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New Cortisol Schiff Base Synthesis and Some Complexes: Anti-inflammatory studies in silico and in vitro

Amira Salama Soliman[a,*], Abbas Mamdoh Abbas[a], Mohamed Fathy Youssef[a], Ahmed Rifaat Gardouh[b,c], Adel Sayed Orabi[a]

[a] *Chemistry department, faculty of science, Suez Canal University, Ismailia, Egypt*
[b] *Department of pharmaceutics and industrial pharmacy, Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt.*
[c] *Department of Pharmacy, Faculty of Pharmacy, Jadara University, Irbid 221110, Jordan*

Abstract

Schiff base compounds demonstrated exceptional efficiency and activity in the synthesis of several important drugs, including anti-tumor and anti-bacterial agents. Schiff base compounds proved their great efficiency and activity in the synthesis of some important drugs, such as anti-tumor, anti-bacterial, and anti-inflammatory agents. A new Schiff base (HEA) was synthesized from hydrocortisone and ethanol amine. The metal complexes were then formed. The metals selected for the preparation of complexes were derived from copper chloride, nickel chloride, magnesium sulfate, and vanadium sulfate. Fourier transform infrared (FTIR), nuclear magnetic resonance (^{1}HNMR), mass, ultra-visible spectroscopy, and thermal studies were used to characterize the synthesized Schiff base ligand. While FTIR spectra, metal percent, UV-visible spectra, thermal analysis, magnetic measurements, and kinetic measurements were used to characterize the prepared complexes. We evaluated the application of biological docking using COX2 (PDB code: 5IKT (Homo sapiens)). The binding energy of HEA was -14.45 kcal/mol, slightly higher than hydrocortisone. The anti-inflammatory activity of hydrocortisone and HEA was tested in vitro and found to be selective for COX2 with IC50 values of 1.72 and 2.29, respectively.

Complexes, Hydrocortisone, Ethanol Amine, Schiff base, In silico

Keywords: Complexes, Hydrocortisone, Ethanol Amine, Schiff base, In silico

1. Introduction

Hydrocortisone, often known as cortisol, is a glucocorticoid generated by the adrenal glands. It performs a variety of physiological roles, including inflammation suppression. Synthetic hydrocortisone is available over the counter as a topical anti-inflammatory and anti-itch medication. Its structure is shown in Figure 1.

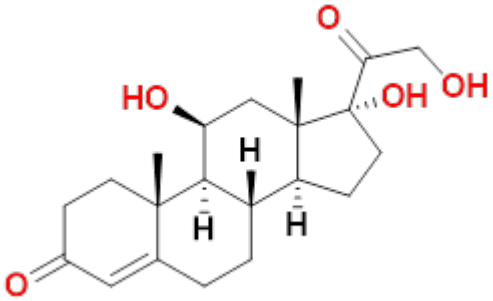

Figure 1: Hydrocortisone structure

In 1933, American chemists successfully isolated cortisol from extracts from animals' adrenal glands. For the first time, a patient who has rheumatoid arthritis was treated with the cortisone issued from these animals' glands. Soon after, the team observed a remarkable improvement in the patient's health [1].

* Corresponding author.
Email address: amiira.salama@gmail.com (Amira Salama Soliman)
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Al-Hakeim et al. assessed a novel hydrocortisone derivative's biological efficacy against several pathogenic skin fungi. The study aims to determine the efficacy of a novel compound (HCX) synthesized by the reaction of HC and cefotaxime as an in vitro antifungal medication. In this investigation, five pathogenic fungal samples were obtained. The antifungal activities of HCX were measured using the agar dilution method with three different HCX concentrations (1, 2, and 3 g/mL, respectively). The novel drug, produced by combining cefotaxime with hydrocortisone (HCX), can prevent the growth of five different dermatophytes in modest dosages compared to other antifungal medicines [2].

Preparing Schiff base ligands and their various metal complexes has recently become popular. It has been used in many fields, like medicine and industry. According to studies, it has a vital role in the biological aspect as anti-fungal, anti-malarial, antibacterial, anti-inflammatory, anti-viral, and antipyretic properties. [3–7]. Schiff bases are compounds with a carbon-nitrogen double bond, such as imine or azomethine functional groups, in which the general formula is $R_1N{=}CR_2R_3$ (S1). To make the Schiff base stable, the nitrogen atom is bound to R_1, an aryl or alkyl group, rather than hydrogen [8].

In a Schiff base reaction, an aldehyde or ketone is condensed with a primary amine (not ammonia) under the influence of an acid catalyst, where the reaction is reversible. The nitrogen counterpart of aldehyde or ketone is Schiff base, where the carbonyl group is substituted by the imine group (C=N-R); it is shown in S2, where R can be an alkyl or an aryl group.

Orabi et al. [9] published ternary complexes of amoxicillin with amino acids and metal ions that were studied potentiometrically to determine their stability constants, which could provide information about the ability of ligands to form complexes and the activities of the formed complexes, used for biological applications. Ternary complexes were formed in an associational manner. For transition metal ions, stability constants were discovered to be in the following order: Zn(II) Cu(II) > Ni(II) > Co(II), and for lanthanide metal ions, Eu(III) > Tb(III).The stability of some ternary complexes is higher than that of analogous binary complexes. The concentration distribution diagrams of all species formed in the solution were investigated and discussed.

