## ABSTRACT

In this study, three functionalized dithiocarbamate ligands [-S<sub>2</sub>CN(R)CH<sub>2</sub>CH<sub>2</sub>OH] were reacted with phosphanegold(I), silver(I) and copper(I) precursors resulting in their respective dithiocarbamate complexes. The molecular structures of these compounds were elucidated using various spectroscopic methods: NMR (<sup>1</sup>H, <sup>13</sup>C{<sup>1</sup>H} and <sup>31</sup>P{<sup>1</sup>H}), IR, as well as by X-ray methods: powder X-ray diffraction (PXRD) and single crystal X-ray diffraction (SCXRD). These compounds were also tested for their biological efficacies in order to identify potential candidates as anti-cancer and anti-bacterial agents.

The successful synthesis of the complexes was determined by NMR and IR methods whereby the interpretation agrees well with literature. The powder patterns of the compounds are of similar to the single crystal data, when crystal structures obtained showed that the gold compounds had linear coordination geometries while the copper and silver compounds has tetrahedral geometries.

Phosphanegold(I) derivatives with isopropyl dithiocarbamate ligand group were found to exhibit excellent anti-proliferative activity against MCF-7R breast cancer cells and anti-bacterial activity against almost a wide spectrum of Gram-positive and Gramnegative pathogens. Nevertheless, the phosphanegold(I) derivatives with the isopropylsubstituted dithiocarbamate were also toxic to non-cancerous human cells.

The phosphanesilver(I) and -copper(I) dithiocarbamates for all series of ligands also showed promising anti-proliferative activity against a number of cancer cell lines: liver (HepG2), breast (MCF-7R), ovarian (A2780), colon (HT-29), lung (A549) and thyroid (8505C) cancer cells. However, these series were also toxic on non-cancerous human cells with the exception on human embryogenic kidney (HEK293) cells.

### ABSTRAK

Kajian ini melibatkan tindak balas antara tiga ligan ditiokarbamat dengan bahan pemula argentum(I), kuprum(I) dan aurum(I)fosfana bagi menghasilkan kompleks ditiokarbamat bagi argentum(I), kuprum(I) dan aurum(I). Struktur molekul sebatian-sebatian ini telah ditentukan melalui beberapa teknik spektroskopi: NMR (<sup>1</sup>H, <sup>13</sup>C{<sup>1</sup>H} and <sup>31</sup>P{<sup>1</sup>H}), IR, pembelauan X-ray serbuk (PXRD) dan pembelauan X-ray kristal tunggal (SCXRD). Semua sebatian ini juga telah diuji untuk bioaktiviti bagi menentukan sebatian yang sesuai sebagai agen anti-kanser dan anti-bakteria.

Penghasilan kompleks telah disahkan melalui kaedah NMR dan IR yang mana interpretasi spektrum adalah berpadanan seperti yang tercatat di dalam kajian sebelumnya. Corak spektrum serbuk sebatian didapati menyamai dengan data kristal tunggal apabila struktur kristal menunjukkan bahawa sebatian aurum mempunyai geometri linear manakala sebatian kuprum dan argentum mempunyai geometri tetrahedral.

Terbitan fosfanaaurum(I) yang mengandungi kumpulan tertukarganti isopropil ditiokarbamat didapati menunjukkan aktiviti anti-proliferatif yang amat baik terhadap sel kanser payudara MCF-7R dan aktiviti anti-bakteria terhadap kebanyakan bakteria Gram-positif dan Gram-negatif. Walau bagaimanapun, sebatian ini adalah toksik terhadap sel manusia yang sihat.

Semua fosfanakuprum(I) dan argentum(I) ditiokarbamat bagi semua siri ligan juga berpotensi dalam aktiviti anti-proliferatif terhadap beberapa sel kanser; hati (HepG2), payudara (MCF-7R), ovari (A2780), kolon (HT-29), peparu (A549) dan tiroid (8505C).

v

Walaupun begitu, semua siri ini adalah selamat hanya ke atas sel ginjal embriogenik manusia (HEK293) tetapi toksik tehadap sel manusia sihat yang lain.

#### ACKNOWLEDGEMENTS

My highest gratitude goes to my supervisor, Prof. Dr Edward R. T. Tiekink for allowing me to work on his project on coinage metals, which has long been my interest and his guidance in the chemistry work was priceless. It was from him that I learned to have patience in my research work and continuous hard work. Dr Siti Nadiah Abdul Halim, my co-supervisor was so generous in providing her attention and ideas in performing laboratory work and collecting single crystal data.

Many thanks to Dr Seng Hoi Ling (University of Malaya) and Dr Cheah Yoke Kqueen (University of Putra Malaysia) who were responsible for conducting biological tests and mechanistic studies for anti-cancer and anti-microbial activities. Other than that, I am grateful to my colleagues and the instrument laboratory staff for their support and time for discussion and assistance in operating the instruments for chemical analyses.

In addition, I wish to dedicate my special thanks and appreciation to my parents and brother for their continuous support. They were always there during my hardest and happiest moments throughout the entire of my research.

Finally, the Ministry of Higher Education (MOHE) and University of Malaya (UM) were thanked for the scholarship provided through Skim Latihan Akademik IPTA (SLAI) for me to carry out my PhD work at the Department of Chemistry, University of Malaya. Also research grants provided by High Impact Research (HIR) Unit and Institute of Research, Management and Monitoring (IPPP) are gratefully acknowledged.

# TABLE OF CONTENT

Abstract	iii
Abstrak	v
Acknowledgement	vii
Table of Content	viii
List of Figures	xiii
List of Schemes	xvi
List of Tables	xvii

# **CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW**

1.1 The Chemistry and Biochemistry of Dithiocarbamates	1
1.2 The Chemistry and Biochemistry of Gold, Silver and Copper	2
1.2.1 Gold	2
1.2.2 Silver	9
1.2.3 Copper	11
1.3 Objective of Research	13

# CHAPTER 2: EXPERIMENTAL METHODOLOGY

2.1 Chemicals
2.2 Synthetic Methodology16
2.2.1 Schematic diagram for the preparation of (hydroxyethyl)dithiocarbamates; KL1,
NaL2 and KL316
2.2.2 Syntheses of (hydroxyethyl)dithiocarbamates; KL1, NaL2 and KL317
2.2.2.1 Synthesis of potassium N-(hydroxyethyl)-N-methyldithiocarbamate, KL1.17
2.2.2.2 Synthesis of sodium N-(hydroxyethyl)-N-isopropyldithiocarbamate, NaL2.17
2.2.2.3 Synthesis of potassium N,N-bis(hydroxyethyl)dithiocarbamate, KL318
2.2.3 Schematic diagram for the preparation of sodium diethyldithiocarbamate,
NaL4
2.2.4 Synthesis of sodium N,N-diethyldithiocarbamate, NaL4

2.2.5 Schematic diagram for the preparation of phosphanegold(I) chloride	
precursors	9
2.2.6 Syntheses of trialkyl / triarylphosphanegold(I) chloride precursors	9
2.2.6.1 Synthesis of (triphenylphosphane)gold(I) chloride, Ph <sub>3</sub> PAuCl	9
2.2.6.2 Synthesis of (tricyclohexylphosphane)gold(I) chloride, Cy <sub>3</sub> PAuCl20	0
2.2.6.3 Synthesis of (triethylphosphane)gold(I) chloride, Et <sub>3</sub> PAuCl20	0
2.2.7 Schematic diagram for the preparation of triphenyl- and tricyclohexyl-	
phosphanegold(I) dithiocarbamate complexes of L1, L2 and L3	1
2.2.8 Syntheses of triphenylphosphanegold(I) dithiocarbamates of L1, L2 and L3. 2.	1
2.2.8.1 Synthesis of Ph <sub>3</sub> PAu(L1)	1
2.2.8.2 Synthesis of Ph <sub>3</sub> PAu(L2)	2
2.2.8.3 Synthesis of $Ph_3PAu(L3)$	2
2.2.9 Syntheses of tricyclohexylphosphanegold(I) dithiocarbamates of L1, L2 and	
L3	3
2.2.9.1 Synthesis of Cy <sub>3</sub> PAu(L1)23	3
2.2.9.2 Synthesis of Cy <sub>3</sub> PAu(L2)23	3
2.2.9.3 Synthesis of $Cy_3PAu(L3)$ 23	3
2.2.10 Schematic diagram for the preparation of triethylphosphanegold(I)	
dithiocarbamates of $L1 - L5$	4
2.2.11 Syntheses of triethylphosphanegold(I) dithiocarbamates of $L1 - L5$ 24	4
2.2.11.1 Synthesis of Et <sub>3</sub> PAu(L1)24	4
2.2.11.2 Synthesis of Et <sub>3</sub> PAu(L2)25	5
2.2.11.3 Synthesis of Et <sub>3</sub> PAu(L3)25	5
2.2.11.4 Synthesis of Et <sub>3</sub> PAu(L4)	5
2.2.11.5 Synthesis of Et <sub>3</sub> PAu(L5)	5
2.2.12 Schematic diagram for the preparation of bis(triphenylphosphino)copper(I)	
dithiocarbamates of L1, L2 and L3	5
2.2.13 Syntheses of bis(triphenylphosphane)copper(I) dithiocarbamates, of L1, L2	
and L3	6
2.2.13.1 Synthesis of (Ph <sub>3</sub> P) <sub>2</sub> Cu(L1)	6
2.2.13.2 Synthesis of (Ph <sub>3</sub> P) <sub>2</sub> Cu(L2)27	7
2.2.13.3 Synthesis of (Ph <sub>3</sub> P) <sub>2</sub> Cu(L3)27	7
2.2.14 Schematic diagram for the preparation of bis(triphenylphosphane)silver(I)	
dithiocarbamates of L1, L2 and L3	7

2.2.15 Syntheses of bis(triphenylphosphane)silver(I) dithiocarbamates of L1,	L2 and
L3.	
2.2.15.1 Synthesis of (Ph <sub>3</sub> P) <sub>2</sub> Ag(L1)	
2.2.15.2 Synthesis of (Ph <sub>3</sub> P) <sub>2</sub> Ag(L2)	
2.2.15.3 Synthesis of (Ph <sub>3</sub> P) <sub>2</sub> Ag(L3)	29
2.3 Instrumentation	29
2.3.1 Nuclear Magnetic Resonance (NMR) Spectrometer	29
2.3.2 Infrared (IR) Spectrometer	29
2.3.3 Melting Point Measurement	29
2.3.4 Elemental Analyser	
2.3.5 Powder X-ray Diffractometer (PXRD)	
2.3.6 Single Crystal X-ray Diffractometer (SCXRD)	
2.4 Biological Assays	31
2.4.1 Anti-cancer Studies	31
2.4.1.1 Cell culture and inhibition of cell growth	31
2.4.1.2 Extraction of RNA, and RT2 Profiler PCR microarray	32
2.4.1.3 Caspase activity (Caspases-3, -7, -8, -9 and -10)	
2.4.1.4 Membrane permeability study by AOPI staining	
2.4.1.5 Intracellular reactive oxygen species (ROS) measurements	
2.4.1.6 Human topoisomerase I inhibition assay	
2.4.2 Anti-bacterial Studies	34
2.4.2.1 Anti-bacterial Activity Assay	34
2.4.2.2 Determination of minimum inhibitory concentration (MIC) and mi	nimum
bactericidal concentration (MBC)	35
2.4.2.3 Time-kill assay	

# CHAPTER 3: RESULTS AND DISCUSSION (PART A: CHEMICAL CHARACTERIZATION)

3.1 Tricyclohexylphosphanegold(I) dithiocarbamate, Cy <sub>3</sub> PAu(dtc)	38
3.1.1 Infra Red (IR) Spectroscopy	39
3.1.3 ${}^{13}C{}^{1}H$ Nuclear Magnetic Resonance ( ${}^{13}C{}^{1}H$ NMR) Spectroscopy	41
3.1.4 ${}^{31}P{}^{1}H{}$ Nuclear Magnetic Resonance ( ${}^{31}P{}^{1}H{}$ NMR) Spectroscopy	42
3.1.5 Powder X-ray Diffraction (PXRD)	43
3.1.5.1 PXRD pattern of compound (b)	43

3.1.6 Single Crystal X-ray Diffraction (SCXRD)	44
3.1.6.1 SCXRD of compound (b)	44
3.2 Triethylphosphanegold(I) dithiocarbamates, Et <sub>3</sub> PAu(dtc)	45
3.2.1 Infra Red (IR) Spectroscopy	46
3.2.2 <sup>1</sup> H Nuclear Magnetic Resonance ( <sup>1</sup> H NMR) Spectroscopy	47
3.2.3 ${}^{13}C{}^{1}H$ Nuclear Magnetic Resonance ( ${}^{13}C{}^{1}H$ NMR) Spectroscopy	49
3.2.4 ${}^{31}P{}^{1}H{}$ Nuclear Magnetic Resonance ( ${}^{31}P{}^{1}H{}$ NMR) Spectroscopy	51
3.2.5 Powder X-ray Diffraction (PXRD)	51
3.2.5.1 PXRD pattern of compound (e)	51
3.2.6 Single Crystal X-ray Diffraction (SCXRD)	52
3.2.6.1 SCXRD of compound (e)	52
3.3 Triphenylphosphanegold(I) dithiocarbamates, Ph <sub>3</sub> PAu(dtc)	54
3.3.2 <sup>1</sup> H Nuclear Magnetic Resonance ( <sup>1</sup> H NMR) Spectroscopy	56
3.3.3 <sup>13</sup> C { <sup>1</sup> H} Nuclear Magnetic Resonance ( <sup>13</sup> C{ <sup>1</sup> H} NMR) Spectroscopy	57
3.3.4 <sup>31</sup> P{ <sup>1</sup> H} Nuclear Magnetic Resonance ( <sup>31</sup> P{ <sup>1</sup> H} NMR) Spectroscopy	58
3.4 Bis(triphenylphosphane)copper(I) dithiocarbamates, (Ph <sub>3</sub> P) <sub>2</sub> Cu(dtc)	59
3.4.2 <sup>1</sup> H Nuclear Magnetic Resonance ( <sup>1</sup> H NMR) Spectroscopy	61
3.4.3 ${}^{13}C{}^{1}H$ Nuclear Magnetic Resonance ( ${}^{13}C{}^{1}H$ NMR) Spectroscopy	62
3.4.4 <sup>31</sup> P{ <sup>1</sup> H} Nuclear Magnetic Resonance ( <sup>31</sup> P{ <sup>1</sup> H} NMR) Spectroscopy	63
3.4.5 Powder X-ray Diffraction (PXRD)	64
3.4.5.1 PXRD patterns of compound (I)	64
3.4.5.2 PXRD patterns of compound (m)	65
3.4.5.3 PXRD patterns of compound (n)	66
3.4.6 Single Crystal X-ray Diffraction (SCXRD)	67
3.4.6.1 SCXRD of compound (I)	67
3.4.6.2 SCXRD for compound ( <b>n</b> )	68
3.5 Bis(triphenylphosphane)silver(I) dithiocarbamates, (Ph <sub>3</sub> P) <sub>2</sub> Ag(dtc)	71
3.5.2 <sup>1</sup> H Nuclear Magnetic Resonance ( <sup>1</sup> H NMR) Spectroscopy	73
3.5.3 ${}^{13}C{}^{1}H$ Nuclear Magnetic Resonance ( ${}^{13}C{}^{1}H$ NMR) Spectroscopy	74
3.5.5 Powder X-ray Diffraction (PXRD)	76
3.5.5.1 PXRD patterns of compound ( <b>p</b> )	76
3.5.5.2 PXRD patterns of compound (q)	77
3.5.6 Single Crystal X-ray Diffraction (SCXRD)	79
3.5.6.1 SCXRD for compound ( <b>p</b> )	79

# **CHAPTER 3: RESULTS AND DISCUSSION** (PART B: BIOLOGICAL ACTIVITIES)

3.6 Anti-proliferative Studies of the $R_3PAuL2$ series ( $R = Ph, Cy, Et$ )
3.6.1 Inhibition of MCF-7R Breast Cancer Cell Proliferation81
3.6.2 Membrane Permeability Study (AO / PI Apoptotic Cell Study)83
3.6.3 Determination of Mode of Cell Death (DNA Fragmentation)86
3.6.4 Analysis of Apoptotic Pathway (PCR Array Study and Caspase Activity)87
3.6.5 Measurement of Reactive Oxygen Species (ROS) Production90
3.6.7 Human Topoisomerase I Inhibition Study92
3.7 Anti-proliferative study of (Ph <sub>3</sub> P) <sub>2</sub> Ag(dtc) and (Ph <sub>3</sub> P) <sub>2</sub> Cu(dtc) series on various
cancer cell lines
3.7.1 Cytotoxicity on normal cell lines
3.7.2 Cytotoxicity on cancer cell lines94
3.8 Anti-bacterial Studies of the $R_3PAu(L2)$ series (R = Ph, Cy, Et)97
3.8.1 Inhibitory activity
3.8.2 Minimum Inhibitory Concentration (MIC)99
3.8.3 Minimum Bactericidal Concentration (MBC)102
3.8.4 Time-kill Assay103
CONCLUSION
REFERENCES
APPENDICES

# LIST OF FIGURES

Figure 1.2: Chemical structures of (a) cyano(triethylphosphino)gold(I), (b) isocyanatobis(triphenylphosphino)gold(I) and (c) isocyanatotris(triphenylphosphino)gold(I)
Figure 1.3: Chemical structures of (a) sodium aurothiomalate and (b) aurothioglucose5
Figure 1.4: Chemical structures of (a) (1-methylthiol)octahydro-1H- quinolizino(triethylphosphino)gold(I), (b) N,N-diethyl-N- ethylthio(triethylphosphino)gold(I) and (c) 1-ethylthio-4- methylpiperazino(triethylphosphino)gold(I)
Figure 1.5: Chemical structure of [3,4,5-triacetyloxy-6-(acetyloxymethyl)oxane-2- thiolato]triethylphosphanegold(I) (auranofin)7
Figure 1.6: Chemical structure of chlorotriphenylphosphinobis(dialkylphosphino)propylgold(I)14
Figure 3.1: Chemical structures of tricyclohexylphosphanegold(I) dithiocarbamate series: (a) Cy <sub>3</sub> PAu(L1), (b) Cy <sub>3</sub> PAu(L2) and (c) Cy <sub>3</sub> PAu(L3)
Figure 3.2: Comparison between the experimental powder pattern (red trace) and simulated pattern collected from the single crystal X-ray data of compound (b)43
Figure 3.3: Molecular structure of ( <b>b</b> )44
Figure 3.4: Chemical structures of triethylphosphanegold(I) dithiocarbamate series; (d) Et <sub>3</sub> PAu(L1), (e) Et <sub>3</sub> PAu(L2), (f) Et <sub>3</sub> PAu(L3), (g) Et <sub>3</sub> PAu(L4) and (h) Et <sub>3</sub> PAu(L5).
Figure 3.5: Comparison between the experimental powder pattern (red trace) and that
calculated from the single crystal data of compound (e)
calculated from the single crystal data of compound (e)
<ul> <li>calculated from the single crystal data of compound (e)</li></ul>
<ul> <li>calculated from the single crystal data of compound (e)</li></ul>
<ul> <li>calculated from the single crystal data of compound (e)</li></ul>

Figure 3.12: Unit cell of compound (I)67
Figure 3.13: Hydrogen bonds for compound (I)
Figure 3.14: Unit cell of compound ( <b>n</b> )69
Figure 3.15: Hydrogen bonds in crystal packing of compound (n)70
Figure 3.16: Chemical structures of the bis(triphenylphosphane)silver(I) dithiocarbamate series: ( <b>o</b> ) (Ph <sub>3</sub> P) <sub>2</sub> Ag(L1), ( <b>p</b> ) (Ph <sub>3</sub> P) <sub>2</sub> Ag(L2) and ( <b>q</b> ) (Ph <sub>3</sub> P) <sub>2</sub> Ag(L3)
Figure 3.17: Comparison between the experimental powder pattern (red trace) and the simulated pattern (blue trace) of the single crystal of compound ( <b>p</b> )76
Figure 3.18: Comparison between the experimental powder pattern (red trace) and the simulated pattern (blue trace) of the single crystal of compound ( <b>n</b> )77
Figure 3.19: Comparison between the experimental powder pattern (red trace) of compound (q) with the simulated pattern (blue trace) of the single crystal data of compound (p)
Figure 3.20: Unit cell of compound ( <b>p</b> )
Figure 3.21: Hydrogen bonds in crystal packing of compound ( <b>p</b> )80
Figure 3.22: MCF-7R cell viability after treatment of NaL2 and its gold(I) thiolate at different concentrations ( $\mu$ M). <i>p</i> value = 0.005 in each case
Figure 3.23: AO/PI staining of MCF-7R cells after being treated at the IC <sub>50</sub> value of each compound: (a) treated with doxorubicin; (b) treated with (j); (c) treated with (b); (d) treated with (e); (e) untreated cells (negative control). Magnification = 100x
Figure 3.24: DNA fragmentation analysis. Formation of ladders on the gels to indicate DNA fragmentation occurs following treatment with (j), (b) and (e) supports cell death by apoptosis
Figure 3.25: Signalling pathway of apoptosis induced by ( <b>j</b> ), ( <b>b</b> ) and ( <b>e</b> ). This diagram collates and summarises the results of the PCR array analysis, caspase activity study, DNA fragmentation and ROS production measurements
Figure 3.26: Production of ROS after treatment of MCF-7R cells with ( <b>j</b> ), ( <b>b</b> ) and ( <b>e</b> ), at doses corresponding to their IC <sub>50</sub> values (4.4, 13.6 and 9.3 $\mu$ M, respectively) for 16 h. After labeling with carboxy-H2DCFDA for 1 h, the fluorescence was measured.
Figure 3.27: Inhibitory activity against Gram-positive bacterial strains measured by zone of inhibition (mm) by NaL2 and its gold(I) complexes; (j), (b) and (e) with ciprofloxacin as the standard drug

Figure 3.28: Inhibitory activity against Gram-negative bacterial strai	ns measured by
zone of inhibition (mm) by NaL2 and its gold(I) complexes; (j)	, ( <b>b</b> ) and ( <b>e</b> ) with
ciprofloxacin as the standard drug.	

Figure 3.29: Time-kill curves of compound (j) against (a) *B. cereus*, (b) *B. subtilis*, (c) *E. faecalis*, (d) *E. faecium*, (e) *L. monocytogenes*, (f) MRSA, (g) *S. aureus*, (h) *S. saprophyticus*, (i) *S. pyogenes*. Note that the bactericidal level is indicated by the dashed lines; ---, growth control; ×, 2 MIC; ▲, MIC; ■ and 1/2MIC; ◆......104

Figure 3.30: Time-kill curves of compound (b) against (a) B. cereus, (b	b) B. subtilis, (c)
E. faecalis, (d) E. faecium, (e) L. monocytogenes, (f) MRSA, (g) S	. pyogenes. Note
that the bactericidal level is indicated by the dashed lines; ; ,	growth control;
×, 2 MIC; ▲, MIC; ■ and 1/2MIC; ◆	

# LIST OF SCHEMES

Scheme 2.1: Preparation of KL1, NaL2 and KL3	16
Scheme 2.2: Preparation of NaL4.	18
Scheme 2.3: Preparation of phosphanegold(I) chloride precursors	19
Scheme 2.4: Preparation of triphenyl- and tricyclohexyl-phosphanegold(I) dithiocarbamate complexes	21
Scheme 2.5: Preparation of triethylphosphinogold(I) dithiocarbamates	24
Scheme 2.6: Preparations of bis(triphenylphosphane)copper(I) dithiocarbamates	26
Scheme 2.7: Preparation of bis(triphenylphosphane)silver(I) dithiocarbamates	27

# LIST OF TABLES

Table 3.1: Selected IR absorption bands of tricyclohexylphosphanegold(I)         dithiocarbamate series.         39
Table 3.2: <sup>1</sup> H chemical shifts, δ (ppm) and multiplicities of tricyclohexylphosphanegold(I) dithiocarbamate series40
Table 3.3: ${}^{13}C{}^{1}H$ chemical shifts, $\delta$ (ppm) and of tricyclohexylphosphanegold(I) dithiocarbamate series
Table 3.4: <sup>31</sup> P{ <sup>1</sup> H} chemical shifts, δ(ppm) of tricyclohexylphosphinogold(I) dithiocarbamate series
Table 3.5: Selected IR absorption bands of the triethylphosphanegold(I) dithiocarbamate series
Table 3.6: <sup>1</sup> H chemical shift, $\delta$ (ppm) and multiplicities of triethylphosphanegold(I) dithiocarbamate series
Table 3.7: <sup>13</sup> C{ <sup>1</sup> H} chemical shifts, δ(ppm) of triethylphosphinogold(I) dithiocarbamate series
Table 3.8: <sup>31</sup> P{ <sup>1</sup> H} chemical shift, δ (ppm) of triethylphosphinogold(I) dithiocarbamate series
Table 3.9: Selected IR absorption bands of triphenylphosphinogold(I) dithiocarbamate series
Table 3.10: <sup>1</sup> H chemical shifts, δ (ppm) and multiplicities of triphenylphosphinogold(I) dithiocarbamate series
Table 3.11: <sup>13</sup> C{ <sup>1</sup> H} chemical shifts, δ (ppm) of triphenylphosphanegold(I) dithiocarbamates series
Table 3.12: <sup>31</sup> P{ <sup>1</sup> H} chemical shifts, δ (ppm) of triphenylphosphanegold(I) dithiocarbamate series58
Table 3.13: Selected IR absorption bands for the bis(triphenylphosphane)copper(I)         dithiocarbamate series.         60
Table 3.14: <sup>1</sup> H chemical shift, δ (ppm), and multiplicities of bis(triphenylphosphane)copper(I) dithiocarbamate series
Table 3.15: <sup>13</sup> C{ <sup>1</sup> H} chemical shifts, δ(ppm) for the bis(triphenylphosphane)copper(I) dithiocarbamate series62
Table 3.16: <sup>31</sup> P chemical shift, δ(ppm), for the bis(triphenylphosphane)copper(I) dithiocarbamate series63
Table 3.17: Selected IR absorption bands for the bis(triphenylphosphino)silver(I)         dithiocarbamate series.         72

Table 3.18: <sup>1</sup> H chemical shift, δ (ppm) and multiplicities of bis(triphenylphosphane)silver(I) dithiocarbamate series	73
Table 3.19: <sup>13</sup> C{ <sup>1</sup> H} chemical shifts, δ (ppm) of bis(triphenylphosphane)silver(I) dithiocarbamate series	74
Table 3.20: <sup>31</sup> P{ <sup>1</sup> H} chemical shift, δ (ppm) of bis(triphenylphosphane)silver(I) dithiocarbamate series	75
Table 3.21: Minimum inhibitory concentration (IC <sub>50</sub> ) of triorganophosphanegold(I) series of L2 and control drugs against MCF-7R.	31
Table 3.22: Cytotoxicity activity of bis(triphenylphosphane)silver(I) and –copper(I) dithiocarbamates against normal cells models at 24 hours treatment	€
Table 3.23: Cytotoxicity activity of bis(triphenylphosphino)silver(I) and –copper(I) dithiocarbamate against human carcinoma cells models at 24 hours treatment9	96

# **CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW**

### **1.1** The Chemistry and Biochemistry of Dithiocarbamates

Dithiocarbamates are versatile ligands with interesting chemical and biological characteristics <sup>1</sup>. This ligand has created vast interest among researchers due to its capability to stabilize transition metals of various oxidation states <sup>2</sup>. Much recent work has been accomplished such as designing new dithiocarbamates with varying substituents in particular, to study chemical properties with transition and non-transition metals. Besides, fungicides and pesticides are some examples of the utilization of dithiocarbamates <sup>3,4</sup>.

