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Anticancer Mechanisms Pd-Phenothiazine Complexes- A Mini Review

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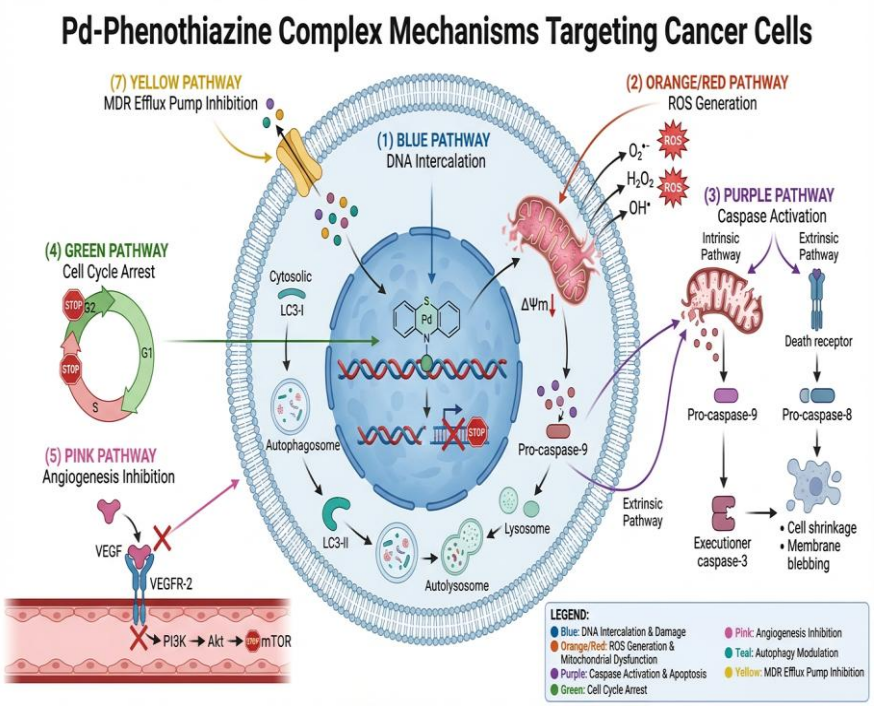
Abstract:
Cancer is a major killer in the world due to its aggressive biology and lack of treatment. There is a high need for alternative, more effective, and safe treatment options. Structurally similar to platinum agents, palladium (Pd) complexes also have the potential to offer improved pharmacological properties. The chemical combination of palladium with phenothiazine derivatives can result in multi-functional drugs. These drugs can exhibit potential anticancer, antioxidant, antibacterial, and antiprotozoal properties. This comprehensive review examines the synthesis, structural characteristics, and biological mechanisms of Pd-phenothiazine complexes, with emphasis on recent advances from 2020-2025. We discuss their anticancer mechanisms including cytotoxicity, apoptosis induction, DNA binding, cell cycle arrest, reactive oxygen species generation, angiogenesis inhibition, and autophagy modulation. The antimicrobial spectrum encompassing antibacterial, antifungal, and antiprotozoal activities is evaluated alongside antioxidant properties. Comparative analyses with platinum-based drugs reveal potential advantages in selectivity and reduced resistance.

Key words: Pd (II) complexes, anticancer activity, antimicrobial activity, phenothiazine derivatives.

Graphical abstract:



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I. Introduction:

When it comes to disease and death, cancer is one of the biggest issues that healthcare is currently facing (Bray et al. 2021). Every year, tens of millions of people receive a cancer diagnosis globally; over half of these patients pass away from this disease, and even with the entrance of many new drugs to the market, treatment response is still below the expected standard of equality (Ma & Yu, 2007). Cancer cells continue to exhibit novel behaviours, such as treatment resistance and dormancy, despite continuous medication. It is critical to understand their inherent and acquired resistance pathways for the development of next-generation targeted therapies (Khan et al., 2024). Identifying an effective anti-cancer pharmaceutical for the efficient treatment of human tumours, such as ovarian, breast, cervical, bladder and head/neck tumours, is the primary target of most researchers (Listro et al., 2022). Recently, different palladium (II) complexes have been synthesised which show promising activity against tumour cell lines (Czarnomysy et al. 2021). It was found that there was a positive correlation between the palladium complexes' cytostatic efficacy and their lipophilicity or solubility. In other words, some complexes of benzene-azo-anilino phosphonate ligands showed the highest activity, which was comparable to that of cisplatin. The acidity of the reaction medium can be utilised to differentiate between molecular and ionic chemicals. At pH 3.5, dihalide adducts with a trans-bonded ligand *via* the quinoline nitrogen were generated. At reduced acidity, a chelate complex was established through coordination involving the nitrogen of quinoline and the phosphonic acid group, while at elevated acidity, salt complexes were produced with the protonated quinolyl methyl phosphonate ligand acting as a cation and the quinolinium methyl phosphonate trihalide complex serving as its counter-ion (Tusek-Bozic et al. 1998).

1.1 Comparison with Platinum-Based Drugs:

In addition, palladium complexes have been revealed recently to have lower side effects as compared to that of cisplatin, which is one of the platinum-metal complexes with significant anti-tumour activity against cancer cells in most used chemotherapeutic clinics. Palladium complexes, as essential metal-based anti-cancer medicines, are anticipated to have less hepatorenal toxicity in comparison to cisplatin. As anticancer drugs, palladium and platinum complexes both show notable cytotoxicity, as shown in **Table 1**; nevertheless, their toxicity profiles, selectivity, and DNA binding mechanisms vary (Jaszczyszyn et al. 2012).

II. Experimental and Analysis:

2.1 Synthesis of palladium with agents:

Many metal-based anticancer drugs with increased activity and reduced toxicity are discovered by investigators. Palladium metal was used in previous research to create several ligands that produced strong complexes that might eventually turn out to be a possible drug. Thus, by thermally treating 200 mg of $PdCl^2$ with 168 mg of KCl and 40 mL of H_2O, the complex [Pd(Glycylglycine)Cl_2] was created. Subsequently, the $[PdCl_4]^{2-}$ solution was filtered, allowed to cool, and then mixed into the solution. A pH adjustment was made to 2 to 3. The precipitate of [Pd(Glycylglycine)Cl_2] was formed, filtered, and then washed thrice using ethanol, diethyl, and distilled water. Two equivalents of $AgNO_3$ were added to the precursor complex to transform it into the di-aqua complexes, and the white AgCl precipitate was then filtered. To ensure complete conversion of the complexes into the di-aqua species and its independence from Ag^+ ions, great care was taken during the synthesis process. Equilibria of complex formation using amides and peptides that create complexes with stoichiometric coefficients are depicted below (**Fig 1**) (Czarnomysy et al. 2021).

