SYNTHESIS AND CHARACTERIZATION OF BIS THIOUREA DERIVATIVES ANOTHER ANTIBACTERIAL ACTIVITY

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(22618)

This project is submitted in partial fulfillment of

the requirement for the degree of Bachelor of Science with Honours

(Resource Chemistry)

Faculty of Resource Science and Technology

University Malaysia Sarawak

2011

Acknowledgement

My gratitude goes first towards my supervisor, Assc. Prof. Dr. Zainab Ngaini, for her constant guidance, generous helps and unfailing enthusiasm in completion of this thesis. Without her advices and full support, I would be in difficulties to completing my research.

The special thank also goes to my co-supervisors, Miss Maya Asyikin Arif and Assc. Prof. Dr. Mohd Hasnain bin Mohd Husin for their helpful advices and guidance.

Great deals appreciated go to all the technical staff and lab assistants for their proficient assistance and providing the necessary laboratory facilities for analysis. I also thankful to my parents and whole family for always believe in me, encourage and support me in my study.

Last but not least, I would like to thank to my fellow friends for supporting me during this research. Their understanding and coorperation have given me pleasant condition during lab works. Thanks for all the memories.

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List of Abbreviations

FTIR = Fourier Transform Infrared

NMR = Nuclear Magnetic Resonance

MIC = Minimum Inhibition Concentrations

THF = Tetrahydrofuran

ppm = part per million

UV = Ultra Violet

LB = Lysogeny broth

List of Figures and Schemes

Synthesis and Characterization of Bis Thiourea Derivatives and their Antibacterial Activity

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ABSTRACT

Thiourea and its derivatives are well known as potent antibacterial and antifungal agents. This research focused on the synthesis of bis thiourea derivatives with two thiourea groups resulting from the reactions of 3-acetylbenzoyl isothiocyanate intermediate with appropriate amine group from certain amino acids and aniline. Five bis thiourea derivatives have been synthesized. The successfully synthesized compounds were characterized using FTIR, ^{1}H NMR and ^{13}C NMR and found to be in highly purity compound. Antibacterial assay was carried out using turbidimetric method against the growth of *Escherichia Coliform sp. (E.coli)*. However, the result indicated that the newly synthesized bis thiourea derivatives showed poor antibacterial activity against *E. coli.* The effect of the structure of the investigated compounds on the antibacterial activity is discussed.

Keywords: Bis thiourea, Antibacterial activity, Amino acid

ABSTRAK

Thiourea dan terbitannya merupakan sebatian yang terkenal sebagai agen anti-bakteria dan anti-fungi. Kajian ini memfokuskan penghasilan sebatian terbitan bis thiourea yang mengandungi dua kumpulan thiourea yang dihasilkan melalui tindakbalas antara sebatian pertengahan 3-asetilbenzoil isothiocianat dan kumpulan amina daripada amino asid dan aniline. Lima sebatian terbitan bis-thiourea telah berjaya dihasilkan. Struktur sebatian yang telah disintesis dikenalpasti dengan menggunakan FTIR, ^{1}H NMR and ^{13}C NMR. Ujian anti-bakteria terhadap tahap pertumbuhan bacteria Escherichia Coliform sp. (E.coli) telah dilakukan menggunakan kaedah turbidimetrik. Walaubagaimanapun, keputusan ujian tersebut menunjukkan bahawa sebatian yang diuji mempamerkan aktiviti anti-bakteria yang lemah. Oleh itu, pengaruh struktur sebatian terhadap tahap aktiviti anti-bakteria telah dibincangkan.

