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# *In silico* determination of fenthion, permethrin, and carbaryl as FFAR2 inhibitors: Type 2 diabetes mellitus pathomechanism study

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## ABSTRACT

**Background:** In the last few decades, many studies have shown that pesticides have a close relationship with increasing blood glucose levels and the incidence of diabetes. Some examples of pesticides include fenthion, permethrin, and carbaryl. Recently, free fatty acid receptor 2 (FFAR2) was identified as having a critical function in preventing insulin resistance. Activation of FFAR2 will reduce fat accumulation and induce glucagon-like peptide 1 (GLP-1) secretion, which plays an important role in regulating type 2 diabetes mellitus (T2DM) prevention.
**Objective:** This study aims to determine a comparison of the binding ability between fenthion, permethrin, and carbaryl to FFAR2 protein for predicting the mechanism of pesticide toxicity to T2DM through an *in silico* study.
**Methods:** This is an exploratory bioinformatic study. The protein structure was FFAR2 receptor (UniProt: O15552), while the ligand was fenthion (PubChem CID: 3346), permethrin (PubChem CID: 40326), and carbaryl (PubChem CID: 6129). This molecular docking was conducted in October 2022 using Asus X202XE with Intel® Core™ i3-3217U CPU equipped with BIOVIA Discovery Studio, AutoDockTools, and AutoDock Vina.
**Results:** The binding affinity values generated after docking between fenthion, permethrin, and carbaryl with FFAR2 indicate that the binding affinity comparison is permethrin < carbaryl < fenthion. This explains that permethrin could form a stronger bond with FFAR2 protein than other pesticides. However, the visualisation results of the form of bond interactions show that permethrin does not bind to the active site of FFAR2, so it could not be called an inhibitor. This is different from fenthion and carbaryl, which could bind to several amino acid residues on the active site of FFAR2 and have the potential to become inhibitors.
**Conclusion:** Carbaryl is a pesticide with the strongest FFAR2 inhibitor. Carbaryl could cause type 2 DM through its inhibitory pathway to FFAR2.

***Latar Belakang**: Dalam beberapa dekade terakhir, banyak studi menunjukkan bahwa pestisida memiliki hubungan yang erat dalam meningkatkan kadar glukosa darah dan angka kejadian diabetes. Beberapa contoh pestisida diantaranya adalah fention, permetrin, dan karbaril. Saat ini, telah ditemukan Free Fatty Acid Receptor 2 (FFAR2) yang berperan penting dalam pencegahan resistensi insulin. Aktivasi terhadap FFAR2 akan menurunkan akumulasi lemak sekaligus menginduksi sekresi GLP-1 sehingga berperan penting dalam regulasi pencegahan diabetes mellitus tipe-2 (DMT2).*
***Tujuan**: Penelitian bertujuan untuk mengetahui dan menjelaskan perbandingan ikatan antara fention, permetrin, dan karbaril sebagai inhibitor FFAR2 melalui uji in silico.*
***Metode**: Penelitian ini adalah penelitian eksplorasi berbasis bioinformatika. Molecular docking ini dilakukan pada bulan Oktober 2022 menggunakan Laptop Asus X202XE dengan spesifikasi Intel® Core™ i3-3217U CPU 1.80 GHz, RAM 4GB, dan sistem operasi Windows 10 64-bit. Laptop yang digunakan dilengkapi berbagai aplikasi,*

*yaitu BIOVIA Discovery Studio, AutoDockTools, dan AutoDock Vina.*

***Hasil**: Nilai binding affinity yang dihasilkan setelah docking antara fenthion, permetrin, dan karbaril dengan FFAR2 menunjukkan bahwa nilai binding affinity permetrin < karbaril < fenthion. Hal ini menjelaskan bahwa permetrin dapat membentuk ikatan yang lebih kuat dengan protein FFAR2 dibandingkan pestisida lain. Meski demikian, hasil visualisasi bentuk interaksi ikatan menunjukkan bahwa permetrin tidak berikatan dengan sisi aktif FFAR2 sehingga tidak bisa disebut sebagai inhibitor. Hal ini berbeda dengan fenthion dan karbaril yang dapat berikatan dengan beberapa residu asam amino pada sisi aktif FFAR2 sehingga berpotensi menjadi inhibitornya.*

***Kesimpulan**: Senyawa karbaril merupakan pestisida dengan kemampuan inhibitor FFAR2 terkuat. Carbaryl dapat menyebabkan DMT2 melalui penghambatan reseptor FFAR2.*

