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Original article

Synthesis and characterization of Cu(II)-based anticancer chemotherapeutic agent targeting topoisomerase Iα: *In vitro* DNA binding, pBR322 cleavage, molecular docking studies and cytotoxicity against human cancer cell lines

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ABSTRACT

New metal-based anticancer chemotherapeutic drug candidates [Cu(phen)**L**]$(NO_3)_2$ (**1**) and [Zn(phen)**L**]$(NO_3)_2$ (**2**) were synthesized from ligand **L** (derived from pharmacophore scaffold barbituric acid and pyrazole). *In vitro* DNA binding studies of the **L**, **1** and **2** were carried out by various biophysical techniques revealing electrostatic mode. Complex **1** cleaves pBR322 DNA *via* oxidative pathway and recognizes major groove of DNA double helix. The molecular docking study was carried out to ascertain the mode of action towards the molecular target DNA and enzymes. The complex **1** exhibited remarkably good anticancer activity on a panel of human cancer cell lines (GI_{50} values < 10 μg/ml), and to elucidate the mechanism of cancer inhibition, Topo-I enzymatic activity was carried out.

1. Introduction

Cancer, a type of malignant growth or tumor, caused by abnormal and uncontrolled cell division is the leading cause of mortality globally. In a recent report in Nature, cancer has surpassed mortality incidence predominating all other health concerns, *viz.*, cardiovascular diseases, cerebrovascular diseases, HIV/AIDS, lower respiratory infection including pneumonia, malaria, cirrhosis and as well as road accidents [1]. It can be cured by preventing the rapid proliferation of cancer cells for which the replication of DNA is to be arrested. Cancer chemotherapy based on metallotherapeutic drugs has gained momentum after the fortuitous discovery of *cis*-diamminedichloroplatinum (II), cisplatin. However, despite its remarkable success for treating solid malignancies, there are well-known drawbacks associated with the platinum drugs such as systemic toxicity, intrinsic acquired drug resistance and patient compliance, which limit their uses [2]. To circumvent such issues, extensive efforts are being made for the development of new metal-based anticancer drugs having maximal curative potential and minimal side effects. Metal-based drugs, in particular, transition metal complexes provides an excellent platform for the rational design of drug candidates, owing to the fact that metal can coordinate ligands in a precise three dimensional configuration, thus allowing: (a) synergistic effects of the ligands and the coordination residue, (b) the tailoring/tuning of the molecule to recognize and interact with specific biological target, (c) protection from the enzymatic degradation of the drug, (d) endogenously biocompatible metal ions present in the living system [3].

There is a considerable interest in the interaction between small molecules and nucleic-acid, not only in the understanding of biological processes such as gene transcription, mutagenesis and carcinogenesis, etc but also in the elucidation of the mechanisms of anti-cancer drugs and the screening of DNA targeted drugs [4]. Many molecules exert their anticancer activities by binding with DNA, thereby altering DNA replication and inhibiting the growth of tumor cells. Moreover, telomerase inhibition has been identified as an attractive target for cancer chemotherapy with the potential for

Abbreviations: UV–vis, UV–visible; CT DNA, Calf thymus DNA; Tris, Tris(hydroxymethyl)aminomethane; EB, ethidium bromide; Phen, 1,10-phenanthroline; Barb, barbituric acid; **L**, 2,4,6 tri(1*H*-pyrazol-1-yl)pyrimidine.

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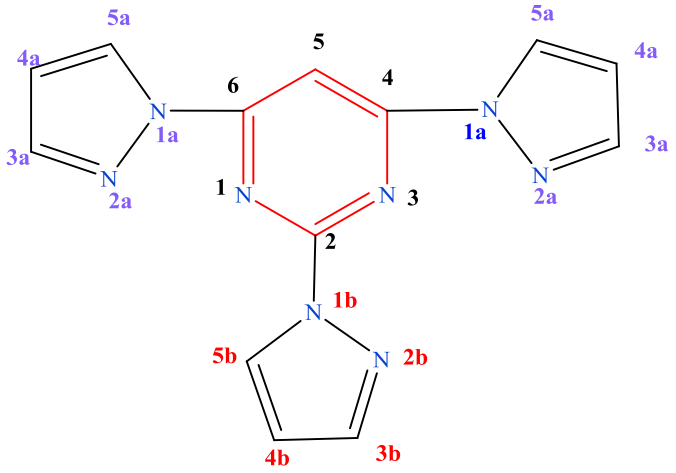


