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Synthesis, Characterization and Anticancer Activity of La(III), Ce(III), Pr(III) and Gd(III) Complexes of 1,1`- Bis(Z)-N- ethyldiene-5- Methylthiazol- 2-Amine Ferrocene

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CONDENSATION of l,l'-diacetyl ferrocene with 2-amino-5-methylthiazole in 1 : 2 molar ratio yields a ferrocenyl Schiff base ligand 1,1`- bis(Z)-N-ethyldiene-5-methylthiazol-2-amine ferrocene (L). This ligand forms 1:1 complexes with La(III), Ce(III), Pr(III) and Gd(III) nitrate in a good yield. Characterization of the ligand and complexes were carried out using infrared, nuclear magnetic resonance, mass spectra, electronic absorption, magnetic susceptibility, molar conductivity and elemental analysis. The cytotoxicity and *in vitro* anticancer evaluation of the ligand and its complexes have been assessed against four different human tumor cell lines (MCF-7, HepG2, A549 and HCT116). The results revealed that the prepared compounds exert their actions in HepG2 and MCF-7 through inhibition of the activity of both urokinase and histone deacetylase (HDAC). Pr-complex revealed promising anticancer activity compared to the activity of the commonly used anticancer drug, doxorubicin.

Keywords: Bioinorganic chemistry, Ferrocene, Schiff –base, Thiazole, Lanthanide complexes anticancer, Urokinase and Histone deacetylase.

Schiff bases form an interesting class of ligands in the field of coordination chemistry mainly due to their facile syntheses, easy availability, electronic properties and good solubility in common solvents[(1)]. Large number of Schiff bases and their complexes show a large variety of catalytic[(2)], biological[(3)], antifungal[(4)] , anti-tumor and anti HIV activities[(5)] . In addition, these compounds have been recently used in metal sensors[(6)] .

Schiff bases of ferrocene derivatives and their metal complexes[(7)] can be regarded as multi-nuclear molecules possessing both the features of organometallics and of coordination chemistry[(8)]. Ferrocene derivatives with a wide range of substitution and functionality have found increasing applications in asymmetric catalysis, material science, bioorganometallic chemistry and pharmaceutics[(9)]. Many compounds containing ferrocene moiety are active to

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inhibit the proliferation of many kinds of tumor cells[10] because they are being readily recognized *in vivo* by amino acids, proteins, DNA, and carbohydrates[10].

On the other hand, much attention has been focused on lanthanide complexes with interesting magnetic and luminescent behaviors, because of their applications in diagnostic medicine (*e.g.* contrast agents for magnetic resonance imaging[11] , radiotherapeutic drugs[12] , fluoro immune assay[13] , in hetero- and homogeneous catalysis, as components of permanent magnetic materials[14] and also lanthanide complexes manifest an antitumor activity and may be developed in the future to be anticancer agents[15] .

In the light of these facts, and in continuation of our program aiming at synthesis of new bioactive compounds[16] ; we intended to study the behaviour of some selected metals with the same ligand and study the cytotoxicity of the prepared new metal complex. On this bases, we report the synthesis and characterization of a ferrocenyl Schiff base ligand and other four lanthanide complexes (La, Ce, Pr, Gd). The study of the proliferative through inhibition of HDAC enzyme activity with investigation of their mechanism of actions on urokinase enzyme inhibition as a marker for cancer invasion and metastatization were carried out.

Materials and Methods

All chemicals and solvents used in this work were of analytical reagent grade. The 1, 1'-diacetylferrocene was prepared by the method described in the literature[16]. The yields refer to analytically pure compounds and were not optimized.

Elemental analyses were determined at the Micro Analytical Center, Cairo University. IR spectra were recorded in the 4000-400 cm^{-1} on a (Jasco FTIR- 6100 Japan**)** spectrometer, using KBr pellets. ^{1}H and ^{13}C NMR were recorded on a Bruker DPX 300, δ values relative to the deuterated DMSO. Mass spectra: Jeol JMS-700 using FAB technique with NBA matrix. Magnetic susceptibilities were measured at 23°C by the Gouy method at the Faculty of Science, Cairo University. The molar conductivity was measured using Metrohem 660 conductivity meter [in solution in DMF (10^{-3})]. Electronic absorptions were recorded on a (PG Instruments ltd., +80+ UV-Vis) automatic spectrophotometer in DMF.

