



Novel Pyrimidine-Triazole Schiff Bases: Synthesis, Antifungal Activities, DFT and Molecular Docking

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Abstract: Seven 4-amino-5-substituted-1,2,4-triazole-3-thione Schiff base compounds were synthesized reacting 4-amino-5-substituted-1,2,4-triazole-3-thione with dichloro-substituted 5-pyrimidines, and the structures were verified by elemental analysis and spectroscopic techniques (FT-IR, ¹H NMR). Additionally, *in vitro* antifungal activities of the compounds (named F1~F2; A1~A5) against Grape anthracnose and Wheat gibberellic have been evaluated. The compounds of F1, A4 and A5 were found to be potentially effective antifungal agents against Grape anthracnose, while the others showed the low bioactivity. The antifungal activity of all compounds against Wheat gibberellic were superior to that of fluconazole (standard drug, SD). Particularly, compounds of F1, A1, A4 and A5 exhibited a broad-spectrum antifungal activity against two fungus as compared to the others. Therefore, molecular docking study was carried out to explore the potential interaction between ligands and *Fusarium graminearum* (PDB ID: 5E9H). The results showed that four compounds had higher affinity compared with fluconazole and form the stable complex with the receptor. Besides, the frontier molecular orbitals (FMOs) and molecular electrostatic potentials (MEP) of four compounds with broad-spectrum antimicrobial activity were also calculated with DFT/ B3LYP /6-31G (d, p) method. The energy gap values ($\Delta E_{LUMO-HOMO}$) of all the synthesized compounds ranged from 3.307-3.375 eV, which was lower than that of SD (6.248 eV). Additionally, according to MEP the electrophile reaction of 5-substituted groups was beneficial to improving the biological activity against Wheat gibberellic and Grape anthracnose.

Keywords: Schiff base, 1,2,4-triazole, DFT, molecular docking, antifungal activity

1. Introduction

The long-term and extensive use of pesticides will significantly increase the side effects and drug resistance of some crops [1]. Therefore, it is necessary and meaningful to design and develop the novel antifungal agents with high efficiency, low toxicity, environmental protection, safety and broad spectrum [2-4]. Nitrogen-containing heterocyclic compounds, such as pyrimidine and triazole, are widely used in pesticides because of their broad-spectrum, high-efficiency and low-toxic biological activities. They can be used as fungicides, insecticides, herbicides, plant growth regulators and so on [5-9]. Triazole antifungal agents play a leading role in the inhibition of agricultural fungal infection, such as Tebuconazole, Epoxiconazole, Triadimenol, Hexaconazole, Bitertanol and Flutriafol, at present [10]. Pyrimidine composed of two nitrogen atoms and four carbon atoms is a kind of six-membered heterocyclic ring with conjugated π bond, which widely exists in human body and organism. Pyrimidine compounds and their derivatives are a kind of very important active substances in the field of pesticides and medicine, with broad-spectrum biological activities. Thus it has been paid special attention in the development of new drugs due to large medicinal potentiality of Pyrimidine-based derivative. The compounds of Schiff base show the extensive applications in medicinal, agricultural and pharmaceutical fields and become one of the important class of organic compounds because of the existence of azomethine group (N=CH) in the structure that is essential for biological activity [11-13].

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The triazole ring, pyrimidine ring and Schiff base groups were linked to increase the diversity of molecular structures and hope to obtain more effective antifungal agents. Our previous studies found that the Schiff base compounds formed by 4,6-dichloropyrimidine ring and 1,2,4-triazole ring have good antifungal activity, and the 5-substituent on the triazole ring were replaced by methyl, it had better biological activity against *Wheat gibberellic* and *Grape anthracnose* [14]. In order to explore the effect of 5-substituent in triazole ring on biological activity, my team and I designed and synthesized seven novel Schiff base compounds with 1,2,4-triazole and pyrimidine ring groups. The *in vitro* antifungal activities against *Grape anthracnose* and *Wheat gibberellic* were evaluated comparing to the standard drug (Fluconazole) [15,16]. Also, the compounds were characterized by melting point, elemental analysis (EA), FT-IR, and ^1H NMR spectroscopic techniques. In addition, the protein of *Fusarium graminearum* was selected as the receptor protein for molecular docking study with Schiff base compounds, and the mechanism of drug-protein binding was analyzed theoretically. Further, the frontier molecular orbitals (FMOs), molecular electrostatic potentials (MEP) and HOMO-LUMO calculations were done for their structural exploration with DFT/ B3LYP /6-31G (d, p) method of Gaussian 09.

