INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2006/08–6–774–777
http://www.fspublishers.org

# Biological Screening of Some Ferrocene Derivative Metal Complexes

M.D. EMDADUL HAQUE, M.D. FRANK HOSSEN†, M.D. SAYEEDUL ISLAM‡ AND ABU SHADAT MOHAMMOD NOMAN[1]¶

*Institute of Biological Sciences, †Department of Chemistry and ‡Genetics and Breeding, University of Rajshahi, Rajshahi-6205, Bangladesh*

*¶Department of Biochemistry and Molecular Biology, University of Chittagong, Chittagong-4331, Bangladesh*

[1]Corresponding author's e-mail: nomanly@yahoo.com; Tel.: +880 31 726311-726314 (ext. 4331); fax: +880 31 726310

## ABSTRACT

The aim of the present study was to investigate the antimicrobial and cytotoxic activities of five newly synthesized ferrocene based complexes [Mn {Fcd $(COO)_2$}, A], [Co {Fcd $(COO)_2$}, B], [Ni {Fcd $(COO)_2$}, C], [Cu {Fcd $(COO)_2$}, D] and [Zn {Fcd $(COO)_2$}, E]. The maximum antibacterial (at the concentration 100 μG/disc) and antifungal (at the concentration 200 μG $disc^{-1}$) activities were shown by the manganese complex A followed by cobalt complex B. The minimum activities were shown by zinc complex E. The minimum inhibitory concentration of the complexes was determined against four pathogenic bacteria *Bacillus subtilis, Staphylococcus aureus, Salmonella typhi* and *Shigella dysenteriae* and the values of complex A were found between 16 - 32 μG $mL^{-1}$. Brine shrimp bioassay lethality was carried out for cytotoxicity measurements of the complexes and the $LC_{50}$ values were calculated after probit transformation of the resulting mortality data. Among the five complexes manganese complex A was showed highest cytotoxic effect, which is indicative of its probable effect on cancer cell lines.

**Key Words:** Coordination complexes; Antibacterial activity; Antifungal activity; Cytotoxicity; Pathogens

## INTRODUCTION

Cancer is caused when genetic damage to the cells prevents them being responsible to normal tissue controls. The cancer spreads when affected cells multiply rapidly, forming tumors of varying degrees. Different therapies can be used, depending on how far the cancer has spread. Anticancer drugs have originated from a variety of sources, including dyestuffs and chemical warfare agents, and from natural products such as plants, microbes and fungi. One of the most potent and effective antitumor agents was discovered in the last century (Alam *et al*., 2004). Transition metal co-ordination complexes have now been widely studied for their antimicrobial and anticancer properties (Beur *et al*., 1966; Amirkhanov *et al*., 1999; Biswas *et al*., 2002). Cisplatin is one of the most potent and effective antitumor agents but it lacks selectivity for tumor tissue and many tumors are growing resistance to this platinum complex (Brachia *et al*., 1999; Britten *et al*., 2005). 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 (Chaudhary *et al*., 2003). The scientists are now engaged to explore other transition based complexes and other complexes (Finncy, 1971; Kelland *et al*., 1994; Friedrich *et al*., 1998; Dentine-Samara *et al*., 2002; Islam *et al*., 2002; Joudah *et al*., 2002; Kamalakannan & Venkappayya, 2002; Hossain *et al*., 2004). In the continuation of this discovery present studies synthesized five new ferrocene-derivative metal complexes and have studied their cytotoxicity, antibacterial and antifungal activities.

## MATERIALS AND METHODS

**Methods.** All chemicals used were of reagent grade. Solvents were distilled and dried before use according to standard procedures. The metal complexes were prepared by the literature methods. The metal salts used were generally in their hydrated form.

**Preparation of [Mn {Fcd $(COO)_2$}, A].** The 5 mL aqueous solution of Fcd $(COONa)_2$ 0.0319 g (0.1 mm) was dropped slowly into the 4 ml $CH_3OH$ solution of $MnCl_2.4H_2O$ 0.0198 g (0.1 mm) contained in a 50 mL round bottom flask, the mixture was stirred and then was put in the dark. About 10 days later, the orange crystals were obtained. Then it was filtered and washed with methanol ($CH_3OH$) and dried under vacuum. The product was free from starting materials (Checked by TLC). Yield: 0.0297 g, 91%. Melting point: > 300°C (decomp).

**Preparation of [Co {Fcd$(COO)_2$}, B].** The 5 mL aqueous solution of Fcd $(COONa)_2$ 0.0319 g (0.1 mm) was dropped slowly into the 4 mL $CH_3OH$ solution of $(CH_3COO)_2Co.4H_2O$ 0.0249 g (0.1 mm) contained in a 50 mL round bottom flask, the mixture was stirred and then was put in the dark. About 10 days later, the yellow crystals were obtained. Then it was filtered and washed with methanol ($CH_3OH$) and dried under vacuum. The product was free from starting materials (Checked by TLC). Yield: 0.0275 g, 83%. Melting point: > 300°C (decomp).

**Preparation of [Ni {Fcd $(COO)_2$}, C].** The 5 mL aqueous solution of Fcd $(COONa)_2$ 0.0319 g (0.1 mm) was dropped slowly into the 6 mL $CH_3OH$ solution of $(CH_3COO)_2Ni.4H_2O$ 0.0248 g (0.1 mm) contained in a 50 mL round

bottom flask, the mixture was stirred and then was put in the dark. About 10 days later, the yellow crystals were obtained. Then it was filtered and washed with methanol ($CH_3OH$) and dried under vacuum. The product was free from starting materials (Checked by TLC). Yield: 0.0261 g, 79%. Melting point: > 300°C (decomp).

**Preparation of [Cu {Fcd $(COO)_2$}, D].** The 5 mL aqueous solution of Fcd $(COONa)_2$ 0.0319 g (0.1 mm) was dropped slowly into the 5 mL $CH_3OH$ solution of $(CH_3COO)_2Cu$. $H_2O$ 0.0171 g (0.1 mm) contained in a 50 mL round bottom flask, the mixture was stirred and then was put in the dark. About 10 days later, the gray crystals were obtained. Then it was filtered and washed with methanol ($CH_3OH$) and dried under vacuum. The product was free from starting materials (Checked by TLC). Yield: 0.0251 g, 75%. Melting point: > 300°C (decomp).

**Preparation of [Zn {Fcd $(COO)_2$}, E].** The 5 mL aqueous solution of Fcd $(COONa)_2$ 0.0319 g (0.1 mm) was dropped slowly into the 5 mL $CH_3OH$ solution of $(CH_3COO)_2$ Zn $2H_2O$ 0.0199 g (0.1 mm) contained in a 50 mL round bottom flask, the mixture was stirred and then was put in the dark. About 10 days later, the reddish yellow crystals were obtained. Then it was filtered and washed with methanol ($CH_3OH$) and dried under vacuum. The product was free from starting materials (Checked by TLC). Yield: 0.0259 g, 77%. Melting point: > 300°C (decomp).

**Antibacterial screening.** *In vitro* antibacterial screening is generally performed by disc diffusion methods for the primary selection of compounds as therapeutic agents (Mayer *et al.*, 1982; Kurbacher *et al*., 1994). In this method activity of the test compounds are expressed by measuring the diameter of zone of inhibition. Generally, the more susceptible the organisms the bigger the zone of inhibition. The method essentially a qualitative or semi quantitative test indicating sensitivity or resistance of microorganisms to the test material as well as bacteriostetic or bactericidal activity of a compound (Kurbacher *et al*., 1994). The antimicrobial activity of the complexes [Mn {Fcd $(COO)_2$}, A], [Co {Fcd $(COO)_2$}, B], [Ni {Fcd $(COO)_2$}, C], [Cu {Fcd $(COO)_2$}, D] and [Zn {Fcd $(COO)_2$}, E] was determined at a concentration of 30 and 200 μG $disc^{-1}$ against six gram positive (*Staphylococcus aureus, Streptococcus-β-heamolyticus, Bacillus megaterium, Bacillus subtilis, Sarcina lutea, Bacillus cereus)* and eight gram negative (*Salmonella typhi, Shigella dysenteriae, Shigella shiga, Shigalla flexneri, Shigella sonnei, Shigella boydii, Escherrichia colli, Klebsiella species*) bacteria. The diameters of the zone of inhibition produced by the complexes were compared with the standard antibiotic (kanamycin 30 μG $disc^{-1}$). The experiment was performed three times to minimize the errors.

**Minimum inhibitory concentration (MIC) determination.** MIC of the compound is defined as the lowest concentration of that compound in a medium without visible growth of the test organisms. MIC of the complexes was determined against two pathogenic bacteria (*B. subtilis, S. aureus, S. typhi & S. dysentariae*) by serial dilution technique (Mellor *et al*., 2005). The results were compared with the standard antibiotic kanamycin. The media used in this respect was nutrient broth (DIFCO).

**Antifungal assay.** The antifungal activities of the complexes were tested against four pathogenic fungi (*Candida albicans, Aspergillus niger, Aspergillus fumigatus & aaspergillus flavus*) at a concentration of 50 and 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. For a better correlation of the anti fungal activities Fluconazole 50 μG $disc^{-1}$ was used as a standard.

**Cytotoxicity bioassay.** Brine shrimp lethality bioassay is a recent development in the assay procedure of bioactive compound, which indicates cytotoxicity as well as a wide range of pharmacological activities (such as anticancer, antiviral, insecticidal, pesticidal, AIDS etc) of the compounds (Mishra *et al*., 1995). Here *in vivo* lethality test was carried out by using brine shrimp nauplii eggs (*Artemia salina* L.). Eggs were hatching 48 h in 3.8% NaCl solution (Sea water) and after two days of hatching, the nauplii were ready for experiment as described previously (Quievryn *et al*., 2003). Standard solutions of the complexes were prepared whose concentration was 5μG $mL^{-1}$ (3 mg of each complex was dissolved in 0.6 mL of Dimethyl Sulfoxide). From the stock solution 5, 10, 20, 40 and 80 μL were placed in 5 different vials and the volume was made up to 5 mL with NaCl (3.8%) solution. Thus the final concentration of the sample in the vials became 5, 10, 20, 40 and 80 μM $mL^{-1}$, respectively. Then 10 brine shrimp nauplii were placed in each vial. For the control of each vial, one vial containing equal volume of Dimethyl Sulfoxide (DMSO) and NaCl solution up to 5 mL. After 24 h of incubation, each vial was observed using a magnifying glass and the number of survivors in each vial was counted and noted. From the data % of mortality was calculated and plotted against Log dose (log C). From the graph $LC_{50}$ values of the complexes were determined using probit analysis (Reiner *et al*., 1982).

## RESULTS AND DISCUSSION

**Antibacterial activity.** The metal complex A show moderate antibacterial activities at the concentration of 30 μG/disc with respect to the standard antibiotic Kanamycin but showed remarkable activities at the high concentration of 100 μG $disc^{-1}$ against both gram positive and gram negative bacteria. The reports listed in Table I show that the other metal complexes (B-E) did not show remarkable activities at the concentration of 30 μG $disc^{-1}$ but show moderate activity at the concentration of 100 μG $disc^{-1}$.

The more antibacterial activity of the complex A may be due to the metal manganese. Further studies were needed to explore the mechanism of antibacterial activity of these

**Table I. *In vitro* antibacterial activities of the coordination complexes A, B, C, D, E and standard Kanamycin**

| Test organisms | Diameter of zone of inhibition (mm) | | | | | | | | | | Kanamycin 30 (µg/disc) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | A(µg/disc) | | B(µg/disc) | | C(µg/disc) | | D(µg/disc) | | E(µg/disc) | | |
| | 30 | 100 | 30 | 100 | 30 | 100 | 30 | 100 | 30 | 100 | |
| **Gram positive bacteria** | | | | | | | | | | | |
| *B. subtilis* | 20 | 35 | 13 | 23 | 00 | 7 | 00 | 9 | 00 | 7 | 30 |
| *S. -β-haemolyticus* | 18 | 32 | 13 | 21 | 00 | 8 | 00 | 10 | 00 | 8 | 29 |
| *B. megaterinun* | 22 | 32 | 13 | 25 | 00 | 8 | 00 | 11 | 00 | 7 | 30 |
| *S. aureus* | 22 | 35 | 14 | 25 | 00 | 9 | 00 | 12 | 00 | 8 | 29 |
| *S. lutea* | 20 | 29 | 12 | 20 | 00 | 10 | 00 | 8 | 00 | 7 | 30 |
| *B. cereus* | 17 | 22 | 12 | 21 | 00 | 9 | 00 | 10 | 00 | 8 | 31 |
| **Gram negative bacteria** | | | | | | | | | | | |
| *E. coli* | 23 | 32 | 12 | 24 | 00 | 10 | 00 | 9 | 00 | 8 | 30 |
| *S. typhi* | 24 | 36 | 12 | 20 | 00 | 9 | 00 | 10 | 00 | 7 | 31 |
| *S. sonnei* | 21 | 30 | 11 | 21 | 00 | 8 | 00 | 9 | 00 | 8 | 30 |
| *S. dysenteriae* | 20 | 35 | 13 | 23 | 00 | 9 | 00 | 10 | 00 | 7 | 32 |
| *S. shiga* | 22 | 32 | 11 | 20 | 00 | 8 | 00 | 9 | 00 | 8 | 31 |
| *S. flexneri* | 20 | 30 | 11 | 20 | 00 | 8 | 00 | 8 | 00 | 7 | 30 |
| *S. boydii* | 21 | 32 | 10 | 21 | 00 | 8 | 00 | 9 | 00 | 7 | 31 |
| *K. species* | 20 | 30 | 11 | 20 | 00 | 9 | 00 | 8 | 00 | 8 | 30 |

A = [Mn(Fcd(COO)$_2$)]; B = [Co(Fcd(COO)$_2$)]; C = [Ni(Fcd(COO)$_2$)]; D = [Cu(Fcd(COO)$_2$)]; E = [Zn(Fcd(COO)$_2$)]

ferrocene derivative compounds. Manganese complexes have been reported for their antibacterial activity (Rosenberg *et al*., 1965; Rios *et al*., 1988; Saglam *et al*., 2002). Many authors also reported antibacterial activity of other transition metal complexes and our present findings supported the previous results of antibacterial activity for both manganese and other metal coordination complexes (Schabel *et al.*, 1979; Shrivastav *et al.*, 2002; Sultana *et al.*, 2003).

**Minimum inhibitory concentration (MIC).** The reports listed in Table II show that the MIC value of the complex A against *B. subtilis, S. aureus, S. typhi and S. dysenteriae* were 16, 16, 16 and 32 µG mL$^{-1}$, respectively; for the complex B, 128, 128, 128, 128, respectively and for other three complexes C, D and E no remarkable MIC values can be found. From the MIC values it was found that the ferrocene coordination complex A was more potent than the other complexes B, C, D and E.

From the MIC values it was found that the ferrocene coordination complex A was more potent than the other complexes B, C, D and E. Earlier three coordination complexes of metals with mixed ligands [Mn (ED) (2-aminophenol), A], [Fe (ED)(2-aminobenzoic acid), B] and [Fe (ED) (2-aminophenol), C] were tested for their antimicrobial activity by disc diffusion and serial dilution methods (Zakaria *et al.*, 2000). The minimum inhibitory concentration of the complex A was determined against four pathogenic bacteria *B. subtilis, S.-β-haemolyticus*, *E. coli* and *S. typhi*. The manganese complex A also showed potent cytotoxic effect, which is indicative of its probable effect on cancer cell lines.

**Antifungal activity.** The antifungal activities of the metal complexes (A-E) and standard Fluconazole (F-50 µG disc$^{-1}$) were determined at the concentration of 200 µG disc$^{-1}$ against four pathogenic fungi are listed in Table III. It was found that the metal complex A was shown greater activity than others against all of the pathogenic fungi. The metal complex B was shown moderate activity.

The antifungal activity of other transition metal complexes also reported by many authors (Friedrich *et al.*, 1998; Hossain *et al.*, 2004). Our present findings supported the previous results.

**Cytotoxic activity.** In the brine shrimp lethality bioassay the synthetic complexes (A - E) showed positive results indicating that the complexes are biologically active. The mortality rate of brine shrimp nauplii was found to increase with the increase of concentration of the sample. The reports listed in Table IV show that the $LC_{50}$ values of the complexes A, B, C, D and E are 5.85, 9.13, 25.10, 21.20 and 31.75 ppm, respectively. The standard anticancer drug gave

**Table II. Minimum inhibitory concentration (MIC values) of the complexes (A- E) and kanamycin**

| Test organisms | Minimum inhibitory concentration (µg/ml) | | | | | |
|---|---|---|---|---|---|---|
| | A | B | C | D | E | Kanamycin |
| *B. subtilis* | 16 | 128 | - | - | - | 4 |
| *S. aureus,* | 16 | 128 | - | - | - | 5 |
| *S. typhi* | 16 | 128 | - | - | - | 4 |
| *S. dysenteriae* | 32 | 128 | - | - | - | 4 |

**Table III. Antifungal activities of the complexes (A-E) and standard Fluconazole**

| Fungal Strains | Diameter of zone of inhibition (in mm ) | | | | | |
|---|---|---|---|---|---|---|
| | A (200/µg/disc) | B (200/µg/disc) | C (200/µg/disc) | D (200/µg/disc) | E (200/µg/disc) | Fluconazole (50µg/disc) |
| **Plant pathogen** | | | | | | |
| *A. fumigatus* | 21 | 11 | 00 | 00 | 00 | 25 |
| *A. flavus* | 22 | 12 | 00 | 00 | 00 | 28 |
| **Human pathogen** | | | | | | |
| *C. albicans* | 21 | 13 | 00 | 00 | 00 | 24 |
| *A. niger* | 16 | 10 | 00 | 00 | 00 | 20 |

**Table IV. The results of cytotoxic effect of complexes A, B, C, D, E and standard Bleomycin and Galic acid**

| Test samples | $LC_{50}$ (ppm) | 90% confidence limit (ppm) | | Regression equation | $\chi^2$ |
|---|---|---|---|---|---|
| | | Lower | Upper | | |
| A | 5.85 | 5.02 | 13.19 | Y=3.7959+1.3224X | 0.3776 |
| B | 9.13 | 8.06 | 19.02 | Y=3.650+1.2353X | 0.1290 |
| C | 25.10 | 12.72 | 30.13 | Y=3.542+1.052X | 0.1651 |
| D | 21.20 | 11.50 | 28.00 | Y=3.644+1.081X | 0.0958 |
| E | 31.75 | 22.71 | 57.16 | Y=3.142+1.19X | 0.1992 |
| Standard bleomycin | 0.41 | 0.276 | 0.620 | Y=3.16+2.99X | 0.62 |
| Galic acid | 4.53 | 3.330 | 6.150 | Y=3.93+1.62X | 1.25 |

its $LC_{50}$ value at 0.41 ppm. The lowest $LC_{50}$ value was found in case of complex A (5.85 ppm) followed by B (9.13 ppm), which is indicative of its higher cytotoxicity and anticancer effect on cancer cell lines.

Many authors explored the cytotoxic properties of ferrocene derivatives compounds and found higher activities in case of manganese complexes (Zakaria *et al*., 2000; Zakaria *et al*., 2001; Vijayalakshmi *et al.*, 2002). Our present results suggested the cytotoxicity of previously reported ferrocene based complexes.

Finally, we may say that, among the five complexes the tested manganese complexes A has strong cytotoxic activity but this investigation is a primary one and farther tests are required to investigate its actual mechanism of cytotoxicity and its probable effects on higher animal model and on cancer cell line. Then we may be explored it as potent cytotoxic agents with the hope of adding arsenal of weapons used against the fatal disease cancer.

**Acknowledgements.** We gratefully acknowledge the continuous help of Dr. Devasish Palit from Chemico Laboratories Limited, Tikapara, Boalia, Rajshahi, in preparation of this article.

## REFERENCES

Alam, M.A., K. -e-Zahan, M.S. Hossain and F.Z. Seema, 2004. Studies on the Antibacterial and Cytotoxic Activities of Three Cobalt µ-Peroxo Complexes and Two Zirconium µ-Peroxo Complexes. *Pakistan J. Biol. Sci.,* 7: 891–4

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 drugs,* 5th International symposium on applied bioinorganic chemistry, Corfu, Greece

Beur, A.W., W.M.M. Jkirby and M. Turck, 1966. Antibiotic susceptibility testing by standardized single disc method, *American J. Clin. Pathol.,* 44: 493–6

Biswas, M.H., A.H.M. Zakaria, A. Farroque, C.M. Zakaria, M. Zakir Sultan, G. Sadik and M.S.A. Bhuiyan, 2002. *In vitro* antibacterial and cytotoxic activity of a benzene sulfonic acid derivative complex compounds. *Bangladesh Pharm. J.,* 12: 43–6

Brachia, A., M. Careelli, P. Pelahatchie, C. Pelizzi, G. Pelizzi and F. Zani, 1999. Antimicrobial and mutagenic activity of some carbono- and thiocarbonohydrazone ligands and their copper (II), iron (II) and zinc (II) complexes. *J. Inorg. Biochem.,* 15: 123–33

