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Biological (Anti-Tumor and Anti Bacterial) Studies of New Halo Complexes

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Abstract: In this research, some of four Halo complexes: $[Fe(pythsalI)(H_2O)_2]Cl_2$, $[Fe(pythsalBr)(H_2O)_2]Cl_2$, $[Fe(pythsalOMe)(H_2O)_2]Cl_2$ and $[Fe(pythsalNO_2)(H_2O)_2]Cl_2$ with the NSNO-donor tetradentate Schiff base ligands pythsalHX [(5-X-N-(2pyridylethylsulfanylethyl) salicylideneimine] (X = I, Br, OMe, NO_2)) obtained from inserted condensation of 1-(2-pyridyl)-3-thia-5amino Pentane with the respective derivative salicylaldehyde in a 1:1 molar ratio were synthesized. Characterizations of the complexes were carried out by elemental analyses, FT-IR and UV-Visible spectroscopic. These new compounds, after 72 hours, show excellent anti-tumor activity against four kinds of cancer cells that are AGS (human gastric carcinoma), HeLa (human cervix carcinoma), K562 (human chronic myeloid leukemia) and Jurkat (human T lymphocyte carcinoma) cells. For the growth of cancer cells, some of doses of compounds also had inhibitor characteristics. The results are completely new and provide new views for using of the mentioned inorganic complexes in chemotherapy. Also Antimicrobial properties of new compounds also have been studied and good results obtained.

Key words: Halo complexes · Cytotoxic · AGS · HeLa · K562 · Jurkat · Anti tumor · Antimicrobial

INTRODUCTION

Most therapeutic agents and drugs will first be tested in tissue culture on a suitable model system. For prospective anticancer drugs, for example, in vitro data obtained by proliferation or colony formation on the cytotoxicity of the agents, the medicinal uses and applications of metals complexes are of increasing clinical and commercial importance [1]. Transition metal complexes with potential biological activity are the focus of extensive investigations [2]. Though transition metals occupy many key positions in biological processes [3], metal-based drugs are traditionally undervalued by the pharmaceutical industry, which is dominated by organic chemistry [4]. The design, synthesis and characterization of iron complexes with Schiff base ligands play a relevant role in the coordination chemistry of iron due to their importance as synthetic models for the iron-containing enzymes [5-7], oxidation catalysts [8, 9] and bistable molecular materials based on temperature-, pressure- or light induced spin-crossover behavior [10, 11]. Nevertheless, a number of coordinated compounds have been applied in the therapy of various diseases [12] (e.g., historically salvarsan against syphilis [13], gold complexes against arthritis [14], bismuth compounds as antiulcer drugs [15, 16], or platinum compounds against cancer [17-21]). Today, chemotherapy with Pt complexes is one of the main pillars in the treatment of cancer. Out of thousands of synthesized and evaluated Pt^{II} complexes, only cisplatin, carboplatin and oxaliplatin have been approved for worldwide clinical practice (in 1978, 1993 and 2002, respectively), while nedaplatin, lobaplatin and heptaplatin have been approved as anticancer agents only in Japan, China and South Korea, respectively [19, 20, 22]. However, severe side effects and activity in a restricted spectrum of tumors as well as acquired or intrinsic resistance limit their successful therapeutic use. Therefore, alternative metal compounds are presently being evaluated in clinical trials [23, 24].

MATERIALS AND METHODS

Chemical Reagents: All chemicals and reagents used for the preparation of the ligands and complexes were commercial products (Merck or Fluka) and were used without further purification. Solvents used for reactions

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were purified and dried by standard procedures. 5-Iodo-salcylaldehyde, 5- phenylazo-salcylaldehide, 1-(2-pyridyl)-3-thia-5amino Pentane were synthesized according to known procedures. 2-Vinylpyridine was distilled in vacuum before using. Elemental analyses (carbon, hydrogen and nitrogen) were determined with an Elemental CHN Analyzer Vario El III. The molar conductance values of the complexes were measured in acetonitrile solution in room temperature with a Jenway 4510 conductometer instrument. Melting points were determined by using an electrothermal apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectroscopic data were recorded on a Bruker spectrospin Avance 400 MHz in $CDCl_3$ and chemical shifts are indicated in ppm relative to tetramethylsilane. The electronic spectroscopic data in 200-900 nm range were recorded in acetonitrile on a Perkin-Elmer lambda 25 spectrophotometer. Infrared spectroscopic data (KBr disc, 4,000-400 cm^{-1}) were recorded on a Shimadzu FT-IR model prestige 21 spectrometer.

