Faculty of Resource Science and Technology

Synthesis, Characterization and Antibacterial Activity of Bis Thiourea Derivatives

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SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF BIS THIOUREA DERIVATIVES

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DECLARATION

I hereby declare that no portion of this dissertation has been submitted in support of an application for another degree of qualification of this or any other university or institution of higher learning.

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(Jayakumar A/l Ponniasalan)

Resource Chemistry Programe

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List of Abbreviations

FTIR	Fourier Transform infrared spectroscopy
1H NMR	Hydrogen Nuclear Magenetic Resonances
13C NMR	Carbon Nuclear Magnetic Resonances
Ppm	part per million
MIC	Minimal Inhibitory Concentration

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Synthesis, Characterization and Antibacterial Activity of BisThiourea Derivatives

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Resource Chemistry

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Abstract

A series of bisthiourea derivatives were succesfully synthesized by reaction of benzene-1, 4-dicarbonyl isothiocyanate intermediates and isophthaloyl dichloride, with a series of aromatic amine at *ortho, meta* and *para* position. The structure of synthesised compound characterized by infra-red spectroscopy (FTIR), ^{1}H Nuclear magnetic resonances, and ^{13}C Nuclear magnetic resonances. The synthesised compounds were screened for their antibacterial properties using Gram-negative bacteria *Escherichia coli* (*E.coli)* by using disc diffusion method. All of synthesized series showed negative results towards antibacterial activity.

Keywords: thiourea, *E.coli,* Antibacterial activity, Amine, Spectroscopy

ABSTRAK

Satu siri bis(tiourea) derivative telah berjaya dihasilkan melalui tindakbalas antara sebatian pertengahan benzene-1,4-dicarbonil isothiosianat, dengan terbitan amina aromatic pada orto, meta dan para. Struktur sebatian disentesis dicirikan menggunakan spektroskopi infra merah (FTIR), resonasmagnetiknuklear(^{13}C NMR dan^{1}H NMR). Sebatian disintesis telah disaring untuk aktiviti anti-bakteria mereka menggunakan bacteria negatif Gram (E.coli) meggunakan kaedah Cakera penyebaran. Kesemua sebatian disintesis tidak menunjukkan perencatanlan gsung terhadap aktiviti anti-bakteria.

Kata Kunci: tiourea, E.coli, aktiviti anti-bakteria, Amina, Spektroskopi

Chapter 1

Introduction

1.1 Thiourea.

Thiourea is an organosulfur compound with chemical formula of CSN_2H_4 (Madan *et al.,* 1991).The major element in thiourea was sulphur, nitrogen and carbon. The basic structure of thiourea is shown in Figure 1 below. The melting point and molar mass of thiourea are 182 ˚C and 76.12 g/mol respectively. Thiourea is white crystalline form which very soluble in polar solvent likes water and insoluble in non-polar solvent (Claude *et al.,* 2013). Thiourea and urea are structurally similar except oxygen atom in urea have been replaced by sulfur atom, thus it contribute to the differences in properties (Loto *et al*, 2012).

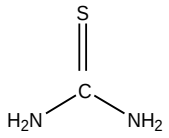
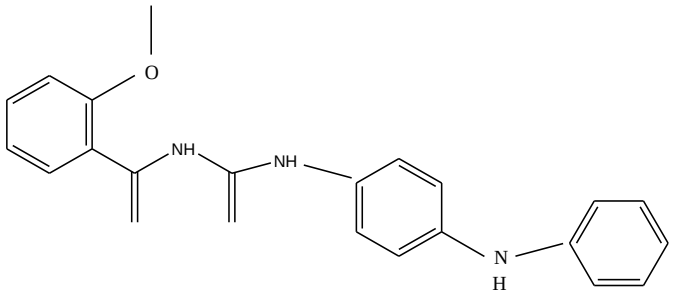


Figure 1 :Thiourea

Thiourea was actively used in organic synthesis due to it versatile reagent properties which able to make coordination bond with metal centres as neutral ligand, monoanions or dianions. These bonding possibilities are due to the oxygen, nitrogen and sulphur donor atom of thiourea derivatives (Yahyazadeh *et al*., 2013).The mechanism in synthesizing thiourea is nucleophilic attack at the electrophilic carbon of thiocyanate ion by amine (McEwen, 1991).

