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Synthesis of Newly Cytotoxic and Antimicrobial Organometallic Complexes

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ABSTRACT

Drugs normally synthesized to use as medication to treat diseases like cancer and microbial infections, these synthesized drugs were interested more than naturally-derived drugs which have been shows low activity or not as efficient against diseases. A new ligand 3-methylbenzyl (2*Z*)-2-[1-(pyridin-4-yl)ethylidene]hydrazine carbodithioate (PE3MBC) and its Cd(II), Cu(II), Co(II) and Zn(II) metal complexes. The new ligand and metal complexes were characterized *via* various physico-chemical and spectroscopic techniques. Cd(II) complex show more activity against microbes and against cancer cell line MCF-7, while other complexes does not shows activity like cadmium complex, all the complexes does not shows any activity against MDAMB-231 cell line. The fatal of the cancer and the microbes cell was due to inhibition of DNA synthesis which was probably due to chelating with metals complexes, or could be referred to lipophilicity, presence of hydrophobic moiety in the complex molecule, also could be due to steric effects and electronic effects.

Introduction

Schiff bases have often been used as chelating ligands in coordination chemistry. Schiff base with donors (N, O, S, etc) have structure similarities with neutral biological systems and due to presence of imine group are utilized in elucidating the mechanism of transformation of rasemination reaction in biological system [1].

Thiosemicarbazide and its derivatives as ligands with potential sulphur and nitrogen bands are interesting and have gained special attention due to their importance in medicinal and pharmaceutical field. They show biological activities including antibacterial antifungal, antidiabetic, antitumor, antiproliferative, anticancer, herbicidal, anticorrosion and anti-inflammatory activities [2].

It is well known that the shape of a certain molecule is the most important factor that affects drug activity [3]. The four major factors that affect the properties of a drug are: its ability to chelate to metal ions, its lipophilicity, steric effects and electronic effects. It is clear that the lipophilicity (Fat-loving) is one of the compound character itself which increases the cell permeability and may cause an intracellular reduction of the active M(II) species which leads to the activation of oxygen that cause lethal for microbes [4, 5].

Heterocycles such as, pyrimidine, pyridine and quinolone have been under investigation as they play important roles in biological systems [5]. The criteria should include the presence of hydrophobic moieties in the synthetic drug molecule.

.A new ligand 3-methylbenzyl (2*Z*)-2-[1-(pyridin-4-yl)ethylidene]hydrazine carbodithioate (PE3MBC) and its Cd(II), Cu(II), Co(II) and Zn(II) metal complexes has been synthesized and characterized successfully. The biochemistry, cytotoxicity and bioactivity of the newly synthesized organic compounds were under focus in this study to discover the factors that affected the antimicrobial and cytotoxic activity.

Material and Methods

Experimental

Carbon, hydrogen, nitrogen and sulfur analyses were carried out using a Leco CHNS-932 analyzer. The molar conductivity measurements were carried out using Wissenschaftlich-TechnischWerk- -statten 8120. Melting points of the synthesized compounds were determined by open capillary and are uncorrected. Melting point apparatus of Gallenkamp M.F.B 600.01 was used. ^{1}H NMR spectra and ^{13}C spectra were recorded on Jeol (JNM-ECA400) 400 MHz NMR spectrometer. Compounds were visualized under UV using UV-Vis 160A in the range of wave number (200-1100 nm). IR spectra were recorded using KBr on Shimadzu FTIR 8300 in the wavelength range of (4000-400) cm^{-1}. The magnetic susceptibility values using (Magnetic Susceptibility Balance), of Johnson mattey catalytic system division using an atomic absorption technique by Perkin-Elmer-5000 to (Cu^{+2}, Ni^{+2} , Cd^{+2} and Zn^{+2}) metal ions.

