Antioxidant, enzyme kinetics, α-amylase, α-glucosidase inhibition and molecular docking studies of (S,N,O$^-$) type Schiff's Base and its Pd(II) complex

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Abstract. The antioxidant properties, molecular docking interactions α-glucosidase inhibition, α-amylase, and enzyme kinetics, of (S,N,O$^-$) type Schiff's base (**L**) and its complex **Pd-L** (**1**) were evaluated. The antioxidant activity was evaluated using various *invitro* assays, revealing significant free radical scavenging potential. Enzyme kinetics studies were conducted to assess the catalytic efficiency and substrate specificity of the compounds, providing insights into their enzymatic interactions. Furthermore, the α-glucosidase inhibition activity was examined, demonstrating promising inhibitory effects against this enzyme implicated in diabetes management. Molecular docking studies were performed. Overall, these findings highlight the potential of **L** and **Pd-L** (**1**) as multifunctional agents with antioxidant and enzyme inhibitory properties, offering prospects for further exploration in drug development and therapeutic interventions.

Keywords: Schiff's base, Pd, antioxidant, enzyme inhibition, molecular docking

1 Introduction

Hydrazone based ONS donor ligands and their metal complexes encompass a wide spectrum of pharmacological and biological studies [1-8]. A wide-range of hydrazide-Schiff's bases and complexes are noticed to possess antidiabetic activities by many research groups. Among the class of hydrazide, Pd(II) complexes are more effective α-glucosidase inhibitors [9]. Several hydrazide Schiff base ligands are reported and extensively evaluated for antidiabetic activity and also antioxidant, antiinflammation, and antimitotic activities[10-13]. The literature reports on hydrazide Schiff's base with various metal complexes revealed the antidiabetic activity studies, including Vanadium(IV/V) complexes of salicylhydrazone Schiff base in the

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inhibition of human tyrosine phosphatises [14], Compared to standard acarbose chromonehydrazones and their metal complexes have shown higher inhibition [15-17].

Also, the chelation of ONS donor atoms to the transition metal complexes has gained remarkable attention owing to the participation in various redox reactions [18-20]. The substrates with NS donor ligand have been investigated as chelating agents to extend effective model complexes and shown effectiveness in biological systems [21-23]. Additionally, the formation of sulfur coordinated transition metal complexes offer stability, and also can be used as economical reagents in stoichiometric percentage of reagent load, low/no toxicity and no requirement of an inert atmosphere.

Owing to the abovementioned facts and in the field of coordination chemistry [24-26], we hereby reported the antioxidant, α-glucosidase inhibition, enzyme kinetics properties and molecular docking study of **L** and complex, **Pd-L** are reported.

2 Experimentation

2.1 Materials and methods

The materials and methods for **L** and complex, as per the reported earlier[24].

2.2 Molecular docking studies

The synthesized **L** and **Pd-L** and standard acarbose were sketched using chemsketch v14.0 and the homology modelling was performed by using MODELLER 9.15program [33]. The docking was conducted to analyze the binding affinity between the **L**, **Pd-L** and acarbose with α-glucosidase using GEMDOCK (Generic Evolutionary Method for Molecular Docking) which works based on evolutionary algorithm [34]. The 3D structures of **L** and **Pd-L** were obtained using Open babel (2.3.0 version) software programs. The default parameters were used for docking. The best-scoring pose was chosen based on binding affinity of the compounds against α-glucosidase, which was predicted by GEMDOCK and visually analyzed using Discovery studio Visualizer 2021 software. Further ADME studies of **L** and **Pd-L** were conducted for the better understanding of pharmacological activity.

2.3 Biological Activity

2.3.1 Antioxidant assays

In this study, ABTS, DPPH and superoxide radical scavenging activities were determined and measured as per the reported procedure [27]. The samples of **L** and **Pd-L** were assessed in varying of concentrations from 0 – 1000 µg/ml.

2.3.2 Inhibition of α–amylase and α–glucosidase

The α-amylase inhibition and the yeast α-glucosidase inhibition of **L** and **Pd-L** were assayed using the substrate pNPG according to the reported method [28]. For α-glucosidase inhibitory activity, the test samples, **L** and **Pd-L** solution prepared in DMSO with phosphate buffer (700 µl 50 mM, pH 6.8). The IC_{50} values of **L**, **Pd-L** and standard (acarbose as a positive control) were reported.

2.3.3 Kinetics of α-glucosidase inhibition

The enzyme kinetics on inhibition of α-glucosidase activity by **Pd-L** was studied using varied IC_{20}, IC_{40} and IC_{60} inhibitory concentrations as per the reported method [29-32].

