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New Coordination Complexes of Chromium as Cytotoxic and Antimicrobial Agents

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Abstract: The aim of the present study was to investigate the biocidal activity of seven new chromium based coordination complexes [$Cr(Pht)_2$ $(Glycine)_2$, S_1], [$Cr(Pht)_2$ $(Leucine)_2$, S_2], [$Cr(Pht)_2$ $(Cystein)_2$, S_3], [$Cr(Pht)_2$ $(Serine)_2$, S_4], [$Cr(Suc)_2$ $(Leucine)_2$, S_5], [$Cr(Suc)_2$ $(Cystein)_2$, S_6] and [$Cr(Suc)_2$ $(Serine)_2$, S_7] against Gram-positive and -negative bacteria, fungi and brine shrimp nauplii. The complexes S_1, S_3, S_4, S_6 showed good antibacterial activity at the concentration of 200 μg $disc^{-1}$ and gave MIC values between 16-64 μg ml^{-1} against the tested bacteria. The complexes gave comparatively better antibacterial activity against the Gram-negatives. S_2 did not show any remarkable antifungal activity but others showed good activity. The brine shrimp were hatched in artificial sea water and exposed to the complexes to determine mortality rate. LC_{50} values were calculated after probit transformation of the resulting data. All the complexes showed better cytotoxic effect but among them S_1 and S_5 having LC_{50} values of 3.31 and 3.63 μg ml^{-1} showed potent cytotoxic activity when compared with the reference standard Gallic acid whose LC_{50} values was 4.53 μg ml^{-1}.

Key words: Chromium coordination complexes, antimicribial, cytotoxicity

INTRODUCTION

Coordination complexes of transition metals have been widely studied for their antimicrobial[1-3] and cytotoxic[4-9] properties. In the history of coordination complexes in cancer therapy, the first clinical trials of cisplatin (a titanium based coordination complex) were carried out in 1971[10]. Cisplatin is one of the most potent and effective antitumor agent but it lacks selectivity for tumor tissue and many tumors are growing resistance to this platinum complex. To address this problem modified versions of cisplatin, leading to second and third generation platinum-based drugs have been synthesized over the past 30 years and have got their less toxic effect to the host tissue[10].

Recently there has been considerable interest and increased research activity in developing other transition metal compounds as anticancer drugs, which are less toxic than platinum-based drugs. In the continuation of the search for new anticancer coordination complexes, titanium complexes have shown better anticancer activity than the cisplatin[11,12]. Besides titanium, other transition metal complexes of iron, nickel, zinc, cobalt and copper showed antitumor properties[13,14]. Chromium complexes have also been reported for their potent cytotoxic activity[15-17].

The aim of the present study was to evaluate the cytotoxic properties of a series of new chromium based coordination complexes with the hope of adding new and potent cytotoxic chemotherapeutic agents to treat mammalian cancer cells. We compared the cytotoxicity of the complexes with the reference standard Gallic acid. We also tested the cytotoxicity of the anticancer drug bleomycin and have compared with the tested chromium complexes. We also studied the antibacterial and antifungal activity of the newly synthesized complexes and have compared the activities with the standard antibacterial and antifungal drugs.

MATERIALS AND METHODS

Preparation of complexes: The complexes were synthesized according to the following procedure; The aqueous solution of chromium (III) chloride and of amino acids containing minimum amount of KOH (to make soluble) were mixed in a molar ratio of 1:2 and then allowed to stand for about 10 min. Two moles of imide salts (potassium phthalimide or potassium succinimide) were then added. To get the precipitates of complexes, the mixture were then heated at 60°C for about 25 min and then allowed to stand for 10 min. The precipitates formed were removed by filtration, washed several times with

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distilled water and finally with alcohol and dried in a vacuum desiccator over anhydrous $CaCl_2$. The prepared complexes were characterized by IR, UV, magnetic moment, melting point and conductivity measurement. According to the following equations the complexes of the chromium (III) were obtained.