Cell Culture: The human gastric carcinoma: AGS cell line(ATCC CRL 1739), the human cervix carcinoma carcinoma: HeLa cell line(Pasteur, C115), the human chronic myeloid leukemia: K562 cell line and the human T lymphocyte carcinoma: Jurkat cell line used for treatment with the drugs, was provided. AGS, HeLa, K562 and Jurkat cells were grown at 37°C in an atmosphere containing 5% CO_2, with RPMI-1640 Medium HEPES Modification with L-glutamine and 25 mM HEPES (Sigma-Aldrich Chemie GmbH, Germany) supplemented with 20% heat-inactivated fetal bovine serum (FBS) (Gibco, USA), 2.7% sodium bicarbonate and 500 mg/L ampicillin. The AGS and HeLa cells were left to adhere for 24 h and they were washed 2 times with sterile PBS.

Antimicrobial Assay: The antibacterial activities of the Halo complexes were determined against the two Gram-positive bacteria: *Staphylococcus aureus (PTCC1189), Micrococcus and* also against the two Gram negative bacteria*: Escherichia coli (PTCC1329), Sodemonas Aerozhinoza (PTCC1447)* by the disc diffusion method (Lorian, 1996). Muller-Hinton agar (MHA) (Oxoid) was used for preparation of the media.

General Synthesis of [Fe (PythsalX)$(H_2O)_2$]Cl_2 Complexes: A solution of 1-(2-pyridyl)-3-thia-5-aminopentane (0.182 g, 1 mmol) in ethanol (5 mL) was added to the required salicylaldehyde (1 mmol) in ethanol (5 mL). The mixture was refluxed for 40 min and then 1 mL of 1 M methanolic NaOH was added and reflux and stirring were continued for a further 5 min. A solution of $FeCl_3.6H_2O$ (0.27 g, 1 mmol) in ethanol 5 mL) was added with stirring and the reaction mixture was stirred under reflux for 50 min. The resultant colored solution was left at room temperature [25]. The resulting precipitate was

