The Evaluation of the Anti-Histone Deacetylase, Antibacterial, Antioxidant and Cytotoxic Activities of Synthetic N,N´-ethylenebis (α methylsalicylideneiminate) Schiff Base Derivatives

Mosalanezhad, Fatemeh
Department of Biology, Jahrom Branch, Islamic Azad University, Jahrom, I.R. IRAN

Asadzadeh, Azizeh
Department of Biology, Faculty of Science, NourDanesh Institute of Higher Education, Mymeh, Isfahan, I.R. IRAN

Ghanbariasad, Ali
Department of Medical Biotechnology, Fasa University of Medical Science, Fasa, I.R. IRAN

Naderpour, Mahboubeh; Bordbar, Razieh
Department of Biology, Jahrom Branch, Islamic Azad University, Jahrom, I.R. IRAN

Ghavamizadeh, Mehdi
Cellular and Molecular Research Center, Gerash University of Medical Science, Gerash, I.R. IRAN

Zolghadri, Samaneh*+
Department of Biology, Jahrom Branch, Islamic Azad University, Jahrom, I.R. IRAN

ABSTRACT: *Recently, Schiff base complexes as synthetic antioxidants are widely used instead of natural antioxidants because they are effective and cheaper. In this study, a series of α,ά-Me_2-salen, (N,N´-ethylenebis(α methylsalicylideneiminate)) Schiff base derivatives have been investigated for their anti-histone deacetylase (HDAC), anticancer, antibacterial, and antioxidant activities. For anti-HDAC studies, AUTODOCK 4.1 and Molecular Dynamics (MD) simulations have been conducted against these combinations. Cytotoxic test, the ferric reducing ability of plasma (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) ABTS assays and Agar diffusion method have been applied to investigate anticancer, antioxidant and antibacterial activities, respectively. Based on the results, the best docking was obtained for α,ά-Me2-salen against HDAC. Also, MD calculation results demonstrated that the α,ά-Me2-salen is a more effective compound for HDAC inhibiting than SAHA as a known enzyme inhibitor. However, α,ά-Me2-salen, and its derivatives didn't display antibacterial activity against any of the microorganisms. Cytotoxic activity analysis toward*

** To whom correspondence should be addressed.*
+ E-mail: szjahromi@yahoo.com
1021-9986/2022/6/1856-1869 14/$/6.04

MCF 7 cell line was apparent that α,ά-Me2-salen and its Ni (II), Co (II), and Cu (II) derivatives manifested high cytotoxic activity with IC50 5, 2, 2, and 3 µg/mL, respectively. The antioxidant results revealed excellent radical scavenging activities of all these compounds against DPPH, ABTS, and FRAP radicals. The antioxidant activity by DPPH, showed Mn(II) complex (IC50 = 0.13 ± 0.50 mg/mL) was the most active. While, α,ά-Me2-salen (IC50 =0.05±0.003 mg/mL) and its Ni(II) derivative (IC50 =0.049 mg/mL) exhibited the highest ABTS scavenging activity. According to the results, all compounds show acceptable anticancer and antioxidant activity and can be used as drug candidates after further investigations.

KEYWORDS: *Schiff base; ABTS; DPPH; FRAP; Anticancer.*

INTRODUCTION

Over the last decades, Schiff base complexes as chemical antioxidants have received considerable attention due to their high potential for radical scavenging activity [1-3]. Free radicals have a profound impact on human health and the pathogenesis of several diseases including cancer, atherosclerosis, renal disease, blood disorders liver injury, cardiovascular diseases, diabetes, neurodegenerative, and another disease [4-6]. So far, large numbers of efficient antioxidants from natural and synthetic sources including several Schiff bases have been investigated, and their antioxidant capacities have been evaluated by different methods [7-16].

In addition to the antioxidant activity of Schiff bases, they can be applied as antimicrobial [17-22], antifungal [7], anti-proliferative [23], antitumor [24-28], enzyme inhibitors [29-31], and selective DNA binding agents [17, 32, 33] and also can be used as an artificial Schiff base-forming enzyme [34, 35]. Therefore, Schiff bases derivatives have different benefits in the food, dye, agrochemical, and pharmaceutical industries.

It has been proved that a carbon-nitrogen double bond in Schiff bases is an essential structural requirement for their biological activities. They are formed by a condensation reaction of a primary amine. An expansion consists of an operating group with a carbon-nitrogen double bond, which is formed by the condensation of first-type amines with aldehydes or ketones (Fig. 1). Schiff bases are commonly flat, triangular, tetrahedral, or multi-dimensional. These molecules can form highly stable derivatives with intermediate metals, which, if a functional group usually has hydroxyl enough to be close to the density site, can act as curative ligands.

Schiff bases and their metal combinations have different applications in biomedicine, analytical chemistry, and the agriculture and food industry [36-38]. Metal complexes of Schiff base derived from the reaction of exchanged salicylaldehydes by aliphatic and aromatic amines have been investigated for their biological activities [39, 40]. In a study, antioxidant, anticancer, and antibacterial properties of some synthetic metal (II) complexes of tetradentate (4 *ethylimino*)-4-[(2-ethyl)imino]pentan-2-one have been investigated by *Ejidike et al* [41].

In this research, α,ά-Me_2-salen, (N,N´-ethylenebis(α-methylsalicylideneiminate)) Schiff base and its metal (Cu (II), Ni(II), Co(II), and Mn(II)) derivatives (Fig. 2) have been synthesized and characterized for their biological activities including anti-HDAC, anticancer, antibacterial and antioxidant activities.

EXPERIMENTAL SECTION

Materials

1,2-diaminoethane, 2-hydroxyacetophenone, methanol, nickel (II) acetatetetrahydrate, copper (II) acetatetetrahydrate, Co(II) acetatetetrahydrate, Mn (II) acetatetetrahydrate, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,4,6- tripyridyl-S-triazine (TPTZ), vitamin C, doxorubicin, iron sulfate ($FeSO_4$), potassium persulfate, Dimethyl sulfoxide (DMSO), ethanol, sodium acetate buffer, phosphate-buffered saline (PBS) and $FeCl_3$ were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Roswell Park Memorial Institute (RPMI-1640) medium, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl

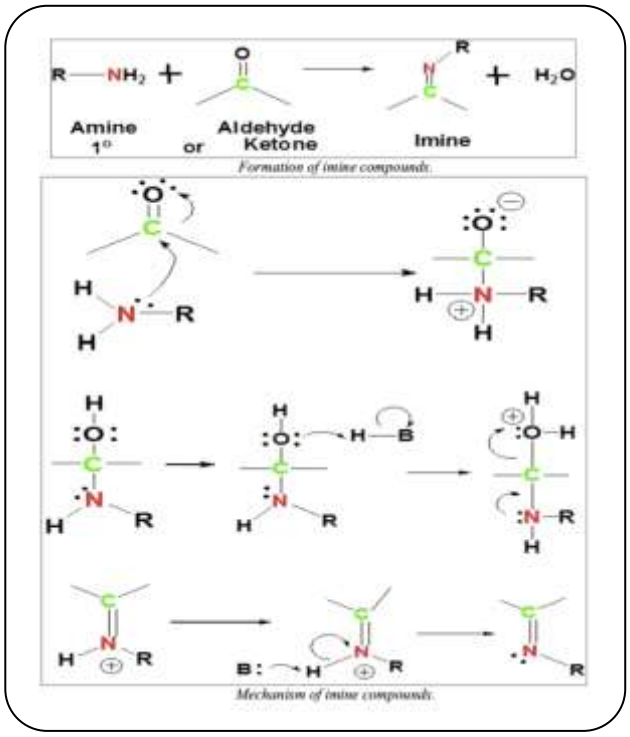


Fig. 1: The formation and mechanism of imine combination [42].

