# DNA Interaction and Biological Activities of Heteroleptic Palladium (II) Complexes

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(Received on 3rd March 2020, accepted in revised form 14th September 2020)

**Summary**: The manuscript describes the binding of DNA as well as biological studies of some mixed ligand dithiocarbamate Palladium (II) complexes (**1-5**). The observed compounds are of general formulae [PdCl(DT)($PR_3$)]. The dithiocarbamate "DT" and "$PR_3$" groups are varied among the studied complexes as DT = *bis*[(2-methoxyethyl) dithiocarbamate)] (**1** and **2**), dibutyl dithiocarbamate (**4** and **5**), bis[(2-ethyl) hexyl dithiocarbamate)] (**3**); $PR_3$ = triphenyl phosphine (**1**), benzy diphenyl phosphine (**2**), diphenyl-*tert*-butyl phpsphine (**3**), diphenyl-*p*-tolyl phosphine (**4**) and diphenyl-2-methoxy phenyl phosphine (**5**). The synthesized complexes were screened for DNA binding study via (UV Visible spectrophotometry and Viscometery) and biological activities such as anti-bacterial and anti-fungal, Molinspiration calculations and antioxidant potencies stimulated by hydrogen peroxide in human blood lymphocytes. In case of drug DNA interaction, complexes showed some sort of interaction with DNA solution. Almost all the complexes exhibited moderate antifungal and antibacterial behavior (against Gram positive and negative bacterial strains). The Molinspiration calculation study revealed that the said Pd (II) mixed complexes are biologically significant drugs having adequate molecular properties regarding drug likeness, except the log P values of complexes **3**-**5** because some structural adjustments must be done for enhancement of their bioavailability and hydrophilic nature. Regarding the antioxidant potential of complexes **1**, **2** and **4,** the $H_2O_2$ treatment of complexes violently decreased the action of antioxidant enzymes, superoxide dismutase and catalase and enhanced the level of thiobarbituric acid-reacting substances. Under experimental conditions, we conclude that all complexes act as anti-mutagens as they significantly suppress $H_2O_2$-induced oxidative damage at non-genotoxic concentrations.

**Keywords**: Dithiocarbamate ligand, Pd (II) complex, DNA interaction study, Biological activity, Molinspiration calculations, Oxidative damage.

## Introduction

Cancer is considered to be one of the greatest troublesome communities health distracts and intensely the main cause of death in entire world. Cisplatin is the most crucial cytotoxic drug employed for treating variety of human cancers [1]. Nowadays there has been a great interest for scientists to discover and develop a new anticancer drug with least side effects, as most of recent anticancer drugs such as platinum based drugs possess severe toxicity issues including nausea, nephrotoxicity, gastrointestinal, and bone marrow toxicity [2]. The DNA molecule is known to be the primary cellular target in treatment of cancer, so understanding the mode of cancer tumor genesis has remain the field of ongoing research [3]. Small ligand molecules interact with DNA in three different kinds of non-covalent manners, which are, (a) intercalating between stacked base pairs of DNA molecule, (b) groove binding and (c) electrostatic interaction with the negatively charged sugar phosphate skeleton [4]. Intercalation leads to introduction of a planar ligand molecule in between DNA complementary base pairs [5], while groove binding involves the binding of drug molecules with minor groove of DNA [6]. A lot of attempts have been made for the reduction of toxic problems originated in platinum anti-cancer complexes, most importantly by using sulfur incorporating ligands as de-toxicant agents contrary to metal-containing drugs [7]. It was determined that employing dithiocarbamate compounds in combination with Cisplatin, appeared to be protective against different animal species from bone marrow, renal and gastrointestinal toxicity, stimulated by Cisplatin [8]. Dithiocarbamate molecules have been found to make complexes with nearly all transition metals with various oxidation states [9]. The chelated sulfur atoms in dithiocarbamate segment of Platinum or Palladium moiety selectively inhibits the nucleophilic attack of enzyme-thiol to the metal center and invariably shields the usual tissue without suppressing their anti-cancer effectuation [10]. Moreover, dithiocarbamate have been employed in arthrosclerosis prevention, waste water treatment where they act as scavengers, floatation agents and metal transportation in membranes [11], also sodium diethyl dithiocarbamate (DDTC) has the potential to cure patients suffering with acute poisoning of thallium, arsenic and nickel carbonyl [12]. In corresponding scene of metal complexes, palladium (II) complexes have attracted profound interest recently in view of their promising biological score [13], as they are regarded to be more utile in treating gastrointestinal region tumors where Cisplatin appears to miscarry [14].

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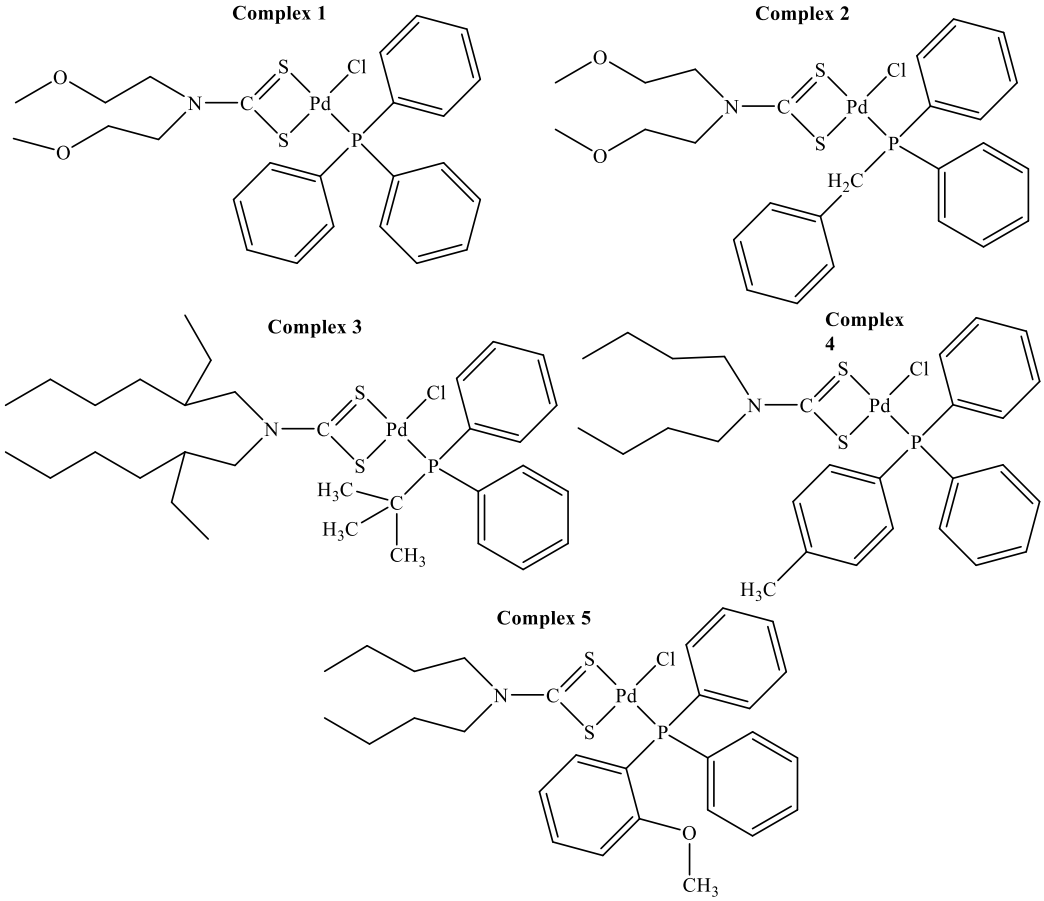
Here in this study we worked on five mixed ligand dithiocarbamate palladium (II) complexes [15] (Scheme 1), having short non polar hydrocarbon sides in their dithiocarbamate moieties that will obviously enhance their water solubility, as the water insolubility is considered to be the main cause of the limited bioavailability and a consequent very low vivo activity of some platinum and palladium complexes [16].

The aim of the study was to execute DNA-binding studies of Pd (II) dithiocarbamate antitumor complexes, which will obviously result to enhance the idea of understanding the mechanisms of action of these complexes with DNA expected to be quite different from that of Cisplatin. The interaction of studying complexes and DNA will be considered promotion in medical science as well as pharmacokinetics. The thermodynamic binding parameters and the modes of binding studied via spectrometry and viscometry are highlighted in the study. All the observed complexes were evaluated for antibacterial and antifungal activities and their Molinspiration calculations were also calculated. We also extend our interest in studying the bio protective power of complexes **1**, **2** and **4** by suppression of oxidative damage induced by $H_2O_2$ in fresh human lymphocytes by finding the potentially positive effect against various sort of cancer and heart diseases as we better know that metal complexes are able to perform as free radical scavengers and neutralize dangerous reactive oxygen species.

## Experimental

*Materials and methods*

The used solvents as ethanol, methanol & DMSO, and Salmon sperm DNA (SS-DNA) were products of Merck and were utilized directly. Singly distilled water was employed in washing and making DNA solution. The UV Visible absorption study was taken on Shimadzu 1800 UV-Visible absorption spectrophotometer by using of 1cm path length cuvettes, while the viscosity readings were exploited by Cannon-Fenske-Opaque viscometer. The overall work was performed at room temperature. The investigated mixed ligand palladium(II) complexes have been synthesized and purified by the reported method[17].




Scheme-1: Proposed structures of complexes **1-5.**

*Drug-DNA interaction study*

*UV Visible absorption study*

The DNA solution was set by overnight stirring of decent quantity of DNA in 25 mL fresh distilled water, which was further and kept at 4°C for one day before use. The concentration of DNA was calculated upon using UV-Visible spectrophotometer following the well-known equation of Beer-lambent law (A = εcl) and was found to be 2.65 mM [18]. Purity of SS-DNA was confirmed from absorbance ratio of DNA at 260 to 280 nm and its value fall in between 1.8-1.9, suggesting that DNA is from protein content DNA [19]. Solutions of compound **1**, **2** & **5** in 50%, while that of **3** & **4** were made in 80% DMSO of 1mM concentration. In both the cuvettes drug concentration was kept constant while SS-DNA solution conc. was diverged continuously during absorption studies from 0-346µM so as to obviate DNA absorbance. The binding constants of drugs with DNA and their Gibbs free energy change values were calculated with the aim to check that to how much extent the observed drug interacts with DNA and to ascertain out the spontaneity factors following Bensei-Hilderbrand and Gibbs free energy change equations respectively.

