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Anticancer activity Studies of Some New Secnidazole-Metal Complexes.

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ABSTRACT

Two New [1-(2-Hydroxypropyl)-2-methyl-5-nitroimidazole-Metal] complex [(Secnidazole-Metal) complex] have been synthesized by reacting Secnidazole with Zr(IV) and Pt(II) salts. The products were characterized by elemental (CHN) analysis, FTIR, electronic spectra, ^{1}H NMR spectra. The two complexes were investigated for their antibacterial activities against Gram-positive and Gram-negative bacteria and were also screened for their in vitro anticancer potential using HeLa and PC3 cells. The complexes were tested for antimicrobial activity and proved to be non-active against *S-aureus, p.aeruginosa* and *E-coli*, However the secnidazole-Pt(II) complex showed a higher cytotoxic activity than secnidazole-Zr(IV) complex towards Hela cells.

Keywords: Secnidazole; Complexes; Antibacterial activity; Anticancer activity

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INTRODUCTION

5-nitroimidazoles are mainly known for their anti-infectious activity [1-5]. Today, there are many medical applications for compounds containing the nitroimidazole moiety such as secnidazole. Secnidazole is an antiprotozoal, anthelmintic and anti-bacterial drugs [6-7].

Many studies have recently stressed the role of metal ions in important biological processes [8-12], whereas the inorganic pharmacology started to be an important field with more than 25 inorganic compounds being used in therapy as anti-bacterial, anti-viral and anticancer drugs [13-17].

This study aims at complexing the antimicrobial drug Secnidazole, with Pt(II) and Zr(IV) metals to study their anti-bacterial and anticancer effect.

EXPERIMENTAL

Chemicals

The drugs, chemicals and solvents used in this study were of analytical grade and used as obtained from Aldrich without further purification:Secnidazole, Zirconium(IV) nitrate $Zr(NO_3)_4$, Potassium tetrachloroplatinate(K_2PtCl_4), Methanol (CH_3OH), deionized water.

Instrumental

The melting points were measured on an electro thermal melting point apparatus and were not corrected. Fourier-transform infrared spectra were recorded using the KBr disc technique on a JASCO 410 FTIR spectrophotometer. Elemental (CHN) analysis was performed using an Exeter CE-440 elemental analyzer. UV-visible absorption spectra were measured in DMF ($\approx 10^{-5}$ $mole^{-1}$) using a Pye–Unicam 8800a UV-visible automatic scanning spectrophotometer.^{1}HNMR spectra of the ligand and its complexes were recorded on a Varian Gemini-200 spectrometer (300MHZ) using DMSO-d_6 as solvent and TMS as internal reference. Microbiological analysis was carried out by the Micro analytical Center, Faculty of Pharmacy, University of Science and Technology, Sana'a, Yemen. Anticancer activity was evaluated at the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

Synthesis of Complexes

Synthesis of Secnidazole:ZirconiumComplex (Secnidazole-Zr)

The Secnidazole-Zr was prepared by mixing 0.339 gm (0.001 mol) of $Zr(NO_3)_4$ was dissolved in 20 ml of methanol and 0.37 gm(0.002 mol) of Secnidazole in 10 ml of methanol. The mixture was refluxing for 24 h, The precipitated complex was filtered, washed twice with methanol, then dried in air for 24 h.

Synthesis of Secnidazole:Platinum Complex (Secnidazole-Pt)

The Secnidazole-Pt was prepared bymixing0.415 gm (0.001 mol) of$K_2PtCl_4$was dissolved in 20 ml of methanol with some amount of deionized water and 0.37 gm (0.002 mol) of Secnidazole in 10 ml of methanol. The mixture was refluxing for 24 h, The precipitated complex was filtered, washed twice with methanol, then dried in air for 24 h.

Cytotoxicity

Cell line Propagation

The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50µg/ml gentamycin. The cells were maintained at 37ºC in a humidified atmosphere with 5% CO_2 and were subcultured two to three times a week.

