



IN-SILICO AND IN-VIVO ANTIDIABETIC STUDIES OF HYDRAZONE, DERIVED FROM 4-AMINOANTIPYRINE AND 1,3-DIPHENYLPROPANE-1,3-DIONE (HPDP) AND ITS Co(II) AND Cu(II) COMPLEXES

AGBO, N.J.^{1*} AND UKOHA, P.O.²

¹Chemistry Advanced Research Centre, Sheda Science and Technology Complex (SHESTCO), Abuja, Nigeria,

²Department of Pure and Industrial Chemistry, Coordination Chemistry and Inorganic Pharmaceutical Unit, University of Nigeria, Nsukka, Nigeria

ABSTRACT

This investigation assesses the *in silico* molecular interactions against target proteins, *in silico* binding affinity of HPDP and its metal complexes against specific molecular targets. toxicity, and antidiabetic potential of 3-[-2-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)hydrazylidene]-1,3-diphenylpropane-1,3-dione] (HPDP) and its Co(II) and Cu(II) complexes, represented as [Co(PDP)₂] and [Cu(HPDP)₂Cl₂] respectively. Acute toxicity investigation was carried out to identify compounds with low toxicity, for *in-silico* analysis and *in-vivo* studies for potential antidiabetic benefits. The two pharmacological targets used were acetylcholinesterase (3LII) and fructose-1,6-biphosphatase (2JJK). A single intraperitoneal dose of 140 mg/kg of alloxan was used to induced diabetes in the experimental Abino rats (120 mg/kg body weight). Rats were divided into nine groups: control (A), diabetic control group (B), diabetic group (C) receiving glibenclamide treatment. The treatment groups (1A, 1B; 2A, 2B and 3A, 3B) received, for a period of 14 days, low and high doses (200 and 400 mg/kg of body weight) HPDP, [Co(PDP)₂] and [Cu(HPDP)₂Cl₂] compounds, respectively. *In silico* molecular docking showed promising binding interactions with important one antidiabetic target. Within 14 days of therapy, the results showed that HPDP, [Co(PDP)₂], [Cu(HPDP)₂Cl₂], and glibenclamide (standard control) decreased the blood glucose levels of the experimental rats by 74.08%, 36.55%, 70.53%, and 60.56 %, respectively. These results imply that these compounds could make interesting candidates for treatments of diabetes.

Keywords: Acute toxicity, Complex, Diabetes, Hydrazone and Molecular docking

***Correspondence:** agbo.ndj@gmail.com, 08057284501

INTRODUCTION

Diabetes is a condition that alters how the body uses sugar [1]. Each kind of diabetes has a different underlying etiology [2]. Whatever the kind, it can cause blood sugar levels to rise too high, which can result in life-threatening health issues. Diabetes cannot be cured with medication, and there are no immunizations available to stop it. Due to less sensitivity and high levels of toxicity of diabetic drugs after extended use, the medications now used to manage disease are losing their efficacy [2].

Millions of people worldwide suffer from diabetes mellitus, a chronic metabolic illness that makes the development of new treatment interventions necessary. Therefore, it is essential to develop new medications that are less harmful and sensitive to disease in order to manage it.

Metal complexes have been investigated for their increased biological activity, and hydrazone derivatives have demonstrated encouraging anti-diabetic potential [3]. Data on hydrazone produced from 1,3-diphenylpropane-1,3-dione and 4-aminoantipyrine combination are few [4]. Hydrazones

are produced when aryl diazonium salts are combined with ketones or aldehydes [3, 4, 5]. Researchers' interest in studying hydrazones has increased due to their versatility as antibacterial, analgesic, anti-inflammatory, antiplatelet, antituberculosis, and anticancer medicines [6, 7, 8].

Since there are evidences supporting the use of these hydrazones as antidiabetic medications, taking resort to the proven activities of hydrazones as cardiac and central nervous system agents and pharmacological activities of 4-aminoantipyrine [9 - 12], development of antidiabetic medicines from two compounds will be extremely feasible.

