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Invitro and Insilico Biological Evolution of Novel Piperidin4-one-thiosemicarbazide Derivatives as Antiplatelet Agent

Zuneera Akram[1*], Rehana Perveen[1], Aisha Noreen[2], Maryam Inayat[3], Sobia Akhter[1], Nousheen Malik[1].

[1]*Department of Pharmacology, Faculty of Pharmacy, Baqai Medical University, Karachi, Pakistan*

[2]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Baqai Medical University, Karachi, Pakistan*

[3]*Department of Pharmacy Practices, Faculty of Pharmacy, Baqai Medical University, Karachi, Pakistan.*

**Corresponding Author: Dr. Zuneera Akram: Department of Pharmacology, Baqai Institute of Pharmaceutical Sciences, Baqai Medical University, Karachi, Pakistan. (dr.zunaira@baqai.edu.pk, +92 332 3099397)*

Abstract: Platelets are thought to have a role in hemostasis, thrombosis, inflammation, wound healing, and immunity. Compound R2 "(Z)-2-(3,3-dimethyl-2,6-diphenylpiperidin-4-ylidene)hydrazinecarbothioamide" is a novel piperidone thiosemicarbazone derivative that is beneficial in the treatment of angina and ischemia. Novel Piperidin4-one-thiosemicarbazide (R2) inhibited platelet aggregation caused by ADP(5 µg/ml) in the blood of healthy volunteers.An aggregometer was used to evaluate the impact of R2 on PRP.R2 significantly decreased platelet aggregation. Without R2, ADP-induced platelet aggregation in PRP was found to be 80%, while R2 prevented platelet aggregation completely at 1.0625 µM. R2 was shown to have an antiplatelet IC50 of 0.2972± 1.10 µM when stimulated with ADP. The binding energy of the selected derivative is -5.44 kcal/mol. A molecular docking study showed strong interactions between Piperidone derivatives and the platelet aggregation active site PDB 3ZDY, suggesting that the medication may be further developed as a platelet aggregation inhibitor. Additionally, the new chemical (R2), a novel thiosemicarbazide derivative, has the potential to be helpful in the treatment of platelet-associated thromboembolic disorders.

Key Word: Platelets, ADP, Aggregation, 4-piperidone, Thiosemicarbazone derivatives, Thrombosis.

INTRODUCTION

Platelets are required for hemostasis, thrombosis, wound healing, atherosclerosis, inflammation, and immunology, among other functions[1–3]. While platelets' primary role after injury or damage is to prevent blood loss, they are often implicated in the development of dysregulated thrombus, which may result in myocardial infarction, acute coronary syndrome, or ischemia [4]. The activation of platelets involves many agonists (Adenosine diphosphate (ADP), collagen, arachidonic acid, platelet activator, thrombin, and thromboxane A2) [5]. Aspirin is used to treat hyperactivity of the platelets caused by increased synthesis of thromboxaneA2 (TxA2) in various coronary condition situations to lower the risk of serious ischemic events [6]. However, between 10% and 20% of those who use aspirin as a secondary prophylactic develop a chronic thrombotic disease after a long period of follow-up. The failure of Aspirin is attributed to aspirin resistance [7–8]. Complications and modes of action are considered by present antiplatelet medications such as acetylsalicylic acid, phosphodiesterase inhibitors, P2Y12 antagonist, and αIIb/β3 major platelet integrin antagonist [9–10]. Medicines produced from natural substances have a minimal risk of adverse effects [11]. Consequently, it is important to improve the effectiveness of these medicines and

to explore new, safe, and more effective non-aspirin antiplatelet inhibitors. As a consequence, some substances from natural or synthetic sources currently utilized in conventional medicine are more carefully investigated to evaluate their antiplatelet action [6].

Piperidine alkaloid products, derived from natural *piper nigrum* (black pepper), provide a broad range of pharmacologically diverse substances [12-15]. Piperidine 4-one or 4-piperidone is the most commonly used piperidine derivative to form the synthesis of many important bioactive molecules available on the market, including propiverine (anticholinergic), piperyle (antipyretic, analgesic), clocapramine (anti-psychotic), fentanyle (anesthetic, analgesic), pimozide, and others (schizophrenia) [16]. The organic reaction of thiosemicarbazide with 4-piperidone carbonyl produces the highly functional intermediate thiosemicarbazone utilized for the production of physiologically active heterocyclic compounds such as thiazole [17-20]. The thiazoles are anti-inflammatory, anti-fungal, antiretroviral, and antihistamine. Many replaced thiazole compounds showed significant analgesic efficacy [21-22] Thiazole, piperidine, and their new derivatives have been bioactive in the development of easy techniques for synthesizing the activity of 4-piperidone-based carbothioamide derivatives. In this research, the significance of the carbothioamide derivative "(Z)-2-(3,3-dimethyl-2,6-diphenylpiperidine-4-ylidene)hydrazinecarbothioamide" for cardio-vascular problems was assessed by in vitro and *in silico* antiplatelet.

MATERIAL AND METHOD

Material

A novel compound, 4-piperidone -based carbothioamide derivative known as “(Z)-2-(3,3-dimethyl-2,6-diphenylpiperidin-4-ylidene)hydrazinecarbothioamide” (R2) was obtained from the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Karachi. Chrono-Log Corp. supplied the adenosine diphosphate (ADP) (Haver-town, PA, USA). The other reagents and solvents used in this study were of analytical grade, including sodium hydroxide, sodium citrate, dimethyl sulfoxide (DMSO), and phosphate buffer solution.

Instruments

The centrifuge machine was used to produce plasma rich in platelets and plasma low in platelets (Eppendorf Centrifuge 5810R and Mini-Spin Eppendorf AG-22331 Hamburg, Germany, respectively). A dual-channel platelet aggregation (Model No. 5490 - 2D Chrono - Log Corp.) linked to a personal computer was used to calculate the platelet aggregation concentration.

Human Subjects

In this study, hundred healthy volunteers (male and female, ages 18–40) were recruited. They had not taken any drug that could affect platelet activity for at least two weeks.

Ethical Approval

The ethical committee of the Karachi Institute of Bioengineering and Genetic Engineering accepted the experimental procedure.

METHOD

1. In Vitro Anti-platelet Assay

PRP and PPP preparation

A total of 30ml venous blood was collected from a healthy volunteer using a 21G butterfly needle and immediately transferred to polypropylene tubes containing 3.8 percent sodium citrate (1:9 V/V). PRP was obtained using a previously reported technique with slight changes by centrifuging citrated blood tubes for 15 minutes at 1400rpm (Eppendorf centrifuge 5810R). PPP was formed after further centrifugation of PRP at 13000 rpm for 15 minutes [23].

Assay for platelets aggregation

The light transmission system was used to determine platelet aggregation responses [24] using a Lumi-aggregometer model (5490–2D) (Chrono–Log, Havertown, PA, USA) at 37°C. PRP (350ul) was incubated in aggregometry sample cuvettes at 1200rpm with continuous stirring. After 1 minute, the 4-piperidone-based carbothioamide derivative

R2 (0.3125µM, and 0.625µM) was added to the ADP-induced platelet aggregations and incubated for an additional 5 minutes. Following that, a concentration of ADP (5µg/ml) was used to induce platelet aggregation. The degree of platelet aggregation was measured for six minutes. Due to platelet clearance in PRP and a greater proportion of light propagation, the turbidity of the platelet sample reduced as platelet aggregation increased. The proportion of platelet aggregation inhibition was calculated using the following formula:

$$\text{Percentage inhibition of platelet aggregation} = \frac{A \times B}{A} \times 100$$

A =by using a control sample, the maximum aggregation was reported.

B= Aggregation was observed following the addition of the test compound "(Z)-2-(3,3-dimethyl-2,6-diphenylpiperidin-4-ylidene)hydrazinecarbothioamide" (R2).

*2. In silico*Anti-platelet Assay

Methodology

Preparation of Inhibitor

ChemOffice 16 was used to create the two-dimensional structure of the chosen chemical, which was then saved in the cdx format. ChemDraw 4D extreme (version 16.0) was used to transform the analog into three-dimensional structures (Chemical Structure Drawing Standard; Cambridge Soft Corporation, USA (2009) [25]. Additionally, using 1000 iterations, the MMFF94X force-field technique was utilized to reduce the energies of all analogs. Finally, a PDB file was created from the processed analog. PDBQT files were generated using the MGL Tools (version 1.5.6) [26].

Preparation of Target Protein

The protein structure for Antiplatelet studies (PDB 3ZDY) was downloaded from RCSB-PDB with a resolution of 2.60 Å (http://www.rcsb.org/pdb/home/home.do). The bound ligand, water molecules, and extra protein chains were deleted using BIOVIA Discovery Studio Visualizer. The polar hydrogen and Kollman Charges were introduced in the receptor file using AutoDockVina Program and generated the PDBQT file.

Molecular Docking Studies

The molecular docking studies were performed using the AutoDockVina version (4.2). The ligand-enzyme interaction was dealtby the Lamarckian genetic algorithm (LGA). The grid box was generated in the middle of active site residues of a receptor with 28.433, 28.311, and 198.790. The AUTOGRID program was used to produce a grid map was 45, 45, and 45 with points spaced at 0.700 Å. The docking parameters were set to the default setting of the genetic algorithm. The best pose of docked ligand-enzyme complexes was predicted by binding energies.

Analysis of Results

The dlg file of the docked complex was used to predict the best pose. Furthermore, the BIOVIA Discovery Studio Visualizer (DSV), PyMol molecular visualization tool [27], and PLIP [28]were used to visualize the interaction of ligand and protein complexes.

Results

In vitro platelet aggregation is inhibited by a novel compound R2.

Figures 1 and 2 illustrate the inhibitory activity of the 4-piperidone-based carbothioamide derivative (R2) on platelet aggregation caused by ADP. The findings indicated that R2 had a strong inhibitory effect on platelet aggregation induced by ADP.

The novel derivative R2 (0.3125µM and 0.625µM) inhibited human platelet aggregation induced by ADP (5µg/ml) in a concentration-dependent manner. The R2 IC50 value for preventing ADP-induced platelet aggregation was 0.2972±1.10 µM. (Table 1, Figure 1).

Table 1.The IC_{50} value and inhibitory action of R2 on platelet aggregation caused by ADP

Compound	Concentration (µM)	Percentage Inhibition	IC_{50} (µM)
R2	0.3125	52.564 ± 2.32	0.2972± 1.10
	0.625	100 ± 0.0	

*** Values are reported as mean ± Standard Error of Mean (SEM), n = 5**

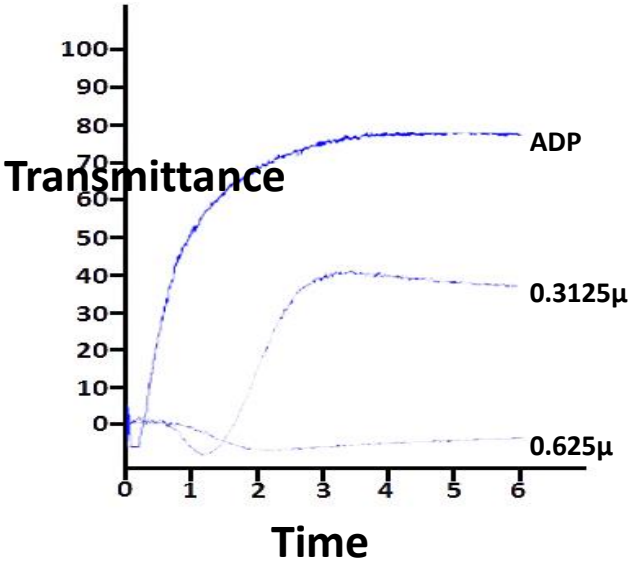


Figure 1. Dose-response curve of R2 novel compound on platelet aggregation induced by ADP

***In silico*platelet aggregation is inhibited by a novel compound R2**.

The binding energy (BE) of a receptor-Ligand complex is often determined by modeling the

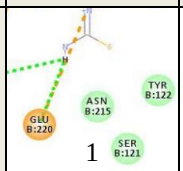
complex's molecular dynamics and computing the free energy of small ligands interacting with biological macromolecules. BE values were computed in this study to quantitatively determine the binding affinity of proteins (PDB 3ZDY) for the target chemical. As shown in Figure 2, the chosen chemical had a negative BE value of -5.44 kcal/mol. Given that a lower BE value typically indicates a higher ligand-binding affinity, the docking findings suggest that all of the test compounds may be very potent ligands with significantly different binding processes.

R2 forms a hydrogen bond and six hydrophobic bonds with the following sites: GLU220 B, PHE160 A, TYR 190 A(3.27), TYR 190 A, ALA 218 B, PHE 231 A, and PHE231A with a binding energy of -5.44 kcal/mol. R2 inhibited PDB 3ZDY at a concentration of 102.3 mM.

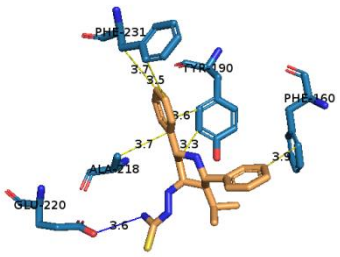
This demonstrates that the newly synthesized carbothioamide compounds possess active binding sites and the ability to inhibit platelet aggregation (Table 2, Figure 2).

The Carbothioamide derivate chosen had higher binding energy. This shows that R2 has potential antiplatelet binding sites and suggests a greater potential for antiplatelet action at PDB 3ZDY

Table 2: Insilico Analysis of Piperidin4-one-thiosemicarbazide derivative.

Derivative	H-Bond	H-Bond (an amino acid with bond length)	No. of Hydrophobic Bond	Hydrophobic Bonding (Amino acid and bond length)	Binding Energy (kcal/mol)
R2	 1	GLU220 B(2.60)	6	PHE160 A(3.87), TYR 190 A(3.27), TYR 190 A(3.61), ALA 218 B (3.75), PHE 231 A (3.69), PHE231A (3.50)	-5.44

A: 2D Image



B: 3D Image

Figure 2: Binding energy and Ligand interactions of Compound R2. A: 2D Image, B: 3D Image of Piperidin4-one-thiosemicarbazide

DISCUSSION

Heart disease and stroke are the main causes of mortality worldwide. They are caused by a blood clot or thrombus formed as a result of platelet and other protein activation. Numerous medications are available for this purpose. However, the researcher continued to work on new compounds to improve the patient's effectiveness and safety profile while minimizing adverse effects.

Diseases caused by blood clots or thrombi, such as heart failure and stroke, are the leading cause of mortality globally. This disease may be treated with a variety of medicines. However, the researcher proceeded to experiment with different medicines to enhance the efficacy and safety profile of the patient by using side effects [29].

Novel synthetic products or natural products derived from plants with antiplatelet activity can be a source of a major compound with significant effectiveness and few adverse effects [30]. At a dosage of 75–150 mg, aspirin is the most often used antiplatelet agent for the prevention of vascular accidents. [7]. Also, at minimal doses, aspirin may induce adverse effects such as gastric erosion and GI bleeding [31]. As a result, novel therapeutic agents are needed for precisely targeting the desired degree of platelet activation while minimizing adverse effects. With high reliability and reproducibility, light transmission aggregometry is used to determine the effect of ADP on platelet aggregation as agonists [32]. The antiplatelet effect of R2 induced by ADP was demonstrated in the current study at a concentration of 0.625µM. (Figure 1). Thus, our novel compound R2 can exert antiplatelet activity by inhibiting the cyclooxygenase and lipoxygenase pathways, as well as platelet aggregation.

New therapeutic options for blood clot-related coronary diseases such as angina, stroke, and MI

have significantly reduced the morbidity profile of CVD patients, either alone or in combination with aspirin and clopidogrel. These therapies are not always successful in lowering mortality in these patients, which may be a consequence of their extended duration of action, which leads to bleeding problems [33–34]. The present approach to platelet activation in CVD therapy places a premium on avoiding adverse events such as bleeding, particularly before and during surgical operations [35]. As a result, other techniques are required that limit platelet activation, thus reducing bleeding complications.

While therapies targeting COX-1 or surface receptors such as PAR1, P2Y12, and integrin receptor IIb3 have been very successful in reducing MI-related morbidity, they have failed to significantly decrease mortality in these patients. This may be because antiplatelet medicines do not block platelet activation, have a prolonged onset and duration of action, and can result in significant morbidity owing to bleeding complications [33–34].

Thus, novel therapeutic approaches are required to inhibit platelet activity when arteries are blocked and stroke occurs without causing bleeding complications. It may be addressed by inhibiting the secondary route of platelet activation, which further reduces coagulation formation without altering the bleeding profile, as shown with inhibition of the COX-1 secondary pathway.

CONCLUSION

Although the exact mechanism of action is unknown, our findings suggest that the novel 4-piperidone-based carbothioamide derivative "(Z)-2-(3,3-dimethyl-2,6-diphenylpiperidin-4-ylidene)hydrazinecarbothioamide" (R2) can inhibit platelet aggregation by inhibiting secondary aggregation pathways such as Lipoxygenase and cyclooxygenase. Based on these findings, further study with other platelet aggregation-inducing drugs, as well as genetic experimentation, is required to investigate broader approaches for treating these disabling medical conditions. Following additional studies, we conclude that a 4-piperidone-based carbothioamide derivative (R2) could be used at defined concentrations as a platelet inhibitor in cardiovascular disorders.

DECLARATION

None

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None

CONFLICTS OF INTEREST

There is no potential for conflict of interest.

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