Dithiocarbamates anions are usually bidentate ligands (Figure 1.1) prepared by reacting carbon disulfide with a primary or secondary amine in the presence of a base at 0 °C. The 'soft' ligand can easily bind to a 'soft' metal yielding the corresponding complex. Bidentate chelation or bidentate bridging and even monodentate-coordination modes are known  $^{5}$ .



Figure 1.1: Delocalization of electrons in dithiocarbamates.

Functionalized dithiocarbamates has been developed recently in pursuance and accomplishment of attaining organic-water solubility of dithiocarbamate complexes. Examples of functionalization include the incorporation of 2-hydroxyethyl, 2-

methoxyethyl and amine in dithiocarbamate substituents, and this indeed conferred some degree of water solubility to the complexes.

The design of such complexes may provide useful attributes in various applications especially in the medicinal area <sup>3</sup>. For example, anti-tubercular activity of some dithiocarbamates with sugar derivatives was investigated; and was found that the substituents, R, on the dithiocarbamates moderated the MIC (minimum inhibitory concentration) in inhibiting the growth of a number of bacteria such as *M. tuberculosis*, *M. bovis* BCG strain and *M. smegmatis* <sup>6</sup>. Due to the lipophilic character conferred by the alkyl substituents of dithiocarbamates, their complexes with bioactive metals can undergo passive diffusion into cell membrane <sup>7</sup>.

# 1.2 The Chemistry and Biochemistry of Gold, Silver and Copper

#### 1.2.1 Gold

Gold is a well-known element with special characteristics such as high chemical and thermal stability, high electrical conductivity and beautiful appearance. It has the lowest electrochemical potential of the metals which means any cationic gold can easily accept an electron from reducing agents to form metallic gold. Being such an inert element, gold is essential in electrical devices as gold is resistant to oxidation and mechanically robust. Gold exists in different oxidation states; -1, 0, +1, +2, +3, +4 and +5. Among these oxidation states, 0, +1 and +3 are the most stable while -1, +2, +4 and +5 are unstable and are not easy to prepare under normal laboratory conditions. Nevertheless, the formation of gold(-1) from gold(0) can be accomplished <sup>8</sup>.

Relativistic effects also play an important role in the physical and chemical properties of gold. The Au(I) radius is smaller (62 ppm) than the radius of Ag(I) (68 ppm). Gold(I) has a  $d^{10}$  configuration that gives rise to the three principal coordination geometries: linear (Figure 1.2a), trigonal (Figure 1.2b) and tetrahedral (Figure 1.2c). Relativistic effects increase the 6s-6p energy gap of gold due to the relative contractions of the s and p shells and the relativistic lowering of gap between 5d and the Fermi level gives gold the vellow colour <sup>9</sup>. The Au-Au bond is stronger than Ag-Ag bond but relatively similar to Cu-Cu bond, in other words, the bond energy is comparable to that of a hydrogen bond <sup>9-11</sup>. Relativistic effects also enhance the stability of the linear geometry over the trigonal and tetrahedral geometries as compared to the other coinage elements, Ag(I) and Cu(I), and the neighbouring  $d^{10}$  metals: Pt(0) and Hg(0)<sup>12</sup>. Interestingly, the presence of a weak Au - - - Au interactions, often makes the gold compound luminous. In the case of monomeric gold(I) compounds with phosphane and thiolates, the maximum emission is affected by the presence of Au - - Au interactions  $^{10,13}$ . Notwithstanding, the inertness and diamagnetic behavior of gold, this precious metal has shown some catalytic properties in catalyzing the decomposition of formic acid <sup>14</sup>.



**Figure 1.2:** Chemical structures of (a) cyano(triethylphosphino)gold(I), (b) isocyanatobis(triphenylphosphino)gold(I) and (c) isocyanatotris(triphenylphosphino)gold(I).

In the pharmaceutical context, gold has been used in the treatment of rheumatoid arthritis <sup>8</sup>. Rheumatoid arthritis is an inflammatory disease characterized by a

progressive erosion of the joints resulting in deformities, immobility and a great deal of pain. It is an autoimmune disease in which the body's immune system mounts a response against itself. The rise in the level of prostaglandins, leukotrienes and cytokines promote the generation of reactive oxygen species OH and  $O_2^-$  and release of collagenase. Earlier, gold(I) thiolates such as sodium aurothiomalate (Figure 1.3a) and aurothioglucose (Figure 1.3b) were used in the treatment of rheumatoid arthritis <sup>15,16</sup>. Unlike other rheumatoid arthritis drugs, auranofin (see later), of which is a phosphane coordinated gold(I) complex, showed some evidence as anti-cancer agent in *in vivo* studies <sup>17</sup>. Gold(III) complexes were the first to be screened for anti-tumour activity owing to its similar structure to dichlorodiaminoplatinum(II) (cisplatin). The gold(III) centre is isoelectronic (5*d*<sup>8</sup>) with platinum(II) and also exists in square planar geometries <sup>18,19</sup>. As such, gold(III) compounds are expected to exhibit similar mechanisms of action as cisplatin.

Most gold(III) compounds are readily reduced to gold(I) compounds in biological media by biologically occurring reductants such as thiols <sup>15</sup>. For instance, stable complexes of gold(I) thiolate can be formed from the reaction of gold(III) compounds with the thiol group of cysteine <sup>20</sup>. From XANES experiments, it is believed that the efficacy of gold(III) is probably due to its gold(I) metabolites, which occur upon reduction *in vivo* by free thiol groups on proteins such as albumin <sup>17</sup>, glutathione and metallothionein <sup>15</sup>. Generally, gold(I) forms linear complexes, possesses biological activities in humans and undergoes associative ligand exchange with biologically active ligands such as cysteinerich peptides and proteins such as glutathione, metallothionein and albumin since gold(I) has high preferences towards 'soft' S- and P- donors rather than 'hard' O- or Ndonors <sup>15,21</sup>.



Figure 1.3: Chemical structures of (a) sodium aurothiomalate and (b) aurothioglucose.

Gold(I) compounds bearing the triethylphosphane ligand exhibit good anti-microbial activities over E. coli, C. albicans and A. niger<sup>22</sup>. The disparities in activity were characterized by the presence of different aminothiol ligands bonded to the metal. Due its bulkiness of the structure in Figure 1.4a, (1-methylthiol)octahydro-1Hto quinolizino(triethylphosphino)gold(I) showed the best activity against C. albicans and A. niger fungi which is comparable to miconazole nitrate and the trend was followed by N.N-diethyl-N-ethylthio(triethylphosphino)gold(I) and 1-ethylthio-4methylpiperazino(triethylphosphino)gold(I), as illustrated in Figure 1.4b and Figure 1.4c respectively. Compound in Figure 1.4b is more hydrophilic than compound in Figure 4a, thus more efficient against E. coli. The anti-fungal activity of (1methylthiol)octahydro-1H-quinolizino(triethylphosphino)gold(I) is reduced upon exchange of the ethyl to phenyl residue on the phosphane. The three compounds were found to inhibit the growth of T. vaginalis at a MIC twice that displayed by metronidazole. (1-methylthiol)octahydro-1H-quinolizino(triethylphosphino)gold(I) and N,N-diethyl-N-ethylthio(triethylphosphino)gold(I) were found to exhibit higher activity than that of auranofin in suppressing the growth of *E. coli* and *C. albicans*  $^{22}$ .





Phosphanegold(I) thiolate derivatives are among the most extensive compounds explored for their anti-cancer behaviour and this includes auranofin (Figure 1.5), an anti-arthritic drug as mentioned earlier. Auranofin was first studied for its anti-arthritic property after which it was discovered to also possess anti-cancer properties against certain cancer cells. This compound has a triethylphosphane unit bonded to the central gold atom with a sugar-derived thiolate unit as its ligand <sup>23</sup>.



**Figure 1.5:** Chemical structure of [3,4,5-triacetyloxy-6-(acetyloxymethyl)oxane-2-thiolato]triethylphosphanegold(I) (auranofin).

Gold has served as a remarkably excellent remedy to cure varieties of disorders and diseases. Treatment using gold known as chrysotherapy, has been applied since antiquity and is very famous amongst the people of the Indian, Chinese and Egypt civilizations. Seeing that gold compounds have not been used in other therapeutic applications at present, this term chrysotherapy is referred to the treatment of rheumatoid arthritis <sup>24</sup>. Gold has gained a lot of interest from microbiologists and pharmacists in investigating its other potential and mechanisms of action in treating diseases other than RA.

In summary, gold(I) thiolates are compounds that were utilized in the treatment of rheumatoid arthritis and have also been tested as anticancer agents <sup>25,26</sup>. The polymeric gold thiolates were the first gold therapeutic drugs (Figure 1.3) <sup>24</sup>. The occurrence of metal-thiolate complexes in the biological systems has created a resurgence interest in metal thiolate chemistry <sup>27</sup>. Metal dithiolate complexes can be synthesized through nucleophilic displacement of the halides or other good leaving groups. This concept was used to prepare heteroleptic dithiolate gold(III) complexes. However, the use of other metal complexes such as [Sn(CH<sub>3</sub>)<sub>2</sub>mnt] as thiolate transfer agents had given a number of gold complexes such as [Au(mnt)ClL] (mnt = maleonitriledithiolate, L = tetrahydrothiophene, PPh<sub>3</sub> or pyridine,) <sup>27</sup>.

Cisplatin has been widely used as one of the prominent cancer drugs for over three decades. The impetus in finding new drugs stems from the severe side-effects associated with the use of cisplatin. The reaction of metabolites of cisplatin with some kidney enzyme sulfhydryl groups was suggested to be primarily responsible for the drug's severe side-effects. A considerable amount of interest has focused on the use of planar ligands such as substituted pyridines in platinum(II) complexes as mimics to cisplatin, which resulted in reduction in rate of deactivation of the sulfhydryl groups without interfering the mode of action of cisplatin <sup>28</sup>.

Several non-platinum metal complexes of iron, cobalt and gold have been studied as potential anti-cancer agents<sup>29</sup> because other metal centres might improve the anticancer activities as well as reducing the severe side-effects associated with cisplatin. As such, gold(III) which typically adopts similar characteristics to isoelectronic and isostructural nature of platinum(II) was therefore studied and was expected to mimic the structural and electronic properties of cisplatin <sup>30,31</sup>. Several gold(III) complexes have shown to be highly cytotoxic against a few tumour cells including the cisplatin-resistant cells lines. Unfortunately, the use of gold(III) complexes are limited as they are unstable under physiological condition. This problem is attributed to their high reduction potential and fast hydrolysis rate. However, the stability can be enhanced by coordinating gold(III) ion to a multidentate nitrogen-donor ligands. The results from the study by Segapelo et al.<sup>28</sup> showed that platinum(II) complexes bearing methyl- and *tert*-butyl- substituents on the pyrazolyl group were less active than those with phenyland para-tolyl- substituents. This might suggest the idea that intercalating DNA of the cancer cells could possibly happen under conditions where the ligands are planar rather than having an alkyl group that interferes the DNA binding. However, the gold(III) complexes of the same ligand exhibited reduced cytotoxicity than platinum(II) complexes but were better than their free ligands, the substituted pyrazoles. The low cytotoxicity might due to their readily reduction of gold(III) to gold(I) ions as mentioned earlier <sup>28</sup>.

There are several gold(I) compounds known to exhibit cytotoxic behavior against several cancer cell lines. For example, the triethylphosphanegold(I) complex of menadione potassium bisulfate thiosemicarbazone (Et<sub>3</sub>PAu(K<sub>3</sub>TSC), was evaluated *in vitro* against the cisplatin-sensitive cell line A2780 and the cisplatin-resistant cell line A2780cis. The IC<sub>50</sub> of the gold(I) complex against A2780 was almost similar to that of cisplatin while the activity displayed against A2780cis was almost 10 times greater than that of cisplatin. The ligand, however, did not show any remarkable activity <sup>32</sup>. Moreover, gold(I) phosphane complexes of N-heterocyclic carbenes also suppressed the proliferation of HT-29 and MCF-7 cells with low IC<sub>50</sub> values in the micromolar range <sup>33</sup>.

#### 1.2.2 Silver

A remarkable diversity of silver(I) in its structural chemistry has been illustrated by the wide variety of structural types that encountered in complexes of silver(I) salts. The coordination of bases such as S- and unsaturated N-containing ligands with silver(I) is also interesting in silver chemistry <sup>13</sup>. In silver(I) mononuclear and multinuclear complexes with neutral phosphane and amine ligands the silver(I) centre exhibits variable coordination numbers. Reactions of AgX (X = NO<sub>3</sub><sup>-</sup>, ClO<sub>4</sub><sup>-</sup>, CN<sup>-</sup>, SCN<sup>-</sup>, SbF<sub>6</sub><sup>-</sup>, Cl<sup>-</sup> and  $\Gamma$  etc.) with monodentate tertiary phosphane and multidentate nitrogen donor bases yielded a diverse array of two-, three- and four-coordinate complexes with structural and spectroscopic properties determined by specific choices of the phosphane

and the ancillary ligands, the stoichiometry and to a lesser extent by the choice of the anion  $^{34,35}$ . The general method of preparation of silver(I) with multidentate ligands is reacting a stoichiometric amount of phosphane with appropriate silver(I) salt. An attempt to prepare a mixed silver(I) complex of triphenylphosphane and 2mercaptothiazoline was performed by Altaf and Stoeckli-Evans<sup>34</sup> but the expected compound achieved instead, unexpected compound, was not an [Ag(PPh<sub>3</sub>)<sub>4</sub>].SbF<sub>6</sub>.CHCl<sub>3</sub> was isolated. The architecture of the geometry around silver(I) was found to be determined by the type of tertiary phosphane and the molar ratio of the reactants used. Reaction of  $AgSbF_6^-$  and  $AgPF_6^-$  with tertiary phosphanes containing bulky groups such as cyclohexylphosphane imposed a linear geometry around the central atom containing non-coordinating counter ions while trigonal planar and tetrahedral geometry were obtained in the case of silver(I) salts with coordinating anions. When triphenylphosphane was used in the reaction, a tetrahedral geometry is confirmed with coordinating or non-coordinating anions  $^{34}$ .

A recent study on silver nanoparticles, AgNPs which was prepared from *I. obliquus* (Chaga mushroom) extract and silver nitrate solution showed remarkable anti-bacterial and anti-cancer activities. The combination of AgNPs with penicillin and tetracycline respectively, increased the antibacterial activity against Gram-positive and Gram-negative bacteria of which the largest inhibition zones observed were against *S. epidermidis* and *E. coli*. The hydroxyl and amido groups of the anti-biotics chelated to silver thus resulted in the better anti-bacterial activity <sup>36</sup>. The anti-bacterial activity of Ag(I) depends on the fast ligand exchange rate with the amino acids or nucleotides. It was also reported that Ag-P complexes synthesized by Ortego *et al.* did not possess any anti-bacterial activity. Additionally, complexes bearing S-donor atom showed a narrower activity range against bacteria. On the other hand, complexes with Ag-N and

Ag-O bonds complexes exhibited a broader spectrum as anti-bacterial agents due to their ability to readily exchange the original ligands with biomolecules. A series of new (aminophosphane)gold(I) and silver(I) complexes were reported to possess moderate anti-microbial activity against certain Gram-positive and Gram-negative bacteria<sup>37</sup>.

AgNPs also serve as a good free radical scavengers. Its free radical scavenging activity increases with the increase in concentration. In addition, the anti-proliferative activity demonstrated by AgNPs was proven against A549 human lung cancer and MCF-7R human breast cancer. The cell proliferation of these cancer cells were significantly suppressed compared to its standard drug. The active physicochemical interaction of silver with the thiol groups of protein is one of the key factor to the cytotoxic effect of silver <sup>36,38</sup>.

### 1.2.3 Copper

Copper(I) has also attracted interest in exploring its structural chemistry with mixed ligands. By employing a strong reducing agent such as tertiary phosphane, numerous compounds of copper(I) and copper(II) were prepared by reaction with neutral phosphane and amine ligands to form mono- or multi-nuclear copper complexes. Extensive studies were made in the case of tertiary phosphane with triphenylphosphane being the most studied phosphane and to a lesser extent, tricyclohexylphosphane. The stoichiometry of the metal salts and ligands as well as the solvent of recrystallization played an important role in determining the geometry around the copper(I) atom <sup>39</sup>. Tertiary phosphanes such as triphenylphosphane are capable to reduce copper(II) to copper(I) thus, much work was carried out by using copper(II) salts as starting materials. Complexes with triphenylphosphane and thiosemicarbazide have been

attempted but the expected products were not obtained. Instead, a tetrahedral complex having a coordinated bromide ion, [CuBr(PPh<sub>3</sub>)<sub>3</sub>].CH<sub>3</sub>CN was obtained <sup>39</sup>. This reaction was done *in situ* and was very similar to that for the preparation of [Ag(PPh<sub>3</sub>)<sub>4</sub>].SbF<sub>6</sub>.CHCl<sub>3</sub>. The dependency on the properties of the ligands, counter anions and crystallization solvents seems to determine the success of producing the desired mixed phosphine-ligand complexes.

Copper is an essential element that acts as a cofactor in an aerobic metabolism that has been applied as long ago as the 5<sup>th</sup> and 6<sup>th</sup> millennia. Copper is also thought to provide benefit in cancer research. Excessive or low amounts of this metal can cause deleterious effects. The generation of reactive oxygen species (ROS) is the main reason of why copper is a toxic element. A redox cycling between Cu(0), Cu(I) and Cu(II) probably explains the generation of ROS <sup>40</sup>. Cu(I) complexes bearing the N-heterocyclic carbenes (NHC) as the ligands display a patent profile of cytotoxicity. The apoptosis and cell homeostasis misregulation due to ROS production was suggested as the plausible mode of action <sup>41</sup>.

Currently, the use of copper has extended to numerous anti-microbial agents including oral hygiene products and anti-septics. However, methicillin-resistant *S. Aureus* (MRSA), *C. difficile*, *E. coli*, *L. monocytogenes*, *Influenza* A (H1N1), *A. niger* and *P. aeruginosa*, to name a few, were tested with copper and the results indicated the potent anti-bacterial effect exhibited upon treatment with copper  $^{40}$ .

For example, when *Burkholderia cepacia* complex (Bcc) was overlaid on a copper surface, a decrease in the viable bacteria count was observed as compared to PVC and stainless steel surfaces. This bacteria count on the copper surface compared with PVC

and stainless steel surfaces was significantly lower after 2 to 4 hours at room temperature and was more pronounced at 6 to 8 hours. Other than that, copper powder also displayed potential anti-microbial activity when applied in combination with different metal alloys. Structural damage and cell lysis in bacteria upon long exposure of bacteria to copper can be explained by the influx of copper ion into bacterial cells. The anti-bacterial properties of copper can also be seen on the copper surface itself where the colour of the copper sheet started to turn to dark brown with time. A pale blue colour due to bacterial suspension on the surface is observed and indicated the release of  $Cu^{2+}$  ion <sup>40</sup>.

### **1.3** Objective of Research

The discovery of new and more potent water-soluble drugs is of interest for most researchers involved in the field of medicinal chemistry in the quest to discover useful bioactivity. In order to achieve this, we therefore endeavoured to synthesize a few series of gold(I) dithiocarbamate complexes bearing three different phosphane derivatives.

Various gold(I) compounds of structural formula R<sub>3</sub>P-Au-X where R = alkyl and X = thiolate demonstrated that the anti-cancer activity is enhanced due to the presence of the P-Au-S moiety. The findings by Keter *et al.* <sup>42</sup> reinforced the concept that it is the P-Au-S motif that enhanced the activity of the phosphane-gold thiolate against cancer cells. In contrast to gold(I) thiolate (AuSR) compounds, the phosphanegold(I) thiolate (R<sub>3</sub>PAuSR') was found to exhibit better activity against cancer cells and inhibit the HIV-1 virus <sup>23,43</sup>. Gold(I) thiolates bearing the phosphane group or other lipophilic substituents increased the lipophilicity of the compounds <sup>44-46</sup>, therefore increasing membrane permeability to make them active <sup>42</sup>. Although the number of phosphanes in

a compound plays a role to improve lipophilicity, general permeabilization caused by non-selective accumulation in mitochondria could also occur due to the high lipophilicity of some compounds. Another consideration that should be taken into account is the solubility of compounds in water. This could be achieved by introducing hydrophilic components in a compound so that a balance of lipophilic / hydrophilic properties is achieved thus, improve assimilation of the drug <sup>23</sup>.

Gold(I) compounds containing mono- and diphosphane ligands were synthesized to increase their potential anti-tumour activities. For instance, the triphenylphosphane group in Figure 1.6 was crucial to protect gold(I) from biological modification that leads to effective anti-cancer activity against different cancer cells<sup>23</sup>.



**Figure 1.6:** Chemical structure of chlorotriphenylphosphinobis(dialkylphosphino)propylgold(I).

In this study, we designed compounds containing monodentate phosphane such as triphenylphosphane (PPh<sub>3</sub>), tricyclohexylphosphane (PCy<sub>3</sub>) and triethylphosphane (PEt<sub>3</sub>). The dithiocarbamate ligands [ $^{-}S_2CNR(CH_2CH_2OH)$ ] will contain both hydrophilic and lipophilic units. The lipophilic group, R was chosen from simple alkyl group while the hydrophilic group will bear a hydroxyl substituent. This is to attempt to synthesize compounds with enhanced water-soluble properties as well as to maintain its efficacy through cell membrane.

The syntheses of unsymmetrical organotin(IV) complexes composed of N-alkyl-Nhydroxyethyldithiocarbamate had been achieved <sup>47</sup> but since no work was carried out on  $R'_{3}PAu[S_{2}CNR(CH_{2}CH_{2}OH)]$  where R' = Ph, Cy and Et, we decided to work on these series by exploiting the substituents on the dithiocarbamate (R = Me, iPr, CH<sub>2</sub>CH<sub>2</sub>OH) and the phosphane unit. Moreover, a series of auranofin-like compounds bearing the  $[Et_3PAu]^+$ group containing two additional dithiocarbamates, namely diethyldithiocarbamate and pyrrolidinodithiocarbamate were also prepared. This work was carried out to investigate and compare the ability of the compounds with auranofin, since auranofin also features a  $[Et_3PAu]^+$  group. We would like to consider the possibility of enhancing the anti-cancer activity of compounds related to auranofin. In addition, series of silver(I) and copper(I) derivatives of similar dithiocarbamate and phosphane ligands;  $(R'_{3}P)_{2}Ag[S_{2}CNR(CH_{2}CH_{2}OH)]$ and  $(R'_{3}P)_{2}Cu[S_{2}CNR(CH_{2}CH_{2}OH)]$  were also prepared in order to compare the influence of the metal centre in their biological efficacies.

# **CHAPTER 2: EXPERIMENTAL METHODOLOGY**

# 2.1 Chemicals

All chemicals and solvents were purchased from Sigma Aldrich (M) Sdn. Bhd., Merck (M) Sdn. Bhd. and Fisher Scientific (M) Sdn. Bhd. and were used without further purification. Ammonium pyrrolidinodithiocarbamate was purchased from Sigma Aldrich (hereafter referred to as L5). Biological kits for anti-cancer and anti-bacterial studies were purchased from local suppliers.