The antidiabetic effects of 3-[-2-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl) hydrazylidene]-1,3-diphenylpropane-1,3-dione (HPDP) and its Cu(II) and Co(II) complexes are examined in this work. The target compounds were synthesized from 4-aminoantipyrine and 1,3-diphenylpropane-1,3-dione following the method described in Agbo and Ukoha (2024) [13]. Complex formation is known to increase the physiological characteristics of ligands [13]. In order to discover possible candidates for the development of novel anti-

diabetic therapies, we assessed the binding interactions and anti-diabetic efficacy of HPDP and its metal complexes using a mix of in vivo experiments and in silico molecular docking investigations.

MATERIALS AND METHODS

Experimental animals

All study with albino rats and mice was approved by the University of Nigeria, Nsukka, Faculty of Veterinary Medicine, Institutional Animal Care and Use Committee (Ethical Approval Reference Number: FVM-UNN-IACUC-2023-11/132). A total of 35 male and female albino rats weighing an average of 120 ± 20 g were used for *in vivo* antidiabetic study; While, 90 mice were used for acute toxicity testing (LD_{50}). These were obtained from the Faculty of Veterinary Medicine, Animal House, University of Nigeria, Nsukka, Enugu State, Nigeria. The animals were housed in standard environmental conditions. They were acclimatized for seven days.

Acute toxicity (LD_{50}) test

The Lorke (1983) method [14] was applied in order to determine LD_{50} . Ninety-nine male and female mice were used after a 24-hour acclimatization period. The mice were placed in three groups of five each and given between 10 to 1000 mg/kg body weight of test compounds separately via a solution of 3% v/v normal saline. After 24 h observation, death pattern was noted and used for the second phase where between 1900 and 5000 mg/kg body weight of compounds were offered. A pattern of lethality after 24-hour was recorded.

In-silico studies

In-silico physicochemical properties of HDPP, $[Co(HDPP)_2]$, and $[Cu(HDPP)_2Cl_2]$ were evaluated. These consist of molecular weight (MW), water/octanol partition coefficient $\log P(o/w)$, total polar surface area (TPSA), number of hydrogen bond acceptors (HBA), number of hydrogen bond donors (HBD), number of rotatable bonds (NoRB), and aqueous solubility ($\log S$).

Drug target

Fructose-1,6-biphosphatase (2JJK) and acetylcholinesterase (3LII) were the two drug targets studied. Human fructose-1,6- biphosphatase is a key gluconeogenic enzyme, responsible for the hydrolysis of fructose-1,6-bisphosphate to fructose-6- phosphate.

It is a potential drug target in the treatment of type II diabetes [15]. This presents an opportunity for the development of novel therapeutics focused on lowering the hepatic glucose production in type 2 diabetics. Epidemiological studies have provided scientific evidence for a significant association between Type 2 diabetes mellitus (T2DM) and Alzheimer's disease (AD) [15]. There are also many clinical and pathological data that suggest a convincing linkage between T2DM and AD [16-18]. Hyperglycemia and insulin dysfunction during diabetes may cause effects on memory, synaptic plasticity and learning which consequently lead to AD [19]. The association between cholinergic neurotransmission deficiency and AD provides a base for the development of acetylcholinesterase (AChE) inhibitors as a therapeutic agent [20].