Synthesis of Ligand, 1

The ferrocenyl ligand 1,1′-bis(Z)-N-ethylidene-5-methylthiazol- 2-amine ferrocene was prepared by addition of ethanolic solutions of 2-amino-5-methyl thiazole (2 mmol, 2.28 gm dissolved in about 20 ml absolute ethanol) slowly to a magnetically stirred solution of 1,1'-diacetylferrocene (1 mmol, 2.70 gm). The mixture was refluxed for three hours, the brown color of the diacetylferrocene started to change to deep brown within the first hour, but reflux was continued to complete the reaction. The solvent was evaporated and the ligand was washed with cold ethanol and dried[17] .

Spectral characterization of the Ligand ($C_{22}H_{22}FeN_4S_2$), 1

Yield 82% ; elemental analysis: calc. for ($C_{22}H_{22}FeN_4S_2$): C, 57.14 H, 4.80; N, 12.08%. Found: C, 56.91; H, 4.94; N, 11.86%. IR: υ = 3093 (C-H, Fc moiety), 1655 (C=N), 1455 (C=C, Fc moiety), 1114 (C–C, Fc moiety), 1049 δ(C-H, Fc moiety), 840(C-S-C), 781 π(C-H, Fc moiety), 501 δ (Fe-ring) cm^{-1}; ^1H NMR (DMSO-d_6) δ = 2.16 (s, 6H, $2CH_3$ in Fc), 2.27 (s, CH_3 in thiazole), 4.58-4.65 (m, 4H, C_5H_4), 4.74-4.80 (m, 4H, C_5H_4), 6.56 (s, H in thiazole);^{13}C NMR (DMSO-d_6): ∂ = 16.6 ($2CH_3$ attached to thiazole rings), 23.1 ($2CH_3$ bonded to the azomethine groups), 68.4, 69.6, 79.5 (ferrocenyl moiety), 120.2, 141.6, 153.7 (thiazole rings), 148.2 (2 C=N) ppm; FAB MS: m/z (%) = 462 (42) [M^+]; UV-Vis. λ = 266, 317, 452 nm (in DMF).

Synthesis of the metal complexes 2-5

The different complexes were prepared by addition of 2.0 mmol of lanthanides nitrate dissolved in 20 ml ethanol, into a warmed solution of the ligand (2.0 mmol in 20 ml ethanol). The mixture was refluxed for five hours. The complexes that separatedout on cooling at 5°C, were filtered, washed twice with n-hexane and dried.

Spectral characterization of La(III) complex ($C_{22}H_{22}FeLaN_7O_9S_2$), 2

Yield 71%; elemental analysis: calc. for ($C_{22}H_{22}FeLaN_7O_9S_2$): C, 35.56; H, 2.82; N, 12.45%. Found: C, 35.45; H, 2.64; N, 12.18%; IR: υ = 3089 (C-H, Fc moiety), 1622 (C=N),1468 (NO_3),1456 (C=C, Fc moiety),1348(NO_3), 1115 (C–C, Fc moiety), 1058 δ(C-H, Fc moiety), 817 (C-S-C), 763 π(C-H, Fc moiety), 528 (La-S), 502 δ (Fe-ring),480(La-N) cm^{-1}; ^1H NMR (DMSO-d_6) δ = 2.18(s, 6H, $2CH_3$ in Fc), 2.27 (s, CH_3 in thiazole), 4.61-4.73 (m, 4H, C_5H_4), 4.80-4.86 (m, 4H, C_5H_4), 7.10 (s, H in thiazole); ^{13}C NMR (DMSO-d_6) : ∂ = 16.8 ($2CH_3$ attached to thiazole rings), 23.4 ($2CH_3$ bonded to the azomethine groups), 68.6, 69.8, 79.7 (ferrocenyl moiety), 122.1, 142.8, 154.7 (thiazole rings), 149.2 (2 C=N) ppm; UV–Vis. λ = 268, 333,461nm; A_M = 23.4 $\Omega^{-1}.mol^{-1}.cm^2$; μ_{eff} = Dia

Spectral characterization of Ce(III) complex ($C_{22}H_{22}CeFeN_7O_9S_2$), 3

Yield 67%; elemental analysis: calc. for ($C_{22}H_{22}CeFeN_7O_9S_2$): C, 33.51; H, 2.81; N, 12.43%. Found: C, 33.47; H, 2.80; N, 12.22%; IR: υ = 3082(C-H, Fc moiety), 1616 (C=N), 1471 (NO_3),1457 (C=C, Fc moiety),1351(NO_3), 1118 (C–C, Fc moiety), 1068 δ(C-H, Fc moiety), 816 (C-S-C), 752 π(C-H, Fc moiety), 533 (Ce-S), 508 δ (Fe-ring), 483(Ce-N) cm^{-1}; UV–Vis λ = 270, 335, 458 nm (in DMF); A_M = 19.8 $\Omega^{-1}.mol^{-1}.cm^2$; μ_{eff} = 2.47BM.

