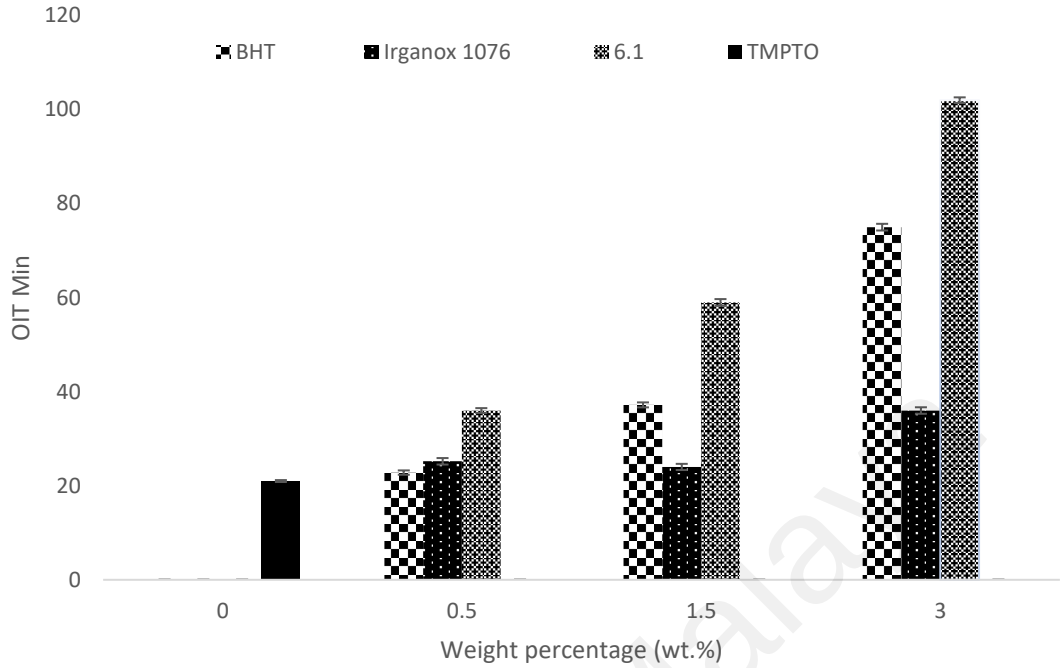


Figure 6.4: OIT obtained by RBOT for TMPTO, BHT, Irganox 1076 and 6.1 at three different wt.%.

Trimethylolpropane trioleate showed 21 min oxidation induction time without any antioxidant. There was no significance chages in OIT of TMPTO for the addition of 0.5 wt.% BHT but it was increased to 36 min for 0.5 wt.% 6.1. At 3 wt.%, Methyl ester of thiolated BHT brought highest oxidation stability 102 min to TMPTO while it was observed 75 min for BHT. Reviewing the RBOT results it is concluded that synthesized compound 6.1 exhibited stronger oxidation stability in TMPTO than the others antioxidant such BHT and Irganox 1076.

We can easily apprehend that our synthesized esters of thiolated BHT (**6.1** and **6.2**) are very successful for increasing the oxidation stability of TMPTO than the commercial antioxidants after evaluating the DSC and RBOT results. Now, for the further assessment of antioxidant blended oil, we carried out the conventional thermogravimetric analysis

(TGA) with TMPTO base oil and TMPTO with 1.5 wt.% experimental antioxidants in order to evaluate the thermal stability.

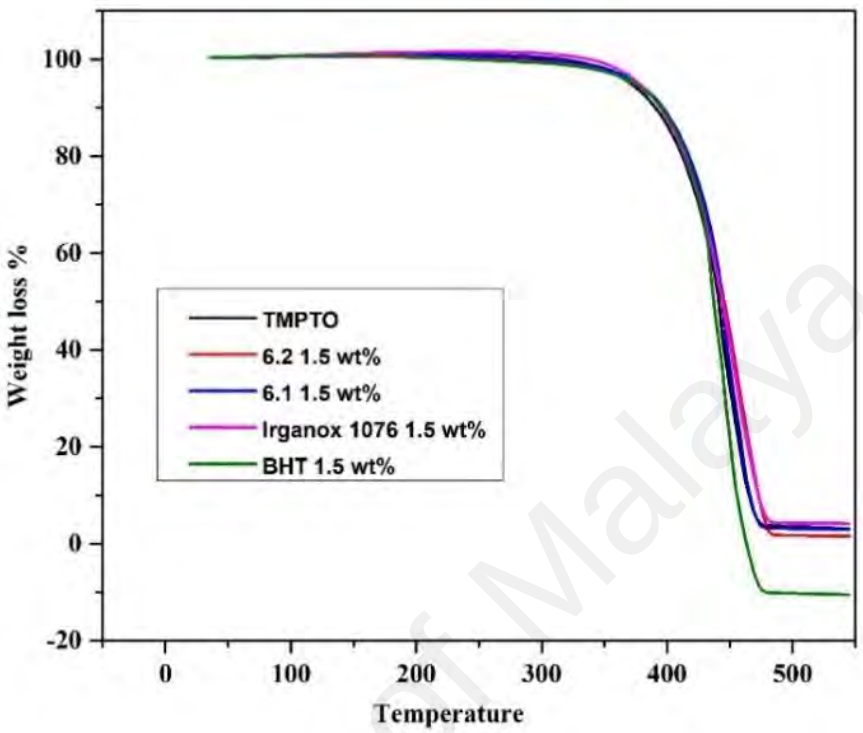
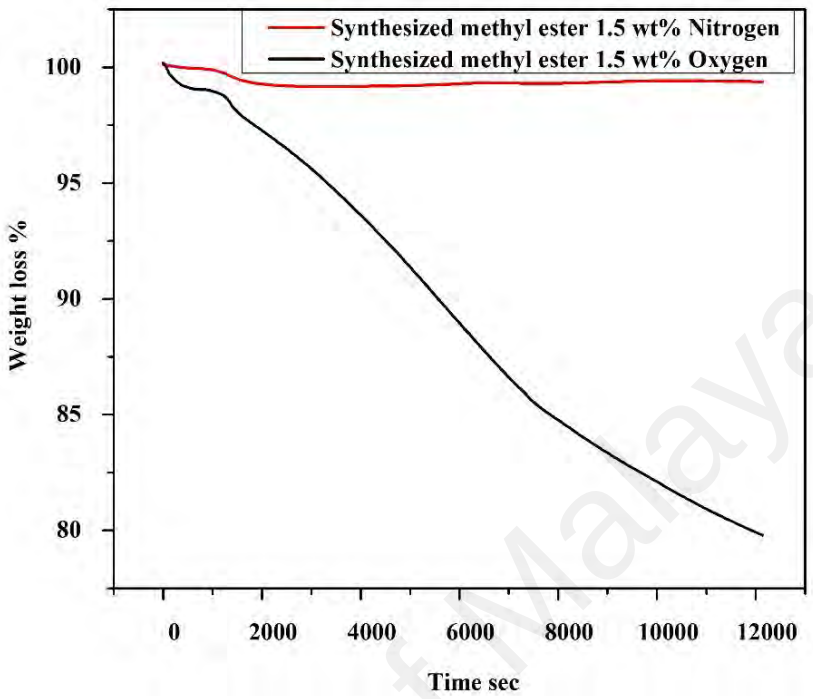


Figure 6.5: Thermal decomposition temperature of TMPTO base oil and TMPTO with 1.5 wt.% antioxidants obtained by thermogravimetric analysis.

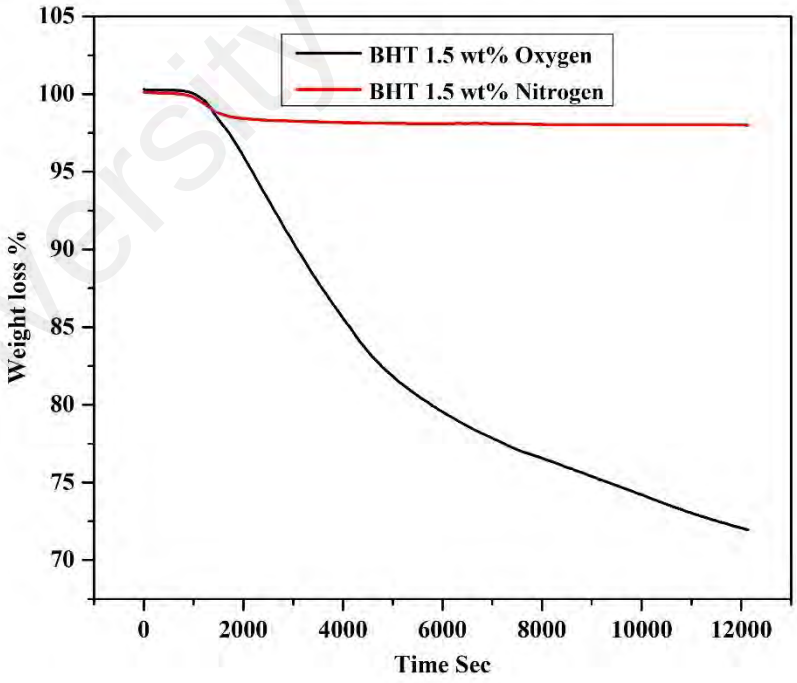
It was seen from Figure 6.5 that, TMPTO with 1.5 wt.% synthesized methyl ester **6.1** started decomposition at 411.551°C whereas only TMPTO decomposed at 398.003°C. On the other hand, 406.43°C, 400.72°C, 400.5°C decomposition temperatures were found for the TMPTO with 1.5 wt.% of ethyl ester **6.2**, BHT and Irganox 1076 respectively which indicates that synthesized methyl ester **6.1** showed the higher thermal stability in contrast with the other antioxidant.

