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Theoretical design and process control of neonicotinoids insecticides suitable for synergistic degradation of rhizobia and carbon-fixing bacteria in soil

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Abstract: This study studied and developed the modification schemes of environmentally friendly substitutes of NNIs along with the regulatory measures that effectively enhanced the synergistic degradation of NNIs by soil rhizobia and carbon-fixing bacteria. Firstly, the binding ability of NNIs to the two key proteins was characterized by molecular docking; Secondly, the mean square deviation decision method, which is a comprehensive evaluation method was used to investigate the binding ability of NNI molecules with the two Rubisco rate-limiting enzymes. The 3D-QSAR model was established for the synergistic degradation and single effect of rhizobia and carbon fixing bacteria, subsequently. Finally, after combining the 3D-QSAR model with a contour maps analysis of the synergistic degradation effect of soil rhizobia and carbon-fixing bacteria, 102 NNI derivatives were designed. 4 NNI derivatives passed the functional and environmentally friendly evaluation. Taguchi orthogonal experiment and factorial experiment assisted molecular dynamics method were used to simulate the effects of 32 regulation schemes on the synergistic degradation of NNIS and its derivatives by rhizobia and carbon fixing bacteria. The synergistic degradation capacity of soil rhizobia and carbon-fixing bacteria was increased to 33.32% after right nitrogen supplementation. This indicated that supplementing the correct amount of nitrogen was beneficial to the microbial degradation of NNIs and their derivatives.

Keywords: NNIs; soil rhizobia and carbon-fixing bacteria; synergistic degradation; 3D-QSAR; molecular docking; molecular dynamics

1 Introduction

Neonicotinoid insecticides (NNIs), a new type of insecticide, are developed based on the structure of nicotine. Compared to the traditional insecticides, they have high selectivity and show relatively low toxicity to the non-target organisms (Matsuda et al. 2005), and have now become one of the world's best-selling pesticides (Bass et al. 2015). The widespread use of NNIs has led to their distribution and accumulation in the global environment (Morrissey et al. 2015). Only about 5% of the active components of NNIs are absorbed by the crops, while most of them are dispersed into a wider range of environmental media and can even be transferred to water bodies, animals, plants, and human bodies through migration (Han et al. 2018; Zhang et al. 2018; Pisa et al. 2017). Li et al. (2018) found that most of the active components of NNIs (usually more than 90%) could enter the soil environment. Horwood (2007) found that the concentration of NNIs in soil decreased rapidly after application due to hydrolysis, photolysis, microbial degradation, plant absorption, and leaching processes. However, Chagnon et al. (2015) proved that the active components of remaining NNIs in the environment could harm the earthworms and other soil invertebrates, along with harming the soil ecosystem. The half-life of NNIs in aerobic soil can be months or even years, and the repeated application can lead to environmental accumulation (Goulson 2013). The main transformation pathways of NNIs in the environment include hydrolysis (Zheng et al. 1999; Karmakar et al. 2009), photolysis (Guo et al. 2021; Chu et al. 2002), and microbial degradation (Chen et al. 2008; Dai et al. 2010; Wang et al. 2013; Zhou et al. 2013; Ma et al. 2014; Akoijam et al. 2015; Andert et al. 2015; Kandil et al. 2015). At present, the traditional physical and chemical methods can only be used as auxiliary means when dealing with soil pesticide residues. Although the treatment effect is significant, the cost is high, and it can also easily cause secondary pollution. Microbial degradation is an effective way for migration, transformation, and elimination of NNIs from the soil environment, which happens due to the strong metabolic activity of microorganisms. The microbial degradation of pesticides has the advantages of safety, high efficiency, no secondary pollution, and low investment (Ding et al. 2019).

NNIs can be used by microorganisms as a carbon source, sole carbon source, or nitrogen source and can be converted into nitroso, imino, urea, and other substances for further degradation (Chen et al. 2008; Dai et al. 2010; Wang et al. 2013; Zhou et al. 2013; Ma et al. 2014; Akoijam et al. 2015; Andert et al. 2015; Kandil et al. 2015). A wide variety of rhizobia and carbon-fixing bacteria exist in the farmland and soil environment, among which soil rhizobium plays an important role in the natural nitrogen cycle.

The available nitrogen fixed by the organisms in the terrestrial ecosystem is about 110 Tg per year (Gruber et al. 2008). Soil carbon-fixing bacteria play a key role in soil carbon transformation and stabilization of soil carbon pool (Trivedi et al. 2013). Stein et al. (2005) found a carbon and nitrogen coupling effect between soil rhizobia and carbon sequestration bacteria during the process of carbon sequestration, while Zhou et al. (2013) isolated a microorganism that could simultaneously fix carbon and nitrogen in the soil. A wide variety of rhizobia and carbon sequestration bacteria are present in the soil environment that contains ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Andersson et al. 2008). Rubisco enzyme plays a key role in the carbon sequestration of the Calvin cycle and has a dominant role in the soil carbon sequestration system (He et al. 2017). At present, based on the molecular biological methods along with the structure, catalytic type, and sensitivity to O₂, Rubisco enzymes are classified into four types (I, II, III, IV). Rubisco I is widely found in photosynthetic organisms, such as higher plants, eukaryotic algae, cyanobacteria, photoautotrophic and chemoautotrophic bacteria along with some other prokaryotes (Selesi et al. 2005; Tolli et al. 2005; Yuan et al. 2012). Myresiotis et al. (2012) reported the NNI degradation rate as 8%-12% within 72 h by *Bacillus subtilis* CB03 that had Rubisco I enzyme.

We aimed to solve the adverse effects of NNIs on the soil environment by enhancing the synergistic degradation of microorganisms in the soil. In this study, the molecular docking technology was used to dock representative NNI molecules with Rubisco rate-limiting enzymes (PDB ID: 3G7F and PDB ID: 1IWA) present in the rhizobia and carbon-fixing bacteria, and the docking score was obtained. Then, the mean square deviation decision method was introduced to calculate the comprehensive effect (D_i) of the two sets of scores, which was taken as the dependent variable while the structural information of NNI molecules was considered as the independent variable. A three-dimensional quantitative model of structure-activity relationship (3D-QSAR) was established on the synergistic degradation effect of NNI molecules by rhizobia and carbon-fixing bacteria in the soil. Based on the three-dimensional equipotential map of the synergistic 3D-QSAR model, the substitution sites and substituent groups were identified. A total of 102 NNI derivatives were designed with flonicamid as the target molecule. Additionally, 102 NNI derivative molecules were evaluated for their toxicity and bio-enrichment. Finally, the environmentally friendly NNI derivative molecules with low biotoxicity and bio-enrichment were screened. Later, based on the molecular dynamic simulation technology assisted by Taguchi orthogonal experiment and factorial experiment, external regulation schemes were screened to effectively improve

the synergistic degradation of NNIs and their derivatives by soil rhizobia and carbon-fixing bacteria in the soil. This study aimed to regulate the environmental risks of NNI insecticides in the source, process, or end process of NNIs production and application while providing theoretical support for the development and design of environmentally friendly alternatives to the NNI compounds.