*Drug-DNA interaction study by viscometer*

The viscosity studies were performed at room temperature of by employing Cannon-fenske opaque viscometer, maintaining the DNA concentration (0.265mM) fixed and vary the compound concentration (0-0.4mM). Samples flow time was noticed in seconds by digital stop watch. The sample solutions were diluted valiantly with whole volume of 5ml, hold up for a while before taking their measurements and then average of triplicate studies of each respective sample was obtained. Ethanol was always used for rinsing the viscometer for each sequential reading. The graphical manipulation from the collected data was made by plotting Drug to DNA concentration ratio ([Drug]/[DNA]) as horizontal and relative viscosity $(\eta/\eta_o)^{1/3}$as vertical axis, where η and $\eta_o$ are viscosities of DNA in presence and absence of drug [20]. Measurements were obtained by the prescribed equations $\eta = t\text{-}t_o/t_o$ (for compound) and $\eta_o = t\text{-}t_o$(for DNA) [21].

*Biological activities*

*Antibacterial study*

Agar well diffusion method was espoused for antibacterial evaluation [22], against 4 bacterial pathogens; three of them were gram positive and one was gram negative. The reference drug taken was Levofloxacin which is known to possess a wide range of anti bacterial action. Nutrient agar media (11.2g/400 mL distilled water) solution was prepared, auto-cleaved and then their relevant amount was poured to sterile Petri plates. Wells were dug with sterile micro-pipette tip after solidification of media. Sterile cotton swabs were used to scatter bacteria in columns on the solidified surface of media. Then DMSO solution of reference drug and test samples (50 µL) at 1 mg/mL was dropped into their labeled wells. The overall work was continued in well-controlled surroundings. Plates were then incubated aerobically at constant temperature of 37 °C for 24 hours. The activity of each complex was characterized by measuring diameter of inhibition zone in mm [22] by a ruler.

*Antifungal screening*

Agar tube dilution method was followed to determine antifungal grade of complexes versus two fungal strains named *Aspergillus Niger* and *Aspergillus Flavus* [22]. Sabouraud dextrose agar media solution was sterilized at 121 °C for fifteen minutes and after reservation to room temperature 4mL media amount was introduced to test tubes. Tubes were loaded with 70µL of each sample from their stock solutions of (12 mg/mL in DMSO) and kept at slanting angle to solidify. Each of the tubes was then inoculated with slice of 5mm diameter of fungal in-column from fungal pathogens of 7 days old cultured. Terbinafine was attempted to be reference drug. The linear growth of fungus in the seven days incubated tubes was measured with ruler. Then percent growth inhibition was evaluated in comparison to reference drug by implementing below urged equation [23].

$$\text{Percent inhibition of fungal growth} = \left[\frac{\text{Growth diameter in test compound (mm)}}{\text{Growth diameter in control (mm)}}\right] \times 100$$

*Molinspiration calculation*

Computer based molecular modeling (bioinformatics and cheminformatics) are completely utile for predicting molecular properties as bioactivity score and drug likeness, as we know that these are quite fast and cheap sources for a compound application even when it is not physically available [24]. SMILES notations of Chem-Draw Ultra 8.0 software structures of all the studied complexes (1-5) as well as the standard drugs were fed in the online software of Molinspiration in order to anticipate their biological properties (Log P, TPSA, no. of hydrogen bond donors and acceptors species, molecular weight

and number of rotatable bonds), and bioactive potencies (GPCR ligands, ion channel modulator, kinase and other enzymes inhibitors & nuclear receptors). Finally, the resultant effectiveness of the complexes was equated with standard drugs, Levofloxacin and Terbinafine.

*Bio protective power of complexes against oxidative damage induced by $H_2O_2$*

*Preparation of complex solution*

1mg of each complex 1, 2 & 4 was dissolved in 1mL DMSO at room temperature.

*Lymphocyte isolation*

Seven healthy male persons of age 24-26 years were subjected for obtaining blood. 4mL of whole blood was taken in sterile heparinized syringes and was kept in sterile 15 mL glass tube. The phosphate buffer saline of pH 7.4 was taken in tube in an equal volume followed by the centrifugation at 200×g for some time. After discarding the supernatant, 3mL of ficoll-hypaque solution/10 ml of blood were added slowly below the blood with the help of a sterile pipette. The renewed mixture was again centrifuged at 2000×g for time interval of 30 minutes and the lymphocytes accumulated just over ficoll-hypaque layer were cautiously isolated [25]. Lymphocytic cells were rinsed via PBS and centrifuged at 600×g for ten minutes. At last the cells were used for further analysis after the supernatant disposal.

*Try pan blue staining*

After isolation, the lymphocytes were immediately debased with the cell culture media (RPMI-1640, thermo scientific). 10µL of each sample and try pan blue stain (0.2%) was taken and mixed. The sample was charged to a haemocytometer and was noticed through microscope to count the stained as well as non-stained cells. Finally the percentage of dead (stained) cells was calculated. The percentage of live cells per sample was more than 85%.

*Cell culturing and treatment*

Cells were diluted with RPMI-1640 and number of cells/mL was enumerated by haemocytometer. The cells were diluted to a systematic concentration of $1\times10^8$ per mL. Cells once with media alone and then combination of complexes (0.5, 5, 10 µg/ml) were incubated for two hours at 37 °C. An aggregate of lymphocytes from each individual sample was used as control to avert variability among them. The incubated cells were subsequently washed with PBS and centrifuged at the rate of 200×g for some time. The supernatant was thrown; their pellet was diluted with PBS in $1\times10^6$ concentration and ultimately placed at -20 °C for ROS and antioxidant enzymes analysis.

*Biochemical analysis*

Resultant lysate for antioxidant enzymes and reactive oxygen species approximation were lysed by ultra-sonication of cells.

*Superoxide dismutase activity*

SOD assay was performed by chasing Marklund assay method of superoxide dismutase [26], which depends upon the auto-oxidation of pyrogallol. The supernatant SOD was evaluated spectrophotometrically at 420 nm. The SOD activity unit can be best described as "the total enzyme requisite to exhibit 50% dismutation of the superoxide radicals per minute". The assay was explicated in international units (or milli-units) mU per $10^6$ cells.

*TBARS*

The TBARS was ascertained by the method of Li *et al*., Hepatic antioxidant status [27], deliberating their strength by noticing absorption at wavelength of 535nm and molar extinction coefficient of 156 mM/cm. The Contents of TBARS were expressed as nano-moles per $10^6$ cells.

*Enzyme catalyze*

CAT was assayed calorimetrically as described by Sinha (1972) [28].

*Reactive oxygen species*

The ROS comprising the cell homogenate were assessed accordingly to the developed methodology of Hayashi *et al*., (2007) [28]. Cell homogenate (5 µL) or $H_2O_2$ standards were taken in to 96 well plates containing 140 µL of 0.1 M sodium acetate buffer of pH 4.8 and incubated for 5 minutes at 37 °C, DEPPD mixed solution (100 µL) & Ferrous sulphate (R1 and R2, at 1:25) was fed to each well followed by incubation for 1 minute at 37 °C. Micro plate reader was implemented to notice absorbance at 505 nm for 180 seconds with 15 seconds break. The standard curve was plated and ROS concentration in Unit/$10^6$ cells was reported.

## Results and Discussion

*UV Visible absorption study of Drug-DNA interaction*

The type and extent of binding mode of a drug with DNA are most commonly studied by electronic absorption spectroscopic proficiency noticing absorption changes. Such as intercalation ordinarily consequences in hypochromism with or without possible small red or blue shift due to expected interaction of aromatic chromophore and DNA base pairs [29]. Drug-DNA interaction was determined by surveying absorption spectrum transitions on sequential increase of DNA solution. Association constant or intrinsic binding constant i.e., $K_b$ was calculated from intercept to slope ratio of the plot drawn between 1/[DNA] and $A_o/A–A_o$. [30]. Free energy of all samples was calculated from Gibb's free energy equation, being acceptable to check the spontaneity of the drug-DNA holding.

*DNA interaction study of complex* **1**

Complex 1 exhibits both hypochromism at 315 nm ($\lambda_1$) as well hyperchromism at 276 nm ($\lambda_2$) in UV region, with the shift change of 1nm on increasing DNA concentration (0-346µM) and idealizing both intercalative and groove binding interactions at relevant wavelengths. Hyperchromism at $\lambda_2$ is the clear clue for partial or non-intercalative binding either because of opposite charges force that takes place between the cation of Pd(II) and the negatively charged phosphate groups of DNA backbone, hydrogen bonding or van der Waals interaction) [31], as hyperchromism when accompanies slight blue shift indicates external adjoin [32]. Slight hypochromism at ($\lambda_1$) gives sense of intercalation between DNA base pairs, as decrease in transition energy levels resulting due to partial occupation of DNA's π-orbital electrons in π* orbital of the Pd(II) complex [33], also the hypochromic effect with bathochromic shift are features of intercalation [34]. The magnitude of $K_b$ and ΔG for corresponding complex was rated as: $5.8\times10^3 M^{-1}$ ($\lambda_1$) and $23.8\times10^4$ $M^{-1}$($\lambda_2$), -21.5 kJmol$^{-1}$ ($\lambda_1$) and -31 kJmol$^{-1}$($\lambda_2$) respectively. Absorption spectrum of complex 1 in the absence and presence of SS-DNA is presented in Fig 1.

