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The dual therapeutic effect of metformin nuclei based drugs modified with one of Tulbaghia violacea extract compounds

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Abstract

Novel Schiff base was synthesized from condensation reaction of metformin with [4-(Diethylamino) benzaldehyde (NBM). Different metal complexes were prepared using Pd(II), Pt(II), Cu(II) and V(IV) metal ions. All complexes showed the non-electrolytic behavior. So, the expected molecular formulas for complexes are [Pd(NBM)Cl₂], [Pt(NBM)Cl₂], [Cu(NBM)₂Cl₂] and [VO(NBM)₂].

The cytotoxicity of (NBM) Schiff base and its metal complexes on human cancer cell line, MCF-7, was investigated. V(IV) and Cu (II) complexes showed potential blood-glucose lowering effect higher than the commercial metformin drug. VO(IV) complex has superior antioxidant activity more than the other synthesized compounds and the standard ascorbic acid. Molecular docking investigation proved the presence of interesting interactions between all synthesized compounds with the active site amino acids of EGFR tyrosine kinase (anticancer activity). The molecular docking of metal complexes observed effective inhibition for the specific mTOR protein that is expected to aid the growth of the COVID-19 virus.

Keywords: Vanadium (IV) and platinum (II) complexes, tyrosine kinase, Metformin, COVID-9 virus.

1. Introduction

Metformin, an antidiabetic inexpensive drug. In 1995, Metformin was approved by the Food and Drug Administration (FDA) as an oral hypoglycemic agent. Recently, several studies reported[1,2] the potential efficacy of metformin as a promising drug for treating polycystic ovary syndrome, cancer, aging, cardiovascular diseases, metabolic syndrome, and neurological diseases. In addition, it is used off-label for weight reduction in the USA [3]. Recent evidence pointed out novel behaviors of metformin in the treatment of reduced macrophage cytokines synthesis and autoimmune disease [3]. Also, it may have an inhibitory effect on the virus, through increasing insulin sensitivity [4]. (Diethylamino)benzaldehyde was identified in GC-MS analysis of *Tulbaghia violacea* extract (TVL), Data obtained demonstrated the hypoglycemic effects of TVL in STZ-induced diabetic rats [5]. The most effective metal ions used are chromium, manganese, copper, cobalt, zinc and vanadium. So, it is expected that Schiff bases produced from the condensation between metformin drug and 4-(Diethylamino)benzaldehyde will be effective hypoglycemic drugs specially when form complexes. Cisplatin is one of the most widely used anticancer drugs [6,7] and highly effective in the treatment of testicular and ovarian cancer. Therefore, complexes like cisplatin which were formed using the previous synthesized Schiff bases with platinum (II) and palladium (II) ions expected to have powerful antitumor effect [8-11]. ‘Metformin in COVID-19: A possible role beyond diabetes’. Because it was discovered that metformin not only has immunomodulatory and antiviral activities but also prevents various acute lung injuries in animal models [3, 12].

So, the present work aims to study, the synthesis of novel Schiff base derived from metformin with (Diethylamino)benzaldehyde and its different metal complexes specially Pt(II) and V(IV) in many bio-application fields as antidiabetics, antitumor, antioxidant and finally their molecular docking as antiviral against COVID-19.

2. Experimental

2.1. *Materials and Reagents*

4-(Diethylamino)benzaldehyde, Metal chloride salts [platinum (II), palladium & copper (II)], vanadyl (IV) sulfate, methanol and all chemicals used in this investigation were provided by Aldrich. Metformin–HCl was purchased from (El-Nasr Company) for Pharmaceutical Chemicals, Cairo, Egypt.

2.2. Synthesis

2.2.1. Synthesis of Schiff-Base and its metal complexes

A methanolic solution of 4-(Diethylamino)benzaldehyde (1 mmol) is added drop wisely to the (1 mmol) metformin solution that is dissolved in (10 ml) methanol. Over a water bath, the mixture is refluxed with continuous stirring for two hours after the addition of a few drops of NaOH. The solution turns to yellow color. The formed pale-yellow product of the synthesized Schiff base (NBM) was washed with a mixture composed of (water and methanol) then dried under vacuum and finally recrystallized from methanol.

Palladium (II) complex $[\text{Pd}(\text{NBM})\text{Cl}_2]$ was prepared by heating PdCl_2 (1.0 mmol) and KCl (2.0 mmol) in the least amount of water to 70°C with stirring. The clear solution of $[\text{PdCl}_4]^{2-}$ solution was cooled to 25°C , filtered and methanolic solution of the prepared Schiff-base (NBM) (1.0 mmol) was added to the stirred solution. An orange crystalline precipitate was obtained; yielded 92%. $\text{C}_{15}\text{H}_{24}\text{Cl}_2\text{N}_6\text{Pd}$ (465.72): calcd. C 38.69, H 5.19, N 18.05; found C: 38.01, H 4.90, N 17.42.