The percentage of weight loss of 1.5 wt.% **6.1** and BHT blended TMPTO occurred by oxidation degradation was investigated by isothermal TGA analysis. In this experiment, the samples were heated at 250°C for 2 hours in the presence of nitrogen and oxygen

separately. The graphs of percentage of residuals versus time in the two-different environment were shown in Figure 6.6.



(a)



(b)

Figure 6.6: (a) weight percentage mass of synthesized methyl ester 6.1 1.5 wt.% formulated TMPTO in nitrogen and air environment (b) weight percentage mass of BHT 1.5 wt.% formulated TMPTO in nitrogen and air environment.

The weight loss during the heating in nitrogen environment was due to the evaporation and decomposition while in oxygen environment it was attributed to the evaporation, decomposition and oxidation. Therefore, comparing the weight loss in the two environments, weight loss due to the oxidation can easily be calculated (Mousavi et al., 2006). In Figure 6.6 (a), the rate of weight loss of 1.5 wt.% 6.1 blended TMPTO in presence of nitrogen was around 99% which was due to evaporation and decomposition whereas in oxygen environment it was about 80% which was occurred by the evaporation, decomposition and oxidation process. So, 19% weight loss was attributed to the oxidation process for 1.5 wt.% synthesized methyl ester formulated TMPTO. Again, according to Figure 6.6 (b), weight loss of 1.5 wt.% BHT formulated TMPTO was around 98% and 70% in nitrogen and oxygen environment respectively, thus around 28% weight loss was occurred due to the oxidation. So, weight loss of TMPTO due to the oxidation process was reduced by 1.5 wt.% **6.1** around 1.5 times better than BHT.

It was observed in the DSC (Figure 6.2) and RBOT (Figure 6.4) results, oxidation stability of trimethylolpropane trioleate (synthetic lubricant oil) increased more due to the presence of ester of thiolated butylated hydroxytoluene (6.1 and 6.2) in comparison with commercial antioxidant BHT and Irganox 1076. Thermogravimetric analysis (TGA) also revealed the greater thermal stability of reporting multifunctional antioxidants in lubricant oil. This higher activity of this reporting compounds (6.1 and 6.2) can be attributed to the presence of two different antioxidant functions in one structure. It was reported that antioxidants containing functional groups that can provide free radical scavenger activity and peroxide decomposer moiety exhibit autosynergy (Rudnick, 2017). The synthesized esters of thiolated butylated hydroxytoluene (6.1 and 6.2) having two functional groups (Figure 6.7 (A)) where, BHT moiety provide the radical scavenging and thioether provides peroxide decomposing functions, offer auto-synergistic antioxidant system.

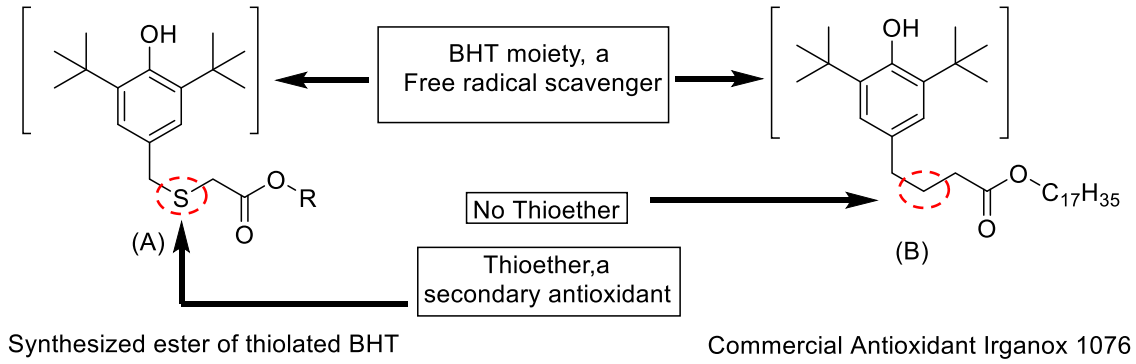


Figure 6.7: Structural comparison between ester of thiolated BHT and commercial AO.

This auto-synergistic effect of the thiolated BHT provide the greater oxidation stability to synthetic lubricant than the individual commercial antioxidant. It was observed in Figure 6.7 that thiolated BHT and Irganox 1076 molecule comprise of BHT and ester moiety, but esters of thiolated BHT becomes more oxidation and thermal resistant due to the presence of thioether. So, it can be said that, presence of thioether in the synthesized compounds provide autosynergy with BHT moiety that make the compounds more efficient to inhibit premature oxidation degradation. Again Irganox 1076 exhibited greater oxidation inhibition than BHT as seen in DSC results, consequently it can also be stated that ether moiety of Irganox 1076 molecule plays significant role to show better oxidation resistance. In addition, it was observed in DSC and TGA results methyl ester of thiolated BHT (6.1) showed more oxidation and thermal stability than ethyl ester of thiolated BHT (6.2), it may anticipate that number of carbons in the ester chain has significance contribution to the activity of our synthesized compounds. Thus, the presence of three important antioxidant components- free radical scavenger (BHT moiety), peroxide decomposer (thioether), and ester moiety in one structure (6.1 and 6.2) can make efficient multifunctional antioxidant.

6.4 Conclusion

Based on the above results and discussion, we can easily conclude that the reported methyl ester of thiolated BHT **6.1** prolonged better oxidation induction time of ester-based lubricant (TMPTO) in any proportion compared to the commercial antioxidants such as BHT and Irganox 1076. In addition, Oxidation Induction Time and Oxidation Decomposition Temperature from DSC and TGA result helped us to make an assumption that lower number of carbons of ester moiety in synthesized structure provides the better oxidation stability. Moreover, weight loss of TMPTO due to the oxidation process was reduced by 1.5 wt.% **6.1** around 1.5 times compared to commercial antioxidants shown in isothermal TGA. This higher oxidation and thermal stability of designated compounds is due to the presence of three important antioxidant moieties in one structure; free radical scavenger (BHT), peroxide decomposer (thioether) and ester radical which give a strong auto-synergistic effect. Further investigations on this multifunctional antioxidant are expected to a promising result which may help the synthetic lubricant oil to get worldwide acceptance.

CHAPTER 7: CONCLUSION AND RECOMMENDATIONS

Based on the rational design, four structures were envisioned as multifunctional antioxidants which were the combination of two or more antioxidant functional groups. First two structures were envisioned as the combination of two types of butylated hydroxyphenyl (BHP) and semicarbazones. Two types of BHP are 3,5-di-tert-butyl-4-hydroxyphenyl and 3,5-di-tert-butyl-2-hydroxyphenyl. The rest two structures were designed by the combination of 3,5-di-tert-butyl-4-hydroxyphenyl, thioether, and secondary aromatic amine. Many reaction protocols were investigated based on the literature review to obtain the designated structure. Most of the contemporary reaction methods were found limited to several aspects such as functional group incompatible, poor yields and others. In an effort to obtain rational designated multipotent or multifunctional antioxidants, a new efficient and robust reaction protocol for the synthesis of semicarbazones was established in this study to skip the limitations of existing reported methods. A variety of semicarbazones were accessed by the established method to ensure the versatility of this method. Few conclusions had been made on the newly established method in this study:

I. A variety of substituted semicarbazides can be obtained by a very simple reaction of acid hydrazide and aryl isocyanate at room temperature without any catalyst. The reaction completion period of this reaction is very short and product yields are noticeably higher.

II. A nucleophilic substitution reaction between substituted semicarbazides and a variety of benzaldehyde upon heating in presence of acidic ethanol leads to obtain an extensive assortment of semicarbazones with greater yields.

III. Since substituted semicarbazides contain no terminal hydrazide group or amine, it can be easily converted into a wide range of semicarbazones bearing

reactive functional groups such as ester, acetyl which are very prone to react with terminal amine.

IV. The presence of (3,5-di-tert-butyl-4-hydroxy-benzylsulfanyl)-acetic acid ethyl ester (8) as by-product in the nucleophilic substitution reactions proves participation of solvent (ethanol) into reaction. And this by-product might be used in the preparation of acid hydrazide 3 following the reaction Scheme 4.2.

