University of Benghazi
Faculty of Education Almarj

جامعة بنغازي
كلية التربية – المرج
ISSN 2518-5845

Global Libyan Journal
المجلة الليبية العالمية

العدد الرابع والأربعون / يوليو / 2019

Synthesis, Characterization and Biological Screening of 3,3-Diethyl Benzoylthiourea derivatives

Halima I. Khalil[1], Aisha AL-abbasi[2], Mohammed B. Kassim[3] and ***Adel M. Najar[1a]**

[1]Department of Botany, Faculty of Arts and Science - El-Marj, University of Benghazi, Benghazi, Libya.

[1a]Department of Chemistry, Faculty of Arts and Science - El-Marj, University of Benghazi, Benghazi, Libya.

[2]Chemistry Department, Faculty of Science, Sebha University, Sebha, Libya.

[3]School of Chemical Sciences and Food Technology, Faculty of Science and Technology, University Kebangsaan Malaysia, UKM 43600 Bangi, Selangor, Malaysia

Synthesis, Characterization and Biological Screening of 3,3-Diethyl Benzoylthiourea derivatives

Abstract

Synthesis, characterization and Antimicrobial Activity of some 3,3-diethyl benzoylthiourea derivatives have been described and tested for in vitro antibacterial activity against Proteus mirabilis, Escherichia coli (gram negative). Bacillus subtilis, Staphylococcus aureus (gram positive) bacteria and in vitro antifungal activity against Candida parapsilosis and Candida albicans by employing agar and potato dextrose agar, respectively, using a paper disc method. The results reveal that the compounds exhibited moderate antibacterial and antifungal activities. The standard antibiotics have been used to compare the results. However, the structural of synthesized compounds were confirmed by spectral data.

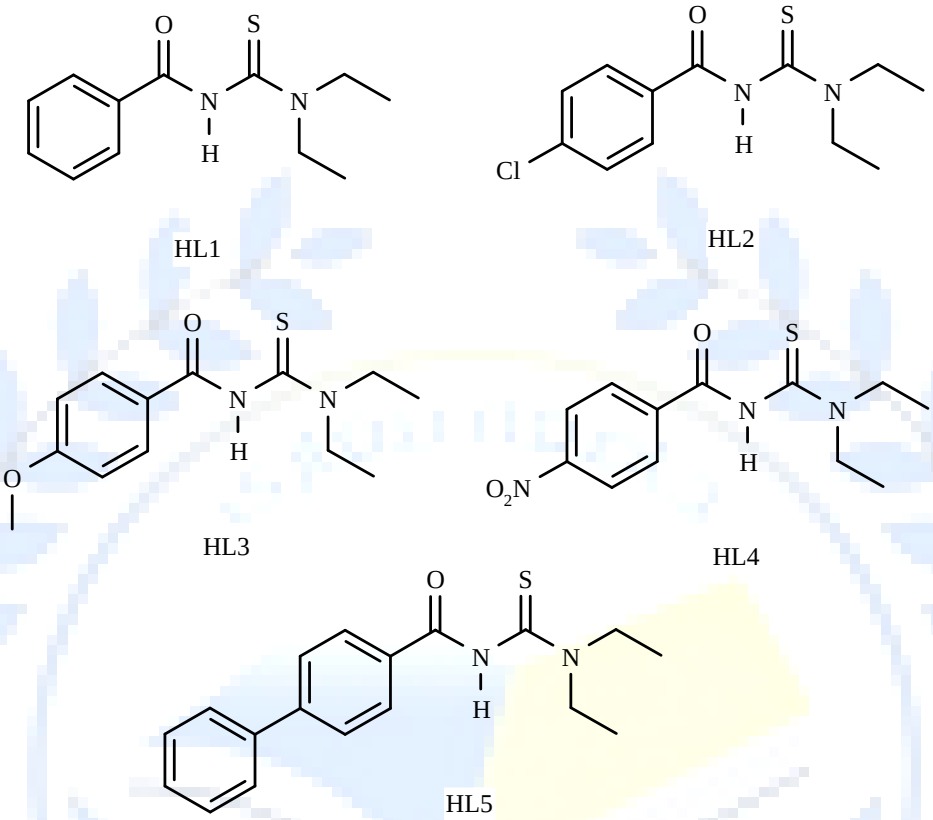
Key words: Antimicrobial activity; Antibacterial; Antifungal; Antibiotic; benzoylthiourea compounds.