2 Materials and methods

2.1 Molecular docking method used for the characterization of molecular ability of soil microorganisms to degrade NNIs

Rubisco is a key enzyme for CO₂ fixation during plant photosynthesis (He et al. 2017), and its activity is positively correlated with the carbon sequestration ability of microorganisms (Ragsdale et al. 1991). Rubisco is widely found in green plants, green algae, cyanobacteria, photoautotrophic and chemoautotrophic bacteria, and other photosynthetic organisms. Also, a variety of soil bacteria can fix CO₂ (Dong et al. 2001; Yuan et al. 2011; Selesi et al. 2005). Okano and Liang et al. (2002; 2017) also speculated rhizobia to be involved in the biological carbon sequestration process showing a relationship between C and N in the microbial carbon sequestration process. The NNIs can be used as carbon and nitrogen sources by many bacterial genera in the laboratory culture or natural soil, which allows their microbial degradation and removal to be achieved (Chen et al. 2008; Dai et al. 2010; Wang et al. 2013; Zhou et al. 2013; Ma et al. 2014; Akoijam et al. 2015; Andert et al. 2015; Kandil et al. 2015).

Based on the Protein Data Bank (PDB) database, the Rubisco rate-limiting enzyme (PDB ID:3G7F) (Tian et al. 2007) is hereafter referred to as 3G7F, while the Rubisco rate-limiting enzyme in red algae *Galdieria Parta* (PDB ID:1IWA) (Ponomarenko et al. 2009) is referred to as 1IWA. Since the rhizobia and carbon-fixing bacteria represent the same ability to biodegrade NNI molecules in the soil, NNIs molecules were docked with 3G7F and 1IWA of the rhizobia and carbon-fixing bacteria in the soil, respectively. The structures of 3G7F and 1IWA are shown in Figure 1.

Place Figure 1 here

In this study, 26 kinds of NNI molecules [42–45] (Tian et al. 2007; Shao et al. 2008; Shao et al. 2010; Zhao et al. 2010) were loaded as ligand molecules into Discovery Studio 4.0 (DS) software, and the selected proteins were defined as receptor molecules by the Libdock module. The Find Sites from the Receptor Cavities that look for the receptor may define the module combined with the site (Ren et al. 2019), and by modifying binding sites and the definition, the final blend in the ligand molecule is formed using the cavity of docking protein and the receptor protein. The binding ability of ligand and

receptor was expressed in terms of a docking score. The higher the docking score, the stronger was the binding ability of the NNI molecules to 3G7F or 1IWA, which indicated higher microbial degradation ability.

2.2 Construction of NNIs collaborative microorganic degradation system using a mean square deviation decision method

We used the mean square deviation decision method (Wang et al. 1999) to deal with the joint score of 26 kinds of NNI molecules, i.e., 3G7F and 1IWA, which characterized the synergistic degradation ability of 3G7F and 1IWA. The specific calculation steps are as follows:

$$\mathbf{X} = \begin{bmatrix} X_{11} & X_{12} \\ X_{21} & X_{22} \\ \vdots & \vdots \\ M & M \\ X_{ij} & X_{ij} \end{bmatrix} (i = 1, 2, \Lambda, m; j = 1, 2, \Lambda, n; m = 26; n = 2) \quad (1)$$

where, X_{ij} is the docking score of type i NNI molecule and degrading enzyme of item j .

The average score of 26 NNI molecules docking with the degrading enzyme of item j was calculated to reflect its average level, which was as follows:

$$\bar{X}_{ij} = \frac{1}{m} \sum_{i=1}^m X_j (i = 1, 2, \Lambda, m; j = 1, 2, \Lambda, n; m = 26; n = 2) \quad (2)$$

where, X_j is the docking score of the microbial degradability index of item j .

Considering the difference between each docking score value and the mean docking score value, the mean square error $\sigma(X_j)$ of each docking score value X_j was calculated to reflect its absolute variation degree, and was as follows:

$$\sigma(X_j) = \sqrt{\sum_{i=1}^m (X_{ij} - \bar{X}_j)^2} (i = 1, 2, \Lambda, m; j = 1, 2, \Lambda, n; m = 26; n = 2) \quad (3)$$

The mean square error $\sigma(X_j)$ was normalized to obtain the weight coefficient of the scoring value of the item j :

$$W_j = \frac{\sigma(X_j)}{\sum_{j=1}^n \sigma(X_j)} (i = 1, 2, \Lambda, m; j = 1, 2, \Lambda, n; m = 26; n = 2) \quad (4)$$

According to the weight of each index, the static individual index of the docking score was calculated between each NNI molecule and the degrading enzyme j , and the calculation was as follows:

$$H_{ij} = \frac{X_{ij}}{X_j} (i = 1, 2, \Lambda, m; j = 1, 2, \Lambda, n; m = 26; n = 2) \quad (5)$$

The value D_i is the synergistic degradation ability of the NNIs by the two degrading enzymes present in microorganisms, and was calculated as follows:

$$D_i = \sum_{j=1}^n H_{ij} \times W_j (i = 1, 2, \Lambda, m; j = 1, 2, \Lambda, n; m = 26; n = 2) \quad (6)$$

2.3 Molecular modification of NNIs suitable for synergistic degradation by soil rhizobia and carbon-fixing bacteria through 3D-QSAR model

In this study, we selected the flupyradifurone molecule as the template molecule since it had the largest value for microbial synergistic degradation ability. Based on the superimposed framework labeled in Figure 2, the Alignment Database module was used for automatic superimposing, (Gu et al. 2017) which ensured the consistency of orientation in each molecular field. In the subsequent modification attempts, the NNI derivative molecules designed according to the template molecule flupyradifurone could not meet the requirements of the study. After several attempts, we selected the target molecule flonicamid with a low comprehensive degradability of microorganisms for further study.

Place Figure 2 here

To facilitate the 3D-QSAR analysis, the logarithmic value of the collaborative degradation ability of NNIs by molecular microorganisms was taken as the dependent variable (Zhao et al. 2020) while the structural parameters of NNIs were taken as the independent variable. Thus, a 3D-QSAR model was constructed using the synergistic degradation ability between NNIs molecular structure and microorganisms (Qu et al. 2012; Zhao et al. 2019). Further, the 3D-QSAR model was constructed using 21 kinds of NNI molecules randomly selected as the training set and six kinds of NNI molecules chosen as the test set (among which template molecules existed in both the training set and the test set).

