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# Interaction Study of 2-Benzoxazolinone Derivatives with DPP-4 and Aldose Reductase Enzymes: Discovery of Novel Antidiabetic Agents

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**ABSTRACT**

Diabetes mellitus (DM) is a metabolic disorder that represents a major global health burden. Its pathophysiology involves impaired insulin function, including pancreatic β-cell dysfunction, insulin resistance, defective insulin secretion, and autoimmune-mediated β-cell destruction. The identification of effective therapeutic agents and novel molecular targets remains a priority in antidiabetic drug discovery. Among potential targets, Aldose Reductase (ALR2) and Dipeptidyl Peptidase-4 (DPP-4) have attracted increasing interest because of their roles in DM progression and complications. Previous studies have suggested that 2-benzoxazolinone derivatives possess promising biological activities and may act as inhibitors of diabetes-related molecular targets. This study aimed to evaluate the interaction of selected 2-benzoxazolinone derivatives with ALR2 and DPP-4 using an in silico approach. Molecular docking was performed using PyRx integrated with AutoDock Vina, while BIOVIA Discovery Studio Visualizer and MarvinSketch were utilized for ligand preparation and interaction analysis. In addition, absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties were predicted using the pKCSM platform. The results showed that compounds 6b (-11.1 kcal/mol; 0.01 μM), 6d (-11.1 kcal/mol; 0.01 μM), and bv10 (-10.8 kcal/mol; 0.01 μM) exhibited binding affinities against ALR2 comparable to the reference inhibitor Zopolrestat (-12.2 kcal/mol; 0.001 μM). For DPP-4, compounds c26 (-7.9 kcal/mol; 1.62 μM), c4 (-7.8 kcal/mol; 1.91 μM), and c6 (-7.7 kcal/mol; 2.27 μM) demonstrated better docking performance than Vildagliptin (-6.6 kcal/mol; 14.5 μM). These compounds also showed generally favorable ADMET profiles. Therefore, they warrant further in vitro and in vivo studies as potential antidiabetic drug candidates.

Keywords: Pancreatic β-cell dysfunction, ALR2, DPP4, 2-Benzoxazolinone

## Introduction

Degenerative diseases remain a major challenge for global healthcare systems, among which diabetes mellitus (DM) is characterized primarily by chronic hyperglycemia (Hakim et al., 2023; Suastika et al., 2021). Diabetic hyperglycemia is strongly associated with various complications, particularly cardiovascular disorders (Fiorentino et al., 2013). Based on insulin deficiency and underlying pathophysiology, DM is generally classified into two main categories, namely type 1 and type 2 diabetes

(Sulastri, 2021). In 2019, the International Diabetes Federation (IDF) reported that approximately 463 million people worldwide were living with diabetes mellitus. This number is projected to increase substantially if current trends persist, reaching an estimated 578 million by 2030 and 700 million by 2045 (International Diabetes Federation, 2019; Y. Yang et al., 2020).

In Indonesia, DM represents the third leading cause of mortality. In 2021, approximately 19.5 million individuals were reported to be living with diabetes, and this number is expected to rise to 28.6 million by 2045(International Diabetes Federation, 2021). Data from the 2018 Basic Health Research (Riskesdas) survey indicated a national diabetes prevalence of 8.5%, reflecting an increase of 1.5% compared with 2013. By 2022, the recorded number of diabetes cases had reached 41,817 individuals (Indonesian, 2018).

Type 1 diabetes is characterized by autoimmune-mediated destruction of insulin-producing pancreatic β-cells, primarily driven by T-cell responses (CD4+ and CD8+) targeting pancreatic tissue (Asmat et al., 2016; Baynest, 2015; Janež et al., 2020; Pathak et al., 2019). In contrast, type 2 diabetes is associated with insulin resistance and defects in insulin secretion resulting from β-cell dysfunction. Although both types require significant clinical attention, type 2 diabetes is far more prevalent, accounting for approximately 90% of all cases, while type 1 diabetes represents about 10% (Julaiha, 2019).

The development of effective therapeutic agents for Diabetes Mellitus continues to advance, with growing interest in molecular targets involved in disease progression and complications. DM is associated with interconnected pathways, including the polyol pathway mediated by Aldose Reductase (ALR2), formation of Advanced Glycation End Products, and reactive oxygen species generation. Epalrestat, an ALR2 inhibitor, has shown potential in managing diabetic complications such as retinopathy and neuropathy, supporting the design of novel inhibitors through *in silico* approaches. In addition, Dipeptidyl Peptidase-4 (also known as CD26) has emerged as an important target in metabolic regulation. DPP-4 is a multifunctional membrane protein widely expressed in various tissues and present in soluble form in plasma. It regulates incretin hormones, particularly Glucagon-like Peptide-1, which are essential for glucose homeostasis. Despite its hypoglycemic potential and role in stimulating insulin secretion, DPP-4 remains relatively underexplored compared with other therapeutic targets, highlighting its promise for novel antidiabetic drug discovery (Deacon, 2019; Husna, 2021; Rendi et al., 2021; Hakim et al., 2023; Rahayu, 2025).

