

ANTICANCER ACTIVITY OF HETEROCYCLIC LIGAND-PLATINUM METAL COMPLEXES

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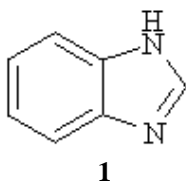
ABSTRACT:

A wide range of research activities have been carried out so far on benzimidazole and pyridine derivatives to evaluate their *Antiviral, Anticancer, Anticonvulsant, Anthelmintic Activity* and many promising results are obtained with respect to the anticancer activity. Out of the various ligands used, the alkylating agents are found to be highly active with respect to cancer chemotherapy. Nitrogen mustards, ethylenemines, alkylsulfonates, nitrosourea, and triazines are all members of the alkylating agents. The presence of active nitrogen atom in a particular structure enhances the binding efficacy of number of metals and hence forms metal complexes. The overall aim of the review is to study cytotoxicity of various platinate and other metal complexes which are effectively formed by platinum and other metals respectively by obtaining the lone pair of electrons from nitrogen. Also in support of the anticancer activity, the derivatives obtained by metal complexation with number of nitrogen containing heterocyclic ligands have been shown.

Keyword: Benzimidazole, Pyridine, Platinum Complexes, Anticancer Activity, Cytotoxic Activity

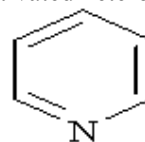
1. Introduction¹⁻⁶

The Benzimidazole (1) heterocycle is represented in nature as an integral part of the structure of vitamin B₁₂ and has been incorporated into pharmaceutical agents to form enzyme inhibitors, and DNA intercalators. Benzimidazoles are very useful intermediates subunits for the development of molecules of pharmaceutical or biological interest. The substituted benzimidazole derivatives have found applications in diverse therapeutic areas including antiulcer, antihypertensive, antifungal, anticancer, and antihistaminic. Some benzimidazole derivatives show diverse biological activities with significant clinical potential, including the treatment of leukemia and cancer.



The pyridine (2) substructure is one of the most prevalent heterocycle found in natural products, pharmaceuticals, and functional materials. Many powerful methodologies for the synthesis of these heterocycles rely on condensation of amines and carbonyl compounds or cycloaddition reactions. Cross-coupling

chemistry also allows introduction of substituents to activated heterocycles.



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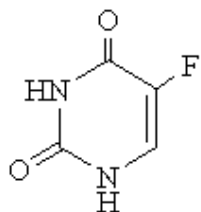
2. Cancer chemotherapy^{7, 8, 9, 10}

The initial signs of cancer were detected in the bones of ancient Egyptian and Peru mummies, who lived in the 3000 BC. The high mortality rate associated with cancer worldwide is the reason for the ongoing research for the cure for this disease. Four methods of treatment have been discovered; these are: 1) surgery 2) radiotherapy 3) hormonal therapy and 4) chemotherapy. Surgery has been the most popular form of treatment, but over the last five years there has been an increase in the use of chemotherapy. Surgery involves careful removal of the malignant cells from the patient's body and have been reported very effective for removal of small tumors. Surgery treatment has also shown effectiveness when used in combination with radiotherapy or chemotherapy. Radiotherapy, which involves destroying cellular components of tumor cells by high energy waves, has also been reported highly effective for cancer treatment though it is limited

to treating small tumors. Another form of cancer treatment is hormonal therapy which involves starving tumor cells of hormones, subsequently inhibiting cell growth of small tumor cells compared to large ones. Although cancer can be treated by one or combination of the above methods, chemotherapy is still the most effective line of treatment.

The use of chemicals to kill malignant cells dates back to 1946. Nitrogen mustards were the first chemotherapeutic agents discovered by Louis Goodman. Research conducted on the mode of action of these drugs, lead to their division into eight main categories. These are alkylating agent, antimetabolites, antitumor antibiotics, topoisomerase inhibitors, spindle inhibitors, miscellaneous agents, biological response modifiers and hormones.