We used the comparative molecular field analysis (CoMFA) distribution for the molecular structure and surrounding molecular field and for the compound activity analysis of the relationship. The Partial Least Square (PLS) method was used to establish the relationship between compound activity and molecular field characteristics. Firstly, the compounds in the training set were cross-validated using the Leave-One-Out method. Once the cross-validated value (q^2) and the optimal number of components (n) were obtained, no cross-regression validation was used to perform the regression analysis. Also, the standard error of estimation (SEE), Non-cross-validated correlation coefficient (r^2), and Fischertest value

(F) were obtained. In terms of external verification, the following formula was used to calculate the mean square error of prediction Q^2_{ext} ($Q^2_{ext} > 0.5$) (Golbraikh et al. 2002) to meet the requirements. Thus, the complete construction of a comprehensive model for molecular microbial degradation of NNIs was obtained.

$$Q^2_{ext} = 1 - \frac{\sum (y_i - \hat{y}_i)^2}{\sum (y_i - \bar{y}_{tr})^2} \quad \square 7 \square$$

where, y_i : the experimental value of the test set; $\hat{y}_i$: estimated value of the test set; $\bar{y}_{tr}$: the average of the experimental values of the training set.

Using the above methods, the single-effect 3D-QSAR model was constructed based on the molecular structure of NNIs and the degradation ability of rhizobia and carbon-fixing bacteria in the soil, where the logarithmic butt-score values of NNIs, 3G7F, and 1IWA was taken as the dependent variable, and the molecular structure parameters of NNIs were taken as the independent variable. This was used to verify the effectiveness of the 3D-QSAR model for the collaborative degradation of NNIs by molecular microorganisms.

2.4 Regulation measures to promote the synergistic degradation of NNIs and their derivatives by rhizobia and carbon-fixing bacteria in soil

2.4.1 Designing of Taguchi orthogonal experiment to screen the regulatory factors that promote the synergistic degradation of NNIs and their derivatives by soil microorganisms

Taguchi orthogonal experimental design is an efficient, rapid, and economical experimental design method (Gu et al. 2018) that can realize the critical ranking and screening of many experimental factors. Here, we adopted the Taguchi orthogonal experimental design of L_{12} . The regulatory factors of N (urea), P (phosphate ester), S (methionine), and ionic forms of Ca, Mg, Fe, and Cu (Li et al. 2018; Yu et al. 2020) were selected as the variables for the orthogonal experiment, whereas their addition and non-addition were taken as the experimental levels (1, 0). Orthogonal experiments were conducted to improve the synergistic degradation of NNIs and their derivatives by soil microorganisms, along with the screening of the main regulatory factors.

2.4.2 Designing of factorial experiments to promote the synergistic degradation of NNIs and their derivatives by microorganisms in the soil

In this study, a factorial experiment was designed based on the regulatory factors screened and described in Section 2.4.1 to promote the synergistic degradation of NNIs and their derivatives by soil

microorganisms. Thus, the effects of adding different combinations of regulatory factors were analyzed on improving the synergistic degradation of NNIs and their derivatives by soil microorganisms. The factorial experiment can effectively test the differences at various levels in each factor and also the interaction between each factor (Ren et al. 2020). This can effectively screen the key factors to improve the synergistic degradation ability of soil microorganisms on NNIs and their derivatives. In the factorial experiment design, the fixed-effect model in the Minitab DOE software (Design of Experiment) was used to analyze the role, and contribution of each regulator in the simulation added regulator system.

2.4.3 Molecular dynamic simulation for verifying the regulation measures of synergistic degradation of NNIs and their derivatives by microorganisms in the soil

This paper is based on the Gromacs software from the Dell Power Edge R7425 server. The molecular dynamics method (Ren et al. 2020) was used to simulate the effects of different combinations of nine influencing factors in the soil environment, including N (urea), P (phosphate ester), S (methionine), and ionic forms of Ca, Mg, Fe, and Cu, on the degree of binding between flonicamid and its derivatives, namely Flonicamide-85, 3G7F, and 1IWA. To verify and supplement the results of synergistic degradation of NNIs and their derivatives by rhizobia and carbon-fixing bacteria in the soil, the composite systems of 3G7F and 1IWA, and flonicamid and flonicamid-85 were placed in 12 periodic cubes with a side length of 15nm, respectively. GROMOS9643a1 force field was used to inhibit molecules while charges of Na⁺ and Cl⁻ were added to neutralize the system. The steepest gradient method was used to simulate energy minimization, and the size of the pressure bath was set at a constant standard atmospheric pressure of 1bar. The binding energy (G_{bind}) was analyzed by the Molecular mechanics Poisson-Boltzmann surface area (MMPBSA). The effects of different combinations of nine influencing factors on the binding ability of flonicamid and flonicamid-85 with 3G7F and 1IWA were also reflected in the form of binding energy. The smaller the value of binding energy, the stronger the interaction between the Rubisco enzyme and NNIs and its derivatives molecules, which indicates a stronger synergistic degradation of NNIs and their derivative molecules by rhizobia and carbon-fixing bacteria. The binding energy calculation formula was as follows:

$$G_{\text{bind}} = G_{\text{complex}} - G_{\text{free-protein}} - G_{\text{free-ligand}} \quad (8)$$

$$G = E_{\text{gas}} - TS_{\text{gas}} + G_{\text{solvation}} \quad (9)$$

$$G_{\text{solvation}} = G_{\text{polar}} + G_{\text{nonpolar}} \quad (10)$$

$$E_{\text{gas}} = E_{\text{bond}} + E_{\text{angle}} + E_{\text{dihedral}} + E_{\text{vdw}} + E_{\text{coulomb}} \quad (11)$$

where, G_{bind} —The binding energy of the composite system, $\text{kJ}\cdot\text{mol}^{-1}$;
 G_{complex} —The binding energy of the complex, $\text{kJ}\cdot\text{mol}^{-1}$;
 $G_{\text{free-protein}}$ —The binding energy of the enzyme, $\text{kJ}\cdot\text{mol}^{-1}$;
 $G_{\text{free-ligand}}$ —The binding energy of the molecule, $\text{kJ}\cdot\text{mol}^{-1}$;
 G —The binding energy of the molecules in solution, $\text{kJ}\cdot\text{mol}^{-1}$;
 T —The gas-phase temperature, K;
 E_{gas} —Energy in the gas phase, J;
 S_{gas} —The gas-phase entropy, $\text{kJ}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$;
 $G_{\text{solvation}}$ —Solvent binding energy, $\text{kJ}\cdot\text{mol}^{-1}$;
 G_{polar} —The polar part in the binding energy of the solvent, $\text{kJ}\cdot\text{mol}^{-1}$;
 G_{nonpolar} —The binding energy of the polar part of the solvent, $\text{kJ}\cdot\text{mol}^{-1}$;
 E_{bond} —Bond interaction, kJ;
 E_{angle} —Bond-angle interaction, kJ;
 E_{dihedral} —Dihedral Angle interaction, kJ;
 E_{vdw} —Van der Waals action, kJ;
 E_{coulomb} —Coulomb electrostatic interactions, kJ.