The remaining metal complexes were formed by using the following procedure. Solution of metal chloride (1 mmol) in 5 mL of methanol was added dropwise to a methanolic solution of the prepared Schiff-base (NBM) (2 mmol) for (Cu(II) & V(IV)) and (1 mmol) in case of Pt(II). Every mixture was refluxed on a water bath for two hours with stirring. The three metal complexes prepared are $[\text{Pt}(\text{NBM})\text{Cl}_2]$, $[\text{Cu}(\text{NBM})_2\text{Cl}_2]$ and $[\text{VO}(\text{NBM})_2]$.

All complexes were filtered, washed and dried as described in Schiff base preparation.

First one $[\text{Pt}(\text{NBM})\text{Cl}_2]$ was a brown crystalline precipitate; yielded 80 %. $\text{C}_{15}\text{H}_{24}\text{Cl}_2\text{N}_6\text{Pt}$ (554.383): calcd. C: 32.50, H 4.36, N 15.16; found C: 31.71, H 4.20, N 14.42.

The second $[\text{Cu}(\text{NBM})_2\text{Cl}_2]$ was a pale blue crystalline precipitate; yielded 75 %. $\text{C}_{30}\text{H}_{48}\text{Cl}_2\text{CuN}_{12}$ (711.244): calcd. C: 50.66, H 6.80, N 23.63; found C: 50.56, H 6.10, N 22.78.

The third $[\text{VO}(\text{NBM})_2]$ was a green crystalline precipitate; yielded 80 %. $\text{C}_{30}\text{H}_{46}\text{N}_{12}\text{OV}$ (641.723): calcd. C: 56.15, H 7.23, N 26.19; found C: 55.56, H 6.10, N 25.78.

The suggested chemical structures of Schiff base and its complexes were represented in (Figure 1).

(Figure 1) The chemical structures of (NBM) and its metal complexes

2.3. Apparatus and programs

Automatic analyzer CHNS Vario EL III-Elementar, Germany. Test scan Shimadzu FTIR spectrometer using KBr disc technique. The spectra were collected in the range 250-4000 cm^{-1} . ^1H -NMR spectra were recorded using 300 MHz Varian-Oxford in DMSO-d_6 as a solvent and TMS as an internal standard. Automated UV/Vis-NIR 3101 PC Shimadzu spectrophotometer ranged from 200-900 nm for measuring electronic absorption spectra in DMF. The conductivity meter ORION model 150 of 0.6 cell constant for measuring molar conductivity of metal complexes. Mass GC-2010 Shimadzu instrument for determination mass spectra of the synthesized compounds. The molar magnetic susceptibility was measured on powdered samples using the Faraday method. The diamagnetic corrections were made by Pascal's constant and $\text{Hg}[\text{Co}(\text{SCN})_4]$ was used as the calibrant. Molar conductivity of metal complexes was measured for 1.00×10^{-3} M DMSO solutions at $25 \pm 1^\circ\text{C}$ using the conductivity meter.

MOE 2009 (Molecular Operating Environment) software was used to simulate every enzyme inhibitor. EGFR tyrosine kinase is the selected protein for anticancer activity (PDB ID: 1M17).

The cytotoxicity of all complexes was determined by Vichai and Kirtikara assay [13] using Sulphorhodamine-B (SRB) is a bright pink aminoxanthrene dye has two sulphonic groups. SRB is a protein stain that attaches to the amino groups of intracellular proteins under mildly acidic conditions to provide a sensitive index of cellular protein content. The Cells were seeded in 96-well Microtiter plates at an initial concentration of 3×10^3 cell/well in a 150 μl fresh medium and left for 24 hours to attach to the plates. 0, 5, 12.5, 25, 50 $\mu\text{g/mL}$ five different concentrations of each drug were added using three wells for each drug concentration. The incubation time for plates was 48 hours. The cells

fixation time was 1 hour at 4 °C with 50 µl cold trichloroacetic acid 10% final concentration. At room temperature, the plates stained with 50 µl 0.4 % SRB dissolved in 1 % acetic acid for 30 minutes. The wells measurements spectrophotometrically at 570 nm were done by using an ELISA micro plate reader (Sunrise Tecan reader, Germany). The mean value of the background absorbance was automatically subtracted and of drug concentration was calculated. Doxorubicin was used as standard liver antitumor drug. The cell survival percent was calculated by:

Surviving fraction = optical density (O. D.) (Treated cells) / O. D. (Control cells).

The IC₅₀ values (the concentrations required to produce 50% inhibition of cell growth) were also calculated.