3 Results and discussions

3.1 Synthesis

The ligand, **L** and its complex **Pd-L** were prepared as per our earlier reported literature [24] and the structures of are given in **Chart 1**.

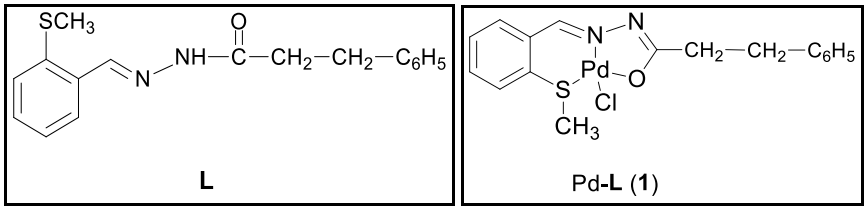

Chart 1 Structure of **L** and Pd-**L** (**1**)

3.2 Molecular Docking

. In our study, **L** and **Pd-L** with noteworthy α-glucosidase inhibition was subjected for docking studies against α-glucosidase which was homology built using MODELLER 9.15 [35]. The binding interactions of acarbose, **L** and **Pd-L** with α-glucosidase enzyme are given in **Figure 1a-1c** respectively. The interactions with the **L** revealed that it forms a hydrogen bond with HIS279. It also binds through 2 π-sulphur bond with HIS239 and PHE311. Further, hydrophobic bonds like π-π stacked with HIS239, π-alkyl bond with LYS155 and ARG312 are showed. The ligand also forms an electrostatic bond with HIS239. Along with 8 non-bonding interactions, the total binding energy estimated of **L** is found to be -79.7716. On the whole molecular docking studies reveal the similar binding pattern was observed with the known drug acarbose. Whereas, the complex **Pd-L** was predicted with 13 non-bonding interactions and a total energy -77.5547. **Pd-L** formed four hydrogen bonds with HIS239 (2 bonds), GLU3014, and SER308. It also formed metal-acceptor bonds including Pd-S, Pd-N, Pd-O, and Pd-Cl. In addition, an electrostatic bond with HIS279 was also formed. In case of hydrophobic bonds **Pd-L** formed 2 π-π stacked interactions with HIS279 and PHE311. Also, π-alkyl bonds with PRO309 and ARG312 were formed.

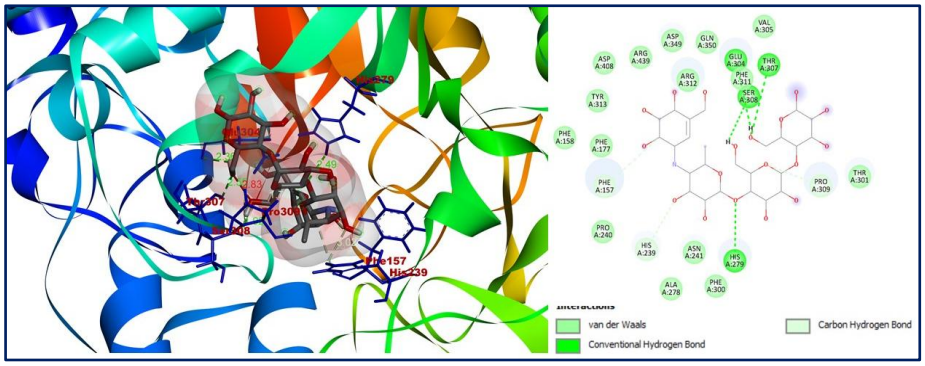

Figure 1a Visualization of acarbosebinding with yeast α-glucosidase enzyme (3D and 2D interactions)

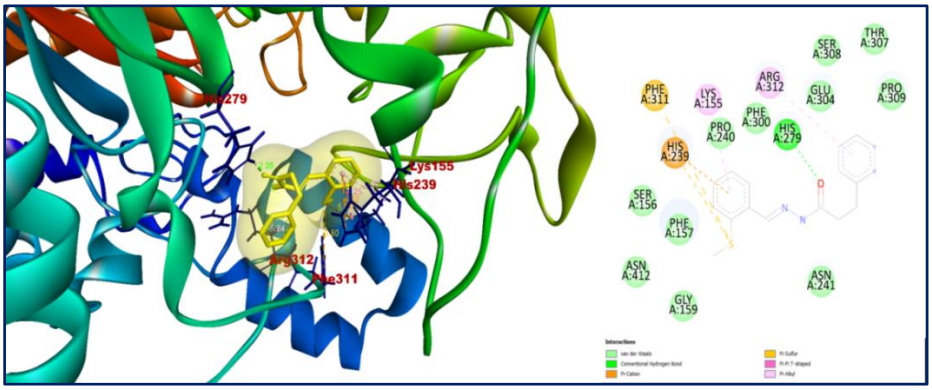

Figure 1b Visualization of **L** binding with yeast α-glucosidase enzyme (3D and 2D interactions)

