Journal of Institute of Science and Technology, *26*(1), 128-140 (2021)
ISSN: 2469-9062 (print), 2467-9240 (e)
https://doi.org/10.3126/jist.v26i1.37840

Review Article

ANTICANCER POTENCY OF COPPER(I) COMPLEXES AGAINST A RANGE CANCER CELL LINES: A REVIEW

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(***Received**: March 01, 2021; **Revised**: May 26, 2021; **Accepted**: June 04, 2021)*

ABSTRACT

The copper(I) complexes of N,N-diimine, N,O- and/or N,S- bidentate systems perform significant dose-dependent anticancer activity toward various cell lines *viz.* MCF-7, LNCap, PSN-1, A431, BxPC3, H157, A2780, HeLa, MDA-MB231, MGC-803 etc. The copper(I) complexes can cross the cellular plasmalemma that results in the accumulation of copper ion in the cancer cells, exhibit significant anticancer effect and overcome the multidrug resistance because these can slightly induce the DNA cleavage as a result of limited generation of reactive oxygen species (ROS). Copper(I) complexes exhibit significantly higher broad-spectrum antiproliferation and cell apoptosis *via* mitochondrial pathway than that of their corresponding Cu(II), Co(II), Pd(II), and Ni(II) complexes. The copper(I) complexes inhibit the cancer cells not only *via* ROS generation but also *via* DNA interactions possibly by attacking the sugar-phosphate backbone of DNA due to their oxidative and partial dissociation behavior. Copper(II/I) complexes are also able to cleave DNA by hydrolytic pathway and induce caspase-dependent-mitochondrial-mediated cell apoptosis by ROS production or blocking the progression of cell cycles. In many cases, the modification in organic moiety and the placement of electronegative substituent near the metallic center of complexes have been found to enhance their anticancer potency in a significant manner. Thus, copper(I) complexes may be used as the better anticancer drugs with multiple modes of action compared to the copper(II) complexes due to having oxidative behavior and generation of empty site on copper(I) ion during partial dissociation.

Keywords: Antibiotics, Anticancer activity, Cisplatin, Copper(I) complex, Diphosphane.

INTRODUCTION

An essential trace element copper act as catalytic and structural cofactors and have importance in human life because of its nature of accumulation in the tumor cells due to their selective permeability and its key role in DNA synthesis, enzymatic functions, intracellular redox potential regulation (Daniel *et al.*, 2005; Lopes *et al.*, 2017; Singh *et al.*, 2020). In the serum of living organisms, Cu(I) and Cu(II) bind to ceruloplasmin and generate the active center in metalloproteins (Lopes *et al.*, 2017; Singh *et al.*, 2020). The redox behavior of copper ($Cu^{I/II}$) plays an important role to provoke anticancer potency, diminish the toxicity, and overcome the resistance activity of the drug (Gunasekaran *et al.*, 2011, Gandin *et al.*, 2015; Singh *et al.*, 2020).

Many copper complexes have been found to exhibit anticancer activities for example copper(II) complex of 5-nitroisatin-4-(1-(2-pyridyl)piperazinyl)-3-thiosemicarbazone exhibited better anticancer potency toward breast cancer cell, MDA-MB-231 (IC_{50} 0.85 μM) (Singh *et al.*, 2021a). Copper complexes have been found to perform different anticancer mechanism of action than that of platinum complex (cisplatin) for example casiopeins has been found to exhibit high antineoplastic activity by respiratory inhibition and ATP synthesis (Marin-Hernandez *et al.*, 2003). Copper complexes of many ligands such as acetyl pyridine, dipyridyl ketone, acetyl pyrazine, etc. have high potency of cancer cell inhibition due to their interaction with DNA either by the covalent or noncovalent way (Miller III *et al.*, 1999; Jansson *et al.*, 2010).

The thiocarbamides with two different substituents on N-atoms exhibit hydrogen bonding between the carbonyl group of substituent and HN group of amide that helps the existence of coordinate covalent bond directly through S-atoms, increases lipophilicity and anticancer potency of ligands and their complexes *via* effective interaction with DNA (Xian *et al.*, 2004; Saeed *et al.*, 2010; Singh *et al.*, 2015; Saswati *et al.*, 2015; Mahendiran *et al.*, 2018b; Pandey *et al.*, 2018). Copper(I) ion, obtained by the reduction of copper(II) ion with metallo-reductase at the cell surface of living organisms, before uptake into the cell can be transported into the living cells by their specific transporters (Puig & Thiele 2002; Puig *et al.*, 2002; Marzano *et al.*, 2009). Copper(I) ion tends to undergo oxidation to form copper(II) ion so there is difficulty in stabilizing the copper(I) complexes. That's why copper chemistry has been dominated by copper(II) complexes. Copper(I) ion with $3d^{10}$ configuration has been stabilized by phosphane molecule due to having soft base P-atom and N,N-diimine systems and it performed significant cytotoxic activity against various cancer cell lines (Santini *et al.*, 2013; Gandin et al., 2017; Lopes *et*

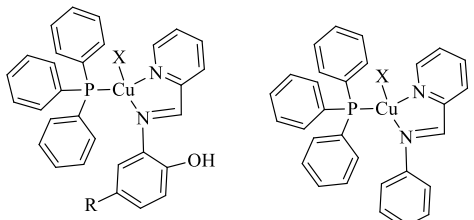
al., 2017; Bravo *et al.,* 2019). Copper(I) complexes dissociate partially by generating an empty site on Cu^{+} ions that can facilitate the complexes to interact with the biological target (Santini *et al.,* 2013). The modification in the structure of organic moiety by different substituents affects the anticancer activity of copper complexes (Saswati *et al.,* 2015; Mahendiran *et al.,* 2018b; Singh *et al.,* 2021b).

Having a tendency to transport into living cells by specific transporters, different anticancer mechanism, oxidative behavior, and ability to generate an empty site by partial dissociation made copper(I) complexes better anticancer agents than Cu(II), Zn(II), Pd(II), etc complexes. Thus the study of copper(I) complexes with different modifications in aromatic moiety will be a milestone in the field of pharmaceutical research.

Copper(I) complexes as anticancer agents

Pyridinyl copper(I) complexes

The copper(I) complexes of imine ligands *viz.* (E)-2-((pyridin-2-ylmethylene)amino)phenol (C_1-C_4), (E)-4-methyl-2-((pyridin2-ylmethylene)amino)phenol (C_5-C_6), and (E)-4-((Pyridin-2-ylmethylene)amino)phenol (C_7) (Fig. 1) against human glioblastoma (U87) cell showed remarkable anticancer potency at micromolar concentration (IC_{50} 20-46.7 μM). The order of complexes toward cytotoxic activity: C_3>C_7>C_2>C_6>C_1>C_5>C_4 confirmed that the copper(I) complexes with halide ions performed higher anticancer potency than that with isothiocyanate (NCS^{-}) ion and the effect of anticancer potency also increased with the increasing mass of halide ions. The complexes showed inhibition toward growth, migration ability, cell cycle progression, and expression level of anti-apoptotic genes but induced necrosis, the expression level of apoptotic genes, and apoptosis in a dose-dependent manner (Milani *et al*., 2020).