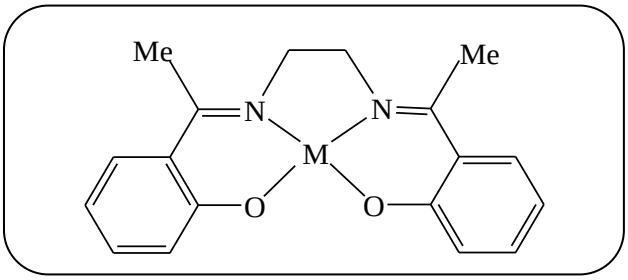


Fig. 2: The structure of metal derivatives of M(α,ά-Me2-salen), M = Ni(II),Cu(II), Co(II),Mn(II)

tetrazolium bromide (MTT) and fetal bovine serum (FBS) were purchased from Gibco (London, UK). All the reagents were used without any further refinements.

Synthesis of α,ά-Me2-salen, and its Schiff base complexes

N,N´-ethylenebis(α-methylsalicylideneiminate (α,ά-Me_2-salen) as the ligand and its Schiff base combinations were synthesized and evaluated through the Nuclear Magnetic Resonance (NMR), UV–Visible, Infrared (IR) spectroscopy, and mass spectrum, melting point, and elemental analysis **(see appendix)** according to our previous publication [43]. Briefly, for the synthesis of α,ά-Me2-salen, a methanol solution of the 2-hydroxyacetophenone (5 mmole, 0.6 mL) in 25 mL of dried methanol was added to a solution of the 1,2-diaminoethane (2.5 mmole, 0.16 mL). For 30 minutes, the reaction mixture was refluxed and then it was cooled. The Schiff base was separated as yellow needles. Also, Ni (II), Co (II), Mn (II), and Cu(II) Schiff Base complexes were synthesized according to the method described by Batley and Graddon [44]. α,ά-Me2-salen and its metal combinations have stability at room temperature and can be solved in conventional organic solvents including the MeOH and DMSO. For biological experiments, all compounds were diluted in DMSO to obtain 16.6 and 33.2µg/100µL.

Anti HDAC studies Schiff base and its derivatives by molecular docking

In this study, the protein–Schiff base complexes were analyzed by the AutoDock Suite version 4.2 software package [45]. Lamarckian Genetic Algorithm (LGA) was used to get the best docking conformations. The crystal structure of histone deacetylase (PDB code: 1T69) was extracted from RCSB (https://www.rcsb.org). Both dimensional structures of Schiff base compounds were built by ChemDraw (ChemDraw Ultra 10.0, Cambridge soft.), and they were transferred into Hyperchem 8.0 (HyperChem, Release 8.0 for Windows, Molecular Modeling System: HyperCube, 2007). Then, energy minimization was done by using MM+ force field and Polak-Ribiere conjugate gradient algorithm and root mean square gradient termination of 0.01 kcal/Å mol.

As inputs, these optimized structures were introduced to AutoDock tools. Then by using the Gasteiger-Marsili procedure the partial charges were calculated. Non-polar hydrogens were merged and then rotatable bonds were assigned. By using Discovery Studio Visualizer, co-crystallized ligands, SuberoylAnilide Hydroxamic Acid (SAHA), and water molecules were removed [46]. All missing hydrogen atoms were added and non-polar hydrogens were merged to their related carbons after determining the Kolman united atom charges [47].

Using Autogrid, electrostatic interactions and desolvation parameters were assigned to each atom of protein. The grid points were adjusted as 40 × 40 × 40 (xyz) with a 0.375 spacing value to the histone deacetylase catalytic site. The search algorithm LGA, was selected for finding the global optimum binding position. Finally, the result of docking poses was analyzed in DS Visualizer 3.5 and by AutoDockTools.

Molecular dynamics

The dynamics of the interactions between HDAC and drugs (SAHA as standard drug and α,ά-Me2-salen (selected from docking results due to best docking score) were investigated using Molecular Dynamics (MD) calculations. The topology file for studied drugs was created by Automated Topology Builder (ATB) server [48]. All processes of the simulations were accomplished using the newest version of GROMACS package by grooms 53a6 force field. The simulation boxes were fulfilled with a SPC/E model of water and by adding appropriate numbers of sodium or chlorine ions, the neutralization of the simulation system was performed [49]. After that, the energy minimization process was performed by the steepest descent algorithm. Then, in the equilibrium process, NVT ensemble for temperature and NPT ensemble for pressure were coupled in 310 K and 1bar, respectively using a v-rescale thermostat and Parinello-Rahman barostat. LINCS algorithm was used for all bonds. The cut-off was 1 nm for Electrostatics and Van der Waals interactions. The interaction energies during the simulation time were obtained using Molecular Mechanics/Poisson–Boltzmann Surface Area (MM/PBSA) method [50, 51]. Finally, the leapfrog algorithm was exerted for the production process of MD simulations [52].

Cytotoxic activity of Schiff base derivatives on breast tumor cells of MCF-7 (Michigan Cancer Foundation-7)

A human breast cancer cell line (MCF7) was obtained from the Fasa university of Medical science, Fars, Iran. The cells were cultured in RPMI-1640 medium accompanied with 10% (v/v) heat-inactivated FBS and 1% streptomycin/penicillin. Cells were grown to the confluence at 37 °C in a 5% CO_2–95% air-humidified incubator[53].

Cytotoxicity test was performed by MTT assay. Briefly, cells (1×10^6 cells /mL) were seeded into 96 well plates and incubated 24 h at 37°C in 5% CO_2 atmosphere to allow them to develop to 90% confluence. After incubation, the culture medium was removed, and 100 µl of different concentrations of compounds ranging from 6 to 330 µg/mL were added to the triplicate well. After 24 hours of incubation, the samples were washed with PBS (pH 7.4). Then, the incubated cells were stained with 20 µL of MTT solution (5 mg/mL) for 3 hours in the dark, and then 100 µL of DMSO was added for dissolving the formazan crystals formed after the addition of MTT, and left for 30 min in the CO_2 incubator. Finally, absorbance was measured at 570 nm *via* ELISA reader. The percentage of cell viability of these compounds was calculated by using the formula:

$$\left(\left[A_s - A_b\right]/\left[A_c - A_b\right]\right)\times 100$$

Where A_c is the absorbance of the control, A_b is the absorbance of the blank, and A_s is the absorbance of the sample. The 50% of inhibited cell growth was denoted as the IC_{50}; the minimum concentration that reduces the absorbance of the treated cells by 50% with reference to the untreated cells. Doxorubicin was used as a control. All data were reported as the average of three replicates [54].

Antibacterial activity of Schiff base derivatives

Four pathogenic microorganisms were applied to test the antibacterial potentials of Schiff base derivatives including *Escherichia coli* (ATCC25922), *Staphylococcus aureus* (ATCC25923), methicillin-resistant *Staphylococcus aureus* (MRSAATCC25923), and *Klebsiella pneumonia* (PTCC1290). The antibacterial activity of each sample was qualitatively determined according to the method explained by Bauer et al [55]. Microorganisms were grown in Petri plates on a nutrient agar medium. Schiff base derivatives (330 µg/mL) were dissolved in DMSO and soaked in a filter paper disc (5 mm diameter and 1 mm thickness (100 mg/cm^3)) and dried under sterile conditions for removing DMSO. Then dried discs were placed on the agar surface inoculated in advance. The plates were inverted and incubated for 24 h at 37 °C. Antimicrobial activity was measured by the diameter of the inhibition zone around each disc after 24 h for bacteria.

Assessment of Antioxidant Activity by the 2,2-diphenyl-1-picrylhydrazyl, (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), and Ferric Reducing Antioxidant Power Radical Scavenging Measures

The ability of α,ά-Me_2-salen derivatives as free radical scavenging agents was investigated through in-vitro antioxidant experiments including DPPH, ABTS, and Ferric-reducing antioxidant power (FRAP) assays. Finally, obtained results were compared to related standard antioxidants such as ascorbic acid and $FeSO_4$.

DPPH assay

As explained by Hu et al [56], the DPPH radical scavenging activity of the compounds was measured.

