SYNTHESIS, X-RAY STRUCTURE CHARACTERIZATION AND ANTIOXIDANT ACTIVITIES OF SOME CHALCONE DERIVATIVES CONTAINING 2-CHLOROTHIOPHENE MOIETY

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by

NG WENG ZHUN

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LIST OF ABBREVIATIONS

3-D	Three-Dimensional
$CuSO_4 \cdot 5H_2O$	Copper Sulfate Pentahydrate
C_4H_4S	Thiophene
DNA	Deoxyribonucleic Acid
FT-IR	Fourier Transform Infrared
NMR	Nuclear Magnetic Resonance
DPPH	Diphenyl-2-picrylhydrazyl
NO	Nitric Oxide
FIC	Ferrous Ion Chelating
H_2O_2	Hydrogen Peroxide
CCD	Charge-Coupled Device
R	Residual-factor
GooF/S	Goodness of Fit
TMS	Tetramethylsilane
I	Characteristic Spin
δ	Chemical Shift
Ppm	Parts per Million
^1H	Proton
^{13}C	Carbon-13
J	Coupling Constant
PBS	Phosphate Buffer Saline
Fe^{2+}	Ferrous ions

OH•	Hydroxyl Radical
UV	Ultraviolet
$CDCl_3$	Deuterated chloroform
ATR	Attenuated Total Reflection
MHz	Mega Hertz
SMART	Siemens Molecular Analysis Research Tools
$\bar{\upsilon}$	Wavenumber
GUI	Graphical User Interface
BIS	Bruker Instrument Service
SAINT	SAX Area-detector Integration (SAX-Siemens Analytical X-ray)
SADABS	Siemens Area Detector Absorption Correction
2-D	Two-Dimensional
vdW	Van der Waals
µg/mL	Microgram per Milliliter
mg/mL	Milligram per Millilitre
EDTA	Ethylenediaminetetraacetic Acid
$FeCl_2$	Ferrous Chloride
wR	Weighted Reliability Index
ORTEP	Oak Ridge Thermal Ellipsoid Plot
IC_{50}	Half Maximal Inhibitory Concentration
DFT	Density Functional Theory
NLO	Nonlinear Optics

SINTESIS, PENCIRIAN STRUKTUR SINAR-X DAN KAJIAN ANTIOKSIDAN TERHADAP BEBERAPA TERBITAN KALKON MENGANDUNGI 2-KLOROTHIOPHENE

ABSTRAK

Dalam projek ini, satu siri yang terdiri daripada dua belas sebatian heterosilik kalkon (**1a**-**1l**) yang mengandungi 2-klorothiophene telah disintesis dan dihaburkan melalui penyejatan secara perlahan. Semua struktur hablur telah dicirikan dengan analisis spektroskopi FTIR, NMR, dan seterusnya ditentukan dengan teknik kristalografi sinar-X hablur tunggal. Selain itu, penilaian kegiatan aktiviti antioksidan bagi semua sebatian telah dijalankan dengan empat ujian termasuk 2, 2-difenil-1-picrylhydrazyl (DPPH), ferrous ion chelating (FIC), nitrik oksida (NO) and hidrogen peroxide (H_2O_2). Sembilan sebatian telah dicirikan dalam kumpulan ruang monoklinik $P2_1/c$ ($P2_1/n$), *Cc* dan *Pc*. Dua sebatian telah dicirikan dalam kumpulan ruang triklinik $P\bar{1}$ dan satu dalam ortorombik *Pbca*. Semua sebatin kalkon yang membina daripada dua fenil yang disambung dengan pengambung C=C−C(=O)−C dan membentuk konformasi trans dari ikatan berganda (C=C). Majoriti sebatian adalah penghubung hampir mendatar kecuali sebatian (*E*)-1-(5-klorothiophen-2-yl)-3-mesitilprop-2-en-1-one (**1e**) dan (*E*)-3-(5'-bromo-[2,2'-bithiophen]-5-yl)-1-(5-klorothiophen-2-yl)prop-2-en-1-one (**1g).** Sebatian (*E*)-1-(5-klorothiophen-2-yl)-3-(thiophen-2-yl)prop-2-en-1-one (**1b**) dan (*E*)-1-(5-klorothiophen-2-yl)-3-(4-(trifluorometil)fenil)prop-2-en-1-one (**1h**) yang mengandungi struktur bercelaru. Semua sebatian mempamerkan kedua-dua ikatan hidrogen konvensional dan bukan konvensional kecuali sebatian (*E*)-1-(5-klorothiophen-2-yl)-3-(4-(metilthio)fenil)prop-2-en-1-one (**1d**) dan (*E*)-1-(5-klorothiophen-2-yl)-3-(4-(trifluorometoksi)fenil)prop-2-en-1-one (**1i**). Berdasarkan

keputusan penghambatan antioksidan, sebatian (*E*)-1-(5-klorothiophen-2-yl)-3-(piridin-2-yl)prop-2-en-1-one (**1a**) dan (*E*)-1-(5-klorothiophen-2-yl)-3-(4-(metilthio)fenil)prop-2-en-1-one (**1d**) aktif terhadap ujian FIC. Manakala, sebatian (*E*)-1-(5-klorothiophen-2-yl)-3-(4-(metilthio)fenil)prop-2-en-1-one (**1d**) menunjukkan kekuatan kelat yang lebih tinggi daripada asam etilenadiaminatetraasetat (ubat standard).

SYNTHESIS, X-RAY STRUCTURE CHARACTERIZATION AND ANTIOXIDANT ACTIVITIES OF SOME CHALCONE DERIVATIVES CONTAINING 2-CHLOROTHIOPHENE MOIETY

ABSTRACT

A total of twelve heterocyclic chalcone compounds (**1a-1l**) containing 2-chlorothiophene moieties were synthesized using Claisen Schmidt Condensation process and were crystallized by slow evaporation methods. The postulate structure of these heterocyclic chalcone compounds were elucidated by FT-IR and NMR spectral analyses, followed by single crystal X-ray diffraction technique. Besides, the antioxidant potential of all compounds was evaluated using four different types of antioxidant assay which include 2, 2-diphenyl-1-picrylhydrazyl (DPPH), ferrous ion chelating (FIC), nitric oxide (NO) and Hydrogen Peroxide (H_2O_2) radical scavenging assay. Nine compounds are crystallized in monoclinic space group with four compounds in $P2_1/c$, three compounds in $P2_1/n$ and two compounds in *Cc* and *Pc*. Another, two compounds are crystallized in triclinic space group $P\bar{1}$ and one in orthorhombic space group *Pbca*. All the chalcone molecules are constructed by two individual rings which are interconnected by a C=C−C(=O)−C enone bridge and form a *trans* configuration with respect to the C=C double bond. The majority of the compounds is adopted to a nearly-planar conformation except compound (*E*)-1-(5-chlorothiophen-2-yl)-3-mesitylprop-2-en-1-one (**1e**) and (*E*)-3-(5'-bromo-[2,2'-bithiophen]-5-yl)-1-(5-chlorothiophen-2-yl)prop-2-en-1-one (**1g**). Two molecular structures, (*E*)-1-(5-chlorothiophen-2-yl)-3-(thiophen-2-yl)prop-2-en-1-one (**1b**) and (*E*)-1-(5-chlorothiophen-2-yl)-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-one **(1h)** are disordered structure. All compounds exhibit both conventional and non-conventional

hydrogen bonds except compounds (*E*)-1-(5-chlorothiophen-2-yl)-3-(4-(methylthio)phenyl)prop-2-en-1-one (**1d**) and (*E*)-1-(5-chlorothiophen-2-yl)-3-(4-(trifluoromethoxy)phenyl)prop-2-en-1-0one (**1i**). Based on the antioxidant inhibition results, it shows that compounds (*E*)-1-(5-chlorothiophen-2-yl)-3 (pyridin-2-yl)prop-2-en-1-one (**1a**) and (*E*)-1-(5-chlorothiophen-2-yl)-3-(4-(methylthio)phenyl)prop-2-en-1-one (**1d**) are active toward FIC assay. Meanwhile, compound (*E*)-1-(5-chlorothiophen-2-yl)-3-(4-(methylthio)phenyl)prop-2-en-1-one (**1d**) shows higher chelating power than the standard drugs (EDTA) in IC_{50} test.