Since semicarbazones are well-known for their several important biological properties such as anticonvulsant, anticancer, antitumor and others., so such important properties can easily be expected from these synthesized semicarbazones (28 compounds) which were prepared for method validation and this would be one of the future researchs works of this present study.

3,5-di-tert-butyl-4-hydroxyphenyl and 3,5-di-tert-butyl-2-hydroxyphenyl incorporated semicarbazones were prepared with good yields by following newly established reaction method. DPPH assay for antioxidant evaluation and differential scanning calorimeter test for oil oxidative stability assessment have drawn the following conclusions:

I. 3,5-di-tert-butyl-4-hydroxyphenyl incorporated semicarbazones (5.7a - 5.7j) showed excellent free radical scavenging properties in comparison with BHT and 3,5-di-tert-butyl-2-hydroxyphenyl incorporated semicarbazones (5.10a - 5.10j).

II. In 3,5-di-tert-butyl-2-hydroxyphenyl incorporated semicarbazones structure, position of hindered hydroxyl group and donor nitrogen atom are favorable for the formation of hydrogen bond. Due to the formation of Hydrogen bond, antioxidant activity of these semicarbazones decrease significantly in

comparison with 3,5-di-tert-butyl-4-hydroxyphenyl incorporated semicarbazones.

III. Electron withdrawing substituents at N-4 ring of semicarbazones increase the antioxidant activity while electron donating substituents decrease the activity.

IV. Oxidation stability assessment of some of the 3,5-di-tert-butyl-4-hydroxyphenyl incorporated semicarbazones (5.7a, 5.7b, 5.7c, 5.7e, 5.7g, 5.9a) were done blending in trimethylolpropane trioleate (TMPTO) by deferential scanning calorimeter test. Compounds having electron withdrawing substituents at ortho or meta position of N-4 ring were found better oxidation stability of TMPTO oil than BHT.

V. Higher oxidation stability of the N^1-(2,4-di-tert-butyl-4-hydroxybenzylidene)-N^4-(3-methoxyphenyl)-semicarbazone (5.7e) and N^1-(2,4-di-tert-butyl-4-hydroxybenzylidene)-N^4-(3-methoxyphenyl)-semicarbazone (5.7b) in TMPTO can be attributed for the better metal deactivating properties. This is because that the multiple donor atoms reside at the favorable position of this structure that can deactivate metal ions by the formation of coordinate bond with metal ions.

These synthesized BHP incorporated semicarbazones can be expected to possess others important biological properties and can also be studied further on detailed biological activities of these compounds since DPPH assay showed their promising antioxidant properties. Therefore, incorporation of BHP into semicarbazones would make the important multipotent antioxidants.

Esters of thiolated butylated hydroxytoluene is the last designated multifunctional structure in this study that is combination of BHP, thioether and ester moiety. Methyl and ethyl ester of thiolated butylated hydroxytoluene (6.1 and 6.1) were obtained in the early

steps of the established semicarbazones method and elaborately investigated their oxidation stability in synthetic lubricant oil (TMPTO) and following conclusions were obtained:

I. Methyl ester of thiolated BHT (6.AO1) showed excellent oxidative stability in every oxidation and thermal stability test such as differential scanning calorimeter (DSC), Rotary bomb oxidation test (RBOT) and thermogravimetric analysis (TGA) in comparison with ethyl ester of thiolated BHT (6.2) and commercial antioxidants namely. BHT and Irganox 1076.

II. Combination of primary and secondary antioxidant (BHT and thioether) in one structure exhibit auto-synergistic effect that are responsible for the higher oxidative stability of these compounds.

Therefore, compounds having multiple different antioxidants function show higher oxidation inhibition due to the auto-synergism. In addition, substituted semicarbazides (5.3a - 5.3j) obtained in the semicarbazone reaction protocol are the combination of 3,5-di-tert-butyl-4-hydroxyphenyl (BHP), thioether and secondary aromatic amine those are the one of the designated multifunctional antioxidant structures in this study. So, substituted semicarbazide can easily be anticipated to have excellent multifunctional antioxidant activity because of their combined structure of primary and secondary antioxidants.

In the future study, several biological properties such as anticonvulsant, anticancer and antitumor properties can be studied with these synthesized semicarbazones in this study. Furthermore, nucleophilic substitution reaction of substituted semicarbazide can be investigated with several nucleophilic molecules.

REFERENCES

Abraham, W. D., Adams, P. E., Lamb, G. D., Wolak, T. J., Denis, R. A., Kocsis, J. A., . . . Cowling, S. V. (2003). Lubricant compositions containing ester-substituted hindered phenol antioxidants: Google Patents.

Afrasiabi, Z., Sinn, E., Lin, W., Ma, Y., Campana, C., & Padhye, S. (2005). Nickel (II) complexes of naphthaquinone thiosemicarbazone and semicarbazone: Synthesis, structure, spectroscopy, and biological activity. *Journal of inorganic biochemistry, 99*(7), 1526-1531.

Aggarwal, B. B., Kumar, A., & Bharti, A. C. (2003). Anticancer potential of curcumin: preclinical and clinical studies. *Anticancer research, 23*(1/A), 363-398.

Aguilar, G., Mazzamaro, G., & Rasberger, M. (2010). Oxidative degradation and stabilisation of mineral oil-based lubricants *Chemistry and Technology of Lubricants* (pp. 107-152): Springer.

Ahsan, M., Samy, J., Khalilullah, H., & Nomani, M. S. (2011). Semicarbazone analogues: A mini review. *Der Pharmacia Sinica, 2*(6), 107-113.

Alamed, J., Chaiyasit, W., McClements, D. J., & Decker, E. A. (2009). Relationships between free radical scavenging and antioxidant activity in foods. *Journal of Agricultural and Food Chemistry, 57*(7), 2969-2976.

Alias, N. H., Yunus, R., Idris, A., & Omar, R. (2009). Effects of additives on oxidation characteristics of palm oil-based trimethylolpropane ester in hydraulics applications. *European journal of lipid science and technology, 111*(4), 368-375.

Anderson, D. J. (1969). Polyalkylene sulfides, sulfoxides and sulfones: Google Patents.

Andersson, D. C., Fauconnier, J., Yamada, T., Lacampagne, A., Zhang, S. J., Katz, A., & Westerblad, H. (2011). Mitochondrial production of reactive oxygen species contributes to the β-adrenergic stimulation of mouse cardiomycytes. *The Journal of physiology, 589*(7), 1791-1801.

André, C., Castanheira, I., Cruz, J., Paseiro, P., & Sanches-Silva, A. (2010). Analytical strategies to evaluate antioxidants in food: a review. *Trends in food science & technology, 21*(5), 229-246.

Anitha, P., Chitrapriya, N., Jang, Y. J., & Viswanathamurthi, P. (2013). Synthesis, characterization, DNA interaction, antioxidant and anticancer activity of new ruthenium (II) complexes of thiosemicarbazone/semicarbazone bearing 9, 10-phenanthrenequinone. *Journal of Photochemistry and Photobiology B: Biology, 129*, 17-26.

Ariffin, A., Rahman, N. A., Yehye, W. A., Alhadi, A. A., & Kadir, F. A. (2014). PASS-assisted design, synthesis and antioxidant evaluation of new butylated hydroxytoluene derivatives. *Eur. J. Med. Chem., 87*, 564-577.

Asis, S., Bruno, A., Molina, D., Conti, G., & Gaozza, C. (1996). Synthesis, DNA interaction and antineoplastic activity of semicarbazone derivatives. *Farmaco (Societa chimica italiana: 1989), 51*(6), 419-423.

Asís, S. E., Bruno, A. M., Martínez, A. R., Sevilla, M. a. V., Gaozza, C. H., Romano, A. M., . . . Ciccia, G. (1999). Diarylsemicarbazones: synthesis, antineoplastic activity and topoisomerase I inhibition assay. *Il Farmaco, 54*(8), 517-523.

Azam, F., El-gnidi, B. A., & Alkskas, I. A. (2010). Combating oxidative stress in epilepsy: design, synthesis, quantum chemical studies and anticonvulsant evaluation of 1-(substituted benzylidene/ethylidene)-4-(naphthalen-1-yl) semicarbazides. *Eur. J. Med. Chem., 45*(7), 2817-2826.

Bala, V., Hartley, R. J., & Hughes, L. J. (1996). The influence of chemical structure on the oxidative stability of organic sulfides. *Lubrication engineering, 52*(12), 868-874.

Barnes, A. M., Bartle, K. D., & Thibon, V. R. (2001). A review of zinc dialkyldithiophosphates (ZDDPS): characterisation and role in the lubricating oil. *Tribology International, 34*(6), 389-395.