الملخص بالعربي

تم تحضير وتشخيص ودراسة النشاط البيولوجي لبعض مشتقات مركب 3و3- ثنائي بينزويل اليوريا حيث تم الاختبار البيولوجي لهذا المركب في المختبر ضد انواع البكتريا التالية : البروتي ميرايبلس والاشركية القولونية(سالبة الجرام) وسيبلس سوبتيلس و ستربتوكوكس(موجبة الجرام) كذلك تم اختبار المركبات علي انواع من الفطريات مثل كانديدابارابيسلوساس و كانديداالبيكانس باستخدام الاجار وديكستروس البطاطا علي التوالي باستخدام طريقة قرص الورق. كشفت النتائج ان المركبات اظهرت نشاط مضاد معتدل ضد البكتريا والفطريات كما استخدمت المضادات الحيوية القياسية لمقارنة النتائج المتحصل عليها. كما يمكننا القول ان المركبات المستخدمة بعد تحضيرها تم التأكد من الحصول عليها وتشخيصها باستخدام تقنيات الطرق الطيفية مثل مطياف الكتلة وتحليل عدد البروتونات و طيف الاشعة فوق البنفسجية وتحت الحمراء .

1-Introduction

Chemistry of Benzoylthiourea has developed an increasing interest due to the prospective use of these compounds in the field of medicine, as an antitumour activity [5,6,7] , agriculture [4,15], as well as their biologically relevant properties [3,11] . The influence of substituents on the physical and chemical properties of compounds has long been an important focus of interest in chemistry [10,14]. The introduction of various substituents onto the compounds (such as methoxy group, nitro group, phenyl fragment and chlorine atom in the para position of benzoyl moiety in benzoylthiourea compound) may increase their activity and tolerate their recognition by living organisms. The structural modifications on the compounds are expected to enhance the solubility (hydrophobic/ hydrophilic) of the benzoylthiourea compounds and increase their effectiveness.Thus, this paper will discuss the biological screening of some benzoylthiourea compounds (Scheme 1) for their in-vitro growth inhibiting activity against different strains of Proteus mirabilis, Escherichia coli,Bacillus subtilis, Staphylococcus aureusbacteriaand Candida parapsilosis and Candida albicans fungi. The results were compared with the standard antibiotic.



Scheme 1.The chemical structure of benzoylthiourea compounds

2-Materials and Methods

2.1-Materials

Organic reagents were purchased from Aldrich and used as received. The HL1, HL2, HL3, HL4 and HL5 2 were prepared according to the literature methods [1,13].

2.2-Apparatus

Melting points were determined by using an electro thermal digital apparatus model H025550/IA91000. Micro elemental analysis for CHN (and S) was carried out by using a Carlo ErbaEA 1108 elemental analyser. The IR- spectra of the products were recorded on a Nicolet 6700FT-IR spectrometer, in the range of 200-4000 cm-1. Samples were prepared as (KBr). Functional groups that were expected to be featured by benzoylthiourea ligands are

C=O, C=S and N–H.The electronic absorption spectra were recorded using a 1650 PC Shimadzu spectrophotometer, in the range 200-400nm in a quartz cell. Each sample was dissolved in dichloromethane (DCM) to obtain a 1×10-4 M solution for ligands.