Besides, thiourea derivative are capable in wide range of biological activities such as antibacterial, antiviral, anticancer, anticonvulsion, analgesic and HDL-elevating properties (Yahyazadeh *et al*., 2013). Moreover, thiourea was also reported was used in controlling plant pathogen (Fernandez *et al*., 2005).

In a study done by Mohamad Halim (2011), monothiourea compound *N*-(2-methoxybenzoyl)-*N*'-(4-diphenylamine)thiourea (**1)** showed good antibacterial properties against *Staphylococcus aureus* with 9.5 mm zone of inhibition. Acyl thiourea exhibit superior pesticides, fungicidal, antiviral and involve in plant grow activity (Saeed et al., 2009).



(1)

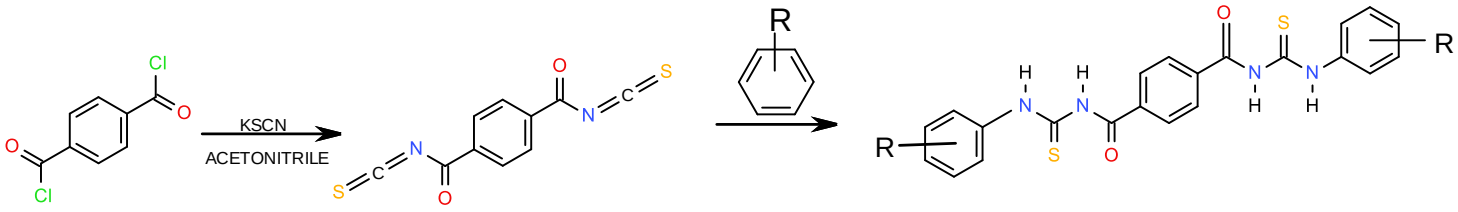
Figure 2: *N*-(2-methoxybenzoyl)-*N*'-(4-diphenylamine)thiourea**1**

1.1 Problem Statement

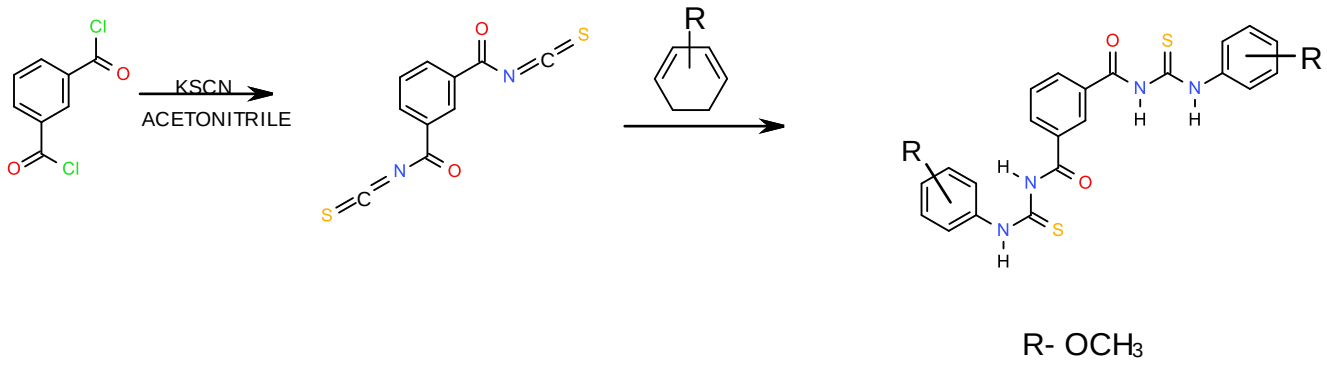
Many researchers have been reported on the synthesis of mono-thiourea. Due to the fact, mono thiourea exhibit good antibacterial properties (Saeed *et al*., 2009). Bisthiourea is now widely explore to study the effectiveness of its antibacterial properties. Therefore in this, research bisthiourea derivative bearing amine at O, P and M position were synthesised and tested for antibacterial properties against *E.coli*.

