SYNTHESIS OF NEW 1,2,4-TRIAZOLE AND 1,3,4-OXADIAZOLE DERIVATIVES DERIVED FROM 2-ETHYLSULFANYL BENZOHYDRAZIDE AND EVALUATION OF THEIR BIOLOGICAL ACTIVITIES

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

FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
KUALA LUMPUR

2016

UNIVERSITI MALAYA

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ABSTRACT

The new 1-acylthiosemicarbazides derivatives **3.3-3.7** were synthesised by the reaction of active intermediate 2-(ethylsulfanyl)benzohydrazide **3.2** with various aryl isothiocyanates. The cyclisation of thiosemicarbazide derivatives in a basic medium (aqueous NaOH, 4 N) yielded compounds **3.8-3.12**, while another method was used to prepare 4-amino-1,2,4-triazole-5-thione **3.13**. All of the thiosemicarbazide derivatives showed better antioxidant activity than 1,2,4-triazole derivatives in both assays DPPH and FRAP. Hydrogen Atom Transfer (HAT) mechanism was proposed and the Density Functional Theory (DFT) method was used to rationalise the experimental results. 5-[2-(Ethylsulphanyl)phenyl]-1,3,4-oxadiazole-2(3*H*)-thione **3.14** was prepared by the reaction of 2-(ethylsulphanyl)benzohydrazide with CS_2 in KOH. On alkylation of compound **3.14** gave 2,5-disubstituted-1,3,4-oxadiazole derivatives **3.20-3.24**. Compound **3.14** showed excellent antioxidant activity in DPPH assay while its alkylated derivatives did not display any antioxidant activity either in DPPH or FRAP assays. Six new hydrazones **3.25-3.30** were prepared and only compounds with substituents 3-OEt-4-OH and 3-OMe-4-OH showed precise antioxidant activity in DPPH assay and in FRAP assay as well. New 2-aryl-5-hydrazino-l,3,4-oxadiazole **3.31** was also synthesized from the reaction of ethyl acetate derivative **3.24** with hydrazine hydrate in dioxane. The reaction of compound **3.31** with some substituted aromatic aldehydes in the presence of ethanol gave new 2,5-disubstituted-1,3,4-oxadiazole derivatives **3.32-3.37**. Compound **3.31** showed strong antioxidant activity while oxadiazole derivatives **3.32-3.37** showed some variability in their antioxidant activities. Eight selected 1,3,4-oxadiazoles derivatives were tested against various cancer cell lines. Results revealed some intriguing unexpected levels of activity and the phenolic OH was found to be responsible for the cytotoxicity of oxadiazole derivatives **3.32** and **3.34-3.37**. Hydrazone **3.25** substituted with 3-OMe-4-OH was further tested for its antiulcerogenic activity and the result from antiulcerogenic activity was reliable to its

DPPH and FRAPs activity, compound **3.25** revealed a significant effect in reducing gastric lesions.

ABSTRAK

Sebatian terbitan baru 1-asiltiosemikarbazida **3.3–3.7** telah disintesiskan dengan menindakbalaskan bahantara aktif 2-(etilsalfanil)benzohidrazida **3.2** dengan pelbagai aril isotiosianida. Pensiklikan terbitan tiosemikarbazida dalam media berbes (akues NaOH, 4N) menghasilkan sebatian-sebatian **3.8–3.12**, manakala kaedah lain telah digunakan untuk menyediakan 4-amino-1,2,4-triazole-5-thione, **3.13**. Kesemua terbitan tiosemikarbazida menunjukkan aktiviti aktioksida yang lebih tinggi daripada terbitan 1,2,4-triazola pada kedua dua kaedah kajian DPPH dan FRAP. Mekanisme perpindahan atom hydrogen (HAT) telah dicadangkan dan kaedah "Density Functional Theoty (DFT)" telah diguna untuk menyokong dapatan dari kajian sebenar. 5-[2-etilsalfanil)fenil]-1,3,4-oksadiazola-2(3*H*)-thione, **3.14**, disediakan melalui tindak balas antara 2-(etilsalfanil)benzohidrazida dengan CS_2 dalam KOH. Pengalkilan sebatian **3.14** memberikan terbitan 2,5-tertukargantidua-1,3,4-oksadiazola, **3.20–3.24**. Sebatian **3.14** menunjukkan aktiviti antioksida yang luarbiasa pada assay DPPH sementara terbitan teralkil tidak menunjukkan sebarang aktiviti antioksida sama ada melalui assay DPPH atau FRAP. Enam hidrazon baru **3.25–3.30** dihasilkan dan hanya sebatian dengan penukarganti 3-OEt-4-OH dan 3-OMe-4-OH menunjukkan aktiviti antioksida yang tinggi dalam assay DPPH dan FRAP. Sebatian baru 2-aril-5-hidrazino-1,3,4-oksadiazola, **3.31**, juga telah disintesiskan dari tindakbalas terbitan etil asetat, **3.24**, dengan hidrazina hidrat dalam dioksana. Tindak balas sebatian **3.31** dengan beberapa aldehid aromatik tertukarganti dalam etanol menghasilkan terbitan baru 2,5-ditertukarganti-1,3,4-oksadiazola, **3.32–3.37**. Sebatian **3.31** menunjukkan aktiviti antioksida yang tinggi sementara terbitan oksadiazola **3.32-3.37** menunjukkan aktiviti antioksida yang pelbagai. Lapan terbitan terpilih 1,3,4-oksadiazola diuji ke atas pelbagai sel kanser. Keputusan kajian menunjukkan beberapa aktiviti di luar jangkaan dan OH dari fenol dipercayai menjadi penyumbang kepada ketoksikan sebatian terbitan **3.32** dan **3.34-3.37**. Hidrazon **3.25** tertukarganti dengan 3-OMe-4-OH seterusnya diuji untuk

aktiviti antiulser dan hasil kajian dari aktiviti antiulser adalah selari dengan assay DPPH dan FRAP dan sebatian **3.25** menunjukkan kesan perlindungan yang tinggi dalam penurunan rembesan gastrik.

DEDICATION

To the spirit of the Prophet Muhammad and Muslims who sacrifice their lives for Islam.

ACKNOWLEDGEMENTS

"He who follows a path in quest of knowledge, Allah will make the path of Jannah easy to him".

With these words of The Messenger of Allah Mohammed, I would like to take the privilege to thank the selfless people from the bottom of my heart who with their constant support, inspiration and encouragement made me feel comfortable to successfully complete this venture.

I wish to express my sincere thanks, with a deep sense of gratitude, to my respected Supervisor ***Associate Professor Dr. Azhar Ariffin,*** for his immense guidance, help, dedicated support and intellectual supervision until the completion of this work. Special thanks to my supervisor ***Prof. Dr. Zanariah Abdullah*** for her professional expertise, trust and successful opinions as well as my grateful gratitude to ***Prof. Dr. Mahmood Ameen Abdulla*** my supervisor for his constant support, opinions and for the use of facilities for the biological activity experimentation.

I would like to express my gratitude to ***Dr. Kok Hoong Leong***, Center for Natural Product and Drug Discovery (CENAR), Department of Chemistry, Faculty of Science, University of Malaya for his help in cytotoxicity study. I would like to extend my thanks and gratitude to **Miss: Kumutinic** for the valuable assistance provided for the completion of my thesis. Also, I wish to express my appreciation to NMR staff, **Miss Norzalida Zakaria**, **Mr. Fateh Ngaliman** and a special thanks to **Mr. Nordin Mohamad**. Also I would like to acknowledge the financial, academic and technical support of the University of Malaya, especially for the UMRG grant (RG149-11AFR and RG233-12AFR) and a PPP grant (PG033-2012B). A special thanks to College of

Health and Medical Technologies, Baghdad for giving me the opportunity to do this research and the Ministry of Higher Education of Iraq for the financial support.

Great thanks to ***Prof. Dr. Noel Francis Thomas,*** for providing me with the mechanism of my synthesized compound as well as to ***Prof. Dr. Ng Seik Weng*** and ***Dr. Nadia Halim*** for the X-ray analysis. I am especially thankful to **Dr. Wageeh A. Yehya** and his wife **Dr. Abeer al-Hadi**. Also to all my friends in chemistry lab especially **Miss. Nurdiana Nordin**, **Miss. Hassna** and also **Miss. Shimar** for their help, support and sweet memories. I am also grateful to **Dr. Farqad al-Hadi**. She guided me to write on the anti-ulcer study in my thesis.

I am fortunate to have parents like mine. My sincere thanks and appreciation to my father Mr. Nazar Bahjat, my mother **Sanaa Jalal** and to my sister **Hala Nazar** and her husband **Mohammed Salah** for their unconditional true love, blessing and support.

A special thanks to ***Dr. Atheer Awad Mehde*** who's always helped me a lot in my life and without him I could not achieve my dreams. My special thanks to my son Ali and to my daughter Ola. I would like to thank everybody who was important to the successful realization of this dissertation as well as expressing my apology as I could not mention everyone personally one by one.

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Å	Angstrom(s)
aq	Aqueous
Ar	Aryl
BDH	Bond-dissociation enthalpies
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
Bs	Broad singlet
BxPC-3	Human primary pancreatic adenocarcinoma
Cat	Catalyst
CoQ	Coenzyme Q10
δ	Chemical shift in parts per million
J	Coupling constant (in NMR spectrometry)
DFT	Density functional theory
DPPH	2,2-diphenyl-1-picrylhydrazyl radical
FRAP	Ferric ion reducing antioxidant power
GSH	Reduced glutathione
GPx	Glutathione peroxidase
GST	Glutathione S-transferase
HOMO	Highest occupied molecular orbital
HBA	Number of hydrogen bond acceptor
HBD	Number of hydrogen bond donor
HREIMS	High-resolution electron ionization mass spectrometry
HAT	Hydrogen atom transfer
H&E	Hematoxyline and eosin
hTERT-HPNE	Normal pancreas cell line

IC_{50}	Half-maximum inhibitory concentration
IP	Ionization potential
IHC	Immunohistochemistry
LogP	Logarithm of partition coefficient
LUMO	Lowest unoccupied molecular orbital
MCF-7	Human breast adenocarcinoma cells
MDA-MB-231	Human breast adenocarcinoma
MTS assay	Cell Proliferation Colorimetric Assay
MWt	Molecular weight
μ	Micro
NSAID	Non-steroidal anti-inflammatory drugs
α	Observed optical rotation in degrees
PPI	Proton-pump inhibitors
PAS	Periodic Acid-Schiff -
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SOD	Superoxide dismutase
S_N2	Bimolecular nucleophilic substitution
THF	Tetrahydrofuran

CHAPTER 1: INTRODUCTION

1.1. Introduction

Reactive oxygen species (ROS), known as mediators of intracellular signalling cascades, are chemically reactive molecules containing oxygen.[1] Most living organisms can produce ROS and metabolize excessive amounts through normal physiological processes. Accumulation of excessive ROS is generally responsible for damaging lipids, proteins and the DNA of cells, leading to oxidative stress. Free radical-induced damage in oxidative stress has been confirmed as a contributor to the pathogenesis and pathophysiology of many chronic health problems such as neurodegenerative conditions (Parkinson, Alzheimer, cardiovascular diseases, cataracts, cancer, autoimmune gastrointestinal inflammation and gastric ulcer.[2]

As scientific research continues to disentangle the root cause of cancer, evidence of oxidative stress leading to chronic inflammation of cells is believed to perpetuate the visibility of this genetic instability.[3] In particular, chronic excessive ROS is one of the underlying reasons for the development of cancer.[4] Cancer is still a global health concern, with an approximate 12.7 million new cases reported in 2008, and is predicted to reach 22.2 million cases by 2030.[5] Genetic instability is also recognized as the feature of cancer development, resulting in an abnormal cell growth. In the cases where a cancerous tumour has metastasized, the survival rate is significantly slim.[6] Gastric ulcer is an illness that affects a considerable number of people worldwide. The pathological basis for the development of gastric ulcer is multifactorial. It includes factors which disturb gastric mucosal integrity, such as mucus secretion, mucosal barrier, acid-pepsin secretion, blood flow, cellular regeneration and endogenous protective agents.[7]

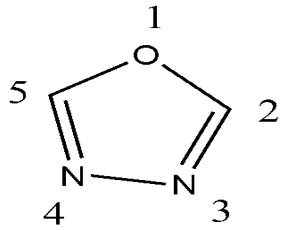
Previously, it was thought that the life's stress is one of the etiological factors in the incidence of gastric ulcer by increasing catecholamine level which causes vasoconstriction.[8] A recent study has also shown that the involvement of oxidative stress in the pathogenesis of stress-induced gastric ulcer.[9] In an effort to diminish the damaging effects of ROS, antioxidant molecules either natural or synthetic, which are capable of interacting with free radicals and ROS has the potential for inhibiting chain reactions before damage the main substantial molecules.[10] Anti-oxidants capable of scavenging excess ROS have received much attention in cancer therapy.[11]

Antioxidants can be classified as primary and secondary molecules depending on their respective mode of operation. The primary antioxidants comprise essentially sterically hindered phenols and secondary aromatic amines.[12] Phenolic compounds represent an important class of antioxidants.[13] The ability of phenolic compounds to quench free radicals which arises from both acidity and their delocalized π-electrons plays an important role in antioxidant behaviour.[14] For examples, curcumin, resveratrol and epigallocatechin, possessing both antioxidant and anticancer properties, have received great scientific interest. Recent research indicates that the inhibitory effects of phenolic antioxidant compounds are mainly from their free radical scavenging properties, and a large amount of evidence supports of their chemo-protective effects against oxidative stress-mediated disorders.[15] The inhibitory effects of phenols are further demonstrated by observing the presence of sulphur compounds classified as secondary antioxidants. Sulphides, for example, can decompose peroxides that are intermediates in the oxidation reactions and regenerate the primary antioxidants.[16]

Several assays have been used to estimate antioxidant capacities. The existing antioxidant activity or the assay capacity methods in literature depends on the consumption of chromogenic radicals, i.e., ABTS[17] and DPPH[18], Oxygen Radical Absorption Capacity:

ORAC[19] or Ferric-Reducing/Antioxidant Power: FRAP.[20] Antioxidant capacity methods are generally divided into two categories: the Hydrogen Atom Transfer (HAT) reaction and Electron Transfer (ET) reaction-based methods. From a mechanistic standpoint, the DPPH assay involves both hydrogen atom transfer (HAT) and ET mechanisms, whereas FRAP assays involves (ET) mechanism.[21]

Heterocyclic chemistry is an integral part of the chemical sciences which constitutes a major area of research touching the boundaries of other sub-categories (e.g) organic chemistry, medicinal chemistry, biochemistry and pharmacology. The chemistry of heterocyclic compounds (is the chemistry of aliphatic or aromatic compounds). The study of heterocyclic systems is of great interest for both theoretical and practical point of view.[22] The wide occurrence of heterocycles in bioactive natural products, pharmaceuticals, and agrochemicals has made them important synthetic targets.[23] 1,3,4-Oxadiazoles, 1,2,4-triazoles, 1,3,4-thiadiazoles, pyrazoles, and isoxazoles represent a class of heterocyclic compounds of great importance in biological chemistry.[24] The derivatives of oxadiazoles plays a very significant role in medicinal chemistry.[22 ,25] Due to the increased hydrolytic and metabolic stabilities in the oxadiazole ring, an improved pharmacokinetic and *in vivo* performance is often observed, which makes these heterocycles an important skeleton for the pharmaceutical industry. 1,3,4-Oxadiazoles (**1.1**) are derived from an important class of heterocyclic compounds with a variety of biological activities.[26]



1.1

Substituted 1,3,4-oxadiazoles have demonstrated on antibacterial and antifungal,[27] antioxidant,[28] anticancer,[29] antimalarial,[30] anticonvulsant,[31] muscle relaxant,[32] and genotoxic activities.[33] They are also used extensively in the symptomatic treatment of rheumatic fever, arthritis (rheumatoid, osteo and jaundice arthritis), and management of primary dysmenorrhea. Oxadiazole pharmacophore has been the key property that influences the ability of the drug to reach the target by transmembrane diffusion which showed potent antimicrobial activity.[34]

The chemistry of 1,2,4-triazole derivatives has received considerable attention in recent years due to their usefulness in different areas of biological chemistry and as industrial intermediates. 1,2,4-triazole derivatives are known to exhibit antimicrobial,[35] antibacterial,[36] antifungal,[37] anti-inflammatory [38] and analgesic activities.[39] 1,2,4-Triazole nucleus has been incorporated into a wide variety of therapeutically plus interesting drug candidates. Some of the modern day drugs that were found to fuse heterocycles with a triazole moiety are alprazolam, triazolam, estazolam (hypnotic, sedative, tranquilizer), trazodone (antidepressant, anxiolytic), trapidil (hypotensive), terconazole (antifungal), hexaconazole (antifungal), etizolam (amnesic, anxiolytic, anticonvulsant, hypnotic, sedative and skeletal muscle relax-ant), rilmazafon (hypnotic, anxiolytic) and rizatriptan (anti-migrane agent).[25] In addition, thiosemicarbazides are also versatile precursors in organic synthesis and these derivatives have been used as intermediates for preparing various heterocyclic derivatives.[40]

1.2. Objectives of the study

1,3,4-Oxadiazole and 1,2,4-triazole derivatives have shown very interesting pharmacological properties. Thus the aims of this research project is;-

1. To synthesise a series of 1,3,4-oxadiazole, 1,2,4-triazole derivatives and Schiff bases.
    i. Synthesise of a series of compounds with 1-acylthiosemicarbazides moieties and with 1,2,4- triazole-3-thiones.
    ii. Synthesis of the second series of compounds by coupling of 1,3,4-oxadiazole with phenol, Schiff bases and thioether group respectively.
    iii. Preparation of a series of new hydroxyl-substituted Schiff bases.
2. Characterisation of synthesized compounds using spectroscopic techniques.
3. Study of biological activities.
    i. Study of *in vitro* antioxidant activities of all the newly synthesized compounds
    ii. Study on their *in vitro* cytotoxic and *in vivo* antiulcer activities for selected compounds.

1.3. Outline of thesis structure

This thesis is divided into six chapters. *Chapter one* is introduction. *Chapter two* deals with literature review. *Chapter three* discusses the detailed synthetic results for 1,2,4-triazole and 1,3,4-oxadiazole derivatives and some new Schiff's bases. *Chapter four* focuses on the biological activity studies of the synthesized compounds. *Chapter five* presents the conclusion of this study and shares some ideas for future work. Finally, *Chapter six* describes the methodology used in the present work and explains the different types of experimental methods employed in the synthesis.

CHAPTER 2: LITERATURE REVIEW

2.1. Free radicals and antioxidants

Oxygen is the essential element for life but on the other hand, under certain conditions oxygen can create unstable free radical intermediates. Free radicals are chemical substances that have one or more unpaired electrons resulting in donation of their electrons to other molecules causing chain reactions and oxidative damage.[41, 42] Free radicals and related molecules are classified as Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS). These radicals are produced during normal metabolism in biological systems, and are simultaneously counterbalanced by cellular antioxidant mechanisms. However, excess ROS and RNS decreases antioxidant defense systems at the cellular level causing oxidative stress, which can induce damage by peroxidation of cellular structures, protein oxidation, DNA damage and inhibition of the electron transport chain in mitochondria.[43, 44]

The formation of free radicals during physiological processes results in the development of antioxidant defense mechanisms. Antioxidants are able to inhibit or prevent oxidation processes of vital molecules in cellular processes and can be produced either in the human body or absorbed from the diet.[45] Production of antioxidants inside the body include; dismutase, peroxidase, and catalase enzymes, as well as glutathione (GSH) and cytochrome.[46]

2.2.The role of antioxidants

Antioxidants play as preventive role during the two pathways for oxidation process, in case of lipid peroxidation of free radical chain reactions happens through three distinct steps described in Scheme 2.1; the first path is H-atom transfer and formation of radicals which are considered as the initiation step. A swift addition of O_2 in step (2) or (propagation step), involves free radical regeneration via a chain reaction which is completed by the termination step. The role of the antioxidant ArOH is to interrupt the chain reaction at step (4). To be effective the ($ArO^{\cdot}$) must be a relatively stable free radical, so that it reacts slowly with the substrate, (RH) but rapidly with ($RO_2^{\cdot}$).[47]

$$R\text{-}H \longrightarrow R^{\cdot} + H^{\cdot} \quad \text{(initiation)} \quad (1)$$

$$R^{\cdot} + O_2 \longrightarrow RO_2^{\cdot} \quad \text{(addition of } O_2\text{)} \quad (2)$$

$$RO_2^{\cdot} + R\text{-}H \longrightarrow ROOH + R^{\cdot} \quad \text{(H-atom exchange)} \quad (3)$$

$$RO_2^{\cdot} + ArOH \longrightarrow ROOH + ArO^{\cdot} \quad (4)$$

Scheme 2.1: Oxidation and hydrogen atom transfer mechanism of antioxidant

The second mechanism for free radical deactivation is the electron transfer reactions[47] Scheme 2.2

$$RO_2^{\cdot} + ArOH \longrightarrow RO_2^{-} + ArOH^{+} \quad \text{electron transfer}$$

$$ArOH^{+} + H_2O \rightleftharpoons ArO^{\cdot} + H_3O^{+} \quad \text{deprotonation equilibrium}$$

$$RO_2^{-} + H_3O^{+} \rightleftharpoons ROOH + H_2O \quad \text{Hydroperoxide formation}$$

Scheme 2.2: Steps of electron transfer mechanism

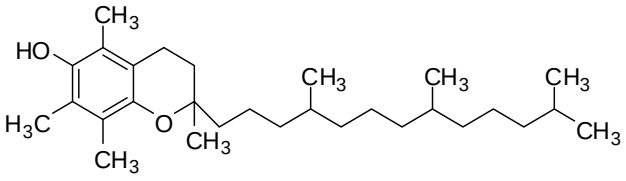
The role of antioxidant in both mechanisms is to deactivate free radicals by protonation or electron donation step (4) a general process in the body.[48]

2.3. Antioxidant system

Diversity of endogenous and exogenous antioxidants act against free radicals and exert their activity by different mechanisms.[49] The human body has developed antioxidant systems which can be divided into two major groups, non-enzymatic and enzymatic oxidants. The natural primary enzymatic antioxidants act as chain breaking antioxidants through their direct reaction with lipid and peroxyl radicals which interrupts the propagation step of auto-oxidation.[50] Secondary enzymatic antioxidants on the other hand, supports an indirect role by retarding lipid oxidation through either oxygen scavenging, or chelating of transition metal ions, thus replenishing hydrogen to primary antioxidants.[51] Metabolic and nutrient antioxidants are the major constituents of non enzymatic antioxidants. Metabolic antioxidants are the resultant of body metabolism such as coenzyme Q10, and glutathione.[51] On the contrary, nutrient antioxidants are not body products but supplied through diet consumption such as flavonoids, omega-3 and trace metals.[52]

2.3.1. Natural antioxidants

The intake of mixed fruits and vegetables which provide protection against several diseases has been attributed to a variety of antioxidants such as vitamin C, vitamin E, β-carotene and polyphenolic compounds.[53] Vitamin E (α-tocopherol) (**2.1**), is the most predominant and potent amongst the set of eight fat soluble related tocopherols and tocotrienols.[54] Thus α-tocopherol can inhibit lipid peroxidation[55] by the donation of phenolic hydrogen to the peroxyl radical to form unreactive tocopheroxyl radicals. These radicals are capable of quenching further propagation interfering with the oxidative chain reaction and regeneration of α-tocopherol.[56]



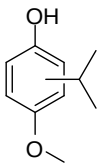
2.1

In vivo vitamin C (ascorbic acid) antioxidant activity comes from the scavenging of superoxide radical anion, hydrogen peroxide and hydroxyl radical.[57] Antioxidant behavior of vitamin C is dependent on singlet oxygen quenching and molecular oxygen removal by donating its hydrogen atom to the lipid radicals.

Many of the antioxidants other than vitamin C, and vitamin E are present in body cells and membranes and each plays a specific role as for example coenzyme Q10 which has the very important function of preventing lipid peroxyl radicals formation.[58] Bioactive compounds such as flavonoids and phenolic acids posses a significant antioxidative effect and health benefits. Flavonoids for example, a chain breaking antioxidant which donates its hydrogen atom to quench lipid radicals and avoid lipid peroxidation.[59] Phenolic antioxidant properties are responsible for the inhibition of oxidation of low-density lipoprotein cholesterol.[60]

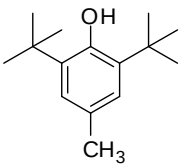
2.3.2. Synthetic antioxidants

The primary industrial concern is the prevention or inhibition of food oxidation and a variety of antioxidants have been used as commercial products. Sterically hindered phenols butylated hydroxyanisole (BHA) (**2.2**) and butylated hydrogenoxytoluene (BHT) (**2.3**) are the most widely used synthetic antioxidants for food preservation.[61]



(BHA)

2.2



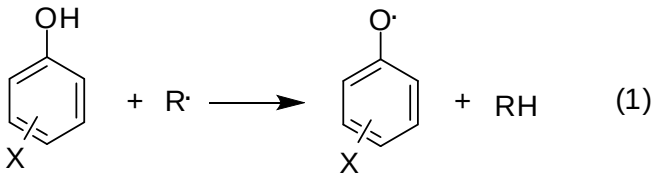
(BHT)

2.3

Their use is not limited only to food preservation, nutrition value, and animal feed products but includes antioxidant capabilities that retard oxidation in cosmetics, inhibiting rancidity, and extending product shelf life.[62] Food antioxidants such as (BHA), and (BHT) are able to inhibit the formation of carcinogenic products of lipid peroxidation in the diet and also protect against chemically induced neoplasia.[63] Propyl gallate (PG) is also an antioxidant additive in both food and pharmaceutical industry, and hydrolysis of Gallic acid derivatives to natural products is found in many plants which acts as a safe food antioxidant.[64]

2.4. Phenolic antioxidants

Among the important compounds that are able to inhibit the oxidation of important biological and commercial materials are phenolic compounds which contain at least one hydroxyl group attached to benzene ring.[65] Simple phenols are typical primary chain breaking antioxidants through the attack and scavenge of free radicals via their hydrogen atom donation[66] as shown in Scheme 2.3.

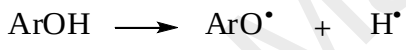


Scheme 2.3: Reaction of phenols with free radicals

The electronic properties of a chromophoric group attached to the phenol skeleton affect the antioxidant reactivity[66] by delocalization of unpaired electrons on the aromatic ring.[67, 68] In case of hindered phenols such as BHA and BHT they are considered as radical-trapping antioxidants for oxy, and especially peroxy radicals. The phenoxy radicals with their bulky substituents are stabilized by sterical hindrance.[69]

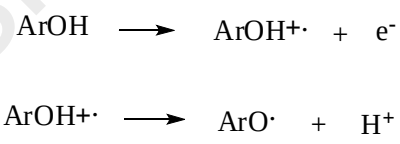
2.4.1. Mechanism of action of phenolic antioxidants

According to Wright *et al.*[47] the two accepted mechanisms of phenolic antioxidants are (HAT) shown below in Scheme 2.4



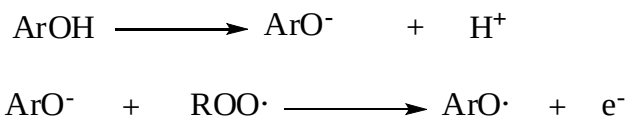
Scheme 2.4: HAT mechanism

and Single-Electron Transfer followed by Proton Transfer (SET-PT) shown in Scheme 2.5



Scheme 2.5: SET-PT mechanism

Recently, sequential Proton Loss Electron Transfer (SPLET) mechanism in Scheme 2.6 was reported by[70-73] which comprises of two steps, the first step is the formation of the phenoxide anion (ArO^-) and the second is electron transfer from phenoxide anion ArO^- to $ROO^{\cdot}$ and the phenoxy radical is formed.[74]

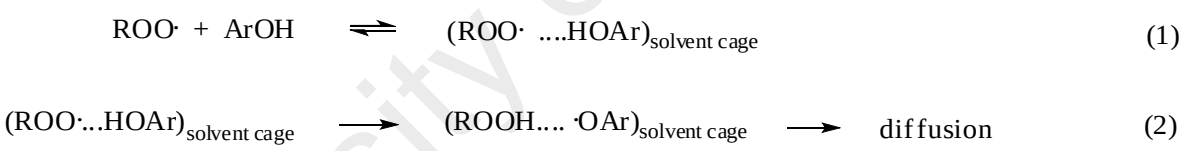


Scheme 2.6: SPLET mechanism

The net result of SPLET mechanism is the same as in the mechanisms of HAT.[74]

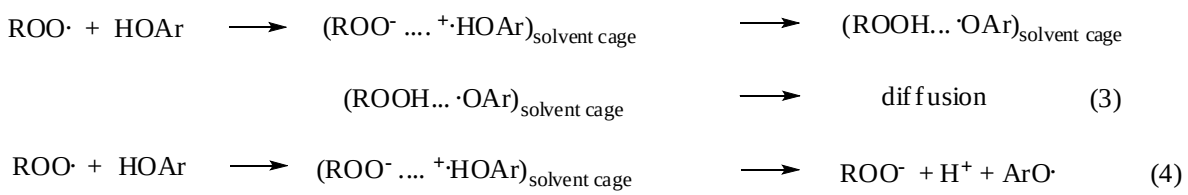
2.4.2. Solvent effect on phenolic antioxidants

Determination of antioxidant activity not only depends on its structure but also on other factors such as the solvent, temperature, concentration and etc. The most important factor is the solvent's role. In the case of HAT mechanism in phenol and peroxyl radical, a H-bonded complex forms between reaction species followed by direct transfer of hydrogen, yielding products with suitable orientation[75] as shown in Scheme 2.7



Scheme 2.7: Solvent effect on H-atom transfer mechanism

In solvents with high dielectric constants, a one-electron-transfer mechanism is involved since both SET and HAT mechanisms are always in parallel but with different reaction rates[76] as shown in Scheme 2.8.

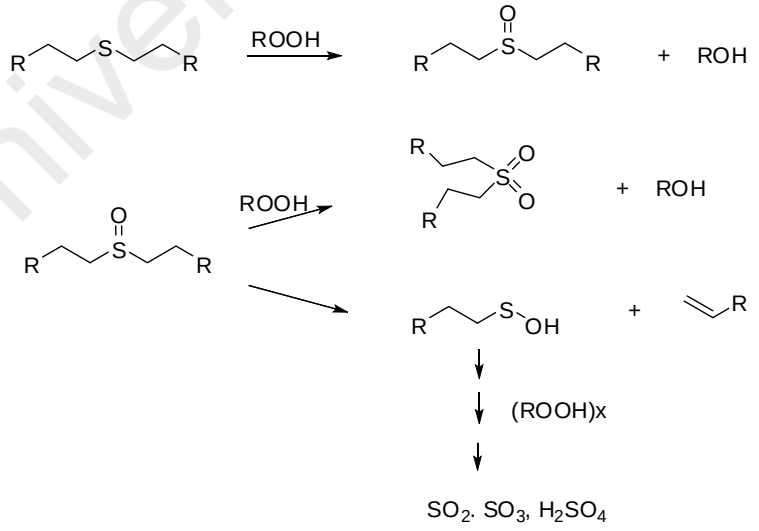


Scheme 2.8: Solvent effect on (SET) mechanism

The peroxyl-radical-trapping antioxidant activity of phenols is dependent on the hydrogen bond acceptor activity of the solvent. Polar solvents balance the ionic pair and build the SET reaction mechanism easily, while HAT mechanism is not solvent dependant.[77]

2.5. Secondary antioxidants

The most important secondary antioxidants are the trivalent phosphorous compounds and thioethers. They act by reducing hydroperoxides. The trivalent phosphorous compounds can be used as long-term heat stabilizer, and are highly polymer dependent. Another class of stabilizers that is able to reduce hydroperoxides is the thioethers. These stabilizers act mainly as long-term heat stabilizers. Combination of thioethers with phenolic antioxidants have a big influence on the long-term heat stability of polyolefins.[78] One of the reasons thioethers are effective stabilizers is due to their oxidation products which act as long-term heat stabilizers.[79-81] They can be even more effective hydroperoxide decomposers than the original compounds.[82] A proposed mechanism of action of thioethers is shown in Scheme 2.9.[83]



Scheme 2.9: Mechanism of action of thioethers

2.6. Thiol-based antioxidants

Thiol-type antioxidants constituting a class of organic sulfur derivatives (mercaptants) having the sulfhydryl (-SH) groups. This type of antioxidant plays a crucial role in protecting cells from oxidative damage through interaction with the electrophilic groups of ROS. They are considered as the first and major member of the physiological antioxidant defense system.[84] ROS frequently react with cellular thiols under 'oxidative stress' conditions and are then converted into a relatively less toxic byproduct at the expense of the reducing power of thiol, which gets oxidized to a disulfide (R-S-S-R).[85] In general thiols undergo one electron oxidation to form thiyl radicals (R-S$^{\cdot}$) through losing of H atom from the –SH group or an electron from the sulfur atom. A proton or two electron oxidation results in the formation of (R-SOH) as shown in Scheme 2.10.

$$R\text{-}SH \longrightarrow R\text{-}S^{\cdot} + H^{+} + e^{-} \quad (1)$$

$$R\text{-}SH + H_2O \longrightarrow R\text{-}SOH + 2H^{+} + 2e^{-} \quad (2)$$

Scheme 2.10: Oxidation of thiols

Under the physiological conditions such as pH, thiyl radicals are unstable and may recombine to form the corresponding disulfide.[85]

2.6.1. Tripeptide γ-glutamylcysteinylglycine (GSH)

Among the living organisms, radical scavenging antioxidants glutathione or GSH occupies a central place in the world of cellular thiols. The liver is a primary exporter of GSH to the plasma, both cytoplasmic and plasma GSH levels are changed with diet and oxidants stress.[86-89] GSH serves as a storage form of cysteine, a reducing agent that can protect protein sulfhydryls from irreversible oxidation to form mixed disulfides, which serve as a recyclable source of reducing equivalents for the glutathione peroxidase

(GPase) reaction. In (GPase) reaction, the cells convert H_2O_2 or lipid peroxides to H_2O or lipid hydroxyl compounds as shown in equation 2 of Scheme 2.11.[90]

$$2\ GSH + ROOH \xrightarrow{(GPase)} GSSG + ROH + H_2O \qquad (1)$$

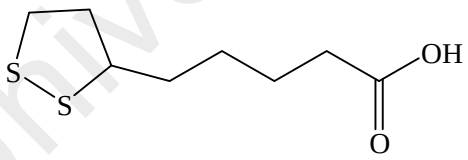
$$GSSG + NADPH + H^+ \xrightarrow{(GRase)} 2\ GSH + NADP^+ \qquad (2)$$

Scheme 2.11: GSH enzymatic reaction to form GSSG

Another reaction of GSH transferase is in the formation of glutathione thioethers (GS-R) with a wide variety of electrophilic compounds, such as important metabolic intermediates, or intermediates in the elimination of toxic xenobiotics.[91]

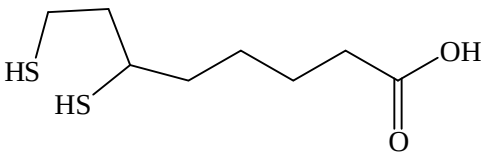
2.6.2. Lipoic acid as a thiol antioxidant

Thiols are principal agents to antioxidant defense in brain and other tissues. The metabolic antioxidant α-lipoate (thioctic acid, 1, 2-dithiolane-3-pentanoic acid; 1, 2-dithiolane-3 valeric acid (**2.4**) and 6,8-dithiooctanoic acid (**2.5**))[92] which is absorbed from the diet and travels through the blood-brain barrier. After its inclusion it reduced into cells and tissues to dihydrolipoate.[93]



Lipoic acid

2.4



Dihydrolipoic acid

2.5

Both forms act as antioxidants the oxidized form α-Lipoic acid (**2.4**) is a scavenger of hydroxyl radical, hypochlorous acid, and singlet oxygen. While it's highly reactive thiol form dihydrolipoic acid (**2.5**) is able to reduce GSSG to GSH as well as affecting the oxidation of thioredoxin and other thiol-containing proteins.[94]

Both forms also function as antioxidants which regenerate other antioxidants like vitamin C and vitamin E and also involve in raising intracellular glutathione levels through redox cycling[93] as shown in Figure 2.1.

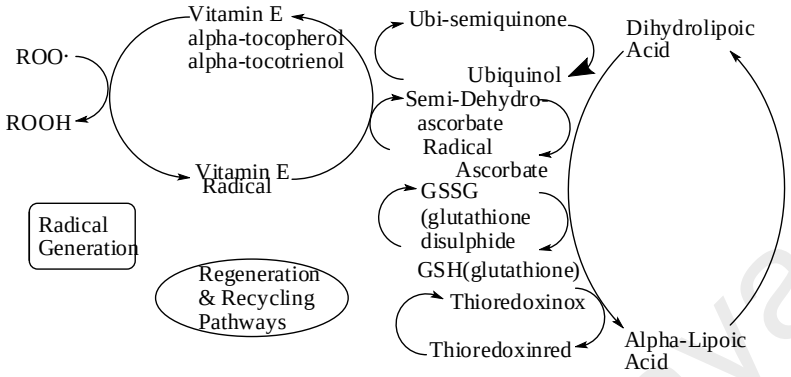


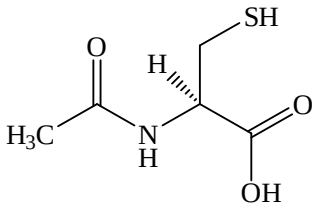
Figure 2.1: Pathways by which dihydrolipoate recycles vitamin E and other antioxidants[93]

It can be seen from Figure 2.1 dihydrolipoate is certainly regenerate ascorbate, either by direct reaction with semiascorbate or through the reduction of GSSG into GSH. Thus, the ascorbate is able to reduce the tocopheroxyl radical which are formed during the oxidation of α-tocopherol.[95, 96]

2.6.3. Clinical importance of sulfur-containing antioxidants

The variety of thiol antioxidants action mechanism meets the outlay on the possibility of using these antioxidants as supplementary for disease prevention and treatment. Sulfur containing compounds or thiol antioxidant intracellular GSH play a vital role in antioxidant cell defense and redox regulation.[97]

Compounds such as NAC *N*-Acetyl cysteine (**2.6**), has been approved for clinical use as mucolytic agent for cystic fibrosis patients.[98] Together with methionine it raises the intracellular GSH concentration and strengthening the natural cellular antioxidant system.[99]



2.6

Some multifunctional synthesized co-drugs related with sulfur antioxidants such as cysteine, methionine and bucill amine, show potentials for anti Parkinson because of their worthy radical scavenging activity.[99]

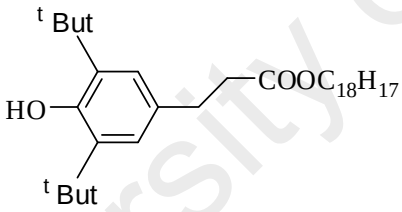
2.6.4. Instability of thiols in biological matrixes and their stabilization via derivatization

The analysis of thiols in biological matrixes usually involves derivatization of the sulfhydryl groups to prevent their oxidation to disulfides.[100] Different alkyl groups such as S-ethyl or thioether group had been used to stabilize thiols.[101] Aromatic or heteroaromatic thioether groups play an important role in antimicrobial activity. A number of these compounds have been synthesized and evaluated as antibacterial and antifungal agents. It has been reported that the highest antimicrobial activity had been observed for the structures bearing a thioethereal group at position 2 of aromatic or heteroaromatic ring.[102] In case of antioxidants, which contain a thioether bond, they usually are more effective than the simple compounds from which they are derived.[103]

In case of polymers, majority of polymers such as polyolefins, polydienes undergo autooxidation to produce carbon centered polymer radicals ($P^{\cdot}$) which undergo a fast reaction with oxygen. The resulting macroalkylperoxy radicals ($POO^{\cdot}$) abstract hydrogen atoms from the polymer backbone to form hydroperoxides (POOH). Chain propagation leads to accumulation of radicals which can trigger off various types of detrimental reactions in the polymer chain.[104] Interception of macroalkylperoxy radicals ($POO^{\cdot}$) with

chain breaking donors,[105] for instance, sterically hindered phenols and primary antioxidants, they inhibit the radical chain reaction. Further stabilization is also obtained if secondary antioxidants, such as sulfides, are used to prevent radical reactions of the decomposition products of nacroalkylhydroperoxides POOH by reduction of the hydroperoxides to alcoholic species POH.[105-106]

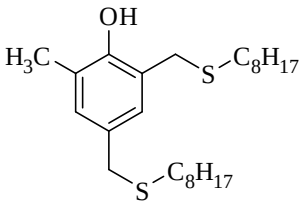
In classical phenols, compound (**2.7**) transforms macroalkylperoxyl radicals ($POO^{\cdot}$) into corresponding hydroperoxide (POOH). In most unsaturated polymers the presence of a sulfur compound such as DLTDP (dilauryl thiodipropionate) provide sufficient stabilization in a static ageing test (oven ageing at 80^0C). In contrast to this, the same combinations (**2.7**) + DLTDP) exhibit only limited activity under dynamic test conditions (Brabender-Test, 160^0C).[106]



2.7

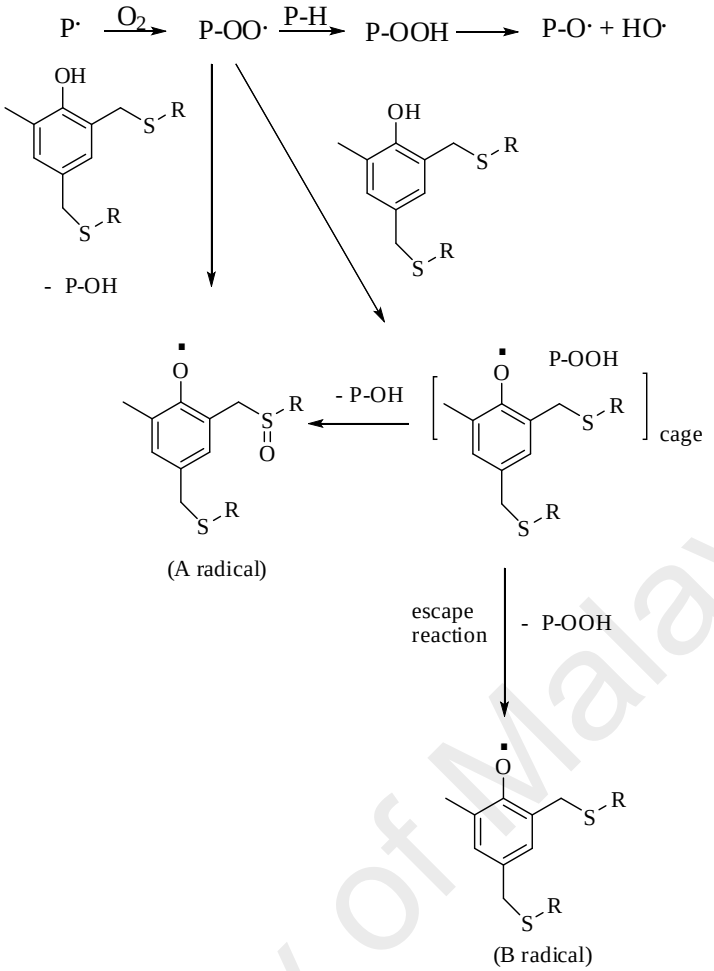
$S\text{-}(CH_2CH_2COOC_{12}H_{25})_2$ DLTDP (dilauryl thiodipropionate)

While the new commercial antioxidant (**2.8**), outperforms classical stabilizer systems in comparable concentration by a factor of 10, the Barbender test indicated the presence of both phenolic OH group and thioether group within the same molecule is mandatory to maintain an efficient stabilization.[106]



2.8

Optimum synergistic cooperation occurs between the step that involves the chain breaking donor phenolic OH-group and hydroperoxide decomposition step (involves the thioether moiety. This 'tandem reaction' shown in Scheme 2.12 was assumed to be either concerted with both reaction centres of the stabilizer involved simultaneously or consists of a two steps process starting with formation of a cage product between the initially formed phenoxy radical and the macroalkylhydroperoxide. The latter implies a high preference for the cage reaction leading to the radical A rather than B.[107]



Scheme 2.12: Reactions of the peroxy radicals

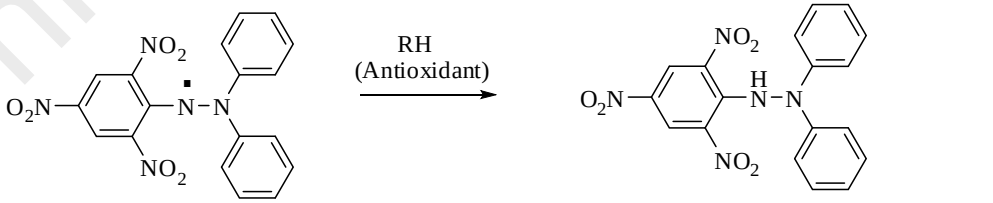
2.7. Antioxidants capacity measurements

Antioxidant capacity is related to compounds which are capable of protecting a biological system against the potential of harmful effect of processes or reactions involving reactive oxygen and nitrogen species. These protective effects of antioxidants have received increasing attention within biological, medical, nutritional, and agrochemical fields and that have resulted in the requirement of simple, convenient, and reliable antioxidant capacity determination methods. The total antioxidant capacity value should include methods applicable to both lipophilic and hydrophilic antioxidants, with regards to the similarity and differences of both HAT and ET mechanisms. In order to evaluate the antioxidant capacity and its quantitative effectiveness in preventing several human diseases such as atherosclerosis, diabetes mellitus, chronic inflammation and certain

types of cancer, there are two types of assays, either based on HAT or based on ET.[108] However, Prior and co-workers[21] have reported that 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity assay was considered to utilize both ET and HAT mechanisms.