Bec, S., Tonck, A., Georges, J.-M., Coy, R., Bell, J., & Roper, G. (1999). *Relationship between mechanical properties and structures of zinc dithiophosphate anti–wear films.* Paper presented at the Proceedings of the Royal Society of London A: Mathematical, Physical and Engineering Sciences.

Becker, R., & Knorr, A. (1996). An evaluation of antioxidants for vegetable oils at elevated temperatures. *Lubrication Science, 8*(2), 95-117.

Biparva, P., Ehsani, M., & Hadjmohammadi, M. R. (2012). Dispersive liquid–liquid microextraction using extraction solvents lighter than water combined with high performance liquid chromatography for determination of synthetic antioxidants in fruit juice samples. *Journal of Food Composition and Analysis, 27*(1), 87-94.

Blois, M. S. (1958). Antioxidant Determination by the Use of a Stable Free Radical. *Nature, 181*(4617), 1199-1200.

Bradley, D. G., & Min, D. B. (1992). Singlet oxygen oxidation of foods. *Critical Reviews in Food Science & Nutrition, 31*(3), 211-236.

Braid, M. (1985). Phenol-hindered phenol borates and lubricant compositions containing same: Google Patents.

Bridgewater, A. J., & Sexton, M. D. (1978). Mechanism of antioxidant action: reactions of alkyl and aryl sulphides with hydroperoxides. *Journal of the Chemical Society, Perkin Transactions 2*(6), 530-536.

Brigati, G., Lucarini, M., Mugnaini, V., & Pedulli, G. F. (2002). Determination of the substituent effect on the O− H bond dissociation enthalpies of phenolic antioxidants by the EPR radical equilibration technique. *The Journal of organic chemistry, 67*(14), 4828-4832.

Buck, D. (1984). Food antioxidants: applications and uses in snack foods. *Cereal Foods World (USA)*.

Casas, J., Garcıa-Tasende, M., & Sordo, J. (2000). Main group metal complexes of semicarbazones and thiosemicarbazones. A structural review. *Coordination Chemistry Reviews, 209*(1), 197-261.

Chalk, A., & Smith, J. (1957). Catalysis of cyclohexene autoxidation by trace metals in non-polar media. Part 2. Metal salts in the presence of chelating agents. *Transactions of the Faraday Society, 53*, 1235-1245.

Chatterjee, R., Bandyopadhyay, U., Mazumdar, A., & Banerjee, R. K. (1996). Lactoperoxidase-catalysed oxidation of indomethacin, a nonsteroidal antiinflammatory drug, through the formation of a free radical. *Biochemical pharmacology, 52*(8), 1169-1175.

Chen, H., Yan, J., Ren, T., Zhao, Y., & Zheng, L. (2012). Tribological behavior of some long-chain dimercaptothiadiazole derivatives as multifunctional lubricant additives in vegetable oil and investigation of their tribochemistry using XANES. *Tribology Letters, 45*(3), 465-476.

Chen, J., Xu, X., Zeng, X., Feng, M., Qu, R., Wang, Z., . . . Sharma, V. K. (2018). Ferrate (VI) oxidation of polychlorinated diphenyl sulfides: Kinetics, degradation, and oxidized products. *Water research*.

Coker, S. B. (1986). The use of phenacemide for intractable partial complex epilepsy in children. *Pediatric neurology, 2*(4), 230-232.

Colclough, T. (1993). Lubricating oil oxidation and stabilisation. *Atmospheric oxidation and antioxidants, 2*.

Colclough, T., Beltzer, M., & Habeeb, J. J. (1996). Lubricating compositions: Google Patents.

David, M. (2002). GF-4 engine oil spec unveiled. *Lube Report, 3*(31), 1.

de Guzman, R., Tang, H., Salley, S., & Ng, K. S. (2009). Synergistic effects of antioxidants on the oxidative stability of soybean oil-and poultry fat-based biodiesel. *Journal of the American Oil Chemists' Society, 86*(5), 459.

de Oliveira, R. B., de Souza-Fagundes, E. M., Soares, R. P., Andrade, A. A., Krettli, A. U., & Zani, C. L. (2008). Synthesis and antimalarial activity of semicarbazone and thiosemicarbazone derivatives. *European journal of medicinal chemistry, 43*(9), 1983-1988.

Denisov, E., & Denisova, T. (2013). Dissociation energies of OH and NH bonds in hybrid antioxidants. *Kinetics and Catalysis, 54*(6), 677-685.

DeVries, L., & King, J. M. (1981). Process of preparing molybdenum complexes, the complexes so-produced and lubricants containing same: Google Patents.

Dexter, M., Thomas, R. W., & King III, R. E. (2002). Antioxidants. *Encyclopedia of Polymer Science and Technology*.

Dimmock, J., & Baker, G. (1994). Anticonvulsant Activities of 4–Bromobenzaldehyde Semicarbazone. *Epilepsia, 35*(3), 648-655.

Dimmock, J. R., Puthucode, R. N., Smith, J. M., Hetherington, M., Quail, J. W., Pugazhenthi, U., . . . Stables, J. P. (1996). (Aryloxy) aryl semicarbazones and related compounds: a novel class of anticonvulsant agents possessing high activity in the maximal electroshock screen. *Journal of medicinal chemistry, 39*(20), 3984-3997.

Dimmock, J. R., Puthucode, R. N., Tuchek, J., Baker, G. B., Hinko, C. N., Steinmiller, C. L., & Stables, J. P. (1999). Anticonvulsant activities of 4-(4′-fluorophenoxy) benzaldehyde semicarbazone. *Drug development research, 46*(2), 112-125.

Dolatabadi, J. E. N., & Kashanian, S. (2010). A review on DNA interaction with synthetic phenolic food additives. *Food Research International, 43*(5), 1223-1230.

Donaldson, M. S. (2004). Nutrition and cancer: a review of the evidence for an anti-cancer diet. *Nutrition Journal, 3*(1), 19.

Douchez, A., & Lubell, W. D. (2015). Chemoselective alkylation for diversity-oriented synthesis of 1, 3, 4-benzotriazepin-2-ones and pyrrolo [1, 2][1, 3, 4] benzotriazepin-6-ones, potential turn surrogates. *Org. Lett., 17*(24), 6046-6049.

Dresel, W. (2007). *Lubricants and lubrication*: John Wiley & Sons.

Droge, W. (2002). Free radicals in the physiological control of cell function. *Physiological reviews, 82*(1), 47-95.

Drummond, J. T., & Johnson, G. (1988). Selective method for the preparation of isomeric N-alkyl and N-aryl-3 (5)-amino-5 (3)-hydroxy-1H-pyrazole-1-carboxamides. *Journal of heterocyclic chemistry, 25*(4), 1123-1127.

Duangkaewmanee, S., & Petsom, A. (2011). Synergistic and antagonistic effects on oxidation stability of antioxidants in a synthetic ester based oil. *Tribology International, 44*(3), 266-271.

Dugger, R. W., Ragan, J. A., & Ripin, D. H. B. (2005). Survey of GMP bulk reactions run in a research facility between 1985 and 2002. *Org. Process Res. Dev., 9*(3), 253-258.

Dunn, R. O. (2005). Effect of antioxidants on the oxidative stability of methyl soyate (biodiesel). *Fuel Processing Technology, 86*(10), 1071-1085.

Dutta, S., Padhye, S., Priyadarsini, K. I., & Newton, C. (2005). Antioxidant and antiproliferative activity of curcumin semicarbazone. *Bioorganic & medicinal chemistry letters, 15*(11), 2738-2744.

Elger, C., Halász, P., Maia, J., Almeida, L., Soares-da-Silva, P., & Group, B. I. S. (2009). Efficacy and safety of eslicarbazepine acetate as adjunctive treatment in adults

with refractory partial-onset seizures: a randomized, double-blind, placebo-controlled, parallel-group phase III study. *Epilepsia, 50*(3), 454-463.

Enyedy, É. A., Bognár, G. M., Nagy, N. V., Jakusch, T., Kiss, T., & Gambino, D. (2014). Solution speciation of potential anticancer metal complexes of salicylaldehyde semicarbazone and its bromo derivative. *Polyhedron, 67*, 242-252.

Erhan, S. Z., & Asadauskas, S. (2000). Lubricant basestocks from vegetable oils. *Industrial crops and products, 11*(2), 277-282.

Farzaliev, V., Fernando, W., & Scott, G. (1978). Mechanisms of antioxidant action: Auto-synergistic behaviour of sulphur-containing phenols. *European Polymer Journal, 14*(10), 785-788.

Field, I. P. (1998). Lubricating compositions: Google Patents.

Finkel, T., & Holbrook, N. J. (2000). Oxidants, oxidative stress and the biology of ageing. *Nature, 408*(6809), 239.

Fox, N., & Stachowiak, G. (2007). Vegetable oil-based lubricants—a review of oxidation. *Tribology International, 40*(7), 1035-1046.