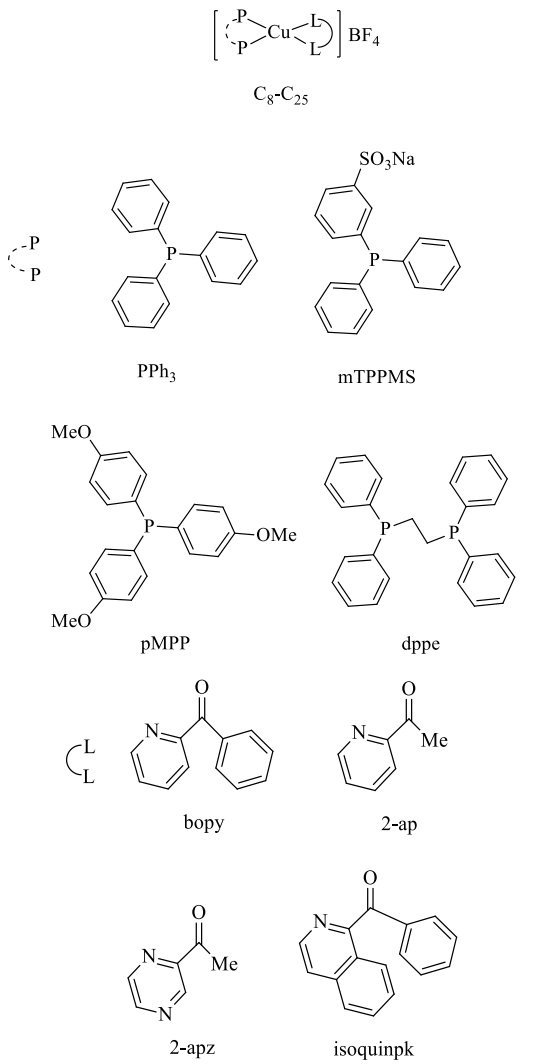


C_1(R=H, X=Cl^{-}), C_2(R=H, X=Br^{-}),
C_3(R=H, X=I^{-}),C_4(R=H, X=NCS^{-}),
C_5(R=CH_3, X=Cl^{-}),C_6(R=CH_3, X=Br^{-}) C_7

Fig. 1. Copper(I) complexes of ((Pyridin-2-ylmethylene)amino)phenol and it's derivative (Milani *et al*., 2020)

Diphosphane and bidentate N, O-heteroaromatic Cu(I) complexes

The *in vitro* cytotoxic study of copper(I) complexes of mixed ligands *viz.* diphosphane (PP) and an N, O-heteroaromatic bidentate ligand (LL), [Cu(PP)(LL)][BF_4] (C_8-C_{25}) (Fig. 2) against cancer cell lines MCF-7 (breast cancer), LNCap (human prostate cancer) and normal prostate cell line (RWPE) exhibited anticancer activity but showed significant activity toward LNCap (IC_{50} 0.20-1.96 μM).



C_8(PP=2*m*TPPMS, LL=2NCMe),
C_9(PP=2*p*MPP, LL=2NCMe),
C_{10}(PP=2PPh_3, LL=bopy), C_{11}(PP=2PPh_3, LL=2-ap),
C_{12}(PP=2PPh_3, LL=2-apz), C_{13}(PP=2PPh_3, LL=isoquinpk),
C_{14}(PP=2*m*TPPMS, LL=bopy), C_{15}(PP=2*m*TPPMS, LL=2-ap),
C_{16}(PP=2*m*TPPMS, LL=2-apz),
C_{17}(PP=2*m*TPPMS, LL=isoquinpk),
C_{18}(PP=2*p*MPP, LL=bopy), C_{19}(PP=2*p*MPP, LL=2-ap),
C_{20}(PP=2*p*MPP, LL=2-apz), C_{21}(PP=2*p*MPP, LL=isoquinpk),
C_{22}(PP=dppe, LL=bopy), C_{23}(PP=dppe, LL=2-ap),
C_{24}(PP=dppe, LL=2-apz), C_{25}(PP=dppe, LL=isoquinpk),

Fig. 2. Copper(I) complexes of diphosphane and bidentate N, O-heteroaromatic ligand (Machado *et al*., 2020)

The order of cytotoxicity of the complexes toward the LNCap cell was found to be

C_{23}>C_{10}>C_{25}>C_{11}>C_{12}>C_{17}>C_{13}>C_{19}>C_{21}>C_{18} thereby indicating the larger extent of contribution by 1,2-(bis(diphenylphosphano) ethane), diphenylphosphane, and 2-acetyl pyridine than that by other phosphane and heterocyclic ring in the cytotoxicity. The most active complex C_{23} (70 fold higher activity toward LNCap than normal prostate cell RWPE) exhibited a high level of cellular internalization, intracellular reactive oxygen species (ROS) generation, and cell death activation mechanism *via* apoptosis/necrosis (Machado *et al*., 2020).

Phosphane and pyrazolyl copper(I) complexes

The *in vitro* cytotoxic study of copper(I) complexes of N-methyl-d-aspartated bis(pyrazol-1-yl) acetate and phosphane ligands (C_{26}-C_{33}) (Fig. 3) against human pancreatic cancer; PSN-1, BxPC3, breast cancer; MCF-7, oral squamous; H157, skin cancer; A431, A431-Pt, ovarian cancer; A2780, A2780cis, A2780ADR reported higher anticancer potency (IC_{50} 0.01-5.9 μM) than that of their corresponding ligands and standard drug cisplatin (IC_{50} 0.45-26.7 μM).

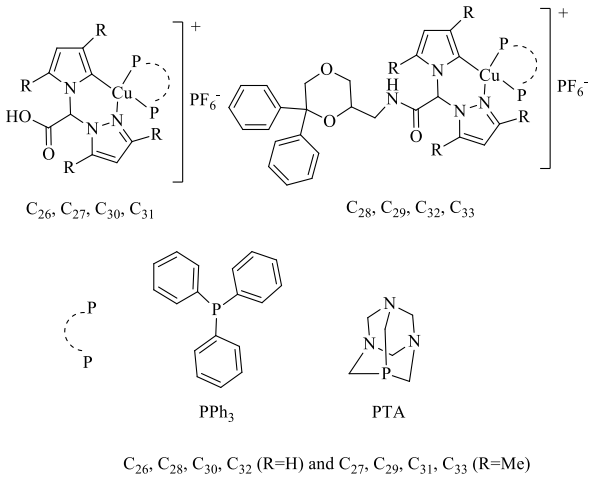


Fig. 3. Copper(I) complexes of phosphane (triphenyl-phosphine, PPh_3 or 1,3,5-triaza-7-phosphaadamantane, PTA) and bis(pyrazol-1-yl) acetate ligands functionalized with an N-methyl-d-aspartate (NMDA) receptor antagonist (Pellei *et al*., 2020)

All the complexes exhibited very high cytotoxicity even in three dimensional (3D) cell culture of H157 (IC_{50} 2.7-13.0 μM) and BxPC3 (IC_{50} 1.2-10.2 μM) cancer cells compared with cisplatin against H157 (IC_{50} 52.51μM) and BxPC3 (IC_{50} 100.5 μM). The highest inhibitory effect by the complexes C_{29} (IC_{50} 2.7 μM) and C_{33} (IC_{50} 1.2μM) in 3D spheroid indicated that triphenyl phosphane and PTA have a large contribution to anticancer potency. The ability of the complexes to cross the cellular plasmalemma resulted in the accumulation of copper in cancer cells showed a significant anticancer effect and overcome the cisplatin and multidrug resistance because they were able to slightly induce the DNA cleavage due to the limited generation of ROS (Pellei *et al*., 2020).