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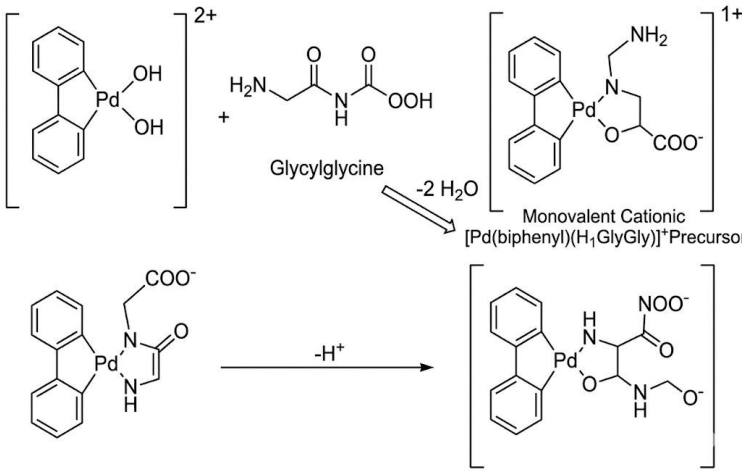

Figure 1: Synthetic pathway for the preparation of the palladium (II) complex. The reaction outlines the initial condensation of the di-hydroxy precursor [Pd(biphenyl)(OH)_2]$^{2+}$ with glycylglycine under the elimination of two water molecules to yield the monovalent cationic intermediate, [Pd(biphenyl)(H_1GlyGly)]$^{+}$. Subsequent deprotonation (-H) $^{+}$ leads to the formation of the thermodynamically stable, charge-neutral square-planar complex.

2.2 Palladium metal efficiency and their molecular structure effect:

Palladium's (Pd) efficiency in various applications, particularly catalysis and other chemical reactions, is significantly influenced by its molecular structure and the specific chemical environment. Numerous palladium complexes demonstrating potent antitumor efficacy both *in vivo* and *in vitro*, utilising nitrogen donor ligands including diverse alkyl-substituted amines and imine derivatives, have recently been synthesised for the exploration of anticancer agents. The existence of a planar and exceptionally stable aromatic metallacycle can induce intercalative cytotoxic effects of palladacycles. Cyclopalladated compounds with planar structures, including aliphatic as well as aromatic amines, have demonstrated cytotoxic effects on some cancer cells, resulting in intercalative damage to DNA. On the other hand, serum albumins exhibit exceptional binding properties, thereby serving a significant and effective function in drug delivery. Also, it has been shown that bovine serum albumin (BSA) has been used widely for protein binding studies because of its low cost and availability. The tertiary structure of BSA shares over 76% similarity with that of HSA in its overall configuration, so much research has used them with the interaction of some small molecules (Arsalan et al. 2020).

2.3 Coordination of Pd metal and Protein binding:

Protein binding is a process where proteins interact with and attach to other molecules, including other proteins, DNA, RNA, or small molecules. This interaction can be specific, like a protein binding to a particular DNA sequence, or non-specific, like a drug binding to albumin in the blood. The coordination of DNA with metal ions can be improved by altering the therapeutic and pharmacological efficacy and structural properties of substances (Arsalan et al. 2020). This may result in a substantial decrease in the activity of nucleic acids in physiological processes. The incorporation of glycine or 1,1-CBDCA enhances the ligand's affinity for DNA, hence modifying the coordinating characteristics of the 2,2'-bipyridine ligands to create a more adaptable ligand system. The amino group in glycine stabilised the DNA adduct with Pd (II) complexes by engaging with the negatively charged phosphate backbone, which is present at the peripheral side of the double helix of CT-DNA. The complexes [Pd(byp)(gly)] and [Pd(byp)CBDCA] have been synthesised and characterised. The bipyridine ring interacted aggressively with the sugar group of the linked DNA in addition to the cyclobutene ring. Interestingly, the computed binding constants for both complexes were higher than those of previously studied complexes, suggesting that both complexes have superior binding. The increased binding capacity of one complex may result from the coordinated opening of the CBDCA ring in [Pd(byp)(CBDCA)] and the formation of a highly stable adduct, maintained by

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stacking interactions between the DNA sugar moiety and the bipyridine ring, along with the coordinated CBDCA ring, similar to the mechanism observed with carboplatin capabilities (Shamsi & Kraatz, 2013).

2.4 Cytotoxicity of palladium metal among different human cancer cell lines:

In terms of their anticancer activity, the Pd (II) complexes that have been investigated up until this point have mostly been described in terms of their cytotoxic and anti-properties against various tumour cell lines (Carneiro et al. 2020).

The testing of a newly synthesised palladium (II) complex, (saccharinato-κN) (2,2′:6′,2″-terpyridine-κ³N, N′, N″) palladium (II)saccharinate tetrahydrate as shown in **(Fig 2)**, against human breast cancer cell lines MDA-MB-231 and MCF-7 was developed to determine its anti-cancer properties. Following treatment with varying complex concentrations (0.09–25 μM) for 24, 48, and 72 hours, the viability was evaluated using the MTT and ATP tests. The combination was demonstrated to have an antiproliferative impact that was dependent on both time and dose. Additionally, the previous combination used the DR4 and DR5 cell death genes to trigger apoptosis, which in turn led to cell death. To sum up, the recently created Pd (II) complex showed potent antiproliferative properties *in vitro* that changed depending on dosage and time, and this suggests that it could function as a promising new medication for the treatment of breast cancer (Ulukaya et al. 2011).