## INTRODUCTION

Based on data from the World Health Organisation (WHO), the prevalence of people with diabetes melitus (DM) has reached around 422 million worldwide.[1] Mortality due to DM is reported to reach around 1.5 million people yearly.[1] It is predicted that the number of people with diabetes will increase to 578 million in 2030.[2] About 90% of DM patients are type 2 DM.[3] In this condition, increased blood glucose levels occur due to resistance or decreased sensitivity to insulin.[4] Risk factors commonly known to cause insulin resistance are obesity, smoking, dyslipidaemia, family history of diabetes, and low physical activity.[5] A new risk factor that could cause insulin resistance is pesticide exposure.[6] In the last few decades, study on the relationship between pesticides and the incidence of T2DM has often been carried out. These studies show that pesticides have a close relationship with increasing blood glucose levels and the incidence of diabetes.[7,8] A cross-sectional study on a rural population with the majority of agricultural workers in Korea showed that 9.3% or 238 of 2559 individuals had diabetes. Of 238 patients, 165 worked as farmers, and 129 had a history of using pesticides.[9]

Organophosphates, pyrethroids, and carbamates are some of the frequently used pesticide groups.[10] One example of these pesticide groups is fenthion, permethrin, and carbaryl. Pesticides are considered to cause insulin resistance through the induction of inflammation, oxidative stress, intestinal microbiota dysbiosis, and endocrine disorders.[11–13] Inflammation and oxidative stress will activate the serine kinase pathway, inhibiting phosphorylation of insulin receptor substrate 1 (IRS-1).[11] Disruption of the gut microbiota could alter intestinal barrier function and host metabolism, which is sufficiently related to insulin resistance.[14] In addition, the disruption of pesticides to the intestinal microbiota also causes changes in the composition of intestinal metabolites that are essential in preventing diabetes, one of which is short-chain fatty acid (SCFA).[15]

The SCFA are metabolic byproducts originating from the gut microbiota's processing of polysaccharides, playing a pivotal role in maintaining the health of their host.[16] In human physiology, one of the specific receptors targeted by SCFAs is free fatty acid receptor 2 (FFAR2).[17] Activation of SCFA by FFAR2 has an anti-diabetic effect through a series of mechanisms, mainly inhibiting fat accumulation in adipose cells and stimulating GLP-1 hormone secretion.[17] This is because the release of GLP-1 in adipose cells could reduce fat accumulation so that it could trigger an increase in insulin sensitivity.[18] In contrast, FFAR2 inhibition leads to various effects that could actually trigger diabetes, inhibition of GLP-1 secretion, and increased fat accumulation.[19]

The role of pesticides on FFAR2 is still unknown, but these compounds have been shown to act as inhibitors on other receptors. A study about the toxicity mechanism of a compound in causing its effects is urgently needed. A study approach that could predict the mechanism of action of a compound cheaply and quickly is to use in silico methods.[20] In silico could be defined as a study that uses a computer as its experimental location. This method significantly differs from other methods generally carried out in laboratories or the natural world, such as in vitro and in vivo. In the in silico method, researchers could predict a chemical substance in terms of modelling, validation, optimisation, design, and the shape of the bond between a ligand and its receptor. In addition, this test could also characterise pharmacological properties ranging from absorption, distribution, metabolism, excretion, and toxicity (ADMET).

This computational-based study has various advantages over other studies, including being fast, cheap, and not requiring living beings as objects. Therefore, in silico testing could be a study solution that is needed quickly or does not have sufficient resources to fulfil the requirements of tools, materials, and costs. Researchers also use in silico studies because they could also be preliminary study that supports further experimental study in the manufacture of new drugs and the identification of toxic compounds.[20]

Molecular docking, a commonly used in silico method, predicts the bonding potential between a compound (ligand) and a protein (receptor). It determines the optimal bonding conformation, allowing to gauge the strength of activation or inhibition by the compound. The outcome of this interaction is reflected in the binding affinity, with lower values indicating more stable interactions. In this context, we explore the potential of pesticides to induce an increased risk of DM by inhibiting FFAR2. Surprisingly, no prior study has comprehensively assessed and compared the inhibitory effects of various pesticides, such as fenthion, permethrin, and carbaryl, on the FFAR2 protein through an in silico analysis. Consequently, we conducted an in silico study to compare the binding affinities of these pesticides with FFAR2, aiming to elucidate the mechanisms underlying pesticide-induced toxicity in the context of T2DM.

## METHODS

### Tools and materials

The instrument used in the study was the Asus X202XE Laptop with Intel® Core™ i3-3217U CPU 1.80 GHz, 4GB RAM, and Windows 10 64-bit operating system. This instrument was equipped with various applications to support the course of study from preparation to implementation. Some applications used were BIOVIA Discovery Studio, AutoDockTools, and AutoDock Vina. Receptors and ligands were prepared using the BIOVIA Discovery Studio and AutoDockTools applications and then docked with AutoDock Vina. The docking configuration was recorded in the Notepad application. After that, the results of the interaction of ligands and proteins after the docking process were visualised using the BIOVIA Discovery Studio.