1.2 Objective

1. To synthesis new bis(thiourea) derivatives bearing aromatic substituted alkyl groups at Ortho, Meta and Para position as shown in scheme 1 and 2.



Scheme 1: Reaction pathway of synthesis N1,N4-bis thiourea derivatives



Scheme 2: Reaction pathway of synthesis N1,N3-bis thiourea derivatives

2. To characterize bisthiourea derivatives using FTIR, ^{1}H NMR and ^{13}C NMR,

3. To study the antibacterial properties of the synthesized bisthiourea derivatives against *Escherichia coli.*

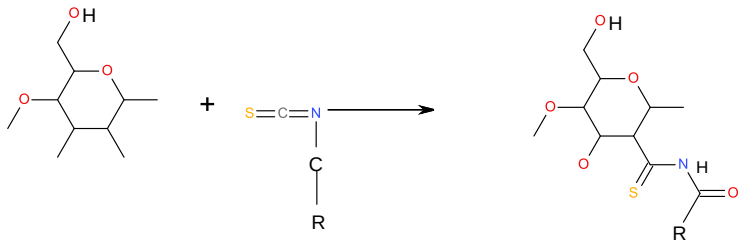
Chapter 2

Literature Review

2.1 Thiourea

Thiourea derivatives is versatile ligand and able to coordinate as neutral ligand, monoanions or dianions to metal centres (Arslan, 2009). The oxygen, nitrogen and sulphur act as donor atom thus providing wide range multitude of bonding possibilities (Arslan,2009). Thiourea derivative coordinate to some transition metal ion to form stable complexes (Alkherraz,2014). Thiourea was used in the reductive work-up of ozonolysis to produce carbonyl compound because of thiourea is odourless and non-volatile due to its polarity (Alkan,2011).

Thiourea is very effective in control plant pathogen (Fernandez,2005). Acyl thiourea also was used in fungicidal, antiviral and regulation activities for plant growth in planting industry. According to, Zhong *et al*, (2008) undergoes research on synthesis of acyl thiourea derivatives of chitosan (CS) and their properties with bacterial. They obtained a clear agar when tested with bacteria such as *Echerichia coli, Pseudomonas aeruginosa, Staphylococcus aures* and *Sarcina.*The test proved that this compound produce a clear agar, which can be conclude that this compound does exhibit antibacterial properties. The pathway of synthesis of acyl thiourea derivatives illustrated in scheme **(3)** below.

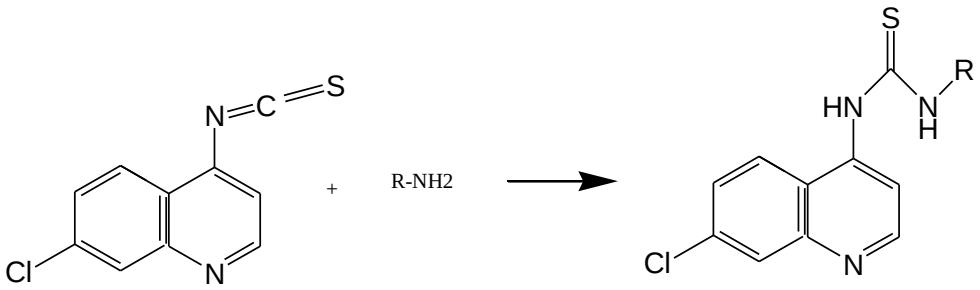


(2)