INTRODUCTION

1.1 X-ray Crystallography

In general, crystals are formed from basis such as atoms, molecules and ions that fit into lattice, the three-dimensional repeating patterns. The word of "crystallography" in science refer to determination of the arrangement of atoms in a crystalline solid. With the invention of X-ray, the inner structure of crystalline material which includes types and positions of all atoms can be analysed through X-ray diffraction technique. The main purpose of using X-ray is due to its wavelength is comparable to the size of atoms and it possesses high energy that enable it to penetrate the matter. This X-ray crystallography technique helps the scientist to investigate the structures and therefore to understand and study the behaviour of the matters. Modification of structures is widely done in the fields of chemistry, pharmacology, molecular biology, materials science and mineralogy to enhance the properties of the structure.

X-ray crystallography has been using for more than a hundred year. In 1895, Wilhelm Roentgen, a German professor of physics, who is the first person to discover electromagnetic radiation in a short wavelength range which known as X-rays. He was then honoured with the first Nobel Prize in Physics in 1901 for his remarkable achievement in discovery of X-rays (Wilhelm Röntgen, 2010). In 1912, another German physicist, Max von Laue, continues to work on the development of X-ray crystallography. He postulated that atoms in a single crystal have a regular and periodic pattern with interatomic distances in the order of about 1 Å. He also made an assumption that structure of a single crystal can be discovered using diffraction patterns of X-rays, much like a gradient in an infrared spectrometer that can diffract

infrared light. This statement was then confirmed by two remarkable researchers, Walter Friedrich and Paul Knipping, who successfully photographed the diffraction pattern associated with the X-ray radiation of crystalline structure, $CuSO_4 \cdot 5H_2O$. A few years later in north England, the British physicist Sir William Henry Bragg and his son William Lawrence Bragg were jointly awarded Nobel Prize in Physics in year 1915 due to their prestige contribution in development of X-ray crystallography. They discovered that diffracted X-rays can be utilized to detect the arrangement of atoms inside a crystal through the famous simple mathematical equation, Bragg's law (*Bragg & William Lawrence*, 2009). This discovery gets the science of X-ray crystallography started. The X-ray crystallographic technique was then refined by the blooming of theories and mathematic equations such as, structure factor, Fourier synthesis, direct methods, Patterson method etc. In recent year, X-ray crystallographic technique is widely employed in characterizing the structure of new compounds.

Spectroscopy is a technique to study the interactions occur between the matter and the electromagnetic radiation. It enables researcher to characterize the studied compounds through combination of different spectroscopy tools. Therefore, the invention of spectroscopy tools has enhanced the development of organic chemistry and they are widely used in the determination of the structure of organic compounds. Two types of spectroscopy tools are used in this research project to characterize the molecular structure which include Fourier transform infrared (FT-IR) spectroscopy and the nuclear magnetic resonance (NMR) spectroscopy (proton and carbon-13).

Antioxidant is related to free radical in human body cell. Free radicals is an unstable atom that it shells is not fully filled up by electron and this atom may bond with another atom to complete the shell to reach stable state. One of the examples is

oxygen molecules in human body split into unpaired electrons (free radicals), and bond with other atoms or molecules. This continuous reaction process is oxidative stress, which can damage human body cells and tend to a range of diseases such as central nervous system diseases, cardiovascular diseases and cancer. To reduce the free radicals in human body, antioxidant is needed to prevent the oxidation of other molecules. To prevent this, antioxidant will donate an electron to free radicals and reducing their reactivity to others atoms or molecules (V. Lobo *et. al*., 2010). To develop the new antioxidant agent, there are many standardized analytic methods such as 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, nitric oxide (NO) scavenging assay, ferrous ion (FIC) chelating assay and hydrogen peroxide (H_2O_2) radical scavenging assay.

The chalcone derivatives containing 2-chlorothiophene moiety which consist of aromatic (thiophene) ketone and an enone that forms the central core for a variety of important biological compounds, which are known collectively as chalcones or chalconoids. The thiophene is a heterocyclic compound that consists of a planar five members ring bearing the formula C_4H_4S with four carbon atoms and one sulphur atom. Thiophene can be obtained through the isolation of natural material such as coal tar and petroleum (National Centre for Biotechnology Information, 2019). Nowadays, thiophene can be prepared commercially from the chemical process by using butane and sulphur dioxide. On the other hand, chalcone can be prepared by an aldol condensation between benzaldehyde and acetophenone in the presence of sodium hydroxide as a catalyst.

1.2 Background and Problem Statement of the Research

Nowadays, several lifestyle, stress and environment factors will boost the number of free radicals in human body and cause oxidative stress which can potentially damage body DNA, trigger heart disease and other diseases (V. Lobo *et. al*., 2010). To prevent free radical exceed antioxidants, the development of better antioxidant agents is still carried on by worldwide researchers (Kurutas, 2016). Heterocyclic chalcone is one of the well-known compounds which have attracted the attentions of scientists who work in the field of pharmaceuticals especially antioxidant assay (Wang *et. al*., 2018). The antioxidant abilities of these heterocyclic chalcone compounds can be improved through the modification of structures and compounds to offer a higher degree of diversity (Yerragunta *et. al*., 2013). A non-destructive method (X-ray crystallography method) is selected for determining the crystal structures of these modified heterocyclic chalcone compounds because it able to generates three-dimensional structure to provide physical properties of the crystal structure. The physical properties of the crystal packing is directly impact to the polymorphism of the drugs design.

In this research project, twelve new heterocyclic thiophene chalcone compounds are synthesized and crystallized in order to study the interactions between the molecules inside the crystal structures as well as the effect of different substituent groups towards the antioxidant abilities. Hirshfeld surface study serves as a visualisation tool to study the interactions and connections between molecules.

1.3 Research Objectives

There are 3 objectives in this research which include:

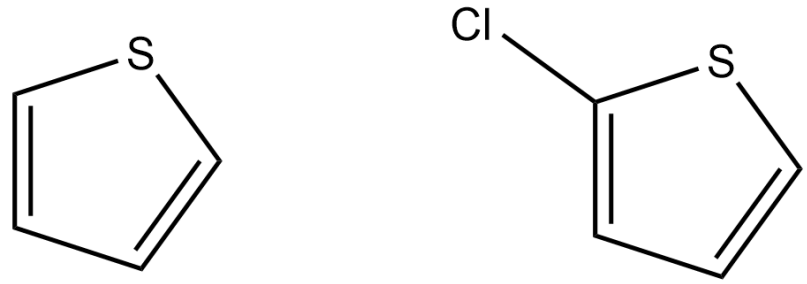
i. To synthesize and characterize the chlorothiophene derivatives using spectroscopic techniques.

ii. To analyze the three-dimensional crystal structures using X-ray crystallography method and Hirshfeld surfaces analysis with fingerprint plots.

iii. To assess the antioxidant potential on these novel chlorothiophene chalcones.

Literature Review

2.1 Thiophene and Chlorothiophene



(a) **(b)**

Figure 2.1 The chemical structures of (a) thiophene and (b) chlorothiophene.