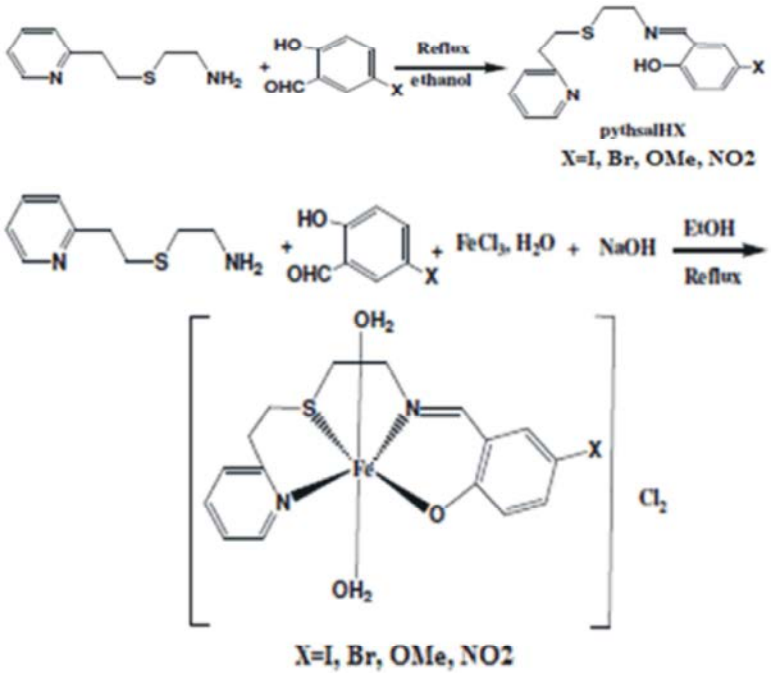

Fig. 1: Synthesis route of Halo Complexes

Table 1: The data of Elemental Analyses (CHN) of Halo complexes

Compound	N	C	H
	(Anal) Found (%)		
$[Fe(pytsalI)]Cl_2.2H_2O$	33.30(33.47)	3.50(3.51)	4.92(4.88)
$[Fe(pytsalBr)]Cl_2.2H_2O$	36.68(36.46)	3.77(3.82)	5.52(5.31)
$[Fe(pythsalNO_2)(H_2O)_2]Cl_2$	39(38.8)	4.1(4.3)	8.50(8.5)
$[Fe\ (pythsalOMe)(H_2O)_2]Cl_2$	42.70(43.1)	4.8(4.82)	5.9(5.8)

Table 2: The data of FT-IR spectra of Halo complexes

Compound	ν (O-H)	ν $(C-H)_{arom.}$	ν $(C-H)_{alip.}$	ν (C = N)	ν (N-O)
$[Fe(pytsalI)]Cl_2.2H_2O$	3420	3030-3080	2870-2910	1617	-
$[Fe(pytsalBr)]Cl_2.2H_2O$	3450	3030-3080	2840-2950	1618	-
$[Fe(pythsalNO_2)(H_2O)_2]Cl_2$	3400	3050-3120	2830-2950	1627	1442
$[Fe\ (pythsalOMe)(H_2O)_2]Cl_2$	3425	370	2840-2920	1621	1445

Table 3: Transitions specifications of Halo complexes

Compound	λ_{max} (nm) (ε, $M^{-1}cm^{-1}$)	λ (nm) (ε, $M^{-1}cm^{-1}$)	λ (nm) (ε, $M^{-1}cm^{-1}$)	λ (nm) (ε, $M^{-1}cm^{-1}$)	λ (nm) (ε, $M^{-1}cm^{-1}$)
$[Fe(pytsalI)]Cl_2.2H_2O$	564(143)	399(4200)	325(4550)	230(28500)	-
$[Fe(pytsalBr)]Cl_2.2H_2O$	450(143)	380(30800)	255(sh)	235(20512)	-
$[Fe(pythsalNO_2)(H_2O)_2]Cl_2$	508(174)	350(13100)	260(sh)	245(15420).	-
$[Fe\ (pythsalOMe)(H_2O)_2]Cl_2$	568(228)	360(4225)	310(4262)	258(13830)	240(13890)

Table 4: 72 hour IC_{50} and IC_{90} values (μM) obtained for compounds

	IC_{50} for Cell line				IC_{90} for Cell line			
Compound	AGS	HeLa	K562	Jurkat	AGS	HeLa	K562	Jurkat
$[Fe(pythsalI)(H_2O)_2]Cl_2$	>101	>98	>53	>50	>161	>120	>100	>99
$[Fe(pythsalBr)(H_2O)_2]Cl_2$	>130	>55	>85	>83	>184	>100	>175	>170
$[Fe(pythsalOMe)(H_2O)_2]Cl_2$	>99	>44	>74	>69	>156	> 92	>152	>140
$[Fe(pythsalNO_2)(H_2O)_2]Cl_2$	>76	>52	>41	>40	>110	>95	>98	>95

filtered off, washed with cold absolute ethanol, recrystalized from methanol or acetonitrile and dried in vacuum (Figure 1). Characterizations of the complexes were carried out by elemental analyses, FT-IR and UV-Visible spectroscopic (Table 1-3).

Cytotoxicity Studies: Halo complexes were assayed for cytotoxicity in vitro against AGS, HeLa, K562 and Jurkat cells.

The four cell lines were provided by the Pastour Instutiute Laboratory of natural and Biomimetic in Iran. The procedure for cytotoxicity studies was similar to that reported earlier [26-33]. Briefly, in order to calculate the concentration of each drug that produces a 50% inhibition of cell growth (IC_{50}), 190 mL of cell suspension (5×10^4 cell/mL) were exposed to various concentrations of complexes dissolved in sterile DMSO. After incubation periods 72 h for all cell lines, the cell concentrations were determined both in control and in drug-treated cultures. All experiments were repeated six times.

