Pakistan J. Zool., vol. 50(6), pp 2391-2393, 2018. **DOI**: http://dx.doi.org/10.17582/journal.pjz/2018.50.6.sc7

Short Communication

In-vitro Anticancer Activity of Copper (II) and Zinc (II) Complexes with Derived Schiff Base Ligands

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ABSTRACT

Derivatives of Schiff bases have been considerably screened for their anticancer potential. We aimed to investigate in-vitro anticancer activity of two transition metal compounds of derived Schiff bases; NM-3 (Tetrakis (2-{(E)-[(2- {[(Z)-(2-hydroxynapthyl) methylidene]amino} phenyl)imino] methyl}phenol) Copper(II) and NM-4 (Tetrakis (2-{(E)-[(2- {[(Z)-(2-hydroxynapthyl) methylidene] amino}phenyl) imino]methyl} phenol) zinc(II)), against human THP-1 leukemia cell line in-relation to miltefosine (standard chemotherapy). Mean % inhibition for NM-3 against THP-1 was higher than NM-4, although both were less effective than miltefosine. IC_{50} for miltefosine, NM-3 and NM-4 against THP-1was 0.000347µM, 44.9µM and 119µM; whereas, their cytotoxicity was 72.26µM, 255µM and 293.8µM, respectively against peripheral blood lymphocytes. Based on the findings of present study the compounds can be put in to list of candidacy for anticancer activity.

Article Information
Received 17 March 2017
Revised 12 April 2018
Accepted 28 April 2018
Available online 11 October 2018

Authors' Contributions
QJ planned and supervised and wrote the manuscript. SA performed the experiments. SA, NHK, SBR and SW analyzed the data, reviewed and rectified the manuscript. MI and SR synthesized the compounds and AS provided lab requirements and overall supervision of the study.

Key words
Schiff base ligands, Anti-cancer activity, THP1 cells.

Cancer treatment typically consists of surgery, radiations, chemotherapy or a combination of these. Chemotherapy remains the mainstay means for treatment of malignancies either by cytotoxicity or cytostasis. DNA damage and subsequent induction of apoptosis is the primary mechanism of cytotoxic drugs like antimetabolites and alkylating agents (Rixe and Fojo, 2007). Most of these chemotherapeutic agents suffer from poor therapeutic index. Genotoxic effects of anticancer drugs to normal cells are considered one of the most serious issues of chemotherapy, possibly due to risk of inducing secondary malignancies. Their side effects may vary with the formulation used, its dosage, duration of treatment and immune status of patient (Partridge *et al*., 2001). Although active cancer research has led to a number of novel and targeted solutions, many have clear limitations and there is still a dire need for discovering potent, safe and selective anticancer agents (Shashidhara *et al*., 2010).

Pyrimidinyl Schiff bases are indicated for their *in-vitro* antitumor activity (Osowole *et al*., 2010). In this study we aimed to assess the anti-cancer activity and associated cytotoxicity by two novel synthetic derivatives of Schiff bases namely NM-3 (Tetrakis(2-{(E)-[(2-{[(Z)-(2-hydroxynapthyl)methylidene]amino}phenyl)imino] methyl}phenol)Copper(II) and NM-4 (Tetrakis(2-{(E)-[(2-{[(Z)-(2-hydroxynapthyl)methylidene]amino}phenyl) imino]methyl}phenol)zinc(II)) (Fig. 1) in-comparison to the standard drug miltefosine (Zentaris, Frankfurt, Germany) on THP-1 leukemia cell line. Cytotoxic effects of these drugs were assessed using peripheral blood lymphocytes (PBLs) as a control cell line.

Materials and Methods

PBLs were isolated from fresh blood of a healthy volunteer using density gradient centrifugation. Four serially diluted concentrations of each compound (100µM, 75µM, 50µM, and 25µM) were tested for their in-vitro activity against THP-1 cells. The study methods were approved by Ethics Committee University of Peshawar. About 6 x 10^4 THP-1 cells/ml were re-suspended in RPMI-1640 (Sigma Aldrich, UK) growth medium and counting was done *via* haemocytometer after 24, 48 and 72 h. Viability of cells was determined using trypan blue exclusion technique. THP-1 cells, with 96 percent viability, were plated at 5x10^4cells/200 uL medium/ well in 96 well flat-bottom micro titer plate. Each drug