Molecular docking

The 3D structures of fructose-1, 6- bisphosphatase 1, (PDB ID:2JJK) [21] and human brain AChE in apo form (PDB ID: 3LII) [22] were used to elucidate the enzyme's regulation, including substrate binding, catalysis, and inhibition and these were retrieved from the Protein Data Bank (PDB) (<http://www.pdb.org>). The structure of the ligand glibenclamide (CID: 91826496) was drawn using molecular modeling software. The substrates: N, N'-(heptane-1,7-diyldicarbamoyl) bis (3- chlorobenzenesulfonamide) and N-acetyl D-glucosamine were separated from 2JJK and 3LII respectively. MMFF94 force field was used for energy minimization of the ligand molecules. The docking of compounds into the binding sites was performed with the default options of the AutoDock Vina package (1.5.7 version) [23]. For the docking calculations, a cube grid box was prepared with dimensions of 3.391, 1.310, and -8.362 \AA in x, y, and z-directions. Furthermore, the grid box position was centered on the middle of compounds under docking. The AutoGrid Tools was used to form grid maps, and spacing within the grid was selected as 0.375 \AA . The grid consists of 40 points along x, y, and z-directions. We focused on the amino acids that play a key role in the binding with the active site of targeted compounds, as we listed in Table 5.

Experimental design

The animal experiments adhered to the Guide for the Care and Use of Laboratory Animals. Animals were weighed and randomly assigned to seven groups and treated as follows:

Table 1: Experimental design

Rat groups	Number of rats	Treatment
Group A	5	Normal control (No diabetes + No Treatment)
Group B	5	Positive Control (Induced diabetes + No treatment)
Group C	5	Standard Control (Induced diabetes + 200 mg/kg of b. w glibenclamide/standard drug treatment)
Group 1A	5	Induced diabetes + 200 mg /kg b. w of HPDP treatment

Group 1B	5	Induced diabetes + 400 mg /kg b. w of HPDP treatment
Group 2A	5	Induced diabetes + 200 mg /kg b. w of [Co(PDP) ₂] treatment
Group 2B	5	Induced diabetes + 400 mg /kg b. w of [Co(PDP) ₂] treatment
Group 3A	5	Induced diabetes + 200 mg /kg b. w of [Cu(HPDP) ₂ Cl ₂] treatment
Group 3B	5	Induced diabetes + 400 mg /kg b. w of [Cu(HPDP) ₂ Cl ₂] treatment

Diabetes induction

Rats in groups 1 through 5 were intraperitoneally given 120 mg/kg body weight of alloxan monohydrate (dissolved in 0.9% sterile NaCl solution of pH 7) in order to induce diabetes; the rats' blood glucose levels had already been measured.

The animals' blood was then extracted from their tail arteries, and the animals' blood glucose levels were determined using an Accu-check active glucometer (produced by Roche Diabetes Care, India). Rats were designated as diabetics for the purposes of the study if they showed clear signs of polyuria, polyphagia, and polydipsia after just one day and had serum glucose levels between 250 and 400 mg/dl [24, 25]. On days 7 and 14, the animals' blood glucose levels were monitored during the 14-day treatment and feeding period.

Blood sample collection

Rats were given a mild anesthetic with chloroform, permitted to starve for the duration of the experiment, and then had their jugular veins cut. Five milliliters of blood were collected from each rat, which was then transferred to a dry centrifuge tube and allowed to clot at room temperature (24–26°C). Following that,

centrifugation was used to separate the serum and clot for 15 minutes at 1000–2000 revolutions per minute. The obtained serum was refrigerated at 4°C in sterile vials.

Statistical analysis

The IBM version 20 of the Statistical Product and Services Solution (SPSS) was used for the descriptive analysis, and the results are presented as mean ± Standard deviation. A one-way analysis of variance ANOVA was used to determine significance, and $p < 0.05$ was the accepted threshold for significance.

RESULT AND DISCUSSION

Acute toxicity tests

Acute toxicity tests results (Table 2) show that for Mice administered with acute dose (1900 – 5000 mg/kg body weight of compounds), of HPDP, [Cu(HPDP)₂Cl₂] and [Co(PDP)₂] suffered no mortalities. Hence, the three compounds are safe and are within the range of toxicity category rating dose. They were employed as drug candidates for *in silico* and *in vivo* studies.