Notably, the group names of these chemotherapeutic agents are derived from their mode of action. For example, the antimetabolite 5-fluorouracil (**3**) inhibits DNA synthesis by mimicking the structure of uracil, a pyrimidine base required for DNA replication.

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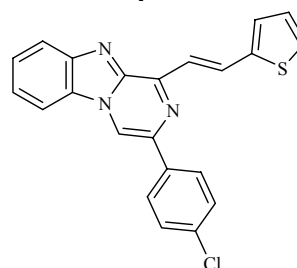
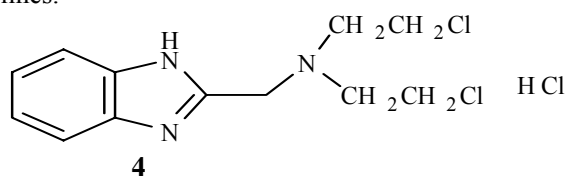
3. Alkylating Agents^{10, 11, 12}

Alkylating agents are the oldest group of chemotherapeutic drugs. Nitrogen mustards, ethyleneamines, alkylsulfonates, nitrosourea, and triazines are all members of alkylating agents. For the purposes of this study, we will focus on nitrogen mustards. Nitrogen mustards and other alkylating agents damage the DNA by attacking oxygen or nitrogen atom of the nucleobases and phosphodiester bonds between bases. However, limitation such as, toxicity and low drug efficacy lowers the use of nitrogen mustard. Many studies have been conducted till now, to develop nitrogen mustard derivatives showing high efficacy and low toxicity. Derivatives were synthesized by changing the alkylating portion or the carrier of the alkylating portion of the mustard.

Inspired by the structural similarity between benzimidazole nucleus and the purine bases of the DNA, Hirschberg, et al. studied the use of

benzimidazole as carriers for the alkylating portion in the synthesis of nitrogen mustard derivatives. The hypothesis behind this study was that the cellular uptake of these new compounds will increase as a result of the benzimidazole nucleus. Benzimidazole mustard, [2-(di-(2-chloroethyl) aminomethyl) benzimidazole hydrochloride (**4**) was synthesized. This compound was found to inhibit mammary Adenocarcinomas 755, E 0771, and Sarcoma 180 when tested against various mouse tumors.

Pyrazino[1, 2-a] benzimidazole derivative (**5**) had shown anti-cancer activity when in vitro tests were done against 60 human tumor cell lines.

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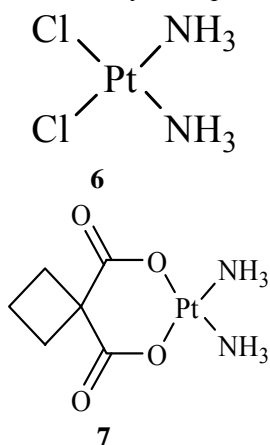
4. Platinum Complexes¹³⁻³⁵

Recently, the wide spectrum of biological activities associated with benzimidazole have been of wide interest. This has included activity against viruses such as HIV, human cytomegalovirus, herpes (HSV-1), RNA, and influenza. These compounds have also been reported to act as anti-inflammatory, anthelmintic, antiparasitic, topoisomerase inhibitors, selective neuropeptide YY1 receptor antagonist, 5-lipoxygenase inhibitors, and factor Xa inhibitors. These properties, coupled with the findings indicate that benzimidazole compounds show antitumor activity.

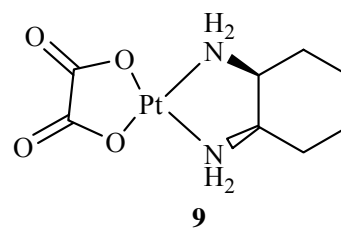
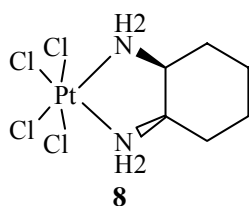
Although benzimidazole compounds have proven active against various cancers, there are recent reports on their combinatorial use with platinum and other metal to treat cancer. Platinum are chemotherapeutic drugs consisting of platinum (II) and platinum (IV) complex with various donor ligands. Cisplatin [cis-diammine dichloroplatinum (II)] (**6**) is one of the platinum complex that has gained worldwide popularity since 1970s. Cisplatin is reported active against testicular, ovarian, bladder, head and neck

cancers. Despite these antitumor activities, neurological disorders such as nephrotoxicity, myelosuppression, ototoxicity greatly limit the use of cisplatin. Moreover, it has been reported that various cancer cells become resistant to this drug, for instance, murine ADJ/PC6 plasmacytoma.

