Novel synthesis of 5-((4-phenylpiperazin-1-yl) methyl)-1H-pyrrole-2-carbaldehyde metal-complexes using Mannich reaction and investigate their cytotoxicity against MCF-7 cell line

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Abstract

An acidic proton next to a carbonyl functional group undergoes a multicomponent process known as the Mannich reaction, which results in aminoalkylation. It requires a suitable carbonyl molecule, such formaldehyde, and either a primary, secondary, or ammonia amine. in this study mannich reaction was used to prepare 5-((4-phenylpiperazin-1-yl) methyl)-1H-pyrrole-2-carbaldehyde (L1) through the reaction between Phenylpiperazine react with formalin and pyrolcarbaldehyde. the (L1) ligand furthermore react with, Ni and Pd to produce C2 and C3 complexes respectively. the prepared compounds were characterized by elemental analysis, ^{1}HNMR, ^{13}CNMR spectroscopy and FTIR spectroscopy. the anti-cancer activity of these compounds were investigated *invitro* at different concentrations against breast cancer MCF-7 cell line, the results show that the C2 and C3 achieved 72.6 and 57.15% cytotoxicity in MCF-7 cell line.

key words: Mannich reaction, Phenylpiperazine, and MCF-7

1- introduction

The traditional three-part Mannich reaction, which involves a primary or secondary amine, a nonenolizable aldehyde (an aldehyde whose molecule contains no alpha hydrogens), and a C-H acidic carbonyl compound, is still a very helpful method for forming C-C bonds [1]–[3]. A chemical known as a Mannich base, or -amino-carbonyl, is the end result [4]. The nucleophilic addition of an amine to a carbonyl group, which is later dehydrated to the Schiff base, initiates the Mannich reaction. The Mannich reaction is a condensation reaction, and the Schiff base is an electrophile that combines in a subsequent step with an enol generated from a carbonyl molecule containing an acidic alpha-proton[5]. The first step in the Mannich reaction is the creation of an iminium ion from amine and formaldehyde[6]. Phenylpiperazine is a basic chemical compound that contains a phenyl group linked to a piperazine ring. In the last decade, phenylpiperazine has gained increasing interest in biological activities, such as a novel family of prospective anticancer drugs, antihistaminic, anthelmintic, and antidepressant agents. They have intriguing anatomical, pharmacological, and cardiovascular characteristics. They are used to treat illnesses like anxiety disorders, Parkinson's, and schizophrenia as well as ailments associated to dopaminergic and/or serotoninergic system abnormalities. Phenylpiperazine derivatives have been the focus of extensive scientific research because of its numerous applications[7]. Cancer is characterized by unchecked cell multiplication and spread. Like in healthy tissues, there is still a balance between the rates of new cell formation and old cell death. This balance is disturbed by cancer. The equilibrium could become out of whack due to unchecked cell proliferation or a lack of apoptosis in the cells [12]. Cancer is brought on by both modifiable factors like genetic mutation, hormones, and immunological status as well as unmodifiable factors like lifestyle factors like smoking and obesity. Risk factors may work in concert or in succession during the onset and spread of cancer[13]. Breast cancer is fatal if the spread of the disease is not controlled. Breast cancer was the second leading cause of mortality in the United States, after lung cancer [14]. in this study Phenylpiperazine react with formalin and pyrolcarbaldehyde through Mannich reaction to prepare a new ligand (L1), which in turn react with some of metals (Pd and Cu) to produce C2 and C3 complexes respectively and investigate the biological activity of prepared compounds as anti MCF-7 cancer cell line.

2. materials and Methods

2.1 chemicals

all chemicals, Absolute ethanol, Absolute methanol,

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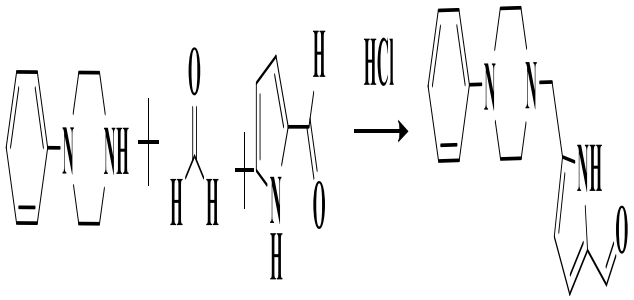
Chloroform, Dichloromethane, Formaldehyde, Hydrochloric acid, Phenylpiperazine, Palladium (II) acetate and Nickel (II) acetate were collected from sigma Aldrich.