Thiophene and its substituted derivatives are very important class of heterocyclic compound that consists of a planar five members ring bearing the formula C_4H_4S with four carbon atoms and one sulphur atom (Figure 2.1a) which showed interesting applications in the field of medicinal chemistry. It has made an indispensable anchor for medicinal chemists to produce combinatorial library and carry out exhaustive efforts in the search of lead molecules. It has been reported to possess a wide range of therapeutic properties with diverse applications in medicinal chemistry and material science, attracting great interests in industry as well as academia (Shah & Verma, 2018). Thiophene can be obtained through the isolation of natural material such as coal tar and petroleum (National Centre for Biotechnology Information, 2019). Nowadays, thiophene can be prepared commercially from the chemical process by using butane and sulphur dioxide. The sulphur element in the thiophene has been well recognized as an antifungal agent through many studies (Richard *et. al*., 2004). Besides, thiophenes are widely used as building block in many agrochemicals and pharmaceuticals (Chu *et. al.*, 2018; Modzelewska *et. al.*, 2006; Debrashi Kar *et. al.*, 2017; Lahtchev *et. al.*, 2008 and Gopi *et. al.*, 2016). Besides, the

sulphur element in the thiophene containing heterocyclic scaffold, has emerged as one of the relatively well-explored scaffold for the development of library of molecules having potential anticancer profile. Thiophene analogs have been reported to bind with a wide range of cancer-specific protein targets, depending on the nature and position of substitutions (Archna *et. al.*, 2020). Furthermore, thiophene moiety has been recognized as a toxicophore because of the potential of oxidative bio-activation leading to electrophilic species. The introduction of bulky or electron-withdrawing groups at the α-carbon to the sulphur atom has the potential to reduce or eliminate bio-activation (Chen *et. al*., 2011).

On the other hand, the synthesis of chlorothiophene (Figure 1.1b) from the substitution of chlorine atom into the thiophene ring enhances the pharmaceutical properties such as anti-inflammatory (Revannasiddaiah, *et. al.*, 2014), analgesic activity (Surendra & Muni, 2003) and antioxidant agent (Chidan *et. al*., 2013). The pharmacological potential of chlorothiophene in the medicinal chemistry encourages the scientists to study on its derivatives. Besides, chlorothiophene-amides exhibit a strong anti-thrombotic effect which may use for the therapy and prophylaxis of cardio-vascular disorders like thromboembolic diseases or restenosis (Steinhagen *et. al*., 2009). Furthermore, the derivatives of chlorothiophene chalcone is one of the fascinating material which exhibits high nonlinear optical coefficient and good crystallizability (Ganapayya *et. al.*, 2012). Therefore, the synthesis and characterization of novel thiophene moieties with wider therapeutic activity is a topic of interest for the medicinal chemist to synthesize and investigate new structural prototypes with more effective pharmacological activity.

2.2 Chalcone

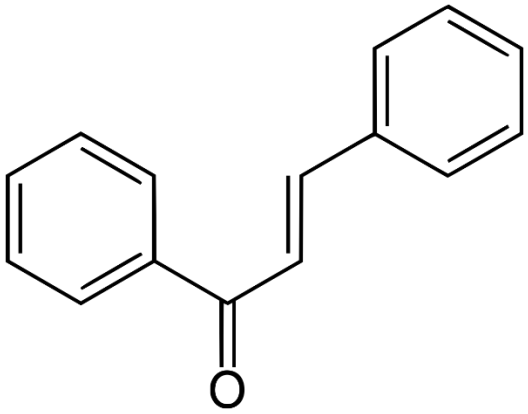


Figure 2.2 The chemical structure of chalcone

Chalcones are a group of plant-derived polyphenolic compounds belonging to the flavonoids family and which construct by aromatic ketones and enones that forms the central core for a variety of important biological compounds, which are known collectively as chalcones or chalconoids. Their physicochemical properties seem to define the extent of their biological activity. One of the physicochemical properties of chalcones is anticancer effects. Although parent chalcones consist of two aromatic rings joined by a three-carbon α,β-unsaturated carbonyl system, various synthetic compounds possessing heterocyclic rings like pyrazole and indole are well known and proved to be effective anticancer agents. (Valavanidis & Vlachogianni, 2013). Chalcone as one of the member of flavonoids, they are considered as open-chain flavonoids found abundantly in edible plants. Many heterocycles of biological importance, such as pyrazolines, flavones, 1,4-diketones and benzothiazepine can be synthesized using chalcones as key precursors. The two rings A and B which are linked by α, β-unsaturated carbonyl system are the unique features of chalcones as antidiabetic drugs (Raut *et. al*., 2016).

Detsi *et. al*. (2020) had synthesized a series of 2-hydroxychalcones and evaluated their inhibitory activity against soybean lipoxygenase. Lipoxygenases are iron-containing enzymes widely distributed in plant and animals. They catalyse the oxidation of polyunsaturated fatty acids such as linoleic acid (plant) and arachidonic acid (mammals) at specific positions to hydroperoxides. Lipoxygenase inhibitors are of interest due to the implication of the enzyme to various pathophysiological conditions. The majority of lipoxygenase inhibitors are antioxidants or free radical scavengers, since lipoxygenation occurs via a carbon centered radical and therefore these compounds can inhibit radical formation or trap it once formed. The lipoxygenase inhibition revealed compounds 119b, 119c and 119j (Figure 2.3) as the most potent having IC_{50} of 52.5, 56 and 53 µM, respectively. This result is comparable with nordihydroguaiacetic acid (IC_{50} of 40 µM) and far better than caffeic acid (IC_{50} of 600 µM).

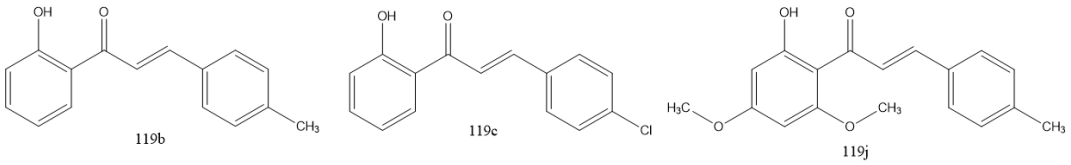


Figure 2.3 Compounds structure of 119b, 119c and 119j.

Kumar *et. al*., (2013) have studied a series of 5-chlorothiophene chalcones and evaluate their in vitro antioxidant activity which includes DPPH Radical Scavenging Assay, ABTS Radical Scavenging Assay, Ferric Reducing Antioxidant Power (FRAP) Assay and Cupric Ion Reducing Antioxidant Capacity (CUPRAC) Assay. The new series of chalcones have been designed (Figure 2.4) and synthesized by the reaction of 2-acetyl-5-chlorothiophene with substituted benzaldehydes in presence of catalytic

amount of NaOH in methanol. The molecular structures of compounds **4a**, **4b**, **4c**, **4d**, **4e** and **4f** with atom numbering schemes is shown in Figure 2.5.