Briefly, the reaction mixture, a total volume of 3.0 mL, with different concentrations of samples solution (0.02, 0.1, 0.2, 0.24, 0.27, 0.28, 0.29 mg/mL) and DPPH (2 mL, 180 μmol/L) at 25 °C for 30 min were incubated. Then, the absorbance of the remained DPPH radical was measured at 517 nm against a control. The control experiment was carried out as above without the test samples. Three replicates for each sample concentration were tested and the reduction of DPPH was calculated relative to the measured absorbance of the control. The same procedure was applied to vitamin C as standard. Radical Scavenging Activity (RSA) was calculated using the following formula:

$$\% \text{ Scavenging of DPPH} = [(\text{Control OD} - \text{Sample OD}) / \text{Control OD}] \times 100$$

$ABTS^+$ radical scavenging activity

ABTS scavenging ability of the M(α,ά-Me_2-salen) Schiff base complexes was evaluated by the following method [57] with minor modifications. ABTS radical cation ($ABTS^+$) was produced by the reaction 7 mM ABTS solution and potassium persulfate solution (2.45 mM) at room temperature in equal amounts (1:1) and for 12 h was stored in the dark. 1 mL of $ABTS^+$ solution was diluted by ethanol to an absorbance of 0.706 ± 0.001 units at 734 nm required for the analysis. After the addition of test samples (1 mL) or standard to 1 mL of the $ABTS^+$ solution, the absorbance was measured spectrophotometrically. For calculating radical scavenging activity, the decrease in absorption was determined and the percentage of the ABTS radical scavenging activity of compounds was obtained from the following equation.

$$\% \text{ Scavenging of ABTS} = [(\text{Control OD} - \text{Sample OD}) / \text{Control OD}] \times 100$$

Ferric-Reducing Antioxidant Power (FRAP)

According to the procedure explained by Benzie and Strain, the reducing power of the complexes was measured [58] with some modifications. Briefly, FRAP solution was mixed with sodium acetate buffer solution (10 mL, 0.3 M, pH 3.6), TPTZ (1 mL, 10 mM) in 40 mM HCl, and $FeCl_3$ (1 mL, 20 mM). Different concentrations (0.006-1 mg/mL) of the samples dissolved in DMSO (0.2 mL) were added with FRAP solution (1.8 mL) as an oxidizing reagent. The mixture was incubated at 50°C for 30 min. The increase in the absorbance of the ferrous tripyridyltriazine complex (the colored product) was measured at 593 nm. FRAP value was calculated from a standard curve by using $FeSO_4$. Experiments were performed in triplicate, and the results are expressed as mM $FeSO_4 \cdot 7H_2O$ equivalent according to the following equation:

$$\text{mM Ferrous Equivalent (nmol/μL or mM Fe2+equivalents)} = (B \times D)/V$$

Where: *B*= Ferrous ammonium sulphate amount from a standard curve (nmol), D= Sample dilution factor, *V* = Sample volume added into the reaction.

Statistical analysis

All determinations were investigated in triplicate and mean values were reported for each case along with standard deviations (±SD). The antioxidant activity of the compounds was compared by ANOVA one-way test, using SPSS program ($P \leq 0.05$).

RESULTS AND DISCUSSION

In the present study, Schiff base metal combinations were synthesized and evaluated by different physiochemical and spectrophotometry approaches as reported in our previous work, and then they were characterized for their biological potency.

Anti-HDAC studies by molecular docking

Gene expression regulation is mediated by different mechanisms including post-translational modifications of histones, ATP-dependent chromatin remodeling, DNA methylation, and DNA acetylation. HDACs, important targets for cell function, are divided into three classes including HDACs I, II, and III. Overexpression of HDACs leads to the deregulation of many target genes which are responsible for cell growth and differentiation. Therefore, substantial efforts have been made to regulate HDACs activities by using various inhibitors with high sensitivity toward target cells. HDAC inhibitors by inducing hyperacetylation of histones can modulate the structure of chromatin and gene expression regulation resulting in cell differentiation, growth arrest, and apoptosis [59, 60].

In this study, molecular docking was successfully performed on these compounds for targeting HDAC8 isoform by using AUTODOCK 4.2. At first, for method validation, SAHA was redocked into HDAC active site.

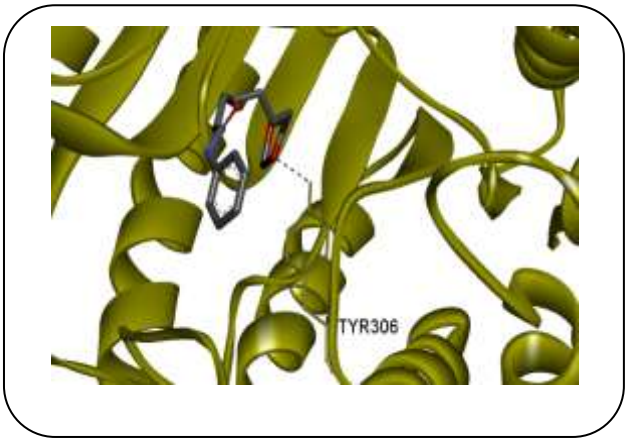


Fig. 3: Reattachment of the SAHA in the active site of HDAC. The image was provided using the Discovery Studio Visualizer application (*Accelrys Software *Inc., USA).

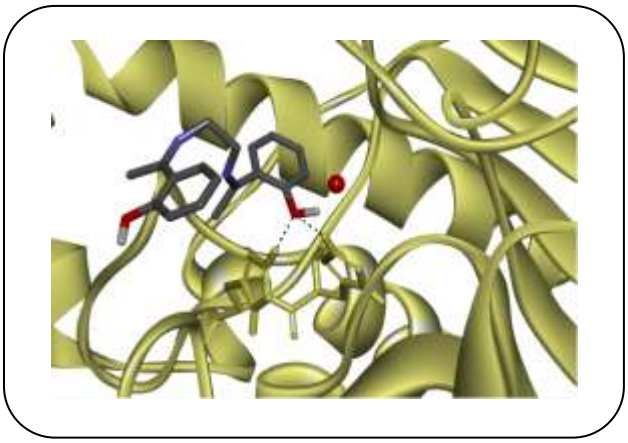


Fig. 4: Binding model of α,ά-Me2-salen for the best-docked pose in the HDAC active site. Zn (II) metal has been shown as a red sphere.

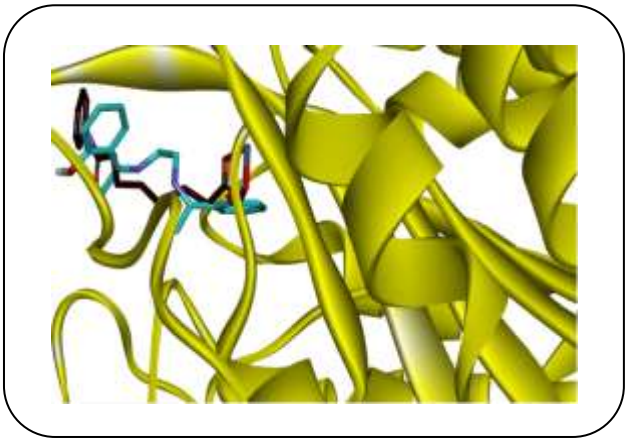


Fig. 5: Superimposition of the best docking poses for α,ά-Me2-salen (cyane) with SAHA (black) in the active site of HDAC.

The molecular interactions of SAHA with HDAC are illustrated in Fig. 1. As seen in this figure, SAHA interacts with TYR306 in HDAC via hydrogen binding.

After protocol validation, the 3D structures of compounds were docked into the HDAC active site. Based on our docking study, the best result was obtained for α,ά-Me2-salen compared to other derivatives. Actually, this compound showed the most negative ΔG (-7.51kcal/mol) of binding with favorable interactions and hydrogen binding to the key amino acid residues (His142 and His143) at the active site of HDACs (Fig. 2). Superimposition of α,ά-Me2-salen and compound SAHA implies that the main scaffolds and the substituents have almost the same orientations (Fig. 3) and may be acted as the HDAC inhibitor and could be resulted to growth arrest, cell differentiation, and apoptosis of MCF7 cell lines.