Preparation of 3-methylphenylhydrazinecarbodithioate (3MBC)

Potassium hydroxide (0.2 mol) was dissolved in absolute ethanol (70 ml). To this solution, hydrazine hydrate (0.2 mol) was added and the mixture was cooled in an ice-salt bath to 0°C. Carbon disulphide(0.2 mol) was added dropwise with constant stirring over a period of one hour. The two layers that subsequently formed were separated using a separating funnel. The light-brown lower layer was dissolved in 40% ethanol (60 ml) below 5°C. The mixture was kept in an ice-bath and to it, 3-methylbenzyl chloride (0.2 mol) was added dropwise with vigorous stirring of the mixture. The sticky white product, (S3MBDTC), which formed was filtered and left to dry overnight in a desiccator over anhydrous silica gel. (Yield: 75%, m.p. 130°C). IR (KBr cm^{-1}): ν(C=N) 1612, ν(N=N) 1046, ν(C=SS) 975 ν (NH)/NH_2) 3398.

Preparation of 3-methylbenzyl (2*Z*)-2-[1-(pyridin-4-yl)ethylidene]hydrazinecarbod ithioate (PE3MBC)

3-methylbenzyl hydrazinecarbodithioate (0.01 mol) was dissolved in hot acetonitrile (100ml). This was added to an equimolar solution of 1-(pyridin-4-yl)ethanone in ethanol (10 ml). The mixture was heated and stirred for 30 minutes and then allowed to stand for a few hours, after which yellow crystals formed, which were filtered off and recrystallised from

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acetonitrile. Yields were fairly high, ca. 85%, C: 59.23(60.29), H: 5.54(5.43), N: 12.65(13.32) and S: 22.03(20.33).

IR (KBr cm-1): ν(C=N) 1592, ν(N=N) 1060, ν(C=SS) 824 ν (NH)/NH_2) 3081.

1H-NMR (DMSO δ ppm): 13.52 (singlet 1H, NH), 7.06-8.55 (multiple 13H, Ar-H), 4.42 (singlet 2H, S-CH_2). 2.29 (singlet 3H, –CH_3)

Preparation of Metal Complexes

A hot ethanolic solution of M[acetate/nitrate] .nH_2O[M = Cu(II), Ni(II), , Cd(II) and Zn(II)] (0.001 mol in 25 ml) were mixed with a solution of PE3MBC in 1:1 acetonitrile: ethanol (0.002mol in 50 ml) and the resulting mixture were heated for ~30 minutes. The solids precipitated were filtered, washed with cold ethanol and dried in a desiccator over silica gel. Yields: 62-74%.

IR (KBr cm-1) for Cd(II) metal complex: ν(C=N) 1601, ν(N=N) 1083, ν(C=SS) 827.

IR (KBr cm-1) for Cu(II) metal complex: ν(C=N) 1591, ν(N=N) 1089, ν(C=SS) 821.

IR (KBr cm-1) for Ni(II) metal complex: ν(C=N) 1573, ν(N=N) 1081, ν(C=SS) 832.

IR (KBr cm-1) for Zn(II) metal complex: ν(C=N) 1585, ν(N=N) 1090, ν(C=SS) 880.

Results and Discussion

All the synthesized compounds were purified by recrystallization in ethanol. The sharp melting point and the of matching the experimental CHNS data with the theoretical. The characterization of the synthesized compounds and their structures was confirmed using, UV-visible, magnetic susceptibility and molar conductivity. Table 1 shows the physical constant data of synthesized compounds, while Table 2 shows the characterization data as depicted below.

Table 1: Physical properties of the synthesized compounds

Compound	Color	Melting Point	Molecular Formula	Yield%
3MBC	White	131	$C_9H_{12}N_2S_2$	75%
PE3MBC	Yellow	208	$C_{16}H_{17}N_3S_2$	85%
Cu(PE3MBC)$_2$	Dark Brown	184	$C_{32}H_{32}CuN_6S_4$	67%
Ni(PE3MBC)$_2$	Golden brown	215	$C_{32}H_{32}NiN_6S_4$	68%
Zn(PE3MBC)$_2$	Golden Yellow	190	$C_{32}H_{32}ZnN_6S_4$	71%
Cd(PE3MBC)$_2$	Yellow	205	$C_{32}H_{32}CdN_6S_4$	74%

