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# Pyrazine and Furan Derivative Activity Prediction on Type 2 Diabetic Mellitus: *In silico* Study

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Abstract

Diabetes Mellitus (DM) is a chronic disease that occurs when the pancreas does not produce enough insulin, or the body cannot use insulin effectively. Type 2 DM treatment can be done using antidiabetic drugs, but the continuous use of synthetic drugs will cause side effects. Empirically, the people of Nias Indonesia use palm juice (*Arenga pinnata* Merr.) as an antidiabetic, which can reduce blood glucose levels. This study aimed to find the active compounds in palm juice that can potentially be an antidiabetic type 2 using an *in silico* approach. The methods used were toxicity screening, profile pharmacokinetics, drug scanning, docking, and molecular dynamics simulation. Screening, molecular docking, and molecular dynamics of 30 compounds generated from pyrazine and furan revealed that two compounds, PF 16 and PF 30, can bind to receptors and produce lower ΔG values than metformin HCl. Molecular dynamics simulation results using the MM-GBSA calculation method showed that the PF 16 compound was more selective to the 2PDY (aldose reductase) with a value of -39.23 kcal/mol, while compound PF 30 was more selective to 1Z89 (aldose reductase) with a value of -7.36 kcal/mol. It can be concluded that the level of affinity of the PF 30 compound to the 1Z89 receptor and the PF 16 compound to the 2PDY were predicted to have the potential as antidiabetic (DM type 2).

## 1. Introduction

According to the World Health Organization (2021), Diabetes Mellitus (DM) is a chronic disease that occurs when the pancreas does not produce enough insulin or when the body does not produce insulin effectively [1]. Globally, DM was included in the top 10 causes of death and became a serious threat to human health. Based on data from the International Diabetes Federation (IDF), the prevalence of DM (combined type 1 and type 2) in people aged 20-79 years has increased from 151 million (4.6% of the global population in 2000) to 463 million (9.3%) in 2019. Of 10 countries with the highest number of DM sufferers, Indonesia ranked 7th with 10.7 million sufferers [2].

The American Diabetes Association (ADA) classified DM into four clinical statuses: type 1 diabetes, type 2 diabetes, gestational diabetes, and other specific diabetes [3]. The number of DM type 2 patients was more common in Indonesia, reaching 90-95% compared to type 1 DM, which is estimated to be less than 5-10% of all DM patients [4]. Some commonly used antidiabetic drugs were sulfonylureas, thiazolidinediones, meglitinides, biguanides, dipeptidyl peptidase-IV inhibitors, and α-glucosidase inhibitors [5]. Continuous use of antidiabetic drugs can cause side effects such as headache, diarrhea, nausea, vomiting, and hypoglycemia [6]. Therefore, to reduce the side effects of synthetic drugs, herbal medicines can be used as an alternative to type 2 DM treatment [7, 8, 9].

The palm plant (*Arenga pinnata* Merr.) is a plant used to treat diabetes mellitus. Empirically, most people process palm juice (*Arenga pinnata* Merr.) into palm sugar as a sweetener. In addition, the people of Nias Indonesia used palm juice as an antidiabetic by assuming that alcohol in sugar palm juice (*Arenga pinnata* Merr.) can reduce glucose levels in DM patients [10]. Sugar palm juice (*Arenga pinnata* Merr.) contains N-Heterocyclic

compounds (Pyrazine), O-Heterocyclics (furans), aldehydes, and ketones [11]. Pyrazine and furan are aromatic heterocyclic compounds.

According to the Australian Product Information (API), heterocyclic rings can be antidiabetic [12, 13], effectively controlling blood glucose levels in patients with type 2 diabetes [14]. Because of the antidiabetic activity of the compounds contained in palm juice, it is necessary to have a specific study to develop antidiabetic drugs. This can be learned by *in silico* prediction.

*In silico* is a computational-based method used for screening tests on compounds suspected of having pharmacological activity to determine which compounds bind best to receptors [15]. Based on this background, the researcher intends to predict the activity of pyrazine and furan derivative compounds in palm juice (*Arenga pinnata* Merr.) against DM type 2 enzyme through an *in silico* study. There are nine types of receptors used in this docking to selectively investigate receptor and ligand interactions.