Frei, B. (1994). Reactive oxygen species and antioxidant vitamins: mechanisms of action. *The American journal of medicine, 97*(3), S5-S13.

Fuller, M. F. (2004). *The encyclopedia of farm animal nutrition*: Cabi.

Garland, K., Gan, W., Depatie-Sicard, C., & Beauchemin, A. M. (2013). A Practical Approach to Semicarbazone and Hydrazone Derivatives via Imino-isocyanates. *Organic letters, 15*(16), 4074-4077.

Garland, K., Gan, W., Depatie-Sicard, C., & Beauchemin, A. M. (2013). A Practical Approach to Semicarbazone and Hydrazone Derivatives via Imino-isocyanates. *Org. Lett., 15*(16), 4074-4077.

Genestra, M. (2007). Oxyl radicals, redox-sensitive signalling cascades and antioxidants. *Cellular signalling, 19*(9), 1807-1819.

Ghaemi, S. N., Berv, D. A., Klugman, J., Rosenquist, K. J., & Hsu, D. J. (2003). Oxcarbazepine treatment of bipolar disorder. *The Journal of clinical psychiatry, 64*(8), 943-945.

Ghosal, S. (2005). Phenolic antioxidant-chromium complexes for treatment or prevention of type 2 diabetes or glucose intolerance: Google Patents.

Gryglewicz, S., Piechocki, W., & Gryglewicz, G. (2003). Preparation of polyol esters based on vegetable and animal fats. *Bioresource Technology, 87*(1), 35-39.

Habeeb, J., Rogers, W., & May, C. (1987). The role of hydroperoxide in engine wear and the effect of ZnDDP/dispersant/detergent interactions. *SAE transactions*, 998-1009.

Haidasz, E. A., Shah, R., & Pratt, D. A. (2014). The catalytic mechanism of diarylamine radical-trapping antioxidants. *Journal of the American Chemical Society, 136*(47), 16643-16650.

Hakan, K. (2001). The impact of crankcase oil containing phosphorus on catalytic converters and engine exhaust emissions. *Industrial Lubrication and Tribology, 53*(6), 237-255.

Halliwell, B. (2007). Biochemistry of oxidative stress: Portland Press Limited.

Hancsók, J., Bubálik, M., Beck, Á., & Baladincz, J. (2008). Development of multifunctional additives based on vegetable oils for high quality diesel and biodiesel. *Chemical Engineering research and design, 86*(7), 793-799.

Hand, C. E., & Honek, J. F. (2005). Biological chemistry of naturally occurring thiols of microbial and marine origin. *Journal of natural products, 68*(2), 293-308.

Hanneken, A., Lin, F.-F., Johnson, J., & Maher, P. (2006). Flavonoids protect human retinal pigment epithelial cells from oxidative-stress–induced death. *Investigative ophthalmology & visual science, 47*(7), 3164-3177.

Hardman, J., & Limbird, L. (2001). Goodman and Gilman's the pharmacological basis of therapeutics. 10th edn McGraw-Hill. *New York*.

Hawkins, W. L. (1984). Polymer degradation *Polymer Degradation and Stabilization* (pp. 3-34): Springer.

He, Z., Rao, W., Ren, T., Liu, W., & Xue, Q. (2002). The tribochemical study of some N-containing heterocyclic compounds as lubricating oil additives. *Tribology Letters, 13*(2), 87-93.

Hertog, M. G., Feskens, E. J., Kromhout, D., Hollman, P., & Katan, M. (1993). Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *The lancet, 342*(8878), 1007-1011.

Hilton, J. W. (1989). Antioxidants: function, types and necessity of inclusion in pet foods. *The Canadian Veterinary Journal, 30*(8), 682.

Holzinger, R. J. (1964). Extreme pressure lubricants: Google Patents.

Horodysky, A. G. (1983). Borated epoxides and lubricants containing same: Google Patents.

Horodysky, A. G. (1988). Borated hydroxyl-containing compositions and lubricants containing same: Google Patents.

Horodysky, A. G., & Herd, R. S. (1985). Etherdiamine borates and lubricants containing same: Google Patents.

Horodysky, A. G., & Kaminski, J. M. (1981). Friction reducing additives and compositions thereof: Google Patents.

Horodysky, A. G., & Kaminski, J. M. (1984). Friction reducing additives and compositions thereof: Google Patents.

Hovorka, S. W., & Schöneich, C. (2001). Oxidative degradation of pharmaceuticals: theory, mechanisms and inhibition. *Journal of pharmaceutical sciences, 90*(3), 253-269.

Hu, J.-Q., Hu, Y.-Q., Qiu, Z.-H., & Sun, Y.-B. (2007). *Evaluation on synergistic antioxidation of molybdenum dialkydithiocarbamate with arylamine antioxidant* (0148-7191). Retrieved from

Hu, J.-Q., Wei, X.-Y., Dai, G.-L., Fei, Y.-W., Liu, C.-C., Zong, Z.-M., & Yao, J.-B. (2007). Synergistic antioxidation of organic molybdenum complex with dithiocarbamate antioxidant evaluated by differential scanning calorimetry and thin film micro oxidation test. *Thermochimica Acta, 453*(1), 21-26.

Hu, J.-Q., Wei, X.-Y., Yao, J.-B., Han, L., & Zong, Z.-M. (2006). Evaluation of molybdate ester as a synergist for arylamine antioxidant in lubricants. *Tribology International, 39*(11), 1469-1473.

Huang, L., Ma, J., Wang, X., Zhang, P., Yu, L., & Zhang, S. (2018). Mesoporous silica nanoparticles-loaded methyl 3-(3, 5-di-tert-butyl-4-hydroxyphenyl) propanoate as a smart antioxidant of synthetic ester oil. *Tribology International, 121*, 114-120.

Huang, W., Hou, B., Zhang, P., & Dong, J. (2004). Tribological performance and action mechanism of S-[2-(acetamido) thiazol-1-yl] dialkyl dithiocarbamate as additive in rapeseed oil. *Wear, 256*(11-12), 1106-1113.

Hudson, B. J. (2012). *Food antioxidants*: Springer Science & Business Media.

Is, Y., & Woodside, J. (2001). Antioxidant in health and disease. *J Clin Pathol, 54*(3), 176-186.

Jafri, L., Ansari, F. L., Jamil, M., Kalsoom, S., Qureishi, S., & Mirza, B. (2012). Microwave-assisted Synthesis and Bioevaluation of Some Semicarbazones. *Chemical biology & drug design, 79*(6), 950-959.

Jain, J., Kumar, Y., Sinha, R., Kumar, R., & Stables, J. (2011). Menthone aryl acid hydrazones: a new class of anticonvulsants. *Medicinal Chemistry, 7*(1), 56-61.

Jones, M. C., Jones, A. R., & Strickland, B. R. (1952). 2, 6-Di-tert-butyl-4-methylphenol as a gasoline antioxidant. *Industrial & Engineering Chemistry, 44*(11), 2721-2725.

Jung, M. E., Chamberlain, B. T., Ho, C.-L. C., Gillespie, E. J., & Bradley, K. A. (2014). Structure–Activity Relationship of Semicarbazone EGA Furnishes Photoaffinity Inhibitors of Anthrax Toxin Cellular Entry. *ACS Med. Chem. Lett., 5*(4), 363.

Kar, S., Subbaram, S., Carrico, P. M., & Melendez, J. A. (2010). Redox-control of matrix metalloproteinase-1: a critical link between free radicals, matrix remodeling and degenerative disease. *Respiratory physiology & neurobiology, 174*(3), 299-306.

Karavalakis, G., & Stournas, S. (2010). Impact of antioxidant additives on the oxidation stability of diesel/biodiesel blends. *Energy & Fuels, 24*(6), 3682-3686.

Karol, T. J., Donnelly, S. G., & Hiza, R. J. (2004). Antioxidant additive compositions and lubricating compositions containing the same: Google Patents.

Kashanian, S., & Dolatabadi, J. E. N. (2009). DNA binding studies of 2-tert-butylhydroquinone (TBHQ) food additive. *Food Chemistry, 116*(3), 743-747.

Kato, T., Ozaki, T., Tamura, K., Suzuki, Y., Akima, M., & Ohi, N. (1999). Novel calcium antagonists with both calcium overload inhibition and antioxidant activity. 2. Structure− activity relationships of thiazolidinone derivatives. *Journal of medicinal chemistry, 42*(16), 3134-3146.

Khalil, Z., & Khodr, B. (2001). A role for free radicals and nitric oxide in delayed recovery in aged rats with chronic constriction nerve injury. *Free Radical Biology and Medicine, 31*(4), 430-439.

Kiriliauskaitė, V., Bendikienė, V., & Juodka, B. (2011). Synthesis of trimethylolpropane esters of oleic acid by Lipoprime 50T. *Journal of industrial microbiology & biotechnology, 38*(9), 1561-1566.