Spectral characterization of Pr(III) complex ($C_{22}H_{22}FeN_7O_9PrS_2$), 4

Yield 70%; elemental analysis: calc. for ($C_{22}H_{22}FeN_7O_9PrS_2$): C, 33.48; H, 2.81; N, 12.42%. Found: C, 33.22; H, 2.74; N, 12.25%; IR: υ = 3091 (C-H, Fc moiety), 1609 (C=N), 1471 (NO_3),1457 (C=C, Fcmoiety),1350(NO_3), 1117 (C –C, Fc moiety), 1048 δ(C-H, Fc moiety), 816 (C-S-C), 765 π(C-H, Fc moiety), 533 (Pr-S), 504 δ (Fe-ring), 482(Pr-N) cm^{-1}; UV–Vis. λ = 268, 334, 462 nm (in DMF); A_M= 24.7 $\Omega^{-1}.mol^{-1}.cm^2$; μ_{eff} = 3.54BM.

Spectral characterization of Gd(III) complex ($C_{22}H_{22}FeGdN_7O_9S_2$), 5

Yield 75%, deep brown; elemental analysis: calc. for ($C_{22}H_{22}FeGdN_7O_9S_2$): C, 32.80; H, 2.75; N, 12.17%. Found: C, 32.67; H, 2.54; N, 12.01%; IR: υ = 3085 (C-H, Fc moiety), 1614 (C=N), 1469 (NO_3),1455 (C=C, Fc moiety),1348(NO_3), 1117 (C–C, Fc moiety), 1079 δ(C-H, Fc moiety), 812(C-S-C), 788 π(C-H, Fc moiety), 532 (Gd-S),507 δ (Fe-ring), 478 (Gd-N) cm^{-1}; UV–Vis. λ = 269, 338, 460 nm; A_M= 25.2 $\Omega^{-1}.mol^{-1}.cm^2$; μ_{eff} = 7.87BM.

Anticancer activity

Chemicals

Fetal bovine serum (FBS) and L-glutamine, were obtained from Gibco Invitrogen Company (Scotland, UK). Dulbecco's modified Eagle's (DMEM) medium was provided from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were obtained from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals and reagents used in this study were of analytical grade and purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Cell lines and culturing

Anticancer activity screening for the tested compounds utilizing four different human tumor cell lines including breast adenocarcinoma MCF-7; human hepatocellular carcinoma HepG2, human lung cancer A549 and human colon cancerHCT116 cell lines were obtained from the American Type Culture Collection (Rockville, MD, USA). The tumor cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated fetal calf serum (GIBCO), penicillin (100 U/ml) and streptomycin (100 µg/ml) at 37 ^oC in humidified atmosphere containing 5% CO_2. Cells at a concentration of 0.50 x 10^6 were grown in a 25 cm^2 flask in 5 ml of complete culture medium.

In vitro

Antiproliferative assay : The antiproliferative activity was measured *in vitro* using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay according to the previous reported standard procedure[18]. Cells were inoculated in 96-well microtiter plate (10^4 cells/ well) for 24 hr before treatment with the tested compounds to allow attachment of cell to the wall of the plate. Test compounds were dissolved in DMSO and diluted with saline to the appropriate volume.

Different concentrations of the compounds under test (0, 4, 8, 16, 32, 64 and 128 µg/ml) were added to the cells. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 hr at 37°C and in atmosphere of 5% CO_2. After incubation, media were removed and 40 µl MTT solution/well were added and incubated for an additional 4 hr. MTT crystals were solubilized by adding 200 µl of DMSO/well and plate was shacked gently for 10 min at room temperature. The absorbance was measured with

microplate reader (AsysHitech, Austria) at 570nm. The relation between surviving fraction and drug concentration is plotted to get the survival curve for each cell line after the specified time.

Determination the level of uPA protein expression : The level of urokinase (uPA) protein expression was determined using Assay Max human uPA ELISA kit (Assaypro, USA) according to manufacturer's instructions. The prepared compounds as well as standard drug, doxorubicin were incubated for 48 hr with, HepG2 and A549 cells at concentration of 1/10 of the IC_{50} values of each compound .

After 48 hr from compounds treatment, medium was collected and centrifuged at 2000 xg for 10 min to remove cellular debris. Add 50 µl of the cell extract per well and incubate for 2 hr. Wells were washed with 200 µl of wash buffer then add 50 µl of biotinylateduPA antibody to each well and incubate for 1 hr at 25ºC. After washing, plates were incubated with 50 µl of streptavidin-peroxidase conjugate per well and incubate for 30 min then wash the microplate as described above. Add 50 µl of chromogen substrate per well and incubate for about 10 min or till the optimal blue color density develops. Then add 50 µl of stop solution to each well. The color will change from blue to yellow. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately and the concentrations of uPA in the samples were determined and the percentage of uPA inhibition for each compound was calculated as compared with control cancer cells (DMSO treated).