# 2.2 Synthetic Methodology

2.2.1 Schematic diagram for the preparation of (hydroxyethyl)dithiocarbamates; KL1, NaL2 and KL3.

The preparation of KL1, NaL2 and KL3 is summarized in the scheme below.



M: Na or K R': Me, i-Pr, CH<sub>2</sub>CH<sub>2</sub>OH

Scheme 2.1: Preparation of KL1, NaL2 and KL3.

2.2.2 Syntheses of (hydroxyethyl)dithiocarbamates; KL1, NaL2 and KL3.

#### 2.2.2.1 Synthesis of potassium N-(hydroxyethyl)-N-methyldithiocarbamate, KL1.

KL1 was prepared *in situ* by mixing equimolar amounts of carbon disulfide in diethyl ether with potassium hydroxide in cold condition. The temperature of reaction was maintained at below 10 °C in an ice-bath. N-(hydroxyethyl)-N-methylamine in diethyl ether was added drop-wise into the reaction mixture <sup>5,48,49</sup>. Approximately 500 ml of diethyl ether was poured into the reaction mixture and stirring was continued for 2 hours. Solvent extraction was carried out to isolate the dithiocarbamate from diethyl ether and the liquid compound was used immediately.

## 2.2.2.2 Synthesis of sodium N-(hydroxyethyl)-N-isopropyldithiocarbamate, NaL2.

NaL2 was prepared *in situ* by mixing equimolar amounts of carbon disulfide in acetone with sodium hydroxide in cold condition. The temperature of reaction was maintained at below 10 °C in an ice-bath. N-(hydroxyethyl)-N-isopropylamine in acetone was added drop-wise into the reaction mixture and was stirred until precipitation occurred <sup>5,48,49</sup>. Stirring was continued for 2 hours and the precipitates formed were isolated upon filtration, washed several times with acetone and was stored in a desiccator over silica gel.

KL3 was prepared *in situ* by mixing equimolar amounts of carbon disulfide in acetone with potassium hydroxide in cold condition. The temperature of reaction was maintained at below 10 °C in an ice-bath. N,N-bis(hydroxyethyl)amine in acetone was added drop-wise into the reaction mixture and was stirred until precipitation occurred <sup>5,48,49</sup>. Stirring was continued for 2 hours and the precipitates formed were isolated upon filtration, washed several times with acetone and was stored in a desiccator over silica gel.

2.2.3 Schematic diagram for the preparation of sodium diethyldithiocarbamate, NaL4.

The preparation of NaL4 is summarized in the scheme below.



Scheme 2.2: Preparation of NaL4.

2.2.4 Synthesis of sodium N,N-diethyldithiocarbamate, NaL4.

NaL4 was prepared *in situ* by mixing equimolar amounts of carbon disulfide in acetone with sodium hydroxide in cold condition. The temperature of reaction was maintained at below 10 °C in an ice-bath. N,N-diethylamine in acetone was added drop-wise into the reaction mixture and was stirred until precipitation occurred <sup>5,48</sup>. Stirring was continued

for 2 hours and the precipitates formed were isolated upon filtration, washed several times with acetone and was stored in a desiccator over silica gel.

2.2.5 Schematic diagram for the preparation of phosphanegold(I) chloride precursors.

The preparation of trialkyl/arylphosphinogold(I) chloride is summarized in the scheme below.

Et<sub>3</sub>PAuCl (2) 0.48 M HCl (2) 0.48 M HCl  $(3) N_2 (g), 2PEt_3 \text{ solution}$   $(3) C_2 (g), 2PEt_3 \text{ solution$ 

Scheme 2.3: Preparation of phosphanegold(I) chloride precursors.

2.2.6 Syntheses of trialkyl / triarylphosphanegold(I) chloride precursors.

### 2.2.6.1 Synthesis of (triphenylphosphane)gold(I) chloride, Ph<sub>3</sub>PAuCl.

A modification in the literature synthetic procedure from  $^{17,50}$  was employed. Potassium tetrachloroaurate, KAuCl<sub>4</sub> (1 mmol, 0.30 g) was stirred in a mixture of water : acetone (90 ml : 30 ml). Sodium sulfite solution (2 mmol, 0.20 g, 30 ml) was added drop-wise into the gold solution until the yellow solution turned colourless. Triphenylphosphane (1 mmol, 0.21 g, 5 ml) in acetone was added and stirring was continued for 1 hour. The white precipitate was extracted with chloroform / water, dried over anhydrous sodium sulfate and was allowed to dry at room temperature. Recrystallization was performed in chloroform : ethanol (1 : 1).

A modification in the literature synthetic procedure from  $^{17}$  was employed. Potassium tetrachloroaurate, KAuCl<sub>4</sub> (1 mmol, 0.30 g) was stirred in a mixture of water : acetone (90 ml : 30 ml). Sodium sulfite solution (2 mmol, 0.20 g, 30 ml) was slowly added into the gold solution until the yellow solution turned colourless. Tricyclohexylphosphane (1 mmol, 0.22 g, 5 ml) in acetone was added and stirring was continued for 1 hour. The white precipitate was extracted with chloroform / water and was allowed to dry at room temperature. Recrystallization was performed in ethanol.

### 2.2.6.3 Synthesis of (triethylphosphane)gold(I) chloride, Et<sub>3</sub>PAuCl.

Potassium tetrachloroaurate, KAuCl<sub>4</sub> (1 mmol, 0.30 g) was dissolved in acetone (10 ml). The solution was acidified with equimolar amount of 0.48 M hydrochloric acid and was purged with nitrogen gas for 1-2 minutes. 1 M triethylphosphane solution in THF was added (2 mmol) and the colourless solution was stirred at room temperature for 1 hour. Solvent extraction was carried out using chloroform : water (1 : 3). The solution was allowed to dry over anhydrous sodium sulfate and allowed to stand at room temperature to yield white solids.
2.2.7 Schematic diagram for the preparation of triphenyl- and tricyclohexyl-phosphanegold(I) dithiocarbamate complexes of L1, L2 and L3.

The preparation of Ph<sub>3</sub>PAu(dtc) and Cy<sub>3</sub>PAu(dtc) is summarized in the scheme below.



Scheme 2.4: Preparation of triphenyl- and tricyclohexyl-phosphanegold(I) dithiocarbamate complexes.

2.2.8 Syntheses of triphenylphosphanegold(I) dithiocarbamates of L1, L2 and L3.

2.2.8.1 Synthesis of  $Ph_3PAu(L1)$ .

# **Preparation 1**

KL1 (1 mmol, 0.20 g) in water (30 ml) was added drop-wise to a suspension of Ph<sub>3</sub>PAuCl (1 mmol, 0.49 g) in acetone (5 ml). The solution was stirred for 1 hour to give a yellow gum. Water was decanted and after several washing with water, the gum was later dissolved in a minimum volume of methanol and was added drop-wise into 1 litre of diethyl ether while stirring vigorously until precipitation occurred. The yellow precipitates were separated using a separating funnel. The yellow compound that deposited at the bottom of the separating funnel was isolated and was allowed to dry at room temperature.

#### **Preparation 2**

KL1 (1 mmol, 0.20 g) in water (30 ml) was added drop wise to a suspension of Ph<sub>3</sub>PAuCl (1 mmol, 0.49 g) in acetone (5 ml). The solution was stirred for 1 hour to give a yellow gum. Water was decanted and after several washing with water, the gum was later dissolved in a minimum volume of methanol, poured into a mortar and the solvent was allowed to evaporate at ambient temperature. Yellow solids were collected after 1-2 days.

Melting point: 144.5 °C. Yield (%): 54%. Elemental analyses (%): Found: C; 43.73, H; 3.46, N; 2.55%. Calculated: C; 43.35, H; 3.80, N; 2.30%.

2.2.8.2 Synthesis of  $Ph_3PAu(L2)$ .

A similar procedure as in 2.2.8.1 was repeated by changing the ligand to NaL2 to form Ph<sub>3</sub>PAuL2. Melting point: 136 °C. Yield (%): 54%. Elemental analyses (%): Found: C; 45.22, H; 4.24, N; 2.20%. Calculated: C; 45.34, H; 4.06, N; 2.07%.

2.2.8.3 Synthesis of Ph<sub>3</sub>PAu(L3).

A similar procedure as in 2.2.8.1 was repeated by changing the ligand to KL3 to form Ph<sub>3</sub>PAuL3. Melting point: 150 °C. Yield (%): 66%. Elemental analyses (%): Found: C; 41.65, H; 3.65, N; 2.01%. Calculated: C; 41.25, H; 3.46, N; 2.29%.

2.2.9 Syntheses of tricyclohexylphosphanegold(I) dithiocarbamates of L1, L2 and L3.

2.2.9.1 Synthesis of Cy<sub>3</sub>PAu(L1).

KL1 (1 mmol) in water (20 ml) was added drop-wise to a suspension of Cy<sub>3</sub>PAuCl (1 mmol, 0.51 g) in acetone (10 ml). The mixture was stirred for 1 hour giving a yellow solid, which was filtered, washed with water, and allowed to dry at room temperature. Melting point: 159 °C. Yield (%): 60%. Elemental analyses (%): Found: C; 41.69, H; 6.75, N; 1.85%. Calculated: C; 42.10, H; 6.58, N; 2.23%.

2.2.9.2 Synthesis of  $Cy_3PAu(L2)$ .

The synthetic method as in 2.2.9.1 was repeated by replacing KL1 with NaL2 to give  $Cy_3PAuL2$ . Crystals of this compound were formed from the slow evaporation technique in THF. Melting point: 146 °C. Yield (%): 53%. Elemental analyses (%): Found: C; 43.78, H; 7.15, N; 2.16%. Calculated: C; 43.96, H; 6.92, N; 2.14%.

2.2.9.3 Synthesis of Cy<sub>3</sub>PAu(L3).

The synthetic method as in 2.2.9.1 was repeated by replacing KL1 with KL3 to give Cy<sub>3</sub>PAuL3. Melting point: 162.5 °C. Yield (%): 67%. Elemental analyses (%); Found: C; 40.46, H; 6.64, N; 2.62%. Calculated: C; 40.06, H; 6.24, N; 2.22%.

2.2.10 Schematic diagram for the preparation of triethylphosphanegold(I) dithiocarbamates of L1 - L5.

The preparation of triethylphosphanegold(I) dithiocarbamates is summarized in the scheme below.



Scheme 2.5: Preparation of triethylphosphinogold(I) dithiocarbamates.

2.2.11 Syntheses of triethylphosphanegold(I) dithiocarbamates of L1 – L5.

# 2.2.11.1 Synthesis of $Et_3PAu(L1)$ .

KL1 (1 mmol) in a minimum amount of water (20 ml) was added drop-wise to a suspension of  $Et_3PAuCl$  (1 mmol, 0.35 g) in acetone (10 ml). The mixture was stirred for 1 hour after which a bright-yellow gum was obtained. Solvent extraction was performed in chloroform : water (1 : 3). The chloroform layer was filtered off, dried over anhydrous sodium sulfate and was allow to dry at room temperature to yield as yellow gum.

A similar procedure as in 2.2.11.1 was carried out by changing the ligand to NaL2 to yield Et<sub>3</sub>PAu(L2). This compound underwent recrystallization in chloroform, giving yellow, block crystals. Melting point: 80 °C. Yield (%): 63%. Elemental analyses (%): Found: C; 29.30, H; 5.93, N; 2.70%. Calculated: C; 29.21, H; 5.52, N; 2.84%.

#### 2.2.11.3 Synthesis of $Et_3PAu(L3)$ .

A similar procedure as in 2.2.11.1 was carried out by changing the ligand to KL3 to yield  $Et_3PAu(L3)$ . No crystals were obtained as the compound was a gum.

# 2.2.11.4 Synthesis of $Et_3PAu(L4)$ .

A similar procedure as in 2.2.11.1 was applied by changing the ligand to NaL4 to give Et<sub>3</sub>PAu(L4) <sup>51</sup>. Melting point: 91.2 °C. Yield (%): 80%. Elemental analyses (%): Found: C; 28.91, H; 5.10, N; 3.42%. Calculated: C; 28.51, H; 5.44, N; 3.02%.

#### 2.2.11.5 Synthesis of $Et_3PAu(L5)$ .

A similar method as in 2.2.11.1 was performed by changing the ligand to NH<sub>4</sub>L5 to yield Et<sub>3</sub>PAu(L5). Melting point: 76.2 °C. Yield (%): 85%. Elemental analyses (%): Found: C; 29.04%, H; 5.06%, N; 2.93%. Calculated: C; 28.64%, H; 5.02%, N; 3.04%. 2.2.12 Schematic diagram for the preparation of bis(triphenylphosphino)copper(I) dithiocarbamates of L1, L2 and L3.

The preparation of (Ph<sub>3</sub>P)<sub>2</sub>Cu(dtc) is summarized in the scheme below.



Scheme 2.6: Preparations of bis(triphenylphosphane)copper(I) dithiocarbamates.

2.2.13 Syntheses of bis(triphenylphosphane)copper(I) dithiocarbamates, of L1, L2 and L3.

# 2.2.13.1 Synthesis of $(Ph_3P)_2Cu(L1)$ .

A modified procedure from <sup>52</sup> was employed. CuCl (1 mmol) was stirred with triphenylphosphane (2 mmol) in acetone (20 ml) at 50 °C until a white precipitate was obtained. An aqueous solution of KL1 (1 mmol) was added to the reaction mixture followed by stirring for 1 hour. The product underwent solvent extraction with chloroform : water (1 : 3), filtered and allowed to dry at room temperature. The precipitate was then washed in diethyl ether under vigorous stirring and filtered. Recrystallization was performed in acetone via a fast evaporation technique at ambient temperature to yield white solids of  $(Ph_3P)_2Cu(L1)$ . The crystals were then obtained from the same solvent via slow evaporation. Melting point: 155 °C. Yield (%):78%. Elemental analyses (%): Found: C; 64.99, H; 5.04, N; 1.78%. Calculated: C; 64.98, H; 5.32, N; 1.89%.

A similar method as in 2.2.13.1 was applied by changing the ligand to NaL2 to give  $(Ph_3P)_2Cu(L2)$ . Melting point: 175.2 °C. Yield (%): 70%. Elemental analyses (%): Found: C; 65.56, H; 5.51, N; 1.69%. Calculated: C; 65.73, H; 5.65, N; 1.83%.

# 2.2.13.3 Synthesis of $(Ph_3P)_2Cu(L3)$ .

A similar method as in 2.2.13.1 was applied by changing the ligand to KL3 to give  $(Ph_3P)_2Cu(L3)$ . Crystals were obtained as colourless blocks from slow evaporation of acetone at room temperature. Melting point: 163.6 °C. Yield (%): 75%. Elemental analyses (%): Found: C; 63.39, H; 5.28, N; 1.61%. Calculated: C; 64.00, H; 5.37, N; 1.82%.

2.2.14 Schematic diagram for the preparation of bis(triphenylphosphane)silver(I) dithiocarbamates of L1, L2 and L3.

The preparation of  $(Ph_3P)_2Ag(dtc)$  is summarized in the scheme below.





2.2.15 Syntheses of bis(triphenylphosphane)silver(I) dithiocarbamates of L1, L2 and L3.

# 2.2.15.1 Synthesis of $(Ph_3P)_2Ag(L1)$ .

A modified method from <sup>52</sup> was employed. AgNO<sub>3</sub> (1 mmol) was stirred with triphenylphosphane (2 mmol) in acetone (20 ml) in a 1 : 2 ratio <sup>53</sup> at room temperature until a suspension was obtained. KL1 (1 mmol) in water (20 ml) was added to the reaction mixture and this was allowed to stir for 1 hour. Chloroform was added and stirring was continued for another 1 hour, after which the yellow solution was separated from the aqueous layer. After drying over anhydrous sodium sulfate, the solution was filtered and allowed to undergo fast evaporation to yield white solids. The solid compound was washed with diethyl ether and was isolated through filtration. Recrystallization was performed in acetone via slow evaporation, yielding colourless blocks. Melting point: 170.5 °C. Yield (%): 76%. Elemental analyses (%): Found: C; 61.30, H; 4.86, N; 1.87%. Calculated: C; 61.30, H; 5.02, N; 1.79%.

#### 2.2.15.2 Synthesis of $(Ph_3P)_2Ag(L2)$ .

A similar procedure as in 2.2.15.1 was applied by changing the ligand to NaL2 to yield (Ph<sub>3</sub>P)<sub>2</sub>Ag(L2). Recrystallization was performed in acetone via slow evaporation technique; yielding colourless blocks. Melting point: 151.9 °C. Yield (%): 70%. Elemental analyses (%): Found: C; 62.13, H; 4.95, N; 1.73%. Calculated: C; 62.14, H; 5.34, N; 1.73%.

A similar procedure as in 2.2.15.1 was applied by changing the ligand to KL3 to yield (Ph<sub>3</sub>P)<sub>2</sub>Ag(L3). Recrystallization was performed in chloroform : acetone (1 : 1). Melting point: 162 °C. Yield (%): 77%. Elemental analyses (%): Found: C; 60.34, H; 5.00, N; 1.44%. Calculated: C; 60.52, H; 5.08, N; 1.72%.

# 2.3 Instrumentation

2.3.1 Nuclear Magnetic Resonance (NMR) Spectrometer

<sup>1</sup>H and <sup>31</sup>P{<sup>1</sup>H} NMR were measured using a Bruker Avance III 400 MHz instrument while <sup>13</sup>C{<sup>1</sup>H} NMR were acquired using a Bruker Avance III 400 MHz and Jeol ECA 400 MHz instruments. All experiments were carried out in deuterated chloroform, deuterated acetone or deuterated DMSO. The <sup>1</sup>H and <sup>13</sup>C{<sup>1</sup>H} NMR were referenced to internal TMS. The <sup>31</sup>P{<sup>1</sup>H} was internally referred to 85% phosphoric acid.

# 2.3.2 Infrared (IR) Spectrometer

Infrared spectra were collected using an Attenuated Technique Reflection (ATR) on a Perkin Elmer Spectrum 2000 Spectrophotometer.

#### 2.3.3 Melting Point Measurement

Melting points were recorded by using DSC on a TA Instrument Q500.

#### 2.3.4 Elemental Analyser

Analyses of the composition of C, H and N were performed on a Perkin Elmer CHN Analyzer 2400.

#### 2.3.5 Powder X-ray Diffractometer (PXRD)

The powder X-ray diffraction patterns were recorded on a PANalytical Empyrean XRD system with Cu-K $\alpha$ 1 radiation ( $\lambda = 1.54056$  Å) in the 2 $\theta$  range 5° to 40° with a step size of 0.026° per second. Experimental data from PXRD pattern was compared with the calculated data obtained from the single crystal X-ray data (CIF file) using PANalytical HighScore Plus software <sup>54</sup>.

# 2.3.6 Single Crystal X-ray Diffractometer (SCXRD)

X-ray measurements of suitable crystals were performed at 100 K on an Agilent Supernova dual wavelength diffractometer with an Atlas (Mo) detector employing the  $\omega$  scan technique and using graphite monochromatized Mo K $\alpha$  radiation so that  $\theta_{max}$  was 27.6°. The structures were solved by direct methods (SHELXS97) through the WinGX Interface and refined (anisotropic displacement parameters, H atoms in the riding model approximation and a weighting scheme of the form w =  $1/[\sigma^2(F_o^2) + aP^2]$  where P =  $(F_o^2 + 2F_c^2)/3)$  with SHELXL97 on F<sup>2</sup>[x2]. The molecular structures were drawn with 50% displacement ellipsoids and the crystal packing diagrams with Mercury 3.5<sup>55</sup>.

#### 2.4 Biological Assays

#### 2.4.1 Anti-cancer Studies

## 2.4.1.1 *Cell culture and inhibition of cell growth*

The selected cancer cell line was obtained from ATCC: The Global Bioresource Center and maintained in culture as described by the provider. The cells were routinely grown in RPMI 1640 medium containing 10% foetal calf serum (FCS) and antibiotics at 37 °C and 6% CO<sub>2</sub>. For evaluation of growth inhibition tests, the cells were seeded in 96-well plates (Techno Plastic Products, TPP, Plastik für die Zellkultur, Switzerland) and grown for 24 h in complete medium. The stock solutions of the trial compounds were prepared by dissolving the compounds in 1 ml of DMSO to reach a concentration of  $10^{-2}$  M. They were then diluted in RPMI medium and added to the wells (100 µl) to obtain a final concentration ranging between 0 and 80 µM. DMSO at comparable concentrations did not show any effects on cell cytotoxicity. Stock solutions of the compounds were diluted directly in culture medium to the required concentration and added to the cell culture. After 24 h incubation at 37 °C, 20 µl of a solution of MTT (3-[4,5dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) in PBS (2 mgml<sup>-1</sup>) was added to each well, and the plates were then incubated for 2 h at 37 °C. The medium was then aspirated and DMSO (100 µl) was added to dissolve the precipitate. The absorbance of each well was measured at 580 nm using a 96-well microplate reader and compared to the values of control cells incubated without test compound. The IC<sub>50</sub> values for the inhibition of cell growth were determined by fitting the plot of the percentage of surviving cells against the drug concentration using a sigmoidal function (Origin v7.5) 55

Total RNA was extracted from cultured MCF-7R cells using a high-purity RNA Isolation Kit (Qiagen, Germany). The real-time PCR for microarray assay was performed using the RT<sup>2</sup> Profiler PCR microarray according to the manufacturer's protocol (Qiagen, USA). Gene expression was compared according to the CT value. Results are based on <sup>55</sup>.

# 2.4.1.3 *Caspase activity (Caspases-3, -7, -8, -9 and -10)*

Caspase activity was assayed by measuring the light intensity using a kit (Caspase Assay, Milipore) and a luminometer (Perkin Elmer HTS 7000, France). Briefly, cells were cultured in 96-well plates in a final volume of 200 ml. Then 50 ml caspase reaction buffer was added and incubated at room temperature for 1 h before measurement <sup>55</sup>.

#### 2.4.1.4 Membrane permeability study by AOPI staining

The MCF-7R cells at a concentration of 5 x  $10^3$  cells/well in 96-well plates were treated with the IC<sub>50</sub> concentration of each compound and incubated for 24 h. Untreated cells were included as a negative control. Treated cells were harvested from the culture flask. 1 x EDTA free-PBS was used to wash the cells twice before transferring to a microcentrifuge tube. The cells were centrifuged at 1000 g for 10 min. Subsequently, the cells were suspended in 100  $\mu$ L 1 x PBS. Then, a 5 mg/ml acridine orange (AO) (Sigma) and propidium iodide (PI) (Sigma) mixture was added to the cells at 1:1 ratio for staining. This was followed by chilling on ice for 10 min. The mixture (20  $\mu$ L) was aliquoted onto a slide and covered with a cover slip and viewed under an Olympus BX- 51 fluorescence microscope. Images were captured by an attached Olympus CMAD-2 camera. The mode of cell death was then determined <sup>55</sup>.

# 2.4.1.5 Intracellular reactive oxygen species (ROS) measurements

5-(and-6)-carboxy-2',7'-dichlorodihydrofluorescein diacetate (carboxy-H2DCFDA) (Sigma, USA) was used to detect intracellular ROS according to the manufacturer's instructions. For ROS quantification, cells were seeded in 96-well black plates (Greiner Bio-One, France) and treated with the trial compound at the indicated  $IC_{50}$  concentrations for 24 h. Afterwards, cells were washed with PBS and incubated with 10  $\mu$ M carboxy-H2DCFDA in DPBS for an hour. Cells were then washed and fluorescence was measured by a plate reader (Perkin Elmer, France) with an excitation wavelength of 535 nm <sup>55</sup>.

# 2.4.1.6 Human topoisomerase I inhibition assay

The human DNA topoisomerase I inhibitory activity was determined by measuring the relaxation of supercoiled plasmid DNA pBR322. Each reaction mixture contained 10 mM Tris-HCl, pH 7.5, 100 mM NaCl, 1 mM phenylmethylsulfonyl fluoride,  $\alpha$ -toluenesulfonyl fluoride, PMSF, and 1 mM 2-mercaptoethanol, 0.25 µg plasmid DNA pBR322, 1 unit of human DNA topoisomerase I, and the test compound at a specified concentration. The total volume of each reaction mixture was 20 µL and these mixtures were prepared on ice. Upon enzyme addition, reaction mixtures were incubated at 37 °C for 30 min. The reactions were terminated by the addition of 2 µL of 10% SDS, and then followed by 3 µL of dye solution comprising 0.02% bromophenol blue and 50% glycerol. SDS is required to denature topoisomerase I, preventing further functional

enzymatic activity. Each mixture was applied to 1.2% agarose gel and underwent electrophoresis for 5 h at 33 V with running buffer of Tris-acetate EDTA, TAE. The gel was stained, destained and photographed under UV light using a Syngene Bio Imaging system and the digital image was viewed with Gene Flash software <sup>55</sup>.