Bis(diphenylphosphino)ferrocene and bidentate heteroaromatic copper(I) complexes

The *in vitro* cytotoxic study of copper(I) complexes of 1,1-bis(diphenylphosphino)ferrocene and N,N-, N,O- and N, S-heteroaromatic bidentate ligands, (C_{34}-C_{41}) (Fig. 4) against human breast cancer cells; MCF7, MDA-MB231 exhibited very high anticancer activity with far lower IC_{50} values (6 to 76 folds against MCF) compared with standard drug cisplatin. All the complexes performed good action toward both cancer cells but slightly better action toward MCF-7 cell. As compared to the precursor, the presence of ferrocene moiety in the phosphane copper(I) complexes did not show any improvement in cytotoxicity indicating that only phosphane ligands have a significant contribution to enhance the anticancer activity during coordination with copper(I) ion (Bravo *et al.*, 2019).

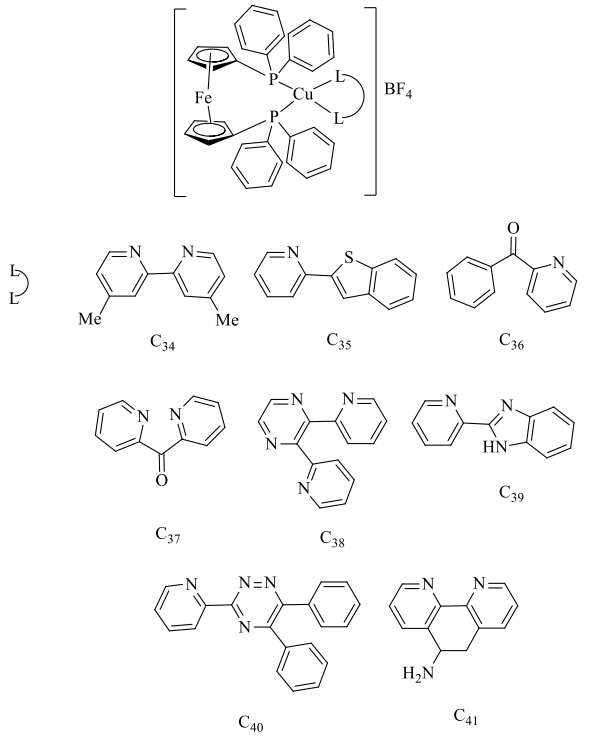
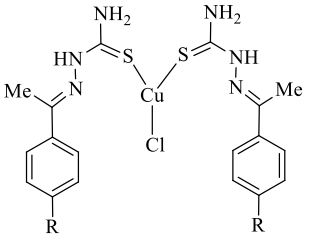


Fig. 4. Copper(I) complexes of 1,1-Bis(diphenyl-phosphino)ferrocene and N,N-, N,O- and N,S-heteroaromatic bidentate ligands (Bravo *et al.,* 2019)

Bis(acetophenone thosemicarbazone) copper(I) complexes

The *in vitro* and *in vivo* studies of copper(I) complexes of bis(acetophenone thosemicarbazone)/bis(p-substituted acetophenone thiosemicarbazone) (C_{42}-C_{47}) (Fig. 5) against cancer cell lines- MCF-7 (human breast adenocarcinoma), HeLa (cervical), Hep-2 (epithelioma),

Ehrlich ascites carcinoma (EAC) and normal cell lines; NHDF (normal human dermal fibroblasts) and L6 myotubes showed their anticancer activity at the micro molar concentration (IC_{50} 10.9-18.9 μM, MCF-7) as comparable to cisplatin (IC_{50} 12.1 μM, MCF-7) by DNA (pBR322) cleavage through a hydrolytic pathway and caspase-dependent- mitochondrial-mediated cell apoptosis *via* ROS production (Mahendiran *et al.,* 2018a). These complexes were able to reduce the volume of tumors in female Swiss albino mice by inducing cell apoptosis and S phase (DNA synthesis) arrest with the mitochondrial controlled pathway. The complexes strongly killed the EAC tumor cell line with no toxicity against normal cells (Mahendiran *et al.,* 2018a).



C_{42}(R=H), C_{43} (R=CH_3), C_{44} (R=OCH_3), C_{45}(R=OH), C_{46} (R=NO_2), C_{47} (R=Cl)

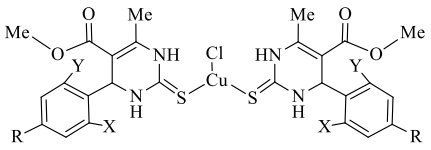
Fig. 5. Copper(I) bis(acetophenone thosemicarbazone) /bis(p-substituted acetophenone thiosemicarbazone) (Mahendiran *et al.,* 2018a)

Copper(I) complexes of dihydropyrimidine derivative with phenyl moiety

The *in vitro* cytotoxic study of copper(I) complexes of methyl 4-aryl-6-methyl-3,4-dihydropyrimidine-2(1H) thione-5-carboxylate (C_{48}-C_{50}) (Fig. 6) [aryl = 4-(*tert*-butyl)phenyl (C_{48}), 2,6-dichloro phenyl (C_{49}), 2,4-dichloro phenyl (C_{50})] against human colorectal cancer (CRC) cell line, Caco-2 exhibited higher antitumor activity (IC_{50} 25.03-38.83 μM) than that of the standard drug cisplatin (IC_{50} 35.42 μM). The complexes C_{48} and C_{49} strongly induced the apoptosis of Caco-2 cell model. The order of anticancer activity of the complexes was found to be C_{49}> C_{48}> C_{50} (Gonzalez-Ballesteros *et al.,* 2016).

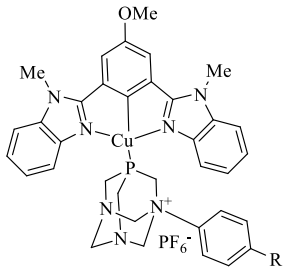
Copper(I) complexes of N-aryl triaza phosphaadamantane and imidazolyl benzene derivative

The study of *in vitro* cytotoxic activity of copper(I) complexes), [Cu(L)(PTA-PhR)](PF_6) (C_{51}-C_{53}) (Fig. 7) having NCN pincer (L) and N-aryl-1,3,5-triaza-7-phosphaadamantane (PTA-PhR) ligand [L = 5-methoxy-1,3-bis (1-methyl-1H-benzo[d]imidazol-2-yl)benzene)] against A549 (human lung), A375 (melanoma), MCF-7 (breast), LoVo (colon adenocarcinoma), HeLa cervical cancer cell lines, and HEK293 (non-malignant fibroblasts) showed higher antitumor activity (IC_{50} 0.98-21.57 μM) than that of the standard drug cisplatin (IC_{50} 2.19-23.87 μM) and their corresponding ligand (IC_{50} >100 μM).