For phthalimide based complexes

$$\text{H-pim} + \text{KOH} \longrightarrow \text{K-pim} + H_2O$$
$$CrCl_3 + \text{A-H} + \text{K-pim} \longrightarrow K[Cr(pim)_2(A)_2]$$

and for succinimide based complexes

$$\text{H-Sim} + \text{KOH} \longrightarrow \text{K-Sim} + H_2O$$
$$CrCl_3 + \text{A-H} + \text{K-sim} \longrightarrow K[Cr(Sim)_2(A)_2]$$

Where,
Pim = anions of phthalimide,
Sim = anions of succinimide
A = amino acids e.g., glycin, alanine, phenylalanine, cystein, cystine, serine, leucine.

Antibacterial assay: *In vitro* Antibacterial screening is generally performed by disc diffusion method[18-19] for primary selection of the compounds as therapeutic agent. Disc diffusion method is highly effective for rapidly growing microorganisms and the activities of the test compounds are expressed by measuring the diameter of the zone of inhibition. Generally the more susceptible the organism the bigger is the zone of inhibition. The method is essentially a qualitative or semi quantitative test indicating sensitivity or resistance of microorganisms to the test materials as well as bacteriostatic or bactericidal activity of a compound[20]. The antibacterial activity of the complexes S_1, S_2, S_3, S_4, S_5, S_6 and S_7 was determined at a concentration of 30 μg $disc^{-1}$ and 200 μg $disc^{-1}$ against two gram-positive (*Bacillus subtilis* and *Streptococcusβ-haemolyticus*) and three gram-negative (*Shigella dysenteriae, Pseudomonas aeruginonosa and Escherichia coli*) bacteria. The diameters of zones of inhibition produced by the complexes were compared with the standard antibiotic (Amoxicillin 30 μg $disc^{-1}$). The experiment was performed in triplicate to minimize errors. The media used in this respect was nutrient agar (DIFCO).

Minimum inhibitory concentration (MIC) determination: MIC of a compound is defined as the lowest concentration of that compound in a medium without visible growth of the test organisms. The minimum inhibitory concentration of the complexes was determined against four pathogenic bacteria *Bacillus subtilis*, *Streptococcus-β-haemolyticus*, *Escherichia coli* and *Salmonella typhi* by serial dilution technique[20]. The results were compared with the standard antibiotic, amoxicillin. The media used in this respect was nutrient broth (DIFCO).

Antifungal assay: The antifungal activity of the complexes were tested against the three pathogenic fungi *Candida albicans, Aspergillus niger* and *Aspergillus fumigatus* at a concentration of 200 μg $disc^{-1}$ for each. The media used in this respect was Potato Dextrose Agar (PDA). The activity was determined after 72 h of incubation at room temperature (30°C).

Cytotoxicity bioassay: Brine shrimp lethality bioassay[21-24] is a recent development in the assay procedure of bioactive compounds which indicates cytotoxicity as well as a wide range of pharmacological activities (e.g. anticancer, antiviral, insecticidal, pesticidal, AIDS, etc.) of the compounds. Here, *in vivo* lethality test were carried out using brine shrimp nauplii eggs (*Artemia salina L.*). Eggs were placed in one side of a small tank divided by a net containing 3.8% NaCl solution for hatching. In other side of the tank, a light source was placed in order to attrack the nauplii. After two days of hatching period the nauplii were ready for the experiment. 3 mg of the complexes were accurately measured and dissolved in 0.6 ml (600 μl) of DMSO to get a concentration of 5 mg ml^{-1}. From the stock solutions 2, 4, 8, 20 and 40 μg ml^{-1} were placed in 6 different vials making the volume up to 5 ml. The final concentration of the samples, in the vials became 2, 4, 8, 20 and 40 μg ml^{-1}, respectively.