2.7.1. DPPH Radical Scavenging Capacity Assay

A simple and extremely sensitive, stable method based on hydrogen donor is a measurement of antioxidant reducing ability toward DPPH radical.[109] DPPH is a stable free radical molecule that can accept an electron or hydrogen radical to form a stable, diamagnetic molecule. DPPH has an odd electron and has a strong absorption band at 518 nm. When this electron is paired off, the absorption intensity decreases stoichiometrically with respect to the number of electrons that have been taken up as in Scheme 2.13. A change in the absorbance produced in this reaction has been widely applied to test the capacity of numerous molecules which act as free radical scavengers.[111] monitoring the DPPH radical with UV spectrometer. The percentage of DPPH remaining is calculated and is found to be proportional to the antioxidant concentration that causes a decrease in the initial DPPH radical concentration by 50% where it is calculated as EC_{50}.[112]



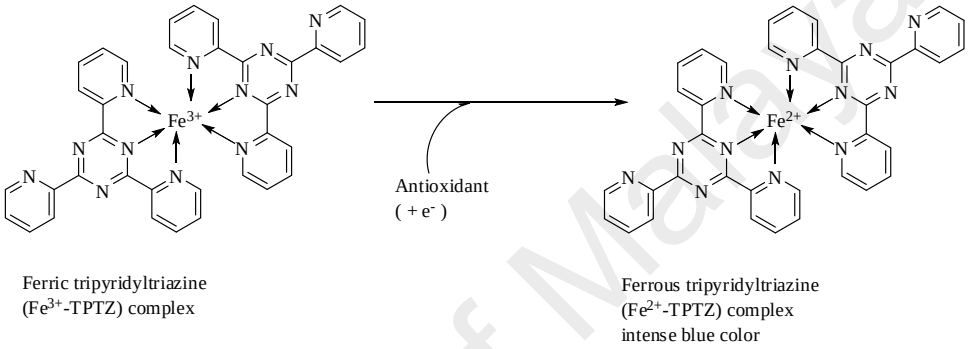
2,2-Diphenyl-1-picrylhydrazyl free radical (DPPH·)

2,2-Diphenyl-1-picrylhydrazyl (DPPH)

Scheme 2.13: Reaction between (DPPH·) and antioxidant to form DPPH[110]

2.7.2. FRAP Ferric Reducing Power Assay

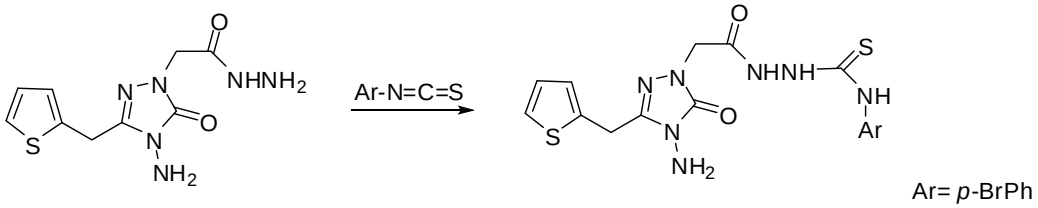
This assay involves electron transfer[113] that rely on the ability of the antioxidant to reduce the yellow ferric tripyridyltriazine complex (Fe(III)-TPTZ) to blue ferrous complex (Fe(II)-TPTZ) by the action of electron-donating antioxidants[20] as shown in Scheme 2.14. The antioxidant effect or its reducing ability is monitored by the formation of Fe^{2+}-TPTZ complex and the redox reactions. These reactions are completed within 4-6 minutes under acidic conditions.[20]



Scheme 2.14: Formation of Fe^{2+}-TPTZ complex from Fe^{3+}-TPTZ complex by antioxidant.[110]

2.8. Thiosemicarbazide derivatives

Thiosemicarbazides have attracted much attention in recent years because they are considered as a functional intermediates and subunits for the development of molecules that have pharmaceutical and/or biological interest. Thiosemicarbazides and their derivatives have been reported as potential antibacterial[113] and antimicrobial[114] which exhibit anti-inflammatory[115] and antioxidant activities.[116] For example, the reaction of isonicotinic acid hydrazides with 2-(4-amino-5-oxo-3-(thiophene-2-ylmethyl)-4,5-dihydro-1,2,4-tiazole-1-yl) acetohydrazide (**2.9**) in Scheme 2.15 gave compound (**2.10**) which shows both antibacterial and antioxidant activities.[117]



2.9 **2.10**

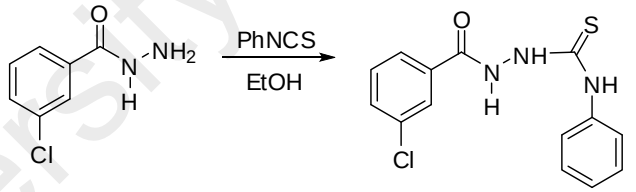
Scheme 2.15: Synthesis of thiosemicarbazide derivative **2.10**

Thiosemicarbazides have also been reported as potential antibacterial and topoisomerase IV inhibitors.[118]

2.8.1. Synthesis of thiosemicarbazides

2.8.1.1. Using isothiocyanates

Synthesis of thiosemicarbazides may be carried out in several ways. The general method involves treatment of carbohydrazides (**2.11**) with aryl isothiocyantes in the presence of ethanol to give 1,4-disubstituted thiosemicarbazide derivatives (**2.12**) as shown in Scheme 2.16.[119]

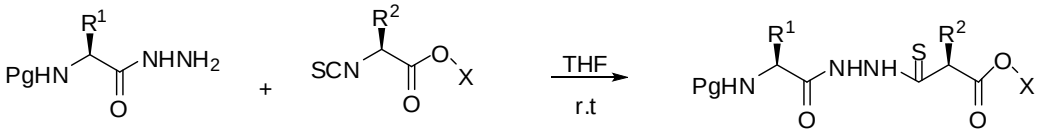


2.11 **2.12**

Scheme 2.16.: Synthesis of 1, 4-disubstituted thiosemicarbazides **2.12**

2.8.1.2. Using isothiocyanate ester

Synthesis of dipeptidyl thiosemicarbazides by the reaction of compound (**2.13**) with isothiocyanato ester (**2.14**) in THF yielded Boc/Z-peptidyl thiosemicarbazides (**2.15**) as shown in Scheme 2.17.[120]

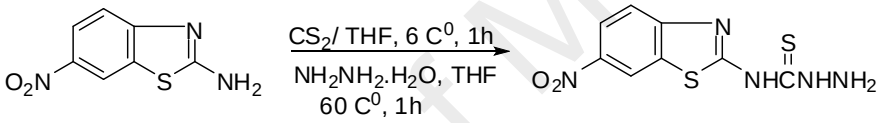


2.13 **2.14** **2.15**

Scheme 2.17: Synthesis of Boc/Z-peptidyl thiosemicarbazides **2.15**

2.8.1.3. Using carbon disulfide

The base-catalyzed nucleophilic addition of arylamines (**2.16**) to carbon disulfide gave potassium arylcarbamodithioates which further reacted with methyl iodide to produce *N*-aryl methyldithiocarbamates. Hydrazinolysis give 4-arylthiosemicarbazides (**2.17**) as shown in Scheme 2.18.[121]

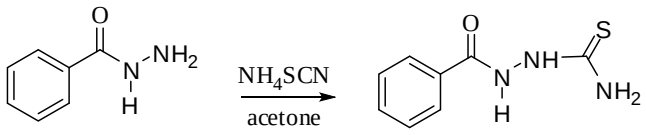


2.16 **2.17**

Scheme 2.18: Synthesis of 4-arylthiosemicarbazides **2.17**

2.8.1.4. Using ammonium thiocyanate

Heating of carbohydrazides (**2.18**) with ammonium thiocyanate in acetone afforded 1-acylthiosemicarbazides (**2.19**) as shown in Scheme 2.19.[122]

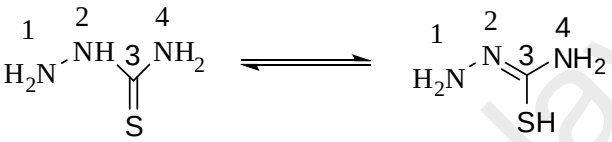


2.18 **2.19**

Scheme 2.19: Synthesis of 1-aroylthiosemicarbazides **2.19**

2.8.2. Reactivity of thiosemicarbazide derivatives

A previous study suggested that reactivity of 4-arylthiosemicarbazides is connected with electronic structure rather than the geometry of the molecule.[123] Thus the nucleophilic properties of thiosemicarbazides is correlated with the three NH- groups existing in the molecule with the reactivity order NH_2 (1) > NH (2) > NH_2 (4). Thiosemicarbazides also exist in the tautomeric thiol form (B) as shown in Scheme 2.20.

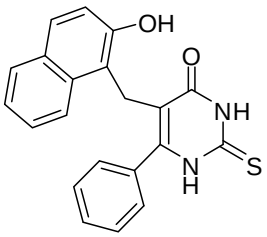


(A) Thione form (B) Thiol form

Scheme 2.20: Tautomeric structures of thiosemicarbazide

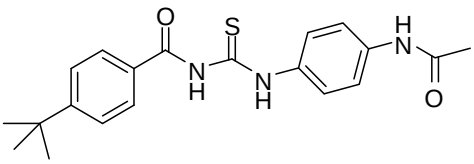
The presence of thiourea in thiosemicarbazide skeleton enhances the possible biological activities. The thioureas are described as most useful class of agents with large number of activities.[124, 125]

The acylthiourea group which is considered as a pharmacophore with a wide diversity of applications in heterocyclic chemistry.[126, 127] Acylthiourea derivatives have been employed as antimicrobial,[128] parasiticidal[129] and antitumoral agent.[130] Some bioactive acyl thiourea compounds such as cambinol (**2.20**) and tenovin-1 (**2.21**) are small molecule inhibitors of the NAD+-dependent family of protein deacetylases known as the sirtuins and there is considerable interest in inhibitors of this enzyme family due to possible applications in both cancer and neurodegenerative disease therapy.[131, 132]



Cambinol

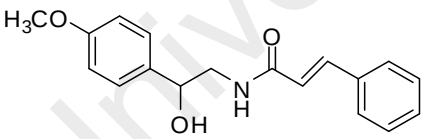
2.20



Tenovin-1

2.21

Another bioactive group present in thiosemicarbazide skeleton is the amide or carboxamide group. Recently Rajan *et al.*[133] in their study had chosen amide group to enhance the antioxidant activity and also the metabolic stability. Another study reported that the synthesis of new *N*-alkyl-*N*-aryl-3-(3,4-dihydroxyphenyl) propan amides was justified because of their superior antioxidant activities, higher lipophilicity and thermal stability. These amide derivatives have become more desirable than the precursor phenolic acid.[134] Furthermore, the natural product amide, aegeline (**2.22**) was reported to exhibit antidyslipidemic activity,[135] while carboxamides lidoderm (**2.23**) and benzafibrate (**2.24**) were used as therapeutic agents.[136]



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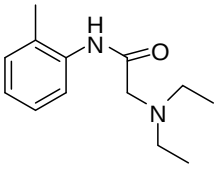

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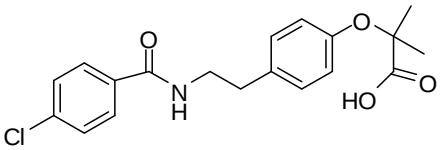


2.24

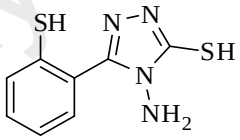
2.9. Heterocyclic compounds

2.9.1. 1,2,4-triazole derivatives

Triazoles are among the heterocyclic compounds that feature a five membered ring of two carbon atoms and three nitrogen atoms as part of the aromatic five-membered ring. Triazole refers to either one of the pair of isomeric compounds Figure 2.2.[25]

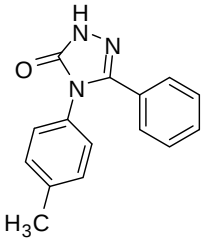
Figure 2.2: Structures of triazoles

Reports on 1,2,4-triazoles strongly indicated the importance of these compounds owing to their wide range of pharmacological activities such as nematicidal activity of compound (**2.25**).[137]



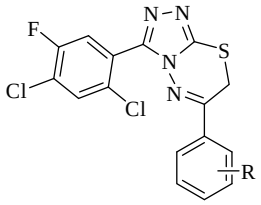
2.25

A series of 4,5-diphenyl-2*H*-1,2,4-triazol-3(4*H*)-ones have been reported and eight of them possessed anticonvulsant activity such as compound (**2.26**).[138]



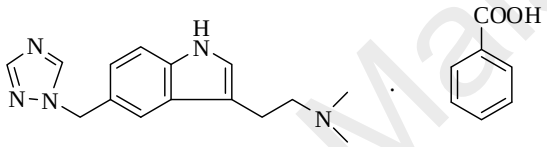
2.26

Bhat *et al.*[139] synthesized a series of 3-(2,4-dichloro-5-fluorophenyl)-6-(substituted phenyl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines (**2.27**) which were evaluated for their anticancer activity



2.27

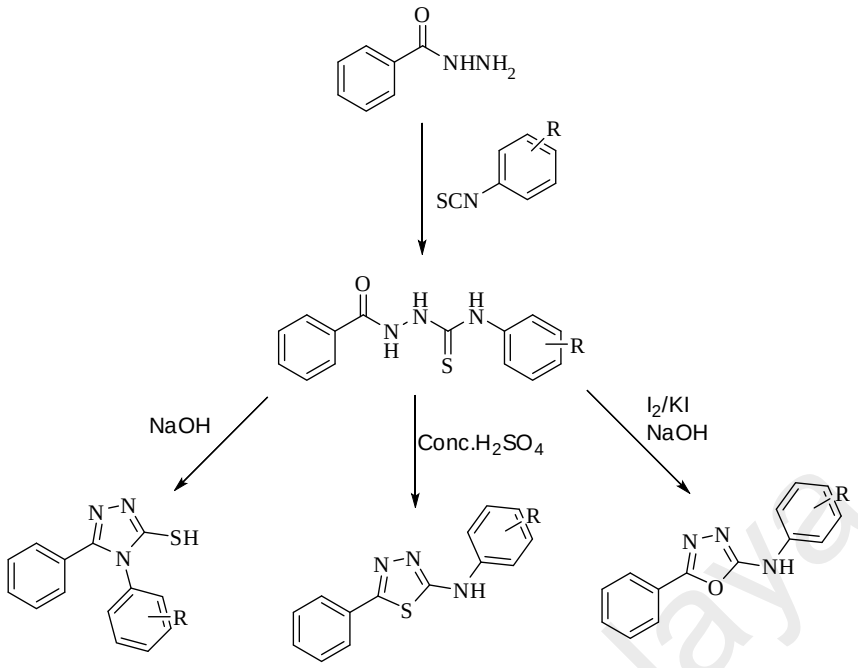
Some recent drugs such as Ribavirin (antiviral agent), Rizatriptan (**2.28**) (antimigraine agent), Alprazolam (anxiolytic agent), Fluconazole and Itraconazole (antifungal agents) are examples of potent molecules possessing a triazole nucleus.[140]



2.28

2.9.1.1. Synthesis of 1,2,4-triazoles

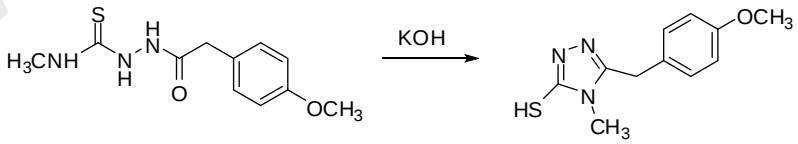
Many approaches to the synthesis of 1,2,4-triazoles have been reported in the literature. Among these, intramolecular dehydrative cyclization reactions of 1,4-disubstituted thiosemicarbazides have revealed to be an excellent strategy for the synthesis of 1,2,4-triazole derivatives. In these reactions five membered heterocycles with three heteroatoms are formed such as, 1,3,4-oxadiazoles, 1,3,4-thiadiazoles, and 1,2,4-triazoles as shown in Scheme 2.21.[141]



Scheme 2.21: Acid-(base-) catalyzed intramolecular dehydrative cyclization reactions of 1,4-disubstituted thiosemicarbazides[141]

2.9.1.1.1. Base-catalysed cyclization

The base-catalysed cyclization of 4-substituted 1-arylacetyl- and 1-aryloxyacetylthiosemicarbazide (**2.29**) by using KOH gave 3-arylmethyl- and 3-aryloxymethyl-5-mercapto-1,2,4-triazoles (**2.29**) as shown in Scheme 2.22.[142]

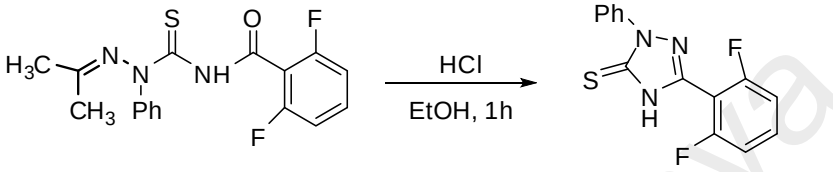


2.29 **2.30**

Scheme 2.22: Base-catalyzed cyclization of compound **2.30**

2.9.1.1.2. Acid-catalysed cyclization

The acid-catalysed cyclization of 2,6-difluoro-*N*-(1-phenyl-2-(propan-2-ylidene)hydrazinecarbonothioyl)benzamide (**2.31**) in refluxing ethanol produced 3-(2,6-difluorophenyl)-1-phenyl-1*H*-1,2,4-triazole-5(4*H*)-thione (**2.32**) as shown in Scheme 2.23.[143]

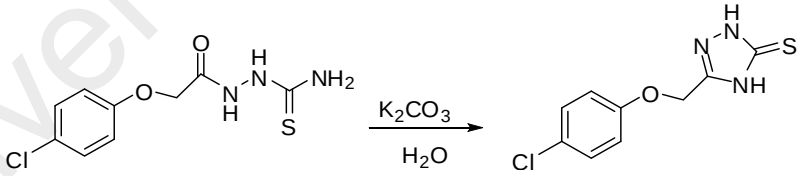


2.31 **2.32**

Scheme 2.23: Synthesis of compound **2.32**

2.9.1.1.3. Using aqueous solution of K_2CO_3

Rao *et al.*[144] have reported that 5-(4-chlorophenoxymethyl)-2,4-dihydro-1,2,4-triazole-3-thione (**2.34**) were synthesized from the treatment of 1-(2-(4-chlorophenoxy)acetyl) thiosemicarbazide (**2.33**) with an aqueous solution of K_2CO_3 as shown in Scheme 2.24.



2.33 **2.34**

Scheme 2.24: Synthesis of compound **2.34**

2.9.1.2. Reactivity of 1, 2, 4-triazole derivatives

Structural properties of triazoles, like moderate dipole character, hydrogen bonding capability, rigidity and stability are the main reasons for their superior pharmacological activities. Many experimental and theoretical studies have been performed to enrich the information regarding the possible mechanisms of proton transfer, tautomeric equilibrium, and relevant properties associated with proton transfer.[145] 1,2,4-Triazole-3-thione and its disubstituted derivatives can exist in two major tautomeric forms and exhibit different reactivities. The thione-thiol tautomerism of 4-ethyl-5-(2-hydroxyphenyl)-*2H*-1,2,4-triazole-3(*4H*)-thione in Figure 2.3 was studied by Özdemir and Türkpençe,[146] at the B3LYP level of theory using 6-311++G(d,p) basis function. The results from this study showed that the thione-enol form is the predominant tautomer among the tautomeric forms both in the gas phase and in solution phase as shown in Figure 2.3. The predicted energy difference between thione-enol and thiol-enol tautomers is within the range of ca. 64–66 kJ mol^{-1}, while the activation energy is within the range of ca. 188–241 kJ mol^{-1} for thione-enol to thiol-enol reaction, and within the range of ca. 124–176 kJ mol^{-1} for thiol-enol to thione-enol reaction. The energy difference between thione-keto and thiol-keto tautomers is very small which is found within the range of ca. 1–2 kJ mol^{-1}, while the activation energy is within the range of ca. 161–171 kJ mol^{-1} for thioneketo to thiol-keto reaction, and by the range of ca. 163–170 kJ mol^{-1} for thiol-keto to thione-keto reaction. In both cases, the barrier height for both the forward and the reverse single proton transfer reaction increases upon the shifting from the gas phase to solution phase. Thus, the tautomerization is not from the thermodynamic and kinetic points of both in the gas and solution phase. These findings are also confirmed by large positive standard enthalpy and free energy changes. Although the participation of a single molecule of the same solvents in the proton-transfer reaction has significantly reduced the barrier height, the values obtained still showed that the tautomerization is unfavorable.

However, it can be deduced by the inclusion of more molecules of solvent as well as the effect of the bulk which possibly reduces more of these barriers.

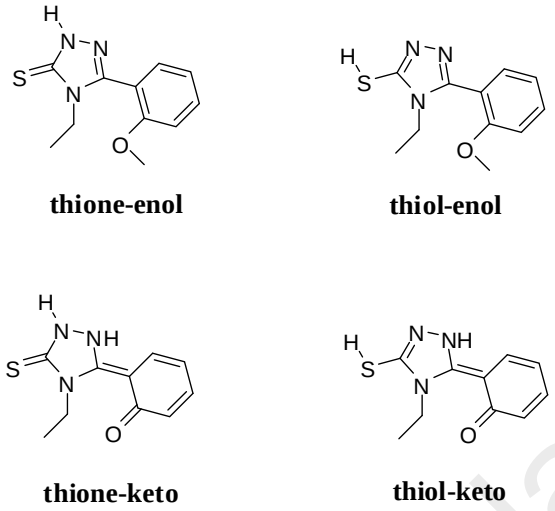


Figure 2.3: Optimized molecular structures of the tautomeric forms of 4-ethyl-5-(2-hydroxyphenyl)-2*H*-1,2,4-triazole-3(4*H*)-thione[146]

2.9.2. 1,3,4-Oxadiazole derivatives

Oxadiazole, a heterocyclic nucleus has attracted considerable attention among chemists in the search of new therapeutic molecules. Oxadiazoles is considered to be derived from furan by replacement of two methylene (-CH=) group by two pyridine type nitrogen (-N=). There are four possible isomers of oxadiazole depending up on the position of nitrogen atom in the ring and are numbered accordingly as shown in Figure 2.4.[147]

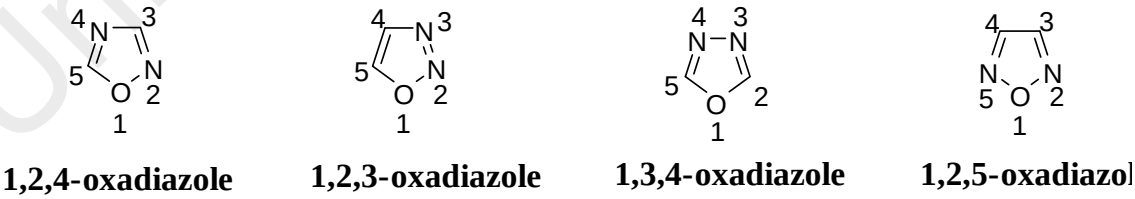
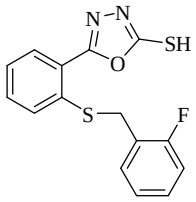


Figure 2.4: Isomers of oxadiazole

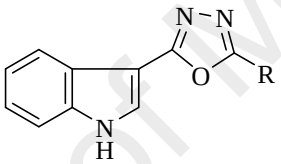
Out of the four possible isomers in Figure 2.4, 1,3,4-oxadiazole is the most widely exploited for various applications.[147] Literature survey reveals that compounds bearing 1,3,4-oxadiazole nucleus are known to have a broader spectrum of biological activities.

For example Zarghi *et al*.[31] showed that the introduction of fluoro substituent at *ortho* position of benzylthio moiety (**2.35**) had the best anticonvulsant activity.



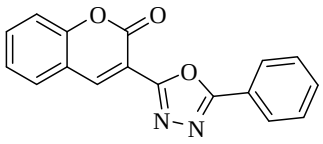
2.35

Another study showed that 5-(3-indolyl)-2-(substituted)-1,3,4-oxadiazoles (**2.36**) possess *in vitro* anticancer activity.[148]



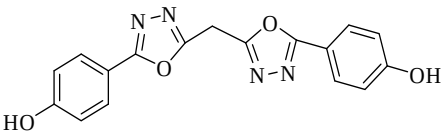
2.36

New 2,5-disubstituted 1,3,4-oxadiazoles showed that the dual inhibition of COX and LOX are promising strategies for treating inflammatory conditions due to their reduced side effects. Compound (**2.37**) is an example.[149]



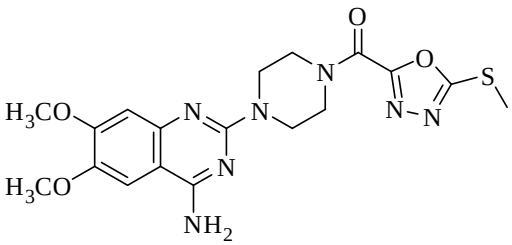
2.37

In the search for antioxidant drugs, Musad *et al*.[150] reported that the preparation of bis(heterocycle) bearing 1,3,4-oxadiazole moieties (**2.38**) resulted in some of these new derivatives demonstrating potent antioxidant activity.



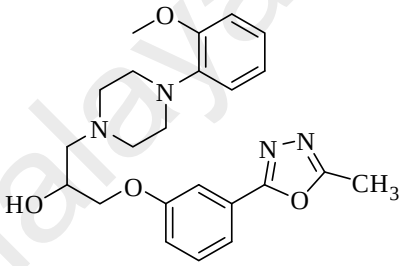
2.38

Another application of 1,3,4-oxadiazole derivatives (**2.39**, **2.40**) is as vasodilators exemplified by the commercially available antihypertensive agents Tiodazosin[151] and Nesapidil.[152]



Tiodazosin

2.39



Nesapidil

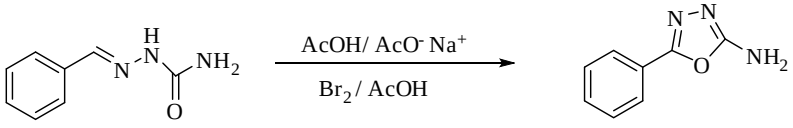
2.40

2.9.2.1. Synthesis of 1,3,4-oxadiazole derivatives

The first method for synthesizing 1,3,4-oxadiazole was reported in 1955.[153] Many other methods have been published since then.

2.9.2.1.1. Oxidative cyclization of semicarbazones

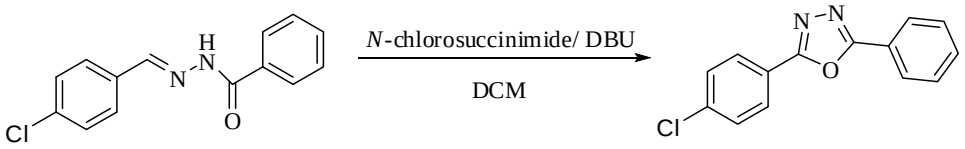
The cyclization of the semicarbazone shown in Scheme 2.25 with bromine in acetic acid is one of the approaches that is frequently used for the preparation of 1,3,4-oxadiazoles. Rajak *et al.*[154] have reported the formation of 2-amino-5-Aryl-1,3,4 oxadiazole (**2.42**) from the reaction of compound (**2.41**) with bromine in the presence of sodium acetate.



2.41 **2.42**

Scheme 2.25: Oxidative cyclization of semicarbazone **2.42**

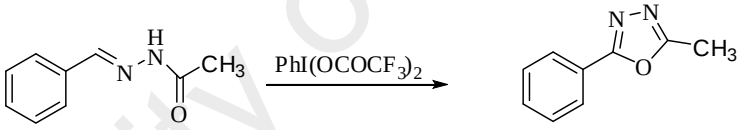
Another example of oxidative cyclization of acyl hydrazone (**2.43**) using *N*-chlorosuccinimide1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as an effective oxidant in the preparation of 2,5-di-substitued-1,3,4-oxadiazole (**2.44**) is illustrated in Scheme 2.26.[155]



2.43 **2.44**

Scheme 2.26: Oxidative cyclization of hydrazone **2.44**

Cyclization of hydrazone (**2.45**) using bis (trifluoroacetoxy) iodobenzene as an oxidizing agent in chloroform or DMSO at room temperature shown in Scheme 2.27 also gave 1,3,4-oxadiazole derivatives.[156]

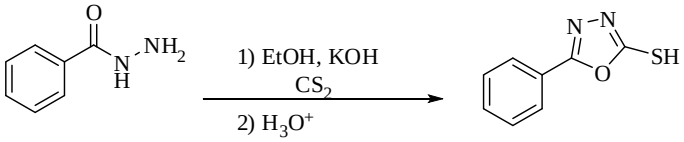


2.45 **2.46**

Scheme 2.27: Cyclization of hydrazone **2.45**

2.9.2.1.2. Synthesis of 5-substituted-1,3,4-oxadiazole-2-thiol(thione)s

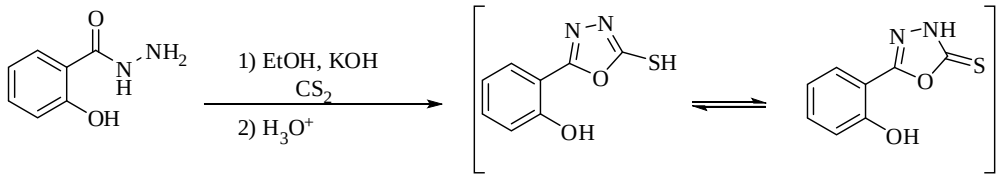
The main synthetic route for 5-substituted-1,3,4-oxadiazole-2-thiol (thione)s involves an initial reaction between an acylhydrazide (**2.47**) and carbon disulfide in a basic alcohol solution, followed by acidification as shown in Scheme 2.28.[157]



2.47 **2.48**

Scheme 2.28: Synthesis of 5-substituted phenyl-1,3,4-oxadiazole-2-thiol **2.48**

Another example is the synthesis of 5-(2-hydroxyphenyl)-1,3,4-oxadiazole-2-thione (**2.50**) from salicylic acid hydrazide (**2.49**) as shown in Scheme 2.29.[158]

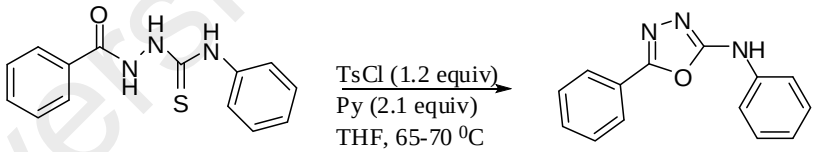


2.49 **2.50**

Scheme 2.29: Synthesis of 5-(2-Hydroxyphenyl)-1,3,4-oxadiazole-2-thiol (thione)s **2.50**

2.9.2.1.3. Synthesis of 1,3,4-oxadiazoles from acylsemicarbazides

Dolman and co-workers[159] reported a new method of synthesis for 5-aryl(alkyl)-2-amino-1,3,4-oxadiazoles (**2.52**) from acylsemicarbazides (**2.51**) (X=O) mediated by tosyl chloride. Yields of 97%–99% were obtained while using thiosemicarbazide derivatives which are more reactive than the corresponding semicarbazide derivatives shown in Scheme 2.30.



2.51 **2.52**

Scheme 2.30: Synthesis of 5-aryl-2-amino-1,3,4-oxadiazole **2.52** from acylthiosemicarbazide **2.51** and tosyl chloride

CHAPTER 3: RESULTS AND DISCUSSION

3. Results and Discussion

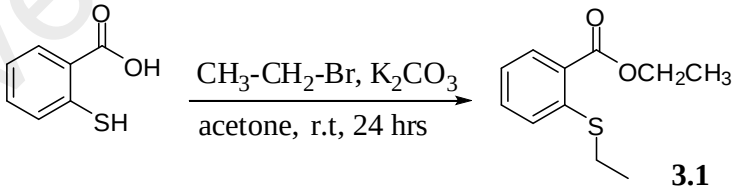
This chapter is divided into two parts; the first part is on the synthesis of thiosemicarbazide and 1,2,4-triazole derivatives, while the second part focuses on the synthesis of 1,3,4-oxadiazole derivatives and also preparation of some new Schiff's bases substituted with different aryl hydroxyl groups.

All spectral data of synthesized compounds are presented in Appendix (1) and (2).

3.1. Thiosemicarbazide and 1,2,4-triazole derivatives

3.1.1. Synthesis of ethyl 2-(ethylsulfanyl)benzoate (3.1) and 2-(ethylsulfanyl)benzohydrazide (3.2)

A simple and well known procedure was used to prepare ethyl 2-(ethylsulfanyl)benzoate **3.1**.[160] Bromoethane and K_2CO_3 were added to a solution of commercially available thiosalicylic acid and dry acetone as shown in Scheme 3.1. After 12 hours at 70˚C, a 75% yield of compound **3.1** was obtained. An improved percentage yield i.e 95% was obtained when the reaction mixture was left for 24 hours at room temperature.



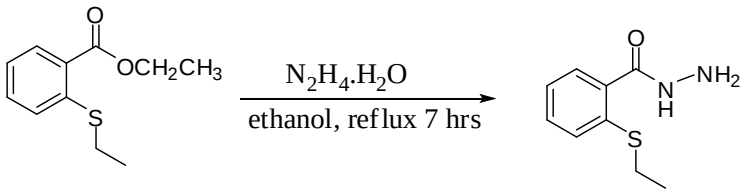
3.1

(95%)

Scheme 3.1: Preparation of compound **3.1**

The reduction of percentage yield with temperature is probably due to the heat sensitivity of S-H bond in thiols, whereby at a higher temperature, heterolytic dissociation of the S-H bond occurrs to give either thiolate anion or sulfanyl cation.[160] These species can directly participate in various transformations to give side products thus a reduction in percentage yield was recorded.

2-(Ethylsulfanyl)benzohydrazide **3.2** was prepared by reacting compound **3.1**, and hydrazine hydrate in ethanol for 7 hours as shown below in Scheme 3.2.



3.1 **3.2** (97%)

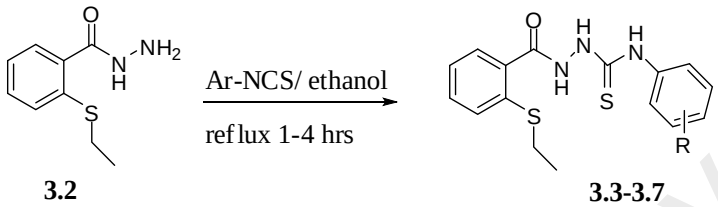
Scheme 3.2: Preparation of the active intermediate **3.2**

Higher percentage yield was obtained when hydrazine hydrate was added in portions and the heating was containued for 7 hours. The product was obtained in 97% yield.

The compounds **3.1** and **3.2** which were used as the key intermediates for the synthesis of thiosemicarbazides and 1,2,4-triazoles had their strucures confirmed by spectroscopic methods. The spectras are given in Appendix. The IR spectrum of compound **3.1** shows the disappearance of an absorption peak at 3435 cm^{-1} which was attributed to hydroxyl OH group of thiosalicylic acid. IR spectrum of acid hydrazide **3.2** shows the presence of NH, NH_2 at 3323-3290 and 3220-3179 cm^{-1} respectively. The ^1H NMR spectrum of compound **3.1** showed signals resulting from ethyl group as a triplet at 1.39 ppm for CH_3 and quartet at 4.38 ppm for CH_2, while ^1H NMR spectrum of compound **3.2** display a broad peak for 2 protons of NH_2 at 4.24 ppm and a broad singlet at 9.46 ppm due to NH's proton.

3.1.2. Synthesis of 1-acylthiosemcarbazides (3.3-3.7)

Reaction of 2-(ethylsulfanyl)benzohydrazide **3.2** with various aryl isothiocyanates, gave a series of 1-acylthiosemicarbazides **3.3-3.7**.[119] The reactions were carried out between 1 to 4 hours as shown in Scheme 3.3. The percentage yield of the products is shown in Table 3.1.



3.2 **3.3-3.7**

Scheme 3.3: Synthesis of 1-acyl thiosemicarbazide derivatives **3.3-3.7**

Table 3.1: Percentage yield of compounds **3.3-3.7**

Compound No.	**R**	**(%) Yeild**
3.3	H	96
3.4	*p*-Cl	93
3.5	*p*-CH_3	95
3.6	*p*-OCH_3	94
3.7	3,4,5-$(OCH_3)_3$	93

This reaction was also carried out in various solvents and the results are shown in Table 3.2.

Table 3.2: Effect of solvent on reaction time and percentage yield of compounds **3.3-3.7**

Solvent	**Reaction time (hrs)**	**(%) Yield**
Ethanol	1-4	>90
DMA	24	75-83
DMF	10	80-88
(DMF)/ethanol	8	65-88

It can be seen from Table 3.2 that when DMA was used as solvent, 75-83 % yield was obtained when the reaction was carried out for 24 hours. Reaction in DMF was carried out for 10 hours and 80-88% yield were obtained. The lowest yield (65-88%) was obtained for the reaction in DMF/EtOH mixture with reaction duration of 8 hours. Table 3.2 also indicates that the synthesis which was carried out in ethanol has the shortest reaction duration and gave the highest yield.

The IR spectrum of thiosemicarbazide derivatives **3.3-3.7** shows NH stretching bands at 3179-3283 cm^{-1}. The IR spectra of these compounds also indicated the presence of C=O stretching bands at 1670-1640 cm^{-1}. The presence of a strong band at 1244-1250 cm^{-1} assigned to the C=S stretching which indicates that **3.3-3.7** exist in the thione form. ^{1}H NMR spectrum shows that the signal of the proton linked to NH -ph was recorded at 9.41–9.57 ppm. While the proton for NH-C=S and NHC=O appears at 9.71–9.92 and 10.42–10.73 ppm respectively.

The ^{13}C NMR spectra of **3.3-3.7** confirmed the presence of C=O and C=S groups of thiosemicarbazides which appears in the region of 167.15 and 181.02-181.12 ppm, respectively. Mass spectrometry confirmed the molecular mass of the synthesized compounds **3.3-3.7** as recorded in Appendix. The X-ray structure of compound **3.3** is as illustrated in Figure 3.1.

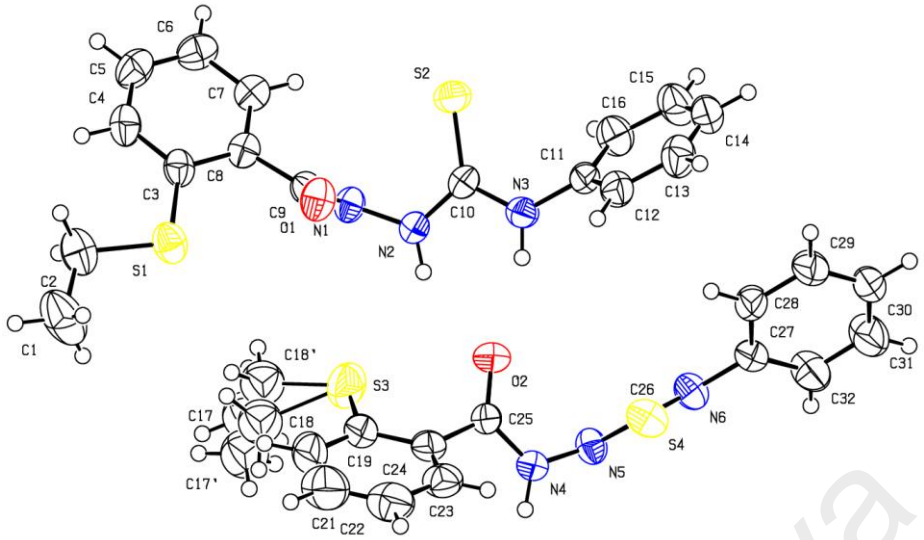
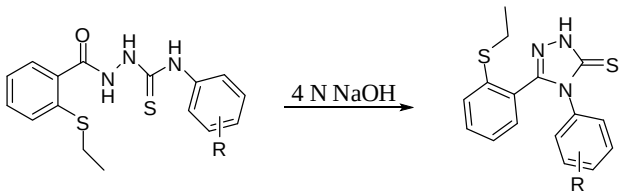


Figure 3.1: Molecular structure of compound (**3.3**), showing the atomic numbering scheme.