Despite the availability of various antidiabetic agents, developing novel therapeutics that target alternative molecular pathways with improved safety remains a major challenge. Current antidiabetic drugs are often associated with long-term adverse effects, including fluid retention, gastrointestinal disturbances, and increased cardiovascular risk. Moreover, most existing therapies primarily focus on glycemic control and inadequately address the molecular mechanisms underlying diabetic complications. Therefore, identifying compounds that can modulate both glucose homeostasis and complication-related pathways has become an attractive strategy in antidiabetic drug discovery (Masdianto et al., 2020).

Among emerging molecular targets, **Dipeptidyl Peptidase-4** and **Aldose Reductase (ALR2)** represent complementary therapeutic strategies. DPP-4 inhibition enhances endogenous incretin activity, particularly **Glucagon-like Peptide-1**, improving insulin secretion and glycemic control. In contrast, ALR2 inhibition suppresses the polyol pathway, thereby reducing diabetic complications such as neuropathy, nephropathy, and retinopathy. Thus, compounds with dual affinity toward DPP-4 and ALR2 may provide broader therapeutic benefits by targeting both hyperglycemia and its complications.

**2-Benzoxazolinone derivatives** have gained attention due to their privileged heterocyclic scaffold and diverse pharmacological activities, including antioxidant, anti-inflammatory, and antidiabetic effects. Previous studies have reported their potential as inhibitors of diabetes-related molecular targets and oxidative stress pathways disorders (Vyas et al., 2018). However, systematic evaluation of their binding potential against both DPP-4 and ALR2 remains limited, and their pharmacokinetic and toxicity profiles are still insufficiently characterized. Therefore, this study aims to evaluate the interactions of 2-benzoxazolinone derivatives with DPP-4 and ALR2 using molecular docking and to assess their drug-likeness and safety through **pkCSM**-based ADMET prediction, with the goal of identifying potential multitarget antidiabetic candidates.

## Methodology

### Materials

The three-dimensional crystal structures of the target macromolecules, namely DPP-4 (PDB ID: 6B1E) and ALR2 (PDB ID: 2PDB) enzymes, were retrieved from the Protein Data Bank (PDB) available at https://rcsb.org/. The ligand models of the tested 2-benzoxazolinone derivatives were obtained from secondary data based on previously published studies, including compounds C1–26 reported by Zhu et al. (2021) and compounds 6a–g reported by Siugzdait et al. (2024) (Šiugždait et al., 2024; Vyas et al., 2018; Zhu et al., 2021).

### Instruments and Software

Computational analyses were performed using a personal computer with the following specifications: AMD Ryzen™ 7 6800H Mobile Processor (8-core/16-thread, 20 MB cache, up to 4.7 GHz max boost), 16 GB DDR5-4800 SO-DIMM memory (×2), 512 GB PCIe® 4.0 NVMe™ M.2 SSD storage, NVIDIA® GeForce RTX™ 3050 Laptop GPU, and a 15.6-inch OLED FHD (1920 × 1080) display. The system operated on Windows 11 (64-bit). Software utilized in this study included PyRx integrated with AutoDock Vina, BIOVIA Discovery Studio Visualizer, MarvinSketch, and the web-based pKCSM platform.

### Ligand Modeling

Two-dimensional and three-dimensional molecular structures of the 2-benzoxazolinone derivatives were constructed and geometry-optimized using MarvinSketch software.

### Target Protein Preparation

The crystal structures of DPP-4 (PDB ID: 6B1E) and ALR2 (PDB ID: 2PDB) enzymes were downloaded from the Protein Data Bank (http://www.rcsb.org/pdb).

Protein preparation involved the removal of water molecules and separation of co-crystallized reference ligands using BIOVIA Discovery Studio Visualizer (DSV). The prepared protein structures were subsequently saved in *.pdb format for further analysis (Saleh, 2015).

**Docking Validation**

Docking protocol validation was performed through re-docking of the native/reference ligands into the active sites of the target enzymes using PyRx and AutoDock Vina. The docking parameters applied were as follows: center_x = 38.602; center_y = 51.144; center_z = 36.819; size_x = 28; size_y = 18; size_z = 16; with an exhaustiveness value of 200 for DPP-4 target and center_x = 17.109; center_y = 9.646; center_z = −11.584; size_x = 15.161; size_y = 13.736; size_z = 9.538, with an exhaustiveness value of 200 for ALR2 target. The docking procedure was considered valid when the Root Mean Square Deviation (RMSD) between the predicted docking pose and the crystallographic conformation was ≤ 2 Å. Additional validation criteria included similarity in amino acid binding profiles and intermolecular interactions between the native ligand and protein compared to crystallographic data (Dwivedi et al., 2014; Prayogi et al., 2023).

**Molecular Docking**

The modeled compounds were subjected to molecular docking simulations to evaluate their interaction with the target protein structures. Docking simulations were conducted using AutoDock Vina to predict binding affinity and interaction profiles within the receptor active sites.

