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Bioinorganic Applications of Some Thiamine Cl HCl Complexes

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ABSTRACT

The effect of thiamine Cl HCl and its synthesized VO^{2+}, Fe(III) and Se(IV) complexes were examined for antimicrobial, anticancer, antiviral, anti-inflammatory, antioxidant and diabetic/antidiabetic activities and inhibitions of acetylcholine sterase, monoamine oxidase (MAO-A) and DNA cleavage inhibitions were carried out in vitro. The results revealed that selenium complex showed antibacterial activity against S. aureus, B. subtilis and E. coli and vanadium complex against S. aureus. The iron and selenium complexes acted as anticancer agent with IC_{50} equals 19.10 and 11.80 μg/ml respectively. Also, antiviral activity was tested by Se(IV) complex with moderate activity (++), while Fe(III) complex showed weak activity (+). DNA cleavage was examined by the three complexes at different concentrations (10- 0.01) mg/ml. It was found that all concentrations prevented the adverse effect of H_2O_2 on DNA fragmentation except (0.01 mg/ml) Se(IV) complex and (10 mg/ml) VO^{2+} complex. From sucrase, maltase and lactase activity results, it is suggested that VO^{2+}, Fe(III) and Se(IV) complexes are effective anti-diabetic compounds. Inhibition of lipase activity was observed for VO^{2+} complex with $IC_{50} = 2 \times 10^{-4}$ mg/ml, while, for Fe(III) and Se(IV) complexes are 0.82 and 9.30 mg/ml, respectively. For antioxidant effect, significant drug concentration was recorded for Se(IV) and VO^{2+} complexes with IC_{50}=0.082 mg/ml and IC_{50} =0.086 mg/ml, respectively, while Fe(III) complex with IC_{50}=0.11 mg/ml. The results showed that AChE was inhibited by Fe(III), Se(IV) and VO^{2+} complexes with IC_{50} values, 0.011, 1.4×10^{-3} and 4×10^{-4} mg/ml, respectively. MAO-A was inhibited by Se(IV) and VO^{2+} complexes with IC_{50} values 0.45 and 3.2×10^{-4} mg/ml.

Keywords: Thiamine; Anticancer; Alzheimer; DNA; SAR

Abbreviations: AChE: Acetyl Cholinesterase; ACTI: Acetylcholin thioiodide; AD: Alzheimer's disease; AO: Aldehyde oxidase; DM: Diabetes mellitus; DNA: Deoxy ribonucleic acid; DPPH: 2,2-diphenyl-1-picrylhydrazyl; DTNB: 5,5'-2-dithiobis nitrobenzoic acid; EDX: Energy Dispersive X-Ray; Hb: Hemoglobin; HIV: Human Immunodeficiency Virus; HSV-1: Herpes Simplex Type-1 Virus; LD_{50}: Lethal Dose; MAO: Monoamine Oxidase; NO: Nitric Oxide; NOS: Nitric Oxide Synthase; RBCs: Red Blood Cells; RDA: Recommended Dietary Allowance; ROS: Reactive Oxygen Species; SAR: Structure Activity Relationship

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INTRODUCTION

Pyrimidines [1-12] are one of the most important compounds because of their biological activity and their involvement in the structure of nucleic acid in the living systems [13,14]. Compounds having nitrogen and sulphur as donor atoms have a significant role as anticancer and antiviral agents [15,16]. Thiamine is very important for all living cells and organisms [17]. It is an indispensable molecule for intermediate metabolism in all life forms. This is mainly because its diphosphorylated derivative (thiamine pyrophosphate or diphosphate (ThDP) that required as an essential cofactor involved in several metabolic sequences [18], as it has key roles in carbohydrate metabolism, amino acid metabolism [19] and pyruvate metabolism [20]. Metals and metal complexes had played an important

role in the development of the pharmaceutical drugs and modern chemotherapy. Most of drugs have different physical and pharmacological properties leading to an enhancement of some drugs of poor activity or high toxicity in pharmacology [21].

The human body has a complex system of natural enzymatic and non-enzymatic antioxidant defenses which prevent the harmful effects of free radicals, like reactive oxygen species (ROS) and other oxidants and prevent the cell damage. Free radicals are responsible for causing a large number of diseases including cancer [22] cardiovascular disease [23], neural disorders [24], Alzheimer's disease [25], Parkinson's disease [26] and alcohol induced liver disease [27]. The oxidative stress is mainly related to the carcinogenesis process [28] and played a role in cerebral damage associated with thiamine deficiency and alcohol toxicity [29].

Some investigations confirmed the beneficial role of thiamine in cancer chemotherapy [30,31]. It was reported that the major moiety pyrimidine ring involved in thiamine possessed antitumor activity against a lot of tumor strains [32]. In addition, thiamine showed a role in inhibition of DNA fragmentation [33]. Nitric oxide synthase (NOS) is an enzyme responsible for generation of NO species which regulates a lot of physiological responses, such as, immune response and cell migration [30]. The inhibition of NO production was observed in Alzheimer's disease and Parkinson's disease patients due to the low levels of the NO synthase enzyme [34]. Thiamine had acute and chronic effects as an anti-inflammatory agent on the skin lesions, and vascular inflammation [35].

AChE is an enzyme needed in biological systems, due to its role in nerve impulse transmission [36], where it inhibits the acetylcholine which causes termination of the neurotransmission process [37]. There was a relation between the pathogenesis of Alzheimer's disease and the deficiency in the brain neurotransmitter acetyl choline [38]. AChEIs act as Anti-inflammatory agent through action against free radicals in the brain and blood which may cause toxicity [38]. The role of thiamine in forms TPP and TTP in the nerve system was observed, to enhance the normal brain metabolism [39], which participates in Acetyl Choline synthesis that controls the nerve transmissions.

Monoamino oxidase (MAO) is an enzyme that regulates several physiological and pathological monoamine neurotransmitters and hormones, such as dopamine, adrenaline, serotonin, and noradrenaline [40,41]. Its defects have been linked to depression and abnormally aggressive behavior [42]. MAO's inibitors (MAOIs) were suggested to be the drug development for these diseases [40]. Diabetes mellitus (DM) is a metabolic disorder condition characterized by hyperglycemia because of the impairment of insulin action or secretion at peripheral tissues [43]. Thiamine cured diabetes with beneficial role [44]. The glucose metabolism was considered to be dependent on thiamine, where insulin synthesis and secretion had impairment due to thiamine deficiency [35].

Metal ion complexes played an essential role in the development of antimicrobial [45]. Some complexes of thiamine have been prepared and screened for their antimicrobial compared with the parent thiamine [46]. The aim of this work is to develop a new metal complexes derived from thiamine Cl HCl for medical applications with better physicochemical properties and studying the antimicrobial, antiviral, antitumor, antioxidant, anti-inflammatory, anti-diabetic activities and inhibition of DNA fragmentation, acetylcholine sterase and MAO-A and the anti-hemolytic effect of some metal complexes of thiamine.

EXPERIMENTAL SECTION

Chemicals

Vitamin B_1 was obtained from German Roth company, Acetylcholin thioiodide, Benzaldehyde, DPPH, Potassium ferricyanide solution, Tris buffer and Sulphanilic acid were purchased from Sigma-Aldrich, USA. 5,5'-2-dithiobis nitrobenzoic acid (DTNB), Lactose, Lipase, Maltose, Olive oil, P-tyramine, Pencreatine, Sucrose and Naphthyl ethylene diamine dihydrochloride, was obtained from Diamond, Egypt. Glucose kit from Diamond, Egypt.

Syntheses of Complexes

The metal thiamine complexes were prepared by mixing 1 mmol (0.337 g) of thiamine hydrochloride dissolved in 10 ml of distilled water (ammonia solution was added to thiamine solution) with 10 ml of aqueous solution of 1 mmol of metal salts. In case of Fe(III) thiamine complex, the metal salt $FeCl_3.6H_2O$ was added directly to thiamine solution without addition of ammonia solution. All complexes were heated at 70ºC for about 20 min leading to precipitation. The complexes then cooled, collected and dried in oven at 60ºC. The analytical data showed the formation of all complexes with stoichiometry 1:1, except vanadium complex with molar ratio (2:1).

Instruments and Working Procedures

Instruments:

Spectrophotometer from Optima, Japan, ELISA reader from Bio-Tek, Electrophoresis unit from Maxfill, Japan and Centrifuge from Hettich, Germany.

Thermal analysis:
Thermogravimetric analysis (TGA) and Differential thermal analysis (DTA) were measured for thiamine ligand and its complexes using the LINSEIS STA PT1000 in temperature range 25-600ºC at 10 k/min. The analysis was performed under oxygen medium.

Antimicrobial activity:
The antimicrobial activity examination based on the well agar diffusion method [47]. The ligand and its complexes were tested for antibacterial activity against several standard microorganisms of Gram positive bacteria of *Staphylococcus aureus*, *Bacillis subtilis*, and Gram negative bacteria of *Pseudomonas aeruginosa*, *Escherichia coli*. For the antifungal activities of the samples, one type of fungi was used, *Candida albicans*. Stock solution (1 mg/ml) was prepared by dissolving the compounds in DMSO. The diameter of the inhibition zones was measured in millimeters at 37ºC after 24 h. The controls were tested in DMSO [47,48], where Ciprofloxacin and Clotrimazole, were used as positive control for microorganisms of bacteria and fungi organism.