3.1.3. Synthesis of 5-[2-(ethylsulfanyl)phenyl]-4-substituted-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones (3.8-3.12)

Ring closure of acylthiosemicarbazides in alkaline medium is a well known method for the synthesis of 1,2,4-triazoles.[161] Thus, compounds **3.8-3.12** were obtained when compounds **3.3-3.7** were refluxed in aqueous NaOH with ethanol as a solvent for 3 hours as shown in Scheme 3.4. The percentage yield of these compounds is shown in Table 3.3.



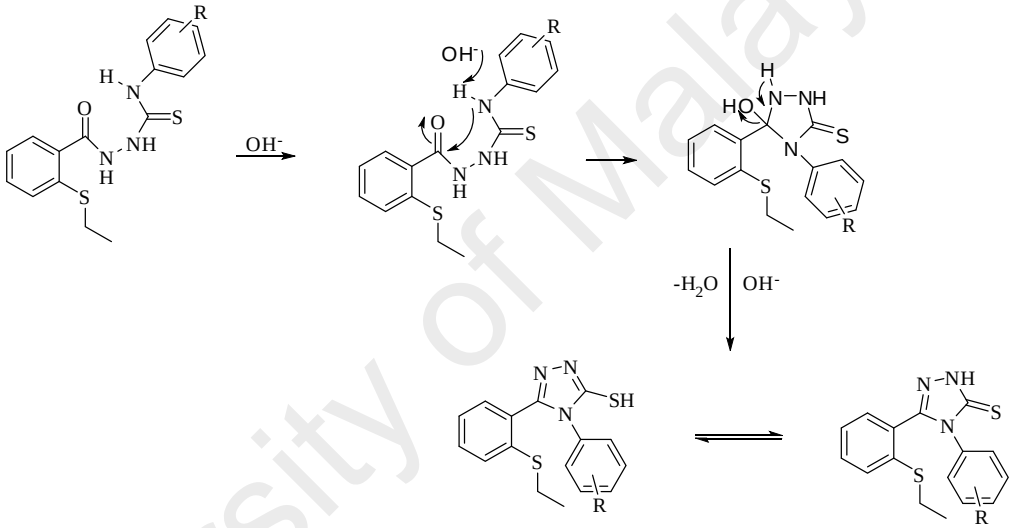
3.3-3.7 **3.8-3.12**

Scheme 3.4: Synthesis of 1,2,4-triazole derivatives **3.8-3.12**

Table 3.3: Percentage yield of compounds **3.8-3.12**

Compound No.	R	(%) Yield
3.8	H	75
3.9	*p*-Cl	73
3.10	*p*-CH_3	70
3.11	*p*-OCH_3	75
3.12	3,4,5-$(OCH_3)_3$	69

The mechanism for the formation of 1,2,4-triazoles **3.8-3.12** as shown in Scheme 3.5 was suggested by Guda *et al*.[161]



Scheme 3.5: Mechanism of the formation of 1,2,4-triazoles **3.8-3.12**

The cyclization of **3.3-3.7** was carried out under alkaline conditions; so that the nucleophilicity of N-4 was enhanced and led to cyclization with the carbonyl carbon atom to give 1,2,4-triazole-5(4*H*)-thiones, **3.8-3.12**.

The disappearance of C=O stretching band of acylthiosemicarbazides in IR spectra and the appearance of strong C=N stretching band at 1607-1642 cm^{-1} are found to be the evidence of ring closure occurrence to form 1,2,4-triazoles. ^{1}H NMR spectra of

compounds **3.8-3.12**, show signals of NH of 1,2,4-triazole-5(4*H*)-thione derivatives in the 14.11-14.83 ppm range.

X-ray analysis confirmed that compound **3.8** exists in the thione form as shown in Figure 3.2.

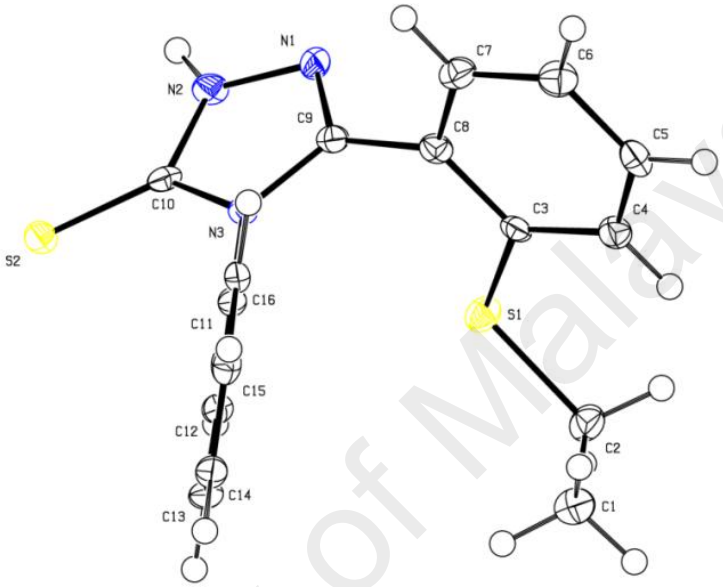
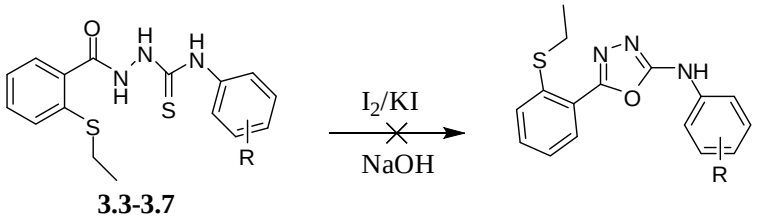


Figure 3.2: Molecular structure of **3.8** showing the atomic numbering scheme.

3.1.4. Cyclization of thiosemicarbazides (3.3-3.7) with iodine in alkaline medium

Omar *et al*.[162] has reported that cyclization of thiosemicarbazide derivatives **3.3-3.7** with iodine in potassium iodide in the presence of sodium hydroxide gave the corresponding 1,3,4-oxadiazole derivatives. However, our attempts to synthesizes 1,3,4-oxadiazole derivatives from the corresponding thiosemicarbazides **3.3-3.7** using Omar's method failed to materialized. For example; reaction of methanolic iodine with solution of **3.3-3.7** as shown in Scheme 3.6 in excess sodium hydroxide caused an immediate colour change but with no detectable product.



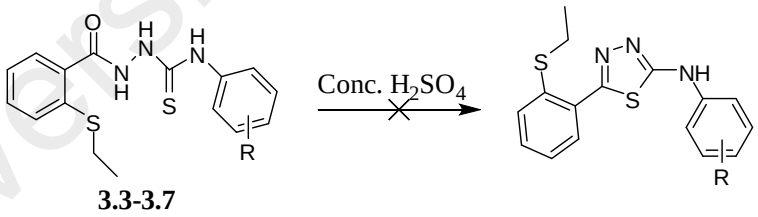
3.3-3.7

Scheme 3.6: Reaction of compounds **3.3-3.7** with methanolic iodine in NaOH

Ruff and Kucsman,[163] studied the effect of sodium periodate on a solution containing aliphatic or aromatic thioethers and concluded that oxidation would occur by a one-step electrophilic oxygen transfer from periodate to the thioether through a polar transition state. Following from Ruff and Kucsman's finding, we believed that the formation of periodate solution may occur as over-oxidation of thioether group in compounds **3.3-3.7** which prevent the cyclization of these compounds to the corresponding 1,3,4-oxadiazole derivatives. In addition, we repeated the reaction several time but still no expected product we don't know exactly why it didn't work with our system so we proceed with a different reactions and stoped further investigations.

3.1.5. Cyclization of thiosemicarbazides (3.3-3.7) under acidic conditions

One of the most widely used method to prepare the 1,3,4-thiadiazoles is the cyclization of the thiosemicarbazide derivatives using concentrated sulfuric acid as reported by Desai, *et al*.[164] Our attempts to prepare 1,3,4-thiadiazole derivatives as shown in Scheme 3.7 using this method however was also unsuccessful.



3.3-3.7

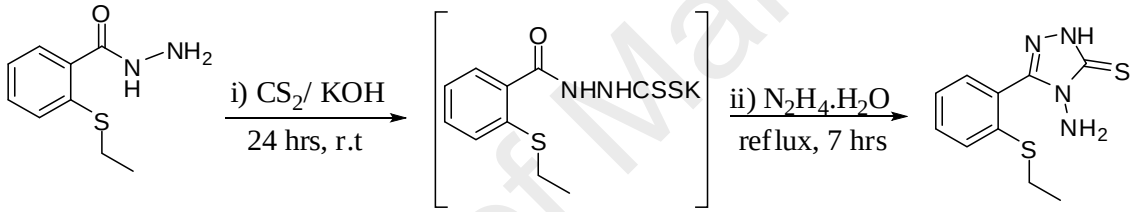
Scheme 3.7: Reaction of compounds **3.3-3.7** with concentrated sulfuric acid

In our attempt, thiosemicarbazide derivatives **3.3-3.7** were dissolved with cooling in concentrated H_2SO_4 and the contents were kept at room temperature for 3 hours and then poured on to crushed ice. As the reaction proceeded, the color of the reaction mixture changed from brown to brownish-red and finally black. This observation could be due to the sulfuric acid acting as both an oxidizing agent and an electrophile as reported by

Nehlsen, *et al.*[165] whereby the oxidized products are likely to be the sulfoxides and other oxidized sulfur species.

We thus conclude that in our experiment, an oxidation reaction may occur between the sulfuric acid and the thiosemicarbazide derivatives bearing thioether group **3.3-3.7**, but unfortunately we were not able to determine the products formed.

3.1.6. Synthesis of 4-amino-5-[2-(ethylsulfanyl)phenyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (3.13)



3.2 **3.13** **(65%)**

Scheme 3.8: Synthetic scheme of the formation of compound **3.13**

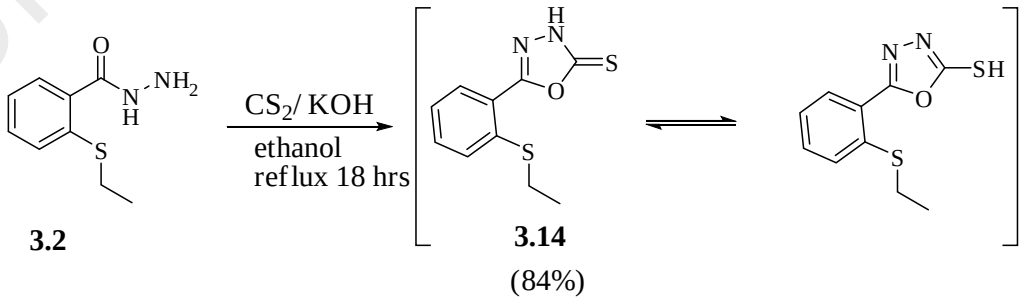
1,2,4-Triazole ring containing amino group at position 4 was reported to be a good nucleophile in most reactions. Several synthetic protocols have been reported in the literature, but the most common synthetic pathway to prepare 4-amino-1,2,4-triazole is through the formation of potassium hydrazine carbodithioate salt followed by reaction with hydrazine hydrate[166] We have applied the same procedure to synthesis compound **3.13** as demonstrated in Scheme 3.8. Acid hydrazide **3.2** was first mixed with CS_2 and stirred for 24 hrs at room temperature. After that $NH_2.NH_2.H_2O$ was added and the mixture was refluxed for 7 hours. After purification, 65% yield of compound **3.13** was obtained.

The IR spectrum of compound **3.13** shows the disappearance of C=O stretching band at 1630 cm^{-1} and the appearance of C=N stretching band at 1607 cm^{-1} which is a first evidence for ring formation as discussed earlier. ^{1}H NMR spectrum of the compound **3.13** revealed the presence of aromatic protons at 7.33-7.53 ppm. The two protons recorded as a singlet at 5.47 ppm corresponds to the NH_2 group at position 4. ^{13}C NMR spectrum of the compound **3.13** confirmed the presence of four sp^2 methines of C4, C5, C6, and C7 at 125.78, 130.92, 125.14 and 128.10 ppm respectively, and the presence of two quaternary carbons C3 and C8 at 137.89 and 131,31 ppm respectively. Quaternary carbons of C9 and C10 were recorded at 149.61 and 166.18 ppm, respectively.

3.2. 1,3,4-oxadiazole derivatives and new hydroxyl-substituted Schiff bases

3.2.1. Synthesis of 5-(2-(ethylsulfanyl)phenyl]-1,3,4-oxadiazole-2(3*H*)-thione (3.14)

Synthesis of 5-(2-(ethylsulfanyl)phenyl]-1,3,4-oxadiazole-2(3*H*)-thione **3.14** was done according to the procedure reported by Koparir and co-workers.[157] An initial reaction between hydrazide **3.2** and carbon disulfide in a basic alcohol solution, followed by acidification of the reaction mixture to produce compound **3.14** as demonstrated in Scheme 3.9. Compound **3.14** was isolated in the thione form, which was confirmed by the IR and NMR data.

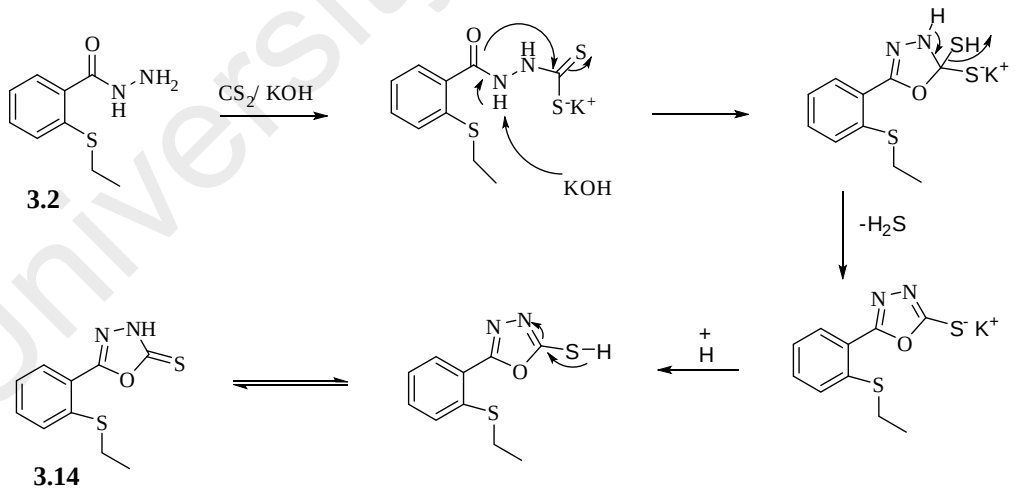


3.2 **3.14** (84%)

Scheme 3.9: Synthesis of compound **3.14**

The IR spectrum of the compound **3.14** showed a broad absorption peak at 3314 cm^{-1} due to N-H stretching and an absorption peak at 1602 cm^{-1} due to C=N stretching of the 1,3,4-oxadiazole ring. The ^{1}H NMR spectrum shows the signals of ethyl group as a triplet at 1.28 ppm and a quartet at 3.06 ppm, and a broad singlet at 14.83 ppm due to NH group. In addition, aromatic protons were recorded between 7.35-7.81 ppm. ^{13}C NMR spectrum of compound **3.14** confirmed that the cyclization has taken place and 1,3,4-oxadiazole ring was formed through the presence of a peak at 159.46 ppm assigned to 1C of C=N of oxadiazole ring, and also a peak at 176.99 ppm due to C of (C=S).

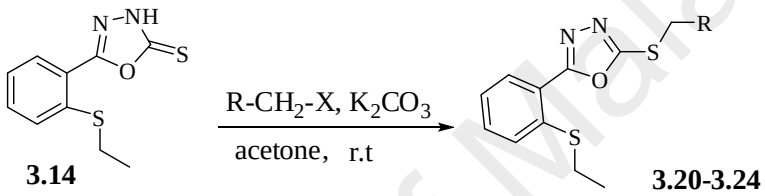
The suggested mechanism for the formation of compound **3.14** is illustrated in Scheme 3.10. The first step involves the reaction of acid hydrazide **3.2** with CS_2 to produce the unstable dithiocarbamate salt. Abstraction of a proton by KOH followed by the loss of H_2S gave 5-(2-(ethylsulfanyl)phenyl]-1,3,4-oxadiazole-2(3*H*)-thione **3.14** as shown in Scheme 3.10.[167]



Scheme 3.10: Mechanism of formation of compound **3.14**

3.2.2. Synthesis of new 2,5-disubstituted-1,3,4-oxadiazole derivatives (3.20-3.24)

Alkylation of compound **3.14** was carried out accordingly to the procedure reported by Trivedi *et.al.*[168] with modification. A mixture of anhydrous potassium carbonate and the appropriate aryl halide in presence of dry acetone were allowed to react at room temperature to yield new 2,5-disubstituted-1,3,4-oxadiazole derivatives **3.20-3.24** as shown in Scheme 3.11. Compounds **3.15**, **3.16** and **3.19** were commercially available while compounds **3.17** and **3.18** were synthesized according to the procedure reported by Akhter *et. al.*[169] The alkylation of compound **3.14** follows the S_N2 mechanism.

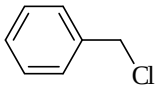
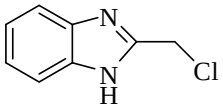
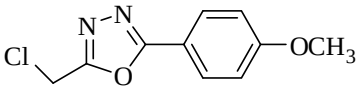
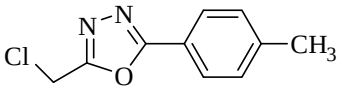
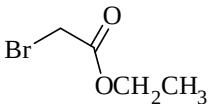


3.14 **3.20-3.24**

Scheme 3.11: Synthesis of 2,5-disubstituted-1,3,4-oxadiazole derivatives **3.20-3.24**

Reaction of compound **3.14** with benzyl chloride **3.15** and benzimidazole **3.16** required 6-7 hrs stirring at room temperature and the product was obtained in a yield of 85-86 %. Reaction of **3.14** with compounds **3.17** and **3.18** on the other hand, lasted 24 hours and 78-80 % yield was obtained. The shortest reaction time was observed when compound **3.14** was reacted with compound **3.19** with 78 % yield as shown in Table 3.4. It can be seen from the Table that bulkier R-CH_2-X has a significant effect on the duration of the reaction and percentage yield.

Table 3.4: Effect of R-CH_2-X on reaction time and percentage yield of compounds **3.20-3.24**.[170]

Compound No.	R-CH_2-X	Reaction time (hrs)	(%) Yield
3.20		6	85
3.21		7	86
3.22		24	80
3.23		24	78
3.24		5	78

^{1}H NMR spectra of compounds **3.20-3.24** shows a singlet peak at 4.49-4.95 ppm, which was assigned to S-CH_2 group. ^{1}H NMR spectrum of compound **3.20** shows an absorption band due to aromatic protons in the region of 7.32-7.81 ppm. While ^{13}C NMR of the same compound shows a peak at 163.16 ppm due to C atom of C=N of oxadiazole ring, and a peak at 164.25 for C atom of S-C=N of oxadiazole.

^{1}H NMR spectrum of compound **3.21** shows a singlet at about 11.07 ppm due to NH proton of imidazole in addition to the aromatic protons of the aromatic rings as discussed earlier. ^{13}C spectrum of **3.21** shows a great difference from compound **3.20** which was due to the presence of imidazole ring whereby the two protons of S-CH_2 was recorded as a singlet at 24.58 ppm.

^{1}H NMR spectrum of compound **3.22** shows a singlet at 3.85 which was assigned to OCH_3 protons. The aromatic protons were recorded as previously discussed. The ^{13}C spectrum shows a peak at about 164.47 ppm for CH_2-C=N of oxadiazole and a peak at

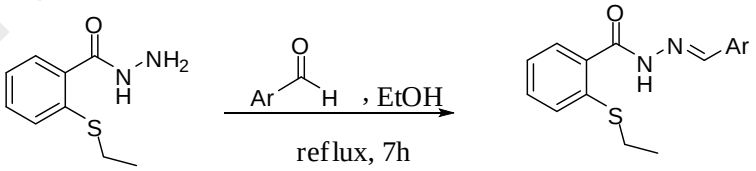
162.54 ppm assigned to the C=N-Ar-OCH_3 and C atom of C-OCH_3 appeares at 161.78 ppm.

Compound **3.23** on the other hand showed a singlet peak at about 2.39 ppm for the protons of the methyl group in the ^{1}H NMR spectrum, and the C-CH_3 appears at about 131.96 ppm in ^{13}C NMR spectrum.

Compound **3.24** with ethyl acetate analogues shows the signals of two CH_3 groups at 1.31 ppm and 1.39 ppm. Three signals of CH_2 protons at 3.02 ppm, 4.13 ppm and 4.29 ppm, in addition to the aromatic protons at about 7.26-7.86 ppm. ^{13}C NMR spectrum shows the C atom of C=O peak at 167.45 ppm, C atom of C=N-Ar at 162.72 ppm and C atom of C=N-S at 165.22 ppm. The C atom of -S-CH_2 was recorded at 34.42 ppm.

3.2.3. Synthesis of the Schiff's bases (3.25-3.30)

Schiff bases, **3.25-3.30** were synthesized by the reaction of 2-(ethylsulfanyl)benzohydrazide **3.2**) with substituted aromatic aldehydes in the presence of ethanol as reported by Despaigne *et.al*.[171] as shown in Scheme 3.12.

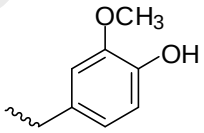
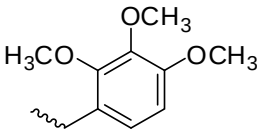
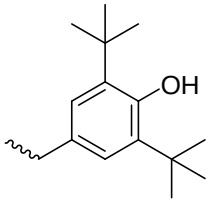


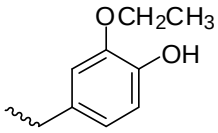
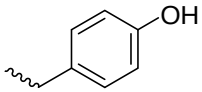
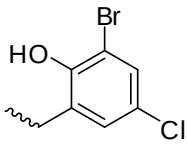
3.2 ***E*/*Z* mixture**
3.25-3.30

Scheme 3.12: Synthesis of new Schiff's bases **3.25-3.30**

According to Palla *et. al.*,[172] hydrazones may exist as *E*/*Z* geometrical isomers with C=N double bonds and cis/trans amide conformers. Thus, reaction of hydrazide **3.2** and p-4-hydroxy-3-methoxybenzaldehyde in the presence of ethanol gave derivative **3.25** in 90 % yield as a mixture of *E* and *Z*-isomers in which *E*-isomer prevails. The ratio of *E*- and *Z* isomer is 3 to 1. Under the same experimental conditions, reaction of hydrazide **3.2** with 2, 3, 4-trimethoxybenzaldehyde gave compound **3.26** in 90 % yield. The ratio of *E*- and *Z* isomer is 4 to 1. Treatment of compound **3.2** with 3, 5-di-*tert*-butyl-4-hydroxybenzaldehyde, gave **3.27** in 90 % yield, also as a mixture of *E*- and *Z*-isomers whereby the ratio of *E*- and *Z* isomer is 3 to 1. The composition of *E*-isomer in a mixture of **3.28** which was formed from 4-hydroxy-3-ethoxybenzaldehyde is higher, i.e. 5 to 1. Similarly with compound **3.29** which was formed from 4-hydroxybenzaldehyde. When the halogenated hydroxybenzaldehyde **3.30** was subjected to the reaction conditions indicated in Scheme 3.12 then mixtures of *E*- and *Z*-isomers were obtained in which the ratio of *E*- and *Z* isomer is 3 to 1 as shown in Table 3.5.

Table 3.5: *E*/*Z* ratio and percentage yield of compounds **3.25-3.30**

Compound No.	Ar-	*E*/*Z* ratio	(%) Yield
3.25		3:1	90
3.26		4:1	90
3.27		3:1	90

3.28		5:1	90
3.29		5:1	90
3.30		3:1	90

The hydroxyl group vibration of Schiff bases **3.25** and **3.27-3.30** was recorded between 3174 cm^{-1} and 3690 cm^{-1}, the imine group stretching vibration and the C=O absorption in were recorded at 1579-1611 cm^{-1} and 1601-1661 cm^{-1} respectively in the IR spectra. ^{1}H and ^{13}C NMR spectra of compounds **3.25-3.30** contain two sets of signals due to the presence of *E* and *Z* isomers in solution[171] and their assignments in DMSO-d_6 are reported in the experimental chapter.

The ORTEP diagram of compound **3.30** is as shown in Figure 3.3, which was determined from ethanol solution at room temperature. It is the monoclinic system, with *Pbca* space group.

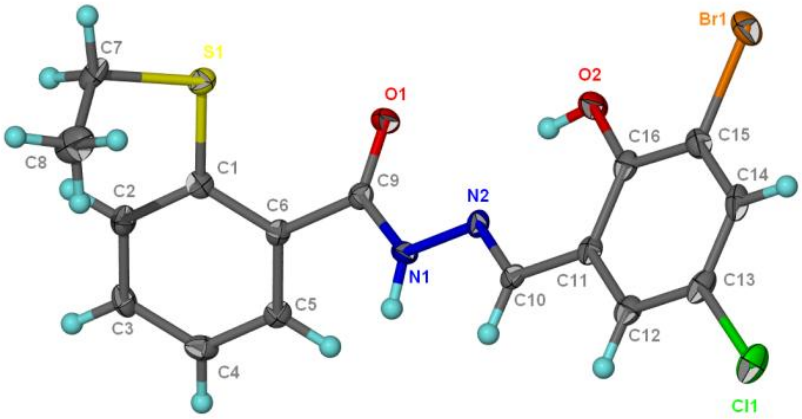
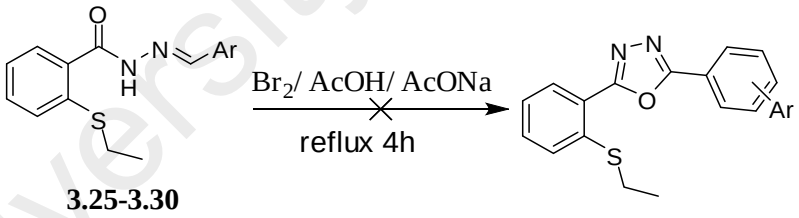


Figure 3.3: The ORTEP diagram of derivative **3.30**, showing 70% probability displacement ellipsoids and the atom numbering scheme.

3.2.4. Synthesis of 1,3,4-oxadiazole derivatives from 2-(ethylsulfanyl)-N'-(substitutedphenyl)methylidene]benzohydrazide using bromine

The oxidative cyclization reaction of the hydrazones **3.25-3.30** with bromine in glacial acetic acid and sodium acetate was carried out according to the procedure reported by Rajak *et al*.[154] to form the corresponding 1,3,4-oxadiazoles as shown in Scheme 3.13.



3.25-3.30

Scheme 3.13: The unsuccessful oxidative cyclization reaction of **3.25-3.30**

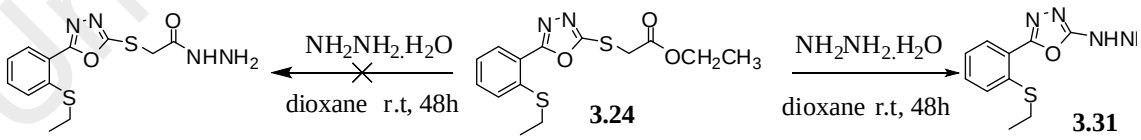
Variations were applied to the concentrations of bromine/ sodium acetate and the temperatures of the reactions, but no products were detected. The detailed results are presented in Table 3.6.

Table 3.6: Effect of bromine/ sodium acetate and the temperature on the cyclization reaction of **3.25**-**3.30**

Br_2	Sodium acetate	Temperature	Product
0.1 eq	1 eq	r.t	No reaction
0.1 eq	1 eq	80 ˚C	No reaction
0.2 eq	2 eq	r.t	No reaction
0.2 eq	2 eq	80 ˚C	No reaction

3.2.5. Synthesis of new 1,3,4-oxadiazole derivatives

Since our efforts to synthesized 1,3,4-oxadiazole derivatives from the corresponding hydrazones **3.25-3.30** have failed, we decided to take advantage of **3.24** as the starting material. In our opinion we could react compound **3.24** with hydrazine hydrate to form a hydrazide. However, when compound **3.24** was reacted with hydrazine hydrate in dioxane at room temperature, the expected hydrazide was not isolated. Instead an unexpected product from the cleavage of C-S bond was isolated as shown in Scheme 3.14.



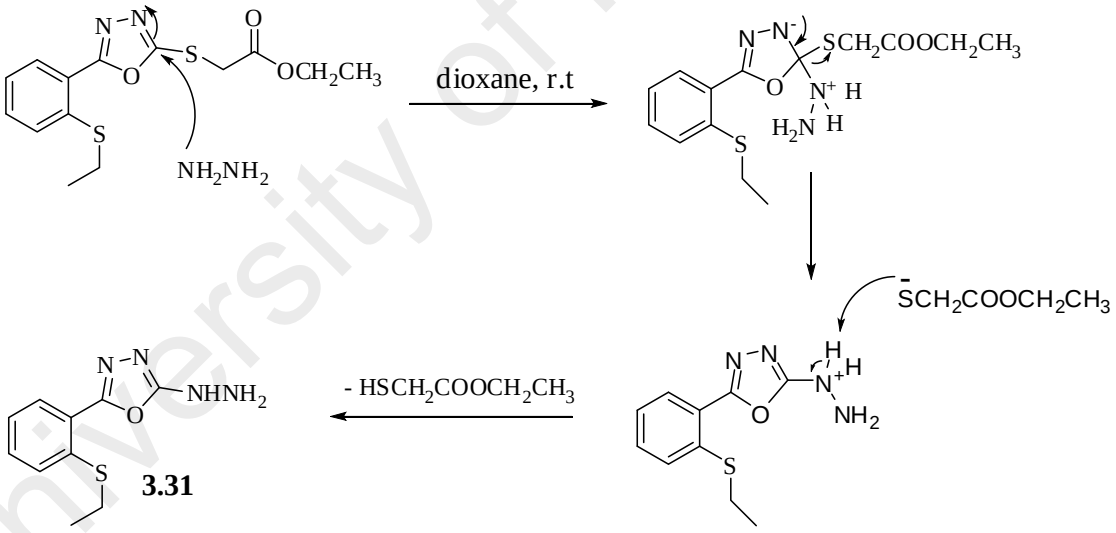
Scheme 3.14: Synthetic route of compounds **3.31**

[From literature survey, El-Sayed and co-workers[173] observed hydrazide which one might have expected from the reaction of the ethyl acetate derivative with hydrazine hydrate]. Also from the literature we have noted that only one paper by Turner *et. al.*[174] who

reported the synthesis of some 2-aryl-5-hydrazino-1,3,4-thiadiazoles but without any explanation regarding the reaction conditions and mechanism. Hence, we proposed two mechanisms to explain the formation of compound **3.31** as shown in Scheme 3.15 and Scheme 3.16.

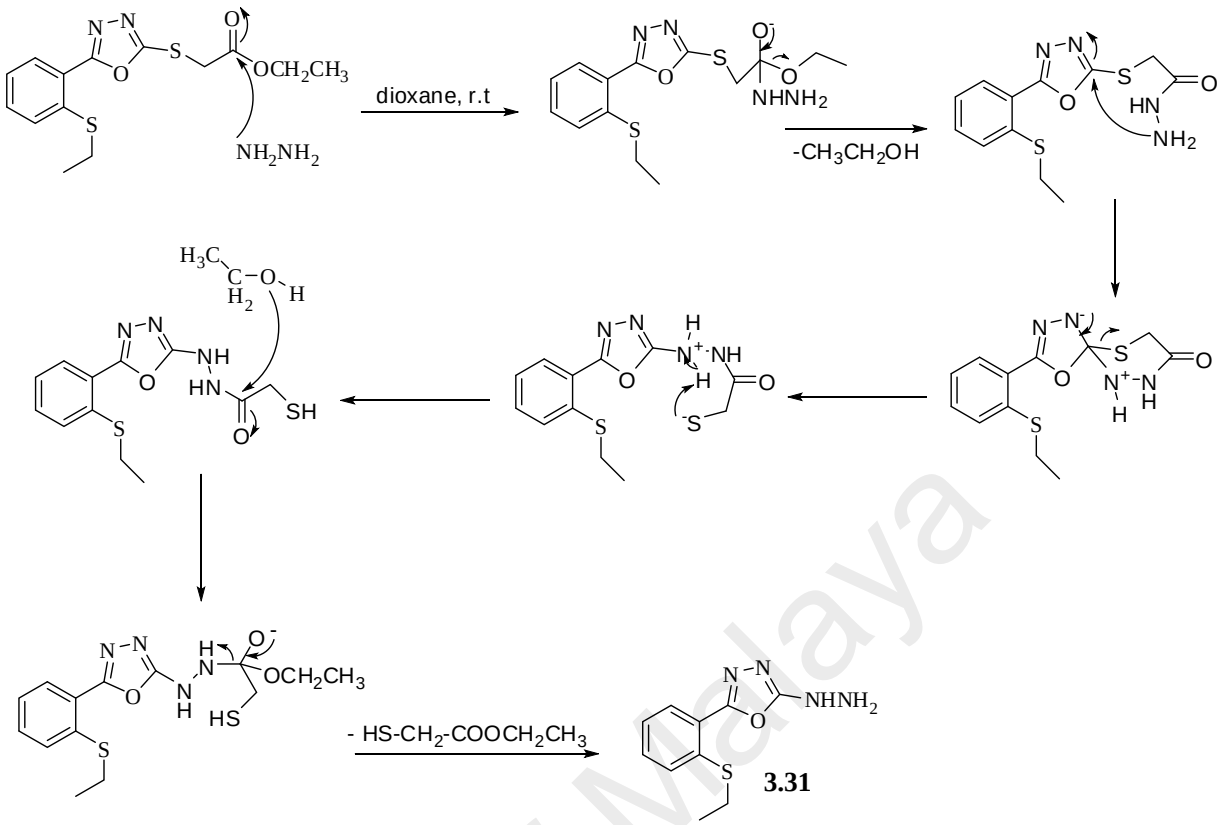
The first proposed mechanism consists of two steps:

The first step was the attack of hydrazinic NH_2 on the more electrophilic C atom of (N=C-S) of the oxadiazole ring to form an intermediate. In the second step deprotonation with the aid of sulfur atom of (S-CH_2-$COOCH_2CH_3$) group occurred to form the oxadiazole ring as depicted in Scheme 3.15.



Scheme 3.15: Mechanism proposed for the formation of compound **3.31**

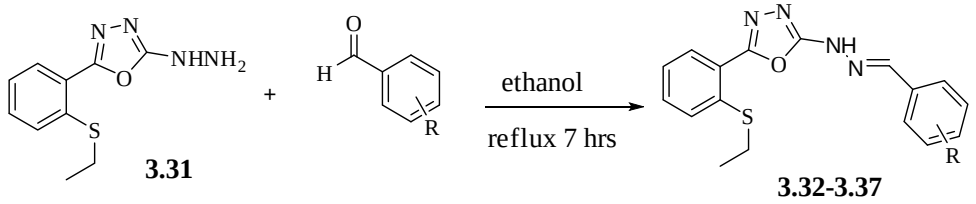
The second proposed mechanism is as shown in Scheme 3.16. It begines with the nucleophilic attack of hydrazinic NH_2 on the ester carbonyl and transfer of a proton followed by the loss of (CH_3CH_2OH). An intramolecular attack of the NH_2 on the electrophilic C atom (N=C-S) of the oxadiazole ring could possibly be a part of the reaction leading to the formation of an intermediate, which is then followed by the loss of (HS-$CH_2$$COOCH_2CH_3$) to form hydrazino-1,3,4-oxadiazole compound.



Scheme 3.16: Second proposed mechanism for the formation of compound **3.31**

3.2.6. Synthesis of new 2,5-disubstituted-1,3,4-oxadiazole derivatives (3.32-3.37)

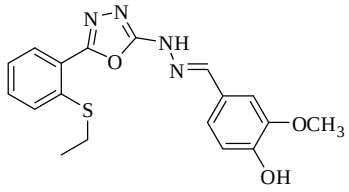
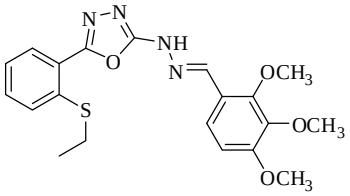
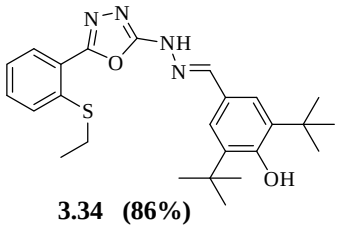
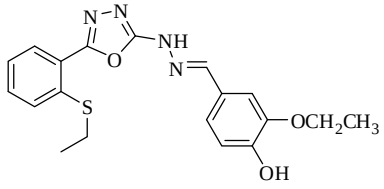
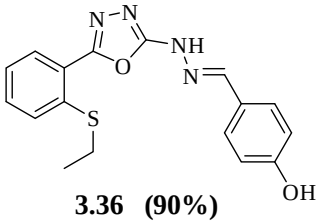
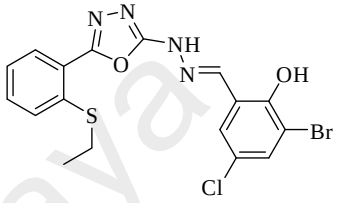
The new 2,5-disubstituted-1,3,4-oxadiazole derivatives **3.32-3.37** were synthesized by the reaction of acid hydrazide **3.31** with substituted aromatic aldehydes in the presence of ethanol according to the procedure reported by Despaigne *et.al*.[171] as shown in Scheme 3.17



Scheme 3.17: Synthesis of compounds **3.32-3.37**

The percentage yield of compounds **3.32-3.37** is as shown in Table 3.7.

Table 3.7: Chemical structure and percentage yield of compounds **3.32-3.37**

 **3.32 (85%)**	 **3.33 (88%)**	 **3.34 (86%)**
 **3.35 (89%)**	 **3.36 (90%)**	 **3.37 (88%)**

The IR spectrum of compound **3.31** showed the appearance of an absorption band in the range of 3490-3081 cm^{-1} due to the NH-NH_2 group. The ^1H NMR of compound **3.31** shows only a signal of ethyl group as a triplet at about 1.26 ppm and a quartet at 3.01 ppm and the appearance of NH_2 signal as a singlet at about 4.51 and another singlet at 8.62 ppm due to NH proton in addition to the aromatic protons at 7.3-7.68 ppm. Additional evidence for the formation of **3.31** is ^{13}C NMR of this compound possesses only the absorption peaks of the original ethyl group at 13.21 ppm and 25.59 ppm respectively in addition to the peaks of C9 at 157.17 ppm and C10 at 165.72 ppm.

The IR spectra of compounds **3.32-3.37** with the exception of compound **3.33** show the absorption bands for hydroxyl groups at 3676-3380 cm^{-1}. All the synthesized compounds show an absorption band of C=N group at 1643-1616 cm^{-1} while the absorption band for N-H was recorded at 2980-3322 cm^{-1}. The ^1H NMR and ^{13}C NMR and mass spectral data of compounds **3.32-3.37** were in accordance to their structures and discussed in details in the experimental part.

CHAPTER 4: BIOLOGICAL ACTIVITIES

This chapter is divided into three parts; the first part consists of verifying the antioxidant activity of the synthesized compounds using two different established *in vitro* antioxidant assays, i.e. the radical scavenging ability (DPPH) and the Ferric Reducing Antioxidant Power (FRAP). The second part involves the evaluation of *in vitro* cytotoxic activity of selected 1,3,4-oxadiazole derivatives against three human cancer cell lines, (-BxPC-3, MCF-7, MDA-MB-231-) and one normal cell line (-hTERT-HPNE) using the MTS assay. The third part focuses on the investigation of the anti-ulcer activity of hydrazone 2-(Ethylthio)-*N'*-(4-hydroxy-3-methoxyphenyl)benzylidene]benzohydrazide **3.25** on ethanol-induced gastric mucosal lesions in rats.

4.1. *In vitro* antioxidant activity using DPPH Radical Scavenging Assay of the synthesized compounds

As pointed out in Chapter 2, the DPPH molecule has the ability to accept an electron or hydrogen radical and converted it into a stable diamagnetic molecule. In order to avoid thermal degradation of the tested compounds, the DPPH free radical method is measured at ambient temperature. All compounds studied were screened to evaluate their radical scavenging activities. Most of the compounds showed significant levels of inhibition of DPPH radical compared to the standard antioxidants used in the study as shown in Figure 4.1.

