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Synthesis, Spectral Characterization and Biological Evaluation of Platinum (II) Complexes of 1,3,4-oxadiazole-2-thione from Fatty Acids

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Summary: The fatty acid can be separated by separation techniques according to degree of unsaturated and chain length. In this paper fatty acid methyl esters are separated by Argentation Chromatography then converted to 1, 3, 4-oxadiazole. Platinum complexes of 1, 3, 4-oxadiazole are synthesized and Oxadiazole-2-thione ligands containing C=N and C=S groups as coordination sites may behave as a monobasic monodentate ligand. Antioxidant activity of complexes are measured by DPPH assay and in vitro cytotoxicity of the synthesized complexes measured against two human cancer cell line by MTT assay. In general, both MCF7 and A549 cell lines are sensitive to all the tested compounds. The Pt-complexes that contain unsaturated fatty acid are most active on the A549 cell line and important for human lung cancer, while the Pt4 has activity on MCF7 cell line and is important for human breast cancer.

Key words: Platinum (II) complex, Oxadiazole-2-thione, anticancer activity, MTT assay.

Introduction

Lipids are one of the three important biomolecules (lipid, carbohydrate and protein) and the fatty acids are the important part of lipid (triglyceride). Fatty acids have important role from physiological functions. Based on the number of double bonds, fatty acids can be classified into three types like saturated fatty acid, monounsaturated fatty acid and polyunsaturated fatty acids. Lauric acid, palmatic acid and stearic acid are the most significant saturated fatty acids while oleic acid and linoleic acid are examples of monounsaturated and polyunsaturated fatty acids respectively [1]. Monounsaturated fatty acid shows broad spectrum of biological activity such as help in promoting healthy blood lipid profiles and mediated blood pressure [2]. Polyunsaturated fatty acid have role in lipid metabolism and can decrease very low density lipoprotein (VLDL) by increasing activity of lipoprotein lipase [3]. The fatty acid can be separated by separation techniques according to degree of unsaturated and chain length. A variety of methods have been reported to separate fatty acids such as GC-MS [4], HPLC [5] and Ag-Si column chromatography. The Ag-Si column chromatography is the most effective technique, which separates fatty acids based on the number of double bonds [6]. Another important bioactive chemical compound is oxadiazole. Oxadiazole is a heterocyclic five-membered ring that contains two nitrogen, two carbon and one oxygen atoms in the ring. The 1,3,4-oxadiazole is the most important isomer of oxadiazole. 1, 3, 4-Oxadiazoles have biological activity properties such as antioxidant [7], anticancer [8], analgesic and antiinflammatory [9]. Mohamed Gaber et al. reported that the biological activity of metal complexes is higher than free ligand. The biological activity of platinum complexes have been reported in several literatures [10]. Jungang Deng et al. reported that the Pt-complexes show antioxidant, anticancer and antimicrobial activity and Pt-complexes have anticancer property higher than cis-platin, the well-known anticancer drug [11].

The aim of this paper is to separate fatty acids from oil and then convert them to 1, 3, 4-oxadiazoles and subsequently their platinum complexes. The antioxidant activity of the complexes and their in vitro cytotoxicity against two human cancer cell lines have been assessed.

Experimental

Materials

The fatty acid methyl esters of sunflower oil and olive oil were used as starting material which were previously prepared in our lab. This work was previously published as conference paper [12]. The

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standards of methyl laurate (12:0), methyl stearate (18:0), silver nitrate, hydrazine hydrate, platinum chloride were purchased from Sigma Aldrich-USA. The MCF7 and A549, human cell lines were purchased from the Shanghai Institute for Biological Science (Iran). All other chemicals were obtained from the store at University Technology Malaysia (UTM). All chemicals were of reagent grade.

Separation Fatty Acid Methyl Ester by Argentation Chromatography

Preparation of Silver Impregnated Silica

Sliver nitrate solution ($AgNO_3$) was prepared by dissolving 10g of $AgNO_3$ in 60 mL ethanol. Silica gel (0.06–0.2 mm, 70–230 mesh ASTM; mean pore diameter of 6 nm, specific surface area of 500 m^2 /g) in 100 ml ethanol (95%) was added to the silver nitrate solution under stirring for 2 h. then the ethanol was evaporated on a rotary evaporator at 60°C and the residual (silver impregnated silica) was activated by heating overnight (110 ± 2 °C) in the hot air oven to prepare Ag-silica powder. Ag-silica powder was cooled and kept in a dessicator in the dark for future use.

Argentation Chromatography Ag-Silica

The water-jacketed column (45 cm 50 mm i.d.) was half-filled with n-hexane, and Ag-silica (5 g) in n-hexane (5 mL) was poured into the column. Mixtures of fatty acid methyl esters – obtained after trans-esterification of oils (sunflower oil and olive oil) (5 g) (which was previously prepared in our lab.) – In n-hexane were loaded onto the chromatography column. The fatty acid methyl esters was eluted with three different organic solvents, first 100% n-hexane, second 3% acetone:n-hexane, third 5% acetone:n-hexane. The fractions were collected in fraction tubes (5 ml in tube), each being checked by TLC (TLC, 5 cm 20 cm). The fractions which contain fatty acid methyl ester were mixed then concentrated and purity of the concentrated methyl esters eluted with the most efficient method. The final product has been checked and validated by using GC-FID and GC-MS.

Gas Chromatography Mass Spectrum

The fatty acid composition from each type of oil was estimated by GC-FID and GC-MS. GC-instrument condition are: column: Elite 5 MS with dimension 30.0 m x 250 µm, oven temperature: initially held at 140 °C for 5 min, increased to 240 °C at 4 °C/min, and then held for 5 min, injector, transfer and source temperatures: 250 °C, 200 °C and 150 °C respectively, Carrier gas: helium and total scan time 50 min. The individual peaks of the gas chromatogram were analyzed by NIST, NBS and Wiley GC-MS library and the relative percentage of fatty acid esters was calculated from total ion chromatography by computerized integrator.

General Procedure for Preparation of Fatty Acid Hyrazides

Hydrazine hydrate (0.02 mol.) was added to separate fatty acid methyl esters (0.010 mol.) in absolute ethanol (20 mL). The reaction mixture was reflexed for 5h. After cooling, the solid separated was filtered and washed with water. The dried solid was crystallized from ethanol. The fatty acid hydrazides were characterized on the basis of spectral data which were in agreement with reported literature.

(9e,12e)-octadeca-9,12-dienehydrazide (linoleic acid hydrazide)

White solid; Yield= 70%; M.P. = 110 – 112 °C; IR (KBr), (v, cm-1): 3318 (NH in NH_2) , 3291 (NH in NH_2, 3223 (NH), 2955 (CH in HC=CH), 1629 (C=O), 2850–2919, (C-H aliphatic) ; ^{1}H NMR: δ 9.04 (1H s, NH), 5.35-5.39 (4H, m, in two CH=CH), 3.91 (2H s, NH_2), 2.78 2H (CH_2 in CH=CH-CH2-CH=CH), 2.05 (4H, m, CH_2 α to CH=CH), 1.66 (2H, m, CH_2 β to carbonyl group), 1.31 (14H, br.s, (7 (CH_2)), 0.86 (3H, t, CH_3). ^{13} C-NMR (400 MHz, $CDCl_3$): δ, ppm 174 (1C, C=O), 128-130 (4C, C=C), 34.56 (1C, C2), 31.5 (1C, C11), 29.32 (2C, C14, C8), 29.32 (1C, C16), 27.10 (5C, C4,C5,C6,C15), 25.47 (1C, C3), 25.37 (1C, C17), 13 (1C, C18).

(E)-octadec-9-enehydrazide (oleic acid hydrazide)

White solid; Yield= 78%; M.P. = 112 – 113 °C; IR (KBr), (v, cm-1): 3318 (NH in NH_2), 3292 (NH in NH_2), 3223 (NH), 3006 (CH in HC=CH), 1629 (C=O), 2850–2920, (C-H aliphatic) ; ^{1}H NMR: δ 6.7 (1H s, NH), 5.0-5.5 (2H, bro, in CH=CH), 3.90 (2H, s, NH_2), 2.15 (6H, m, two CH_2 α to CH=CH, CH_2 α to carbonyl group), 1.64 (2H, m, CH_2 β to carbonyl group), 1.25 (20 H, br.s, (10 (CH_2)), 0.88(3H, t, CH_3). ^{13}C-NMR (400 MHz, $CDCl_3$): δ, ppm 174 (1C, C=O), 129-130 (2C, C=C), 34.02 (1C, C2), 31.7 (2C, C8, C11), 29.32 (9C, C4, C5, C6, C7, C12, C13, C14, C15, C16), 29.00 (1C, C3), 25.4 (1C, C17), 13 (1C, C18).

Stearohydrazide (Stearic Acid Hydrazide)

White solid; Yield= 75%; M.P. = 112 – 113 °C; IR (KBr), (v, cm-1): 3318 (NH in NH_2), 3290 (NH in NH_2), 3201 (NH), 1629 (C=O), 2849–2956, (C-H aliphatic), 1535 (C-N); ^{1}H NMR: δ 6.71 (1H s, NH),

3.69 (2H, s, NH_2), 2.16 (2H, m, CH_2 α to group), 1.65 (2H, m, CH_2 β to carbonyl group), 1.27 (28 H, br.s, (14 (CH_2 aliphatic)), 0.88 (3H, t, CH_3). ^{13} C-NMR (400 MHz, $CDCl_3$): δ, ppm 174 (1C, C=O), 34.62 (1C, C2), 31.93 (1C, C16), 29.32 (12C, C4 – C15), 25.51 (1C, C3), 22.71 (1C, C17), 14 (1C, C18).