**Analysis of Docking Results and ADMET Prediction**

Docking results were visualized using BIOVIA Discovery Studio 2021, and inhibition parameters were calculated accordingly. Visual analysis focused on intermolecular interaction profiles between the test compounds and target enzymes, including hydrogen bonding, hydrophobic interactions, and electronic interactions. Selected compounds were further evaluated for their pharmacokinetic properties through absorption, distribution, metabolism, excretion, and toxicity (ADMET) predictions using the web-based pKCSM platform (Prayogi et al., 2025). The inhibition constant (Ki) was estimated from the binding free energy (ΔG, kcal/mol) obtained from molecular docking simulations according to the thermodynamic relationship:

$$Ki = e^{\frac{\Delta G}{RT}}$$

where Ki is the inhibition constant (M), ΔG is the binding free energy (cal/mol), R is the universal gas constant (1.987 cal·mol$^{-1}$·K$^{-1}$), and T is the absolute temperature (298.15 K).

**Result and Discussion**

A total of **43 test compounds representing 2-benzoxazolinone derivatives** were used in this study.

**Target Protein Preparation and Docking Validation**

**Validation of the DPP-4 Target Protein**

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Docking simulations were performed by positioning the grid box at the center of the reference ligand. The docking parameters applied were as follows: center_x = 38.602; center_y = 51.144; center_z = 36.819; size_x = 28; size_y = 18; size_z = 16; with an exhaustiveness value of 200. The results of protein preparation, visualization of amino acid residue interactions with the ligand, and the overlay between ligands before and after docking are presented in Figure 1. The obtained RMSD value was 1.839 Å, indicating that the docking protocol successfully reproduced the original binding pose. The majority of amino acid residues involved in the co-crystallized complex were consistent with those observed in the re-docking results, confirming that the docking method used in this study is valid.

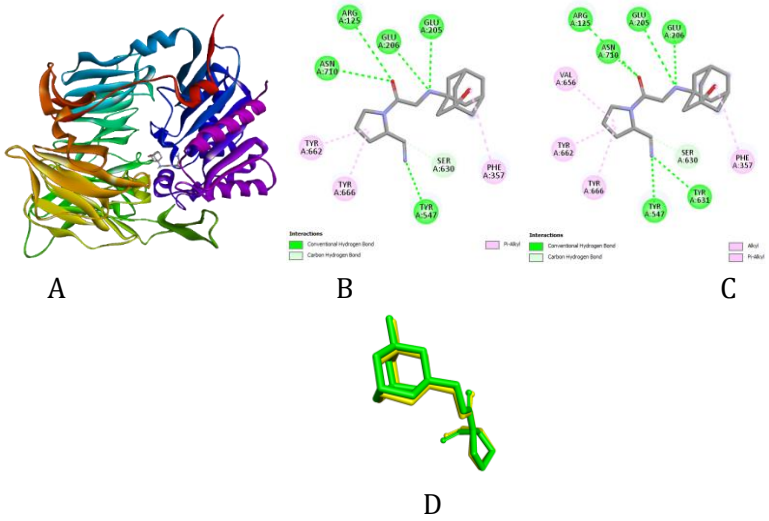


**Figure 1. Re-docking of Vildagliptin with DPP-4 (PDB ID: 6B1E): (A) Target protein structure; (B) Re-docking interaction profile; (C) Co-crystallized interaction profile; (D) Overlay of re-docked ligand (green) and co-crystallized ligand (yellow)**

**Validation of the ALR2 Target Protein**

Docking simulations were performed by positioning the grid box at the center of the reference ligand. The docking parameters applied were as follows: center_x = 17.109; center_y = 9.646; center_z = −11.584; size_x = 15.161; size_y = 13.736; size_z = 9.538, with an exhaustiveness value of 200. The results of protein preparation, visualization of amino acid residue interactions with the ligand, and the overlay between ligands before and after docking are presented in Figure 2.

The obtained RMSD value was 0.3 Å, indicating that the docking protocol successfully reproduced the native binding pose with high accuracy. The majority of amino acid residues involved in the co-crystallized complex were consistent with those observed in the re-docking results. Therefore, the docking method applied in this study was considered valid.

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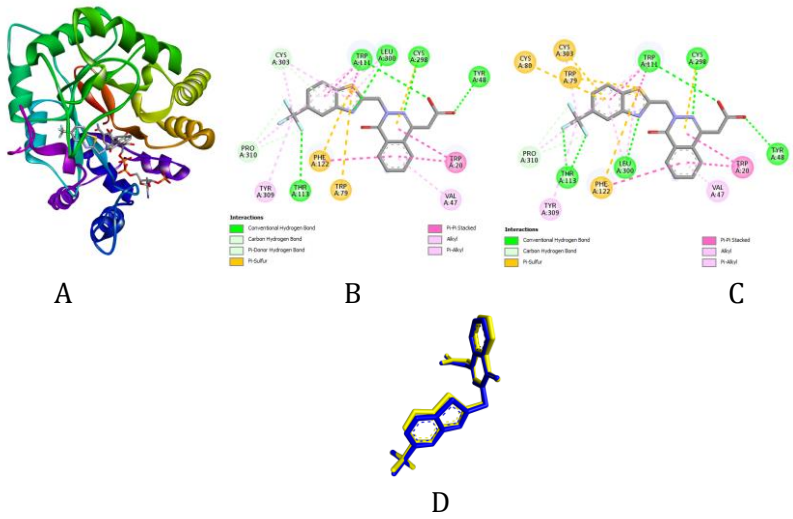