Determination of histone deacetylase (HDAC) activity : Histone deacetylase inhibitors represent a promising new class of compounds for the treatment of cancer[19]. The activity of HDAC in the lysate of hepatic HepG2 and lung A549 cancer cells treated with the prepared compounds 1-5 was measured using a colorimetric assay kit (BioVision, Mountain View, kit no. K331-100). The procedure involves the use of the HDACcolorimetric substrate (Boc-Lys (Ac)-pNA), which comprises an acetylated lysine side chain and is incubated with a sample containing nuclear extract.

Deactivation sensitizes the substrate, and treatment with the lysine developer produces a chromophore, which can be analyzed using a colorimetric plate reader. A sample containing HDAC activity (*e.g.* HeLa cell nuclear extract) was used as a positive control. A standard curve was prepared using the known amount of the deacetylated standard (Boc-Lys-pNA). A similar volume of control sample was added to 100 ng/ml trichostatin A (TSA), as a known inhibitor of HDAC activity. The activity was recorded as µM deacetylated substrate/mg protein from standard curve and was expressed as percentage of control untreated cells.

Statistical analysis : The results are reported as Mean ± Standard error (S.E.) for at least four times experiments. Statistical differences were analyzed

according to followed by one way ANOVA test followed by student's *t* test wherein the differences were considered to be significant at $p < 0.05$.

Results and Discussion

Chemistry

Synthesis and characterization of Ligand, 1

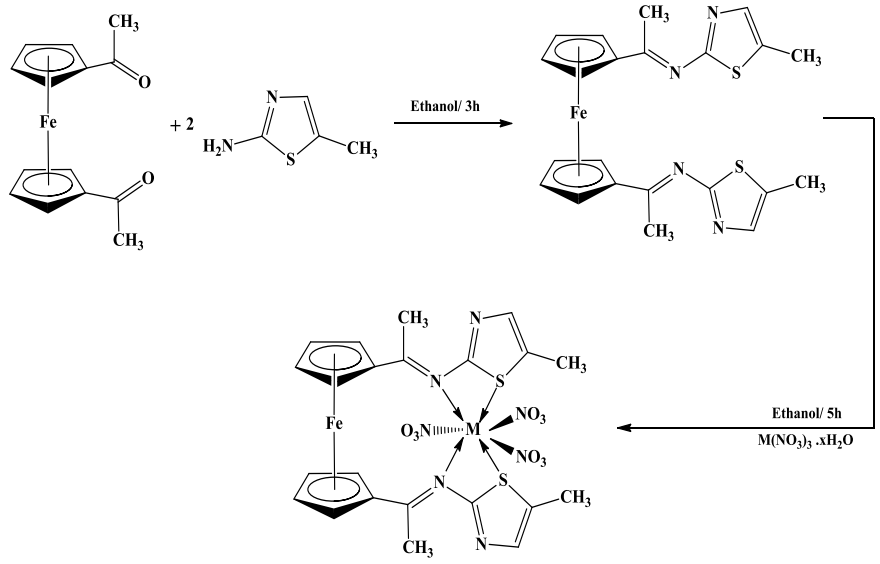
The ferrocenyl ligand, {1,1'-bis (Z)-N-ethylidene-5-methylthiazol-2-amine ferrocene}, 1 (Scheme 1) was prepared by addition of ethanolic solution of 1,1'-diacetylferrocene to 2-amino-5-methylthiazole in 1: 2 molar ratio with reflux for three hours, where the brown color of the diacetylferrocene started to change to a deep brown within the first hour. The ligand was isolated in a good yield and it is soluble in common organic solvents such as ethanol, methanol, acetone, and chloroform. The elemental analysis of the ligand 1 is consistent with the calculated results. The ligand 1 was characterized using different spectroscopic tools. The IR spectra of the prepared ligand 1 showed a strong band at 1655 cm^{-1} which was assigned to the formation of the azomethine (C=N) bond[16] . Another medium band appeared at 840cm^{-1} which was assigned to C-S-C (ring) stretching vibration[16]. The characteristic peaks of the ferrocenyl moiety were observed at 3093, 1455, 1114, 1049, 781, and 501 cm^{-1}. These bands were attributed to υ(C-H), υ(C=C), υ(C-C), δ(C-H), π(C-H) and δ(Fe-ring), respectively [16] .