3 Results and analysis

3.1 Calculation of the D_i value representing the synergistic ability of microorganisms in the soil to degrade NNI molecules based on the molecular docking method and mean square deviation decision method

We used the DS software to conduct molecular docking on 26 NNI molecules with 3G7F and 1IWA, which resulted in the LibDock scores of the two groups. Then, these docking score values were comprehensively calculated based on the mean square deviation decision method to obtain the collaborative ability of microbes to degrade NNI molecules, which was represented as D_i and the logarithmic value was denoted as $\log D_i$ (Table 1).

Place Table 1 here

3.2 Evaluation and Analysis of 3D-QSAR Model for microbial synergistic degradation of NNIs

The evaluation parameters constructed in this study were different for the three 3D-QSAR models, but the evaluation and analysis standards remained consistent. The evaluation and analysis standards of the 3D-QSAR model were briefly described by considering the collaborative degradation model as an

example. The cross-validation coefficient q^2 of the synergistic degradation model was 0.794 (>0.5) while the optimal principal component coefficient (n) was 3, indicating a good predictive ability of the model (Qu et al. 2012); the non-cross validation coefficient R^2 of the collaborative degradation model was 0.934 (>0.8) while the standard deviation SEE was 0.021, and the test value F was 80.774, indicating a good fitting and prediction ability of the constructed collaborative degradation model (Ren et al.2021). After being externally verified by equation (7), the prediction results showed that the mean square error Q^2_{ext} was 0.619 (>0.6), which indicated a good external prediction ability of the collaborative degradation model (Zhao et al. 2018) (Table 2). In the synergistic degradation model, the contribution rates of stereoscopic field and static electric fields were found to be 71.20% and 28.80%, respectively, indicating a greater spatial effect impact of groups on NNIs. Table 2 shows the parameters of the 3D-QSAR model for the synergistic degradation and single degradation effects of microorganisms on NNIs.

Place Table 2 here

3.3 Molecular design of NNI derivatives suitable for synergistic degradation by microbes, based on 3D-QSAR model

In this study, flonicamid was selected as the target molecule for molecular modification. The structural formula of flonicamid is shown in Figure 3. Based on the three-dimensional Contour maps of the constructed microbial collaborative degradation model (Fig.4), the green region in the three-dimensional field indicates that introducing large groups in the region can improve the microbial degradability of the compound while the yellow region indicates that introducing large groups in the region can reduce the microbial degradability of the compound. Similarly, in the electrostatic field, the blue region indicates that the addition of positively charged groups shows conduciveness to improve microbial degradation while the red region indicates that the addition of negatively charged groups is conducive to improve microbial degradation (Wang et al. 2017). The three-dimensional Contour maps of the collaborative microbial degradation of the NNIs model are shown in Figure 4.

Place Figure 3 here

Place Figure 4 here

As seen from Figure 4, the green region distributed at the end of 10 substituents of the target molecule indicates that introducing large groups here can be conducive to enhance the microbial degradation of NNI molecules. The electrostatic field shows that the red region wrapped around the end of the 4-position substituent of the target molecule indicates enhanced microbial degradation of NNI

molecules by introducing electronegative groups at the site. Therefore, the target sites 4 and 10 were selected for molecular modification based on the principle of single-point and double-point substitution. As a result, 35 NNI derivatives were designed by single point substitution modification at site 4 of the target molecule, while another 35 NNI derivatives were designed by single point substitution modification at site 10 of the target molecule. Similarly, 32 NNI derivatives were designed by two-point molecular substitution modification at sites 4 and 10 of the target molecule, which overall constituted a total of 102 NNI derivatives (Table 3).

Place Table 3 here

The microbial synergistic and single degradation effects on the 102 NNI derivative molecules designed by single and double substitutions were predicted in this study, which is shown in Table 4.

Place Table 4 here

As seen in Table 4, the microbial synergistic degradation ability of 102 NNI derivative molecules designed based on the synergistic degradation model showed an increase of varying degrees (1.39%-25.60%) compared to that of the target molecule. Among these, the microbial synergistic degradation ability of flonicamid-85 was the highest (25.6%). Considering the microbial synergism and single degradation ability of NNI derivative molecules, nine of them (Flonicamid-36, Flonicamid-37, and Flonicamid-38, etc.) were selected for further study from three models with similar modifications and improved effects.

3.4 Evaluation of the molecular environmental friendliness of NNI derivatives based on EPI database prediction

In this study, EPIWEB 4.1 database was used to predict the half-lethal concentration (LC_{50}) and biological enrichment ($\log BCF$) of nine NNI derivative molecules (Table 5) in fish, which evaluated the biotoxicity and biological enrichment of the designed NNI derivative molecules.

Place Table 5 here

As seen in Table 5, the LC_{50} values of the four NNIs derivative molecules, Flonicamid-36, Flonicamid-78, Flonicamid-85, and Flonicamid-95 were lower than those of flonicamid, showing a reduction in the toxicity from 90.06% to 65.57%. This suggested that the toxicity of flonicamid derivatives in fish was significantly lower than that of flonicamid. The $\log BCF$ values of the four NNI derivatives also decreased compared to the flonicamid showing a reduction from 31.8% to 0%, indicating weakened biological enrichment ability compared to the target molecule. Also, they did not easily

accumulate in various environmental media.

3.5 Analysis of Molecular Mechanism underlying the Microbial Synergistic Degradation of NNIs Based on Molecular Docking

In this study, flonicamid-85 with significantly improved LC_{50} and $\log BCF$ values was selected as the representative to analyze the mechanism of docking results with 3G7F and 1IWA molecules. Molecular docking showed that when flonicamid and its derivative flonicamid-85 were bound to the Rubisco rate-limiting enzyme, the amino acid residues around the receptor molecule played a major role by binding to the receptor through hydrogen bonding, charge, or polar interaction (Fig. 5 and Fig. 6).

Place Figure 5 here

Place Figure 6 here

Place Table 6 here

The docking results of flonicamid and the two enzymes before and after modification are shown in Table 6. As seen in Figure 5 and Table 6, compared to flonicamid, the combination of flonicamid-85 with 3G7F resulted in the number of amino acid residues around the molecule to be increased by 1 number through electrostatic field force. The number of amino acid residues that were combined with the molecule by van der Waals force did not change significantly, which enhanced the docking effect. This was due to the effect of the electrostatic field force being far greater than that of the van der Waals force, which proved that flonicamid-85 was much easily degraded by rhizobia than flonicamid. The interaction between the amino acid residues and NNI derivatives was speculated to be the main factor affecting the binding ability of the enzyme to NNI derivatives.

According to the analysis shown in Figure 6 and Table 6, compared to flonicamid, the combination of flonicamid-85 with 1IWA resulted in the residual base of amino acids around the molecule to increase by 1 number through electrostatic field force while the residual base of amino acids bound to the molecule was increased by 3 through the van der Waals force. Simultaneously, two new hydrogen bonds were formed and bound to the molecule. Therefore, the intermolecular force between flonicamid-85 and 1IWA was stronger (Tong et al. 2017), making its degradation easier by carbon fixation bacteria than flonicamid.