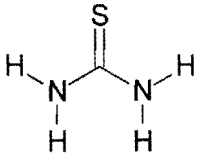
Kata kunci: Bis-thiourea, Aktiviti anti-bakteria, Amino asid

Chapter 1

Introduction

1.1 Background of study

Thiourea **1** is an organic compound that consists of carbon, nitrogen, sulfur and hydrogen atoms. It is also known as thiocarbamide or sulfourea. It is a white solid compound with molecular formula of CH_4N_2S and molecular weight of 76.12 g/mol. The structure of thiourea is shown in Figure 1. Thiourea occurs as the mixture of two tautomers, $S=C(NH_2)_2$ (thiourea) **2** and $HS=CNHNH_2$ (isothiourea) **3** as shown in Figure 2. Thiourea can be synthesized by reacting amino group and thiocyanato group in a suitable solvent. Thiourea has been reported to posses pharmacological properties such as antibacterial (Saeed et al., 2009) and antitumor property (Mahjula et al., 2008).



(1)

Figure 1: Thiourea compound

Thiourea Isothiourea

(2) **(3)**

Figure 2: Tautomeric forms of thiourea

1.2 Problem statement

There are various types of thiourea derivatives reported and study on the biological properties. But most of the compound only contained one unit thiourea. Besides, many studies reported on analyzing the pharmacological activities of thiourea complexes derivatives. The main purpose of this project is to prepare new bis thiourea derivatives which contain two thiourea groups using isophthaloyl dichloride and study on the pharmacological properties against *E.coli.*

1.3 Research Objectives

The objectives of this research are:

1. to synthesis a new bis thiourea compounds by reacting different types of amino acids and appropriate thiocyanate group.
2. to characterize bis thiourea derivatives using FTIR, ^{1}H NMR and ^{13}C NMR.
3. to perform the antimicrobial activities of bis thiourea derivatives against *E.coli.*

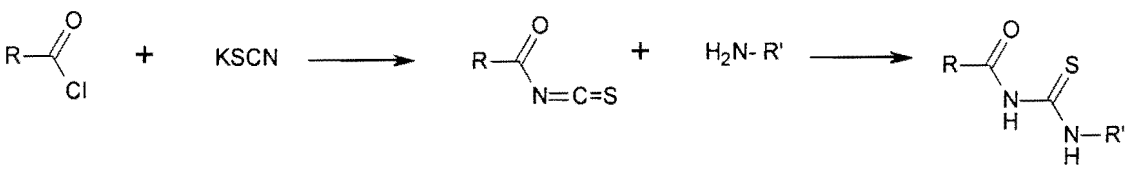
Chapter 2

Literature review

2.1 Thiourea

Thiourea is an organic compound with molecular formula of CH_4N_2S. Thiourea is soluble in water and insoluble in non polar solvent. It also soluble in protic and aprotic organic solvents. Thiourea is well known compound with various types of usage such as herbicides, pharmaceuticals, pesticides, rodenticides, vulcanization accelerator, and as building agent in organic synthesis reaction (Mohanta *et al.,* 1999).

Thiourea can be synthesized by reacting amino group and thiocyanate group in an appropriate solvent. The general reaction is shown in Scheme 1.

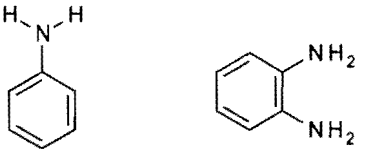


R= aryl or alkyl group

Scheme 1: The general reaction for synthesis of thiourea group.

2.1.1 Amine groups in synthesis of thiourea derivatives.

The synthesis of thiourea and its derivatives performed by Mohanta *et al.* (1999) had used aniline **4**, a primary amine group to synthesize symmetrical thiourea derivatives. They were also used o-pheylenediamine **5** as shown in Figure 3 to synthesize 2(1H)-benzimidazolinethione, a heterocyclic thiourea compound **5** was reacted with 1-(methyldithiocarbonyl)imidazole, a transfer reagent, in ethanol reflux to give 2(1H)-benzimidazolinethione in high yield.