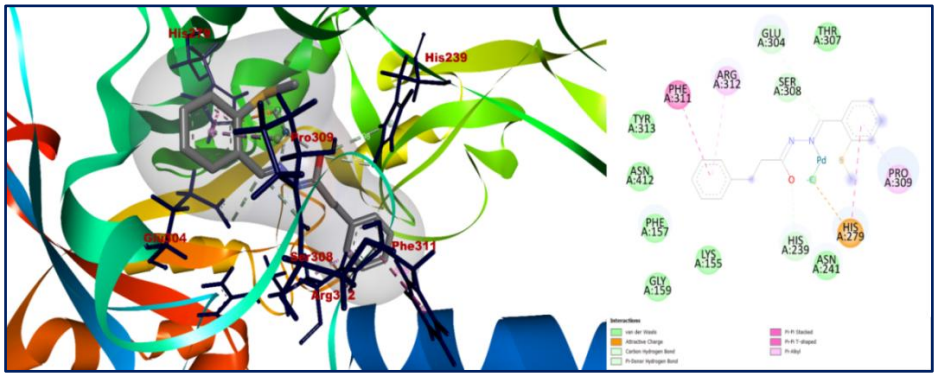

Figure 1c Visualization of **Pd-L** binding with yeast α-glucosidase enzyme (3D and 2D interactions)

3.3 Biological Activity

3.3.1 Antioxidant activity

The various free radical scavenging activities were carried out and measured as per the standard reported procedure and the results were tabulated as follows [27].

Table 1 Antioxidant activity of **L** and **Pd-L**

Compound	$EC_{50}^{*,\#}$ (mg/ml)		
	Radical scavenging activities		
	DPPH	ABTS	Superoxide
L	2.60 ± 0.22^{c}	3.47 ± 1.36^{c}	3.40 ± 0.79^{c}
[Pd(**L**)Cl]	1.68 ± 1.43^{b}	2.30 ± 0.17^{b}	2.02 ± 0.86^{b}
Standard ^	0.65 ± 0.06^{a}	0.50 ± 0.04^{a}	0.70 ± 0.32^{a}

From all the scavenging activities, it was concluded that the **Pd-L was effective than L.** When the results were compared with standard BHA, it was in the following order Butylated hydroxyl anisole (BHA) > **Pd-L** >**L.**

3.3.2 Inhibitory effects on yeast α-glucosidase and α-amylase

In the present analysis, determination of *in vitro* inhibition potential demonstrated that **Pd-L** was more effective in the inhibition of the enzyme over that of its ligand, **L** with an IC_{50} values of 2.50 and 1.45 mg/mL for **L** and **Pd-L** , respectively. From the results it can be concluded that **Pd-L** having the strong inhibition than **L** against α-glucosidase and α-amylase.

Table 2 Inhibitory activities of **L** and **Pd-L**

Compound	$IC_{50}^{x,y}$ (mg/mL)	
	α-amylase	α-glucosidase
L	2.26 ± 0.85^{b}	2.50 ± 0.52^{c}
[Pd(**L**)Cl]	1.96 ± 0.27^{b}	1.45 ± 0.26^{b}
Standard^	0.50 ± 0.21^{a}	0.70 ± 0.24^{a}

[x]*Values are expressed as mean ± SE.*

3.3.3 Kinetic analysis of α-glucosidase inhibition

Ligand, **L** and metal complex **Pd-L** were evaluated for the kinetics of inhibition at different concentration of substrate and mode of inhibition and determined [K_m] and [V_{max}] values. The plots of inhibition for L and complex **1** were shown in **L** (**Figure 2a**) and **Pd-L** (**Figure 2b**). From the plots, it indicates the mechanism of inhibition reversible.