A B C



D

**Figure 2. Re-docking of Zopolrestat with ALR2 (PDB ID: 2PDB): (A) Target protein structure; (B) Re-docking interaction profile; (C) Co-crystallized interaction profile; (D) Overlay of re-docked ligand (blue) and co-crystallized ligand (yellow)**

**Molecular Docking**

The modeled compounds were evaluated for their interactions with target proteins through molecular docking simulations using three-dimensional crystal structures. This approach aimed to predict binding affinity, inhibition constants (Ki), and intermolecular interaction profiles within the active sites of the target enzymes.

The inhibitory potential of a ligand toward its receptor can be assessed by the inhibition constant (Ki), which is directly related to binding affinity and the thermodynamic stability of the ligand–protein complex. Lower Ki values indicate stronger binding interactions and lower ligand concentrations required to inhibit enzymatic activity. In molecular docking and structure-based drug design, Ki is widely used to estimate ligand potency because it correlates with binding free energy and reflects the strength and specificity of molecular recognition. Therefore, lower Ki values generally indicate stronger ligand–protein interactions and greater inhibitory potential (Ferreira et al., 2015; Klebe, 2013; Pantsar & Poso, 2018; Torres et al., 2019).

Recent studies emphasize that although docking-derived binding affinity and predicted Ki values offer valuable preliminary screening metrics, their interpretation should be complemented by detailed interaction analysis and pharmacokinetic evaluation to ensure biological relevance and translational potential in drug discovery pipelines (Pantsar & Poso, 2018; Torres et al., 2019).

**Table 2. Binding interaction profile of the test compounds against the target proteins**

| Compound | DPP4 Target (PDBID: 6B1E) | | ALR2 Target (PDBID: 2PDB) | |
|---|---|---|---|---|
| | Binding-Energy (kcal/mol) | Ki (µM) | Binding-Energy (kcal/mol) | Ki (µM) |
| Zopolrestat | - | - | -12.2 | 0.001 |
| Vildagliptin | -6.6 | 14.5 | - | - |
| bv9 | -7.1 | 6.24 | -10.2 | 0.03 |
| c17 | -7.6 | 2.68 | -9.8 | 0.07 |
| c13 | -7.2 | 5.27 | -10.2 | 0.03 |
| c8 | -7.1 | 6.24 | -10 | 0.05 |
| c6 | -7.7 | 2.27 | -10.5 | 0.02 |

| Compound | DPP4 Target (PDBID: 6B1E) | | ALR2 Target (PDBID: 2PDB) | |
|---|---|---|---|---|
| | Binding-Energy (kcal/mol) | Ki (µM) | Binding-Energy (kcal/mol) | Ki (µM) |
| c4 | -7.8 | 1.91 | -9.5 | 0.11 |
| bv10 | -7.2 | 5.27 | -10.8 | 0.01 |
| 6b | -7 | 7.39 | -11.1 | 0.01 |
| c23 | -7.4 | 3.76 | -10.3 | 0.03 |
| 6d | -7.4 | 3.76 | -11.1 | 0.01 |
| bv8 | -7.3 | 4.45 | -10.1 | 0.04 |
| 6g | -7.2 | 5.27 | -10.8 | 0.01 |
| c24 | -6.8 | 10.35 | -10.1 | 0.04 |
| c7 | -7.3 | 4.45 | -10.3 | 0.03 |
| c5 | -7.1 | 6.24 | -10 | 0.05 |
| c13 | -7.2 | 5.27 | -10.1 | 0.04 |
| c9 | -7.5 | 3.18 | -10 | 0.05 |
| bv3 | -6.8 | 10.35 | -10.6 | 0.02 |
| c21 | -6.9 | 8.74 | -9.3 | 0.15 |
| c19 | -7.5 | 3.18 | -9.4 | 0.13 |
| c13 | -7.3 | 4.45 | -10.1 | 0.04 |
| c10 | -7.4 | 3.76 | -10.1 | 0.04 |
| bv3 | -6.8 | 10.35 | -10.4 | 0.02 |
| 6f | -6.6 | 14.51 | -10.4 | 0.02 |
| 6c | -6.7 | 12.26 | -10.5 | 0.02 |
| c25 | -6.7 | 12.26 | -9.9 | 0.06 |
| c22 | -6.7 | 12.26 | -9.7 | 0.08 |
| 6e | -6.4 | 20.34 | -9.9 | 0.06 |
| c26 | -7.9 | 1.62 | -9.4 | 0.13 |
| c16 | -7.4 | 3.76 | -9.9 | 0.06 |
| c15 | -7.1 | 6.24 | -10.1 | 0.04 |
| bv3 | -6.7 | 12.26 | -9.9 | 0.06 |
| c14 | -7.1 | 6.24 | -10.1 | 0.04 |
| bv7 | -6.9 | 8.74 | -10.3 | 0.03 |
| c20 | -6.7 | 12.26 | -9.3 | 0.15 |
| c18 | -7.4 | 3.76 | -9.5 | 0.11 |
| bv5 | -6.7 | 12.26 | -10.2 | 0.03 |
| bv6 | -6.7 | 12.26 | -10.3 | 0.03 |
| bv5 | -6.6 | 14.51 | -10.2 | 0.03 |
| c3 | -5.8 | 55.99 | -8.7 | 0.42 |
| c1 | -5.8 | 55.99 | -8.2 | 0.97 |