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0030-9923/2018/0006-2391 $ 9.00/0

was applied in 4 concentrations in triplicate. Cells were incubated in humidified carbon dioxide incubator with 5% CO_2 at 37°C. The test formulations were then applied on peripheral blood lymphocytes (PBLs) under the same conditions as described for THP1 cells. Cells (THP-1 and PBLs) were counted after 48 h after trypan blue exclusion. Percentage inhibition was interpreted by viable cell count (Lavanya *et al*., 2010) using:

% Inhibition = Treated – Control / Control x 100

IC_{50} values for each concentration of NM-3, NM-4 and miltefosine were estimated in GraphPad Prism (V.4) by non-linear sigmoidal curve-fitting of cell-line's %inhibition versus log-transformed drug concentration values (Miller, 2003). Therapeutic index (TI) was also calculated. TI is a simple ratio of 50% toxic concentration to macrophages to 50% efficacy concentration against cells and is a better parameter for such comparisons (Grogl *et al*., 2013).

Results and discussion

Both the test compounds in our study showed a concentration dependent inhibition of the THP1 cells.

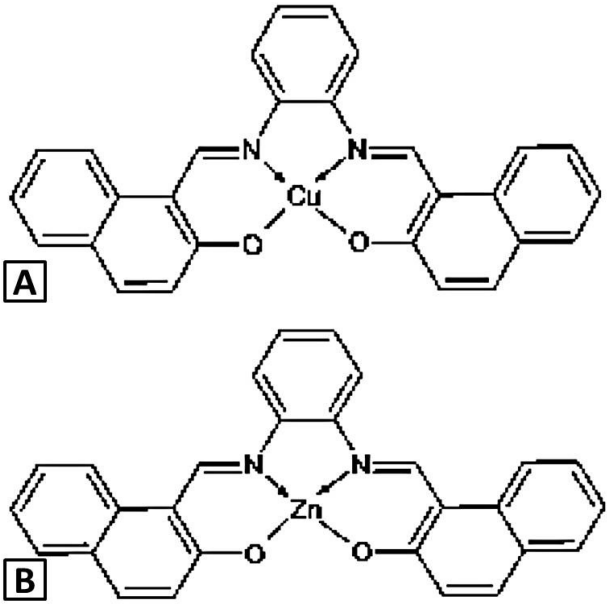

Fig. 1. Structure of test complexes with Schiff base derived ligands: **A**, NM-3 Tetrakis(2-{(E)-[(2-{[(Z)-(2-hydroxynapthyl)methylidene]amino}phenyl)imino]methyl}phenol)Copper(II). Formula: $C_{28}H_{18}CuN_2O_2$, Mol. Wt: 478gms. **B**, NM-4 Tetrakis (2-{(E)-[(2-{[(Z)-(2-hydroxynapthyl)methylidene]amino}phenyl)imino]methyl}phenol)zinc(II). Formula: $C_{28}H_{18}N_2O_2Zn$; Mol. Wt: 480gms.

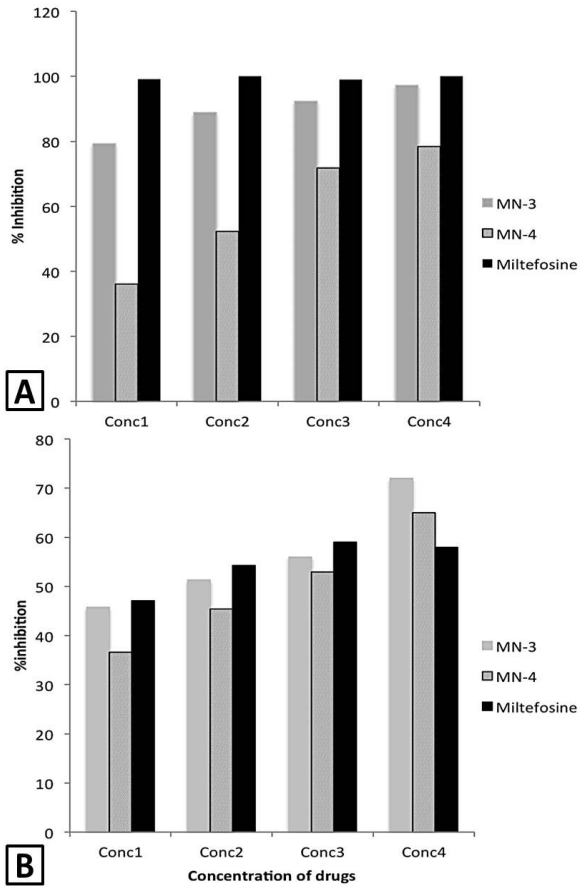

Fig. 2. Percent inhibition by THP1 cells (A) PBLs (B) against four concentrations of NM3, NM4 and miltefosine.