Scheme 3 : Pathway of synthesis of acyl thiourea derivatives**2**

2.2 Synthesis of thiourea derivative

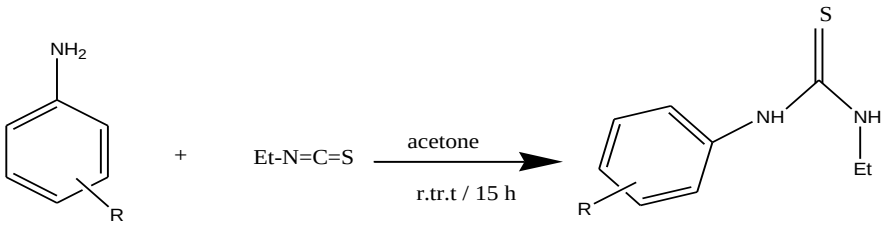
Thiourea derivative (**5)** 7-chloroquinolinyl thioureas usually synthesis by the action of nucleophilic amine (**4)** reactivity toward electrophilic carbon of thiocyanate ion (**3) (**Mahajan *et al.*, 2007). The general mechanism is shown in Scheme 4.



(3) **(4)** **(5)**

Scheme 4: Mechanism to synthesis thiourea**4**

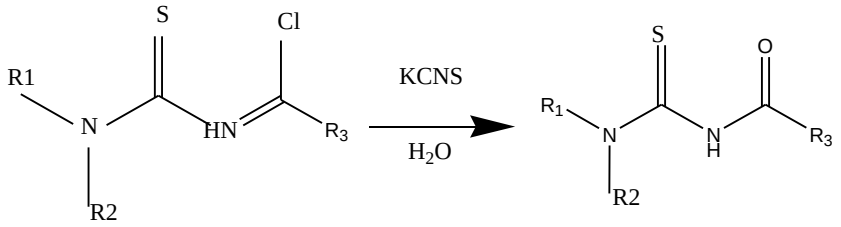
One of the example synthesis of unsymmetrical thiourea derivatives as shown in Scheme 5. 1-Ethyl-3-(3-hydroxyphenyl)thiourea (**6**) was successfully synthesised by the reaction of aromatic amine with ethylisothiocyanate in acetone. Aromatic amine was used to produce 67% of yield of compound after purification (Yahyazadeh *et al.*, 2013).



(6**)**

Scheme 5: Synthesis of thiourea from aromatic amines **6**

Thiourea was also reported as N,N-disubstituted thiourea and N-acylthiourea. Acyl thiourea was widely used in numerous transformations. For example acylthiourea were used to synthesise of four-, five-,six-,and seven- membered heterocyclic ring system. The compound was studied for good antibacterial, antiarthrictic and anticoagulant agent. According to Alan *et al.,* (2004) acylthiourea was believed to increase antifungal activities in plant. Pathogen that causes infection and alteration of development stages of plant can be inhibited. These infected plants have high tendency to cause serious health problem to consumers (Paola *et al.,* 2011*).* For example acylthiourea (**7)** was synthesised by the reaction of aminothiocarbonylimitoyl chlorides with potassium thiocyanate (Alan *et al.,* 2004). The reaction pathway is shown in Scheme **6**.

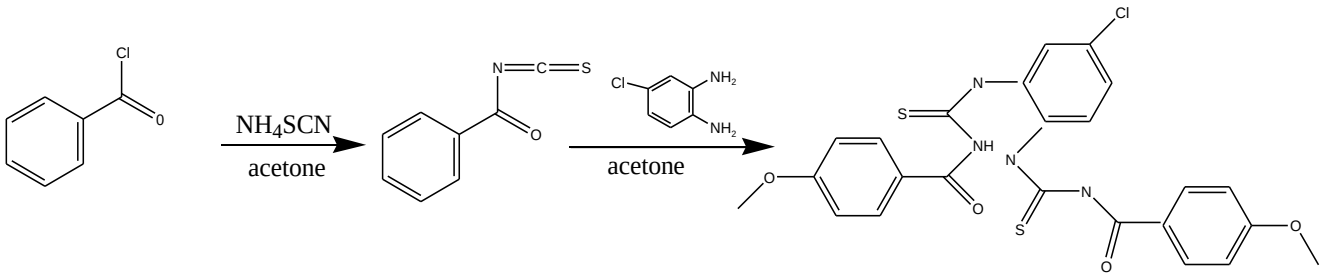


(**7)**

Scheme 6 : Reaction pathway of synthesis acylthiourea**7**

Other than that, 1,2-Bis[N'-(2-methoxybenzoyl)thioureido]-4-nitrobenze (**8)** was synthesised from the reaction of benzoyl isothiocyante with 4-chloro-1,2-phenylenediamine as shown in Scheme **7**. This reaction produced 69% yield. This compound contain two thiourea moieties