#### 2.4.2 Anti-bacterial Studies

#### 2.4.2.1 Anti-bacterial Activity Assay

The disk diffusion method was applied to screen for anti-bacterial activity in accordance with the National Committee for Clinical Laboratory Standards (NCCLS) guidelines. A total of 25 bacterial strains were included in this study, namely Aeromonas hydrophilla (A. hydrophilla) ATCC 35654, Acinetobacter baumannii (A. baumannii) ATCC 19606, Bacillus cereus (B. cereus) ATCC 10876, Bacillus subtilis (B. subtilis) ATCC 6633, Citrobacter freundii (C. reundii) ATCC 8090, Enterobacter cloacae (E. cloacae) ATCC 35030, Enterobacter aerogenes (E. aerogenes) ATCC 13048, Enterococcus faecalis (E. faecalis) ATCC 29212, Enterococcus faecium (E. faecium) ATCC 19434, Escherichia coli (E. coli) ATCC 25922, Klebsiella pneumoniae (K. pneumoniae) ATCC 700603, Listeria monocytogenes (L. monocytogenes) ATCC 19117, Proteus mirabilis (P. mirabilis) ATCC 25933, Proteus vulgaris (P. vulgaris) ATCC 13315, Pseudomonas aeruginosa (P. aeruginosa) ATCC 27853, Salmonella paratyphi A (S. paratyphi A) ATCC 9150, Salmonella typhimurium (S. typhimurium) ATCC 14028, Shigella flexneri (S. flexneri) ATCC 12022, Shigella sonnei (S. sonnei) ATCC 9290, Staphylococcus aureus (S. aureus) ATCC 25923, methicillin resistant Staphylococcus aureus (MRSA) ATCC 43300, Staphylococcus saprophyticus (S. saprophyticus) ATCC 15305, Stenotrophomonas maltophilia (S. maltophilia) ATCC 13637, Streptococcus pyogenes (*S. pyogenes*) ATCC 49399 and *Vibrio parahaemolyticus* (*V. parahaemolyticus*) ATCC 17802. All bacterial cultures were purchased from American Type Culture Collection (ATCC). The inoculum suspension of each bacterial strain was adjusted to 0.5 McFarland standard turbidity (corresponding to approximately  $10^8$  CFU/ml) by adding Mueller-Hinton broth. This suspension was then swabbed on the surface of Mueller-Hinton agar (MHA) plates using a sterile cotton swab. The test compounds were dissolved in DMSO to achieve a test concentration of 2 mg/ml. Sterile 6 mm filter paper discs were aseptically placed on MHA surfaces and 5 µl of the dissolved test compound was immediately added to the discs. Each plate contained one standard anti-biotic paper disc, serving as the positive control, one disc served as negative control (5 µl broth) and one disc served as solvent control (5 µl DMSO). The plates were incubated at 37 °C for 24 hours. Anti-bacterial activity was evaluated by measuring the diameter of inhibition zone against each bacterial strain. Each experiment was performed in duplicate <sup>54</sup>.

# 2.4.2.2 Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

A broth micro-dilution method was used to determine the MIC and MBC values according to the NCCLS guidelines. The test compounds were serially twofold diluted in DMSO to achieve the range of test concentrations of 2000-0.06  $\mu$ g/ml and then placed into each well of a 96-well microplate. An inoculum suspension with density of  $10^5$  CFU/ml exponentially growing bacterial cells was added into each well. The 96-well microplates were incubated at 37 °C for 24 hours. All tests were performed in triplicate. Four controls comprising medium with standard anti-biotic (positive control), medium with DMSO (solvent control), medium with inoculums bacterial cells (negative control) and medium with broth only (negative growth control) were included in each test. Bacterial growth was detected by adding 50  $\mu$ l of a 0.2 mg/ml

*p*iodonitrotetrazolium violet (INT) indicator solution into each of the microplate wells and incubated at 37 °C for 30 minutes under aerobic agitation. Where bacterial growth was inhibited, the suspension in the well remained clear after incubation with INT. The INT will react in the presence of bacterial activity, as indicated by a change from clear to a red colour. The lowest concentration of the test compound which completely inhibited bacterial growth was considered as the MIC. After MIC determination, an aliquot of 100  $\mu$ l from each well which showed no visible growth was spread onto MHA and further incubated at 37 °C for 30 minutes. The MBC was defined as the lowest concentration of the tested compound that produced a 99.9% reduction in bacterial viable count on the MHA <sup>54</sup>.

#### 2.4.2.3 Time-kill assay

Time-kill assays were performed by the broth macro-dilution method in accordance with the NCCLS guidelines. Inoculum suspensions with approximately  $10^5$  CFU/ml of exponentially growing bacterial cells were used in this study. The test compound was added to 10 ml of inoculum suspensions with final concentrations corresponding to ½ x MIC, MIC and 2 x MIC. A growth control comprising the bacterial strain without the test compound was included in each trial. The inoculum cultures were incubated at 37 °C on an orbital shaker at 200 rpm. Aliquots were removed from the inoculum culture after timed intervals of incubation (i.e. 0, 1, 2, 3 and 4 hours, and 0, 4, 8 and 24 hours), and serial tenfold dilutions were prepared in saline as needed. The numbers of viable cells were determined by the plate count technique which involved plating 25 µl of each dilution on a MHA plate. All plates were incubated at 37 °C for 24 hours. The experiments were performed in triplicate. Data were analysed as killing curves by plotting the log<sub>10</sub> colony forming unit per mililitre (cfu/ml) versus time (hours) and the

change in bacterial concentration was determined. The viable bacterial cell count for the time-kill end point determination, i.e. bactericidal activity, is defined as a reduction of  $\geq$  3 log<sub>10</sub> cfu/ml relative to the initial inoculum, whereas bacteriostatic activity corresponds to < 3 log<sub>10</sub> cfu/ml decrease relative to the initial inoculums <sup>54</sup>.

# CHAPTER 3: RESULTS AND DISCUSSION (PART A: CHEMICAL CHARACTERIZATION)

# 3.1 Tricyclohexylphosphanegold(I) dithiocarbamate, Cy<sub>3</sub>PAu(dtc)

Three compounds of the Cy<sub>3</sub>PAu(dtc) series were synthesized and the atom labels are shown in Figure 3.1. The structural elucidations were carried out based on infra red spectroscopy, nuclear magnetic resonance ( ${}^{1}H$ ,  ${}^{13}C{}^{1}H$ ,  ${}^{31}P{}^{1}H$ ) spectroscopy, powder X-ray diffraction and single crystal X-ray diffraction.



**Figure 3.1:** Chemical structures of tricyclohexylphosphanegold(I) dithiocarbamate series: (a) Cy<sub>3</sub>PAu(L1), (b) Cy<sub>3</sub>PAu(L2) and (c) Cy<sub>3</sub>PAu(L3).

#### 3.1.1 Infra Red (IR) Spectroscopy

Table 3.1 showed the absorption bands of the tricyclohexylphosphanegold(I) dithiocarbamate series. The compounds of this series were successfully obtained as important diagnostic bands were observed.

Compounda	Wavenumber, v (cm <sup>-1</sup> )						
Compounds	О-Н	С-Н	C-N	C-S (asym)	C-S (sym)		
Cy <sub>3</sub> PAuCl	-	2848, 2925 (sh, s)	-	-	-		
(a)	3367	2848, 2924	1443	1175	1004		
	(br, m)	(sh, s)	(m, sh)	(m)	(m)		
(b)	3437	2850, 2925	1440	1051	965		
	(br, s)	(sh, s)	(m, sh)	(sh, s)	(m, sh)		
(c)	3228	2844, 2924	1458	1065	985		
	(br, m)	(sh, s)	(m)	(s)	(m, sh)		

 Table 3.1: Selected IR absorption bands of tricyclohexylphosphanegold(I) dithiocarbamate series.

\*br = broad, sh = sharp, s = strong, m = medium, w = weak

The interpretation of infrared bands shown for the precursor,  $Cy_3PAuCl$  gave only one vibrational band corresponding to C-H stretching mode at 1680 and 1101 cm<sup>-1</sup> respectively. The presence of broad O-H vibrational bands were observed in all three compounds of the tricyclohexylphosphanegold(I) dithiocarbamate series. Broad bands of OH in compound (**b**) vibrated at the highest energy region, 3437 cm<sup>-1</sup> followed by O-H stretch in compound (**a**) at 3367 and (**c**) at 3228 cm<sup>-1 56</sup>. The C-N stretching band was absent in the precursor but observed in the complexes as medium and sharp bands, in the range of 1440-1460 cm<sup>-1</sup>. The finger print region demonstrated the asymmetric and symmetric bands. A medium asymmetric stretching C-S band was seen at 1175 cm<sup>-1</sup> for (**b**) and (**c**) respectively. Symmetric stretching bands of C-S were found at approximately

965-1000 cm<sup>-1</sup>. The C-S stretching bands frequencies were quite close to the frequencies reported for mono- and bis-(dithiolate)gold(III) complexes  $^{57}$ .

3.1.2 <sup>1</sup>H Nuclear Magnetic Resonance (<sup>1</sup>H NMR) Spectroscopy

From Table 3.2, the multiplicity of the protons in the cyclohexyl ring was observed as a multiplet integrating with 33 protons, indicating the presence of the phosphane in the complexes. The OH signal for compound (a) was found to appear at 2.55 ppm of which is upfield compared to compounds (b) and (c) at 3.37 ppm. This may due to the bulkiness of compounds (b) and (c), with *i*Pr and CH<sub>2</sub>CH<sub>2</sub>OH respectively, that may lead to the formation of hydrogen bond, thus making the OH slightly deshielded.

Compound	Chemical Shift, δ (ppm) and Multiplicity
(a)	1.56 ppm, 33H (H1', H2', H3' and H4'), m;
	2.55 ppm, 1H (OH), s;
	3.44 ppm, 3H (H1), s;
	3.93 ppm, 2H (H3), s;
	$4.09 - 4.12$ ppm, 2H (H2), t, $J_{H-H} = 6$ Hz
<b>(b)</b>	1.27 ppm, 6H (H2), d;
	1.76 ppm, 33H (H1', H2', H3' and H4'), m;
	3.37 ppm, 1H (OH), t, J <sub>H-H</sub> = 6.0 Hz;
	3.92 ppm, 2H (H4), q, J <sub>H-H</sub> = 5.3 Hz;
	4.02 ppm, 2H (H3), t, $J_{H-H} = 6.0$ Hz;
	5.40 ppm, 1H (H1), sept, $J_{H-H} = 6.0 \text{ Hz}$
(c)	1.64 ppm, 33H (H1', H2', H3' and H4'), m;
	3.37 ppm, 2H (OH), s;
	4.08 ppm, 4H (H2), t, $J_{H-H} = 6.0$ Hz;
	4.20 ppm, 4H, (H1), t, $J_{H-H} = 6.0 \text{ Hz}$

**Table 3.2:** <sup>1</sup>H chemical shifts,  $\delta$  (ppm) and multiplicities of tricyclohexylphosphanegold(I) dithiocarbamate series.

The success in making compound (**a**) can also be confirmed with the appearance of a singlet, corresponding to methyl bonded to the N atom. This is observed at 3.44 ppm due to the deshielding effect of the nitrogen atom. For compound (**b**), a septet was

observed in the downfield region compared to other protons of the same compound. Similarly, the integration of H1 and H2 for compound (c) also showed expected number of protons.

3.1.3  ${}^{13}C{}^{1}H$  Nuclear Magnetic Resonance ( ${}^{13}C{}^{1}H$  NMR) Spectroscopy

All carbon signals correspond to carbons in the tricyclohexylphosphanegold(I)

dithiocarbamate series were shown in Table 3.3.

**Table 3.3:**  ${}^{13}C{}^{1}H$  chemical shifts,  $\delta$  (ppm) and of tricyclohexylphosphanegold(I) dithiocarbamate series.

Compound	Chemical Shift, δ (ppm) and Multiplicity
(a)	25.92 ppm (C4');
	27.09 ppm (C3'), d, J <sub>P-C</sub> = 52 Hz;
	30.60 ppm (C2');
	33.47 ppm (C1'), d, J <sub>P-C</sub> = 192 Hz;
	44.26 ppm (C1);
	58.70 ppm (C3);
	61.32 ppm (C2);
	209.50 ppm (C4)
<b>(b)</b>	20.18 ppm (C2);
	25.91 ppm (C4');
	27.09 ppm (C3'), d, J <sub>P-C</sub> = 44 Hz;
	30.47 ppm (C2');
	33.31 ppm (C1'), d, $J_{P-C} = 112$ Hz;
	49.62 ppm (C1);
	55.71 ppm (C4);
	63.00 ppm (C3);
	208.88 ppm (C5)
(c)	25.96 ppm (C4');
	27.13 ppm (C3'), d, $J_{P-C} = 48$ Hz;
	30.55 ppm (C2');
	$33.37 \text{ ppm}$ (C1'), d, $J_{P-C} = 112 \text{ Hz}$ ;
	59.14 ppm (C1);
	61.31 ppm (C2);
	210.51 ppm (C3)

Two doublets for each compound were observed for C1' and C3', indicating the occurrence of carbon-phosphorus coupling. C4' was found at the most upfield region

for compounds (**a**) and (**c**) but not in compound (**b**) as the methyl groups in compound (**b**) were more shielded than C4' (Table 3.3). Other carbon signals appeared at their expected chemical shifts and the quaternary carbons were observed at almost the same chemical shift for all compounds.

3.1.4  ${}^{31}P{}^{1}H$  Nuclear Magnetic Resonance ( ${}^{31}P{}^{1}H$  NMR) Spectroscopy

The phosphorus signals of the tricyclohexylphosphanegold(I) dithiocarbamates were displayed in Table 3.4. The tricyclohexylphosphanegold(I) chloride, Cy<sub>3</sub>PAuCl, showed a signal at 53.99 ppm.

**Table 3.4:** <sup>31</sup>P{<sup>1</sup>H} chemical shifts,  $\delta$ (ppm) of tricyclohexylphosphinogold(I) dithiocarbamate series.

Compound	Chemical Shift (ppm)
Cy <sub>3</sub> PAuCl	53.99
(a)	55.38
(b)	55.43
(c)	55.42

Upon complexation, a single signal was observed in each spectrum, indicating complete reaction had occurred. The chemical shifts of the corresponding phosphorus atom for each complex appear slightly downfield compared with signal of the precursor. There was no systematic difference in chemical shifts observed among the complexes.

#### 3.1.5 Powder X-ray Diffraction (PXRD)

# 3.1.5.1 PXRD pattern of compound (b)

The powdered form (red trace) of compound (**b**) was compared with the simulated pattern (blue trace) of its single crystal (see later) after underwent Lebail fitting during the analysis, as displayed in Figure 3.2.



Figure 3.2: Comparison between the experimental powder pattern (red trace) and simulated pattern collected from the single crystal X-ray data of compound (b).

Apparently, the powdered compound (**b**) matched the simulated pattern, indicating that the structure of bulk compound (**b**) is the same with that obtained from the crystal grown in THF via slow evaporation. Only crystals of compound (**b**) were successfully obtained while compounds (**a**) and (**c**) resulted in gummy substances upon contact with solvent.

# 3.1.6.1 SCXRD of compound (b)

Compound (**b**) crystallized as yellow, block shaped crystals from slow evaporation in tetrahydrofuran after a week. The crystals crystallized in the  $P2_1/n$  space group (Figure 3.3). Gold is coordinated in a distorted linear manner where the S1-Au-P1 angle is 170.79(3)°. The deviation from the linear angle 180° is ascribed to the presence of the intramolecular Au - - - S2 contact of 3.0386(9) Å<sup>55</sup>.



Figure 3.3: Molecular structure of (b).

In this structure, a longer bond length was observed between S1-C19, of 1.751(3) Å, while S2-C19 displayed a shorter bond length of 1.698(3) Å. A monodentate mode of coordination by the dithiocarbamate reflected the discrepancy in the C-S bond lengths with the shortest of these being associated with the weakly binding S2 atom  $^{55}$ .

# **3.2** Triethylphosphanegold(I) dithiocarbamates, Et<sub>3</sub>PAu(dtc)

Five compounds of the  $Et_3PAu(dtc)$  series were synthesized and the atom labels are shown in Figure 3.4. The structural elucidations were carried out based on infra red spectroscopy, nuclear magnetic resonance (<sup>1</sup>H, <sup>13</sup>C{<sup>1</sup>H}, <sup>31</sup>P{<sup>1</sup>H}) spectroscopy, powder X-ray diffraction and single crystal X-ray diffraction.



**Figure 3.4:** Chemical structures of triethylphosphanegold(I) dithiocarbamate series; (d) Et<sub>3</sub>PAu(L1), (e) Et<sub>3</sub>PAu(L2), (f) Et<sub>3</sub>PAu(L3), (g) Et<sub>3</sub>PAu(L4) and (h) Et<sub>3</sub>PAu(L5).

#### 3.2.1 Infra Red (IR) Spectroscopy

The selected IR absorption bands of triethylphosphanegold(I) dithiocarbamate series are given in Table 3.5. This table only presents four compounds of the series including the precursor, as other two compounds; compound (d) and (f) were obtained in as gummy substances.

Compounda	Wavenumber, v(cm <sup>-1</sup> )						
Compounds	О-Н	С-Н	C-N	C-S (asym)	C-S (sym)		
Et <sub>3</sub> PAuCl	-	2964	-	-	-		
		(sh, m)					
(e)	3360	2964	1452 (sh, s)	1038 (sh, s)	968 (m, sh)		
	(m, br)	(sh, w)					
( <b>g</b> )	-	2969	1484 (sh, s)	1047 (s, sh)	982 (s, sh)		
		(sh, m)					
( <b>h</b> )	-	2958	1432 (s, m)	1040 (s, sh)	999 (s, sh)		
		(sh, m)					

 Table 3.5: Selected IR absorption bands of the triethylphosphanegold(I) dithiocarbamate series.

\*br = broad, sh = sharp, s = strong, m = medium, w = weak

The stretching mode of C-N for compounds (e), (g) and (h) were observed in similar range from 1432-1452 cm<sup>-1</sup> and is absent in the precursor. A sharp and strong band at 1452 and 1484 cm<sup>-1</sup> indicated the existence of amide bond in (e) and (g), respectively. For (h), the amide stretching mode was seen at 1432 cm<sup>-1</sup> as a strong band  $^{56}$ .

Remarkable differences in the C-N stretching bands for the compounds (except the precursor) in Table 3.5, indicated the strength of the vibrations. Larger wavenumbers signify the bond strength of a functional group therefore, related to the frequency of vibration. The C-N bond in (g) is stronger than other derivatives collected in Table 3.5, thus the vibration frequency is stronger. In this table, (h) showed the weakest vibration frequency of C-N bond.

The substituent effect, hydrogen bond and rigidity of ligand on the nitrogen atom of dithiocarbamate may explain the large differences in wavenumber of C-N stretching frequencies. The electron donating group in (g) contributed to the increasing bond order of C-N bond. Hence, vibration frequency increases. The frequency in (e) is lower than that observed in (g) mainly because of an intermolecular hydrogen bond which reduces the frequency to a slightly lower wavenumber. For (h), the ligand itself is rigid and this may suggest in decreasing of C-N bond order. Therefore, vibration frequency is the lowest.

The occurrence of the hydroxyl stretching band was seen in (e) at 3360 cm<sup>-1</sup> as a medium and broad band. The C-H stretching bands for (e) and (g) were displayed at around 2964 and 2969 cm<sup>-1</sup>. However, due to the cyclic conformation of pyrrolidine in (h), the C-H band was shown at a slightly lower waveumber, 2958 cm<sup>-1</sup>. Other than that, asymmetric C-S stretching bands vibrate at higher frequency than symmetric C-S, as presented in Table 3.5.

# 3.2.2 <sup>1</sup>H Nuclear Magnetic Resonance (<sup>1</sup>H NMR) Spectroscopy

The proton NMR data in Table 3.6 shows that the series of triethylphosphanegold(I) dithiocarbamates were successfully obtained. Signals of the corresponding protons of the dithiocarbamates appeared in the spectra of each compound.

Compound (d) showed two different methyl groups; one group of three methyl units bound to phosphane (H2') at the most upfield region while another methyl unit is bound to the nitrogen of the dithiocarbamate (H1) at slightly downfield region. However, compounds (e) and (g) presented their methyl units bound to both phosphane (H2') and

dithiocarbamate (H2) groups at the same chemical shift.

Table 3.6: <sup>1</sup> H chemical shift,	$\delta$ (ppm) and multiplicities of triethylphosphanegold(I)
	dithiocarbamate series.

Compound	Chemical Shift, δ (ppm) and Multiplicity
Et <sub>3</sub> PAuCl	1.14 ppm, 9H (H1'), dt, J <sub>H-H</sub> = 20 Hz, 16 Hz;
	1.78 ppm, 6H (H2'), q, J <sub>H-H</sub> = 5.3 Hz
(d)	1.09 ppm, 9H (H1'), dt, J <sub>H-H</sub> = 20 Hz, 12 Hz;
	1.71 ppm, 6H (H2'), q, $J_{H-H} = 4.6$ Hz;
	3.10 ppm, 1H (OH), s;
	3.37 ppm, 3H (H3), s;
	$3.84$ ppm, 2H (H1), t, $J_{H-H} = 6$ Hz;
	4.00 ppm, 2H (H2), t, $J_{H-H} = 4$ Hz
(e)	1.13 ppm, 15H (H2' and H4), m;
	1.87 ppm, 6H (H1'), q, J <sub>H-H</sub> = 7.2 Hz;
	3.67 ppm, 2H (H1), t, J <sub>H-H</sub> = 6 Hz;
	3.73 ppm, 2H (H2), t, J <sub>H-H</sub> = 6 Hz;
	4.77 ppm, 1H (OH), s;
	5.30 ppm, 1H (H3), sept, $J_{H-H} = 6 Hz$
( <b>f</b> )	1.16 ppm, 9H (H2'), q
	1.81 ppm, 6H (H1'), s
	3.98 ppm, 4H (H1), s
	4.06 ppm, 4H (H2), d
( <b>g</b> )	1.17 ppm, 15H (H2 and H2'), m, $J_{H-H} = 7$ Hz;
	1.74 ppm, 6H (H1'), pent, $J_{H-H} = 8 \text{ Hz}$ ;
	$3.82 \text{ ppm}, 4\text{H} (\text{H1}), q, J_{\text{H-H}} = 6.7 \text{ Hz}$
( <b>h</b> )	$1.17 \text{ ppm}, 9 \text{H} (\text{H2'}), \text{dt}, \text{J}_{\text{H-H}} = 20 \text{ Hz}, 14 \text{ Hz};$
	1.77 ppm, 6H (H1'), q, J <sub>H-H</sub> = 4.6 Hz;
	1.93 ppm, 4H (H2), pent, $J_{H-H} = 3$ Hz;
	3.76 ppm, 4H (H1), t, J <sub>H-H</sub> = 8 Hz

The signals of methylene protons bound to phosphane (H1') are slightly downfield than compared to the methyl groups. Other methylene units in compounds (d)-(h) showed their signals at almost similar range of 3.80-4.13 ppm, indicating the electronegativity effect of the neighbouring atom, thus deshielding these  $\alpha$ -protons next to the oxygen and nitrogen atoms.

On the other hand, the methyne proton in compound (e) displayed its signal at the most deshielded region. Compound (f) did not show a signal for the OH proton, probably due to the occurrence of rapid exchange of hydrogen and deuterium atoms in solution.

3.2.3  ${}^{13}C{}^{1}H$  Nuclear Magnetic Resonance ( ${}^{13}C{}^{1}H$  NMR) Spectroscopy

All carbon signals correspond to carbons in the triethylphosphanegold(I) dithiocarbamate series are listed in Table 3.7. The methyl carbon of the phosphane group, C2' for all complexes in this series showed a signal in the upfield region between 8.0-9.0 ppm.

A signal of C1 for complex (**d**) was observed at the same chemical shift of C2'. Signals of the adjacent carbon to phosphorus, C1' gave a doublet due to carbon-phosphorus coupling, yielding a coupling constant of 136 Hz. Two signals of C1 and C2 in compounds (**d**)-(**f**) represented the signals of carbon-nitrogen and carbon-oxygen, respectively. The chemical shift of both signals showed no significance but the signals of C1 and C2 were observed at slightly upfield for compound (**e**) compared to compounds (**d**) and (**f**).

Compound	Chemical Shift, $\delta$ (ppm) and Multiplicity
(d)	9.00 ppm (C3 and C2');
	18.38 ppm (C1'), d, J <sub>P-C</sub> = 136 Hz;
	58.72 ppm (C1);
	60.73 ppm (C2);
	209.09 ppm (C4)
(e)	9.0 ppm (C2');
	18.7 ppm (C1');
	20.3 ppm (C2);
	49.7 ppm (C4);
	56.0 ppm (C3);
	62.9 ppm (C1);
	208.5 ppm (C5)
( <b>f</b> )	8.04 ppm (C2');
	17.30 ppm (C1'), d, J <sub>P-C</sub> = 136 Hz;
	58.32 ppm (C1);
	59.79 ppm (C2)
	208.35 ppm (C3)
( <b>g</b> )	8.82 ppm (C2');
	12.06 ppm (C2);
	18.43 ppm (C1'), d; $J_{P-C} = 136 \text{ Hz}$
	50.03 ppm (C1);
	202 ppm (C3)
( <b>h</b> )	8.46 ppm (C2');
	17.80 ppm (C1'), dt, $J_{P-C} = 132$ Hz, 53 Hz;
	25.62 ppm (C2);
	53.53 ppm (C1);
	202.14 ppm (C3)

**Table 3.7:**  ${}^{13}C{}^{1}H$  chemical shifts,  $\delta(ppm)$  of triethylphosphinogold(I) dithiocarbamate series.

Carbon signals for methyl in (g) and methylene in (h) were labeled as C2. These signals appeared at 12.1 and 25.6 ppm for compounds (g) and (h), respectively. However, the carbon-nitrogen signals which were represented as C1 were clearly seen upfield compared with that observed in compounds (d), (e) and (f). Quartenary carbons for all compounds in this series were displayed as smaller signals than the other carbon signals in their respective complexes. These signals were also seen at a highly downfield region at around 200-210 ppm.