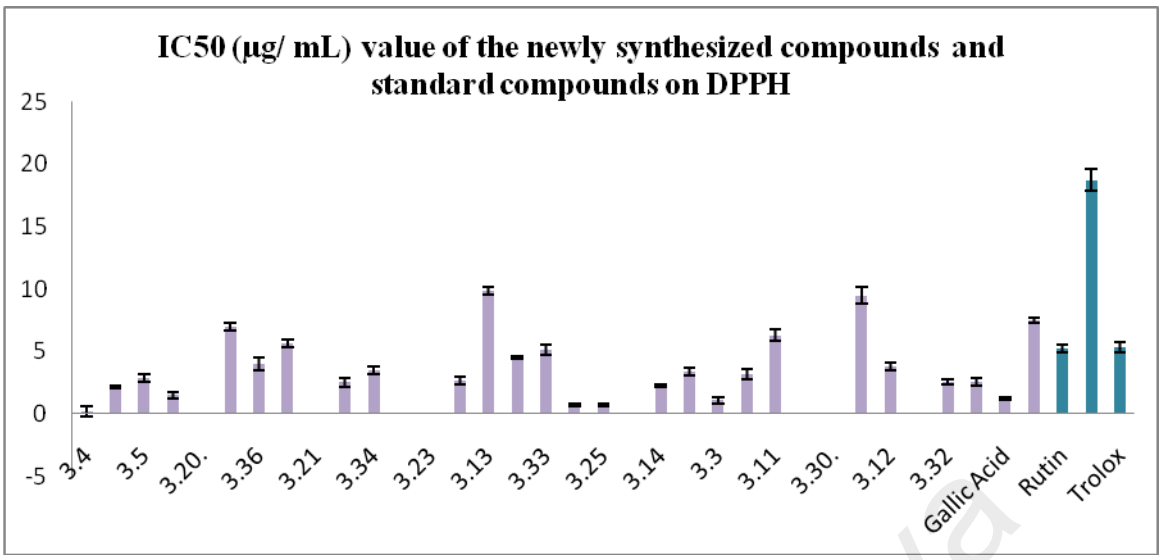


Figure 4.1: IC_{50} value of the stable DPPH radical of the newly studied compounds and the standard references

4.1.1. *In vitro* DPPH radical scavenging activity of the thiosemicarbazide and 1,2,4-triazole derivatives (3.3-3.13)

The free radical scavenging activities of the thiosemicarbazide derivatives **3.3-3.7**, along with those of the reference standards are shown in Figure 4.2 and Table 4.1. It can be seen from Table 4.1 that all of thiosemicarbazides possess greater scavenging effects in the DPPH assay than standard BHT compounds and ascorbic acid. Compounds **3.3** and **3.4** possessed greater scavenging effect than standard compounds, with IC_{50} values of (1.08 ± 0.02) µg/ml and (0.22 ± 0.01) µg/ml, respectively. The corresponding value for the standard antioxidant gallic acid, on the other hand, was (1.20 ± 0.13) µg/ml. It can also been from Table 4.1 that the presence of an electron-withdrawing group (*Cl*) on the phenyl ring of the thiosemicarbazides increases the scavenging ability of thiosemicarbazide (**3.4**, IC_{50} = 0.22 ± 0.01 µg/ml) in comparison to that of compound **3.3** (IC_{50} = 1.08 ± 0.02 µg/ml). By contrast, the presence of an electron-donating group (*OMe*) decreases the scavenging ability (**3.7**, IC_{50} = 4.50 ± 0.01 µg/ml)

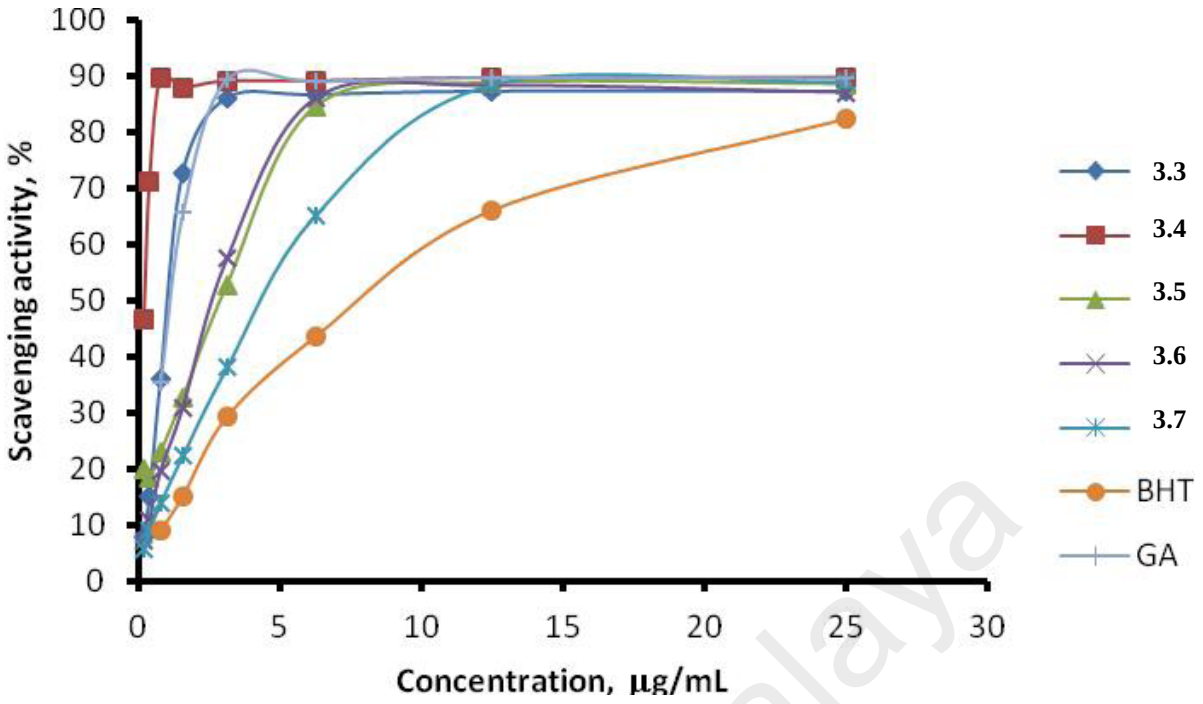


Figure 4.2: Scavenging activity of compounds **3.3-3.7** on DPPH radical[175]

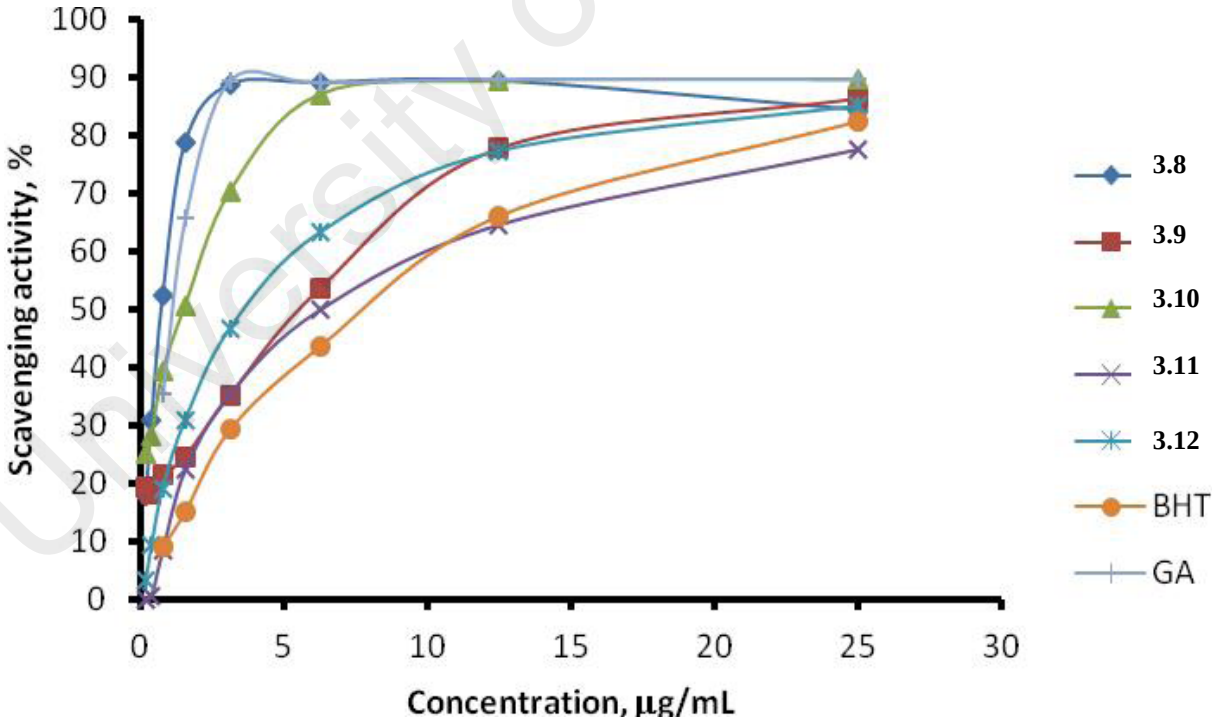
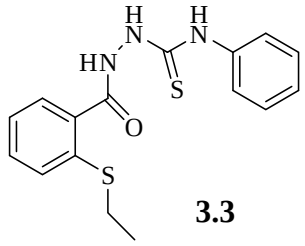
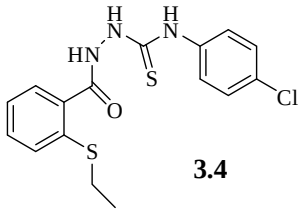
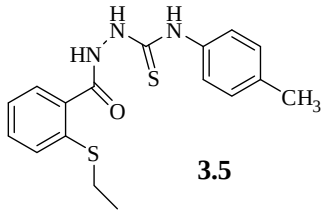
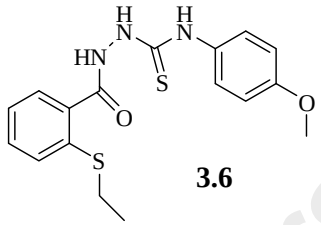
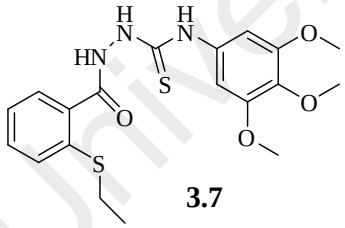
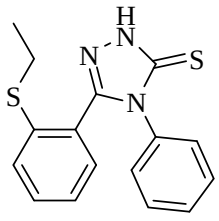
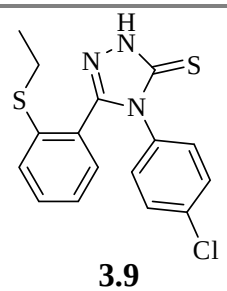
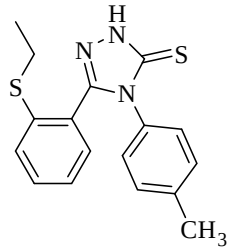
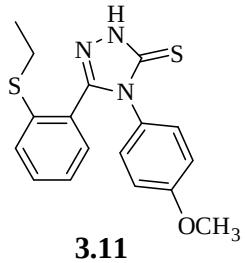
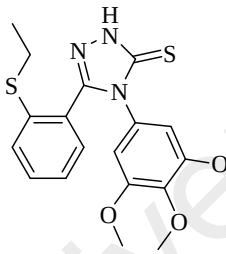
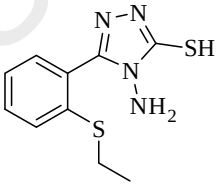


Figure 4.3: Scavenging activity of compounds **3.8-3.12** on DPPH radical [175]

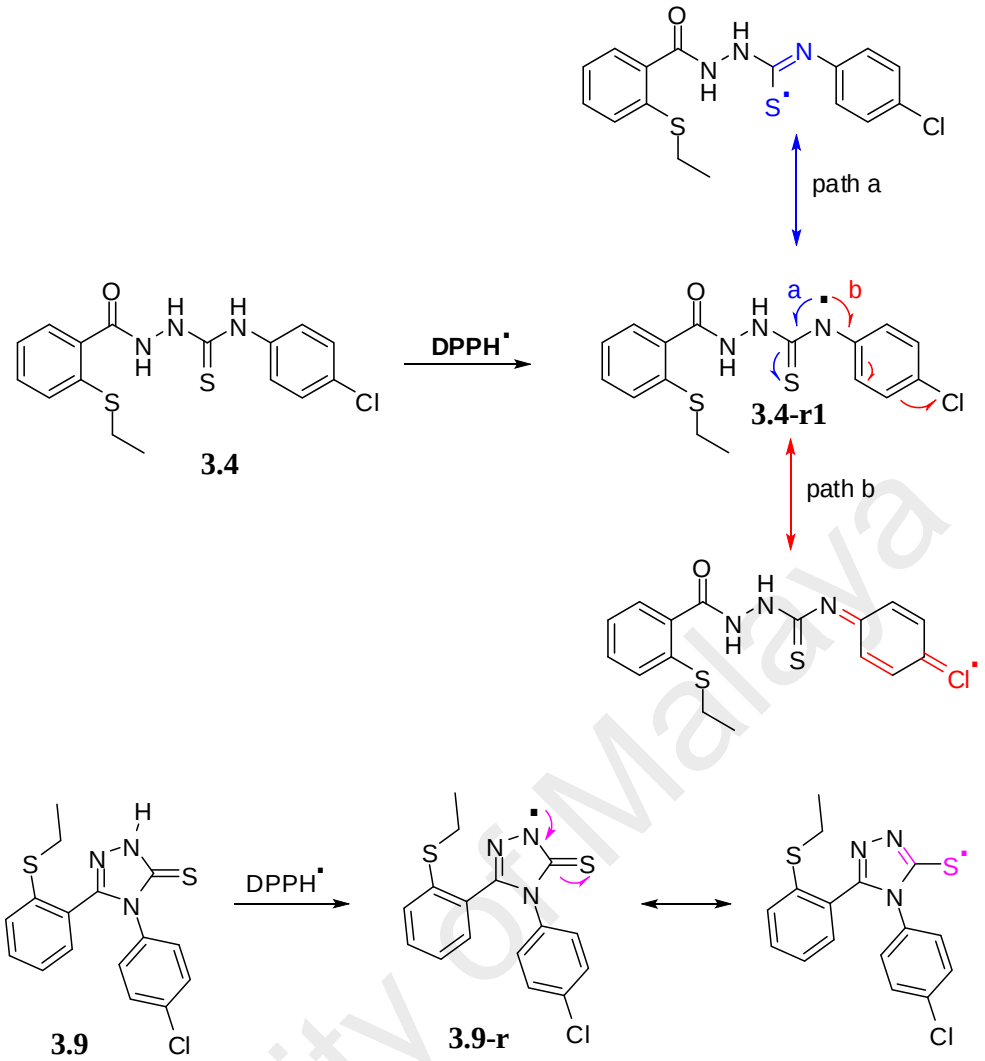
Table 4.1: Percentage inhibition and IC_{50} values of DPPH assay for compounds **3.3-3.13**

Compounds structure	**% Inhibition ± S.E.M.**	**IC_{50} µg/ml ± S.E.M. DPPH assay**
 **3.3**	89.8 ± 0.05	1.08 ± 0.02
 **3.4**	89.7 ± 0.09	0.22 ± 0.01
 **3.5**	88.6 ± 0.08	2.91 ± 0.05
 **3.6**	87.1 ± 0.1	2.69 ± 0.21
 **3.7**	89.1 ± 0.12	4.50 ± 0.01
 **3.8**	84.8 ± 0.24	0.74 ± 0.15

 **3.9**	86.5 ± 0.38	5.64 ± 0.03
 **3.10**	89.7 ± 0.45	1.51 ± 0.08
 **3.11**	77.6 ± 0.09	6.3 ± 0.13
 **3.12**	85.1 ± 0.31	3.75 ± 0.01
 **3.13**	84.5 ± 0.43	9.87 ± 0.04
Quercetin	88.5 ± 0.003	2.54 ± 0.07

BHT	82.3 ± 0.002	18.71 ± 0.01
Trolox	91.7 ± 0.05	5.35 ± 0.64
Rutin	86.3 ± 0.09	5.25 ± 0.01
Gallic acid	89.6 ± 0.12	1.2 ± 0.13
Ascorbic acid	90.6 ± 0.22	7.52 ± 0.08

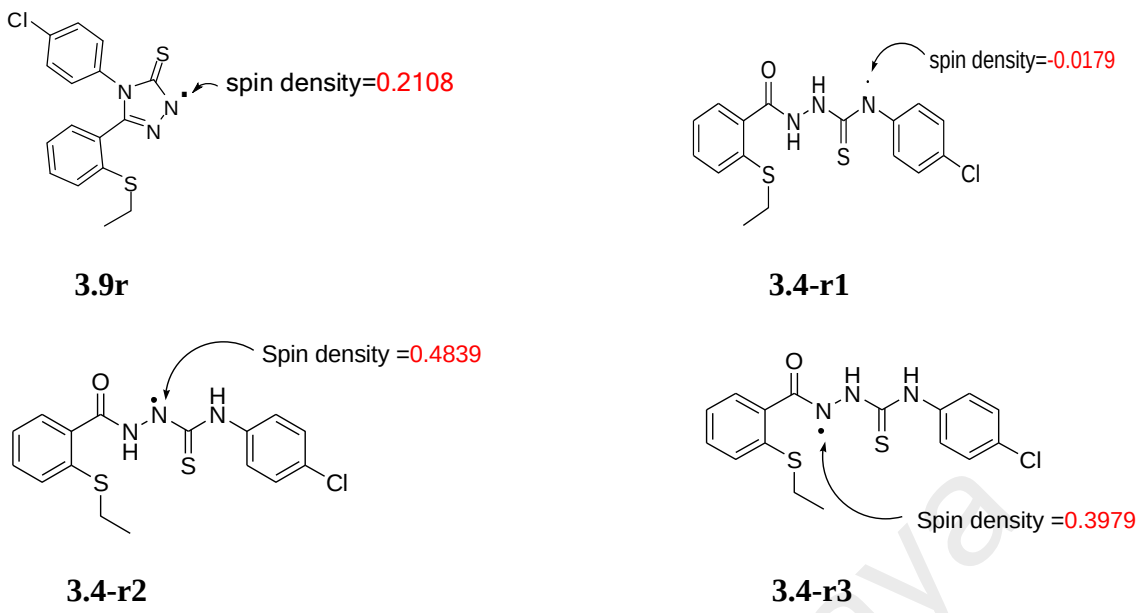
It was also found that the cyclization of thiosemicarbazides to their corresponding 1,2,4-triazole thione derivatives did not improve the radical-scavenging activity relative to acyclic compounds, as in the case of compounds **3.8** and **3.10** shown in Figure 4.3 and Table 4.1. Compounds **3.8** and **3.10** however showed improvement in the radical scavenging activity, with IC_{50} values of 0.74 ± 0.15 μg/ml and 1.51 ± 0.08 μg/ml, respectively, compared to their corresponding thiosemicarbazides **3.3** and **3.5** (IC_{50} 1.08 ± 0.02 and 2.91 ± 0.05 μg/ml, respectively). Compound **3.4** possessed better scavenging properties than its cyclic product, **3.9**. In addition, compound **3.13** showed radical scavenging activity only higher than that of BHT. Unlike thiosemicarbazides, there was no correlation between the substituent group on the phenyl ring and the scavenging ability of 1,2,4-triazole thione compounds. This may be explained by the mechanism shown in Scheme 4.1. The electron-withdrawing substituent on the phenyl ring has an influence on the radical-scavenging effects of thiosemicarbazide **3.4** by delocalisation of electrons of nitrogen centered radical. In 1,2,4-triazole thione derivatives, delocalisation of electrons across the phenyl ring is not an option. The chlorine atom is a strong electron-withdrawing atom by induction, whereas the –OMe group is a strongly electron-donating group by resonance.



Scheme 4.1: Proposed mechanism to account that thiosemicarbazide **3.4** has superior radical scavenging effects compared to **3.9** [175]

This observation is further supported by the computational analysis of the relative radical stabilities and bond-dissociation enthalpies (BDH_{298}) based on DFT (uB3LYP/6-31G (d, p)) calculations which enable us to rationalise the experimental results.[176, 177] By calculating the spin density on the radical intermediate, we could predict which intermediate would be expected to be more stable. All calculations were performed using Gaussian 09W based on DFT.[176, 177]

The spin density values were useful in understanding the difference on the antioxidant activities among the compounds. The results are represented in Figure 4.4.



3.9r **3.4-r1**

3.4-r2 **3.4-r3**

Figure 4.4: Spin density in the -N radical of compound **3.9r** and compound **3.4** radicals at uB3LYP/6-31G (d, p).[175]

The sequence $\eta_{\mathbf{3.4\text{-}r1}} < \eta_{\mathbf{3.9r}} < \eta_{\mathbf{3.4\text{-}r3}} < \eta_{\mathbf{3.4\text{-}r2}}$ indicates that antioxidant ability of compound **3.4** is higher than that of compound **3.9**. This can be explained by the low spin density on -N radical in compound **3-r1**.[178] the single electron in compound **3.4** can be dispersed to the benzene ring.[178] the low antioxidant activity of compound **3.9** may also be due to the less resonating radical in the system. A relative low spin density on N atom in compound **3.4r1** implicates that opened chain are much more electron rich than closed ring in compound **3.9**.[178]

Because the DPPH assay involves the HAT mechanism, calculations of BDH_{298} could further support the experimental data. Using this method, we calculated the BDH_{298} for compounds **3.4-r1**, **3.4-r2**, **3.4-r3** and **3.9-r**, and the results are summarised in Table 4.2. Table 4.2 shows the value of BDH for compounds **3.4** and **3.9**. The DFT results appear to be quite realistic for phenol compounds. In this case, the BDH values for the NH sites on the open chained system, **3.4r1** (414.8133 kcal/mol) is lower than the closed chain (425.7250 kcal/mol). This clearly confirms that HAT from the open chain system of compound **3.4r1** is easier than the closed system of compound **3.9**. The results indicate

that the reactivity of the open chain system for compound **3.4r1** is higher than the reactivity for closed chain system for compound **3.9**. Thus, compound **3.4** has the capacity to significantly impact activity by acting as a hydrogen atom donor and also by enabling the formation of a relatively stable radical when formed through electron delocalization.

Table 4.2: Optimized geometries and BDH298 values of radicals derived from **3.4** and **3.9** for gas phase calculations

Compd	ΔH^{rxn} (kcal/mol)	ΔG^{rxn} (kcal/mol)	BDH_{298} (kcal/mol)
3.4r1	7.4370	8.9157	414.8133
3.4r2	7.4546	8.9520	426.4630
3.4r3	7.4470	7.3645	436.3319
3.9r	8.4776	7.9895	425.7250

Compound **3.4r1** is known to be stable as they benefit from inductive effects as well as from orbital interactions of the p-type lone pair of sulphur atom with the half-filled p-orbital of the mainly sp^2 hybridized radicals.[178] The picture of the SOMO and the mapped out spin density of **3.4r1** illustrate the final effect as shown in Figure 4.5. On top of that, the position of the radical help stabilize the radical further, mainly by hyperconjugation. Addition of –Cl as a withdrawing electron group may also improve the stability of the conjugation.

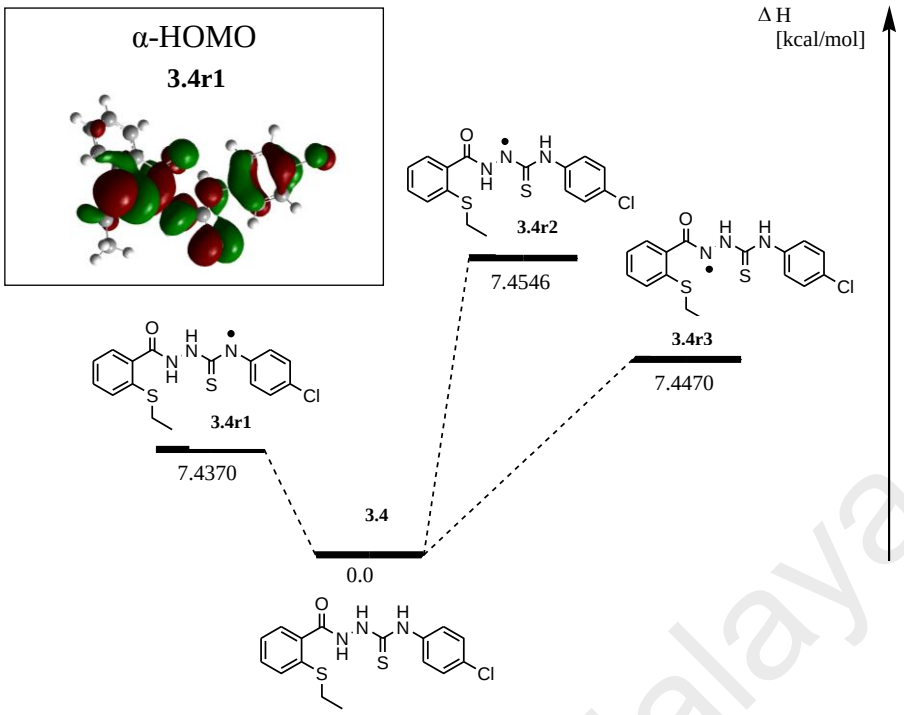


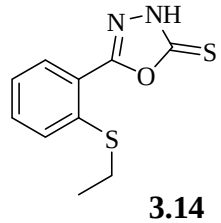
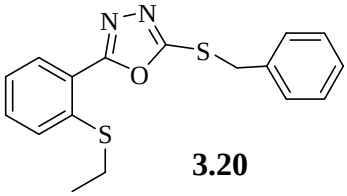
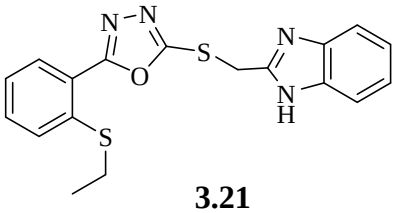
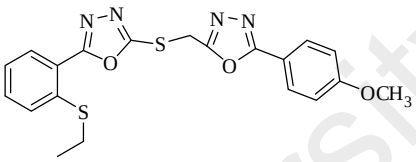
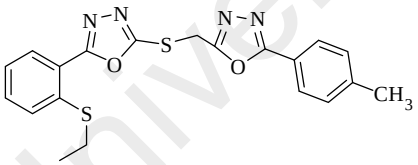
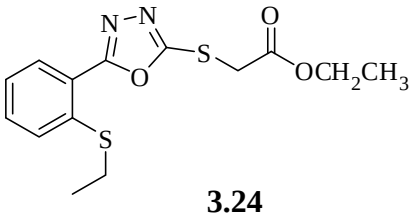
Figure 4.5: Bond-dissociation enthalpies for three radicals in compound **3.4**.

Stabilization of the radical in **3.4r1** is longer due to the adjacent sulphur and hyperconjugation leading to a BDH of 7.4370 kcal/mol (uB3LYP/6-31 G (d,p)).

4.1.2. *In vitro* DPPH radical scavenging activity of the 1,3,4-oxadiazole derivatives 3.14 and (3.20-3.24)

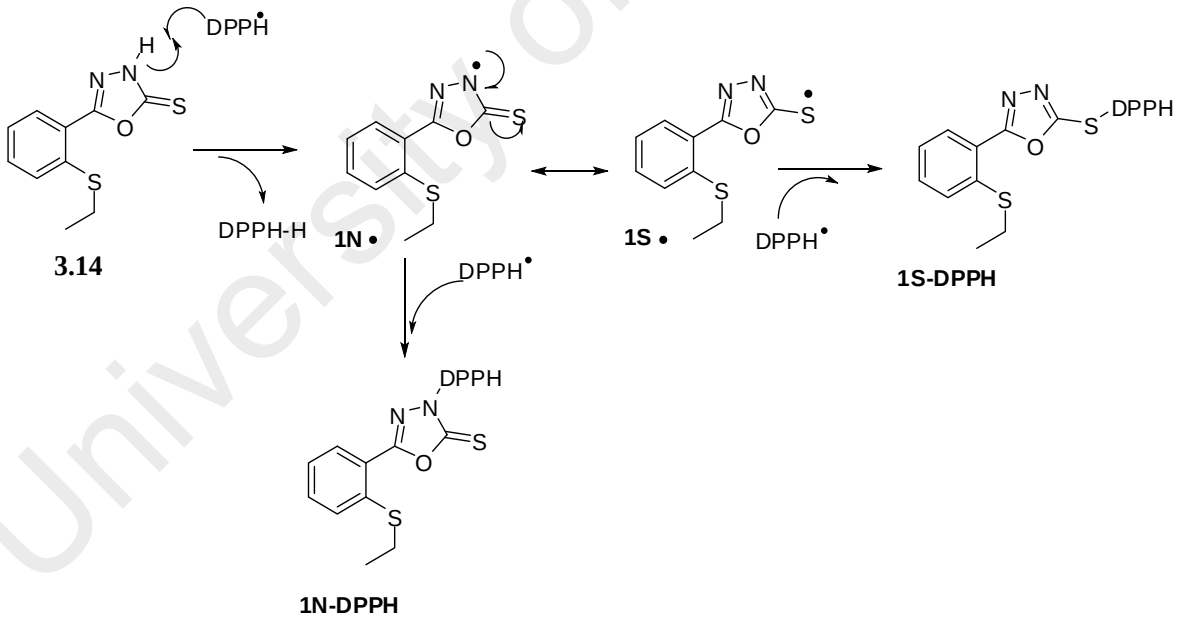
Starting with the parent compound, **3.14** containing 1,3,4-oxadiazole-2-thione moiety, this compound exhibited significant radical scavenging potential with IC_{50} (2.22 ± 0.01 µg/ml) as shown in Table 4.3. On alkylation of compound **3.14** to form compounds **3.20-3.24**, it can be seen from Table 4.3 that compound **3.20** with phenyl ring did not show any radical scavenging activity. Similar observation was recorded with derivatives **3.21-3.24**.

Table 4.3: Percentage inhibition and IC_{50} values of DPPH assay for compounds **3.14** and **3.20-3.24**

Compound	% inhibition ± S.E.M.	IC_{50} µg/ml ± S.E.M. DPPH assay
 **3.14**	89.8 ± 1.06	2.22 ± 0.01
 **3.20**	3.7 ± 0.1	> 25 (poor activity)
 **3.21**	7.7 ± 1.2	> 25 (poor activity)
 **3.22**	20.1 ± 0.1	> 25 (poor activity)
 **3.23**	14.55 ± 0.12	> 25 (poor activity)
 **3.24**	6.0 ± 0.01	> 25 (poor activity)

Quercetin	88.5 ± 0.003	2.54 ± 0.07
BHT	82.3 ± 0.002	18.71 ± 0.01
Trolox	91.7 ± 0.05	5.35 ± 0.64
Rutin	86.3 ± 0.09	5.25 ± 0.01
Gallic acid	89.6 ± 0.12	1.2 ± 0.13
Ascorbic acid	90.6 ± 0.22	7.52 ± 0.08

Based on these results, we are proposing HAT mechanism occurs in the DPPH assay as proposed in Scheme 4.2. It is suggested that hydrogen radicals are extracted from the N-H of the oxadiazole ring on to DPPH radicals. This mechanism shows that compound **3.14** is able to suppress two DPPH• radicals.

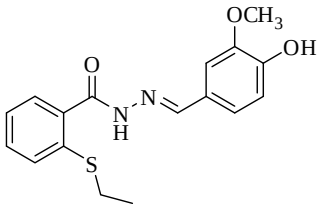
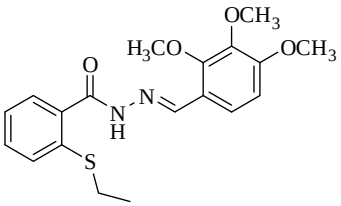
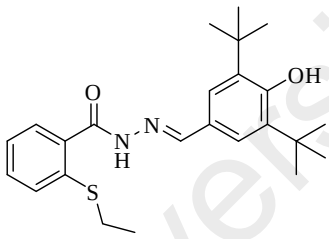
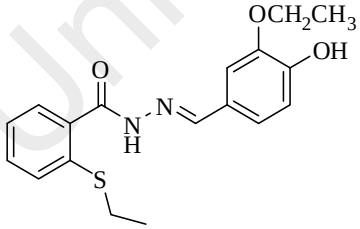
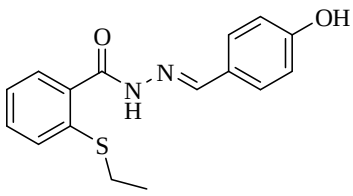


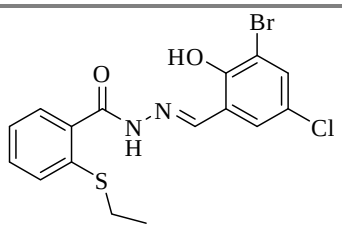
Scheme 4.2: Proposed HAT mechanism between compound **3.14** and the DPPH radical.

4.1.3. *In vitro* DPPH radical scavenging activity of the Schiff's bases (3.25-3.30)

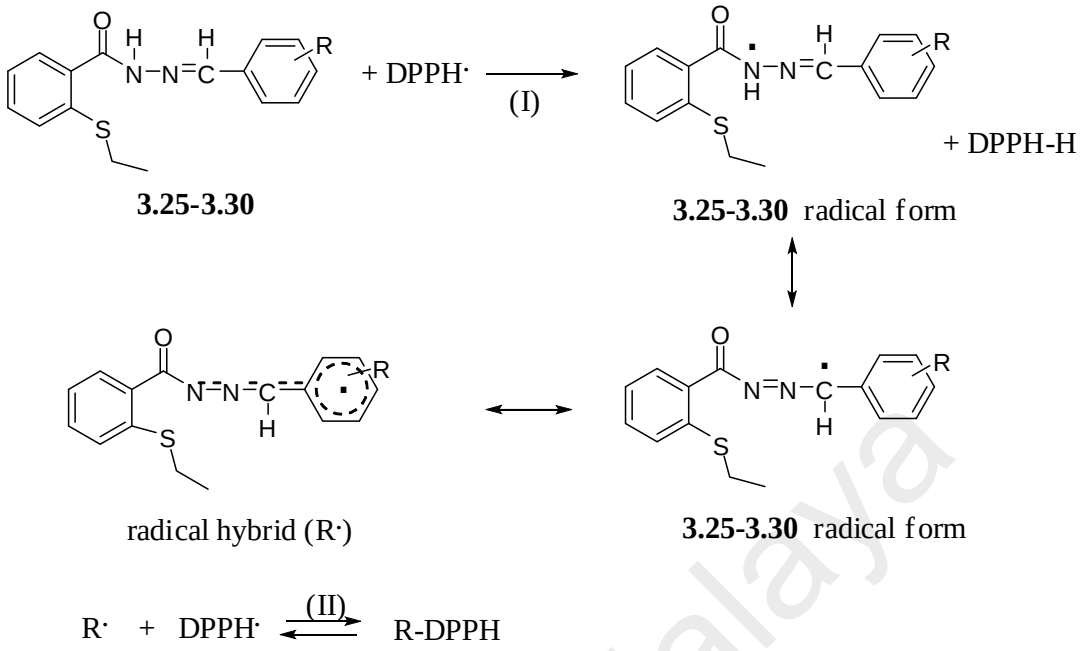
The scavenging effect of Schiff's bases **3.25-3.30** on the DPPH radical is as shown in Table 4.4.

Table 4.4: Percentage inhibition and IC_{50} values of DPPH assay for compounds **3.25-3.30**

Compound	% inhibition ± S.E.M.	IC_{50} µg/ml ± S.E.M. DPPH assay
 **3.25**	79.3 ± 0.1	0.69 ± 0.08
 **3.26**	5.1 ± 0.3	> 25 (poor activity)
 **3.27**	7.7 ± 1.2	2.52 ± 0.11
 **3.28**	81.9 ± 0.001	3.34 ± 0.55
 **3.29**	20.2 ± 0.17	> 25 (poor activity)

 **3.30**	8.0 ± 0.21	> 25 (poor activity)
Quercetin	88.5 ± 0.003	2.54 ± 0.07
BHT	82.3 ± 0.002	18.71 ± 0.01
Trolox	91.7 ± 0.05	5.35 ± 0.64
Rutin	86.3 ± 0.09	5.25 ± 0.01
Gallic acid	89.6 ± 0.12	1.2 ± 0.13
Ascorbic acid	90.6 ± 0.22	7.52 ± 0.08

Amongst the compound screened for their scavenging activity shown in Table 4.4, compound **3.25** showed excellent radical scavenging activity with IC_{50} (0.69 ± 0.08 μg/ml) better than the value of gallic acid IC_{50} (1.20 ± 0.13 μg/ml). Compounds **3.27** and **3.28** showed very good radical scavenging activity on DPPH radical. Compounds **3.26**, **3.29** and **3.30** did not show any antioxidant activity. The results obtained were similar to the results reported by Nguyen and co-workers[110] as they have reported that the compounds with substituents such as 3-OEt-4-OH and 3-OMe-4-OH exhibit very good antioxidant activity. Thus, this trend of antioxidant activity observed is due to the presence of an N-H group, which can donate a hydrogen to the DPPH radical. Compounds **3.25-3.30** existed in a radical form, and the radical could delocalize to the benzene ring to produce stable resonance hybrid as shown in Scheme 4.3. The electron conjugation in the structure stabilizes the radical preventing it from participating in a destructive biochemical reaction.



Scheme 4.3: Reaction of compounds **3.25-3.30** with DPPH radical

4.1.4. *In vitro* DPPH radical scavenging activity of hydrazide (3.31) and the 2,5-disubstituted-1,3,4-oxadiazole derivatives (3.32-3.37)

The radical scavenging activities for compound **3.31** and its derivatives **3.32-3.37** were shown in Figure 4.6 and Table 4.5.

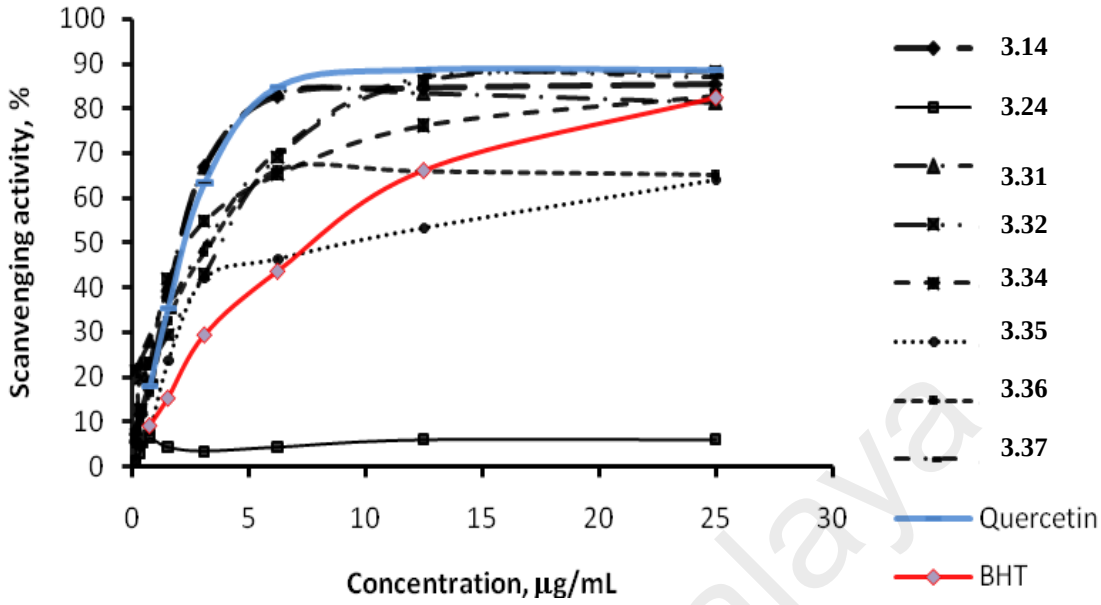
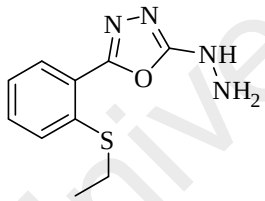
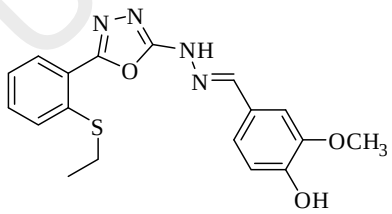
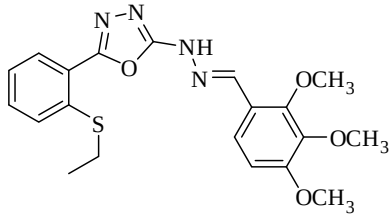
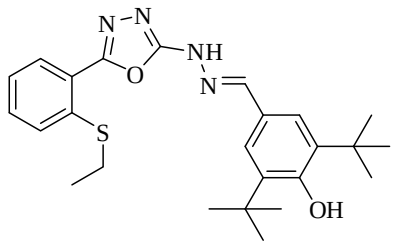
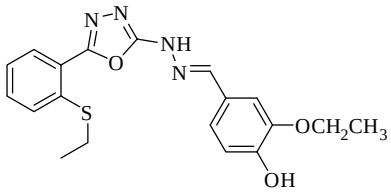
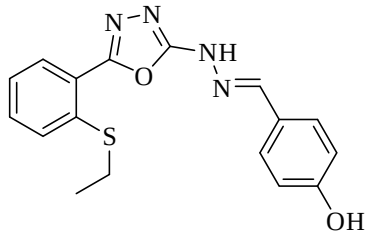
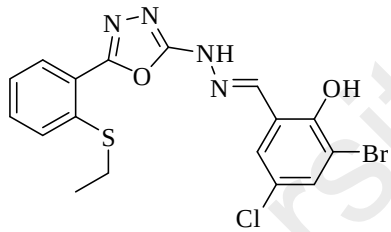


Figure 4.6: Scavenging activity of compounds **3.14, 3.24**, **3.31**, **3.32 and 3.34-3.37** on DPPH radical

Table 4.5: Percentage inhibition and IC_{50} values of DPPH assay for compounds **3.31** and **3.32-3.37**

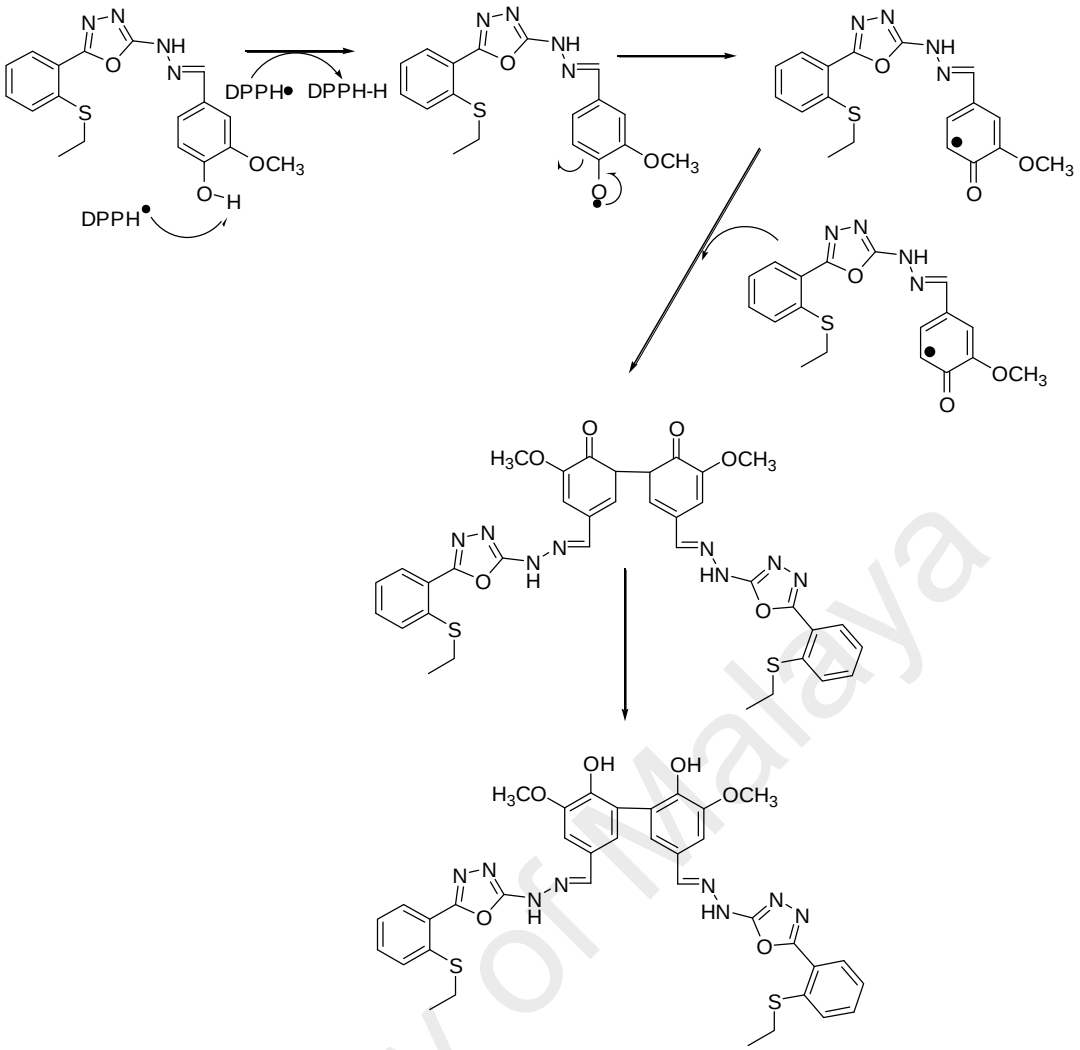
Compound	% inhibition ± S.E.M.	IC_{50} µg/ml ± S.E.M. DPPH assay
 **3.31**	86.6 ± 1.2	2.21 ± 2.3
 **3.32**	82.8 ± 1.4	2.55 ± 2.3
 **3.33**	83.4 ± 1.8	5.09 ± 0.81

 **3.34**	81.9 ± 0.001	3.49 ± 0.95
 **3.35**	65.2 ± 0.17	9.48 ± 1.56
 **3.36**	88.3 ± 0.53	3.96 ± 0.07
 **3.37**	87.1 ± 0.89	3.19 ± 0.58
BHT	82.3 ± 0.002	18.71 ± 0.01
Trolox	91.7 ± 0.05	5.35 ± 0.64
Rutin	86.3 ± 0.09	5.25 ± 0.01
Gallic acid	89.6 ± 0.12	1.2 ± 0.13
Ascorbic acid	90.6 ± 0.22	7.52 ± 0.08

It is well established that organic molecules incorporating electron donating groups (amine, hydroxyl and methoxy) can act as free radical trapping agents and are capable of opposing oxidative challenges. It can be seen from Table 4.5 that compounds **3.31** and **3.32** showed the highest scavenging activity on DPPH·, whereas compounds **3.34**, **3.36** and **3.37** exhibited moderate activity while **3.33** and **3.35** showed very low scavenging

activity. Compounds **3.31** and **3.32** bearing an electron donating group showed dominant DPPH activity with IC_{50} values of 2.21± 2.3 and 2.55 ± 2.3 μg/mL, respectively. The presence of chloro and bromo in **3.37** exhibited less activity compared to compounds **3.31** and **3.32**. This observed activity may be correlated with the introduction of electron donor substituent which stabilizes the generated radical during oxidation. Compound **3.35** demonstrated the lowest radical scavenging activity. We could not offer any explanation at the moment as to why **3.35,** which is very similar in structure with **3.32** but, show poor radical scavenging activity. Further study thus will have to be carried out. Medium activity recorded by compound **3.34**, is probably due to the presence of di-*tert*-butyl moiety beside the phenolic OH causing steric hindrance to the hydrogen donating ability of this compound.