(4) (5)

Figure 3: Primary amine compounds

Instead of using an amine group, Bayer *et al.* (1996) have introduced amino acid in form of methyl ester L-valine to produce thiourea derivatives, 2{[(3,3-Diethylthioureido)phenylmethyl]amino}-3-methylbutyric acid **6** that functioned as selective ligands for the concentration and separation of metal cations of Pt group. The used of amino acid in this research is based on its recognition by living things and its ability to rise the selectivity toward targeted cations. Compound **6** prepared by reacting 3-(chlorophenylmethylene)-1,1-diethylthiourea with methyl ester of L-valine in acetone reflux for 1 h.

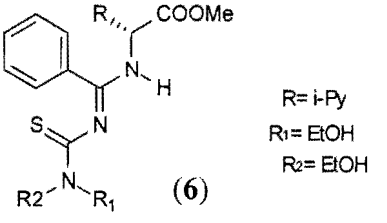
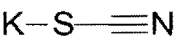


Figure 4: 2{[(3,3-Diethylthioureido)phenylmethyl]amino}-3-methylbutyric acid methyl ester

2.1.2 Thiol groups in synthesis of thiourea derivatives

The thiol group that required in synthesis of thiourea derivatives basically is a group having sulphur atom bonded to carbon atom. It is mainly introduced in formed of isothiocyanate or thiophosgene (Mohanta, et al., 1999). The thiocyante group is usually used when it react with other compound containing good leaving group as it will produce the desire structure of

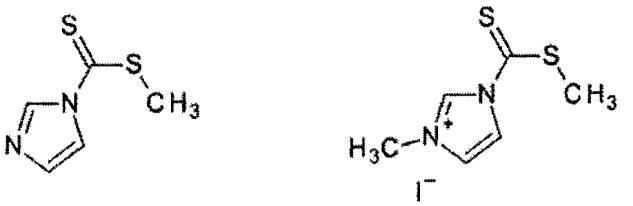
intermediate compound before it react with appropriate animo compound to produce thiourea derivatives. For instance, Arslan *et al.* (2009) reported on the reaction of potassium thiocyanate **7** that reacted with cyclohexanecarbonyl chloride to form thiocyanatocarbonyl compound that will undergo further reaction with a series of secondary anime compounds to produce (*N*-(diethylcarbamothioyl)cyclohexanecarboxamide thiourea derivatives.



(7)

Figure 5: The structure of Potassium thiocyanate

Mohanta *et al.* (1999) had synthesized a useful thiocarbonyl transfer reagent and used it instead of isothiocyanate or thiophosgene as a source of thiol group to produce substituted thiourea compounds. They synthesized 1-(methyldithiocarbonyl)imidazole **8** and its salt, 3-methyl-1-(methyldithiocarbonyl)-imidazolium iodide **9** and then these compounds had been reacted with amino acids to produce thiourea derivetives, benzimidazoline-2-thione and imidazolidine-2-thione.



(8) **(9)**

Figure 6: The thiocarbonyl transfer reagents

2.1.3 Solvent used in the synthesis of thiourea

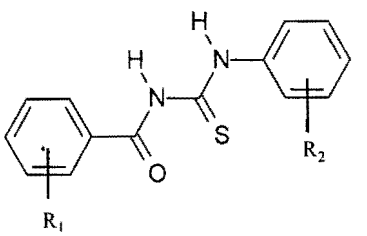
Solvent has also played an important role in the synthesis of thiourea. There are various types of solvent has been reported in the synthesis of thiourea. For example, Kaswala *et al.*, (2010) had used ethanol as a solvent in synthesis of *s*-triazinyl thoiurea derivatives. Acetone is also a well

known solvent in the synthesis of thiourea. Didier *et al.* (2009) had reported to use THF as a solvent to dissolve diamine solution and aryl isothiocyanate in process to synthesize thiourea. Rauf, *et al.* (2009) had widely used acetone in his research to produce some *N-N'*-disubtituted thiourea derivatives by reaction between prepared benzoyl isothiocyanate and substituted aniline. Acetone is considered as a better choice for solvent as it gave very high yield of the disubtituted thiourea products (89-92%).