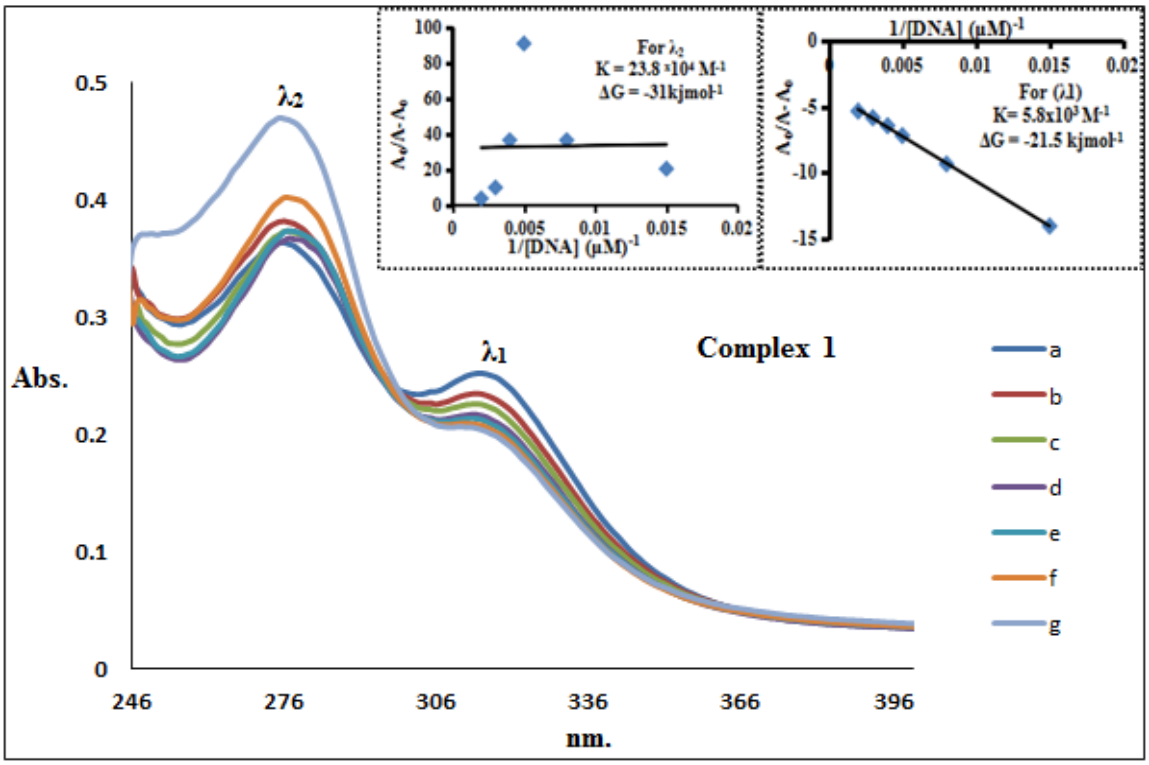



Fig. 1: Absorption spectrum of 1mM complex 1 in absence (a) and existence of 65 µM (b) 126 µM (c), 185 µM (d) 241 µM (e), 294 µM (f) and 346 µM (g) DNA. The arrow focuses on increased concentration of DNA.

*DNA interacting study of complex* **2**

The maximal absorption position of complex 2 was noticed at wavelength of 275nm, arguing π-π* electronic excitation and shows hyperchromism with a slight blue shift of 3 nm on continuous increase in DNA concentration, pointing external mode of binding [32]. $K_b$ and ΔG were established to be 26 x $10^3$ $M^{-1}$ and -25 $kJmol^{-1}$ respectively (Fig 2).

*DNA interaction study of complex* **3**

Complex 3 locates hypochromism with a red shift of 2nm at 294nm on the incremental addition of DNA (0-241 µM) follows the partial intercalation binding, as hypochromism in addition to red shift of lower than 15nm featuring the partial intercalation [34], because the bulky structure of dithiocarbamate portion of compound prevents the entire drug molecule to be intercalated, lowers the transition energy required for electrons to excite from π-π* orbital of the complex due to occurring conjugation between highest occupied π-orbital of DNA base pairs to complex lowest empty molecular orbital [35]. $K_b$ and ΔG were resulted as 9.7x $10^2$ $M^{-1}$ and -17 kJ $mol^{-1}$ respectively highlighted in Fig 3.

*DNA interaction study of complex* **4**

Complex 4 maximum absorption position established at 274nm attributing π-π* transition of the coordinated ligands and an increased DNA concentration (0-294 µM) demonstrates hypochromism alongside hypsochromic effect to 9nm, establishing sufficient denotation of electrostatic binding. The possibility of hydrogen bonding formation between nitrogen in dithiocarbamate ligand and DNA base pairs is more likely to occur, as DNA double helix possesses many hydrogen-bonding sites [36], which is strengthen by lone pair of nitrogen atom owns intense inductive effect in presence of attached butyl group. The ΔG and $K_b$ values proved as: -18 $kJmol^{-1}$ and 13x$10^2M^{-1}$ (Fig 4).

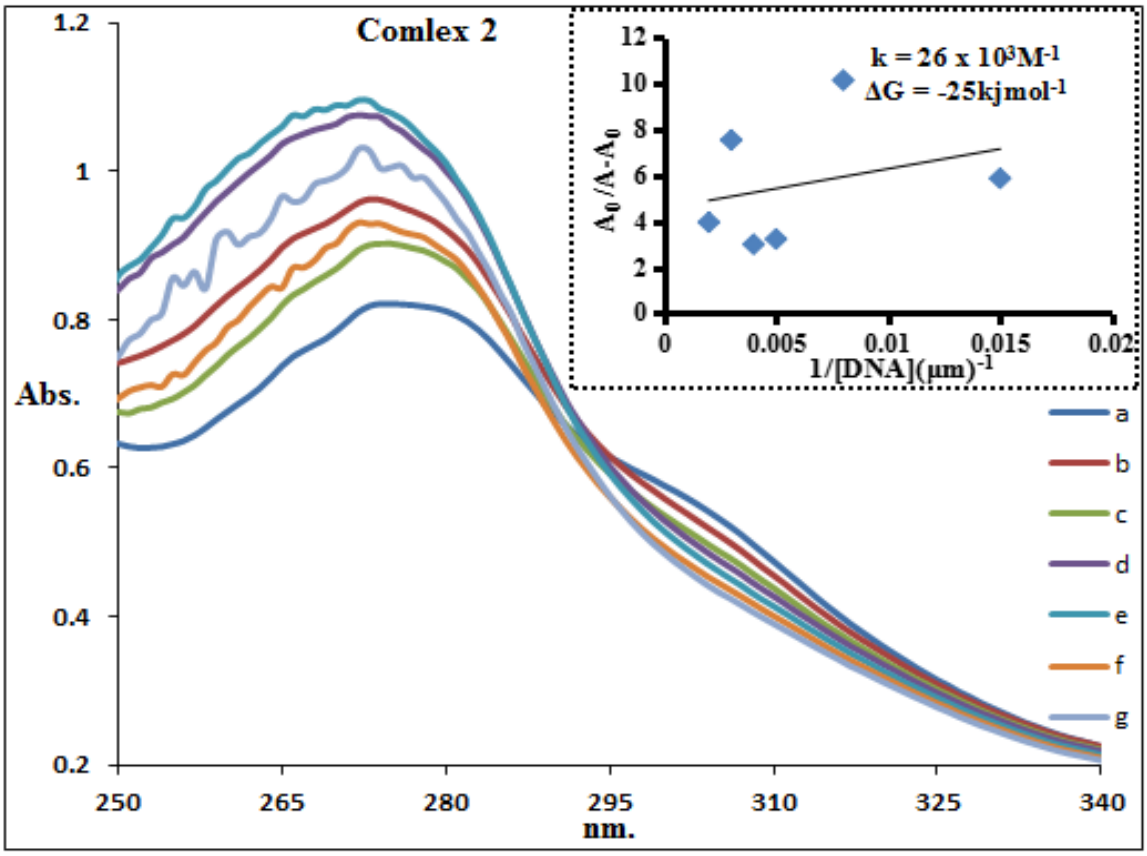



Fig. 2: Electronic spectra of complex 2 only (a) and in presence of 65 µM (b) 126 µM (c) 185 µM (d) 241 µM (e) 294 µM (f) and 346 µM (g) DNA. Arrow highlights sequential increase in DNA concentration.

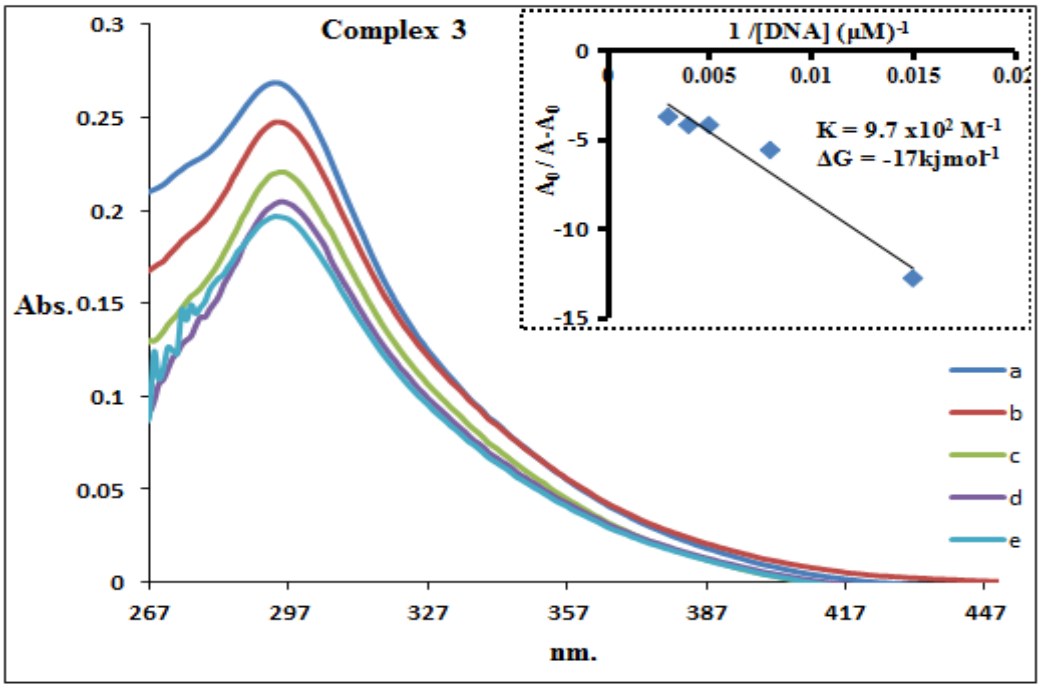



Fig. 3: Absorption spectrum of the complex 3 in absence (a) and presence of 65 µM (b) 126 µM (c) 185 µM (d) and 241 µM (e) DNA. The arrow direction follows increased DNA concentration