However, the molecular docking method is a valuable simulation method for the investigation of protein ligands interaction within the active region but all docking methods are probabilistic approaches. Therefore, further simulations by MD were applied to docking results in order to validate and compare the obtained results from docking[61].

Molecular dynamics simulation

In order to more accurate investigation, the extent of changes in protein structure in combination with α,ά-Me2-salen, and SAHA, dynamics of the interactions between HDAC and α,ά-Me2-salen were investigated via MD simulations,

RMSD of the protein backbone depending on time for the free protein and protein in complex with mentioned drugs were performed for further investigating the accuracy of the simulation (Fig. 6). The numerical value of RMSD is balanced after 20 ns. The mean RMSD values of about 0.25 nm are fluctuating in free protein. In the protein-α,ά-Me2-salen system, the mean RMSD (0.38 nm) was similar to the protein-SAHA system (0.38 nm). These results indicate that the most severe fluctuation in RMSD and consequently more instability in protein is observed for the protein-(α,ά-Me2-salen) and protein-SAHA systems.

As can be seen from Fig. 7, SAHA in the locations around the residue Tyr 200-300 makes the highest fluctuation in the protein which can further put out the instability in its structure. Also in the location of residues

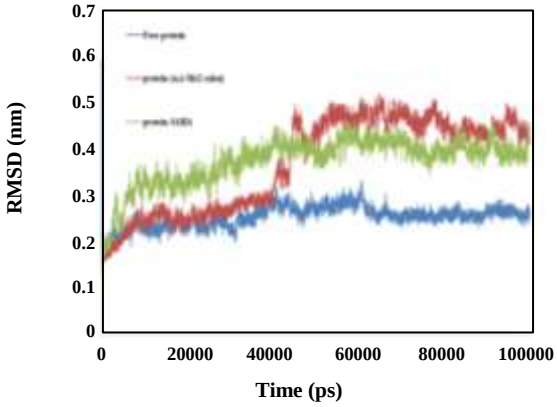


Fig. 6: Time evolutions of RMSD of C-alpha atoms for free protein and protein in complex with α,ά-Me2-salen, and SAHA in 60 ns of simulations.

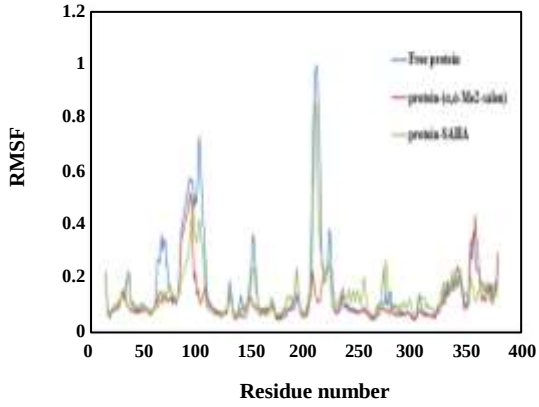


Fig. 7: Time evolutions of RMSF of C-alpha atoms for free protein and Protein in complex with SAHA and α,ά-Me2-salen in 60 ns of simulations.

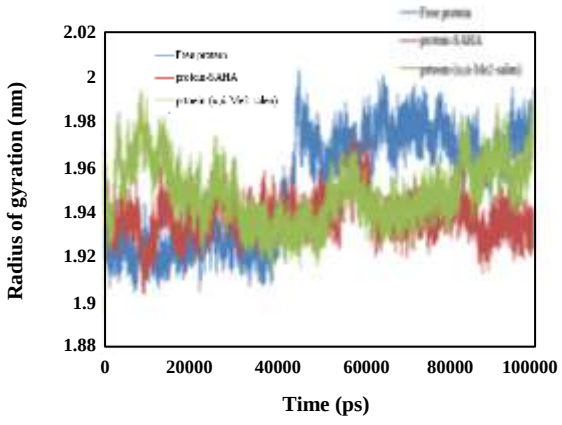


Fig. 8: Time evolutions of the Radius of Gyration for free protein and Protein in complex with SAHA and α,ά-Me2-salen in 60 ns simulations.

350-360 in the α,ά-Me2-salen containing system the protein has higher fluctuations than in other systems. (Fig.7).

The radius of gyration represents the protein compactness degree. As shown in Fig. 8, the radius of gyration has decreased in the protein in the complex with SAHA and α,ά-Me2-salen (<1.93 nm) rather than free protein (1.96 nm). The decrease in Rg indicates more compassion for the protein in the presence of these compounds. This condensation can tighten the opening of the active site and prevent the substrate from correctly binding to the enzyme.

Hydrogen bond analysis is an important indicator to evaluate the stability of the protein-drug complex. As shown in Table.1 the higher mean number of H-bond formations is seen in the complex of protein with SAHA (0.97), rather than α,ά-Me2-salen (0.2) which indicates the more stability of the protein-drug complex in combination with SAHA.

One of the main analyses for investigation of the secondary structure of the proteins is DSSP (Dictionary of the Secondary Structure of the Protein). The secondary structures of free protein and protein in complex with SAHA and α,ά-Me2-salen during 60-ns of simulation are depicted in Fig.9. From this figure no significant difference was observed in helical content and beta-sheet regions of the HDAC complexes with α,ά-Me2-salen, and SAHA.

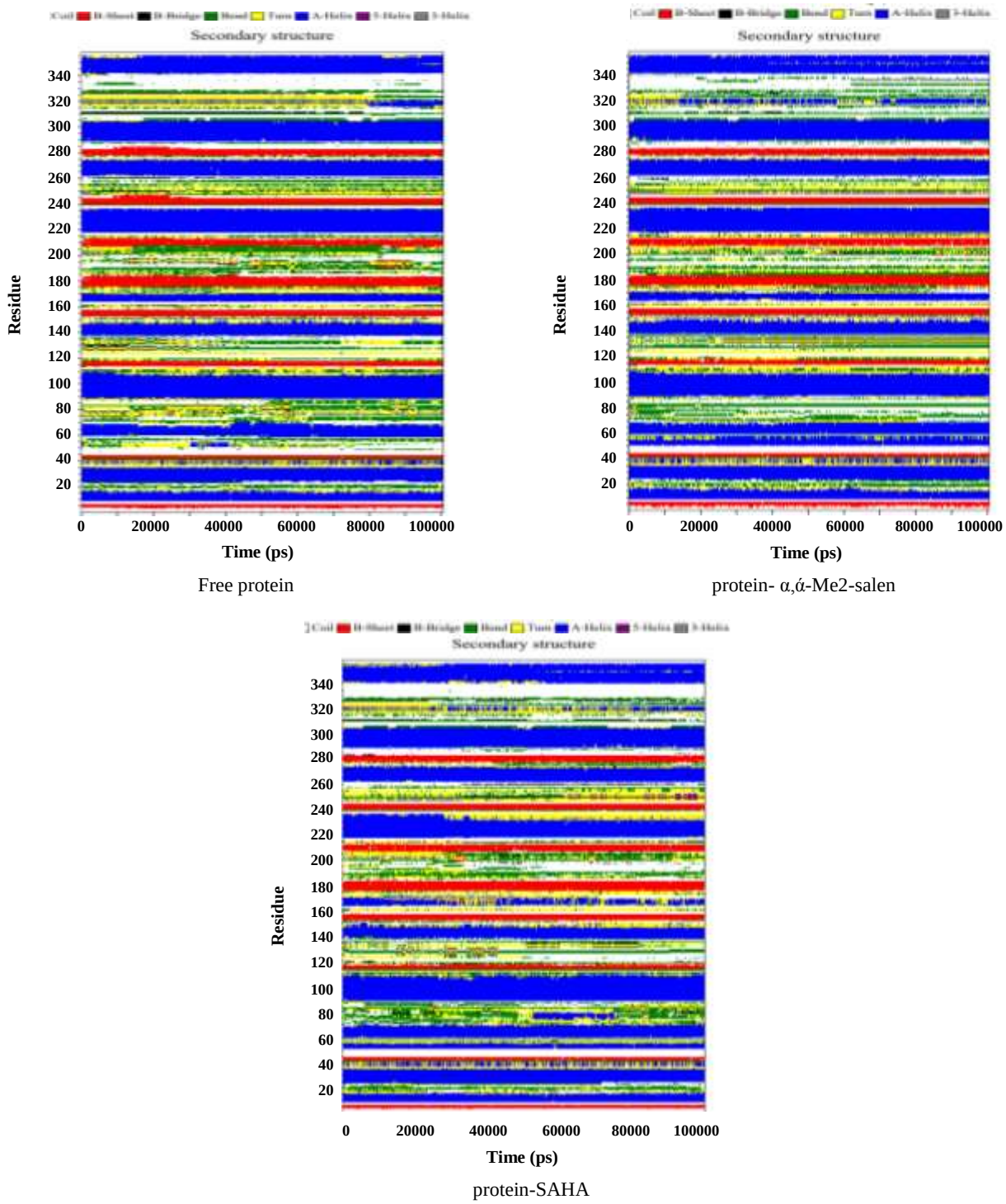
The interaction energies for different protein-SAHA and protein-α,ά-Me2-salen complexes were obtained during the simulation time using MMPBSA method, and their mean values are reported in Table 2. As can be seen from the table, in the dynamic state, α,ά-Me2-salen has conducted a more stable complex with HDAC compared to SAHA and it has interacted with protein in both electrostatic and Vander wales manner. Comparatively, the interaction energies obtained here have a good agreement with those obtained from docking results in which the docking data show that the α,ά-Me2-salen forms a more stable complex than SAHA.