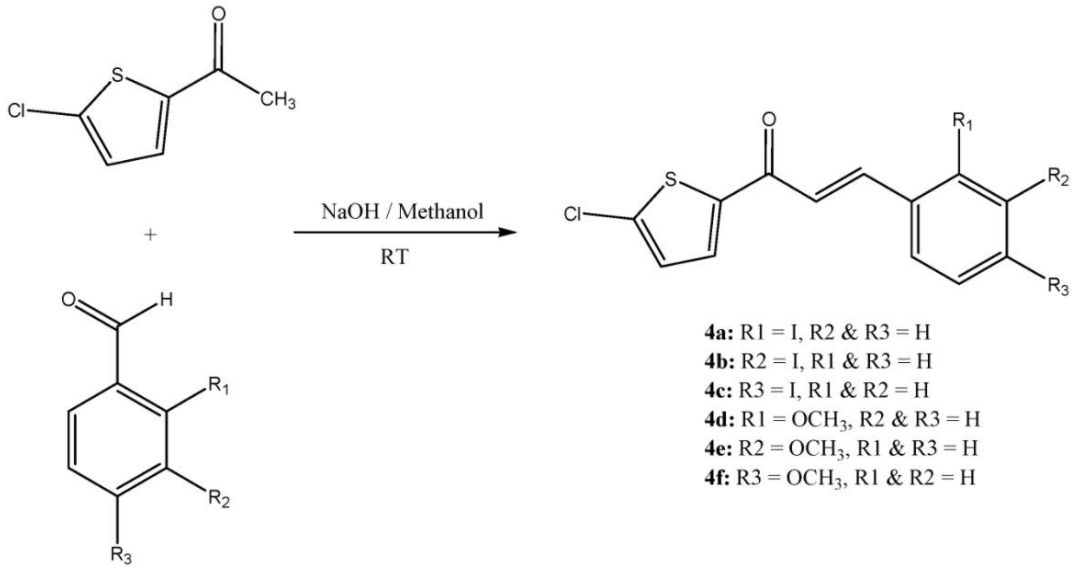


Figure 2.4 The reaction scheme a series of 5-chlorothiophene chalcones.

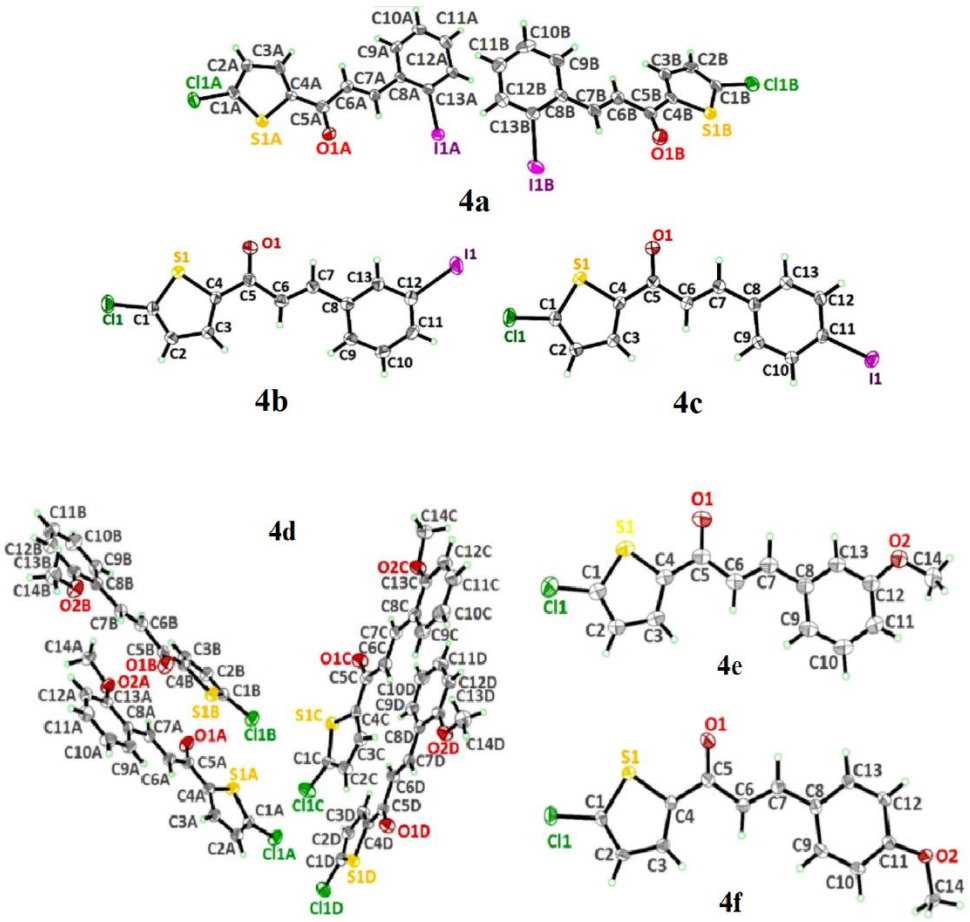


Figure 2.5 Molecular structures of compounds **4a**, **4b**, **4c**, **4d**, **4e** and **4f** with atom numbering schemes.

The report showed the compounds display mild to good antioxidant properties, which is due to the presence of electronegative *–I* and electron donating -OCH_3 substituent at different positions on the phenyl ring. The increasing order of antioxidant activity of the synthesized compounds follows the order **4f** > **4d** > **4e** > **4b** > **4a** > **4c**. The present study revealed that (1) the influence of the nature of the functional linkage (electron withdrawing and electron donating groups) and (2) the position of the substituent on the phenyl ring of 5-chlorothiophene chalcones are crucial for the exhibited antioxidant activities.

2.3 Spectroscopic Studies

A series of heterocyclic chalcones containing halogen thiophene was discuss by Al-Maqtari *et. al.* (2015). The Fourier Transform Infrared (FT-IR) spectroscopy and the Nuclear Magnetic Resonance (NMR) spectroscopy (proton and carbon-13) data for these series of chalcone was shown in Table 2.1. Based on the results, sp^2 aromatic carbon (=C-H) are presence in all compounds with the absorption bands higher than 3000 cm^{-1} (3078 - 3102 cm^{-1}). A very strong band of the carbonyl group (C=O) is shifted to lower frequencies (1634 cm^{-1} - 1645 cm^{-1}) which is due to the conjugation of C=C in the enone bridge. The C=C stretching bands for aromatic rings of the thiophene and benzene rings occur in pairs at the frequency's range of 1512 – 1562 cm^{-1} and 1524 – 1585 cm^{-1}, respectively. Meanwhile, at the low region, a strong C-Cl stretching absorption band for all compounds are observed in the frequency ranges of 1013 - 1020 cm^{-1}.

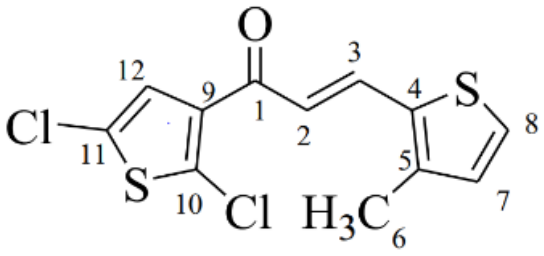


Figure 2.6 The compound **(9)** structure with atomic labelling scheme.

The 1H NMR spectrum exhibited six signals integrating for eight protons (3 for the thiophene rings, 2 for α,β-unsaturated double bond and 3 for methyl protons). The vinylic proton, H-3 at δ 7.98 (1H, d, J = 15.4 Hz) was coupled with H-2 at δ 7.12 (1H, d, J = 15.4 Hz suggested that they are in trans-orientation. The 13C NMR and DEPT NMR spectra of chalcone **(9)** (Figure 2) showed the presence of twelve carbons, consisted of five quaternary (C-4, C-5, C-9, C-10, C-11) and deshielded carbon signal

at δ 183.0ppm was assigned to carbonyl group (C=O) at (C-1).The olefinic carbon C-2 and C-3 were observed at 121.4 and 136.2 ppm, respectively.

Table 2.1 The FT-IR bond values for heterocyclic chalcones containing halogen thiophene.