2.2 Biological properties of thiourea derivatives

2.2.1 Thiourea derivatives with antibacterial activity

Thiourea derivatives have been reported to have antibacterial activities. Saeed *et al.* (2009) reported that the 1-aroyl-3-aryl thiourea **10** (as shown in Figure 7) with chlorine substituted synthesized by reacting benzoyl isothiocyanate and aniline. Compound **10** showed just moderate activity against *Staphyloccus aureus, Basillus subtilis, Pseudomonas aueroginosa* and *Escherichia coli*. The *in vitro* evaluation of antibacterial activity against those four strains was performed using Kirby-Bauer method. The presence of halo group in thiourea **10** gave enhancement of inhibitory activity.

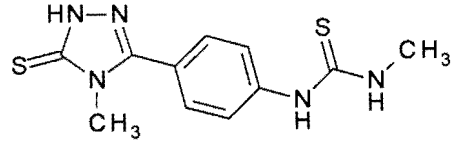


(10)

R1=H, R2=3-Cl

Figure 7: 1-Aroyl-3-aryl thiourea with antibacterial activity

However, in 2005, Fernandez *et al.* have synthesized some 3-thioxo/alkylthio-1,2,4-triazoles with a substituted thiourea moiety that reported as excellent antimycobacterial agent against *M. tuberculosis* in monolayers of mouse bone marrow macrophages. 1-methyl-3-[4-(4-methyl-5-thioxo-1H-1,2,4-triazol-3-yl)phenyl]thiourea **11** (as shown in Figure 8) is the synthesized bis thiourea derivative that reported to inhibit 90% of the mycobacterial growth. Compound **11** is synthesized from reaction of methyl thiocyanate and 3-(4-aminophenyl)-4-methyl-1H-1,2,4-triazole-5-thione in the present of methanol as a solvent. The methyl substituent at position of triazole ring or the one substituted at the therminal nitrogen of thioureas was reported to enhance the antimycobacterial activity of compound **11**. The more bulky substituent in other synthesized thiourea derivatives had showed lower antimycobacterial activity.



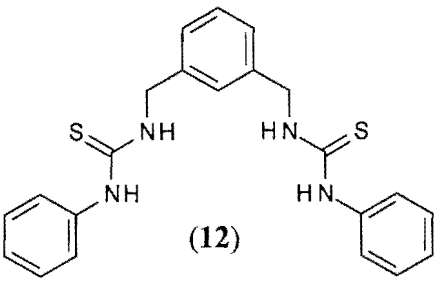
(11)

Figure 8: 1-methyl-3-[4-(4-methyl-5-thioxo-1H-1,2,4-triazol-3-yl)phenyl]

2.2.2 Thiourea derivatives with ion-selective activity

Other than possessing pharmaceutical properties, thiourea derivatives were also reported for their ability as ion-selective compound. Based on this property, thiourea derivatives are widely used in production of ion electrode and receptor. Nishizawa *et al.* (1998) has synthesized series of neutral bis thiourea ionophore which functioned as ion-selective electrodes. For example, *α,α'-bis(N'*-phenylthioureylene)-*m*-xylene **12** is one of the synthesized bis thiourea which is very

sensitive and selective electrode toward sulfate ion. Thiourea **12** is synthesized from reaction between prepared *n*-phenylthiocyanato and *m*-phenylenedimethanamine in acetone reflux for 4 h.