In the ^{1}H NMR spectra of the ligand 1, the signal of the two methyl groups in the ferrocenyl moiety was observed at 2.16 ppm whereas the two methyl groups of the thiazole ring appeared at 2.27 ppm. The protons of the ferrocenyl moiety appeared as two multiplets at 4.74-4.80 and 4.58-4.65 ppm. These bands were assigned to the α- and β-protons for the substituted cyclopentadienyl rings[16] . The spectra showed also a band at 6.56 ppm, which was assigned to the proton of thiazole ring.

The ^{13}C NMR of the ligand 1 showed a signal at 16.6 ppm which assigned to the two methyl group attached to the thiazole rings, a signal at 23.1 ppm due to the two methyl groups bonded to the azomethine groups[16] , and three signals at 68.4, 69.6 and 79.5 ppm due to the ferrocenyl moiety. The signal that appeared at 148.2 ppm was assigned to the two carbons of the azomethine groups, and three signals at 120.2, 141.6 and 153.7 ppm were assigned to the thiazole rings[16] .

Synthesis and characterization of the complexes 2-5

The complexes of La(III), Ce(III), Pr(III), and Gd(III) ions 2-5 were prepared in a good yield from the equimolar ratio of the ligand and the corresponding metal salts in ethanol with reflux for five hours (Scheme 1). All the complexes are stable in air and light. The elemental analysis of the complexes is consistent with the calculated results from the empirical formula of each compound.



Scheme 1

All the complexes possess similar IR spectra, indicating that they have similar structures. It was found that the characteristic band of the C=N group in the free ligand (at 1655 cm^{-1}) was shifted to a lower frequency (1609 -1622 cm^{-1}) in the complexes. This shift indicates coordination of the azomethine nitrogen to the metal ions in the complexes[16]. The medium-intensity band at 840 cm^{-1} observed in the free ligand was assigned to C-S-C (ring) stretching vibration. This band shifted to lower values by 24-28 $cm^{-1}$for all complexes, which indicates the coordination of the C-S-C group in the bonding with the metal ions. The IR spectra of the complexes showed also four additional bands, which can not be found in the spectrum of the ligand. The first two bands observed at 1468 and 1348 cm^{-1} are assigned to the vibrations of nitrate groups. The difference between these two absorption bands is 120 cm^{-1}, which indicates that the nitrate ions are coordinated as a monodentate group to the lanthanide (III) complexes[20] (Scheme 1). The other two bands in the ranges 528-533 and 483-476 cm^{-1} are attributed to Ln-S and Ln-N bonds in the complexes respectively. The characteristic frequencies of the ferrocenyl moiety in the spectra of the complexes appeared at nearly the same position in the ligand which indicates that the cyclopentadienyl ring of the ferrocene is not directly coordinated to the metal ions[16] .

The ^1H NMR spectra of the diamagnetic La-complex 2 was recorded at room temperature using DMSO-d_6 as a solvent (Table 1). The spectra showed nearly the same signals appeared in the spectra of the ligand with slightly downfield shift, which may be due to complexation of nitrogen atoms with metal ion[16] .

The spectra of 2 showed a signal at 2.18 ppm which assigned to the methyl groups for the Fc moiety and another one at 2.29 attributed to the methyl groups of thiazole rings. The spectra showed also two multiplets for the α- and β-protons for the substituted cyclopentadienyl rings appeared at the range 4.80-4.86 and 4.61-4.73 ppm. The signal appeared at 7.10 ppm was attributed to the proton of thiazole ring in the complexes. The ^{13}C NMR signals of the La-complex were shifted slightly downfield compared with that of the ligand, which may be due to coordination of the ligand with the metal ions (Table 1).

TABLE 1. ^{1}HNMR and ^{13}CNMR data of the ligand 1 and La (III) 2 complex.

Ligand/ complex	^{1}H NMR (DMSO-d6), d (ppm)	^{13}C NMR (DMSO-d6), d (ppm)
1	2.16 (s, 6H, $2CH_3$ in Fc), 2.27 (s, CH_3 in thiazole), 4.58-4.65 (m, 4H, C_5H_4), 4.74-4.80 (m, 4H, C_5H_4), 6.56 (s, H in thiazole)	16.6 ($2CH_3$ attached to thiazole rings), 23.1 ($2CH_3$ bonded to the azomethine groups), 68.4, 69.6, 79.5 (ferrocenyl moiety), 120.2, 141.6, 153.7 (thiazole rings), 148.2 (2C=N) ppm
2	2.18 (s, 6H, $2CH_3$ in Fc), 2.29 (s, CH_3 in thiazole), 4.61-4.73 (m, 4H, C_5H_4), 4.80-4.86 (m, 4H, C_5H_4), 7.10 (s, H in thiazole)	16.8 ($2CH_3$ attached to thiazole rings), 23.4 ($2CH_3$ bonded to the azomethine groups), 68.6, 69.8, 79.6 (ferrocenyl moiety), 120.5, 141.8, 154.1 (thiazole rings), 148.6 (2C=N) ppm