The antioxidant activity of all complexes was studied using spectrophotometric technique with 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. It gives a purple solution in methanol. The radical scavenging potentials of the complexes with DPPH radical were evaluated as described [14]. The tested drugs are prepared in different concentrations. Spectrophotometrically at 517 nm, the absorbance of the mixture produced from mixing DPPH and each drug was measured. All test analysis was performed in triplicate. Ascorbic acid (Vitamin C) was taken as the standard drug. DPPH radical scavenging activity (%) was calculated.

2.4. *Animals*

60 Female Wistar rats (8 weeks, 180–250 g), were kept in climate-controlled room in the animal house of National Research Centre at air-conditioned room a maintained temperature and relative humidity (23 ± 25°C, 35– 60%) respectively with 12h light- dark cycle, The rats were fed with pellet diet and water.

2.4.1. Induction of diabetes

Diabetes mellitus (type 2 diabetes) was induced by intraperitoneal (i.p.) single dose of streptozotocin (STZ) in overnight fasted animals. STZ was freshly prepared (40 mg/Kg body weight) dissolved in 0.1M citrate buffer (pH 4.5) immediately before use [15-17]. As seen in (Figure 2), after injection, animals had free access to food and water and were given 5% glucose solution to drink overnight to counter hypoglycemic shock[18]. After 48hrs, body weights as well as blood glucose concentrations of the STZ-injected rats were measured. The samples were taken from the tail vein after 12hrs overnight fasting conditions. Hyperglycemia was achieved by high glucose level in plasma using a digital glucometer (One Touch Ultra). Rats with blood glucose concentrations greater than 250

mg/dl were considered to be diabetic. Also, blood glucose level and body weights of the rats were measured by the same method every week during the period of experiment.

(Figure 2) Streptozotocin (STZ) injection in rats.

2.4.2. Experimental design

Rats were divided into eight groups (10 rats in each group) according to the protocol mentioned in (Table 1). Groups 4 - 6 orally injected with the understudied complexes for 30 days (50 mg/Kg body weight; the same dose of metformin). At the end of the experiment, the animals were fasted overnight (12-14 hours), the blood samples were taken from each animal in all groups into sterilized tubes for serum separation. Serum was separated by centrifugation at 3000×g at 4°C for 10 minutes using refrigerated centrifuge (sigma 2K15 U.S.A.) and stored at (−80°C) for further biochemical analysis. After blood collection, all rats of each group were sacrificed under ether anesthesia, the organs (pancreas, liver and kidney) from different animals were immediately removed weighed and washed from blood by ice-cold isotonic saline. A piece of liver was immediately frozen at (−80°C) for biochemical analyses.

2.4.3. Preparation of liver homogenate

Liver tissues were homogenized in either 10 volumes of ice cold bidistilled water (for reduced glutathione "GSH") or 10% trichloroacetic acid (for nitric oxide "NO" and lipid peroxidation "MDA") using an electrical homogenizer (Janke & Kunkel, IKA-WERK, Germany). The homogenates were centrifuged at 3000×g for 15 minutes at 4 °C, the supernatant was collected and aliquoted in Eppendorf tubes and stored at (−80°C). The supernatants were used for different biochemical tissue analysis.

2.5. Biochemical studies

Nitric oxide (NO), lipid peroxidation (MDA) and reduced glutathione (GSH) were measured in liver homogenate using Biodiagnostic kit. Determination of creatinine and urea were assayed in serum by biodiagnostic kit methods.

2.6. Statistical Analysis

Data were analyzed by comparing values for different treatment groups with the values for individual control. All data were expressed as mean $\pm$ standard error of 5 rats in each group. Significant differences between the groups were statistically analyzed using one-way analysis of variance, ANOVA using the spss16 computer program. Differences were considered significant at $p \leq 0.05$.