Table 2: LD₅₀ results of HPDP and its complexes

Phase 1	Dosages mg/kg	Mortality			Behavioural changes
		HPDP	V	Y	
Grp 1	10	0/5	0/5	0/5	Nil
Grp 2	100	0/5	0/5	0/5	Nil
Grp 3	1000	0/5	0/5	0/5	Nil
Phase 2					
Grp 4	1900	0/5	0/5	0/5	Nil
Grp 5	2600	0/5	0/5	0/5	Nil
Grp 6	5000	0/5	0/5	0/5	Weakness, drowsiness

Where V = [Co(PDP)₂], Y = [Cu(HPDP)₂Cl₂]

In-silico studies

Certain physicochemical factors impact a drug's bioavailability in the systemic circulation, which in turn determines its capacity to bind to the intended receptor and elicit pharmacological activity. Lipinski defined the rule of five (ro5) as a metric for determining a molecule's drug ability. A molecule must therefore have MW < 500, logP ≤ 5, HBD ≤ 5, and HBA ≤ 10 in order to be considered drug-like. Compounds that fail to meet multiple

physicochemical characteristics are deemed unlikely candidates for drugs [26].

Table 3 demonstrated that, the complexes exhibited a single deviation from the requirements, whereas the ligand fully complied with all requirements, showing no deviations. They are therefore probably drug candidates.

In rats, the bioavailability of a chemical is influenced by the number of rotatable bonds (NoRB). It has been demonstrated that NoRB with ≤ 10 has

good oral bioavailability [27]. NoRB < 10 for these synthesized compounds indicates a possible good oral bioavailability. The measurement of cell permeability is called total polar surface area, or TPSA. A molecule

that has a TPSA of less than 140 Å will not have trouble passing through the cell membrane [28 &29]. Every compound met these criteria.

Table 3: Physicochemical properties of HPDP and its complexes

Comp	MW	HBA	HBD	NoRB	logP(o/w)	logS	TPSA	LNV
HPDP	438.48	4	1	7	6.03	-6.373	82.08	1
[Co(PDP) ₂]	941.95	8	0	8	9.71	-11.41	78.3	2
[Cu(HPDP) ₂ Cl ₂]	1019.49	8	2	8	4.01	-12.85	85.88	2

MW: molecular weight; HBA: hydrogen bond acceptor; HBD: hydrogen bond donor; TPSA: total polar surface area; NoRB: number of rotatable bonds; logP(o/w): log octanol/water partition coefficient; logS: log solubility in water; LNV: Lipinski's number of violations

Molecular docking

The two pharmacological targets studied were fructose-1,6-biphosphatase (2JJK) and acetylcholinesterase (3LII). Five amino acid residues have been found to mediate glibenclamide interaction with the catalytic active site (CAS) of 2JJK: LYS 112, ARG 140, THR 27, LEU 30, and GLY 26 (Fig. 1;

Table 4). When compared to [Co(PDP)₂], [Cu(HPDP)₂Cl₂], and the co-crystallized inhibitor, the ligand exhibited the highest binding energy (-6.65 kcal/mol). This interaction was observed with three amino acid residues on the CAS: LEU 30, ARG 140, and GLY 21.

Table 4: Free binding energy (ΔG, kcal/mol) of ligands and some complexes and the interacting amino acid residues

Target	Compound	Binding energy, ΔG (kcal/mol)	Interacting amino acid residues
2JJK	Glibenclamide	-12.01	LYS 112, ARG 140, THR 27, LEU 30, GLY 26
	Co-crystallized ligand	-9.39	GLY 26, MET 18, THR 31, GLY 28, GLY 21, LEU 30
	[Co(PDP) ₂]	-7.07	2(THR 27), GLN 32
	[Cu(HPDP) ₂ Cl ₂]	-6.90	4(ASP 178), LYS 290
	HPDP	-8.65	GLY 21, LEU 30, ARG 140
3LII	Glibenclamide	-9.84	GLU 358, GLY 345, PRO 344
	Co-crystallized ligand	-9.55	ND
	[Co(PDP) ₂]	-8.87	GLY 345, 3(GLU 358)
	[Cu(HPDP) ₂ Cl ₂]	-12.51	GLY 345, 4(GLU 358), PRO 344
	HPDP	-9.55	SER 347, GLY 342