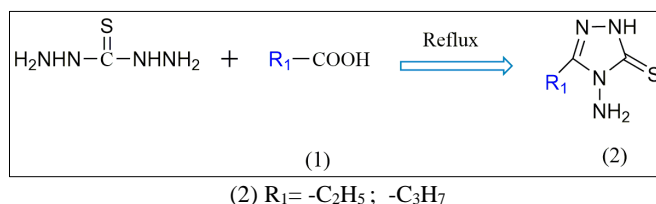
2. Materials and methods

All the solvents and chemicals were of commercial reagent grade and used as received without further purification. Elemental analysis of C, H and N was performed on a Vario EL III elemental analyzer from ELEMENTAR Company. Fourier infrared spectrum (FT-IR) were recorded in the range of 400-4000 cm^{-1} on an EQUINOX 55 FT-IR spectrometer from Bruker Company. ^1H NMR spectroscopy was recorded with AV400 and 600 NMR spectrometer (Bruker) and proton chemical shifts are recorded in ppm relative to tetramethylsilane as an internal standard using DMSO- d_6 as solvent.

2.1. Synthesis

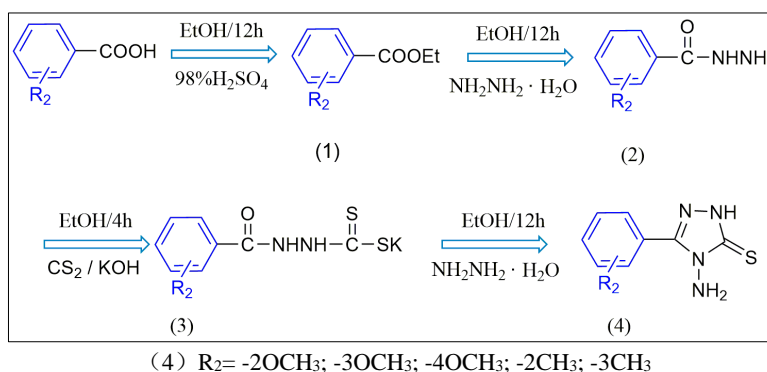
Step- I Synthesis of 4-amino-5-substituted-1,2,4-triazole-3-thione (F, A)

The intermediates of F were synthesized according to the literature [15,17,18], and propionic acid or n-butyric acid was used as reactant and solvent. The synthetic route was illustrated in Scheme 1.



Scheme 1 Synthesis of the intermediates

Based on the reported method, the intermediates of 4-amino-5-aryl-2,4-dihydro-3H-1,2,4-triazole-3-thiones (A) were prepared via four steps that includes esterification (1), hydrazide reaction (2), salt formation (3), and cyclization (4) (Scheme 2) [19-22].

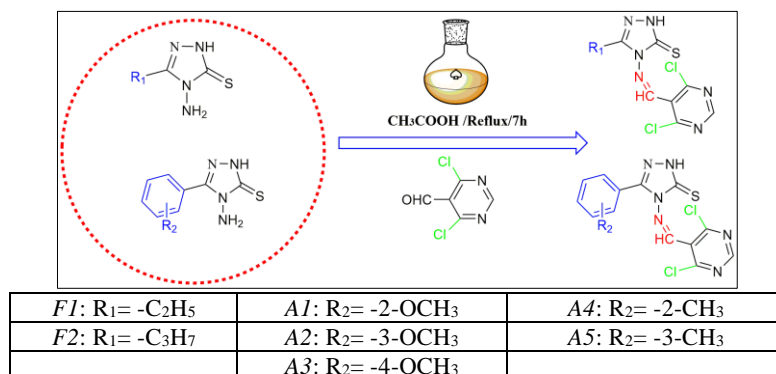


Scheme 2 Synthesis of second type of intermediates

The crude intermediates were purified using absolute ethanol, and the physicochemical properties and the results of EA analysis were summarized in Table S1.

Step- II General synthetic procedure for Schiff base compounds (*F1~F2*, *A1~A5*)

A series of Schiff base compounds were synthesized by reacting 4-amino-5-substituted-1,2,4-triazole-3-thiones and chloro-substituted pyrimidine formaldehydes. The mixture was stirred and heated to 110°C, and then refluxed in the glacial acetic acid and the synthesis of process was shown in Scheme 3. The completion of reaction was checked by TLC. The reaction mixture was cooled and poured into the crushed ice. The precipitated solid was filtered, washed with ice water, purified in absolute ethanol and dried to obtain the yellow powder [19, 23, 24].



Scheme 3 Synthetic route of the Schiff base compounds

4-((4,6-dichloropyrimidin-5-yl)methylene amino)-5-ethyl-2*H*-1,2,4-triazole-3(4*H*)-thione (*F1*): Yield: 54.2 % (light yellow power); m.p. >290.0°C; IR (KBr, cm⁻¹): 3083 (N-H), 2995 (C-H), 1687 (C=N), 1374 (C=S); ¹H NMR (DMSO-*d*₆, δ ppm): 8.33 (Py-H), 7.95 (N=CH), 2.78 (CH₂), 1.18 (CH₃); Anal.: Calcd for C₉H₈Cl₂N₆S (303.17); Calcd.:C, 35.66; H, 2.66; N, 27.72 %; Found: C, 35.59; H, 2.61; N, 27.81 %.