The brine shrimp nauplii 10 in number were then placed in each vial. For the control test of each vial, one vial containing the same volume of DMSO plus water up to 5 ml was used. After 24 h of incubation, the vials were observed using a magnifying glass and the number of survivors in each vial were counted and noted. The resulting data were transformed to the probit analysis[25] for the determination of LC_{50} values for the complexes.

RESULTS AND DISCUSSION

Antibacterial activity: The chromium complexes were slightly active in the concentration of 30 μg $disc^{-1}$ but showed good activity in the increased concentration of 200 μg $disc^{-1}$ compared with the standard antibiotic amoxicillin. Among the complexes S_1, S_3, S_4 and S_6 showed better activity against Gram-negative bacteria than positive ones. The complex S_4 showed maximum activity against *Bacillus subtilis*, *Streptococcus-β-haemolyticus*, *Shigella dysenteriae* and *Pseudomonas aeruginosa* and the complex S_3 showed maximum activity against

Table 1: *In vitro* antibacterial activity of the coordination complexes S_1, S_2, S_3, S_4, S_5, S_6, S_7 and standard amoxicillin

	Diameter of zone of inhibition (in mm)														
	S_1		S_2		S_3		S_4		S_5		S_6		S_7		Amoxicillin
µg disc^{-1} →	30	200	30	200	30	200	30	200	30	200	30	200	30	200	30
Gram positive bacteria															
Bacillus subtilis	10	17	09	15	11	19	10	21	09	16	11	17	00	15	21
Streptococcus-β-haemolyticus	11	16	12	16	10	21	12	20	00	15	10	19	00	14	20
Gram negative bacteria															
Shigella dysenteriae	12	18	11	17	12	18	13	23	00	15	13	20	10	18	21
Pseudomonas aeruginosa	14	22	10	15	11	19	12	22	10	17	12	18	09	15	21
Escherichia coli	13	21	00	16	12	22	13	19	00	18	14	21	00	15	23

Table 2: Minimum Inhibitory Concentration (MIC) values of the compounds S_1, S_2, S_3, S_4, S_5, S_6, S_7 and standard amoxicillin

	Minimum inhibitory concentration (µg ml^{-1})							
Test organisms	S_1	S_2	S_3	S_4	S_5	S_6	S_7	Amoxicillin
Bacillus subtilis	64	64	32	32	32	16	64	4
Streptococcus-β-haemolyticus	32	64	32	16	64	32	64	4
Escherichia coli	32	32	16	16	32	16	64	8
Salmonella typhi	64	64	16	16	32	16	64	4

Table 3: *In vitro* antifungal activity of the complexes S_1, S_2, S_3, S_4, S_5, S_6, S_7 and standard nystatin

	Diameter of zone of inhibition (in mm)							
	S_1	S_2	S_3	S_4	S_5	S_6	S_7	Nystatin
µg disc^{-1} →	200	200	200	200	200	200	200	30
Candida albicans	15	00	16	16	18	12	16	17
Aspergillus niger	17	09	18	15	20	17	18	16
Aspergillus fumigatus	18	10	14	12	19	19	14	16

Table 4: The results of cytotoxic effect of the complexes S_1, S_2, S_3, S_4, S_5, S_6, S_7 and standard bleomycin and gallic acid

		95% confidence limit			
Test samples	LC_{50} (ppm)	lower	upper	Regression equation	x^2
$[Cr(Pht)_2 (Glycine)_2, S_1]$	3.31	12.21	4.98	Y = 3.453331+2.989771X	2.51
$[Cr(Pht)_2 (Leucine)_2, S_2]$	7.71	3.55	16.75	Y = 3.119999+2.126059X	4.91
$[Cr(Pht)_2 (Cystein)_2, S_3]$	3.63	2.22	5.93	Y = 3.740003+2.27554X	0.11
$[Cr(Pht)_2 (Serine)_2, S_4]$	11.67	2.92	46.65	Y = 3.180002+1.710808X	2.24
$[Cr(Suc)_2 (Leucine)_2, S_5]$	5.60	2.89	10.85	Y = 3.63+1.81047X	8.29
$[Cr(Suc)_2 (Cystein)_2, S_6]$	4.36	2.91	6.53	Y = 3.230003+2.807054X	0.24
$[Cr(Suc)_2 (Serine)_2, S_7]$	5.62	3.71	8.49	Y = 2.773334+2.989765X	8.63
Standard bleomycin	0.41	0.27	0.62	Y = 3.163565+2.989771X	0.62
Gallic acid	4.53	3.33	6.15	Y = 3.93+1.62X	1.25