2.2 Procedure for preparation of 5-((4-phenylpiperazin-1-yl) methyl)-1H-pyrrole-2-carbaldehyde (L1)

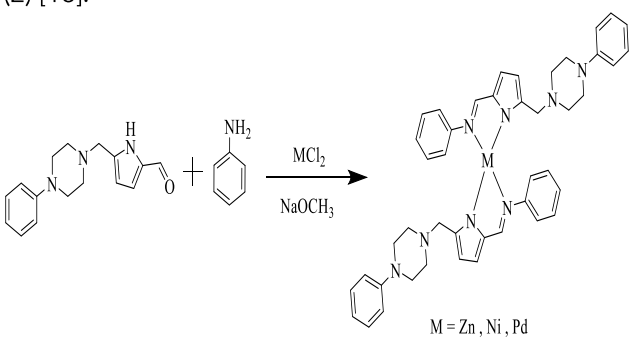
the L1 ligand was prepared according to our previous study as a following procedure. Conc HCl (12 N, 0.83 mL, 10.00 mmol) was added at room temperature to a methanol/water (20 mL, V/V 2:1) suspension of phenylpiperazine (1.62 g, 10.00 mmol) and formaldehyde (38 percent, 0.80 mL, 10.00 mmol). A homogeneous solution was created from the reaction mixture. In order to create a colorless precipitate, pyrrole-2-carbaldehyde (0.95 g, 10.00 mmol) was dissolved in 5 mL of methanol and added to the mixture. The mixture was then agitated overnight (15 H). The reaction mixture was then supplemented with solid NaOH (0.40 g, 10.00 mmol), and agitated for a further hour. The methanol was partially extracted while under vacuum, and the reaction mixture was filtered. After being dried under vacuum and rinsed with water (2*15 mL), the precipitate produced L1 (2.13 g, 7.89 mmol, 79 percent), a colorless solid scheme (1) [15]



Scheme (1) Synthetic route for L1

2.3 General procedure for preparation of complexes C2 and C3

A methanolic solution (10 mL) of MCl_2 (0.25 mmol) was mixed with a suspension of L**1** (0.135 g, 0.5 mmol) and aniline (0.045 mL, 0.5 mmol) in 10 mL of methanol in presence of sodium methoxide (0.027 g, 0.5mmol). The resulting solution was kept aside at room temperature and the color of the solution changed over 1 hour. By slow evaporation, precipitate was obtained after two days. These were filtered off, washed with water and air dried scheme (2) [16].



Scheme (Error! No text of specified style in document.)

Synthetic route of C2 and C3 complexes

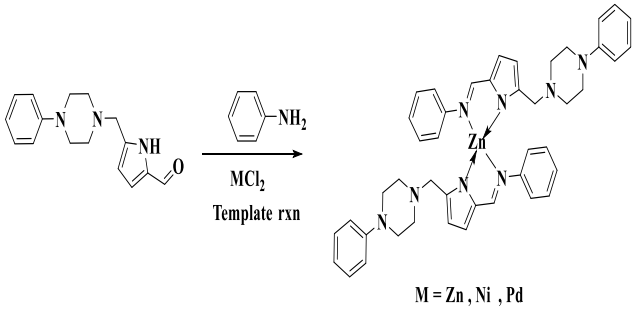
2.4 Anticancer activity procedure

the prepared compounds were tested against, MCF-7 cells, and PDL cells are used as a control cell. An MTT (dimethyl–2–thiazolyl–2,5–diphenyl–2H–tetrazolium bromide) based cell viability assay was used to determine the cytotoxic potential of the test analogs in human breast cancer cells MCF-7 and. The effect of these compounds on normal cells, namely Periodontal Ligament cells (PDL), was also investigated. The cells were cultured in a volume of 100 µL in each well of a 96-well plat and incubated at 37 °C. After 3 hours, the cells were treated with the test compounds (100 µL) with concentration (1mg/mL) in DMSO and incubated for another 24 hours. After pipeting the media, 10 µL MTT and 90 µL media were added to each well. The plate was incubated for 4 hours at 37°C and, then 100 µL of DMSO was added. The plate was then incubated for 30 minutes at 37 °C, and the absorbance was measured at 570 nm while a background measurement was taken at 620 nm using a microplate reader. The experiment was repeated three times, and the findings were determined as IC_{50} values based on the mean of three independent values.