Based on the molecular docking (showed Table 2.) analysis performed against the ALR2 target, compounds coded 6b, 6d, and bv10 demonstrated binding affinity values and predicted inhibition constants comparable to those of the reference inhibitor Zopolrestat, suggesting a similar interaction profile within the active site and potential inhibitory capability. These findings indicate that the selected derivatives may effectively stabilize key interactions with catalytic residues involved in the polyol pathway, which is known to play a critical role in the progression of diabetic complications (Grewal, 2021; J. et al. Yang, 2022).

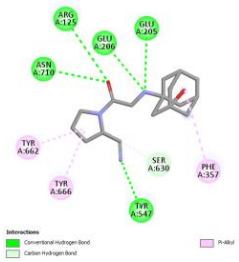
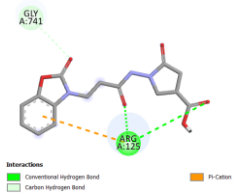
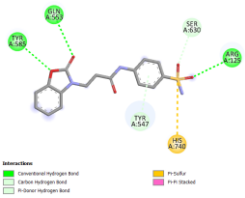
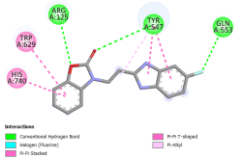
Furthermore, compounds c26, c4, and c6 were identified as the most promising 2-benzoxazolinone derivatives targeting DPP-4, as reflected by their lower predicted binding affinity values and inhibition constants relative to the reference drug Vildagliptin. In addition, these compounds exhibited generally favorable ADMET profiles, suggesting suitable pharmacokinetic properties and potential drug-likeness characteristics. Given that DPP-4 inhibition enhances incretin activity and improves glycemic control through increased GLP-1 signaling, the observed interaction profiles support their potential as novel antidiabetic candidates (Mulvihill & Drucker, 2021; Röhrborn et al., 2021).

Collectively, these results highlight compounds c26, c4, c6, 6b, 6d, and bv10 as promising lead structures for further optimization. Subsequent in-depth analyses, including interaction mapping, dynamic stability evaluation, and pharmacokinetic

prediction, were therefore conducted to better characterize their therapeutic potential within a structure-based drug discovery framework (Cavasotto & Aucar, 2020; Lionta et al., 2021). Subsequently, these selected compounds were subjected to further in-depth analyses.

**ADMET Prediction and Results Analysis**

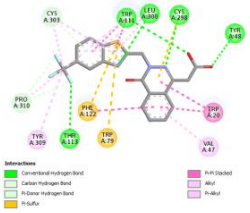
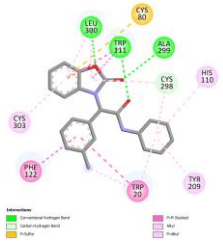
**Tabel 4. Binding Interaction Profile of the Tested Compounds Against the DPP4 target (PDBID: 6B1E)**

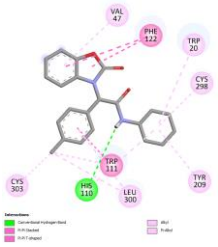
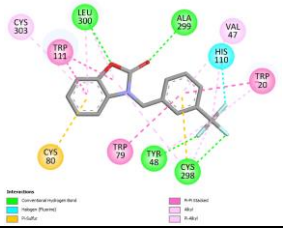
| Compound | Binding affinity (kkal/moL) | Ki (µM) | Bonding Hydrogen bonding | Hydrophobic interaction | Electrostatic interactions | Other interactions |
|---|---|---|---|---|---|---|
| Vildagliptin | -6.6 | 14.51 | ARG A:125<br>ASN A:710<br>GLU A:205<br>GLU A:206<br>TYR A:547<br>SER A:630 | TYR A:662<br>TYR A:666<br>PHE A:357 | - | - |
| | | |  | | | |
| C26 | -7.9 | 1.61 | ARG A:125<br>GLY A: 741 | - | ARG A:125 | - |
| | | |  | | | |
| C4 | -7.8 | 1.91 | TYR A:585<br>GLN A:553<br>SER A:630<br>TYR A:547 | HIS A:740 | - | HIS A:740 |
| | | |  | | | |
| C6 | -7.7 | 2.26 | ARG A:125<br>TYR A:547<br>GLN A:553 | HIS A:740<br>TRP A: 629<br>TYR A: 547 | - | - |
| | | |  | | | |