2.3-Synthesis of Benzoylthiourea compounds

Benzoylthiourea compounds were prepared according to the procedures reported in the literature [1,2,13] .Typically, a solution of benzoyl chloride or its derivatives (0.01 moles) in acetone was added dropwise to a solution of potassium thiocyanate (0.01 moles) in acetone, with continuous stirring. A white solid of ammonium chloride immediately formed and the reaction mixture was stirred for 30 minutes. The precipitate was removed by filtration and washed several times with cold acetone. The filtrate was cooled in an ice bath (5 to 10oC) for 15min. A cold solution of secondary amine (0.01 moles) in acetone was added and the resulting mixture was stirred for 2 to 5 hours at room temperature to obtain a clear yellow solution.The solvent (acetone) was reduced to one-third of the original volume using a rotatory evaporator and the residue was poured onto ice water with vigorous stirring. Solid benzoylthiourea was formed immediately. The mixture was stirred for several minutes before the precipitate was filtered, washed with water, and dried. The reaction was monitored and purity was checked using TLC, on Merck TLC plates (10 x 20 cm) with acetone as an eluent for most cases. The final products were recrystallized from mixed solvents of ethanol/acetone (2:1 v/v) or acetone/dichloromethane (3:1 v/v). The compounds were subjected to elemental analysis, infrared spectroscopy.

1-benzoyl-3,3-diethylthiourea (HL1): Yield (90%); mp 98-100°C, elemental analysis for C12H16N2OS: Calculated (anal.): C, 60.99 (60.68); H, 6.82 (6.61); N, 11.85 (12.37) %. FT-

IR (KBr, cm-1): 1652 (C=O), 3268 (N–H). UV (DCM, λmax (nm), ε x 103 (L mole-1 cm-1)):244(26.9), 282(15.510) and 342(1.134).

1-(4-chlorobenzoyl)-3,3-diethylthiourea (HL2): Yield (89%); mp 151-152°C, elemental analysis for C12H15ClN2OS: Calculated (anal.): C, 53.23 (52.69) H, 5.58 (5.57); N, 11.21 (11.06) %. FT-IR (KBr, cm-1): 1643 (C=O), 3279 (N–H). (DCM, λmax (nm), ε x 103 (L mole-1 cm-1)):250(36.085) and 345(1.3).

1,1-diethyl-3-(4-methoxybenzoyl)thiourea (HL3): Yield (90%); mp 134-135°C, elemental analysis for C13H18N2O2S: Calculated (anal): C, 58.62 (59.60); H, 6.81 (7.30); N, 11.10 (10.90) %. FT-IR (KBr, cm-1): 1634 (C=O), 3300 (N–H). UV (DCM, λmax (nm), ε x 103 (L mole-1 cm-1)):251(21.666).

1,1-diethyl-3-(4-nitrobenzoyl)thiourea (HL4): Yield (84%); mp 161-162°C, elemental analysis for C12H15N3O3S: Calculated (anal.): C, 51.23 (51.47); H, 5.37 (5.08); N, 14.94 (15.06) FT-IR (KBr, cm-1): 1648 (C=O), 3284 (N–H). UV (DCM, λmax (nm), ε x 103 (L mole-1 cm-1)):267(10.690).

1,1-diethyl-3-(4-phenylbenzoyl)thiourea (HL5):Yield: (80%); mp 145-146°C, elemental analysis for C18H20N2OS: Calculated (anal.): C, 69.20 (68.80); H, 6.45 (7.21); N, 8.97 (8.92) %. FT-IR (KBr, cm-1): 1651 (C=O), 3266, 3229 (N–H). UV (DCM, λmax (nm), ε x 103 (L mole-1 cm-1)):281(14794).

2.4-Experimental procedure for Antibacterial Activities

Antibacterial activity was evaluated using a paper disc plate method [8,9]. The nutrient agar medium was prepared in a 6.0 mm diameter paper (Whatman No. 1) discs. One mg of each compound was dissolved in one mL of DMF solution, to make a stock solution. New bacterial cultures of all examined organisms were subcultured from the old culture

(previously prepared) by picking one colony from the old one and spreading it onto a new agar plate. These plates were incubated for 24 h at 37 ºC and then each new bacterium was refrigerated (2−8 °C) prior to use. The agar medium was prepared as per the manufacturer's instructions and sterilized in an autoclave at 120 °C for 2 h and then cooled to 45-60 °C. The medium was then poured into several Petri dishes and allowed to solidify. The bacteria were subcultured in the agar medium, and incubated for 24 h at 37 °C. The 6.0 mm diameter discs were placed on Petri dishes, which were previously seeded with organisms. These discs were soaked by 10 µg/10mL of tested compounds and incubated at 37 °C for 24 h. Standard antibacterial drug (Tobramycine) 6.0 mm diameter discs were used for comparisonunder similar conditions, and DMF solvent was also examined to know the activity of the solvent. The experiment was carried out in three replicates per treatment and the antibacterial activity of each compound was estimated on the basis of the size of the inhibition zone formed around the paper discs on the seeded agar plates. The inhibition zone around each disc was measured and the results were recorded in the form of inhibition zones (in millimetres and percentages).