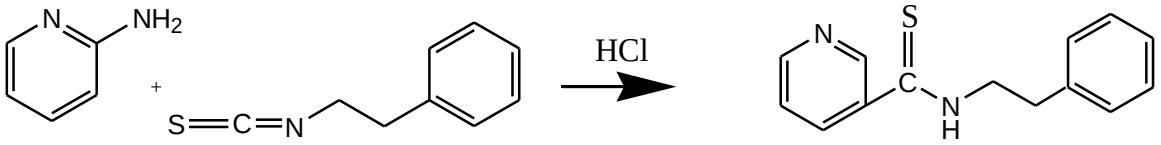
that contribute to good antibacterial activities against *Staphylococcus aueus.*Moreover, the this compound exhibit good antibacterial properties due to that it contain three penyl group and an halogen atom (Shaikh*et al.,* 2014).



(8)

Scheme 7: Reaction pathway of 1,2-Bis[N'-(2-methoxybenzoyl)thioureido]-4-nitrobenze **8**

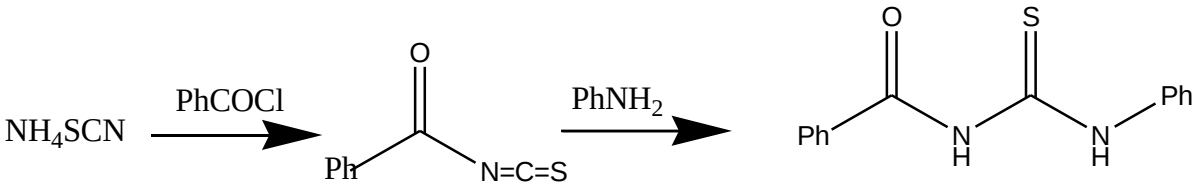
Venkatesh (2009) synthesised heterocyclic based thiourea derivatives. 1-pyridin-2yl-thiourea (**9)** was synthesised reaction of 2-aminopyridine on to ammonium thiocyanate in methanol with the yield ranging from 64-85 %. Compound 8 exhibit good anti-oxidant with 76.7% inhibition on lipid peroxide level. The reaction pathway was shown in scheme **8**.



(9)

Scheme 8 : Reaction pathway of N-(2-methoxy(benzoyl)-N'-4(4-diphenylalamine)thiourea **9**

Frank *et al.,* (1995) reported on α-benzoyl-β-phenylthiourea **10** when synthesised by the reaction of ammonium thiocyanate and benzoyl chloride. Aniline that act as amine was added and yellow precipitate compound **10** formed with 85% of yield as shown in Scheme **9**.



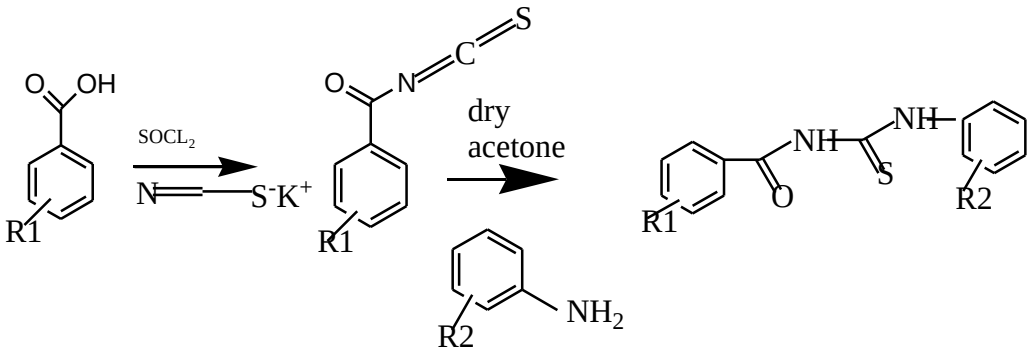
(10)