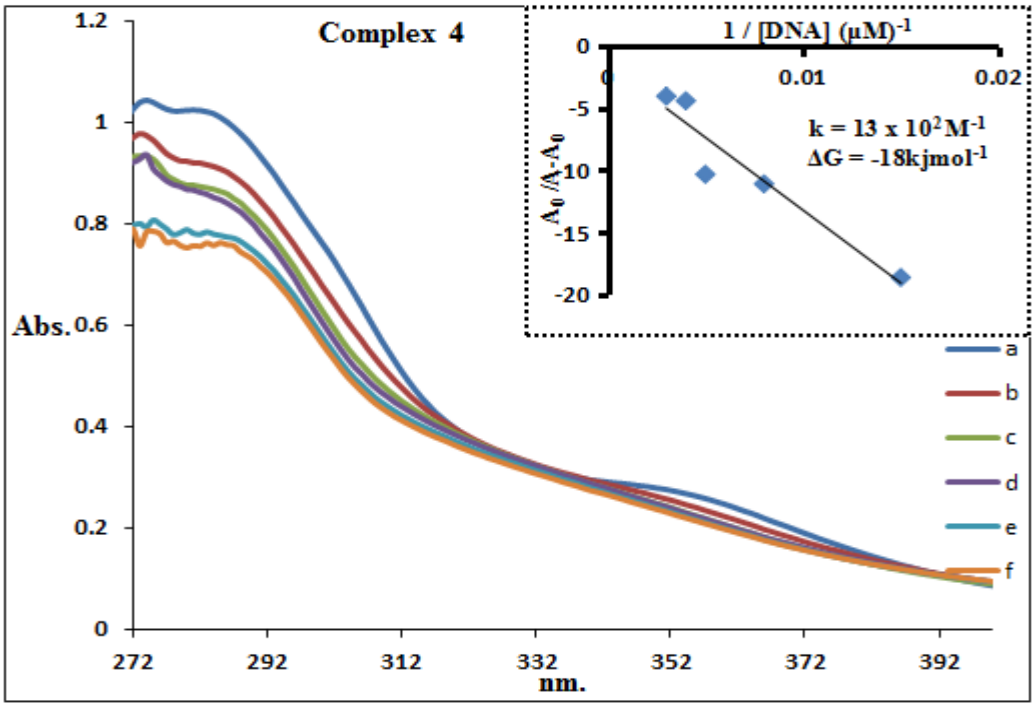



Fig. 4: Electronic absorption spectrum of complex 4 in the absence and presence of DNA, [complex] = 1mM; [DNA] = 0–294µM(b-f). Arrow shows the absorbance change upon the increasing DNA concentrations.

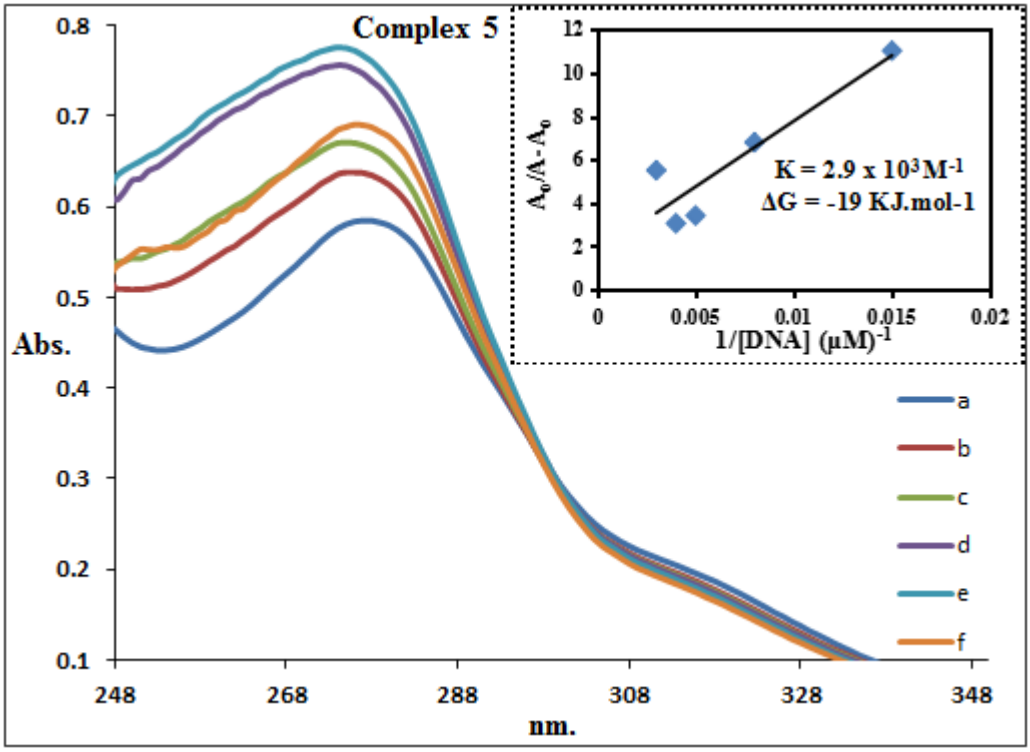



Fig. 5: Absorption spectrum of the complex 5 in the absence (a) and the presence of 65 µM (b) 126µM (c) 185 µM (d) 241 µM (e) and 294 µM (f) DNA. The arrow is focusing on the increased DNA concentration.

*DNA interaction study of complex* **5**

The complex 5 exhibits hyperchromism with hypsochromic effect of 3nm maximum at 277nm in UV region, proposing partial or non-intercalative interaction [31]. The absorption spectrum, Gibb's free energy alteration (-19 kJmol$^{-1}$) and $K_b$ (2.9 x $10^3$ $M^{-1}$) values are focused in Fig 5.

Table-1: Binding constants and Gibb's free energies data of complexes **1-5.**

| Compound no. | $K_b$ ($M^{-1}$) | -ΔG (KJ $mol^{-1}$) |
|---|---|---|
| 1 | $5.8\times10^3(\lambda_1)$ and $23.8\times10^4(\lambda_2)$ | $21.5(\lambda_1)$ and $31(\lambda_2)$ |
| 2 | $26\times 10^3$ | 25 |
| 3 | $9.7 \times 10^2$ | 17 |
| 4 | $13 \times 10^2$ | 18 |
| 5 | $2.9 \times 10^3$ | 19 |

*DNA interaction study by viscometer*

Optical photo physical probes permit essential but not efficient evidence to endorse a binding type, so the hydrodynamic measurement (viscosity) which tender to length change is viewed to be the least equivocal and critical test to elaborate a binding model in solution [36]. The ultimate increase in DNA viscosity occurs due to lengthening of DNA molecule in intercalative binding to accommodate the bound ligand between base pairs, whereas a sequential decrease in viscosity is caused in non-classical or partial intercalation because of decreased effective length upon bending in DNA [35, 36]. Fig 6(a) depicts the effects of complex 1, 2 and 5 on DNA viscosity respectively making sure the slight increase in viscosity of DNA content predicting the intercalation, predicting hypochromic establishment in of (300-350nm), (290-350nm) and (300-350nm) ranges orderly.

The partial intercalative binding was suggested for complexes 3 and 4, as the viscosity first increases and then drops, Fig 6(b). The decrease in viscosity can be assigned to aggregation in DNA molecule, which minimizes the amount of independently moving DNA molecules in solution [37].