## 2. Experimental

### 2.1. Tools and Materials

The tools used in this research were a set of PC (specification: intel® Core™ i2-6400 CPU @ 3.90 GHz (4CPUs), GPU Nvidia Geforce GTX 970 Gigabyte OC Edition, RAM 8GB DDR4), as well as software such as ChemDraw Ultra 8.0, MarvinSketch 5.2.5.1, Molegro Molecular Viewer 2.5, Toxree, AutodockTools 1.5.6, PyRx 0.9.8, BIOVIA Discovery Studio 2017, AMBER 16 and some programs web server such as RSCB PDB, PDBSum, PreADMET, and PubChem.

The materials used in this study were the structure of the enzyme as a diabetic target, which was downloaded from http://www/rscb.org/. There were nine enzymes with code PDB 1IR3 (insulin receptor tyrosine kinase), 1Z89 (aldose reductase), 2IIT (Human dipeptidyl peptidase), 2PDY (aldose reductase), 3PCU (human retinoic X receptor alpha), 4GQR (Human Pancreatic alpha-amylase), 4Y29 (PPAR gamma), 4ZZJ (sirtuin 1), 5NN5 (human lysosomal acid-alpha-glucosidase), and 30 ligand structures where 28 ligands were obtained from the PubChem web server and two ligands from the journal of the isolation of sugar palm (*Arenga pinnata*. Merr), and positive control (metformin HCl).

### 2.2. Receptor Analysis and Preparation

The receptors were analyzed using the website http://www.ebi.ac.uk/pdbsum. A protein structure has good quality if the value of most favored regions is >90% and disallowed regions are <0.8%. The receptor was downloaded on https://www.rcsb.org and separated from the water molecule, cofactor, and natural ligand [16].

### 2.3. Method Validation

The method was validated by redocking the molecule with natural ligands. The docking method [17] is valid if the RMSD (root mean square deviation) value is below 2 Å [18].

### 2.4. Ligand Preparation

The pyrazine and furan derivatives were downloaded on the PubChem web server or drawn using the ChemDraw Ultra 8.0 application. Optimization of geometry, protonation at pH 7.4, and conformation search were carried out using MarvinSketch 5.2.5.1 [19].

### 2.5. Toxicity Prediction

Toxicity studies were carried out using Toxtree 3.1.0 software. The parameters were the Cramer Rules, Benigni/Bossa rule base, and Kroes TTC decision tree [20].

### 2.6. Pharmacokinetic Screening

Pharmacokinetic studies were carried out to predict absorption, distribution, metabolism, and elimination processes in the human body through an *in silico* study using the PreADMET website [21].

### 2.7. Lipinski's Rule of Five Study

Drug analysis was conducted by considering the Lipinski Rule of Five rules, which included molecular weight <500 g/mol, partition coefficient log P <5, hydrogen bond donor <5, hydrogen bond acceptor <10, and molar refractory falls within 40-130 [22].

**Table 1**. Receptor analysis

| No. | Receptor | Resolution (Å) | Most favored regions (%) | Disallowed regions(%) |
|---|---|---|---|---|
| | | (< 2 Å) | (> 90 %) | (< 0.8 %) |
| 1 | 1IR3 | 1.9 | 93.3 | 0.0 |
| 2 | 1Z89 | 1.43 | 91.7 | 0.0 |
| 3 | 2IIT | 2.35 | 86.0 | 0.0 |
| 4 | 2PDY | 1.65 | 91.7 | 0.0 |
| 5 | 3PCU | 2.0 | 95.4 | 0.0 |
| 6 | 4GQR | 1.2 | 88.3 | 0.0 |
| 7 | 4Y29 | 1.98 | 90.9 | 0.4 |
| 8 | 4ZZJ | 2.74 | 89.2 | 0.0 |
| 9 | 5NN5 | 2.0 | 90.8 | 0.0 |

**Table 2**. Method validation results

| No. | ID PDB | Grid Box | | | Run | RMSD (Å) | Binding energy (Kcal/mol) |
|---|---|---|---|---|---|---|---|
| | | X | Y | Z | | | |
| 1 | 1IR3 | -23.18 | 30.109 | 7.562 | 98 | 1.59 | -5.18 |
| 2 | 1Z89 | 16.879 | -5.797 | 14.287 | 3 | 1.36 | -9.95 |
| 3 | 2PDY | 20.467 | 6.132 | 27.205 | 89 | 1.91 | -13.69 |
| 4 | 3PCU | -19.188 | 4.065 | 3.995 | 6 | 0.87 | -10.47 |
| 5 | 4Y29 | 5.18 | -3.368 | 22.54 | 83 | 1.97 | -7.89 |
| 6 | 5NN5 | -13.854 | -31.335 | 96.509 | 65 | 1.46 | -5.46 |