3. Results and discussion

IR spectrum of novel Schiff base in NBM shows the lack of the NH_2 stretching band characteristic of metformin at 3371 cm^{-1} with the presence of new strong $\text{C}=\text{N}$ stretching band at 1670 cm^{-1} . In addition to strong splitted band at $(1643 \text{ \& } 1597)\text{ cm}^{-1}$ due to $\nu(\text{C}=\text{NH})$ imine group of metformin. This suggests the condensation of the aldehyde group and the metformin's primary amino group forming the desired Schiff base (NBM) [19]. (NBM) ligand showed a strong band at $(3332\text{--}3043)$ is associated with the presence of three imine groups. Previously, these bands were observed in the spectra of some complexes with biguanide Schiff-bases [20]. Further, bands at $(1485 \text{ \& } 1269)\text{ cm}^{-1}$ in the IR spectrum of the prepared ligand were observed due to asymmetric and symmetric $\nu(\text{N}-\text{C}-\text{N})$ [19] while, the aromatic $\nu(\text{C}-\text{H})$ are observed at 2974 cm^{-1} . IR spectra of all complexes sthe how intense band at about 3200 cm^{-1} region assignable to the $\text{N}-\text{H}$ group stretching vibration [21and 20]. The presence of the IR spectral bands of the imine group ($-\text{C}=\text{NH}$) in complexes are shifted in comparison to the free ligand with significant intensity. This indicates that the ligand is linked to the metal ions through the nitrogen atom of the imine group. The strong band related to $\nu(\text{C}=\text{N})$ is shifted to a smaller frequency value in all synthesized complexes, meaning its involvement in coordination [22]. Vanadyl complex has a new band at 1701 cm^{-1} that isn't observed in its parent Schiff base or other complexes. It can be explained due to the induced ionization for one of the imine group ($-\text{C}=\text{NH}$). A band appearing at $1571\text{--}1556$ and $1276\text{--}1275\text{ cm}^{-1}$ interval has been specified to $\text{N}-\text{C}-\text{N}$ stretching of symmetric and asymmetric types respectively [23]. The confirmation of metal-ligand bonding is observed by the newly formed bands which are tentatively assigned to $\nu(\text{M}-\text{N})$ and $\nu(\text{M}-\text{Cl})$ [24]. $\nu(\text{M}=\text{O})$ band was observed in case of Vanadyl complex only [19 27]. All characteristic bands were observed in (Table 2).

$^1\text{H NMR}$ for the suggested ligand gave two types of aromatic protons at $7.137\text{--}7.108$ and $6.661\text{--}6.605$ ppm while the NH proton is given as a sharp singlet peak at 9.6 ppm (D_2O exchangeable). The peaks obtained at 7.6 and 8.2 ppm are attributable to the two amide protons $\text{C}=\text{NH}$ (D_2O exchangeable).

The peak recorded at 5.57 ppm is assigned to the azomethine proton [25] while the signal for the methyl protons was observed as a singlet at $\delta=3.25$ ppm. The peaks recorded at $\delta=3.55$ and 1.112 ppm are assigned to the CH_3 and CH_2 of N(Ethyl) group respectively.

After the complexation of NBM ligand, we have two changes in the chemical shifts corresponding to one of the amide protons $\text{C}=\text{NH}$ that placed at $\delta=8.2$ ppm and the azomethine proton. It may be explained due to the involvement of both sites of donation in the coordination process. The new values of the amide protons present become at $\delta=8.53$ and 8.456 ppm for Pd(II) and Pt(II) complexes respectively. But their azomethine protons become at 5.62 and 5.73 ppm respectively.

All (Pd(II) & Pt(II)) complexes are characterized using ^1H NMR technique with the comparison between the complex chart and its parent Schiff base as shown in (Figures 3 and 4). The paramagnetic properties of Cu(II) and VO(IV) are troublemakers due to the fast-nuclear spin relaxation induced from their unpaired electrons that significantly broadens their NMR resonances[26 and 27].

The mass spectra are represented to confirm the coincidence between the molecular ion peaks and the formula weights. The results are tabulated in (Table 3), the equations represent the most important fragmentations are seen in (Figure 5). The complexes continue $\text{HN}(\text{CH}_3)_2$ species, Cl^- and the rest of the Schiff-base. The most abundant fragmentations are mentioned in (Figure 6)

Based on the **conductivity**, values below $50 \Omega^{-1} \text{ ohm}^{-1} \text{ cm}^2$ in DMSO solution are for nonelectrolytes [28 and 29]. The molar conductance (Λ_M) values for all prepared complexes are very low indicating that, these complexes are nonelectrolytes and the chloride ion is coordinated in Pd(II), Pt(II) and Cu(II) complexes. For vanadyl complex, the NBM ligand was undergo induced ionization of the amide proton $\text{C}=\text{NH}$ to make complete neutralization for the metal cationic charge. It was supported before in IR study.

Pt(II) and Pd(II) complexes have 0.0 B.M. magnetic moment values due to the diamagnetic properties of the d^8 square planar geometry[30 and 31]. Copper(II) complex is expected to be in the distorted Octahedral geometry[32] because its measured magnetic moment value is (1.91 B.M.). The measured value is slightly higher than the spin-only value 1.73 B.M. expected for one unpaired electron but falls within the range normally observed for hexa-coordinate Cu(II) complexes[33].

Vanadyl complex is paramagnetic in the solid-state with the electronic configuration $[\text{Ar}]3d^1$. Vanadium(IV) has one unpaired electron with a spin-only formula predicting a magnetic moment of 1.73 B.M. The experimental values are in the range of 1.79 B.M. Some monomeric pentacoordinate vanadyl Schiff-bases complexes recorded the previous value [34-37] and are consistent with square pyramidal geometry around the central metal ion [38].