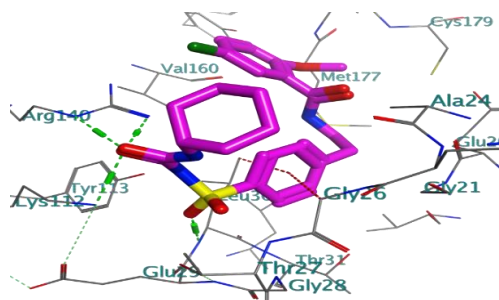


Figure 1: Interaction of Glibenclamide docked to fructose-1, 6-bisphosphatase 1 (2JJK). The ligand (Glibenclamide) has been shown in 'stick' representation (green dotted line = H-bond; red dotted line = van der Waal bond).

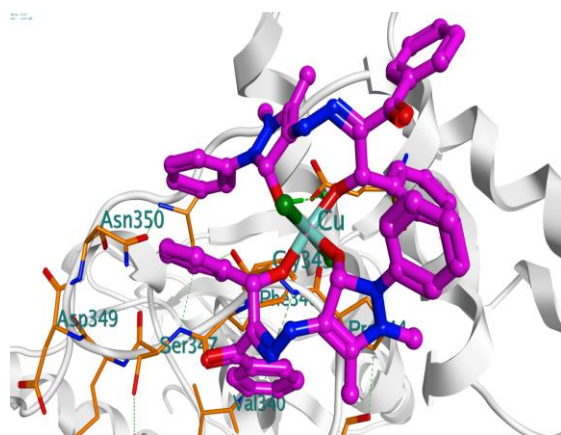


Figure 2: Binding pose of [Cu(HPDP)₂Cl₂] in the binding cavity of 3LII. The compound is shown in 'ball and stick' representation (green dotted line = H-bond)

Effect of HPDP, [Cu(HPDP)₂Cl₂] and [Co(PDP)₂] on blood glucose concentration of experimental diabetic rats.

Table 5: Effect of HPDP and its Cu(II) and Co(II) complexes on blood glucose concentration of Experimental Diabetic rats.

Group	Before Induction (mg/dL)	After Induction (mg/dL)	After 7 Days Treatment (mg/dL)	After 14 Days Treatment (mg/dL)	% decrease (7 days)	% decrease (14 days)
A	71.60±6.84 ^{Abc}	72.40±7.57 ^{Aa}	74.00±12.85 ^{Aa}	74.40±7.23 ^{Aa}		
B	73.40±9.91 ^{Abc}	308.00±65.36 ^{Bb}	414.00±45.67 ^{Cf}	527.80±37.12 ^{Df}		
C	76.40±5.32 ^{Ac}	294.60±77.75 ^{Cb}	175.00±60.38 ^{Bbcde}	116.20±18.23 ^{ABabc}	40.60	60.56
1A	76.40±6.80 ^{Ac}	326.40±74.97 ^{Cb}	142.60±33.82 ^{Babc}	84.60±21.65 ^{Aab}	56.31	74.08
1B	68.80±13.94 ^{Aa} _{bc}	333.20±65.42 ^{Cb}	195.40±69.51 ^{Bbcde}	92.40±13.35 ^{Aab}	41.36	72.27
2A	77.40±10.09 ^{Ac}	319.00±78.71 ^{Cb}	266.20±65.04 ^{BCe}	202.40±111.34 ^{Bd}	16.55	36.55
2B	75.80±7.53 ^{Ac}	361.40±78.07 ^{Bb}	245.80±128.61 ^{Bde}	268.60±125.02 ^{Be}	31.99	25.68
3A	59.00±8.06 ^{Aab}	343.40±68.94 ^{Cb}	226.00±84.04 ^{Bcde}	112.00±13.93 ^{Aabc}	34.19	67.38
3B	57.40±13.63 ^{Aa}	374.00±75.46 ^{Cb}	244.40±71.44 ^{Bde}	110.20±14.87 ^{Aab}	34.65	70.53

Results are expressed as mean \pm SD (n = 5). Values with different lower-case letters as superscripts down the column are significant at $p < 0.05$. Values with different upper-case letters as superscripts across the rows are significant at $p < 0.05$.