Based on table 4. amino acid residues ARG A:125, HIS A:740, GLN A:553, and TYR A:547 were consistently identified as the most frequent interaction partners for both the tested ligands and the reference compounds. The recurrence of these interactions suggests that these residues play a critical role in stabilizing ligand binding within the active site and may represent key determinants governing the ligand-binding mode. Such conserved interactions are often indicative of structurally and functionally important regions within the binding pocket, contributing to specificity, affinity, and catalytic modulation. Therefore, these residues are proposed to act as essential anchoring points in the ligand–protein interaction network and may serve as strategic targets for structure-based optimization of novel inhibitors (Ferreira & Andricopulo, 2022; Pinzi & Rastelli, 2021; Torres et al., 2021).

Recent advances in structure-based drug design emphasize that recurrent contacts between ligands and conserved residues within the active site frequently correlate with improved binding stability and enhanced inhibitory potential. Mapping these interaction hotspots is thus crucial for rational ligand optimization, enabling the identification of key pharmacophoric features and guiding the design of derivatives with improved potency and selectivity (Ferreira & Andricopulo, 2022; Pinzi & Rastelli, 2021).

**Table 5. Binding Interaction Profile of the Tested Compounds Against the ALR2**

| Compound | Binding affinity (kkal/moL) | Ki (µM) | Bonding Hydrogen bonding | Hydrophobic interaction | Electrostatic interactions | Other interactions |
|---|---|---|---|---|---|---|
| Zopolrestat | -12.2 | 0.001 | CYS A:303<br>PRO A:310<br>THR A:113<br>TRP A:111<br>LEU A:300<br>CYS A:298<br>TYR A:48 | VAL A:47<br>TRP A:20<br>PHE A:122<br>LEU A:300<br>TRP A:111<br>CYS A:303<br>PRO A:310<br>TYR A:309 | - | PHE A:122<br>TRP A:79<br>CYS A:298 |
| | | |  | | | |
| 6b | -11.1 | 0.01 | LEU A:300<br>TRP A:111<br>ALA A:299<br>CYS A:298 | TRP A:20<br>TYR A:209<br>HIS A:110<br>CYS A:298<br>TRP A:111<br>LEU A:300<br>CYS A:303<br>PHE A:122 | - | CYS A:80 |
| | | |  | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| 6d | -11.1 | 0.01 | HIS A:110 | CYS A:303<br>LEU A:300<br>TRP A:111<br>CYS A:298<br>TRP A:20<br>PHE A:122<br>VAL A:47 | - | - |
| | | | |  | | |
| Bv10 | -10.8 | 0.01 | LEU A:300<br>ALA A:299<br>TYR A:48<br>CYS A:298 | CYS A:303<br>TRP A:111<br>LEU A:300<br>CYS A:298<br>VAL A:47<br>TRP A:20 | - | CYS A:80<br>CYS A:298<br>HIS A:110 |
| | | | |  | | |

The interaction profiles between the tested compounds and the ALR2 target were predominantly characterized by hydrogen bonding and hydrophobic interactions, as summarized in Table 5. Notably, amino acid residues CYS A:303, LEU A:300, TRP A:111, TRP A:20, and CYS A:298 were consistently involved in all interaction patterns across the three selected compounds. The recurrent involvement of these residues strongly suggests their critical role as key anchoring points within the binding pocket, contributing to ligand stabilization and potentially influencing inhibitory activity.

From a structure-based drug design perspective, conserved hydrogen bond networks and hydrophobic contacts within the active site are widely recognized as essential determinants of binding affinity and specificity. Residues such as tryptophan and cysteine frequently participate in π-interactions, van der Waals contacts, and stabilizing polar interactions, which collectively enhance ligand orientation and binding stability. Therefore, the consistent interaction with these residues indicates that they may represent crucial determinants governing the ligand-binding mode and could serve as strategic targets for future lead optimization of ALR2 inhibitors (Mishra, 2023; Pinzi & Rastelli, 2021; Zhang, 2022).

Recent studies on aldose reductase inhibitors further emphasize that stable engagement with conserved residues within the catalytic pocket is strongly correlated with improved inhibitory potency and selectivity, supporting the hypothesis that these identified amino acids play a pivotal role in mediating effective ligand binding (Chen, 2023; Grewal, 2021).