4-((4,6-dichloropyrimidin-5-yl)methylene amino)-5-propyl-2*H*-1,2,4-triazole-3(4*H*)-thione (*F2*): Yield: 52.1 % (light yellow power); m.p. >290.0 °C; IR (KBr, cm⁻¹): 3075 (N-H), 2961 (C-H), 1671 (C=N), 1369 (C=S); ¹H NMR (DMSO-*d*₆, δ ppm): 8.32 (Py-H), 7.95 (N=CH), 1.71-1.43 (CH₂), 0.83 (CH₃); Anal.: Calcd for C₁₀H₁₀Cl₂N₆S (317.2); Calcd.:C, 37.87; H, 3.18; N, 26.49 %; Found: C, 37.76; H, 3.12; N, 26.39 %.

4-((4,6-dichloropyrimidin-5-yl)methylene amino)-5-(2-methoxyphenyl)-2*H*-1,2,4-triazole-3(4*H*)-thione (*A1*): Yield: 67.8 % (light yellow power); m.p. 262.0-263.1 °C; IR (KBr, cm⁻¹): 2988 (N-H), 2921 (C-H), 1671 (C=N), 1369 (C=S); ¹H NMR (DMSO-*d*₆, δ ppm): 8.32 (Py-H), 7.96 (N=CH), 7.84 (Ar-H), 7.52-7.25 (Ar-H), 4.40 (-OCH₃); Anal.: Calcd for C₁₄H₁₀Cl₂N₆OS (381.24); Calcd.:C, 44.11; H, 2.64; N, 22.04 %; Found: C, 44.05; H, 2.58; N, 22.11 %.

4-((4,6-dichloropyrimidin-5-yl)methyleneamino)-5-(3-methoxyphenyl)-2*H*-1,2,4-triazole-3(4*H*)-thione (*A2*): Yield: 71.2 % (light yellow power); m.p. 283.5-284.4°C; IR (KBr, cm⁻¹): 3070 (N-H), 2989 (C-H), 1673 (C=N), 1386 (C=S); ¹H NMR (DMSO-*d*₆, δ ppm): 8.35 (Py-H), 8.06 (N=CH), 7.99-7.85 (Ar-H), 7.71-7.15 (Ar-H), 4.41 (-OCH₃); Anal.: Calcd for C₁₄H₁₀Cl₂N₆OS (381.24); Calcd.:C, 44.11; H, 2.64; N, 22.04 %; Found: C, 44.15; H, 2.61; N, 21.98 %.

4-((4,6-dichloropyrimidin-5-yl)methyleneamino)-5-(4-methoxyphenyl)-2*H*-1,2,4-triazole-3(4*H*)-thione(*A3*): Yield: 70.3 % (light yellow power); m.p. 279.8-280.7°C; IR (KBr, cm⁻¹): 2989 (N-H), 2900 (C-H), 1669 (C=N), 1255 (C=S); ¹H NMR (DMSO-*d*₆, δ ppm): 13.99 (N-H), 9.79 (Py-H), 8.33 (N=CH), 7.96-7.65 (Ar-H), 7.19-6.88 (Ar-H), 3.76 (-OCH₃); Anal.: Calcd for C₁₄H₁₀Cl₂N₆OS (381.24); Calcd.:C, 44.11; H, 2.64; N, 22.04 %; Found: C, 44.02; H, 2.59; N, 21.97 %.

4-(((4,6-dichloropyrimidin-5-yl)methylene amino)-5-(*o*-tolyl)-2*H*-1,2,4-triazole-3(4*H*)-thione(*A4*): Yield: 68.4 % (light yellow power); m.p. 285.4-286.3 °C; IR (KBr, cm⁻¹): 3070 (N-H), 2929 (C-H), 1675



(C=N), 1388 (C=S); $^1\text{H NMR}$ (DMSO- d_6 , δ ppm): 8.31 (Py-H), 7.95 (N=CH), 7.78-7.30 (Ar-H), 7.30-6.94 (Ar-H), 2.59-2.54 (-CH₃); Anal.: Calcd for C₁₄H₁₀Cl₂N₆S (365.24); Calcd.:C, 44.04; H, 2.76; N, 23.01 %; Found: C, 46.00; H, 2.71; N, 23.09 %.