Anticancer activity:
The antitumor activity was examined on tumor cells of (HepG-2 cell line). The cells were grown as monolayers on RPMI-1640 medium with addition of 10% inactivated fetal calf serum and 50 µg/ml gentamycin. The cells were holded in a 96-well microtiter plate at the bottom of wells and were incubated at 37ºC for 24 h in a humidified atmosphere with 5% CO_2, then washed with sterilized phosphate buffer (0.01 M, pH 7.2). The cells were instantaneously treated with 100 µl of six different dilutions (50, 25, 12.5, 6.25, 3.125, 1.56 µg/ml) of tested ligand and its complexes in fresh medium and were incubated at 37ºC. A control with untreated cells was used without addition of tested samples. Doxroubcin drug was used as a positive control and also was tested as a reference for comparison. The cells were subcultured from two to three times a week. The number of viable cells was evaluated using ELISA reader. The absorbance values were detected at 590 nm and the viability percentage was determined from the following formula [49,50].

$$\text{Viability \%} = [1- A_t / A_c] \times 100\%$$

Where:
A_t: the mean absorbance of cells treated with the tested samples.
A_c: the mean absorbance of the untreated cells.

The untreated cells showed absorbance of 100%. The 50% inhibitory concentration (IC_{50}) was the concentration needed to reveal toxic effects on 50% of unaffected cells and was obtained from the plots of the graph [49,50].

Antiviral activity:
The antiviral activity was based on the inhibition of cytopathic effect method [51] and evaluated against herpes simplex type-1 virus (HSV-1)**.** The vero cells, derived from a kidney tissue, were grown in Dulbecco's modified Eagle's medium (DMEM) and 10% inactivated fetal bovine serum (FBS), 1% L-glutamine, HEPES buffer and 50 µg/ml gentamycin were added. The cells were introduced into each well of a 96-well microtiter plate and incubated at 37ºC for 24 h in a humidified medium with 5% CO_2. A fresh medium DMEM was applied into the wells, then the tested samples were added into each well. The control of the untreated cells was incubated in absence of the tested samples. After that, the microplate was incubated at 37ºC in humidified medium with 5% CO_2 for 3 days. The cells were subcultured two times a week and checked daily under the microscope detection until the virus in control well showed a complete viral-induce cytopathic effects (CPE). The antiviral activity was determined by the inhibition of CPE compared with the control and treatment of cells by the tested samples was enabled [52].

DNA cleavage:
The examination of DNA cleavage was performed using the agarose gel electrophoresis technique with the ligand and metal complexes [53] at different concentrations (10, 5, 1, 0.5, 0.1, 0.05 and 0.01 mg/ml) using H_2O_2 as oxidant [54]. A 2 µl of compound and 10 µl of H_2O_2 were added to 20 µl of DNA (1 mg was dissolved in 10 ml buffer (50 mM Tris-HCL-18 mM NaCl buffer pH=7.2)). The mixture was incubated for 2.30 h at 25ºC.

Agarose gel electrophoresis protocol:
The agarose gel tray was prepared as follow, 1% agarose gel was prepared containing 5 µl ethidium bromide, then cooled and introduced into the electrophoresis chamber which was covered with 1x TBE buffer. The DNA samples (5 µl sample with 2 µl bromo phenol blue) and 3 µl DNA ladder were loaded separately into the electrophoresis chamber wells. DNA samples were electrophoreesed at 100 V for 1 h. The DNA bands were visualized using UV

light box and then photographed to evaluate the DNA cleavage of the tested samples compared to the DNA ladder and DNA control.

Diabetic/antidiabetic effect:

Sucrase, maltase and lactase activity: Activities of sucrase, lactase, maltase were determined by the described method of Dahlqvist [55]. 10 µl of the compound was mixed with 20 µl pencreatine (0.1 g/10 ml H_2O with drops of NaOH) and 100 µl of 0.1 M phosphate buffer (pH= 7.4), the mixture was incubated at ºC for 45 min. Then 20 µl of (1 g%) substrate sucrose, maltose or lactose was added and the mixture was incubated again for 20 min. After that 200 µl of glucose reagent was added to the mixture and finally incubated at 25ºC for 20 min, then the absorbance was measured spectrophotometrically at 490 nm [55].
Specific activity was expressed as U= µg of glucose per min per mg of protein.

Lipase activity: 250 µl of the tested compound was mixed with 0.5 ml (1 g%) lipase, 2.5 ml olive oil (100 ml olive oil and 2 bitterness bass) and 1.25 ml 0.2 M Tris buffer (pH=8). The mixture was incubated at 37ºC for 2 h. Then 1.5 ml ethanol was added to the mixture. The previous mixture was titrated against 0.2 M NaOH using phenol phthaline (ph.ph) as indicator. The color changed from colorless to faint pink [56]. Lipase activity was expressed as U= µg of 0.2 M NaOH per mg of protein.

Antioxidant activity:

For the scavenging activity of DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical in the ligand and prepared complexes, DPPH was prepared in methanol. 100 µl of ligand and complexes were separately mixed with 100 µl of DPPH. The mixture was incubated in dark at 25ºC for 30 min [57,58]. The absorbance was detected at 490 nm by ELISA reader. The DPPH radical scavenging activity of the compounds was determined using the following equation:

$$\% \text{ DPPH radical scavenging} = [A_{control} - A_{sample}] / A_{control} \times 100$$

Nitric oxide scavenging activity (anti-inflammatory):

The method is based on decomposition of sodium nitroprusside in a physiological pH (7.2) yielding NO• that may cause tissue damage. NO• reacts with oxygen to produce nitrite and nitrate ions which can be evaluated by Griess reagent [59,60]. Scavengers of NO• compete with oxygen which lead to inhibition of nitrites ions production [59]. 40 µl of (10 mM) sodium nitroprusside, 10 µl phosphate buffer saline (pH= 7.4) and 10 µl of compound were mixed and incubated at 25ºC for 2.30 h. Then 20 µl of Griess reagent (0.1% naphthyl ethylene diamine dihydrochloride + 1% sulphanilic acid in 5% phosphoric acid) was added to the mixture and incubated again for 30 min at 25ºC, similar steps were repeated with distilled H_2O instead of the compound for the control. The absorbance was recorded at 540 nm by ELISA reader [59,60].

$$\% \text{ Nitric oxide radical scavenging activity} = [\text{Acontrol} - \text{Asample}] / \text{Acontrol} \times 100$$

Acetyl cholinesterase activity (AChE):

Preparation and purification of brain homogenate: A fresh brain tissue was obtained, weighed and washed with 0.1 M phosphate buffer saline (containing 0.2 M NaCl; pH=7.6). The brain tissue then homogenized in 5 ml of 0.1 M phosphate buffer saline (containing 0.2 M NaCl; pH=7.6). After that the brain homogenate was centrifuged at 4ºC for 15 min at 6000 rpm, the supernatant was cleared and collected for purification. purification process was carried out under 4ºC, where solid ammonium sulphate was added to the supernatant stepwise with continuous stirring for about 1 h. Solution was stored at 4ºC for 3 h then centrifuged at 4ºC for 20 min at 6000 rpm till formation of precipitate. The obtained precipitate was equilibrated with 0.1 M phosphate buffer (pH=7.6), then a sucrose dialysis process was made for 24 h at 4ºC. The dialyzed solution was passed on Sephadex G-25 column (15 × 1cm) and eluted with the same equilibration buffer [36].

Activity assay: AChE activity was determined according to the assay of Ellman et al. [61]. For tested compounds wells, 130 µl of 0.1 M phosphate buffer (pH=8.0) was added to 20 µl of compound and 20 µl of enzyme. But for the blank test a 150 µl of 0.1 M phosphate buffer (pH=8.0) was added without both enzyme and tested compound, the mixtures of blank and activity samples were incubated for 45 min at 37ºC. After that 5 µl of 75 mM acetylcholin thioiodide (ACTI) substrate was added for all wells, mixed well, and incubated again for 15 min at 37ºC. 60 µl of 0.32 mM DTNB (5,5'-2-dithiobis nitrobenzoic acid) was added for all wells. The absorbance was detected at 405 nm and the specific activity of AChE was determined [62,63].

Specific activity = [A] × [total volume in cuvette (µl)] / [Molar extinction coefficient of DTNB × volume of brain extract (µl)].

Monoamine oxidase activity (MAO-A):

Preparation of brain homogenate: Fresh brain tissue was obtained and washed with cold saline (0.9% NaCl), then homogenized in 9 ml of 0.1 M sodium phosphate buffer (pH=7.4). The homogenate was centrifuged at 6000 rpm for 10 min. The supernatant was cleared and collected to be used in determination of activity [64].