Thousand of analogues of cisplatin have been made and evaluated, with two major driving forces. The first has been to seek compounds with lower neurotoxicity than cisplatin. Whereas better clinical management has improved things, one of the main drivers of analogue development has been agents with less neurotoxicity. Carboplatin (**7**) has carboxylate instead of chloride-leaving groups. These hydrolyze much less rapidly, resulting in lower nephrotoxicity and neurotoxicity (the dose-limiting toxicity of carboplatin is myelosuppression), while retaining the broad spectrum of activity of cisplatin.

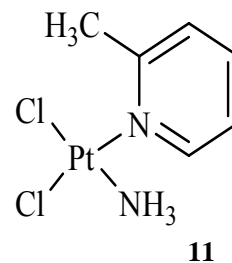
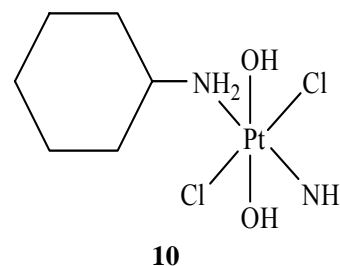


The second impetus to analogue development has been to seek agents active in cell lines that become resistant to cisplatin. One mechanism of resistance to cisplatin is an increased ability to repair the DNA adducts formed. However, analogues such as tetraplatin (**8**; Ormaplatin) and Oxaliplatin (**9**), with trans-1, 2-diaminocyclohexane (DACH) ligand, were shown to be more effective against such resistant cell lines. These compounds proved to be neurotoxic but Tetraplatin was difficult to formulate, but Oxaliplatin have shown promising results.



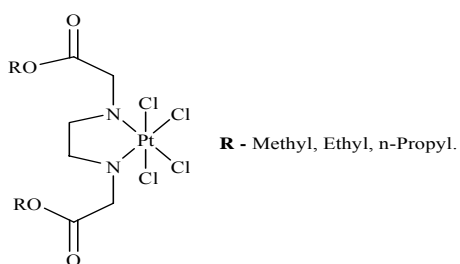
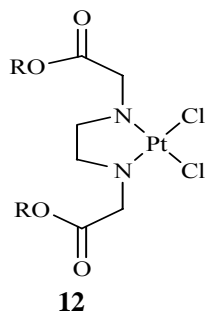
For more than three decades, many research groups have focused on developing cisplatin analogues with less toxicity, improved activity and active on cisplatin resistant tumors.

Cisplatin analogues, such as, JM 335 (trans-ammine (cyclohexylaminedichlorodihydroxy) platinum (IV) (**10**) and ZD0473 (cis-amminedichloro(2-methylpyridine) platinum (II) (**11**) were synthesized. JM 335 tested positive for antitumor activity against human ovarian carcinoma xenografts, cisplatin-resistant murine ADJ/PC6 plasmacytoma and L 1210 leukaemia models.

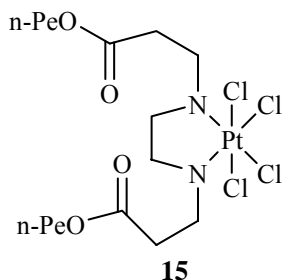
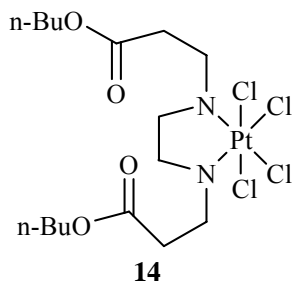