Table 2: Molar conductivity, Magnetic Susceptibility and UV- Vis Spectroscopy of metal complexes

Complex	Λ (S cm^2 mol^{-1})	μ_{eff} (BM) at 298K	λ_{max} (Log ε) nm)
PE3MBC	-	-	326 (2.91)
Cu(PE3MBC)$_2$	5.03	1.79	285 (3.45), 427 (3.63), 641 (0.31)
Ni(PE3MBC)$_2$	8.45	Diamagnetic	329 (3.19), 445 (2.65), 885 (0.31)
Zn(PE3MBC)$_2$	1.27	Diamagnetic	252 (3.45), 291 (3.12)
Cd(PE3MBC)$_2$	3.98	Diamagnetic	305 (3.02)

Antimicrobial activity

The antimicrobial activities of the compounds were qualitatively determined by a modified disc diffusion method. A lawn of microorganisms was prepared by pipetting and evenly spreading inoculum (10-4 ml, adjusted turbidometrically to 105–106 cfu/ml (cfu: colony forming units) on to agar set in Petri dishes, using nutrient agar (NA) for the bacteria and potato dextrose agar (PDA) for fungi. Whatman No. 1 filter paper discs of 6 mm diameter were impregnated with dimethyl sulphoxide (DMSO) stock solution of the compound (100 mg/ml) and dried under sterile conditions. The dried discs were then placed on the previously inoculated agar surface. The plates were inverted and incubated for 24 h at 37°C for bacteria and 30°C for fungi. Antimicrobial activity was indicated by the presence of clear inhibition zones around the discs.

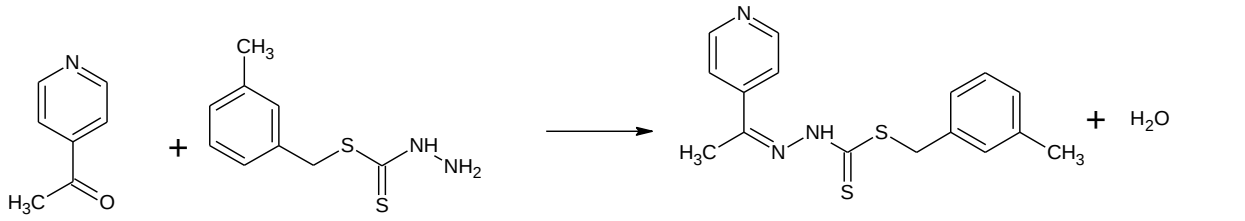
Seven pathogenic microbes were used to test the biological potential of the compounds: (i) Methicillin resistant Staphylococcus (MRSA), (ii) Bacillus subtilis-wild type (B29) (B. Sabtilis), (iii) Pseudomonas aeruginosa (60690) (P. aeruginosa), (iv) Candida albicans (CA), (v) Aspergillusochraceous (398) (A. Ochraceous), (vi) Saccharomyces cereviceae (20341) (S. cereviceae) and Salmonella choleraesuis, P.aeruginosa – Pseudomonas aeruginosa (60690).The sources of microbes and culture maintenance were as previously described. The antimicrobial activity of the extracts was qualitatively determined by a modified disc diffusion method [6, 7]. Streptomycin was used for antibacterial control while Nystatin was used as antifungal control. Measurement of inhibitory activity as described by Hufford and Clark, the lowest concentration that completely inhibited microbial growth recorded as the minimum inhibitory concentration (MIC, µg,cm^{-3}) [8]. Table 3 shows the antimicrobial activity of the compounds.

It has been observed that the Schiff bases were not active against the tested bacteria and fungi the inhibition diameters (18 mm) which is less than that of the commercially available standard, Nystatin (22 to 27 mm), indicating that they were less effective. However Metal complexes were found to be more active against the selected bacteria and fungi compare to the ligand. Streptomycin (22–24 mm), especially that of the Cd(II) complex, which had inhibition diameters as high as 26 mm. The Cadmium is well known with its toxicity, in addition to that, the methyl group in PE3MBC also reduces the water solubility of the complex and its ability to hydrogen bond.