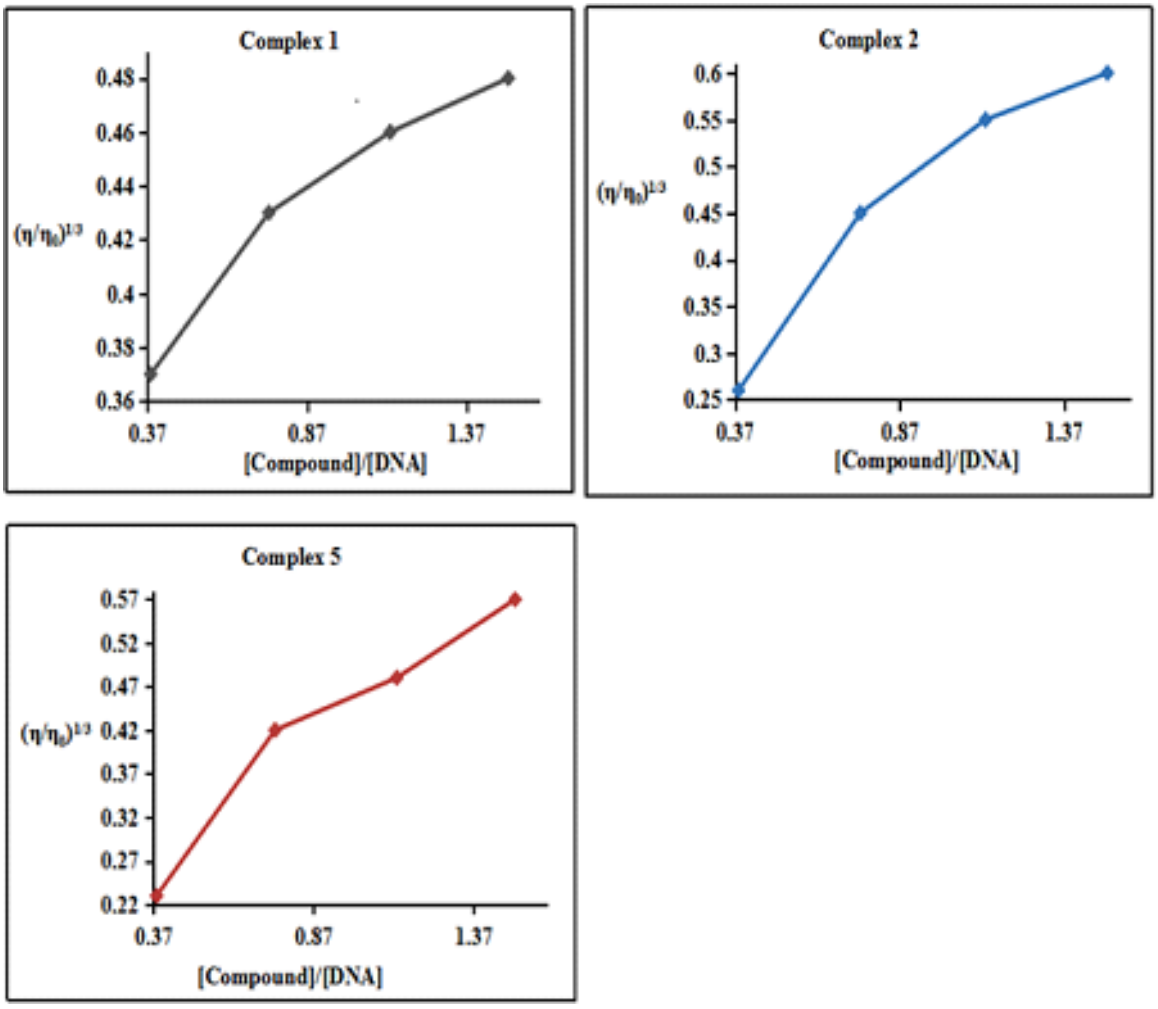



Fig. 6: (a): Results of incremental addition of complex 1, 2 & 5 on relative viscosity of DNA at room temperature 15 ± 1 °C. [DNA; 0.265mM].

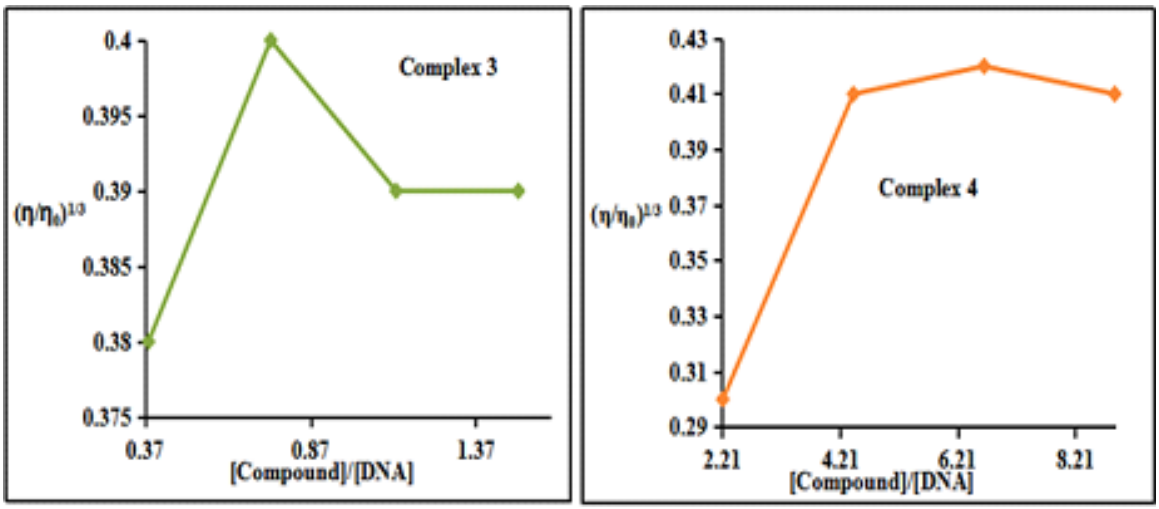



Fig. 6: (b) Consequences of increasing the concentration of complex 2 and 3 on the relative viscosity of SS-DNA at 15 ± 1 °C. [DNA] = 0.265mM (Complex 3), [DNA] = 0.045mM (Complex 4).

Table-2: Antibacterial score of Levofloxacin and Pd (II) complexes (1-5)* and standard drug.

| Compound no. | Zone of inhibition in mm | | | |
|---|---|---|---|---|
| | *Proteus vulgarus (-ve)* | *Staphylococcus Epidermides (+ve)* | *Micrococcus leutus (+ve)* | *Staphylococcus aurus (+ve)* |
| **1** | **11** | **12** | **12** | **10** |
| **2** | **12** | **11** | **8** | **10** |
| **3** | **10** | **12** | **7** | **8** |
| **4** | **9** | **15** | **10** | **10** |
| **5** | **8** | **12** | **12** | **7** |
| **Levofloxacin** | **17** | **12** | **20** | **18** |

* Numbering of the compounds is according to that given in scheme 1.

*Antibacterial evaluation*

The efficiencies of the ligands and the complexes have been tested against 3 gram positive (*Staphylococcus epidermides*, *Micrococcus luteus*, *Staphylococcus aureus*) and one gram negative (*Proteus vulgarus*) microorganisms. The results of the zone of inhibition are presented in Table 2. The agar well diffusion method [23, 24] as used in this assay. All the complexes (especially Complex 4, shows the maximum activity even higher than the standard drug in case of *Staphylococcus epidermides*) were found fairly good antibiotics showing maximum activity against *Staphylococcus epidermidis*.

*Antifungal activity*

Agar tube dilution method [23, 24], was followed for antifungal assay of complexes with reference to standard drug Terbinafine taking notice of their percent growth inhibition. The effect of the five studied complexes on colony growth of the two test fungi strains is shown in Table 3. The results revealed that almost all observed complexes shows that they are of good antifungal grade and were more predominant against *Aspergillus Niger* as compare to *Aspergillus Flavus*. The highest inhibitory effects were observed for complex 1 against *Aspergillus Niger* (90%) and *Aspergillus Flavus* (80%).

Table-3: Zone of inhibition in (mm) of antifungal assay of complexes (1-5)* and standard drug.

| Compound no. | Percent growth inhibition | |
|---|---|---|
| | *Aspergillus niger (A)* | *Aspergillus flavus (B)* |
| **1** | **90** | **80** |
| **2** | **98** | **62** |
| **3** | **90** | **48** |
| **4** | **93** | **63** |
| **5** | **88** | **60** |
| **Terbinafine** | **100** | **100** |

* Numbering of the compounds is according to that given in scheme 1.

*Molinspiration calculation*

The drug likeness parameters (known to be a composite balance regarding various properties and structural characteristics) of complexes are aggregated in the Table 4. The well-known Lipinski's rule of five which assumes a molecule to possess an orally active potential if it has no more than one intrusion to this criteria, (i) molecular weight is lower than 500a.m.u,(ii) the octanol/water partition coefficient (log P) is less than 5, (iii) not more than 5 hydrogen bond donors (OH and NH sets) and (iv) equal or less than 10 hydrogen bond acceptors (N and O atoms) [39], was chased to predict molecular properties essential for molecule pharmacokinetic in vivo. The molecular hydrophobicity measurement relays on Log P factor in rational drug designing. Also the significance of hydrophilic/lipophilic quality of drug can be explained from its intervening affects in drug bioavailability, absorption, drug-receptor interactions, molecular metabolism and toxicity. The Log P estimations of complexes were observed against the rule (as ideal ranges of lipophilicity for orally administrated drugs should be $0 \leq \log P \leq 3$). Complexes 3, 4 and 5 were found lipophilic so they will be highly toxic because the drug is predicted to be accumulated in the mitochondria [40], and will need some structural adjustment while complexes 1 and 2 were hydrophobic to some extent. The violation of rule was seen in corresponding issue of molecular weights for all tested drugs (greater than 500) and hence will not be easily transported, diffused and absorbed, while the numbers of hydrogen bond donor and acceptor atoms were well within Lipinski's limit. Total polar surface area (TPSA) being a good predictor of drug transport properties and owning to strong hydrogen bonding potential of all the tested compounds was found (21.71-75.01) well below the 160 Å limit. All the predicted compounds were flexible with enough number of rotatable bonds and hence will have oral bioavailability [40].The predicted bioactivity scores of tested complexes and standard drugs are summarized in Table 5. It is widely accepted that the drug having larger bioactivity score will have higher activity. Hence, a molecule will be substantially active if (bioactivity score > 0.00), moderately active (bioactivity score lies in between -0.50 to 0.00) and inactive if (bioactivity score < -0.50) [41]. The results discovered that the investigated compounds (particularly complex 2) can be almost of considerable biological importance and will create physiological actions by inhibiting protease, other enzymes and will produce moderate activity while interacting with GPCR and nuclear receptor ligand.

Table-4: Drug likeness features of complexes (1-5)* and standard drugs Levofloxacin and Terbinafine.

| Compound no. | milog P | TPSA | nAtoms | nON | nOHNH | n violation | n rotb | Volume | MW |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 5.58 | 21.71 | 33 | 3 | 0 | 2 | 11 | 472.83 | 612.49 |
| 2 | 5.48 | 21.71 | 34 | 3 | 0 | 2 | 12 | 489.63 | 626.52 |
| 3 | 9.50 | 3.24 | 39 | 1 | 0 | 2 | 17 | 617.23 | 700.77 |
| 4 | 8.66 | 3.24 | 34 | 1 | 0 | 2 | 11 | 505.02 | 622.58 |
| 5 | 8.40 | 12.47 | 35 | 2 | 0 | 2 | 12 | 514.01 | 638.58 |
| Levofloxacin | -0.26 | 75.01 | 26 | 7 | 1 | 0 | 2 | 311.15 | 361.37 |
| Terbinafine | 5.72 | 3.24 | 22 | 1 | 0 | 1 | 4 | 306.73 | 291.44 |

* Numbering of the compounds is according to that given in scheme 1.