Table I.- Percent inhibition and IC_{50} values of compounds onTHP1 and PBLs.

Formulation	IC_{50} (µM) THP1 (95%CI*)	IC_{50} (µM) PBLs (95%CI)	Therapeutic index (TI)
NM-3	44.91 (0.06-3.27x10^4)	255 (0.87-7.48x10^4)	5.67
NM-4	119.7 (0.14-1.05 x10^4)	293.8 (0.15-5.95x10^4)	2.45
Miltefosine	0.000347	72.26	2.07x10^5

*Confidence interval.

NM-3 demonstrated an effective growth inhibition of 79.3%, 88.9%, 92.3% and 97.3% at 100µM, 75µM, 50µM, and 25µM, respectively (Fig. 2). *In-vitro* anti-cancer effect of NM3 and NM4 on THP1 and toxicity in PBLs control cell line was evaluated in terms of IC_{50}s (Table I). NM3 proved to be a more potent formulation (IC_{50}=44.99µM) compared to NM4 (IC_{50}=119.7µM), although with an ef-

ficacy less than miltefosine (IC_{50}=0.000347µM). Metal complexes of Schiff base ligands have been demonstrated to exhibit improved biopotency than the free ligands (Kumar-Naik *et al*., 2014). Many have been tested for their bioactivity as antioxidants, antimicrobials (Singh, *et al*., 2007) and anticancer activity against certain types of leukemia (Aliyu and Ado, 2011; Srivastva *et al*., 2014). Cu (II) and Pd (II) complexes of these bases are demonstrated to be effective against MCF-7 (human breast adenocarcinoma) and HT-29 (colon carcinoma) cell lines (Osowole and Akpan, 2012). Fluorescence and electronic spectra studies indicate that complexes of copper (I), nickel (I) and zinc (I) operate by intercalating DNA. Complexes of copper (I), nickel (I) and zinc (I) have reported cancer inhibition rates of 53.3%, 51.7% and 32.2%, respectively to EAC (Ehrlich ascites carcinoma) *in-vivo* (Chunhua *et al*., 1993).

Miltefosine was the most cytotoxic drug with an IC_{50} of 72.26 in PBLs and a consistently higher % inhibition through all the four studied concentrations. NM4 had the least cytotoxic effect followed by NM3. However, in terms of percent inhibition, at higher concentrations NM4 showed higher cytotoxicity than miltefosine (Table I; Fig. 2). Though the MN3 and MN4 show much higher IC50 values than the standard drug, their cytotoxicity is much less than the standard drug. Considering the lower cytotoxicity, the compounds get at least to the list of candidacy at this stage of experimentation. The standard being with higher toxicity is still used as a drug. Other studies like Dorlo et al. (2012) have also reported Miltefosine to be causing testicular atrophy and teratogenic effects. Complexes containing zinc and copper ligands are known to show marked cytotoxicity to human promyolicytic leukemia (HL60) cell line (Khoo *et al*., 2014). Studies have reported a comparatively higher cytotoxicity of Cu (II) containing complexes to cancer cells (Osowole *et al*., 2010; Osowole and Akpan, 2012).

As discussed above, derived Schiff base complexes have effectual anticancer activity against leukemia. In this study, copper-containing complex (NM-3) (TI=5.67) apparently showed better performance than the containing zinc (II) (NM-4) (TI=2.45) (Table I). Drug development for cancer, in general, is an area of active research for scientists and pharmaceutical innovationists. Our findings not only present the compounds as potential candidates for anticancer activity, but also prove them less cytotoxic to the normal cells.

Acknowledgments

We are thankful to volunteers and staff at Department of Zoology, University of Peshawar for their unprecedented support.

Statement of conflicts of interest

Authors bear no conflict of interest.

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