This higher antibacterial activity of the metal complexes compared to ligand is may be due to the change in structure due to coordination and chelating tends to make metal complexes act as more powerful and potent bacteriostatic agents, thus inhibiting the growth of the bacteria. Furthermore, chelation reduces the polarity of the metal ion mainly due to the partial sharing of its positive charge with the donor groups within the chelate ring system. Such chelation increases the lipophilic nature of the central metal atom, which favors its permeation more efficiently through the lipid layer of the microorganism, thus destroying them more forcefully. Thus all complexes showed more increased activity than the corresponding ligand and the two antibacterial drugs [9-11].

Cytotoxic Activity

The MCF-7 (Human Breast cancer cells with positive estrogen receptor) and the MDA-MB-231 (Human Breast cancer cells with negative estrogen receptor) cell lines were obtained from the National Cancer Institute, U.S.A. The cells were cultured in RPMI-1640/DMEM (Sigma) medium supplemented with 10% fetal bovine serum. Cytotoxicity was determined using the microtitration of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (Sigma, USA) as reported by Mosmann [6] . Controls that contained only cells were included for each sample. Cytotoxicity was expressed as IC_{50}, i.e. the concentration that reduced the absorbance of treated cells by 50% with reference to the control (untreated cells). Tamoxifen was used as a standard cytotoxin. Table 4 shows the activity of the compounds against 2 cancer cell lines.



(PE) 3-methylbenzyl hydrazinecarbodithioate (3MBC) 3-methylbenzyl (2*Z*)-2-[1-(pyridin-4-yl)ethylidene]hydrazinecarbodithioate (PE3MBC)

Scheme 1: Condensation reaction of PE3MBC

Table 3: Qualitative antimicrobial analysis of the ligand and its metal complexes

Complex	Inhibition diameters (mm)						
	Bacterial Strains			Fungal Strains			
	MRSA	P.Aer	S.Cho	B.Sub	C.Alb	A.Och	S.Cer
3MBC	17	17	16	18	18	18	18
PE3MBC	-	-	-	12	-	-	-
$Cd(PE3MBC)_2$	21	16	17	26			
$Cu(PE3MBC)_2$	-	-	-	-	-	-	-
$Ni(PE3MBC)_2$	14	-	-	14	-		
$Zn(PE3MBC)_2$	10	14	10	10	-	-	-
Streptomycin	24	22	23	22	-	-	-
Nystatin	-	-	-	-	22	23	27

Code: *MRSA* – Methicillin resistant *Staphylococcus Aureus, P.aer – Pseudomonas aeruginosa, S.cho – Salmonella cholerasuis, B.Sub – Bacillus subtilis*- wild type, *C.alb – Candida albicans, A.och – Aspergillusochraceous, S.cer – Saccharomyces cerevisiae.* Inhibition diameter > 15 mm is strongly active; - indicates 'not active'

Table 4: Cytotoxic Data of the ligand and its Transition Metal complexes

Complex	**IC50(µg/ml)**	
	MCF-7	**MDA-MB-231**
3MBC	8.7	Inactive
P33MBC	Inactive	Inactive
$Cd(PE3MBC)_2$	4.7	4.5
$Cu(PE3MBC)_2$	7.3	15.2
$Ni(PE3MBC)_2$	17.8	Inactive
$Zn(PE3MBC)_2$	5.9	17
Tamoxifen	**5.1**	**5.4**

IC50 < 5.0 µg cm^{-3}- strongly active, IC50 5.0 < 10.0 µg cm^{-3}- moderately active, IC50 10.0 < 25.0 µg cm^{-3}- weakly active, IC50 > 25.0 µg cm^{-3}- not active. IC50 (µg cm^{-3}) = Cytotoxic dose at 50% i.e. the concentration to reduce growth of cancer cells by 50%. MCF-7= Human Breast Carcinoma Cells with Positive Estrogen Receptor, MDA-MB-231 = Human Breast Carcinoma Cells with Negative Estrogen Receptor