Dodecanehydrazide (lauric acid hydrazide)

White solid; Yield= 73%; M.P. = 112 – 113 °C; IR (KBr), (v, cm-1): 3316 (NH in NH_2), 3293 (NH in NH_2), 3201 (NH), 1629 (C=O), 2850–2956, (C-H aliphatic), 1536 (C-N) ; ^{1}H NMR: δ 6.74 (1H s, NH), 3.88 (2H, s, NH_2), 2.16 (2H, m, CH_2 α to group), 1.65 (2H, m, CH_2 β to carbonyl group), 1.27 (16 H, br.s, (8 (CH_2 aliphatic)), 0.88 (3H, t, CH_3). ^{13} C-NMR (400 MHz, $CDCl_3$): δ, ppm 174 (1C, C=O), 34.62 (1C, C2), 31.93 (1C, C16), 29.32 (12C, C4 – C15), 25.51 (1C, C3), 22.71 (1C, C17), 14 (1C, C18). ^{13} C-NMR (400 MHz, $CDCl_3$): 174 (C=O), 29.64 (CH2).

General Procedure For Preparation Of 1,3,4 Oxadiazole From Fatty Acid Hydrazides

Carbon disulphide (0.01 mol.) was added drop wise to the stirred solution of fatty acid hydrazide (0.01) and potassium hydroxide (0.015 mol.) in 30 mL ethanol at room temperature with stirring for two more hours to ensure the fatty acid hydrazide totally converted to its potassium salt (white precipitate). After obtained white precipitate, the mixture was heated under reflux (80 – 90 °C) until the evolution of H_2S ceased (using lead acetate paper). The reaction mixture was concentrated under reduced pressure, then cooled to room temperature. The reaction mixture was added to acid ice solution. The solid product was filtered, washed with cold water,dried, and recrystallized from ethanol. The products were identified by spectral data.

5-undecyl-1,3,4-oxadiazole-2(3h)-thione (lauric acid oxadiazole) **(L1)**

White solid; Yield= 80%; IR (KBr) (v, cm-1): 3201 (NH), 2918(CH-aliphatic), 1619 (C=N), 1469 (C-N), 1174(C=S), 1063 (C-O-C). ^{1}H NMR: δ ppm: 11.17 (1H s, NH), 2.71 (2H, m, CH_2 α to ring), 1.76 (2H, m, CH_2 β to ring), 1.37 (16 H, br.s, (8 ($CH_{2\ aliphatic}$)), 0.89 (3H, t, CH_3). ^{13} C-NMR (400 MHz, $CDCl_3$): δ, ppm 178.61 (1C, C13), 164.82 (1C, C1 cyc. N-**C**-O), 34.46 (1C, C2), 29.55 (1C,C10), 29.68 (6C, $C_{aliphatic}$), 25.70 (1C, C11), 25.55 (1C, C3) 14.09 (1C, C12).

5-heptadecyl-1,3,4-oxadiazole-2(3h)-thione (stearic acid oxadiazole) **(L_2)**

White solid; Yield= 80%; IR (KBr) (v, cm-1): 3143 (NH), 2917(CH-aliphatic), 1613 (C=N), 1469 (C-N), 1166 (C=S), 1072 (C-O-C). ^{1}H NMR: δ ppm: 10.89 (1H s, NH), 2.71 (2H, m, CH_2 α to ring), 1.76 (2H, m, CH_2 β to ring), 1.30 (28 H, br.s, (14 ($CH_{2\ aliphatic}$)), 0.901 (3H, t, CH_3). ^{13} C-NMR (400 MHz, $CDCl_3$): δ, ppm 178.66 (1C, C19), 164.79 (1C, C1 cyc. N-**C**-O), 31.91 (1C, C2), 29.68 (12C, $C_{aliphatic}$), 22.67 (1C, C3), 25.70 (1C, C17) 14.09 (1C, C18).

(E)-5-(Heptade-8-En-1-Yl)-1,3,4-Oxadiazole-2(3H)-Thione (Oleic Acid Oxadiazole) **(L3)**

White solid; Yield= 73%; IR (KBr) (v, cm-1): 3217 (NH), 2920(CH-aliphatic), 1612 (C=N), 1485 (C-N), 1165 (C=S), 1053 (C-O-C). ^{1}H NMR: δ ppm: 11.46 (1H s, NH), 5.35-5.34 (2H, m, in two CH=CH), 2.71 (2H, m, CH_2 α to ring), 2.31 (2H, m, CH_2 α to CH=CH), 2.03 (2H, m, CH_2 β to ring), 1.30 (20 H, br.s, (10 ($CH_{2\ aliphatic}$)), 0.86 (3H, t, CH_3). ^{13} C-NMR (400 MHz, $CDCl_3$): δ, ppm 178.66 (1C, C19), 164.81 (1C, C1 cyc. N-**C**-O), 129-130 (2C, C=C), 34.17 (2C, C8, C11), 29.24 (9C, $C_{aliphatic}$), 28.78 (1C, C3), 25.54 (1C, C17) 13.08 (1C, C18).

5((8e,11e)-heptadeca-8,11-dien-1-yl)-1,3,4-oxadiazole-2(3h)-thione (linoleic acid oxadiazole) **(L4)**

White solid; Yield= 78% IR (KBr) (v, cm-1):, 3204 (NH), 3008 (CH in C=C), 2921 (CH-aliphatic), 1623 (C=N), 1485 (C-N), 1164 (C=S), 1052 (C-O-C). ^{1}H NMR: δ ppm: 11.67 (1H s, NH), 5.30-5.39 (4H, m, in two CH=CH), 2.78 2H (CH2 in CH=CH-CH2-CH=CH), 2.36 (2H, m, CH_2 α to ring), 1.75 (2H, m, CH_2 α to CH=CH), 1.31 (16 H, br.s, (8 ($CH_{2\ aliphatic}$)), 0.86 (3H, t, CH_3). ^{13} C-NMR (400 MHz, $CDCl_3$): δ, ppm 178 (1C, C19), 164.86 (1C, C1 cyc. N-**C**-O), 127-130 (4C, C=C), 31.5 (1C, C11), 29.67.56 (1C, C2), 29.01 (8C, $C_{aliphatic}$), 28.9 (1C, C17), 25.6 (1C, C3), 13 (1C, C18).

General procedure for synthesis of met al complexes with oxadiazole ligands

M *et al* complexes were prepared by adding a stoichiometric amount of a $PtCl_2$ solution (0.025 mmol, 0.0660 g) in ethanol (10 cm^3) to ligand (0.05 mmol) (10 cm^3) in the same solvent in 1:2 molar ratio. The reaction mixture was heated with stirring at 40C for 1h. The result solid were filtered off, washed several time with methanol and dry under air. The products were identified by spectral data and element analysis [13].

$[Pt(L_1)_2]$ $3H_2O$ (Pt1)

Brown solid; Yield= 80%; IR (KBr) (v, cm-1): 3220 (NH), 2919 (CH-aliphatic), 1598 (C=N), 1459 (C-N), 1183 (C=S), 1035 (C-O-C), 421 (Pt-N), 398 (Pt-S), 316 (Pt-Cl). ^{1}H NMR: δ ppm: 2.83 (2H, m, CH_2 α to ring), 1.71 (, s, OH), 1.27 (16 H, br.s, (8 ($CH_{2\ aliphatic}$)), 0.90 (3H, t, CH_3). UV abs. (221, 321 nm). Mass spectrum (ESI) [M+] calculated= 761, found = 759.5 mass fragments: 102, 128, 390, 453, 610, 684.

[Pt(L$_2$)$_2$] H$_2$O$_2$EtOH (Pt$_2$)

Brown solid; Yield= 80%; IR (KBr) (v, cm-1): 2919 (CH-aliphatic), 1602 (C=N), 1463 (C-N), 1166 (C=S), 1091 (C-O-C). ^{1}H NMR: δ ppm, 2.67 (2H, m, CH_2 α to ring), 1.72 (s, OH),, 1.66 -1.20 (28 H, br.s, (14 ($CH_{2\ aliphatic}$)), 0.90 (3H, t, CH_3). UV abs. (260 nm). Mass spectrum (ESI) [M+] calculated= 981, found = 980.5 mass fragments: 102, 304, 403, 610, 684,760, 832, and 902.

[Pt(L$_3$)$_2$]H$_2$O2EtOH (Pt$_3$)

Brown solid; Yield= 78%; IR (KBr) (v, cm-1): 2918 (CH-aliphatic), 1598 (C=N), 1462 (C-N), 1189 (C=S), 1034 (C-O-C), 461 (Pt-S), 324 (Pt-Cl). ^{1}H NMR: δ ppm: 5.4-5.3 (4H, m, in two CH=CH), 2.83 2H (CH2 in CH=CH-CH2-CH=CH), 2.67 (2H, m, CH_2 α to ring), 2.02 (2H, m, CH_2 α to CH=CH), 1.98 (16 H, br.s, (8 ($CH_{2\ aliphatic}$)), 1.59 (s, OH), 0.88 (3H, t, CH_3). Mass spectrum (ESI) [M+] calculated= 979, found = 981.8 mass fragments: 223, 322, 610, 684,758, 832, 906. UV abs.

[Pt(L$_4$)] 4H$_2$O EtOH (Pt$_4$)

Brown solid; Yield= 78%; IR (KBr) (v, cm-1): 3010 (CH in C=C), 2921 (CH-aliphatic), 1601 (C=N), 1458 (C-N), 1188 (C=S), 1032 (C-O-C), 420 (Pt-S), 323 (Pt-Cl). (^{1}H NMR: δ ppm: 5.36-5.39 (4H, m, in two CH=CH), 2.85 2H (CH2 in CH=CH-CH2-CH=CH), 2.68 (2H, m, CH_2 α to ring), 2.07 (2H, m, CH_2 α to CH=CH), 1.27 (16 H, br.s, (8 ($CH_{2\ aliphatic}$)), 1.60 (s, OH), 0.88 (3H, t, CH_3). Mass spectrum (ESI) [M+] calculated= 983.39, found = 984.3.mass fragments: 140, 203, 453,684,758, 850, 906.