Molar conductance and magnetic behavior of compounds 2-5

The molar conductivities of 10^{-3} M of the complexes (dissolved in DMF) were measured at room temperature. The results were in the range [19.8-25.2] $\Omega^{-1}.mol^{-1}.cm^2$. These values suggest that all the Ln-complexes arenon-electrolytes 2-5 [21] , implying that all nitrate groups are in coordination sphere.

Magnetic moments of the lanthanide (III) complexes showed that the La complex is diamagnetic; whereas complexes 3-5 have the values between 2.47 and 7.87 BM. These measured values are very close to the theoretical values calculated for the free corresponding ions. This result indicates minor participation of 4f electrons in bond formation because they are well shielded $5s^2$-$5p^6$ electrons[22] .

Electronic spectra of compounds 1-5

The electronic spectra of 1-5 compounds were measured in DMF. The ligand showed two absorption bands at 266 and 317 nm, these bands can be assigned to π-π* and n-π transitions, respectively. Comparing the spectra of the complexes with those of the ligand revealed that the absorption band at 266 nm of the free ligand shows a slightly bathochromic shift to this band by 2 to 4 nm upon coordination. The band at 317 nm of the free ligand shows a pronounced bathochromic shift in the complexes and appears at 333nm. Another broad band

appeared at 452-462 nm in the spectra of the ligand and its complexes. This band was attributed to the charge-transfer from iron to either the nonbonding or anti-bonding orbitals on cyclopentadienyl ring[23]. No absorption bands due to the f-f transitions of the lanthanide ions could be identified in the visible region of these complexes. This may be probably attributed to the fact that the f-f transitions are very weak and obscured by the strong charge-transfer transition bands of the ligand[24]. On the basis of the above observed spectral data, it is suggested the compounds have the structure in Scheme 1.

Anticancer activity

In vitro antiproliferative activity

The antiproliferative activity of 1-5 compounds were evaluated against breast adenocarcinoma MCF-7; human hepatocellular carcinoma HepG2, human lung cancer A549 and human colon cancer HCT116 cell lines using MTT colorimetric assay, in comparison with doxorubicin as a reference drug. The results revealed that, all the tested compounds did not exert any activity against human A549 and colon HCT116 cell lines. The antiproliferative activities are expressed by median growth inhibitory concentration (IC_{50}) and provided in Table 2. From the results, it is evident that the tested compounds displayed potent growth inhibitory activity against only two tested cell lines (human hepatocellular carcinoma HepG2 and human lung cancer A549).

TABLE 2. Cytotoxicity (IC_{50}, µg/ml) of the ligand and its complexes against human malignant cell lines after 48 hr of incubation as measured with MTT assay method.

Compound	Cell line			
	HepG2	**A549**	**MCF-7**	**HCT116**
Doxorubicin	18.60 ± 1.90	23.00±2.70	20.00±1.90	19.25±2.00
1	40.00±4.30	NA	48.60±4.50	NA
2	22.00±2.50	NA	24.80±2.60	NA
3	31.00±3.20	NA	34.00±3.65	NA
4	19.90±2.00	NA	21.20±2.20	NA
5	23.70±2.63	NA	29.00±2.80	NA

Data are expressed as means ± S.E. of four separate experiments. (NA is no activity).

The IC_{50} of the 1-5 compounds for HepG2 cell line was 40.00±4.30, 22.00±2.50, 23.70±2.63, 19.90±2.00 and 31.00±3.20 µg/ml, respectively. It is clear that, while IC_{50} of 4 (19.90±2.00 µg/ml) is closed to the value of the reference drug, doxorubicin (18.60 ± 1.90 µg/ml), followed by 2,5,3,1. Similarly, the IC_{50} of the 1,2,5,4,3 compounds for MCF-7 cell line was 48.60±4.50, 24.80±2.60, 29.00±2.80, 21.20±2.20 and 34.00±3.65µg/ml, respectively, it is clear that, compound 1 was more potent similar to reference drug, doxorubicin (20.00±1.90 µg/ml), followed by 2,5,3,1 compounds.