The complexes were more cytotoxic towards the MCF-7 cells than the MDA-MB-231 cells (Table 4) meaning that the structure or part of the structure of the complexes is complementary to the stereo electronic structure of the positive estrogen receptor responsible for the desired biological action. Receptors are specific areas of proteins and glycoproteins embedded in cellular membranes or in the nuclei of living cells[10] which may open ion channels or release secondary messengers when bonded to a favourably shaped molecule. If a tumor is estrogen-receptor positive (ER-positive), it has receptors that react with estrogen and it is more likely to grow in a high-estrogen environment. ER-negative tumors do not have any receptors that react with estrogen and are usually not affected by the levels of estrogen and are not inhibited by well-known anti-cancer drug, Tamoxifen [6, 12, 13]. Tamoxifen has been used to treat breast cancer by blocking the binding of estrogens to the estrogen receptor [13].

The increased activity of the metal chelates can be explained on the basis of chelation potent bactericidal agents and to antifungal agent, thus killing more of the bacteria than the ligand. It is observed that, in a complex, the positive charge of the metal is partially shared with the donor atoms present in the ligand, and there may be π-electron delocalization over the whole chelating. This increases the lipophilic character of the metal chelate and favors its permeation through the lipoid layer of the bacterial membranes. Also, there are other factors which also increase the activity, such as solubility, conductivity and bond length between the metal and the ligand. The mode of action may involve the formation of a hydrogen bond through the azomethine nitrogen and oxygen atom with the active centers of the cell constituents, resulting in interference with the normal cell process. The variation in the effectiveness of different compounds against different organisms depend either on the impermeability of the cells of the microbes or the difference in ribosomes of microbial cells [14].

A complex that is used as a therapeutic chelating agent should have the following characteristics. Chelates occur naturally in biological systems as enzymes and involve mainly transition metal ions. A pathogenic organism can be killed or inhibited by introducing a ligand having a greater affinity for an essential metal ion than the natural ligand, forming a stable, inert chelate [15]. Chelation reduces the polarity of the molecule making it more lipophilic and hence allowing for more binding sites and interactions in the cell membranes, as cell membranes consists of lipids, polysaccharides and proteins amongst others. This would allow the molecule to penetrate the cell membrane easily to reach the cell's DNA, to bind to the nucleoside bases in the DNA and inhibiting its biosynthesis [16, 17]. Hence, it is probably the complex is unable to hydrogen bond due to the presence of different substituents; it is unable to interact with the DNA and proteins in the cell and cause cell apoptosis and is less effective as an antimicrobial or anti-cancer agent [11]. The increase in the size of the complex allows for a better interaction with the hydrophobic pocket in the target site of the bacteria/fungi which might strengthen the binding of the complex to the microbe, destroying the cell wall and ultimately

killing the microbe [10]. Cadmium has the largest size comparing with other metals used in this study.

There are many factors that affect potential drug behaviour in the biological system. Some of these include the rigidity of the molecule, the number of rings in the system, nature of bonding, the conformation and stereochemistry, presence of a pharmacophore, number of methyl groups and the degree of unsaturation. All of these affect the lipophilicity and hydrophobicity of the complexes. This in turn plays a huge role in determining whether the complexes would be able to interact with the receptors on the cell membrane, and with the DNA in the nuclei of the cell, ultimately leading to cell death. It is of interest to note that the complexes exhibit approximately equal inhibition to the standard antibiotic which reveals the biological efficiency of these complexes and showed the possibility to be useful as new drug [18].

Conclusion

The chelating of DNA with metal complexes will lead to the inhibition of DNA synthesis in cancer cells and microbes which result to apoptosis of the cell, this could be probably due to electronic effect, lipophilicitym steric effect or could be to the hydrophobic behaviors to the molecule of the complex.

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