Klaus, E., Duda, J., & Wang, J. C. (1992). Study of copper salts as high-temperature oxidation inhibitors. *Tribology transactions, 35*(2), 316-324.

Klein, E., Lukeš, V., Cibulková, Z., & Polovková, J. (2006). Study of N–H, O–H, and S–H bond dissociation enthalpies and ionization potentials of substituted anilines, phenols, and thiophenols. *Journal of Molecular Structure: THEOCHEM, 758*(2-3), 149-159.

Koch, F. W. (1993). Boron-containing compositions and lubricants containing them: Google Patents.

Koleva, I. I., Van Beek, T. A., Linssen, J. P., Groot, A. d., & Evstatieva, L. N. (2002). Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques, 13*(1), 8-17.

Korcek, S., Johnson, M., Jensen, R., & Zinbo, M. (1986). Determination of the High Temperature Antioxidant Capability of Lubricants and Lubricant Components. *Industrial & engineering chemistry product research and development, 25*(4), 621-627.

Kouřimská, L., Pokorný, J., & Tirzitis, G. (1993). The antioxidant acitivity of 2, 6-dimethyl-3, 5-diethoxycarbonyl-1, 4-dihydropyridine in edible oils. *Food/Nahrung, 37*(1), 91-93.

Kühnau, J. (1976). The flavonoids. A class of semi-essential food components: their role in human nutrition *World review of nutrition and dietetics* (Vol. 24, pp. 117-191): Karger Publishers.

Kunchandy, E., & Rao, M. (1990). Oxygen radical scavenging activity of curcumin. *International Journal of Pharmaceutics, 58*(3), 237-240.

Layer, R. W. (1963). The Chemistry of Imines. *Chemical Reviews, 63*(5), 489-510.

Lee, J., Koo, N., & Min, D. B. (2004). Reactive oxygen species, aging, and antioxidative nutraceuticals. *Comprehensive reviews in food science and food safety, 3*(1), 21-33.

Lewis, C. P., Dinsdale, D., & Nemery, B. (1994). Potentiation of oxidant-induced toxicity in hamster lung slices by dimethylthiourea. *Free Radical Biology and Medicine, 16*(5), 561-569.

Li, J., Xu, X., Wang, Y., & Ren, T. (2010). Tribological studies on a novel borate ester containing benzothiazol-2-yl and disulfide groups as multifunctional additive. *Tribology International, 43*(5-6), 1048-1053.

Li, Y., & Schellhorn, H. E. (2007). New developments and novel therapeutic perspectives for vitamin C. *The Journal of nutrition, 137*(10), 2171-2184.

Liang, Y. C., May, C. Y., Foon, C. S., Ngan, M. A., Hock, C. C., & Basiron, Y. (2006). The effect of natural and synthetic antioxidants on the oxidative stability of palm diesel. *Fuel, 85*(5), 867-870.

Liu, Z., Wu, S., Wang, Y., Li, R., Wang, J., Wang, L., . . . Gong, P. (2014). Design, synthesis and biological evaluation of novel thieno [3, 2-d] pyrimidine derivatives possessing diaryl semicarbazone scaffolds as potent antitumor agents. *Eur. J. Med. Chem., 87*, 782-793.

Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy reviews, 4*(8), 118.

Logan, A. C. (2004). Omega-3 fatty acids and major depression: a primer for the mental health professional. *Lipids in health and disease, 3*(1), 25.

Majumdar, P., Pati, A., Patra, M., Behera, R. K., & Behera, A. K. (2014). Acid hydrazides, potent reagents for synthesis of oxygen-, nitrogen-, and/or sulfur-containing heterocyclic rings. *Chemical Reviews, 114*(5), 2942-2977.

Makahleh, A., Saad, B., & Bari, M. (2015). Synthetic phenolics as antioxidants for food preservation *Handbook of antioxidants for food preservation* (pp. 51-78): Elsevier.

Mare, H. D. L., & Coppinger, G. (1963). Oxidation of N, N-dialkyl hydroxylamines with t-butyl hydroperoxide. A new synthesis for nitrones. *The Journal of organic chemistry, 28*(4), 1068-1070.

Mark, H. F. (2013). *Encyclopedia of polymer science and technology, concise*: John Wiley & Sons.

May, J. M. (1999). Is ascorbic acid an antioxidant for the plasma membrane? *The FASEB journal, 13*(9), 995-1006.

Miller, A. L. (1996). Antioxidant flavonoids: structure, function and clinical usage. *Alt Med Rev, 1*(2), 103-111.

Mishra, V., Pandeya, S., DeClercq, E., Pannecouque, C., & Witvrouw, M. (1998). Synthesis of aryl semicarbazone of 4-aminoacetophenone and their anti-HIV activity. *Pharmaceutica Acta Helvetiae, 73*(4), 215-218.

Mishra, V., Pandeya, S., Pannecouque, C., Witvrouw, M., & De Clercq, E. (2002a). Anti-HIV Activity of Thiosemicarbazone and Semicarbazone Derivatives of (±)-3-Menthone. *Archiv der Pharmazie, 335*(5), 183-186.

Mishra, V., Pandeya, S., Pannecouque, C., Witvrouw, M., & De Clercq, E. (2002b). Anti-HIV Activity of Thiosemicarbazone and Semicarbazone Derivatives of (±)-3-Menthone. *Archiv der Pharmazie: An International Journal Pharmaceutical and Medicinal Chemistry, 335*(5), 183-186.

Moran, R. C., Evers, W. L., & Fuller, E. W. (1936). Petroleum product and method of making same: Google Patents.

Moran, R. C., & Kozacik, A. P. (1939). Mineral oil composition: Google Patents.

Morris, J. R. (1956). Lubricant containing a phosphorus acid ester-aldehyde condensation product: Google Patents.

Mortier, R. M., Orszulik, S. T., & Fox, M. F. (2010). *Chemistry and technology of lubricants* (Vol. 107115): Springer.

Mousavi, P., Wang, D., Grant, C. S., Oxenham, W., & Hauser, P. J. (2006). Effects of antioxidants on the thermal degradation of a polyol ester lubricant using GPC. *Industrial & engineering chemistry research, 45*(1), 15-22.

Mukhopadhyay, A. K. (2006). Antioxidants-natural and Synthetic: Amani Int'l Publishers.

Nagendramma, P. (2011). Study of pentaerythritol tetraoleate ester as industrial gear oil. *Lubrication Science, 23*(8), 355-362.

Naidu, K. A. (2003). Vitamin C in human health and disease is still a mystery? An overview. *Nutrition Journal, 2*(1), 7.

Nakao, K., Kubota, H., Yasuhara, M., Saito, K., Suzuki, T., Ohmizu, H., & Shimizu, R. (2001). Novel hydroxyphenylurea dual inhibitor against acyl-CoA: cholesterol acyltransferase (ACAT) and low density lipoprotein (LDL) oxidation as antiatherosclerotic agent. *Bioorganic & medicinal chemistry, 9*(4), 853-861.

Nanditha, B., & Prabhasankar, P. (2008). Antioxidants in bakery products: a review. *Critical reviews in food science and nutrition, 49*(1), 1-27.

Nath, A. R., & Yehye, W. A. (2018a). Acid Hydrazide: A Potential Reagent for the Synthesis of Semicarbazones. *Synthesis, 50*(21), 4301-4312.

Nath, A. R., Yehye, W. A., Zulkifli, N., & Johan, M. R. (2018). Ester of Thiolated Butylated Hydroxytoluene: Potential Antioxidant for Synthetic Lubricant Oil. *Thermochimica Acta*. 670, 7-12

Nath Pandeya, S. (2012). Semicarbazone–a versatile therapeutic pharmacophore for fragment based anticonvulsant drug design. *Acta Pharm., 62*(3), 263-286.

Nivas, D., Gaikwad, D., & Chavan, P. (2010). Antiradical activity of medicinally important Morinda pubescens fruits. *International Journal of Pharma and Bio Sciences, 1*(3).

Nixon, A., Minor, H., & Calhoun, G. (1956). Effect of Alkyl Phenols on the Storage and Manifold Stability of Gasolines. *Industrial & Engineering Chemistry, 48*(10), 1874-1880.

Noblía, P., Baran, E. J., Otero, L., Draper, P., Cerecetto, H., González, M., . . . Adachi, Y. (2004). New Vanadium (V) Complexes with Salicylaldehyde Semicarbazone Derivatives: Synthesis, Characterization, and in vitro Insulin-Mimetic Activity− Crystal Structure of [VvO2 (salicylaldehyde semicarbazone)]. *European Journal of Inorganic Chemistry, 2004*(2), 322-328.

O'Keefe, C. A., Johnston, K. E., Sutter, K., Autschbach, J., Gauvin, R. g., Trébosc, J., . . . Oudatchin, K. (2014). An investigation of chlorine ligands in transition-metal complexes via 35Cl solid-state NMR and density functional theory calculations. *Inorganic chemistry, 53*(18), 9581-9597.