2.5-Experimental procedure of Antifungal screening

The antifungal activity of the benzoylthiourea compounds were evaluated against Candida parapsilosis and Candida albicans by the agar plate technique [9,12] The fresh growth fungi cultures of both examined fungi were subcultured from the old culture by picking one colony from the old one and spreading it onto a new potato dextrose agar plate. These plates were incubated for 48 h at 37 ºC, and then each new fungus was refrigerated prior to use. The potato dextrose agar medium was prepared as per the manufacturer's instructions and sterilized in an autoclave at 120 oC for 2 h and then cooled to 45-60 oC. The medium was then poured into several Petri dishes and allowed to solidify. The subcultured fungi were

distributed into the potato dextrose agar media, and incubated for 48 h for the bacteria and 48 h for the fungi at 37 ^{0}C. After 24 h, the 6 mm diameter discs were placed onto an appropriate medium previously seeded with fungi in Petri plates; these discs were soaked by 10 µg/10 mL of tested compounds and incubated at 37 °C for 48 h. Standard antifungal drug (Nystatine) 6.0 mm diameter discs were used for comparison under similar conditions, and DMF solvent was also examined to know the activity of the solvent. The experiment was carried out in three replicates per treatment. The linear growth of the fungus was obtained by measuring the diameter of the inhibition zone formed around the paper discs on the seeded agar plates.

3-Results and Discussion

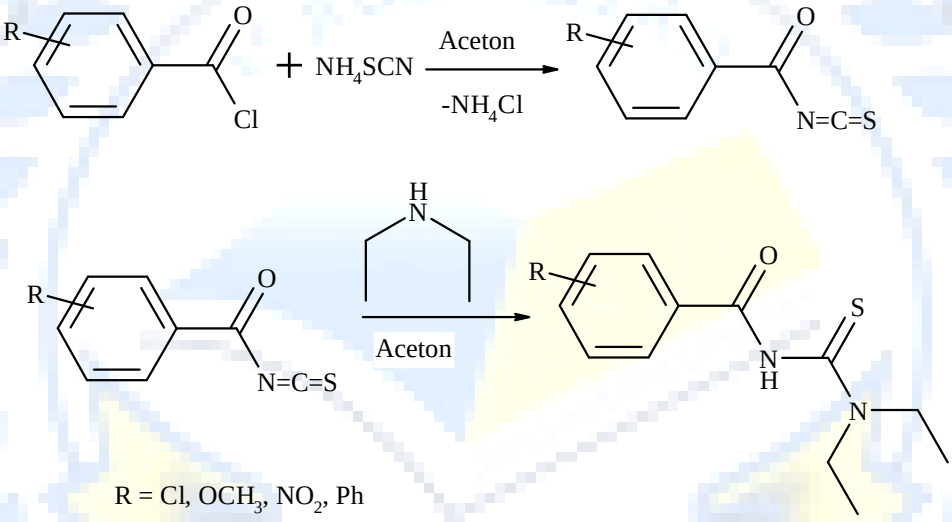
3.1- Synthesis and Physical Properties

Benzoylthiourea compounds were synthesized in good yield (Scheme 2) and their structures were confirmed by elemental analysis and IR-spectrafor the presence the C=S, N-H and C=O function groups table 1.

The solid state infrared spectra of benzoylthiourea were recorded in the range of 200-4000 cm-1. Table 1 shows the characteristic bands for C=O, C=S, and N–H. The profiles of the infrared spectra of all benzoylthiourea compounds appear quite similar. The presence of benzoylthiourea moiety is shown by the presence of stretching frequencies of (N–H), (C=O), and (S=C) bands at approximately 3384-3268, 1651-1634, and 1224-1229 cm-1, respectively. The high intensity and low frequency values of the ν(C=O), due to the resonance effect, as a result of the conjugated resonance with the phenyl ring. The electronic conjugation results in the delocalization of the π electrons of both C=O and phenyl group. Since C=S group is less

polar than the C=O group with higher oscillator mass expected to appear at a lower energy which encroaches onthe fingerprint region and, therefore, is difficult to identify.