Bahron et al. [10] reported the preparation of a Schiff base ligand, (LA), and its uninuclear and dinuclear Ni(II) and Co(II) complexes. To elucidate the structural details, some important spectroscopic analysis, physical analysis, TGA, and magnetic measurement were used. The Ni(II) has a square planar form, with the ligand behaving as a tetradentate ONNO chelation, according to single crystal X-ray diffraction of the Ni(LA) complex. A unit cell contains four asymmetric units, each containing one crystallized water molecule and one Ni(LA) complex. All of the compounds were examined for anticancer activity, and Ni(LA) was shown to be the most effective, with an IC_{50} value of 0.81 mM. The sequence of anticancer effectiveness was: Ni_2(LA) > Ni(LA) > LA > Co_2(LA) > Co(LA).

2. Experimental

2.1. Materials and methods

All starting materials and solvents used in the procedure were highly qualified and bought from Sigma-Aldrich in Burlington, MA; copper ($CuCl_2.2H_2O$), nickel ($NiCl_2.6H_2O$), magnesium ($MgSO_4.7H_2O$), and vanadium ($VOSO_4.5H_2O$). Hydrocortisone was received in the purest form from a pharmaceutical company and utilized without further purification. The solvent used in the preparation was methanol HPLC. Distilled water was used to rinse the necessary equipment, which was dried in the oven before being used.

2.2. Synthesis of HEA Schiff base

An equimolar ratio (1:1) of hydrocortisone and ethanol amine in methanol was refluxed. One mmol (0.365 g) hydrocortisone in 20 ml of methanol was whirled for 35 min. on a thermal magnetic stirrer and one mmol (ethanol amine) was added, followed by dropping glacial acetic acid. The solution was refluxed for 3 - 5 hours on a heater while its color changed from its previous appearance. Then Schiff base ligand was

evaporated to a quarter of its starting wieght. At ambient temperature, the precipitate was formed. Then the formed solution was exposed to filtering and washing with methanol and diethyl ether to vacuum-drying and storage in a vacuum desiccator. The final product, Schiff base, was colored and solid. The physical characteristics of the obtained products were determined visually. The purity of the synthesized compounds was assessed by thin-layer chromatography on a silica gel plate with the solvent system (90 % Chloroform: 10% Ethanol). Next, put the plate in a UV chamber. The result demonstrates the prepared component's purity.

2.3. Synthesis of metal complexes

To make the complexes, a warm methanolic solution of metal salts ($CuCl_2.2H_2O$ (0.0852 g), $NiCl_2.6H_2O$ (0.0648 g), $MgSO_4.7H_2O$ (0.12324 g), and $VOSO_4.5H_2O$ (0.085 g) in 20 ml of methanol) was added drop by drop to a hot solution of 2 mmol of the ligand HEA in 20 m. After that, a homogeneous solution was obtained, which helped the ligand participate in the complexation process. They continued refluxing on the thermal magnetic stirrer until the reaction was complete (1-2 h). By evaporating a portion of the solution, complexes of various colours were formed in the solid phase. The solid compounds were then filtered, washed with methanol, and kept in a desiccator**.**

2.4. Instrumentation

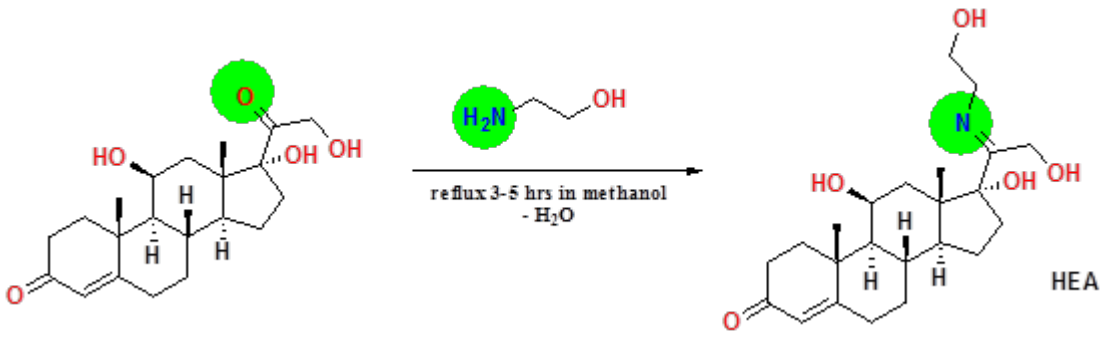
An electrical melting point device measured the melting points using transparent capillaries. A WTW digital conductivity probe was used for electrical conductivity measurements of solid complexes, which were done at 25-27 °C with 10^{-3} M methanol solution. The metal percentage was observed using an ammonia buffer and EDTA titration with an indicator (murexide) at pH 10. FT-IR spectra were recorded on a Bruker Tensor 27 spectrophotometer (4000–400 cm^{-1}) in KBr discs. For ^{1}H NMR spectra, a solution of the Schiff base ligand in DMSO- d_6 was obtained on a 300 MHz Varian-Oxford Mercury at ambient temperature, and TMS was used as an internal standard. The EI technique obtained mass spectra at 70 eV, and the MS-5988 GS-MS Hewlett-Packard instrument was used at the Microanalytical Center, National Center for Research, Egypt. The metal complexes' UV-Visible spectra were observed by UV-1800-Shimadzu spectrophotometer (double beam spectrophotometer) in the 800-200 nm region. A Gouy balance was used to record magnetic moments on the MSB-MK1 balance at ambient temperature using mercury(II) tetra-thio-cyanate-cobaltate (II). The thermogravimetric analyses (TG) of the solid ligand and complexes were carried out in a dynamic nitrogen atmosphere (40 ml/min) from room temperature to 800 °C with a linear heating rate of 20 C/min. by using a Shimadzu TG-50H thermal analyzer. The prepared compounds' molecular docking was applied by studying energy efficiency improvement operations in Chem Office utilizing the MM2 computation. UV-visible, molar conductivity, FTIR, and thermal analyses were studied at Suez Canal University, Ismailia.The efficacy of the Schiff base ligand to prevent inflammatory COX-2 (IC_{50} value, M) was confirmed using an enzyme immunoassay (EIA) kit (item no. 560131, Cayman Chemical, Ann Arbor, MI, USA) regarding Cayman Chemical protocols [11], and it was evaluated at Cairo University.