Type of bond / Compound	Ar-H (cm^{-1})	C=O (cm^{-1})	Ar-C=C (cm^{-1})	C-Cl (cm^{-1})	1H NMR (CDCl3)	13C NMR (CDCl3)
(*E*)-3-(5-Bromothiophen-2-yl)-1-(2,5-dichlorothiophen3-yl)-2-propen-1-one **(8)**	3078	1644	1512, 1579	1020	δ 7.07 (1H, d, J =4 Hz, H-5), δ 7.08 (1H, d, J=15.2 Hz, H-2), δ 7.12 (1H, d, J=4 Hz, H-6), δ 7.19 (1H, s, H-11), δ 7.76 (1H, d, J =15.2 Hz,H-3)	δ 117.2 (C-7), δ 122.6 (C-5), δ127.1 (C-11), δ 127.1 (C-8), δ 131.3 (C-9), δ 131.5 (C-2),δ 132.9 (C-6), δ 136.6 (C-3), δ 137.6 (C-10), δ 141.6(C4), δ 182.8 (C-1)
(*E*)-1-(2,5-Dichlorothiophen-3-yl)-3-(3-methylthiophen2-yl)-2-propen-1-one **(9)**	3102	1640	1562, 1524	-	δ 2.39 (3H, s, H-6), δ 6.92 (1H, d, J = 4.8 Hz, H-7), δ 7.12 (1 H, d, J = 15.4 Hz, H-2), δ 7.2 (1H, s, H12), δ 7.35 (1H, d, J = 4.8 Hz, H-8), δ 7.9 (1H, d, J = 15.4 Hz, H-3)	δ 14.3 (C-6), δ 121.4 (C-2), δ 126.9 (C-9), δ 127.1 (C-12), δ 128.2 (C-8), δ 131.0 (C-7), δ 131.6 (C-5), δ 134.3 (C-10), δ 136.2 (C-3), δ 137.9 (C 11), δ 143.5 (C-4), δ 183.0 (C-1)
(*E*)-1-(5-Chlorothiophen-2-yl)-3-(3-methylthiophen-2-yl)-2-propen-1-one **(10)**	3079	1634	1523, 1572	1013	δ 2.42 (3 H, s, H-6), δ 6.94 (1H, d, J =4.8 Hz, H-11), δ 7.02 (1H, d, J = 4.0 Hz, H-8), δ 7.07 (1H, d, J = 15.2 Hz, H-2), δ 7.35 (1H, d, J = 5.2 Hz, H7), δ 7.64 (1H, d, J = 4.0 Hz, H-10), δ 8.06 (1H, d, J = 15.2 Hz, H-3)	δ 14.3 (C-6), δ 118.2 (C-2), δ 127.6 (C-7, C-8), δ 130.8 (C-10), δ 131.5 (C-11), δ 134.2 (C-3), δ 135.3 (C-5), δ 139.4 (C-12), δ 143.3 (C-4), δ 144.4 (C-9), δ 180.7 (C-1)
(*E*)-3-(5-Bromothiophene-2-yl)-1-(5-chlorothiophen-2-yl)-2-propen-1-one **(11)**	3083	1645	1528, 1585	1014	δ 7.12 (1H, d, J = 4.8 Hz, H-6), δ 7.19 (1H, d, J = 15.2 Hz, H-2), δ 7.21(1H, s, H-11), δ 7.39 (1H, d, J = 3.6 Hz, H-5), δ 7.47 (1H, d, J = 5.2 Hz, H7), δ 7.90 (1H, d, J = 15.2 Hz, H-3)	-

Table 2.1 *Cont.*

Type of bond / Compound	Ar-H (cm^{-1})	C=O (cm^{-1})	Ar-C=C (cm^{-1})	C-Cl (cm^{-1})	1H NMR (CDCl3)	13C NMR (CDCl3)
(*E*)-1-(2,5-Dichlorothiophen-3-yl)-3-(thiophen-2-yl)-2- propen-1-one **(12)**	3091	1643	1525, 1567	1018	δ 7.12 (1H, d, J= 5.0 Hz, H-6), δ 7.19 (1H, d, J = 15.2 Hz, H-2), δ 7.21(1H, s, H-11), δ 7.39 (1H, d, J = 3.6 Hz, H-5), δ 7.47 (1H, d, J = 5.0 Hz, H-7), δ 7.90 (1H, d, J = 15.2 Hz, H-3)	-
(*E*)-1-(5-Chlorothiophen-2-yl)-3-(thiophen-2-yl)-2-propen-1-one **(13)**	3080	1637	1528, 1574	1014	δ7.03 (1H, d, J = 4 Hz, H-5), δ 7.13 (1H, t, J =Hz, H-6), δ 7.134 (1H, d,J = 15.2 Hz, H-2), δ 7.40 (1H, d, J = 3.6 Hz, H-10), δ 7.47 (1H, d, J = 4.8 Hz, H-7), δ 7.65 (1H, d, J = 4.0 Hz, H-9), δ 7.98 (1H, d, J = 15.2 Hz, H-3).	-

Chapter 3

METHODOLOGY

3.1 Introduction

In this research, 12 chalcone compounds were synthesized through Claisen-Schmidt condensation reaction and were recrystallized into single crystals by slow evaporation process. The crystal structures for all the compounds were characterized using several methods such as X-ray crystallography, FT-IR, NMR spectroscopy and Hirshfeld surface analysis. Next, all compounds were evaluated by 4 types of antioxidant assays which include 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, nitric oxide (NO) scavenging assay, ferrous ion (FIC) chelating assay and hydrogen peroxide (H_2O_2) radical scavenging assay.

The research process for this project is classified into five steps which are sample preparation, spectroscopy studies, single crystal analysis, Hirshfeld surface studies and antioxidant analysis (Figure 3.1). Each step of the research activities will be further deliberated in the following sections.

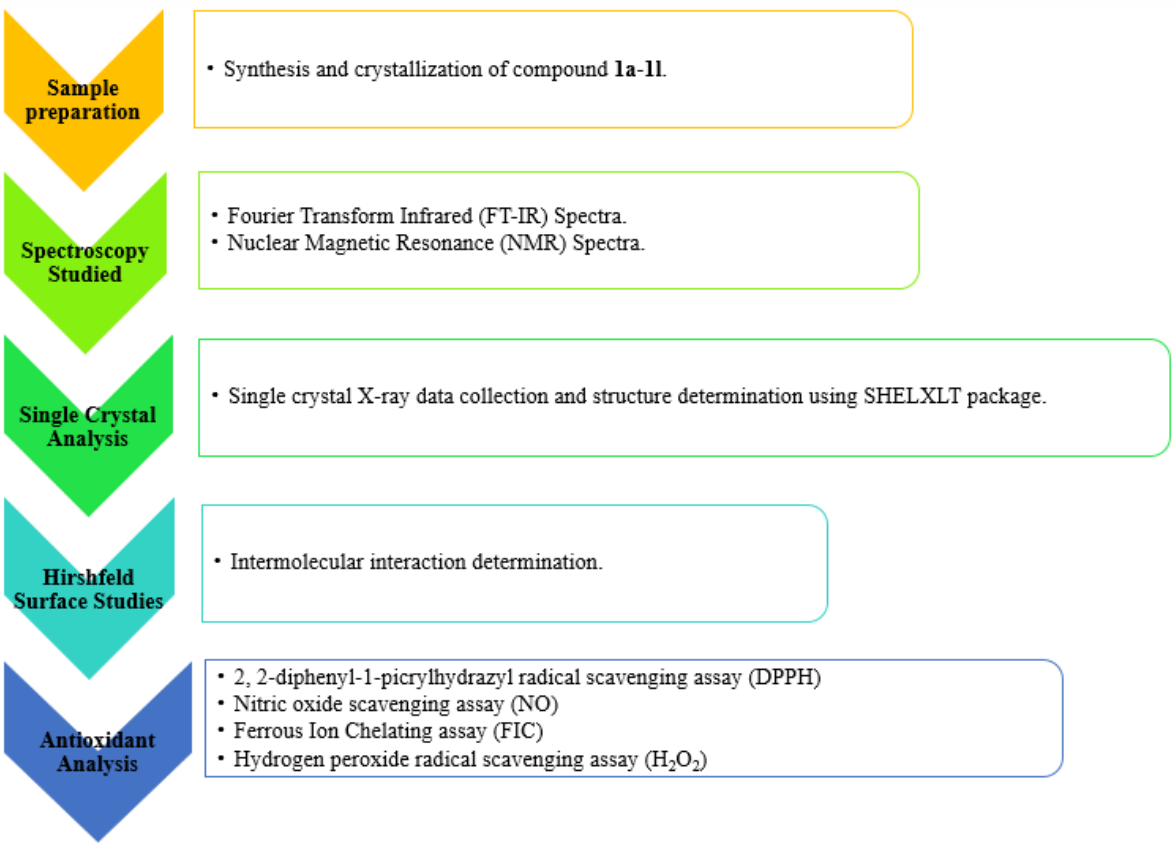


Figure 3.1 Flow chart of research activities.