# 3.2.4 ${}^{31}P{}^{1}H$ Nuclear Magnetic Resonance ( ${}^{31}P{}^{1}H$ NMR) Spectroscopy

The triethylphosphanegold(I) chloride precursor, Et<sub>3</sub>PAuCl showed a signal at 31.41 ppm (Table 3.8). Upon complexation, a single signal was observed in each spectrum of the triethylphosphanegold(I) dithiocarbamate derivatives, indicating complete reaction had occurred.

**Table 3.8:** <sup>31</sup>P{<sup>1</sup>H} chemical shift,  $\delta$  (ppm) of triethylphosphinogold(I) dithiocarbamate series.

Compound	Chemical Shift (ppm)
AuPEt <sub>3</sub> Cl	31.41
(d)	34.12
(e)	36.17
( <b>f</b> )	34.37
(g)	33.99
(h)	34.31

The values in chemical shifts of the synthesized compounds (d)-(h) were demonstrated downfield from the precursor. Compounds (d), (f), (g) and (h) shared almost similar chemical shift at approximately 34 ppm but higher than that of the precursor. However, compound (e) has an isopropyl group attached to the nitrogen atom of the dithiocarbamate ligand exhibited the most downfield chemical shift at around 36 ppm.

# 3.2.5 Powder X-ray Diffraction (PXRD)

# 3.2.5.1 PXRD pattern of compound (e)

The powdered form (red trace) of compound (e) was compared with the simulated pattern (blue trace) calculated from its single crystal after underwent Lebail fitting during the analysis, as shown in Figure 3.5.



**Figure 3.5:** Comparison between the experimental powder pattern (red trace) and that calculated from the single crystal data of compound (e).

The powdered compound (e) matched the calculated pattern, indicating that the structure of bulk compound (e) is the same as that obtained from the crystal grown in chloroform via slow evaporation. Only crystals of compound (e) were successfully obtained. Compounds (d) and (f) resulted in yielding gummy substance upon contact with solvent.

3.2.6 Single Crystal X-ray Diffraction (SCXRD)

# 3.2.6.1 SCXRD of compound (e)

Compound (e) crystallized as yellow, block shaped crystals from slow evaporation of its chloroform solution after a week. The crystals crystallized in the  $P2_1$  space group. The crystallographic asymmetric unit comprises two independent molecules (Figure 3.6)<sup>55</sup>.



Figure 3.6: Molecular structure of the two independent molecules of (e).

It is of interest to look at the magnitude of the intramolecular interaction of Au1 - - - S2 bond and correlate it with the linearity of the P1-Au-S1 bond. In this structure, the interaction of Au1 - - S2 is longer, resulting in deviation from the ideal linear angle, 180°. In comparison to compound (**b**) in Figure 3.3, the P1-Au-S1 bond angle, 176.23(6)° in compound (**e**) is closer to linearity. The S1-C1 bond is longer than that of S2-C1 bond by 0.02 Å. The shortest C-S bond is unlikely to bind to gold atom, therefore a monodentate mode of coordination is assigned. The Au1 - - S2 and Au2 - - S4 bond lengths are calculated as 3.112 Å each while Au1-S1 and Au2-S3 having bond lengths of 2.338 and 2.335 Å, respectively.

# **3.3** Triphenylphosphanegold(I) dithiocarbamates, Ph<sub>3</sub>PAu(dtc)

Three compounds of the  $Ph_3PAu(dtc)$  series were synthesized and atom labels are shown in Figure 3.7. The structural elucidations were carried out based on infra red spectroscopy, nuclear magnetic resonance (<sup>1</sup>H, <sup>13</sup>C{<sup>1</sup>H}, <sup>31</sup>P{<sup>1</sup>H}) spectroscopy, powder X-ray diffraction and single crystal X-ray diffraction.



**Figure 3.7:** Chemical structures of the triphenylphosphanegold(I) dithiocarbamate series; (i) Ph<sub>3</sub>PAu(L1), (j) Ph<sub>3</sub>PAu(L2) and (k) Ph<sub>3</sub>PAu(L3).

#### 3.3.1 Infra Red (IR) Interpretation

Selected IR absorption bands for the triphenylphosphanegold(I) dithiocarbamate series are displayed in Table 3.9. The infrared absorption bands shown for the precursor, Ph<sub>3</sub>PAuCl, gave two vibrational bands corresponding to aryl and P-aryl stretching modes at 1680 and 1101 cm<sup>-1</sup>, respectively.

	Wavenumber, υ (cm <sup>-1</sup> )						
Compounds	О-Н	С-Н	Aryl	P-Aryl	C-S (asym)	C-S (sym)	C-N
Ph <sub>3</sub> PAuCl	-	-	1680	1101	-	-	-
			(w)	(sh, m)			
(i)	3342	2924	1574	1099	1026	997	1436
	(br)	(w)	(w)	(sh, m)	(w)	(w)	(sh, m)
(j)	3311	2972	1585	1101	1027	997	1436
	(br, s)	(w)	(w)	(sh, m)	(w)	(w)	(sh, m)
(k)	3315	2924	1586	1099	1027	997	1436
	(br)	(w)	(w)	(sh, m)	(w)	(w)	(sh, m)

**Table 3.9:** Selected IR absorption bands of triphenylphosphinogold(I) dithiocarbamate series.

\*br = broad, sh = sharp, s = strong, m = medium, w = weak

The presence of broad O-H vibrational bands is observed in the spectra of all three compounds comprising the triphenylphosphanegold(I) dithiocarbamate series. Broad bands due to OH in compound (i) vibrated at 3342 cm<sup>-1</sup> and in a higher energy region compared to similar bands observed in compounds (j) and (k) at 3311 and 3315 cm<sup>-1</sup>, respectively. The stretching of C=C bonds of the aryl group were found to shift to lower energy from their precursor. All compounds in this series shifted their aryl vibrational bands to approximately 1570-1586 cm<sup>-1</sup> from 1680 cm<sup>-1 56</sup>.

There are three assigned bands displayed in the finger print region. A sharp and medium vibration due to P-aryl stretching was observed in all spectra around 1100 cm<sup>-1</sup> which is less intense to the band appearing in the spectrum of the precursor. Other than that,

weak asymmetric and symmetric stretching C-S bands were apparent at 1027 and 997 cm<sup>-1</sup>, respectively, indicating the formation of compounds of triphenylphosphanegold(I) dithiocarbamate.

# 3.3.2 <sup>1</sup>H Nuclear Magnetic Resonance (<sup>1</sup>H NMR) Spectroscopy

The proton NMR data of compounds (i)-(k) are shown in Table 3.10, signifying the success in obtaining the compounds. As presented in Table 3.10, the OH protons for compounds (i) and (j) were shown but not for compound (k). The reason is mainly because of the rapid intermolecular exchange of the OH proton with the deuterium atom in solution. The OH shifts for (i) and (j) were demonstrated at 4.33 and 3.22 ppm, respectively.

**Table 3.10:** <sup>1</sup>H chemical shifts,  $\delta$  (ppm) and multiplicities of triphenylphosphinogold(I) dithiocarbamate series.

Compound	Chemical Shift, δ (ppm) and Multiplicity
(i)	3.53 ppm, 3H (H1), s;
	$4.00 \text{ ppm}, 2 \text{H} (\text{H3}), \text{t}, \text{J}_{\text{H-H}} = 4 \text{ Hz};$
	4.16 ppm, 2H (H2), t, $J_{H-H} = 6$ Hz;
	4.33 ppm, 1H (OH), s;
	7.41-7.63 ppm, 15H (H1', H2', H3' and H4'), m
( <b>j</b> )	1.25 ppm, 6H (H2), d, J <sub>H-H</sub> = 4 Hz;
	3.18 ppm, 1H (OH), s;
	3.92 ppm, 2H (H4), t, J <sub>H-H</sub> = 4 Hz;
	4.01 ppm, 2H (H3), t, J <sub>H-H</sub> = 5.6 Hz;
	5.39 ppm, 1H (H1), sept, $J_{H-H} = 6.8$ Hz;
	7.44-7.63 ppm, 15H (H aromatic), m
( <b>k</b> )	$4.10 \text{ ppm}, 4\text{H} (\text{H1}), t, J_{\text{H-H}} = 6 \text{ Hz};$
	$4.22 \text{ ppm}, 4\text{H} (\text{H2}), t, J_{\text{H-H}} = 6 \text{ Hz};$
	7.45-7.58 ppm, 15H (H1', H2', H3' and H4'), m

The effect of donating group on the nitrogen atom may also explain the appearance of OH signals at different values. Compound (j) has a larger donating group (isopropyl) as
compared to compound (i). The isopropyl group in compound (j) may contribute to a relatively higher shielding effect on the OH than in compound (i).

Similarly, the resonance of methyl group in compound (i) is seen upfield compared to that for (j), also because of the deshielding effect of the neighbouring atom. All aromatic protons in this series were found in the range 7.41-7.63 ppm. As for methine proton (H1) in compound (j), this signal is observed upfield but more downfield than the aromatic region due to its position next to the nitrogen atom.

3.3.3 <sup>13</sup>C {<sup>1</sup>H} Nuclear Magnetic Resonance (<sup>13</sup>C{<sup>1</sup>H} NMR) Spectroscopy

Carbon-13 resonances of compounds (i)-(k) are displayed in Table 3.11. The phenyl carbons are present in the  $sp^2$  region around 128-135 ppm. A small signal at 209 ppm was noted for the quartenary carbon of each compound.

**Table 3.11:** <sup>13</sup>C{<sup>1</sup>H} chemical shifts,  $\delta$  (ppm) of triphenylphosphanegold(I) dithiocarbamates series.

Compound	Chemical Shift, δ (ppm) and Multiplicity
(i)	40.23 ppm (C1);
	55.70 ppm (C3);
	61.71 ppm (C2);
	131.56 ppm (C aromatic);
	209.50 ppm (C4)
(j)	20.37 ppm (C2);
_	49.80 ppm (C1);
	56.29 ppm (C4);
	62.73 ppm (C3);
	131.69 ppm (C aromatic);
	208.79 ppm (C5)
(k)	59.46 ppm (C1);
	61.17 ppm (C2);
	131.34 ppm (C aromatic);
	209.86 ppm (C3)

A methyl (C1) signal of (i) was seen at 40 ppm while signals due to the methylene (C2 and C3) nuclei were found in the downfield region. The methylene signals were observed at a similar chemical shift for all compounds in this series. Compound (j) presented the isopropyl unit. The methine carbon was shifted downfield compared with that seen in compound (i). The methyl units appeared in the upfield region at 20 ppm.

3.3.4  ${}^{31}P{}^{1}H$  Nuclear Magnetic Resonance ( ${}^{31}P{}^{1}H$  NMR) Spectroscopy

The triphenylphosphanegold(I) dithiocarbamate series exhibited moderate chemical shifts compared to the precursor,  $Ph_3PAuCl$ , as displayed in Table 3.12. These signals are evidence for the formation of the gold dithiocarbamate complexes. A signal at 31.1 ppm <sup>58</sup> will be observed if triphenylphosphane oxide is present. However, no signal at this chemical shift is seen, indicating the absence of triphenylphosphane oxide.

**Table 3.12:** <sup>31</sup>P{<sup>1</sup>H} chemical shifts,  $\delta$  (ppm) of triphenylphosphanegold(I) dithiocarbamate series.

Compound	Chemical Shift (ppm)
Ph <sub>3</sub> PAuCl	33.22
(i)	35.62
(j)	36.30
(k)	34.56

A single signal was observed at 33.22 ppm for the precursor, triphenylphosphanegold(I) chloride, Ph<sub>3</sub>PAuCl. The phosphorus shifted to downfield region upon complexation between the precursor and the corresponding dithiocarbamate ligands. Complexation of Ph<sub>3</sub>PAuCl with L3, of which produced compound (**k**), gave most upfield chemical shift, at 34.56 ppm. This was followed by compound (**i**), showing a signal at 35.62 ppm and most downfield shift was presented by compound (**j**), with a signal at 36.30 ppm.

# **3.4** Bis(triphenylphosphane)copper(I) dithiocarbamates, (Ph<sub>3</sub>P)<sub>2</sub>Cu(dtc)

Three compounds of the  $(Ph_3P)_2Cudtc$  series were synthesized and atom labels are shown in Figure 3.8. The structural elucidations were carried out based on infra red spectroscopy, nuclear magnetic resonance (<sup>1</sup>H, <sup>13</sup>C{<sup>1</sup>H}, <sup>31</sup>P{<sup>1</sup>H}) spectroscopy, powder X-ray diffraction and single crystal X-ray diffraction.



**Figure 3.8:** Chemical structures of bis(triphenylphosphino)copper(I) dithiocarbamate series; (**l**) (Ph<sub>3</sub>P)<sub>2</sub>CuL1, (**m**) (Ph<sub>3</sub>P)<sub>2</sub>CuL2 and (**n**) (Ph<sub>3</sub>P)<sub>2</sub>CuL3.

The selected IR absorption bands of all important functional groups for compounds (I)-(n) are presented in Table 3.13. Similar frequencies of vibrations were obtained for all functional groups. A strong and sharp band is seen at 978-998 cm<sup>-1</sup> indicating the presence of a symmetric C-S vibration while C-N vibrates at around 1432 cm<sup>-1</sup> to give a medium and sharp band.

Compounda	Wavenumber, v (cm <sup>-1</sup> )				
Compounds	О-Н	Aryl	C-S (asym)	C-S (sym)	C-N
(PPh <sub>3</sub> ) <sub>2</sub> CuCl	-	1584 (w)	-	-	-
(l)	3353 (m)	1582 (w)	1092 (m, sh)	992 (m)	1432 (m)
(m)	3467 (m)	1586 (w)	1092 (m, sh)	978 (m)	1432 (m)
( <b>n</b> )	3353 (m)	1584 (w)	1094 (m, sh)	990 (m)	1432 (m)

 Table 3.13: Selected IR absorption bands for the bis(triphenylphosphane)copper(I) dithiocarbamate series.

\*br = broad, sh = sharp, s = strong, m = medium, w = weak

In addition, vibration at approximately 1584 cm<sup>-1</sup>, due to the phenyl group, and this weak vibration frequency is only evident in compounds of this series. Other than that, the O-H bands were observed at above 3300 cm<sup>-1</sup> <sup>56</sup>. A similar O-H vibration frequencies in (**l**) and (**n**) were seen but the O-H vibration for compound (**m**) was at higher wavenumber. The electron donating effect contributed by the isopropyl group could influence the strength of O-H vibration in compound (**m**). In addition, all three O-H bands showed a sharp band and not the usual broad bands. Possibly, a hydrogen bond occurred in the compounds hence the broad bands of O-H are not seen.

The asymmetric and symmetric C-S vibrational bands were observed between 1090-1095 cm<sup>-1</sup> and 990-1000 cm<sup>-1</sup>, respectively. The vibrational frequencies of C-S in

compounds (l)-(n) were reduced to a lower frequency. However, the strength of the bands were increased in compounds (l)-(n) compared to the precursor.

3.4.2 <sup>1</sup>H Nuclear Magnetic Resonance (<sup>1</sup>H NMR) Spectroscopy

The proton NMR data are shown in Table 3.14. All compounds in this series showed a

signal due to OH proton at a relatively similar chemical shift for compounds (m) and

(n) while the OH proton of compound (l) appeared slightly upfield.

Compound	Chemical Shift, $\delta$ (ppm) and Multiplicity
(1)	3.40 ppm, 3H (H1), s;
	3.83 ppm, 2H (H2), q, J <sub>H-H</sub> = 5.3 Hz
	4.01 ppm, 2H (H3), t, $J_{H-H} = 6$ Hz
	7.14-7.33 ppm, 30H (H aromatic), m
(m)	1.13 – 1.15 ppm, 6H (H2), d, J <sub>H-H</sub> = 8 Hz;
	$2.93 - 2.96$ ppm, 1H (OH), t, $J_{H-H} = 6$ Hz;
	3.77 – 3.81 ppm, 2H (H3), q, J <sub>H-H</sub> = 8 Hz;
	3.95 – 3.98 ppm, 2H (H4), t, J <sub>H-H</sub> = 6 Hz;
	$5.52 - 5.62$ ppm, 1H (H1), sept, $J_{H-H} = 6.7$ Hz;
	7.17-7.40 ppm, 30H (H aromatic), m
( <b>n</b> )	3.01 ppm, 2H (OH), s;
	$3.92 \text{ ppm}, 4 \text{H} (\text{H1}), \text{t}; \text{J}_{\text{H-H}} = 4 \text{ Hz}$
	4.10 ppm, 4H (H2), t; $J_{H-H} = 4 Hz$
	7.22-7.36 ppm, 30H (H aromatic), m

**Table 3.14:** <sup>1</sup>H chemical shift,  $\delta$  (ppm), and multiplicities of bis(triphenylphosphane)copper(I) dithiocarbamate series.

Aromatic protons were recognized as multiplet signals in the aromatic range. Methylene protons,  $(N-CH_2)$  and  $(O-CH_2)$  for all copper(I) compounds are observed in range 3.80-4.00 ppm. As discussed in the proton NMR of the earlier compounds, the methine proton of compound (**m**) is positioned at the most downfield region after the methylene protons.

3.4.3  ${}^{13}C{}^{1}H$  Nuclear Magnetic Resonance ( ${}^{13}C{}^{1}H$  NMR) Spectroscopy

The <sup>13</sup>C{<sup>1</sup>H} NMR data, as presented in Table 3.15, of compounds (I), (m) and (n) showed signals of the dithiocarbamate unit as well as the precursor  $(Ph_3)_2Cu$  entity.

Compound	Chemical Shift, δ (ppm)
(1)	31.05 ppm (C1);
	56.71 ppm (C3);
	61.10 ppm (C2);
	128.33 – 133.96 ppm (C aromatic);
	206.97 ppm (C4)
( <b>m</b> )	20.27 ppm (C2);
	47.75 ppm (C1);
	52.11 ppm (C4);
	63. 04 ppm (C3);
	128. 35 – 134.36 ppm (C aromatic);
	209.81 ppm (C5)
<b>(n)</b>	56.01 ppm (C1);

58.70 ppm (C2);

208.73 ppm (C3)

128.88 – 134.46 ppm (C aromatic);

**Table 3.15:** <sup>13</sup>C{<sup>1</sup>H} chemical shifts,  $\delta$ (ppm) for the bis(triphenylphosphane)copper(I) dithiocarbamate series.

A quartenary carbon signal is shown for each compound in this series in range of 205-210 ppm. These signals appeared as the least intense signals among all signals. The signals in range of 128-134 ppm are dominated by phenyl groups from the phosphane ligands. Other signals are due to the dithiocarbamate ligand. Compound (I) exhibited methyl carbon signal, C1, at 31 ppm while both methylene groups, C2 and C3, were found at a higher field due to the electronegativity effect.

In compound (**m**), C2, a methyl group, showed a signal at 20 ppm. This is followed by the methine carbon, C1, at an upfield region at 48 ppm. The methylene carbons, C3 and C4, were presented at a similar region as found in compound (**l**). Compound (**n**) showed

a close value in chemical shift for both methylene carbons, C1 and C2 at a 56.01 and 58.70 ppm.

3.4.4  ${}^{31}P{}^{1}H$  Nuclear Magnetic Resonance ( ${}^{31}P{}^{1}H$  NMR) Spectroscopy

The  ${}^{31}P{}^{1}H$  NMR of the synthesized phosphane-bound copper(I) dithiocarbamate showed chemical shifts below 0 ppm (Table 3.16). The precursor,  $(Ph_3P)_2CuCl$ , showed a  ${}^{31}P{}^{1}H$  signal at -4.63 ppm.

**Table 3.16:** <sup>31</sup>P chemical shift, δ(ppm), for the bis(triphenylphosphane)copper(I) dithiocarbamate series.

Compound	Chemical Shift (ppm)
(Ph <sub>3</sub> P) <sub>2</sub> CuCl	-4.63
(1)	-1.26
(m)	-1.78
(n)	-1.03

The  ${}^{31}P{}^{1}H{}$  signals of the synthesized copper(I) compounds shifted to slightly downfield than the precursor. Compound (**n**) has two hydroxyl groups, thus the electron withdrawing effect is greater than in compounds (**l**) and (**m**). Therefore, a downfield shift in the signal is expected.

Both compounds (**l**) and (**m**) have one hydroxyl group. Compound (**m**) has isopropyl as a substituent attached to the nitrogen while compound (**l**) has methyl. Hyperconjugation effect is more preferred in methyl than in isopropyl hence, methyl has a stronger electron donating ability than isopropyl. Although the increment in chemical shift is influenced by the electronegativity effect of the OH group, the isopropyl in (**m**) also donates electrons. This made the  ${}^{31}P{}^{1}H{}$  in (**m**) more shielded than in (**l**). In line with this, the  ${}^{31}P{}^{1}H$  in (l) is expected to resonate slightly upfield at -1.26 ppm than in compound (m), at -1.78 ppm.

### 3.4.5 Powder X-ray Diffraction (PXRD)

#### 3.4.5.1 PXRD patterns of compound (l)

The powdered form (red trace) of compound (I) was compared with the simulated pattern (blue trace) for its single crystal after underwent Lebail fitting during the analysis, as presented in Figure 3.9.



Position [°2Theta] (Copper (Cu))

**Figure 3.9:** Comparison between the experimental powder pattern (red trace) and that calculated for the single crystal data of compound **(l)**.

The powder pattern (red trace) for compound (**l**) matched the calculated pattern (blue trace). The major signals were similar, indicating that the structure of bulk compound (**l**) is the same with that obtained from the crystal grown in acetone via slow evaporation.

Crystals of compound (**m**) was obtained but the diffraction pattern was too poor and precluded data collection, hence no unit cell data were obtained. In order to confirm its molecular geometry, the powder pattern of compound (**m**) was compared with the single crystal data of compound (**p**), a silver(I) complex of L2, which will be discussed later on, since both compounds differ only by the nature of the metal atom (Figure 3.10). Compound (**m**) is isostructural to compound (**p**) in such a way that both compounds possess similar chemical structures.



Figure 3.10: Comparison between the experimental powder pattern (red trace) of compound (m) with the simulated pattern (blue trace) of compound (p).

The powder pattern of compound (**m**) is comparable to the single crystal data of compound (**p**), a silver(I) complex. Figure 3.10 displayed similarities in major signals between both compounds since the complexes were formed from the same ligand, NaL2. Hence, (**m**) is isostructural with (**p**).

#### 3.4.5.3 PXRD patterns of compound (n)

The powder pattern (red pattern) of compound (**n**) was compared to the simulated pattern of its single crystal data (blue pattern) after underwent Lebail fitting during the analysis, as displayed in Figure 3.11.



**Figure 3.11:** Comparison between the experimental powder pattern (red trace) and that calculated for the single crystal X-ray data of compound (**n**).

The bulk material (red trace) of compound (**n**) showed similarities with that shown the simulated pattern of its single crystal X-ray data. The major signals in the powder pattern matched with the signals shown in the calculated pattern. The disparities in intensities in the powder pattern were due to the sample preparation during which the analysis was performed.

# 3.4.6.1 SCXRD of compound (1)

The crystal obtained for compound (I) was a yellow block and was cut into  $0.05 \ge 0.09 \ge 0.11$  mm. It crystallized in the triclinic space group, *P*-1. The central copper atom is coordinated by two monodentate and a bidentate ligand to yield a tetrahedral geometry. In Figure 3.12, the triphenylphosphane is displayed as a monodentate ligand where P1 and P2 were bonded to copper(I) while the dithiocarbamate is a bidentate ligand with S1 and S2 bonded to the metal centre.



Figure 3.12: Unit cell of compound (I).



Figure 3.13: Hydrogen bonds for compound (l).

A classical hydrogen bond was observed, indicating an O1-H1A - - - S1 interaction. The hydrogen bond is indicated with a blue dotted line, as displayed in Figure 3.13. Besides, the presence of five non-classical hydrogen bonds was also observed. The interactions were noticed between the methylene hydrogen atoms, H2A and H3A with S1 and H2A with O1. There were also two interactions that involved two hydrogen atoms of the phenyl groups, namely H6A and H38A, with O1.

#### 3.4.6.2 SCXRD for compound (n)

The crystal obtained for compound (**n**) was a yellow, block crystal and was cut into 0.07 x 0.07 x 0.12 mm. It crystallized in a triclinic space group, P-1. The central copper atom is coordinated by two monodentate and a bidentate ligand to yield a tetrahedral geometry. The triphenylphosphane is the monodentate ligand where P1 and P2 were

bonded to copper while the dithiocarbamate is a bidentate ligand with S1 and S2 bonded to the metal centre.



Figure 3.14: Unit cell of compound (n).

The crystal packing is stabilized with chloroform as the solvate molecule. Based on the data obtained, compound (**n**) is composed of two classical and two non-classical hydrogen bonds (Figure 3.14).



Figure 3.15: Hydrogen bonds in crystal packing of compound (n).

As displayed in Figure 3.15, there were two classical hydrogen bonds between O1-H1 - - O2 and O2-H2 - - O1. The blue dotted lines represented the classical hydrogen bonds. Each of the hydrogen bonds made up the intramolecular and intermolecular interactions between the two molecules of (**n**). The non-classical hydrogen bonds present were C2-H2B - - S1 and C3-H3A - - S2. These hydrogen bonds deviate from the classical concept and involved the interaction between the C-H and sulfur atom. The C-H - - S interaction is a weak interaction because sulfur is weak hydrogen acceptor due to its low electronegativity <sup>59</sup>.

