



Alpha amylase and Alpha glucosidase Enzymes inhibitory assay of newly synthesized zinc complexes

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Abstract: A total of five Zinc(II) complexes of 2-(2-Methoxyphenoxy)acetic acid were prepared by using Methyl alcohol as a solvent, in the absence (C1), and in the presence of pyridine (C2), 3-Methyl pyridine (C3), 1,10-phenanthroline (C4) and 2,2'-bipyridine (C5). Moderately good yields of the complexes were obtained. The synthesized compounds were characterized by FT-IR and UV-Visible spectrophotometry. Complexes showed inhibition activity towards Alpha-amylase and Alpha- glucosidase enzymes. Their increasing order of activity in Alpha-amylase inhibition was C5 > C3 > C1 > C2 > C4 > C and that of Alpha-glucosidase inhibition C4 > C2 > C > C3 > C1 > C5.

Keywords: zinc complexes, alpha glucosidase, alpha amylase, inhibition.

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1. Introduction

Enzymes serve as biological catalysts that accelerate metabolic reactions in living organisms [1]. They are integral to physiological metabolism, regulating homeostasis, development, and reproduction [2]. Aberrant enzyme regulation can lead to various diseases in humans, including inflammation, tumours, central nervous system disorders, cardiovascular ailments, and infections [3]. Medicinal inorganic chemistry is an emerging field that use transition metal complexes as innovative methods for diagnosing and treating diseases. The structural diversity of transition metal complexes enables their prospective application in various domains, including catalysis, biology, and photoluminescence [4]. Metal complexes have been thoroughly investigated for their enzyme inhibition capabilities due to their subsequent qualities:

i) Their three-dimensional design can accurately fit into the hydrophobic pocket of the enzyme.

ii) The M-L bond associated with a metal complex that is prone to hydrolysis may also participate actively as donor groups present on the enzyme.

iii) Besides that, metal complexes have additional photodynamic and photoactive functions including redox activity as well [5]. For many years, metal carboxylate complexes have been synthesized which are of great interest from the point of view of chemistry, have unusual geometric structures and have a wide range of

applications in the most important areas. They are also very important in the areas of medicine and catalysis, electrochemistry, magnetics and electronics. It is the transition metals which are important such as carboxylates complexation, headily because of thier lewis acid properties, large amount of oxidation state ranges and redox potential [6]. It is also possible to incorporate various ancillary functional ligands to the metal carboxylates, including N-donor ligands. It is also possible to vary the physical and chemical characteristics of metal carboxylates based on the modification of the substituents at the aromatic nuclei. However, carboxylate complexes can form good hydrogen contacts, which are required in order to create stable supramolecular structures that are temperature-independent. These properties make metal carboxylates the better choice in creating metal based pharmaceuticals [7,8]. Carboxylic acids are considered strong organic ligands because of the strong coordinating properties of these functional groups. In addition, they have been shown to possess the capacity to build molecular structures of structural complexity [9]. Carboxylic acids affinities to a metal atom are carried out through different ways. They can attached to the metal centre in a symmetrical, bridging, monodentate or bidentate manner [10]. The final crystalline network provides the necessary flexibility which allows one to alter the physical chemical characteristics of a metal complex [8]. Zinc is considered to be the second most abundant bio-essential trace metal in human body.