For the monophenolic compounds **3.32-3.37** with the exception of **3.33**, a different mechanism is proposed. Compound **3.32** demonstrated the highest radical scavenging activity. The proposed mechanism involves the dimerisation between the resulting radicals as shown in Scheme 4.4. Brand-Williams have proposed a similar mechanism for phenoxy compounds with free *ortho*- and *para*- positions.[179] After dimerisation, the two hydroxyl groups were able to regenerate intramolecularly and could again interact with the DPPH radical.



Scheme 4.4: Proposed HAT mechanism between compound **3.32** and the DPPH radical.

4.2. *In vitro* Ferric ions Reducing Antioxidant Power (FRAP) assay of the synthesized compounds

This method was carried out at pH 3.6 in order to maintain iron solubility.[20] All the compounds studied were subjected to this method.

4.2.1. Ferric ions reducing antioxidant power of thiosemicarbazide and 1,2,4-triazole derivatives (3.3-3.13)

FRAP reducing power of thiosemicarbazides and 1,2,4-triazoles are shown in Figure 4.7 and Table 4.6.

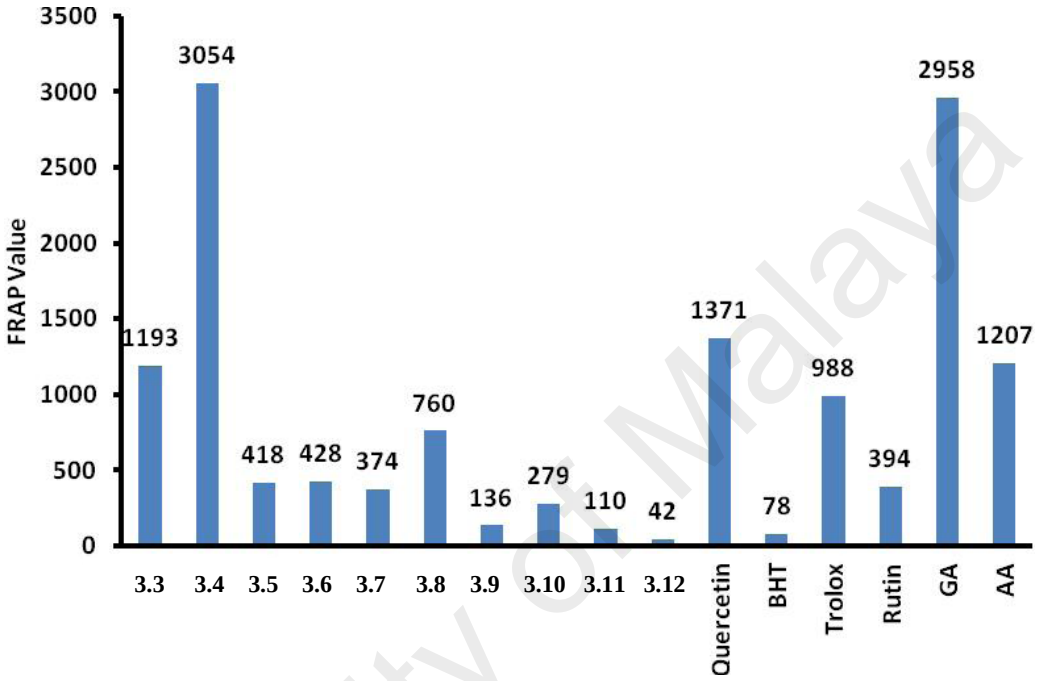
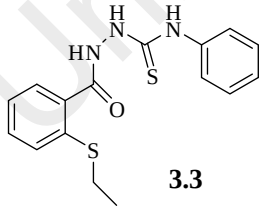
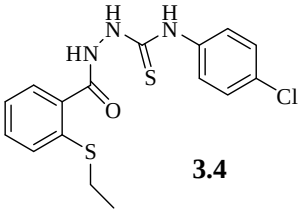
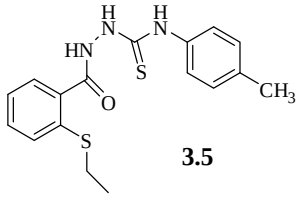
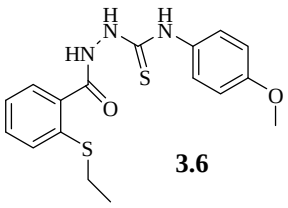
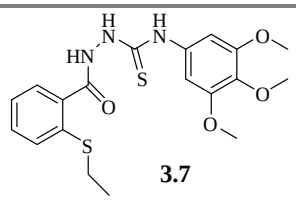
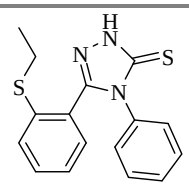
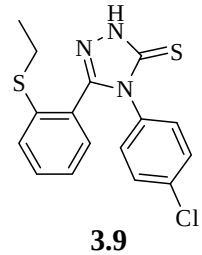
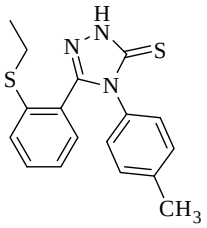
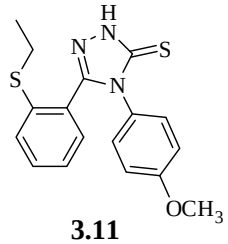
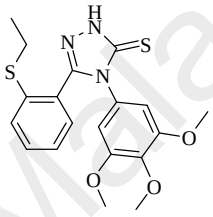
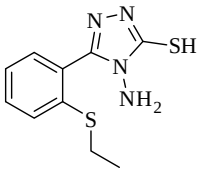


Figure 4.7: FRAP value for compounds **3.3-3.12** and reference standards[175]

Table 4.6: FRAP values for compounds **3.3-3.13**

Compounds structure	FRAP values (µM/100 g)	Compounds structure	FRAP values (µM/100 g)
 **3.3**	1193.3 ± 0.05	 **3.4**	3054.4 ± 0.01
 **3.5**	418.16 ± 0.19	 **3.6**	427.8 ± 0.15

 **3.7**	374.4 ± 0.14	 **3.8**	760 ± 0.03
 **3.9**	135.7 ± 0.19	 **3.10**	278.8 ± 0.15
 **3.11**	109.7 ± 0.05	 **3.12**	418 ± 0.1
 **3.13**	252 ± 0.1		
Quercetin	1371.1 ± 0.26	**Rutin**	393.89 ± 0.02
BHT	77.83 ± 0.08	**Gallic acid**	2957.78 ± 0.05
Trolox	987.78 ± 0.14	**Ascorbic acid**	1206.67 ± 0.02

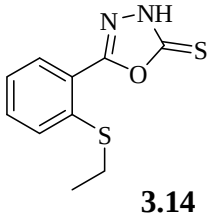
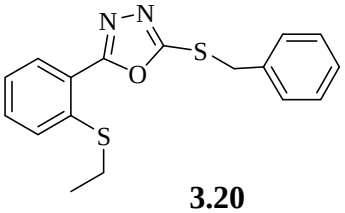
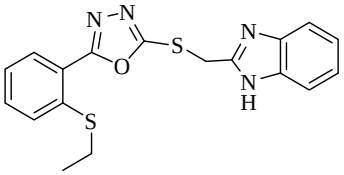
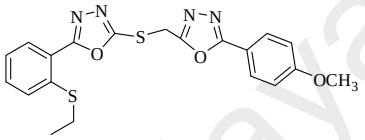
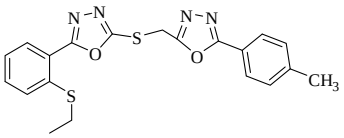
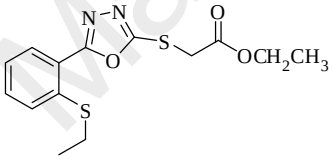
The result from Table 4.6 showed that the phenyl derivative **3.3** exhibited FRAP value (1193.33 ± 0.05) comparable with ascorbic acid (1206.67 ± 0.02), while the *p*-chlorophenyl derivative compound **3.4** has the highest FRAP value of (3054.44 ± 0.01), which is also above the value obtained for gallic acid (2957.78 ± 0.05). Compounds **3.5**, **3.6**, **3.8** and **3.12** displayed FRAP values (418.16 ± 0.19, 427.83 ± 0.11, 760.00 ± 0.03 and 418. ± 0.1 respectively), which are higher than Rutin (393.89 ± 0.02) but lower than Trolox (987.78 ± 0.14).

In addition, compounds **3.7**, **3.9**, **3.10, 3.11** and **3.13** possessed FRAP reducing power values of (374.4 ± 0.14, 135.7 ± 0.19, 278.8 ± 0.15, 109.7 ± 0.05 and 252 ± 0.1, respectively) which is higher than that of BHT (77.83 ± 0.08). The results obtained from the FRAP assay clearly indicates that all of thiosemicarbazide derivatives showed better FRAP reducing power than 1,2,4-triazoles. As in the case of the DPPH assay, the FRAP assay of the thiosemicarbazides also shows a similar pattern. The presence of an electron withdrawing group (*Cl*) increases the reducing power of the thiosemicarbazides (**3.4**, 3054.44 ± 0.01) in comparison to that of compound **3.3** (1193.33 ± 0.05), which lacks substituents. By contrast, the presence of an electron-donating group (*OMe*) decreases the reducing power (**3.7**, 374.4 ± 0.14). As for the 1,2,4-triazoles, there was no direct correlation between the substituent on the phenyl ring and the reducing power ability of the compounds.

4.2.2. Ferric ions reducing antioxidant power of the 1,3,4-oxadiazole derivatives 3.14 and (3.20-3.24)

The results of reducing power ability of compounds **3.14** and **3.20-3.24**, is as shown in Table 4.7.

Table 4.7: FRAP values for compounds **3.14** and **3.20-3.24**

Compounds structure	FRAP values (µM/100 g)	Compounds structure	FRAP values (µM/100 g)
 **3.14**	438.33 ± 0.01	 **3.20**	139.0 ± 0.7
 **3.21**	14.0 ± 0.11	 **3.22**	402.4 ± 1.95
 **3.23**	312.1 ± 1.86	 **3.24**	230.4 ± 0.002
Quercetin	1371.1 ± 0.26	**BHT**	77.83 ± 0.08
Trolox	987.78 ± 0.14	**Rutin**	393.89 ± 0.02
Gallic acid	2957.78 ± 0.05	**Ascorbic acid**	1206.67 ± 0.02

It can be seen from the Table 4.7 that, in contrary to the DPPH result, compound **3.14** exhibited very poor FRAP reducing power value (438.33 ± 0.01). The same was observed with compounds **3.20-3.24**, which possessed very poor values compared to the reference compounds. The reason behind these poor FRAP values is that the FRAP assay cannot measure the antioxidant capacity of certain antioxidants accurately. This may be due to the species present in FRAP assay, can react with ferrous ion (Fe^{+2}) and antioxidants containing SH group.[180] Also the FRAP assay involves only the ET mechanism. ET-based methods detect the ability of a potential antioxidant to transfer one electron and

reduce any compound including metals, carbonyls, and radicals.[108] In the case of synthesized derivatives **3.20-3.24** they have no free electron that can undergo such reactions.

4.2.3. Ferric ions reducing antioxidant power of the Schiff's bases (3.25-3.30)

For Schiff's base derivatives **3.25-3.30**, FRAP results is as shown in Figure 4.8 and Table 4.8.

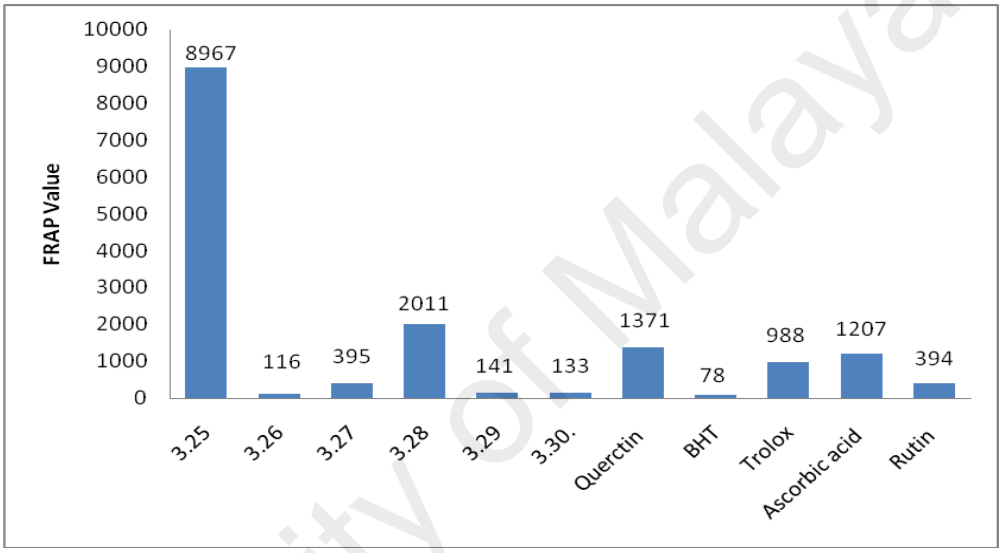
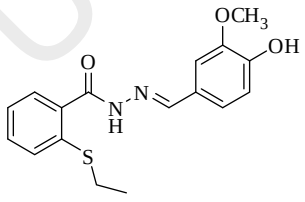
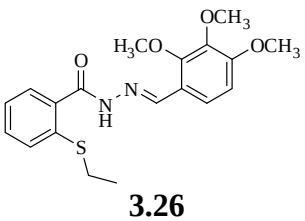
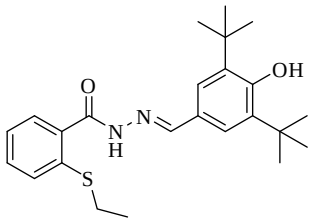
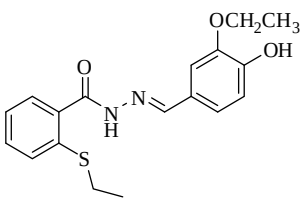
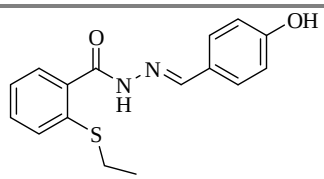
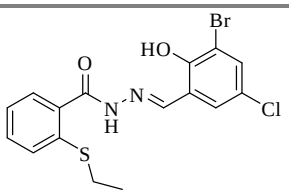


Figure 4.8: FRAP value for compounds **3.25-3.30** and reference standards

Table 4.8: FRAP value for compounds **3.25-3.30**

Compounds structure	FRAP values (µM/100 g)	Compounds structure	FRAP values (µM/100 g)
 **3.25**	8966.7 ± 0.03	 **3.26**	115.55 ± 1.7
 **3.27**	394.9 ± 0.77	 **3.28**	2011.1 ± 0.03

 **3.29**	140.7 ± 1.01	 **3.30**	132.7 ± 0.3
Quercetin	1371.1 ± 0.26	**BHT**	77.83 ± 0.08
Rutin	393.89 ± 0.02	**Trolox**	987.78 ± 0.14
Gallic acid	2957.78 ± 0.05	**Ascorbic acid**	1206.67 ± 0.02

Compounds **3.25** and **3.28** display excellent FRAP values (8966.7 ± 0.03 and 2011.1 ± 0.03, respectively), compared to the reference, whereas compound **3.27** possessed a greater FRAP reducing power than Rutin and BHT (395), while compounds **3.26**, **3.29** and **3.30** show FRAP values (116, 141 and 133, respectively) which are higher than BHT as shown in Figure 4.8 and Table 4.8.

The trend for the ferric ion reducing activities of compounds **3.25-3.30** did not vary markedly from their DPPH scavenging activities. This result is in agreement with previous reports that ferric reducing potential can be related to phenolic content[181, 182] as phenolics are capable to quenching free radicals by forming resonance-stabilized phenoxyl radicals. Thus the introduction of electron donor substituent which stabilizes the generated radical during oxidation[183] as in the case of compounds **3.25** and **3.28** had a great advantage to the biological activities.

4.2.4. Ferric ions reducing antioxidant power of hydrazide (3.31) and the 2,5-disubstituted-1,3,4-oxadiazole derivatives (3.32-3.37)

Results from Table 4.9 and Figure 4.9 show that compounds **3.31**, **3.32**, **3.34**, and **3.35** displayed excellent FRAP values compared to the reference compounds

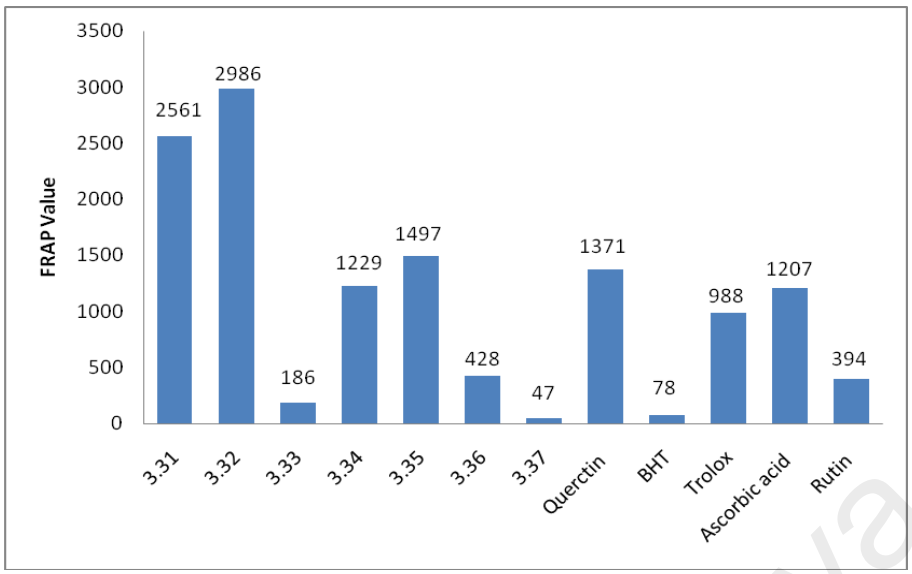
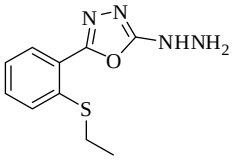
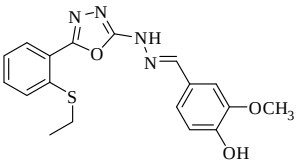
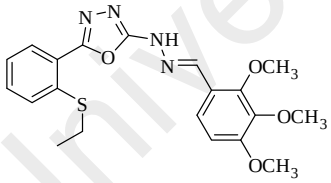
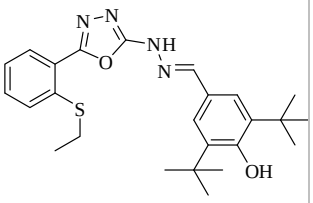
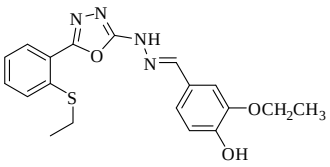
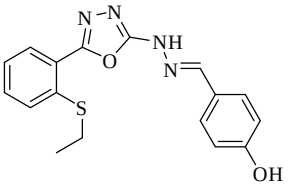
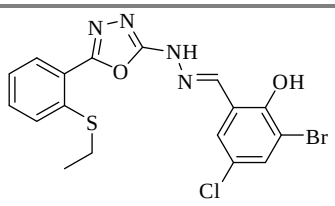


Figure 4.9: FRAP value for compounds **3.31-3.37** and reference standards

Table 4.9: FRAP value for compounds **3.31** and **3.32-3.37**

Compounds structure	FRAP values (µM/100 g)	Compounds structure	FRAP values (µM/100 g)
 **3.31**	2561.1 ± 0.4	 **3.32**	2985.5 ± 0.11
 **3.33**	185.7 ± 1.2	 **3.34**	1228.8 ± 1.85
 **3.35**	1497.2 ± 0.68	 **3.36**	428.4 ± 1.3

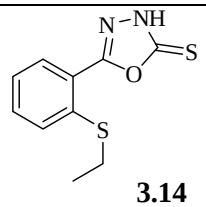
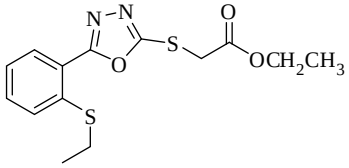
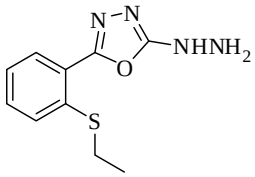
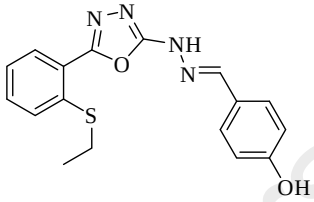
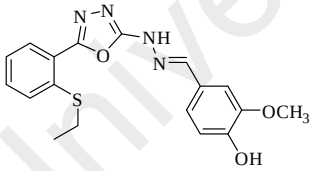
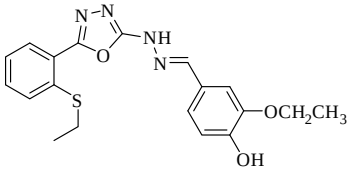
 **3.37**	46.8 ± 0.45		
Quercetin	1371.1 ± 0.26	**BHT**	77.83 ± 0.08
Trolox	987.78 ± 0.14	**Gallic acid**	2957.78 ± 0.05
Rutin	393.89 ± 0.02	**Ascorbic acid**	1206.67 ± 0.02

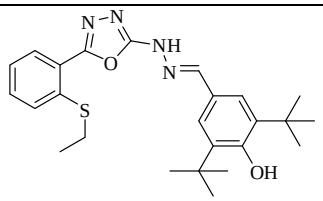
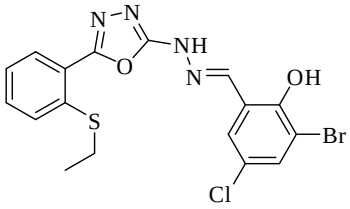
Compounds **3.33** and **3.36** possessed greater FRAP reducing power than BHT, while compound **3.37** showed very poor FRAP values when compared to the reference compounds. From the FRAP assay data in Table 4.9, comparison between compounds **3.32-3.37** with exception of compound **3.33** shows that the presence of an electron donating group on the phenolic ring increases the reducing power of the compounds. On the other hand, the presence of electron withdrawing groups reduces the reducing power of the compounds. Compounds **3.32** and **3.35** has a strong electron donating ability through induction effect of OMe and OEt groups, respectively that results in strong reducing power. Compound **3.36**, which does not possess a substituent, shows a lower reducing property similar to **3.34**, which possesses a weak electron donating group. In contrast, compound **3.37** with two strong electrons withdrawing groups (Br and Cl) also showed weak reducing power.

4.3. Cytotoxic activity

Selected 1,3,4-oxadiazole derivatives were evaluated for their cytotoxic activity *in vitro* against three cancer cell lines BxPC-3, MCF-7, and MDA-MB-231 and the hTERT-HPNE normal cell line using the MTS assay. This colorimetric assay measures the percentage of viable cells after exposure to a test compound.[184] Adose-response curve is recorded from cytotoxic compounds. Our studies with selected 1,3,4-oxadiazole derivatives showed that the percentage of viable cells decreases with increasing concentrations of the compounds. The 50% inhibitory concentration (IC_{50}) is obtained from the dose-response curve at the concentration where 50% of the cells remain viable as shown in Table 4.10. A positive control, curcumin, was used for comparison. Curcumin was selected because it contains two benzene rings that are substituted with –OH and –OMe groups, which has structural resemblance to our compounds **3.32** and **3.34-3.37**. The derivatives studied revealed a moderate inhibitory effect against the cancer cell lines, comparable to curcumin was observed. The monophenolic derivatives **3.32** and **3.34-3.37** gave an inhibitory effect against the cell lines, with the lowest being compound **3.37** against MDA-MB-231 (IC_{50} = 21.40 ± 1.22 µM) and **3.35** against BxPC-3 (IC_{50} = 26.17 ± 1.10 µM). However, the halogenated compound **3.37** showed higher cytotoxicity against normal pancreatic cells (hTERT-HPNE IC_{50} = 1.88 ± 0.29 µM) than pancreatic cancer cells (BxPC-3 IC_{50} = 31.07 ± 2.76 µM). Compounds **3.14**, **3.24** and **3.31** did not exhibit any cytotoxic activity against the cell lines tested, as their IC_{50} values were greater than 100 µM.

Table 4.10: IC_{50} values of 1,3,4-oxadiazole derivatives and curcumin obtained from their respective dose-response curves.

Compounds	IC_{50} µM (Mean ± SD, n=3)			
	BxPC-3	**hTERT-HPNE**	**MCF-7**	**MDA-MB-231**
 **3.14**	> 100	> 100	> 100	> 100
 **3.24**	> 100	> 100	> 100	> 100
 **3.31**	> 100	> 100	> 100	67.79 ± 9.22
 **3.36**	46.68 ± 1.38	60.45 ± 3.49	46.76 ± 1.01	63.07 ± 1.05
 **3.32**	43.85 ± 3.10	75.59 ± 0.26	53.90 ± 1.07	> 100
 **3.35**	26.17 ± 1.10	39.04 ± 3.01	36.49 ± 0.60	80.24 ± 1.50

 **3.34**	> 100	> 100	> 100	> 100
 **3.37**	31.07 ± 2.76	1.88 ± 0.29	39.54 ± 2.23	21.40 ± 1.22
Curcumin	47.85 ± 0.17	39.22 ± 0.25	61.20 ± 0.53	34.82 ± 1.36

* BxPC-3 is a pancreatic cancer cell line, hTERT-HPNE, is a normal pancreatic cell line, MCF-7 is a non-metastatic breast cancer cell line, and MDA-MB-231 is a metastatic breast cancer cell line.

Studies shows that parent compound **3.14** containing only a 1,3,4-oxadiazole moiety, did not show any cytotoxic potency against the tested cell lines. The same observation was recorded with compound **3.24**, which contains an ethyl ester moiety and compound **3.31** with a hydrazide group**.** Compounds **3.32** and **3.34-3.37** are derivatives of compound **3.31**, which was formed after the introduction of the phenolic OH moiety and varying substitutions on the aromatic ring. Interestingly, the introduction of the phenolic OH confers cytotoxic potency against the tested cell lines, with an exception of compound **3.34** as shown in Table 4.10. It was reported that the presence of phenolic OH moiety in natural polyphenolic compounds, such as trolox and quercetin, is vital in suppressing reactive oxygen species (ROS).[185,186] Excessive ROS can lead to chronic inflammation which is one of the reasons for the cancer development, by which overpowers its potential strategy for cancer therapy.

4.4. Anti-ulcer study

Based on our *in vitro* antioxidant results from both DPPH and FRAP assays, hydrazone 2-(Ethylthio)-*N'*-(4-hydroxy-3-methoxyphenyl)benzylidene]benzohydrazide **3.25** exhibited excellent antioxidant activity in comparison to standard compounds quercetin, BHT, Trolox, Rutin, ascorbic acid and gallic acid. Gastric ulcer study was carried out to investigate the gastroprotective effect of compound **3.25** on ethanol-induced gastric mucosal lesions in rats.

4.4.1. Acute Toxicity test

Acute toxicity is a study in which the animals were treated with compound **3.25** at a dose 500 mg/kg and were kept under observation for 14 days. All the animals remained alive and did not manifest any significant visible sign of toxicity at these doses. Clinical observations, serum biochemistry, and histopathology data did not show any significant differences between control and treated groups as shown in Table 4.11 and Figure 4.10. We concluded that oral administration of compound **3.25** to rats is safe and that no drug-related toxicity was detected.

Table 4.11: Acute toxicity test

A. Effects of compound 3.25 on renal function tests in rats

Dose	Sodium (mmol/L)	Pottasium (mmol/L)	Chloride (mmol/L)	CO_2 (mmol/L)	Anion gap (mmol/L)	Urea (mmol/L)	Creatinine (µmol/L)
Vehicle (10% Tween 20)	141.53 ± 0.42	4.65 ± 0.11	105.63 ± 0.98	23.05 ± 0.44	18.50 ± 0.25	7.71 ± 0.33	41.280 ± 2.88
(500mg/kg)	143.01 ± 0.69	4.55 ± 0.13	103.38 ± 0.85	22.84 ± 0.42	17.38 ± 0.30	7.97 ± 0.28	43.09 ± 4.17

Values expressed as mean ± S.E.M. There are no significant differences between groups. Significant value at $p<0.05$

B. Effects of compound 3.25 on liver function tests in rats

Dose	Total protein (g/L)	Albumin (g/L)	Globulin (g/L)	TB (µmol/L)	CB (µmol/L)	AP (IU/L)	ALT (IU/L)	AST (IU/L)	GGT (IU/L)
Vehicle (10% Tween 20)	64.23 ± 1.28	11.257 ± 0.28	53.57 ± 1.79	2.00 ± 0.00	1.00 ± 0.00	111.72 ± 6.22	43.41 ± 1.97	169.10 ± 6.82	3.78 ± 0.39
(500mg/kg)	62.15 ± 1.19	10.78 ± 0.31	52.43 ± 1.63	2.00 ± 0.006	1.00 ± 0.00	98.81 ± 5.51	40.27 ± 2.16	174.25 ± 8.65	3.53 ± 0.51

Values expressed as mean ± S.E.M. There are no significant differences between groups. Significant value at $p<0.05$
TB: Total bilirubin; CB: Conjugated bilirubin; AP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase;GGT: G-Glutamyl Transferase .

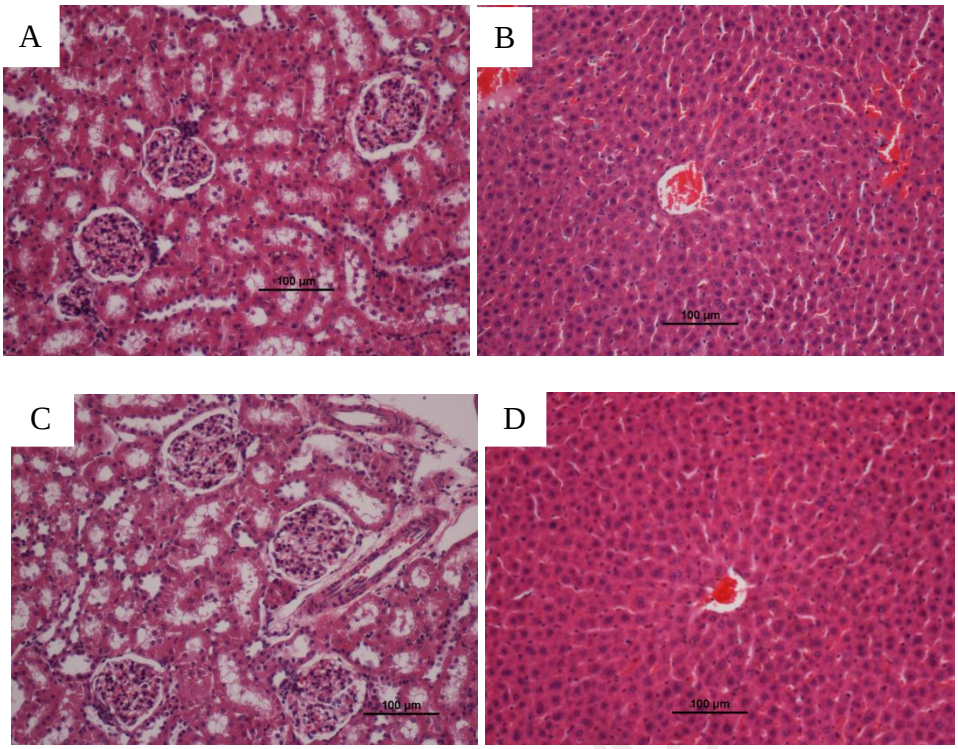


Figure 4.10: Histological sections of liver and kidney in acute toxicity test.
(A and B) Rats treated with 5 ml/kg vehicle (10% Tween 20).
(C and D) Rats treated with compound **3.25** (500mg/kg).
There is no significant difference in structures of liver and kidney between treated and control groups (H & E stain, magnification ×20).

4.4.2. pH of Gastric Content and Mucus Production

The acidity of gastric content in experimental animals pretreated with compound **3.25** was studied and found that the acidic content was decreased significantly compared to that of the ulcer control group ($p<0.05$). The mucus production of gastric mucosa significantly ($p<0.05$) increased in animals pretreated compared to the ulcer control group as shown in Table 4.11.

4.4.3. Gross Evaluation of Gastric Lesions

The anti-ulcer activity of compound **3.25** in ethanol-induced gastric lesion model is shown in Table 4.12. The lesions were long, hemorrhic and confined to the glandular portions. Results showed that rats pre-treated with omeprazole or compound **3.25** before being given absolute alcohol had significantly reduced areas of gastric ulcer formation

compared to rats pre-treated with ulcer control group as shown in Figure 4.11. Absolute ethanol produced extensive visible haemorrhagic lesions of gastric mucosa. Moreover, compound **3.25** significantly suppressed the formation of the ulcers and it was interesting to note the flattening of gastric mucosal folds in rats pre-treated with compound **3.25** (50 mg/kg). It was also observed that protection of gastric mucosa was most prominent in rats pre-treated with 100 mg/kg of compound **3.25** as shown in Table 4.12. In addition, the ethanol-induced mucosal damage was significantly reduced in the size and severity by pre-treated of the animals with compound **3.25**. The significant inhibition of gastric ulcer in rats pretreatment with this compound **3.25** (100 mg/kg) was comparable with omeprazole which is a standard drug used for curing gastric ulcer as shown in Table 4.12 and Figure 4.11.

Table 4.12: Effect of compound **3.25** on mucus production, acidity, ulcer area and inhibition percentage in rats

Animal group	pretreatment (5ml/kg)	pH of Gastric tissue	Mucus weight (g)	Ulcer Area (mm)(mean±S.E.M)	% Inhibition
1	10% Tween20 (ulcer control)	2.95±0.40	0.512±0.015	850.00±14.43	-
2	Omeprazole (20mg/kg)	5.60±0.50*	0.46±0.025*	178±9.6*	79
3	Compound **3.25** (50 mg/kg)	5.02 ± 0.48*	0.50 ± 0.098	63.36 ± 34.80*	92.55
4	Compound **3.25** (100 mg/kg)	3.9±0.9*	0.44 ± 0.06*	18.72 ± 5.42*	97.8
5	Normal	7.05±0.6*	0.586±0.031	0	-

Values are assumed as mean ± S.E.M. The statistical analysis was assessed with one-way ANOVA (post hoc analysis) with $P < 0.05$. *Significant differences when compared to group 1

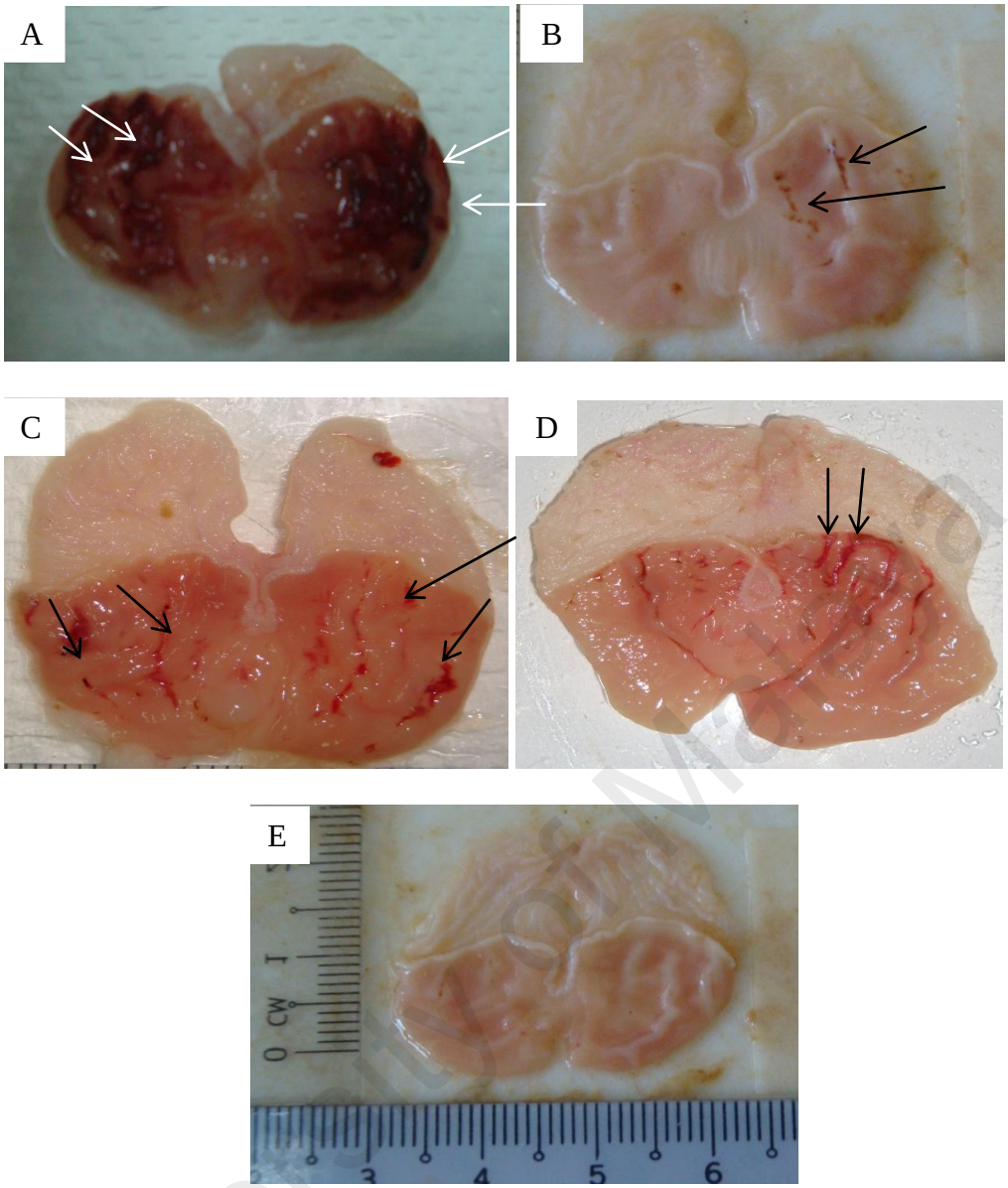
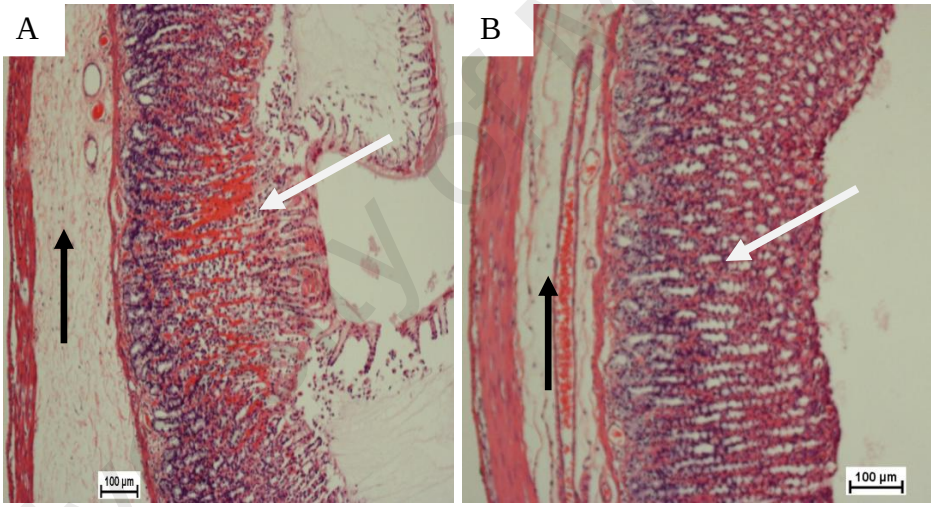


Figure 4.11: Gross appearance of the gastric mucosa in rats.
(A) Rats pre-treated with 5 ml/kg 10% Tween 20 (ulcer control). Severe injuries are seen in the gastric mucosa (arrow). Absolute ethanol produced extensive visible haemorrhagic necrosis of gastric mucosa.
(B) Rats pre-treated with of omeprazole (20 mg/kg). Injuries to the gastric mucosa are very milder (arrow) compared to the injuries seen in the ulcer control rats.
(C) Rats pre-treated with compound **3.25** (50 mg/kg). Moderate injuries are seen in the gastric mucosa (arrow).
(D) The compound reduces the formation of gastric lesions induced by absolute ethanol. Rats pre-treated with (100 mg/kg) of compound **3.25**, mild injuries are seen in the gastric mucosa (arrow).
(E) Rats in the normal control group showed intact gastric mucosa.