Scheme 9 : reaction pathway of synthesis α-benzoyl-β-phenylthiourea **10**

2.3 Biological Activities of Thiourea Derivatives

2.3.1 Antibacterial activities

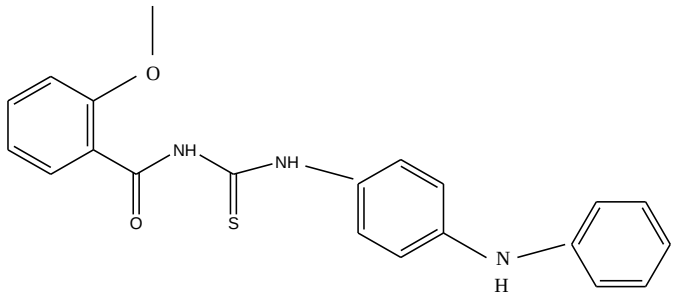
Saeed*et al* (2009) reported, 1-aroyl-3-aryl thiourea (**11)** showed antibacterial properties against *Escherichia coli* and *Staphylococcus aureus.*1-aroyl-3-aryl thiourea (**11)** was synthesised by the reaction of aryl chlorides and potassium thiocyanate with addition of substituted anilines. The substituent like halo groups result in effectiveness in antibacterial properties (Saeed *et al*., 2009). The reaction pathway is shown in Scheme **10**.



(11)

Scheme 10: Reaction pathway of 1-aroyl-3-aryl thiourea **11**

In 2011, Halim*et al.,* synthesised N-(2-methoxybenzoyl)-N'-(4-diphenylamine)thiourea (**12)** and the compound was reported to show antibacterial properties against *Staphylococcus aureus* with 9.5mm zone of inhibition. The Antibacterial activity of the thiourea was believed to enhance with the metal such as copper.

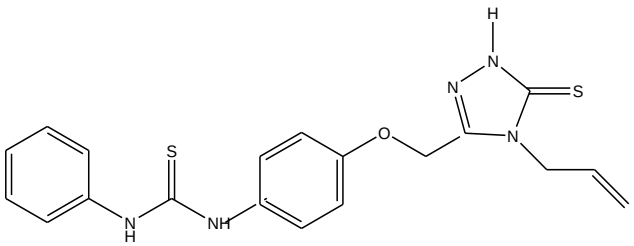


(12)

Figure 3 :N-(2-methoxybenzoyl)-N'-(4-diphenylamine)thiourea**12**

2.3.2 Antiviral Activities

According to Chen (2010) the substitution place, electronic, steric effect and linker length greatly affect the antiviral activities. Compound 1-[4-[(4-allyl-5-methylene-1,24-triazolidin-3-yl)methoxy]phenyl]-3-phenyl-thiourea (**13)** reported to exhibit antiviral characteristic against Coxsackie virus B4 due to the ally group at the N-4 of the 1,2,4-triazole ring and phenyl moiety at the terminal nitrogen (Kucukguzel *et al*.,2008).

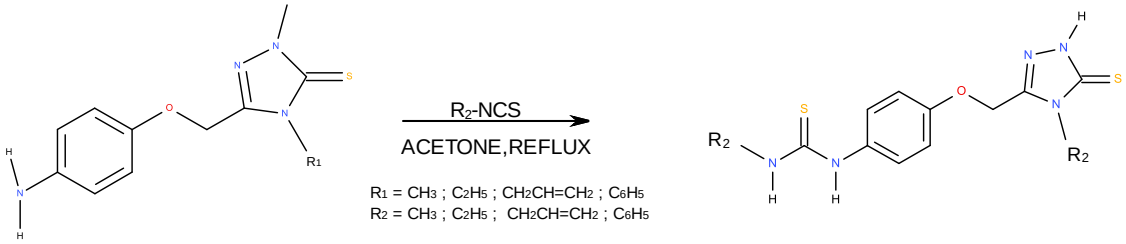


(13)