Activity assay: 150 µl of compound was mixed with 300 µl of brain homogenate and incubated for 45 min. 150 µl of the previous mixture was taken and mixed with 133 µl of 0.1 M phosphate buffer (pH=7.6) and 667 µl of 500 µM P-tyramine. The absorbance was recorded at 250 nm after 1 min and 2 min, and the MAO activity was calculated [64-66].

MAO activity = [ΔA × total volume x 1000] / 32.2 × sample volume × 0.5

Cytotoxicity effect on red blood cells (RBCs):

Preparation of RBCs sample: Fresh RBCs sample was obtained from a healthy donor. The blood sample was mixed with phosphate buffer saline containing NaCl, pH=7.4 (working buffer) and then centrifuged at 3000 rpm for 10 min. The supernatant liquid containing plasma and erythrocytes was drawn off and the RBCs were washed with phosphate buffer, pH=7.4 several times at 25ºC and re-suspended in phosphate buffered saline [67].

Hemolysis assay: This method is based on the detection of light absorbance at 450 nm which assigned to the hemolysis where, hemoglobin (Hb) yielded an enhanced/inhibited reaction due to the effect of the compound that started or inhibited the peroxide oxidation of lipids through the erythrocytic membranes [67]. The test sample was prepared by mixing 1 ml of erythrocyte suspension with 4 ml of compound prepared in (distilled H_2O or working buffer), the control sample of E_o (normal blood hemolysis) or E_{max} (complete blood hemolysis) was prepared by mixing 1 ml erythrocyte suspension with 4 ml of working buffer or distilled H_2O, respectively. All samples content were shaken well, then incubated at 37ºC for 1 h. After incubation, shaking again for all samples then centrifuged at 1000 rpm for 10 min. 200 µl of supernatant of each sample was introduced into ELISA microplate wells. The absorbance was recorded at 540 nm by ELISA reader. Hb released from cells shows color absorbance in the supernatant [67].

% Anti-hemolytic effect = [E_{max} - (Test- Blank) / E_{max}] × 100

Computer Programs

Molecular docking was performed by ACD/Labs and Molsoft L.L.C. softwares for *in vivo* examination [68].

RESULTS AND DISCUSSION

The structures illustrated in Figure 1, of some synthesized metal complexes were proposed according to the discussion of results of analytical data obtained from the metal, elemental and chloride content analyses, infrared spectra, UV-Vis, NMR, molar conductance, magnetic properties, EDX and thermal analysis which reported before [69].

Thermal Analysis

TGA thermogram of $[Fe(th)Cl_3(H_2O)_2]2Cl.H_2O$ complex, Figure 2, showed well defined five thermal decomposition steps in the range 22.8-499.1ºC. The first two successive steps occur at 22.8-109.2ºC and 109.3-216.8ºC which attributed to the removal of the crystallized water and coordinated water molecules with mass loss of 1.68% (1.63%) and 6.07% (6.50%) and activation energies 59.06 and 17.28 KJ/mole, respectively. The third step in the range 216.8–297.6ºC is attributed to the removal of latter coordinated H_2O and the complex starts to decompose with removal of outer 2Cl. The fourth and fifth steps are due to the further decomposition of the complex with formation of Fe metal and the rest of the ligand as a residue with percent 40.92% (41.01%).

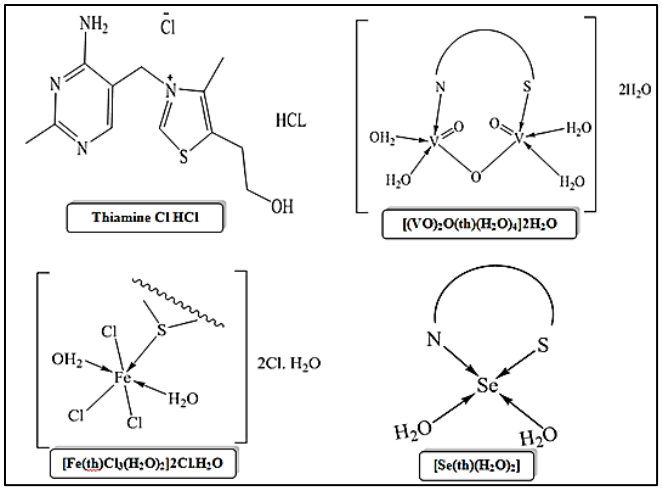
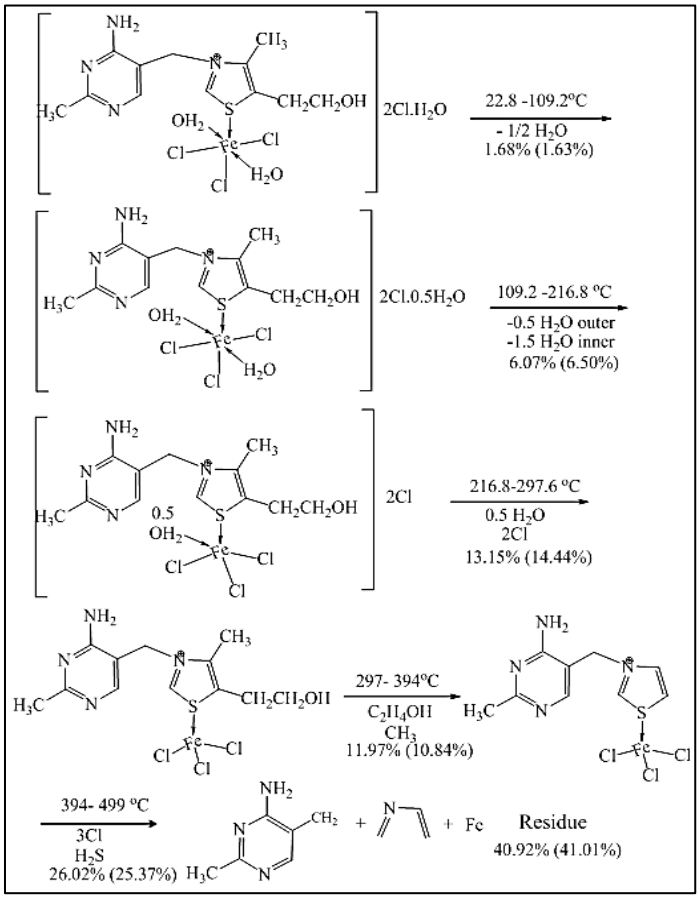


Figure 1: Proposed structures of thiamine Cl HCl and some of its metal complexes

DTA curve of $[Fe(th)Cl_3(H_2O)_2]2Cl.H_2O$ complex, Figure 2, showed three endothermic peaks at 229.9°C, 394.7°C and 455ºC with activation energies 113.07, 98.85 and 168.77 KJ/mole and reaction orders of 1.34, 0.95 and 0.63, respectively, which means that those peaks are of the first order. The first endothermic peak in DTA is attributed to the first two successive TGA steps due to dehydration of water molecules. Second and third endothermic DTA ones are due the further decomposition of complex. The mechanism of decomposition could be represented as follows (Scheme 1):



Scheme 1: Proposed degradation pathway for $[Fe(th)Cl_3(H_2O)_2]2Cl.H_2O$ complex

Biological Studies

Antimicrobial activity:

Thiamine Cl HCl ligand and its VO^{2+}, Fe(III) and Se(IV) complexes were investigated for their antimicrobial activity. The antibacterial and antifungal activities are summarized in Table 1 showing that thiamine Cl HCl ligand was not effective toward the all four bacterial species *S. aureus, B. subtilis, P. aeruginosa* and *E. coli* and the fungal organism *Candida albicans*. VO^{2+} complex exhibited activity toward only *S. aureus* with inhibition zone 13 mm. Se(IV) complex with good effect toward the two Gram positive, *S. aureus* with inhibition zone 15 mm and *B. subtilis* (16 mm) and one Gram negative bacteria *E. coli* (12.5 mm). Fe(III) complex inactive toward all the tested bacterial species. The metal coordination converted the ligand from inactive form to active one as the presence of metal enhanced the antimicrobial action for thiamine.