Additionally, a comparison of the hydrophobicity of amino acid residues around flonicamid and flonicamid-85 showed that the ratio of the number of hydrophobicity and hydrophilicity of amino acid residues around flonicamid-85 was higher than that of flonicamid. The results suggested that this ratio was also one of the factors affecting the microbial degradability of NNI derivatives.

To further investigate the mechanism of docking between amino acid residues and NNI derivative molecules, the distance between the amino acid residues was calculated around flonicamid-85 (Table 6). Chu et al. (2019) believed that the average distance between amino acid residues and the molecule directly affected the molecular docking results. The smaller the average distance, the better the docking effect, and vice versa. In molecular docking with 3G7F and 1IWA, the average distance between the hydrophilic groups was smaller around flonicamid-85 and 3G7F and 1IWA. Contrastingly, when docked with flonicamid, the hydrophilic groups around the flonicamid bonded at a greater average distance. When flonicamid and flonicamid-85 were docked with the two enzymes, the mean distance between the hydrophobic groups acting around the molecules and the two enzymes also showed different results, i.e., the mean distance between flonicamid and flonicamid-85 was larger than being smaller. Therefore, it was concluded that the average distance between amino acid residues around flonicamid-85 directly affected the docking between the molecule and the enzyme.

3.6 Molecular dynamics simulation-based process regulation to promote the synergistic degradation of NNI molecules by microorganisms

3.6.1 Screening of regulatory factors for microbial synergistic degradation of NNI molecules based on molecular dynamics simulations

Studies have shown a significant positive correlation between Rubisco enzyme activity and available phosphorus and nitrate in the soil (Li et al. 2018). Also, a significant correlation was found between the content of sodium and magnesium in soil and the abundance of some fungi (Yu et al. 2020). In agricultural production, rational application of pesticides and chemical fertilizers is a necessary means to improve the nutrient level in crops and soil, which facilitates crop production by preventing pests and treating diseases. Common fertilizers provide essential beneficial elements such as N, P, Ca, Mg, S, Fe, and Cu to agricultural products. Therefore, in this study, we used flonicamid and its derivative flonicamid-85 as the representatives of NNI molecules for analysis. Also, we tried to introduce different regulatory factors to further improve the binding ability of NNI derivative molecules to rhizobia and carbon-fixing bacteria in the soil. In this study, L_{12} Taguchi orthogonal experiment and molecular dynamics simulation method were used to screen out the regulatory factors that significantly improved the ability of rhizobia and carbon-fixing bacteria in the soil to biodegrade NNI derivatives, thus, providing basic data for subsequent designing of the factorial experiment.

In the preliminary screening of regulatory factors, we selected eight regulatory factors, including

nitrogen, phosphorus, sulfur, sodium, magnesium, calcium, copper, and zinc. The average SNR and the range of SNR at the level of 2 (0 represents no addition and 1 represents addition) were used as the evaluation criteria for verification (Table 7).

Place Table 7 here

The SNR response results, shown in Table 7, indicated the degree of importance of the regulatory factors that enhanced the biodegradation ability of NNI derivatives by rhizobia and was as follows: Na, Ga, Zn, Mg, Cu, P, N, S. Similarly, the degree of importance of regulatory factors that enhanced the ability of carbon-fixing bacteria to biodegrade NNI derivatives was as follows: Ca, Zn, Cu, S, P, N, Mg, Na. Based on the ranking results of various regulatory factors listed in Table 7, Ca, Zn, Cu, N, and P ranked first in the two groups of regulatory schemes. These five factors were selected as regulatory factors to promote the collaborative degradation of NNI derivatives by soil microorganisms, which were used for further design of the factorial experiment.

3.6.2 Regulation scheme based on the mean square deviation decision method to promote synergistic degradation of NNI derivative molecules by microorganisms in the soil

The Taguchi orthogonal screening was used to improve rhizobia and carbon sequestration in soil bacteria for biodegradation of NNI derivatives, where five regulatory factors (Ca, Zn, Cu, N, P) were used to design the factorial experiment based on two levels (0: no addition, 1: addition). A total of 64 regulatory combinations, including the blank control group, were generated (Table 8). Simultaneously, the derivative molecule flonicamid-85 was taken as an example to simulate the molecular dynamics of flonicamid-85 with 3G7F and 1IWA under different combinations of regulatory factors. The mean square deviation decision method was used to calculate the effect of synergistic degradation of NNI derivatives by rhizobia and carbon-fixing bacteria in the soil.

Place Table 8 here

Molecular dynamics simulation results showed that the binding energy of the control combination was -98.406 kJ/mol. In the simulated experimental group, the combination of No. 5, 6, 14, and 23 was not ideal in promoting the synergistic degradation of NNI derivatives by soil rhizobia and carbon-fixing bacteria (4.64%, 6.31%, 1.42%, and 10.26%, respectively). The binding ability of the other 27 experimental combination groups showed improvement (the average increase range was between 2.15% and 33.32%), among which the experimental combination no. 3 (adding only nitrogen) had a significant effect on promoting the synergistic degradation of NNI derivatives by rhizobia and carbon-fixing bacteria

with a binding energy of -147.585 kJ/mol. Also, the statistical results of the binding energy in 32 experimental combinations showed that in both rhizobia and carbon fixers, the average binding energy with combinations containing calcium was -110.211 kJ/mol while in the combinations containing Cu, it was -116.324 kJ/mol, and in the combinations containing Zn, it was -112.760 kJ/mol. The mean binding energy of the combination containing N was -113.097 kJ/mol, and in the combination containing P, it was -110.102 kJ/mol. Overall, the results showed a lower synergistic binding energy in the four groups compared to that of the control group, which suggested that P was a secondary factor in promoting the synergistic degradation of NNI derivatives by soil rhizobia and carbon-fixing bacteria.

The Pareto diagram reflects the major factors that affect a particular reason, shown in Figure 7. Here, the microbial synergy required to promote the degradation of NNI derivatives by soil rhizobia and carbon-fixing bacteria had a significant influence on the degradation capability of NNIs by the second-order interaction $A \times D \times E$ ($Ca \times N \times P$), followed by another second-order interaction $A \times C \times E$ ($Ca \times Zn \times P$) along with a single factor group B (Cu).

Place Figure 7 here

In conclusion, the combination of regulatory factors $Ca \times P$ ($A \times D$) was the most critical among all regulatory combinations. Similarly, the addition of regulatory factor N (D) or Zn (C) also showed a significant promoting effect on the synergistic degradation of NNI derivatives by soil rhizobia and carbon-fixing bacteria. In the univariate group, the addition of only Cu had the most significant improving effect on the biodegrading ability of NNI derivative molecules by soil rhizobia and carbon-fixing bacteria while the addition of only N (D) or P (E) in the last two of the five univariate groups indicated avoiding the addition of regulatory factors N (D) and P (E) alone. Also, the continuous addition of Cu in a certain range could promote the activities of some soil enzymes (Tang 2014). Guo (2020) found that adding a certain amount of Ca to the soil could promote soil respiration and improve soil bacterial diversity. The Ca and Zn plasma ions were important to maintain soil acid-base balance, and the addition of Zn in a certain concentration range could promote soil enzyme activity (Li et al. 2013).