Benzoylthiourea compounds exhibited a strong intensity band for the π→π* transitions of the benzoyl group and the two absorption bands assigned to the n→π* transitions for the carbonyl and thiocarbonyl chromophores (Table 1). It was observed that band II (n→π* transition exhibits a blue shift to the extent that it appears as a shoulder of n→π* band.



Scheme 2. General reaction procedures for synthesis of benzoylthiourea.

Table 1 Physical properties of benzoylthiourea compounds

Comp.	Colour	IR (cm-1)			CHN calc.(Exp.)		
		C=O	C=S	N-H	C%	H%	N%
HL1	White	1652	1224	3268	60.99 (60.68)	6.82 (6.61)	11.85 (12.37)
HL2	White	1643	1226	3279	53.23 (52.69)	5.58 (5.57)	11.21 (11.06)

HL3	Yellow	1634	1225	3300	58.62 (59.60)	6.81 (7.30)	11.10 (10.90)
HL4	Yellow	1648	1227	3384	51.23 (51.47)	5.37 (5.08)	14.94 (15.06)
HL5	White	1651	1228	3266	69.20 (68.80)	6.45 (7.21)	8.97 (8.92)

Table 2 Electronic spectral for benzoylthiourea compounds (λmax,nm)

Comp.	R	n→π* (ε,L mol-1 cm-1)	n→π* (ε,L mol-1 cm-1)	n→π* (ε,L mol-1 cm-1)
HL1	H	244(26900)	282(15500)	342(1100)
HL2	p-Cl	250(36100)	Shoulder	345(1300)
HL3	p-OCH3	251(21700)	Shoulder	shoulder
HL4	p-NO2	267(10700)	Shoulder	shoulder
HL5	p-Ph	Shoulder	281(14800)	Shoulder

3.2- Antimicrobial activities

The in-vitro antimicrobial screening effects of the benzoylthiourea compounds were carried out using a zone inhibition technique against four bacterial strain namely Bacillus subtilis (+), Staphylococcus aureus (+), Eschirichiacoli (-). Proteus mirabilis (-) and two fungal strains, namely Candida parapsilosis and Candida albicans. The study employs a disc diffusion method, using a nutrient agar medium for antibacterial activity and potato dextrose agar

media for antifungal activity. The results of the in-vitro antibacterial and antifungal activities promisingand were summarized in Tables 3 and 4.

Based on the results, all compounds showed moderate antibacterial and fungi activities against those selected organisms. The comparison between the anti-bacterial and anti-fungal activities of the compounds are presented in Figures 1 and 2. The data reveals that the tested benzoylthiourea and their free compounds have approximately equivalent activities. Considering both results obtained from antifungal and antibacterial tests, the tested compounds are more active towards fungi than bacteria. On the basis of the observed zones of inhibition, compound with chloro-substituent was particularly active against all tested microorganisms.

Table 3 Antibacterial activities of benzoylthiourea(Inhibition zone (mm))

Bacteria	Tobramycin	HL1	HL2	HL3	HL4	HL5
B.S.	34	9	10	10	11	10
S.A	12	10	11.5	10.3	9.5	10
E. Coli	16	10.5	12	11	11.5	11
P.M.	15	9	11	9	10	9

Table 4Aantifungalactivities of benzoylthiourea (Inhibition zone (mm))

Fungi	Nystatine	HL1	HL2	HL3	HL4	HL5
C.alb.	13	8	8	7.5	8.5	8
C. paraps.	15	8.5	9	9	9.5	9