3. Results and Discussion

3.1 Template reaction for fictionalization of Mannich base

The prepared L1 posses only one donor atom represented as the nitrogen of pyrole group, so it cannot form chelating with the metal to form complex, therefore we suggest to add another donor atom through template reaction using aniline. Palladium (II) and zinc (II) complexes, with the general formula L_2M, were synthesis by the reaction of Mannich bases L**1** suspended in methanol, in presence of aniline to form Schiff base, the metal chloride was added in a 2:1 ligand to metal ratio. A base (sodium methoxide) was needed to dissociate the proton in the Pyrole ring and offer a vacant site for the incoming metal ion, for the less basic amines, this took several hours, whereas for the more basic (pKa > 4), aqueous metal (II) acetate could be used[17] . Thus, the Schiff base ligand works as a bidentate ligand. The obtained metal complexes are colored solids and stable to the presence of air. These complexes are soluble in $CDCl_3$, DMSO and DMF[18] **,** Scheme (3).



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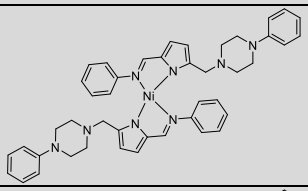
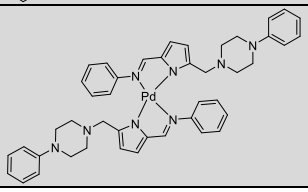
Synthesis of Mannich base complexe

3.2 Elemental analysis for C2 and C3 complexes

In this work, the elemental analysis method is used to determine the elemental ratios of the synthesized complexes. Tables (1) show the theoretical and experimental values of complex elements, as well as the physical properties of compounds. The experimental results were in good agreement with the theoretical values. This indicates the high purity of our products.

Table(1): Lists some of the physical properties includes the elemental analysis values of all synthesized compounds

Name	Structure	Melting point°C	yield	Elemental analysis(calculated)		
				C%	H%	N%
C2		201-202	61%	(70.88)70.86	(6.22)6.33	(15.03)15.16
C3		211-213	49%	(66.62)66.50	(5.84)5.92	(14.12)14.24

3.3 FTIR spectroscopy for C2, and C3complexes

IR spectrum of each free ligand is compared to the spectra of its corresponding complexes to investigate the binding mode of Schiff base to metal complexes. Table (2) shows the important IR bands.

Table (2) FT- IR bands values of the ligands and Their complexes

Compounds	$\nu(\mathbf{C=N})$	$\nu(\mathbf{M-N})$
C2	1609	484 (Ni-N)
C3	1596	467 (Pd-N)

3.4 Anticancer activity of C2, and C3 complexes

Many chemotherapeutic drugs used to treat cancer work by inhibiting certain cellular division processes in order to kill malignant tumor cells. Because these anti-tumor compounds are cytostatic or cytotoxic to all proliferating cells, including normal cells, they are consequently nonspecific [21]. Mannich anti-cancer compounds have recently gotten a lot of interest because of their biodegradability, biocompatibility, and applicability. The MTT assay is a colorimetric assay used to assess the metabolic activit.y of cells. MMT assay experiments are used to estimate the IC_{50} value for compounds C2, and C3 against PDL and MCF7 cells. The MTT assay is a quick colorimetric assay with a 96-well design for cell viability assessment. This test assesses the ability of mitochondrial succinate dehydrogenase to convert yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) into an insoluble blue formazan material. Since dying cells are incapable of converting MTT into formazan, color formation is used to identify living cells. In this work, the cytotoxicity of MCF-7. cancer cell line for 72 hours using the MTT method. Ni and Pd complexes were applied to the human MCF-7 cancer cell line at various concentrations to ascertain their anti-proliferative effects.

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay was adopted to evaluate the in vitro cytotoxicity of compounds against tow human cancer cell lines namely

human breast cancer (MCF-7) cell lines.