4 Conclusion

In this study, a set of molecular design schemes for NNI derivatives suitable for the synergistic degradation by soil rhizobia and carbon-fixing bacteria were constructed by 3D-QSAR, molecular docking technology, and mean square deviation decision method.

A regulatory scheme of soil nutrients that could effectively improve the ability of soil rhizobia and

carbon-fixing bacteria to synergistically degrade NNI derivatives was proposed by molecular dynamics simulation technology, Taguchi orthogonal experimental design, and factorial experimental design.

Among these, the molecular design of NNI derivatives and their soil nutrient regulation programs significantly reduced the ecological and environmental problems caused by NNIs, through their source and process control, in the soil. Also, the ability of NNIs to diffuse to other environmental media was controlled.

The green alternative technology method constructed in this study for the neonicotinoids can provide theoretical support for upgrading NNIs.

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624

625 **Tables and Figures**626 **Table 1** Calculation of synergistic ability of microorganisms to degrade NNI molecules

No.	Compounds	LibDock Score		Synergy	Synergy
		3G7F	IIWA	degradation (D_i)	degradation ($\log D_i$)
1	Compound 1	55.767	42.995	48.800	1.69
2	Compound 2	56.790	41.187	48.279	1.68
3	Compound 3	52.346	45.778	48.763	1.69
4	Compound 4	67.445	47.001	56.293	1.75
5	Compound 5	52.644	39.120	45.267	1.66
6	Compound 6	58.207	40.824	48.725	1.69
7	Compound 7	59.642	34.066	45.691	1.66
8	Compound 8	48.152	38.919	43.116	1.63
9	Compound 9	53.736	43.938	48.391	1.68
10	Compound 10	49.554	37.338	42.890	1.63
11	Compound 11	48.494	44.967	46.570	1.67
12	Compound 12	50.278	38.988	44.120	1.64
13	Compound 13	45.775	36.849	40.906	1.61
14	Compound 14	45.651	33.636	39.097	1.59
15	Compound 15	51.634	44.492	47.738	1.68
16	Compound 16	50.955	41.404	45.745	1.66
17	Compound 17	63.426	41.190	51.297	1.71
18	Flonicamid	54.949	36.924	45.117	1.65
19	Flupyradifurone	75.816	58.522	66.383	1.82
20	Compound 18	64.741	47.501	55.337	1.74
21	Compound 19	67.376	56.687	61.545	1.79
22	Compound 20	66.435	62.598	64.342	1.81
23	Compound 21	66.912	55.885	60.897	1.78

24	Compound 22	65.389	66.367	65.922	1.82
25	Compound 23	64.350	63.295	63.775	1.80
26	Compound 24	64.948	61.820	63.242	1.80

Table 2 The evaluation parameters of the 3D-QSAR model for synergistic degradation and single degradation effect of microorganisms on NNI molecules

Model	q^2	n	R^2	SEE	F	Q^2_{ext}
Synergy degradation model	0.794	3	0.934	0.021	80.774	0.619
Single activity model (for 3G7F)	0.859	7	0.991	0.007	314.280	0.678
Single activity model (for 1IWA)	0.783	7	0.976	0.016	93.843	0.709

Table 3 Molecular designing of NNI derivatives suitable for microbial synergistic degradation.

Compounds	Structure	Compounds	Structure
Flonicamid-1		Flonicamid-52
Flonicamid-2		Flonicamid-53
Flonicamid-3		Flonicamid-54
Flonicamid-4		Flonicamid-55
Flonicamid-5		Flonicamid-56

Flonicamid-6		Flonicamid-57
Flonicamid-7		Flonicamid-58
Flonicamid-8		Flonicamid-59
Flonicamid-9		Flonicamid-60
Flonicamid-10		Flonicamid-61
Flonicamid-11		Flonicamid-62
Flonicamid-12		Flonicamid-63
Flonicamid-13		Flonicamid-64
Flonicamid-14		Flonicamid-65

Flonicamid-15

Flonicamid-66

Flonicamid-16

Flonicamid-67

Flonicamid-17

Flonicamid-68

Flonicamid-18

Flonicamid-69

Flonicamid-19

Flonicamid-70

Flonicamid-20

Flonicamid-71

Flonicamid-21

Flonicamid-72

Flonicamid-22

Flonicamid-73

Flonicamid-23

Flonicamid-74

Flonicamid-24		Flonicamid-75	
Flonicamid-25		Flonicamid-76	
Flonicamid-26		Flonicamid-77	
Flonicamid-27		Flonicamid-78	
Flonicamid-28		Flonicamid-79	
Flonicamid-29		Flonicamid-80	
Flonicamid-30		Flonicamid-81	
Flonicamid-31		Flonicamid-82	
Flonicamid-32		Flonicamid-83	

Flonicamid-33

Flonicamid-84

Flonicamid-34

Flonicamid-85

Flonicamid-35

Flonicamid-86

Flonicamid-36

Flonicamid-87

Flonicamid-37

Flonicamid-88

Flonicamid-38

Flonicamid-89

Flonicamid-39

Flonicamid-90

Flonicamid-40

Flonicamid-91

Flonicamid-41

Flonicamid-92

Flonicamid-42

Flonicamid-93

Flonicamid-43		Flonicamid-94	
Flonicamid-44		Flonicamid-95	
Flonicamid-45		Flonicamid-96	
Flonicamid-46		Flonicamid-97	
Flonicamid-47		Flonicamid-98	
Flonicamid-48		Flonicamid-99	
Flonicamid-49		Flonicamid-100	
Flonicamid-50		Flonicamid-101	
Flonicamid-51		Flonicamid-102	

Table 4 The prediction of synergistic degradation and single-effect degradation ability of NNI derivatives