### Study subject

The 3D structure of the FFAR2 receptor (UniProt: O15552) was obtained and downloaded via the UniProt website (https://www.uniprot.org/), while the 3D structure of the fenthion ligand (PubChem CID: 3346), permethrin (PubChem CID: 40326), and carbaryl (PubChem CID: 6129) downloaded from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/). The docking method was validated by re-docking the main ligand with FFAR2. The main inhibitor ligand used was (S)-3-(2-(3-Chlorophenyl)acetamido)-4-(4-(trifluoromethyl)phenyl)butanoic acid (CATPB). Redocking is done to calculate the value of RMSD (root mean square deviation) to determine whether the docking process has been running accordingly. The docking method is considered valid if the value of RMSD ≤ 2. This is because the smaller RMSD value indicates the position of natural ligand results, and crystallographic docking is also getting closer. Researchers have done docking in this study using natural ligands as validation and control. The RMSD value found by researchers is 1.969.

### Study procedure

Subjects were downloaded from UniProt and PubChem sites as *.pdb or *sdf files. Then, the structure was prepared using the BIOVIA Discovery Studio application by removing water molecules and natively attached ligands. Before docking, protein and compound files must be prepared and exported in PDBQT format using AutoDockTools. This study follows the docking steps by Joshi and Kaushik with modifications.[21] In this preparation step, all files with charges and hydrogen atoms were added. The ligand torque of the compound file is set according to its normal state.

A grid box was meticulously designed to encompass the entire docking region, guaranteeing precision in the ligand-receptor interaction. Active site amino acid residues were tagged to demarcate the grid box's dimensions and placement. This designated area encompasses the ligand and the protein's active site. Vital structural details of the ligand, receptor, and the grid box's parameters are documented in a configuration file, which is generated using the Notepad application and saved in the .config.txt

format. This file is indispensable for facilitating the subsequent docking process.

**Molecular docking and data analysis**

Docking was conducted using the AutoDock Vina application. All files, such as compound structure (*.pdbqt), protein (*.pdbqt), and configuration files (*.txt) were stored in the same folder. Then, docking could be executed by sending commands through the command prompt.

Molecular docking was performed using the AutoDock Vina application. All necessary files, including compound structure files (.pdbqt), protein files (.pdbqt), and configuration files (*.txt), were stored within the same directory. Subsequently, the docking process was initiated by issuing commands via the command prompt. The outcome of the docking process yields a binding affinity value, which is recorded in the log file. The optimal binding affinity value is selected from the various bond conformations. Visualising the results is accomplished through the BIOVIA Discovery Studio application, aiding in elucidating the interaction model between the ligand and the receptor. Binding affinity serves as the key parameter in the docking analysis. A lower binding affinity value indicates a greater ease in forming bonds between the compound (ligand) and the protein (receptor), signifying a higher potential for interaction with the target protein. Additionally, the analysis examines the visualised interactions between the ligand and the receptor. The more and stronger the types of bonds formed on the active amino acid residues of a receptor, the stronger the ligand bond will be.[23]

**RESULTS**

**Native ligand and FFAR2**

The AutoDock Vina application was employed to dock CATPB compounds with FFAR2 proteins, yielding a set of nine distinct interaction models, each distinguished by its unique binding affinity. Among these models, the lowest binding affinity value recorded was -7.5 Kcal/mol. Figure 1 depicts the interaction model between CATPB and FFAR2 at this minimum binding affinity value. To exert an inhibitory effect on the target receptors, CATPB must effectively bind to specific amino acids within the active sites of the FFAR2 protein. These crucial amino acids include Lys65, Ser86, Gln148, Glu166, Tyr238, Arg255, and Ser256. The molecular docking results reveal that the native ligand could establish hydrogen bonds with Lys65 and Ser256 amino acids, form a hydrophobic bond with Arg255, and engage in an attractive charge interaction (Pi-anion) with Glu166.