# **3.5** Bis(triphenylphosphane)silver(I) dithiocarbamates, (Ph<sub>3</sub>P)<sub>2</sub>Ag(dtc)

Three compounds of the  $(Ph_3P)_2Ag(dtc)$  series were synthesized and atom labels are shown in Figure 3.16. The structural elucidations were carried out based on infra red spectroscopy, nuclear magnetic resonance (<sup>1</sup>H, <sup>13</sup>C{<sup>1</sup>H}, <sup>31</sup>P{<sup>1</sup>H}) spectroscopy, powder X-ray diffraction and single crystal X-ray diffraction.



**Figure 3.16:** Chemical structures of the bis(triphenylphosphane)silver(I) dithiocarbamate series: (**o**)  $(Ph_3P)_2Ag(L1)$ , (**p**)  $(Ph_3P)_2Ag(L2)$  and (**q**)  $(Ph_3P)_2Ag(L3)$ .

#### 3.5.1 Infra Red (IR) Spectroscopy

Three compounds of the bisphosphanesilver(I) dithiocarbamate series displayed essentially similar vibrational bands in their IR spectra (Table 3.17). The formation of complexes was determined by the presence of O-H, C-S and C-N bands contributed by the ligands.

**Table 3.17:** Selected IR absorption bands for the bis(triphenylphosphino)silver(I) dithiocarbamate series.

Compounda	Wavenumber, υ (cm <sup>-1</sup> )				
Compounds	О-Н	Aryl	C-S (asym)	C-S (sym)	C-N
(Ph <sub>3</sub> P) <sub>2</sub> AgNO <sub>3</sub>	-	1572 (w)	-	-	-
(0)	3360 (br)	1584 (w)	1094 (m, sh)	983 (m)	1432 (m, sh)
<b>(p</b> )	3384 (br)	1586 (w)	1092 (m, sh)	971 (w)	1434 (m, sh)
( <b>q</b> )	3322 (br)	1584 (w)	1094 (m, sh)	983 (w)	1434 (m, sh)

\*br = broad, sh = sharp, s = strong, m = medium, w = weak

All silver(I) complexes prepared showed vibrations of O-H bands at over 3300 cm<sup>-1</sup> where O-H of (**q**) vibrated at a lower frequency as compared to O-H of compounds (**o**) and (**p**). Vibrational bands due to C-N were observed at approximately 1430 cm<sup>-1</sup> for compounds (**o**), (**p**) and (**q**) <sup>56</sup>. The stretching of the symmetric C-S bonds vibrate at lower frequencies and were observed at 970-983 cm<sup>-1</sup> for all silver(I) compounds. The asymmetric C-S stretching bands were observed between 1090-1100 cm<sup>-1</sup> for all compounds but the vibration strength is reduced from strong to medium as the complexations occurred.

Similarly, a vibrational band was apparently due to stretching of the C=C of phenyl groups. The stretching due to phenyl rings was observed around 1584 cm<sup>-1</sup> for compounds (**o**) and (**q**) but compound (**p**) showed a vibrational band at 1586 cm<sup>-1</sup>. These bands appeared as weak and least intense bands.

# 3.5.2 <sup>1</sup>H Nuclear Magnetic Resonance (<sup>1</sup>H NMR) Spectroscopy

The <sup>1</sup>H NMR data of bisphosphanesilver(I) dithiocarbamate compounds are collected in Table 3.18. All compounds of this series showed the presence of signals due to  $(Ph_3P)_2AgNO_3$  precursor of the corresponding dithiocarbamate ligands.

Compound	Chemical Shifts, $\delta$ (ppm) and Multiplicity
(0)	$2.95 - 2.98$ ppm, 1H (OH), t, $J_{C-H} = 6$ Hz;
	3.53 ppm, 3H (H1), s;
	3.94 – 3.98 ppm, 2H (H3), q, J <sub>C-H</sub> = 8 Hz;
	$4.19 - 4.21$ ppm, 2H (H2), t, $J_{C-H} = 4$ Hz;
	7.02 – 7.11 ppm, 30H (aromatic H), m
<b>(p)</b>	1.17 – 1.19 ppm, 6H (H2), d, J <sub>C-H</sub> = 8 Hz;
	$3.25 - 3.28$ ppm, 1H (OH), t, $J_{O-H} = 6$ Hz;
	3.89 – 3.93 ppm, 2H (H3), q, J <sub>C-H</sub> = 5.3 Hz;
	$4.04 - 4.07$ ppm, 2H (H4), t, $J_{C-H} = 6$ Hz;
	$5.66 - 5.76$ ppm, 1H (H1), sept, $J_{C-H} = 6.7$ Hz;
	7.25 – 7.42 ppm, 30H (H aromatic), m
( <b>q</b> )	3.13 ppm, 2H (OH), s;
	$3.96 - 3.98$ ppm, 4H (H2), t, $J_{C-H} = 4$ Hz;
	$4.16 - 4.18$ ppm, 4H (H1), t, $J_{C-H} = 4$ Hz;
	7.23 – 7.33 ppm, 30H (H aromatic), m

**Table 3.18:** <sup>1</sup>H chemical shift,  $\delta$  (ppm) and multiplicities of bis(triphenylphosphane)silver(I) dithiocarbamate series.

The aromatic hydrogens were observed to resonate downfield in the region 7.00-7.42 ppm. There were significant differences in the values in chemical shift for the OH resonances of each compound. Compound (**o**) exhibited a OH signal at a slightly upfield compared to that shown by (**p**) and (**q**), pronouncing the non-existence of hydrogen bonding. For compound (**p**), the OH resonated at downfield than the OH resonance in compounds (**o**) and (**q**), probably due to the effect of hydrogen bond that made the hydroxyl hydrogen more deshielded. Other protons such as methyl, methylene and methine protons were seen at their expected chemical shifts.

As presented in Table 3.19, all phosphane-bound silver(I) dithiocarbamate compounds exhibited aromatic carbon signals in range of 127-135 ppm. The least intensity signal at the most downfield region, at approximately 208-211 ppm in the spectra was associated to quartenary carbon of each compound.

Compound	Multiplicity
(0)	29.05 ppm (C1);
	54.71 ppm (C3);
	60.10 ppm (C2);
	127.33 – 134.96 ppm (C aromatic);
	207.96 ppm (C4)
<b>(p</b> )	20.33 ppm (C2);
	49.50 ppm (C1);
	54.81 ppm (C4);
	63.07 ppm (C3);
	128.27 – 134.21 ppm (C aromatic);
	211.21 ppm (C5)
(q)	58.81 ppm (C2);
	60.01 ppm (C1);
	128.70 – 133.89 ppm (C aromatic);
	210.96 ppm (C3)

**Table 3.19:** <sup>13</sup>C{<sup>1</sup>H} chemical shifts,  $\delta$  (ppm) of bis(triphenylphosphane)silver(I) dithiocarbamate series.

Signals of methyl carbon in (**o**), C1 and methyl carbons in (**p**), C2 were assigned at 29.05 and 20.33 ppm, respectively. This was followed by methine carbon, C1 in (**p**) where the signal appeared slightly down-field than the methyl groups. Besides, methylene carbons of each compound that are bonded to N and O atoms were observed at a higher field around 55-60 ppm.

3.5.4  ${}^{31}P{}^{1}H$  Nuclear Magnetic Resonance ( ${}^{31}P{}^{1}H$  NMR) Spectroscopy

The  ${}^{31}P{}^{1}H{}$  signal of the precursor prepared for the syntheses of bis(triphenylphosphane)silver(I) dithiocarbamate series, was observed at 8.61 ppm, as stated in Table 3.20. Unlike the phosphanegold(I) dithiocarbamate series, the silver precursor's signal is in the upfield region.

**Table 3.20:** <sup>31</sup>P{<sup>1</sup>H} chemical shift,  $\delta$  (ppm) of bis(triphenylphosphane)silver(I) dithiocarbamate series.

Compound	Chemical Shift (ppm)
$(Ph_3P)_2AgNO_3$	8.61
(0)	4.43
( <b>p</b> )	5.30
(q)	5.80

A single signal was observed in the spectra of each synthesized complex although two phosphane molecules were used in the preparation. The phosphorus atom experienced the same environment, exhibiting only a single resonance due to the rapid exchange of PPh<sub>3</sub> at various positions in solution. The free triphenylphosphane has a <sup>31</sup>P resonance at -5.5 ppm. The <sup>31</sup>P resonances of the precursor and compounds (**o**), (**p**) and (**q**) were significantly shifted downfield compared to the free triphenylphosphane.

However, unlike the three phosphanegold(I) and bis(triphenylphosphane)copper(I) compounds series discussed earlier, the  ${}^{31}P{}^{1}H{}$  signals of the silver(I) compounds shifted upfield than the precursor  ${}^{60}$ . These phenomena might result from the  $\pi$  back bonding from the central metal atom towards phosphorus, therefore shielding the phosphorus atom.

#### 3.5.5.1 PXRD patterns of compound (p)

Figure 3.17 presented the comparison of the experimental powder patterns of compound (**p**) with simulated pattern of its single crystal data obtained.



Figure 3.17: Comparison between the experimental powder pattern (red trace) and the simulated pattern (blue trace) of the single crystal of compound (**p**).

The powdered form (red trace) of compound (**p**) was compared with the calculated pattern (blue trace) of its single crystal after underwent Lebail fitting during the analysis. The major signals were similar, indicating that the structure of bulk compound (**p**) is the same with that obtained from the crystal grown in acetone via slow evaporation.

Crystals of compound (**q**) was obtained and but the crystal quality was too poor, hence no unit cell was obtained. In order to confirm its molecular geometry, the experimental powder pattern (red pattern) of compound (**q**) was compared with the simulated pattern (blue pattern) of the single crystal data of compound (**n**) since both compounds are isostructural (Figure 3.18). Compound (**q**) is isostructural to compound (**n**) in such a way that both compounds possess the same ligand structures.



**Figure 3.18:** Comparison between the experimental powder pattern (red trace) and the simulated pattern (blue trace) of the single crystal of compound (**n**).

The powder pattern of compound (**q**) is comparable to the single crystal data of compound (**n**), a copper(I) compound with the R value obtained as 9.6%. Figure 3.18 displayed some similarities in major signals between both compounds since the compounds were formed from similar ligand, KL3. Therefore, (**q**) is isostructural with (**n**).

If the experimental powder patterns (red trace) of compound (**q**) are compared with the calculated pattern (blue trace) of the single crystal data of compound (**p**), as exhibited in Figure 3.19, similar patterns were also observed for certain signals. Both (**p**) and (**q**) are of the same series but differ in the ligand composition.



Figure 3.19: Comparison between the experimental powder pattern (red trace) of compound (q) with the simulated pattern (blue trace) of the single crystal data of compound (p).

The experimental powder pattern (red trace) was analyzed at the same angle of diffraction as collected in the single crystal data (blue trace). Most signals in red were combined to become a broad signal as described in blue. Since the major patterns in both red and blue traces are obtained at similar position, compound ( $\mathbf{q}$ ) is isostructural to compound ( $\mathbf{p}$ ) which means compound ( $\mathbf{q}$ ) adopts a tetrahedral geometry.

#### 3.5.6.1 SCXRD for compound (**p**)

The crystal obtained for complex (**p**) was a colourless block and was cut into 0.11 x 0.13 x 0.14 mm. It crystallized in a triclinic space group, *P*1. As presented in Figure 3.20, the unit cell is occupied with two molecules of (**p**) solvated with two molecules of acetone; acetone was used as the medium to grow crystal (**p**). The central silver atom is coordinated by two monodentate and a bidentate ligand to yield a tetrahedral geometry.



Figure 3.20: Unit cell of compound (p).

Triphenylphosphane is a monodentate ligand where P1 and P2 were bonded to silver while dithiocarbamate is a bidentate ligand with S1 and S2 bonded to the metal. The presence of classical and non-classical hydrogen bonds was observed. This is pictured in Figure 3.21.



Figure 3.21: Hydrogen bonds in crystal packing of compound (p).

Compound (**p**) is solvated with a molecule of acetone in the crystal packing. There is a classical hydrogen bond attaching O2-H(2A) - - O3. This interaction is linked between the hydroxyl unit and the carbonyl oxygen of the acetone. Additionally, four non-classical hydrogen bonds were present as intermolecular interactions between C34-H34A - - S2, C72-H72A - - S2, C73-H73A - - S2 and C76-H76A - - S1. All non-classical hydrogen bonds except that for C34-H34A - - S2, were interactions between the methylene hydrogen atoms with S2.

# CHAPTER 3: RESULTS AND DISCUSSION (PART B: BIOLOGICAL ACTIVITIES)

# 3.6 Anti-proliferative Studies of the R<sub>3</sub>PAuL2 series (R = Ph, Cy, Et)

3.6.1 Inhibition of MCF-7R Breast Cancer Cell Proliferation

The triorganophosphanegold(I) series of L2 demonstrated significant inhibition of cell proliferation of MCF-7R cells and their potential were comparable or greater than that of cisplatin. The ligand, which is the sodium salt of L2 (NaL2), was non-cytotoxic. The  $IC_{50}$  values of the tested compounds are presented in Table 3.21.

**Table 3.21:** Minimum inhibitory concentration (IC50) of triorganophosphanegold(I)series of L2 and control drugs against MCF-7R.

Compound	IC <sub>50</sub> (μM)
$Ph_3PAu(L2)$ (j)	$4.4 \pm 0.4$
$Cy_3PAu(L2)$ (b)	$13.6 \pm 0.2$
$Et_3PAu(L2)$ (e)	$9.3 \pm 0.3$
NaL2	>80
Doxorubicin	$5.1 \pm 0.2$
Cisplatin	$19.6 \pm 0.4$

Compound (j) inhibited the proliferation of MCF-7R cancer cells with a comparable  $IC_{50}$  value to that exhibited by doxorubicin but displayed 4.5 times greater activity compared to cisplatin under similar experimental conditions. Compounds (b) and (e) were less potent than (j) and doxorubicin. The  $IC_{50}$  value demonstrated by complex (e) was twice greater than cisplatin but (b) showed almost a comparable  $IC_{50}$  value with cisplatin. The  $IC_{50}$  values displayed by cells treated with the ligand, NaL2, was far larger than other values, indicating the non-cytotoxic behavior of the ligand. Exposure to higher concentration resulted in a significant in dose-dependent inhibition of cell proliferation, as presented in Figure 3.22.



**Figure 3.22:** MCF-7R cell viability after treatment of NaL2 and its gold(I) thiolate at different concentrations ( $\mu$ M). p value = 0.005 in each case.

When the cancer cell is treated with 1  $\mu$ g/ml of the corresponding compounds, no significant difference in cell viability was observed. However, the viability of cell was seen to decrease after treatment with 5  $\mu$ g/ml of test compounds. According to Figure 3.22, at this concentration, (e) exhibited similar viability to that shown by cells treated with NaL2. A huge difference was noticed when the three gold(I) compounds were compared. It seemed that the type of phosphane bound to gold(I) may play a major role here. Cyclic conformation type of substituent is more favourable thus, increases the suppression of cancer growth.

As the concentration is increased to 10  $\mu$ g/ml, (**b**) and (**j**) showed the same viability. The percentage of viability dropped drastically for cells treated with (**e**) at 20  $\mu$ g/ml, indicating that gold compound containing ethyl-bound phosphane is more toxic at this concentration than the other two gold(I) derivatives. As the concentration increases up to 80  $\mu$ g/ml, the cancer cells viability approaches zero. The reduction in cell growth or the efficacy of suppressing the cancer cells was not observed for NaL2.

When a cell undergoes programmable cell death or apoptosis, the plasma membrane increases its permeability which allows certain fluorescent dyes to diffuse into the cell. In this study, we used Acridine Orange (AO), a membrane-permeable, monovalent and cationic dye that binds to nucleic acid of a cell. This will give a green fluorescence if a low concentration of AO diffuses into the cell and red fluorescence is observed if the concentration is higher.

When cells with intact membrane are viewed under a fluorescence microscope, the cell's nucleus will appear in plain bright-green while apoptotic cells will have a bright-green nucleus with additional features. A dense-green feature is apparent in an apoptotic cell is due to the condensation of chromatin <sup>55</sup>. Another dye used in this study is Propidium Iodide (PI). Unlike AO, PI is impermeable to plasma membrane but easily penetrates plasma membrane of a dead or dying cell that will eventually produce a bright red fluorescence upon intercalation with the DNA or RNA. In other words, cells that have lost their membrane integrity will show red staining throughout the cytoplasm and a halo of green staining on the plasma membrane. Nuclear condensation with cytoplasm alteration and nuclear fragmentation are some ways to identify apoptotic cells <sup>55</sup>. The image in Figure 3.23 presents the characteristic appearance of a healthy, necrotic and apoptotic cells. The green arrow shows healthy cell, red arrow indicates necrotic cell while blue arrow shows apoptotic cell.

In this study, the majority cancer cells underwent apoptosis after treatment with (j) whereas (b) and (e) induced a reduced extent of apoptosis. That apoptotic cells are evident based on the morphological characteristics including the condensation of

83

chromatin and cytoplasm, membrane blobbing and the formation of apoptotic bodies <sup>61</sup>. Apoptotic bodies are round or oval masses of cytoplasm and are smaller than the cells of origin. By contrast to the cells treated with (**j**), a large amount of necrotic cells were observed upon treatment with (**b**) and (**e**), particularly about 70% of necrosis was observed after cells underwent treatment with (**b**).

Enlargement of cell volume and multinucleated cells were noticed for cells treated with (**j**) and (**e**). Most of the cells were clumped together in colonies. Interestingly, this is not seen in cells treated with doxorubicin. By contrast, the control cells were shiny, clear and healthy but a smaller number of necrotic cells with ruptured plasma membrane was observed. Among all compounds, the number of apoptotic cells shown after treatment with (**j**) is higher than doxorubicin-treated cells (Figure 3.23).







(c)



(d)



Figure 3.23: AO/PI staining of MCF-7R cells after being treated at the IC<sub>50</sub> value of each compound: (a) treated with doxorubicin; (b) treated with (j); (c) treated with (b); (d) treated with (e); (e) untreated cells (negative control). Magnification = 100x.

#### 3.6.3 Determination of Mode of Cell Death (DNA Fragmentation)

The DNA fragmentation analysis in Figure 3.24 showed three columns; L1 (a-c) are 1 kb DNA ladder, L2 (a-c) are the negative control (untreated cells) and L3 (a-c) are MCF-7R cells cultured for 24 h in RPMI 1640 control media in the presence of 4.4 mM of compound (j) in (a), 13.6 mM of compound (b) in (b) and 9.3 mM of compound (e) in (c).



**Figure 3.24:** DNA fragmentation analysis. Formation of ladders on the gels to indicate DNA fragmentation occurs following treatment with (**j**), (**b**) and (**e**) supports cell death by apoptosis.

One of the hallmarks of the terminal stages of apoptosis is the fragmentation of chromosomal DNA. The DNA was initially cleaved into 50-300 kb fragments and eventually into oligonucleosomal pieces <sup>55,62</sup>.

The absence of DNA laddering was observed in L2 in which shown by smearing effect. L3, on the other hand, displayed characteristic DNA laddering with some smearing effect, confirming apoptosis-induced cell death <sup>63</sup> of MCF-7R cells after exposure to trial gold compounds. This smearing effect is due to the cleavage of some DNA during extraction from the cultures.

#### 3.6.4 Analysis of Apoptotic Pathway (PCR Array Study and Caspase Activity)

The PCR array study is to ascertain the pathways leading to cell death. There are two recognized cellular processes that result in necrosis and apoptosis. Cell necrosis is usually triggered by direct physical injury, acute changes in environmental conditions and pathogenic activity. The integrity of the cell is collapsed and concurrently affects cell insult within tissues <sup>64</sup>. Cell apoptosis, on the other hand, is caused by the release of caspases. There are two pathways involved to achieve apoptosis. First, caspases operate through the receptor-mediated pathway containing members of the tumor necrosis factor (TNF) family of death receptors. Second, the mitochondrial-mediated pathway, involves the release of cytochrome c in mitochondria <sup>55</sup>.

The cytotoxicity of the present gold compounds is related to induction of a p53 / p73dependent activation of the mitochondrial pathway, demonstrating the anti-cancer potential of these gold compounds. The PCR array analysis also displayed three distinct pathways induced by the gold compounds involving both intrinsic and extrinsic pathways as summarized in Figure 3.25. Intrinsic pathway of apoptosis involves mitochondrial pathway while the extrinsic pathway is initiated at the plasma membrane stimulated by death receptors <sup>65</sup>.

In this study, treatment with (j), (b) and (e) triggered the up-regulation of p53 / p73 genes which in turn, induced apoptosis. This occurred due to overburdened on the DNA repair system that leads to DNA damage. Both p53 and p73 genes were expressed when cancer cells were treated with (e). On the other hand, expression of only p53 gene and its binding protein were evident after treatment with (j) and the expression of only p73 gene was triggered by (b).

Secondly, p53 / p73-induced apoptosis was mediated by induction of BAX and BCL-2associated X protein. Therefore, Bax mitochondrial translocation and cytochrome c release were promoted. Mitochondrial membrane is regulated through proto-oncogenes. BCL-2 is one of the family of proto-oncogenes that comprises of anti-apoptotic (BCL-2) or pro-apoptotic (BAD, BAX) proteins. It was found that as the genes were activated, BCL-2 was down-regulated. This resulted in allowing insertion of Bax into mitochondria membrane to increase its permeability, thus leading to apoptosis.





Alteration in mitochondria resulted in the opening of the permeability transition pore (PTP). The pore opening will permit water into mitochondria, leading to swelling and causes the outer membrane to rupture <sup>66</sup> which then releases cytochrome c <sup>67</sup>. The MCF-7R cells treated with the three trial gold compounds were shown to express significant amount of cytochrome c. Cytochrome c, which binds to apoptotic activating factor-1 (APAF-1), is known to activate caspase-9, of which in turn activates caspase-3 <sup>68</sup>. Low expression of caspase-3 results in lower cytotoxicity, which is seen for (**b**), exhibiting the lowest cytotoxicity against MCF-7R cells.

In the extrinsic pathway, the death pathways involved death receptors such as FAS and TNFR1. From this study, treatment of MCF-7R cells with the gold compounds increased FAS gene expression which in turn, activated caspase-8, caspase-9, caspase-10 and caspase-3. Another possible pathway was that caspase-8 and caspase-10 could also cleave Bid, a molecular linker bridging death receptor and mitochondria pathway and released cytochrome c<sup>68</sup>. The gold compounds up-regulated the expression of BID in MCF-7R cells thus, provided additional evidence for the proposed apoptotic pathways.

# 3.6.5 Measurement of Reactive Oxygen Species (ROS) Production

The reactive oxygen species (ROS) generation is believed to correlate to the cytotoxicity demonstrated by the gold compounds. As presented in Figure 3.26, cellular ROS were generated 5-fold upon treatment with the gold compounds compared to the untreated group. This indicated that the trial compounds induced significant amount of hydrogen peroxide.

Excessive ROS will enhance the mitochondrial membrane permeability and causes damage to the respiratory chain that will results in further in ROS production. Increasing of ROS in the mitochondria caused the release of cytochrome c that initiated the cell to undergo apoptosis.



**Figure 3.26:** Production of ROS after treatment of MCF-7R cells with (**j**), (**b**) and (**e**), at doses corresponding to their IC<sub>50</sub> values (4.4, 13.6 and 9.3  $\mu$ M, respectively) for 16 h. After labeling with carboxy-H2DCFDA for 1 h, the fluorescence was measured.

The switch mechanism of cell death mode from apoptosis to necrosis and from necrosis to apoptosis has considerably attracted attention in research of cell death mode. The levels of ATP supply will determine the switch mechanism. High ATP levels enable a cell to perform apoptosis while low ATP levels switch the energy-requiring apoptotic cell death to necrosis. <sup>69,70</sup>. Similarly, when the ATP supply increases, necrosis will be switched to apoptosis <sup>71</sup>. Some chemicals are thought to cause apoptosis changes to necrosis <sup>55</sup>.

Phagocytes engulfed apoptotic cells before they leak their contents <sup>64</sup> whereas necrotic cells caused general and localized reactions. Necrotic cells starts from acute disruption of cellular metabolism leading to the depletion of ATP, ion dysregulation, cell and mitochondria swelling, activation of degradative enzymes, failure of plasma membrane and cell lysis <sup>72</sup>. Switch mechanism can also occur as a result of burst in the intracellular level of ROS.

A burst in the intracellular level of superoxide was suggested to cause majority of MCF-7R treated with complexes (**b**) and (**e**) to undergo switch cell death mode from apoptosis to necrosis. However, necrosis was not evident for cells treated with complex (**j**) although an oxidative burst was observed. These results may indicate the role of the trial compounds in controlling the supply of ATP. It seemed that cells underwent treatment with (**b**) and (**e**) may cause some depletion of ATP levels in mitochondria, thus switched the cell death mode to necrosis from apoptosis mode.

#### 3.6.7 Human Topoisomerase I Inhibition Study

DNA topoisomerases are vital enzymes that responsible for solving topological problems related to nuclear processes such as DNA replication, transcription, repair and chromosomal segregation. Topoisomerases comprise two classes: Type I is monomeric and transiently nicking only one of the two DNA strands. It allows adjustment in helical winding. Type II is dimeric, promotes nicking double-stranded DNA in the same or different molecule and dependent on the presence of ATP <sup>55,73</sup>.