However, testing with the ethylenediamine-N, N'-diacetato ester type complexes of platinum(II) (**12**) and platinum(IV) (**13**) halide shown that the most efficient complexes against human adenocarcinoma HeLa cells (ca. five times less active than cisplatin) and human myelogenous leukemia K562 cells (comparable with cisplatin) were the tetrachloroplatinum(IV) complexes acting through apoptotic cell death. It could be seen that the analyzed agents shown a dose-dependent antiproliferative effect toward 1411HP, H12.1 and DLD-1 cell lines. The IC₅₀ values of cisplatin are also included for comparison. The IC₅₀ values demonstrated that the replacement of the methyl group in

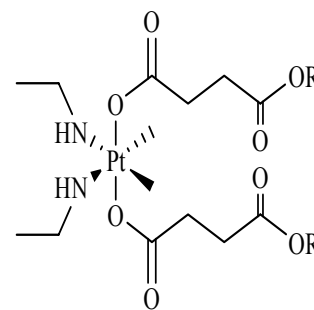
substance (**13**) with ethyl or n-propyl increased the cytotoxic activity of the Pt (IV) complexes against both cisplatin-resistant 1411HP and cisplatin-sensitive H12.1, but decreased their activity in the cisplatin-resistant colon carcinoma cell line DLD-1.



In vitro antitumor activity by two platinum (IV) complexes with bidentate dibutyl and dipentyl esters of ethylenediamine-N, N'-di-3-propionate, [PtCl₄ (Bu₂eddp)] (**14**) and [PtCl₄ (Pe₂eddp)] (**15**) respectively, was demonstrated on L929 fibrosarcoma and U251 astrocytoma tumor cells. The kinetics of the tumor cell death process induced by these complexes was considerably faster in comparison to that induced by the classical platinum (II) - based drug cisplatin.



Four novel bis(carboxylate)platinum(IV) complexes were investigated for their in vitro cytotoxicity in four human tumor cell lines, originating from ovarian carcinoma (CH1), colon carcinoma (SW480) and non-small cell lung cancer (A549) with the help of the colorimetric microculture MTT assay. Remarkably, IC₅₀ values down to the nanomolar range, up to 32 times lower compared to cisplatin, were found. The Platinum complex (**16**) is as cytotoxic as cisplatin in the cisplatin-sensitive CH1 cell line; complexes (**17-19**) are 3 to 17 times more cytotoxic than cisplatin in the same cell line.



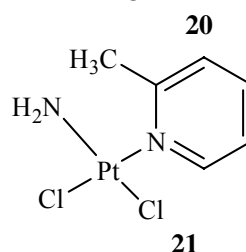
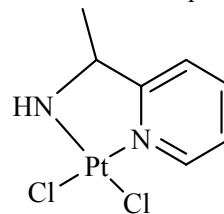
16 R = CH₃

17 R = CH₂CH₃

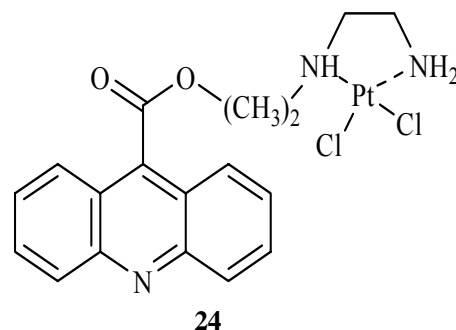
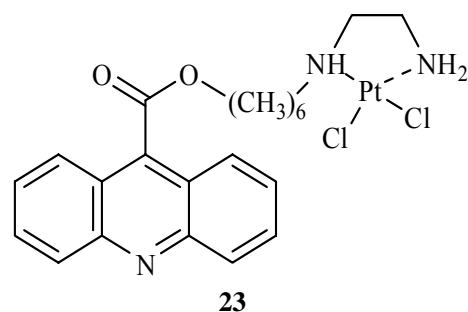
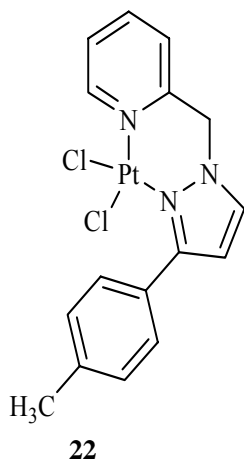
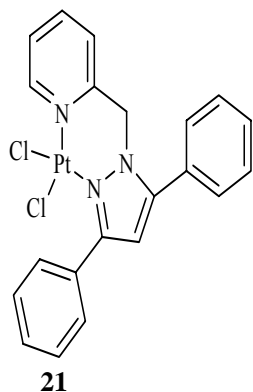
18 R = CH₂CH₂CH₃