Compounds	Synergy degradation model	Single factor model (for 3G7F)	Single factor model (for 1IWA)
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	Pred.	Change rate (%)	Pred.	Change rate (%)	Pred.	Change rate (%)
Flonicamid	1.655	-	1.734	-	1.567	-
Flonicamid-1	1.684	6.91	1.775	8.40	1.575	1.79
Flonicamid-2	1.685	7.15	1.796	13.77	1.615	11.61
Flonicamid-3	1.683	6.66	1.788	11.70	1.624	13.94
Flonicamid-4	1.682	6.41	1.782	10.16	1.580	2.97
Flonicamid-5	1.688	7.89	1.782	10.16	1.585	4.16
Flonicamid-6	1.682	6.41	1.774	8.15	1.570	0.62
Flonicamid-7	1.671	3.75	1.772	7.66	1.568	0.16
Flonicamid-8	1.683	6.66	1.781	9.91	1.582	3.44
Flonicamid-9	1.684	6.91	1.777	8.90	1.583	3.68
Flonicamid-10	1.681	6.17	1.777	8.90	1.571	0.85
Flonicamid-11	1.688	7.89	1.787	11.44	1.581	3.20
Flonicamid-12	1.679	5.68	1.778	9.15	1.571	0.85
Flonicamid-13	1.688	7.89	1.785	10.93	1.584	3.92
Flonicamid-14	1.688	7.89	1.776	8.65	1.587	4.64
Flonicamid-15	1.693	9.14	1.782	10.16	1.592	5.85
Flonicamid-16	1.684	6.91	1.773	7.91	1.581	3.20
Flonicamid-17	1.683	6.66	1.771	7.41	1.583	3.68
Flonicamid-18	1.671	3.75	1.773	7.91	1.570	0.62
Flonicamid-19	1.671	3.75	1.769	6.92	1.567	-0.07
Flonicamid-20	1.669	3.28	1.766	6.18	1.566	-0.30
Flonicamid-21	1.690	8.39	1.771	7.41	1.587	4.64
Flonicamid-22	1.690	8.39	1.771	7.41	1.592	5.85
Flonicamid-23	1.690	8.39	1.776	8.65	1.595	6.58
Flonicamid-24	1.685	7.15	1.760	4.72	1.581	3.20
Flonicamid-25	1.690	8.39	1.774	8.15	1.587	4.64
Flonicamid-26	1.690	8.39	1.768	6.67	1.585	4.16

Flonicamid-27	1.671	3.75	1.773	7.91	1.567	-0.07
Flonicamid-28	1.661	1.39	1.748	1.87	1.554	-3.02
Flonicamid-29	1.665	2.33	1.748	1.87	1.558	-2.12
Flonicamid-30	1.689	8.14	1.763	5.45	1.592	5.85
Flonicamid-31	1.662	1.62	1.750	2.34	1.557	-2.34
Flonicamid-32	1.662	1.62	1.749	2.10	1.554	-3.02
Flonicamid-33	1.692	8.89	1.758	4.24	1.588	4.88
Flonicamid-34	1.690	8.39	1.751	2.58	1.581	3.20
Flonicamid-35	1.675	4.71	1.752	2.81	1.556	-2.57
Flonicamid-36	1.712	14.02	1.784	10.67	1.624	13.94
Flonicamid-37	1.712	14.02	1.783	10.42	1.607	9.57
Flonicamid-38	1.712	14.02	1.785	10.93	1.606	9.32
Flonicamid-39	1.699	10.66	1.789	11.95	1.597	7.08
Flonicamid-40	1.711	13.76	1.788	11.70	1.603	8.57
Flonicamid-41	1.738	21.06	1.744	0.94	1.706	37.62
Flonicamid-42	1.711	13.76	1.789	11.95	1.610	10.33
Flonicamid-43	1.711	13.76	1.790	12.21	1.608	9.82
Flonicamid-44	1.712	14.02	1.782	10.16	1.628	15.00
Flonicamid-45	1.716	15.08	1.781	9.91	1.617	12.12
Flonicamid-46	1.716	15.08	1.783	10.42	1.612	10.84
Flonicamid-47	1.695	9.65	1.790	12.21	1.594	6.34
Flonicamid-48	1.693	9.14	1.789	11.95	1.589	5.12
Flonicamid-49	1.702	11.43	1.793	12.99	1.589	5.12
Flonicamid-50	1.696	9.90	1.790	12.21	1.592	5.85
Flonicamid-51	1.698	10.41	1.788	11.70	1.598	7.32
Flonicamid-52	1.704	11.94	1.790	12.21	1.606	9.32
Flonicamid-53	1.697	10.15	1.790	12.21	1.590	5.36
Flonicamid-54	1.691	8.64	1.790	12.21	1.586	4.40
Flonicamid-55	1.686	7.40	1.791	12.47	1.576	2.02

Flonicamid-56	1.698	10.41	1.784	10.67	1.599	7.57
Flonicamid-57	1.699	10.66	1.780	9.66	1.609	10.08
Flonicamid-58	1.696	9.90	1.787	11.44	1.593	6.09
Flonicamid-59	1.695	9.65	1.791	12.47	1.586	4.40
Flonicamid-60	1.690	8.39	1.790	12.21	1.584	3.92
Flonicamid-61	1.690	8.39	1.791	12.47	1.584	3.92
Flonicamid-62	1.700	10.92	1.790	12.21	1.592	5.85
Flonicamid-63	1.704	11.94	1.755	3.52	1.634	16.60
Flonicamid-64	1.704	11.94	1.792	12.73	1.598	7.32
Flonicamid-65	1.699	10.66	1.789	11.95	1.594	6.34
Flonicamid-66	1.665	2.33	1.763	5.45	1.610	10.33
Flonicamid-67	1.717	15.35	1.756	3.76	1.669	26.38
Flonicamid-68	1.680	5.93	1.757	4.00	1.606	9.32
Flonicamid-69	1.687	7.65	1.745	1.17	1.621	13.16
Flonicamid-70	1.706	12.46	1.787	11.44	1.606	9.32
Flonicamid-71	1.711	13.76	1.779	9.41	1.614	11.35
Flonicamid-72	1.711	13.76	1.770	7.16	1.634	16.60
Flonicamid-73	1.716	15.08	1.769	6.92	1.623	13.68
Flonicamid-74	1.715	14.82	1.770	7.16	1.618	12.38
Flonicamid-75	1.702	11.43	1.780	9.66	1.595	6.58
Flonicamid-76	1.704	11.94	1.777	8.90	1.612	10.84
Flonicamid-77	1.712	14.02	1.771	7.41	1.630	15.53
Flonicamid-78	1.742	22.18	1.797	14.04	1.677	28.73
Flonicamid-79	1.704	11.94	1.779	9.41	1.604	8.82
Flonicamid-80	1.707	12.72	1.738	-0.45	1.630	15.53
Flonicamid-81	1.705	12.20	1.774	8.15	1.612	10.84
Flonicamid-82	1.712	14.02	1.770	7.16	1.613	11.10
Flonicamid-83	1.712	14.02	1.772	7.66	1.611	10.58
Flonicamid-84	1.711	13.76	1.776	8.65	1.609	10.08