The human body has an average of about 3 milligrams of zinc [11]. The average male is believed to require 11 mg of zinc daily, whereas the average female requires 9 mg per day. Numerous bodily enzymes incorporate zinc as both a structural and catalytic element. It has a crucial role in stabilizing enzymes [12]. It sustains and modulates gene expression in humans. Zinc possesses distinctive chemical features that render it more significant than other metals. Zinc (II) exhibits robust binding affinity because to its diminutive size and positive charge [11]. Zinc acts as enzyme inhibitors because it has a very strong binding affinity with most protein components of the enzymes [12]. Zn has been implicitly recognized to play an important role in transcription, translation and apoptosis. Also, zinc plays the extraordinary non-catalytic function as an antioxidant [13]. Zinc boosts the body's defense mechanism. Animal foods for instance beef, poultry, spices for cereals such as peanuts, almonds, walnuts, oats and dairy foods like yoghurt, cheese and milk also contain zinc [14]. A deficiency of zinc affects about 25% of the human population worldwide with the rates among children aged two and younger. Zinc deficiency has been associated with many health ailments. Zinc deficiency stimulates the degeneration of bone formation. Nutritional outcomes associated with zinc deficiency in man include; memory impairment, alopecia, deficiency, muscular weakness and fatigue, dermatitis, delayed puberty, lower fertility, retarded growth, less appetite, diarrhea, strange behaviors, and loss of taste [15,16]. Deficiency of zinc in the plants has many negative effects on the plants themselves which include diseases like chlorosis, necrotic lesions, bronzing foliation, stunted leaves and overall weakening of the plant bodies [17]. Zinc(II) carboxylate complexes are common among metal carboxylates due to their structural and chemical difference. The carboxylate ligand is versatile and can be monodentate, bridging, and chelating, bidentate to zinc [18]. Several binding modes are distinguished by single crystal analysis, spectroscopic methods, and NMR experiments [19]. In these complexes, the carboxylate ligand can show a different type of spectroscopic behaviour just due to chemical shift. The technique is particularly important in catalytic events such as enzymatic reactions that involve zinc in biological systems [20]. These variables are the role of Lewis bases as co-ligands, hydrogen bonding, the interaction between carboxylate and other co-ligands and pH [21]. Various geometries of zinc carboxylate complexes including tetrahedral, octahedral, trigonal bipyramidal and square pyramidal are produced through carboxylate shift process in one-dimensional two-dimensional and three-dimensional structures [22]. Heteroleptic complexes of zinc carboxylates are well characterized with nitrogen donor co-ligands including substituted pyridines and polypyridyl ligands. N-donor co-ligands can affect the biological properties of zinc(II) carboxylates. For example, when polypyridyl co-ligands including 1,10-phenanthroline and 2,2'-bipyridyl were incorporated into zinc(II)carboxylate complexes the biological activity was enhanced compared to activity of simple zinc(II) carboxylates [23,24]. The inclusion of N-donor co-ligands in zinc carboxylate complexes has appreciable effects on their structure, physicochemical properties and outcome[19]. Heteroleptic zinc(II) complexes ligands, acting as lewis bases, are important because they can be used in catalysis, hybrid organic inorganic materials and biological applications [21]. Both accelerated and retarded reactions are achieved when nitrogen donor ligands are employed as co-ligands for varying chemistries of zinc(II) carboxylates. Coordination compounds with nitrogen

donor ligands exhibit distinct beneficial actions with regard to their catalytic activity in enzyme-catalyzed reactions. They are scrutinized for their astonishing biological efficacy such as for their anticancer, antiviral, and antimicrobial properties [24].

In the current study alpha amylase and alpha glucosidase enzymes inhibitory assay were assessed for the five synthesized zinc complexes.

2. Materials and Methods

2.1 Chemicals

Zinc (II) acetate dihydrate, 2-(2-methoxyphenoxy)acetic acid, pyridine, 3-methylpyridine, 2,2'-bipyridine, 1,10-phenanthroline, alpha-amylase enzyme, starch, dinitrosalicylic acid and DMSO were purchased from Sigma Aldrich.

2.2 Synthesis of the Complexes

a) C1

0.241g of zinc(II) acetate dihydrate (0.001 mol) was dissolved in 20 mL methanol in round-bottomed reaction flask. To the stirred zinc(II) acetate dihydrate solution in methanol (50 mL) was added a solution of 2-(2-methoxyphenoxy)acetic acid (0.400 g, 0.002 mol, in 20 mL of methanol) in aliquots with stirring. The reaction mixture was allowed to stir and was refluxed for six hours. After the reaction, the flask was cooled before the solution was filtered. Then the filtrate was allowed to stand for crystallisation at room temperature.

Color: white, Composition: $\text{ZnC}_{18}\text{H}_{22}\text{O}_{10}$; Formula mass: 463.75; M. Point: 76°C; Yield: 65%;

b) C2

The synthesis of the second complex, complex C2, was formulated following a preliminary procedure that was implemented for complex C1. After that, a nitrogen donor co-ligand, pyridine (176μL), was added into the reaction flask, and the resulting solution was refluxed for another six hours. The further cooling and crystallisation of the solution/filtrate were done as the procedure applied for the synthesis of the C1 complex. The molar ratio was 1:2:2 for zinc(II) acetate dihydrate, 2-(2-methoxyphenoxy)acetic acid and pyridine.