For C2, Table (3), it was found that in 100 µg/mL and 200 µg/mL a significant cytotoxicity against PDL (31.87 and 36.60) and MCF-7 (61.41and 72.61). the low cytotoxicity in PDL cell line and good cytotoxicity at 100 µg/mL and 200 µg/mL make this compound has very good properties as anti-cancer Figure (1).

according to result C3 It was discovered that at 100 g/mL and 200 g/mL a moderate cytotoxicity against PDL (19.89 - 26.16) and MCF-7 (47.03 - 57.15). therefore, cannot be conceder C3 as anti-cancer Figure (2).

Figure (3) show the morphological change in MCF7 and PDL cell line under effect of C2 and C3.

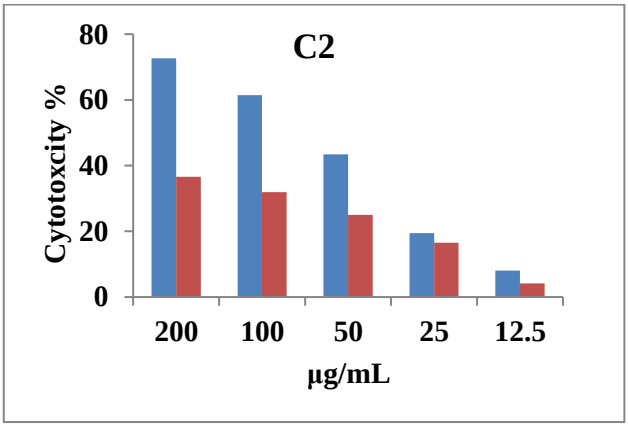
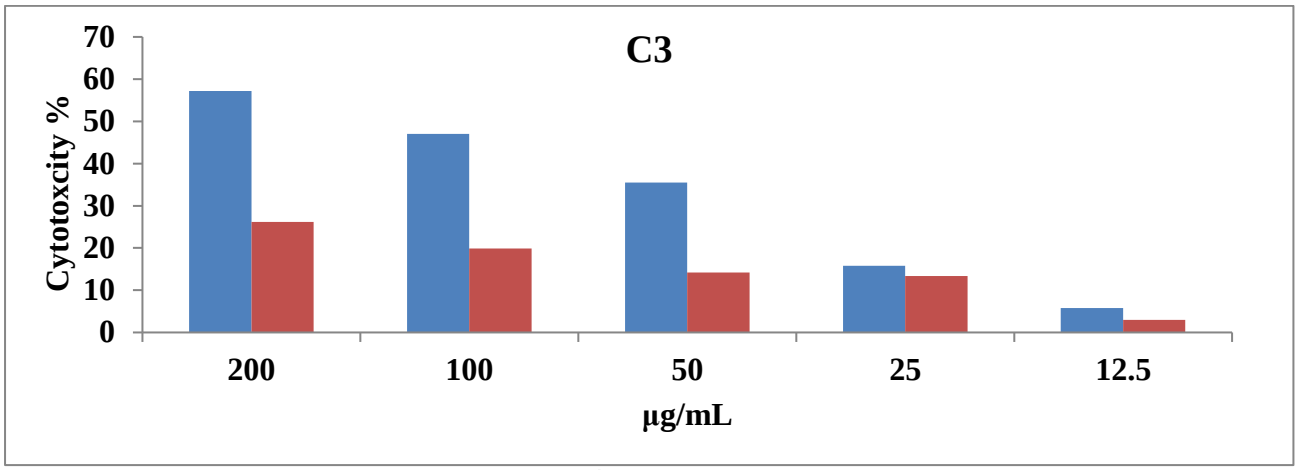


Figure (1) Cytotoxicity % of C2 against MCF-7 and PDL cell lines



Figure(2) Cytotoxicity % of C3 against MCF-7 and PDL cell lines

Table (3) Cytotoxicity % of C2 and C3 against MCF-7 and PDL cell lines				
Conc.µg/mL	C2 Cytotoxicity %		C3 Cytotoxicity %	
	Normal	MCF7	Normal	MCF7
12.5	4.152794	8.02876	2.960091	5.811863
25	16.47662	19.41282	13.39795	15.81786
50	25.02851	43.37927	14.19612	35.53026
100	31.87001	61.41402	19.89738	47.03415
200	36.60205	72.61833	26.16876	57.15998