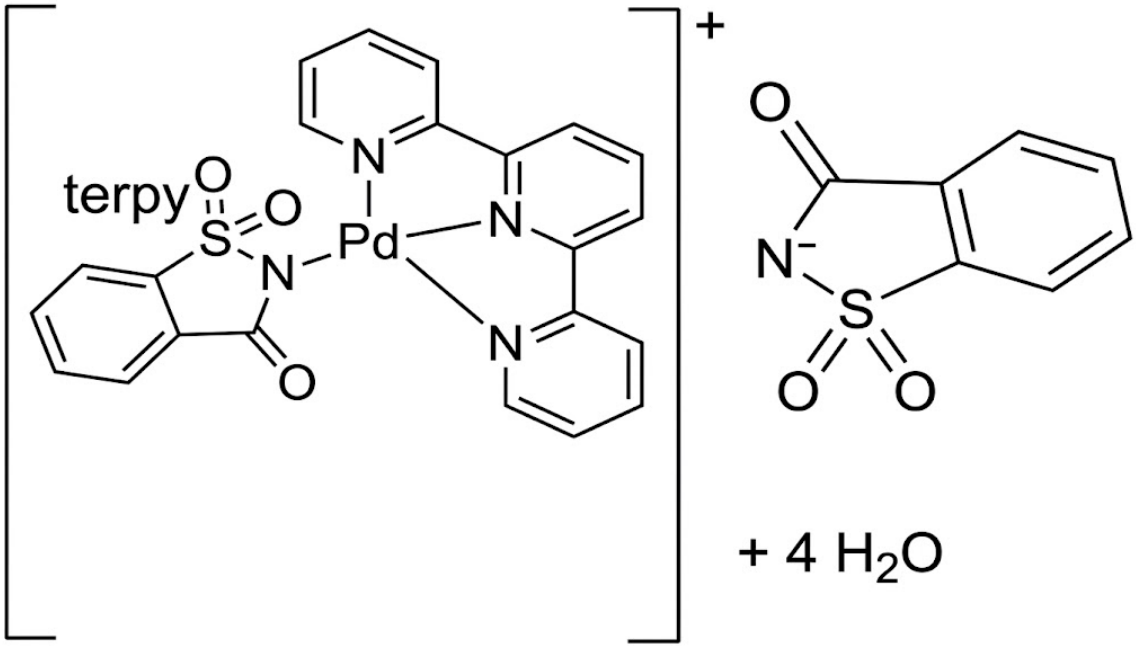

Figure 2: Molecular structure of the anticancer complex (saccharinato-KN)(2,2′:6′,2″-terpyridine-k³N,N′,N″)palladium(II) saccharinate tetrahydrate. The square-planar complex cation inside the brackets carries an overall positive charge (+), balanced by a free, deprotonated saccharinate counter-anion (N^-) and four water molecules (+ 4 H_2O).

2.5 Synthesis and Structural Characteristics of Pd-Phenothiazine Complexes:

Phenothiazine (PTZ) belongs to a major class of three-ring, heterocyclic compounds: two benzenoid-rhombic and one single para-thiophene ring. It is widely used in medicinal chemistry. At present, it has been proven to possess various biological effects: antihistamine, antitussive, antiemetic, antibacterial, and antioxidant. Since the PTZs and congeners are also well known for their anti-cancer activity in various cancers, including ovarian, cervical and glioblastoma, as a result of inhibiting cancer stem cells, inducing DNA damage, inducing cell cycle arrests and generating reactive oxygen species-induced cell death. Some drugs of PTZ have also shown anticancer efficiency through inducing apoptosis. Apoptotic and autophagy programmed cell death is induced by different PTZ-derived compounds in diverse cancer cell types such as leukaemia, invasive oral, breast, and oesophageal cancers (Varga et al. 2017). The use of phenothiazine derivatives in animal models has been the subject of

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recent research, which has shown promising results with higher survival rates. For example, chlorpromazine is being examined in clinical research for glioblastoma, showing effectiveness in traversing the blood-brain barrier and particularly targeting tumour metastases in the central nervous system. A limited number of metal complexes have been analysed for their reactivity with BSA relative to the quantity of organic molecules. Recent studies indicated that palladacyclic complexes had significant DNA/BSA binding affinity and moderate cytotoxic effects against many tumour-cell lines (Vanneste et al. 2023).

2.6 Antioxidant activity of phenothiazine:

The interaction of phenothiazine complexes with oxygen and its radicals becomes crucial in all aspects. Antioxidant qualities have previously been demonstrated in several phenothiazine derivatives. *In vitro*, several phenothiazine derivatives demonstrated a significant capacity to scavenge H_2O_2 free radicals and a reducing power potential to convert Fe^{3+} to Fe^{2+}. These properties were similar to those of ascorbic acid, a common antioxidant. It is well known that covalently bound sulphur in phenothiazine derivatives has a high oxidisability. Sulphur dioxide is an example of an oxidation product that can be produced by a series of oxidation processes. The molecular structure of propenyl phenothiazine dimer, which is composed of two heterocycle fragments of phenothiazine, and its derivative, pyrido phenothiazine, which only contains one heterocycle, is closely related to their strong antioxidant activity (Voronova et al. 2022). The thiazine heterocycle's two benzene rings and its chemical structure were linked to the observed dependence. In this regard, these substances readily participate in oxidation-accompanied processes and are excellent electron donors. The antioxidant properties of phenothiazine are based on the molecular properties of its parent molecule. However, the inclusion of functional groups significantly improves the antiradical properties of phenothiazine derivatives.

2.7 Antibacterial activity of phenothiazine:

Phenothiazine therapy may be a useful choice in the treatment of several bacterial infections, as evidenced by the many studies that have examined the antibacterial activity of phenothiazine to date. Phenothiazine derivatives have the potential to have both direct antibacterial effects and effects that reduce or inhibit antibiotic resistance. The suppression of bacterial multidrug resistance (MDR) efflux pumps is one of the most significant of the various potential pathways that have been previously described. Because MDR proteins on bacteria prevent medications from having the desired bactericidal effect and concentration in the cytoplasm, these pumps play a critical role in the development of antibiotic resistance in bacteria. By blocking these pumps, bacteria can be made susceptible to the corresponding antibiotics. Phenothiazine utilises energy from ATP hydrolysis to inhibit multidrug resistance efflux pumps and to obstruct calcium binding to calcium-dependent proteins like calmodulin. Antibiotic medications can accumulate in the cytoplasm of bacteria due to the suppression of multidrug resistance pumps. Phenothiazines, like chlorpromazine, have antibacterial properties at *in vitro* concentrations of 25 µg/ml, which markedly exceeds the clinically applicable plasma value of 0.5 µg/ml. Nonetheless, it has been shown that phenothiazine may attain concentrations up to a hundredfold within macrophages, effectively eliminating phagocytosed germs. A plasma concentration below the threshold for antipsychotic treatment may nonetheless have a therapeutic benefit (Voronova et al. 2022).