Color:White, Composition: $\text{ZnC}_{28}\text{H}_{28}\text{N}_2\text{O}_8$; Formula mass: 585.92; M. Point: 88°C, Yield:71%;

C) C3

Just like C2 the complex C3 was synthesised by making the two end aryl rings analogous to the meta-substituted phloroglucine through the procedure stated. However, 3-methyl pyridine equivalent to 231 μL was used instead of pyridine as the N-donor co-ligand. The molar ratio was 1:For zinc(II) acetate dihydrate as the metal source, 2-(2-methoxyphenoxy)acetic acid as the ligand, and 3-methylpyridine as the coligand the ratio used in the coordination was 2:2:2 respectively.

Color: White, Composition: $\text{ZnC}_{30}\text{H}_{32}\text{N}_2\text{O}_8$; Formula mass: 613.97; M. Point: 104°C, Yield: 82%;

d) C4

For synthesis of C4, the procedure that was followed for the synthesis of C2 was repeated, with a slight variation in the fact that

1,10-phenanthroline (0.1964g, 0.001mol) was used in place of pyridine as the N-donor co-ligand. The molar ratio was 1:A molar ratio of 2:1:1 for $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$, 2-(2-methoxyphenoxy)acetic acid, and 1,10-phenanthroline was used.

Color: Brown, Composition: $\text{ZnC}_{30}\text{H}_{26}\text{N}_2\text{O}_8$; Formula mass: 607.93; M. Point: 110°C, Yield: 62%;

e) C5

The synthesis of Complex C5 was made using the same procedure and mole ratios that were used during the synthesis of C4. The nitrogen donor co-ligand used was 2,2'-bipyridine 0.1710g, 0.001 mol.

Color: Reddish, Composition: $\text{ZnC}_{28}\text{H}_{26}\text{N}_2\text{O}_8$; Formula mass: 583.90; M. Point: 118°C, Yield: 52%;

2.3 Alpha-Amylase Inhibition Assay

The synthesised compounds were tested using a procedure reported in the literature for the alpha-amylase inhibition schedule [25]. Twenty microlitres of the test compound mixtures at various concentrations were added with ten microlitres of Alpha-amylase (2 U/ml), and sixteen microlitres of phosphate buffer (100 mM, pH 6.8) and were incubated in a 96-well plate for twenty minutes at 37°C. A quantity of twenty microlitres of the substrate, 1% soluble starch dissolved in 100 mM phosphate buffers were used. The incubation was performed for 30 minutes at 37°C for the incubation. The DNS colour reagent was boiled for 10 minutes then 100µl was pipetted into the tube. The absorbance of the milk blend that formed was determined at 540 nm. Glibenclamide was used at different concentrations as a reference while Acarbose was also used in equal concentrations. The findings were presented as percent inhibition using the relationship as stated below.

$$\text{Percentage inhibition} = (1 - \text{As}/\text{Ac}) \times 100$$

Where, As = Absorption of the sample

Ac = Control absorption.

2.4 Data Statistics

All the responses were done in triplicate. The calculation of the IC₅₀ and SEM values included two essential statistical concepts: column statistics and linear regression software was utilized from Graph Pad Prism version 6. The statistical evaluation of variance through one-way analysis of variance (ANOVA) using Graph Pad Prism software was used.

2.5 Alpha-Glucosidase inhibition Assay

A modified method for screening Alpha-glucosidase inhibitors was used here [26]. In this test, 210 µL of total volume of each plate was retained in each of the 96 wells. For each well, the reaction medium was made up of 20 µL of the sample, which was prepared by diluting a 2 µL aliquot in 18 µL of dimethyl sulfoxide, 20 µL of alpha glucosidase solution, 130 µL of PBS and 40 µL of the substrate p Nitrophenyl alpha D glucopyranoside. A total of five fold dilution of 20µL in DMSO is made on each sample by serial dilution. Further 130µL of phosphate buffer with pH 6.8 and 20µL of enzyme Alpha-glucosidase were added to the above reaction mixture. The above mixture was then shaken for 15 minutes at 37°C. The absorbance was determined at 405 nm by using the Elx 800 Reader.