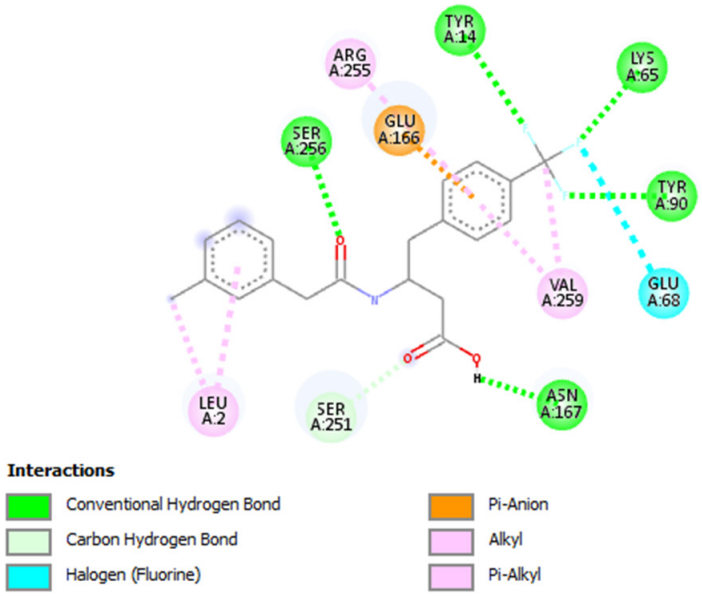


Figure 1. Bond interaction model between CATPB and FFAR2

**Fenthion and FFAR2**

The AutoDock Vina application was utilised to dock fenthion compounds with FFAR2 proteins, generating nine distinct interaction models and their corresponding binding affinities. The minimum recorded binding affinity value was

-4.9 Kcal/mol. Figure 2 illustrates the interaction model between fenthion and FFAR2 at this lowest binding affinity value. In order to enact an inhibitory impact on the target receptors, fenthion must establish binding with specific amino acids constituting the active sites of the FFAR2 protein, namely, Lys65, Ser86, Gln148, Glu166, Tyr238, Arg255, and Ser256. The molecular docking results demonstrate fenthion's capability to form hydrogen bonds with the Ser256 amino acids, engage in van der Waals interactions with Arg255, and establish attractive charge interactions (Pi-anion) with Glu166.

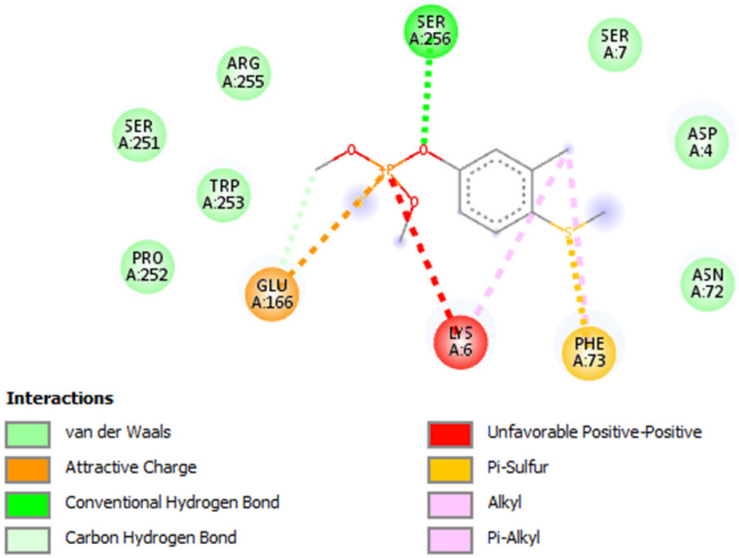


Figure 2. Bond interaction model between fenthion and FFAR2

**Permethrin and FFAR2**

The minimum recorded binding affinity value observed between permethrin and FFAR2 was -7.1 kcal/mol. Figure 3 illustrates the interaction model between permethrin and FFAR2 at this lowest binding affinity value. According to the molecular

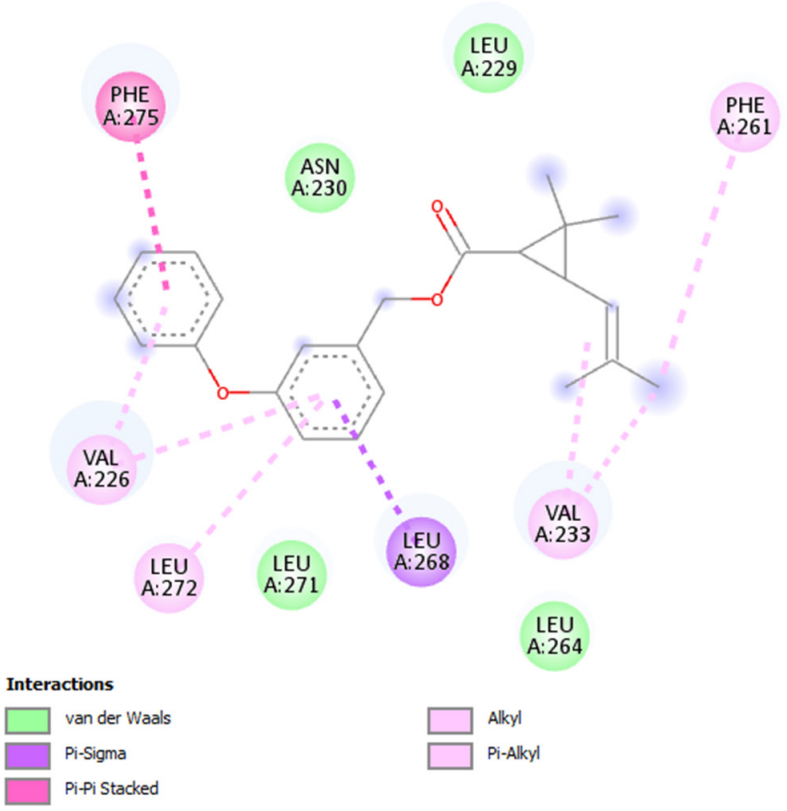


Figure 3. Bond interaction model between permethrin and FFAR2

docking results, it is evident that permethrin does not establish bonds with the active site of the FFAR2 protein. Nevertheless, it demonstrates the capability to bind with alternative sites.