2.8 Antifungal activity of phenothiazine:

The antifungal qualities of phenothiazine have been mentioned in the literature on occasion since it was first described in the 1930s. Because phenothiazine can pass across the blood-brain barrier, some research teams were looking for new ways to treat Cryptococci-induced meningitis. The antifungal activity of the phenothiazine scaffold was thus the focus of the first structure-activity relationship (SAR) analysis, which produced derivatives of the antipsychotic trifluoperazine with enhanced antifungal activity against C. neoformans, C. albicans, and calmodulin. The latter inhibition had a major impact on the mechanism of phenothiazine's antifungal action. Significantly, reference clinical strains and strains resistant to fluconazole, one of the most crucial medications for the treatment of candidiasis, are active against the phenothiazine derivatives (Shamsi & Kraatz, 2013).

2.9 Anti-protozoal activity of phenothiazine:

Ehrlich initially documented the antimalarial characteristics of methylene blue in the 1910s. Nonetheless, phenothiazines might once again assume a crucial role in the treatment of malaria patients due to the emergence of Plasmodium strains resistant to chloroquine. Numerous studies have demonstrated that chlorpromazine possesses

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antimalarial qualities, including the capacity to reduce resistance to chloroquine. Currently, amphotericin B is thought to be a sufficient treatment for naeglerias. Nevertheless, studies have indicated that mice which were treated with chlorpromazine after contracting naeglerias have had 35% greater survival rates than mice treated with amphotericin B (Jaszczyszyn et al. 2012)

III. Mechanisms of targeting cancer cells by phenothiazine:

3.1 Phenothiazine's interactions with lipid bilayers and their function in reversing multidrug resistance:

The direct effect of phenothiazine derivatives on the plasma membrane, as well as their potential as anti-cancer agents, are illustrated in many research papers. The disruption of the plasma membrane is the most direct consequence of phenothiazines. This results in a sudden surge of Ca^{2+}, depolarising the actin filaments and triggering the machinery responsible for repairing damaged membranes. Phenothiazines can cause cell death by delaying the resealing of the membrane (Carneiro et al. 2010). Phenothiazines, on the other hand, mainly cause membrane thinning and enhanced permeabilization, both of which might result in membrane disruption. Unlike the elevated Ca^{2+} inflow linked to membrane rupture, phenothiazines can also deactivate the Ca^{2+} channels, resulting in Ca^{2+} dysregulation that impacts several cellular processes, such as growth. Phenothiazine and K-RAS interact to cause K-RAS to separate from the plasma membrane and accumulate in the cytosol, which in turn causes cell cycle arrest or apoptosis.

3.2 Pro-apoptotic activity and caspase activation:

Apoptosis is essential for the removal of damaged or superfluous cells. An essential component of this process is the activation of caspases, which is a group of proteolytic enzymes (Zong et al. 2011). Caspases serve as crucial mediators of programmed cell death in reaction to harmful stimuli. Some phenothiazines, like trifluoperazine (TFP), possess chemotherapeutic sensitising properties. TFP and CPZ both have shown the ability to enhance caspase-3 activation in H23 cells when they are exposed to bleomycin. The cleavage of caspase-3's substrate PARP demonstrated the protein's nuclear activity. Notably, U1810 cells with 4n DNA content had the highest levels of active caspase, indicating that TFP may promote apoptosis because of aberrant mitosis. Many initiator caspases, including caspases-2, -8, and -9, can trigger the activation of caspase-3. Following DNA damage to U1810 cells, TFP markedly increased the proteolytic cleavage of caspase-8 and caspase-9. In TFP-cotreated U1810 cells, there was a significant increase in catalytically active caspase-9, which was linked to mitochondrial depolarisation and Bak and Bax conformational activation. Consequently, by increasing the activation of intrinsic as well as extrinsic pathways, TFP enhanced DNA damage-induced apoptosis (Zong et al. 2011).

3.3 DNA repair inhibition:

The storage and expression of genetic information in a cell is a fundamental role of the anionic polyelectrolyte DNA. During recent decades, the investigation of the interaction between cationic metal complexes and DNA and the cytotoxic effects of such interactions has been very active. The complexes can attach to nucleic acids both covalently and non-covalently, which depends on the specific characteristics of the metal as well as the ligand. The interaction of DNA with transition metal complexes induces DNA damage in cancer cells, inhibiting their proliferation and resulting in cell death. A drug molecule was inserted between two head-to-head base pairs of nucleic acids during this interaction. Researchers' groups agreed that several small molecules can cause mutagenesis and carcinogenesis in the intercalation state. Consequently, numerous studies on DNA intercalation *via* square-planar complexes have been applied (Zang et al., 2012).

3.4 Angiogenesis inhibition:

Angiogenesis is a multi-step physiological process that involves the proliferation and migration of endothelial cells. It is crucial for both the healing of wounds and the extracellular matrix breakdown mediated by endothelial cells. Vascular endothelial growth factor VEGF stimulates endothelial cell migration and proliferation during the production of micro-vessels throughout organ development, which contributes to tumour angiogenesis. Endothelial cell activity is essential for controlling a variety of vascular physiological and pathological processes in cancer (Byun et al., 2012). Thioridazine's anti-angiogenic effects on tumour angiogenesis *in vivo*, along with the expression of angiogenesis-related proteins and the assessment of tumour cell lysates through immunohistochemistry and immunoblotting, have been examined. The quantity of blood vessels identified with CD31 was approximately four times lower in tumour sections of mice treated with thioridazine, according to immunohistochemical

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labelling of endothelial cells. In agreement with this, immunoblotting demonstrated that thioridazine-treated tumours had lower levels of phosphorylation of VEGFR-2 and VEGF expression in comparison to the controls. Next, immunoblotting was used to examine the activation of phosphatidylinositol-3'-kinase (PI3K) downstream targets following thioridazine administration. Thioridazine treatment decreased the phosphorylation of PDK1, Akt, and mTOR (phosphorylation of the signalling molecules), but not their overall levels. These findings imply that thioridazine induces modifications in the PI3K/Akt signalling pathway, cell cycle progression, and apoptotic cell death (Colturato-Kido et al., 2021).