Table-5: Bioactivity score of employed standard drugs and complexes (**1-5**)*

| Compound no. | GPCR ligand | Ion channel modulator | Kinase inhibitor | Nuclear receptor ligand | Protease inhibitor | Enzyme inhibitor |
|---|---|---|---|---|---|---|
| 1 | -0.09 | -0.14 | -0.07 | -0.14 | -0.01 | 0.00 |
| 2 | -0.07 | -0.05 | -0.05 | -0.19 | 0.09 | 0.06 |
| 3 | -0.04 | -0.26 | -0.21 | -0.16 | 0.02 | -0.04 |
| 4 | -0.02 | -0.11 | -0.12 | -0.09 | 0.00 | 0.02 |
| 5 | -0.02 | -0.11 | -0.05 | -0.09 | 0.00 | 0.03 |
| Levofloxacin | 0.23 | -0.14 | -0.06 | -0.13 | -0.26 | 0.35 |
| Terbinafine | 0.29 | 0.37 | 0.10 | 0.44 | 0.19 | 0.68 |

* Numbering of the compounds is according to that given in scheme 1.

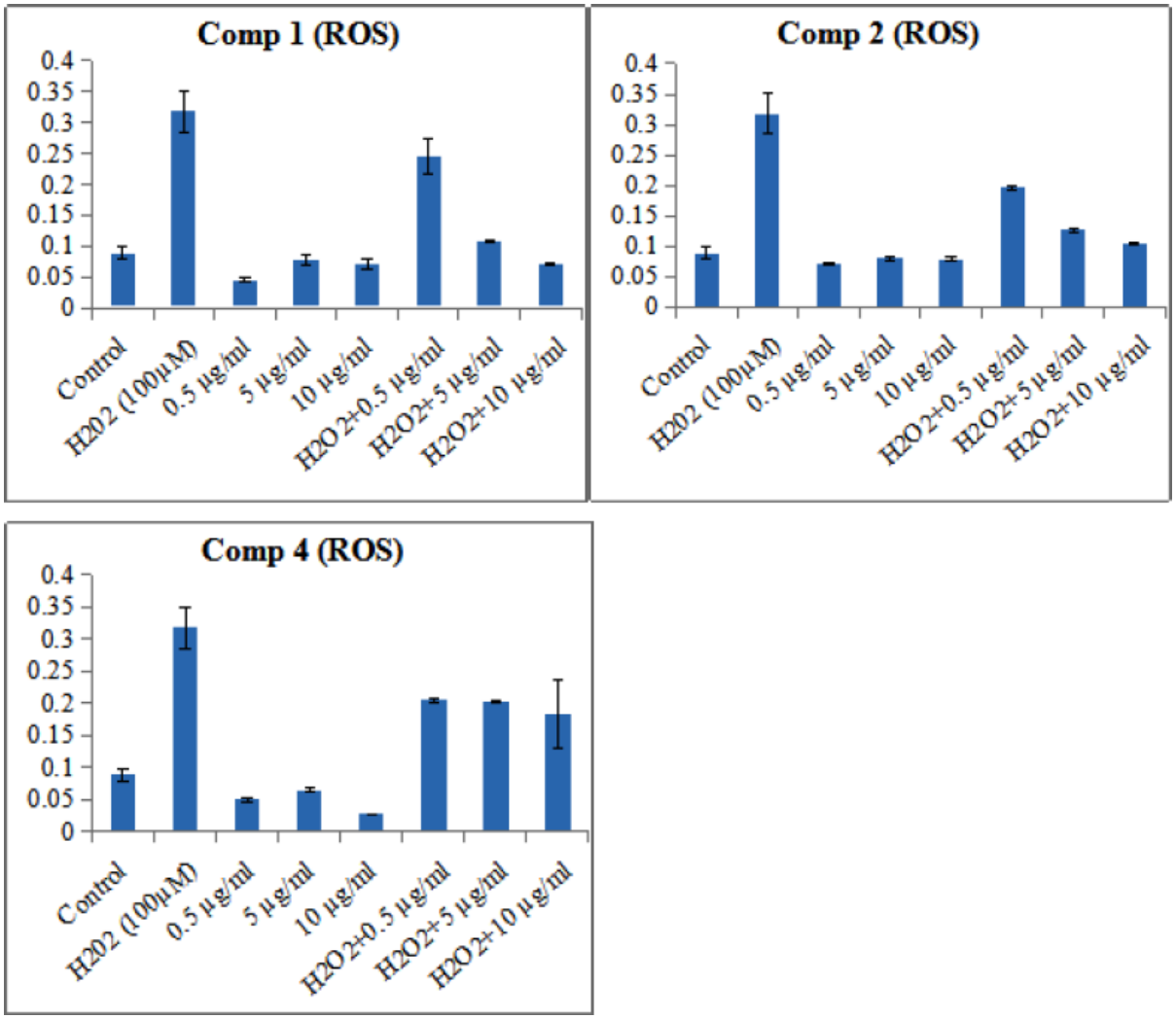



Fig. 9: a): Effects of complex 1, 2 and 4 on activity of ROS in lymphocytes. PBS = negative control, $H_2O_2$ = positive control, 0.5–10 = samples treated with (a) complex 1, 2 and 5 only (b) in presence of $H_2O_2$ (concentration in $\mu g.mL^{-1}$), activity of ROS in presence of $H_2O_2$ only, free complex and of complex + $H_2O_2$ are compared to negative control and activity of ROS in presence of free complex and of complex + $H_2O_2$ are compared to positive control.

*Bio protective power of complexes against oxidative damage induced by $H_2O_2$*

The possible benefits of antioxidants of different antioxidant components have been examined in many studies by reversing, fixing or preclusion the bad effects of oxidative stress. In this regard, the antioxidant enzyme system plays defensive role for cells against oxidative damage. We examined the ameliorating effect of mixed ligand Pd (II) complexes (1, 2 and 4) against the oxidative stress stimulated in human lymphocytes by $H_2O_2$. First, the non-genotoxic concentrations of complexes were tested in order to examine their potential to protect human DNA of pre-treated human lymphocytes objected to hydrogen peroxide. Lymphocytes without treatment were considered the negative control and those processed with hydrogen peroxide only considered the positive control. From results we propose that the studied complexes might be considered as desmutagens, are well capable to intervene mutation either by decreasing levels of DNA single-strand break up lesions through various pathways or to suppress mutations via their antioxidant properties, or they might be able to stimulate corresponding enzymes which can detoxify mutagen anterior to reach DNA [42]. The reactive oxygen species that can be superoxide anions, $H_2O_2$ or both are furnished throughout the cells during the normal aerobic metabolism and the intracellular conc. depends on both production and their removal by variety of antioxidant. Fig 9(a) shows that the concentration of ROS has been minimized to normal level by almost all the three studied complexes at 10µg/mL concentration induced by $H_2O_2$, in which complex **1** was found to be the most profound in bringing the ROS concentration even below than the normal level.

Three basic enzymes naming superoxide catalase (CAT), dismutase (SOD), and glutathione peroxidase (GPx) make up the greater contribution in antioxidant organization in mammalian cell that work in concert to detoxify superoxide anions and $H_2O_2$ in cells [43]. The results declared that pretreatment of complexes caused an increase in antioxidant enzymes activity in $H_2O_2$-challenged lymphocytes while ultimate significant decrease was observed in the activities of superoxide dismutase and catalase in $H_2O_2$ treated lymphocytes to normal control comparatively (Fig 9(b)). Also, the activities of antioxidant enzymes were importantly increased in all treated complex samples by comparing them to $H_2O_2$ control and were significantly regenerated to normal control levels being correlated with the reduction in lipid peroxides [44]. Complex **1** merged to be most efficient fraction in all complexes to bring superoxide dismutase and catalase relevant activities towards normal level

The TBARS levels approached almost the normal control in case of all the corresponding complex subjected lymphocytes exposed to $H_2O_2$ and it might because of an enhanced action of antioxidant enzymes [45]. A predicted increase in the concentration of TBARS levels was noticed in the $H_2O_2$control in equation to normal control being diminished by all treated complex samples [Fig 9(c)], reminding complex **1** of better ameliorative effect. Differences between the results of observed complexes might be due to the difference in their chemical composition.

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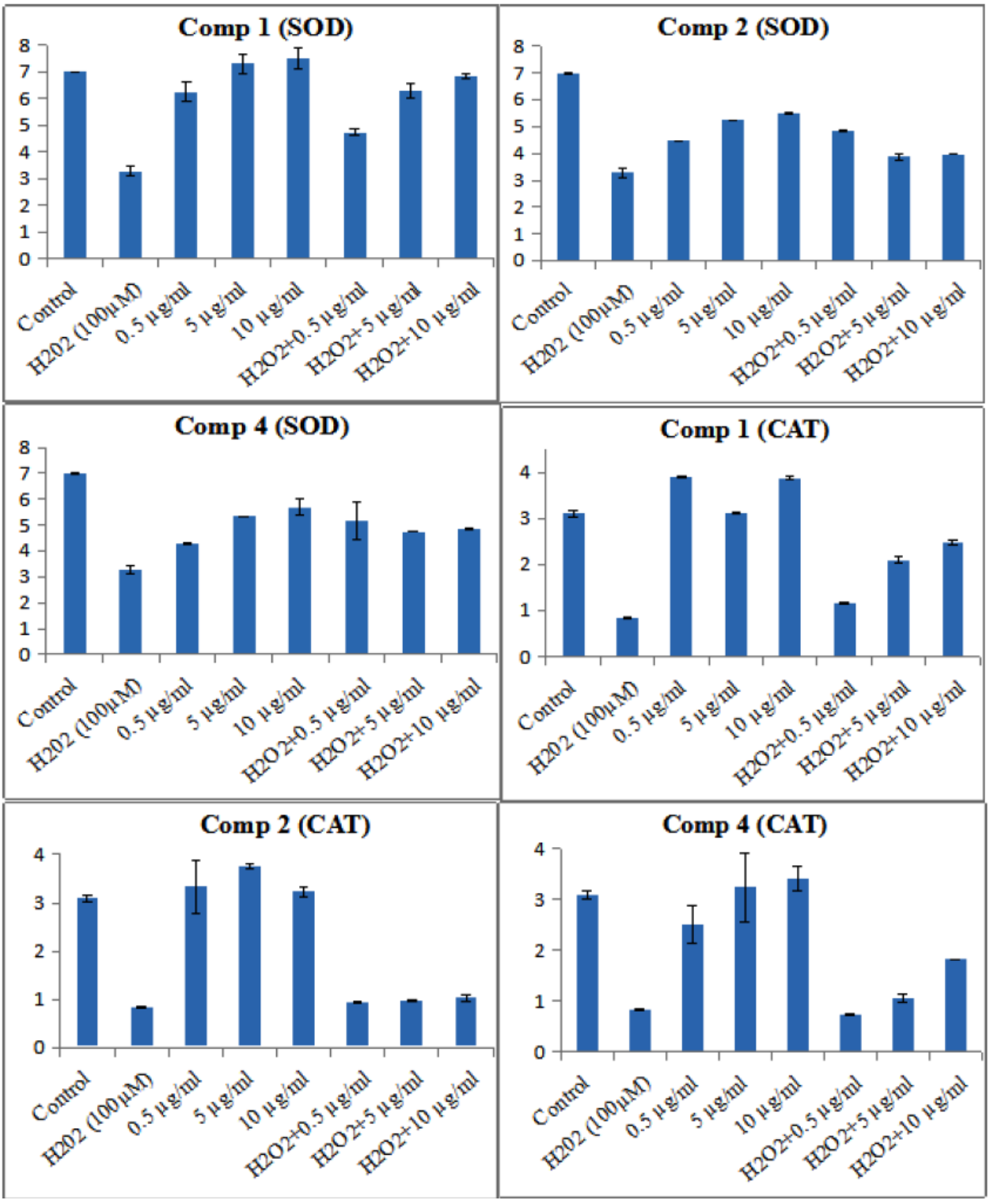