C_{48}(X=Y=H, R= -$C(CH_3)_3$, C_{49}(X=Y=Cl, R=H), C_{50}(X=R=Cl, Y=H)

Fig. 6. Copper(I) complexes of methyl 4-aryl-6-methyl-3,4-dihydropyrimidine-2(1*H*)thione-5-carboxylate (Gonzalez-Ballesteros *et al.,* 2016)



C_{51}(R=CH_3), C_{52}(R=CH_3CH_2-), C_{53}(R=$(CH_3)_2$CH-)

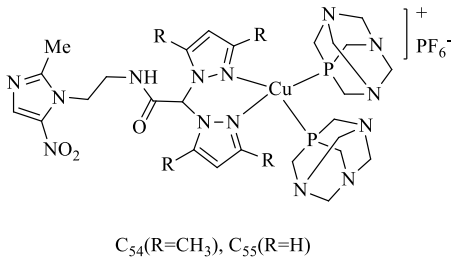
Fig. 7. Copper(I) complexes of N-aryl triaza phosphaadamantane and imidazolyl benzene derivative (Tabrizi & Chiniforoshan, 2017)

All the complexes showed a lower value of malignant behavior (IC_{50} 75.52-92.13 μM) than that of cisplatin (IC_{50} 19.32 μM) when tested against HEK293. The experimental results showed that the complexes (C_{51}-C_{53}) bind to circulating tumor deoxyribonucleic acid (CT DNA) through intercalation mode and hence the order of hypochromism magnitude and antitumor activity was found to be C_{53} (IC_{50} 0.98-13.21 μM) >C_{52} (IC_{50} 2.45-18.32 μM) >C_{51} (IC_{50} 3.61-21.57 μM) (Tabrizi & Chiniforoshan, 2017).

5-Nitroimidazole conjugated heteroscorpionate copper(I) complexes

The study of *in vitro* cytotoxic activity of copper(I) complexes of 5-nitroimidazole conjugated heteroscorpionate ligands *viz.* 2,2-bis(pyrazol-1-yl)-N-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)acetamide (C_{54}-C_{55}) (Fig. 8) against A431 (cervical), BxPC3 (pancreatic), HCT-15 (colon), MCF-7 (breast), A549 (lung), and 2008 (ovarian) cancer cell lines exhibited about 2.5 folds higher antitumor activity (IC_{50} 1.4-15.8 μM) than that of the standard drug CDDP (cisplatin) and their corresponding ligands (IC_{50} >50 μM) by constantly inducing a massive cytoplasmic vacuolization and induction of paraptotic-like cancer cell death. The complex C_{54} was found to have a higher antitumor activity (about 1.4-fold) than that of the compounds C_{55} and cisplatin. The cytotoxic results also

exhibited a higher antitumor activity for copper(I) complexes (about 2 folds) than that of their corresponding copper(II) complexes (Pellei *et al.,* 2018).

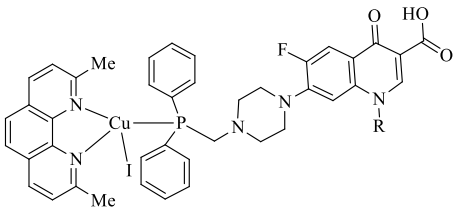


C_{54}(R=CH_3), C_{55}(R=H)

Fig. 8. Copper(I) complexes of 2,2-bis(pyrazol-1-yl)-N-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)acetamide ligand (Pellei *et al.,* 2018)

Fluoroquinolones *viz.* ciprofloxacin and norfloxacin copper(I) complexes

The *in vitro* cytotoxic study of copper(I) complexes of 2nd and 3rd generation antibiotics (fluoroquinolones) *viz.* ciprofloxacin (C_{56}) and norfloxacin (C_{57}) (Fig. 9) against CT26 (mouse colon carcinoma) and A549 (human lung adenocarcinoma) showed pronounced antitumor activity (IC_{50} 2.9-5.0 μM, A549 and IC_{50} 2.4-5.2 μM, CT26) than that of their corresponding Cu(II) ones *via* ROS generation, DNA interactions and the sugar-phosphate backbone of its chain due to their redox reactivity. The complex C_{56} have higher antitumor activity than that of the complex C_{57} (Bykowska *et al.,* 2018).

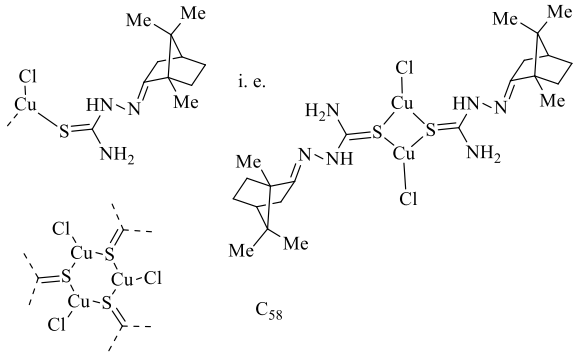


C_{56}(-R= ), C_{57}(R=CH_3CH_2-)

Fig. 9. Copper(I) complexes of fluoroquinolones *viz.* ciprofloxacin and norfloxacin (Bykowska *et al.,* 2018)

(-)-Camphor thiosemicarbazone copper(I) complex

The *in vitro* cytotoxic study of (-)-camphor thiosemicarbazone copper(I) complex (C_{58}) (Fig. 10) against MCF-7 cell line showed that the Cu(I) complex experienced remarkable dose-dependent cytotoxic activity through apoptosis (IC_{50} 9.8 μM) as that of the standard cisplatin and higher than that of uncomplexed ligand (IC_{50} >50 μM), mononuclear Zn(II) complexes (IC_{50} 14-20 μM) and binuclear Pd(II) complexes (IC_{50} 12.5-12.9 μM). The complexation of ligand to metal ion significantly enhanced its anticancer activity and hence the order of anticancer activity was found to be- Cu(I) complex>Pd-complex>Zn-complex>Ligand (Kokina *et al.,* 2019).

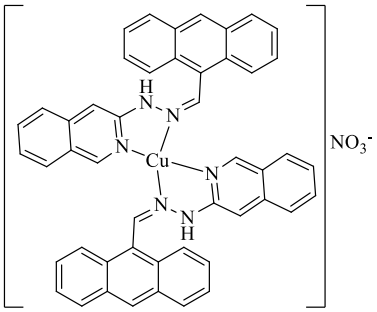


C_{58}

Fig. 10. Copper(I) complex of (-)-camphor thiosemicarbazone in different possible forms (Kokina *et al.,* 2019)

9-Quinolylanthrahydrazone copper(I) complex

The *in vitro* cytotoxic study of copper(I) complex of 9-quinolylanthrahydrazone (9-AQH), $[Cu^{I}(9\text{-}AQH)_2]NO_3$, ($C_{59}$) (Fig. 11) against MGC-803 (human gastric cancer) and other typical cancer cell lines exhibited remarkably higher broad-spectrum antiproliferation and cell apoptosis *via* mitochondrial pathway than that of their corresponding Cu(II), Co(II) and Ni(II) complexes (Liu *et al.,* 2021).



C_{59}

Fig. 11. Copper(I) complexes of 9-quinolylanthrahydrazone (Liu *et al.,* 2021)

Diphosphane and bidentate N, N-, N,O- and N, S-heteroaromatic copper(I) complexes

The *in vitro* cytotoxic activity of copper(I) complexes of triphenyl phosphane and N,N-, N,O-, and N,S- bidentate ligands (C_{60}-C_{65}) (Fig. 12) [L= 2-(2-pyridyl)benzo[b] thiophene (pbt) (C_{60}), 2- benzoylpyridine (bopy) (C_{61}), di(2-pyridyl)ketone (dpk) (C_{62}), 2,3-bis(2-pyridyl)pyrazine (dpp) (C_{63}), 2,2'-bipyridine (2,2'-bipy) (C_{64}) and 2,2′-bipyridine-4,4′-dicarboxylic acid (dcbipy) (C_{65}) against MCF-7 (human breast cancer) cell line exhibited significant anticancer activity with the far lower IC_{50} values (6.6-17.7 μM) than that of cisplatin (IC_{50} 59.0 μM). The complexes C_{60} and C_{62} were found to induce the apoptosis of MCF-7 cell death and a cell cycle arrest in the G2/M phase but complex C_{64} exhibited a significant

cytostatic effect, resulting cell cycle arrest at the S phase. The order of anticancer activity was found to be- C_{60}>C_{64}>C_{62}>C_{61}>C_{65}>C_{63} (Morais *et al.,* 2018b).

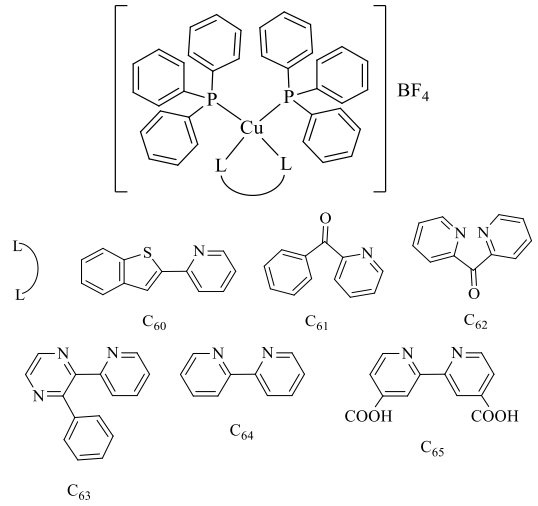


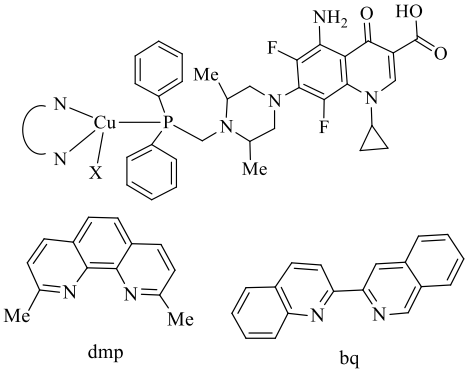
Fig. 12. Copper(I) complexes of triphenyl phosphane and N,N-, N,O- and N,S- bidentate ligands (Morais *et al.,* 2018)

Copper(I) complexes of biquinoline/substituted phenanthroline and sparfloxacin derivative

The *in vitro* cytotoxic study of copper(I) complexes of a 3rd generation fluoroquinolone antibiotic agent (phosphine derivative of sparfloxacin) and 2,2'-biquinoline (bq) or 2,9-dimethyl-1,10-phenanthroline (dmp) (C_{66}-C_{69}) (Fig. 13) against CT26 (mouse colon carcinoma) and A549 (human lung adenocarcinoma) exhibited higher anticancer activity (IC_{50} 7.51-33.79 μM) than that of their corresponding ligands (IC_{50} 104.08-264.28 μM) *via* reactive oxygen species (ROS) generation, plasmid DNA damage and the induction of cell apoptosis. The order of anticancer activity was found to be-C_{67}>C_{66}>C_{68}>C_{69} (Komarnicka *et al.,* 2016b).

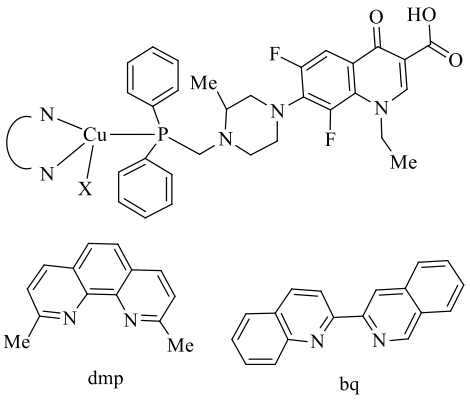
Copper(I) complexes of biquinoline/substituted phenanthroline and lomefloxacin derivative

The *in vitro* cytotoxic study of copper(I) complexes of a 2nd generation fluoroquinolone antibiotic agent (phosphine derivative of lomefloxacin) and 2,2'-biquinoline (bq) or 2,9-dimethyl-1,10-phenanthroline (dmp) (C_{70}-C_{73}) (Fig. 14) against CT26 (mouse colon carcinoma), A549 (human lung adenocarcinoma) and MCF-7 (breast cancer) exhibited higher anticancer activity (IC_{50} 5.8-38.0 μM) than that of their corresponding ligands (IC_{50} 176.0-326.0 μM) and cisplatin (IC_{50} 51.0-242.0 μM) *via* single-stranded cleavage of the sugar-phosphate backbone of plasmid DNA but the complexes C_{72} and C_{73} also intercalate in DNA. The complexes showed the interaction with human serum albumin (HAS) without any change in its secondary structure. The cleavage in single- and/or double-strain plasmid was observed after the addition of H_2O_2. The order of anticancer activity was found to be- C_{70}>C_{71}>C_{72}>C_{73} in CT26 and A549 cells but C_{71}>C_{70}>C_{73}>C_{72} in MCF-7 cells (Komarnicka *et al.,* 2016a).