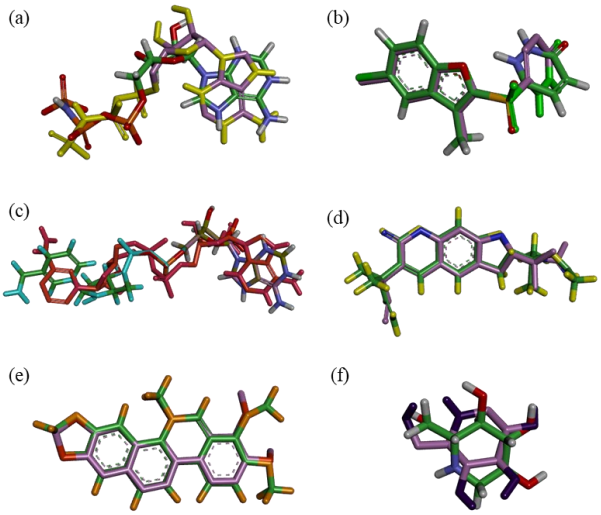


**Figure 1**. Overlay form between (a) 1IR3 initial conformation (blue) and after re-docking (yellow), (b) 1Z89 initial conformation (purple) and after re- docking (green), (c) 2PDY initial conformation (blue) and after re-docking (pink), (d) 3PCU initial conformation (green) and after re-docking (purple), (e) 4Y29 initial conformation (orange) and after re- docking (purple), (f) 5NN5 initial conformation (green) and after re-docking (purple)

### 2.8. Docking

The PyRx 0.9.8 was used for molecular docking between the receptor and the ligand. The grid box settings were determined using the validation results of the method. The analysis was carried out by selecting the conformational compound that had the smallest Gibs free energy (ΔG) and inhibition constant (Ki) [23].

### 2.9. Molecular Dynamics

Molecular dynamics simulations were performed using AMBER 16 software [24]. The results of the AMBER analysis focused on three aspects: Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), and Molecular Mechanics-Poisson Boltzmann Surface Area (MM-PBSA) [25]. The MM-PBSA method is favored in molecular dynamics due to its effective balance of computational demand and accuracy. MMPBSA in molecular dynamics is a computational method used to estimate the binding free energy of ligands to biological molecules by considering molecular mechanics, solvation effects, and the solvent-accessible surface area [26, 27, 28, 29].

## 3. Results and Discussion

### 3.1. Receptor Analysis and Preparation

In selecting the receptor to be used in molecular docking, there are several conditions for the receptor. Conditions that must be met include resolution and the most favored and disallowed regions. The data in Table 1 collectively suggest that the analyzed receptor structures predominantly occupy the most favored regions of conformational space, implying a high degree of structural reliability and precision within the resolution limits assessed; they are 1IR3, 1Z89, 2PDY, 3PCU, 4Y29, and 5NN5. The receptor was prepared by separating the complex receptor from natural ligands, water molecules, and residues. The goal is to optimize the interaction of the ligand and receptor by preventing hydrogen bonds from forming between the ligand and the water molecule.

### 3.2. Method Validation Results

The method was validated by re-docking the receptor with natural ligands. The method is valid if it produces a value of RMSD ≤ 2 Å. The method validation results in Table 2 show that all six receptors meet the redocking requirements by producing a value of RMSD ≤ 2 Å. Receptors that met the requirements were visualized as an overlay using BIOAVIA Discovery Studio 2017, as shown in Figure 1.

Based on the examination of binding energy values, four receptors with the lowest binding energies were identified: receptors 1Z89, 2PDY, 3PCU, and 2Y29. Consequently, these receptors were selected for utilization in the *in silico* docking process.

### 3.3. Ligand Preparation

Ligand preparation includes a geometric optimization process to obtain the most stable structure of the test compound, protonation to adjust conditions according to a human physiological pH of 7.4, and conformational adjustments to ensure effective interaction between the ligand and the active site on the receptor.

### 3.4. Toxicity Screening

Toxicity screening was carried out to determine a compound's toxic potential as a consideration when selecting new drug candidates. Some parameters analyzed in the toxicity screening were Cramer Rules, Kroes TTC decision tree, and Benigni/Bossa Rulebase.