(Figure 3) ¹H NMR chart of NBM Schiff base

(Figure 4) ^1H NMR chart of Pd-NBM and Pt-NBM complexes respectively

(Figure 5) Mass spectral charts

(Figure 6) Some mass fragmentations

Antidiabetic Investigation

Hyperglycemia is a state in which a large amount of glucose circulates in the blood plasma. The blood sugar level is more than 11.1mmol/L (200mg/dK), but symptoms may not start to be noticeable until even higher values such as 15-20mmol/L (~ 250-300 mg/L) [39]. Hyperglycemia treatment needs to remove the underlying cause, example diabetes. Direct administration of insulin; in most cases; is used for treatment of acute hyperglycemia while oral hypoglycemic therapy and lifestyle modification is used for severe hyperglycemia [39].

The results in (Table 4) show a significant 4-fold increase in blood glucose levels in STZ administrated rats in comparison to normal control rats. Elevation in blood glucose was decreased; nearly normalized; in the treated animals with metformin, NBM Schiff base and its, Vanadyl (IV) and Copper (II) complexes. STZ- diabetic animals had significantly lower ($P \leq 0.05$) body weight and higher kidney weight as well as kidney hypertrophy as compared to those in the normal control group (Table 5). In addition, the different treatment given to diabetic animals ameliorated kidney hypertrophy index and kidney weight after the comparison with STZ-diabetic rats.

The elevation in blood glucose level in STZ-diabetic rats may be attributed to the loss of insulin effect on liver leads to glycogenolysis and increase in hepatic glucose production [39]. Moreover, STZ- diabetes induction leads to inflate and ultimately degenerate the Langerhans islets of β -cells and consequently, hydrogen peroxide and hydroxyl radicals are generated which have an effective role in the cytotoxicity of STZ. The blood glucose levels reduction in all treated groups may be due to gluconeogenesis and the regulation of serum lipid levels. As displayed in (Table 6), the levels of NO and MDA in STZ-diabetic group were significantly increased ($P \leq 0.05$) compared with normal control group but the level of GSH decreased significantly ($P \leq 0.05$) in diabetic animals. The elevation of NO probably due to hyperglycemia-induced oxidative stress. Also, the increase in the levels of NO might be via apoptosis.

In a good agreement with our results El-Baz et al. declared that free radicals act by lipid peroxidation led to release of MDA in a large amount. Thus, MDA concentration in the liver and plasma can detect about the cell damage and apoptosis in diabetic patient and/or animals, which in turn effect on liver and pancreatic tissues showing marked hepatocyte ballooning and hydropic degeneration as well as disarrangement changes in pancreatic blood vessels and interlobular duct. GSH is considered one of the most known non-enzymatic bio-molecules found in tissues that reduce the effect of diabetes via scavenging of free radicals or alter their conversion to hazard products.

After the treatment of diabetic animals with different drug supplementations, the levels of both NO and MDA were significantly decreased, whereas GSH levels were significantly increased ($P \leq 0.05$)

(Table 6). This improvement may be explained due to the elimination of the oxidative stress produced during induction of diabetes. The study of renal function (creatinine and urea) shows highly significant differences in renal function results between the STZ-diabetic rats and normal control group (Figure 7). The present results are in accordance to the work done by Al-Ali et al. [40]. The increase in urea levels may be referred to stimulated protein catabolism and elevation of amino acids for gluconeogenesis. It may also be signs of injury at the glomerular and tubular levels of the kidney [41]. Also, the elevation of creatinine levels in diabetic rats may be accompanied by impaired role of the nephrons [42]. The compounds under test effectively down-modulate renal function. The amelioration in the studied parameters may be referred to controlling blood glucose levels leading to remove of reactive oxygen species (ROS); which are involved in the etiology of several diabetic complications including diabetic nephropathy.

(Figure 7) Effect of new metformin complexes on kidney function of STZ- diabetic rats. Data are expressed as mean $\pm$ SE 5 rats in each group, data are expressed as mg/dl for creatinine (mean $\times$ 100) and urea, a is the level of significance at $P \leq 0.05$ compared with control group, b is the level of significance at $P \leq 0.05$ compared with diabetic group.

Antioxidant Activity

The attack of reactive species like free radicals for body cells causing several oxidative damages diseases.

The importance of studying the antioxidants was increased with increasing the human exposure to free radicals' pollution [43]. Metal-derived antioxidant is a recent type that received attention to prove these compounds have a high capacity in scavenging free radicals.