3. Result and discussion

3.1. Characterization of drug derivatives (HEA)

The ligand HEA has the molecular formula $C_{23}H_{35}NO_5$ (M.wt =405.54). HEA was formed with a reddish-orange crystalline appearance, and M.P. was over 280 °C. The compound was soluble in some common solvents (DMSO and DMF and partially soluble in (Ethanol and Methanol)). The CHN% of the target compound is C% = 68.42. H% = 8.49, N% = 3.27.

The ^{1}HNMR spectra of the HEA Schiff base reveal some resulting shifted values listed in S3 and Figure 2. The characteristic bands of hydrocortisone, with some shifts, have appeared. Also, new bands approved that the new ligand had been formed. The methylene group's protons in N=CR-CH_2-OH showed shifted doublet peaks appearing at δ (4.12, 4.18) ppm. At 5.58, 4.54 and 4.27 ppm the singlet peak of the three OH groups of HC behaves in some shifted bands. The ligand spectra exhibited a new singlet peak at δ 5.23 ppm, which could belong to



Scheme 1: The postulated structure of the Schiff base: HEA

the OH group, while ethanolamine's (CH_2-N=) proton appeared as a doublet at δ (3.51-3.52) ppm.

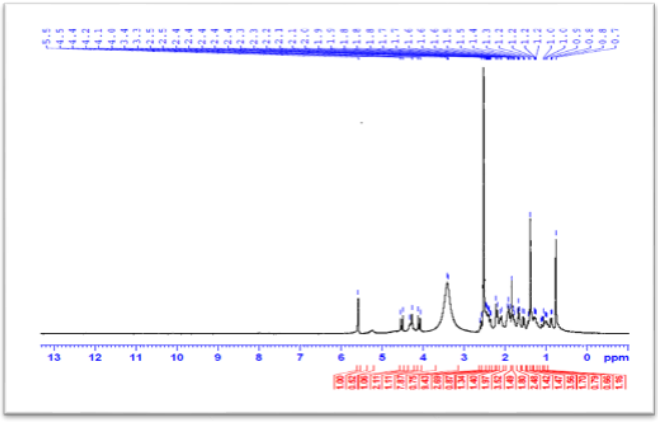


Figure 2: ^{1}HNMR spectrum of the HEA ligand

The chromatogram of the mass spectra proved the purity of the Schiff base. Also, the ligand's experimental formula was approved. The molecular ion peak was noticed = 414 m/z, intensity (48%). The fragmentations of the HEA ligand were shown in S4.

The FTIR spectrum of HC reveals a broad band at 3442 cm^{-1} which may be belong to the stretching vibration of the OH function group; the bands' broadness would be related to inter-hydrogen bonding. The band at 1711 cm^{-l} indicated υ(C=O) stretching. Figures 3 and S5 show the significant IR frequencies of HC and the Schiff base HEA.

There was a broadband placed at 3341 cm1 for the Schiff base HEA, referred to as the stretching vibration of (O-H); this band is broad because of the inter- or intra-hydrogen bonding, as in HC. The IR spectrum also shows that the band at 1715 cm^{-1} could belong to the C=O group. The band that appeared at 1634 cm^{-1} could belong to the azomethine group's stretched vibration υ(C=N).

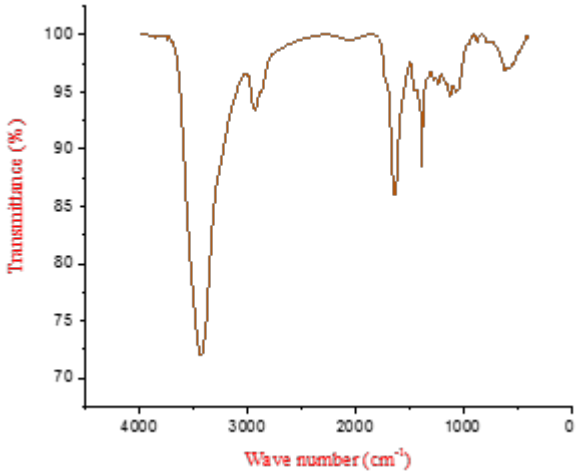


Figure 3: FTIR spectrum of the ligand HEA

In order to characterize the thermal behavior of HEA during the thermal degradation process, TG and DTA analysis were done. The thermogram is shown in Figure 4.

The TG curve for Ligand HEA contains two stages of decomposition. The first decomposing step was ranged at 19–121 °C with a DTA endothermic temperature peak at 56 °C, accompanying a mass loss of 6.77%, referred to as the "hydrated water loss" (calc. 6.24%). The other step lies in the 230–581 °C range with three DTG temperature peaks at 307, 488, and 522 °C and a mass loss of 93.23%, owing to the loss of a part of the ligand (calc. 93.75%). The DTA results at the first step show an endothermic peak at 60 °C. The other step has two exothermic peaks at 347 and 532 °C.

