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# Design and synthesis of enantiomeric (R)- and (S)-copper(II) and diorganotin(IV)-based antitumor agents: Their *in vitro* DNA binding profile, cleavage efficiency and cytotoxicity studies

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ABSTRACT

New chiral reduced Schiff base ligands (R)/(S)-2-(2-hydroxy-1-phenylethylaminomethyl)phenol (**L**), (R)/(S)-2-(benzylamino)-2-phenylethanol (**L′**) and their Cu(II)/organotin(IV) complexes (**1**–**4**) were synthesized and thoroughly characterized. Preliminary *in vitro* DNA binding studies of (R)- and (S)-enantiomeric pairs of ligands **L**, **L′** and complexes **1**–**4** were carried out employing UV–vis, fluorescence and circular dichroic techniques to evaluate their enantioselective DNA binding potential, thereby to act as antitumor chemotherapeutic drug entities. The observations demonstrated that S-enantiomer of Cu(II) complex, **1** binds more avidly to DNA in comparison to its R-enantiomeric form and organotin(IV) complex **2**. This was further established by $K_b$ and $K_{sv}$ values of ligands **L** and **L′** and (S)-/(R)-**1**–**4** complexes, which demonstrated multifold increase in case of S-enantiomer of copper complex **1** in comparison to its R-enantiomeric form. This clearly demonstrates the chiral preference of S-enantiomer over R-enantiomer and its potency to act as a chemotherapeutic agent. Cleavage studies of **1**–**4** with pBR322 plasmid DNA were carried out, noticeably, S-enantiomer of complex **1** exhibited effective DNA cleavage efficiency in absence of external agents. The cytotoxicity of ligands **L** and **L′** and (S)-/(R)-**1**–**4** complexes was examined on a panel of 19 human tumor cell lines of different histological origins by SRB assay. In the both the cases, the S-enantiomer of complex **1** and **3** revealed remarkably good cytotoxic activity ($GI_{50}$ values <10) against T24 (Urinary Bladder), DU145 (Prostate), U373MG (Astrocytoma) and HCT15, SW620 (Colon) clearly underlining the influence of enantiomeric discrimination. Interestingly, ligands **L**, **L′** and rest of the complexes demonstrated moderate cytotoxic activity ($GI_{50}$ values <40).



## 1. Introduction

The metal complex-DNA interactions are of paramount importance for the development of new metal-based chemotherapeutic drugs [1]. Understanding the binding modes *viz*., covalent or non-covalent play a pronounced influence on the efficacy of a drug or chemical entity as a potential drug; among the factors governing the binding modes, the most significant and fascinating criteria for drug design is the molecular shape-chiral preference or enantioselectivity. Those complexes that fit best against the DNA helical structure display the highest binding affinity [2]. The structure–function relationship in nature is so powerful that, when a functional disorder is manifested in the form of a disease, it can be handled in many cases by using a molecule of specific chiral structure [3]. The use of stereochemistry can give clear insight into the mechanism of action allowing the discrimination between unspecific interactions, which are common to both enantiomers and specific contacts that give rise to enantioselectivity. This approach includes the possibility of introducing chiral centers in either metal complexes or chelating ligand scaffold and even using achiral fragments to obtain chiral networks [4]. Owing to this, there is a huge demand for enantiomeric pharmaceuticals that have proven to be more efficacious, exhibiting less systemic toxicity and also possess high specificity. Chiral molecules therefore, play a critical role in the exploitation of three-dimensional space at the target site and regulate stereoselectively in a highly organized fashion and were assumed to be therapeutically active as most of the biotargets of drugs are chiral in nature.

To design efficient metal-based anticancer strategy, the chemical framework and ligand donor atom set is of crucial importance since it can modulate the hard/soft properties of the metal, the lipophilic/hydrophilic balance of the resulting complexes, and their solubility in extracellular fluids as well as the ability to permeate

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the bilayer lipid membrane. Our approach has been to design complexes of reduced Schiff base of (S)/(R)-2-phenylglycinol with salicylaldehye/benzaldehyde which is an attractive chiral ligand scaffold and its metal complexes could interact enantioselectively and stereoselectively with the ultimate genetic DNA target. Upon binding to DNA, these conformations give rise to the preferential binding of one conformation over another, which is termed as enantioselectivity [5], because the DNA double helix also has two possible conformations; right-handed B- and A-form DNA, and left-handed Z-form DNA. The best way to interact with nature is by using chiral molecules [6]. The selective binding of chiral molecules is regiodirectional and in pharmaceuticals, one chiral form is more dominant than another [7].

Copper complexes are known to have a broad spectrum of biological action as anti-inflammatory, antimicrobial and antitumor agents [8]. Copper-based complexes have been investigated on the assumption that endogenous metals may be less toxic for normal cells with respect to cancer cells. It has been further shown that copper accumulates in tumor due to selective permeability of the cancer cell membranes to copper compounds; thus, a number of copper complexes have been screened for anticancer activity, and some of them were found to be active *in vivo* and *in vitro* [9,10].

On the other hand, organotin compounds display a wide spectrum of antitumor activity; the cytotoxicity induced by the different kinds of organotins has been related to several mechanisms. Recently, a large number of organotin derivatives have been prepared and were tested *in vitro* and *in vivo*, first against murine leukemia cell lines (P388 and L1210) and later against different panels of human cell lines [11]. The diorganotin(IV) complexes act as an anchoring site by specifically binding to phosphate group of DNA backbone [12].