DPPH (1,1-diphenyl-2-picrylhydrazyl) is stable nitrogen radical and the most famous free radicals used in vitro antioxidant activity where it has an odd electron number with a strong absorption band at 517 nm. The absorption decreases stoichiometrically relative to the number of electrons taken up [44 and 45] by pairing off this electron. On the other hand, DPPH also is decolorized by reducing agents as well as H-transfer. So, we can use DPPH as a substrate to determine the antioxidant activity. We used ascorbic acid (Vitamin C) as standard.

The antioxidant activities of our compounds with ascorbic acid as the standard drug were performed depending on the free radical scavenging effect of the stable DPPH free radical activity. Also, we must calculate IC_{50} values to test the real strength of tested samples, which define as the concentration needed to diminish certain activity at its half. The investigated changes in the free radical scavenging activity of the test compounds on the basis of percent inhibition are displayed in (Figure 8). From these data, it is clear that the superior complex in antioxidant activity is VO(IV) complex and its activity is more than that of the standard (ascorbic acid). Generally, vanadium element has the highest number of the oxidation states where it contains four common oxidation states (+2, +3, +4 and +5), so, it facilitates the donation of an electron to the free radical in vitro therefore, it acts as the strongest reducing agent and oxidized easily. The oxidation state of vanadium element is +4 and acts as an electron donor compound. It can reduce the DPPH radical to DPPH (α -diphenyl- β -picrylhydrazine) compound, and the VO(IV) ion will be oxidized to its +5 state (from $V(IV)O^{2+}$ to $V(V)O^+$) easily [46]. So, this result approves that the modifying of the nucleus of free ligand by the metal has high effective in improvement of the antioxidant activity subsequently, treatment of cancer. The increased antioxidant activity of our metal complexes than parent Schiff base ligand due to electron withdrawing effect of metals which facilitates the discharge of hydrogen to reduce the DPPH. Specially, our compounds have many proton sources. The hydrogen atom will then be abstracted by free radicals, resulting in a stable radical. It is further confirmed by the observed yellow solution produced from the discharge of purple DPPH radical solution, showing scavenging of the DPPH radicals by hydrogen donation.

(Figure 8) Scavenging activity of NBM compounds on DPPH radical.

Cytotoxicity and molecular docking Investigation:

Generally, Cancer is a generic term for a large group of diseases that can affect any part of the body. One of the most frequent malignancy in females is breast cancer. Platinum (Pt) drugs are the most successful class of inorganic medicinal compounds used to treat cancer [47 and 48]. Numerous metals specially Pd (II) and Cu (II) complexes have promising activity against tumor cell lines have been synthesized and published [49-54]. In addition to vanadium which has a potential role in tumor growth inhibition and in prophylaxis against different experimental cancer models like; liver cancer, colon cancer, breast cancer and others and in various types of malignant cell lines.

The cytotoxicity of Schiff bases and their metal complexes on human cancer cell line, MCF-7, was ascertained by exposing cells to the medium with varying concentrations of compound (1–50 $\mu\text{g mL}^{-1}$). The results can be seen in (Figure 9). After incubation with complexes a decrease in cell proliferation, it's clear that the activity of metal complexes is higher than that of free ligand (NBM). This indicated an amelioration of the antitumor activity after coordination. This improvement may be related to the positive charge of the metal increasing the acidity of the coordinated ligand that bears protons, causing stronger hydrogen bonds which enhance the biological activity. It seems that

changing the coordination sites and the nature of the metal ion has a clear effect on the biological activity by altering the binding ability of DNA. Mechanistically, these drugs forming M-DNA complexes that cause DNA damage that accumulate to a point that is beyond repair, finally, leading to cell death [55 and 56]. Vanadium and copper complexes show a higher cytotoxic efficiency by their lower IC_{50} than the other investigated metal complexes. We can suggest, due to the larger probability of increasing the hydrogen bonds as both complexes were formed as 1 (M) to 2 (L). we can conclude from our results that vanadium complex have a stronger desired anti-tumor activity. So, it provides a guide to design new Synthetic complexes with effective results.

The antitumor docking study of the ligand and complexes are reported. The most important interaction is the formation of hydrogen bond. The different types of interactions are mentioned in (Table 7) and seen in (Figure 10).

(Figure 9) The surviving fraction of NBM ligand and its metal complexes against MCF7 breast cells.

(Figure 10) Binding affinity against EGFR tyrosine kinase receptor (PDB Code: 1M17)

Molecular docking study of inhibition effect of our compounds against mTOR protein.

mTOR, is a protein that helps the COVID-19 virus grow so contribute to its severity. So, our target to inhibit mTOR protein by our synthesized compounds. The inhibition was investigated by the study the interaction types and scoring energy between each compound by (4jsv) the protein downloaded from protein data bank. The different interactions are mentioned in (Table 8) and seen in (Figure 11). After investigation, we found that all derived compounds from metformin show better scoring energy and more inhibition of mTOR enzyme than the parent metformin. We noticed metal complexes were more effective in mTOR inhibition that expected to help in COVID-19 virus growing specially vanadium and copper complexes with different types of interactions as arene-cation or side chain acceptor explored the more effective interaction results to the selected protein.