3.2 Sample Preparation

In this research, twelve samples have been synthesized with good yield through Claisen Schmidt Condensation process at School of Physics (X-ray Crystallography Unit), Universiti Sains Malaysia. All of these samples were crystallized using slow evaporation methods.

3.2.1 Chemicals

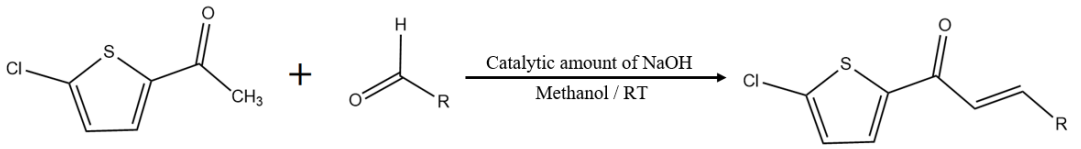
All chemicals consumed in this research project are used directly without any purification and are listed in Table 3.1.

Table 3.1 List of chemicals used for this research project.

Chemical name	Molecular Formula	Purity (%)	Manufacturer
2-Acetyl-5-chlorothiophene	C_6H_5ClOS	99 %	Sigma Aldrich
2-Pyridinecarbaldehyde	C_6H_5NO	99 %	Merck
Thiophene-2-carbaldehyde	C_5H_4OS	98 %	Merck
5-Methylthiophene-2-carbaldehyde	C_6H_6OS	98 %	Merck
4-Methylthiopbenzaldehyde	C_8H_8OS	≥ 97 %	Merck
Mesitaldehyde	$C_{10}H_{12}O$	≥ 90 %	Fluka
5-Bromo-2-thiophenecarboxaldehyde	C_5H_3BrOS	95 %	Sigma Aldrich
5'-Bromo-2,2'bithiophene-5-carboxaldehyde	$C_9H_5BrOS_2$	97 %	Sigma Aldrich
4-(Trifluoromethyl)benzaldehyde	$C_8H_5F_3O$	98 %	Merck
4-(Trifluoromethoxyl)benaldehyde	$C_8H_5F_3O$	96 %	Sigma Aldrich
4-(Dimethylamino)benzaldehyde	$C_9H_{11}NO$	99 %	Merck
4-(1-Piperidinyl)benzaldehyde	$C_{12}H_{15}NO$	97 %	Sigma Aldrich
2,3-Dihydrobenzofuran-5-carboxaldehyde	$C_9H_8O_2$	97 %	Sigma Aldrich
Acetone	C_3H_6O	99.5 %	Qrec
Ethanol	C_2H_5OH	99.5 %	Qrec
Methanol	CH_3OH	99.5 %	Qrec
Ethyl acetate	$C_4H_8O_2$	≥ 99 %	Qrec
L-Ascorbic acid	$C_6H_8O_6$	99 %	Sigma Aldrich
2,2-Diphenyl-1-picrylhydrazyl	$C_{18}H_{12}N_5O_6$	95 %	Sigma Aldrich
Ethylenediaminetetraacetic acid	$C_{10}H_{16}N_2O_8$	99 %	Sigma Aldrich
Ferrous chloride	$FeCl_2$	98 %	Sigma Aldrich
Ferrozine	$C_{20}H_{12}N_4Na_2O_6S_2$	97 %	Sigma Aldrich
3,4,5-Trihydroxybenzoic acid	$C_{10}H_{12}O_5$	98 %	Sigma Aldrich
Phosphate buffer saline pellet	$Cl_2H_3K_2Na_3O_8P_2$	≥ 99.9 %	Sigma Aldrich
Hydrogen peroxide	H_2O_2	30 %	Merck
Sodium hydroxide pellet	NaOH	97 %	Merck
Deuterated chloroform	$CDCl_3$	≥ 99.9 %	Sigma Aldrich

3.2.2 General Procedure for the Synthesis of 5-Chlorothiophen-2-yl Chalcone (Compounds 1a-1l)

The synthesis process for heterocyclic chalcone analogous (**1a** – **1l**) is shown in Figure 3.2. All compounds were obtained expectedly from a mixture of 2-acetyl-5-chlorothiophene (0.01 mol) and substituted benzaldehyde (0.01 mol) in 8 ml methanol. While the mixture is completely dissolved, catalytic amount of sodium hydroxide was added drop by drop to the mixture solution with vigorous stirring in a 50 ml round-bottom flask. The reaction mixture was stirred for about 6 hours at room temperature. After the stirred process was completed, the mixture was poured into cold water and stirred for 10 minutes. Next, the resultant precipitate products were filtered, washed a few times with distilled water and allow to dry. Single crystals were obtained using the suitable solvent in the crystallization and recrystallization process. Melting points for samples **1a** – **1l** were recorded by using Stuart SMP10 digital melting point apparatus.



Where R is, for

1a = 2-Pyridine
1b = 2-Thiophene
1c = 5-Methyl-2-thiophene
1d = 4-(Methylthio)benzene
1e = Mesitylene
1f = 5-Bromo-2-thiophene
1g = 5'-Bromo-2,2'bithiophene
1h = 4-(Trifluoromethyl)benzene
1i = 4-(Trifluoromethoxyl)benzene
1j = 4-(Dimethylamino)benzene
1k = 4-(1-Piperidinyl)benzene
1l = 2,3-Dihydrobenzofuran

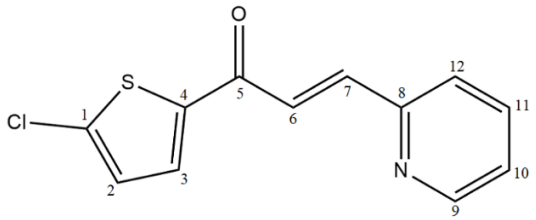
Figure 3.2 Schematic diagram for synthesis of heterocyclic chalcone derivatives.

3.3 Spectroscopic Studies

Spectroscopy is a technique to study the interactions occur between the matter and the electromagnetic radiation. It enables researcher to characterize the studied compounds through combination of different spectroscopy tools. Therefore, the invention of spectroscopy tools has enhanced the development of organic chemistry and they are widely used in the determination of the structure of organic compounds. Two types of spectroscopy tools are used in this research project to characterize the molecular structure which include Fourier transform infrared (FT-IR) spectroscopy and the nuclear magnetic resonance (NMR) spectroscopy (proton and carbon-13).