19 R = CH₂CH₂(CH₃)₂

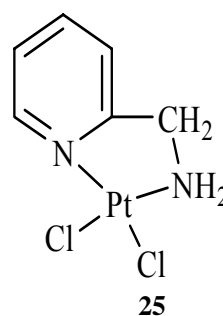
Over the years considerable amount of interest has focused on the use of pyridine platinum (II) complexes as mimics of cisplatin. These studies have shown that the use of planar ligands, such as the substituted pyridines (**20**, **21**) in platinum (II) complexes, can reduce the rate of deactivation by sulfhydryl groups without interfering with DNA binding, considered to be the mode of action of cisplatin.



The platinum complexes bearing alkyl substituents on the pyrazolyl ring were less active than those with aryl substituents. Although in terms of IC_{50} values, activity of **(22)** ($IC_{50} = 3.849$ IM) and **(23)** ($IC_{50} = 8.920$ IM) were approximately eight and nineteen times lower than that of cisplatin.

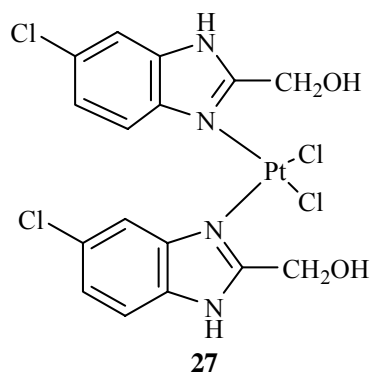
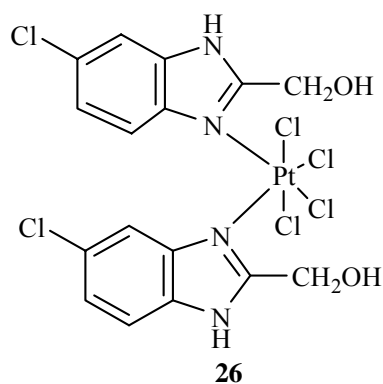


It has been found that compounds with the following structure **(25)** have potent antitumor activity against sarcoma-180 cells.



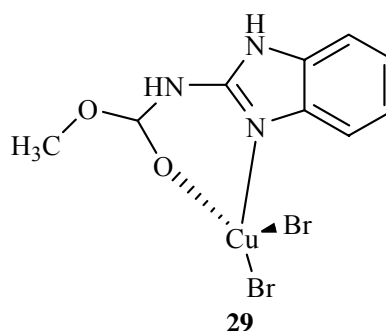
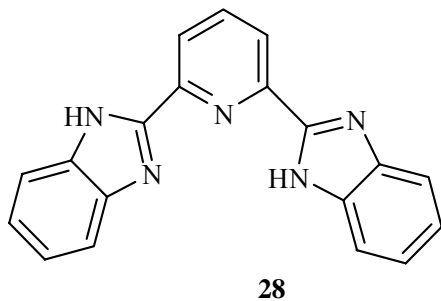
Interestingly, the replacement of amide by ester functionalities in the series of novel acridine-9-carboxylate tethered (ethane-1,2-diamine) platinate complexes and connected by a polymethylene chain increased their cytotoxic effect which was higher than cisplatin or oxaliplatin in the three colic HCT 116, SW480 and HT-29 cell lines. The influence of the polymethylene linker in platinum complexes was dependent on the cell line: in HCT 116 and SW480, **(23)** having the hexamethylene chain, remained the most cytotoxic, whilst in HT-29, the most sensitive cell line to reference Pt compounds, **(24)** with the dimethylene linker was the most active. A relationship between cytotoxicity and polymethylene chain length in platinum compounds was also reported by Silva *et al.*