(Figure 11) Binding affinity against mTOR protein receptor (PDB Code: 4jsv)

Table.1 Rats were divided into eight groups (10 rats in each group) according to the following protocol:

Group 1 (NC)	Healthy untreated rats as normal control
Group 2 (DC)	diabetic untreated control
Group 3 (M)	Diabetic rats treated orally with metformin (50mg/Kg body weight for 30days) ^[57]
Group 4 (NBM)	Diabetic rats +[NBM]
Group 5 (VO-NBM)	diabetic rats +[VO-NBM]
Group 6 (Cu-NBM)	diabetic rats +[Cu-NBM]

Table 2. The most important IR frequencies bands of NBM ligand and complexes (200- 4000 cm⁻¹).

Compound	$\nu(\text{C}=\text{N})$	$\nu(\text{C}-\text{N})$	$\nu(\text{N}-\text{C}-\text{N})$	$\nu(\text{M}-\text{N})$	$\nu(\text{M}-\text{Cl})$	$\nu(\text{M}-\text{O})$
NBM	1670, 1643, 1597	1269	1485,1442	-----	-----	-----
Pd(NBM)Cl₂	1597, 1527	1265	1411, 1357	308, 293	270,239	-----
Pt(NBM)Cl₂	1639, 1620, 1566	1284	1373, 1354	351, 324	266,246	-----
Cu(NBM)₂Cl₂	1604, 1527	1242	1381, 1334	339, 308	246,216	-----
VO(NBM)₂	1701, 1612, 1562	1296	1504	601	-----	983

Table 3. Some mass fragmentation patterns of ligand and its complexes.

Compound	m/z (Wt. loss)		Lost species
	Calcd.	Found	Ligand (L), Complex(C)
NBM	288.399	-----	-----
	72.111	70.96	0.24(L)
	141.178	140.05	0.48(L)
	163.264	162.09	0.55(L)
	260.345	260.09	0.89(L)
	274.372	271.12	0.94(L)
Pd(NBM)Cl₂	465.719	-----	-----
	44.050	44.08	0.09(C)

	69.938	69.17	0.15(C)
	72.081	71.15	0.15(C)
	202.134	202.22	0.43(C)
	449.024	448.43	0.96(C)
Pt(NBM)Cl₂	554.383	-----	-----
	69.938	69.17	0.12(C)
	72.08	71.15	0.13(C)
	148.113	148.29	0.26(C)
Cu(NBM)₂Cl₂	711.244	-----	-----
	72.131	69.35	0.10(C)
	98.072	96.9	0.13(C)
	369.005	369.9	0.51(C)
	413.055	412.8	0.58(C)
	513.136	514.21	0.72(C)
	551.242	551.34	0.77(C)

Table 4. Effect of new metformin derivative compounds on blood glucose level of STZ –diabetic rats

Blood glucose level (mg/dl)					
Compound	After Induction	1 st Week	2 nd Week	3 rd Week	4 th Week
g NC	99.2 ± 1.6	98.2 ±1.4	95.2 ± 1.6	97.0 ± 1.5	96.7 ± 1.7
g DC	373.0 ±15.2*	361.7 ± 12.6*	383.5 ± 5.9*	384.0 ± 9.9*	386.2 ± 7.8*
gM	390.0 ± 8.9*	334.7± 17.0*	248.2 ± 14.6 ^{*a}	172.2 ± 4.3 ^{*a}	129.2 ± 2.6 ^{*a}
g NBM	391.5 ± 9.3*	369.5 ± 9.1*	252.5 ± 8.4 ^{*a}	164.2 ± 3.1 ^{*a}	132.2 ± 2.7 ^{*a}
gVO-NBM	391.2 ± 5.5*	361.0 ± 7.2*	238.7 ± 7.1 ^{*a}	154.0 ± 3.3 ^{*a}	125.2 ± 2.2 ^{*a}
g Cu-NBM	373.3 ± 4.9*	356.7 ± 8.2*	237.7 ± 6.9 ^{*a}	188.5 ± 4.3 ^{*a}	132.2 ± 3.4 ^{*a}

Data are expressed as mean ±SE 5 rats in each group, * is the level of significance at P≤0.05 compared with normal control group, ^a is the level of significance at P≤0.05 compared with diabetic group.