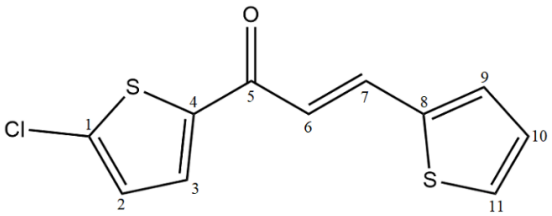
All compounds were subjected to Fourier transform infrared (FT-IR) and nuclear magnetic resonance (NMR) spectroscopy to ensure the chemical structure of these synthesized products are as postulated. The Fourier transform infrared spectroscopy (FT-IR) spectra were analysed by Perkin Elmer Frontier FTIR Spectrometer equipped with attenuated total reflection (ATR) in frequency range of 600 - 4000 cm^{-1}. Whereas, ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were determined by Bruker Avance III 500 spectrometer with deuterated chloroform ($CDCl_3$) as solvent in frequencies of 500 MHz and 125 MHz, respectively. Spectrum (*Spectrum*, 2011) was used to analyse the raw data of FT-IR, while Delta (*Delta 5.0.4*, 2014) was applied to classify the atoms in NMR spectra. The spectra results were obtained as following:

(E)-1-(5-chlorothiophen-2-yl)-3-(pyridin-2-yl)prop-2-en-1-one **(1a):**



Solvent used to form single crystal: Acetone; Yield: 66%; M.P.: 127 ˚C -129 ˚C; FT-IR (ATR(solid)cm^{-1}): 3047 υ(Ar C-H), 1651.5 υ(C=O), 1596.4, 1420.5 υ(Ar C=C), 1227.6 υ(C-N), 774 υ(C-Cl), 679 υ(C-S); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.683-8.671 (d, 1H, J= 4.6 Hz, ^{3}CH), 7.935-7.897 (d, 1H, J= 15.6 Hz ^{7}CH), 7.793-7.723 (m, 3H, ^{6}CH, ^{9}CH and ^{11}CH overlapped), 7.466-7.447 (d, 1H, J=7.2 Hz, ^{12}CH), 7.323-7.289 (t, 1H, J=7.2 Hz, ^{10}CH), 7.013-7.002 (d, 1H, J= 4.6 Hz, ^{2}CH); ^{13}C-NMR (125 MHz, $CDCl_3$): 181.39 (C5), 152.87 (C8), 150.28 (C9), 144.31 (C4), 142.44 (C7), 140.43 (C1), 137.06 (C3), 132.09 (C11), 127.90 (C2), 125.90 (C6), 124.72 (C12), 124.15 (C10)

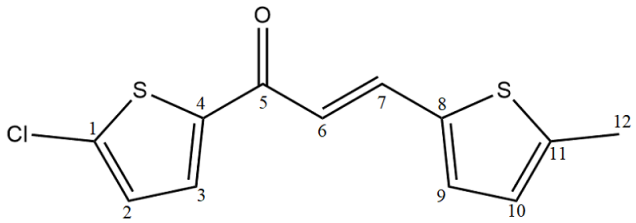
(E)-1-(5-chlorothiophen-2-yl)-3-(thiophen-2-yl)prop-2-en-1-one **(1b)**



Solvent used to form single crystal: Acetone; Yield: 70%; M.P.: 92 ˚C -94 ˚C; FT-IR (ATR(solid)cm^{-1}): 3081 υ(Ar C-H), 1639.2 υ(C=O), 1571.6, 1413.6 υ(Ar C=C), 799.9 υ(C-Cl), 694.1 υ(C-S); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.965-7.924 (d, 1H, , J=16.4 Hz, ^{7}CH), 7.617-7.608 (d, 1H, J= 3.4 Hz, ^{11}CH), 7.438-7.425 (d, 1H, J= 4.6 Hz ^{3}CH), 7.367-7.359 (d, 1H, J= 3.4 Hz, ^{9}CH), 7.117-7.079 (m, 2H, ^{6}CH and ^{10}CH overlapped), 7.000-6.990 (d, 1H, J= 4.6 Hz, ^{2}CH); ^{13}C-NMR (125 MHz, $CDCl_3$): 180.68 (C5),

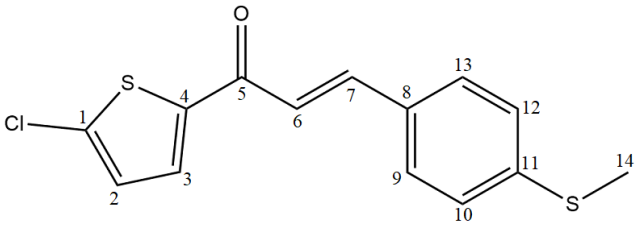
144.30 (C4), 140.05 (C1), 139.77 (C8), 136.95 (C3), 132.59 (C9), 131.14 (C7), 129.26 (C2), 128.55 (C11), 127.78 (C6), 119.17 (C10).

(E)-1-(5-chlorothiophen-2-yl)-3-(5-methylthiophen-2-yl)prop-2-en-1-one **(1c)**



Solvent used to form single crystal: Acetone; Yield: 64%; M.P.: 108 ˚C -110 ˚C; FT-IR (ATR(solid)cm^{-1}): 3078 υ(Ar C-H), 2916.8, 2857.9 υ(CH_3), 1652.2 υ(C=O), 1574.8, 1419.8 υ(Ar C=C), 793.7 υ(C-Cl), 706.9 υ(C-S); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.882-7.844 (d, 1H, J= 15.2 Hz, ^{7}CH), 7.584-7.574 (d, 1H, J= 4.4 Hz, ^{3}CH), 7.171-7.162 (d, 1H, J= 3.6 Hz, ^{9}CH), 6.986-6.938 (m, 2H, ^{6}CH and ^{10}CH overlapped), 6.755-6.743 (d, 1H, J=4.4 Hz, ^{2}CH), 2.523 (s, 3H, $^{12}CH_3$); ^{13}C-NMR (125 MHz, $CDCl_3$): 180.73 (C5), 145.17 (C4), 144.49 (C11), 139.43 (C1), 138.11 (C8), 137.41 (C3), 133.54 (C9), 130.88 (C7), 127.73 (C2), 127.09 (C6), 117.82 (C10), 16.04 (C12)

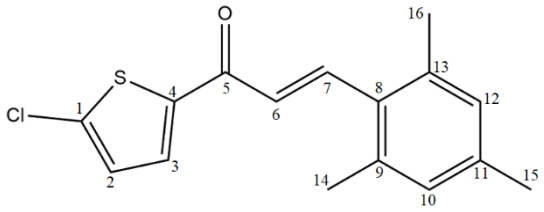
(E)-1-(5-chlorothiophen-2-yl)-3-(4-(methylthio)phenyl)prop-2-en-1-one **(1d)**



Solvent used to form single crystal: Acetone; Yield: 68%; M.P.: 129 ˚C -131˚C; FT-IR (ATR(solid)cm^{-1}): 3078 υ(Ar C-H), 2985, 2832 υ(CH_3), 1645 υ(C=O), 1575, 1420 υ(Ar C=C), 794 υ(C-Cl), 729 υ(C-S); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.794-7.756 (d, 1H, J= 15.2 Hz, ^{7}CH), 7.622-7.612 (d, 1H, J= 4.0 Hz, ^{3}CH), 7.536-7.515 (d, 2H, J= 8.4 Hz ^{9}CH and ^{13}CH overlapped), 7.275-7.228 (m, 3H, ^{6}CH, ^{10}CH and ^{12}CH

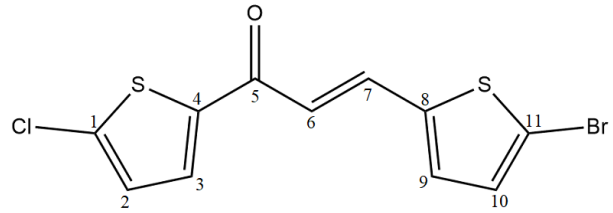
overlapped), 6.995-6.985 (d, 1H, J=4.0 Hz, ^{2}CH), 2.506 (s, 3H, $^{14}CH_3$); ^{13}C-NMR (125 MHz, $CDCl_3$): 181.07 (C5), 144.44 (C4), 144.07 (C7), 142.91 (C1), 139.69 (C11), 131.11 (C3), 131.02 (C8), 128.98 (C9 and C13 overlapped), 127.77 (C2), 126.00 (C10 and C12 overlapped), 119.27 (C6), 15.17 (C14)