**Carbaryl and FFAR2**

The lowest binding affinity value for carbaryl bonds to FFAR2 was -5.9 kcal/mol. The interaction model between carbaryl and FFAR2 at the lowest binding affinity value is shown in Figure 4. Molecular docking results show that carbaryl could form hydrogen bonds in the amino acids Ser256, van der Waals in Lys65 and Arg255, and Pi-anion in Glu166.

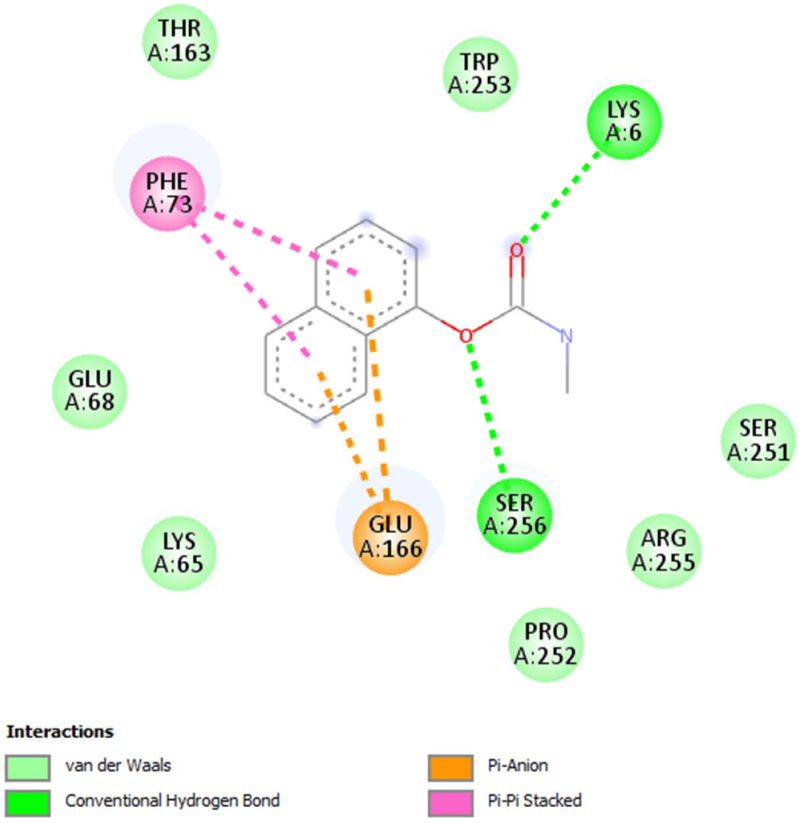


Figure 4. Bond interaction model between carbaryl and FFAR2

**Comparison of in silico test between fenthion, permethrin, and carbaryl**

The binding affinity values resulting from the docking process of fenthion, permethrin, and carbaryl with the target protein FFAR2 reveal a hierarchy of affinities: permethrin (-7.1) < carbaryl (-5.9) < fenthion (-4.9). This hierarchy demonstrates that permethrin could establish a more robust bond with the FFAR2 protein compared to carbaryl and fenthion. Such enhanced stability in the bond augments the ligand-protein interaction. However, the visualisation results of the bond interactions of permethrin indicate that this compound does not bind to the active site of FFAR2, rendering it ineligible for the title of an inhibitor. This is in contrast to fenthion and carbaryl, both of which display the capacity to bind to various amino acid residues constituting the active sites of FFAR2, endowing them with the potential to serve as inhibitors. A detailed comparison of the binding affinity values and the nature of bond interactions between fenthion, permethrin, and carbaryl with the FFAR2 protein is presented in Table 1.