C_{66} (X=I, NN=dmp), C_{67} (X=NCS, NN=dmp), C_{68} (X=I, NN=bq), C_{69} (X=NCS, NN=bq)

Fig. 13. Copper(I) complexes of phosphine derivative of sparfloxacin and 2,2'-biquinoline or 2,9-dimethyl-1,10-phenanthroline (Komarnicka *et al.,* 2016b)



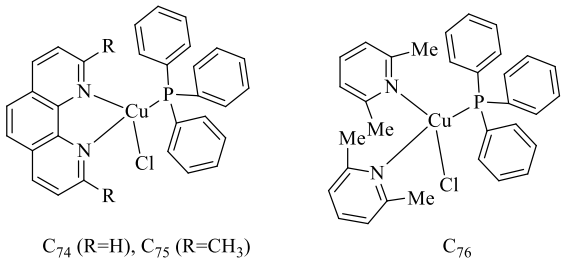
C_{70} (X=I, NN=dmp), C_{71} (X=NCS, NN=dmp), C_{72} (X=I, NN=bq), C_{73} (X=NCS, NN=bq)

Fig. 14. Copper(I) complexes of phosphine derivative of lomefloxacin and 2,2'-biquinoline or 2,9-dimethyl-1,10-phenanthroline (Komarnicka *et al.,* 2016a)

Phosphane and bidentate N, N- or monodentate N-heteroaromatic copper(I) complexes

The study of *in vitro* cytotoxicity of copper(I) complexes of triphenylphosphine (PPh_3) and phenanthroline (phen) (C_{74}) neocuproine (neo) (C_{75}) or dimethylbipyridine (dmbpy) (C_{76}) (Fig. 15) toward MDA MB 231 (human metastatic breast adenocarcinoma), A459 (human lung epithelial carcinoma) and HeLa (human cervical adenocarcinoma) cell lines exhibited much higher anticancer activity (IC_{50} 1.3-8.4 μM) than that of the standard drug cisplatin (IC_{50} 30-50 μM). The molecule having a higher extent of lipophilicity was observed as a

strong anti-cancer agent. The anticancer activity was observed in the order of; C_{75}>C_{74}>C_{76} (Alvarez *et al.*, 2017).

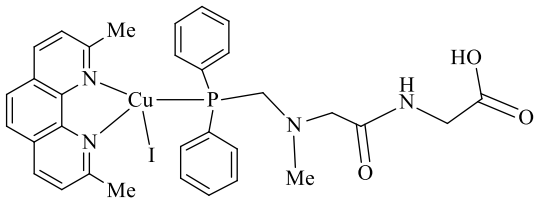


C_{74} (R=H), C_{75} (R=CH_3) C_{76}

Fig. 15. Copper(I) complexes of triphenylphosphine and phenanthroline, neocuproine, or dimethylbipyridine (Alvarez *et al.*, 2017)

Copper(I) complex of substituted phenanthroline and sarcosine-glycine derivative

The *in vitro* cytotoxic study of copper(I) complex of phosphine-peptide conjugate (PPh_2CH_2Sar-Gly-OH) derived from sarcosine-glycine (SarGly) and 2,9-dimethyl-1,10-phenanthroline (dmp), [CuI(dmp)($P(Ph)_2CH_2$-Sar-Gly-OH)] (C_{77}) (Fig. 16) toward CT26 (mouse colon carcinoma), A549(human lung adenocarcinoma) and MCF-7 (human breast adenocarcinoma) exhibited higher anticancer activity (IC_{50} 0.98-3.12μM) than that of its corresponding ligand (IC_{50} >100 μM) and standard drug cisplatin (IC_{50} 50.9 to > 100 μM) *via* a high level of ROS generation that resulted to oxidative damages of the sugar-phosphate backbone of plasmid DNA and *via* apoptotic cell deaths in MCF-7 cells with the simultaneous decrease of mitochondrial membrane potential and increase of caspase-9 and -3 activities.



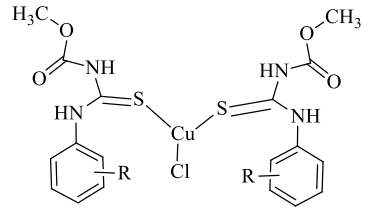
C_{77}

Fig. 16. Copper(I) complex of phosphine-peptide conjugate derived from sarcosine-glycine and 2,9-dimethyl-1,10-phenanthroline (Komarnicka *et al.*, 2018)

The IC_{50} value of the complex against normal cell line MRC5 (primary line of human pulmonary fibroblasts) was found to be 78.56 μM and that of cisplatin was 31.48 μM. The accumulation of the complex C_{77} in cells increased with increasing time such as 96 % inside MCF-7 and only 20% in MRC5 cell indicating that the complex C_{77} experienced higher anticancer potency and less toxicity in comparison to the standard drug (Komarnicka *et al.*, 2018).

Copper(I) complexes of phenyl-N'(methoxycarbonyl)thiocarbamides derivative

The *in vitro* cytotoxic study of copper(I) complexes of N-(2-chloro-4-nitro/2/4 methoxy)phenyl-N'(methoxycarbonyl)thiocarbamides (C_{78}-C_{81}) (Fig. 17) toward human ovarian cancer; IGROV-1, A2780/CP, A2780), cervical cancer; 2008, C13* and human normal hepatic cell; WRL-68 showed that the coordination of ligands to the metal ion substantially increased (2 -3 folds) the growth inhibition toward cancer cell but decreased the effect toward normal cell through the DNA damage. Thus the complexes were found to be less toxic toward normal cells (Pandey *et al.*, 2019a). The redox behavior of Cu(I) ion in the complexes caused to produce a large quantity of highly reactive ·OH (hydroxyl) radical that is responsible for the damages of DNA, lipid, or protein thereby showing a higher anticancer potency of complexes than their respective ligands (Mosmann, 1983, Lopes *et al.*, 1997, Lee & Steinert, 2003, Stepanenko & Dmitrenko, 2015, Pandey *et al.*, 2019ba).

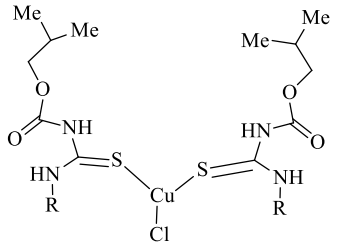


C_{78} (R=2-OMe), C_{79} (R=4-OMe),
C_{80} (R=2-Cl, 4-NO_2), C_{81} (R=2-OMe, 4-NO_2)

Fig. 17. Copper(I) complex of N-(2-chloro-4-nitro/2/4 methoxy)phenyl-N' (methoxycarbonyl) thiocarbamides (Pandey *et al.*, 2019)

N, N′-disubstituted isobutoxycarbonyl thiocarbamide copper(I) complexes

The results of *in vitro* cytotoxic assay of copper(I) complexes of N, N′-disubstituted isobutoxycarbonyl thiocarbamide (C_{82}-C_{85}) (Fig. 18) against cancer cells; IGROV-1, A2780, A2780/CP, C13* (cervical), 2008 (cervical) and normal hepatic cell; WRL-68 showed that all the complexes with redox behavior have better anticancer potency (9-10 folds) than their corresponding ligands may be due to having a significant DNA cleavage activity. The complexes performed well on cervical cancer, C13* compared to cisplatin. All the complexes induced cell apoptosis by blocking the progression of cell cycles of 2008 (cervical cancer), C13*(cervical cancer), and IGROV-1 cancer cells in G_0/G_1 phase. The presence of a highly electronegative atom or group of atoms near the metallic center helped to exhibit good anticancer potency by complexes (Pandey *et al.*, 2019b).