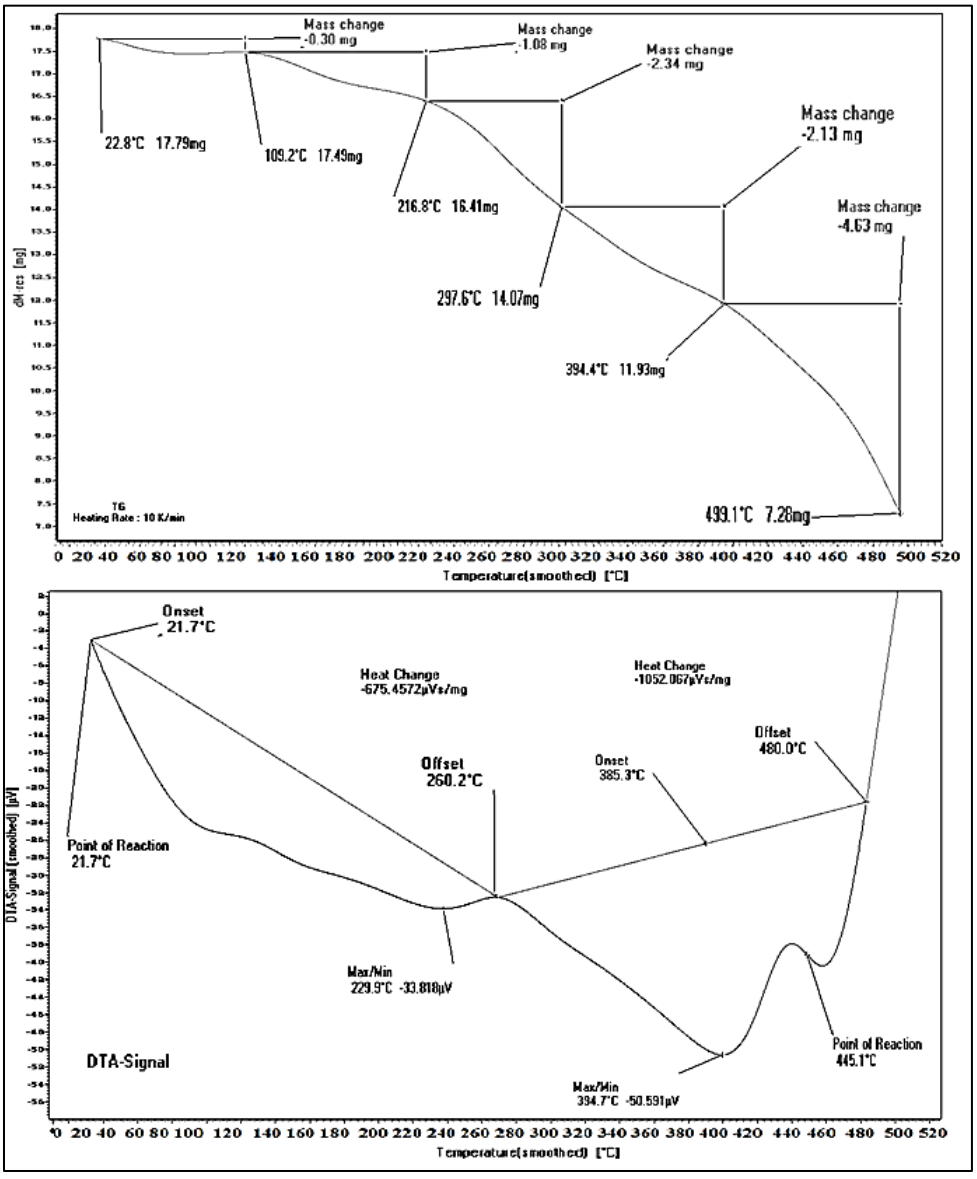


Figure 2: TGA/ DTA curves for $[Fe(th)Cl_3(H_2O)_2]2Cl.H_2O$ complex

Antiviral activity:

Thiamine Cl HCl ligand and its VO^{2+}, Fe(III) and Se(IV) complexes with concentration (10 mg/ml) were tested as antiviral agents against herpes simplex virus type-1 (HSV-1)**.** Antiviral activities are shown in Table 2 and Figure 3. Fe(III) showed weak antiviral activity (+) and Se(IV) complexes showed moderate antiviral activity (++) compared to thiamine ligand that did not show activity. VO^{2+} complex had no antiviral activity as the same manner of ligand.

Anticancer activity:

Anti-hepatocellular carcinoma activity of thiamine Cl HCl ligand and its metal complexes, Fe(III), Se(IV) and VO^{2+} were tested at different concentrations (50, 25, 12.5, 6.25, 3.125 and 1.56 µg/ml). The results are listed in Table 3. The viability of hepatocellular carcinoma cells by thiamine ligand is 100% at zero concentration, while as concentration increased (1.56, 3.125, 6.25, 12.5, 25 and 50 µg/ml), the viability of tumor cells decreased as follows 96.49, 91.52, 80.13, 65.64, 52.57 and 37.82%, respectively. Different concentration of Fe(III) and Se(IV) complexes decreased in tumor viabilities. While VO^{2+} complex had anticancer effect but lower than that of ligand. Most significant effect was observed for Se(IV) complex. Drug effect was represented by inhibitory concentration (IC_{50}) which is the concentration of drug required to inhibit 50% of cell growth. The most effective anticancer was Se(IV) complex (IC_{50}=11.80 µg/ml) and the lowest one was VO^{2+} complex (IC_{50}=39.10 µg/ml). This could be due to the antioxidant properties of Se(IV) which promoted the anticancer effect [30].

Table 1: Antimicrobial activities of thiamine Cl HCl and its complexes

Compound	Antibacterial activity zone of inhibition in (mm)				Antibacterial activity zone of inhibition in (mm)
	Gram +ve		Gram -ve		
	S. aureus	*B. Subtilis*	*P. aeruginosa*	*E. coli*	*C. albicans*
Thiamine HCl Cl	-	-	-	-	-
$[Fe(th)Cl_3(H_2O)_2]2Cl.H_2O$	-	-	-	-	-
$[Se(th)(H_2O)_2]$	15	16	-	12.5	-
$[(VO)_2O(th)(H_2O)_4]2H_2O$	13	-	-	-	-

Table 2: Antiviral activity of thiamine Cl HCl and its complexes

Compound (10 mg/ml)	Antiviral activity
Thiamine hydrochloride	-ve
$[Fe(th)Cl_3(H_2O)_2]2Cl.H_2O$	+
$[Se(th)(H_2O)_2]$	++
$[(VO)_2O(th)(H_2O)_4]2H_2O$	-ve

+: weak; ++: moderate; +++: highly active; -ve: no antiviral activity

Table 3: Anticancer activity of thiamine Cl HCl and its complexes

Compound	Cell viability %							IC_{50}
Concentration (µg/ml)	50	25	12.5	6.25	3.125	1.56	0	(µg/ml)
Thiamine Cl HCl	37.82	52.57	65.64	80.13	91.52	96.49	100	29.4
$[Fe(th)Cl_3(H_2O)_2]2Cl.H_2O$	31.89	43.62	57.24	71.39	84.78	91.31	100	19.1
$[Se(th)(H_2O)_2]$	25.43	36.91	48.75	59.47	72.38	87.56	100	11.8
$[(VO)_2O(th)(H_2O)_4]2H_2O$	41.57	60.84	73.95	82.34	95.68	99.72	100	39.1

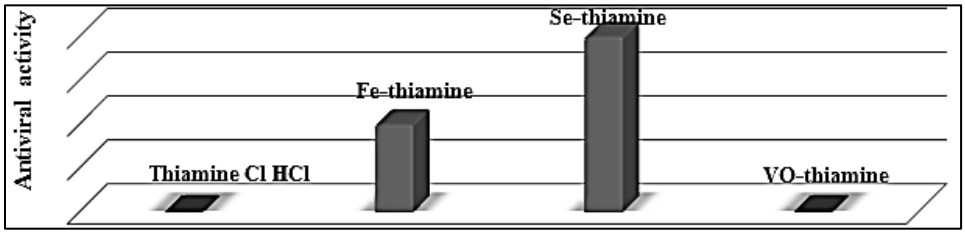
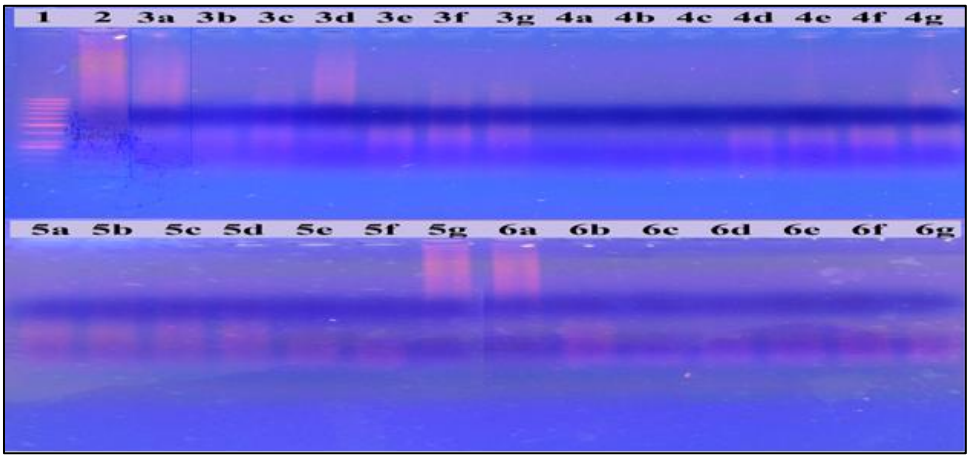


Figure 3: Antiviral activity of thiamine Cl HCl and its complexes

DNA cleavage:

DNA cleavage was examined by thiamine Cl HCl ligand and three complexes, Fe(III), Se(IV) and VO^{2+} at different concentrations 10, 5, 1, 0.5, 0.1, 0.05 and 0.01 mg/ml. From Figure 4, it was found that the presence of H_2O_2 caused DNA fragmentation which indicated by the DNA smearing in the control, Lane (2). Thiamine ligand and all concentrations of the tested three complexes prevented the adverse effect of H_2O_2 on DNA fragmentation as the smearing decreased, except Lane (5g) and Lane (6a) which attributed to (0.01 mg/ml) Se(IV) complex and (10 mg/ml) VO^{2+} complex which did not show effect on DNA fragmentation as shown by the intense smearing. The presence of smear in the gel diagram indicates the presence of radical cleavage [70].