Table 5 presents a comparison between the blood glucose levels of diabetic rats treated with HPDP, $[\text{Cu}(\text{HPDP})_2\text{Cl}_2]$, and $[\text{Co}(\text{PDP})_2]$ with those of untreated rats and candidates for a normal control group.

Compared to the glucose concentration 24 hours after the induction of diabetes, the glucose concentration following seven days of HPDP treatment was shown to be significantly ($P < 0.05$) lower. Following a week of treatment, the glucose level was about 56% lower when a low dose of HPDP (20 mg/kg.b.w.) was administered. However, after a 14-day therapy period, the glucose level decreased by over 74%. The result is better than with Glibenclamide (61%), the traditional medicine. This suggests that HPDP outperforms glibenclamide in terms of effectiveness [18]. Using a larger dose of HPDP (400 mg/kg) only produced a 72% decrease rather than any discernible improvement.

$[\text{Co}(\text{PDP})_2]$ (200mg/kg b.w.) decreased glucose level of diabetic rats from 319.00 ± 78.71 mg/dL to 202.40 ± 111.24 mg/dL. After 14 days treatment only about 37% decrease in glucose level was observed compared to 61% decrease caused by standard drug. It is very likely that low dose of $[\text{Co}(\text{PDP})_2]$ has minimal antidiabetic property. After dose (400mg/dL b.w.) did perform any better hence it may not be used as drug for diabetes.

The blood glucose level was significantly ($p < 0.05$) reduced by both low and high doses of $[\text{Cu}(\text{HPDP})_2\text{Cl}_2]$. After 14 days of treatment with 200 mg/dL b.w. $[\text{Cu}(\text{HPDP})_2\text{Cl}_2]$, the glucose level decreased from 343.40 ± 68.94 mg/dL to 112.00 ± 13.93 mg/dL. This 67.35% reduction is far better than that which is achievable (60.56%) with standard drug; after 14 days, the high dose (400 mg) treatment was very effective, resulting in a 70.55% reduction in glucose levels as opposed to the 60.56% reduction caused by the regular medication. Therefore, it was assumed that by controlling blood glucose levels, both high and low doses of HPDP, $[\text{Cu}(\text{HPDP})_2\text{Cl}_2]$ and $[\text{Co}(\text{PDP})_2]$ would improve diabetes mellitus and its related complications. Glibenclamide did, however, function in the short term.

Prior to the induction of diabetes, the rats in all the groups used in the study had normal blood glucose levels, showing that they were healthy and free of diabetes. However, all animals treated with Glibenclamide and HPDP, at both low and high dosages, showed raised blood glucose levels, suggesting that the rats had developed alloxan-induced diabetes. The diabetic control group's blood glucose levels continued to rise after seven and fourteen days after the diabetes was produced, showing persistent hyperglycemia. This result is in line with previously published methodologies [19, 20,

25, 26]. The persistently high blood sugar levels in the diabetic control rats increase the risk that they would develop severe hyperglycemia and associated complications, which might drastically lower their odds of survival and, in the worst-case scenario, result in death.

CONCLUSION

HPDP, $[\text{Co}(\text{PDP})_2]$, and $[\text{Cu}(\text{HPDP})_2\text{Cl}_2]$ were shown to be non-toxic in the acute toxicity (LD_{50}) test, and the antidiabetic studies show great promise as potential treatments for diabetes. They were considerably more effective in curing diabetes in rats with induced diabetes than the antidiabetic drug Glibenclamide

in-vivo.

They appear to be promising medication candidates with strong oral bioavailability and no trouble penetrating cell membranes, according to molecular docking stimulation.

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