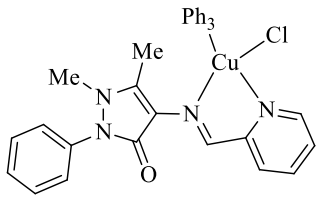


C_{82} (R=2,4-di-ClC_6H_3), C_{83} (R=2-Cl-4-$NO_2C_6H_3$), C_{84} (R=2-$OCH_3C_6H_4$), C_{85} (R=4-Cl-2-$NO_2C_6H_3$)

Fig. 18. Copper(I) complexes of N, N′-disubstituted isobutoxycarbonyl thiocarbamide (Pandey *et al.,* 2019b)

Copper(I) complex of Phosphane and pyridine carboxaldehyde derivative

The study of *in vitro* cytotoxicity of copper(I) complex of 5-dimethyl-2-phenyl-4-[(pyridin-2- ylmethylene)-amino]-1,2-dihydropyrazol-3-one (C_{86}) (Fig. 19) against Hep2 (laryngeal epithelial), HeLa (cervical) and MCF-7 (breast) cancer cell lines assessed tremendous cell inhibitory activity (IC_{50} 3.04-19.25 μM) compared to its ligand (IC_{50} >100 μM) and cisplatin (IC_{50} 12.52-13.84 μM) *via* plasmid DNA (pBR322) cleavage by hydrolytic pathway where binding with CT-DNA occurs through intercalative mode (Sathiyaraj *et al.,* 2013).

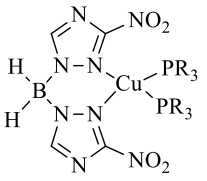


C_{86}

Fig. 19. Copper(I) complex of 5-dimethyl-2-phenyl-4-[(pyridin-2- ylmethylene)-amino]-1,2-dihydropyrazol-3-one (Sathiyaraj *et al.,* 2013)

Copper(I) complexes of phosphane and triazolyl derivative

The *in vitro* cytotoxic study of copper(I) complexes of phosphane and dihydridobis(3-nitro1,2,4-triazolyl)borate (C_{87}–C_{89}) (Fig. 20) against HL60 (promyelocytic leukemia), A549 (lung cancer), A375 (melanoma), A431 (cervix carcinoma) and 2008 (ovarian cancer) reported higher cell inhibitory activity (especially in case of A549) than that of their corresponding ligands and cisplatin. In the case of A549 the IC_{50} of the complexes (IC_{50} 1.52-6.88 μM) has been reported appreciably higher than that of their ligands (IC_{50} 68.12-71.6 μM) and standard drug cisplatin (IC_{50} 39.27 μM) through oxidative phosphorylation uncoupling by mitochondrial membrane potential dissipation (Marzano *et al.,* 2006).



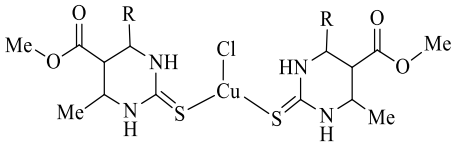
C_{87} - C_{89}

C_{87} (PR_3 = P(m-tolyl)$_3$, C_{88} (PR_3 = $P(C_6H_5)_2$ (p-C_6H_4COOH), C_{89} (P(p-$C_6H_4F)_3$

Fig. 20. Copper(I) complexes of phosphane and dihydridobis(3-nitro1,2,4-triazolyl)borate (Marzano *et al.,* 2006)

Copper(I) complexes of dihydropyrimidine derivative with phenyl or naphthalenyl moiety

The *in vitro* cytotoxic study of copper(I) complexes of methyl 4-aryl (phenyl, 1-napthalenyl, 2-bromophenyl or 2-chlorophenyl) -6-methyl-3,4-dihydropyrimidine-2(1H)thione-5-carboxylate (C_{90}-C_{93}) (Fig. 21) against breast cancers; HCC1806 and MCF-7 showed that the complexes have highly aggressive anticancer potency toward HCC1806 (IC_{50} 5.30-7.96 μM) compared to their corresponding ligands (IC_{50} 25.07->100 μM) with the order of C_{92}>C_{90}>C_{91}>C_{93} and MCF-7 (IC_{50} 19.62-25.96 μM; complexes, IC_{50} 80.72->100 μM; ligands) with the order of; C_{90}>C_{92}>C_{91}>C_{93} may be due to different activities of the same substituent on different cell lines. These complexes also exhibited a higher cell inhibitory property than the standard drugs; doxorubicin and docetaxel (Gonzalez-Ballesteros *et al.,* 2015).



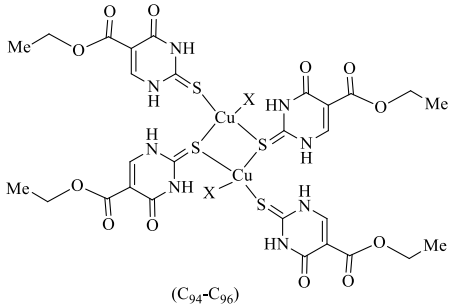
C_{90} (R = C_6H_5), C_{91} (R = $C_{10}H_7$), C_{92} (R = 2-BrC_6H_4), C_{93} (R = 2-ClC_6H_4)

Fig. 21. Copper(I) complexes of methyl 4-aryl (phenyl, 1-napthalenyl, 2-bromophenyl or 2-chlorophenyl) -6-methyl-3,4-dihydropyrimidine-2(1H)thione-5-carboxylate (Gonzalez-Ballesteros *et al.,* 2015)

5-Carbethoxy-2-thiouracil copper(I) complexes

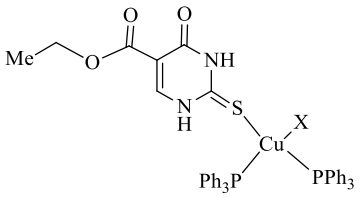
The *in vitro* cytotoxic study of copper(I) complexes of 5-carbethoxy-2-thiouracil with or without diphosphane (C_{94}–C_{99}) (Fig. 22) against cancer cells; A549 (pulmonary), HeLa (epithelial), and non-cancer cell; MRC5 (fetal lung fibroblast) reported that the copper(I) complexes with highly toxic triphenylphosphine counterparts (C_{97}–C_{99}) have substantial anticancer potency (IC_{50} 2.55-5.55 μM) may be due to the stability of complexes by phosphane group. The complexes (C_{94}–C_{96}) also reported a higher action of growth inhibition (IC_{50}

41.3-110 μM) than that of free phosphane ligand (IC_{50} 105-118 μM). The anticancer potency of these drugs may be performed by DNA cleavage and excess ROS production (Papazoglou *et al.*, 2014).