Cytotoxicity evaluation using viability assay

For antitumor assays, the tumor cell lines were suspended in medium at concentration $5x10^4$ cell/well in Corning® 96-well tissue culture plates, then incubated for 24 hr. The tested compounds were then added into 96-well plates (three replicates) to achieve twelve concentrations for each compound. Six vehicle controls with media or 0.5 % DMSO were run for each 96 well plate as a control. After incubating for 24 h, the numbers of viable cells were determined by the MTT test. Briefly, the media was removed from the 96 well plate and replaced with 100 µl of fresh culture RPMI 1640 medium without phenol red then 10 µl of the 12 mM MTT stock solution (5 mg of MTT in 1 mL of PBS) to each well including the untreated controls. The 96 well plates were then incubated at 37°C and 5% CO_2 for 4 hours. An 85 µl aliquot of the media was removed from the wells, and 50 µl of DMSO was added to each well and mixed thoroughly with the pipette and incubated at 37°C for 10 min. Then, the optical density was measured at 590 nm with the microplate reader(SunRise, TECAN, Inc, USA)to determine the number of viable cells and the percentage of viability was calculated as [(ODt/ODc)]x100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound.The 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each conc. using Graph pad Prism software (San Diego, CA. USA)**[18-19].**

Antimicrobial activity

For antimicrobial activity, a filter paper sterilized disk saturated with a measured quantity of the sample is placed on the plate containing solid bacterial medium (nutrient agar broth) which has been heavily seeded with spore suspension of the tested organism. After inoculation, the diameter of the clear zone of inhibition surrounding the sample is taken as a measure of the inhibitory power of the sample against the particular test organism **[20-21]**.

RESULT AND DISCUSSION

Synthesis and characterization

The Secnidazole-Zr and Secnidazole-Pt complexes were prepared by the reaction of Secnidazole (2 moles) with $Zr(NO_3)_4$ and K_2PtCl_4(1 mole) in methanol under reflux for 24 h, respectively.Table1 summarized the physical properties (melting point, color, percentage yield, and elements analysis) of the two complexes. The proposed structure of Secnidazole-Zr and Secnidazole-Pt Complexes were shown in Figure 1.

Table1: Physical properties of Secnidazole complexes.

No	Complex Unit formula	F. wt.	Color	Yield	M.P.°C	CHN cal/(f)		
						C	H	N
1	Secnidazole-Zr $C_{14}H_{22}N_6O_6.Zr(NO_3)_4.H_2O$	727.62	Whit e	%60	171-170	23.11 (23.07)	3.32 (3.01)	19.25 (19.34)
2	Secnidazole-Pt $C_{14}H_{22}Cl_2N_6O_6Pt$	.63635	Black	%50	250<	26.42 (25.39)	3.48 (3.24)	13.21 (13.50)

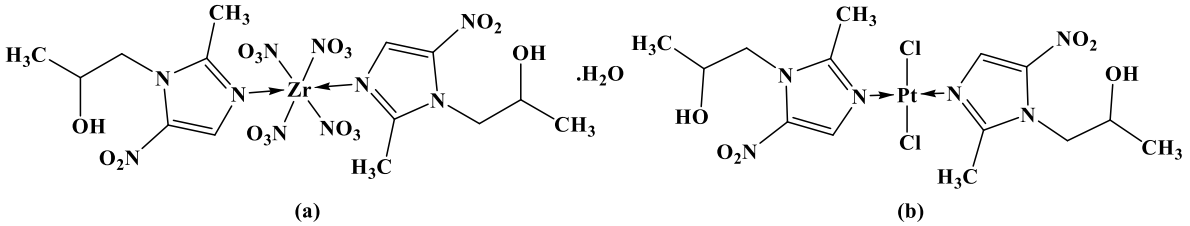

Fig 1: The proposed structure of Complexes:(a) Secnidazole-Zr and (b)Secnidazole-Pt.

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IR spectra

The main IR Spectra of compounds are summarized in Table 2,Secnidazole has potential binding sites for transition metal-ions Secnidazole has tow -nitrogen atoms, which can donate electron pairs nitrogen of imidazole ring. The IR Spectral data of Secnidazole and its complexes showed intense absorption band in the range 3367-3506 cm^{-1} due to asymmetric and symmetric OH stretching vibration of the hydroxyl groups. The Zr(IV) and Pt(II) complexes displays the frequency shifts and intensity changes of the C=N group on complexation suggested that the C=N group is involved in coordination in Zr and Pt complexes the strong band at 1652 cm^{-1} assigned to C=N in free ligand was shifted to lower wave number in the complexes(ca. 32 cm^{-1}), which indicating participation of the C=N group in coordination and confirms complexation**[22-25]**.