**DISCUSSION**

Free fatty acid receptor2 is a class of G protein-coupled receptors (GPCR) widely expressed in entero-endocrine, adipose, pancreas, and inflammatory cells.[24] In various scientific studies, FFAR2 is also known as GPR43. In humans, FFAR2 is formed by 330 amino acids arranged in the structure of 7 transmembrane-spanning proteins (7TM). The FFAR2 consists of several parts: backbone, sidechain, hydrophobic, neutral, acidic, basic, and disulfide residues (Figure 5). The active

Table 1. Comparison of the bond between fenthion, permethrin and carbaryl with FFAR2

| Pesticide compounds | Binding affinity score (Kkal mol) | Type of binding to the active site of FFAR2 | | |
|---|---|---|---|---|
| | | Hydrogen | van der Waals (v) or hydrofobic (h) | Pi-anion |
| CATPB | -7.5 | Lys65, Tyr90, Ser256 | Ser256 (h) | Glu166 |
| Fenthion | -4.9 | Ser256 | Arg255 (v) | Glu166 |
| Permethrin | -7.1 | - | - | - |
| Carbaryl | -5.9 | Ser256 | Lys65, Arg255 (v) | Glu166 |

CATPB, (S)-3-(2-(3-Chlorophenyl)acetamido)-4-(4-(trifluoromethyl)phenyl)butanoic acid; Lys, lysine; Tyr, tyrosine; Ser, serine: Glu, glutamic acid; Arg, arginine; (v), van der Waals; (h), hydrophobic.

sites for the agonist ligand attachment of FFAR2 are the sites with amino acid residues Arg180, Tyr238, His242, and Arg255, while the active sites for the antagonistic ligands are Lys65, Ser86, Gln148, Glu166, Tyr238, Arg255, and Ser256.[25]

The FFAR2 could only be activated by SCFA. More specifically, SCFAs that could activate FFAR2 include acetate (C2), propionate (C3), butyrate (C4), and valerate (C5).[24] The SCFA results from intestinal microbiota fermentation of undigested food that transits the lower digestive tract.[26] Foods that human enzymes cannot digest are also known as prebiotics.[27] The FFAR2 regulation through SCFA could regulate glucose levels and lipid metabolism through the effects of hormone secretion and inflammatory pathways.

Activation of FFAR2 by SCFA could induce various chemical pathways that are beneficial for metabolic regulation and prevention of T2DM. The agonist interaction between the two molecules could provide an anti-diabetic effect by inhibiting fat accumulation in adipose cells while simultaneously stimulating GLP-1 hormone secretion.[17] The FFAR2 also has an important role in inhibiting inflammation, one of the pathways that trigger insulin resistance. Activation of FFAR2 with SCFA could reduce the expression of TNF and NOS. This activation could also down-regulate IL-8 as well as an inhibitory effect on NF-κB.[28]

Inhibition of FFAR2 has the potential to have the opposite effect of its activation. It could trigger various effects associated with the occurrence of diabetes mellitus.[19] Various effects that inhibit FFAR2 could trigger T2DM through several pathways, including inhibition of GLP-1 hormone secretion, increased TNF, inhibition of interleukin down-regulation, and inhibition of NF-κB inhibition. The GLP-1 pathway could cause T2DM through obesity, whereas the TNF, interleukin, and NF-κB pathways may trigger T2DM through oxidative stress and inflammation.

Based on molecular docking results, it is evident that permethrin exhibits the lowest binding affinity value among the tested pesticides. These findings signify that permethrin possesses a more potent binding capability in comparison to fenthion and carbaryl. The fundamental criterion for evaluating binding affinity is that a lower value corresponds to a stronger interaction between two compounds. This principle holds in the reverse as well. In a separate in silico examination, permethrin demonstrated its potential to inhibit androgen receptors, with the recorded values reaching -10.57.[29] It is noteworthy that variations in binding affinity values occur due to differences in ligands, receptors, and the specific computational tools employed. The binding affinity value in the interaction between a ligand and its receptor is primarily influenced by the quantity and nature of bonds formed, especially non-covalent bonds.

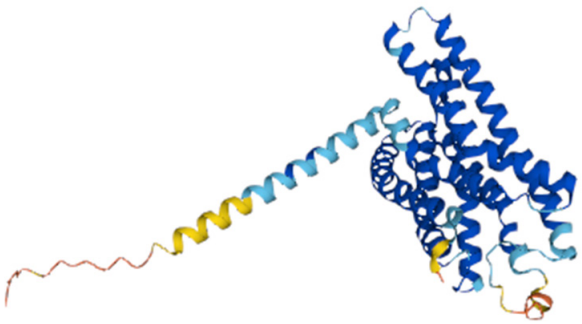

Figure 5. FFAR2 structure.