Antioxidant screening in vitro (dpph assay)

Total antioxidant scavenging for complexes were measured according to reference 37 with simple modification. Briefly 1mL of various concentration (25, 50, 75, 100 µg/mL) of samples and ascorbic acid (as reference) in dichloromethane were added to 4 ml of DPPH solution (80µg/mL). After 30 min incubation at room temperature, the absorbance was measured at 517 nm. The percentage of inhibition scavenging was assessed using the following equation.

$$\% \text{ of scavenging} = \frac{A\ control - A\ sample}{A\ control} * 100 \quad \text{equation 1}$$

where A control is the absorbance of the control reaction (containing all reagents except the test compound) and A sample is the absorbance of the test compound. Tests were carried at in triplicate.

Cytotoxicity Assay in Vitro

The A549 and MCF7 cell lines were maintained in cell culture medium at 37 °C and 5% CO_2 atmosphere. The Pt(II) complexes were individually dissolved in DMSO using ultrasonic bath to prepare 1mg/mL stock solutions. Each solution was diluted to the desired concentration (500, 250, 125, 62.5 and 15.65 µg/mL) in cell culture medium. The cell toxicity investigation was conducted using the 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) assay. Briefly, the cells were seeded in plates and put in an incubator for 24 h. All complexes at various concentrations were individually dispensed in the wells and incubated for 48 h. MTT solution (10 L) was added to each well and incubated for 4 h. The culture medium was removed and 100 µL of DMSO was added to each well. An enzyme labeling instrument was used to measure the absorbance at 570 nm with a reference filter of 630 nm through the Elisarid machine. IC_{50} values were calculated. All the tests were repeated at least three times. [14]

Result and Discussion

Separation fatty acid methyl ester by argntation chromatography:

In this method, the fatty acid methyl esters (FAMEs) are separated according to the polarity of the fatty acids, which depend on the number of double bonds they contain. High polarity fatty acids have more double bonds. For example linoleic acid is more polar then oleic acid because of the number of double bonds. Ag-Si column chromatography has been used for separation of FAMEs based on the number of double bonds. In this method, activated silver impregnated silica is used as a stationary phase. When the mixture of the FAME passed through the column, the silver (Ag) ion forms polar complexes with the fatty acids through the double bonds. The FAMEs are selectively eluted by changing the polarity of the

eluent solvent mixture. Acetone-hexane mixture are used as eluent and the polarity of this eluent increased by increased acetone content. The ratio of the acetone-hexane in the eluent is shown in the Table-1:

Table-1: Solvent system for elution.

	Hexane (V)	Acetone (V)	% Hexane	% Acetone
A	100	0	100	0
B	99	1	99	1
C	98	2	98	2
D	95	5	95	5

The result shows that the saturated FAMEs eluted with 100% of hexane and oleic acid (monounsaturated fatty acid) are separated when the polarity of the solvent increased by 2% of acetone and linoleic acid are eluted by more polar eluent (5% acetone). The fatty acid profile of each fraction obtained by Ag-Si column chromatography are confirmed by TLC and GC-MS.

All column fractions eluted with Solvent C and D are checked by TLC to detect which fractions contain FAME (Fig 1). This Fig indicates that the fraction 6 – 29 of sunflower contain FAME and the fraction 5 – 40 of olive contain FAME. The mixture of the fractions applied to GC-FID to check purity.

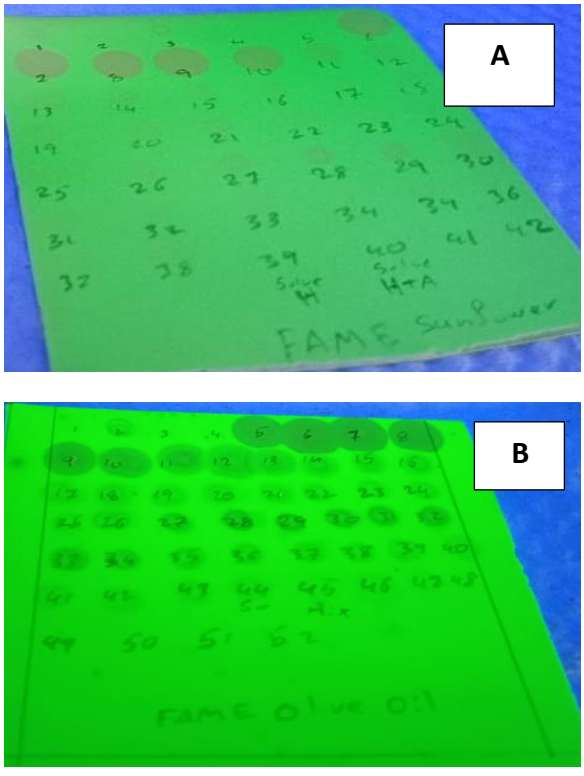


Fig. 1: TLC of all fractions of A: Sunflower FAME and B: olive oil FAME.

The GC results show that the fractions of sunflower oil which eluted by solvent D contain 95.8% of linoleic acid (see Fig 2) and the fractions of olive oil which eluted by solvent C contain 97.9% of oleic acid (see Fig 3). The fatty acid profiles of each fraction are shown in the table 2, the two fractions were used for preparing the further compounds.

Characterizations of Fatty Acid Hydrazide (Fah)

FAHs were prepared by the reaction between FAME such as methyl Laurate, Methyl Stearate, Methyl olate and methyl Linolate with hydrazine hydrate in boiling ethanol. This reaction condition gave yields ≥ 70. The structures of FAHs were confirmed by using spectral data, IR, ^{1}H-NMR and ^{13}C-NMR spectra shown the FAHs are successfully produced from FAME.

IR spectra of FAHs showed the absorption band at (3316 and 3293 for Lauric acid hydrazide, 3318, 3290 for Stearic acid hydrazide, 3318, 3292 for Oleic acid hydrazide and 3318 and 3291 for Linoleic acid hydrazide) which can be assigned to NH_2 groups. The absorption band of (NH) starching is at 3201 - 3223 cm^{-1}. The C-N stretching hydrazide shows absorption band at 1460 cm^{-1}. Additionally, the (C=O) in FAHs is at 1629 cm^{-1}. IR spectral shows the FAHs are successfully produced because both band of NH_2 and (NH) is not found in FAME. All IR spectra data for FAHs and FAMEs are shown in the table 3. ^{1}H-NMR and ^{13}C-NMR were used to confirm the structure FAH. In ^{1}H-NMR spectrum of FAH, the signals at 9.04 - 6.7 for NH and the two protons of NH_2 in the FAHs were observed in the region (3.6–3.9 ppm). Moreover, in ^{13}C-NMR carbon for (C=O) is observed in the region (174 ppm). The NMR result show that the hydrazine hydrate successfully react with FAME to form FAHs. The all ^{1}H-NMR and ^{13}C-NMR data are shown in the table 3.

Characterizations of Fatty Acid Oxadiazole

The oxadiazoles were prepared by two steps, first the nucleophile addition of carbon disulphide in alcoholic (KOH) solution to produce potassium salt from fatty acid hydrazides, second cyclization of potassium salt by HCl at 0°C. The structures of the oxadiazoles have been confirmed by IR, ^{1}H-NMR and ^{13}C-NMR spectra that are shown in the table 4 and table 5 respectively.

Table-2: Fatty acid profiles before and after using Ag-Si chromatography.

Fatty acid	Sunflower oil		Olive oil	
	%FA[a]	%FA[bD]	%FA[a]	%FA[bB]
Palmitic acid	11.8	0.12	5.13	0.24
Linoleic acid	80.99	95.8	----	----
Oleic acid	---	---	89	97.93
Stearic acid	5.03	3.55	2.8	0.15
Cis-13-Eicosenoic acid	0.38	---	1.3	---
Other	1.8	0.52	1.77	1.60

% Yield	47.1	43.3

a:Fatty acid composition before fractionation by Ag-Si chromatography (This work previously published as conference paper), b:Fatty acid composition after fractionation by Ag-Si chromatography C: eluted solvent is (2%acetone and 98%hexan) D: eluted solvent is (5%acetone and 95%hexan)

Table-3: IR and NMR spectrum of Fatty acid hydrazides.

	NH		NH_2		C=O	
	IR / ν cm^{-1}	^{1}H-NMR	IR / ν cm^{-1}	^{1}H-NMR	IR / ν cm^{-1}	^{13}C-NMR
Lauric acid hydrazide	**3201**	**6.74**	**3316**	**3.88**	**1629**	**174**
Staric acid hydrazide	**3201**	**6.71**	**3318**	**3.96**	**1629**	**174**
Oleic acid hydrazide	**3223**	**6.7**	**3318**	**390**	**1629**	**174**
Linoleic acid hydrazide	**3318**	**9.04**	**3318**	**3.91**	**1629**	**174**

Table-4: IR spectra of met al complexes with Oxadiazole ligands

Compounds	OH (ν cm^{-1})	NH (ν cm^{-1})	C=N (ν cm^{-1})	C-N (ν cm^{-1})	C=S (ν cm^{-1})	M-N (ν cm^{-1})	M-S (ν cm^{-1})
L_1	-----	3201	1619	1469	1177	-----	-----
L_2	-----	3143	1613	1469	1166	-----	-----
L_3	-----	3217	1620	1485	1165	-----	-----
L_4	-----	3204	1623	1485	1164	-----	-----
Pt1	**3492**	-----	**1597**	**1460**	**1186**	**501**	**420**
Pt2	**3463**	-----	**1602**	**1461**	**1189**	**508**	**421**
Pt3	**3463**	-----	**1598**	**1462**	**1189**	**461**	**352**
Pt4	**3485**	-----	**1601**	**1458**	**1187**	**503**	**420**

Table-5: ^{1}H-NMR data of met al complexes with Oxadiazole ligands.