Anticancer capability

The results of cytotoxic screening are presented in Table.3. Among the present compounds, Co(II), Ni(II), Cu(II) Schiff base complexes are more effective than doxorubicin towards MCF7 cells and their IC_{50} values of are comparable with doxorubicin. However, Mn(II) is less effective than others towards breast cancer cells.

Table 1: The mean of H-bond numbers values.

	protein + SAHA	protein + α,ά-Me2-salen
h-bond No.	0.97	0.2



Free protein

protein- α,ά-Me2-salen

protein-SAHA

Fig. 9: The Secondary structure of free protein and protein in complexes with SAHA and α,ά-Me2-salen during 60 ns MD simulation.

Table 2: Means of dynamic interaction energies (kJ/mol) for protein-SAHA and Protein-(α,ά-Me2-salen).

	VdW	Electrostatic	Total
protein - (α,ά-Me2-salen)	-158.156	-88.7091	-246.865
Protein-SAHA	-193.0818	121.604	-71.47829

Table 3: Cytotoxicity for the combinations.

Schiff bases	(α,ά-Me_2-salen)	Mn(α,ά-Me_2-salen)	Co(α,ά-Me_2-salen)	Ni(α,ά-Me_2-salen)	Cu (α,ά-Me_2-salen)	doxorubicin
IC50 (μg/mL)	5.35±0.2	330±2.5	2.2±0.06	2.10±0.01	3.15±0.1	4.45±0.1

Antibacterial capability

Synthesized Schiff base derivatives were evaluated for antibacterial activity against Gram-positive and Gram-negative bacteria. Primary screening for qualitative study on antimicrobial activities of compounds was carried out via disc diffusion assay (Table.4). Neither (α,ά-Me2-salen) nor its metal complexes showed significant activity against any of the above bacteria.

The free ligand α,ά-Me2-salen, and its metal complexes of Schiff base revealed inhibitory zones ranging 10-11 mm. It means that the ligand and the related complexes did not exhibit an effective and significant antimicrobial activity over the range of these considered concentrations as compared to standard drugs.

According to these data, even α,ά-Me2-salen complexation with metals didn't enhance their antimicrobial potential and indicated no significant difference between α,ά-Me2-salen, and its metal derivatives. These results were out of our expectations and unlike other research. However, based on the last reports, several synthetic Schiff bases including amino acids, sulfonamides, resacetophenones, aminothiazolyl bromocoumarins, O-phthaldehyde, did not exhibit any notable antibacterial activity [62]. It is not clear to us why these compounds didn't show potent antibacterial activity while they have excellent anticancer and antioxidant properties. Based on the research, upon metal complexation, the metal ion polarity will be reduced and the lipophilicity of the complexes increases due to the partial sharing of positive charges with donor groups. Therefore, it is expected that the diffusion of the complexes increases through the lipid membranes and the allocated metal binding sites of the targeted enzymes of the microorganisms blocks [63] and hence it is expected that metal (II) complexes be more effective than the α,ά-Me2-salen. But, in this research, an unexpected result was obtained. However, antimicrobial activity depends on several factors including the concentration, molecular structure, and geometries of the compound and the bacterial strain, and the polarity of the solvent [64].

Antioxidant assays

Antioxidant properties of α,ά-Me_2-salen, and its derivatives were determined by three different methods including DPPH and ABTS, and FRAP.

DPPH radical scavenging assay

The DPPH assay is a prevalent method to determine the radical scavenging ability of various compounds. Most investigations are widely measured the hydrogen-donating activity, using DPPH radicals as a hydrogen acceptor, to estimate the antioxidant activities of samples due to its simplicity and high validity compared to other methods [65]. The radical scavenging activity of the DPPH by Schiff base derivatives has been summarized in Table 5. According to the table, all compounds (with IC_{50} values 0.13-0.24 mg/mL) demonstrated significant DPPH free radical scavenging activity compared to vitamin C (IC_{50}=0.44 mg/mL). However, among them, Mn (α, ά-Me2-salen) showed the highest activity with an IC_{50} value of 0.13 ± 0.50 mg/mL.

ABTS

As listed in Table 5, all compounds represented better ABTS scavenging activity compared to vitamin C (IC_{50}=0.26±0.001). Among the studied complexes, α,ά-Me2-salen and Ni (II) complex exhibited the highest ABTS scavenging activity with IC_{50} values of 0.05±0.003 and 0.049 mg/mL, respectively. While Mn (II), Co (II) and Cu (II) complexes had an IC_{50} value of 0.12±0.002, 0.14±0.009, 0.13±0.001 milligram per milliliter, correspondingly.

FRAP

FRAP assay is a convenient test among the most widely used methods for the measurement of antioxidant

Table 4: Qualitative antimicrobial assay (100 mg/cm^3); Inhibition diameter in millimeters. Values >15 millimeters represent high antibacterial activity. Values are expressed as mean ± SD of three replicates.

Compounds	Escherichia coli(ATCC25922)	Staphylococcus aureus(ATCC25923)	methicillin-resistant Staphylococcus aureus (MRSAATCC25923)	Klebsiella pneumonia(PTCC1290)
(α,ά-Me2-salen)	10.333±0.9	10.677±0.2	10.667±0.15	10.667± 0.73
Mn (α, ά-Me$_2$-salen)	10.33 ±0.1	10.67± 0.13	11 ±0.21	10 ±0.31
Co (α,ά-Me2-salen)	10.33 ±0.25	10.67 ±0.1	10.33 ±0	10 ±0.12
Ni (α,ά-Me2-salen)	10.67 ±0.3	10.33 ±0.14	10 ±0.25	10.67 ±0.43
Cu (α,ά-Me2-salen)	11 ±0.54	10.33 ±0.36	10 ±0.3	11 ±0.22
Streptomycin (control)	-	24±2.1	31 ±1.7	-

Table 5: Antioxidant activity of* Schiff base complexes *by DPPH, ABTS [IC$_{50}$ (mg/mL)], and FRAP [mM Fe (II)/g equivalent] methods. Values are expressed as mean ± SD of three replicates. The values with different letters (a - e) are significantly different at p < 0.05.