Table 5. Effect of new metformin derivative compounds on body weight, kidney weight and kidney hypertrophy of STZ –diabetic rats

Groups	Body weight (gm) 4th week	Kidney weight (gm)	Kidney hypertrophy (kidney wt / body wt) %
<i>g NC</i>	188.7 ± 2.5	1.23 ± 0.015	0.65 ± 0.011
<i>g DC</i>	122.5 ± 1.8*	1.64 ± 0.025*	1.33 ± 0.006*
<i>gM</i>	162.7 ± 3.4 ^a	1.32 ± 0.022 ^a	0.81 ± 0.025 ^a
<i>g NBM</i>	156.5 ± 3.6 ^a	1.45 ± 0.023 ^a	0.93 ± 0.034 ^a
<i>g VO-NBM</i>	166.0 ± 2.1 ^a	1.36 ± 0.014 ^a	0.81 ± 0.011 ^a
<i>g Cu-NBM</i>	165.7 ± 2.7 ^a	1.29 ± 0.035 ^a	0.77 ± 0.024 ^a

Data are expressed as mean ±SE 5 rats in each group, * is the level of significance at P≤0.05 compared with normal control group, ^a is the level of significance at P≤0.05 compared with diabetic group

Table 6. Effect of new metformin derivative compounds on antioxidant markers of STZ- diabetic rats

Groups	NO (μmol / ml)	MDA (nmol / ml)	GSH (mmol / ml)
<i>g NC</i>	13.74± 0.68	0.288±0.006	110.5± 4.11
<i>g DC</i>	34.00± 1.47*	0.682±0.003*	43.49±2.73*
<i>gM</i>	21.10± 1.09 ^a	0.342±0.003 ^a	93.50± 2.39 ^a
<i>g NBM</i>	23.70± 1.98 ^a	0.402±0.003 ^a	85.50±4.94 ^a
<i>g VO-NBM</i>	22.50± 1.44 ^a	0.354±0.006 ^a	93.50±2.39 ^a
<i>g Cu-NBM</i>	22.75± 1.65 ^a	0.350±0.003 ^a	87.50±1.84 ^a

Data are expressed as mean ±SE 5 rats in each group, * is the level of significance at P≤0.05 compared with control group, ^a is the level of significance at P≤0.05 compared with diabetic group.

Table 7. Binding affinity of complexes against EGFR tyrosine kinase receptor (PDB Code: 1M17)

Antitumor docking 1M17		
Compound	Involved amino acids	Type of interaction
NBM	Thr-830 and Lys-721	Side chain donor & side chain acceptor
Pd-NBM	Lys-721	Side chain acceptor
Pt-NBM	Phe-699 and Asp-831	Arene-cation interaction and (side chain donor& metal contact)
Cu-NBM	Leu-694	backbone donor
VO-NBM	Arg-817	Arene-cation interaction and backbone donor

Table 8. Binding affinity of complexes against mTOR protein receptor (PDB Code: 4jsv).

Docking 4jsv		
Compound	Involved amino acids(scoring energy kcal/mol)	Type of interaction
Metformin	Asp-1632 and Ser-1584(-1.63)	Side chain acceptor and backbone acceptor
NBM	Ser1582 and lys1422(-4.57)	Metal solvent contact, Side chain acceptor and Backbone acceptor
Pd-NBM	Ser-1584, Glu-1631and Tyr-1587(-6.80)	Metal solvent contact, Side chain acceptor and Arene-arene interaction
Pt-NBM	Ser-1584 (-5.99)	Metal solvent contact
Cu-NBM	Ser-1584 and Lys-1635(-9.56)	Side chain acceptor and Arene-cation interaction
VO-NBM	His-1454, Asn-1421 and Pro-1426(-9.04)	Side chain acceptor and Backbone acceptor

4. Conclusion

In this work the Schiff bases were prepared from condensation reaction of metformin non-expensive drug with aldehydic compound [4-(Diethylamino)benzaldehyde(NBM)]. Different metal complexes were prepared using Pd(II), Pt(II), Cu(II) and V(IV) metal ions. Vanadium (IV) and Cu (II) complexes derived from (NBM) Schiff base showed higher cytotoxic activity against breast cancer cell lines than the parent ligand or other metal complexes. Also, both complexes have a potential blood-glucose-lowering effect higher than the commercial metformin drug. VO(IV) complex has superior antioxidant activity more than the other synthesized compounds and the standard ascorbic acid. The high activity can be related to the large number of its variable oxidation states. Molecular docking investigation proved that; all synthesized compounds had interesting interactions with the active site amino acids of EGFR tyrosine kinase (anticancer activity). The most interesting result in this work is the pioneer of vanadium, copper, platinum and palladium complexes in inhibition of mTOR protein expected to be responsible for COVID-19 virus growing.

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