The UV-visible spectrum of the HEA was measured in a methanolic solvent at 25°C. (Dipole mo-

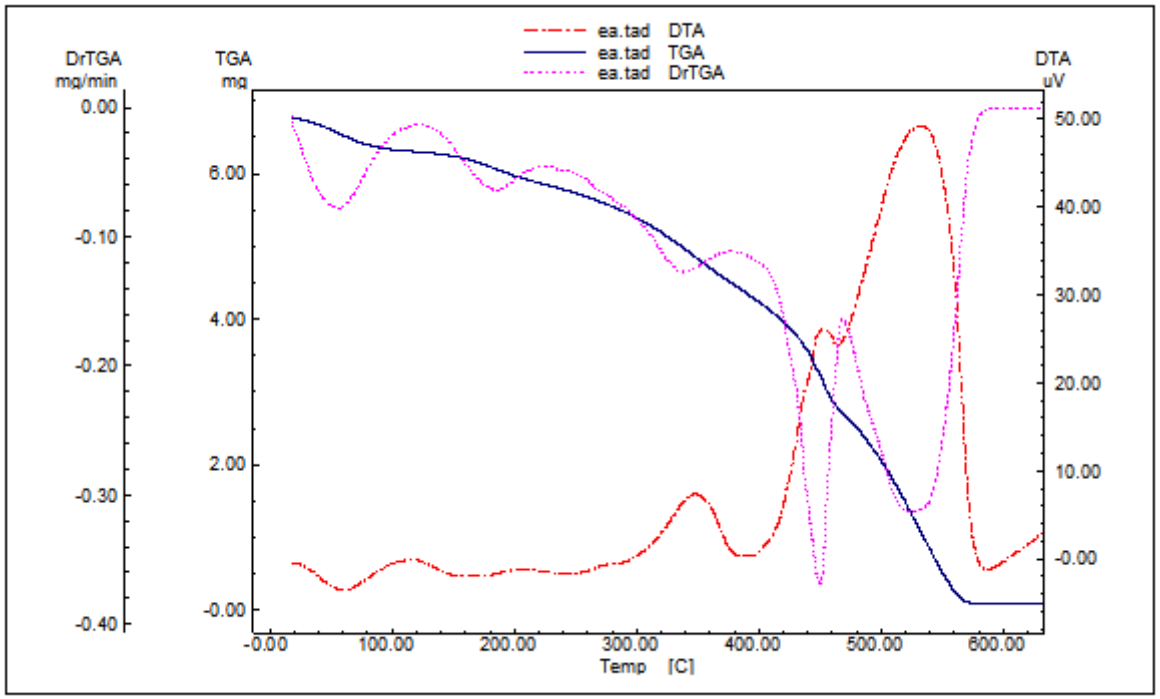


Figure 4: Thermogram of HEA Schiff base

ment = 1.70 D) and are depicted in Figure S6**.** The absorbance in the spectrum is a specific property of the groups; (-CH=N-), C=O, and C=C [12]. The spectrum of HEA has a band observed at 242 nm and a shoulder at 290 nm in a methanolic solvent, which refers to the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ electronic transitions.

3.2. Complexes of HEA ligand

When the ligand HEA interacted with the divalent Cu(II) ions, yellowish orange crystals with the molecular formula: $[Cu(HEA)_2Cl_2].3H_2O$ were formed. Ni(II) ion gave reddish-orange crystals with the formula: $[Ni(HEA)_2Cl_2].3.5H_2O$, the dark yellow crystals formed with Mg(II) ions had the formula: $[Mg(HEA)_2\ (H_2O)(SO_4)].7H_2O$. VO(II)-HEA complex formed as yellowish green crystals with the formula: $[VO(HEA)_2(SO_4)].4H_2O$. The conductivity was obtained in a methanolic solution (0.001 M). All complexes have results with range: 6-16 $ohm^{-1}.cm^2.mol^{-1}$, which proves the complexes' non-electrolytic behavior (Table 1).

The FTIR spectroscopic analysis of the HEA ligand and its complexes are listed in Table 2 and shown in S7-10. A robust broadband of υ(O-H) and water molecules appeared for all complexes, which ranged at 3100 – 3720, 3100 – 3750 cm^{-1}, 3050 – 3700 cm^{-1}, 3070 – 3690 cm^{-1}, 3200 – 3650 cm^{-1} for the ligand HEA and its complexes; Cu(II), Ni(II), Mg(II) and VO(II), respectively. The carbonyl group was at 1715, 1711, 1720, 1712, and 1722 cm^{-1} for HEA, Cu(II), Ni(II), Mg(II), and VO(II) complexes, respectively, which explain the unparticipating of this group in the complexation. The bands at 1634, 1646, 1638, 1633, and 1629 cm^{-1} for the ligand HEA, Cu(II), Ni(II), Mg(II), and VO(II), respectively, may be attributed to the presence of υ(C=N), in which the complexation was approved.

The complexes have new bands in the fingerprint area, where υ(M-O) band appeared at 610, 617, 613, and 594 cm^{-1}, the υ(M-N) band was around 559, 585, 569, and 473 cm^{-1} for Cu(II), Ni(II), Mg(II), and VO(II) complexes, respectively. Mg(II) and VO(II) gave new bands at (613, 1116) and (679, 1160) cm^{-1}, respectively, related to υ(S=O), confirming the sulfate group's the unidentate behavior [13].