**Table 3**. Screening toxicity

| No. | Compound | Parameter | | |
|---|---|---|---|---|
| | | Cramer Rule | Kroes TTC decision tree | Benigni/Bossa Rulebase |
| 1 | PF 1 | High (Class III) | + | + |
| 2 | PF 2 | Intermediate (Class II) | + | + |
| 3 | PF 3 | High (Class III) | + | + |
| 4 | PF 4 | High (Class III) | + | + |
| 5 | PF 5 | High (Class III) | + | + |
| 6 | PF 6 | High (Class III) | + | + |
| 7 | PF 7 | High (Class III) | + | + |
| 8 | PF 8 | High (Class III) | + | + |
| 9 | PF 9 | High (Class III) | + | + |
| 10 | PF 10 | High (Class III) | + | + |
| 11 | PF 11 | High (Class III) | + | + |
| 12 | PF 12 | High (Class III) | + | + |
| 13 | PF 13 | High (Class III) | + | + |
| 14 | PF 14 | High (Class III) | + | + |
| 15 | PF 15 | High (Class III) | + | + |
| 16 | PF 16 | High (Class III) | + | + |
| 17 | PF 17 | High (Class III) | + | + |
| 18 | PF 18 | High (Class III) | + | + |
| 19 | PF 19 | Intermediate (Class II) | + | + |
| 20 | PF 20 | High (Class III) | + | + |
| 21 | PF 21 | High (Class III) | + | + |
| 22 | PF 22 | High (Class III) | + | + |
| 23 | PF 23 | High (Class III) | + | + |
| 24 | PF 24 | High (Class III) | + | + |
| 25 | PF 25 | High (Class III) | - | - |
| 26 | PF 26 | High (Class III) | - | - |
| 27 | PF 27 | High (Class III) | + | + |
| 28 | PF 28 | High (Class III) | - | - |
| 29 | PF 29 | High (Class III) | + | + |
| 30 | PF 30 | High (Class III) | + | + |
| 31 | Metformin HCl | High (Class III) | + | + |

Note:
(+): No potential to cause cancer
(-): Potential to cause cancer

The results of the toxicity screening based on Table 3, of the 30 compounds derived from pyrazine and furan in the Cramer Ruler parameter, overall the compounds were at a high toxicity level or High (Class III), which means they have high toxicity, but can be used as new drug candidates by adjusting the dose, to minimize the toxic effects that may occur. However, there were two compounds, PF 2 and PF 19, at moderate or intermediate toxicity levels (Class II). In the Kroes TTC Decision Tree parameters, most compounds were at a safe threshold in the body. However, there were three compounds, namely PF 25, PF 26, and PF 28, within the cancer risk threshold but with a low occurrence risk.

The Benigni/Boss Rulebase parameters indicate no potential for mutagenicity or carcinogenicity, suggesting these compounds are safe for human use. However, three compounds—PF 25, PF 26, and PF 28—were identified as potentially carcinogenic. In comparison with the positive control, 25 compounds exhibited similar toxicity properties, characterized by high toxicity levels (Class III), a safe threshold within the body, and no potential for mutagenicity.

### 3.5. Pharmacokinetic Prediction

Pharmacokinetic prediction was carried out on 25 compounds that met the toxicity requirements. The parameter analyzed was Caco-2, which is related to the permeability of the compound. HIA (Human Intestinal Absorption) to predict drug absorption in the intestine. PPB (Plasma Binding Protein) is used to predict binding to plasma proteins [30].

**Table 4.** Screening pharmacokinetic

| No. | Compound | Caco-2 (nm/sec) | HIA (%) | PPB (%) |
|---|---|---|---|---|
| 1 | PF 1 | 30.26 | 100.00 | 65.40 |
| 2 | PF 3 | 27.82 | 100.00 | 9.37 |
| 3 | PF 4 | 23.74 | 100.00 | 83.11 |
| 4 | PF 5 | 23.63 | 100.00 | 58.01 |
| 5 | PF 6 | 23.63 | 100.00 | 57.98 |
| 6 | PF 7 | 23.61 | 100.00 | 24.28 |
| 7 | PF 8 | 23.61 | 100.00 | 24.26 |
| 8 | PF 9 | 22.19 | 100.00 | 89.97 |
| 9 | PF 10 | 22.19 | 100.00 | 60.66 |
| 10 | PF 11 | 22.19 | 100.00 | 90.07 |
| 11 | PF 12 | 23.53 | 100.00 | 51.90 |
| 12 | PF 13 | 45.06 | 100.00 | 17.25 |
| 13 | PF 14 | 22.11 | 100.00 | 97.27 |
| 14 | PF 15 | 22.11 | 100.00 | 97.26 |
| 15 | PF 16 | 29.37 | 98.54 | 48.62 |
| 16 | PF 17 | 23.69 | 100.00 | 84.09 |
| 17 | PF 18 | 23.52 | 100.00 | 58.91 |
| 18 | PF 20 | 25.67 | 100.00 | 96.03 |
| 19 | PF 21 | 55.17 | 100.00 | 69.33 |
| 20 | PF 22 | 28.90 | 100.00 | 79.51 |
| 21 | PF 23 | 29.49 | 100.00 | 83.78 |
| 22 | PF 24 | 11.91 | 88.80 | 82.06 |
| 23 | PF 27 | 29.49 | 100.00 | 83.78 |
| 24 | PF 29 | 36.84 | 100.00 | 100.00 |
| 25 | PF 30 | 57.83 | 100.00 | 69.01 |
| 26 | Metformin HCl | 21.08 | 45.66 | 3.95 |