Escherichia coli. The concentration of 30 µg disc^{-1} the complex S_5 did not show any activity against *Streptococcus-β-haemolyticus*, *Shigella dysenteriae* and *Escherichia coli* and the complex S_7 did not show activity against *Bacillus subtilis*, *Streptococcus-β-haemolyticus* and *Escherichia coli*. Against *Escherichia coli* we did not find any activity for the complex S_2 at the concentration of 30 µg disc^{-1} (Table 1).

We found good antibacterial activity of the complexes S_1, S_3, S_4 and S_6 against the tested bacteria and comparatively maximum activity were found in case of Gram-negative bacteria which is an interesting finding of our present study. In the literature survey we did not find any report of antibacterial activity of chromium complexes and for why this is probably the first report of antibacterial activity of chromium complexes. Previous works showed chromium complexes as to cause oxidation of DNA by binding with guanine[17]. So, our present findings suggest the previous reports. The mechanism of antibacterial activity of our complexes may be due to oxidative DNA damage as the previous reports[15,17] but further studies are needed to confirm the mechanism of antibacterial action.

Minimum inhibitory concentration: The MIC values of the complex S_1 against *Bacillus subtilis*, *Streptococcus β-haemolyticus*, *Escherichia coli* and *Salmonella typhi* were 64, 32, 32 and 64 µg ml^{-1}, respectively (Table 2), for the complex S_2 64, 64, 32 and 64 µg ml^{-1}, respectively for complex S_3 32, 32, 16 and 16 µg ml^{-1}, respectively for complex S_4 16, 32, 16 and 16 µg ml^{-1}, respectively for complex S_5 32, 64, 32 and 32 µg ml^{-1}, respectively for complex S_6 16, 32, 16 and 16 µg ml^{-1}, respectively and that for complex S_7 64, 64, 64 and 64 µg ml^{-1}, respectively.

From the MIC results we can conclude that the chromium complexes are more active against the Gram-negative rather than Gram-positives. The MIC values of the complexes are higher than the values for the standard antibiotic amoxicillin which indicate the less activity of chromium complexes compared with the amoxicillin but it as an interesting findings as the chromium complexes are being reported for their antibacterial activities for the first time.

Antifungal activity: Against *Candida albicans* the complex S_2 did not show any activity in the concentration of 200 μg $disc^{-1}$ but other complexes showed good activity and maximum activity was shown by the complex S_5 (Table 3). Against *Aspergillus niger* the complex S_2 showed poor activity and the complex S_4 showed moderate activity but the others showed good activity comparatively with the standard antifungal nystatin and maximum activity was shown by the complex S_5. Against *Aspergillus fumigatus* the complexes S_3 and S_7 showed same activity and the complexes S_2 and S_4 showed relatively poor activity but the complexes S_1, S_5 and S_6 showed good activity. Many coordination complexes of different transition metals had been reported for their antifungal activity[2,3] but still we did not find any previous antifungal activity report for chromium complexes. It is the first antifungal report for chromium complexes and it supports the previous results of other coordination complexes. The probable mechanism of fungicidal activity is the oxidative DNA damage as previous report[15,17] but further studies are needed to confirm the mechanism of fungicidal activity of theses new chromium based complexes.