The TG/DTG and DTA thermograms of the com-

Table 1: Analytical data and conductivity measurements of HEA Schiff base and its complexes

Compounds	Molecular weight	Color	Melting point (°C)	Ω* (μS)	M% analysis	
					found	calculated
HEA	405.54	Reddish orange	>280	-	-	-
$[Cu(HEA)_2Cl_2]3H_2O$	999.56	Yellowish orange	>280	6.5	6.28	6.36
[Ni(HEA)$_2$Cl$_2$]3.5H2O	1003.72	Reddish orange	>280	15.7	5.89	5.85
$[Mg(HEA)_2(H_2O)(SO_4)]7H_2O$	1075.55	Dark yellow	>280	8.8	2.29	2.26
$[VO(HEA)_2(SO_4)]4H_2O$	1046.13	Yellowish green	>280	-	4.89**	4.87

* 10^{-3} M in methanol, ohm^{-1} cm^2 mol^{-1}
** for V(IV) isICP analysis was used

Table 2: Significant IR frequencies (cm^{-1}) for HEA Schiff base and its complexes.

Compound	υ(O-H)	υ(C=O)	υ(C=N)	υ(M-O)	υ(M-N)	υ(S=O)
HEA	3441(s, br)	1715(sh)	1634(m)	-	-	-
Cu-HEA	3440(s, br)	1711(sh)	1646(s)	610(w)	559(w)	-
Ni-HEA	3425(s, br)	1720(sh)	1638(m)	617(w)	585(w)	-
Mg-HEA	3441(s, br)	1712(sh)	1633(m)	463(w)	569(w)	613(w), 1116(w)
VO-HEA	3441(s, br)	1722(sh)	1629(m)	594(m)	473(w)	679(w), 1160(w)

s: strong m: medium w: weak and sh: shoulderbr: broad

plexes derived from HEA are shown in S 11-14 and listed in S15-16. All the complexes were decomposed at well-defined stages. The initial decomposition stage of the Cu-HEA complex ranged at 13-109 °C with a maximum of 56 °C, the weight loss of this step was 5.77%, which indicates the dehydration process. The DTA peak for this step was at 58°C, indicating endothermic behavior. The next decomposition stage for Cu-HEA indicates partial or final decomposition of the Schiff base ligand. It ranged at 109-218 °C with a maximum at 164 °C and a weight loss of 8.89% (Calcd. 8.93%). The last decomposition stage was at 218–600 °C with two peaks at 430 and 541 °C and a weight loss of 85.11% (calculated at 85.61%). Ni-HEA, Mg-HEA, and VO-HEA were decomposed in two steps. The first step involves the loss of hydrated water at temperatures ranging from 29 to 129, 15 to 130, and 15 to 153 degrees Celsius, with maxima of 66, 70, and 52 degrees Celsius and mass losses of 6.03 (calculated 6.23%), 13.21 (calculated 12.92%), and 6.9% (calculated 6.9%) for complexes Ni-HEA, Mg-HEA, and VO-HEA, respectively. This step had an endothermic behaviour where the DTA peaks were at 69, 85, and 48 °C, respectively. The final step of decomposition for Ni-HEA was 129-600 °C with three peaks at 239, 384, and 497 °C and weight loss of 85.85% (calculated at 84.86%), while Mg-HEA had a final step of 130-600 with three peaks at 223, 350, and 514 °C and mass reduction of 76.99% (calculated at 77.4%). Finally, the VO-HEA complex's last decomposition took place at 153-535 °C with three maxima at 240, 352, and 489°C, accompanied by a weight loss of about 77.86% (calculated at 78.48%).

The final decomposition of these complexes was accompanied by an endothermic DTA peak, which was centered at 574, 687, 539, and 498 °C for the Cu(II), Ni(II), Mg(II), and VO(II) complexes, respectively. The residue % agrees with the calculated one, and the postulated formulas were NiO + C, MgO + 4C, and VO + 7C. The copper complex has no residue, which may result in low values for the copper content [14]. DTA data in S16 show the thermal stability behaviour of the different species, where the arrangement of the complexes according to thermal stability is: Mg-HEA > VO-HEA > Ni-HEA > Cu-HEA. While the arrangement of enthalpy H for the dehydration step is: VO-HEA > Ni-HEA >

Cu-HEA > Mg-HEA

The DTA data explains the thermal behavior of the complexes, where the complexes behaved as an endothermic reaction for the dehydration process steps. The positive values of ΔH of the dehydration step reveals the non-spontaneity of this process. VO complexes collectively gave the biggest positive ΔH value for the dehydration process

Thermodynamic parameters of HEA complexes are shown in S17**.** An example of the linearization relation of the complexes calculated by Coats-Redfern equations is presented in S18.

Some selected decomposition steps from the thermogram of the complexes: Cu(II), Ni(II), Mg(II), and VO(II) (depending on the appearance and intensity of the curves) were chosen to evaluate and discuss the kinetic and thermodynamic parameters. The parameters ΔH*, ΔG*, and ΔS* were shown in S17.

Depending on the collision theory, we can explain the results of various values of ΔS* showed the disorder during the decomposition process. Mg(II) complex had the most significant pre-exponential factor (Z) = ($1.447*10^{13}$) and also the most extensive E_a (196.35 J/mol). All complexes have negative ΔH* results, indicating the exothermic behavior for these processes. ΔG* positive values for all complexes showed the non-spontaneously behavior, under consideration with two exceptions gave negative value, cited in S17. The regular E_a value showed the reactivation behavior of these complexes for thermal decomposition reactions.

Electronic spectra analysis for the synthesized complexes (in methanolic solution) and their assignments and magnetic measurements are given in Table 3, Figures 5 and S19-21.

Ligand HEA shows an electronic transitions band at 242 nm and shoulder at 290 nm showing the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions, which undergo blue shift (hypsochromic) accompanied by hyperchromic or hypochromic shifts after complexation, indicating complex formation.