**Tabel 6. Physicochemical Properties, Lipinski's Rule, and Toxicity Classification of Ligands**

| Compound | Molecular Weight (Da) | Log P | Hydrogen Bond Donors | Hydrogen Bond Acceptors | Refractivity Molar | Lipinski's Violation | LD50 (mol/kg) | AMES Toxicity |
|---|---|---|---|---|---|---|---|---|
| Vildagliptin | 303.4 | 1.174 | 2 | 4 | 86.18 | 0 | 2.674 | No |
| Zopolrestat | 418.3 | 2.365 | 0 | 7 | 99.53 | 0 | 2.798 | No |
| C26 | 333.3 | -0.051 | 2 | 6 | 85.06 | 0 | 1.904 | No |
| C4 | 361.3 | 1.270 | 6 | 2 | 91.37 | 0 | 1.711 | Yes |
| C6 | 297.2 | 2.852 | 1 | 4 | 80.64 | 0 | 2.379 | No |
| 6b | 384.8 | 4.286 | 1 | 4 | 105.77 | 0 | 2.189 | No |
| 6d | 364.4 | 3.941 | 1 | 4 | 105.73 | 0 | 3.042 | No |
| Bv10 | 293.2 | 3.661 | 0 | 3 | 71.23 | 0 | 2.084 | No |

The physicochemical characterization showed that all investigated compounds comply with Lipinski's Rule of Five, indicating acceptable preliminary drug-likeness for oral administration. This suggests that their molecular size, polarity, and hydrogen bonding capacity are within ranges associated with favorable absorption and permeability. However, Lipinski compliance alone is insufficient to predict pharmacokinetic performance, as factors such as molecular flexibility, polar surface area, and metabolic stability also influence in vivo bioavailability. Therefore, although these compounds demonstrate promising initial drug-likeness, further pharmacokinetic validation is still required (Benet et al., 2020; Shityakov & Förster, 2021).

The molecular weight distribution (293–418 Da) falls within the optimal physicochemical range for many small-molecule drugs, balancing structural complexity for selective target binding with favorable drug-like properties. Derivatives such as 6b approach the upper limit, potentially improving binding through greater receptor surface contact. However, increased molecular weight and complexity may also impair metabolic clearance and raise the risk of off-target interactions, emphasizing pharmacokinetic trade-offs during lead optimization.

Lipophilicity analysis further clarifies the structure–activity relationship among the derivatives. Compound C26 shows a low LogP value, indicating better aqueous solubility but potentially reduced membrane permeability, which may limit intracellular target access. In contrast, 6b and 6d exhibit higher lipophilicity, likely enhancing hydrophobic interactions within the enzyme binding pocket and contributing to favorable docking scores. However, excessive lipophilicity may increase nonspecific binding and metabolic liability. Moreover, docking affinity can be biased toward lipophilic compounds, highlighting the importance of integrating ADMET analysis to minimize false-positive lead selection (Cavasotto & Aucar, 2020).

Hydrogen bonding characteristics significantly influence ligand binding and pharmacokinetic behavior. The higher number of hydrogen bond donors in C4 may strengthen polar interactions with the target but can also reduce membrane

permeability and increase desolvation penalties during binding. Excessive hydrogen bonding is often associated with lower oral bioavailability and higher toxicity risk, indicating that structural optimization may be needed to balance polarity and permeability without compromising target affinity.

Molar refractivity analysis showed that all compounds possess suitable molecular volume and polarizability, which contribute to dispersion forces and stabilization of ligand–protein complexes. Compounds 6b and 6d exhibited higher molar refractivity, potentially enhancing van der Waals interactions within hydrophobic regions of the binding pocket. This observation aligns with structure-based drug design principles, where optimal polarizability supports stronger binding stability and favorable interaction networks within the active site (Pinzi & Rastelli, 2021).

Toxicity prediction through AMES analysis revealed predominantly favorable safety profiles; however, compound C4 was identified as potentially mutagenic. Early identification of mutagenicity risk is critical for reducing late-stage attrition in drug development. Structural alerts associated with mutagenicity typically require targeted modification strategies, such as removal or replacement of reactive functional groups, to retain biological activity while improving safety. Integrating toxicity assessment at early discovery stages is increasingly recognized as essential for efficient lead prioritization (H. Yang et al., 2022).

Analysis of predicted LD50 values provides additional insight into the relative safety of the candidates. Compound 6d showed the highest LD50, indicating lower predicted acute toxicity and supporting its potential as a lead candidate. In contrast, C4 exhibited a lower LD50 and positive AMES test result, suggesting greater safety concerns. These findings emphasize the importance of integrating physicochemical, docking, and toxicity data for a comprehensive evaluation of drug potential.

Overall, compounds 6d, 6b, and C6 emerged as the most promising candidates due to balanced lipophilicity, favorable safety profiles, and acceptable drug-likeness. Compound C26 may require structural optimization to improve permeability, while C4 presents toxicity-related limitations. These results highlight the value of combining physicochemical analysis, structure-based modeling, and early ADMET prediction to improve lead selection in early-stage antidiabetic drug discovery and increase the likelihood of successful therapeutic development.