4.4.4. Histological Evaluation of Gastric Lesions

Our studies has shown that histological observation of ethanol induced gastric lesions in ulcer control group pre-treated with 10% Tween 20, showed comparatively extensive damage to the gastric mucosa and necrotic lesions penetrating deeply into mucosa, extensive oedema and leucocytes infiltration of the submucosal layer are present as shown in Figure 4.12. Rats that received pretreatment with compound **3.25** had comparatively better protection of the gastric mucosa as seen by reduction of ulcer area, submucosal oedema and leucocytes infiltration as shown in Figure 4.12. This compound has been shown to exert cytoprotective effects in a dose-dependent manner.



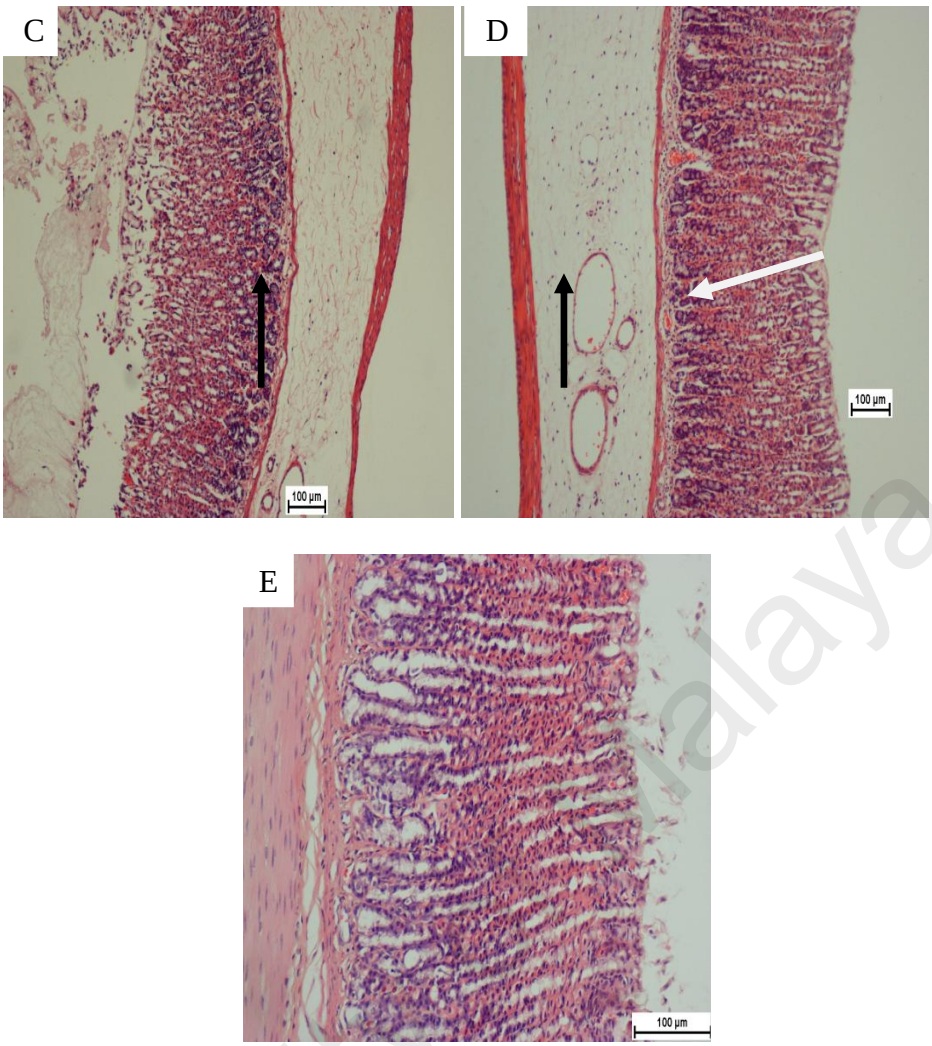


Figure 4.12: Histological study of the absolute ethanol-induced gastric mucosal damage in rats.

(A) Rats pre-treated with 5 ml/kg of 10% Tween 20 (ulcer control). There is severe disruption to the surface epithelium and necrotic lesions penetrate deeply into mucosa (white arrow) and extensive edema of submucosa layer and leucocyte infiltration are present (black arrow).

(B) Rats pre-treated with omeprazole (20 mg/kg). Mild disruption of the surface epithelium mucosa is present (white arrow) but deep mucosal damage is absent. Reduction of submucosal edema and leucocytes infiltration (black arrow).

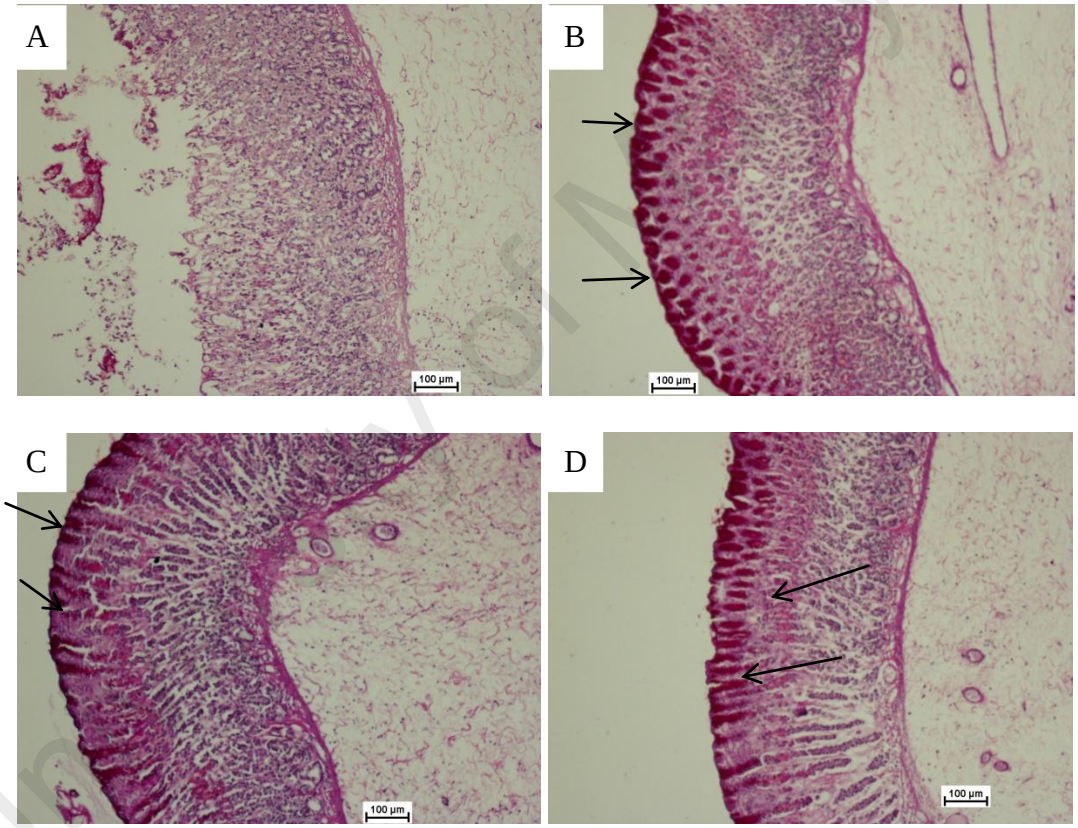
(C) Rats pre-treated with compound **3.25** (50 mg/kg), mild disruption of surface epithelium is present but deep mucosal damage is absent. Reduction of submucosal edema and leucocytes infiltration (black arrow).

(D) Rats pre-treated with compound **3.25** (100 mg/kg), mild disruption of surface epithelium is present but deep mucosal damage is absent. Reduction of submucosal edema and leucocytes infiltration (black arrow).

(E) Rats in the normal control group showed intact gastric mucosa.

4.4.5. Mucus Staining

Periodic acid–Schiff (PAS) staining was used to observe the glycogen level in control and pre-treated animals in our work. Compound **3.25** pre-treatment shown in Figure 4.13 resulted in the expansion of a substantially continuous PAS-positive mucous gel layer lining the entire gastric mucosal surface observed as the magenta colour. However, gastric specimen from ulcer control group didn't exhibit this magenta staining colour of PAS.



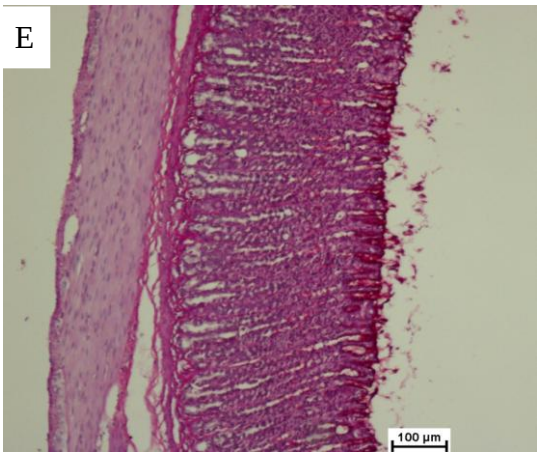


Figure 4.13: Effect of the compound **3.25** on gastric tissue glycoprotein-PAS staining (×20).

(A) The ulcer control group,
(B) The reference group (omeprazole, 20 mg/kg),
(C) Rats received 50 mg/kg of the compound **3.25**
(D) Rats received 100 mg/kg of the compound **3.25**. Magenta colour in the apical epithelial cells in the treated groups with the compound shows gradual increase in mucosal secretion of gastric glands. The intense secretion of mucus in gastric glands is demonstrated in (D). The arrow points to the glycoprotein accumulation.
(E) Rats in the normal control group showed intact gastric mucosa.

4.4.6. HSP-70 and Bax Immunohistochemistry

Using immunhistochemistry staining, the immunostained localization of HSP-70 was up regulated in compound **3.25** pretreated-animals more than that observed in ulcer control group as shown in Figure 4.14. This result indicates the possible participation of this protein in protective effect of compound **3.25**. On the other hand, the immunostained localization of the pro-apoptotic Bax protein in all experimental animals was down regulated compared to the ulcer control group as shown in Figure 4.15. Hence, the suppressive effect on Bax protein in treatment group might have contributed in the gastroprotective activity of compound **3.25**. The antigen site in immunohistochemistry appears as brown-colored.

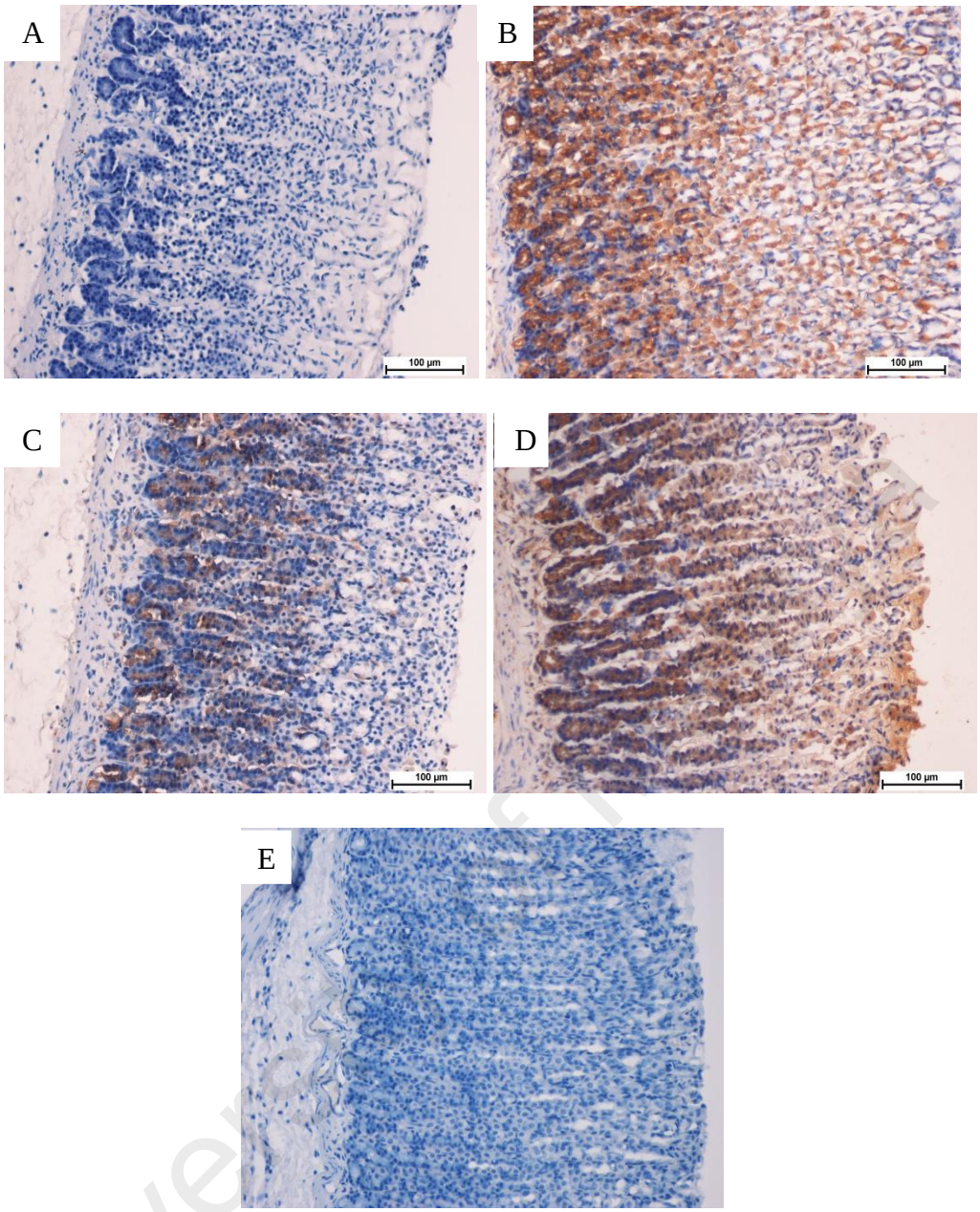


Figure 4.14: Immunohistochemical analysis of HSP-70 protein. HSP-70 expression in the gastric tissue of rats submitted to ethanol-induced gastric mucosal lesions at different groups where:

(A) Ulcer control group,
(B) Omeprazole group,
(C and D) the pretreated groups with compound **3.25** at doses 50 and 100 mg/kg, respectively.
(E)Rats in the normal control group showed intact gastric mucosa. The antigen site appears as a brown color (IHC: ×20).

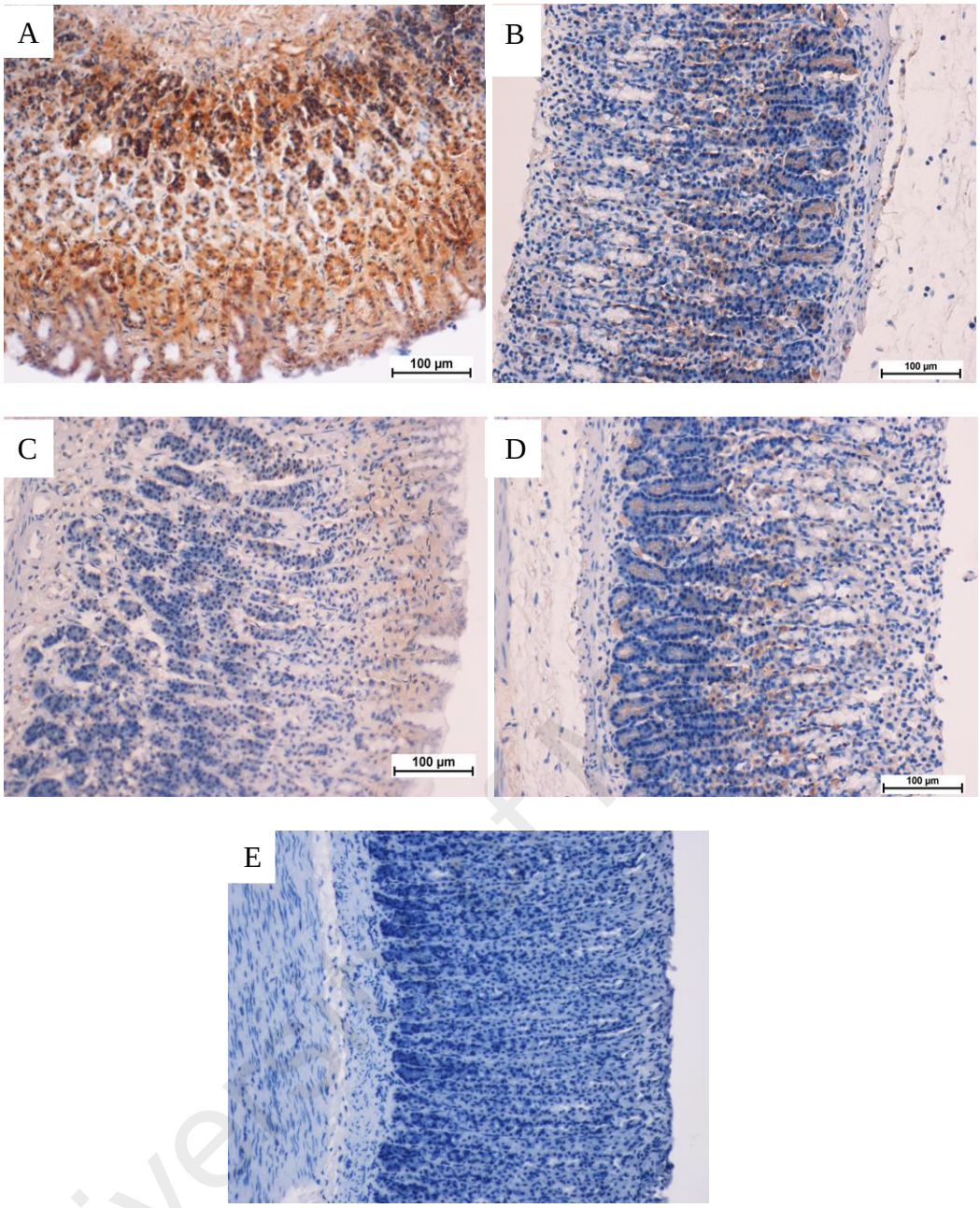


Figure 4.15: Immunohistochemical analysis of Bax protein. Bax expression in the gastric tissue of rats submitted to ethanol-induced gastric mucosal lesions at different groups where:

(A) Ulcer control group,
(B) Omeprazole group,
(C and D) the pre-treated groups with compound **3.25** at doses 50 and 100 mg/kg,
(E) Rats in the normal control group showed intact gastric mucosa. The antigen site appears as a brown color (IHC: ×20).

In this study, the gastric gavage of absolute alcohol caused a visible gastric mucosal injury grossly and histologically. Alcohol-induced gastric lesions severely impaired gastric defensive elements such as mucus and mucosa circulation. Ethanol creates necrotic lesions of the gastric mucosa in a multifactorial way. It can reach the mucosa by disruption of the mucus-bicarbonate barrier and cause cell damage in the wall of blood vessels. These effects are presumably due to biological actions, such as of lipid peroxidation, free radicals formation, oxidative stress, alterations in permeability and depolarization of the mitochondrial membrane preceding to cell death.[187] In the present study, compound **3.25** has an effective antisecretory and anti-ulcer action against ethanol-induced gastric mucosal injury, it decreased the acidity and increase the mucus of gastric content. This finding is consistent with results of a previous report.[187] Similarly, mucus secretion is regarded as an essential defensive factor in the protection of the gastric mucosa from gastric lesions.[188] Pretreatment with compound **3.25** could to some extend reduce the ulcer area and prevent gastric ulceration. Although ulcer etiology is unknown in most cases, it is generally agreed that an imbalance between acid and pepsin production and mucosal integrity would be causative factor acting via endogenous defense mechanisms. Peptic ulcers are caused by an imbalance between the protective and the aggressive mechanisms of the mucosa, and are the result of the association of several endogenous factors and aggressive exogenous factors that are related to living conditions.[189, 190] It has been reported that significant antiulcer effects could be due to strengthening of gastric mucosa with the enhancement of mucosal defence.[191, 192] Acute toxicity test did not show any signs of toxicity and mortality. Behavioural changes like irritation, restlessness, respiratory distress, and abnormal locomotion over a period of 14 days were not observed. This revealed that compound **3.25** is safe and has no toxicity when administered orally up to 500mg/kg. Omeprazole exhibits an anti-secretory and protective effect,[193] and this drug protected the stomach against ethanol's necrotic damage and its effect was more obvious than omeprazole, a cytoprotective agent. Ulcer

area parameter was used for the evaluation of antiulcer activity since ulcer formation is directly related to factors such as gastric volume and acidity. In case of vehicle (ulcer control), increasing the acid secretion, which in turn increases gastric volume, decreases pH and increases acidity resulting into increase in ulcer area.[194] Omeprazole, a proton pump inhibitor (PPI) showed a fairly protected gastric mucosa and has been widely used as an acid inhibitor agent for the treatment of disorders related to gastric acid secretion for about 15 years.[195] PPIs are capable of producing almost complete suppression of acid secretion. The mechanism of action of omeprazole is such that it binds very specifically to a single subunit of the H^+, K^+-ATpase (adinosine triphosphatase) at the secretory surface of parietal cell and inactivates it and reduces acid secretion regardless of the source of secretory stimulation. By increasing intragastric pH through inhibition of acid secretion, PPIs inhibit activation of pepsin. PPI is highly selective for the proton pump and undergoes catalyzed conversion into an active form within the acid-forming space. The active inhibitors react with SH (thiol) group of the proton pump, resulting in inhibition of acid formation.The long and short term use of them is extremely effective in treating peptic ulcer disease and gastroesophageal reflux.[195] The pathogenesis of mucosal damage in the stomach includes the production of reactive oxygen species (ROS) that seem to play a crucial role in the formation of lipid peroxides, accompanied by a defect of anti-oxidative enzyme activity of cells.[195]

Oxidative stress plays important role in the pathogenesis of various diseases, including gastric ulcer, with antioxidants being reported to play a significant role in protection of the gastric mucosal injury against various necroticagents.[196] Antioxidants could help to protect gastric cellular damage caused by oxidative stress and enhanced the body's defence systems against degenerative diseases. Administration of antioxidants inhibits ethanol-induced gastric injury in rat.[197, 198] Compound **3.25** in this study has been shown to contain antioxidants and it is likely that gastroprotective effect exerted by this

compound could be attributed to its antioxidant property. The result of the present study also revealed protection of gastric mucosa and inhibition of leucocytes infiltration of gastric wall in rats pretreated with compound **3.25**. Activation and infiltration of neutrophils attend to be involved in the initial processes of formation of the lesion. Similarly, Mahmood *et. al*.,[199] demonstrated that the reduction of neutrophil infiltration into ulcerated gastric tissue actively promotes the prevention of gastric ulcers in rats. Absolute alcohol would extensively damage the gastric mucosa leading to increased neutrophil infiltration into the gastric mucosa. Oxygen free radicals derived from infiltrated neutrophils in ulcerated gastric tissues have inhibitory action on gastric ulcers healing in rats. Neutrophils mediate lipid peroxidation through the production of superoxide anions.[200] Neutrophils are a major source of inflammatory mediators and can release potent reactive oxygen species such as superoxide, hydrogen peroxide and myeloperoxidase derived oxidants. These reactive oxygen species are highly cytotoxic and can produce tissue damage.[201, 202] In the present study, we observed flattening of the mucosal folds which suggests that gastroprotective effect of compound **3.25** might be due to a decrease in gastric motility. It is reported that the changes in the gastric motility may play a role in the progression and prevention of experimental gastric lesions.[203] Relaxations of circular muscles may protect the gastric mucosa through flattening of the folds. This will increase the mucosal area exposed to necrotizing agents and reduce the volume of the gastric irritants on rugal crest.[203] Ethanol produces a marked contraction of the circular muscles of rat fundic strip. Such a contraction can lead to mucosal compression at the site of the greatest mechanical stress the mucosal folds leading to necrosis and ulceration.[199]

CHAPTER 5: CONCLUSION

5.1. Conclusion

For the growing need and use of new, safe and effective potential drug candidates new synthetic strategies and biological evaluation approaches arise. In this study a new series of thiosemicarbazides **3.3-3.7** were prepared by reaction of hydrazide **3.2** with various aryl isothiocyanates in the presence of ethanol. Variation of solvents in this method led to a dramatic improvement in yield of **3.3-3.7** from 65-88% to > 90% shown in Table 3.1. The base-intramolecular dehydrative cyclization reaction of 1-acylthiosemcarbazides **3.3-3.7** using 4 N NaOH gave good yield of 1,2,4-triazoles **3.8-3.12** and their mechanistic are hypotheses discussed.

The use of different protocols to prepare the 1,2,4-triazoles was also tested. From literature, the most common synthetic pathway to prepare 4-amino-1,2,4-triazole derivative was through the formation of potassium hydrazine carbodithioate salt followed by reaction with hydrazine hydrate to afford compound **3.13** in 65% yield.

Contrary to the protocol used in the literature, the acid-intramolecular dehydrative cyclization reaction of 1-acylthiosemcarbazides **3.3-3.7** using concentrated H_2SO_4 did not undergo cyclization. Also the common procedure to synthesizes 1,3,4-oxadiazole derivatives from their corresponding thiosemicarbazides **3.3-3.7** using iodine in potassium iodide in presence of sodium hydroxide had failed even after examination of a variety of reaction conditions.

5-[2-(Ethylsulphanyl)phenyl]-1,3,4-oxadiazole-2(3*H*)-thione **3.14** was prepared by the reaction of 2-(ethylsulphanyl)benzohydrazide with CS_2 in KOH with 84% yield. By attempting to introduce the substituents using alkylation reaction, a new 2,5-disubstituted-1,3,4-oxadiazole derivatives **3.20-3.24** were prepared via reaction of

compound **3.14** with appropriate alkyl and aryl halide in presence of K_2CO_3 and acetone at room temperature.

Reaction of acid hydrazide **3.2** with different substituted aromatic aldehydes in presence of ethanol afforded new Schiff's bases **3.25-3.30** in 90% yields as a mixture of *E*- and *Z*-isomers in which *E*-isomers were predominant. The X-ray structure of compound **3.30** revealed that the *E*-isomer is more stable than others in the solid phase at RT.

The reaction of ester **3.24** with hydrazine hydrate in the presence of an alcohol solvent did not undergo hydrazination to form the expected hydrazide in contrast to the common method. A dioxane solvent at RT gave unexpected hydrazide **3.31** with a 69% yield. Two mechanistic hypotheses were proposed to explain the formation of the new hydrazide **3.31**. Reaction of hydrazide **3.31** with different substituted aromatic aldehydes in presence of ethanol afforded a new 2,5-disubstituted-1,3,4-oxadiazole derivatives **3.32-3.37** with a very good yield 85-90%.

The antioxidant activities of the newly synthesis compounds were studied using DPPH and FRAP assays and the following conclusions were determined. Results from the antioxidant activity for the first series of compounds revealed that the thiosemicarbazides have shown better antioxidant activities rather than their cyclised 1,2,4-triazoles in both assays. From the DPPH assay data, the presence of an electron-withdrawing group i.e. (*Cl*) on the phenyl ring of thiosemicarbazides improved their scavenging ability over their cyclic product. This result was further confirmed using the (DFT) method. The latter function was found to provide an accurate calculations on the spin density of the radical intermediate and bond-disociation enthalpies. The DFT results showed that the lowest spin density and bond dissociation enthalpies calculated on the radical formed in

thiosemicarbazide derivative bearing electon with drawing group gave more stable compound and the highest antioxidant activity.

In the second series the synthesized compound bearing 1,3,4-oxadiazole-2-thiol moiety showed promising radical scavenging activity in the DPPH assay, while its alkylated derivatives did not possess any antioxidant activity either in DPPH or FRAP assays. The proposed HAT mechanism occuring in the DPPH assay suggested that hydrogen radicals are extracted from the N-H of the oxadiazole ring on to DPPH radicals which enables this compound to suppress two DPPH• radicals.

The antioxidant activities of hydrazones **3.25-3.30** were comparable to the DPPH radical scavenging and FRAP. The results showed that the presence of electron donating *o*-substituents on phenolic ring possessed the most significant activity among the compounds tested.

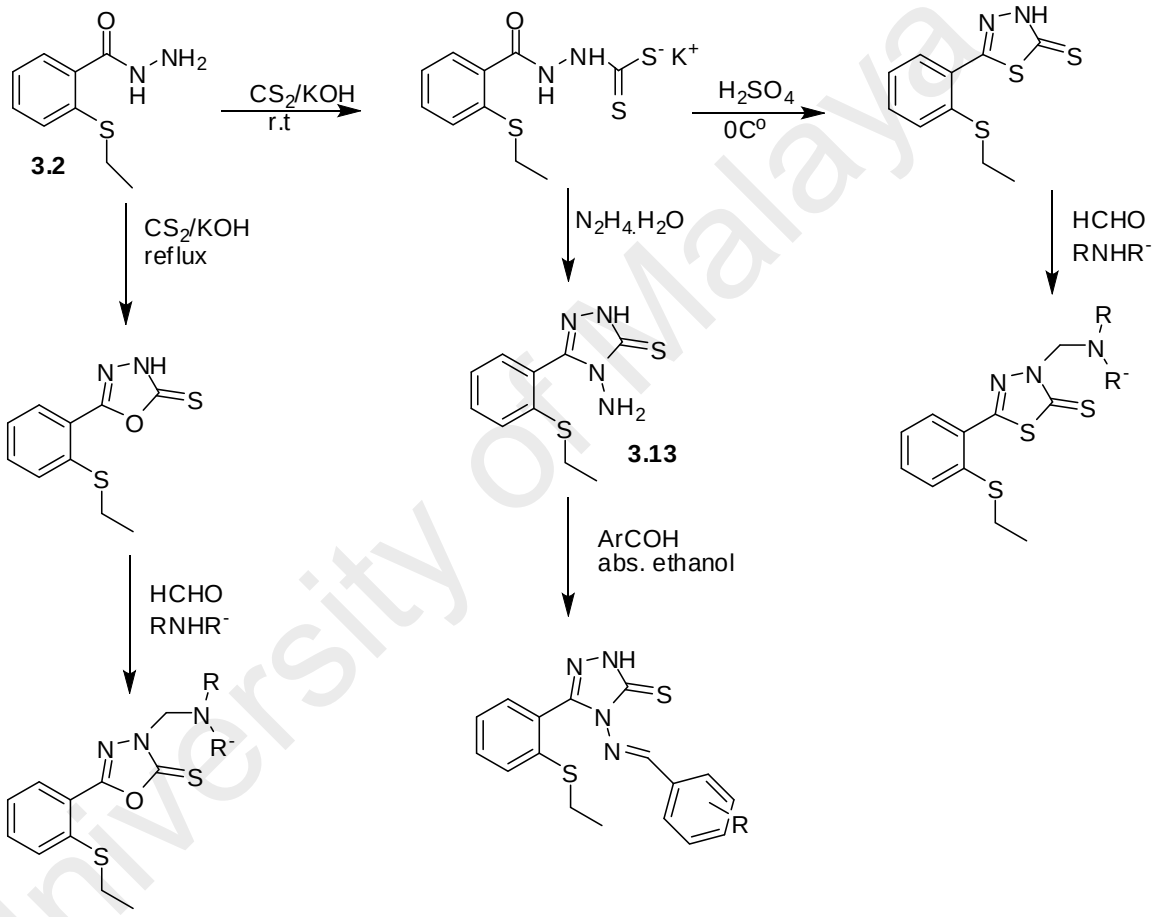
The 2-aryl-5-hydrazino-l,3,4-oxadiazole **3.31** showed a high antioxidant activity compared with standard compounds. This observed activity may be correlated with the presence of electron donor substituent which stabilizes the generated radical during oxidation. However, its oxadiazole derivatives **3.32** and **3.34-3.37** showed variability in their antioxidant activities. Based on the antioxidant results, the presence of higher activity in the DPPH is observed. Therefore, researchers infer that the scavenging activity of the reactive species by compounds **3.32** and **3.34-3.37** are from the hydrogen donating ability of the phenolic OH moiety. With regards to compound **3.34**, the di-*tert*-butyl moiety flanking the phenolic OH may have caused steric hindrance to the hydrogen donating ability of this compound.

Eight new 1,3,4-oxadiazoles were selected for further studies on their *in vitro* cytotoxic activities. The results revealed that in spite of their strong antioxidant activities compounds **3.14** and **3.31** display low cytotoxic potency. In oxadiazole derivatives **3.32** and **3.34-3.37**, compound **3.37** showed the most potent cytotoxic activity against MDA-MB-231 and compound **3.35** showed the most potent activity against BxPC-3. The results also revealed that phenolic OH was responsible for the cytotoxicity of these derivatives and the introduction of steric (di-*tert*-butyl) or electron withdrawing (Cl and Br) groups to the aromatic ring causing reduced selectivity in the cancer cell lines.

In vivo antiulcer study of hydrazone **3.25** revealed that this compound did not cause any sign of acute toxicity in rats. This compound could significantly protect the gastric mucosa against ethanol-induced injury. Such protection was ascertained grossly by a significant increase in the gastric wall mucus in comparison with the ulcer control group. Furthermore, the reduction of haemorrhagic mucosal areas in the gastric wall as well as the reduction or inhibition of edema and leukocytes infiltration of the sub mucosal layers was shown histologically. The *in vivo* antiulcer study provided evidence on gastro protective property of compound **3.25** suggested that the complex preserved gastric mucus secretion. The exact mechanism underlying this anti-ulcerogenic effect of this compound is due to potent antioxidant activity.

5.2. Future work

The future work consists of three parts. The first and third part will start from compound **3.2** that is to synthesize new 1,3,4-oxadiazole and 1,3,4-thiadiazole bearing Mannich bases to be screened for biological activity. The second part is to synthesize new derivatives of compound **3.13** as shown in Scheme 5.1 and conduct further study on their anticonvulsant activity.



Scheme 5.1: Outline of future work

CHAPTER 6: MATERIALS AND METHODS

6.1. General

All chemicals and solvents were of analytical grade acquired from Aldrich and Merck. Thin layer chromatography was performed on pre-coated silica gel plates (Si, 60, F254) for monitoring reactions and determining purity. Melting points were determined using a MEL-TEMP II apparatus and are uncorrected. IR spectra were recorded from 4000-400 cm^{-1} using a Perkin Elmer 400 Fourier transforms infrared (FTIR) Spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a BRUKER-AVN III 400 MHz instrument using DMSO-d_6 and $CDCl_3$ as solvents and TMS as an internal standard. Mass spectra were recorded on an Agilent5975 for EI/MS and Finnegan TSQ7000 for HREI/MS. Crystal data collection: APEX2' cell refinement; SAINT; data reduction; SAINT; programs (s) to solve structure; SHELXL97; molecular graphics; XSEED. Crystal data could be obtained from http://www.ccdc.cam.ac.uk/conts/ retrieving.html.

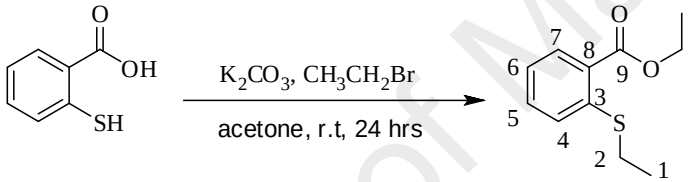
1, 1-Diphenyl-2picryl-hydrazyl (DPPH) was purchased from Sigma-Aldrich. Reference standards such as, ascorbic acid, gallic acid, quercetin, Trolox, Rutin and butylated hydroxytoluene (BHT) were purchased from E.Merch and were used to record FRAP and DPPH assay.

Human cancer and normal cell lines were obtained from American Type Culture Collection (ATCC) (Manassas, Virginia, USA). Dulbecco's modified Eagle's medium (DMEM), Hanks' Balanced Salt Solution (HBSS), 100 mM non-essential amino acids, phosphate buffer solution (pH 7.2), gentamycin and amphotericin B were purchased from Invitrogen Corporation (Carlsbad, California, USA). L-glutamine, fetal bovine serum (FBS), 0.25% trypsin-EDTA, dimethyl sulphoxide (DMSO), curcumin and 4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA).

Omeprazole was used as the reference anti-ulcer drug, and was obtained from the University Malaya Medical Centre (UMMC) Pharmacy.

Female ICR mice (30-40g) and albino *Sprague Dawley* rats (200-225g) were obtained from the Animal House Experimental Unit, Faculty of Medicine, University of Malaya. The animals were then placed individually in cages and acclimatized under standard conditions of humidity, lighting and temperature and were fed with standard rat pellets and tap water.

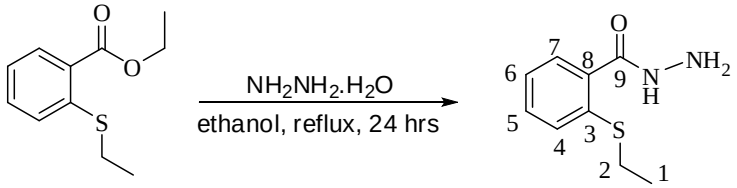
6.2. Synthesis of ethyl 2-(ethylsulfanyl)benzoate (3.1)



3.1

Thiosalicylic acid (4 g, 1mmol) dissolved in dry acetone and potassium carbonate anhydrous (1 g, 1mmol) was added and stirred at room temperature for 15 minutes. Bromoethane (20 ml) was then added and the mixture was stirred at room temperature for 24hours. The solvent was evaporated and the residue was extracted with ethyl acetate (25 ml). The organic layer was washed with saturated solution of sodium carbonate (5 g) and dried with an-hydrous sodium sulfate. Evaporation of solvent gave an oily product, (3.8 gm, yield: 95%). IR (KBr) (ν, $cm^{-1)}$ 3061 (C-H aromatic) 2975-2871 (C-H aliphatic), 1707 (C=O). ^{1}H NMR ($CDCl_3$, 400 MHz), δ, ppm: 1.38 (t, 3H, $SCH_2\underline{CH_3}$) 1.39 (t, 3H, $OCH_2\underline{CH_3}$), 2.95 (q, J=7.4, 2H, SCH_2), 4.38 (q, 2H, OCH_2), 7.14 (t, J=7.9, 1H, H6), 7.31 (d, J=8, 1H, H4), 7.42 (t, J=7.7, 1H, H5), 7.96 (d, J=7.8, 1H, H7). ^{13}C NMR ($CDCl_3$, 100 MHz), δ, ppm: 13.18 (1C, $OCH_2\underline{CH_3}$), 14.31 (1C, CH_3), 25.99 (1C, SCH_2), 61.06 (1C, OCH_2), 123.64 (1C, C6), 125.45 (1C, C8), 127.88 (1C, C4), 131.20 (1C, C7), 132.14 (1C, C5), 141.90 (1C, C3) and 166.53 (1C, C9).

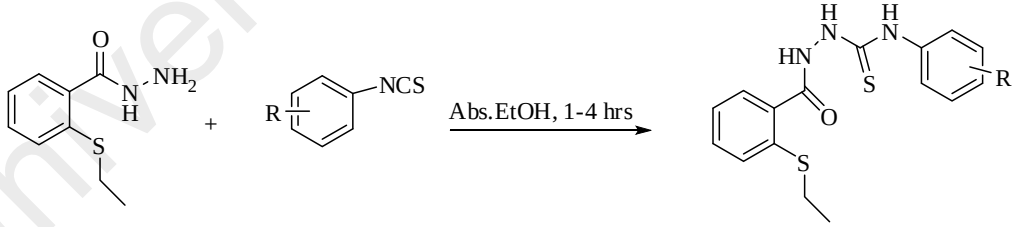
6.3. Synthesis of 2-(ethylsulfanyl)benzohydrazide (3.2)



3.1 **3.2**

Hydrazine hydrate 85% (5ml) was added to (5g, 15mmole) of ethyl 2-(ethylsulfanyl) benzoate in (2ml) ethanol. The mixture was refluxed 24 hours, after cooling the precipitate was collected as solid powder (4.8 g, yield: 97%) m.p. 82 ^{0}C. IR (KBr) (ν, cm^{-1}), 3280 (NH, NH_2), 2972, (C-H aromatic), 1601 (C=O). ^{1}H NMR (DMSO-d_6) δ ppm: 1.21 (t, J=7.3, 3H), 2.92 (q, 2H, SCH_2), 4.24 (br.s., 2H), 7.19 (m, 1H, H6), 7.32 (d, J=6.4, 1H, H5), 7.4 (d, J=3.4, 2H, H4, H7), 9.46 (br.s., 1H). ^{13}C NMR (DMSO-d_6) δ ppm : 13.69 (1C, CH_3), 25.97 (1C, CH_2), 124.69 (1C, C4), 127.38 (1C, C7), 127.73 (1C, C6), 129.98 (1C, C5), 135.42 (1C, C8), 135.79 (1C, C3) and 167.13 (1C, C9). HREI/MS m/z = 196.0665 [M^+] (calc. for $C_9H_{12}N_2O_1S_1$, 196.0670).

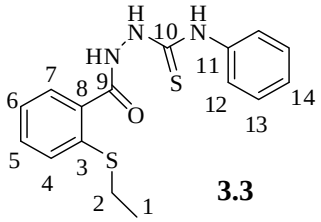
6.4. General synthesis of 2-[2-(ethylsulfanyl)benzoyl]-*N*-(4-subsetuted phenyl)hydrazinecarbothioamide (3.3-3.7)



R = H, Cl, CH_3, OCH_3, $(OCH_3)_3$

A solution of 1 mmol of 2-(ethyl sulfanyl)benzohydrazide (1 mmol) and appropriate amount of isothiocyanate (0.21 g, 1.39 mmol) in 15 ml of anhydrous ethanol was heated under reflux for 1-4 hrs. The solution was cooled and the solid formed was filtered off, washed with diethyl ether, dried, and recrystallized from EtOH.