The magnetic measurements of Cu(II) and Ni(II) complexes showed results at 2.20 and 3.60 BM, respectively, which may referred to the formation sp^3d^2 configuration with an octahedral structure.

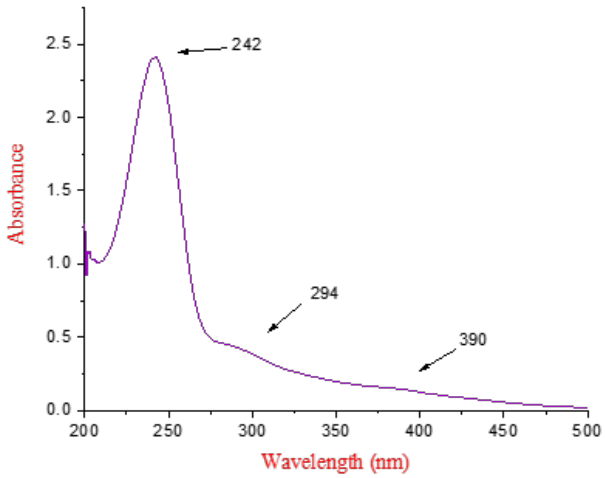


Figure 5: UV-Vis. Spectrum of $[Cu(HEA)_2Cl_2]3H_2O$ complex

The electronic transition assignments of these octahedral complexes are shown in Table 3. The Mg(II) complex showed a UV-Vis spectrum band at 242 nm, which indicated to $\pi \rightarrow \pi^*$ transition, and another band at 291 nm, which assigned to $n \rightarrow \pi^*$ transition. The VO(II) complex shows a magnetic moment of 1.56 BM, indicative of an octahedral structure. The electronic spectrum showed bands at 260, 290, and 400 assigned for $\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$, and $2T_{2g} \rightarrow 2E_g$.

According to the all previous characterization, the postulated structures of HEA complexes are shown in Figures 6 and S22.

3.3. Docking and biological application

3.3.1. Docking of the Hydrocortisone drug and its derivatives with COX2 (PDB code: 5IKT (Homo sapiens))

To reduce the gap between the theoretical and real versions and better comprehend the target drug's interaction with specific proteins. Molecular docking was applied to of the Hydrocortisone and HEA into the target receptor was delivered, covering the three-dimensional projection.

COX2 crystal structures were obtained from the RCSB Protein Data Bank. All bound water and ligands were released from the protein, and polar hydrogen was added. COX2 (S23) was characterized as a receptor, and the site sphere was chosen ac-

Table 3: The magnetic properties and electronic spectra of HEA ligand and its complexes.

Compounds	Peak		Assignment	ε^*	10Dq		CFSE	μ_{eff}	PostulateStructure
	nm	cm^{-1}		$(M^{-1}cm^{-1})x10^4$	cm^{-1}	kJ/mol	(kJ/mol)	(B.M)	
HEA	242	41322	$\pi\rightarrow\pi^*$	0.77	-	-	-	-	-
	290	34482	$n\rightarrow\pi^*$	0.30					
	242	41322	$\pi\rightarrow\pi^*$	2.40					
$[Cu(HEA)_2Cl_2].3H_2O$	294	34013	$n\rightarrow\pi^*$	0.42	25641	307	-184+4P	2.20	Octahedral
	390	25641	$^2E_g\rightarrow{}^2T_{2g}$	0.01					
	242	41322	$\pi\rightarrow\pi^*$	2.37					
$[Ni(HEA)_2Cl_2]3.5H_2O$	328	30487	$n\rightarrow\pi^*$	0.32	14697	176	-106+3P	3.60	Octahedral
	378	26455	$^3A_{2g}\rightarrow{}^3T_{1g}(P)$	0.02					
	458	21834	$^3A_{2g}\rightarrow{}^3T_{1g}(F)$	0.01					
$[Mg(HEA)_2(SO_4)(H_2O)].7H_2O$	242	41322	$\pi\rightarrow\pi^*$	1.98	-	-	-	-	Octahedral
	291	34364	$n\rightarrow\pi^*$	0.32					
	260	38461	$\pi\rightarrow\pi^*$	3.96					
$[VO(HEA)_2(SO_4)].4H_2O$	290	34482	$n\rightarrow\pi^*$	3.85	25000	299	-120	1.56	Octahedral
	400	25000	$^2T_{2g}\rightarrow{}^2E_g$	0.01					

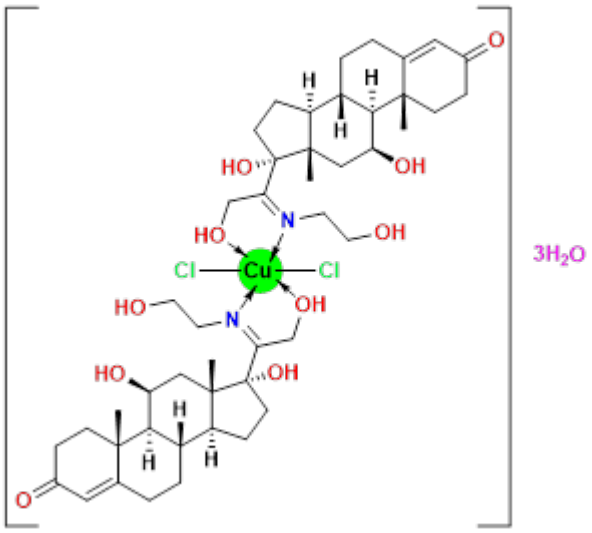


Figure 6: The postulated structures of complexes derived from HEA ligand Cu-HEA

cording to the binding sites of the ligands Tolfenamic acid and COH (Protoporphyrin IX containing Co), These molecules were subsequently eliminated, and Hydrocortisone and HEA were docked in their place. The types of interactions between the docked protein and targeted drugs were investigated [15]. All the studies were done on Hewlett-Packard Pentium Dual-Core T4300 2.10 GHz running Windows 10 Ultimate using autodock software.