The hydrogen bond is a bond of electrostatic attraction between a hydrogen atom (H) that has previously been bonded to an atom in a more electronegative group with another atom with a lone pair of electrons. Therefore, this bond will be formed if the hydrogen atom meets F, O, S, and N atoms.[30] The free energies of hydrogen bonds generally range from -12 to -20 kJ/mol but could range from -4 to -30 kJ/mol.[31] Van der Waals or London forces are the universal attractive forces responsible for the interactions of non-polar molecules. This interaction occurs because each atom could have a limited dipole moment due to the movement of the atoms around the nucleus. When molecules approach each other, a temporary dipole of one molecule induces an opposite dipole in another molecule approaching so that an attractive force arises. The binding energy value of this bond ranges from -2 to -4 kJ/mol.[31] A hydrophobic bond is a short-term attractive interaction between non-polar molecules instead of water molecules. The hydrophobic effect is a non-polar relationship in aqueous solutions that is advantageous and often dominates the bonds between proteins and their ligands.[32] The range of binding energy values from this bond is relatively weak, namely 0.1 to 0.2 kJ/mol.[31] The pi-anion interaction is a beneficial non-covalent bond between electron-deficient (π-acid) aromatic systems and anions. This bond plays an important role in chemistry, with energy values ranging from 4 to 16 kcal/mol.[33]

The results from bond visualisation indicate that permethrin is incapable of binding to the amino acid constituting the active site of FFAR2. Due to its inability to bind to the active site, permethrin fails to inhibit FFAR2 despite its relatively strong binding compared to other pesticide variants. This finding aligns with a separate study conducted by Tufail Chaudhary and Hasnain, in which they observed that an alkylating agent named S-303 could not establish binding with the active site of CD-61, the primary platelet antigen involved in blood clotting.[34] This led the two researchers to conclude that S-303 cannot influence the CD-61 receptor to perform its platelet function despite its negative binding energy.[34] This underscores the significance of the bond formed between a compound and the target protein's active site in determining the desired biological effect.

This molecular docking test also shows that carbaryl has a lower binding affinity value than fenthion. These results indicate that carbaryl could bind FFAR2 more strongly and stably than fenthion. Fenthion is the compound with the highest binding affinity even though it has more bonds than carbaryl because it has positive-positive unfavourable bonds indicating repulsion between the two atoms.[35] No data has been found that compares the binding affinity of these two compounds to the same receptor. The in silico study regarding these two compounds was carried out separately. Fenthion has been tested in silico and was found to have a potentially toxic effect on the human metabolic system through its inhibition of glutathione S-transferases (GST) with a value of -32.45.[36] Carbaryl pesticides have also been tested by molecular docking on human melanocyte receptors and have a binding affinity value of -7.7.[37] The variance in binding affinity, with fenthion exhibiting a lower value compared to carbaryl, arises from the distinct receptors used in the docking process. Consequently, it is not suitable for direct benchmarking or comparison. In contrast, the researchers in our study conducted the binding analyses of fenthion and carbaryl using the same receptor, specifically FFAR2.

Based on the docking and visualisation results, it has been established that both fenthion and carbaryl pesticides exhibit the capability to bind effectively to the active site of the FFAR2 protein. Carbaryl compounds, in particular, demonstrate the highest number of bonds within the FFAR2 active site, forming one hydrogen bond, two Van der Waals forces, and one pi-anion bond. Conversely, fenthion compounds form one hydrogen bond, one Van der Waals force, and one pi-anion bond with FFAR2. These findings strongly suggest the potential of fenthion and carbaryl to function as inhibitors of the FFAR2 protein. Significantly, prior to this study, no previous in silico studies had examined the interaction of these pesticides with the FFAR2 receptor, making it a novel and pioneering contribution to the understanding of the pathomechanism of T2DM. The presence of fenthion and carbaryl's ability to bind to the active site of the FFAR2 receptor holds paramount importance in defining their nature as FFAR2 inhibitors. The binding of these two pesticides to the active site, where the FFAR2 inhibitor operates, indirectly implies that these compounds possess similar inhibitory capabilities. It is worth

noting that the natural inhibitors of FFAR2 are represented by CATPB, involving the active sites Lys65, Ser86, Gln148, Glu166, Tyr238, Arg255, and Ser256.[25] This molecular docking study has revealed that fenthion binds to the amino acids Glu166, Arg255, and Ser256, while carbaryl binds to Lys65, Glu166, Arg255, and Ser256.

Determining fenthion and carbaryl as FFAR2 inhibitors is an important finding regarding the pathomechanism of pesticides in the incidence of type 2 DM. Inhibition of FFAR2 has the potential to trigger various effects associated with diabetes mellitus.[19] Various pathways that could cause this event include inhibition of GLP-1 hormone secretion, increased TNF, inhibition of interleukin down-regulation, and inhibition of inhibition of NF-κB.[17,28] The GLP-1 pathway could cause T2DM through obesity-related fat accumulation, while the tumor necrosis factor (TNF), interleukin, and NF-κB pathways could potentially trigger T2DM through oxidative stress and inflammation (Figure 6).