3.5 Autophagy in cancer cells:

Determining the effectiveness of targeting autophagy in the treatment of cancer and other disorders requires an understanding of how these proteins, together with other elements of the autophagy and apoptosis pathways, might tip the scales between survival and death (Park & Thayer, 2014)(23). To preserve cell homeostasis, autophagy is a biological process that renews or eliminates proteins and organelles by forming autophagosomes and lysosomal hydrolase-mediated destruction. Autophagy can function as a "double-edged sword", influencing the survival of cells and proliferation or triggering autophagy cell death, contingent on the kind of cell and the stimuli. Autophagy may be triggered in cancer cells as a means of surviving chemotherapy drugs that cause apoptosis. As a result, several investigations suggested that chemically induced autophagy inhibition enhanced medication anticancer efficacy. In this instance, it was demonstrated that the phenothiazine-derived thioridazine caused an autophagy reaction in acute T cells (Park et al. 2014).

Post-TR treatment, the expression of LC3-II, an indicator of the autophagic processes essential for autophagosome elongation, exhibited a time-dependent increase. Moreover, there was an increase in the expression of LAMP2, which is a protein of the lysosomal membrane that is involved in the fusion of autophagosomes with lysosomes to create an autolysosome. To ascertain if autophagy produced by TR in Jurkat cells is linked to apoptosis or functions as a pro-survival mechanism, pharmacological agents such as 3-MA, chloroquine and bafilomycin A1 were utilised to suppress autophagy. All inhibitors used to restrict autophagy greatly increased the apoptosis in Jurkat cells which was induced by TR. Accordingly, earlier research in leukaemia demonstrated that autophagy suppression boosted the anticancer effects of masitinib, daunorubicin, and asparaginase in leukaemia models. Therefore, autophagy stimulation is a prospective therapeutic target since it is one of the pro-survival strategies employed by cancer cells to guard against cellular stress. Cells adjust their autophagy flux in response to cytotoxic stimuli to protect themselves and increase their chances of surviving. Nevertheless, excessive autophagy activation can lead to cellular death. Western blot was used to analyse the expression of many autophagy-related proteins during 12 and 24 hours of incubation with 10 μM TR to determine whether autophagy modulation was implicated in the cytotoxicity that was caused by TR in Jurkat cells. TR induced the time-dependent conversion of full-length LC3-I to LC3-II. Additionally, LAMP2, which is a lysosomal membrane protein implicated in autophagy and lysosomal integrity, was also expressed more frequently in response to TR. Since lysosomes combine with autophagosomes throughout the autophagy process *via* the fluorescent dye Lysotracker, which is permeable to cells and accumulates to nanomolar concentration levels inside lysosomes (Park et al. 2014).

IV. Phenothiazine derivatives as promising for cancer treatment:

Phenothiazine plays an important role in drug discovery. In addition to their basic action, they also have a variety of biological activities that contribute to their chemotherapeutic effect against cancer. These activities include their inhibitory effects on calmodulin and protein kinase C, their anti-proliferative properties, their inhibitory properties of P-glycoprotein transport function, and their ability to reverse multidrug resistance. (**Fig3)** and **Table 2** show the phenothiazine's general chemical formula.

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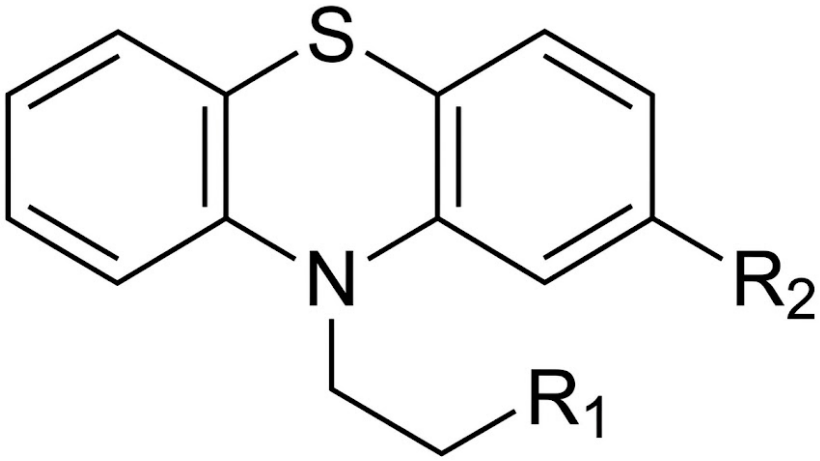

Figure 3: General chemical structure of the phenothiazine core pharmacophore.

The side chain's terminal amine group and the alkyl bridge connecting the nitrogen atom at position (N-10) of the tricyclic ring, as well as the substituents at position C-2 of the phenothiazine ring, as per literature data, influence the anticancer activity of phenothiazines (PhTs) (Jaszczyszyn et al. 2012). The primary compound of interest was thioridazine; at clinically relevant dosages, trifluoperazine and chlorpromazine also showed comparable anticancer efficacy. Phenothiazine slightly exhibits selective toxicity, as proved by the fact that leukaemia cells underwent apoptosis induction while healthy lymphocytes were left intact. Melanoma cells have demonstrated similar anticancer activity both *in vivo* and *in vitro* after being injected into mice. Transition metal-catalysed processes are typically used to create phenothiazine–palladium complexes, and some procedures are described for efficiently synthesising these molecules. Typically, the procedure starts with the corresponding halogenated and phenothiazine compounds being dissolved in a solvent. The palladium precursor is mixed with potassium carbonate or other comparable bases. Until full conversion is verified, thin-layer chromatography (TLC) can be used to track the reaction's development. After finishing, the reaction mixture is usually chilled and given a water treatment to cause the product to precipitate (Varga et al. 2017)**.**

4.1 Biological effect of aliphatic moiety [synthesised Pd-chlorpromazine] complex:

Chlorpromazine (CPZ) is a popular antipsychotic drug. It is used to treat schizophrenia and other mental diseases (**Fig 4**). Many studies have indicated that CPZ affects various molecular oncogenic targets *via* different pathways, which include cell cycle regulation, cancer spreading, chemoresistance, and the stemness of cancerous cells.