3.3.1. Docking of Hydrocortisone with COX2 (PDB code: 5IKT (Homo sapiens

The molecular docking of hydrocortisone with COX2 was shown in S24, and the resulting data are summarized in Table 4. The results validate the binding of the target ligand positions O39 and O51 with the protein through the amino acids N Thr198(A) and N2 Gln275(A). The interacting bonds were observed as hydrogen bonds. S24 shows the postural form with the highest binding energy. The binding energy was calculated to be -14.02 kcal/mol.

The molecular docking of the drug with COX2 was shown in Figure 7 and S25; the results are in Table 5. The selected pose gave the interaction of the target ligand O33 and O39 with the target protein through H-bond with the O_1 Thr198(A) and $N_{\varepsilon 2}$ His193(A) amino acid respectively, and C9 of

Table 4: The apparent interaction parameters of the Hydrocortisone with COX2 [PDB 5IKT (Homo sapiens)].

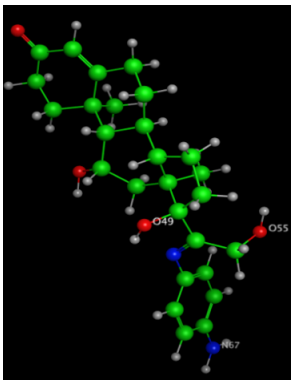
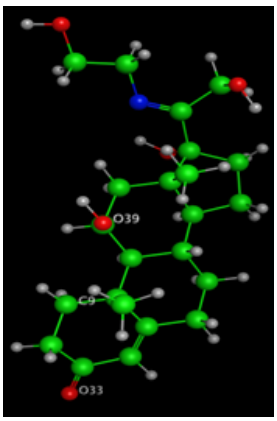
Ligand structure	Ligand	Receptor	Interaction	Distance	Energy (kcal/mol)	Nearby pose	Binding energy (kcal/mol)
	O39	N Thr198(A)	H-acceptor	3.03	-1.00	His200 –	-14.02
	O51	N_2Gln275(A)	H-acceptor	2.95	-1.90	His374 –	
						Tyr371 –	
						Phe196 –	
						His372 –	
						Lys197 –	
						Asn368 –	
						His193 –	
						Val277 –	
						Glu276	

Table 5: The apparent interaction parameters of the HEA with COX2 [PDB code: 5IKT (Homo sapiens)].

Ligand structure	Ligand	Receptor	Interaction	Distance	Energy (kcal/mol)	Nearby pose	Binding energy (kcal/mol)
	O33	O1Thr198(A)	H- acceptor	3.09	-0.70	Val430 –	-14.45
	O39	N2His193(A)	H-acceptor	2.95	-3.70	Tyr134 –	
	C9	5-ringHis193(A)	H-pi	4.67	-0.70	Phe381 –	
						Tyr371 –	
						Ala185 –	
						Leu376 –	
						Leu377 –	
						Phe186 –	
						Tyr134 –	
						Val433 –	
						Val281	

methyl group formed Arene-H bond with 5-ring His193(A) amino acid. The types of the interacted bonds were showen as hydrogen bonds and hydrophobic interactions (ex., Arene-H bonds); and the hydrogen bond type is predominant. The binding energy was -14.45 kcal/mol, slightly higher than hydrocortisone.

3.3.2. In vitro anti-inflammatory activity of Hydrocortisone derivative and its complexes (Cyclooxygenase Inhibition Assay

Anti-inflammatory activity of the ligands by enzyme immunoassay (EIA)

The goal of the in vitro biological activity experiment was to determine whether a synthetic ligand might prevent bovine COX-2 activity using a colorimetric enzyme immunoassay (EIA) kit to search for isozyme-specific inhibition. Calculating the dose at which enzymes performed 50% inhibition (IC_{50}) allowed researchers to gauge a drug's potency [16]. The measured COX-2 inhibition effect of the ligand was comparable to those of the reference medication hydrocortisone (HC). The results showed that the prepared ligand HEA is a promising inhibitor of the COX-2 enzyme that showed an IC_{50} average = 2.29, but it is a bit weak inhibitor compared to the

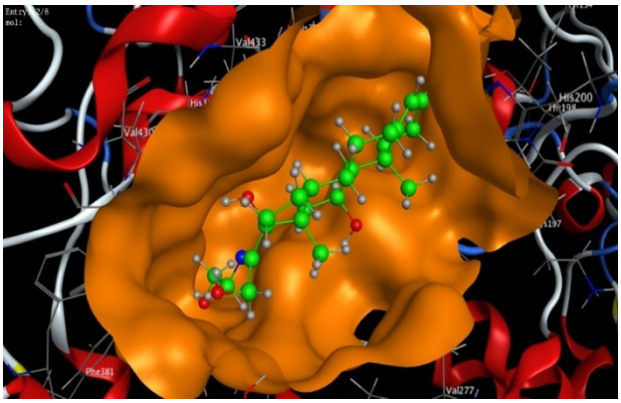


Figure 7: Docking model of the interaction of HEA with COX2 [PDB 5IKT (Homo sapiens)] bonding sites. 3D cavity interaction diagram

hydrocortisone, which had an IC_{50} average=1.72 pg/ml.