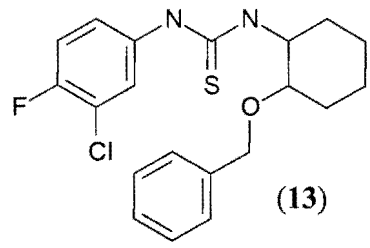


(12)

Figure 9: The structure of neutral ionophore, *α,α'-bis(N'*-phenylthioureylene)-*m*-xylene

2.2.3 Thiourea derivatives with antitumor activity

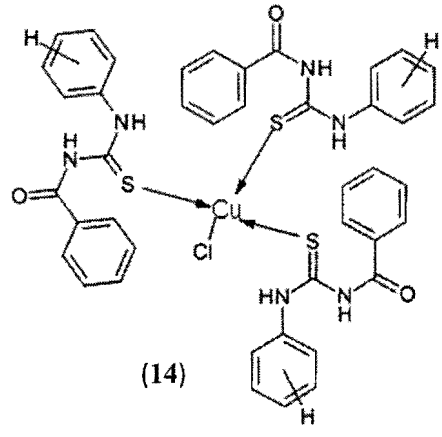
Besides showing antibacterial and antifungal activities, thiourea derivatives also found to be a potent antitumor agent. Manjula *et al.* (2008) reported that optically active thiourea and its 2-aminobenzothiazol derivatives have shown moderate antitumor activity against two human cancer cell lines namely as MCF-7 and HeLa. The optically active amine was reacted with thiophosgene to obtain optically active isothiocyanates which then been condensed with 4-fluoro-3-chloro aniline yielded various optically active thiourea derivatives. 1-(2-(benzyloxy)cyclohexyl)-3-(3-chloro-4-fluorophenyl)thiourea **13** is one of the derivative that gave the higher IC50 values for MCF-7 and HeLa cells in range of 15–30 μM and 33–48 μM, respectively. Thiourea **13** is shown in Figure 10.



(13)

Figure 10: The structure of 1-(2-(benzyloxy)cyclohexyl)-3-(3-chloro-4-fluorophenyl)thiourea

Complexes of thiourea derivatives have also show antitumor activity. Rauf *et al.* (2009) has synthesized complexes of copper(I) with *N,N'*-disubtituted thioureas for antitumor behavior against human cell lines such as carcinomas A498 (Renal), EVSA-T (Breast), H226 (Lung), IGROV (Ovarian), M19 (Melanoma-Skin), MCF-7 (Breast) and WIDR (Colon). The complexes are reported to exhibit a moderate cytotoxic activity against all the cancer cell lines used. As shown in Figure 11, [CuCl(1-Phenyl-3-benzoylthiourea)$_3$] **14** is one of *N,N'*-disubtituted thiourea complexe that reported to give good antitumor activity. The result is due to directional migration of endothelial cell in angiogenesis by stimulation of copper(II) atom. The antitumor activity for ligand for the complexes is not reported.



(14)

Figure 11: Complex of copper(I) with *N,N'*-disubtituted thiourea

2.2.4 Thiourea derivatives with antiviral activity.

In 2008, Kucukguzel *et al.* (2008) has synthesized novel thiourea derivatives which analyzed on antiviral activity. A bis thiourea derivative, 1-[4-[(4-allyl-5-thioxo-1H-1,2,4-triazol-3-yl)methoxy]phenyl]-3-phenyl-thiourea **15** is reported as the most active derivatives against Coxsackie virus B4 and thymidine kinase positive Varicella-zoster virus. The compound **15** was obtained from reaction of 5-[(4-aminophenoxy) methyl]-4-alkyl/aryl-2,4-dihydro-3H-1,2,4-triazole 3-thiones and benzoyl thiocyanate under acetone reflux. The good antiviral activity was resulted by the presence of allyl group at N-4 of the triazole ring and a phenyl moiety at terminal nitrogen of thiourea in the compound **15**.