The level of uPA protein expression

Progression and dissemination of human cancers are characterized by the acquisition of novel functional competences by malignant cells including self-sufficiency in growth signals, insensitivity to anti-growth signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis and tissue invasion and metastasis[25]. The aberrant expression of urokinase system in malignant tissues contributes to the acquisition of some of these new cell capabilities. In fact, beside its role in extracellular matrix degradation allowing tumor progression and metastasis, extensive experimental evidence has been accumulated over the last years documenting the relevance of urokinase in multiple aspects of the neoplastic evolution, including tumor cell proliferation, adhesion and migration, intravasation and extravasation, growth at the metastaticsites and tumor neoangiogenesis[26]. To identify the mechanism of action responsible for the cytotoxicity of prepared compounds in both HepG2 and MCF-7, the level of uPA protein expressed in the two cell lines were estimated. The results revealed that the data of uPA expression were consistent with the cytotoxicity activity.

In case of hepatic cancer cell line HepG2 and breast cancer cell line MCF-7, ligand and Ce-complex have a little effect on the expression of uPA, only 6 and 9% inhibition were obtained in HepG2, while 6 % and 9% inhibition were obtained in MCF-7 cells. On the other hand, 2,5,4 compounds inhibited uPA expression by 63, 52 and 70% , respectively in HepG2. While in case of MCF-7 uPA expression were inhibited by 58, 53 and 63% , respectively. From these results, the compound 4 exhibited a good activity in HepG2 and MCF-7 near to doxorubicin (86% and 80%, respectively) (Fig. 1). In both HepG2 and MCF-7 the inhibition of uPA activity of the tested compounds was in accordance with the cytotoxicity activity.

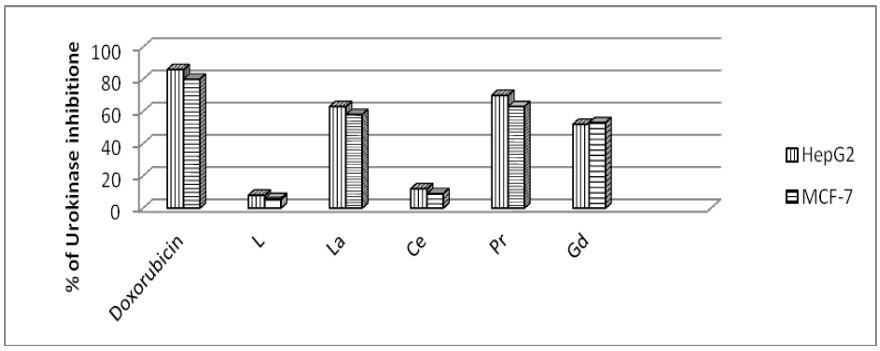


Fig. 1. *In vitro* the percent of uPA inhibition of the compounds 1-5 comparing to doxorubicin in different cell lines.

The previous results were in consistence with the report of Gutierrez *et al.*[27] who mentioned that in experimental animal models, urokinase inhibition considerably showing that the formation of metastasis and that tumor growth were retarded. From the foregoing results we can identify the urokinase inhibition as a suitable target for anti-cancer therapies.

Histone deacetylase (HDAC) activity

Histone deacetylase (HDAC) inhibitors are a new class of targeted anticancer agents, which are potent inducers of growth arrest, differentiation, and/or apoptotic cell death of transformed cells *in vitro* and *in vivo*[28]. Several HDAC inhibitors are in clinical trials and have shown significant activity against a spectrum of both haematological and solid tumors at doses that are well tolerated by patients. HDACs and histone acetyl transferasescan, by reversible acetylation, modify the structure and function of histones and proteins in transcription factor complexes, which are involved in the regulation of gene expression, as well as many non-histone proteins that are involved in regulating cell proliferation and cell death[28] . HDAC inhibitors are a group of structurally diverse molecules, which selectively altered the expression of genes. HDAC inhibitors can induce cancer cell death, whereas normal cells are relatively resistant to HDAC inhibitor-induced cell death[29]. It has been confirmed that HDAC inhibitors could be used to induce differentiation of leukemia cells or solid tumor cells, for example, sodium butyrateand trichostatin A (TSA) could increase the remission rate of many kinds of tumors obviously by cooperation with other anticancer drugs[29]. Thereby, we examined the effect of prepared compounds as histone deacetylase inhibitors in an attempt to use them in treatment of tumor.

In this work the activity of HDAC in the lysate of hepatic HepG2 and breast MCF-7 cancer cells treated with the prepared compounds as well as Trichostatin, as a known inhibitor was measured and the data were calculated as percentage of inhibition as compared to the control untreated cancer cells. While, treatment of hepatic HepG2 and breast MCF-7 cancer cells with Trichostatin resulted in 65% and 60% inhibition, respectively as compared with control untreated cancer cells, the treatment with 1-5 compounds resulted in 10, 40, 13, 38 and 58 % , respectively inhibition in HDAC activity in hepatic HepG2 cells. Similarly, the treatment of breast MCF-7 cells with the tested compounds 1-5 resulted in inhibition of the activity of HDAC by 8, 35, 11, 30 and 52% , respectively (Fig. 2).