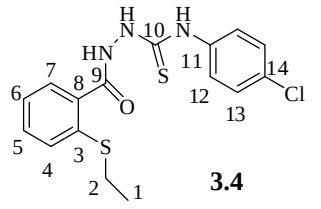
6.4.1. 2-[2-(Ethylsulfanyl)benzoyl]-*N*-phenylhydrazinecarbothioamide (3.3)



3.3

Colorless solid (0.65 g, yield: 96%) m.p.120 ^{0}C, IR. (KBr), (ν, cm^{-1}): 3260 (N-H st), 2972 (C-H st), 1643(C=O st), 1598(C=N st), 1239(C=S st). ^{1}H NMR (DMSO-d$_6$, 400 MHz), δ, ppm: 1.2 (t, 3H, CH_3), 2.95 (q, 2H, -CH_2), 7.18 (m, 1H, H14) 7.29 (m, 1H, H5), 7.37 (t, J=7.8, 2H, H13), 7.51 (m, 4H, 2H, H12, H4, H5), 7.74 (d, J=7.5, 1H, H7), 9.53 (br.s., 1H, NHPh), 9.84 (br.s., 1H, NHCS), 10.42 (s;1H,NHCO). ^{13}C NMR (DMSO-d$_6$, 100 MHz), δ, ppm : 13.64 (1C, CH_3), 26.56 (1C, CH_2), 124.93 (3C, C10, C3), 128.23 (2C, C13), 128.63 (1C, C14), 130.82 (2C, C6, C7), 133.93 (1C, C5), 136.24 (1C, C8), 138.97 (2C, C3, C11), 167.02 (1C, C9), 180.97 (1C, C10). HREIMS m/z 331.0816 [M $^{+}$] (calc. for $C_{16}H_{17}N_3O_1S_2$ 331.0813).

6.4.2. 2-[2-(Ethylsulfanyl)benzoyl]-*N*-(4-chlorophenyl)hydrazinecarbothioamide (3.4)

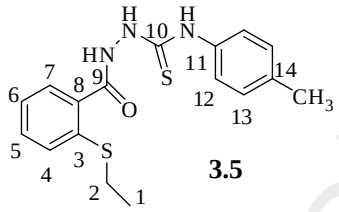


3.4

Colorless solid (0.55 g, yield: 93%) m.p.150 ^{0}C, IR. (KBr) (ν, cm^{-1}): 3179 (N-H st), 1666 (C=O st), 1587 (C=N st), 1223(C=S st). ^{1}H NMR (DMSO-d$_6$, 400 MHz), δ, ppm: 1.21 (t, 3H, CH_3), 2.95 (q, 2H, -CH_2), 7.28 (m, 1H, H6), 7.43 (m, 4H, 2H12, 2H13), 7.55 (d, J=7.8, 2H, H4, H5), 7.75 (d, J= 7.7, 1H, H7), 9.57 (br.s., 1H, NH-Ph), 9.92 (br.s., 1H, NHCS), 10.41 (s., 1H, NHCO). ^{13}C NMR (DMSO-d$_6$, 100 MHz), δ, ppm: 13.63 (1C,

CH_3), 26.37 (1C, CH_2), 124.76 (2C, C13), 126.99 (1C, C4), 127.85 (1C, C6), 128.107 (2C, C12), 128.70 (1C, C7), 130.88 (1C, C5), 133.50 (1C, C14), 136.56 (2C, C8, C11), 138.03 (1C, C3), 166.99 (1C, C9), 180.86 (1C, C10). HREIMS m/z 365.0419 [M^+] (calc. for $C_{16}H_{16}N_3O_1S_2$ 365.0423).

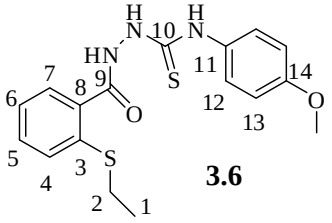
6.4.3. 2-[2-(Ethylsulfanyl)benzoyl]-*N*-(4-methylphenyl)hydrazinecarbothioamide (3.5)



3.5

Colorless solid (0.62 g, yield: 95%), m.p.120 ^{0}C, IR. (KBr) (ν, cm^{-1}): 3240 (NH st), 1641 (C=O st), 1615 (C=N st), 1237 (C=S st). ^{1}H NMR (DMSO-d_6, 400 MHz), δ, ppm: 1.19 (t, 3H, CH_3), 2.3 (s, 3H, -CH_3), 2.94 (q, 2H, -CH_2), 7.16 (m, J=8.2, 2H, H12), 7.28 (dt, 1H, H6), 7.37 (m, J=8, 2H, H13), 7.48 (d, J=3.9, 2H, H4, H5), 7.72 (d, J=7.5, 1H, H7), 9.44 (br.s., 1H, NHPh), 9.76 (br.s., 1H, NHCS), 10.38 (s. 1H, NHCO). ^{13}C NMR (DMSO-d_6, 100 MHz), δ, ppm: 13.65 (1C, CH_3), 20.50 (1C, $\underline{CH_3}$), 26.65 (1C, CH_2), 125.02 (2C, C12), 125.24 (1C, C4), 128.18 (1C, C7), 128.158, 128.71 (2C, C11), 130.82 (1C, C6), 134.05 (1C, C5), 134.27 (1C, C8), 136.09 (1C, C11), 136.32 (1C, C3), 138.36 (1C, C14), 167.15 (1C, C9), 181.02 (1C, C10). HREIMS m/z 345.0956 [M^+] (calc. for $C_{17}H_{19}N_3O_1S_2$ 345.0970).

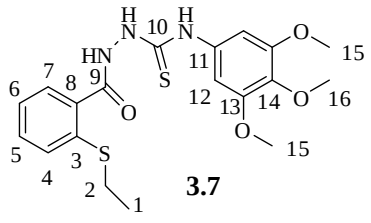
6.4.4. 2-[2-(Ethylsulfanyl)benzoyl]-*N*-(4-methoxyphenyl)hydrazinecarbothioamide (3.6)



3.6

Colorless solid (0.59 g, yield: 94%), m.p.172-174 ^{0}C, IR. (KBr) (ν, cm^{-1}): 3267 (NH st), 1667 (C=O st), 1607 (C=N st), 1354 (C=S st). ^{1}H NMR (DMSO-d_6, 400 MHz), δ, ppm: 1.19 (t, 3H, CH_3), 2.94 (q, 2H, -CH_2), 3.76 (s, 3H, -OCH_3), 6.93 (m, 2H, H12), 7.29 (m, 1H, H4), 7.35 (m, J=8.5, 2H, H13), 7.48 (d, J=4, 2H, H5, H6), 7.73 (d, J=7.5, 1H, H7), 9.41 (br.s., 1H, NH-Ph), 9.71 (br.s., 1H, NHCS), 10.37 (s, 1H, NHCO). ^{13}C NMR (DMSO-d_6, 100 MHz), δ, ppm: 13.65 (1C, CH_3), 26.61 (1C, CH_2), 55.22 (1C, O$\underline{CH_3}$), 113.43 (2C, C13), 124.96 (1C, C4), 126.79 (1C, C7), 128.13 (1C, C6), 128.64 (2C, C12), 130.81 (1C, C11), 131.74 (1C, C5), 134.0 (1C, C8), 136.15 (1C, C3), 156.79 (1C, C14), 167.05 (1C, C9), 181.16 (1C, C10). HREIMS m/z 361.0907 [M $^{+}$] (calc. for $C_{17}H_{19}N_3O_2S_2$ 361.0919).

6.4.5. 2-[2-(Ethylsulfanyl)benzoyl]-*N*-(3,4,5-trimethoxyphenyl)hydrazinecarbothioamide (3.7)

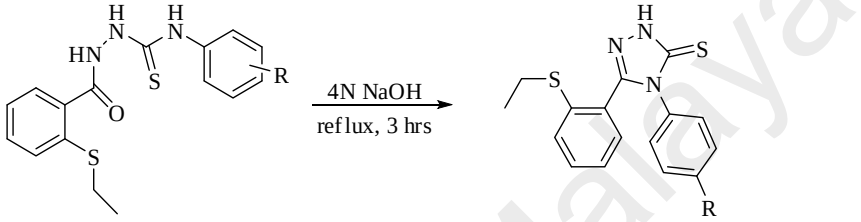


3.7

Colorless solid (0.54 g, yield: 93%), m.p. 156-158 ^{0}C, IR. (KBr) (ν, cm^{-1}) 3545, 3355, 3283 (NH st), 2969 (C-H st), 1651 (C=O st), 1594 (C=N st), 1339 (C=S st). ^{1}H NMR (DMSO-d_6, 400 MHz), δ, ppm: 1.21 (t, 3H, CH_3), 2.95 (q, 2H, -CH_2), 3.66-3.76 (s, 9H, -OCH_3), 6.91 (s, 2H, H12), 7.28 (dt, J=7.5, 1H, H6), 7.48 (br.s., 2H, H4, H5), 7.73 (d, J=7.4, 1H, H7), 9.41 (br.s., 1H, NHPh), 9.79 (br.s.; 1H, NHCS), 10.37 (s., 1H, NHCO). ^{13}C NMR (DMSO-d_6, 100 MHz), δ, ppm: 13.68 (1C, CH_3), 26.61 (1C, CH_2), 55.85 (2C,

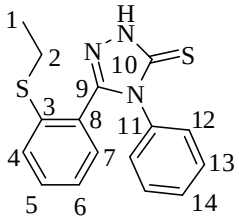
C15, O$\underline{CH_3}$), 60.07 (1C, C16, O$\underline{CH_3}$), 102.25 (2C, C12), 124.90 (1C, C4), 128.05 (1C, C7), 128.66 (1C, C6), 130.84 (1C, C11), 133.88 (1C, C5), 134.67 (1C, C8), 134.79 (1C, C14), 136.30 (1C, C3), 152.33 (2C, C13), 166.96 (1C, C9), 180.47 (1C, C10). HREI/MS m/z 421.1114 [M^+] (calc. for $C_{19}H_{23}N_3O_4S_2$ 421.1130).

6.5. General synthesis of 5-[2-(ethylsulfanyl)phenyl]-4-(4-subsetutedphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (3.8-3.12)



Appropriate substituted thiosemicarbazides (0.01mol) were dissolved in 4N aqueous sodium hydroxide (15 ml) and were refluxed for 3 hrs. After cooling, the mixture was neutralized with 3M hydrochloric acid (5 ml). The precipitate formed was filtered and washed several times with distilled water.

6.5.1. 5-[2-(Ethylsulfanyl)phenyl]-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (3.8)

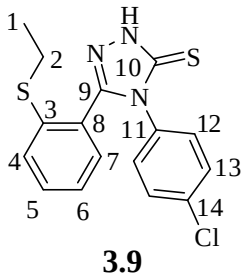


3.8

Colorless solid (0.43 g, yield: 75%), m.p.180 ^{0}C, IR. (KBr) (ν, cm^{-1}) 3260, 3031 (NH st), 2970 (C-H st), 1634 (C=N st), 1596 (C=C st), 1326 (C=S st). ^{1}H NMR (DMSO-d_6, 400 MHz), δ, ppm: 1.1 (t, 3H, CH_3), 2.86 (q, 2H, -CH_2), 7.19 (m, 1H, H14), 7.29 (m, 2H, H12), 7.37 (m, 5H, 2H13, H4, H5, H6), 7.46 (dd, J=7.6, 1H, H7), 14.14 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 100 MHz), δ, ppm: 13.63 (1C, CH_3), 26.52 (1C, CH_2), 125.31 (1C,

C6), 126.06 (1C, C7), 127.94 (1C, C4), 128.07 (2C, C12), 128.66 (2C, C13), 128.97 (1C, C14), 131.19 (1C, C5), 131.65 (1C, C8), 133.66 (1C, C11), 137.65 (1C, C3), 149.82 (1C, C9), 167.64 (1C, C10). HREI/MS m/z 313.0712 [M^+] (calc. for $C_{16}H_{15}N_3S_2$ 313.0707).

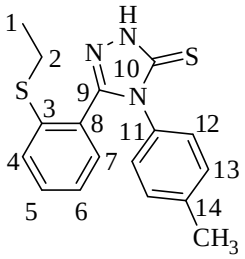
6.5.2. 4-(4-Chlorophenyl)-5-[2-(ethylsulfanyl)phenyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (3.9)



3.9

Colorless solid (0.4 g, yield: 73%), m.p.230-232 ^{0}C, IR. (KBr) (ν, cm^{-1}) 3194 (NH st), 1557 (C=C st), 1245 (C=S st). ^{1}H NMR (DMSO-d_6, 400 MHz), δ, ppm: 1.07 (t, 3H, CH_3), 2.83 (q, 2H, -CH_2), 7.21 (td, J=7.6, 1H, H6), 7.29 (m, 2H, H12), 7.37(d, J=7.5, 1H, H4), 7.43 (m, 4H, 2H, H13, H5, H6), 14.23 (br.s, 1H, NH). ^{13}C NMR (DMSO-d_6, 100 MHz), δ, ppm: 14.01 (1C, CH_3), 26.90 (1C, CH_2), 125.96 (1C, C6), 128.33 (1C, C4), 129.33 (2C, C12), 130.41 (2C, C11, C14), 132.01 (1C, C5), 132.10 (1C, C8), 132.84 (2C, C12), 134.31 (1C, C7), 138.01 (1C, C3), 150.29 (1C, C9), 167.95 (1C, C10). HREI/MS m/z 347.0316 [M^+] (calc. for $C_{16}H_{14}N_3S_2Cl$ 347.0318).

6.5.3. 5-[2-(Ethylsulfanyl)phenyl]-4-(4-methylphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (3.10)

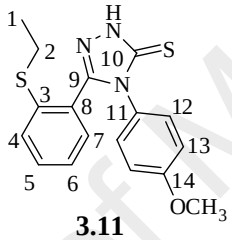


3.10

Colorless solid (0.38 g, yield: 70%), m.p.220 ^{0}C, IR. (KBr) (ν, cm^{-1}) 3091 (NH st), 2968 (C-H st), 1642 (C=N st), 1599 (C=C st), 1329 (C=S st). ^{1}H NMR (DMSO-d_6, 400 MHz), δ, ppm: 1.12 (t, 3H, CH_3), 2.26 (s, 3H, -CH_3), 2.87 (q, 2H, CH_2), 7.16 (s, 4H, 2H, H12,

2H, H13), 7.19 (td, J=7.3, 1H, H6), 7.41 (m, 3H, H4, H5, H7), 14.11 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 100 MHz), δ, ppm : 14.13 (1C, CH_3), 21.09 (1C, CH_3), 27.04 (1C, CH_2), 125.82 (1C, C6), 126.65 (1C, C7), 128.29 (2C, C13), 128.45 (1C, C4), 129.68 (2C, C12), 131.59 (1C, C5), 131.69 (1C, C8), 132.09 (1C, C11), 138.17 (1C, C14), 139.09 (1C, C3), 150.40 (1C, C9), 168.20 (1C, C10). HREI/MS m/z 327.0869 [M^+] (calc. for $C_{17}H_{17}N_3S_2$ 327.0864).

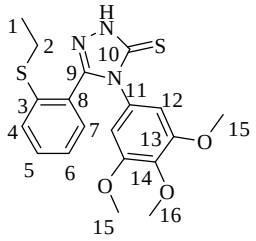
6.5.4. 5-[2-(Ethylsulfanyl)phenyl]-4-(4-methoxyphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (3.11)



3.11

Colorless solid (0.42 g, yield: 75%), m.p.184 ^{0}C, IR. (KBr) (*v*, cm^{-1}) 3328 (NH st), 1607 (C=N st), 1593 (C=C st), 1346 (C=S st). ^{1}H NMR (DMSO-d_6, 400 MHz), δ, ppm: 1.11 (t, 3H, CH_3), 2.87 (q, 2H, -CH_2), 3.71 (s, 3H, -OCH_3), 6.9 (m, 2H, H12), 7.19 (m, 3H, 2H13, 1H, H4), 7.42 (m, 3H, H5, H6, H7) 14.12 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 100 MHz), δ, ppm: 13.63 (1C, CH_3), 26.49 (1C, CH_2), 55.27 (1C, OCH_3), 113.83 (2C, C13), 125.32 (1C, C6), 126.09 (1C, C4), 126.17 (1C, C11), 127.89 (2C, C12), 129.28 (1C, C5), 131.21 (1C, C8), 131.57 (1C, C7), 137.63 (1C, C3), 150.02 (1C, C9), 159.22 (1C, C14), 167.80 (1C, C10). HREI/MS m/z 343.0811 [M^+] (calc. for $C_{17}H_{17}N_3OS_2$ 343.0813).

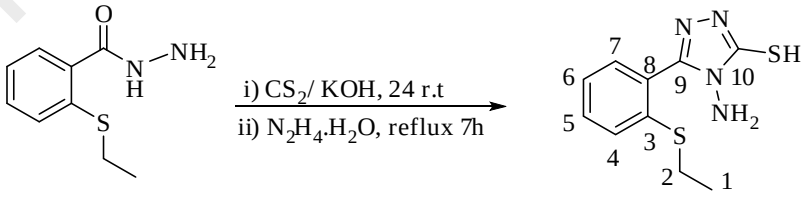
6.5.5. 5-[2-(Ethylsulfanyl)phenyl]-4-(3,4,5-trimethoxyphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (3.12)



3.12

Colorless solid (0.34 g, yield: 69%), m.p.180-182 ^{0}C, IR. (KBr) (ν, cm^{-1}) 3329 (NH st), 1555 (C=C st), 1251 (C=S st). ^{1}H NMR (DMSO-d_6, 400 MHz), δ, ppm: 1.11 (t, 3H, CH_3), 2.89 (q, 2H, -CH_2), 3.34-3.63 (s, 9H, -OCH_3), 6.65 (s, 2H, H12), 7.23 (t, J=7.4, 1H, H6), 7.44 (m, 2H, H4, H5), 7.52 (d, J=7.5, 1H, H7), 14.13 (br.s., 1H, NH). ^{13}C NMR (DMSO-d_6, 100 MHz), δ, ppm: 13.66 (1C, CH_3), 26.43 (1C, CH_2), 55.98 (2C, C15), 59.99 (1C, C16), 105.98 (2C, C12), 125.21 (1C, C6), 126.13 (1C, C7), 127.73 (1C, C4), 129.03 (1C, C11), 131.23 (1C, C5), 131.71 (1C, C8), 137.41 (1C, C16), 137.82 (1C, C3), 149.89 (1C, C8), 152.34 (2C, C13), 167.44 (1C, C10). HREI/MS m/z 403.1030 [M $^{+}$] (calc. for $C_{19}H_{21}N_3O_3S_2$ 403.1024).

6.6. Synthesis of 4-amino-5-[2-(ethylsulfanyl)phenyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (3.13)

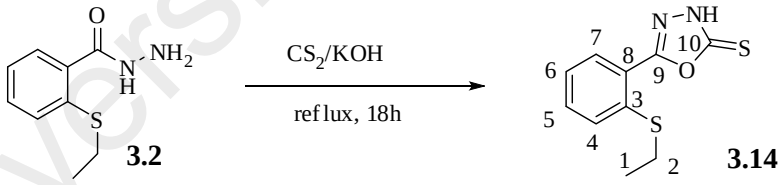


3.2 **3.13**

To a solution of 2-(ethylsulfanyl)benzohydrazide (1 gm, 2 mmole) in absolute ethanol (10ml), carbon disulphide (0.23 gm, 3 mmole) and potassium hydroxide (0.35 g, 2mmol) was added at room temperature. The mixture was stirred for 24 hrs, then dry ether (25ml) was added and the mixture was stirred further for 2 hours. The precipitate was then

filtrated and washed with dry ether. The product was dried at 80 °C to give white solid of potassium 2-(2-(ethylsulfanyl)benzoyl)hydrazine carbodithioate salt. Without purification the salt (0.7 gm, 2 mmol) was dissolved in excess of hydrazine hydrate 80% (10ml). The mixture was heated and refluxed for 7 h, cooled and poured into ice water. The pH was adjusted to 5 by using HCl (10%, 5ml). The precipitate was filtrated, washed with (30ml) water, dried and recrystallized from ethanol to obtain 0.5 gm of pure white crystals, product. Yield: (65%), mp.46 ^{0}C. IR (cm^{-1}): 3354, 3293, 3190 (NH, NH2), 2933 (C-H aliphatic), 1644 (C=N), 1595, 1561 (C=C). ^{1}H NMR (DMSO-d$_6$, 400 MHz), δ, ppm: 1.17 (t, 3H, CH_3), 2.94 (q, 2H, CH_2), 5.47 (br.s, 2H, NH_2), 7.33 (m, 1H, H7), 7.53 (m, 3H, H4, H5, H6). ^{13}C NMR (DMSO-d$_6$, 100 MHz), δ, ppm: 13.74 (1C, CH_3), 26.69 (1C, CH_2), 125.14 (1C, C6), 125.78 (1C, C4), 128.10 (1C, C7), 130.92 (1C, C5), 131.31 (1C, C8), 137.89 (1C, C3), 149.61 (1C, C9), 166.18 (1C, C10). HREIMS m/z: 252.0498 [M^{+}] (calc. for $C_{10}H_{12}N_4S_2$ 252.0503).

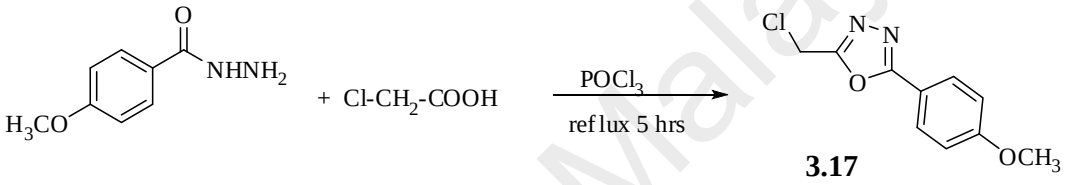
6.7. Synthesis of 5-[2-(ethylsulfanyl)phenyl]-1,3,4-oxadiazole-2(3*H*)-thione (3.14)



Potassium hydroxide (0.21 g, 3.75 mmol) was added to a solution of 2-(ethylsulfanyl)benzohydrazide (1 g, 3.75 mmol) and an excess of carbon disulphide (0.8 g, 0.6 ml) in absolute ethanol (15ml). The mixture was allowed to reflux for 18 hrs and the solvent was evaporated. Distilled water (25 ml) was added to the resulting residue, which was filtered. Hydrochloric acid (5%, 2 ml) was added and the filtered white precipitate was washed with water and recrystallised from ethanol to give pure compound. Fine powder (0.8 g, yield: 84%), m.p. 178-180 °C, IR. (KBr) (*v*, cm^{-1}) 3314, 3219 (br, NH), 3059 (C-H aromatic), 2959 (C-H aliphatic), 1602 (C=N), 1558 (C=C),

1337 (C=S). ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 1.28 (t, 3H, CH_3), 3.06 (q, 2H, CH_2), 7.35 (ddd, 1H, H6), 7.57 (m, 2H, H4, H5), 7.81 (dd, 1H, H7), 14.83 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6, 100 MHz), δ, ppm: 13.27 (1C, CH_3), 25.67 (1C, CH_2), 119.93 (1C, C6), 124.87 (1C, C4), 126.70 (1C, C8), 129.51 (1C, C7), 132.06 (1C, C5), 138.18 (1C, C3), 159.46 (1C, C=N), 176.99 (C=S). HREIMS (ESI): m/z = 237.0155 [M-H] - (calc. for $C_{10}H_9N_2OS_2$ 237.0162).

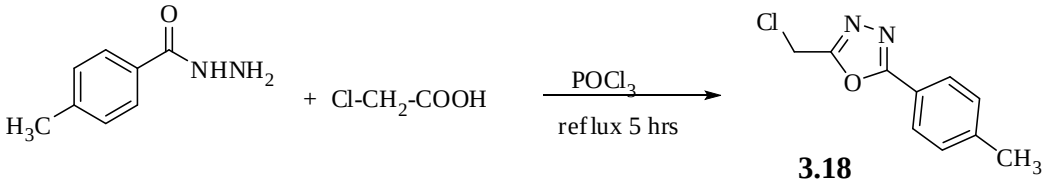
6.8. Synthesis of 2-(chloromethyl)-5-(4-methoxyphenyl)-1,3,4-oxadiazole (3.17)[204]



3.17

4-Methoxybenzohydrazide (1mmol) was dissolved in phosphorus oxychloride (5mL) and chloro-acetic acid (1mmol) was added to the above mixture. The reaction mixture was refluxed for 5 h, cooled to room temperature and poured on to crushed ice. The contents were neutralized with sodium bicarbonate solution (20%, 15ml), a solid mass separated out and was filtered, washed with water and dried. It was then recrystallized from methanol/ethanol. Fine powder (0.45 g, yield: 84%), the ^{1}H NMR and ^{13}C NMR and mass spectral data of the compound were in accordance to the literature.

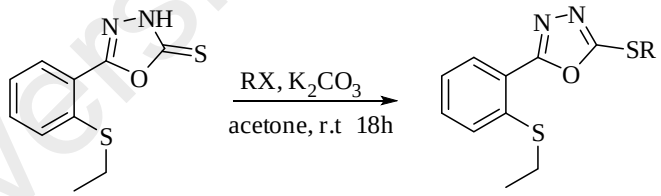
6.9. Synthesis of 2-(chloromethyl)-5-(4-methoxyphenyl)-1,3,4-oxadiazole (3.18)[204]



3.18

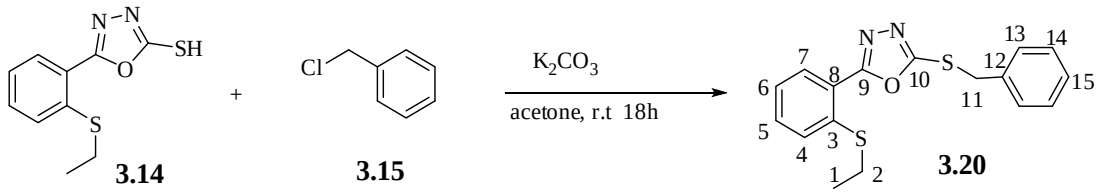
4-Methylbenzohydrazide (1mmol) was dissolved in phosphorus oxychloride (5mL) and chloro-acetic acid (1mmol) was added to the above mixture. The reaction mixture was refluxed for 5 h, cooled to room temperature and poured on to crushed ice. The contents were neutralized with sodium bicarbonate solution (20%, 15ml), a solid mass separated out and was filtered, washed with water and dried. It was then recrystallized from methanol/ethanol. Fine powder (0.4 g, yield: 80%), the ^{1}H NMR and ^{13}C NMR and mass spectral data of the compound were in accordance to the literature.

6.10. General alkylation of 5-(2-(ethylsulfanyl)phenyl]-1,3,4-oxadiazole-2(*3H*)-thione (3.20-3.24)



Substituted benzyl halide (1mmol) was added in small portion to a stirred suspension of 1, 3, 4-oxadiazole (0.31 gm, 1mmol) in dry acetone (20ml) and anhydrous potassium carbonate (0.14gm, 1mmol). The mixture was left overnight with strirring at ambient temperature. After that the solvent was evaporated and the residue was extracted with ethyl acetate (25ml), dried under anhydrous magnesium sulfate, filtrated and left to be crystallized.

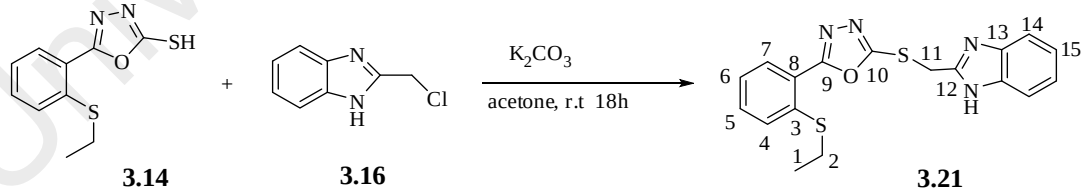
6.10.1. 2-(Benzylsulfanyl)-5-[2-(ethylsulfanyl)phenyl]-1,3,4-oxadiazole (3.20)



3.14 **3.15** **3.20**

The product was recrystallized from methanol to obtained white crystal (0.5gm, yield: 85%) m.p. 79 ^{0}C. IR (KBr) (ν, cm^{-1}) 2996 (C-H aromatic), 2973 (C-H aliphatic), 1593(C=N), 1570 (C=C). ^{1}H NMR (DMSO-d$_6$, 400 MHz), δ, (ppm): 1.26 (t, J=7.3, 3H), 3.03 (q, 2H, CH_2), 4.59 (s, S-CH_2-ph, 2H), 7.32 (m, 4H, 1H, H6, 2H, H14, 1H, H15), 7.53 (m, 4H, 2H, H13, H4, H5), 7.81 (dd, J=7.8, 1.1, 1H, H7). ^{13}C NMR(DMSO-d$_6$, 100 MHz), δ, ppm: 13.33 (1C, CH_3), 25.77 (1C, CH_2), 35.89 (1C, C11), 120.93 (1C, C6), 124.86 (1C, C15), 126.83 (1C, C7), 127.75 (1C, C4), 128.55 (2C, C13), 129.01 (2C, C14), 129.77 (1C, C5) 131.81 (1C, C8), 136.49 (1C, C3), 138.22 (1C, C12), 163.16 and 164.25 (C9, C10, C=N oxadiazole) respectively. HREIMS m/z 328.0701 [M $^{+}$] (calc. for $C_{17}H_{16}N_2O_1S_2$ 328.0704).

6.10.2. 2-[({5-[2-(ethylsulfanyl)phenyl]-1,3,4-oxadiazol-2-yl}sulfanyl)methyl]-1H-benzimidazole (3.21)

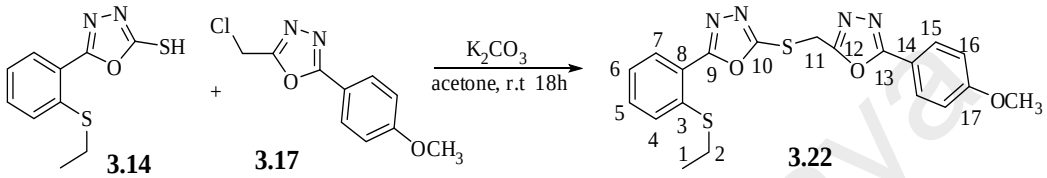


3.14 **3.16** **3.21**

The result mass was recrystallized from ethanol to obtain white crystal (0.3gm, yield: 86%) m.p. 154 °C. IR (KBr) (ν, cm^{-1}) 3203 (br.NH), 2971 (C-H aromatic), 2928 (C-H aliphatic), 1621 (C=N), 1590, 1567 (C=C), 1093 (C-O-C). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.43 (t, J=7.4, 3H, CH_3), 3.07 (q, 2H, CH_2), 4.72 (s, 2H, H11), 7.26 (m, 3H, H5, H13, H14), 7.46 (m, 4H, H3, H4, H12, H15), 7.86 (dd, J=7.9, 1.4, 1H, H6), 11.07 (br.s, 1H, N-H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm: 8.16 (1C, CH_3), 21.51 (1C, CH_2), 24.58

(1C, CH_2, C11), 115.37 (1C, C6), 117.76 (1C, C7), 119.40 (2C, C14, 1C, C4), 121.22 (2C, C15), 124.49 (2C, C8, C5), 126.45 (2C, C13), 134.12 (1C, C3) 144.51 (1C, C12), 160.25 (C9 and C10). HREI/MS m/z 368.0755 [M^+] (calc. for $C_{18}H_{16}N_4O_1S_2$ 368.0766).

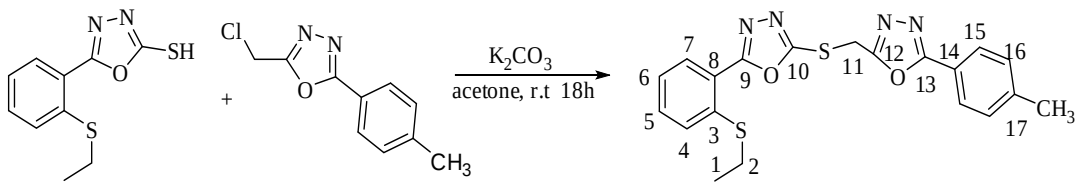
6.10.3. 2-[2-(Ethylsulfanyl)phenyl]-5-({[5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl]methyl}sulfanyl)-1,3,4-oxadiazole (3.22)



3.14 **3.17** **3.22**

White precipitate (0.4gm, yield: 80%) m.p. 106-108 ^{0}C, IR. (KBr) (ν, cm^{-1}) 2976 (C-H aromatic), 2930 (C-H aliphatic), 1610 (C=N), 1905, 1787 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.23 (t, J=7.3, 3H, CH_3), 3.01 (q, 2H, CH_2), 3.85 (s, 3H, OCH_3), 4.49 (s, 2H, CH_2, C11), 7.12 (m, 2H, H16), 7.33 (m, 1H, H6), 7.57 (m, 2H, H5, H4), 7.87 (m, 3H, 2H, H15, 1H, H7). ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm) 13.29 (1C, CH_3), 25.70 (1C, CH_2), 30.64 (1C, CH_2, C11), 55.51 (1C, OCH_3), 114.89 (2C, C16), 115.33 (2C, C15), 120.72 (1C, C14), 124.84 (1C, C6), 126.83 (1C, C7), 128.31 (1C, C4), 129.88 (1C, C5), 131.99 (1C, C8), 138.37 (1C, C3), 161.78 (1C, C17), 162.13 (1C, C9), 162.54 (1C, C13), 164.47 (1C, C12), 164.72 (1C, C10). HREI/MS: m/z = 427.0879 [M + H] $^+$ (calc. for $C_{20}H_{19}N_4O_3S_2$ 427.0899).

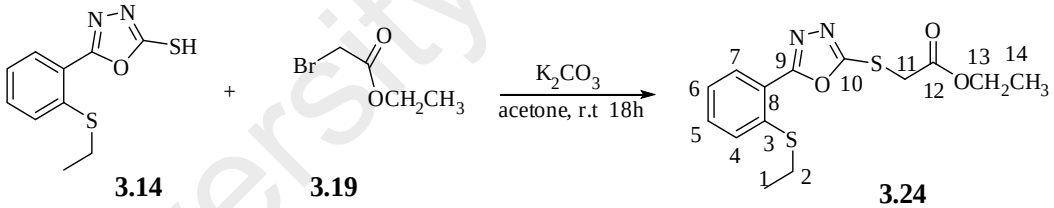
6.10.4. 2-[2-(Ethylsulfanyl)phenyl]-5-({[5-(4-methylphenyl)-1,3,4-oxadiazol-2-yl]methyl}sulfanyl)-1,3,4-oxadiazole (3.23)



3.14 **3.18** **3.23**

The solid product was recrystallized from acetone: water to obtained a white precipitate, (0.39 gm, yield: 78%) m.p.96-98^{0}C, IR. (KBr) (ν, cm^{-1}) 2970 (C-H aromatic), 2927 (C-H aliphatic), 1613 (C=N), 1787 (C=C). ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ ppm: 1.23 (t, J=7.3, 3H, CH_3), 2.39 (s, 3H, $\underline{CH_3}$), 3 (q, 2H, CH_2), 4.95 (s, 2H, CH_2, C11), 7.32 (t, J=7.5, 1H, H6), 7.38 (d, J=8.1, 2H, H4, H5), 7.56 (m, 2H, H16), 7.84 (m, 3H, 2H, H15, 1H, H7). ^{13}C NMR (DMSO-d$_6$, 100 MHz) δ ppm: 13.25 (1C, CH_3), 21.07 (1C, $\underline{CH_3}$), 25.69 (1C, CH_2), 25.84 (1C, CH_2, C11), 120.22 (1C, C14), 120.25 (1C, C6), 120.66 (1C, C7), 124.81 (1C, C4), 126.39-126.78 (2C, C16), 129.85-129.95 (2C, C15), 131.96 (1C, C17), 138.36 (1C, C5), 142.36 (1C, C8), 142.38 (1C, C3), 161.75 (1C, C13), 162.85 (1C, C9), 164.62 (1C, C12), 164.70 (1C, C10). HREIMS m/z 411.0950 [M $^+$] (calc. for $C_{20}H_{19}N_4O_2S_2$ 411.0944).

6.10.5. Ethyl ({5-[2-(ethylsulfanyl)phenyl]-1,3,4-oxadiazol-2-yl}sulfanyl)acetate (3.24)



3.14 **3.19** **3.24**

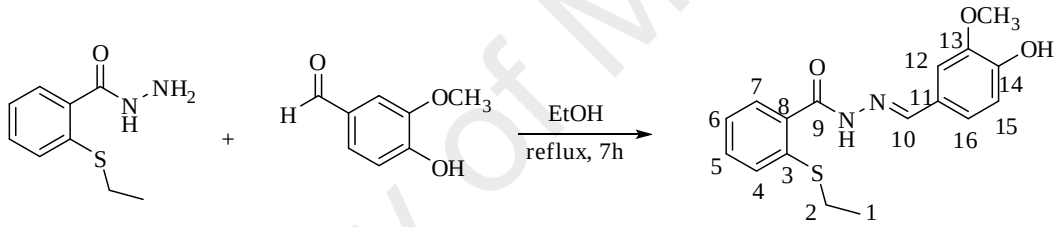
The solid product was recrystallized from ethanol-ethyl acetate (1:2) to obtain a pale yellow precipitate (0.9gm, yield: 78%), m.p. 46 ^{0}C, IR. (KBr) (ν,cm^{-1}) 2981(C-H aromatic), 2931, 2871(C-H aliphatic), 1729 (ester -C=O), 1591(C=N). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.31 (t, 3H, CH_3), 1.39 (t, J= 7.4, 3H, $OCH_2\underline{CH_3}$), 3.02 (q, J=7.3, -$O\underline{CH_2}$), 4.13 (s, S-CH_2), 4.29 (q, 2H, SCH_2), 7.26 (m, 1H, H6) 7.44 (m, 2H, H4, H5) 7.85 (dd; J=7.8, 1H, H7). ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm: 13.45 (1C, CH_3), 14.08 (1C, C14, S-CH3), 26.91 (1C, $S\underline{CH_2}CH_3$), 34.42(1C, C11, CH_2), 62.39 (1C, C13, -OCH_2), 121.64 (1C, C6), 124.69 (1C, C7), 126.93 (1C, C4), 129.81 (1C, C5), 131.38 (1C, C8),

139.07 (1C, C3), 162 (1C, C9), 165.22 (1C, C10), 167.45 (1C, C12). HREIMS (ESI): m/z = 325.0687 $[M + H]^+$ (calc. for $C_{14}H_{17}N_2O_3S_2$ 325.0675).

6.11. General synthesis of 2-(ethylsulfanyl)-N'-(substitutedphenyl)methylidene]benzohydrazide (3.25-3.30)

To a warmed stirred solution of aryl aldehyde 3mmol in 20 ml absolute ethanol 2-(ethylsulfanyl)benzo hydrazide (1 gm, 3mmol) was added in small portion, and refluxed for 7 hours, the mixture was cooled, precipitate was filtered and washed with cold ethanol and recrystallized from ethanol.

6.11.1. Synthesis of 2-(ethylsulfanyl)-N'-(4-hydroxy-3-methoxyphenyl)methylidene]benzohydrazide (3.25)

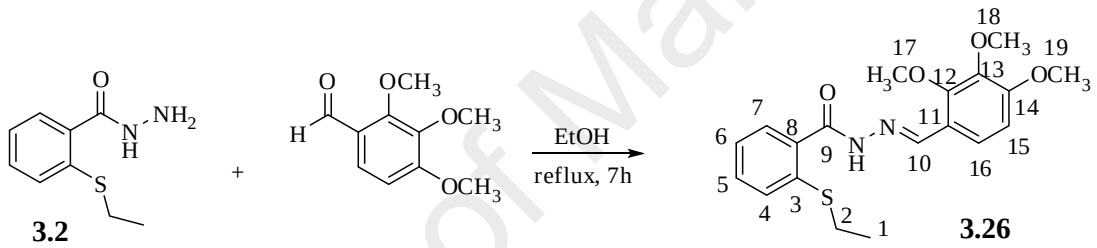


3.2 **3.25**

Using 4-hydroxy-3-methoxybenzaldehyde, colourless solid (0.9 gm, yield: 90%). m.p. 236-238 ^{0}C. IR (KBr) (v, cm^{-1}) 3545 (OH phenol), 3158 (NH), 2961 (C-H aromatic), 1616 (C=O), 1579 (C=N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.06 [t, 3H, CH_3, (Z isomer)], 1.24 (E isomer), 2.9 [q, 2H, CH_2, (Z isomer)], 2.98 (E isomer), 3.64 [s, 3H ,O$\underline{CH_3}$, (Z isomer)], 3.85 (E), 6.84 (Z) [d, J=7.9, H15, (Z isomer)], 6.86 (E), 7.07 (Z) [d, J=8.1, 1H, H6, (Z isomer)], 7.09 (E), 7.29 (Z) [m, H12, H4, (Z isomer)], 7.32 (E), 7.46 [m, 3H, H16, H5, H7, (Z isomer)], 7.48 (E), 7.90 [s, 1H, (CH=N-), (Z isomer)], 8.18 (E), 9.39 [s, 1H, OH, (Z isomer)], 9.55 (E), 11.58 (E) [s, 1H, NH, (E isomer)], 11.64 (Z isomer). ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 13.83 [1C, CH_3, (E isomer)], 14.31 (Z isomer), 26.29 [1C, CH_2, (E isomer)], 27.34 (Z isomer), 55.20 [1C, OCH_3, (Z isomer)], 55.53 (E isomer), 108.94 (E) [1C, C12, (E isomer)], 109.58 (Z), 115.41 [1C, C15, (E and Z isomers)], 120.65 (E) [1C, C16, (E isomer)], 122.09 (Z), 125.07 (E) [1C, C4, (E

isomer)], 125.51 (Z), 125.61 (E) [1C, C6, (E and Z isomers)], 127.68 (E) [1C, C7, (E isomer)], 127.90 (Z), 127.98 (E) [1C, C11, (E isomer)], 129.17 (Z), 129.46 (E) [1C, C5, (E isomer)], 130.27 (Z), 133.46 [1C, C8, (E isomer)], 135.65 (Z), 135.95 (E) [1C, C3, (E isomer)], 138.42 (Z), 143.18 (Z) [1C, C=N, C10, (Z isomer)], 147.89 (E isomer), 147.66 [1C, C14, (Z isomer)], 148.02 (E isomer), 148.41 (E) [1C, C13, (E isomer)], 148.96 (Z), 163.67 [1C, C=O, C9, (E isomer)], 169.93 (Z isomer). HREIMS m/z: 330.1035 [M^+] (calc. for $C_{17}H_{18}N_2O_3S_1$ 330.1036).