(E)-1-(5-chlorothiophen-2-yl)-3-mesitylprop-2-en-1-one **(1e)**



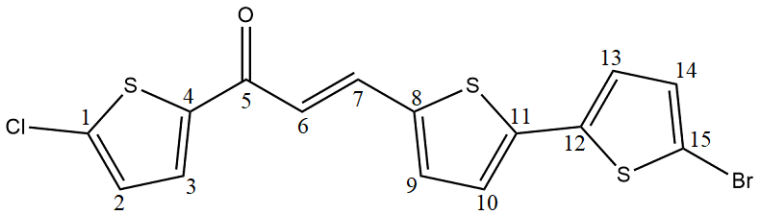
Solvent used to form single crystal: Acetone; Yield: 74%; M.P.: 107 ˚C -109 ˚C; FT-IR (ATR(solid)cm^{-1}): 3078 υ(Ar C-H), 2916.7, 2864 υ(CH_3), 1643.8 υ(C=O), 1591.4, 1417.6 υ(Ar C=C), 799.9 υ(C-Cl), 719.3 υ(C-S); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.014-7.975 (d, 1H, J= 15.6Hz, ^{7}CH), 7.551-7.541 (d, 1H, J= 4.0 Hz, ^{3}CH), 6.992-6.921 (m, 4H, ^{2}CH, ^{6}CH, ^{10}CH and ^{12}CH overlapped), 2.379 (s, 6H, $^{14}CH_3$ and $^{16}CH_3$ overlapped), 2.299 (s, 3H, $^{15}CH_3$); ^{13}C-NMR (125 MHz, $CDCl_3$): 181.23 (C5), 144.36 (C4), 143.12 (C7), 139.78 (C1), 138.97 (C11), 137.39 (C3), 131.27 (C10 and C12 overlapped), 129.47 (C8, C9 and C13 overlapped), 127.81 (C2), 125.66 (C6), 21.34 (C14 and C16 overlapped), 21.206 (C15).

(E)-3-(5-bromothiophen-2-yl)-1-(5-chlorothiophen-2-yl)prop-2-en-1-one **(1f)**



Solvent used to form single crystal: Ethyl Acetate; Yield: 70%; M.P.: 135 ˚C -137 ˚C; FT-IR (ATR(solid)cm^{-1}): 3084.1 υ(Ar C-H), 1646 υ(C=O), 1584, 1416.7 υ(Ar C=C), 790.6 υ(C-Cl), 703.8 υ(C-S), 610.8 υ(C-Br); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.820 – 7.782 (d, 1H, J= 15.2 Hz, ^{7}CH), 7.589 – 7.578 (d, 1H, J= 4.4 Hz, ^{3}CH), 7.101 – 7.091 (d, 1H, J= 4.0 Hz ^{9}CH), 7.054 – 7.044 (d, 1H, J= 4.0 Hz, ^{10}CH), 6.998 – 6.959 (m, 2H, ^{2}CH and ^{6}CH overlapped); ^{13}C-NMR (125 MHz, $CDCl_3$): 180.38 (C5), 144.10 (C4), 141.590 (C1), 140.06 (C8), 135.98 (C9), 132.84 (C3), 131.53 (C7), 131.254 (C10), 127.84(C2), 119.41 (C6), 116.91 (C11).

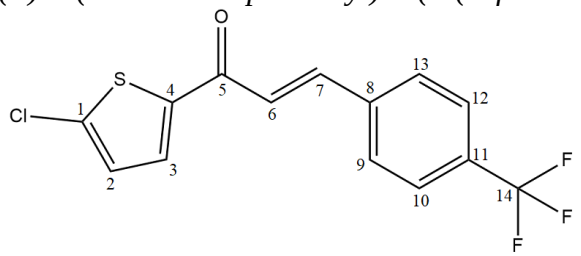
(E)-3-(5'-bromo-[2,2'-bithiophen]-5-yl)-1-(5-chlorothiophen-2-yl)prop-2-en-1-one **(1g)**



Solvent used to form single crystal: Acetone; Yield: 78%; M.P.: 189 ˚C -190 ˚C; FT-IR (ATR(solid)cm^{-1}): 3074.8 υ(Ar C-H), 1635 υ(C=O), 1577.8, 1419.8 υ(Ar C=C), 781.3 υ(C-Cl), 703.8 υ(C-S); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.889 – 7.851 (d, 1H, J= 15.2 Hz, ^{7}CH), 7.612 – 7.601 (d, 1H, J= 4.4 Hz, ^{3}CH), 7.103 – 7.056 (m, 2H, ^{9}CH and ^{10}CH overlapped), 7.034 – 6.994 (m, 4H, ^{2}CH, ^{6}CH, ^{13}CH and ^{14}CH overlapped); ^{13}C-NMR (125 MHz, $CDCl_3$): 180.43 (C5), 144.31 (C4), 139.91 (C1), 138.99 (C12),

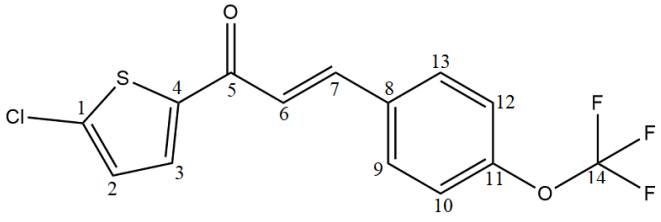
138.12 (C11), 136.52 (C8), 133.87 (C3), 131.10 (C7), 127.81 (C10), 126.31 (C14), 125.11 (C9), 124.92 (C2), 124.48 (C13), 119.04 (C6), 112.91 (C15)

(E)-1-(5-chlorothiophen-2-yl)-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-one **(1h)**



Solvent used to form single crystal: Acetone; Yield: 72%; M.P.: 124 ˚C -126 ˚C; FT-IR (ATR(solid)cm^{-1}): 3081 υ(Ar C-H), 1647.6 υ(C=O), 1593.3, 1416.8 υ(Ar C=C), 1111.6 υ(C-F), 799.9 υ(C-Cl), 740.6 (C-S); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.832 – 7.793 (d, 1H, J= 15.6 Hz, ^{7}CH), 7.723 – 7.702 (d, 2H, J= 8.4 Hz, ^{9}CH and ^{13}CH overlapped), 7.670 – 7.648 (m, 3H, ^{3}CH, ^{10}CH and ^{12}CH overlapped), 7.385 – 7.346 (d, 1H, J= 15.6 Hz, ^{6}CH), 7.017 – 7.007 (d, 1H, J= 4.0 Hz, ^{2}CH); ^{13}C-NMR (125 MHz, $CDCl_3$): 180.69 (C5), 143.91 (C4), 142.54 (C7), 140.54 (C1), 137.96 (C8), 132.34 (C14), 132.09 (C14), 131.71 (C3), 128.67 (C6, C10 and C12 overlapped), 127.93 (C9 and C13 overlapped), 126.01 (C11), 125.22 (C14), 122.65 (C2), 122.51 (C14).

(E)-1-(5-chlorothiophen-2-yl)-3-(4-(trifluoromethoxy)phenyl)prop-2-en-1-one **(1i)**



Solvent used to form single crystal: Acetone; Yield: 66%; M.P.: 119 ˚C -121 ˚C; FT-IR (ATR(solid)cm^{-1}): 3082.3 υ(Ar C-H), 1652.2 υ(C=O), 1579.3, 1419.8 υ(Ar C=C), 1156.3 υ(C-F), 1016.5 υ(C-O), 799.9 υ(C-Cl), 750.3 υ(C-S); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.798 – 7.759 (d, 1H, J= 15.6 Hz, ^{7}CH), 7.650 – 7.626 (m, 3H, ^{3}CH, ^{9}CH