In this investigation, carbaryl has emerged as the pesticide exhibiting the most robust FFAR2 inhibition capability compared to other compounds. Notably, carbaryl can bind to four amino acid residues, constituting the active site of FFAR2, outperforming fenthion, which binds to only three amino acid residues. Despite Autodock Vina's calculations indicating a lower binding affinity for carbaryl compared to fenthion, it is evident that carbaryl stands as the most potent FFAR2 inhibitor among the three types of pesticides scrutinised in this study. This fact bears significant importance, underscoring the need for vigilance in monitoring carbaryl as a potential contributor to T2DM, as the molecular docking study suggested. However, it is imperative to acknowledge that the study has certain limitations. It does not account for factors that might influence the interaction between proteins and their ligands, such as pH, temperature, and substrate. Furthermore, the

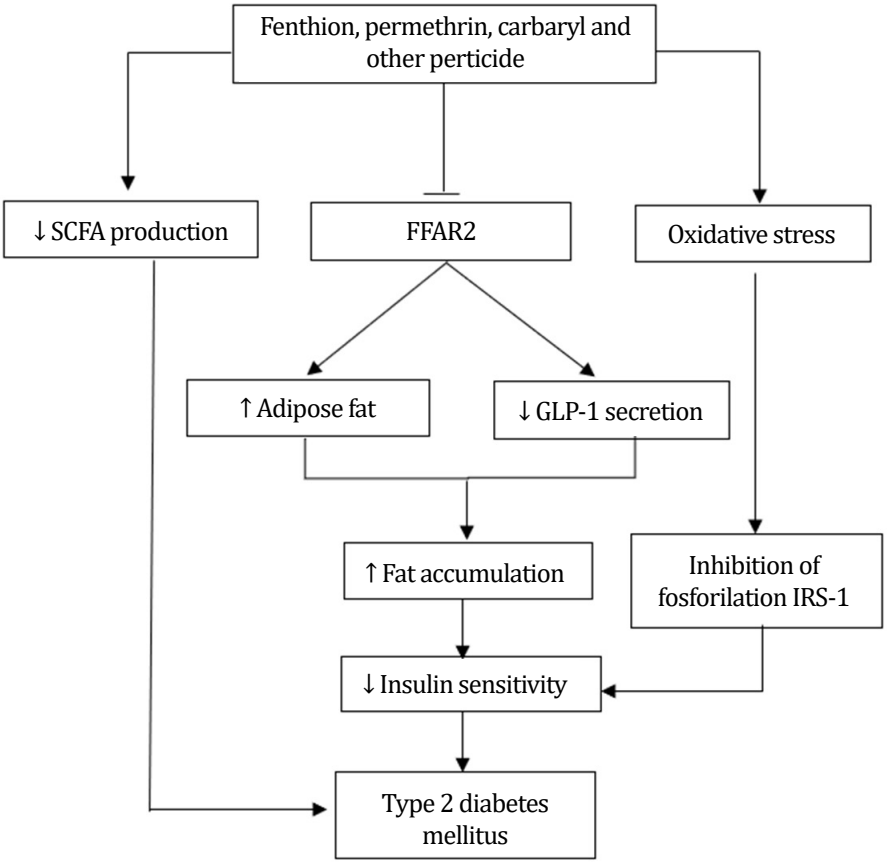


Figure 6. Mechanism of type 2 diabetes mellitus toxicity by pesticides
SCFA: Short Chain Fatty Acid;
FFAR2: Free Fatty Acid Receptor;
GLP-1: Glucagon-Like Peptide 1;
IRS-1: Insulin Receptor Substrate 1

study's scope is constrained to specific compounds or proteins for which crystal structures are known.[38]

## CONCLUSION

A comparison of the binding affinity values for the three pesticides, in descending order of strength, reveals that permethrin, carbaryl, and fenthion exhibit varying abilities to form bonds with the FFAR2 protein. Notably, carbaryl stands out as the most potent FFAR2 inhibitor among them. The inhibitory pathway of carbaryl towards FFAR2 suggests its potential role in contributing to T2DM. To establish the veracity of the in silico predictions regarding the inhibitory potential of fenthion, permethrin, and carbaryl compounds against FFAR2 and the impact of FFAR2 inhibition on the pathomechanisms of other metabolic diseases, further study utilising animal models is imperative.

## CONFLICT OF INTEREST

All the authors in this computational study report that no relationships could be considered as a conflict of interest.

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## AUTHOR CONTRIBUTION

ENS and NA perform the docking, visualisation, and finalise the manuscript and article guarantor. KYWP and YA helped to conduct the manuscript draft.

## LIST OF ABBREVIATION

FFAR2: Free Fatty Acid Receptor; GLP-1: Glucagon-Like Peptide 1; T2DM: Type 2 Diabetes Mellitus; IL-8: Interleukin 8; IRS-1: Insulin Receptor Substrate 1; NF-κB: Nuclear Factor Kappa B; NOS: Nitric Oxide Synthase; SCFA: Short Chain Fatty Acid; TNF: Tumor Necrosis Factor; WHO: World Health Organization; CATPB: (S)-3-(2-(3-Chlorophenyl)acetamido)-4-(4-(trifluoromethyl)phenyl)butanoic acid

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