Fig. 9: (b): Effect of complexes 1, 2 and 4 on behavior of superoxide dismutase and catalase. PBS = negative control, $H_2O_2$ = positive control, 0.5–10 = samples tempered with (a) complex 1, 2 and 5 only (b) in presence of $H_2O_2$ (conc. in $\mu g.mL^{-1}$), activity of SOD and CAT in presence of $H_2O_2$ only, free complex and of complex + $H_2O_2$ samples were compared to negative control while the activity of SOD and CAT in presence of free complex and of complex + $H_2O_2$ were compared to positive control.

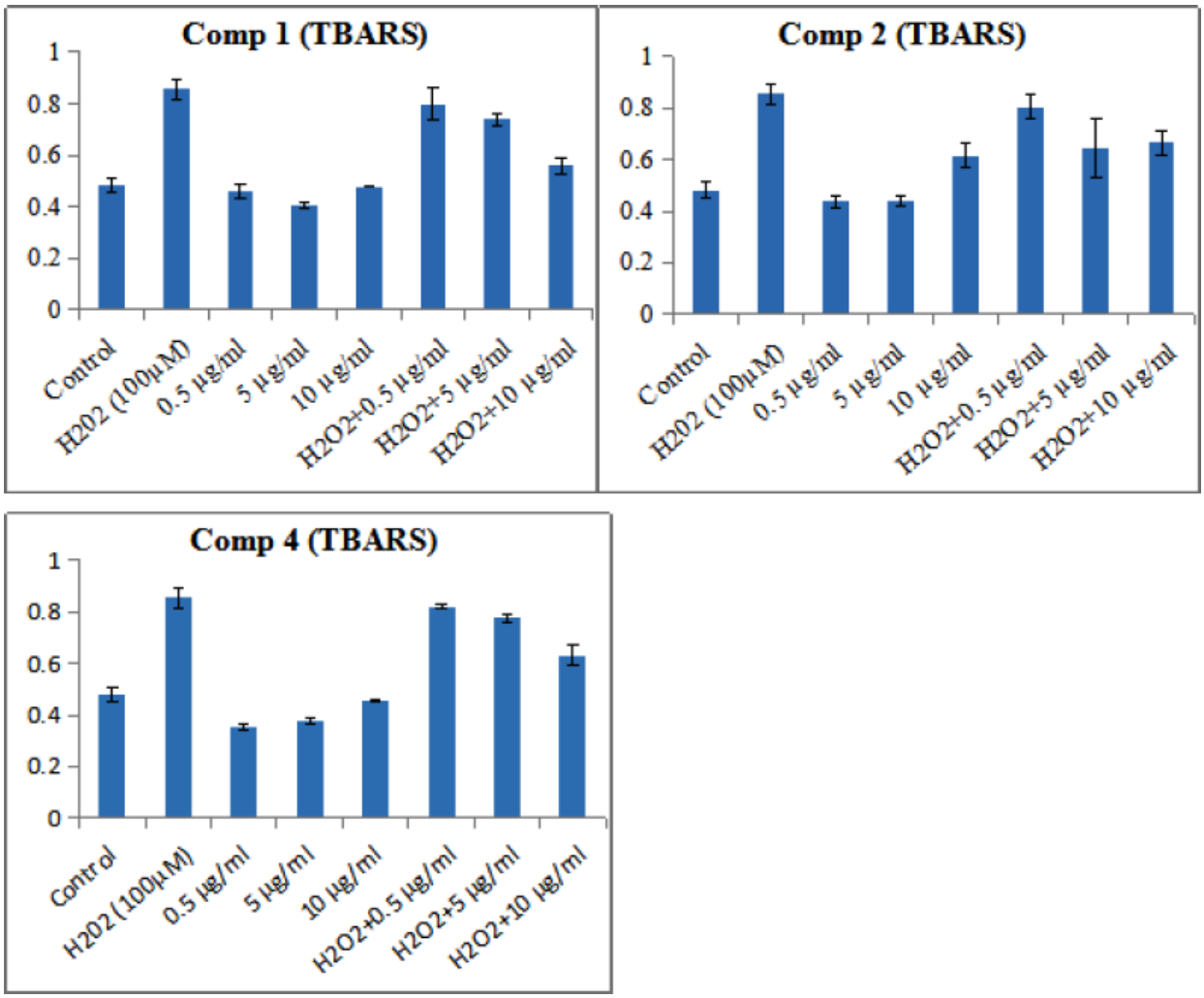


Fig. 9: (c): Effect of complexes 1, 2 and 4 on the levels of TBARS. Legend for the x axis: PBS = negative or normal control, $H_2O_2$ = positive control, 0.5–10 = samples treated with (a) complex 1, 2 and 5 only (b) in presence of $H_2O_2$ (concentration in $\mu g.mL^{-1}$), activity of TBARS in presence of $H_2O_2$ only, free complex and of complex + $H_2O_2$ are compared to negative control and activity of TBARS in presence of free complex and of complex + $H_2O_2$ are compared to positive control.

## Conclusion

All complexes (1-5) flourish certain kinds of interaction with DNA that will in turn result cleavage or improper functioning of DNA. Groove binding for complexes (2 and 5), intercalation (3 and 4) while 1 appeared to own both these interactions at particular wavelengths with decent $K_b$ and $\Delta G$ grades. Complex 1revealed strong interaction with DNA molecule, higher $K_b$ values both at $\lambda_1$ and $\lambda_2$, strong bio protective efficiency and sound anti-fungal and antibacterial conduct in regard of implied strains. Complex 4 testified maximum zone of inhibition against the Staphylococcus Epidermis bacteria, even higher than Levofloxacin. The Molinspiration forecasting concluded all complexes of biological importance attributing satisfactory molecular properties of drug likeness exclude log P values of complex 3, 4 and 5. Complex 2 ascertained to be the one of most bright on behalf of its bioactivity score holding higher extent of protease and other enzyme inhibition comparatively to standard drug Levofloxacin. Complexes (1, 2 and 4) are capable to diminish the negative impacts induced in human lymphocytes due to various reasons and can be actually useful in pharmacology and medicine.

## Acknowledgement

The authors are highly thankful to the higher education commission (HEC) of Pakistan, for providing the financial assistance (Project number:

10669/KPK/NRPU/R&D/HEC/2017) for the performed research work.

**References**


1 J. Reedijk, J. Teuben and B. Lippert, *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*, B, Lippert, Verlag Helvetica Chimica Acta/WILEY-VCH, Zürich, Switzerland/Weinheim, Germany, p. 339 (1999).

2 G. D'addario, M. Pintilie, N. B. Leighl, R. Feld, T. Cerny and F. A. Shepherd, Platinum-based versus non-platinum-based chemotherapy in advanced non-small-cell lung cancer: a meta-analysis of the published literature, *J. Clin. Oncol.*, **23**, 2926 (2005).

3 J. H. Hoeijmakers, DNA damage, aging, and cancer, *N. Eng. J. Med.*, **361**, 1475 (2009).

4 4. E. C. Long and J. K. Barton, On demonstrating DNA intercalation, *Acc. Chem. Res.*, **23**, 271 (1990).

5 R. Palchaudhuri and P. J. Hergenrother, DNA as a target for anticancer compounds: methods to determine the mode of binding and the mechanism of action, *Curr. Opin. Biotechnol.*, **18**, 497 (2007).

6 M. Sirajuddin, N. Uddin, S. Ali and M. N. Tahir, Potential bioactive Schiff base compounds: synthesis, characterization, X-ray structures, biological screenings and interaction with Salmon sperm DNA, *Spectrochim. Acta A Mol. Biomol Spectrosc.*, **116**, 111 (2013).

7 D. Fregona, L. Giovagnini, L. Ronconi, C. Marzano, A. Trevisan, S. Sitran, B. Biondi and F. Bordin, Pt (II) and Pd (II) derivatives of ter-butylsarcosinedithiocarbamate: synthesis, chemical and biological characterization and in vitro nephrotoxicity. *J. Inorg. Biochem.*, 93, 181 (2003).

8 S. Hidaka, M. Tsuruoka, T. Funakoshi, H. Shimada, M. Kiyozumi and S. Kojima, Protective effects of dithiocarbamates against renal toxicity of cis-diamminedichloroplatinum in rats, *Ren. Fail.*, **16**, 337 (1994).