Osawa, T. (1992). *Phenolic antioxidants in dietary plants as antimutagens*. Paper presented at the ACS symposium series.

Padmaja, K. V., Rao, B. V., Reddy, R. K., Bhaskar, P. S., Singh, A. K., & Prasad, R. B. (2012). 10-Undecenoic acid-based polyol esters as potential lubricant base stocks. *Industrial crops and products, 35*(1), 237-240.

Pandeya, S., Aggarwal, N., & Jain, J. (1999). Evaluation of semicarbazones for anticonvulsant and sedative-hypnotic properties. *Die Pharmazie, 54*(4), 300-302.

Pandeya, S. N. (2012). Semicarbazone–a versatile therapeutic pharmacophore for fragment based anticonvulsant drug design. *Acta Pharmaceutica, 62*(3), 263-286.

Pandeya, S. N., Yogeeswari, P., & Stables, J. P. (2000). Synthesis and anticonvulsant activity of 4-bromophenyl substituted aryl semicarbazones. *Eur. J. Med. Chem., 35*(10), 879-886.

Patsalos, P. N. (2005). Properties of antiepileptic drugs in the treatment of idiopathic generalized epilepsies. *Epilepsia, 46*, 140-148.

Pelosi, G., Bisceglie, F., Bignami, F., Ronzi, P., Schiavone, P., Re, M. C., . . . Pilotti, E. (2010). Antiretroviral Activity of Thiosemicarbazone Metal Complexes. *J. Med. Chem., 53*(24), 8765-8769.

Pham-Huy, L. A., He, H., & Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. *International journal of biomedical science: IJBS, 4*(2), 89.

Pokorny, J. (2007). Antioxidants in food preservation. *Handbook of food preservation, 2*, 259-286.

Popp, F. D. (1984). Potential anticonvulsants. VIII. Some hydrazones of indole-3-carboxaldehyde. *Journal of heterocyclic chemistry, 21*(2), 617-619.

Pospíšil, J. (1988). Mechanistic action of phenolic antioxidants in polymers—A review. *Polymer degradation and stability, 20*(3-4), 181-202.

Pospisil, J., & Klemchuk, P. P. (1989). *Oxidation inhibition in organic materials* (Vol. 1): CRC Press.

Qiao, S., Shi, Y., Wang, X., Lin, Z., & Jiang, Y. (2017). Synthesis of Biolubricant Trimethylolpropane Trioleate and Its Lubricant Base Oil Properties. *Energy & Fuels, 31*(7), 7185-7190.

Qiu, C., Han, S., Cheng, X., & Ren, T. (2006). Determining the antioxidant activities of organic sulfides by rotary bomb oxidation test and pressurized differential scanning calorimetry. *Thermochimica Acta, 447*(1), 36-40.

Quinchia, L. A., Delgado, M. A., Valencia, C. n., Franco, J. M., & Gallegos, C. (2011). Natural and synthetic antioxidant additives for improving the performance of new biolubricant formulations. *Journal of Agricultural and Food Chemistry, 59*(24), 12917-12924.

Rahman, T., Hosen, I., Islam, M. T., & Shekhar, H. U. (2012). Oxidative stress and human health. *Advances in Bioscience and Biotechnology, 3*(07), 997.

Rajendiran, A., Sumathi, A., Krishnasamy, K., Kabilan, S., & Ganguli, D. (2016). Antiwear study on petroleum base oils with esters. *Tribology International, 99*, 47-56.

Rawat, D. S., Joshi, G., Lamba, B. Y., Tiwari, A. K., & Kumar, P. (2015). The effect of binary antioxidant proportions on antioxidant synergy and oxidation stability of Jatropha and Karanja biodiesels. *Energy, 84*, 643-655.

Reid, L. C. (1953). Effect of thiourea and thyroxine on oxygen uptake of tissue homogenates. *Naunyn-Schmiedebergs Archiv für experimentelle Pathologie und Pharmakologie, 219*(5), 466-468.

Rohrbach, P., Hamblin, P., & Ribeaud, M. (2005). Benefits of antioxidants in lubricants and greases assessed by pressurised differential scanning calorimetry. *Tribotest, 11*(3), 233-246.

Roper, G., & Bell, J. (1995). Review and evaluation of lubricated wear in simulated valve train contact conditions. *SAE transactions*, 1478-1494.

Roveda, J.-G., Clavette, C., Hunt, A. D., Gorelsky, S. I., Whipp, C. J., & Beauchemin, A. M. (2009). Hydrazides as tunable reagents for alkene hydroamination and aminocarbonylation. *J. Am. Chem. Soc., 131*(25), 8740-8741.

Rudnick, L. R. (2009). *Lubricant additives: chemistry and applications*: CRC press.

Rudnick, L. R. (2017). *Lubricant additives: chemistry and applications*: CRC press.

Salem, I. A., El-Maazawi, M., & Zaki, A. B. (2000). Kinetics and mechanisms of decomposition reaction of hydrogen peroxide in presence of metal complexes. *International Journal of Chemical Kinetics, 32*(11), 643-666.

Sandoval, M., Okuhama, N., Zhang, X.-J., Condezo, L., Lao, J., Angeles, F., . . . Miller, M. (2002). Anti-inflammatory and antioxidant activities of cat's claw (Uncaria tomentosa and Uncaria guianensis) are independent of their alkaloid content. *Phytomedicine, 9*(4), 325-337.

Sangeetha, P., Das, U., Koratkar, R., & Suryaprabha, P. (1990). Increase in free radical generation and lipid peroxidation following chemotherapy in patients with cancer. *Free Radical Biology and Medicine, 8*(1), 15-19.

Saravanan, S., Sridharan, V., & Muthusubramanian, S. (2006). Chemoselective Addition of Hydrazine to δ-Keto Esters and Dimethylformamide-Mediated Deesterification. *Synth. Commun., 36*(7), 849-858.

Schroeder, H. A., & Adams, M. H. (1941). The effect of tyrosinase on experimental hypertension. *Journal of Experimental Medicine, 73*(4), 531-550.

Schumacher, R., Evans, S., & Dubs, P. (1991). Lubricant composition: Google Patents.

Scott, G. (2012). *Developments in Polymer Stabilisation—8*: Springer Science & Business Media.

Sedlar, J., Marchal, J., & Petruj, J. (1982). Photostabilising mechanisms of HALS: A critical review. *Polymer Photochemistry, 2*(3), 175-207.

Selby, T., Bosch, R., & Fee, D. (2005). Phosphorus additive chemistry and its effects on the phosphorus volatility of engine oils. *Journal of ASTM International, 2*(9), 1-16.

Seren, S., Lieberman, R., Bayraktar, U. D., Heath, E., Sahin, K., Andic, F., & Kucuk, O. (2008). Lycopene in cancer prevention and treatment. *American journal of therapeutics, 15*(1), 66-81.

Shahidi, F. (2004). Quality characteristics of edible oils *Quality of fresh and processed foods* (pp. 239-249): Springer.

Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects–A review. *Journal of functional foods, 18*, 820-897.

Shahidi, F., Janitha, P., & Wanasundara, P. (1992). Phenolic antioxidants. *Critical Reviews in Food Science & Nutrition, 32*(1), 67-103.

Shahidi, F., & Naczk, M. (2004). Phenolic in food and nutraceutical (pp. 1–558): Boca Raton, FL: CRC Press.

Sharma, B. K., Perez, J. M., & Erhan, S. Z. (2007). Soybean oil-based lubricants: a search for synergistic antioxidants. *Energy & Fuels, 21*(4), 2408-2414.

Sharma, O. P., & Bhat, T. K. (2009). DPPH antioxidant assay revisited. *Food Chemistry, 113*(4), 1202-1205.

Sheets, R. M., Guinther, G. H., & Loper, J. T. (2010). Lubricant formulations and methods of lubricating a combustion system to achieve improved emmisions catalyst durability: Google Patents.

Shelton, J. R. (1959). Mechanism of antioxidant action in the stabilization of hydrocarbon systems. *Journal of Applied Polymer Science, 2*(6), 345-350.

Singh, R. K., Kukrety, A., & Singh, A. K. (2014). Study of novel ecofriendly multifunctional lube additives based on pentaerythritol phenolic ester. *ACS Sustainable Chemistry & Engineering, 2*(8), 1959-1967.

Singhal, M., Paul, A., & Singh, H. P. (2014). Synthesis and reducing power assay of methyl semicarbazone derivatives. *Journal of Saudi Chemical Society, 18*(2), 121-127.

Singhal, M., Paul, A., Singh, H. P., Dubey, S. K., & Songara, R. K. (2011). Synthesis and evaluation of antioxidant activity of semicarbazone derivatives. *Int J Pharmaceut Sci Drug Res, 3*, 150-154.

Soleimani, M., Dehabadi, L., Wilson, L. D., & Tabil, L. G. (2018). Antioxidants Classification and Applications in Lubricants.