(C_{94}-C_{96})

C_{94} (X = Cl), C_{95} (X = Br), C_{96} (X = I)



(Ph = C_6H_5)

(C_{97}-C_{99})

C_{97} (X = Cl), C_{98} (X = Br), C_{99} (X = I)

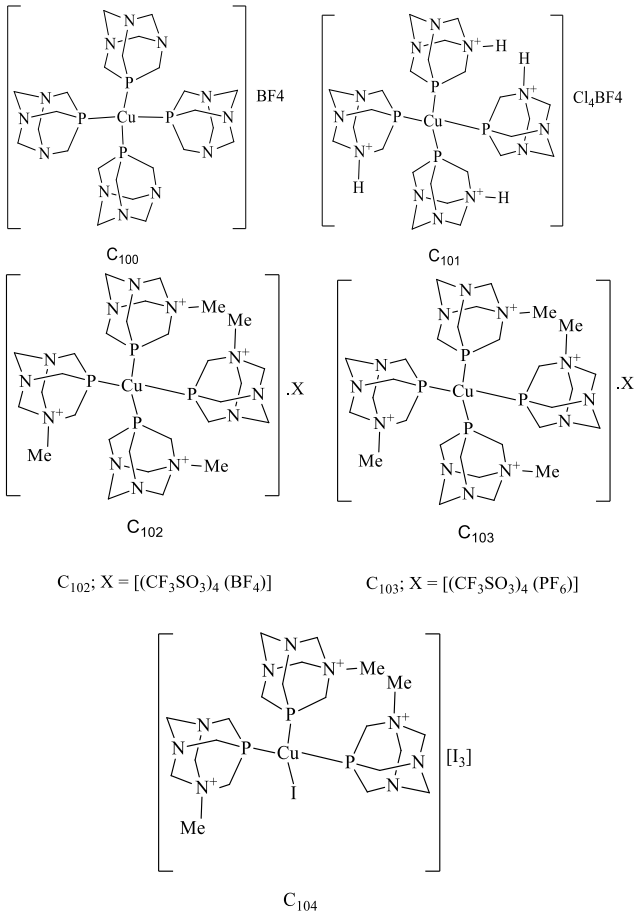
Fig. 22. Copper(I) complexes of 5-carbethoxy-2-thiouracil with or without diphosphane (Papazoglou *et al.*, 2014)

N-methyl triaza phosphaadamantane copper(I) complexes

The *in vitro* cytotoxic test of copper(I) complexes of N-methyl-1,3,5-triaza-7-phosphaadamantane (mPTA) phosphine (C_{100}–C_{104}) (Fig. 23) against MCF-7 (breast), HeLa (cervix), A549 (pulmonary), HCT-15 (colon) and A375 (melanoma) cancers showed significant anticancer potency (IC_{50} 7.58-29.3 μM) comparable to cisplatin. These complexes also showed remarkably lower resistance than cisplatin when tested against ovarian cancer 2008/C13* or 2008/C13*-Pt (resistance factor; < 7 fold) and A431/A431-Pt cancer (resistance factor; < 2.5 fold) indicating the different mechanism of action from the cisplatin (Porchia *et al.*, 2009).

Copper(I) complexes of N, N'-disubstituted thioamides

The *in vitro* cytotoxic results of copper(I) complexes of N-aryl, N'-isobutoxy/methoxycarbonyl thiocarbamide (C_{106}–C_{108}) (Fig. 24) against A2780, A2780/CP, IGROV-1, 2008 (cervical), and C13* (cervical) cancer cell lines showed the higher anticancer potency than that of their coordinating ligands through the mechanism of DNA damage (Singh *et al.*, 2015, Pandey *et al.*, 2018). The copper complexes tend to break the strand of induced DNA *via* highly reactive hydroxyl radical (·OH) and superoxide anion (O_2^-) (Mosmann, 1983, Lopes *et al.*, 1997, Lee & Steinert, 2003, Stepanenko & Dmitrenko, 2015). The presence of highly electronegative atoms or group of atoms on organic moiety has been found to cleave DNA more effectively (Shao *et al.*, 2014, Stepanenko & Dmitrenko, 2015) so the complexes C_{105} and C_{106} showed significant anticancer effect toward IGROV-1, 2008 (cervical) and C13* (cervical) cell lines.

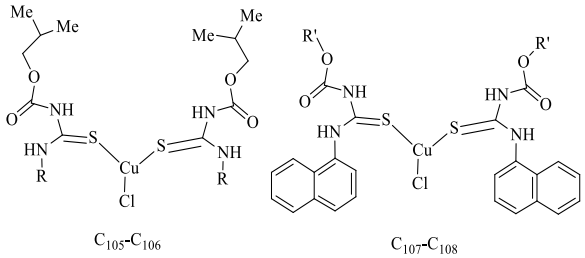


C_{100} C_{101} C_{102} C_{103}

C_{102}; X = [$(CF_3SO_3)_4$ (BF_4)] C_{103}; X = [$(CF_3SO_3)_4$ (PF_6)]

C_{104}

Fig. 23. Copper(I) complexes of N, N'-disubstituted thioamides (Porchia *et al.*, 2014)



C_{105}-C_{106} C_{107}-C_{108}

C_{105} (R=4-$OCH_3C_6H_4$), C_{106} (R=2-OCH_3-4-$NO_2C_6H_3$),
C_{107} (R'=-$CH_2CH(CH_3)_2$, C_{108} (R'=-CH_3)

Fig. 24. Copper(I) complexes of N-aryl, N'-isobutoxy/methoxycarbonyl thiocarbamide (Singh *et al.*, 2015, Pandey *et al.*, 2018)

A unique copper(I/II) complex of hydroxyl naphthalene derivative

In vitro cytotoxic study of a unique complex, 3-hydroxy naphthalene-2-carboxylic acid thiophene-2-ylmethylene hydrazide copper(I/II) (C_{109}) (Fig. 25) against A549 (lung) and MCF-7(breast) cancer cell lines exhibited almost double antiproliferation activity (IC_{50} 12-15 μM/ml) than its ligand (IC_{50} 25-32 μM/ml) and slightly higher than the standard cisplatin (IC_{50} 15-17 μM/ml) *via* DNA binding with intercalative mode (Anu *et al.*, 2019).

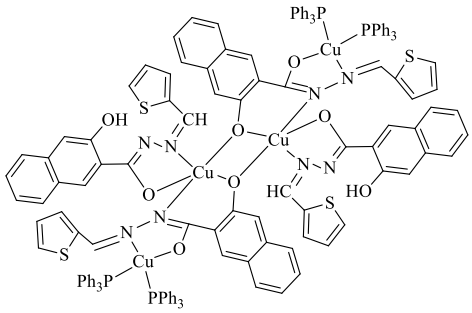


Fig. 25. Copper(I/II) complex of 3-hydroxy naphthalene-2-carboxylic acid thiophen-2-ylmethylene hydrazide (Anu *et al.*, 2019)

CONCLUSION

Copper(I) complexes have been found to dissociate partially by generating an empty site on Cu^+ ions that can facilitate the complexes to interact with the biological target. Copper(I) ion, being stabilized by phosphane and N,N-diimine systems form many complexes with higher anticancer potency, lesser toxicity, and lower drug resistance activity toward various cancer cell lines than that of their corresponding copper(II) complexes as well as standard cisplatin drug. The designation of copper(I) molecules with the modification in N,N-diimine systems by the organic moiety with anticancer potency may bring revolution in the synthesis of anticancer drugs with significant inhibitory activity.

ACKNOWLEDGEMENT

The authors sincerely acknowledge the Nepal Academy of Science and Technology (NAST), Nepal for providing Ph. D. fellowship (2015/2016 AD) to NKS.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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