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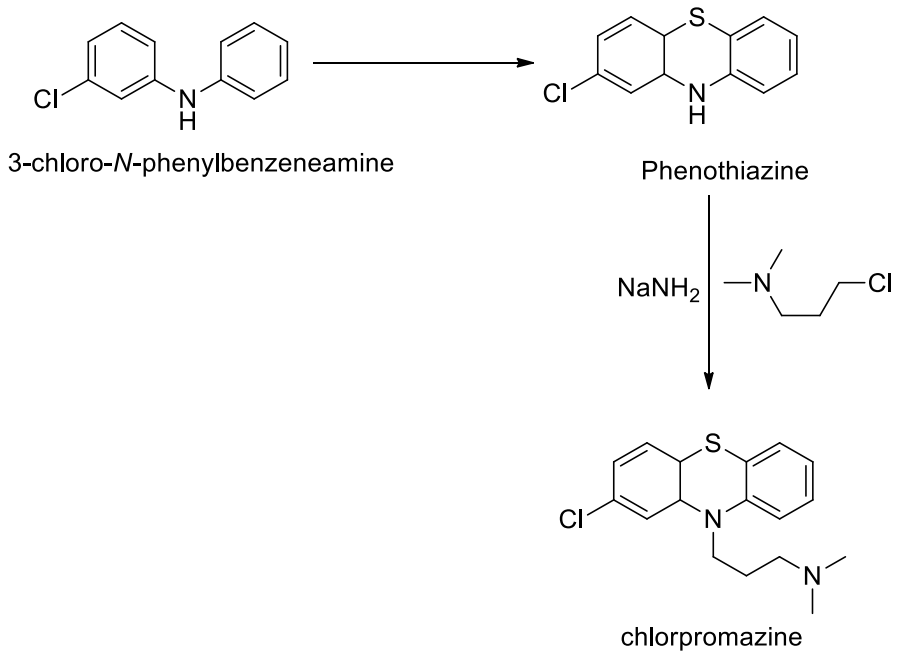

Figure 4: Synthesis of 2-chloro-10H-phenothiazine.

Two mechanisms of CPZ activity in breast cancer have been linked: a decrease in YAP signalling and an increase in membrane permeability that encourages the build-up of chemotherapeutic drugs, such as Taxol and doxorubicin. It has been demonstrated that CPZ inhibits tumour development and triggers apoptosis in colorectal cancer. The investigated impact of CPZ on colorectal cancer was linked to p53 inhibitor down-regulation. It is interesting to note that YAP levels encourage colorectal tumour aggression. Thus, YAP reduction by CPZ in colorectal cancer may contribute to its observed anti-oncogenic action, much like in breast cancer (Chang-En Yang et al. 2019). Antitumour genic impacts of CPZ on various malignancies and their reported cellular pathways are elaborated in the following **Table 3**.

Table 3: Overview of the CPZ's antitumorigenic effects across various malignancies

Type of Cancer	**IC_{50} (μM)**	**Anticipated Mechanism**	**Cell lines**
Breast	10.2	Increases membrane permeability	MCF-7
Colorectal	4 to 10	Inhibition of SIRT1	HCT116
Brain	4.50	Disruption of interactions between REST-mSin3	DAOY
Lung	26.65	Suppression of Akt and mTOR phosphorylation	HSC3
Skin	12.33	Disruption of REST-mSin3 interaction	HPB-ALL
Leukaemia	6.942	Disruption of REST-mSin3 interaction	Ba/F3/KIT
Lymphoma	26	Detaching K-Ras from the plasma cell membrane	PANC-1

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Type of Cancer	IC_{50} (µM)	Anticipated Mechanism	Cell lines
Sarcoma	23	Suppression of Akt phosphorylation	Ca9-22

4.2 Biological effect of Pipradine moiety [synthesised Pd-thiazoline] complex:

Medications of the thiazoline class have a variety of biological effects. A thiazoline derivative ligand called 2-(pyridin-2-ylmethyleneimino)-2,3-dihydro-1,3-thiazole has been synthesised and characterised by some researchers to investigate the possible cytotoxic properties of the complex PdPyTT in human promyelocytic leukaemia as described in(**Fig 5)**. To investigate if the decrease in cell viability was linked to the induction of apoptosis, the degree of apoptotic nuclei in cells stained was computed (Bernalte-Garcia et al. 1999).

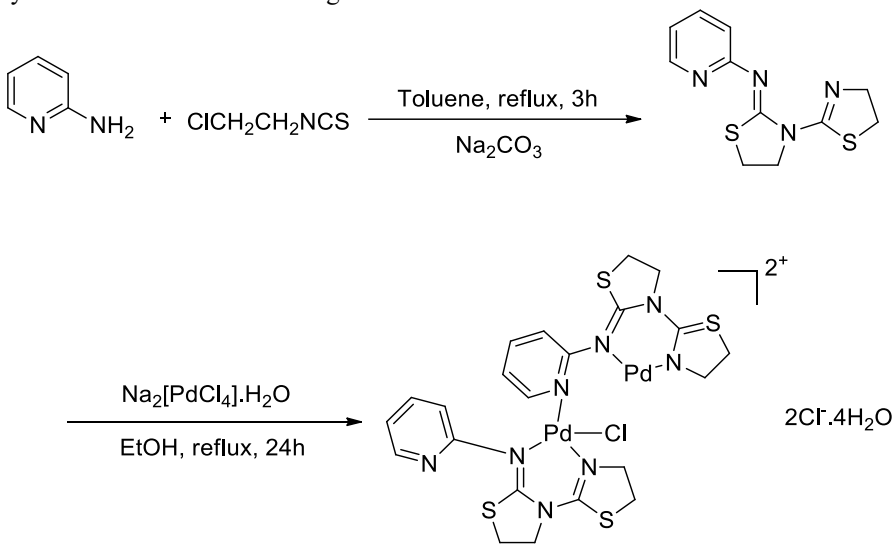

Figure 5: preparation of PyTT and PdPyTT complexes.

The administration of 20.8 µM PdPyTT or 11.4 µM cisplatin to HL-60 cells for a duration of 24 hours resulted in the induction of nuclear fragmentation and condensation, yielding the production of 31.3 ± 6.4% and 41.7 ± 7.2% apoptotic cells for PdPyTT and cisplatin, respectively, as depicted in(**Fig 6)**.