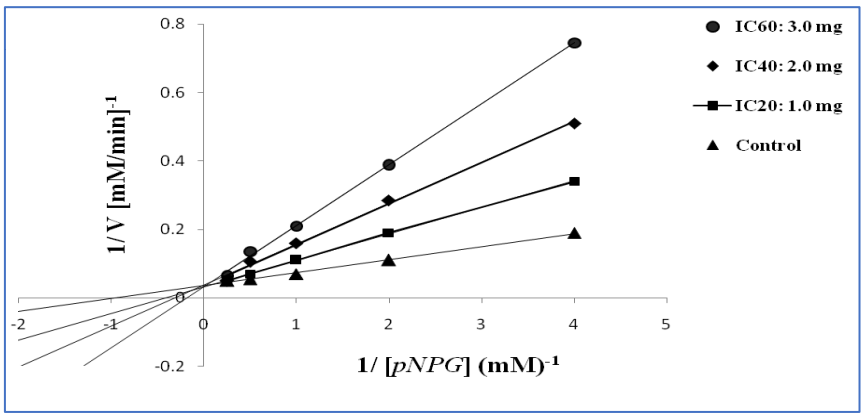

Figure 2a Lineweaver-Burk plot of substrate inhibition by **L**.

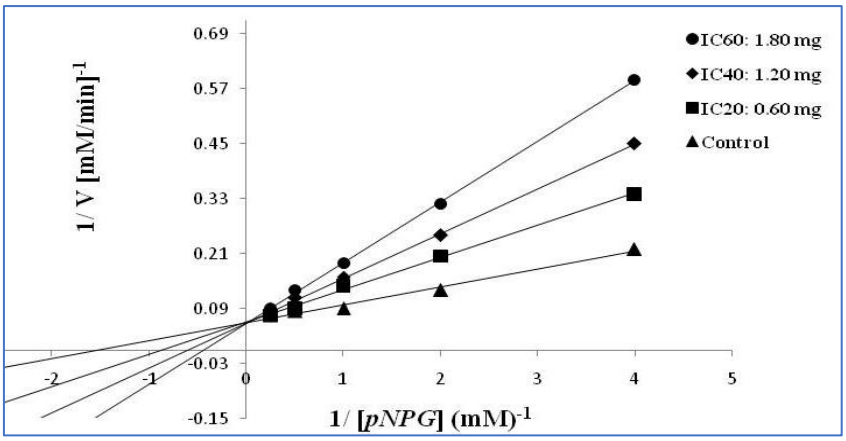

Figure 2b Lineweaver-Burk plot of substrate on inhibition by **Pd-L**

The inhibitory dissociation constant (K_i), were determined as 0.79 mg for **L** and 0.89mg for **Pd-L** as in **Table 3**.

Table 3 Enzyme kinetics of α-glucosidase by **L** and **Pd-L**

Compound	Treatment				
L	Control	Competitive	1.06	30.49	0.79±0.09
	IC_{20} 1.0 mg		2.42	31.25	
	IC_{40} 2.0 mg		3.33	27.75	
	IC_{60} 3.0 mg		5.43	30.49	
[Pd(**L**)Cl]	Control	Competitive	0.65	16.72	0.80±0.16
	IC_{20} 0.60 mg		1.17	16.50	
	IC_{40} 1.20 mg		1.67	17.09	
	IC_{60} 1.80 mg		2.25	17.00	

[x]*inhibition Values are expressed as mean ± SE.*

3.3.4 ADME Study

ADME (absorption, distribution, metabolism and excretion) study was performed using Swiss ADME tool which showed that **L** and **Pd-L** both can be easily passed through blood-brain barrier. The pharmacokinetics calculation for **L** and **Pd-L** show that the GI absorption is high and are poorly soluble in water, whereas skin permeation is moderate **(Table 4)**. In general, the compounds follow all the drug rules and there is no violation of Lipinski rule of five while, in medical Chemistry analysis there is a violation in Brenk and lead likeness.

Table 4 ADME study of **L** and **Pd-L**

Compound	Blood-brain barrier	Water Solubility	GI absorption	Drug likeness	Medical Chemistry	Log K_p

L	Yes	Poor	High	Pass	Violation	-5.59 cm/s
[Pd(**L**)Cl]	Yes	Poor	High	Pass	Violation	-5.57 cm/s

4 Conclusion

In summary, the screening studies showed that **Pd-L** fared better than **L** in terms of IC_{50} values of α-glucosidase inhibition activity, but below the standard. Furthermore, the kinetic studies revealed that the mode of inhibition was similar to the standard drug showing a competitive mode exerted by both the compounds. The docking studies revealed that the binding of **L** and **Pd-L** to the active site of α-glucosidase, signifies that both the **L** and **Pd-L** indeed possesses prospective antidiabetic activity. The activities of **L** and **Pd-L** demonstrated that the complex **Pd-L** identified as a lead candidate which can serve for the development of new class of inhibitors.

Conflicts of Interest

The authors declare that they have no competing interests.

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