1- DNA Ladder	2- Control H_2O		
3- Thiamine Cl HCl	4- Fe(III) complex	5- Se(IV) complex	6- VO^{2+} complex (2:1)
a- (10 mg/ml)	a- (10 mg/ml)	a- (10 mg/ml)	a- (10 mg/ml)
b- (5 mg/ml)	b- (5 mg/ml)	b- (5 mg/ml)	b- (5 mg/ml)
c- (1 mg/ml)	c- (1 mg/ml)	c- (1 mg/ml)	c- (1 mg/ml)
d- (0.5 mg/ml)	d- (0.5 mg/ml)	d- (0.5 mg/ml)	d- (0.5 mg/ml)
e- (0.1 mg/ml)	e- (0.1 mg/ml)	e- (0.1 mg/ml)	e- (0.1 mg/ml)
f- (0.05 mg/ml)	f- (0.05 mg/ml)	f- (0.05 mg/ml)	f- (0.05 mg/ml)
g- (0.01 mg/ml)	g- (0.01 mg/ml)	g- (0.01 mg/ml)	g- (0.01 mg/ml)

Figure 4: DNA cleavage study by thiamine Cl HCl and its complexes in the presence of H_2O_2

Diabetic/antidiabetic effect:

Sucrase activity: The results showed that all concentrations of metal thiamine complexes were sucrase inhibitors except for, concentration of (10 mg/ml) of both Fe(III) and Se(IV) complexes did not show inhibitory effect on sucrase activity. The IC_{50} values are listed in Table 4. The most significant sucrase inhibitor is VO^{2+} complex with IC_{50}=0.53 mg/ml, followed by Se(IV) complex with IC_{50} = 0.58 mg/ml and Fe(III) complex with IC_{50}=3.37 mg/ml compared to ligand (IC_{50}=18.44 mg/ml).

Maltase activity: The results showed that all concentrations of Fe(III), VO^{2+} and Se(IV) complexes act as maltase inhibitors. The IC_{50} values are listed in Table 4. The most potent inhibitory effect on maltase activity was observed for VO^{2+} complex with IC_{50}=6.09 mg/ml, followed by Se(IV) complex with IC_{50}=16.95 mg/ml and Fe(III) complex with IC_{50}=24.89 mg/ml compared to ligand (IC_{50}=27.69 mg/ml).

Lactase activity: The results showed that all concentrations of Fe(III), VO^{2+} and Se(IV) complexes act as lactase inhibitors, except concentrations of 10 and 5 mg/ml of Se(IV) complex did not show inhibitory effect on lactase activity. The IC_{50} values are listed in Table 4. The significant inhibitory concentration was observed for VO^{2+} complex with IC_{50}=0.05 mg/ml followed by Se(IV) complex with IC_{50}=0.1 mg/ml, while Fe(III) complex with IC_{50}=0.72 mg/ml compared to the free ligand which showed activity action.

From sucrase, maltase and lactase activity results, it is suggested that metal complexes of thiamine are effective anti-diabetic compounds that can improve glucose metabolism (transport glucose to cells), treat hyperglycemia, act as a good regulator for insulin and can also alleviate other diabetes mellitus symptoms.

Lipase activity: The results showed that thiamine with concentrations (10, 5, 1 and 0.5 mg/ml), Se(IV) complex with concentrations (1, 0.5, 0.1, 0.05 and 0.01 mg/ml) and VO^{2+} complex (2:1) with concentrations (10, 5 and 1 mg/ml) were lipase activators, while remaining concentrations of these complexes and all concentrations of Fe(III) complex acted as lipase inhibitors. The values of IC_{50} are listed in Table 4. The significant drug effect for inhibition of lipase activity was observed for VO^{2+} complex (2:1) with $IC_{50} = 2 \times 10^{-4}$ mg/ml compared to ligand IC_{50}= 0.005 mg/ml. However, the inhibitory concentration (IC_{50}) for Fe(III) and Se(IV) complexes are 0.82 and 9.30 mg/ml, respectively.

Antioxidant:
Antioxidant activity of thiamine and its metal complexes Fe(III), Se(III) and VO^{2+} on scavenging of free radical DPPH at different concentrations 10, 5, 1, 0.5, 0.1, 0.05 and 0.01 mg/ml are listed in Table 5. The resulted data showed that the complexes have very good activity on scavenging of free radical DPPH compared to Vitamin C as positive control and free thiamine ligand. Very significant activity was recorded for Se(IV) and VO^{2+} complexes at higher concentration (10 mg/ml) with values 321.69% and 322.75%, respectively compared to free thiamine 34.39% at the same concentration. Also concentrations of (5, 1, 0.5, 0.1, 0.05 and 0.01 mg/ml) of both complexes showed great antioxidant activities with values 93.12, 75.32, 68.25, 52.68, 37.50 and 31.22% for Se(IV) complex and 189.95, 106.35, 77.25, 55.68, 39 and 24% for VO^{2+} complex compared to ligand (33.33, 31.81, 31.22, 17.52, 12.17 and 9.63%), respectively. Fe(III) complex showed good antioxidant activities (85.71, 78.31, 85.19, 84.13, 49.6, 38.68 and 21.69%) for concentrations (10, 5, 1, 0.5, 0.1, 0.05 and 0.01 mg/ml) compared to ligand. The IC_{50} values (Table 5), were measured. Significant inhibition for Se(IV) and VO^{2+} complexes with IC_{50}=0.082 mg/ml and IC_{50}=0.086 mg/ml, respectively compared to free thiamine IC_{50}= 14.8 mg/ml, while Fe(III) complex with IC_{50}=0.11 mg/ml. The marked antioxidant activities observed for Se(IV) and VO^{2+} complexes higher than Fe(III) complex may be due to the coordination of metal (Se and V) with $N_{1'}$ site in pyrimidine ring through the deprotonation and S atom in thiazole ring, so proton of nitrogen atom is easily accepted by DPPH free radical and convert itself to free radical, while Fe(III) coordinated to only sulphur atom.

Nitric oxide (NO):
The reduction of nitric oxide radical by thiamine and its metal complexes, Fe(III), Se(IV) and VO^{2+} at different concentrations 10, 5, 1, 0.5, 0.1, 0.05 and 0.01 mg/ml (Table 6). It was observed that all concentrations of the three metal thiamine complexes showed great nitric oxide scavenging activities compared to thiamine ligand. The IC_{50} values are listed in Table 6. The significant inhibitory concentration was recorded for Fe(III) complex with IC_{50} = 5.2×10^{-4} mg/ml followed by VO^{2+} complex (2:1) with IC_{50} = 1.3×10^{-3} mg/ml and finally Se(IV) complex with IC_{50} = 2.67 mg/ml compared to ligand with IC_{50} = 80.42 mg/ml. Those complexes can be used as selective inhibitors for nitric oxide. So, these metal complexes can be used in improvement of immune response and cell migration, cerebral circulation and the memory cellular basis, hence, it can give beneficial treatment for Alzheimer's and Parkinson's disease patients.

Acetyl cholinesterase inhibitor (AChE):
Inhibition or activation of acetyl cholinesterase by thiamine and its metal complexes, Fe(III), Se(IV) and VO^{2+} at different concentrations 10, 5, 1, 0.5, 0.1, 0.05 and 0.01 mg/ml, are listed in Table 7. It was found that all concentrations of the three metal thiamine complexes act as great AChE inhibitors compared to thiamine ligand and the control. The IC_{50} values are listed in Table 7. The significant inhibitory concentration effect on AChE was observed for VO^{2+} complex with IC_{50}= 4×10^{-4} mg/ml followed by Se(IV) complex with IC_{50}=1.4×10^{-3} mg/ml and finally Fe(III) complex with IC_{50}=0.011 mg/ml compared to ligand. These inhibitors can help in treatment of Alzheimer disease (AD) by maintaining the levels of acetylcholine through inhibition of (AChE).

Monoamine oxidase (MAO-A):
Activation or inhibition by thiamine and its metal complexes, Fe(III), Se(IV) and VO^{2+} at different concentrations 10, 5, 1, 0.5, 0.1, 0.05 and 0.01 mg/ml, are listed in Table 8. It was observed that concentrations of 0.5 and 0.1 mg/ml of Se(IV) complex acted as MAO-A inhibitors with inhibitory effect -60.05, -20.10% and concentration of 0.01 mg/ml of VO^{2+} complex (2:1) with inhibition -10%. The remaining concentrations of Fe(III), Se(IV) and VO^{2+} did not show inhibitory effect. Potential inhibitory effect was observed for VO^{2+} complex with IC_{50} = 3.2×10^{-4} mg/ml. While, Se(IV) complex showed inhibitory effect with IC_{50}=0.45 mg/ml compared to ligand. Fe(III) complex had no inhibitory concentration effect.