9 G. Hogarth, Transition metal dithiocarbamates: 1978–2003. *Prog. Inorg. Chem.*, **53**, 71 (2005).

10 H. Mansouri-Torshizi, I. Mahboube, A. Divsalar and A.-A. Saboury, 2, 2′-Bipyridinebutyldithiocarbamatoplatinum (II) and palladium (II) complexes: synthesis, characterization, cytotoxicity, and rich DNA-binding studies, *Bioorg. Med. Chem.*, **16**, 9616 (2008).

11 L. Messori, F. Abbate, G. Marcon, P. Orioli, M. Fontani, E. Mini, T. Mazzei, S. Carotti, T. O'Connell and P. Zanello, Gold (III) complexes as potential antitumor agents: solution chemistry and cytotoxic properties of some selected gold (III) compounds, *J. Med. Chem.*, **43**, 3541 (2000).

12 A. Pasini, G. D'Alfonso, C. Manzotti, M. Moret, S. Spinelli and M. Valsecchi, Cytotoxic diamine-platinum (II) complexes with methylsulfinyl carboxylates as the leaving groups, Synthesis, characterization, and reactivity toward chloride ions, 5'-GMP, and 9-methylguanine, *Inorg. Chem.*, **33**, 4140 (1994).

13 V. Alverdi, L. Giovagnini, C. Marzano, R. Seraglia, F. Bettio, S. Sitran, R. Graziani and D. Fregona, Characterization studies and cytotoxicity assays of Pt (II) and Pd (II) dithiocarbamate complexes by means of FT-IR, NMR spectroscopy and mass spectrometry. *J. Inorg. Biochem.*, **98**, 1117 (2004).

14 M. P. Hacker, E. B. Douple and I. H.Krakoff, *Platinum Coordination Complexes in Cancer Chemotherapy: Proceedings of the Fourth International Symposium on Platinum Coordination Complexes in Cancer Chemotherapy Convened in Burlington, Vermont by the Vermont Regional Cancer Center and the Norris Cotton Cancer Center, June 22–24, 1983*. Springer Science & Business Media, Vol. **17** (2012).

15 H. Khan, *Complexation, Characterization and biological activities of NPS donor ligands with palladium*, Quaid-i-Azam University, Islamabad, (2011).

16 N. Farrell, Current status of structure-activity relationships of platinum anticancer drugs: activation of the trans geometry, *Met. Ions Biol. Syst.*, **32**, 603 (1996).

17 H. Khan, A. Badshah, G. Murtaz, M. Said, C. Neuhausen, M. Todorova, B.J Jean-Claude, and I.S. Butler, 2011. Synthesis, characterization and anticancer studies of mixed ligand dithiocarbamate palladium (II) complexes. *European journal of medicinal chemistry*, *46*(9), pp.4071-4077.

18 C. Chai and W. Armarego, *Purification of laboratory chemicals*. Butterworth-Heinemann, (2003).

19 C. Sastri, D. Eswaramoorthy, L. Giribabu and B. G.Maiya, DNA interactions of new mixed-ligand complexes of cobalt (III) and nickel (II) that incorporate modified phenanthroline ligands, *J. Inorg. Biochem.*, **94**, 138 (2003).

20 F. Asghar, A. Badshah, A. Shah, M. K. Rauf, M. I. Ali, M. N. Tahir, E. Nosheen and R. Qureshi, Synthesis, characterization and DNA binding studies of organoantimony (V)

ferrocenyl benzoates, *J. Organomet. Chem.,* **717**, 1 (2012).

21 F. A. Shah, M. Sirajuddin, S. Ali, S. M. Abbas, M. N. Tahir and C. Rizzoli, Synthesis, spectroscopic characterization, X-ray structure and biological screenings of organotin (IV) 3-[(3, 5-dichlorophenylamido)] propanoates, *Inorg. Chim. Acta.*, **400**, 159 (2013).

22 B. Meyer, N. Ferrigni, J. Putnam, L. Jacobsen, D. j. Nichols and J. McLaughlin, Brine shrimp: a convenient general bioassay for active plant constituents, *Planta Med.,* **45**, 31 (1982).

23 M. Tariq, N. Muhammad, M. Sirajuddin, S. Ali, N. A. Shah, N. Khalid, M. N. Tahir and M. R. Khan, Synthesis, spectroscopic characterization, X-ray structures, biological screenings, DNA interaction study and catalytic activity of organotin (IV) 3-(4-flourophenyl)-2-methylacrylic acid derivatives, *J. Organomet. Chem.,* **723**, 79 (2013).

24 D. F. Veber, S. R. Johnson, H.-Y. Cheng, B. R. Smith, K. W. Ward and K. D. Kopple, Molecular properties that influence the oral bioavailability of drug candidates, *J. Med. Chem.,* **45**, 2615 (2002).

25 M. Noroozi, W. J. Angerson and M. Lean, Effects of flavonoids and vitamin C on oxidative DNA damage to human lymphocytes, *Am. J. Clin. Nutr.,* **67**, 1210 (1998).

26 S. Marklund and G. Marklund, Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase, *The FEBS J.*, **47**, 469 (1974).

27 Z.-H. Li, J. Velisek, V. Zlabek, R. Grabic, J. Machova, J. Kolarova and T. Randak, , Hepatic antioxidant status and hematological parameters in rainbow trout, Oncorhynchus mykiss, after chronic exposure to carbamazepine, *Chem. Biol. Interact.,* **183**, 98 (2010).

28 A. K. Sinha, Colorimetric assay of catalase, *Anal. Biochem.,* **47**, 389 (1972).

29 I. Hayashi, Y. Morishita, K. Imai, M. Nakamura, K. Nakachi and T. Hayashi, High-throughput spectrophotometric assay of reactive oxygen species in serum, *Mutat Res Genet Toxicol Environ Mutagen.,* **631**, 55 (2007).

30 M. Sirajuddin, S. Ali and M. N. Tahir, Pharmacological investigation of mono-, di-and tri-organotin (IV) derivatives of carbodithioates: Design, spectroscopic characterization, interaction with SS-DNA and POM analyses, *Inorg. Chim. Acta.,* **439**, 145 (2016).

31 K. Chichetu, Characterization, *DNA Binding and Cleavage Activities of New Prodigiosin and Tambjamine Analogues and Their Cu 2+ and Zn 2+ Complexes*, Portland State University, (2015).

32 X. Sheng, X. Guo, X.-M. Lu, G.-Y. Lu, Y. Shao, F. Liu and Q. Xu, DNA binding, cleavage, and cytotoxic activity of the preorganized dinuclear zinc (II) complex of triazacyclononane derivatives, *Bioconjugate Chem.,* **19**, 490 (2008) .

33 C. Rajarajeswari, M. Ganeshpandian, M. Palaniandavar, A. Riyasdeen and A. Akbarsha, Mixed ligand copper (II) complexes of 1, 10-phenanthroline with tridentate phenolate/pyridyl/(benz) imidazolyl Schiff base ligands: Covalent vs non-covalent DNA binding, DNA cleavage and cytotoxicity, *J. Inorg. Biochem.,* **140**, 255 (2014).

34 N. Shahabadi, S. Kashanian, M. Mahdavi and N. Sourinejad, DNA interaction and DNA cleavage studies of a new platinum (II) complex containing aliphatic and aromatic dinitrogen ligands, *Bioinorg Chem Appl.,* **2011** (2011).

35 M. Sirajuddin, S. Ali and A. Badshah, Drug–DNA interactions and their study by UV–Visible, fluorescence spectroscopies and cyclic voltametry, *J. Photochem. Photobiol., B.*, **124,** 1 (2013).

36 L. Jia, P. Jiang, J. Xu, Z.-y. Hao, X.-m. Xu, L.-h. Chen, J.-c. Wu, N. Tang, Q. Wang and J. J. Vittal, Synthesis, crystal structures, DNA-binding properties, cytotoxic and antioxidation activities of several new ternary copper (II) complexes of N, N′-(p-xylylene) di-alanine acid and 1, 10-phenanthroline, *Inorg. Chim. Acta.*, **363**, 855 (2010).

37 J. Liu, T.-B. Lu, H. Li, Q.-L. Zhang, L.-N. Ji, T.-X. Zhang, L.-H. Qu and H. Zhou, DNA-binding and cleavage studies of a dinuclear copper (II) complex with a 26-membered hexaazamacrocycle, *Transition Met. Chem.,* **27**, 686 (2002).

38 S. Arikan, V. Paetznick and J. H. Rex, Comparative evaluation of disk diffusion with microdilution assay in susceptibility testing of caspofungin against Aspergillus and Fusarium isolates, *Antimicrob. Agents Chemother.*, **46**, 3084 (2002).

39 C. A. Lipinski, F. Lombardo, B. W. Dominy and P. J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, *Adv. Drug Delivery Rev.*, ***23***, 3 (1997).

40 E. Abas, R. P. Martinez, D. A. Ramírez, A. R. Dieguez, M. Laguna and L. Grasa, New selective thiolate gold (I) complexes inhibit proliferation of different human cancer cells

and induce apoptosis in primary cultures of mouse colon tumors, *RSC Dalton Trans.* (2020).

41 A. Verma, Lead finding from Phyllanthus debelis with hepatoprotective potentials, *Asian Pac. J. Trop. Biomed.*, **2**, S1735 (2012).

42 S. ib Bhattacharya, Natural antimutagens: a review, *Res. J. Med. Plant.*, **5**, 116 (2011).

43 M. Hashim, S. Lincy, V. M. Remya, M. Teena and L. Anila, Effect of polyphenolic compounds from Coriandrum sativum on $H_2O_2$-induced oxidative stress in human lymphocytes, *Food chem.*, **92**, 653 (2005).

44 I. Lang, G. Deak, G. Müzes, L. Pronai and J. Feher, Effect of the natural bioflavonoid antioxidant silymarin on superoxide dismutase (SOD) activity and expression in vitro, *Biotechnol. ther.*, **4**, 263 (1993).

45 B. Halliwell and S. Chirico, Lipid peroxidation: its mechanism, measurement, and significance, *Am. J. Clin. Nutr.*, **57**, 715S (1993).