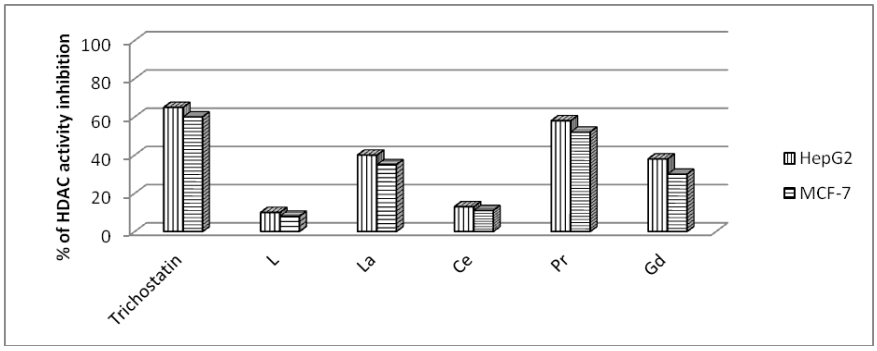


Fig. 2. The effect of prepared compounds 1-5 on HDAC activity of HepG2 and A549 cells after treatment with the prepared compounds and cells treated with Trichostatin, as a known inhibitor. The data compared with the HDAC activity of the control cancer untreated cells.

From the previous results, it was suggested that there is correlation between the cytotoxicity of the tested compounds and inhibition of the urokinase and Histone deacetylase activities. The tested compounds exert anti-carcinogenic activity in hepatic HepG2 and breast MCF-7 cancer cell lines through down-regulation the activity of these enzymes that may reduce the cell proliferation and resulted in significant growth inhibitory.

Conclusion

On the basis of spectral studies, it was found that the ligand forms 1: 1 complexes with La(III), Ce(III), Pr(III), and Gd(III) nitrates. The ligand acts as a tetradentate ligand coordinating through azomethine nitrogen and sulphur atom of thiazole ring. Nitrate ions are coordinated as a unidentate group with all corresponding metals. All spectral data showed that the complexes 2-5 are neutral molecules where the central Ln (III) ions have 7-coordination number.

The cytotoxicity and *in vitro* anticancer evaluation of the ligand and its complexes has been assessed against 4 different human tumor cell lines (MCF-7, HepG2, A549 and HCT116). The results revealed that the prepared compounds exert their actions in HepG2 and MCF-7 through inhibition of the activity of both urokinase and histone deacetylase (HDAC). Pr-complex revealed promising anticancer activity compared to the activity of the commonly used anticancer drug, doxorubicin.

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تحضير وتوصيف ودراسه النشاط المضاد للسرطان لمتراكبات اللانثانم والسيريم والبرازوديميم والجادولينيوم الثلاثي لثنائي إثيلين– 5 –ميثيل-ثيازول-2- اميين فيروسين

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قد تم تحضير مركبا فيروسينيا مخلبيا جديدا من تكثيف 1،1-داي استيل فيروسين مع 2-أمينو-5-ميثيل سيازول بنسبة 2:1مولر. هذا المركب كون متراكبات بنسبة 1:1 مع نترات اللانثانم والسيريم والبريليوم والجادولينيوم الثلاثية نسب جيدة.

وقد تم توصيف المركب المخلبى المحضر والمتراكبات بواسطة طيف الاشعة تحت الحمـراء (IR) ، الـرنين النـووى المغنـاطيسى لنـواة ذرة الهيدروجين (^{1}H NMR) والتحليل العنصرى ، طيف التحليل الكتلى، طيف الامتصاص الالكترونى ، التوصيل المولارى والعزم المغناطيسى. كما تم تعيين النشاط السمي للمركب المخلبى المحضر و متراكباته كمضادات ضد اربعة انواع مختلفة من الخلايا السرطانية (MCF-7, HepG2, A549, HCT116).

وقد اظهرت النتائج ان المركبات المحضرة لها نشاط ملحوظ مع نوعيين من الخلايا السرطانية (MCF-7, HepG2) ، وذلك من خلال تثبيط النشاط الانزيمي لكل من اليوروكاينيز و هيستون ديي اسيتيليز. وقد وجد من النتائج ان متراكب البرازوديميم الثلاثي له نشاط عالى كمضاد للسرطان بالمقارنة بعقار الدوكسوروبسيين المستخدم كدواء .