Information:

■ = qualified

■ = unqualified

According to Table 4, the pharmacokinetic screening results for the 25 compounds, along with metformin HCl, indicate moderate permeability within the range of 4- 70 nm/sec. Metformin HCl shows poor absorption in the small intestine, whereas the 25 compounds exhibit good absorption. Metformin HCl forms weak bonds with plasma proteins, similar to 20 of the compounds. However, the percentage of plasma protein binding for metformin HCl exceeds 90%, indicating strong binding to plasma proteins. Drugs bound to plasma proteins are inactive, as only free drugs can exert a biological response and undergo elimination. Nevertheless, five other compounds—PF 11, PF 14, PF 15, PF 20, and PF 29—display strong bonds with plasma proteins, suggesting poor distribution capabilities within the body and likely difficulty in excretion.

### 3.6. Screening Lipinski's Rule of Five

Lipinski's Rule of Five study was used to predict *in silico* the feasibility of ligands as potential drug candidates in oral dosage forms. This study was conducted on 20 pyrazine and furan derivatives that have met the requirements for toxicity and pharmacokinetics.

Based on Table 5, a comprehensive analysis of pyrazine and furan derivatives was conducted. Six compounds—PF 9, PF 10, PF 12, PF 16, PF 18, and PF 30—meet the established criteria, indicating good permeability and ease of absorption. Conversely, the remaining 14 compounds fail to meet one criterion: molar refractivity. Therefore, their suitability as drug candidates in oral dosage forms warrants reconsideration. However, adhering to Lipinski's rules, drugs may be administered orally if they violate no more than one criterion [31].

**Table 5**. Screening Lipinski's Rule of Five

| No. | Compound | Parameter | | | | |
|---|---|---|---|---|---|---|
| | | MW | Hydrogen donor | Hydrogen acceptor | Log P | Refractory molar |
| | | < 500 g/mol | < 5 | < 10 | < 5 | 40-130 |
| 1 | PF 1 | 80.00 | 0 | 2 | 0.47 | 22.03 |
| 2 | PF 3 | 108.00 | 0 | 2 | 1.03 | 31.40 |
| 3 | PF 4 | 108.00 | 0 | 2 | 1.09 | 31.50 |
| 4 | PF 5 | 121.00 | 0 | 2 | 1.95 | 38.35 |
| 5 | PF 6 | 122.00 | 0 | 1 | 1.34 | 36.14 |
| 6 | PF 7 | 120.00 | 0 | 2 | 1.42 | 36.85 |
| 7 | PF 8 | 120.00 | 0 | 2 | 1.42 | 36.85 |
| 8 | PF 9 | 136.00 | 0 | 2 | 1.65 | 40.88 |
| 9 | PF 10 | 136.00 | 0 | 2 | 1.65 | 40.88 |
| 10 | PF 12 | 136.00 | 0 | 2 | 1.60 | 40.78 |
| 11 | PF 13 | 134.00 | 0 | 2 | 1.52 | 38.76 |
| 12 | PF 16 | 188.00 | 0 | 3 | 2.22 | 53.01 |
| 13 | PF 17 | 108.00 | 0 | 2 | 1.09 | 31.50 |
| 14 | PF 18 | 134.00 | 0 | 2 | 1.81 | 41.47 |
| 15 | PF 21 | 68.00 | 0 | 1 | 1.27 | 18.70 |
| 16 | PF 22 | 82.00 | 0 | 1 | 1.58 | 23.44 |
| 17 | PF 23 | 100.00 | 0 | 2 | 0.36 | 25.03 |
| 18 | PF 24 | 98.00 | 1 | 2 | 0.77 | 24.63 |
| 19 | PF 27 | 138.00 | 0 | 1 | 3.18 | 42.03 |
| 20 | PF 30 | 144.00 | 0 | 1 | 2.94 | 44.14 |
| 21 | Metformin HCl | 130.00 | 6 | 5 | -2.13 | 38.69 |