Flonicamid-85	1.754	25.60	1.786	11.18	1.706	37.62
Flonicamid-86	1.711	13.76	1.778	9.15	1.617	12.12
Flonicamid-87	1.724	17.22	1.772	7.66	1.639	17.95
Flonicamid-88	1.727	18.03	1.771	7.41	1.653	21.81
Flonicamid-89	1.725	17.49	1.774	8.15	1.638	17.68
Flonicamid-90	1.724	17.22	1.773	7.91	1.647	20.14
Flonicamid-91	1.709	13.24	1.771	7.41	1.634	16.60
Flonicamid-92	1.716	15.08	1.781	9.91	1.630	15.53
Flonicamid-93	1.724	17.22	1.776	8.65	1.637	17.41
Flonicamid-94	1.707	12.72	1.783	10.42	1.617	12.12
Flonicamid-95	1.716	15.08	1.783	10.42	1.622	13.42
Flonicamid-96	1.707	12.72	1.774	8.15	1.623	13.68
Flonicamid-97	1.715	14.82	1.784	10.67	1.616	11.87
Flonicamid-98	1.724	17.22	1.775	8.40	1.633	16.33
Flonicamid-99	1.726	17.76	1.774	8.15	1.644	19.31
Flonicamid-100	1.723	16.95	1.773	7.91	1.647	20.14
Flonicamid-101	1.719	15.88	1.773	7.91	1.639	17.95
Flonicamid-102	1.721	16.41	1.779	9.41	1.624	13.94

Table 5 The LC_{50} and $\log BCF$ of flonicamid and its derivatives based on the prediction of the EPI database

Compounds	Predicted values	Change rate	Predicted values	Change rate of
	of LC_{50} *	of LC_{50} (%)	of $\log BCF$	$\log BCF$ (%)
Flonicamid	806.344	-	0.500	-
Flonicamid-36	277.635	-65.57	0.500	0.00
Flonicamid-42	49393.191	6025.57	0.500	0.00
Flonicamid-44	1997.556	147.73	0.500	0.00
Flonicamid-46	2324.106	188.23	0.500	0.00
Flonicamid-78	80.160	-90.06	0.500	0.00

Flonicamid-85	256.805	-68.15	0.341	-31.80
Flonicamid-94	403.479	822.96	0.500	0.00
Flonicamid-95	7442.243	-90.50	0.500	0.00
Flonicamid-97	76.627	5236.32	1.102	120.40

* LC_{50} in fish was selected as the test animal in the EPI database.

Table 6 The analysis of docking results of flonicamid and its derivatives with 3G7F and 1IWA

Docking enzyme		3G7F		1IWA	
Molecular		Flonicamid	Flonicamid-	Flonicamid	Flonicamid-
			85		85
	Electrostatic field force	2	3	5	12
Amino acid	Van der Waals force	10	10	1	4
residue base	Pi bond	4	2	2	0
	Charge interaction	0	0	5	3
Number of hydrogen bonds		0	0	1	5
Number of hydrophobic amino acid residues		10	9	1	11
Number of hydrophilic amino acid residues		0	1	5	5
The average bond length (Å)		3.06	2.80	4.06	3.74

Table 7 The results of eight factors and two levels of SNR with the ranking in the regulatory scheme for promoting synergistic degradation by microorganisms

Type					3G7F					1IWA				
Level	1	0	Delta	Row rank	Level	1	0	Delta	Row rank	Level	1	0	Delta	Row rank
Na	44.51	45.16	0.65	1	Ca	34.76	38.65	3.89	1	Na	44.51	45.16	0.65	1
Ca	44.66	45.02	0.36	2	Zn	38.02	35.39	2.64	2	Ca	44.66	45.02	0.36	2
Zn	45.00	44.68	0.32	3	Cu	35.43	37.98	2.54	3	Zn	45.00	44.68	0.32	3

Mg	44.69	44.98	0.29	4	S	35.84	37.57	1.73	4
Cu	44.71	44.97	0.26	5	P	37.45	35.96	1.50	5
P	44.73	44.95	0.22	6	N	36.10	37.31	1.22	6
N	44.94	44.74	0.20	7	Mg	36.98	36.43	0.55	7
S	44.82	44.85	0.03	8	Na	36.62	36.79	0.18	8

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645 **Table 8** Molecular dynamics simulation of a regulatory scheme suitable for synergistic degradation of

646 NNIs and their derivatives by microorganisms in the soil

Set no.	Factor					Binding energy*(kJ/mol)		Synergistic binding energy (kJ/mol)	Relative error(%)
	Ca	Cu	Zn	N	P	3G7F	1IWA		
1	0	0	0	0	0	-165.535	-64.984	-98.406	-
2	0	0	0	1	1	-173.757	-72.722	-106.305	7.43%
3	0	0	0	1	0	-173.593	-134.636	-147.585	33.32%
4	0	1	1	0	1	-150.455	-75.726	-100.565	2.15%
5	0	0	1	1	1	-166.143	-58.140	-94.0391	-4.64%
6	1	0	0	1	0	-161.130	-58.424	-92.5624	-6.31%
7	0	0	1	0	0	-185.687	-93.521	-124.156	20.74%
8	0	0	1	0	1	-168.067	-78.275	-108.121	8.99%
9	1	0	1	1	1	-177.153	-79.226	-111.776	11.96%
10	1	1	1	1	0	-158.085	-96.977	-117.289	16.10%
11	1	0	0	0	0	-165.149	-74.763	-104.806	6.11%
12	1	1	0	0	1	-151.674	-88.819	-109.711	10.30%
13	0	1	0	0	1	-164.094	-96.933	-119.257	17.48%
14	1	0	0	1	1	-155.906	-67.715	-97.0288	-1.42%
15	1	0	1	0	0	-173.665	-69.458	-104.095	5.47%
16	1	0	1	1	0	-155.754	-90.620	-112.270	12.35%
17	0	1	1	1	0	-182.034	-79.643	-113.677	13.43%
18	0	0	1	1	0	-151.322	-112.110	-125.144	21.37%

19	1	0	0	0	1	-188.225	-65.180	-106.079	7.23%
20	0	0	0	0	1	-175.250	-65.573	-102.028	3.55%
21	0	1	1	1	1	-187.379	-67.643	-107.442	8.41%
22	0	1	1	0	0	-171.013	-70.730	-104.063	5.44%
23	1	1	0	1	0	-176.337	-45.893	-89.2512	-10.26%
24	1	1	1	0	1	-178.322	-84.056	-115.389	14.72%
25	1	0	1	0	1	-163.340	-103.917	-123.669	20.43%
26	1	1	1	1	1	-173.816	-72.692	-106.305	7.43%
27	0	1	0	1	1	-173.085	-101.893	-125.556	21.62%
28	1	1	0	0	0	-184.708	-70.730	-108.615	9.40%
29	1	1	0	1	1	-164.153	-110.559	-128.373	23.34%
30	0	1	0	0	0	-140.145	-146.801	-144.589	31.94%
31	0	1	0	1	0	-172.538	-116.229	-134.946	27.08%
32	1	1	1	0	0	-166.882	-120.871	-136.165	27.73%

* The smaller the value of binding energy, the greater the binding ability.

Fig. 1 Structure of 3G7F(Rhizobia)and 1IWA (Carbon-fixing bacteria) (a) 3G7F; (b) 1IWA

Fig. 2 Molecular structure of template molecule flupyradifurone (*: The basic skeleton for the superposition)

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Fig. 6 Docking results of flonicamid and flonicamid-85 with Rubisco rate-limiting enzyme 1IWA

Fig. 7 Pareto of 3G7F and 1IWA for standardized degradation effect