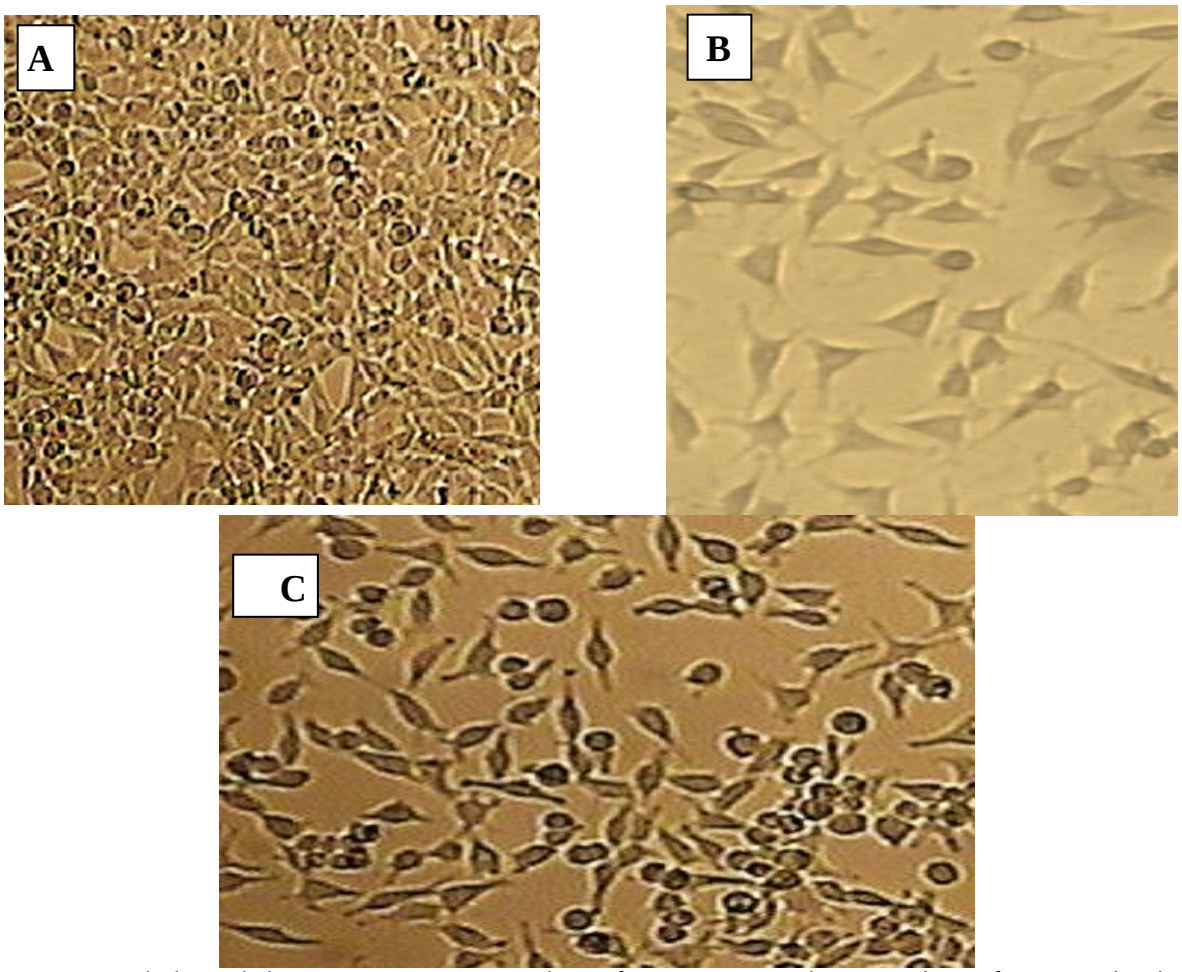


Figure (3) Morphological change MCF-7 , (A) control, (B) after treatment with (C2), and (C) after treated with (C3).

4. Conclusion

The 5-((4-phenylpiperazin-1-yl) methyl)-1H-pyrrole-2-carbaldehyde (L1) was synthesized via the Mannich process by reacting phenylpiperazine with formalin and pyrrole-2-carbaldehyde. Additionally, the (L1) ligand interacts with Ni, and Pd to form the complexes C2, and C3, respectively. By using FTIR spectroscopy, 1HNMR, 13CNMR, and elemental analysis to analyze the produced compounds. When the anti-cancer activity of these substances was

tested in vitro against the breast cancer MCF-7 cell line, the results revealed that the C2 and C3 obtained 72.6 and 57.15% cytotoxicity respectively.

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