Compounds	^{1}H-NMR					^{13}C-NMR				
	NH (1H)	CH=CH (2 or 4 H)	OH in water and EtOH	CH_3 (3H)	CH_2 aliphatic	C=S (1C)	C=N (1C)	C=C (2 or 4 C)	CH_2 α to C=O	CH_2 aliphatic
L_1	11.17	-----	-----	0.89	1.37	178.61	164.82	-----	31.93	29.27
L_2	10.89	-----	-----	0.90	1.30	178.66	164.79	-----	31.90	29.68
L_3	11.46	5.34-5.35	-----	0.86	1.30	178.66	164.81	129-130	31.91	29.24
L_4	11.67	5.30-5.39	-----	0.86	1.37	178	164.86	127-130	31.51	29.01
Pt1	-----	-----		0.9	1.27	-----	-----	-----	-----	-----
Pt2	-----	-----	1.71	0.90	1.27	-----	-----	-----	-----	-----
Pt3	-----	5.36	1.59	0.88	1.98	-----	-----	-----	-----	-----
Pt4	-----	5.36	1.60	0.88	1.27	-----	-----	-----	-----	-----

Table-6: The physical data of metal complexes with Oxadiazole ligands.

Compound	Colour	Yield	Molecular weight (g/mol)	Suggestion formula
Pt1	**brown**	**70**	**759.93**	**$[Pt(L_1)_2].3H_2O$**
Pt2	**brown**	**69**	**984.36**	**$[Pt(L_2)_2].H_2O$ 2EtOH**
Pt3	**brown**	**63**	**980.33**	**$[Pt(L_3)_2].H_2O$ 2EtOH**
Pt4	**brown**	**71**	**980.3**	**$[Pt(L_4)_2].4H_2O$ EtOH**

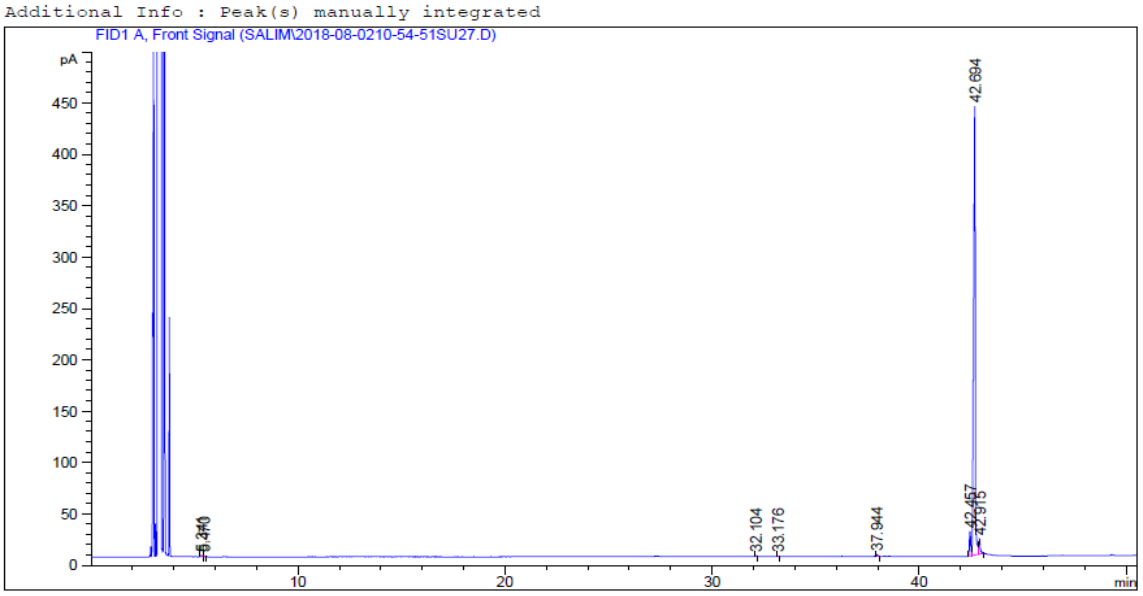


Fig. 2: GC-FID chromatogram of Sunflower FAME Fractions.

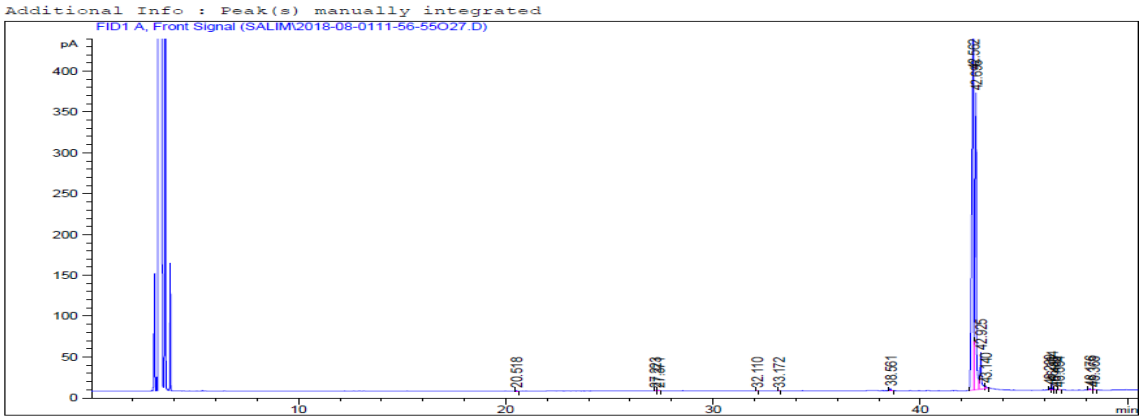
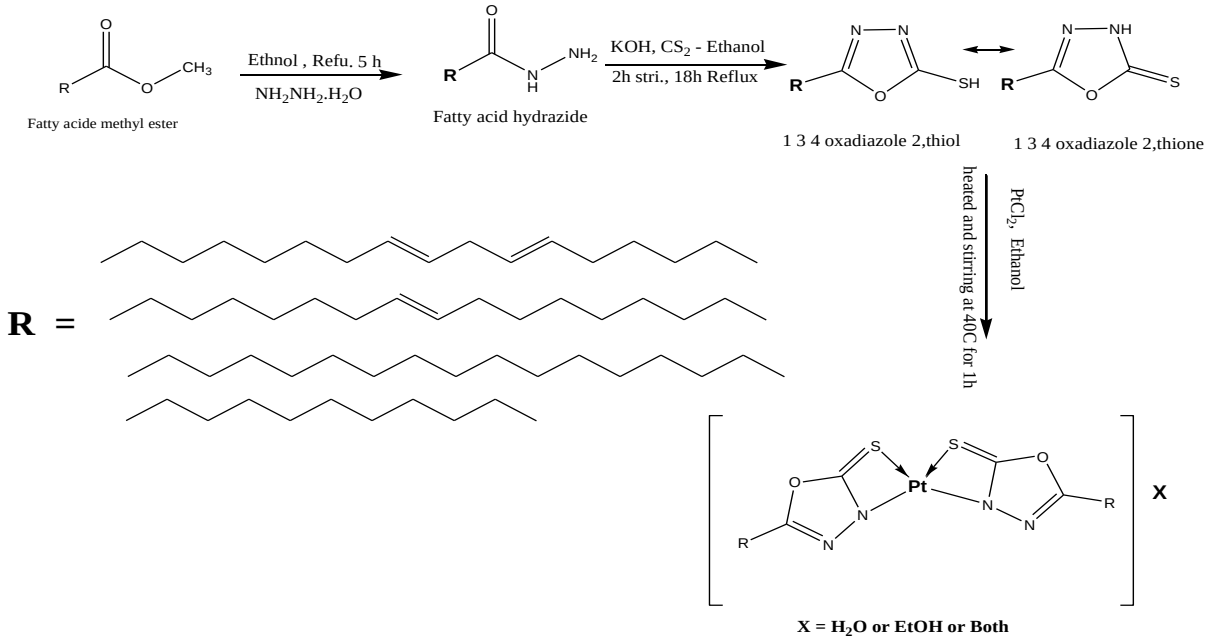


Fig. 3: GC-FID chromatogram of olive FAME Fractions.



Scheme-1: Synthetic pathway for platinum complexes with oxadiazole.

In IR spectral, the appearance of peaks at 1612 – 1623 cm^{-1} (1619 cm^{-1} for Lauric acid Oxadiazole, 1613 cm^{-1} for Staric acid Oxadiazole, 1620 cm^{-1} for Oleic acid Oxadiazole, 1623 cm^{-1} for Linoleic acid Oxadiazole) for C=N group and peak at 1485 – 1513 cm^{-1} (1469 cm^{-1} for Lauric acid Oxadiazole, 1469 cm^{-1} for Stearic acid Oxadiazole, 1485 cm^{-1} for Oleic acid Oxadiazole, 1485 cm^{-1} for Linoleic acid Oxadiazole) for C – N group are proved that the oxadiazole ring has formed. There are no peaks observed around 2550 – 2600 cm^{-1} for thiol group and the peak at 1164 – 1177 cm^{-1} (1177 cm^{-1} for Lauric acid Oxadiazole, 1166 cm^{-1} for Stearic acid Oxadiazole, 1165 cm^{-1} for Oleic acid Oxadiazole, 1164 cm^{-1} for linoleic acid Oxadiazole) for C=S prove that the oxadiazole ring has thione form and it has N-H group, this N-H group shows the peak at 3143 – 3217 cm-1 (3201 cm^{-1} for Lauric acid Oxadiazole, 3143 cm^{-1} for Stearic acid Oxadiazole, 3217 cm^{-1} for Oleic acid Oxadiazole, 3204 cm^{-1} for linoleic acid Oxadiazole). This result shows that oxadiazole is not as thiol isomer. Furthermore, the N-H groups has signal in ^{1}H-NMR at 10.89 – 11.67 ppm (11.17 for Lauric acid Oxadiazole, 10.89 for Stearic acid Oxadiazole, 11.46 for Oleic acid Oxadiazole, 11.67 for Linoleic acid Oxadiazole). The signal of NH_2 is disappear this prove that the oxadiazole ring is formed. The ^{13}C-NMR shows two peaks at 178

ppm for (cyc. O-C=S), and 164 ppm for (cyc. O-C=N). This two peaks related to carbons in oxadiazole ring.

Characterizations Met al complexes with oxadiazole ligands

1,3,4-Oxadiazole-2-thione ligands (L1-L4) contain NH and C=S groups as a coordination sites and behaves as bidentate ligand. The ligands react with metal ion to form mononuclear complexes (Scheme 1). All complexes are brown colour which differs from the ligands. The structure of the complexes have been confirmed by analytical data and spectroscopic studies. The physical data of metal complexes are shown in Table-6.

IR, Nmr And Mass Spectrum

Oxadiazole-2-thione ligands contain C=N and C=S groups as a coordination sites and may behave as a monobasic monodentate ligand. The ligands react with Pt ion to form mononuclear complexes. All complexes are stable and brown colour. The structure of the complexes have been confirmed by analytical data and spectroscopic studies.

Various absorption bands have been detected on the IR spectrum of metal complexes. Some of the bands are shifted to low or high frequency and some bands present on the complexes but absent on the ligands. This phenomenon indicated that the ligands are coordinated with metal. Characteristic infrared (IR) spectral band of 1,3,4-oxadiazole-2-thione complexes are shown in Table-5. In the all complexes, the disappearance the (NH) bands at (3141-3217) cm^{-1} due to de-protonation of (NH) group and then coordinated with metal as C-N group and the band for (C-N) was shifted to lower frequency from the ligand by (8) cm^{-1} at (1460-1461) cm^{-1} for Pt1 and Pt2, and to higher frequency from the ligand by (16) cm^{-1}) at (1458 -1469) cm^{-1} for Pt3 and Pt4. As well as in the all complexes, the band for (C=N) was found to be at lower frequency from the ligand by (11-21) cm-1 at (1597 – 1601) cm^{-1}. This is indicating that the new bond is formed between nitrogen atom and platinum and palladium met als (M-N). In addition, the new band between (461– 580) cm^{-1} supported that the (M-N) is formed. An absorption band of (C=S) shifted to high frequency by (10 – 15) cm^{-1} at (1183– 1189 $cm^{-1)}$ due to coordination through the S atom in the 1, 3, 4-oxadiazol-2-thione ligands. This is indicating that the new bond is formed between sulphur atom and platinum and Palladium met als (M-S). In addition, the new band between (487– 352) cm^{-1} supported that the (M-S) is formed. Abroad band in the metal complexes spectra at (3452-3497) cm^{-1} indicated the present OH group in water and/or ethanol molecular with complexes. The band for (C=N) was found to be at lower frequency from the ligand by (11-21) cm-1 (1597– 1601 cm^{-1}). The IR spectra of complexes are shown in Fig. 4.

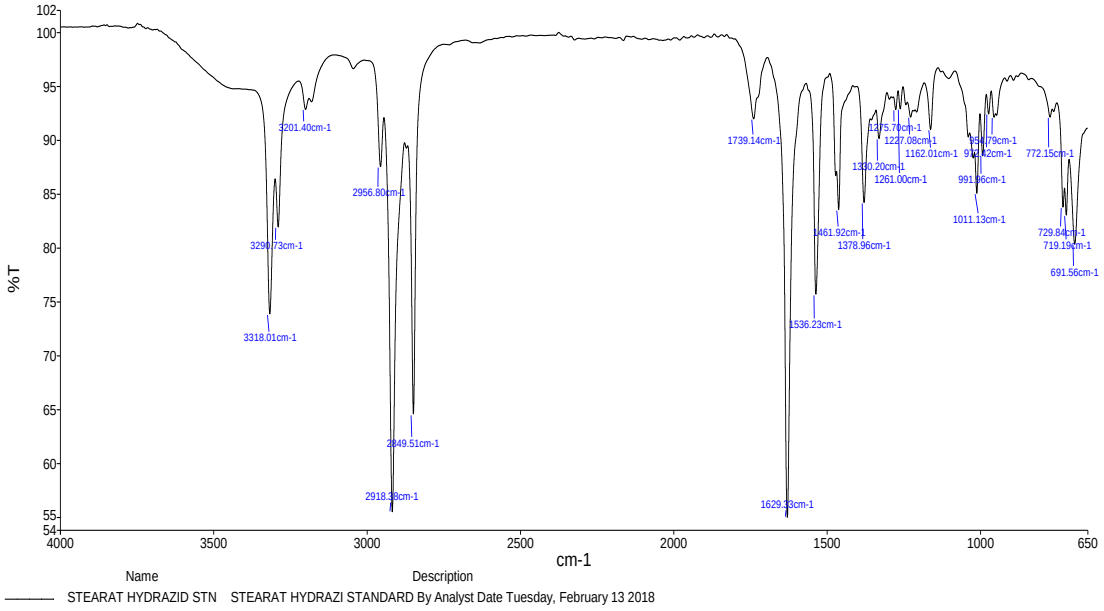


Fig. 4: IR spectrum of Staric acid hydrazide.

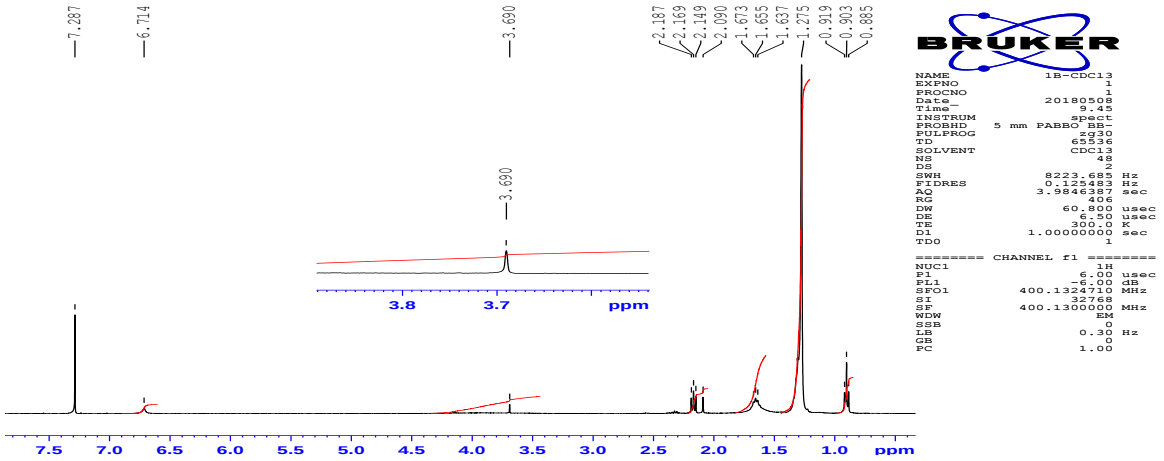


Fig. 5: [1]H-NMR spectrum of Staric acid hydrazide.

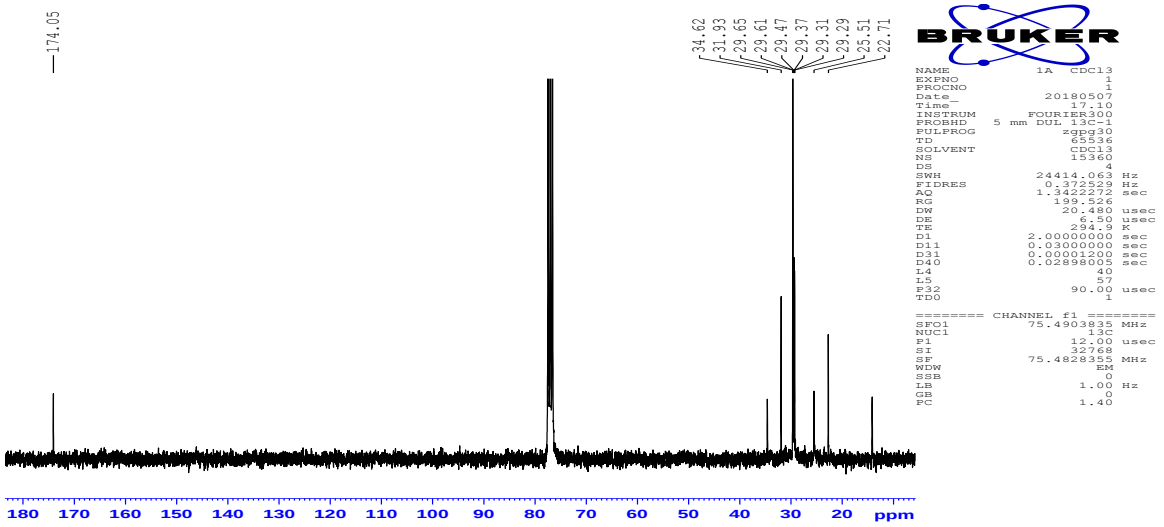


Fig. 6: [1]H-NMR spectrum of Stearic acid hydrazide.

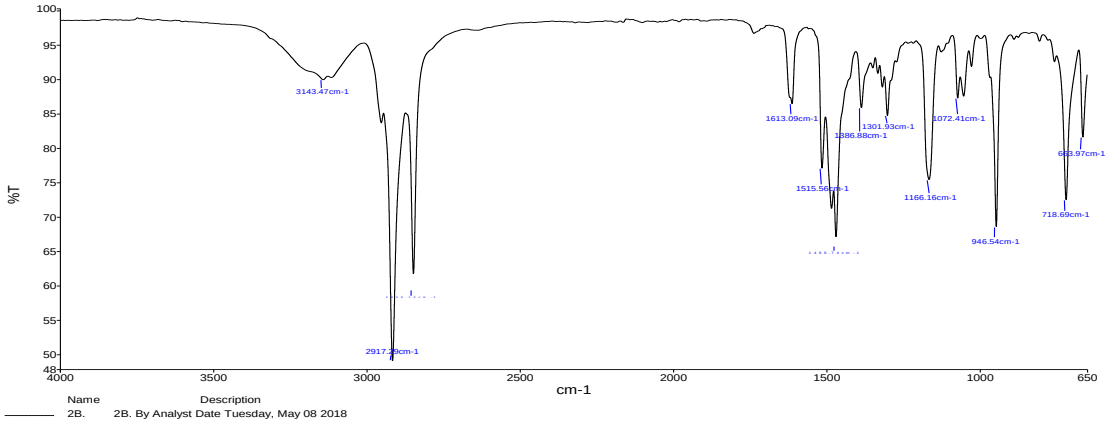


Fig. 7: IR spectrum of Stearic acid Oxadiazole.

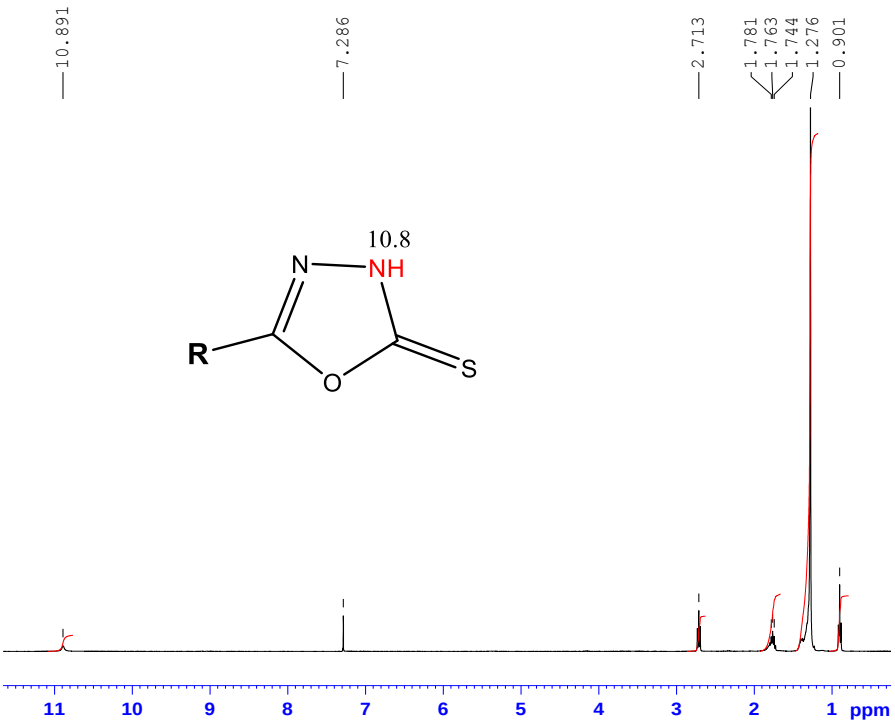


Fig. 8: [1]H-NMR spectrum of Stearic acid Oxadiazole.

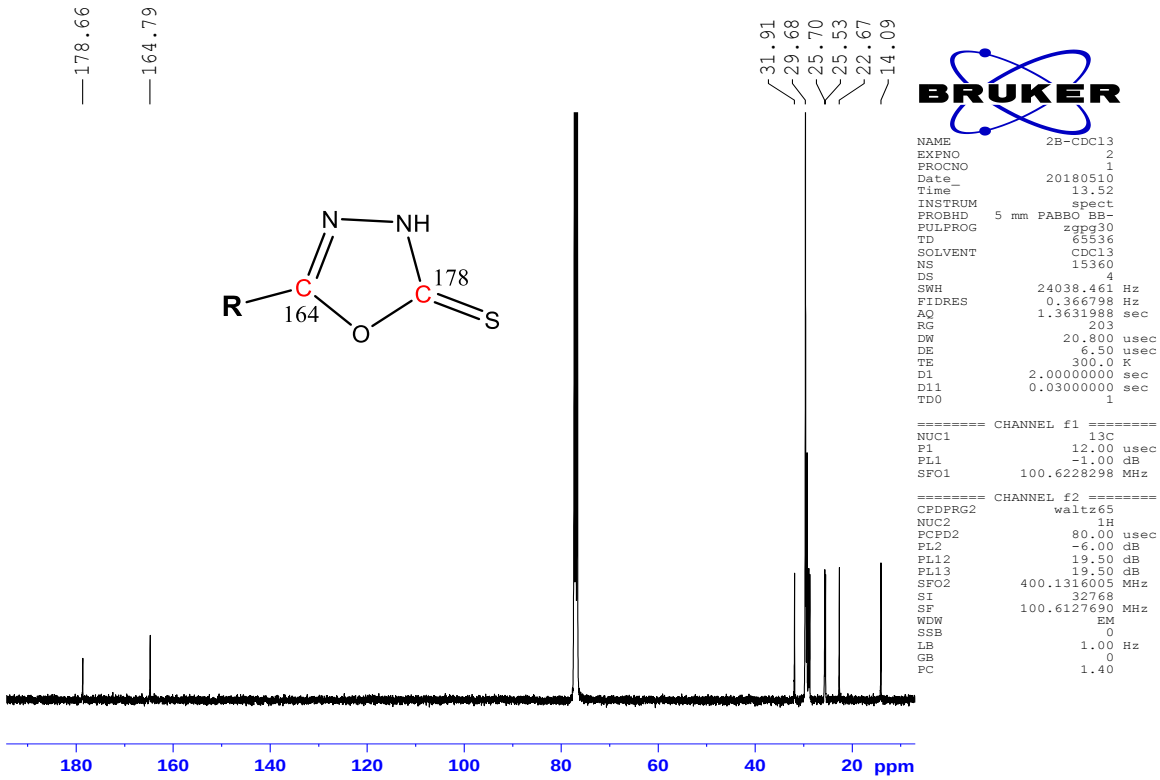


Fig. 9: [13]C-NMR spectrum of Stearic acid Oxadiazole.

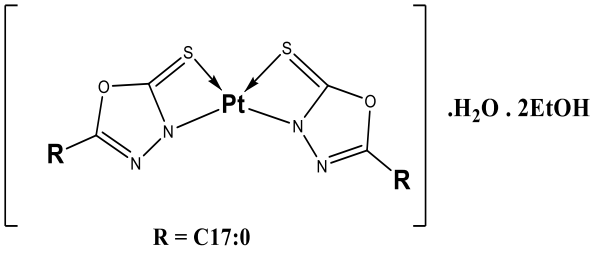


Fig. 10: Proposed structure of Pt_2.

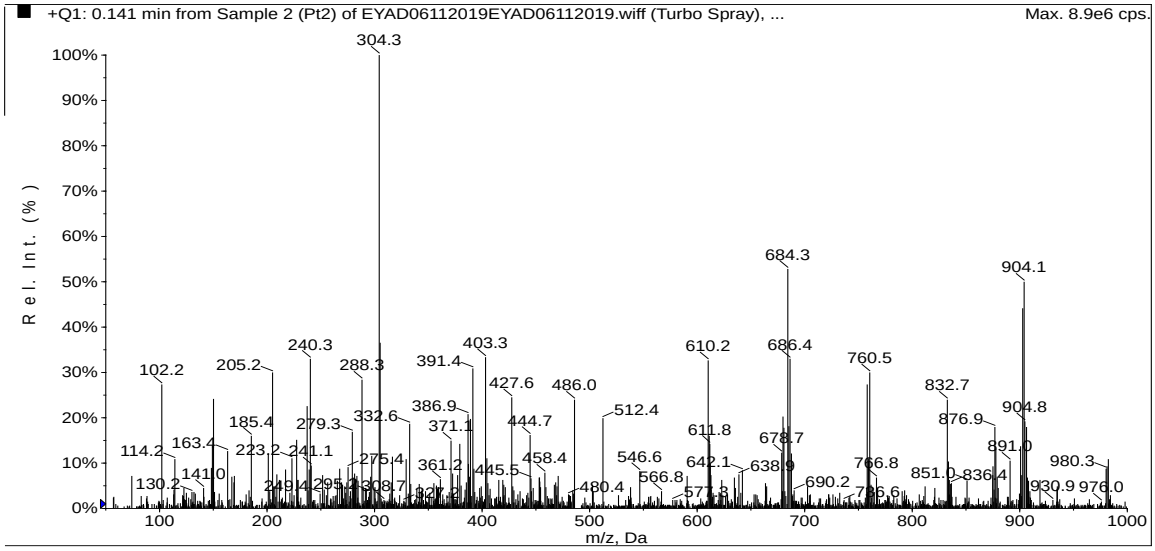


Fig. 11: Mass Spectrum of Pt_2.