Compound	FRAP [mM Fe (II)/g equivalent]	DPPH$^{\bullet}$ [IC$_{50}$ (mg/mL)]	ABTS[IC$_{50}$ (mg/mL)]
Cu (α,ά-Me2-salen)	0.18±0.000 a	0.13±0.001a	130±2.0 a
Co (α,ά-Me2-salen)	0.18±0.001 a	0.14±0.009a	483.3 ±0.0 b
Mn (α,ά-Me2-salen)	0.13±0.000 b	0.12±0.002b	92.5±2.1 c
Ni (α,ά-Me2-salen)	0.24±0.001 c	0.04±0.000c	1000±0.0 d
α,ά-Me2-salen	0.16±.001 d	0.05±0.003c	75±2.3 e
Vitamin C	0.44±0.002 e	0.26±0.001d	----

capacities of natural and synthetic compounds. In this study, the antioxidant power of all compounds was determined by FRAP assay and was expressed as an equivalent of standard antioxidant, FeSO4. FRAP values indicated that all compounds possess the ferric-reducing antioxidant capacity. However, Ni (II) and Co (II) derivatives showed comparatively high antioxidant activity compared to others (Table 5).

Generally, our results represented high DPPH radical scavenging activities of α,ά-Me2-salen, and its derivatives (especially Mn (II)) compared to vitamin C. Also, ABTS and FRAP analysis showed high antioxidant activity of Ni(II) derivative compared to other complexes. Therefore, it may be a result that Ni(II) and Mn(II) complexes have the best antioxidant capacity among other metal complexes. Except for Mn (II) complex, the DPPH scavenging activities of other Schiff base metal complexes are significantly lower than that of α,ά-Me2-salen. Therefore, based on the results, it seems that metal ions have no additional effect on the radical scavenging activity of these compounds.

According to a report published by *Baykara, et al.* (2014), some new metal complexes of the types [ML2(H2O)2]Cl2 and [ML2]Cl2 (M = Mn(II); Co.(II); Ni(II); Cu(II); and Zn(II)) have been found with high antioxidant effects. Furthermore, metal complexes revealed the potent scavenging property as compared to free ligands [66].

In another study, three new synthetic salicylaldehyde Schiff base ligands containing aromatic groups along with aliphatic spacers have been investigated for their antioxidant activities. The results showed that the dinitro compounds and diamine precursors have moderate percentage scavenging activities in DPPH free radical assay while the Schiff base ligands showed significant values [67]. These findings are in accordance with our study which has shown the antioxidant activity of metal Schiff base complexes.

CONCLUSIONS

The synthesized α,ά-Me_2-salen, and its metal derivatives have shown anticancer potential against MCF7 breast cancer cells. Furthermore, a docking study revealed that α,ά-Me2-salen has a high inhibitory potency compared to other derivatives and MD calculations showed that it has conducted a more stable complex with HDAC compared to SAHA. Based on the antioxidant results, all these combinations exhibited excellent radical scavenging activities against DPPH radical and ABTS. They showed major antioxidant activity than oxidation-inhibiting agents like vitamin C and $FeSO_4$. DPPH and ABTS results revealed that these compounds can donate electrons or hydrogen atoms and subsequently are capable of reacting with free radicals or terminating chain reactions in a dose-dependent manner.

Acknowledgments

We would like to appreciate all those who helped us in this work. We are also thankful to Gerash University of Medical Sciences for the financial support of this project.

Received : Dec. 12, 2020 ; Accepted : May 18, 2021

REFERENCES

[1] Abdel Aziz AA., Elbadawy HA., Spectral, Electrochemical, Thermal, DNA Binding Ability, Antioxidant and Antibacterial Studies of Novel Ru(III) Schiff Base Complexes, *Spectrochim. Acta. A. Mol. Biomol. Spectrosc.*, **124**:404-415 (2014).

[2] Sathyadevi P., Krishnamoorthy P., Butorac R.R., Cowley A.H., Dharmaraj N., Synthesis of Novel Heterobimetallic Copper(I) Hydrazone Schiff Base Complexes: A Comparative Study on the Effect of Heterocyclic Hydrazides towards Interaction with DNA/Protein, Free Radical Scavenging and Cytotoxicity, *Metallomics*, **4(5)**:498-511(2014).

[3] Meftah S., Yazdanparast., Razieh: Synthesis and Antioxidant Activities of [5-fluoro N, N'-bis (salicylidene) ethylenediamine] and [3, 5-fluoro N, N'-bis (salicylidene) ethylenediamine] Manganese (III) Complexes, *Iran. J. Chem. Chem. Eng (IJCCE).*, **32(1)**: 67-75 (2013).

[4] Lobo V., Patil A., Phatak A., Chandra N., Free Radicals, Antioxidants and Functional foods: Impact on Human Health. *Pharmacogn. Rev.*, **4(8)**: 118-126 (2010).

[5] Wojtunik-Kulesza K.A., Oniszczuk A., Oniszczuk T., Waksmundzka-Hajnos M., The Influence of Common Free Radicals and Antioxidants on Development of Alzheimer's Disease, *Biomed. Pharmacother.*, **78**:39-49 (2016).

[6] Valko M., Jomova K., Rhodes C.J., Kuca K., Musilek K., Redox- and Non-Redox-Metal-Induced Formation of Free Radicals and Their Role in Human Disease, *Arch. Toxicol.* **90(1)**:1-37 (2016).

[7] Ejidike IP., Cu(II) Complexes of 4-[(1E)-N-{2-[(Z)-Benzylidene-amino]ethyl}ethanimidoyl]benzene-1,3-diol Schiff Base: Synthesis, Spectroscopic, In-Vitro Antioxidant, Antifungal and Antibacterial Studies, *Molecules.* **23(7)** (2018).

[8] Bharathi-Dileepan A.G., Daniel-Prakash T., Ganesh-Kumar A., Shameela-Rajam P., Dhayabaran V., Rajaram R., Isatin based macrocyclic Schiff Base Ligands as Novel Candidates for Antimicrobial and Antioxidant Drug Design: In Vitro DNA Binding and Biological Studies, *J. Photochem. Photobiol. B.* **183**:191-200 (2018).

[9] Revathi N., Sankarganesh M., Rajesh J., Raja J.D., Biologically Active Cu(II), Co(II), Ni(II) and Zn(II) Complexes of Pyrimidine Derivative Schiff Base: DNA Binding, Antioxidant, Antibacterial and In Vitro Anticancer Studies, *J. Fluoresc.*, **27(5)**:1801-1814 (2018).

[10] Palopoli C., Gomez G., Foi A., Doctorovich F., Mallet-Ladeira S., Hureau C., Signorella S., Dimerization, Redox Properties and Antioxidant Activity of Two Manganese(III) Complexes of Difluoro- and Dichloro-Substituted Schiff-Base Ligands, *J. Inorg. Biochem.*, **167**:49-59 (2017).

[11] Gull P., Babgi B.A., Hashmi A.A., Synthesis of Ni(II), Cu(II) and Co(II) Complexes with New Macrocyclic Schiff-Base Ligand Containing Dihydrazide Moiety: Spectroscopic, Structural, Antimicrobial and Antioxidant Properties, *Microb. Pathog.*, **110**: 444-449 (2017).

[12] Zhang Y., Zou B., Chen Z., Pan Y., Wang H., Liang H., Yi X., Synthesis and Antioxidant Activities of Novel 4-Schiff Base-7-Benzyloxy-Coumarin Derivatives, *Bioorganic Med. Chem. Lett.*, **21(22)**: 6811-6815 (2011).

[13] Aswathanarayanappa C., Bheemappa E., Bodke Y.D., Krishnegowda P.S., Venkata SP, Ningegowda R: Synthesis and Evaluation of Antioxidant Properties of Novel 1,2,4-Triazole-Based Schiff Base Heterocycles, *Arch. Pharm.,* **346(12)**: 922-930 (2013).

[14] Li Y.F., Liu Z.Q., Ferrocenyl Schiff Base as Novel Antioxidant to Protect DNA Against the Oxidation Damage, *Eur. J. Pharm. Sci.*, **44(1-2)**: 158-163 (2011).

[15] Chen A.S., Taguchi T., Aoyama S., Sugiura M., Haruna M., Wang M.W., Miwa I., Antioxidant Activity of a Schiff Base of Pyridoxal and Aminoguanidine, *Free Radic. Biol. Med.*, **35(11)**:1392-1403 (2003).

[16] Ejidike I.P., Ajibade P.A., Synthesis, Characterization, Anticancer, and Antioxidant Studies of Ru(III) Complexes of Monobasic Tridentate Schiff Bases, *Bioinorg. Chem. Appl.,* **2016**: 9672451 (2016).

[17] Prasad K.S., Kumar L.S., Prasad M., Revanasiddappa H.D., Novel Organotin(IV)-Schiff Base Complexes: Synthesis, Characterization, Antimicrobial Activity, and DNA Interaction Studies, *Bioinorg. Chem. Appl.*, **2010**:854514 (2010).

[18] Casanova I., Duran M.L., Viqueira J., Sousa-Pedrares A., Zani F., Real J.A., Garcia-Vazquez J.A., Metal Complexes of a Novel Heterocyclic Benzimidazole Ligand Formed by Rearrangement-Cyclization of the Corresponding Schiff Base. Electrosynthesis, Structural Characterization and Antimicrobial Activity, *Dalton. Trans.,* **47(12)**: 4325-4340 (2018).

[19] Chaudhary N.K., Mishra P., Metal Complexes of a Novel Schiff Base Based on Penicillin: Characterization, Molecular Modeling, and Antibacterial Activity Study. *Bioinorg. Chem. Appl.* **2017**:6927675 (2017).

[20] Subbaraj P., Ramu A., Raman N., Dharmaraja J., Novel Mixed Ligand Complexes of Bioactive Schiff base (E)-4-(phenyl (phenylimino) methyl) Benzene-1,3-diol and 2-Aminophenol/2-Aminobenzoic Acid: Synthesis, Spectral Characterization, Antimicrobial and Nuclease Studies, *Spectrochim. Acta A Mol. Biomol. Spectrosc.*, **117**:65-71 (2014).

[21] Nitha L.P., Aswathy R., Mathews N.E., Kumari B.S., Mohanan K., Synthesis, Spectroscopic Characterisation, DNA Cleavage, Superoxidase Dismutase Activity and Antibacterial Properties of Some Transition Metal Complexes of a Novel Bidentate Schiff Base Derived from Isatin and 2-Aminopyrimidine, *Spectrochim. Acta A Mol. Biomol. Spectrosc.* **118**: 154-161 (2014).

[22] Sharaby C.M., Synthesis, Spectroscopic, Thermal and Antimicrobial Studies of Some Novel Metal Complexes of Schiff base Derived from [N1-(4-methoxy-1,2,5-thiadiazol-3-yl) Sulfanilamide] and 2-thiophene Carboxaldehyde. Spectrochim, *Acta A Mol. Biomol. Spectrosc.,* **66(4-5)**: 1271-1278 (2007).

[23] Song W.J., Cheng J.P., Jiang D.H., Guo L., Cai M.F., Yang H.B., Lin Q.Y., Synthesis, Interaction with DNA and Antiproliferative Activities of Two Novel Cu(II) Complexes with Schiff Base of Benzimidazole, *Spectrochim. Acta A Mol. Biomol. Spectrosc.*, **121**: 70-76 (2014).

[24] Geweely N.S., Anticandidal Cytotoxicity, Antitumor Activities, and Purified Cell Wall Modulation by Novel Schiff Base Ligand and Its Metal (II) Complexes Against Some Pathogenic Yeasts, *Arch. Microbiol.*, **191(9)**: 687-695 (2009).

[25] Proetto M., Liu W., Hagenbach A., Abram U., Gust R., Synthesis, Characterization and In Vitro Antitumour Activity of a Series of Novel Platinum(II) Complexes Bearing Schiff Base Ligands, *Eur. J. Med. Chem.*, **53**: 168-175 (2012).

[26] Duff B., Thangella V.R., Creaven B.S., Walsh M., Egan D.A., Anti-Cancer Activity and Mutagenic Potential of Novel Copper(II) Quinolinone Schiff Base Complexes in Hepatocarcinoma Cells, *Eur. J. Pharmacol.,* **689(1-3)**: 45-55 (2012).

[27] Zhong X., Yi J., Sun J., Wei H.L., Liu W.S., Yu K.B., Synthesis and Crystal Structure of Some Transition Metal Complexes with a Novel Bis-Schiff Base Ligand and Their Antitumor Activities, *Eur. J. Med. Chem.*, **41(9)**: 1090-1092 (2006).

[28] Chakraborty A., Kumar P., Ghosh K., Roy P., Evaluation of a Schiff Base Copper Complex Compound as Potent Anticancer Molecule with Multiple Targets of Action, *Eur. J. Pharmacol.,* **647(1-3)**: 1-12 (2010).

[29] You Z.L., Shi D.H., Xu C., Zhang Q., Zhu H.L., Schiff Base Transition Metal Complexes as Novel Inhibitors of Xanthine Oxidase, *Eur. J. Med. Chem.* **43(4)**: 862-871 (2008).

[30] Adsule S., Barve V., Chen D., Ahmed F., Dou Q.P., Padhye S., Sarkar F.H., Novel Schiff Base Copper Complexes of Quinoline-2 Carboxaldehyde as Proteasome Inhibitors in Human Prostate Cancer Cells, *J. Med. Chem.*, **49(24)**: 7242-7246 (2006).

[31] Aslam M.A. Mahmood S.U., Shahid M., Saeed A., Iqbal J., Synthesis, Biological Assay In Vitro and Molecular Docking Studies of New Schiff Base Derivatives as Potential Urease Inhibitors, *Eur. J. Med. Chem.*, **46(11)**:5473-5479 (2011).

[32] Raman N., Sobha S., Thamaraichelvan A., A Novel Bioactive Tyramine Derived Schiff base and Its Transition Metal Complexes as Selective DNA Binding Agents, *Spectrochim. Acta A Mol. Biomol. Spectrosc.*, **78(2)**:888-898 (2011).

[33] Qin D.D., Yang Z.Y., Wang B.D., Spectra and DNA-Binding Affinities of Copper(II), Nickel(II) Complexes with a Novel Glycine Schiff Base Derived from Chromone, *Spectrochim. Acta A Mol. Biomol. Spectrosc.*, **68(3)**:912-917 (2007).

[34] Garrabou X., Wicky B.I., Hilvert D., Fast Knoevenagel Condensations Catalyzed by an Artificial Schiff-Base-Forming Enzyme, *J. Am. Chem. Soc.*, **138(22)**:6972-6974 (2016).

[35] Ohashi M., Koshiyama T., Ueno T., Yanase M., Fujii H., Watanabe Y., Preparation of Artificial Metalloenzymes by Insertion of Chromium(III) Schiff Base Complexes into Apomyoglobin Mutants, *Angew. Chem.*, **42(9)**: 1005-1008 (2003).

[36] Gubendran A., Kesavan M.P., Ayyanaar S., Mitu L., Athappan P., Rajesh J., Non-enolisable Knoevenagel condensate Appended Schiff Bases-Metal (II) Complexes: Spectral Characteristics, DNA-Binding and Nuclease Activities, *Spectrochim. Acta A Mol. Biomol. Spectrosc*, **181**:39-46 (2017).

[37] Sumrra S.H., Ibrahim M., Ambreen S,. Imran M., Danish M., Rehmani F.S., Synthesis, Spectral Characterization, and Biological Evaluation of Transition Metal Complexes of Bidentate N,O Donor Schiff Bases, *Bioinorg. Chem. Appl.*, **2014**: 812924 (2014).

[38] Sherif O.E., Abdel-Kader N.S., Spectroscopic and Biological Activities Studies of Bivalent Transition Metal Complexes of Schiff Bases Derived from Condensation of 1,4-phenylenediamine and Benzopyrone Derivatives, *Spectrochim. Acta A Mol. Biomol. Spectrosc.*, **117**:519-526 (2014).

[39] Neelakantan M.A., Marriappan S.S., Dharmaraja J., Jeyakumar T., Muthukumaran K., Spectral, XRD, SEM and Biological Activities of Transition Metal Complexes of Polydentate Ligands Containing Thiazole Moiety, *Spectrochim. Acta A Mol. Biomol. Spectrosc.*, **71(2)**:628-635 (2008).

[40] Mohammadi K., Azad S.S., Amoozegar A., New Tetradentate Schiff Bases of 2-amino-3,5-Dibromobenzaldehyde with Aliphatic Diamines and Their Metal Complexes: Synthesis, Characterization and Thermal Stability, *Spectrochim. Acta A Mol. Biomol. Spectrosc.*, **146**: 221-227 (2015).

[41] Ejidike I.P., Ajibade P.A., Synthesis, Characterization, Antioxidant, and Antibacterial Studies of Some Metal(II) Complexes of Tetradentate Schiff Base Ligand: (4E)-4-[(2-{(E)-[1-(2,4-Dihydroxyphenyl)ethylidene]amino}ethyl)imino]pentan-2-one, *Bioinorg. Chem. Appl.*, **2015**: 890734 (2015).

[42] Aljamali N., Hadi M., Mohamad M., Salih L., Aljamali N., Review on Imine Derivatives and Their Applications, *Int. J. Photochem.*, **5**: 20-32 (2019).

[43] Asadi Z., Mosalanezhad F., The Interaction of Nickel (II) and Copper (II) Schiff Base Complexes with Diorganotin (IV) Dichlorides in Acetonitrile: A Thermodynamic Study, *Synth. React. Inorg. M.*, **41**: 1158-1166 (2011).

[44] Bathley G.E., Graddo D.P., Nickel(II) Hydrosalicylamide Complexes, *Aust. J. Chem.*, **20**: 1749-1751 (1967).

[45] Morris G.M., Huey R., Lindstrom W., Sanner M.F., Belew R.K., Goodsell D.S., Olson A.J., AutoDock4 and AutoDockTools4: Automated Docking with Selective Receptor Flexibility, *J. Comput. Chem.* 30(16):2785-2791 (2009).

[46] Visualizer D.S., "Release 3.5", Accelrys Inc, San Diego, CA, USA (2012).

[47] Weiner S.J., Kollman P.A., Case D.A., Singh U.C., Ghio C., Alagona G., Profeta S., Weiner P., A New Force Field for Molecular Mechanical Simulation of Nucleic Acids and Proteins, *J. Am. Chem. Soc.* **106(3)**: 765-784 (1984).

[48] Malde A.K., Zuo L., Breeze M., Stroet M., Poger D., Nair P.C., Oostenbrink C., Mark AE., An Automated Force Field Topology Builder (ATB) and Repository: Version 1.0, *J. Chem. Theory Comput.*, **7(12)**: 4026-4037 (2011).

[49] Binder K., Applications of Monte Carlo Methods to Statistical Physics, *Rep. Prog. Phys.*, **60(5)**: 487 (1997).

[50] Kumari R., Kumar R., Open Source Drug Discovery C, Lynn A: g_mmpbsa--a GROMACS Tool for High-Throughput MM-PBSA Calculations, *J. Chem. Inf. Model.*, **54(7)**:1951-1962 (2014).

[51] Baker N.A., Sept D., Joseph S., Holst M.J., McCammon J.A., Electrostatics of Nanosystems: Application to Microtubules and the Ribosome, *Proc. Natl. Acad. Sci. U S A.*, **98(18)**: 10037-10041 (2001).

[52] van Aalten D., Amadei A., Bywater R., Findlay J., Berendsen H., Sander C., Stouten P., A Comparison of Structural and Dynamic Properties of Different Simulation Methods Applied to SH_3, *Biophys. J.* **70(2)**:684-692 (1996).

[53] Bahman Jahromi E., Jafaarnejad L., Vahdani M., Zolghadri S., Toxicity Effect of Bromoacetic Acid on MCF7 Breast Cancer Cell Line and Analysis of Expression of Apoptosis-Associated Genes, *Iran. J. Breast. Dis.*, **13(2)**: 37-48 (2020).

[54] Andiappan K., Sanmugam A., Deivanayagam E., Karuppasamy K., Kim H.S., Vikraman D., In vitro Cytotoxicity Activity of Novel Schiff Base Ligand-Lanthanide Complexes, *Sci. Rep.*, **8(1)**: 3054 (2018).

[55] Bauer A.W., Kirby W.M., Sherris J.C., Turck M., Antibiotic Susceptibility Testing by a Standardized Single Disk Method, *Am. J. Clin. Pathol.*, **45(4)**: 493-496 (1966).

[56] Hu Y., Zhang J., Yu C., Li Q., Dong F., Wang G., Guo Z., Synthesis, Characterization, and Antioxidant Properties of Novel Inulin Derivatives with Amino-Pyridine Group, *Int. J. Biol. Macromol.* 70:44-49 (2014).

[57] Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C., Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay, *Free Radic. Biol. Med.*, **26(9-10)**: 1231-1237 (1999).

[58] Benzie I.F., Strain J.J., The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. *Anal. Biochem.*, **239(1)**: 70-76 (1996).

[59] Noor Z., Afzal N., Rashid S., Exploration of Novel Inhibitors for Class I Histone Deacetylase Isoforms by QSAR Modeling and Molecular Dynamics Simulation Assays, *PloS One*, **10(10)**: e0139588 (2015).

[60] Balakin K.V., Ivanenkov Y.A., Kiselyov A.S., Tkachenko S.E., Histone Deacetylase Inhibitors in Cancer Therapy: Latest Developments, Trends and Medicinal Chemistry Perspective, *Anti-Cancer Agents Med. Chem.*, **7(5)**: 576-592 (2007).

[61] Tazikeh-Lemeski E., Moradi S., Raoufi R., Shahlaei M., Janlou M.M., Zolghadri S., Targeting SARS-COV-2 Non-Structural Protein 16: A Virtual Drug Repurposing Study, *J. Biomol. Struct. Dyn.*, **2020**: 1-14 (2020).

[62] da Silva. C.M.S., Modolo L.V., Alves R.B., de Resende M.A., Martins V.B. , de Fatima A., Schiff Bases: A Short Review of their Antimicrobial Activities, *J. Adv. Res.*, **2**: 1-8 (2011).

[63] Kandile N.G., Mohamed M.I., Ismaeel H.M., Synthesis and In Vitro Biological Evaluation of New Heterocycles Based on The Indole Moiety, *J. Enzyme Inhib. Med. Chem.*, **30(1)**: 140-151 (2015).

[64] Fasina T.O., Ejiah F.N., Dueke-Eze C.U., Biological Activity of Copper (II), Cobalt (II) and Nickel (II) Complexes of Schiff Base Derived from O-Phenylenediamine and 5-Bromosalicylaldehyde, *Int. J. Biol. Chem.*, **6**: 24-30 (2012).

[65] Al-Amiery A.A., Al-Majedy Y.K., Ibrahim H.H., Al-Tamimi A.A., Antioxidant, Antimicrobial, and Theoretical Studies of the Thiosemicarbazone Derivative Schiff Base 2-(2-imino-1-methylimidazolidin-4-ylidene)hydrazinecarbothioamide (IMHC), *Bioorg. Med. Chem. Lett.*, **2(1)**: 4 (2012).

[66] Baykara H., Ilhan S., Levent A., Salih Seyitoglu M., Ozdemir S., Okumus V., Oztomsuk A., Cornejo M., Synthesis, Characterization and Experimental, Theoretical, Electrochemical, Antioxidant and Antibacterial Study of a New Schiff Base and Its Complexes, *Spectrochim. Acta A Mol. Biomol. Spectrosc.*, **130**: 270-279 (2014).

[67] Iftikhar B., Javed K., Ullah Khan M.S., Akhter Z., Mirza B., Mckee V., Synthesis, Characterization and Biological Assay of Salicylaldehyde Schiff Base Cu(II) Complexes and Their Precursors, *J. Mol. Struct.*, **1155**: 337-348 (2018).