Britten, R.A., C. Hardy, A. Vlahou, B. Gregory, P.S. Giri and R. Drake, 2005. Identification of reproducible low mass SELDI protein profiles specific to cisplatin resistance in human ovarian cancer cells. *Oncol. Rep.,* 14: 1323–30

Chaudhary, A., N. Bansal, A. Gajraj and R.V. Singh, 2003. Antifertility, antibacterial, antifungal and % disease incidence aspects of macrocyclie complexes of manganese (ii). *J. Inorg. Biochem.,* 96: 393–400

Dentine-Samara, C., L. Alevizopoulou, L. lordanldis, E. Samaras and D.P. Kessissoglou, 2002. 15 -MC- 5 manganese metallacrowns hosting herbicide complexes, Structure and bioactivity. *J. Inorg. Biochem.,* 89: 89–96

Finncy, D.J., 1971. *Probit Analysis, (3rd ed.)*, Pp: 18, 37, 77. University press, Cambridge, UK

Friedrich, M., C. Villena-Heinsen, C. Farnhammer and W. Schmidt, 1998. Effects of vinorelbine and titanocene dichloride on human tumor xenografts in nude mice. *European J. Gynaccol. Oncol.,* 19: 333–7

Hossain, M.S., K. -e-Zahan, M.A. Islam, S. Sarkar, J. Nassin, A. Islam, M.A. Farooque and A. Alam, 2004. *In vitro* antimicrobial and *in vivo* Cytotoxic activity of three coordination complexes synthesized by mixed ligands. *Pakistan J. Biol. Sci.,* 7: 1113–6

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: 797–9

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

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

Kelland, L.R., C.F.J. Barnard, K.J. Mellish, M. Jones, P.M. Goddard, M. Valenti, A. Bryant, B.A. Murrer and K.R. Harrap, 1994. A novel *trans*-platinum coordination complex possessing *in vitro* and *in vivo* antitumor activity. *Cancer Res.,* 54: 5618–22

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 titanoccnedicholoride in human renal cell carcinoma compared to conventional antincoplastic agents, *Anticancer Res.,* 14: 1529–33

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. *Pl. Medica,* 45: 31–4

Mellor, H.R., S. Snelling, M.D. Hall, S. Modok, M. Jaffar, T.W. Hambley and R. Callaghan, 2005. The influence of tumor micro environmental factors on the efficacy of cisplatin and novel platinum (IV) complexes. *Biochem. Pharmacol.,* 70: 1137–46

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

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. *Biochemistry,* 42: 1062–70

Reiner, R., 1982. *Detection of antibiotic activity in Antibiotics an Introduction,* Pp: 21–5. Roche Scientific Services, Switzerland

Rios, J.J., M.C. Reico and A. Villar, 1988. Antimicrobial screening of natural products. *J. Entho. Pharmacol.,* 23: 127–49

Rosenberg, B., L. Van Acamp and T. Krigas, 1965. Inhibition of cell division in *Escherichia coli* by electrolysis products from a platinum electrode. *Nature,* 205: 698–9

Saglam, N., A. Colak, K. Serbest, S. Dulger, S. Guner, S. Karaboeck and A.O. Belduz, 2002. Oxidative cleavage of DNA by homo-and heteronuclear Cu (ii)-Mn (ii) complexes of an oxime-type ligand. *Biometals,* 15: 357–65

Schabel, F.M.J., M.W. Trader, W.R.J. Laster, T.H. Corbett and D.P.J. Griswold, 1979. Cis-Dichlorodiammineplatinum (II): combination chemotherapy and cross-resistance studies with tumors of mice, *Cancer Treat. Rep.,* 63: 1459–73

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

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. *Pakistan J. Biol. Sci.,* 6: 525–7

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

Zakaria, C.M., A. Farroque, M.R. Islam, M.A. Islam and M.H. Biswas, 2001. Cytotoxic activity of ferrocene derivative compounds. *Oriental J. Chem.,* 17: 47–50

Zakaria, C.M., A. Farroque, M.R. Islam, M.A. Islam and M.H. Biswas, 2000. Antimicrobial screening of ferrocene derivative compounds. *Oriental J. Chem.,* 16: 85–90

**(Received 13 February 2006; Accepted 12 August 2006)**