Information:

= qualified Lipinski's Rule of Five

= unqualified Lipinski's Rule of Five

### 3.7. Docking

Docking is used to predict the binding between the ligand and the receptor with the aim of predicting a model of the binding that occurs between the ligand and the receptor in three-dimensional form [32]. In this study, docking was performed on receptors that fulfilled the validation criteria for the docking method, along with 20 test compounds derived from pyrazine and furan that had previously met the criteria for toxicity and pharmacokinetic screening. Additionally, a positive control, metformin HCl, was included for comparative analysis of the activity generated from the *in silico* tests.

Based on the results of molecular docking in Table 6 of the six compounds derived from pyrazine and furan, which produced a low value of ΔG and Ki, PF 16 and PF 30 showed better effectiveness than metformin HCl. Likewise, the Ki value produced was directly proportional to the ΔG value. Judging from comparing the molecular docking results of the four receptors, compounds PF 16 and PF 30 are more specific to receptors 1Z89 and 2PDY with a marked lower ΔG value. The interaction of the molecular docking results of compounds PF 16 and PF 30 and the positive control, namely metformin HCl, on the 1Z89 and 2PDY receptors is seen in Table 7.

The active site formation in the 1Z89 receptor involves hydrogen bonding with amino acid residues His110, Gln183, and Tyr48 [33], revealing similar amino acid residues binding to the metformin HCl compound. Likewise, in the 2PDY receptor, active site hydrogen bonding occurs with amino acid residues Gln183, Ser159, Ser210, Lys262, Lys21, Thr19, and Thr265 [34], where similar amino acid residues are bound to compounds PF 16, PF 30, and metformin HCl. This alignment of amino acid residues between the receptor and the test compounds suggests effective interaction at the receptor binding site, indicative of comparable activity to the natural ligand. A protein binding site represents an important role where a protein binds to a ligand through specific amino acid residues, thereby influencing the conformation or function of the receptor.

### 3.8. Molecular Dynamics

Molecular dynamics simulations were conducted on PF 16 and 30 compounds and receptors 1Z89 and 2PDY with time 20,000 ps.

**Table 6**. Docking result

| Compound | 1Z89 | | | 2PDY | | | 3PCU | | | 4Y29 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Run | ΔG (kcal/mol) | Ki (μM) | Run | ΔG (kcal/mol) | Ki (μM) | Run | ΔG (kcal/mol) | Ki (μM) | Run | ΔG (kcal/mol) | Ki (μM) |
| PF 9 | 74 | -5.19 | 156.40 | 3 | -5.19 | 156.35 | 55 | -5.00 | 216.40 | 2 | -4.24 | 780.81 |
| PF 10 | 54 | -5.21 | 152.71 | 14 | -5.33 | 132.97 | 98 | -4.79 | 307.37 | 37 | -4.27 | 736.87 |
| PF 12 | 50 | -4.68 | 371.48 | 33 | -5.21 | 151.03 | 72 | -4.65 | 393.16 | 74 | -3.99 | 1180 |
| PF 16 | 100 | -6.06 | 36.15 | 44 | -6.85 | 9.54 | 9 | -6.07 | 35.70 | 65 | -5.33 | 122.99 |
| PF 18 | 48 | -5.02 | 207.67 | 49 | -5.41 | 107.96 | 88 | -5.12 | 177.92 | 31 | -4.37 | 622.95 |
| PF 30 | 96 | -6.09 | 34.35 | 57 | -6.13 | 32.35 | 29 | -5.82 | 54.55 | 5 | -5.15 | 168.25 |
| Metformin HCl | 88 | -4.13 | 934.69 | 54 | -5.59 | 79.67 | 85 | -4.49 | 510.63 | 32 | -363 | 2200 |

**Table 7**. Amino acid interaction of molecular docking result

| Receptor | Compound | Interaction with amino acids | |
|---|---|---|---|
| | | Hydrogen bonds | Hydrophobic bonds |
| 1Z89 | PF 16 | Thr113 | Trp219, Ala299, Pro310 |
| | PF 30 | Thr113 | Cys298, Ala299, Phe311, Tyr309, Pro310, Phe115, Phe122, Trp79 |
| | Metformin HCl | Gln183 | Lys77, Tyr48, Trp20, Trp79, Val47, Trp111, Cys298, Asn160, Tyr209, Ser159 |
| 2PDY | PF 16 | Lys21, Lys262, Ser210 | Asp43, Thr19, Gly18, Ser214, Pro211, Pro261, Tyr209 |
| | PF 30 | Thr19 | Ser214, Leu212, Pro261, Ser210, Tyr209, Tyr48, Gly18, Trp20, Lys21, Pro215 |
| | Metformin HCl | Gln183, Tyr48, Thr19 | Ile260, Lys77, Gly18, Cys44, Lys262, Trp20 |