In ^{1}H-NMR spectral of Platinum with 1,3,4--oxadiazol-2-thione complexes were recorded in CD_2Cl_2. All the protons were found in their expected regions. All of the aliphatic CH protons of ligands downshifted by 0.1 – 0.8 ppm due to coordination with the metal. One proton of NH present in the spectrum of the ligands at (10 -11.5) ppm but disappeared in the spectra of the complexes which were evident de protonation of (NH) group and coordinated through the nitrogen atom. The signal at (1.60-1.71) ppm due to the OH group in water and at (3.6-4.2) ppm in solvent with complexes. ^{1}H-NMR data of Platinum complexes with 1.3,4-Oxadiazole ligands are shown in Table-5.

To find the molecular ion of prepared complexes, the electron impact mass spectrometry (MS-EI) is used and mass spectrum of Platinum complexes showed the molecular ion peak at m/z= 759, 984.3, 981.8 and 980.3 m/z for Pt1, Pt2, Pt3 and Pt4 complexes respectively. Base on the molecular ions, can be expected that two mole of 1, 3, 4-oxadiazole ligands are reacted with Pt (II) met als. According the molecular mass of complexes can be expected that most of the complexes have water or ethanol in the out of the coordination sphere. The mass spectrum of Pt2 shows as a representative example. The molecular peak of Pt2 is 984.3 m/z in mass spectra. The molecular weight of Pt2 with two molecular of L_2 is 876.22 m/z. According to mass spectra the Pt2 may contain one molecular of water and two molecules of ethanol. The mass spectrum of Pt2 shows a molecular ion peak at 984.3 m/z and the molecular mass of Pt2 with two molecules of L_2 one molecular of water and two molecules of ethanol is equal to 984.36 g/mol (see proposed structure of Pt2 in Fig 10). The most important fragments of Pt2 are m/z= 906.7, 884.3, 833.0, 758.0, 140.7 and 102. The spectrum of this complex shows a fragment peak at 906.7 after loss ethanol, water and CH_3. In the mass spectrum of this complex shows another peaks at 833.0 after loss Ethanol and C_2H_5 and at 610.3 after loss C_5H_{11}. The base peak of this complex is 140.7 due to $C_7H_{12}N_2O$ and the peak at 102 m/z due to $C_2H_2N_2OS$. Mass spectrum of Pt2 is shown in Fig. 11. The Fig 12: represents the fragmentation pathways of the Pt2 complex as a representative example.

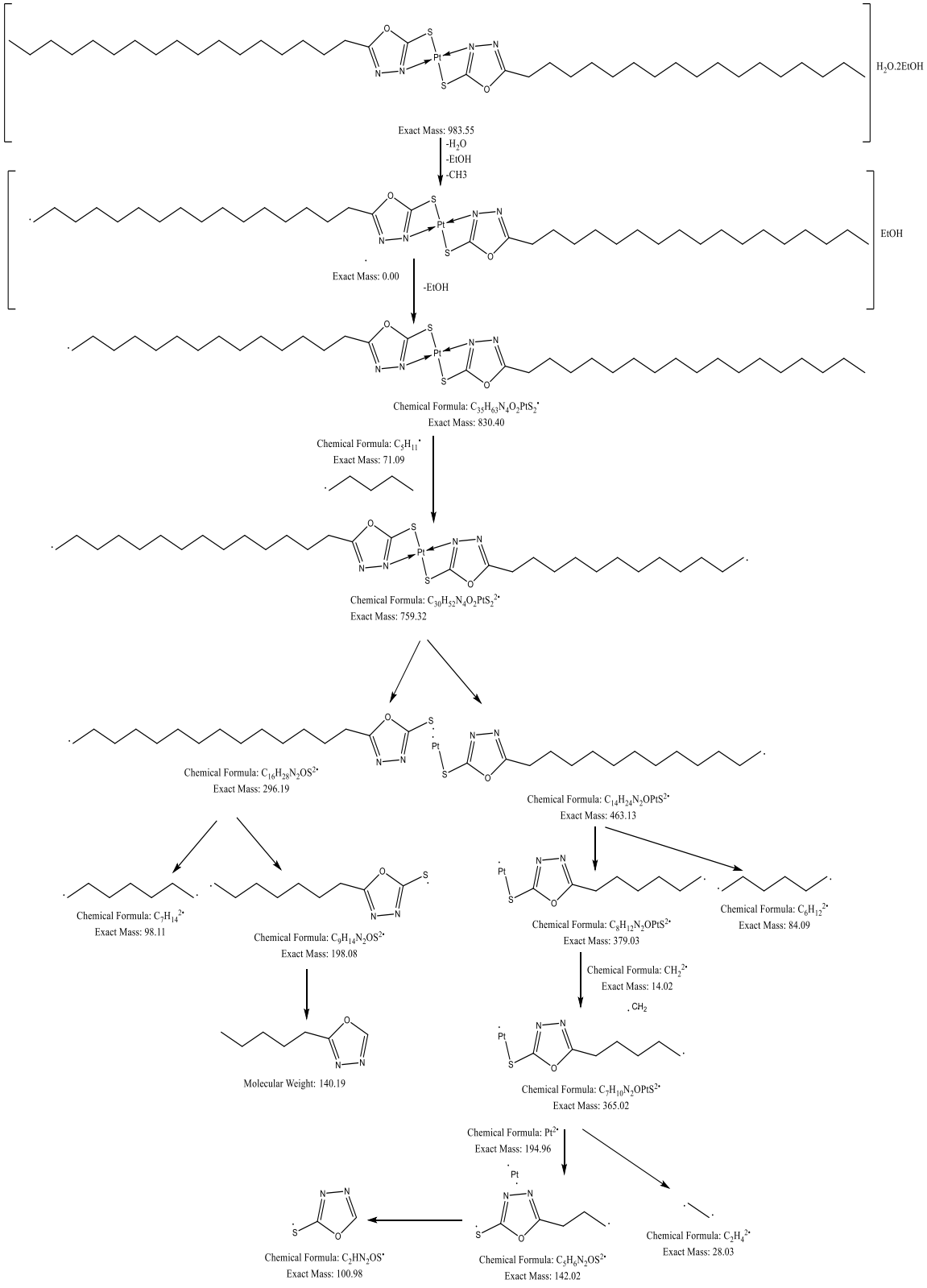


Fig. 12: fragmentation pathways of the Pt2 complex.

Table-7: Electronic spectral and conductivity of ligand and Pt (II) complexes

Name	Conductivity µs	Band absorption		
		nm	cm^{-1}	Assignment transition
L_1	-----	252	39682.54	π-π*
L_2	-----	270	37037.04	π-π*
L_3	-----	256	39062.50	π-π*
L_4	-----	250	40000.00	π-π*
Pt1	2.3	232	43103.45	π-π*
		321	31152.65	$^1A_1g \rightarrow {}^1Eg$
Pt2	0.39	230	43478.26	π-π*
		264	37878.79	$^1A_1g \rightarrow {}^1Eg$
		322	31055.90	MLCT
Pt3	0.15	250	40000.00	π-π*
		322	31055.90	$^1A_1g \rightarrow {}^1Eg$
		390	25641.03	MLCT
Pt4	0.39	256	39062.50	π-π*
		320	31250.00	$^1A_1g \rightarrow {}^1Eg$
		383	26109.66	MLCT

Table-8: Percentage radical scavenging metal complexes with $IC_{50.}$

Conc.	% scavenging				
µg/mL	Pt1	Pt2	Pt3	Pt4	VC
25	5.36	39.83	48.08	14.11	34.71
50	58.92	45.32	52.43	31.21	50.44
75	52.43	67.79	69.16	68.79	64.79
100	76.65	74.66	73.28	77.28	89.76
IC_{50}	56.06	48.91	31.79	45.21	48.68

Electronic Spectral and Conductivity

The electronic spectra of Pt-complexes displayed three bands while the free ligand showed one band. The band for the free ligands at 250 – 270 nm are attributed to intra-ligand π-π* transitions associated with the C=N or C=S function groups in the oxadiazole ligands. The Pt-Oxadiazole complexes showed the three absorption bands except Pt1. First band as a ligands contributed to π-π* transition and second band at (264- 320 nm) which assigned $^1A_1g \rightarrow {}^1Eg$ transition. The third band at (322 – 390 nm) contribute the MLCT. The result of electronic spectra indication the complexes are square planar geometry around the central metal. The electronic spectra data of ligands and metal complexes are illustrated at Table 7.

The conductivity of Pt-complexes were measured in DCM (10^{-3} M) and all complexes showed a small value of conductivity (Table-7). This result indicated that the all complexes are non-ionic in nature and non-electrolyte and the both Chlorides are located in the coordination sphere except Pt1

Antioxidant Screening In Vitro (Dpph Assay)

Antioxidant activity of oxadiazole platinum complexes has been measured by DPPH assay. The DPPH is a stable free radical. The antioxidant power of Pt-oxadiazole complexes is compared with standard ascorbic acid. The DPPH radical scavenging is expressed as IC_{50}. The compound with lower IC_{50} shows more antioxidant potential. The IC_{50} and % scavenging with different concentration of Pt-complexes are shown in Table-8 and Fig. 13 and. The Fig 13 shown that the % scavenging free radical increased with increased concentration of Pt-complexes. Pt3 and Pt4 are shown higher antioxidant activity than standard ascorbic acid. Pt3 has shown highest antioxidant activity while the Pt1 have lowest antioxidant activity. The result shows the oxadizoel with unsaturated fatty acid as a ligands have higher antioxidant activity than oxadiazole with saturated fatty acid ligand

Anticancer Cell Line (Mtt Assay)

In vitro cytotoxicity of the Pt-complexes was determined by MTT assay against two different human cancer cell line (MCF7 human breast adenocarcinoma and A549 human lung cancer cell line). This assay is colorimetric assay to determine the cellular growth. The mitochondrial dehydrogenase enzyme of living cells tetrazalium yellow MTT dye convert to purple colour insoluble formazan. This formazan is dissolved in suitable solvent to measure the absorbance at certain wavelength. The intensity of formazan is proportional with living cancer cell. If the drug can cause the cell death, the intensity of formazan is decreased. The curve of dose-depend effect of Pt-complexes on cell viability of A549 and MFC7 human cell line are shown in Fig 14 and 15 receptively.