2.6 Data statistics

In experiment of alpha-glucosidase inhibition, the IC₅₀ value was used for showing the results. The IC₅₀ value was estimated by non-regression analysis using the mean inhibitory value and the percent inhibition versus the inhibitor concentration on a semi-logarithmic graph. Acarbose was the reference used in the study. In total, each of the tests was repeated three times.

2.7 Characterization

The melting points of the synthesised compounds were determined using Stuart SMP10 in United Kingdom. Fourier transform infrared spectra were obtained using an infrared spectrophotometer. UV-VIS spectra were recorded at the Perkin-Elmer Spectrophotometer.

3. Results and Discussion

3.1 FT-IR Spectroscopy

Comparative analysis of the FT-IR spectra of the synthesised product and that of known metal carboxylates shows that FT-IR spectroscopy is a widely used method for the characterization of metal carboxylates. The whole of the conventional specific peaks are measured within the band of 4000-400 cm^{-1} [27]. The spectra also explain the bonding mode of the carboxylate ligand and confirm the synthesis of zinc(II) carboxylates [28]. The following findings were made while working on the FT- IR spectra of the synthesised compounds. The broad symmetric OH band is seen in the C spectra at 3500-2500 cm^{-1} . The absence of the OH band in the spectra of the complexes confirms a deprotonation of the 2-(2-methoxyphenoxy) acetic acid and its coordination to the Zn(II) ion through the COO group [29].

There is less intense and narrow band around 3352 cm^{-1} in the complex C1, which indicates the interaction of H_2O molecules with Zn(II) [30]. High intensity bands were observed at 1733 cm^{-1} C=O and 1229 cm^{-1} C-O in the C spectra. In complexes, two high intensity bands replaced the asymmetric and symmetric COO vibrations in the regions, 1612-1591 cm^{-1} and 1497- 1419 cm^{-1} [29]. The nature of the carboxylate group interaction with the metal could be determined from the spectrum by finding the difference between the asymmetric and symmetric COO stretching frequencies ($\Delta\nu$). Based on the previously described information, the synthesised complexes C1, C2, C3, C4 and C5 had $\Delta\nu$ of 137, 137, 145, 172 and 115 respectively. As the $\Delta\nu$ value for the complexes was established at less than 200 cm^{-1} , it became clear that the carboxylate ligand is bidentate chelating with the Zn(II) ion [31,32]. The presence of Bands below 600 cm^{-1} which are out of our range and perhaps due to the vibrations of the Zn-O (494-451 cm^{-1}) and Zn-N (548-512 cm^{-1}) bonds conformed the formation of the complexes. Evidence for the N-donor co-ligands in complexes C2, C3, C4 and C5 is supported by observed ring wagging vibrations at 742, 705, 723 and 737 cm^{-1} , respectively.

3.2 UV-Visible Spectra

The obtained complexes were characterized by UV-Vis spectroscopy. The UV-Visible absorption spectra of the complexes (100µM) in CH_3OH are represented in Figures 1. These absorption bands were identified in the range of 350-200 nm. The bands may arise from intra-ligand π to π^* transition. A slight red shift might be due to the extended conjugation of the broad aromatic system of the N-donor co-ligands in all C2-C5 complexes.