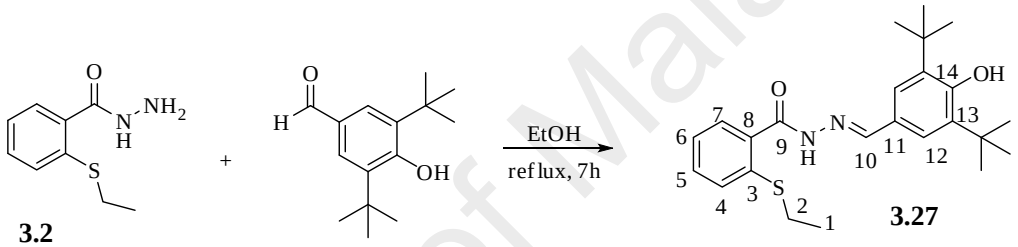
6.11.2. Synthesis of 2-(ethylsulfanyl)-N'-(2,3,4-trimethoxyphenyl)methylidene]benzohydrazide (3.26)



Using 2, 3, 4-trimethoxybenzaldehyde, colorless solid (0.9 gm, yield: 90%). m.p. 182 ^{0}C. IR (KBr) (v, cm^{-1}) 3158(NH), 2969 (C-H aromatic), 1635 (C=O), 1594 (C=N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.12 (Z) [t, 3H, CH_3, (Z isomer)], 1.22 (E isomer), 2.87 (Z) [q, 2H, CH_2, (Z isomer)], 2.99 (E isomer), 3.73 (Z) [s, 9H ,O$\underline{CH_3}$, (Z isomer)], 3.86 (E isomer), 6.98 (E) [m, 1H, H15, (E and Z isomers)], 7.28 (E isomer) [m, 1H, H16, (E and Z isomers)], 7.45 (E isomer) [m, 3H, H4, H5, H6, (E and Z isomers)], 7.93 (E isomer) [d, J=8.8, 1H, H7, (E and Z isomers)], 8.2 [s, 1H, (CH=N-), (Z isomer)], 8.48 (E isomer), 11.68 [s, 1H, NH, (E and Z isomers)]. ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 14.27 (E) [1C, CH_3, (E isomer)], 14.77 (Z isomer), 26.68 (E) [1C, CH_2, (E isomer)], 27.81 (Z isomer), 56.45 (E) [1C, C19, OCH_3, (E and Z isomers)], 60.96 (E) [1C, C18, OCH_3, (E and Z isomers)], 62.29 (E) [1C, C17, OCH_3, (E and Z isomers)], 109.23 (E) [1C, C15, E and Z isomers)], 120.61 (E) [1C, C11, (E isomer)], 120.75 (Z isomer), 121.04 (E) [1C, C16, (E and Z isomers)], 125.46 (E) [1C, C4, (E isomer)], 126.10 (Z isomer), 128.07 [1C,

C7, (E isomer)], 128.28 (Z isomer), 128.44 (E) [1C, C6, (E and Z isomers)], 129.78 [1C, C5, (E isomer)], 130.89 (Z isomer), 136.00 (E) [1C, C8, (E and Z isomers)], 136.33 (E) [1C, C3, (E and Z isomers), 141.99 (E) [1C, C13, (E and Z isomers)], 143.66 (E) [1C, C=N, C10, (E and Z isomers)], 153.12 (E) [1C, C12, (E and Z isomers)], 155.64 (E) [1C, C14, (E and Z isomers)], 164.20 (E) [1C, C=O, C9, (E and Z isomers)]. HREIMS m/z: 374.1299 [M^+] (calc. for $C_{19}H_{23}N_2O_4S_1$ 374.1300).

6.11.3. Synthesis of 2-(ethylsulfanyl)-N'-(3,5-di-tert-butyl-4-hydroxyphenyl)methylidene]benzohydrazide (3.27)

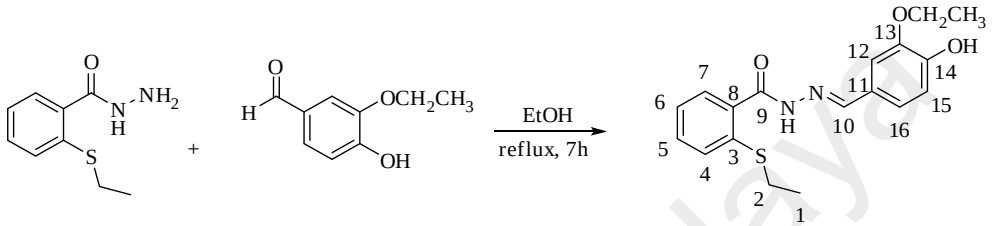


3.2 **3.27**

Using 3, 5-di-*tert*-butyl-4-hydroxybenzaldehyde, colorless solid (0.9 gm, yield: 90%). m.p. 212 ^{0}C. IR (KBr) (v, cm^{-1}) 3630 (OH phenol), 3180 (NH), 2952 (C-H aromatic), 1639 (C=O), 1605 (C=N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.09 (Z) [t, 3H, $SCH_2\underline{CH_3}$)], 1.23 (E), 1.29 (Z) [s, 18H, 2× *t*-Bu, Z isomer)], 2.86 (Z) [q, 2H, CH_2, (Z isomer)], 2.92 (E), 7.17 (E) [s, 1H, OH, (E and Z isomers)], 7.28 (E) [m, 1H, H6, (E and Z isomers)], 7.43 [m, 5H, H4, H5, H12, H7, (E and Z isomers)], 7.88 (Z) [s, 1H, -CH=N, (Z isomer)], 8.19 (E), 11.55 (E) [s, 1H, NH, (E isomer)], 11.63 (Z isomer). ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 13.82 [1C, CH_3, (E isomer)], 14.4 (Z), 34.37 (E) [1C, CH_2, (E isomer)], 34.45 (Z), 34.49 [6C, 2×-$C(CH_3)_3$, E and Z isomers)], 39.03 [2C, 2×-$C(CH_3)_3$, (E and Z isomers)], 123.41 (Z) [2C, C12, (Z isomer)], 123.88 (E), 125.1 (E) [1C, C11, (E isomer)], 125.69 (Z), 127.58 (Z) [1C, C4, (Z isomer)], 127.89 (E), 129.01 (E) [1C, C7, (E isomer)], 129.59 (Z), 130.29 (E) [1C, C6, (E and Z isomers)], 133.31 (E) [1C, C5, (E and Z isomers)], 135.65 (E) [1C, C8, (E and Z isomers)], 135.91 (E) [1C, C3,

(E and Z isomers)], 138.76 (E) (2C, C13, (E isomer)], 139.21 (Z), 143.47 (E) [1C, C=N, C10, (E isomer)], 148.78 (Z), 155.51 [1C, C14, (Z isomer)], 156.13 (E), 163.20 (E) [1C, C=O, C9, (E isomer)], 170.10 (Z). HREIMS m/z: 412.2175 [M^+] (calc. for $C_{24}H_{32}N_2O_2S_1$ 412.2185).

6.11.4. 2-(Ethylsulfanyl)-N'-(4-hydroxy-3-ethoxyphenyl)methylidene]benzohydrazide (3.28)

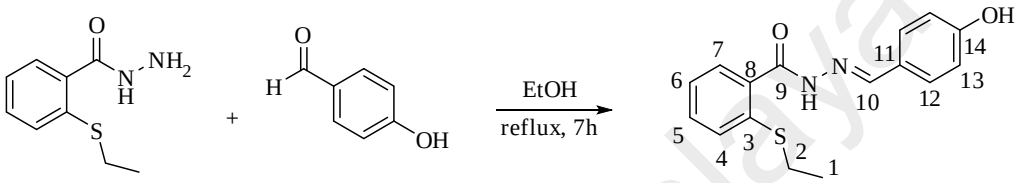


3.2 **3.28**

Prepared from 4-hydroxy-3-ethoxybenzaldehyde, the solid was recrystallized from ethanol: water 5:1 to obtained white solid (0.9 gm, yield: 90%), m.p. 200-202 ^{0}C. IR (KBr) (ν, cm^{-1}), 3174 (OH phenol), 3100 (NH), 2978 (C-H aromatic), 1623 (C=O), 1582 (C=N). ^{1}H NMR (DMSO-d_6, 400MHz) δ (ppm): 1.11 (Z) [t, 3H, $SCH_2\underline{CH_3}$, (Z isomer)], 1.21 (E), 1.25 (t, 3H, $OCH_2\underline{CH_3}$, (Z isomer)], 1.4 (E), 2.88 (Z) [q, 2H ,SCH_2, (Z isomer)], 2.97 (E), 3.84 (Z) [q, 2H, OCH_2, (Z isomer)], 4.09 (E), 6.72 [m, 1H, H15, (Z isomer)], 6.9 (E), 7.06 (E) [d, J=8.1 ,1H, H7, (E and Z isomers)], 7.29 (E) [m, 2H, H6, H16, (E and Z isomers)], 7.45 [m, 3H, H4, H5, H12, E and Z isomers)], 8.14 (Z) [s, 1H, (CH=N-), (Z isomers)], 8.15 (E), 9.42 (Z) [br.s, 1H, OH, (Z isomers)], 9.57 (E), 11.62 (E) [s, (1H) (NH), (E isomer)], 11.66 (Z). ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm): 14.30 (E) [1C,S-$CH_2\underline{CH_3}$, (E isomer)], 15.17 (Z) 26.79 (E) [1C, $OCH_2\underline{CH_3}$), (E and Z isomers)], 27.87 (Z) [1C, SCH_2, (Z isomer)], 31.13 (E), 61.05 (Z) [1C, OCH_2, (Z isomer)], 61.35 (E), 110.79 (E) [1C, C12, (E isomer)], 111.22 (Z), 115.9 [1C, C15, E and Z isomers)], 121.1 [1C, C16, (Z isomer)], 122.5 (E), 125.5 (E) [1C, C4, (E isomer)], 126.0 (Z), 128.1 [1C, C7, (Z isomer)], 128.3 (E), 128.4 [1C, C11, (E and Z isomers)], 129.6 (Z) [1C, C6, (Z isomer)], 129.9 (E), 130.8 [1C, C5, (E and Z isomers)], 136.1 [1C, C8, (Z isomer)],

136.3 (E), 143.7 [1C, C3, (E and Z isomers)], 147.6 [1C, C=N, C10, (E and Z isomers)], 148.4 [1C, C13, (E and Z isomers)], 149.7 [1C, C14, (E and Z isomers)], 164.2 (E) [1C, C=O, C9, (E isomer)], 170.3 (Z isomer). HREIMS m/z: 344.1149 [M^+] (calc. for $C_{18}H_{20}N_2O_3S_1$ 344.1195).

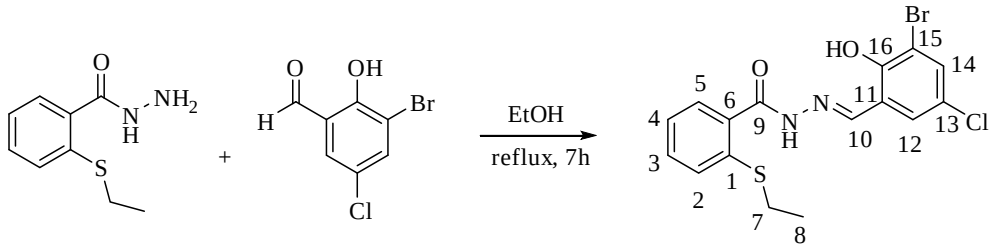
6.11.5. Synthesis of 2-(ethylsulfanyl)-N'-(4-hydroxyphenyl)methylidene]benzohydrazide (3.29)



3.2 **3.29**

Using 4-hydroxybenzaldehyde, colorless solid (0.9 gm, yield: 90%). m.p. 260 ^{0}C. IR (KBr) (v, cm^{-1}) 3690 (OH phenol), 3158 (NH), 2973 (C-H aromatic), 1593 (C=N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.07 (Z) [t, 3H, CH_3, (Z isomer)], 1.23 (E), 2.88 (Z) [q, 2H, CH_2, (Z isomer)], 2.98 (E), 6.71 (Z) [d, 2H , C13, (Z isomer)], 6.85 (E), 7.2 (Z) [d, 2H, H12, (Z isomer)], 7.3 (E), 7.4 (Z) [m, 4H, H4, H5, H6, H7, (Z isomer)], 7.56 (E), 7.9 [s, 1H, -CH=N, (Z isomer)], 8.18 (E), 9.83 [s, 1H, OH, (Z isomer)], 9.96 (E), 11.55 (E) [s, 1H, NH, (E isomer)], 11.57 (Z). ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 13.82 (E) [1C, CH_3, (E isomer)], 14.25 (Z), 26.26 (E) [1C, CH_2, (E isomer)], 27.34 (Z), 115.53 [2C, C13, (E isomer)], 115.69 (Z), 125.04 (E) [2C, C12, (E isomer)], 125.58 (Z), 127.59 (Z) [1C, C11, (Z isomer)], 127.94 (E), 128.24 (Z) [1C, C4, (Z isomer)], 128.83 (E), 129.24 (E) [1C, C7, (E and Z isomers)], 129.54 (Z) (1C, C6, (Z isomer)], 130.28 (E), 133.37 [1C, C5, (E and Z isomers)], 135.65 (Z) [1C, C8, (Z isomer)], 135.88 (E), 138.83 (E) [1C, C3, (E and Z isomers)], 143.62 (Z) [1C, C=N, C10, (Z isomer)], 147.72 (E), 158.98 [1C, C14, (Z isomer)], 159.40 (E), 163.68 [1C, C=O, C9, (E isomer)], 169.84 (Z). HREIMS m/z: 300.0927 [M^+] (calc. for $C_{16}H_{15}N_2O_2S_1$ 300.0932).

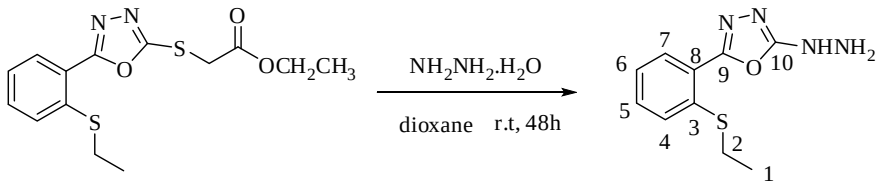
6.11.6. Synthesis of 2-(ethylsulfanyl)-N'-(3-bromo-5-chloro-2-hydroxyphenyl)methylidine]benzohydrazide (3.30)



3.2 **3.30**

Prepared from 3-bromo-5-chloro-2-hydroxybenzaldehyde, the solid was recrystallized from ethanol to obtained yellow crystals (0.9 gm, yield: 90%), m.p. 170 ^{0}C. IR (KBr) (*v*, cm^{-1}) 3302 (OH phenol), 3060 (NH), 2925(C-H aliphatic), 1661(C=O), 1611(C=N). ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ ppm: 1.2 (Z) [t, 3H, CH$_3$, (Z isomer)], 1.23 (E), 2.51 (Z) [q, 2H, CH$_2$, (Z isomer)], 2.97 (E), 7.3 (Z) [m, 1H , H12, (Z isomer)], 7.32 (E), 7.5 (Z) [m, 1H, H2, (Z isomer)], 7.53 (E), 7.55 (E) [m, 1H, H14, (E and Z isomers)], 7.6 (E) [d, 1H, H4, (E and Z isomers)], 7.65 (E) [m, 1H, H3, (E and Z isomers)], 7.72 (E) [d, 1H, H5, (E and Z isomers)], 8.18 (Z) [s, 1H, -CH=N, (Z isomer)], 8.42 (E), 10.41 (Z) [s, 1H, OH, (Z isomer)], 12.23 (E), 12.43 (E) [s, 1H, NH, (E isomer)], 12.55 (Z). ^{13}C NMR (DMSO-d$_6$, 100 MHz) δ ppm: 13.72 (E) [1C, CH$_3$, (E isomer)], 14.11 (Z), 26.22 (E) [1C, CH$_2$, (E isomer)], 27.04 (Z), 110.73 [1C, C15, (Z isomer)], 110.83 (E), 120.32 (Z) [1C, C11, (Z isomer)], 123.31 (E), 125.03 (E) [1C, C2, (E and Z isomers)], 126.15 (E) [1C, C13, (E and Z isomers)], 127.91 (E) [1C, C12, (E and Z isomers)], 128.17 (Z) [1C, C5, (E and Z isomers)], 129.23 (z) [1C, C4, (Z isomer)], 129.46 (E), 130.9 (Z) [1C, C3, (Z isomer)], 132.77 (E), 133.05 (E) [1C, C6, (E and Z isomers)], 134.01 (E) [1C, C14, (E and Z isomers)], 136.37 (E) [1C, C1, (E and Z isomers)], 147.01 (E) [1C, C=N, C10, (E and Z isomers)], 152.04 (Z) [1C, C16, (Z isomer)], 153.20 (E), 163.87 (E) [1C, C=O, C9, (E isomer)], 169.73 (Z). HREIMS m/z: 411.9660 [M^{+}] (calc. for $C_{16}H_{14}N_2O_2BrClS_1$ 411.9648).

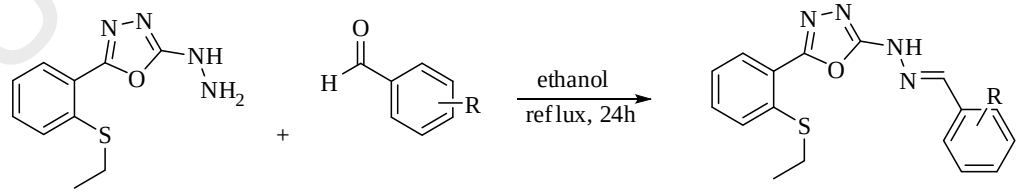
6.12. Synthesis of 2-[2-(ethylsulfanyl)phenyl]-5-hydrazinyl-1,3,4-oxadiazole (3.31)



3.24 **3.31**

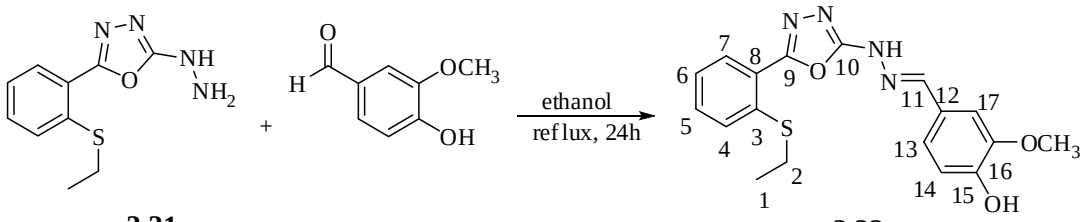
A solution of hydrazine hydrate (80%, 4 ml) was added to ethyl ({5-[2-(ethylsulfanyl)phenyl]-1,3,4-oxadiazol-2-yl}sulfanyl)acetate (2 g, 5 mmol) in dioxane (25 ml) and the mixture was left to stir for 48 hours at room temperature. The solvent was evaporated under reduced pressure resulting in the formation of a pure white solid (1.38 gm, yield: 69%). m.p. 86 ^{0}C. IR (KBr) (ν, cm^{-1}) 3490, 3328, 3020, 3081 (NH_2, NH), 2975 (C-H aliphatic), 1662, 1642 (C=N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm 1.26 (t, J=7.3, 3H, CH_3), 3.01 (q, 2H, CH_2), 4.51 (br.s, 2H, NH_2), 7.3 (m, 1H, H4), 7.49 (m, 2H, H5, H6), 7.68 (d, J=7.7, 1H, H7), 8.62 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 13.21 (1C, CH_3), 25.59 (1C, CH_2), 121.53 (1C, C6), 124.78 (1C, C7), 126.41 (1C, C4), 128.93 (1C, C5), 130.99 (1C, C8), 137.25 (1C, C3), 157.17 (1C, C9) 165.72 (1C, C10). HREIMS: m/z = 236.0733 [M^+] (calc. for $C_{10}H_{12}N_4O_1S_1$ 236.0732).

6.13. General synthesis of 2-[2-substitutehydrazinyl]-5-[2-(ethylsulfanyl)phenyl]-1,3,4-oxadiazole (3.32-3.37)



The appropriate substituted hydroxybenzaldehyde (1 mmol) was added in small portions while stirring to a solution of 2-[2-(ethylsulfanyl)phenyl]-5-hydrazinyl-1,3,4-oxadiazole (0.25 g, 1 mmol) in absolute ethanol (15 ml), and the mixture was refluxed for 6 hrs. The mixture was cooled and the precipitate formed was washed and recrystallised from a suitable solvent.

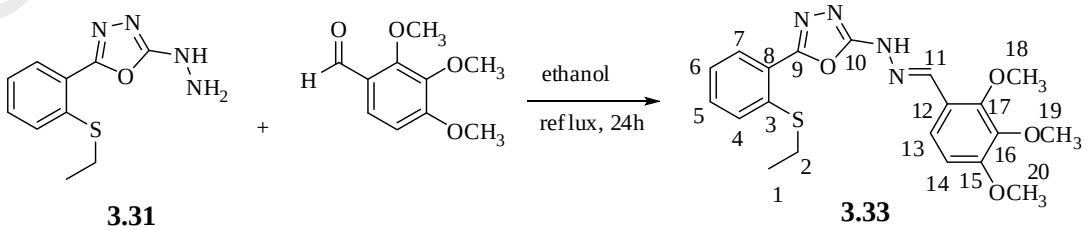
6.13.1. Synthesis of 4-[(2-{5-[2-(ethylsulfanyl)phenyl]-1,3,4-oxadiazol-2-yl}hydrazinylidene)methyl]-2-methoxyphenol (3.32)



3.31 **3.32**

Using 4-hydroxy-3-methoxybenzaldehyde, colourless solid (0.2 gm, yield: 85%). m.p. 100-102 ^{0}C. IR (KBr) (ν, cm^{-1}) 3380 (O-H), 2951 (C-H aliphatic), 1631 (C=N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.28 (t, 3H, CH_3), 3.04 (q, J=7.3, 2H, CH_2), 3.82 (s, 3H ,O$\underline{CH_3}$), 6.83 (d; 1H, H14), 7.1 (dd, 1H, H6), 7.32 (m, 2H, H5, H13),7.52 (m, 2H, H4, H17), 7.79 (d, J=7.7, 1H, H7) 8.05 (s, 1H, CH=N), 9.51 (s, 1H, OH), 11.89 (s, 1H, NH) . ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 18.58 (1C, CH_3), 30.98 (1C, CH_2), 60.76 (1C, OCH_3), 114.36 (1C, C17), 120.68 (1C, C14), 126.44 (1C, C13), 127.07 (1C, C6), 130.14 (1C, C7), 130.76 (1C, C4) 132.01 (1C, C12), 134.35 (1C, C5), 136.27 (1C, C8), 142.70 (1C, C3), 150.79 (1C, C11), 153.17 (1C, C16), 153.72 (1C,C15), 162.73 (1C, C9), 166.65 (1C, C10). HREIMS m/z: 370.1086 [M^{+}] (calc. for $C_{18}H_{18}N_4O_3S_1$ 370.1100).

6.13.2. Synthesis of 2-[2-(2,3,4-trimethoxybenzylidene)hydrazinyl]-5-[2-(ethylsulfanyl)phenyl]-1,3,4-oxadiazole (3.33)

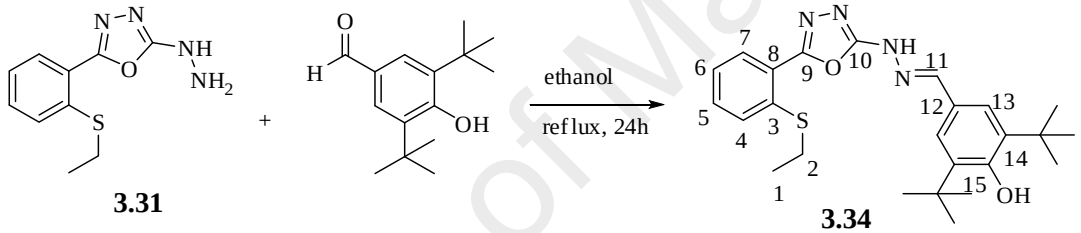


3.31 **3.33**

Using 2, 3, 4-trimethoxybenzaldehyde, colourless solid (0.22, gm, yield: 88%). m.p. 194 ^{0}C. IR (KBr) (ν, cm^{-1}) 2928 (C-H aromatic), 1657 (C=N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.28 (t, J=7.3, 3H, CH_3), 3.04 (q, 2H, CH_2), 3.78 (s, 3H ,O$\underline{CH_3}$), 3.85 (s,

6H ,O$\underline{CH_3}$), 6.92 (d, J=8.9, 1H, H15), 7.34 (m, 1H, H16), 7.54 (m, 3H, H4, H5, H6), 7.78 (d, J=7.5, 1H, H7), 8.33 (s, 1H, CH=N-) 11.99 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 13.3 (1C, CH_3), 25.75 (1C, CH_2), 55.93 (1C, C20), 60.48 (1C, C19), 61.77 (1C, C18), 108.69 (1C, C12), 120.08 (1C, C14), 120.42 (1C, C13), 121.72 (1C, C4) 124.9 (1C, C7), 126.76 (1C, C6), 129.12 (1C, C5), 131.10 (1C, C8), 137.48 (1C, C3), 140.77 (1C, C11), 141.47 (1C,C16), 152.09 (1C, C17), 154.82 (1C, C15), 157.56 (1C, C9), 161.29 (1C, C10). HREIMS m/z: 414.1371 [M^+] (calc. for $C_{20}H_{22}N_4O_4S_1$ 414.1362).

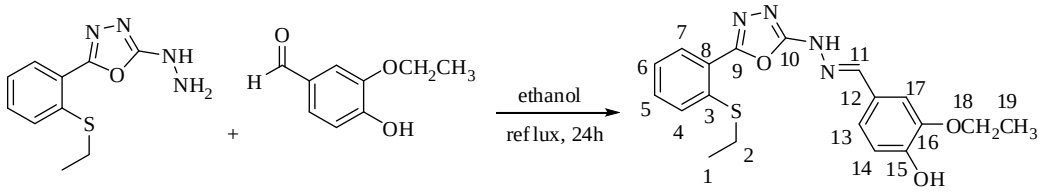
6.13.3. Synthesis of 2,6-di-*tert*-butyl-4-[(2-{5-[2-(ethylsulfanyl)phenyl)-1,3,4-oxadiazol-2-yl)hydrazinylidene)methyl]phenol (3.34)



3.31 **3.34**

Using 3, 5-di-*tert*-butyl-4-hydroxybenzaldehyde, colourless solid, (0.21 gm, yield: 86%). m.p 216 ^{0}C. IR (KBr) (*v*, cm^{-1}) 3630 (OH phenol), 3313, 3214 (NH), 2956 (C-H aromatic), 2868 (C-H aliphatic), 1643, 1616 (C=N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.28 (t, 3H, $SCH_2\underline{CH_3}$), 1.41 (s, 18H, 2× *t*-Bu), 3.04 (q, J=7.4, 2H, CH_2), 7.35 (m, 2H, H phenolic, H4), 7.46 (s, 2H, H13), 7.53 (d, J=3.8, 2H, H5, H6), 7.77 (d, J=7.7, 1H, H7) 8.08 (s, 1H, -CH=N), 11.86 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 13.42 (1C,CH_3), 25.77 (1C, CH_2), 30.11 (6C, 2× -$C(CH_3)_3$), 34.4 (2C, 2× -$C(CH_3)_3$, 122.04 (2C, C13), 123.38 (1C, C12), 124.92 (1C, C6), 125.47 (1C, C7), 126.91 (1C, C4), 129.05 (1C, C5), 130.97 (1C, C8), 137.34 (1C, C14), 139.24 (1C, C3), 146.04 (1C, C=N, C11), 155.37 (1C, C15), 157.53 (1C, C=O, C9), 161.49 (1C, C=N, C10). HREIMS m/z: 452.2239 [M^+] (calc. for $C_{25}H_{32}N_4O_2S_1$ 452.2246).

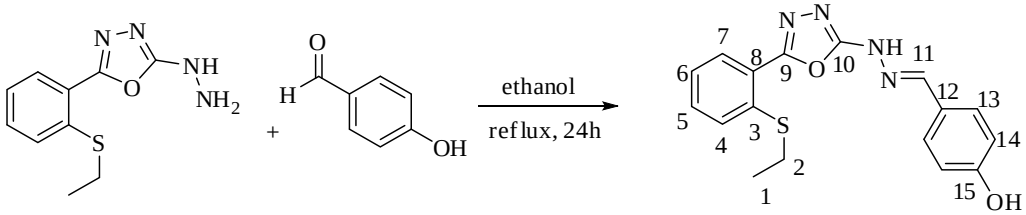
6.13.4. Synthesis of 2-ethoxy-4-[(2-{5-[2-(ethylsulfanyl)phenyl]-1,3,4-oxadiazol-2-yl}hydrazinylidene)methyl]phenol (3.35)



3.31 **3.35**

Using 4-hydroxy-3-ethoxybenzaldehyde, colorless solid (0.2 gm, yield: 89%). m.p. 204 ^{0}C. IR (KBr) (v, cm^{-1}) 3545 (OH phenol), 2980 (NH), 2931 (C-H aromatic), 2870 (C-H aliphatic), 1634 (C=N). ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ ppm: 1.28 (t, J=7.3, 3H, $SCH_2\underline{CH_3}$), 1.36 (t, J=7, 3H, $OCH_2\underline{CH_3}$), 3.04 (q, J=7.3, 2H, $S\underline{CH_2}$), 4.07 (q, J=6.9, 2H, OCH_2), 6.84 (d; J=8.2, 1H, H14), 7.09 (dd, J=8.2, 1H, H6), 7.25 (d, J=1.8, 1H, H5), 7.34 (m, 1H, H13) 7.54 (m, 1H, H4, H17), 7.78 (d, 1H, H7) 8.04 (s, 1H, CH=N), 9.42 (s, 1H, OH), 11.88 (s, 1H, NH). ^{13}C NMR (DMSO-d$_6$, 100 MHz) δ ppm: 13.29 (1C, $SCH_2\underline{CH_3}$), 14.47 (1C, $OCH_2\underline{CH_3}$) 25.77 (1C, SCH_2), 63.92 (1C, OCH_2), 110.27 (1C, C17), 115.45 (1C, C14), 121.33 (1C, C13), 121.65 (1C, C6), 124.97 (1C, C7), 125.49 (1C, C4) 126.80 (1C, C12), 129.18 (1C, C5), 131.21 (1C, C8), 137.37 (1C, C3), 145.93 (1C, C11), 147.01 (1C, C16), 148.57 (1C,C15), 157.57 (1C, C9), 161.40 (1C, C10). HREIMS m/z: 384.1257 [M^{+}] (calc. for $C_{19}H_{20}N_4O_3S_1$ 384.1256).

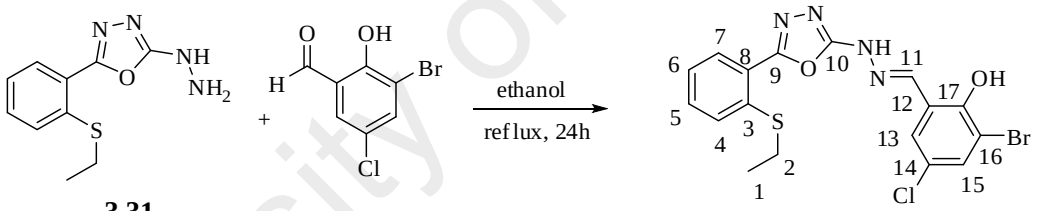
6.13.5. Synthesis of 4-[(2-{5-[2-(ethylsulfanyl)phenyl]-1,3,4-oxadiazol-2-yl}hydrazinylidene)methyl]phenol (3.36)



3.31 **3.36**

Using 4-hydroxybenzaldehyde, colourless solid (0.2 gm, yield: 90%). m.p. 198-200 ^{0}C. IR (KBr) (ν, cm^{-1}) 3676 (OH phenol), 3322 (NH), 2981 (C-H aromatic), 1633 (C=N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.29 (t, J=7.3, 3H, CH_3), 3.04 (q, 2H, CH_2), 6.84 (m, 2H, H14), 7.34 (m, 1H, H6), 7.53 (m, 4H, H4, H5, H13),7.79 (d, J=7.4, 1H, H7), 8.06 (s, 1H, CH=N), 9.9 (s, 1H, OH), 11.88 (s, 1H, NH) . ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 13.84 (1C, CH_3), 26.19 (1C, CH_2), 116.15 (2C, C14), 122.37 (1C, C6), 125.30 (1C, C12), 125.62 (1C, C7), 127.19 (1C, C4), 128.82 (2C, C13) 129.50 (1C, C5), 131.36 (1C, C8), 138.04 (1C, C3), 145.55 (1C, C11), 157.89 (1C, C15), 159.52 (1C, C9), 161.94 (1C, C10). HREIMS: m/z = 340.0992 [M + H] $^{+}$ (calc. for $C_{17}H_{16}N_4O_2S_1$ 340.0994).

6.13.6. Synthesis of 2-bromo-4-chloro-6-[(2-{5-[2-(ethylsulfanyl)phenyl]-1,3,4-oxadiazol-2-yl}hydrazinylidene)methyl]phenol (3.37)



3.31 **3.37**

Using 3-bromo-5-chloro-2-hydroxybenzaldehyde, colourless solid (0.19 gm, yield: 88%). m.p. 202-204 ^{0}C. IR (KBr) (ν, cm^{-1}) 3545(OH phenol), 2949 (C-H aromatic), 1632 (C=N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.3 (t, J=7.3, 3H, CH_3), 3.06 (q, 2H, CH_2), 7.36 (ddd, J=7.9, 1H, H15), 7.55 (m, 2H, H5, H6), 7.69 (dd, J=9.2, 2H, H4, H13), 7.8 (m, 1H, H7), 8.34 (br.s, 1H, CH=N), 11.46 (br.s, 1H, OH), 12.69 (s, 1H, NH) . ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 13.32 (1C, CH_3), 25.75 (1C, CH_2), 111.02 (1C, C16), 121.19 (1C, C12), 121.52 (1C, C6), 123.62 (1C, C7), 124.87 (1C, C4), 126.80 (1C, C14) 128.22 (1C, C13), 129.10 (1C, C5), 131.22 (1C, C8), 132.52 (1C, C15), 137.71 (1C, C3), 144.09 (1C, C11), 152.36 (1C, C17), 158.01 (1C, C9), 160.56 (1C, C10). HREIMS m/z: 451.9707 [M^{+}] (calc. for $C_{17}H_{14}N_4BrClO_2S_1$ 451.9709).

6.14. Biological Activity Studies

6.14.1. Antioxidant Activities

6.14.1.1. DPPH Free Radical Scavenging Activity

The determination of the radical-scavenging activity of the compounds was performed as reported.[4] The 100 µM solution of DPPH (195 µL) in 96% ethanol was added to the tested sample solution (5 µL) in ethanol and mixed in a 96-well plate. Test compounds were allowed to react with the stable free radical 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) for 3 h at 37 °C. After incubation, a decrease in absorption was measured at 515 nm using a spectrophotometer. The percent radical-scavenging activity was calculated using the following equation:

DPPH radical scavenged (%) = [OD Blank − OD Sample]/[OD Blank] × 100%

where the OD blank is the absorbance of the control DPPH solution, and the OD sample is the tested compound absorbance. The IC_{50} (compound concentration required to reduce the absorbance of the DPPH control solution by 50%) value was then calculated.

6.14.1.2. Ferric Reducing Antioxidant Power (FRAP) Assay

The reducing capacities of the prepared compounds were measured by the method of Benzie and Strain with modification.[20] First, 10 mL of acetate buffer (300 mM) was adjusted to pH 3.6 by mixing 3.1 g $CH_3COONa{\cdot}3H_2O$ and 16 mL glacial acetic acid. Next, a TPTZ solution was prepared by dissolving 10 mM TPTZ in 40 mM HCl. Then, 1 mL of the (2,4,6-tripyridyl-s-triazine) TPTZ solution was mixed with the FRAP solution, and 1 mL of ferric chloride hexahydrate (20 mM) in a distilled water. The FRAP solution was warmed to 37 °C, the tested compound was added to it, and the mixture was left to react in the dark. The absorbance was monitored spectrophotometrically at 593 nm. The results were expressed in µM ferrous/g dry mass and compared to those for the reference compounds.

6.14.2. Cytotoxicity assay

Cytotoxicity of the compounds was evaluated against 3 types of cancer cell lines, namely pancreas (BxPC-3), non-metastatic (MCF-7) and metastatic breast (MDA-MB-231) as well as a normal pancreas cell line (hTERT-HPNE). Cell lines were cultured in DMEM media supplemented with 2 mM L-glutamine, 10% FBS, 50 µg/ml gentamycin and 2.5 µg/ml amphotericin B; maintained in a 37 ºC humidified atmosphere of 5% CO_2 cell incubator. Study Compounds and positive control (curcumin) were dissolved in DMSO and further diluted with DMEM media to yield a final DMSO concentration of less than 0.5% v/v. Cells were plated into 96-well microplates at 10^4 cells per well and maintained in the cell incubator for 24 hour. Then, 100µL of samples were introduced in triplicates to a final concentration range of 400 – 1.56 µM. As for the negative control, cells were treated with vehicle (0.5% v/v DMSO) only. Cells were further incubated for 72 hours and at the end of the period, cell viability was determined. Culture media were carefully refreshed with 100µL of HBSS, followed by 50µL per well of MTT reagent (2 mg/ml). Microplates were returned to the incubator for 2 hours, after which the supernatant was removed and the formazan crystals dissolved in 100µL DMSO. The absorbance of the formazan product was read on a microplate reader at 570 nm with 620 nm as the background wavelength (Infinite 200, Tecan, Männedorf, Swizerland).[205]

6.14.3. Acute Toxicity Test

The acute toxic study was used to determine a safe dose for synthesized compound. This study was carried out in adult female albino rats by the "fix dose" method of OECD (2002) (Organization for Economic Co-operation and Development) Guideline No.420.[206] The fixed dose method with a starting dose of 500 mg/kg body weight, was adopted. The animals were fasted overnight and next day the product (suspended in 10% tween 80 solution) was administered orally at a dose level 500 mg/kg. Then the animals were observed continuously for 3 hours for general behavioral, neurological, and

autonomic profiles and then every 30 minutes for next 3 hours and finally for mortality after 24 hours till 14 days.[207] The animals were sacrificed on the 15th day after overnight fasting. Histology and serum biochemical parameters were determined.[192,199]

6.14.4. Antiulcer Test

The rats were divided randomly into 4 groups of 6 rats each. Each rat that weighed between 200-225 g was placed individually in a separate cage (one rat per cage) with wide-mesh wire bottoms to prevent coprophagia during the experiment. The animals were maintained on standard pellet diet and tap water. The study was approved by the Ethics Committee for Animal Experimentation, Faculty of Medicine, University of Malaya, Malaysia. Throughout the experiments, all animals received human care according to the criteria outlined in the "Guide for the Care and Use of laboratory Animals" prepared by the National Academy of Sciences and published by the National Institute of Health.

The rats fasted for 24 hours before the experiment but were allowed free access to drinking water up till 2 hours before the experiment.[208] Gastric ulcer was induced by orogastric intubation of absolute ethanol (5 ml/kg).[203] Ulcer control groups were orally administered vehicle (10%Tween 20, 5ml/kg). The reference group received oral doses of 20 mg/kg omeprazole in CMC (5 ml/kg) as positive control. Experimental groups were orally administered of compound 3.25 in 10%Tween 20 (5 ml/kg) at doses of 50 and 100 mg/kg. One hour after this pretreatment all groups of rats were administered orally with absolute ethanol (5 ml/kg) in order to induce gastric ulcers. The rats were euthanized 60 minutes later,[209] under an overdose of xylazin and ketamine anesthesia and their stomachs were immediately excised.

6.14.5. Morphological changes and acute gastric lesions evaluation

Ulcers of the gastric mucosa appear as elongated bands of haemorrhagic lesions parallel to the long axis of the stomach. Gastric mucosa of each rat was thus examined for damage. The length and width of the ulcer (mm) were measured by a planimeter (10 × 10 mm^2 = ulcer area) under dissecting microscope (1.8x). The ulcerated area was measured by counting the number of small squares, 2 mm × 2 mm, covering the length and width of each ulcer band. The sum of the areas of all lesions for each stomach was applied in the calculation of the ulcer area (UA) where in the sum of small squares × 4 × 1.8 = UA (mm^2). The inhibition percentage (I.0 %) was calculated by the following formula (I%) = $[(UA_{control} - UA_{treated}) \div UA_{control}] \times 100\%$.[187]

6.14.6. Histological evaluation of gastric lesions

Specimens of the gastric walls of each rat were fixed in 10% buffered formalin and processed in a paraffin tissue processing machine. Sections of the stomach were made at a thickness of 5 µm and stained with Hematoxyline and eosin for histological evaluation. Additionally, to evaluate mucus production, some slides were also stained by periodic acid Schiff Base (PAS) following the manufacture instruction (Sigma Periodic Acid-Schiff -PAS Kit). For further analysis, other slides underwent immunohistochemistry (IHC) staining using Dako ARK™ to observed immunhistochemical localization of HSP-70 (1:100) and Bax (1:50) proteins. Both proteins were purchased from Santa Cruz Biotechnology, Inc., California, USA.

6.14.7. Measurement of mucus production

Gastric mucus production was measured in the rats that were subjected to absolute ethanol-induced gastric lesions. The gastric mucosa of each rat was obtained by gentle scraping the mucosa with a glass slide and the collected mucus were weighed by using a precision electronic balance.[210]

6.14.8. Measurement of acid content of gastric juice (pH)

Samples of gastric contents were analyzed for hydrogen ion concentration by pH metric titration with 0.1 N NaOH solutions using digital pH meter.[210]

6.14.9. Statistical analysis

All values were reported as mean $\pm$ S.E.M. The statistical significance of differences between groups was assessed using one-way ANOVA. A value of $p<0.05$ was considered significant.

6.15. Computational Studies

All computations were performed using the GAUSSIAN 09W software package.[211] ChemSketch and GaussView visualisation were used to present the images in the figures. The optimisation structures were calculated by the B3LYP/6-311G (d, p) method.[212, 213] Our calculation includes the frontier orbital HOMO and LUMO energies, BDE on each NH site, and the spin-density distribution for the radicals formed after H-removal. The conformer with the lowest electronic energy was used for calculation. The haemolytic BDE values were calculated by the following relationship, using the standard-state enthalpies at 1 atm and 298.15K:

$$BDE = H_{radical} + H_H - H_{molecule} \quad (1)$$

where $H_{radical}$ is the total enthalpy of the free radical, H_H is the gas-phase total enthalpy of the hydrogen atom, and $H_{molecule}$ is the total enthalpy of the parent molecule.

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PUBLICATIONS

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Oral Presentations

1. Oral presentation for International Conference on Ionic Liquids **2013** Langkawi, Malaysia; 11th to 13th of December, **2013**.