### 3.8.1. Root Mean Square Deviation (RMSD)

RMSD is a measure used to compare distance and ligand conformation changes in 3D geometry [35]. Based on Figure 2, it can be seen that PF 16, PF 30, and metformin HCl experienced an increase in RMSD from the beginning of the simulation. However, for the level of stability, PF 16 tended to have better stability than PF 30 and metformin HCl as a comparison drug.

### 3.8.2. Root Mean Square Fluctuation (RMSF)

RMSF was used to predict the region of protein flexibility by describing conformational changes in the protein chain over simulation time [36]. Based on Figure 3, it can be predicted that the test compounds PF 16, PF 30, and Metformin HCl produced the same fluctuations during the 20,000 ps simulation time. The highest fluctuations in PF 16, PF 30, and metformin HCl to the 1Z89 receptor did not occur in the amino acid residues His110, Gln183, and Tyr48, where these residues were the active sites of the 1Z89 receptor [33]. Likewise, for 2PDY, the highest fluctuations did not occur in the amino acid residues Gln183, Ser159, Ser210, Lys262, Lys21, Thr19, and Thr265, where these residues were the active sites of the 2PDY receptor [34]. This shows that PF 16, PF 30, and metformin HCl have good flexibility and stable interactions with 1Z89 and 2PDY receptors.

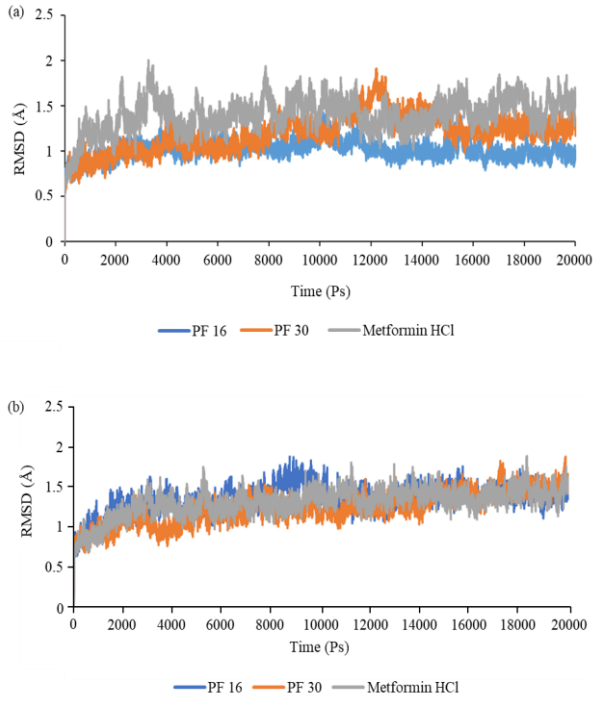


**Figure 2**. Graph of RMSD values from molecular dynamics simulation of PF 16, PF 30, and metformin HCl (a) against 1Z89 (b) against 2PDY

**Table 8**. Binding free energy of each complex 20 ns molecular dynamic simulation (MM-GBSA)

| Parameter (kcal/mol) | System | | | | | |
|---|---|---|---|---|---|---|
| | 1Z89 | | | 2PDY | | |
| | PF 16 | PF 30 | Metformin HCl | PF 16 | PF 30 | Metformin HCl |
| VdW | -1.81 | -18.60 | -13.19 | -28.11 | -11.95 | -18.59 |
| EEL | -67.45 | -63.87 | -180.16 | -227.43 | -226.40 | -85.05 |
| $E_{GB}$ | 69.98 | 78.18 | 192.43 | 219.96 | 233.33 | 79.28 |
| $E_{SURF}$ | -0.24 | -3.07 | -2.49 | -3.64 | -2.65 | -3.29 |
| $\Delta G_{gas}$ | -69.27 | -82.47 | -193.36 | -255.55 | -238.36 | -103.65 |
| $\Delta G_{solv}$ | 69.74 | 75.10 | 189.93 | 216.31 | 230.67 | 75.99 |
| $\Delta G_{total}$ | 0.46 | -7.36 | -3.42 | -39.23 | -7.68 | -27.66 |