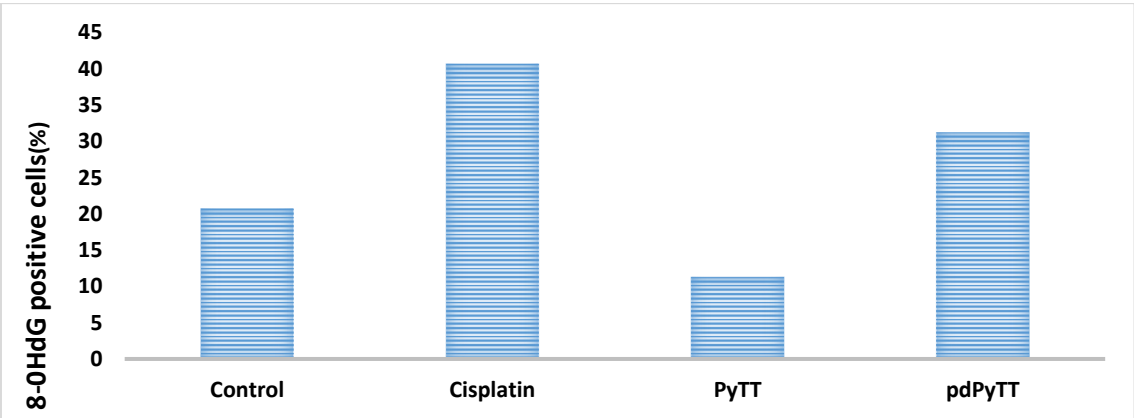

Figure 6: The complex PdPyTT is compared with cisplatin, control, and free ligand PyTT regarding cytotoxicity effect.

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It was suggested that apoptosis in this experiment was the primary mode of cell death. Furthermore, it has been documented that 2-mercaptothiazoline-containing platinum (II) as well as Pd (II) complexes have notable cytotoxic effects on haematoma cells. Recent studies indicate that Pt (II) and Pd (II) complexes with a thiazoline-derived ligand induce apoptosis in HT-29 colon as well as in U-937 lymphoma cell lines. 2-(pyridin-2-ylmethyleneimino)-1,3-thiazolidine, a thiazoline-derived ligand, has been synthesised and characterised in a palladium (II) complex to explore the cytotoxic and pro-apoptotic properties of the PdPyTT complex in human promyelocytic leukaemia HL-60 cells (Chang-En Yang et al. 2019).

4.3 Antitumor activity with piperazine moiety:

Nowadays, piperazine, which is one of the derivatives of phenothiazine, is the most prevalent chemotype in medicine and is also the 3rd most significant nitrogen-containing compound, following piperidine and pyridines. Recently, the MDDR database stated that over 11,000 structures feature the piperazine moiety, which exhibits diverse pharmacological activities such as anti-fungal, anti-depressant, anti-malarial, anti-proliferative, anti-obesity, anti-hypertensive, anti-bacterial, and antioxidant (Zhang et al. 2021). Numerous nitrogen-containing heterocyclic compounds have been advanced as anticancer agents, and a range of piperazine derivatives has been synthesised as promising scaffolds exhibiting enhanced cytotoxicity against various cancer cell lines, including those of the colon, breast, prostate, and leukaemia. In both preclinical and clinical investigations, many compounds containing a piperazine moiety have exhibited anticancer action. However, many industries have synthesised new piperazine derivatives as therapeutic agents because it has shown high performance in reactions. Moreover, piperazine backbones synthesised through rational drug design have shown outstanding performance as anti-cancer medications, like piperazine-linked bisanthrapyrazole derivative and quinazoline-linked substituted piperazine. The previous compound suppresses the propagation of cancer cells and induces apoptosis in the erythroleukemic A-562 cell line, epidermal cervical cancer, and lung cancer cells, respectively (Jeon & Shin 2021). To sustain equilibrium in various organisms Apoptosis represents a fundamental and essential biological process; however, any defect within this mechanism can result in immunodeficiency, alongside genetic and autoimmune disorders, ultimately culminating in the development of cancer. Apoptosis can be initiated through two distinct pathways: the extrinsic pathway, often referred to as the “death receptor pathway,” and the intrinsic pathway, which is mediated by mitochondrial processes. Extracellular surface receptors initiate extrinsic pathways through ligand-binding interactions, while intracellular signals within the mitochondrial intermembrane space are activated in the intrinsic pathway, commonly referred to as the mitochondrial pathway. Indeed, these two pathways are interrelated, and the elements of each pathway can influence the other. Cell contraction, fragmentation of the nucleus and DNA, and chromatin condensation all indicate morphological alterations which are linked with apoptosis in a cell. The two primary signalling mechanisms referenced above, which induce apoptosis, are being employed as chemotherapeutics to inhibit cancer cell viability. 2-(Allylthio) phenyl) (4-(4-methoxyphenyl) piperazin-1-yl) methanone, a piperazine derivative illustrated in (**Fig 7)**, was synthesised at the Korean Institute of Radiological Medical Sciences. It was testified as a possible anticancer agent. A colorimetric test was employed to ascertain the apoptotic pathway activated in CB01-treated cells and to examine the activities of caspase-3, caspase-8, and caspase-9. The activities of caspase-3 and caspase-9 in cells exposed to 40 nM CB01; conversely, the activities of caspase-8 in these cells remained unchanged.Consequently, CB01 seems to induce apoptosis through the intrinsic route. It is anticipated that the basic structural characteristics of piperazine, which can be modified, will enable the development of an additional option for the treatment of solid tumours, especially those found in the breast, pancreas, colon, and lung (Jeon & Shin 2021).

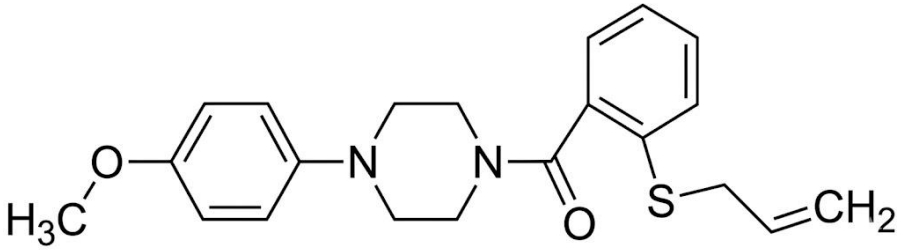

Figure 7: Chemical structure of the anticancer piperazine derivative CB01.