Similar pattern was observed on the banding pattern for incubated pBR322 with trial samples; NaL2, (**j**), (**b**) and (**e**) at the highest concentration (0.125-6.0  $\mu$ M) with the control where no cleavage or unwinding in DNA occurred. Incubating pBR322 with topoisomerase I and increasing the concentration of (**j**) and (**e**) resulted in a reduction of the nicked band and formation of various faster moving bands of topoisomers with different degrees of relaxation while less relaxed topoisomers appeared as slow moving bands. Interestingly, no inhibitory activity was observed for pBR322 incubated with compounds (**b**) and NaL2. As a result, the DNA bands were found to appear the same as the bands for DNA incubated with only topoisomerase I <sup>55</sup>.
These results showed the ability of (**j**) and (**e**) to inhibit topoisomerase I by relaxing the supercoiled pBR322. On top of that, the degree of inhibition is dependent on concentration. Therefore, (**j**) and (**e**) are topoisomerase I inhibitors and not topoisomerase I poisons, which prevents the nicked DNA from relegation. Complexes (**j**) and (**e**) exhibited full inhibitory activity on topoisomerase I at concentration of 4.0  $\mu$ M and 6.0  $\mu$ M, respectively. Compound (**j**) started to show topoisomers at 0.5  $\mu$ M but (**e**) achieved full inhibition on topoisomerase I activity at 50% higher the concentration of (**j**) <sup>55</sup>.

# 3.7 Anti-proliferative study of $(Ph_3P)_2Ag(dtc)$ and $(Ph_3P)_2Cu(dtc)$ series on various cancer cell lines

3.7.1 Cytotoxicity on normal cell lines

All bis(triphenylphosphane)silver(I)- and bis(triphenylphosphane)copper(I) dithiocarbamate series showed high cytotoxic character towards rat cardiac myoblasts cells, H9C2. The IC<sub>50</sub> values shown in Table 3.22, for all compounds except (**n**), were below 1  $\mu$ M.

**Table 3.22:** Cytotoxicity activity of bis(triphenylphosphane)silver(I) and -copper(I) dithiocarbamates against normal cells models at 24 hours treatment.

Call line	(0)	(p)	(q)	(1)	(m)	(n)	
Cen inte	IC <sub>50</sub> (µM)						
Н9С2	*0.76 ±0.1	$*0.69 \pm 0.02$	$*0.87 \pm 0.02$	*0.82 ±0.1	$*0.85 \pm 0.1$	$*19.67 \pm 0.8$	
HEK293	>80	>80	*38.92 ±1.2	>80	>80	>80	

H9C2: Rat cardiac myoblasts

HEK293: Human embryogenic kidney 293

(\*p = 0.005 value)

Compound (**n**) is cytotoxic as the IC<sub>50</sub> value reaches 19.67  $\mu$ M. A different case was observed when human embryogenic kidney cells, HEK293 were treated with the silver(I) and copper(I) series. It is apparent that these compounds were non-cytotoxic at all on healthy kidney cells, with exception for (**q**) of which cell viability was reduced at its IC<sub>50</sub> value of 38.92  $\mu$ M.

#### 3.7.2 Cytotoxicity on cancer cell lines

A pure compound is generally considered as cytotoxic if its  $IC_{50}$  value is 4 µg/ml or less <sup>74</sup>. As indicated in Table 3.23, compound (I) displayed a remarkable cytotoxicity effect compared with compound (**o**) against all tested cancer cell lines. The lowest  $IC_{50}$  value for (I) was observed at 0.54 µM against A2780 cells while the highest was recorded against A549 cells at a concentration 7.4 µM. However, a different result was observed for (**o**) where only two cancer cells were eradicated at its lowest  $IC_{50}$  values. The proliferation of A2780 and A549 cells were inhibited at 2.48 and 4.25 µM respectively. Insignificance in  $IC_{50}$  value was evident between treatments applied on other cell lines.

Like (I), (m) also displayed excellent anti-proliferation activities against HepG2, MCF-7R, A2780 and HT-29 cells with corresponding IC<sub>50</sub> values of 1.0, 7.8, 0.81 and 1.5  $\mu$ M, respectively. Based on these results, (m) was highly cytotoxic towards HepG2 and A2780 cells. However, lower cytotoxicity against 8505C cells was observed with an IC<sub>50</sub> value of 39.3  $\mu$ M. On the other hand, no anti-proliferative effect was seen when this compound was applied to A549 cells, indicating non-cytotoxicity.

Compound (**p**) on the other hand, displayed almost similar behaviour on cancer cells as compound (**o**). Promising anti-proliferative effects were seen against A2780 and A549

cells with IC<sub>50</sub> values of 0.71 and 8.32  $\mu$ M, respectively. The cytotoxicity demonstrated by (**p**) towards the growth of HepG2, MCF-7R and 8505C were insignificant, as shown in Table 3.23. Nevertheless, the concentration applied to kill HT-29 cells was 31.3  $\mu$ M, which was about twice of that required against HepG2, MCF-7R and 8505C cells.

Surprisingly, (**n**) showed similar trend to (**q**) as anti-proliferative agents towards all tested cells. The cytotoxicity of both compounds seemed not to be affected by the presence of different metal but the ligand is essential in determining the value of  $IC_{50}$ . The lowest  $IC_{50}$  value displayed by (**q**) was 3.7  $\mu$ M and the highest was 38.6  $\mu$ M. In addition, the concentrations of (**q**) used to stop the proliferation of HepG2 and A549 were negligible, i.e. 8.85 and 9.03  $\mu$ M, respectively. Similarly, slightly higher concentrations were needed to kill MCF-7R and 8505C, that is 13.6 and 17.5  $\mu$ M, respectively. However, compound (**q**) was less toxic on HT-29 cells with a  $IC_{50}$  value of 38.6  $\mu$ M. Three significant were observed for (**n**) in that the  $IC_{50}$  values recorded against HepG2 and A2780 cells were almost similar. MCF-7R and A549 cells also have similar  $IC_{50}$  values, as were HT29 and 8505C cells.

Call line	IC <sub>50</sub> (µM)								
Cen nne	Cisplatin	Doxorubicin	Paclitaxel	(0)	<b>(p</b> )	(q)	(l)	( <b>m</b> )	( <b>n</b> )
HepG2	*152 ± 2.3	$*4.6 \pm 0.2$	>80	*11.7±0.8	*11.5±0.6	*8.85±0.5	*2.8 ±0.3	*1.0 ±0.	*8.0±0.5
MCF-7R	$*19.6\pm0.7$	$*5.2 \pm 0.4$	*15.9 ± 1.3	$*16.6\pm0.6$	$*14.9\pm0.6$	$*13.6\pm0.4$	$*2.3 \pm 0.2$	$*7.8\ \pm 0.3$	$*12.7 \pm 0.6$
A2780	$*28.8\pm0.7$	$*4.4 \pm 0.2$	*14.2 ± 1.1	$*2.48 \pm 0.4$	$*0.71 \pm 0.03$	*3.7 ± 0.5	$*0.54 \pm 0.04$	$*0.81 \pm 0.03$	$*2.4 \pm 0.3$
HT-29	$*25 \pm 0.8$	$*4.8 \pm 0.3$	>80	$*23.5 \pm 0.7$	*31.3 ± 1.3	*38.6 ± 1.6	$*2.7 \pm 0.4$	$*1.5 \pm 0.2$	$*21.8 \pm 0.9$
A549	*35.4 ± 1.4	>80	>80	$*4.25 \pm 0.6$	*8.32 ± 0.7	*9.03 ± 0.8	$*7.4 \pm 0.7$	>80	$*13.5 \pm 0.8$
8505C	*120 ± 1.8	>80	>80	$*22.8 \pm 0.9$	*15.2 ± 0.9	*17.5 ± 0.4	$*3.4 \pm 0.6$	*39.3 ± 1.3	*25.8 ± 1.0

 Table 3.23: Cytotoxicity activity of bis(triphenylphosphino)silver(I) and -copper(I) dithiocarbamate against human carcinoma cells models at 24 hours treatment.

HepG2: Human liver hepatocellular carcinoma cells

MCF-7R: Human breast carcinoma cells (multidrug resistant strain)

A2780: Human ovarian carcinoma cells

HT-29: Human colon adenocarcinoma cells

A549: Human lung adenocarcinoma epithelial cells

8505C: Human thyroid carcinoma cells

(\* p = 0.005 value)

#### 3.8 Anti-bacterial Studies of the $R_3PAu(L2)$ series (R = Ph, Cy, Et)

#### 3.8.1 Inhibitory activity

This study was performed to measure the zone of inhibition exhibited by the triorganophosphanegold(I) series of L2 and a standard anti-biotic from the quinolone group, ciprofloxacin. The ligand, NaL2, was found to inhibit only the growth of two Gram-positive bacterial strains: *B. subtilis* and *L. monocytogenes* with significantly lower diameters of zone of inhibition than that displayed by ciprofloxacin. Meanwhile, all Gram-positive pathogens were inhibited when treated with the tested gold(I) compounds: (b), (e) and (j). Compound (e) proved to have significantly higher inhibitory activity than ciprofloxacin against *E. faecium*, MRSA, *S. pyogenes* and *S. saprophyticus* (Figure 3.27).



**Figure 3.27:** Inhibitory activity against Gram-positive bacterial strains measured by zone of inhibition (mm) by NaL2 and its gold(I) complexes; (j), (b) and (e) with ciprofloxacin as the standard drug.

Interestingly, compound (e) showed its potential behaviour as an anti-bacterial agent, having either comparable or higher activity than that presented by ciprofloxacin and the other tested gold(I) complexes (Figure 3.28). Not only did (e) inhibit the Gram-positive

strains but also inhibited all tested Gram-negative strains with the exception to *P*. *aeruginosa*. The standard drug, ciprofloxacin, inhibited the growth of all pathogens including *P*. *aeruginosa* and its zone of inhibitions for Gram-negative bacteria were higher than the diameter observed for bacterial strains treated with (e).

Bacteria have different structures of membrane that classify them as Gram-positive and Gram-negative. The thickness of the peptidoglycan layer is the most distinctive in order to differentiate both classes of bacteria. Gram-positive bacteria have a thicker peptidoglycan layer while Gram-negative bacteria have a thinner cell wall of peptidoglycan surrounded by а second layer of membrane containing lipopolysaccharides and lipoprotein<sup>37</sup>. Some studies showed Gram-negative bacteria are more easily killed than Gram-positive bacteria and vice versa <sup>75</sup>. The dissimilarity in inhibitory activity possessed by (e) and ciprofloxacin might be correlated with the type of compound applied and mechanical protection provided by the outer membrane and peptidoglycan which make the Gram-negative bacteria cell more resistant to many chemicals<sup>75</sup>.

The outer membrane has the ability to exclude some hydrophobic and hydrophilic molecules to pass through it but allows only small hydrophilic molecules such as sugar and amino acids to pass through. Therefore, large anti-biotic molecules are harder to diffuse through the membrane into the cell. The low penetration will then lead to anti-biotic resistant Gram-negative bacteria. *P. aeruginosa* is one of the most resistant to anti-bacterial agents as its outer membrane is 100 times less permeable than that of *E. coli*<sup>76</sup>. The trial gold compounds would probably need to have more hydrophobic sites for easy penetration into the cell of *P. aeruginosa*.

#### 3.8.2 Minimum Inhibitory Concentration (MIC)

Three compounds, NaL2, (**j**) and (**b**), were found to inhibit the growth of Gram-positive bacteria but were inactive against Gram-negative bacteria. From the zone of inhibition versus bacterial strains graph, the ligand, NaL2, only inhibited the growth of two Grampositive pathogens, i.e. *B. subtilis* and *L. monocytogenes*. The zone of inhibition is two-to four-fold less than that exhibited by the same pathogens treated by the standard drug, ciprofloxacin, at a concentration of  $5\mu g / disc$ . As for (**j**) and (**b**), the zone of inhibitions were smaller than that shown by ciprofloxacin.

Interesting inhibitory activity was observed for (e) against all Gram-positive and Gramnegative pathogens except *P. aeruginosa*. Compound (e) displayed comparable or higher inhibition compared to ciprofloxacin when the compound was applied to Grampositive bacteria. Nevertheless, lower inhibitory zones were observed when all tested Gram-negative bacteria were treated with the same compound.



Figure 3.28: Inhibitory activity against Gram-negative bacterial strains measured by zone of inhibition (mm) by NaL2 and its gold(I) complexes; (j), (b) and (e) with ciprofloxacin as the standard drug.

The anti-bacterial activity was further assessed by minimum inhibitory concentration (MIC) determination. Lower MIC values indicate greater anti-bacterial activity because a lower amount of compound is needed to inhibit the growth of bacteria. The MIC values for all compounds were in the range of 0.98-2000.00  $\mu$ g/ml whereas ciprofloxacin was active in the range of 0.06-125  $\mu$ g/ml. The low activity displayed by NaL2 against *B. subtilis*, *L. monocytogenes* and *S. pyogenes* were observed at high MIC values of 2000.00, 2000.00 and 500.00  $\mu$ g/ml, respectively. These results indicate the importance of having the phosphanegold(I) unit for anti-bacterial properties.

By contrast, (**j**) and (**b**) exhibited promising MIC values towards all tested Grampositive bacteria in ranges of 7.81-62.5  $\mu$ g/ml and 31.25-125.00  $\mu$ g/ml, respectively. Meanwhile, the inhibitory activity towards Gram-positive bacteria was found to exceed that of (**j**), (**b**) and ciprofloxacin when the bacteria colonies were treated with the lowest MIC, between 0.98-3.91  $\mu$ g/ml of (**e**). The colonies of Gram-negative pathogens however, were inhibited by (**e**) with MIC values in the range of 15.63-1000.00  $\mu$ g/ml. From the MIC tests, few bacterial strains were found to become the most susceptible towards (**e**). These are methicillin-resistant *Staphylococcus aureus* (MRSA) and *Bacillus* spp. (0.98  $\mu$ g/ml), followed by 1.95  $\mu$ g/ml of that particular compound against *Staphylococcus* spp., *L. monocytogenes* and *S. pyogenes* as well as *Enterococcus* spp. (1.95-3.91  $\mu$ g/ml).

The *Staphylococcus* spp., *Enterococcus* spp. and *Bacillus* spp. are known to cause urinary tract infection, nosocomial infection and bacteraemia while *L. monocytogenes* causes listeriosis and meningitis. With this, it is believed that (e) offers potential efficacy for treatment against a variety of ailments caused by these bacterial strains <sup>54</sup>.

The MIC values obtained for (e) were relatively lower than the MIC values reported by Yeo *et al.* <sup>77</sup> for Ph<sub>3</sub>PAu[SC(OR)=N(tol-*p*)], where R = Me, Et and iPr. Compounds prepared by Yeo *et al.* <sup>77</sup> were tested against *Bacillus* spp. and *Staphylococcus* spp. The present study proved significant MIC values indicating efficacy of inhibition on both species as (e) seemed to display lower MIC values compared to the MIC values obtained to inhibit the growth of the tested bacteria reported by Yeo *et al.* <sup>77</sup>. Concisely, (e) could be further developed as a candidate for future anti-microbial agent for MRSA, *Staphylococcus* spp., *Bacillus* spp. and *Enterococcus* spp. as this compound possess high anti-bacterial activity.

#### 3.8.3 Minimum Bactericidal Concentration (MBC)

The bactericidal properties of (j), (b) and (e) against all tested pathogens, excluding *P*. *aeruginosa* were analyzed by measuring the minimum bactericidal concentration (MBC) and were summarized as MBC / MIC ratios. If a compound displays MBC value not more than four-fold higher than the MIC or if the MBC / MIC  $\leq$  4, that compound is consider to be bactericidal. However, if the MBC / MIC  $\geq$  4, the compound is bacteriostatic against pathogens. Bactericidal is characterized as the ability of a compound to kill and stop bacteria from growing while bacteriostatic is a character of slowing the growth of bacteria and not killing them <sup>78</sup>.

The present phosphanegold(I) thiolate compounds except (e) were found to be bactericidal towards susceptible Gram-positive bacteria strains with MBC / MIC  $\leq 2$ . Compound (b) on the other hand, promoted similar bacteriostatic activity against *E*. *faecium* where its MBC value was eigth-fold higher than its MIC value. Meanwhile, bactericidal activity was demonstrated by (e) against *B. cereus*, *B. subtilis*, *L.*  monocytogenes, S. saprophyticus, S. pyogenes, A. hydrophilla, S. paratyphi A, S. flexneri, S. sonnei, P. marabilis and V. parahaemolyticus. Compound (e) also showed bacteriostatic activity towards E. faecalis, E. faecium, MRSA, S. aureus, A. baumanii, C. freundii, E. aerogenes, E. cloacae, E. coli, K. pneumonia, S.typhimurium, S. maltophilia and P. vulgaris. Bacterial strains treated with ciprofloxacin showed higher ratio MBC / MIC values against E. aerogenes, S. paratyphi A, S. maltophilia and P. mirabilis. The behaviour exhibited by the gold(I) compounds suggested similarity to ciprofloxacin and dependency on the bacterial strain determined the bacteriostatic and bactericidal activities.

#### 3.8.4 Time-kill Assay

Gold compounds with high anti-bacterial activity of which MIC < 100  $\mu$ g/ml towards susceptible bacterial strains were selected for time-kill studies. The kinetic interaction between the gold compounds and susceptible pathogens were determined at 3 concentrations; twice MIC (2 x MIC), MIC and one-half MIC (1/2 x MIC).

As discussed earlier, the MBC / MIC ratios play an important role in determining bactericidal properties of the test compounds. From the time-kill assay, (**j**) was found to exhibit bactericidal characteristic after 1 hour towards *B. cereus*, *B. subtilis*, *E. faecalis*, *E. faecium*, *L. monocytogenes*, MRSA, *S. aureus*, *S. saprophyticus* and *S. pyogenes* as summarized in Figure 3.29. A similar behaviour was observed when *B. cereus*, *B. subtilis*, *L. monocytogenes*, MRSA and *S. pyogenes* were treated with (**b**) as presented in Figure 3.30. The bactericidal properties shown by the time-kill profiles indicated that both (**j**) and (**b**) were independent to concentrations.



Figure 3.29: Time-kill curves of compound (j) against (a) B. cereus, (b) B. subtilis, (c) E. faecalis, (d) E. faecium, (e) L. monocytogenes, (f) MRSA, (g) S. aureus, (h) S. saprophyticus, (i) S. pyogenes. Note that the bactericidal level is indicated by the dashed lines; - - - , growth control; ×, 2 MIC; ▲, MIC; ■ and 1/2MIC; ◆.

Nevertheless, a different effect was observed for pathogens treated with (e). Various degrees of bactericidal and bacteriostatic activities were exhibited depending on the strains and concentrations (Figure 3.31). Complex (e) had similar killing rate as (j) and (b) against *B. cereus*, *B. subtilis* and *S. pyogenes* after 1 hour.



Figure 3.30: Time-kill curves of compound (b) against (a) *B. cereus*, (b) *B. subtilis*, (c) *E. faecalis*, (d) *E. faecium*, (e) *L. monocytogenes*, (f) MRSA, (g) *S. pyogenes*. Note that the bactericidal level is indicated by the dashed lines; ; - - - , growth control; ×, 2
MIC; ▲, MIC; ■ and 1/2MIC; ◆.

The killing rate of (e) became slower than the other gold compounds against *E. faecalis*, *E. faecium*, *L. monocytogenes*, *S. aureus* and MRSA in which bactericidal activities were seen after 3 hours interaction at 2 x MIC and started to show bactericidal character against *S. saprophyticus* after 4 hours at  $\frac{1}{2}$  x MIC.



Figure 3.31: Time-kill curves of compound (e) against (a) *B. cereus*, (b) *B. subtilis*, (c) *E. faecalis*, (d) *E. faecium*, (e) *L. monocytogenes*, (f) MRSA, (g) *S. aureus*, (h) *S. saprophyticus*, (i) *S. pyogenes*, (j) *E. coli*, (k) *P. vulgaris*. Note that the bactericidal level is indicated by the dashed lines; ---, growth control; ×, 2 MIC; ▲, MIC; ■ and 1/2MIC; ◆.

Consistent with the MBC / MIC ratios, (e) was bactericidal towards *B. cereus*, *B. subtilis*, *L. monocytogenes*, *S. saprophyticus* and *S. pyogenes* at its MIC value after 24 hours. On the other hand, bacteriostatic character was apparent towards *E. faecalis*, *E. faecalis*, *E. faecuum*, MRSA, *S. aureus*, *E. coli* and *P. vulgaris* at the same concentration. Aggressive bactericidal activities for (e) towards *E. faecalis*, *E. faecium*, MRSA, *S.* 

*aureus* and *E. coli* were achieved at higher concentrations than MIC values over 24 hours.

The time-kill profiles also displayed the bacteriostatic activity shown at its MIC value against *P. vulgaris* after 4 hours contact but the strain was found to regrow to the same level as the control after 24 hours. Regrowth of *E. coli* was seen after 4 hours exposure at its MIC and  $\frac{1}{2}$  x MIC. This phenomenon was not observed for other strains and could be attributed to two distinct sub-populations with different susceptibility in which the selective growing of resistant sub-population take over the preferential killing of the susceptible sub-population at a specified time of interaction <sup>54</sup>.

### **CHAPTER 4: CONCLUSIONS**

All phosphanegold(I), -silver(I) and -copper(I) dithiocarbamates have been successfully synthesized and structurally characterized by employing spectroscopic techniques crystal X-ray diffraction. The substituted including powder and single (hydroxyethyl)dithiocarbamates behave monodentate ligands towards as phosphanegold(I) but as bidentate towards bis(phosphane)silver(I) and -copper(I). None of the Ph<sub>3</sub>PAu(I) derivatives yielded crystals as these compounds turned to sticky compounds in the presence of solvent. However, the Cy<sub>3</sub>PAu(I) and Et<sub>3</sub>PAu(I) derivatives gave crystals, one of each series. In addition, phosphanesilver(I) and copper(I) dithiocarbamates afforded some crystals. For compounds that crystals were not obtained, their powder patterns were compared to known structures to see if they were isostructural.

The tested gold(I) compounds exhibited better cytotoxicity than cisplatin in terms of  $IC_{50}$  values, thus were toxic to tested cancer cells. Nevertheless, these gold(I) compounds were also toxic to non-cancerous human cells. For the anti-bacterial study, all tested gold(I) derivatives were either bacteriostatic or bactericidal towards all Grampositive pathogens. Of the gold(I) compounds, phosphanegold(I) dithiocarbamates bearing the triethylphosphane group showed great potential as anti-cancer agents and bactericidal agents on some Gram-negative bacteria. However, none of these were active in suppressing *P. aeruginosa*. From the present study, it is also apparent that the silver(I) and copper(I) derivatives inhibited the proliferation of cancer cells but their toxic behaviour on non-cancerous human cells mitigated further exploration.

In order to improve the biological efficacies of these compounds, a few modifications on the chemical structure could be applied. A change in substituent or functional groups on the ligand and the type of phosphane also could contribute to the enhancement in bioactivities.

## REFERENCES

- 1 Li, Q.-H., Ding, Y. & Huang, N.-W. Synthesis and biological activities of dithiocarbamates containing 1,2,3-triazoles group. *Chinese Chemical Letters* **25**, 1469-1472, doi:http://dx.doi.org/10.1016/j.cclet.2014.05.022 (2014).
- 2 Ahmed, M. *et al.* Synthesis and Evaluation of Polymeric Gold Glyco-Conjugates as Anti-Cancer Agents. *Bioconjugate Chemistry* **24**, 979-986 (2013).
- 3 Hogarth, G. *et al.* Functionalised Dithiocarbamate Complexes:. *Inorganica Chimica Acta* **362**, 2020-2026 (2009).
- 4 Ahamad, M., Rao, R. M., Rafi, M. M., Mohiddin, G. J. & Sreeramulu, J. Synthesis, Spectral Analysis and Antimicrobial Activity of Novel Dithiocarbamate Schiff Base Metal Complexes. *Archives of Applied Science Research* **4**, 858-862 (2012).
- 5 Onwudiwe, D. C. & Ajibade, P. A. Synthesis and Characterization of Metal Complexes of N-alkyl-N-phenyldithiocarbamates. *Polyhedron* **29**, 1431-1436 (2010).
- 6 Horita, Y. *et al.* Synthesis and Evaluation of Anti-tubercular Activity of New Dithiocarbamate Sugar Derivatives. *Bioorganic & Medicinal Chemistry Letters* **21**, 899-903 (2011).
- 7 Gandin, V. *et al.* Cancer cell death induced by phosphine gold(I) compounds targeting thioredoxin reductase. *Biochemical Pharmacology* **79**, 90-101, doi:10.1016/j.bcp.2009.07.023 (2010).
- 8 Laguna, A. in *Modern Supramolecular Gold Chemistry: Gold-Metal Interactions and Applications* (ed Antonio Laguna) 1-64 (WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, 2008).
- 9 Bond, G. C. Relativistic effects in coordination, chemisorption and catalysis. Journal of Molecular Catalysis A: Chemical **156**, 1-20, doi:http://dx.doi.org/10.1016/S1381-1169(00)00075-3 (2000).
- 10 Tang, S. S., Chang, C.-P., Lin, I. J. B., Liou, L.-S. & Wang, J.-C. Molecular Aggregation of Annular Dinuclear Gold(I) Compounds Containing Bridging Diphosphine and Dithiolate Ligands. *Inorg. Chem.* **36**, 2294-2300 (1997).
- 11 Kaeser, A. *et al.* Homoleptic Copper(I), Silver(I), and Gold(I) Bisphosphine Complexes. *European Journal of Inorganic Chemistry*, 1-12 (2014).
- 12 Shaw III, C. F. Gold-Based Therapeutic Agents. *Chem. Rev.* **99**, 2589-2600 (1999).