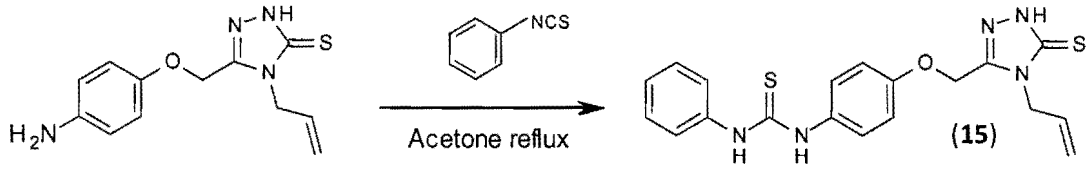
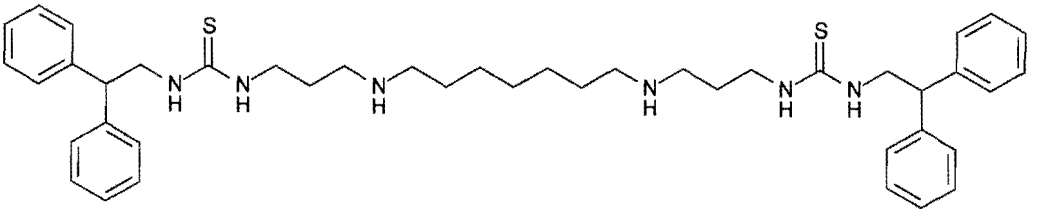


Figure 12: The preparation of 1-[4-[(4-allyl-5-thioxo-1H-1,2,4-triazol-3-yl)methoxy]phenyl]-3-phenyl-thiourea

2.2.5 Thiourea derivatives with anticancer activity

Sharma *et al.* (2010) has synthesized bis thiourea derivatives that shown good anticancer activity. The synthesized 1-(2,2-diphenylethyl)-3-[3-{7-[3-(2,2-diphenylethylcarbamo thioylamino)propylamino]heptylamino}propyl]thiourea **16** has been reported to induce increases in methylation at the histone 3 lysine 4 (H3K4) chromatin mark, a specific target of lysine-specific demethylase , in Calu-6 lung carcinoma cells. Lysine-specific demethylase is the enzyme that can control gene overexpression which can contribute to development of cancer. The compound **16** was prepared by the reaction between prepared *N,N'-bis*(3-aminopropyl)heptane-1,7-diamine and [isothiocyanato(phenyl)methyl]benzene.



(16)

Figure 13: Bis thiourea derivatives with anticancer activity

Chapter 3

Materials and Methods

3.1. Materials

Isophthaloyl dichloride, potassium thiocyanate, glycine, *beta*-alanine, L-alanine, L-phenylalanine and aniline were purchased from Merck and used without purification. Acetone was distilled over magnesium sulfate anhydrous. All other reagents and solvent were used as received.

3.2 Measurement

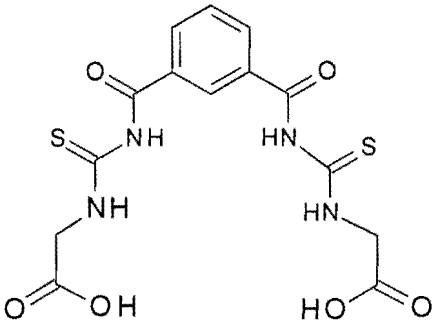
The synthesized compounds were characterized using Perkin Elmer Spectra GX Fourier Transform Spectrometer (FTIR) with pure KBr disc. ^{1}H NMR and ^{13}C NMR spectra were recorded using Joel spectrometer at 500 MHz. Perkin Elemer. Melting points were recorded using on Stuart SMP3 and uncorrected.

3.3 Methodology

3.3.1General procedure for the synthesis of bis thiourea derivatives.

Isophthaloyl dichlorid in dry acetone was added drop wise to a suspension of potassium thiocyanate in dry acetone. The mixture was stirred for 1 h at room temperature. The white potassium chloride (KCl) was filtered. Amino acid in dry acetone was added into the filtrate and the resulting mixture was heated at 50 °C under reflux condition for 7 h. The mixture was cooled to room temperature and filtered. The filtrate was poured into a beaker with ice to form solid. The solid product was washed with ethanol and purified by recrystallization from an ethanol acetonitrile (1:1) mixture.