**Tabel 7. Predicted Absorption**

| Compound | Caco-2 | HIA | Pgp substrat |
|---|---|---|---|
| Vildagliptin | 0.591 | 74.384 | No |
| Zopolrestat | 1.38 | 97.547 | No |
| C26 | 0.646 | 55.76 | Yes |
| C4 | -0.07 | 82.459 | Yes |
| C6 | 0.965 | 84.41 | Yes |
| 6b | 0.558 | 94.059 | Yes |
| 6d | 0.813 | 85.155 | Yes |
| Bv10 | 1.704 | 95.121 | No |

The predicted absorption profiles revealed notable differences in intestinal permeability and transporter interactions among the evaluated compounds (Table 7). Bv10 (1.704) and Zopolrestat (1.38) showed the highest Caco-2 permeability, indicating favorable passive diffusion across intestinal epithelial membranes. In contrast, C4 exhibited a negative Caco-2 value (−0.07), suggesting limited membrane

permeability despite relatively high predicted human intestinal absorption (HIA) (82.46%). This discrepancy may reflect compensatory transport mechanisms or prediction variability, highlighting the need to integrate multiple absorption parameters rather than relying on a single descriptor (Daina et al., 2017; Shityakov & Förster, 2021).

HIA values further support favorable absorption potential for most derivatives, particularly Bv10 (95.12%), 6b (94.06%), and 6d (85.16%). However, C26 demonstrates comparatively lower HIA (55.76%), likely attributable to its high polarity and reduced lipophilicity, which may limit passive membrane diffusion. Notably, several compounds (C26, C4, C6, 6b, 6d) are predicted to be P-glycoprotein (P-gp) substrates. Efflux via P-gp can significantly reduce intracellular drug accumulation and oral bioavailability, particularly for moderately lipophilic compounds (Benet et al., 2020). In contrast, Bv10 and the reference drugs are predicted to be non-substrates, suggesting potentially improved absorption stability.

Overall, Bv10 and 6d demonstrate the most balanced absorption profiles, combining favorable Caco-2 permeability, high HIA, and reduced transporter liability, thereby strengthening their candidacy for further pharmacokinetic evaluation.

**Tabel 8. Distribution Prediction**

| Compound | VDss | BBB | CNS |
|---|---|---|---|
| Vildagliptin | 0.586 | -0.652 | -3.129 |
| Zopolrestat | -0.59 | -1.288 | -2.583 |
| C26 | -1.191 | -1.009 | -3.284 |
| C4 | -0.563 | -0.809 | -2.846 |
| C6 | -0.129 | 0.363 | -2.11 |
| 6b | 0.345 | 0.304 | -1.837 |
| 6d | 0.358 | 0.062 | -1.885 |
| Bv10 | 0.017 | 0.378 | -1.33 |

The predicted distribution parameters in Table 7 provide important insights into systemic exposure and tissue penetration of the investigated compounds. The volume of distribution at steady state (VDss) showed notable variability, with compounds 6b (0.345) and 6d (0.358) exhibiting higher distribution potential than the reference compounds. Higher VDss values generally indicate better tissue penetration and broader systemic distribution, which may enhance pharmacodynamic efficacy but also increase the risk of off-target accumulation (Shityakov & Förster, 2021).

Predicted Blood–brain barrier permeability indicates that most compounds have limited CNS exposure, as shown by negative BBB values. This is favorable for antidiabetic agents, where peripheral targeting is preferred to reduce CNS-related adverse effects. However, C6, 6b, and Bv10 showed positive BBB scores, suggesting moderate brain penetration. Although this may raise safety concerns, moderate BBB permeability can also indicate balanced lipophilicity and membrane permeability, supporting favorable overall pharmacokinetics (Varma, 2021).

CNS permeability predictions further support limited central exposure across most derivatives, with strongly negative values indicating low likelihood of significant CNS accumulation. Notably, Bv10 exhibits the least negative CNS score, suggesting relatively higher brain exposure compared to other candidates. Integrated analysis of VDss, BBB, and CNS parameters suggests that compounds 6b and 6d demonstrate a favorable balance between systemic distribution and controlled CNS penetration,

supporting their prioritization for further pharmacokinetic optimization and experimental validation (Daina et al., 2017).

**Conclusion**

Overall, the integrated *in silico* analyses showed that the investigated 2-benzoxazolinone derivatives interacted with key catalytic residues of DPP-4 and ALR2 and exhibited favorable drug-likeness, balanced physicochemical properties, and promising pharmacokinetic profiles. Compounds 6b, 6d, and C6 emerged as the most promising candidates, showing strong binding affinities, optimal lipophilicity, adequate absorption and distribution, and favorable safety profiles compared with reference drugs. Most derivatives complied with Lipinski's Rule of Five and demonstrated good intestinal absorption, although transporter-mediated efflux and potential CNS exposure require further evaluation. Overall, molecular interactions, binding affinity, inhibition constants (Ki), and ADMET profiles support their potential as ALR2 and DPP-4 inhibitors. Further validation through synthesis and subsequent *in vitro* and *in vivo* studies is needed to confirm their pharmacological activity, safety, and therapeutic potential as novel antidiabetic agents.

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**Declaration of Competing Interest**

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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