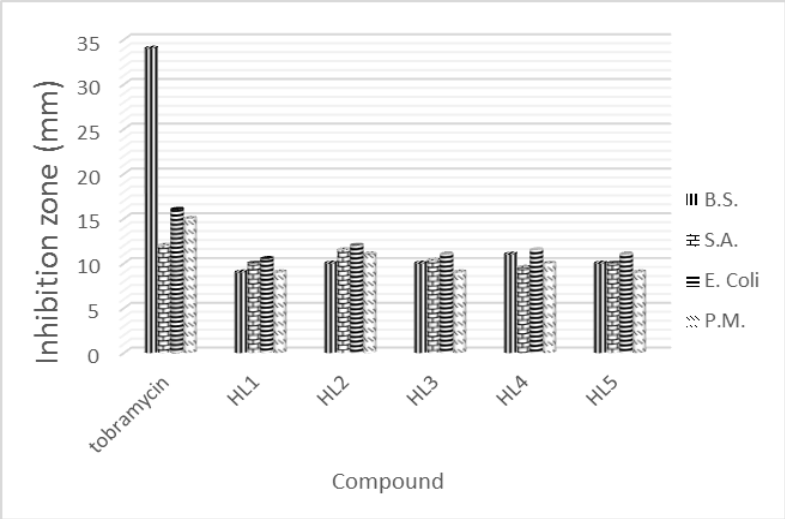


Figure 1 Antibacterial activities of benzoylthiourea

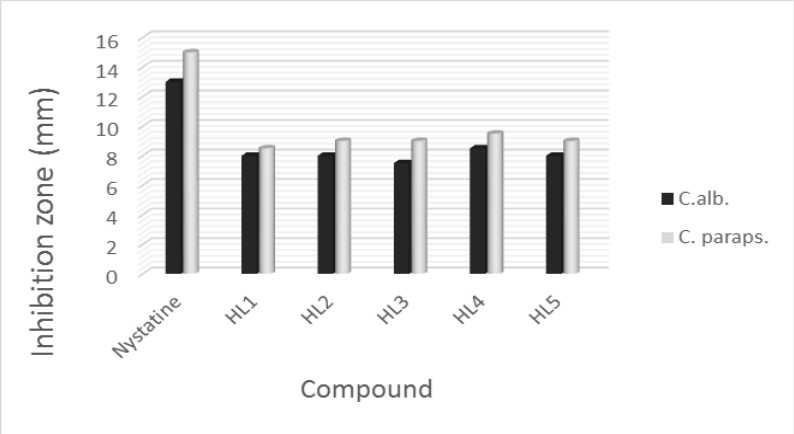


Figure 2 Antifungal activities of benzoylthiourea

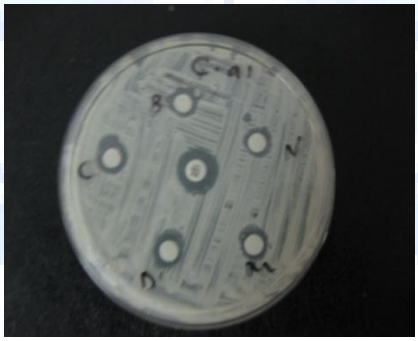


Figure 3 Inhibition zone formed around the paper discs on the seeded potato dextrose agar plates

Conclusion

We have prepared some benzoylthiourea derivatives and their in vitro antimicrobial activities were evaluated. Compounds exhibited equal moderate antibacterial activities against gram-negative and gram-positive, considering the results obtained from antifungal and antibacterial tests together, it is noteworthy to mention that tested compounds are more active towards fungi than bacteria.

Acknowledgements

This study was supported by the UKM/Malaysia (faculty science and technology- chemistry department under prof. Mohammed B. Kassim). The authors Thank Chemistry Department/ Sebha university and Botany/chemistry Department Faculty of Arts and Science, El-Marj, University of Benghazi.

References

[1] AL-abbasi, A. 2013. Structure, spectroscopy and coordination chemistry of oxo-vanadium hydrotris(3,5-dimethyl pyrazolyl)borate With benzoylthiourea ligands, Chemistry, UKM- Malaysia, Malaysia.

[2] Al-Abbasi, A., Mohamed Tahir, M., Kassim, M., . 2011. 1,1-Diethyl-3-(4-methoxybenzoyl)thiourea. *ActaCrystallographica Section E Structure Reports Online* 67:o3414.