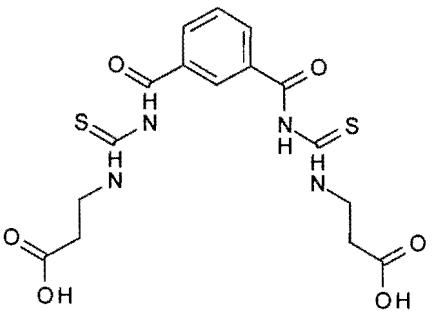
3.3.2 Synthesis of 2-[[3-(carboxymethylcarbamothioylcarbamoyl)benzoyl] carbamothioylamino] acetic acid (18)



(18)

Isophthaloyl dichloride (0.203 g, 1 mmol) in 15 mL of dry acetone was added drop wise to a suspension of potassium thiocyanate (0.194 g, 2 mmol) in of dry acetone (15 mL). The mixture was stirred for 1 h at room temperature and KCl was filtered. Glycine (0.150 g, 2 mmol) in dry acetone (15 mL) was added into the filtered solution and resulting mixture was heated at 50 °C under reflux condition for 7 h. The mixture was cooled to room temperature and filtered. The filtrate was poured into a beaker with ice to form solid. The crude was recrystallized in EtOH : CH_3CN to get (1 : 1) to get compound **18** (0.27 g, 73%) as a yellowish solid, m.p: 226-227 °C; ν_{max} (nujol mull/ cm^{-1}) 3233, 2922, 1729,1679, 1602, 1557, 1231. δ_H (500 MHz, DMSO-D6) 4.23 (4H, d, 2xCH_2), 7.69 (1H, t, Ar-H), 8.14 (2H, d, Ar-H), 8.47 (1H, s, Ar-H), 11.10 (2H, t, 2xNH), 11.40 (2H, s, 2xNH). δ_C (500 MHz, DMSO-D6) 47.82, 128.46, 129.06, 131.95, 133.000, 166.97, 169.47, 179.75.

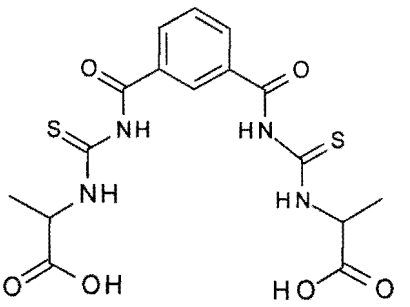
3.3.3 Synthesis of 3-[[3-(2-carboxyethylcarbamothioylcarbamoyl)benzoyl]carbamothioylamino]propanoic acid (19)



(19)

Isophthaloyl dichloride (0.203 g, 1 mmol) in 15 mL of dry acetone was added drop wise to a suspension of potassium thiocyanate (0.194 g, 2 mmol) in 15 mL of dry acetone. The mixture was stirred for 1 h at room temperature. The white precipitate (KCl) was filtered. *Beta*-alanine (0.178 g, 2 mmol) in dry acetone (15 mL) was added into the filtered solution and resulting mixture was heated at 50 °C under reflux condition for 12 h. The mixture was cooled to room temperature and filtered. The filtrate was poured into a beaker with ice to form solid. The crude was recrystallized in EtOH : CH_3CN to get (1 : 1) to get compound **19** (0.18 g, 67%) as a white solid, m.p: 219.1-220.2 °C; ν_{max} (nujol mull/ cm^{-1}) 3082, 2666, 2553, 1691, 1611, 1580, 1520, 1281. δ_H (500 MHz, DMSO-D6) 2.66 (4H, t, 2xCH_2), 3.84 (4H, q, 2xCH_2), 7.63(1H, t, Ar-H), 8.15 (2H, d, Ar-H), 8.48 (1H, s, Ar-H), 11.01 (2H, t, 2xNH), 11.33 (2H, s, 2xNH). δ_C (500 MHz, DMSO-D6) 30.15, 32.50, 128.42, 129.00, 131.89, 132.94, 166.94, 173.15, 179.77.