The anticancer activity of complexes are illustrated as IC_{50} and result summarized in Fig 16. The result shows that the anti-tumour activity of complexes increased by increases concentration of Pt-complex. In general, the both MCF7 and A549 cell line are sensitive to all the tested compounds. The Pt3

exhibited high anti-tumour activity (showing lowest IC_{50}) against both MCF7 and A549 cell line. Base on QSAR, it can be concluded that the anticancer activity of Pt- complexes are depend on the degree of unsaturated of the fatty acid chain. The anticancer activity of Pt-complexes which contain unsaturated fatty acid are higher than Pt-complexes which contain saturated fatty acid. According to the IC_{50} values of each complexes on the both cell lines, the Pt4 and Pt2 are most active on the A549 cell line and important for human lung cancer, while the Pt4 have activity on MCF7 cell line and is important for human breast cancer.

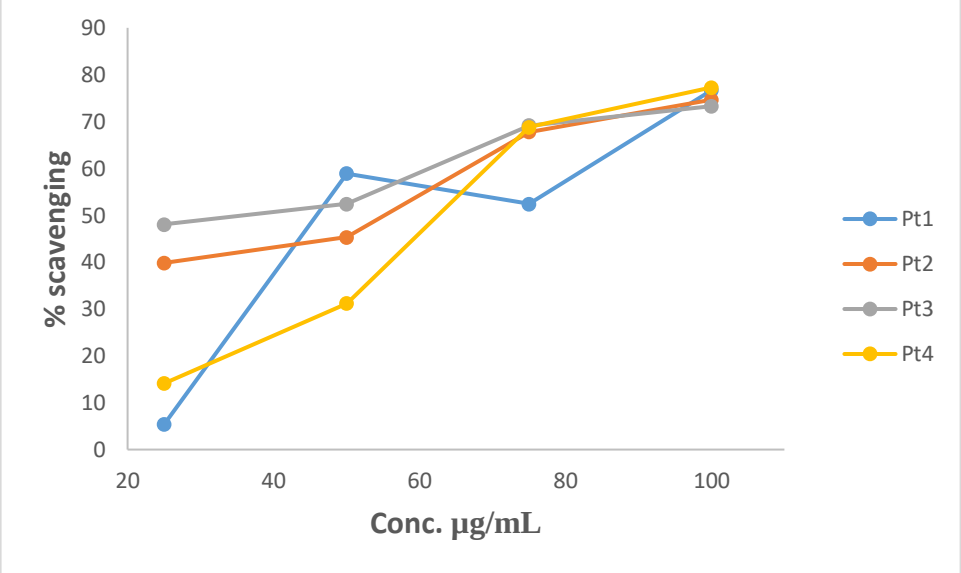


Fig. 13: Percentage radical scavenging metal complexes with IC_{50}

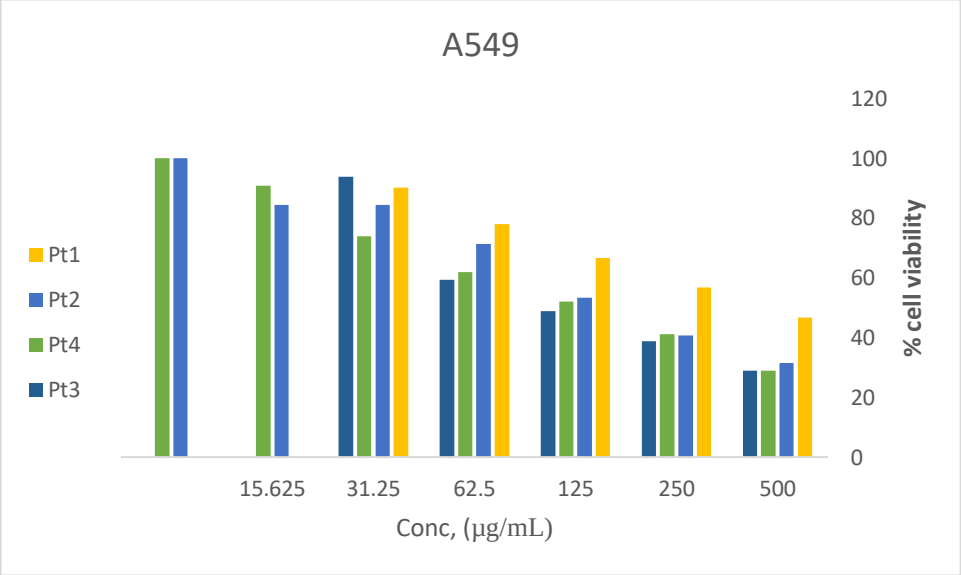


Fig. 14: dose-depend effect of Pt-complexes on cell viability of A549 human cell line.

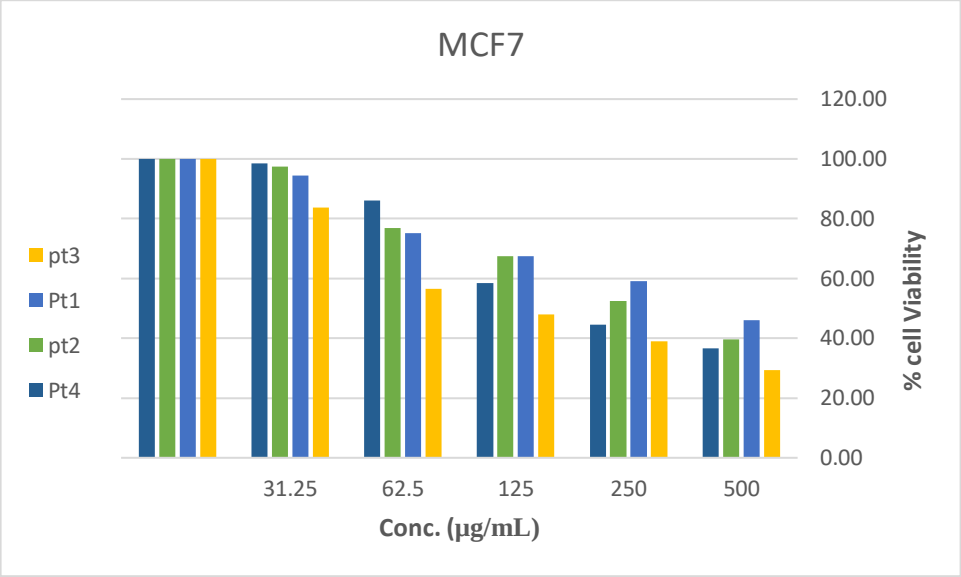


Fig. 15: dose-depend effect of Pt-complexes on cell viability of MFC7 human cell line.

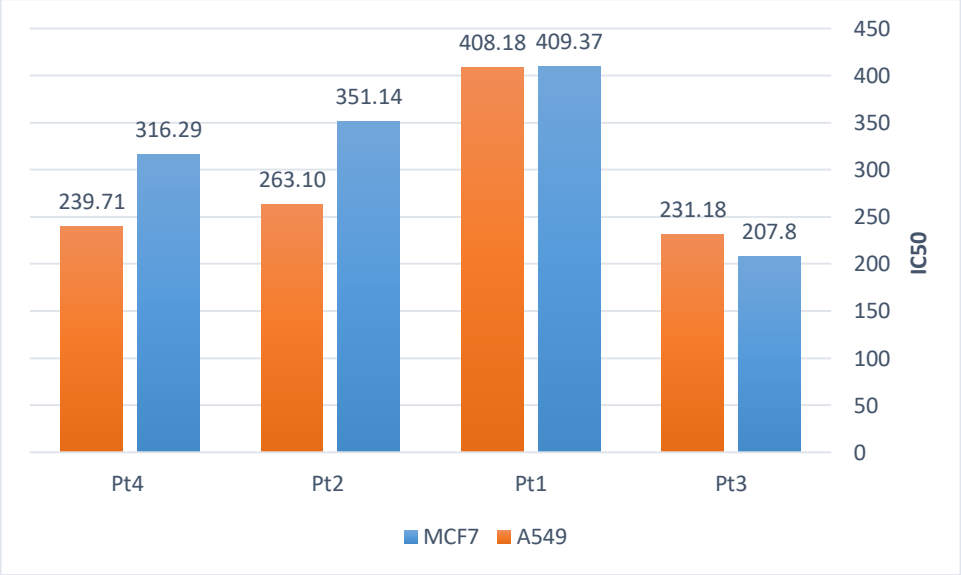


Fig. 16: IC_{50} of platinum complexes against MCF7 and A549 cell lines.

Conclusion

The platinum complexes with differ type of ligand have many biological activity**.** In the present study, platinum complexes of 1, 3, 4-oxadiazole are synthesized and antioxidant activity of complexes are measured by DPPH assay. The in vitro cytotoxicity of the synthesized complexes measured against two human cancer cell line by MTT assay. The both MCF7 and A549 cell line are sensitive to all the tested compounds. The Pt4 and Pt2 are most active on the A549 cell line and important for human lung cancer, while the Pt4 have activity on MCF7 cell line and is important for human breast cancer.

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