***In-Vitro* Anti Bacterial Activity:** The antibacterial activities of the Halo complexes were determined against the two Gram-positive bacteria: *Staphylococcus aureus (PTCC1189), Micrococcus and* also against the two Gram negative bacteria*: Escherichia coli (PTCC1329), Sodemonas Aerozhinoza (PTCC1447)* by the disc diffusion method (Lorian, 1996). Muller-Hinton agar (MHA) (Oxoid) was used for preparation of the media. The filter paper discs (6mm in diameter) were individually impregnated with 10 μL of stock solution of the compounds ($5mgmL^{-1}$) and then placed onto the agar plates, which had been previously inoculated with the tested microorganisms. The plates were inoculated with bacteria and incubated at 37°C for 24 h. The diameters of inhibition zones were measured in millimetres. All the tests were performed in duplicate. Gentamicin and Ofloxacin (30 mg) served as positive control [34, 35] (Table 4).

RESULTS AND DISCUSSION

Preparation for Iron (III) Complexes: We have prepared iron (III) complexes of four Schiff base ligands and characterized them by physico-chemical and spectroscopic means. They are formulated as 1:2 electrolytes of general formula $[Fe(pythsalX)(H_2O)_2Cl_2$. The infrared spectra reveal a common mode of complexation through the nitrogen atoms of the

Table 5: Zones of growth inhibition (mm) in concentration (mg/mL) obtained for compounds

	Bacteria			
Compound	Staphylococcus aureus	Micrococcus	*Escherichia coli*	Sodemonas Aerozhinoza
$[Fe(pythsalI)(H_2O)_2]Cl_2$	10mm	9mm	10mm	-
$[Fe(pythsalBr)(H_2O)_2]Cl_2$	10mm	12mm	5mm	-
$[Fe(pythsalOMe)(H_2O)_2]Cl_2$	8mm	10mm	12mm	-
$[Fe(pythsalNO_2)(H_2O)_2]Cl_2$	-	-	-	-

azomethine and pyridine groups, oxygen atom of deprotonated phenolic group and thioether sulfur atom. The electronic spectra indicate octahedral geometry for the complexes [25].

Cytotoxicity and Anti Bacterial Assays *In Vitro:* The general method used for testing on anti tumor properties of compounds is the standard testing method that has been previously described in greater detail:

After preincubation lasting 12h at 37°C in a 5% CO_2 atmosphere and 100% humidity, the tested compounds in the concentration rang 0.1-200µM for $[Fe(pythsalI)(H_2O)_2]Cl_2$, 0.1-350 µM for $[Fe(pythsalBr)(H_2O)_2]Cl_2$, 0.1-500µM for $[Fe(pythsalOMe)(H_2O)_2]Cl_2$ and 0.1-300µM for $[Fe(pythsalNO_2)(H_2O)_2]Cl_2$ were added. The incubation lasted 72 h and at the end of this period IC_{90} and IC_{50} of the dead cells and live cells was measured by Trypan blu. IC_{90} and IC_{50} values, which are the compounds concentrations lethal for 90% and 50% of the tumour cells, were determined both in control and in compounds concentrations lethal for both in compounds-treated cultures. The complexes were first dissolved in DMSO and then filtrated. The final concentration of DMSO in the growth medium was 2%(v/v) or lower, concentrations without effect on cell replication [26, 33]. The corresponding 50% and 90% inhibitory doses (IC_{50} and IC_{90}) values are shown in Table 5.

CONCLUSION

It is clear from the above discussion that iron (III) complexes offer a new outlook for chemotherapy. The result of anti-tumour activity show that the metal complexes exhibit anti-tumour properties and it is important to note that they show enhanced inhibitory activity compared to the parent ligand. The mechanism by which these complexes act as anti-tumour agents is apoptosis. It has also been proposed that concentration plays a vital role in increasing the degree of inhabitation. Also Anti bacterial properties of new compounds studied and good results obtained [26-35].

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