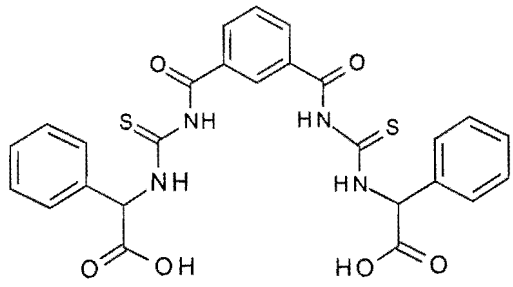
3.3.4 Synthesis of 2-[[3-[(2-hydroxy-1-methyl-2-oxo-ethyl) carbamothioylcarbamoyl] benzoyl]carbamothioylamino]propanoic acid (20)



(20)

Isophthaloyl dichloride (0.203 g, 1 mmol) in 15 mL of dry acetone was added drop wise to a suspension of potassium thiocyanate (0.194 g, 2 mmol) in dry acetone (15 mL). The mixture was stirred for 1 h at room temperature and KCl was filtered. L-alanine (0.178 g, 2 mmol) in dry acetone (15 mL) was added into the filtered solution and resulting mixture was heated at 50 °C under reflux condition for 7 h. The mixture was cooled to room temperature and filtered. The filtrate was poured into a beaker with ice to form solid. The crude was recrystallized in EtOH : CH_3CN to get (1 : 1) to get compound **20** (0.28 g, 70%) as a white solid, m.p: 207-208 °C, ν_{max} (nujol mull/ cm^{-1}) 3365, 3225, 3096, 2992,1730, 1693, 1600, 1514, 1220. δ_H (500 MHz, DMSO-D6) 1.51 (6H, d, 2xCH_3), 4.85 (2H, m, 2xCH), 7.69 (1H, t, Ar-H), 8.14 (2H, d, Ar-H), 8.49 (1H, s, Ar-H), 11.26 (2H, d, 2xNH), 11.49 (2H, s, 2xNH). δ_C (500 MHz, DMSO-D6) 17.15, 53.20, 128.67, 129.08, 131.80, 133.12, 167.43, 172.82, 179.57.

3.3.5 synthesis of 2-[[3-[(2-hydroxy-2-oxo-1-phenylethyl)carbamothioylcarbamoyl]benzoyl] carbamothioylamino]-2-phenyl-acetic acid (21)



(21)

Isophthaloyl dichloride (0.203 g, 1 mmol) in 15 mL of dry acetone was added drop wise to a suspension of potassium thiocyanate (0.194 g, 2 mmol) in dry acetone (15 mL). The mixture was stirred for 1 h at room temperature and KCl was filtered. L-phenylalanine (0.302 g, 2 mmol) in dry acetone (15 mL) was added into the filtered solution and resulting mixture was heated at 50 °C under reflux condition for 7 h. The mixture was cooled to room temperature and filtered. The filtrate was poured into a beaker with ice to form solid. The crude was recrystallized in EtOH : CH_3CN to get (1 : 1) to get compound **21** (0.43 g, 91%) as a yellowish solid, m.p: 229.0-230.2 °C; ν_{max} (nujol mull/ cm^{-1}) 3227, 3027, 2997, 1718, 1690, 1600, 1509, 1417. δ_H (500 MHz, DMSO-D6) 5.14 (2H, d, 2xCH), 7.21-7.30 (10H, m, Ar-H), 7.70 (1H, t, Ar-H), 8.10 (2H, d, Ar-H), 8.48 (1H, s, Ar-H), 11.17 (2H, d, 2xNH), 11.54 (2H, s, 2xNH). δ_C (500 MHz, DMSO-D6) 58.73, 126.96, 128.46, 129.25, 131.69, 133.24, 136.18, 167.41, 171.33, 180.08.