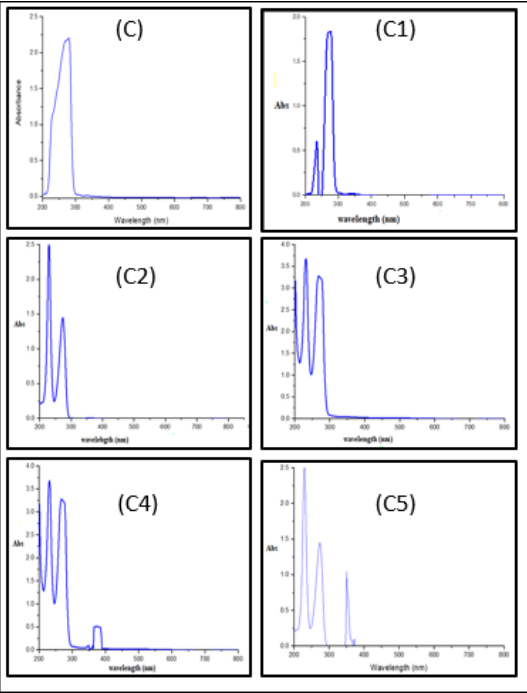


Figure 1. UV-Visible Spectra

3.3 Inhibition action of Alpha-Amylase

The potency of the C and its complexes in the immobilization of alpha-amylase at different concentrations is shown in figure 2. The result shows that the inhibition of alpha-amylase by the test complexes increases with increasing concentration. As shown in Table 1 the complexes possess lower IC50 values reflecting the improved ability of the complexes to inhibit the Alpha-Amylase enzyme. The synthesised complexes exhibit the potential to inhibit alpha-amylase according to the IC50 values in the following order: C5 > C3 > C1 > C2 > C4 > C. The complex C5 is found to provide the maximum percentage of inhibition having an IC50 value of 5.227 and the lowest inhibition is shown by the complex C4 having IC50 value of 10.13. The complexes show a better enzyme inhibition activity than the free C, which has the IC50 value of 11.23. The lower IC50 values, compared to the C, confirming enhanced enzyme inhibition ability of the complexes, evidences the role of zinc in enzyme inhibitory activity. Zinc(II) ion has lewis acid nature and has highly charged density, it can coordinate with the donor groups present in the enzyme’s active sites for inhibitory action.

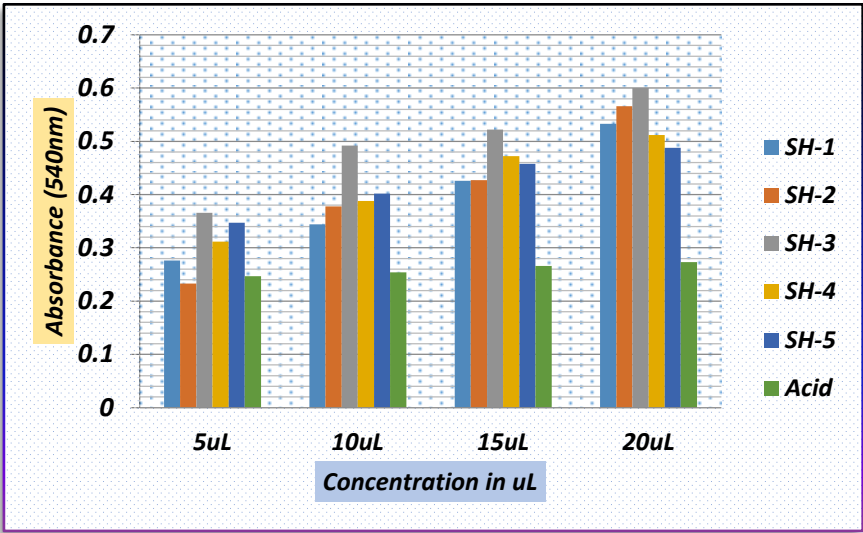


Figure 2 Alpha-Amylase inhibition activity of the C and complexes.

Table 1: IC ₅₀ data for alpha-amylase inhibition potential of the C and complexes		
S.NO.	Compound	IC ₅₀ ± SEM
1	C1	8.02 ±0.072
2	C2	9.17± 0.058
3	C3	6.73± 0.074
4	C4	10.13± 0.061
5	C5	5.227±0.063
6	C	11.23 ± 0.045

3.4 Alpha-Glucosidase Inhibition Activity:

The ability of the synthesised complexes to act as alpha-glucosidase inhibitors was evaluated at various concentrations of the complexes (5-20 μ L). An absorbance versus a logarithm of inhibitor concentration graph was plotted to construct the dose-response curve. The bar graph above underlined the connection between activity and concentration levels. Altogether, the distilled results clearly indicate that the inhibitory activity of complexes rises with higher sample concentrations. The effect of alpha-glucosidase inhibition by the C and its complexes is shown in Figure 3, while Table 2 shows the IC₅₀ values. Acarbose was used the standard drug in the experiment. The C and synthesised complexes demonstrated Alpha-glucosidase inhibitory potential in the following order: C4 > C2 > C > C3 > C1 > C5.