It is generally thought that since platinum (IV) complexes are inert on ligand substitution reactions relative to their platinum (II) analogues they must be reduced to platinum (II) species before binding to DNA. The reduction potentials of diamine platinum (IV) complexes are dependent on the nature of the axial and equatorial ligands, but the axial ligands generally exert the stronger influence. On the other hand, Choi *et al* studied a range of complexes and suggested that the rate of reduction of platinum (IV) complexes depended on the bulkiness of the equatorial ligands. Although more factors are involved for the cytotoxicity of platinum (IV) complexes, including membrane transport, absorption, reduction, and possibly direct interaction with DNA, the steric hindrance of the “non-leaving ligands” of the platinum (IV) complex **(26)** may be related to their lower cytotoxicity than the corresponding platinum (II) complex **(27)**.



4.1. Other metal complexes³⁶⁻⁵²

In last decade, various Benzimidazole substitutions have been complexed with copper. These complexes show prominent cytotoxic, apoptosis activity. 2, 6- bis(benzimidazol-2-yl)pyridine(**12**) have DNA cleaving properties. There are also many examples in the literature of copper complexes of ligands containing a diimino (N=C-C=N) moiety such as Phenantroline that can induce apoptosis. It has been reported that methyl 2-benzimidazolecarbamate complex with copper (II) bromide [Cu (2cmbz) Br₂] (**13**) which shown the activity against MCF-7 cell line [38].



The transition metal complexes of 2-substituted benzimidazole ligands act as anticancer agents. Cancer cells are found to have less superoxide dismutase activity than normal cells and copper(II) complexes are known to mimic activity of copper, zinc-superoxide dismutase (Cu, Zn-SOD), an antioxidant enzyme that protect cells from the toxic effect of superoxide ion by its dismutation into dioxygen and hydrogen peroxide in biological systems. The antitumor activity of SOD metal complexes has been suggested to be due to their superoxide scavenging ability. It's Zn (II), Be (II), and Al (III) derivatives are photoluminescent. Hydroxyl benzimidazole shows excited-state intramolecular transfer (ESIPT) properties due to acidic protons of phenol and imidazole nitrogen. It has been reported that hydroxyl benzimidazole and benzoxazole behave as a structural mimic of DNA base pair for which tautomerism may be initiated at a definite time and position within duplex DNA. Structurally similar natural product bis (benzimidazole) UK-1 has been reported to posses anticancer activity, and the metal-binding studies of UK-1 indicates that benzoxazole-like compound are capable of binding a variety of biologically important metal ions. Benzimidazole derivatives have exhibited significant activity against several viruses such as HIV, human cytomegalovirus (HCMV), herpes (HSV-1) and influenza.

Conclusion

Since past few decades, there has been an increase in the scope of cancer therapy, especially the alkylating agents. Many attempts have been made by the researchers to synthesize and use platinum complexes as alkylating agents which are gaining wide importance in obtaining anticancer effect. Platinum containing complexes such as Cisplatin, Carboplatin, Oxyplatin etc are currently being marketed and are reported to be active against testicular, ovarian, bladder, head and neck cancers. However due to the neurotoxicity that are

reported against these drugs, there is a need to develop more and more analogues with less toxicity, improved activity and active on cisplatin resistant tumors. Also, with an aim for complexation of heterocyclic derivatives, the number of metals such as Pt (II), Zn (II), Be (II) and Al (III) has been used so far to form corresponding metal complexes.

Acknowledgement

The authors thank Dr. Prashant Naik and Dr. Anwar Shaikh, M.Pharm Incharge, M.E.T's Institute Of Pharmacy, Nashik for their excellent technical assistance and critical proof reading of our manuscript.

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