Table 2: Main IR absorption bands Secnidazole and its complexes.

Compound	C=C[a]	CHaliphatic	OH	C= N Ring
secnidazole	1619	2995,2875	3506	1652
Secnidazole-Zr	1542	2995,2854	3367	1619
Secnidazole-Pt	1512 ,1560	2981,2850	3551,3425	1620

[a]Aromaticring stretch

^{1}HNMR

The main^{1}HNMR spectrum of the Secnidazole showed OH proton at 4.8 ppm (S,H,D_2O exchangeable); CH –imidazole ring at 7.81 ppm (S,1H); The signal observed at 2.5 ppm (S,3H) is due to CH_3 imidazole.

In the ^{1}HNMR spectra of the Zr(IV) and Pt(II)complexes an electron density shift from the ligand to the metal was observed .The signals of OH protons appeared at 4.38 and 4.5 ppm in the Zr(IV) and Pt(II) complexes, respectively, as compared to 4.8 ppm in the Secnidazole ligand, also the proton of CH- imidazole ring appeared at 8.15 and 8.02 ppm in the Zr(IV) and Pt(II) complexes, respectively, inferring coordination through the imidazole ring of the ligand .

Electronic spectra

The UV-vis spectra of the ligands Secnidazole and its complexes (Zr(VI)and Pt(II)) were measured in the range 200-800 nm. The UV-visible absorption spectra were record in DMF solution. The spectra of the ligand and its complexes exhibit two π-π*(K-band) λ_{max}at 194–246 nm. The another one charge transfer transition band n-π*(R-band) at λ_{max}312–325 nm band due to the Secnidazole intra-ligand (IL).The d–d electronic transitions for complexes were not observed in their UV–Vis spectra in DMSO solution at ambient temperature**[22,25-27]**.

Antimicrobial activity

For in vitro antimicrobial activity ,the investigated compound were tested against the bacteria. *S-aureus, p.aeruginosa and E-coli.* The ligand and its complexes were showed inactive antimicrobial. Table 3 shows the results of the bioassay.

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Table 3: Antimicrobial activity of the Secnidazole and its complexes.The inhibition zones (IZ) diameter is in mm.

Compound	F.wt	Microorganisms		
		Gram negative		Gram positive
		Escherichia coli	Pseudomonas aeruginosa	Staphylococcus aureus
Secnidazole	158.18	(-)	(-)	(-)
Secnidazole-Zr	727.24	10>	10>	10>
Secnidazole-Pt	636	10>	(1+) 10	10>

(1+)slightly sensitive (15-10mm),(-) noeffect.

Anticancer activity

The results of in vitro anticancer activity of the tested complexes were evaluated for cytotoxicity against PC3 cells and Hela cells of humans in comparison with Cis palatine as a positive control. The Secnidazole-Pt complex showed a higher cytotoxic activity than Secnidazole-Zr complex towards Hela cells. Table 4 represents the cytotoxic activity of the tested compounds.

Table 4: IC_{50}values(µg/ml) for Pt(II) and Zr(IV) complexes of Secnidazole tested against HELAcells and PC3 cells.

Compound	PC3	HELA
	IC ± $_{50}$SD (µg/ml)	**IC ± $_{50}$SD (µg/ml)**
Secnidazole-Zr	28.5±0.7	61.0±1.4
Secnidazole-Pt	28.1±0.2	44.5±0.8
Cispalatine(as control)	6.65±0.31	7.76±0.4

CONCLUSION

The present work describes the synthesis and *in vitro* antimicrobial and anticancer activity of Secnidazole and its complexes Zr(IV),Pt(II). The ligand and its complexes showed inactive antimicrobial activity, However the Secnidazole-Pt complex showed a higher cytotoxic activity than Secnidazole-Zr complex towards Hela cells.

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