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V. Clinical Implications and Future Directions

The translational potential of Pd-phenothiazine complexes is supported by several converging lines of evidence. Chlorpromazine is currently being examined in clinical research for glioblastoma, demonstrating effectiveness in traversing the blood-brain barrier and particularly targeting tumor metastases in the central nervous system (Vanneste et al. 2023). This clinical precedent for phenothiazine-based anticancer therapy strengthens the rationale for developing Pd-phenothiazine hybrids. Several key areas require focused investigation to advance the field toward clinical application. Structure-Activity Relationship (SAR) Optimization: Comprehensive SAR studies are needed to identify the structural features that govern biological activity, selectivity, and pharmacological properties. Systematic variation of the phenothiazine substituents, the coordination environment of palladium, and the overall complex architecture should be correlated with cytotoxicity, mechanism of action, and physicochemical properties. Computational approaches, including molecular docking, molecular dynamics simulations, and quantitative structure-activity relationship (QSAR) modeling, can guide rational design and prioritize synthetic efforts. Mechanistic Elucidation: While multiple mechanisms have been implicated in the biological activity of Pd-phenothiazine complexes, detailed mechanistic understanding remains incomplete. Identification of the primary molecular targets, elucidation of the sequence of events leading from target engagement to cell death, and characterization of resistance mechanisms are essential. Proteomics and transcriptomics approaches can provide unbiased identification of affected pathways. Chemical biology tools, including photoaffinity labeling and activity-based probes, can identify direct protein targets. Genetic approaches, such as CRISPR screens, can reveal genes that modulate sensitivity or resistance. Pharmacokinetics and Drug Delivery**:** The pharmacokinetic properties of Pd-phenothiazine complexes—absorption, distribution, metabolism, and excretion—must be characterized in animal models. The rapid ligand exchange kinetics of palladium complexes pose challenges for systemic delivery, potentially requiring formulation strategies to enhance stability. Nanoparticle encapsulation, liposomal formulation, or conjugation to targeting moieties (antibodies, peptides, small molecules) may improve delivery to tumor tissues while reducing off-target exposure. The Pd_2L_4 cage architecture offers intriguing possibilities for host-guest chemistry, potentially enabling encapsulation of additional therapeutic agents for combination therapy or imaging agents for theranostic applications. Combination Therapies: The multitarget nature of Pd-phenothiazine complexes suggests potential for synergistic interactions with other therapeutic modalities. Combination with conventional chemotherapeutics, targeted therapies, immunotherapies, or radiation could enhance efficacy and overcome resistance. Systematic screening of combination regimens in preclinical models, followed by mechanistic studies to understand the basis of synergy, will inform rational combination strategies for clinical testing. Biomarker Development**:** Identification of predictive biomarkers that identify patients most likely to benefit from Pd-phenothiazine therapy would enable precision medicine approaches. Potential biomarkers include expression levels of DNA repair enzymes, antioxidant defense proteins, apoptosis regulators, or drug transporters. Genomic features such as mutations in DNA damage response genes or mismatch repair deficiency may predict sensitivity. Functional assays measuring cellular redox state or apoptotic threshold could stratify patients. Validation of biomarkers in preclinical models and early-phase clinical trials is essential for their clinical utility. Antimicrobial Development**:** The broad-spectrum antimicrobial activity of Pd-phenothiazine complexes warrants development as antibacterial, antifungal, or antiprotozoal agents. The global crisis of antimicrobial resistance necessitates novel agents with mechanisms distinct from existing antibiotics. Pd-phenothiazine complexes, with their multitarget mechanisms and potential to overcome resistance, represent promising candidates. Development pathways for antimicrobial agents differ from anticancer drugs, with distinct regulatory requirements and clinical trial designs. Topical formulations for skin and soft tissue infections or localized delivery for device-associated infections may represent initial clinical applications, with systemic formulations for invasive infections pursued subsequently. Toxicology and Safety: Comprehensive toxicology studies in multiple species are required to establish safety profiles and identify potential organ toxicities. Acute toxicity, repeat-dose toxicity, genotoxicity, and reproductive toxicity must be assessed according to regulatory guidelines. Comparison with platinum drug toxicity profiles will inform the therapeutic index. Identification of biomarkers of toxicity (e.g., renal function markers, neurological assessments) will enable monitoring in clinical trials. Clinical Trial Design: First-in-human Phase I trials will establish

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maximum tolerated dose, dose-limiting toxicities, pharmacokinetics, and preliminary efficacy signals. Adaptive trial designs that enable dose escalation and expansion cohorts in specific tumor types can accelerate development. Incorporation of correlative studies (pharmacodynamic biomarkers, tumor biopsies, imaging) will provide mechanistic insights and inform subsequent trial design. Phase II trials in selected tumor types, potentially enriched for biomarker-positive populations, will assess efficacy. Ultimately, randomized Phase III trials comparing Pd-phenothiazine complexes to standard of care will be required for regulatory approval. Regulatory Pathway: Engagement with regulatory agencies (FDA, EMA) early in development is advisable to align preclinical and clinical development plans with regulatory expectations. Orphan drug designation for rare cancers or tropical diseases may provide incentives and expedited review pathways. Breakthrough therapy designation or fast track status may be available if preliminary evidence demonstrates substantial improvement over existing therapies. The path from laboratory discovery to clinical application is long and uncertain, with high attrition rates at each stage. However, the unique properties of Pd-phenothiazine complexes—multitarget mechanisms, potential for reduced toxicity, activity against resistant cancers, and antimicrobial properties—justify continued investment in their development. Collaborative efforts integrating synthetic chemistry, structural biology, cell biology, pharmacology, and clinical medicine will be essential to realize the therapeutic potential of this promising class of compounds.

VI. Conclusion:

New biologically active substances are created annually with the goal of treating cancer. The most significant discoveries made in the metal complex to date are reviewed in this review, along with their advantages and disadvantages in terms of the toxicity and efficacy of metal-based cancer treatment options like with palladium complexes. Numerous biological activities, such as psychotropic, anticancer, and other pharmacological effects, have been demonstrated by phenothiazines and their related molecules. It has been demonstrated that phenothiazine derivatives produce significant binding to proteins as well as antibacterial, anti-protozoal, and antioxidant properties.

Supplementary material is available in a separate document.

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Consent for the Publication

Written consent was obtained from all participants involved in this article.

Declaration of competing or financial interest:

The authors declare that they have no known competing financial interest or personal relationship that could have influenced the resulted of this study.

Author Contributions

Reem Almutairi: Investigation, Software and Original Draft Preparation. **Dr. Emad Elzayat**: Supervision and Manuscript Editing. **Dr. Mamdouh I. Nassar:** Review. **Dr. Azza A. Shoukry**: Supervision and Manuscript Review.

Data Availability

All data generated or analysed during this study are included within this article.

Declaration of generative AI and AI assisted technologies:

The authors declare that, during the preparation of this manuscript, AI-assisted tools were used solely for language and grammar revision. All outputs were critically reviewed and edited by the authors, who assume full responsibility for the content.

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