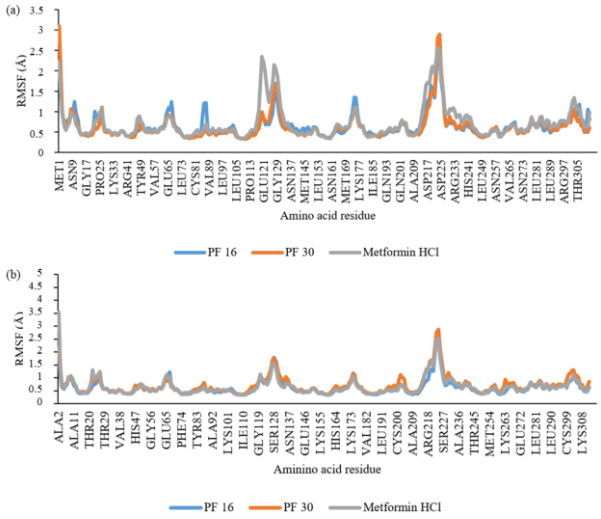


**Figure 3**. Graph of RMSF value from molecular dynamics simulation of PF 16, PF 30, and metformin HCl (a) against 1Z89 (b) against 2PD

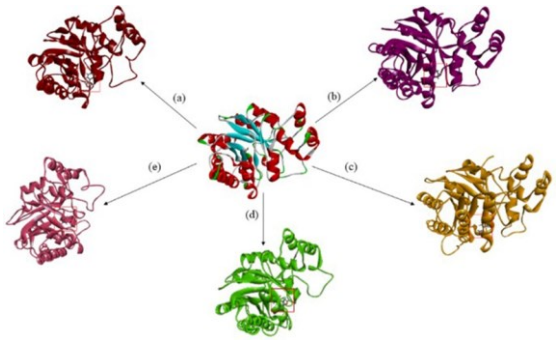


**Figure 4**. The conformational change of the molecular dynamics simulation of PF 30 against 1Z89 at times of (a) 1000 ps, (b) 5000 ps, (c) 10,000 ps, (d) 15,000 ps, and (e) 20,000 ps

### 3.8.3. Molecular Mechanics-Generalized Born Surface Area (MM-GBSA)

MM-GBSA calculates the bond-free energy between the ligand and receptor systems in molecular dynamics simulations. According to the binding free energy data presented in Table 8, the PF 16 compound exhibits higher stability and affinity for the 2PDY receptor, while the PF 30 compound shows greater selectivity for the 1Z89 receptor. Therefore, it can be inferred that the PF 16 and PF 30 compounds have stable and potent interactions, suggesting their potential as effective type 2 antidiabetic agents.

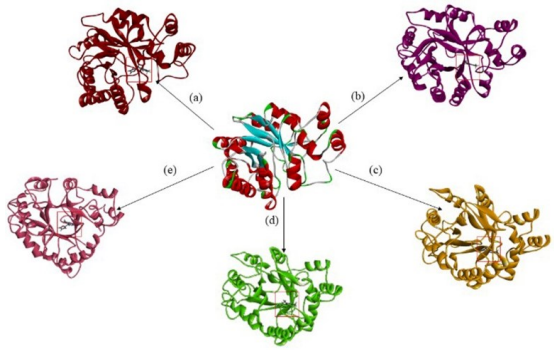


**Figure 5**. The conformational change of the molecular dynamics simulation of PF 16 to 2PDY at times of (a) 1000 ps, (b) 5000 ps, (c) 10,000 ps, (d) 15,000 ps, and (e) 20,000 ps

### 3.9. Visualization

Visualization was conducted to observe the conformational changes of the receptor-ligand complex during the molecular dynamics simulation. As shown in Figure 4, the PF 30 test compound exhibited minimal conformational changes when bound to the 1Z89 receptor over the 20,000 ps simulation period. Likewise, the compound PF 16 against the 2PDY receptor (Figure 5) did not experience much conformational change. Therefore, it can be predicted that the PF 16 compound has good stability with the 2PDY receptor, and the PF 30 compound has good stability with the 1Z89 receptor.

## 4. Conclusion

Based on the screening results, it is found among the pyrazine and furan derivatives in *Arenga pinnata* Merr. palm juice, compounds PF 16 and PF 30 are the most promising candidates for the development of type 2 diabetes drugs due to their favorable toxicity and pharmacokinetic profiles, strong binding affinities to target receptors 1Z89 and 2PDY, and adherence to the Lipinski's Rule of Five, which predicts good oral availability.

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