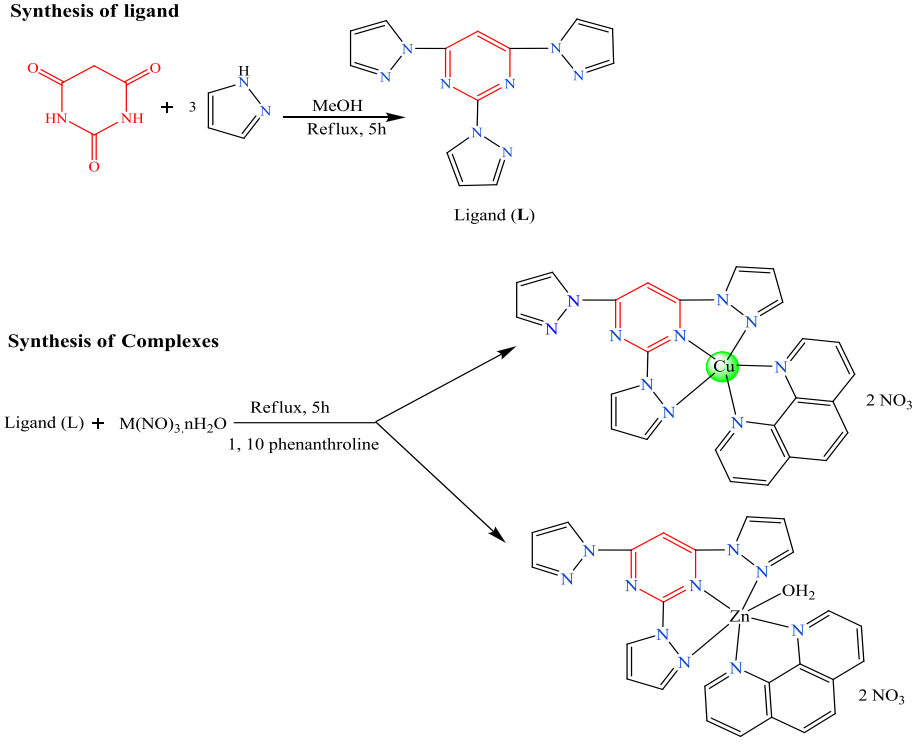
Fig. 1. Molecular structure of ligand (**L**).

selective toxicity for cancer cells over normal ones. Therefore, ligands that stabilize G-quadruplexes DNA are of particular interest because of their possible roles in the inhibition of telomerase activity [5,6]. Similarly, DNA topoisomerases governs the topology of DNA inside cells and are involved in vital cellular processes. Literature revealed that selective DNA–Topo I inhibitors such as camptothecin (anticancer drug), Hoechst 33258 and bleomycin have been approved as antineoplastic and antiproliferative agents [7]. Therefore, topoisomerases have been established as effective chemotherapeutic targets for many antitumor drugs because they can cause permanent DNA damage and can lead to cell cycle arrest and/or cell death by apoptosis [8].

It is well known that heterocyclic compounds containing the pyrazole moiety are gaining immense interest owing to their biological and pharmacological properties *viz*. antimicrobial, antiviral and anticancer, ACE (angiotensin-converting-enzyme) inhibitory, anti-inflammatory activities [9]. On the other hand, barbiturates are clinically used drugs for many ailments as anesthetics, for insomnia, and epilepsy. Moreover, among the Cu(II) complexes explored so far, considerable attention has been focused on 1,10-phenanthroline Cu(II) complexes due to their high nucleolytic efficiency and numerous biological activities such as antitumor, antimicrobial, etc. Several Cu(II) complexes have been described as DNA cleaving agents and the best studied example is Sigman's 1,10-phenanthroline copper complex $[Cu(phen)_2]^{2+}$ [10]. Development of new therapeutic modalities particularly, those possessing biologically active pharmacophore, designed to target DNA non-covalently, capable of inhibiting Topo-I enzyme activity or which could undergo efficient cleavage lead to safer and more rational approaches for cancer chemotherapy. Herein, we describe synthesis, characterization and DNA binding profile of Cu(II) and Zn(II) complexes of ligand **L** (Fig. 1) derived from barbituric acid and pyrazole scaffold. The pBR322 DNA cleavage ability and Topo-I inhibitory activity of complex **1** was investigated by gel electrophoresis. *In vitro* cytotoxicity of complex **1** was tested against a panel of human cancer cell lines of different histological origins, which revealed that **1** can act as anticancer chemotherapeutic agent. Furthermore, molecular docking studies were carried out with DNA and Topo-I enzyme, particularly with G-quadruplex because ligand (**L**) containing planar aromatic moieties selectively bind to G-quadruplex motifs, and therefore can be explored for the rational design of new anticancer therapeutic agents.

2. Results and discussion

Cu(II) and Zn(II) based molecular entities **1** and **2** were synthesized (Scheme 1) were obtained by the reaction of ligand **L** (derived from barbituric acid and pyrazole), phenanthroline and metal nitrates in 1:1:1 molar ratio. The molecular formulas of the complexes **1** and **2** were proposed on the basis of elemental analysis, spectroscopic data and XRPD studies. The complexes were



Scheme 1. Synthetic route to ligand and its metal complexes **1** and **2**.