4. Conclusion

The synthesized compounds (5 compounds) were obtained in pure form and characterized according to modern analytical methods. The new Schiff base ligand, HEA, was prepared, well characterized, and gave promising properties. The formed complexes Cu(II), Ni(II), and VO(II) have octahedral structures; meanwhile, Mg(II) has an octahedral structure. The thermal properties of the formed complexes gave some benefit data: The order of ΔH for the liberation of the water of crystallization of the M(II)-HEA: VO-HEA > Ni-HEA > Cu-HEA > Mg-HEA with the non-spontaneity process**.** The following formula could postulate the formed complexes: $[Cu(HEA)_2Cl_2].\ 3H_2O$, $[Ni(HEA)_2Cl_2].\ 3.5\ H_2O$, $[Mg(HEA)_2(H_2O)(SO_4)].\ 7H_2O$, and $[VO(HEA)_2(SO_4)].\ 4H_2O$. The docking studies were carried out for the Schiff base ligand HEA. The docking work was carried out using COX2 (PDB code: 5IKT (Homo sapiens)). The Hydrocortisone drug gave total binding energy = -14.02 kcal/mol, whereas HEA gave binding energy: -14.45 kcal/mol. The results indicate that HEA is more attached to the target protein, which exceeds the market drug (Hydrocortisone). This result lets us advise making extra medicinal and pharmaceutical studies to make the correct decision about using this compound as a market drug. The anti-inflammatory activity of the Schiff base ligand was tested at the in-vitro level. The results showed that the prepared ligand HEA is a suitable inhibitor of the COX-2 enzyme that showed an IC_{50} average = 2.29, but it is a bit weak inhibitor compared to the hydrocortisone, which had an IC_{50} average=1.72 pg/ml.

References

[1] M. M. S. Yazdan, M. T. Ahad, Z. Mallick, S. P. Mallick, I. Jahan, M. Mazumder, An Overview of the Glucocorticoids' Pathways in the Environment and Their Removal Using Conventional Wastewater Treatment Systems, Pollutants (2021) 141–155.

[2] A. H. Mohsen, I. H. Mohsen, A. N. Al-Khafaji, Compared between the efficiency of chemotherapy and alcoholic extract of plant leaves Melia azedarch L. in the growth of many fungi that cause Tinea capitis in humans in the laboratory, Journal of Pharmaceutical Sciences and Research 11 (2019) 393–397.

[3] P. Anand, V. Patil, V. Sharma, R. Khosa, N. Masand, Schiff bases: A review on biological insights, Int. J. Drug Des. Discov 3 (2012) 851–868.

[4] K. Gupta, A. K. Sutar, C. C. Lin, Polymer-supported Schiff base complexes in oxidation reactions, Coordination Chemistry Reviews 253 (2009) 1926–1946.

[5] A. M. Abbas, S. R. Fisal, A. Radwan, M. Makhlouf, A. S. Orabi, Novel Action for Ampicillin Derivative and Its Complexes: Physicochemical, Thermal analysis, DNA Interaction, Docking with FabH Protein, In silico, and In vitro Studies, Journal of Molecular Liquids (2022) 118333–118333.

[6] A. S. Orabi, Complexes derived from some biologically active ligands, Journal of Coordination Chemistry (2008) 1294–1305.

[7] A. S. Orabi, K. M. A. El-Nour, S. A. Ahmed, A. I. El-Falouji, Novel gold and silver-Sarcophine complexes as antitumor agents against MCF7 and HepG2 cells: synthesis, characterization, in silico, in vitro and docking studies, Journal of Molecular Liquids 273 (2019) 559–575.

[8] W, A. Zoubi, Solvent extraction of metal ions by use of Schiff bases, Journal of Coordination Chemistry 66 (2013) 2264–2289.

[9] A. S. Orabi, M. Abdelhameed, A. M. Abbas, G. M, Mostafa, modern view for binary and ternary complexes of metal ions with amoxicillin and some amino acids, Advances in Environmental and Life Sciences 1 (2022) 22–39.

[10] H. Bahron, S. S. Khaidir, A. M. Tajuddin, K. Ramasamy, B. M. Yamin, Synthesis, characterization and anticancer activity of mono-and dinuclear Ni (II) and Co (II) complexes of a Schiff base derived from o-vanillin, Polyhedron (2019) 84–92.

[11] P. Singh, J. Kaur, H. Kaur, A. Kaur, R. Bhatti, Synergy of physico-chemical and biological experiments for developing a cyclooxygenase-2 inhibitor, Scientific reports 8 (2018) 1–14.

[12] S. Arulmurugan, H. P. Kavitha, B. Venkatraman, Biological activities of Schiff base and its complexes: a review, Rasayan J Chem 3 (2010) 385–410.

[13] M. Hayyan, M. A. Hashim, I. M. Alnashef, Superoxide ion: generation and chemical implications, Chemical reviews 116 (2016) 3029–3085.

[14] E. J. Meehan (1982).

[15] X. L. Wang, Y. B. Zhang, J. F. Tang, Y. S. Yang, R. Q. Chen, F. Zhang, H. L. Zhu, Design, synthesis and antibacterial activities of vanillic acylhydrazone derivatives as potential β-ketoacyl-acyl carrier protein synthase III (FabH) inhibitors, European journal of medicinal chemistry 57 (2012) 373–382.

[16] B. Roschek, R. C. Fink, D. Li, M. Mcmichael, C. M. Tower, R. D. Smith, R. S. Alberte, Pro-inflammatory enzymes, cyclooxygenase 1, cyclooxygenase 2, and 5-lipooxygenase, inhibited by stabilized rice bran extracts, Journal of medicinal food 12 (2009) 615–623.