Anti-hemolytic effect (RBC's cytotoxicity):
Thiamine Cl HCl and its metal complexes, Fe(III), Se(IV) and VO^{2+} at different concentrations 10, 5, 1, 0.5, 0.1, 0.05 and 0.01 mg/ml, were examined in *in vitro* cytotoxicity by HRBC stabilization method (Table 9). The three tested complexes showed anti-hemolytic effect compared to ligand. All concentrations of Fe(III) complex showed significant anti-hemolytic effect, followed by all concentrations of Se(IV) complex. Only concentrations of 0.1, 0.05 and 0.01 mg/ml of VO^{2+} complex showed anti-hemolytic effect and safe, while concentrations of 10, 5, 1 and 0.5 mg/ml showed toxic effect. IC_{50} values are listed in Table 9 showed the most significant anti-hemolytic effect was observed for VO^{2+} complex with IC_{50}=2.3×10^{-3} mg/ml. The inhibitory concentration of Fe(III) and Se(IV) complexes are IC_{50}=1.23 mg/ml and IC_{50}=1.65 mg/ml, respectively.

Table 4: Inhibition or activation of thiamine Cl HCl and its complexes on sucrase, maltase, lactase and lipase activity

Compound (mg/ml)	IC_{50}			
	Sucrase activity	Maltase activity	Lactase activity	Lipase activity
Thiamine Cl HCl	18.44	27.69	-	0.005
$[Fe(th)Cl_3(H_2O)_2]2Cl.H_2O$	3.37	24.89	0.72	0.82
$[Se(th)(H_2O)_2]$	0.58	16.95	16.95	9.3
$[(VO)_2O(th)(H_2O)_4]2H_2O$	0.53	6.09	0.05	$2x10^{-4}$

%= [(test - control)/control] × 100

Table 5: Antioxidant activity of thiamine Cl HCl and its complexes

Compound	DPPH scavenging activity %							IC_{50}
Concentration (mg/ml)	**10**	**5**	**1**	**0.5**	**0.1**	**0.05**	**0.01**	**(mg/ml)**
Control Vitamine C	69.31	75.13	76.71	77.78	77.78	78.31	78.84	33.24
Thiamine Cl HCl	34.39	33.33	31. 81	31.22	17.52	12.17	9.63	14.8
$[Fe(th)Cl_3(H_2O)_2]2Cl.H_2O$	85.71	78.31	85.19	84.13	49.68	38.68	21.69	0.11
$[Se(th)(H_2O)_2]$	321.69	93.12	75.32	68.25	52.68	37.5	31.22	0.082
$[(VO)_2O(th)(H_2O)_4]$ $2H_2O$	322.75	189.95	106.35	77.25	55.68	39	24	0.086

%= [(control-test)/control] × 100

Table 6: Inhibition or activation of thiamine Cl HCl and its complexes on NO scavenging activity

Compound	NO scavenging activity %							IC_{50}
Concentration (mg/ml)	**10**	**5**	**1**	**0.5**	**0.1**	**0.05**	**0.01**	**(mg/ml)**
Thiamine Cl HCl	13.79	10.34	9.19	5.7	4.59	2.29	4.59	80.42
$[Fe(th)Cl_3(H2O)_2]2Cl.H_2O$	208.04	80.45	74.71	78.16	68.96	67.81	66.66	$5.2x10^{-4}$
$[Se(th)(H_2O)_2]$	13.79	29.88	63.21	72.41	72.41	74.71	75.86	2.67
$[(VO)_2(th)(H_2O)_4]2H2O$	106.89	66.67	63.21	57.47	60.91	52.87	56.32	$1.3x10^{-3}$

%= [(control-test)/control] ×100

Table 7: Inhibition or activation of thiamine Cl HCl and its complexes on AChE activity

Compound	Inhibition or activation %							IC_{50}
Concentration (mg/ml)	**10**	**5**	**1**	**0.5**	**0.1**	**0.05**	**0.01**	**(mg/ml)**
Thiamine Cl HCl	19.34	10.46	7.53	10.07	5.55	8.88	8.88	-
$[Fe(th)Cl_3(H2O)_2]2Cl.H_2O$	-61.69	-63.44	-61.61	-56.38	-55.98	-54.32	-49.96	0.011
$[Se(th)(H_2O)_2]$	-74.78	-74.54	-62.25	-60.42	-58.6	-64.39	-54.08	$1.4x10^{-3}$
$[(VO)_2(th)(H_2O)_4]2H2O$	-85.4	-66.53	-69.94	-67.32	-78.98	-66.53	-62.64	$4x10^{-4}$

%= [(test - control)/control] × 100

Table 8: Inhibition or activation of thiamine Cl HCl and its complexes on MAO-A activity

Compound	Inhibition or activation %							IC_{50}
Concentration (mg/ml)	**10**	**5**	**1**	**0.5**	**0.1**	**0.05**	**0.01**	**(mg/ml)**
Thiamine Cl HCl	-19.92	-9.92	40.2	370.48	530.53	750.89	10.17	-
$[Fe(th)Cl_3(H2O)_2]2Cl.H_2O$	830.79	170.23	650.64	530.53	40.2	40.2	10.17	-
$[Se(th)(H_2O)_2]$	80.15	350.38	140.2	-60.05	-20.1	250	270	0.45
$[(VO)_2(th)(H_2O)_4]2H2O$	840	290	270	250	200	160	-10	$3.2x10^{-4}$

%= [(test-control)/control] × 100

Table 9: Anti-hemolytic effect of thiamine Cl HCl and its complexes

Compound	Anti-hemolysis effect %							IC_{50}
Concentration (mg/ml)	**10**	**5**	**1**	**0.5**	**0.1**	**0.05**	**0.01**	**(mg/ml)**
Thiamine Cl HCl	-13.83	-10.64	-9.57	-8.51	-6.38	-5.32	-4.26	-
$[Fe(th)Cl_3(H2O)_2]2Cl.H_2O$	286.17	262.77	164.9	76.59	39.36	38.3	38.3	1.23
$[Se(th)(H_2O)_2]$	285.11	263.83	37.23	36.17	35.11	34.04	25.53	1.65
$[(VO)_2(th)(H_2O)_4]2H2O$	-48.94	-47.87	-23.4	-18.08	26.59	27.66	29.79	$2.3x10^{-3}$

% Anti-hemolytic= $[E_{max}$ - (test-blank)/$E_{max}] \times 100$

Software applications:

Molecular docking softwares, ACD/Labs [68] used for *in vivo* examination of bioavailability, toxicity using, health effects, and LD_{50}. The properties were found for thiamine Cl HCl and Se(IV) complex, while properties of Fe(III) and VO^{2+} complexes showed the same manner of ligand. Molsoft L.L.C. used to evaluate how drug link likness.

Bioavailability: The bioavailability of thiamine and Se(IV) complex, showed oral bioavailability less than 30%. Thiamine Cl HCl and Se(IV) complex are soluble and have stability in pH<2. They are first-pass metabolism and active transport.

Health effect: The health effect of thiamine Cl HCl and Se(IV) complex on blood, cardiovascular system, gastrointestinal system, kidney, liver and lungs was examined (Figures 5a and 5b and Table 10). Se(IV) complex showed health effect on blood lower than that of ligand with probability 0.34 compared to ligand with 0.47, while the health effect of Se(IV) complex on cardiovascular, system gastrointestinal system, kidney, liver and lungs was higher with probabilities of 0.77, 0.95, 0.86, 0.21 and 0.68 compared to ligand.

Table 10: Health effect of thiamine Cl HCl and its complexes

Health effect	Compound	
	Thiamine Cl HCl	Se (IV) thiamine
Blood	0.47	0.34
Cardiovascular system	0.47	0.77
Gastrointestinal system	0.87	0.95
Kidney	0.78	0.86
Liver	0.19	0.21
Lungs	0.66	0.68

LD_{50}: The experimental values of LD_{50} are listed in Table 11. The results showed that the lethal dose (LD_{50}) of thiamine Cl HCl needed to measure the poisining potential on tested mouse/intraperitoneal, mouse/oral, mouse/intravenous, mouse/substaneous, rat/intraperi-toneal and rat/oral is 98, 1800, 110, 420, 230 and 810 mg/kg, while for Se(IV) complex, 56, 140, 48 49 110 and 23 mg/kg.