Figure 4 : Structure of 1-[4-[(4-allyl-5-methylene-1,24-triazolidin-3-yl)methoxy]phenyl]

Phenyl-thiourea **13**

The reaction of 5-[(4-aminophenoxy) methyl]-4-alkyl/aryl-2,4-dihydro-3H-1,2,4-triazole-3-thiones with amines group that contain alkyl or aryl isothiocyanates in the dry acetone as solvent successfully obtained 1-alkyl/aryl-3-{4-[(4-alkyl/aryl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)-methoxy]-phenyl}thiourea derivatives (**14)**. This compound has an ally group at the N-4 of the 1, 2,4-triazole ring and phenyl moiety at the terminal nitrogen is active derivative against viral. This compound show antiviral properties against Coxsackie virus B4.Scheme 11 shows the synthesis of compound (**14).**

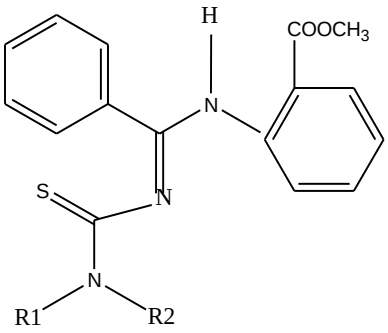


(14)

Scheme 11: Synthesis of thiourea derivative **14**

2.3.3 Antifungal Activities

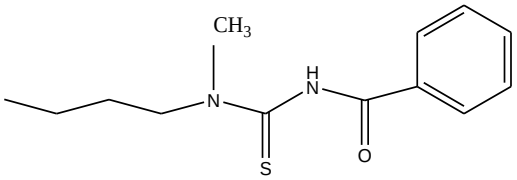
Synthesis of thiourea derivatives of methyl anthranilate as 2-{[(diethyl-thiocarbamoylimino)-phenyl-methyl]-amino}-benzoic acid methyl ester **15** was reported to exhibit antifungal properties and inhibit growth of plant pathogen that induce plant diseases such as *Phoma*betae and *Fusariumoxysporum* (Campo *et al.*, 2004).



(15)

Figure 5 :2-{[(diethyl-thiocarbamoylimino)-phenyl-methyl]-amino}-benzoic acid methyl ester **15**

Del Campo (2004), also reported 3-benzoyl-1-butyl-1-methyl-thiourea (**16)** showed antifungal activity and suppresses the growth of *Penicilliumdigitatum* and *Saccharomyces cerevisiae.*

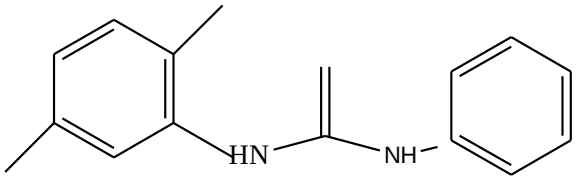


(16)

Figure 6: 3-benzoyl-1-butyl-1-methyl-thiourea **16**

2.3.4 Anticancer

Cancer was reported as world major health problem. Early tumour detection increased the survival rate of cancer patients. Phenyl thiourea derivatives was reported to be use to prevent and treat cancer by inhibit the telomerase activity. 1-(2-, 5-Dimethylphenyl)-3-phenylthiourea (**19)** was synthesised by using phenyl isothiocyanate added with substituted aromatic and fat amine (Gua*et al.,* 2014).



(19)

Figure 7 : 1-(2-,5-Dimethylphenyl)-3-phenylthiourea **19**

Other than that, bisthiourea compound (**20)** 1-(2, 2-diphenylethyl)-3-[3-{7-[3-(2,2-diphenylethylcarbamo thioylamino)propylamino]heptylamino}propyl]thiourea. Compound (**20)** was prepared from the reaction of *N,N'-bis*(3-aminopropyl)heptane-1,7-diamine and [isothiocyanato(phenyl)methyl]benzene (**20)** was also reported to initiate methylation at histone 3 lysine 4 chromatin mark and specific target of lysine-specific demethylase in lung carcinoma cell. Lysine-specific demethylase responsible and control development of cancer (Sharma *et al.,* 2010).