- 13 Rodríguez, A., Sousa-Pedrares, A., García-Vázquez, J. A., Romero, J. & Sousa, A. Synthesis and Structural Characterization of Copper(I), Silver(I) and Gold(I) Complexes with Pyrimidine-2-thionato Ligands and their Adducts with Phosphanes. *European Journal of Inorganic Chemistry*, 3403-3413 (2011).
- 14 Bond, G. C. The Catalytic Properties of Gold: Potential Applications in the Chemical Industry. *Gold Bulletin* **5**, 11-13 (1972).
- 15 Fricker, S. P. Medical Uses of Gold Compounds: Past, Present and Future. *Gold Bulletin* **29**, 53-60 (1996).
- 16 Ott, I. On the Medicinal Chemistry of Gold Complexes as Anticancer Drugs. *Coordination Chemistry Reviews* 253, 1670–1681, doi:10.1016/j.ccr.2009.02.019 (2009).
- 17 Faggiani, R., Howard-Lock, H. E., Lock, C. J. L. & Turner, M. A. The Reaction of Chloro(triphenylphosphine)gold(I) with 1-methylthymine. *Can. J. Chem.* **65**, 1568-1575 (1987).
- 18 Milacic, V. *et al.* A Novel Anticancer Gold(III) Dithiocarbamate Compound Inhibits the Activity of a Purified 20S Proteasome and 26S Proteasome in Human Breast Cancer Cell Cultures and Xenografts. *Cancer Res* 66, 10478-10486 (2006).
- 19 Buckley, R. G. *et al.* Antitumor Properties of Some 2-[(Dimethylamino)methyl]phenylgold(III) Complexes. *J. Med. Chem* **39**, 5208-5214 (1996).
- 20 Bhabak, K. P., Bhuyan, B. J. & Mugesh, G. Bioinorganic and Medicinal Chemistry: Aspects of Gold(I)-protein Complexes. *Dalton Transactions* **40**, 2099 - 2111 (2011).
- 21 Vicente, J., Chicote, M. T., Abrisqueta, M. D., González-Herrero, P. & Guerrero, R. Recent Advances in the Chemistry of Gold(I) Complexes with C-, N- and S-Donor Ligands. Part I: Alkynyl, Amino, Imino and Nitrido Derivatives. *Gold Bulletin* **31**, 83-87 (1998).
- 22 Novelli, F., Recine, M., Sparatore, F. & Juliano, C. Gold(I) Complexes as Antimicrobial Agents. *Il Farmaco* **54**, 232-236 (1999).
- 23 Lima, J. C. & Rodriguez, L. Phosphine-Gold(I) Compounds as Anticancer Agents: General Description and Mechanisms of Action. *Anti-Cancer Agents in Medicinal Chemistry* 11, 921-928 (2011).
- 24 Tiekink, E. R. T. Phosphinegold(I) Thiolates Pharmacological Use and Potential. *Bioinorganic Chemistry and Applications* **1**, 53-67 (2002).
- 25 Meyer, A. *et al.* On the Biological Properties of Alkynyl Phosphine Gold(I) Complexes. *Bioorganometallic Chemistry* **51**, 8895-8899 (2012).

- 26 Rigobello, M. P., Scutari, G., Boscolo, R. & Bindoli, A. Induction of Mitochondrial Permeability Transition by Auranofin, a Gold(I)-phosphine Derivative. *British Journal of Pharmacology* **136**, 1162-1168 (2002).
- 27 Cerrada, E. *et al.* Synthesis and Reactivity of Trinuclear Gold(III) Dithiolate Complexes. X-ray Structure of  $[Au(C_6F_5)(S_2C_6H_4)]_3$  and  $[Au(C_6F_5)(S_2C_6H_4)(SC_6H_4SPPh_3)]$ . Organometallics **14**, 5537-5543 (1995).
- 28 Segapelo, T. V., Guzei, I. A., Spencer, L. C., Zyl, W. E. V. & Darkwa, J. (Pyrazolylmethyl)pyridine platinum(II) and gold(III) Complexes: Synthesis, Structures and Evaluation as Anticancer Agents. *Inorganica Chimica Acta* **362**, 3314-3324 (2009).
- 29 Ott, I. & Gust, R. Non Platinum Metal Complexes as Anti-cancer Drugs. *Archiv der Pharmazie Chemistry in Life Sciences* **340**, 117-126 (2007).
- 30 Garza-Ortiz, A. *et al.* Novel Mononuclear Gold(III) Complexes with Modified 2-(arylazo)pyridine Ligands: Synthesis and Characterization. *Journal of Inorganic Biochemistry* **101**, 1922-1943 (2007).
- 31 Mota, V. Z. *et al.* Gold Complexes with Benzimidazole Derivatives: Synthesis, Characterization and Biological Studies. *Biometals* **27**, 183-194 (2014).
- 32 Casas, J. S. *et al.* A Gold(I) Complex with Vitamin K<sub>3</sub> Derivative: Characterization and Antitumoral Activity. *Journal of Inorganic Biochemistry* **100**, 1858-1860 (2006).
- 33 Rubbiani, R., Salassa, L., de Almeida, A., Casini, A. & Ott, I. Cytotoxic Gold(I) N-heterocyclic Carbene Complexes with Phosphane Ligands as Potent Enzyme Inhibitors. *Chem Med Chem* 9, 1205-1210 (2014).
- 34 Altaf, M. & Stoeckli-Evans, H. Silver(I) Tertiary Phosphine Complexes: Influence of the Anion on the Structural and Spectroscopic Properties. *Polyhedron* **29**, 701-708 (2010).
- 35 Meijboom, R., Bowen, R. J. & Berners-Price, S. J. Coordination Complexes of Silver(I) with Tertiary Phosphine and Related Ligands. *Coordination Chemistry Reviews* **253**, 325-342 (2009).
- 36 Nagajyothi, P. C., Sreekanth, T. V. M., Lee, J. I. & Kap, D. L. Mycosynthesis: Antibacterial, Antioxidant and Antiproliferative Activities of Silver Nanoparticles Synthesized from *Inonotus obliquus* (Chaga Mushroom) Extract. *Journal of Photochemistry and Photobiology B: Biology* 130, 299-304 (2014).
- Ortego, L., Gonzalo-Asensio, J., Laguna, A., Villacampa, M. D. & Gimeno, M. C. (Aminophosphane)gold(I) and Silver(I) Complexes as Antibacterial Agents. *Journal of Inorganic Biochemistry* 146, 19-27 (2015).
- 38 Banti, C. N. & Hadjikakou, S. K. Anti-proliferative and Anti-tumor Activity of Silver(I) Compounds. *Metallomics* **5**, 569-596 (2013).

- 39 Altaf, M. & Stoeckli-Evans, H. Copper(I) and Silver(I) Tertiary Phosphines Complexes: Synthesis, X-ray Structures and Spectroscopic Characterization. *Inorganica Chimica Acta* **363**, 2567-2573 (2010).
- 40 Ibrahim, M. *et al.* Copper as an Antibacterial Agent for Human Pathogenic Multidrug Resistant *Burkholderia cepacia* Complex Bacteria. *Journal of Bioscience and Bioengineering* **112**, 570-576 (2011).
- 41 Oehninger, L., Rubbiani, R. & Ott, I. N-Heterocyclic Carbene Metal Complexes in Medicinal Chemistry. *Dalton Transactions* **42**, 3269-3284 (2013).
- 42 Keter, F. K., Guzei, I. A. G., Nell, M., van Zyl, W. E. & Darkwa, J. Phosphinogold(I) Dithiocarbamate Complexes: Effect of the Nature of Phosphine Ligand on Anticancer Properties. *Inorganic Chemistry* 53, 2058–2067 (2014).
- 43 Foley, J., Jr., R. C. F., McDougal, K., Bruce, M. R. M. & Bruce, A. E. Electronic and Steric Effects in Gold(I) Phosphine Thiolate Complexes. *Metal Based Drugs* **1**, 405-417 (1994).
- 44 Fonteha, P. & Meyer, D. Novel Gold(I) Phosphine Compounds Inhibit HIV-1 Enzymes. *Metallomics* **1**, 427-433 (2009).
- 45 Scheffler, H., You, Y. & Ott, I. Comparative Studies on the Cytotoxicity, Cellular and Nuclear Uptake of a Series of Chloro Gold(I) Phosphine Complexes. *Polyhedron* **29**, 66-69 (2010).
- 46 Serebryanskaya, T. V. *et al.* Gold(I) Thiotetrazolates as Thioredoxin Reductase Inhibitors and Antiproliferative Agents<sup>†</sup>. *Dalton Transactions* **44**, 1161-1169 (2015).
- 47 Srivastava, S. K., Pandey, H. & Sharma, R. Unsymmetrically Substituted Organotin(IV) N-alkyl, N-hydroxyethyl dithiocarbamates: Synthesis and Spectral Aspects. *Indian Journal of Chemistry* **46A**, 1105-1108 (2007).
- 48 Yin, H.-D., Zhai, J., Sun, Y.-Y. & Wang, D.-Q. Synthesis, Characterizations and Crystal Structures of New Antimony(III) Complexes with Dithiocarbamate Ligands. *Polyhedron* **27**, 663-670 (2008).
- 49 Mamba, S. M. *et al.* Spectral, thermal and *in vitro* antimicrobial studies of cyclohexylamine-N-dithiocarbamate transition metal complexes. *Spectrochimica Acta Part A* **77**, 579-587 (2010).
- 50 Abbehausen, C. *et al.* Gold(I)-Phosphine-N-Heterocycles: Biological Activity and Specific (Ligand) Interactions on the C-Terminal HIVNCp7 Zinc Finger. *Inorganic Chemistry* **52**, 11280-11287 (2013).
- 51 de Vos, D., Soo, Y. H. & Tiekink, E. R. T. Cytotoxicity Profiles for a Series of Triorganophosphinegold(I) Dithiocarbamates and Triorganophosphinegold(I) Xanthates. *Bioinorganic Chemistry and Applications* **2**, 141-154 (2004).

- 52 Afzaal, M., Rosenberg, C. L., Malik, M. A., Whiteb, A. J. P. & O'Brien, P. Phosphine Stabilized Copper(I) Complexes of Dithiocarbamates and Xanthates and their Decomposition Pathways. *New Journal of Chemistry* **35**, 2773-2780 (2011).
- 53 Stein, R. A. & Knobler, C. Crystal and Molecular Structure of a 1:1 Complex of Silver Nitrate and Triphenylphosphine, AgNO<sub>3</sub>.P(C<sub>6</sub>H<sub>5</sub>)<sub>3</sub>. *Inorganic Chemistry* **16**, 242-245 (1977).
- 54 Sim, J.-H. *et al. In vitro* Antibacterial and Time-kill Evaluation of Phosphanegold(I) Dithiocarbamates,  $R_3PAu[S_2CN(iPr)CH_2CH_2OH]$  for R = Ph, Cy and Et, Against a Broad Range of Gram-positive and Gram-negative Bacteria. *Gold Bulletin* **47**, 225-236 (2014).
- 55 Jamaludin, N. S. *et al.* Phosphanegold(I) Dithiocarbamates, R<sub>3</sub>PAu[SC(=S)N(<sup>i</sup>Pr)CH<sub>2</sub>CH<sub>2</sub>OH] for R = Ph, Cy and Et: Role of Phosphanebound R Substituents upon *in vitro* Cytotoxicity against MCF-7R Breast Cancer Cells and Cell Death Pathways. *European Journal of Medicinal Chemistry* 67, 127-141, doi:10.1016/j.ejmech.2013.06.038 (2013).
- 56 Gunay, N. S. *et al.* 5-Nitroimidazole Derivatives as Possible Antibacterial and Antifungal Agents. *Il Farmaco* 54, 826-831 (1999).
- 57 Takahashia, M., Robertson, N., Clark, R. J. H. & Underhill, A. E. Isolation and characterisation of a new monodithiolate gold(III) complex. *Polyhedron* **18**, 1545-1548 (1999).
- 58 Bhabak, K. P. & Mugesh, G. A Synthetic Model for the Inhibition of Glutathione Peroxidase by Antiarthritic Gold Compounds. *Inorg. Chem.* **48**, 2449-2455 (2009).
- 59 Francuski, B. M., Novakovic, S. B. & Bogdanovic, G. A. Electronic Features and Hydrogen Bonding Capacity of the Sulfur Acceptor in Thioureido-Based Compounds. Experimental Charge Density Study of 4-methyl-3thiosemicarbazide. *Crystal Engineering Communication* **13**, 3580-3591 (2011).
- 60 Isab, A. A. *et al.* Synthesis, characterization and antimicrobial studies of mixed ligand silver(I) complexes of thioureas and triphenylphosphine; crystal structure of {[Ag(PPh<sub>3</sub>)(thiourea)(NO<sub>3</sub>)]<sub>2</sub> . [Ag(PPh<sub>3</sub>)(thiourea)]<sub>2</sub>(NO<sub>3</sub>)<sub>2</sub>}. *Polyhedron* **29**, 1251-1256 (2010).
- 61 Wang, K. Molecular mechanisms of liver injury: Apoptosis or necrosis. *Experimental and Toxicologic Pathology* **66**, 351-356, doi:<u>http://dx.doi.org/10.1016/j.etp.2014.04.004</u> (2014).
- 62 Zhang, J. & Xu, M. Apoptotic DNA Fragmentation and Tissue Homeostasis. *Trends in Cell Biology* **12**, 84-89 (2002).
- 63 Wetzel, C. *et al.* Gold(I) Complexes of Water-soluble Diphos-type Ligands: Synthesis, Anticancer Activity, Apoptosis and Thioredoxin Reductase Inhibition. *Dalton Transactions* **40**, 9212-9220 (2011).

- 64 Martin, S. J. & Henry, C. M. Distinguishing between apoptosis, necrosis, necroptosis and other cell death modalities. *Methods* **61**, 87-89, doi:<u>http://dx.doi.org/10.1016/j.ymeth.2013.06.001</u> (2013).
- 65 Schug, Z. T., Gonzalvez, F., Houtkooper, R. H., Vaz, F. M. & Gottlieb, E. BID is Cleaved by Caspase-8 within a Native Complex on the Mitochondrial Membrane. *Cell Death and Differentiation* **18**, 538-548 (2011).
- 66 Batandier, C., Leverve, X. & Fontaine, E. Opening of the Mitochondrial Permeability Transition Pore Induces Reactive Oxygen Species Production at the Level of the Respiratory Chain Complex I. *The Journal of Biological Chemistry* **279**, 17197-17204 (2004).
- 67 Winter, E. *et al.* Antitumoral activity of a trichloromethyl pyrimidine analogue: molecular crosstalk between intrinsic and extrinsic apoptosis. *Chemical Research in Toxicology*, 1-35 (2014).
- 68 Nagata, S. Apoptotic DNA Fragmentation. *Experimental Cell Research* **256**, 12-18, doi:<u>http://dx.doi.org/10.1006/excr.2000.4834</u> (2000).
- Nikoletopoulou, V., Markaki, M., Palikaras, K. & Tavernarakis, N. Crosstalk Between Apoptosis, Necrosis and Autophagy. *Biochimica et Biophysica Acta* (*BBA*) - *Molecular Cell Research* 1833, 3448-3459, doi:<u>http://dx.doi.org/10.1016/j.bbamcr.2013.06.001</u> (2013).
- Vianello, A. *et al.* The Mitochondrial Permeability Transition Pore (PTP) An Example of Multiple Molecular Exaptation? *Biochimica et Biophysica Acta* (*BBA*) *Bioenergetics* 1817, 2072-2086, doi:<u>http://dx.doi.org/10.1016/j.bbabio.2012.06.620</u> (2012).
- 71 Kim, J.-S., He, L. & Lemasters, J. J. Mitochondrial permeability transition: a common pathway to necrosis and apoptosis. *Biochemical and Biophysical Research Communications* **304**, 463-470, doi:<u>http://dx.doi.org/10.1016/S0006-291X(03)00618-1</u> (2003).
- 72 Nunes, T., Bernardazzi, C. & de Souza, H. S. Cell Death and Inflammatory Bowel Diseases: Apoptosis, Necrosis, and Autophagy in the Intestinal Epithelium. *BioMed Research International*, 1-12 (2014).
- 73 Carey, J. F., Schultz, S. J., Sisson, L., Fazzio, T. G. & Champoux, J. J. DNA Relaxation by Human Topoisomerase I Occurs in the Closed Clamp Conformation of the Protein. *Proceedings of the National Academy of Sciences* 100, 5640-5645, doi:10.1073/pnas.1031537100 (2003).
- 74 Malek, S. N. A., Chung, W. P., Ibrahim, H., Wahab, N. A. & Kae, S. S. Phytochemical and Cytotoxic Investigations of Alpinia mutica Rhizomes. *Molecules* **16**, 583-589 (2011).
- 75 Barudin, N. H. A., Sreekantan, S., Ong, M. T. & Lai, C. W. Synthesis, Characterization and Comparative Study of Nano-Ag-TiO2 Against Grampositive and Gram-negative Bacteria under Fluorescent Light. *Food Control* **46**, 480-487, doi:<u>http://dx.doi.org/10.1016/j.foodcont.2014.05.046</u> (2014).

- 76 Brooks, G. F., Carroll, K. C., Butel, J., S & Morse, S. A. in *Cell Structure* (ed Stephen A Morse) 769 (The McGraw-Hill Companies, Inc., United States of America, 2007).
- 77 Yeo, C. I. *et al.* Pathogenic Gram-positive Bacteria are Highly Sensitive to Triphenylphosphanegold(O-alkylthiocarbamates),  $Ph_3PAu[SC(OR)=N(ptolyl)]$ (R = Me, Et and iPr). *Gold Bulletin* **46**, 145-152 (2013).
- 78 Pankey, G. A. & Sabath, L. D. Clinical Relevance of Bacteriostatic versus Bactericidal Mechanisms of Action in the Treatment of Gram-Positive Bacterial Infections. *Clinical Infectious Diseases* **38**, 864-870 (2004).

# APPENDICES

Empirical formula	C <sub>24</sub> H <sub>45</sub> AuNOPS <sub>2</sub>
Formula weight	655.66
Temperature/K	100(2)
Crystal system	monoclinic
Space group	P2 <sub>1</sub> /n
a/Å	10.4647(3)
b/Å	10.8268(3)
c/Å	23.5694(6)
α/°	90
β/°	97.936(3)
γ/°	90
Volume/Å <sup>3</sup>	2644.82(13)
Z	4
$\rho_{calc}g/cm^3$	1.647
$\mu/mm^{-1}$	5.797
F(000)	1320.0
Crystal size/mm <sup>3</sup>	$0.12 \times 0.06 \times 0.04$
Radiation	MoKα ( $\lambda$ = 0.71073)
20 range for data collection/°	4.514 to 55.12
Index ranges	$-12 \le h \le 13, -13 \le k \le 13, -20 \le l \le 30$
Reflections collected	10673
Independent reflections	$6068 [R_{int} = 0.0303, R_{sigma} = 0.0492]$
Data/restraints/parameters	6068/0/275
Goodness-of-fit on F <sup>2</sup>	1.148
Final R indexes [I>= $2\sigma$ (I)]	$R_1 = 0.0285, wR_2 = 0.0746$
Final R indexes [all data]	$R_1 = 0.0396, wR_2 = 0.0932$
Largest diff. peak/hole / e Å <sup>-3</sup>	0.80/-0.83

## Appendix A: Crystal data for compound (b)

Empirical formula	C <sub>12</sub> H <sub>27</sub> AuNOPS <sub>2</sub>
Formula weight	493.40
Temperature/K	100(2)
Crystal system	monoclinic
Space group	P21
a/Å	11.5458(12)
b/Å	8.9567(10)
c/Å	17.386(9)
α/°	90.00
β/°	107.35(3)
γ/°	90.00
Volume/Å <sup>3</sup>	1716.1(9)
Z	4
$\rho_{calc}g/cm^3$	1.910
$\mu/mm^{-1}$	8.899
F(000)	960.0
Crystal size/mm <sup>3</sup>	0.10 imes 0.05 imes 0.05
Radiation	MoKα ( $\lambda$ = 0.71073)
2 $\Theta$ range for data collection/°	4.9 to 55.14
Index ranges	$-15 \le h \le 14, -11 \le k \le 11, -21 \le l \le 22$
Reflections collected	12889
Independent reflections	7812 [ $R_{int} = 0.0349, R_{sigma} = N/A$ ]
Data/restraints/parameters	7812/1/327
Goodness-of-fit on F <sup>2</sup>	1.002
Final R indexes [I>= $2\sigma$ (I)]	$R_1 = 0.0339, wR_2 = 0.0717$
Final R indexes [all data]	$R_1 = 0.0386, wR_2 = 0.0748$
Largest diff. peak/hole / e Å <sup>-3</sup>	1.51/-0.76
Flack parameter	-0.023(6)

Appendix B: Crystal data for compound (e)

Empirical formula	$C_{40}H_{38}CuNO\ P_2S_2$
Formula weight	814.44
Temperature/K	100.15
Crystal system	triclinic
Space group	P-1
a/Å	12.3145(7)
b/Å	12.8280(6)
c/Å	13.7989(13)
α/°	97.894(5)
β/°	93.688(5)
γ/°	115.535(3)
Volume/Å <sup>3</sup>	1929.6(2)
Z	2
$\rho_{calc}g/cm^3$	1.402
$\mu/mm^{-1}$	0.899
F(000)	844.0
Crystal size/mm <sup>3</sup>	$0.12 \times 0.07 \times 0.07$
Radiation	MoKα ( $\lambda$ = 0.71073)
$2\Theta$ range for data collection/°	3.58 to 55.46
Index ranges	$-15 \le h \le 16, -16 \le k \le 16, -17 \le l \le 18$
Reflections collected	18663
Independent reflections	8964 [ $R_{int} = 0.0504, R_{sigma} = 0.0815$ ]
Data/restraints/parameters	8964/0/451
Goodness-of-fit on F <sup>2</sup>	1.074
Final R indexes [I>= $2\sigma$ (I)]	$R_1 = 0.0527, wR_2 = 0.1361$
Final R indexes [all data]	$R_1 = 0.0780, wR_2 = 0.1670$
Largest diff. peak/hole / e Å <sup>-3</sup>	1.01/-0.78

Appendix C: Crystal data for compound (I)

Empirical formula	$C_{42}H_{43}ClCuNO_2P_2S_2$
Formula weight	196.95
Temperature/K	100.1(5)
Crystal system	triclinic
Space group	P-1
a/Å	10.7354(5)
b/Å	13.5453(8)
c/Å	15.9766(9)
α/°	67.559(6)
β/°	86.831(4)
γ/°	72.748(5)
Volume/Å <sup>3</sup>	2046.3(2)
Z	9
$\rho_{calc}g/cm^3$	1.4383
$\mu/mm^{-1}$	0.944
F(000)	917.3
Crystal size/mm <sup>3</sup>	$0.05\times0.09\times0.11$
Radiation	Mo Kα ( $\lambda$ = 0.71073)
2 $\Theta$ range for data collection/°	5.96 to 57.9
Index ranges	$-14 \le h \le 14, -15 \le k \le 18, -21 \le l \le 20$
Reflections collected	13941
Independent reflections	8044 [ $R_{int} = 0.0328$ , $R_{sigma} = 0.0588$ ]
Data/restraints/parameters	8044/0/481
Goodness-of-fit on F <sup>2</sup>	1.050
Final R indexes [I>= $2\sigma$ (I)]	$R_1 = 0.0461, wR_2 = 0.0981$
Final R indexes [all data]	$R_1 = 0.0674, wR_2 = 0.1194$
Largest diff. peak/hole / e Å <sup>-3</sup>	1.33/-0.92

Appendix D: Crystal data for compound (n)

Empirical formula	$C_{44}H_{48}AgNO_2P_2S_2$
Formula weight	1640.78
Temperature/K	293(2)
Crystal system	triclinic
Space group	P-1
a/Å	10.816(13)
b/Å	14.042(16)
c/Å	16.214(19)
α/°	65.873(16)
β/°	86.969(17)
γ/°	76.008(17)
Volume/Å <sup>3</sup>	2178(4)
Z	1
$\rho_{calc}g/cm^3$	1.251
$\mu/mm^{-1}$	0.665
F(000)	804.0
Crystal size/mm <sup>3</sup>	$0.20\times0.11\times0.09$
Radiation	MoKα ( $\lambda$ = 0.71073)
2 $\Theta$ range for data collection/°	2.76 to 53
Index ranges	$-13 \le h \le 13, -17 \le k \le 17, -20 \le l \le 20$
Reflections collected	25601
Independent reflections	9017 [ $R_{int} = 0.0401, R_{sigma} = 0.0487$ ]
Data/restraints/parameters	9017/0/478
Goodness-of-fit on F <sup>2</sup>	1.052
Final R indexes [I>= $2\sigma$ (I)]	$R_1 = 0.0407, wR_2 = 0.1077$
Final R indexes [all data]	$R_1 = 0.0670, wR_2 = 0.1369$
Largest diff. peak/hole / e Å <sup>-3</sup>	0.85/-0.66

Appendix E: Crystal data for compound (p)