Somayaji, A., & Aswath, P. B. (2009). The role of antioxidants on the oxidation stability of oils with F-ZDDP and ZDDP, and chemical structure of tribofilms using XANES. *Tribology transactions, 52*(4), 511-525.

Sowbhagya, C., & Bhattacharya, K. (1976). Lipid autoxidation in rice. *Journal of food science, 41*(5), 1018-1023.

Sriram, D., Yogeeswari, P., & Thirumurugan, R. (2004). Antituberculous activity of some aryl semicarbazone derivatives. *Bioorganic & medicinal chemistry letters, 14*(15), 3923-3924.

Stuckey, B. N. (1972). Antioxidants as food stabilizers (Vol. 185): CRC Press, Boca Raton, FL.

Sutton, M. R., Barton, W. R., & Price, D. (2016). Lubricating composition comprising poly (isobutylene)/poly (vinyl aromatic) block copolymer: Google Patents.

Swami, K., Prakash, S., Sarin, R., Tuli, D., & Bhatnagar, A. (2003). Development of ashless multifunctional additives from cashew nutshell liquid. *Lubrication Science, 15*(4), 361-368.

Takao, T., Moriya, M., & Suzuki, H. (2007). Introduction of a Methoxy Group into a Hydrocarbyl Ligand Derived from a Linear Alkane on a Triruthenium Cluster via Chemical Oxidation. *Organometallics, 27*(1), 18-20.

Thendral, M., Devi, S. K., & Maheswari, R. (2015). Journal of Pharmaceutical and Biological Sciences.

Thiel, O. R., & Achmatowicz, M. M. (2003). Palladium-Catalyzed Triazolopyridine Synthesis: Synthesis of 7-Chloro-3-(2-Chlorophenyl)-1, 2, 4-Triazolo [4, 3-a] Pyridine. *Organic Syntheses, 90*, 287-300.

Thomas, R., Dexter, M., & King III, R. (2000). Antioxidants, polymers. *Kirk-Othmer Encyclopedia of Chemical Technology*.

Thorisson, S., Gunstone, F., & Hardy, R. (1992). The antioxidant properties of ethoxyquin and of some of its oxidation products in fish oil and meal. *Journal of the American Oil Chemists Society, 69*(8), 806-809.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M., & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The international journal of biochemistry & cell biology, 39*(1), 44-84.

Valko, M., Morris, H., & Cronin, M. (2005). Metals, toxicity and oxidative stress. *Current medicinal chemistry, 12*(10), 1161-1208.

Valko, M., Rhodes, C., Moncol, J., Izakovic, M., & Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-biological interactions, 160*(1), 1-40.

Varatharajan, K., & Pushparani, D. (2017). Screening of antioxidant additives for biodiesel fuels. *Renewable and Sustainable Energy Reviews*.

Venkatachalam, T. K., Bernhardt, P. V., Noble, C. J., Fletcher, N., Pierens, G. K., Thurecht, K. J., & Reutens, D. C. (2016). Synthesis, characterization and biological activities of semicarbazones and their copper complexes. *Journal of inorganic biochemistry, 162*, 295-308.

Wasson, J., & Smith, W. (1953). Effect of alkyl substitution on antioxidant properties of phenols. *Industrial & Engineering Chemistry, 45*(1), 197-200.

Waynick, J. A. (2001). The development and use of metal deactivators in the petroleum industry: a review. *Energy & Fuels, 15*(6), 1325-1340.

Willcox, J. K., Ash, S. L., & Catignani, G. L. (2004). Antioxidants and prevention of chronic disease. *Critical reviews in food science and nutrition, 44*(4), 275-295.

Wu, Y., Li, W., Zhang, M., & Wang, X. (2013). Improvement of oxidative stability of trimethylolpropane trioleate lubricant. *Thermochimica Acta, 569*, 112-118.

Xiangqiong, Z., Heyang, S., Wenqi, R., Zhongyi, H., & Tianhui, R. (2005). Tribological study of trioctylthiotriazine derivative as lubricating oil additive. *Wear, 258*(5-6), 800-805.

Xu, X., Wan, Y., & Cao, L. (2000). Tribological performance and action mechanism of S-alkyl and S-benzimidazole substituted dialkyldithiocarbamates. *Wear, 241*(1), 41-46.

Yamaguchi, E., Onopchenko, A., Francisco, M., & Chan, C. (1999). The relative oxidation inhibition performance of some neutral and basic zinc dithiophosphate salts. *Tribology transactions, 42*(4), 895-901.

Yao, J. (2006). Recent development of antiwear, extreme pressure (EP) additives for lubricant. *Lubr Oil China, 21*(3), 29-37.

Yao, J., Wang, Q., Chen, S., Sun, J., & Dong, J. (2002). Borate esters used as lubricant additives. *Lubrication Science, 14*(4), 415-423.

Ye, Y., & King III, R. (2003). Additives for polyolefin film products: an overview of chemistry and effects. *Ciba Specialty Chemicals*.

Yehye, W. A., Rahman, N. A., Ariffin, A., Hamid, S. B. A., Alhadi, A. A., Kadir, F. A., & Yaeghoobi, M. (2015). Understanding the chemistry behind the antioxidant activities of butylated hydroxytoluene (BHT): A review. *European journal of medicinal chemistry, 101*, 295-312.

Yen, G. C., & Duh, P. D. (1994). Scavenging effect of methanolic extracts of peanut hulls on free-radical and active-oxygen species. *Journal of Agricultural and Food Chemistry, 42*(3), 629-632.

Yi, W., Fischer, J., Krewer, G., & Akoh, C. C. (2005). Phenolic compounds from blueberries can inhibit colon cancer cell proliferation and induce apoptosis. *Journal of Agricultural and Food Chemistry, 53*(18), 7320-7329.

Yogeeswari, P., Sriram, D., Pandeya, S., & Stables, J. (2004). 4-Sulphamoylphenyl semicarbazones with anticonvulsant activity. *Il Farmaco, 59*(8), 609-613.

Yogeeswari, P., Sriram, D., Thirumurugan, R., Raghavendran, J. V., Sudhan, K., Pavana, R. K., & Stables, J. (2005). Discovery of N-(2, 6-dimethylphenyl)-substituted semicarbazones as anticonvulsants: hybrid pharmacophore-based design. *J. Med. Chem., 48*(20), 6202-6211.

Yogeeswari, P., Sriram, D., Veena, V., Kavya, R., Rakhra, K., Ragavendran, J. V., . . . Stables, J. (2005). Synthesis of aryl semicarbazones as potential anticonvulsant agents. *Biomed. Pharmacother., 59*(1), 51-55.

Yogeeswari, P., Thirumurugan, R., Kavya, R., Samuel, J. S., Stables, J., & Sriram, D. (2004). 3-Chloro-2-methylphenyl-substituted semicarbazones: synthesis and anticonvulsant activity. *Eur. J. Med. Chem., 39*(8), 729-734.

Yosef, H. A. A., & Ibrahim, N. M. (2016). Synthesis, Thermolysis, Photolysis and Antimicrobial Evaluation of some Novel Semicarbazones and Thiosemicarbazones Derived from 3-Methyl--benzothiazolinone Hydrazone. *EGYPTIAN JOURNAL OF CHEMISTRY, 59*(5), 867-886.

Zeng, X., Li, J., Wu, X., Ren, T., & Liu, W. (2007). The tribological behaviors of hydroxyl-containing dithiocarbamate-triazine derivatives as additives in rapeseed oil. *Tribology International, 40*(3), 560-566.

Zhang, H.-Y., Yang, D.-P., & Tang, G.-Y. (2006). Multipotent antioxidants: from screening to design. *Drug Discovery Today, 11*(15-16), 749-754.

Zhang, J., Liu, W., & Xue, Q. (1999). The effect of molecular structure of heterocyclic compounds containing N, O and S on their tribological performance. *Wear, 231*(1), 65-70.

Zurita, J. L., Jos, Á., del Peso, A., Salguero, M., López-Artíguez, M., & Repetto, G. (2007). Ecotoxicological effects of the antioxidant additive propyl gallate in five aquatic systems. *Water research, 41*(12), 2599-2611.

LIST OF PUBLICATIONS AND CONFERENCE

List of Publications

1. Nath, A. R., & Yehye, W. A. (2018). Acid Hydrazide: A Potential Reagent for the Synthesis of Semicarbazones. *Synthesis, 50*(21), 4301-4312.
2. Nath, A. R., Yehye, W. A., Zulkifli, N., & Johan, M. R. (2018). Ester of Thiolated Butylated Hydroxytoluene: Potential Antioxidant for Synthetic Lubricant Oil. *Thermochimica Acta*. 670, 7-12

List of Conference

1. Scholar Summit 2017, Universitas Indonesia, Oral Presentation on "A Novel and Facile Approach to Synthesize Semicarbazones via Nucleophilic Substitution Reaction"