It is seen that the complex C4 has the highest level of inhibition with the IC₅₀ of 6.056 and the least level of inhibition corresponding to the IC₅₀ value of 13.44 for the complex C5.

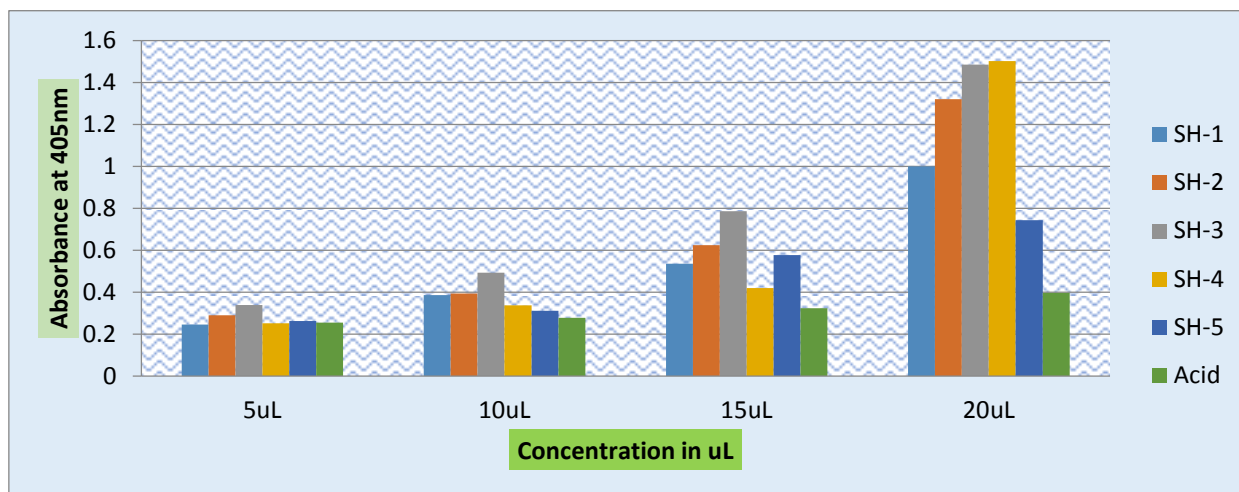


Figure 3 Alpha-Glucosidase inhibition activity of the C and complexes.

S.NO.	COMPOUNDS	IC ₅₀ ±SEM
1	C1	12.07 ±0.085
2	C2	9.09 ±0.032
3	C3	11.67 ±0.078
4	C4	6.056 ±0.052
5	SH-5	13.44±0.098
6	C	10.78 ± 0.084

Conclusion

Zinc(II) complexes of 2-(2-methoxyphenoxy)acetic acid were synthesised without (C1) and with (C2, C3, C4, and C5) N-donor co-ligands in methyl alcohol solvent under reflux conditions. The complexes were obtained in moderate to good yields (52-82%). FT-IR spectra indicated a chelating bidentate coordination of the carboxylate ligand to zinc in the complexes, as evidenced by the $\Delta\nu$ values (less than 200 cm^{-1}).

The UV-Vis spectra exhibit bands within the 350-200 nm region due to intra-ligand $\pi-\pi^*$ transitions. The test compounds shown significant alpha-amylase enzyme inhibition potential in the following order: C5 > C3 > C1 > C2 > C4 > C. The increased activity of the complexes relative to the C underscores the

significance of zinc metal in the suppression of alpha-amylase enzyme activity. The complexes demonstrated significant alpha-glucosidase enzyme inhibition potential in the following order: C4 > C2 > C > C3 > C1 > C5.

List of abbreviations

[Zn(II)-{2-(2-Methoxyphenoxy)acetate}₂(H₂O)₂], C1

[Zn(II)-{2-(2-Methoxyphenoxy)acetate}₂(Py)₂], C2

[Zn(II)-{2-(2-Methoxyphenoxy)acetate}₂(3-Mepy)₂], C3

[Zn(II)-{2-(2-Methoxyphenoxy)acetate}₂(1,10-Phen)], C4

[Zn(II)-(2,2-Methoxy-phenoxyacetate)₂(2,2'-bipy)], C5

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