[3] Arslan, H.; Duran, N.; Borekci, G.; Koray Ozer, C.; Akbay, C. . 2009. Antimicrobial Activity of Some Thiourea Derivatives and Their Nickel and Copper Complexes. *Molecules* 14:519-527.

[4] Brown, B. T. and Harris, R. L. N. . 1973. Derivatives of N-(pyrid-2-yl)thiourea as root growth inhibitors. Pesticide Science. *Pesticide Science* 4:215–225.

[5] Hernández, W., E. Spodine, L. Beyer, U. Schrötider, R. Richter, J. Ferreira, and M. Pavani. 2005. Synthesis, characterization and antitumor activity of copper(II) complexes, [CuL2] [HL1-3=N,N-diethyl-N′-(R-benzoyl)thiourea (R=H, o-Cl and p-NO2)]. *Bioinorganic Chemistry and Applications* 2005 (3-4):299-316.

[6] Hernández, W., E. Spodine, J. C. Muñoz, L. Beyer, U. Schröder, J. Ferreira, and M. Pavani. 2003. Synthesis, characterization and antitumor activity of cis-bis(acylthioureato) platinum(II) complexes, cis-[PtL2][HL 1=N,N-diphenyl-N′-benzoylthiourea or HL2 = N,N-diphenyl-N′-(p-nitrobenzoyl)thiourea]. *Bioinorganic Chemistry and Applications* 1 (3-4):271-284.

[7] Hernández, W., E. Spodine, R. Richter, K. H. Hallmeier, U. Schröder, and L. Beyer. 2003. Platinum(II) Mixed Ligand Complexes with Thiourea Derivatives, Dimethyl Sulphoxide and Chloride: Syntheses, Molecular Structures, and ESCA Data. *Zeitschrift fur Anorganische und Allgemeine Chemie* 629 (14):2559-2565.

[8] Hindler, J. . 1995. In Special Antimicrobial Susceptibility Tests. In *Textbook of Diagnostic Microbiology*, edited by C. C. Mahon: George Manuselis .

[9] King, A. 2001 Recommendations for susceptibility tests on fastidious organisms and those requiring special handling. *J Antimicrob Chemother*. 48 (1):77-80.

[10]Sacht, C., and M. S. Datt. 2000. Synthesis and characterisation of mixed-ligand platinum(II)-sulfoxide complexes, [PtCl(DMSO)(L)], for potential use as chemotherapeutic agents (HL = N,N-dialkyl-N'-(3-R-benzoyl)thiourea). *Polyhedron* 19 (11):1347-1354.

[11] Saeed, A., R. A. Khera, N. Abbas, M. Latif, I. Sajid, and U. Flörke. 2010. Synthesis, characterization, crystal structures, and antibacterial activity of some new 1-(3,4,5-trimethoxybenzoyl)-3-aryl thioureas. *Turkish Journal of Chemistry* 34 (3):335-345.

[12] Sanders, R. 2012. Aseptic Laboratory Techniques: Plating Methods. *J. Vis. Exp.* (63):3064.

[13] Tan, S., Al-abbasi, A., Mohamed Tahir, M., . Kassim, M. . 2014. Synthesis, structure and spectroscopic properties of cobalt(III) complexes with 1-benzoyl-(3,3-disubstituted)thiourea. *Polyhedron* 68 (02):287-294.

[14] Vivas-Reyes, R., E. Espinosa-Fuentes, J. Forigua, A. Arias, R. Gaitán, and E. Arguello. 2008. Theoretical study of a series of N-(N-propyl)-N′-(para-R-benzoyl)-thioureas

with trans[pt(py)2cl2] through chemistry reactivity descriptors based on density functional theory. *Journal of Molecular Structure: THEOCHEM* 862 (1-3):92-97.

[15] You-Ming Zhang, Hai-Xia Pang, Cheng Cao & Tai-Bao Wei. 2007. Synthesis, crystal structure and biological activity of a new complex,+ bis(1,1-diethyl-3-(3-fluorobenzoyl)-thiourea)nickel(II) *Indian Journal of Chemistry*, 46A:1792-1795.