Table 11: LD_{50} of thiamine Cl HCl and its complexes

Species/Administration route	LD_{50} (mg/kg)	
	Thiamine Cl HCl	Se(IV) thiamine
Mouse/Intraperitoneal	98	56
Mouse/ Oral	1800	140
Mouse/ Intravenous	110	48
Mouse/Substaneous	420	49
Rat/Intraperitoneal	230	110
Rat/Oral	810	23

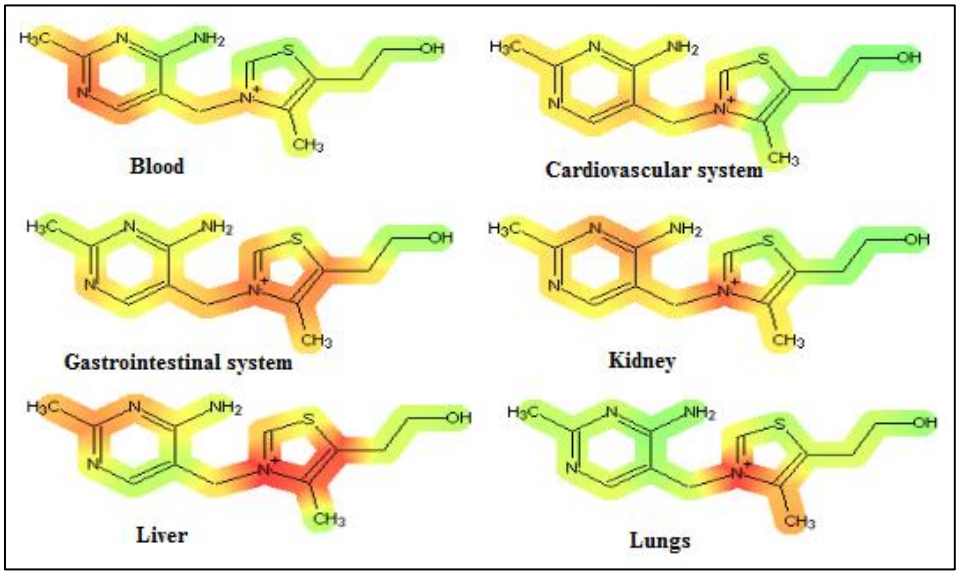


Figure 5a: Probability effect of thiamine Cl HCl on blood, cardiovascular system, gastrointestinal system, kidney, liver and lungs

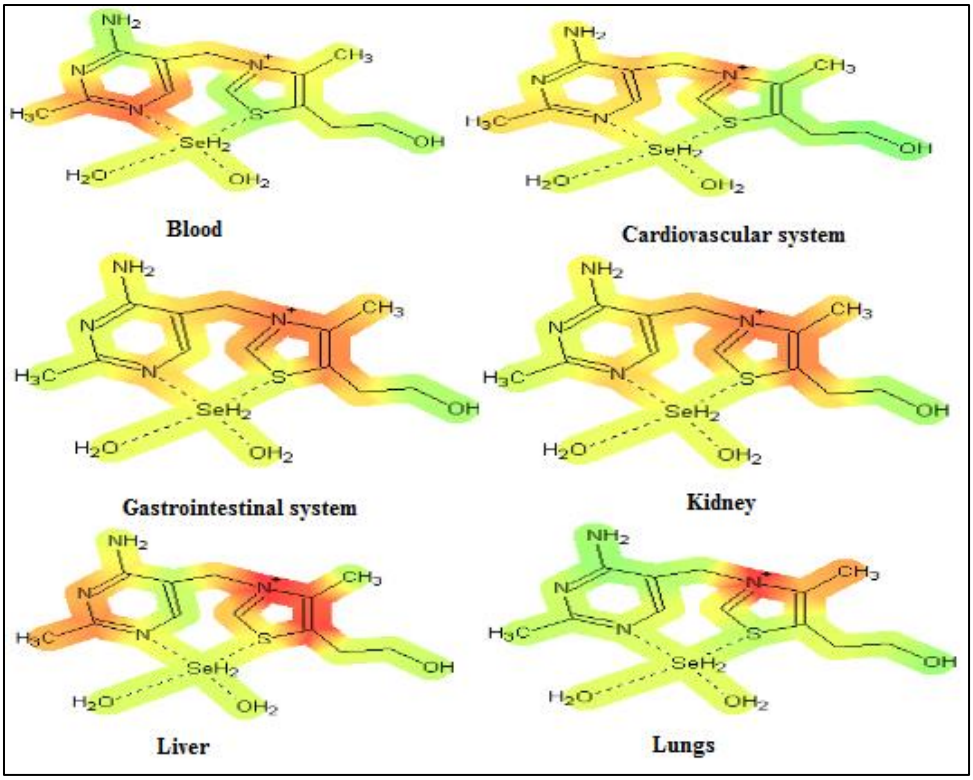


Figure 5b: Probability effect of selenium complex on blood, cardiovascular system, gastrointestinal system, kidney, liver and lungs

Toxicity use: Toxicity use data for thiamine Cl HCl and its selenium complex are listed in Table 12. Se(IV) complex has 82% probability that $LD_{50} \leq 300$ mg/kg and 95% probability that $LD_{50} > 5$ mg/kg compared to ligand which has 82% probability that $LD_{50} \leq 2000$ mg/kg and 87% probability that $LD_{50} > 300$ mg/kg.

Table 12: Toxicity use for thiamine Cl HCl and its complexes

Concentration (mg/kg)	Compound	
	Thiamine Cl HCl	Se(IV) thiamine
≤ 5	0.003	0.055
>5	0.997	0.945
≤ 50	0.017	0.331
>50	0.983	0.669
≤ 300	0.132	0.817
>300	0.868	0.183
≤ 200	0.823	0.987
>2000	0.177	0.013
≤ 5000	0.974	0.998
>5000	0.026	0.002

Drug link likness: All three complexes of $[Fe(th)Cl_3(H_2O)_2]2Cl.H_2O$, $[Se(th)(H_2O)_2]$ and $[(VO)_2O(th)\ (H_2O)_4]2H_2O$ were tested for the drug link likness (Figure 6 and Table 13). The drug link likness score showed that the three complexes range between 0.93-1.25 in the blue range. So, they can be used as drug.

Table 13: Drug link likness score of thiamine and its complexes

Compound	Drug link likness model score
Thiamine Cl HCl	0.93
$[Fe(th)Cl_3(H_2O)_2]2Cl.H_2O$	1
$[Se(th)(H_2O)_2]$	1.15
$[(VO)_2O(th)(H_2O)_4]2H_2O$	1.24

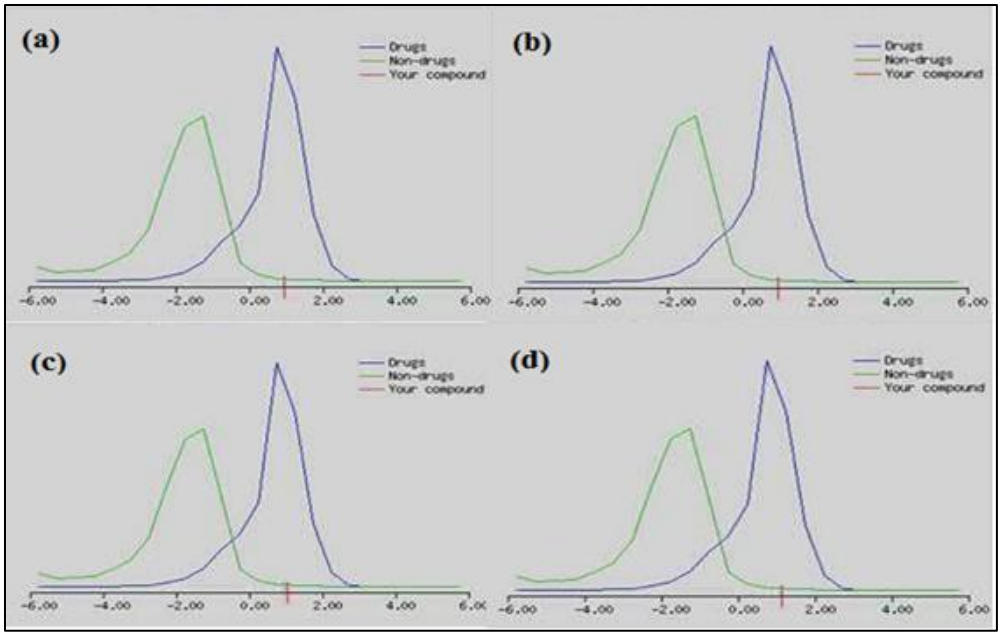


Figure 6: Drug link likness model of thiamine Cl HCl and its complexes

Structure Activity Relationship (SAR)

The process of the developed SAR is an attempt to explain how properties relevant to activity are evaluated by the chemical structure. In the pharmaceutical and chemical fields, SAR is used design chemicals with desirable pharmacologic and therapeutic properties [71]. Actually, it was observed that the chelating behavior is improved for the nitrogen and sulfur containing organic compounds when they are binding with metal ion center [72].