Cytotoxic activity: The mortality rate of brine shrimp napulii was found to increase with concentration of the complexes. The LC_{50} values of the complexes S_1, S_2, S_3, S_4, S_5, S_6 and S_7were found to be 3.31, 7.71, 11.67, 5.60, 4.36 and 5.62 μg ml^{-1}, respectively. The standard anticancer drug bleomycin gave its LC_{50} value at 0.41 μg ml^{-1}. The lowest LC_{50} values for the complexes were found in case of the complexes S_1 and S_3 having the values of 3.31 and 3.63 μg ml^{-1}, respectively. These values are near to the standard bleomycin and indicative of their (S_1 and S_3) potent cytotoxic properties.

Bioassay of the chromium complexes showed significant cytotoxicity with the LC_{50} values between 3.31 to 7.71 ppm except the complex S_4 which showed moderate cytotoxicity with the LC_{50} of 11.67 ppm compared with the control DMSO and gallic acid, used as standard agent[26]. Previously, many authors explored the cytotoxic activity of metal coordination complexes[4-6]. At the present investigation we also found potent cytotoxic activity for the chromium based complexes. The cytotoxicity results in our present investigation suggest the previous report of biocidal activities[5,17] but further investigations are required to confirm the activities for the newly synthesized complexes.

In conclusion, all the chromium complexes showed biocidal activity against bacteria, fungi and brine shrimp nauplii. Further studies of these cytotoxic chromium complexes may explore their clinical implications in the world most life threaten disease cancer.

REFERENCES

1. Kamalakannan, P. and D. Venkappayya, 2002. Synthesis and characterization of cobalt and nickel chelates of 5-dimethylaminomethyl-2-thiouracil and their evaluation as antimicrobial and anticancer agents. J. Inorg. Biochem., 21: 22-37.
2. Islam, M.S., M.A. Farooque, M.A.K. Bodruddoza, M.A. Mosaddik and M.S. Alam, 2002. Antimirobial and toxicological studies of mixed ligand transition metal complexes of schiff bases. Online J. Biol. Sci., 2: 797-799.
3. Sultana, C., M.A.A. Rahman, M.A.A. Al-Bari, M.L.A. Banu, M.S. Islam, N.A. Khatune and G. Sadik, 2003. *In vitro* Antimicrobial screening of three cadmium complexes and two addition compounds of antimony and arsenic. Pak. J. Biol. Sci., 6: 525-527.
4. Rho, Y.S., S.A. Kim, J.C. Jung, C.C. Shin and S.G. Chang, 2002. Anticancer cytotoxicity and nephro-toxicity of the new platinum (II) complexes containing diaminocyclohexane and glycolic acid. Int. J. Oncol., 20: 929-35.
5. Treshchalina, E.M., A.L. Konovalova, M.A. Presnov, L.F. Chapurina and N.I. Belichuk, 1979. Antitumor properties of mixed coordination compounds of copper (II) and alpha-amino acids. Dokl. Akad. Nauk., 248: 1273-6.
6. Kelland, L.R., C.F. Barnard, K.J. Mellish and M. Jones, 1994. Goddard P.M., Valenti M., Bryant A., Murrer B.A. and Harrap K.R. A novel trans-platinum coordination complex possessing *in vitro* and *in vivo* antitumor activity. Cancer Res., 54: 5618-22.
7. Amirkhanov, V.M., E.A. Bundya, V.A. Trush, V.A. Ovchynnikov and V.N. Zaitsev, 1999. Coordination compounds of Co(II), Ni(II), Mn(II) and Zn(II) with new representative of carbacylamidophosphates– potential anticancer drus. 5th International symposium on applied bioinorganic chemistry. Corfu, Greece, pp: 13-17.