In continuation to our search for efficient metal-based therapeutic molecular entities for the treatment of cancer [13], herein, we describe the design, synthesis of (R)- and (S)- Cu(II) and diorganotin(IV) complexes **1**–**4** derived from chiral bioactive precursor ligands, **L** (2-(2-hydroxy-1-phenylethylaminomethyl)phenol and **L′** (2-(benzylamino)-2-phenylethanol). The complexes **1**–**4** were examined for chemotherapeutic drug potential; validated by DNA binding, cleavage efficiency and cytotoxicity activity on a panel of different human cell lines. Our results have demonstrated that Cu(II) based S-enantiomers of complexes **1** and **3** revealed good anticancer compound, and warrant further *in vivo* investigation.

## 2. Experimental section

### 2.1. Materials

All reagents were of the best commercial grade and were used without further purification. (S)/(R)-2-phenylglycinol, $(CH_3)_2SnCl_2$, (Sigma), Salicylaldehyde (Alfa Aesar), $NaBH_4$, $CuCl_2$ .$2H_2O$, Tris-(hydroxymethyl)aminomethane-HCl (Tris–HCl) (E. Merck) were used as received. Disodium salt of Calf thymus DNA (CT DNA) was purchased from Sigma chemical Co and was stored at 4 °C. 6× loading dye (Fermental Life Science), agarose, ascorbic acid, sodium azide ($NaN_3$), DMSO, superoxide dismutase (SOD), methyl green, DAPI, mercaptopropionic acid (MPA) (Sigma–Aldrich) and Super coiled plasmid DNA pBR322 (Genei) were utilized as received.

### 2.2. Physical measurements

Carbon, hydrogen and nitrogen contents were determined using Carlo Erba Analyzer Model 1108. Molar conductance was measured at room temperature on a Digisun Electronic conductivity Bridge. Fourier-transform infrared (FTIR) spectra were recorded on an Interspec 2020 FTIR spectrometer. Electronic spectra were recorded on UV-1700 PharmaSpec UV–vis (Shimadzu) and Lambda 25, UV–vis spectrophotometers. Data were reported in $\lambda_{max}$/nm. CD measurements were carried out with a Jasco spectropolarimeter (J-815) equipped with a Jasco Peltier-type temperature controller (PTC-424S/15).The EPR spectra of the Cu(II) complexes were acquired on a Varian E 112 spectrometer using X-band frequency (9.1 GHz) at liquid nitrogen temperature in solid state. The $^1H$ and $^{13}C$ NMR spectra were obtained on a Bruker DRX-400 spectrometer. Optical rotations of chiral complexes were determined on a Polarimeter Rudolf Autopol III at 25 °C using the sodium D line in DMSO. ESI–MS spectra were recorded on Micromass Quattro II triple quadrupole mass spectrometer.

DNA binding experiments which include absorption spectral titrations, luminescence, circular dichroic experiments and DNA cleavage conformed to the standard methods [14] and practices previously adopted by our laboratory [15]. While measuring the absorption spectra an equal amount of DNA was added to both the compound solution and the reference solution to eliminate the absorbance of the DNA itself.

### 2.3. Anticancer activity

The cell lines used for *in vitro* antitumor screening activity were, MIAPaCa-2 (Pancreatic), MCF-7, ZR-75-1 (Breast), SiHa (Uterine Cervix), Colo205, HCT15, SW620 (Colon), HOP-62, A549 (Lung), DWD (Oral), K562 (Leukemia), DU145, PC-3 (Prostate), A498 (Renal Cell), A2780 (Ovary), T24 (Urinary Bladder), U373MG (Astrocytoma), HT29 (colon adenocarcinoma grade II cell line) and HeLa (Epithelial Carcinoma). G-adriamycin, standard anticancer drug was taken as control. These human malignant cell lines were procured and grown in RPMI-1640 medium supplemented with 10% Fetal Bovine Serum (FBS) and antibiotics to study growth pattern of these cells. The proliferation of the cells upon treatment with chemotherapy was determined using the Sulphorhodamine-B (SRB) semi automated assay [16]. The dose response parameters such as growth inhibition 50% ($GI_{50}$), total growth inhibition (TGI) and lethal concentration 50% ($LC_{50}$) were calculated. $GI_{50}$ is the concentration of drug required to decrease the cell growth to 50%, compared with that of the untreated cell number. TGI is the concentration of drug required to decrease the cell growth to 100%, compared with that of the untreated cell number, during drug incubation. $LC_{50}$ is the concentration of drug required to decrease the cell growth by 50% of the initial cell number prior to the drug incubation. Cells were seeded in 96 well plates at an appropriate cell density to give optical density in the linear range (from 0.5 to 1.8) and were incubated at 37 °C in $CO_2$ incubator for 24 h. Stock solutions of the complexes were prepared as 100 mg/mL in DMSO and four dilutions i.e. 10 μL, 20 μL, 40 μL, 80 μL, in triplicates were tested, each well receiving 90 μL of cell suspension and 10 μL of the drug solution. Appropriate positive control (Adriamycin) and vehicle controls were also run. The plates with cells were incubated in $CO_2$ incubator with 5% $CO_2$ for 24 h followed by addition of drug. The plates were incubated further for 48 h. Termination of experiment was done by gently layering the cells with 50 μL of chilled 30% TCA (in case of adherent cells) and 50% TCA (in case of suspension cell lines) for cell fixation and kept at 4 °C for 1 h. Plates were stained with 50 μL of 0.4% SRB for 20 min. The bound SRB was eluted by adding 100 μL 10 mM Tris (pH 10.5) to each of the wells. The absorbance was read at 540 nm with 690 nm as reference wave length. All experiments were repeated 3 times.

### 2.4. Synthesis

#### 2.4.1. (S)- and (R)-2-(2-hydroxy-1-phenylethylaminomethyl)phenol, (L)

The reduced Schiff base was prepared by the modification of the procedure reported earlier [17] and was carried out by the reduction of Schiff base with sodium borohydride as described below: