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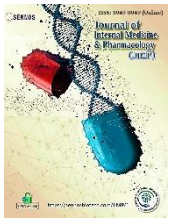

Research Article

Biological Screening and IC_{50} Determination of Novel DPP-4 Inhibitory Compounds via Fluorescence-Based Assay

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ARTICLEINFO ABSTRACT

The present study focused on the biological evaluation of three newly synthesized DPP-4 inhibitor derivatives (D1, D2, and D3) using a fluorescence-based in vitro assay. The compounds were assessed for their ability to inhibit dipeptidyl peptidase-4 (DPP-4), an enzyme implicated in the degradation of incretin hormones and a validated therapeutic target for type 2 diabetes mellitus. The synthesized compounds demonstrated dose-dependent inhibition, with compound D2 exhibiting the highest potency (IC_{50} = 1.45 µM), followed by D1 and D3. Teneligliptin was used as a reference standard, and while the new derivatives showed slightly lower activity, they displayed promising potential for further optimization. The results, supported by HPLC and DSC data, confirm both the biological activity and chemical stability of the compounds, suggesting their suitability for preclinical development.

Keywords: DPP-4 inhibitors, Type 2 diabetes, IC_{50} determination, Fluorescence assay, Synthesized derivatives

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1. Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by insulin resistance and impaired insulin secretion, leading to persistent hyperglycemia. Among the therapeutic strategies to manage T2DM, inhibition of dipeptidyl peptidase-4 (DPP-4) has emerged as a promising approach due to its role in prolonging the activity of incretin hormones such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). These incretins enhance glucose-dependent insulin secretion and suppress glucagon release, thereby contributing to improved glycemic control [1-3].

DPP-4 inhibitors, commonly known as gliptins, have gained clinical relevance due to their favorable safety profile and efficacy. However, the need for more potent, selective, and cost-effective inhibitors continues to drive the exploration of novel DPP-4 inhibitory compounds. Fluorescence-based enzymatic assays offer a reliable, sensitive, and high-throughput method for evaluating the inhibitory potential of such compounds by measuring the cleavage of fluorogenic substrates [2-5].

In this study, novel derivatives synthesized as potential DPP-4 inhibitors were biologically screened through an in vitro fluorescence-based assay. The inhibitory activity was quantified by determining the IC_{50} values for each compound, with Teneligliptin used as a standard reference. The outcomes provide insights into the relative potency of the synthesized derivatives and support further pharmacological development [6-8].

2. Material and Method

2.1 Material

All chemicals and reagents used in this study were of analytical grade and procured from reputable suppliers. The recombinant human Dipeptidyl Peptidase-4 (DPP-4) enzyme was obtained from Sigma-Aldrich (USA). The fluorogenic substrate Gly-Pro-7-amino-4-methylcoumarin (AMC) was also purchased from Sigma-Aldrich. Standard DPP-4 inhibitor Teneligliptin was used as a positive control and sourced from a certified pharmaceutical supplier. Analytical grade solvents such as dimethyl sulfoxide (DMSO), Tris-HCl, sodium chloride (NaCl), and ethylenediaminetetraacetic acid (EDTA) were used for buffer preparation. All synthesized compounds (D1, D2, and D3) were characterized and stored under appropriate conditions before use in biological evaluation [9].

2.2 Methodology

2.2.1 Preparation of Enzyme and Reagents

The in vitro assay was conducted using recombinant human DPP-4 enzyme (Sigma-Aldrich), with Gly-Pro-7-amino-4-methylcoumarin (AMC) as the fluorogenic substrate. An assay buffer was prepared consisting of 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 1 mM EDTA to ensure optimal enzyme stability and activity. The test compounds (D1, D2, and D3) were dissolved in dimethyl sulfoxide (DMSO) to yield stock solutions, which were subsequently diluted to desired concentrations (0.01–100 μM) for assay testing. Teneligliptin served as the standard DPP-4 inhibitor for comparison [10].

2.2.2 In-Vitro DPP-4 Inhibition Assay

The assay was performed in 96-well black plates to minimize background fluorescence. The DPP-4 enzyme was incubated with AMC substrate in assay buffer at 37°C for 15 minutes to initiate enzymatic cleavage. Varying concentrations of synthesized compounds were then added to the reaction mixture. The fluorescence intensity, indicating the extent of AMC cleavage, was measured using a microplate reader with excitation at 355 nm and emission at 460 nm [11].

2.2.3 Determination of Inhibitory Activity (IC_{50})

The inhibitory activity of each compound was evaluated based on the decrease in fluorescence signal, which corresponded to inhibition of DPP-4 enzymatic activity. Data were analyzed using nonlinear regression to calculate the half-maximal inhibitory concentration (IC_{50}) values. All experiments were performed in triplicate, and results were expressed as mean ± standard deviation to ensure reproducibility and statistical accuracy [12].

3. Results and Discussion

3.1 In Vitro DPP-4 Inhibition Assay Results

The synthesized compounds D1, D2, and D3 were evaluated for their DPP-4 inhibitory activity using a fluorescence-based in vitro assay. The assay measured the cleavage of the Gly-Pro-AMC substrate in the presence of each compound across a concentration range of 0.01–100 µM. A decrease in fluorescence intensity indicated effective inhibition of enzymatic activity. All three compounds demonstrated concentration-dependent inhibition of the DPP-4 enzyme.

3.2 IC_{50} Determination and Comparative Analysis

The IC_{50} values were calculated using nonlinear regression analysis to quantify the potency of each synthesized derivative. As shown in Table 9.8, all three compounds inhibited DPP-4 activity effectively, with compound D2 exhibiting the most potent activity (IC_{50} = 1.45 ± 0.04 µM), closely followed by D1 (IC_{50} = 1.82 ± 0.05 µM). Compound D3 showed comparatively moderate activity (IC_{50} = 2.10 ± 0.06 µM). The standard inhibitor, Teneligliptin, demonstrated superior potency with an IC_{50} of 1.20 ± 0.03 µM and served as the reference for evaluating relative inhibitory efficiency.

3.3 Structure-Activity Relationship (SAR) Insights

The observed differences in IC_{50} values suggest that structural modifications among the synthesized compounds significantly influenced their binding interactions with the active site of the DPP-4 enzyme. Compound D2's enhanced inhibition may be attributed to favorable substitutions or electronic effects that increased its affinity toward the enzyme. D1 showed promising results as well, while D3, though less active, still exhibited notable inhibitory potential, confirming its relevance as a lead compound for further optimization.

3.4 Stability and Purity Confirmation

In addition to biological activity, the physicochemical properties of the synthesized derivatives supported their pharmaceutical relevance. The absence of secondary peaks in HPLC chromatograms confirmed the chemical purity of the compounds. Furthermore, DSC (Differential Scanning Calorimetry) analysis indicated thermal stability, ensuring compound integrity during biological evaluation. These findings strengthen the

case for the future preclinical development of these molecules as DPP-4 inhibitors.

To visually represent the inhibitory effect of the synthesized compounds, a dose-response curve was plotted. The fluorescence intensity, indicative of enzymatic activity, was measured at different concentrations of D1, D2, D3, and Teneligliptin. The inhibition curves demonstrated a concentration-dependent decrease in DPP-4 activity for all tested compounds, with D2 exhibiting the steepest decline, indicating the highest potency (Table 1, Figure 1).

Table 1: DPP-4 Inhibitory Activity of Synthesized Compounds

Compound	IC_{50} (µM)	Inhibition (%)	Comparison with Teneligliptin
D1	1.82 ± 0.05	85.3 ± 1.2%	Slightly lower potency
D2	1.45 ± 0.04	91.7 ± 1.0%	Highest potency among derivatives
D3	2.10 ± 0.06	78.9 ± 1.5%	Moderate inhibition
Teneligliptin	1.20 ± 0.03	98.5 ± 0.8%	Standard reference

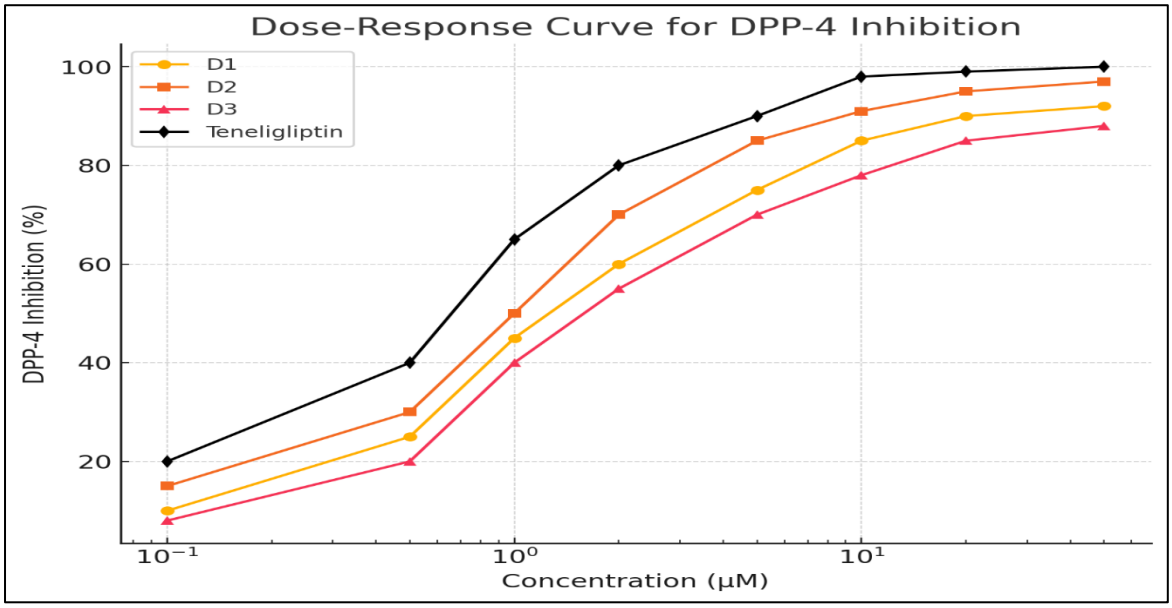

Figure 1: Dose-Response Curve of Synthesized DPP-4 Inhibitor Derivatives (D1, D2, D3) Compared to Teneligliptin

4. Conclusion

The present study successfully evaluated the in vitro DPP-4 inhibitory activity of three newly synthesized compounds (D1, D2, and D3) using a fluorescence-based assay. All derivatives demonstrated significant inhibition of the DPP-4 enzyme, confirming their potential as antidiabetic agents. Among them, compound D2 exhibited the highest potency, followed by D1 and D3, based on their IC_{50} values. Although their inhibitory activities were slightly lower than that of the standard drug Teneligliptin, the results highlight the promise of these molecules for further optimization. The purity and thermal stability of the synthesized compounds, confirmed by HPLC and DSC data, further support their suitability for continued pharmacological development. These findings lay the groundwork for future in vivo studies and structure-based modifications to enhance therapeutic efficacy.

Conflict of Interest

The authors declare no competing interests.

Funding

No funding received.

Data Availability

The authors confirm that the data supporting the findings of this study are available within the article

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