To investigate the relationship between the molecular structure and the biological activities of thiamine complexes such as anti-diabetic, antitumor activity, antioxidant, anti-hemolytic, DNA binding ability and antimicrobial activity, a series of metal thiamine complexes have been synthesized and characterized by IR, UV-Visible spectroscopy, thermal analysis and elemental analysis.

a) Anti-diabetic effects (sucrase, lactase and maltase) gave desirable activities, where for sucrose activity, the inhibitory concentrations of VO^{2+}, Se(IV) and Fe(III) complexes are IC_{50}=0.53, IC_{50}=0.58 and IC_{50}=3.37 compared to ligand (IC_{50}=18.44). For maltase activity, the inhibitory concentrations of VO^{2+}, Se(IV) and Fe(III) complexes are IC_{50}=6.09, IC_{50}=16.95 and IC_{50}=24.89 compared to ligand (IC_{50}=27.69). While for lactase activity, the inhibitory concentrations of VO^{2+}, Se(IV) and Fe(III) complexes IC_{50}=0.05, IC_{50}=0.1 and IC_{50}=0.72 compared to ligand. The potential inhibitory effect was observed for VO^{2+} complex may be due its potent insulin mimetic effects. Lipase activity was tested by thiamine and its metal complexes, where the highest inhibitory effect was recorded for VO^{2+} complex with $IC_{50}=2 \times 10^{-4}$ mg/ml due to the oxidation state V(IV) [73] compared to ligand 0.005 mg/ml. Both Fe(III) and Se(IV) complexes showed inhibitory effect with IC_{50}=0.82 and IC_{50}=9.30 mg/ml, respectively, lower than ligand.

b) In fact, anticancer activity of the metal complexes is related to the type of the ligand and the metal center ion [73] which was explained by Tweedy's chelation theory, also changing of coordination sites and anion. Furthermore, the improvement of anticancer activity upon coordination may be attributed to that the positive charge of the metal ion increases the acidity of coordinated ligand that possess protons leading to formation of strong hydrogen bonds which increased the biological activity [74]. For the anticancer activity results, the Se(IV) complex is a potent anticancer agent with (IC_{50}=19.1 μg/ml), followed by Fe(III) complex with anticancer activity (IC_{50} =11.8 μg/ml) compared to ligand (IC_{50}=29.4 μg/ml). But the square pyramidal VO^{2+} complex did not show anticancer activity with (IC_{50}=39.1 μg/ml) may be due to the redox chemistry of vanadium through generation of reactive oxygen species [73]. However, the Se(IV) complex with square planar geometry involves two water molecules substituents assisting the penetration of complex into tumor cells compared to Fe(III) complex which has octahedral geometry having substituents of three chloride ions and two coordinated water molecules leading to steric effect [75].

c) In oxidative DNA cleavage reaction, metal ions that involved in metal complexes react with H_2O_2 to produce the hydroxyl radical which attacks C_4 site of the sugar moiety and cleaves the DNA. So some metal complexes can induce redox mediated cleavage of DNA [70], like VO^{2+} complex but only at higher concentration 10 mg/ml and Fe(III) complex at lower concentration 0.01 mg/ml. The generated reactive oxygen species lead to oxidative stress and DNA damage [73].

d) Different activated oxygen forms are generated during metabolism involving reactive oxygen species (ROS) like, free radical (O_2^-), non-free radical (H_2O_2) and hydroxyl radicals (OH•). These species are the main cause of cancer, coronary heart and Alzheimer's disease. Antioxidants are considered as species which save human body from oxidation by the reaction with active oxygen species and act a free radical scavenger [76]. Attachment of hydroxyl group on the aromatic rings makes the ligand to be a free radical scavenger to help in treatment of disease in a relation with free radical damage. The antioxidant activity of organic ligand and its metal complexes is dependent on the nature of ligand itself (having conjugation and free radical scavenging ability that represented in type of function group), reactivity, ionization potential and the geometry [75]. Thiamine ligand and its three metal complexes (Fe(III), Se(IV) and VO^{2+}) were examined as antioxidants. The free radical scavenging ability was determined. The antioxidant activity of thiamine ligand cannot be neglected due to its structural features with different coordination sites, like, -OH, -S-, -NH- and C=N which participate in antioxidant activity through donation of an electron or hydrogen [75]. Antioxidant activity results showed significant effects for VO^{2+} complex with (IC_{50} = 0.081 mg/ml) and Se(IV) complex with (IC_{50}=0.086 mg/ml) compared to ligand (IC_{50}=14.8 mg/ml). VO^{2+} is the most potent and considered as a beneficial antioxidant attributed to the presence of four water molecules in VO^{2+} complex (2:1) and two water molecules in Se(IV) complex in the inner sphere, leading to extra proton transfer. While Fe(III) showed effect (IC_{50}=0.11 mg/ml) compared to ligand attributed to the introduction of three chloride ions with two water molecules with a steric effect induced by the octahedral geometry hindering the approach of free DPPH radical toward the active centers of the Fe(III) complex (either Fe(III) metal ion or the functional groups) leading to lower antioxidant effect [75].

e) The observed toxicity of concentrations of 10, 5, 1 and 0.5 mg/ml vanadium complex through the hemolysis examination is mainly related to degree of oxidation state and the type of the coordinated organic ligand [(73)], also its high concentration may cause toxicity, in contrast with the lower concentrations that showed anti-hemolytic effect. From IR spectra one can observe that both Se(IV) (1:1) and VO^{2+} (2:1) complexes coordinated through the ($N_{1'}$) and sulphur atom sites compared with Fe(III) complex which coordinated to only sulphur atom. So, thiamine acted as a bidentate ligand in both Se(IV) and VO^{2+} complexes leading to higher biological activity, while as monodentate in Fe(III) complex with lower activity. The significant activity of VO^{2+} complex (2:1) may be related to the introduction of dinuclear atom to both $N_{1'}$ and sulphur atom sites. According to thermal analysis, the activation energy (ΔE) of Fe(III) complex is higher than that of ligand and VO^{2+} complex (2:1) indicating the thermal stability of Fe(III) complex leading to lower biological activity.

f) The antibacterial and antifungal activity were examined for thiamine ligand and synthesized eleven metal thiamine complexes. Some of the studied complexes showed a moderate antimicrobial activity compared to the free ligand. Thiamine ligand did not show any antimicrobial activity toward all tested microorganisms. Hg(II) complex is the most effective against the two positive, two negative bacteria and fungi than other complexes, due to the ability of the Hg(II) ion and organomercurials (highly lipid soluble) to rapidly passing through the biological membranes. The toxicity effect of mercury specially toward *E. coli* was reported [77]. Se(IV) complex showed moderate activity toward both positive bacteria *S. aureus* and *B. subtilis* and one negative bacteria *E. Coli.* Co(II) complex showed moderate activity towards *S. aureus* and *Candida albicans.* Cu(II) and VO^{2+} complexes exhibited moderate activity towards only *S. aureus*. While, Mn(II) complex showed activity toward only *E. coli.* Fe(III), Ni(II), Zn(II), Pd(II) and tungsten complexes were inactive toward all the tested microorganisms, due to their higher stabilities and decomposition at higher temperatures based on thermal analysis.

These compounds showed great activities as the metal ions enhanced the activity compared to ligand which indicates that the coordinated metals influence the antibacterial effects. The higher activity of some complexes compared to the ligand can be explained by the chelation theory that shows that the decrease in the polarizability of the metal ion gives extent to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with the donor groups. This enhances the delocalization of p- and d-electrons over the whole chelate and increase the lipophilicity of the complexes [78] which enhances the penetration of the complexes into lipid layer of bacterial membranes causing death of the organisms. Also the increased activity of the complexes may be due to the size of metal ion [79].

The variation of the efficiency of the different complexes against different organisms depends either on the impermeability of the microorganism cells or difference in ribosome of microbial cells. Furthermore, metal complexes form hydrogen bonds with the active centers of different cellular constituents resulting disturbance of the normal cellular processes [73,76,80]. So, the results show that the introduction of transition metal ions to the ligand show greater activity than the ligand itself. The biological activity of the tested metal complexes is mainly dependent on the nature of metal ion center and steric effect of the complex.

CONCLUSION

Metals were proved to be a good chelating agents and thiamine was considered as useful ligand in the formation of metal thiamine complexes. Biological changes in thiamine were seen after combining with metals. It was concluded that the new synthesized, Fe(III), VO^{2+} and Se(IV) thiamine complexes can be used as an antimicrobial against different bacterial and fungal species, as anticancer agents in cancer disease treatment. They also are effective anti-diabetic compounds that can improve glucose metabolism (transport glucose to cells), treat hyperglycemia, act as a good regulator for insulin and can also alleviate other diabetes mellitus symptoms and are suggested to improve lipid metabolism, especially Fe(III) complex, avoid abnormal buildup of fats in the body and decrease the triglyceride and cholesterol level in blood. Fe(III), VO^{2+} and Se(IV) thiamine complexes acted as significant antioxidants. Those complexes can be used as anti-inflammatory agent and selective inhibitors for nitric oxide species to avoid inflammations and infections and can help in treatment of Alzheimer disease (AD) by maintaining the levels of acetylcholine through inhibition of acetyl cholinesteras and monoamino oxidase enzyme. The three complexes had no hemolytic effect on the red blood cells, which means that they are safe.

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