8. Brown, D.B., A.R. Khokhar, M.P. Hacker, L. Lokys, J.H. Burchenal, R.A. Newman, J.J. McCormack and D. Frost, 1982. Synthesis and antitumor activity of new platinum complexes. J. Med. Chem., 25: 952-6.
9. Mirabelli, C.K., D.T. Hill, L.F. Faucette, F.L. McCabe, G.R. Girard, D.B. Bryan, B.M. Sutton, J.O. Bartus, S.T. Crooke and R.K. Johnson, 1987. Antitumor activity of bis (diphenylphosphino) alkanes, their gold(I) coordination complexes and related compounds. J. Med. Chem., 30: 2181-90.
10. McGowan, D.P.C., 2001. RSC Education and Professional Development, Cancer chemotherapy gets heavy, school of chemistry, University of Leeds, Leeds.
11. Kurbacher, C.M., W. Nagel, P. Mallmann, J.A. Kurbacher, G. Sass, H. Hubner, P.E. Andreotti and D. Krebs, 1994. *In vitro* activity of titanocenedicholoride in human renal cell carcinoma compared to conventional antineoplastic agents. Anticancer Res., 14: 1529-33.
12. Friedrich, M., C. Villena-Heinsen, C. Farnhammer and W. Schmidt, 1998. Effects of vinorelbine and titanocene dichloride on human tumor xenografts in nude mice. Eur. J. Gynaecol. Oncol., 19: 333-7.
13. Shrivastav, A., N.K. Singh and G. Srivastava, 2002. Synthesis, characterization and antitumor studies of transition metal complexes of o-hydroxydithiobenzoate. Bioorg. Med. Chem., 10: 2693-704.
14. Mishra L., M.K. Said, H. Itokawa and K. Takeya, 1995. Antitumor and antimicrobial activities of Fe(II)/Fe(III) complexes derived from some heterocyclic compounds. Bioorg. Med. Chem., 3: 1241-5.
15. Vijayalakshmi, R.V. Subramanian and B.U. Nair, 2002. A study of the interaction of Cr (III) complexes and their selective binding with B-DNA: a molecular modeling approach. J. Biomol. Struct. Dyn., 19: 1063-71.
16. Quievryn, G., E. Peterson, J. Messer and A. Zhitkovich, 2003. Genotoxicity and mutagenicity of chromium(VI)/ascorbate-generated DNA adducts in human and bacterial cells. Biochem., 42: 1062-70.
17. Joudah, L.S. Moghaddas and R.N. Bose, 2002. DNA oxidation by peroxo-chromium(v) species: oxidation of guanosine to guanidinohydantoin. Chem. Commun., 21: 1742-3.
18. Beur, A.W., W.M.M. Jkirby and M. Turck, 1966. Antibiotic susceptibility testing by standardised single disc method. Am. J. Clin. Pathol., 44: 493-496.
19. Rios, J.J., M.C. Reico and A. Villar, 1988. Antimicrobial screening of natural products. J. Entho. Pharmacol., 23: 127-149.
20. Reiner, R., 1982. Detection of antibiotic activity. In Antibiotics an introduction. Roche Scientific Services, Switzerland, 1: 21-25.
21. Persoone, G. *et al*., 1980. Proceeding the international symposium on brine shrimp *Artemia saline,* volumes 1-3, Universe press. Witteren, Belgium, pp: 1-3.
22. Mayer, B.N., N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols and J.L. Mclaughlin, 1982. Brine shrimp: a convenient bioassay for active plant constituents. Plant Medica., 45: 31-34.
23. Mclaughlin, J.L. and J.E. Anderson, 1988. Brine shrimp and crown gall tumors: simple bioassay for the discovery of plant antitumour agents. Proceeding NIH workshop. Bioassay for discovery of antitumour and antiviral agents from natural sources. Bethesda, pp: 22.
24. Mclaughlin, J.L., 1990. Bench tops bioassay for the discovery of bioactive compounds in higher plants. Brenena, pp: 29.
25. Finney, D.J., 1971. Probit analysis, 3rd Ed. University press, Cambridge, UK., pp: 18, 37, 77.
26. Sarkar, M.K., D. Ergil, A.U. Tamir and N. Sahin, 1988. Antimicrobial activity and cytotoxicity of *Ables nordmannlana subsp.* equi-trojani extract. Fitoterapia, 69: 457-460.