4-((4,6-dichloropyrimidin-5-yl)methylene amino)-5-(*m*-tolyl)-2H-1,2,4-triazole-3(4H)-thione(A5): Yield: 69.5 % (light yellow power); m.p. 257.2-258.1 °C; IR (KBr, cm⁻¹): 3063 (N-H), 2916 (C-H), 1687 (C=N), 1369 (C=S); $^1\text{H NMR}$ (DMSO- d_6 , δ ppm): 8.35 (Py-H), 7.95 (N=CH), 7.57 (Ar-H), 7.56-7.44 (Ar-H), 7.16 (Ar-H), 7.12-6.96 (Ar-H), 2.58-2.54 (-CH₃); Anal.: Calcd for C₁₄H₁₀Cl₂N₆S (365.24); Calcd.:C, 44.04; H, 2.76; N, 23.01 %; Found: C, 46.00; H, 2.71; N, 23.09 %.

2.2. Antifungal activity

The antifungal activity of compounds against *Grape anthracnose* and *Wheat gibberellic* was determined by mycelial growth rate method [25,26]. The synthesized compounds and the standard drug were dissolved in DMF respectively to test their antimicrobial rate at different concentrations (2, 4, 8, 16 mg/L) (Table S2). The toxicity regression equation, correlation coefficient and EC₅₀ were obtained through the logarithm value of the drug concentration and the probability of inhibition rate (Table S3). Two pathogenic fungus were provided by College of life Sciences, Northwest A&F University. Fluconazole was purchased from Shanghai Siyu Chemical Technology Co. Ltd. The technique of antifungal activities and data processing were performed according to the method described in literature [27].

2.3. Molecular docking

Molecular docking is a microscopic docking study based on the principle of complementary geometric, energy and chemical environment to obtain the mode of interaction and binding affinity between ligand and receptor, so as to minimize the free energy of the system [28].

In this context, AutoDockTools-1.5.6 was applied to explore the most possible binding mode between ligand and *Fusarium graminearum* (PDB ID: 5E9H) [29]. Ligand structure of compounds in pdb format was generated by ChemBioDraw Ultra 14.0 and Chem3D pro 14.0 [30]. The results could be viewed through the Discovery Studio 4.5 Visualizer [31, 32].

2.4. Density functional theoretical (DFT) calculation

The density functional theoretical (DFT) calculation of four compounds with broad-spectrum bioactivity was performed with B3LYP/6-31G (d, p) method. The frontier molecular orbitals (FMOs) and molecular electrostatic potentials (MEP) were obtained and analyzed. Vibration analysis showed that the optimized structure was in accordance with the minimum points on the potential energy surface. All calculations were carried out by Gaussian 09 program [33].

3. Results and discussions

3.1. Spectroscopic analysis

FT-IR spectroscopy can be used to determine the absorption vibrational peaks of characteristic functional groups in compounds in the range of 4000-400 cm⁻¹. The appearance of band near 3000 cm⁻¹ is due to stretching vibrations of the N-H group in triazole ring. The characteristic band due to C-H stretching vibration of phenyl ring appeared in the region 2990-2900cm⁻¹. The presence of thiol group wasn't confirmed by S-H stretching vibration band in the range of 2560-2329 cm⁻¹, and there were characteristic peaks of C=S in the range of 1388-1255cm⁻¹, indicating that the compounds are in the form of thione (C=S). A new band appeared in 1690-1640 cm⁻¹ was confirmed to be the azomethine bond (HC=N).

In $^1\text{H NMR}$ spectrum, characteristic singlet due to the HC=N group appeared at $\delta=7.73-8.75$ ppm, which confirmed the condensation between the amino group and the carbonyl group. The protons present in the phenyl ring resonated in the region 6.88-7.96 ppm. Three methyl protons of compounds

(A4, A5) were demonstrated at $\delta=2.58\text{--}2.94$ ppm. The methoxide protons of the compounds were observed around 4.00 ppm (4.40 ppm for A1, 4.41 ppm for A2 and 3.77 ppm for A3).

3.2. Antifungal activity

The EC_{50} values of the synthesized compounds for *in vitro* antifungal activity against *Grape anthracnose* and *Wheat gibberellic* are exhibited in Figure 1.

The preliminary antifungal results indicated that type and location of substituents on triazole ring had different effect on the antifungal properties of compounds. Only the compounds of F1 ($EC_{50}=3.85$ mg L⁻¹), A4 ($EC_{50}=1.68$ mg L⁻¹) and A5 ($EC_{50}=0.88$ mg L⁻¹) showed the higher antifungal activity against *Grape anthracnose* than that of Fluconazole ($EC_{50}=3.91$ mg L⁻¹), while all the tested compounds ($EC_{50}:5.34\text{--}9.08$ mg L⁻¹) were found to be more effective against *Wheat gibberellic* ($EC_{50}:10.82$ mg L⁻¹).

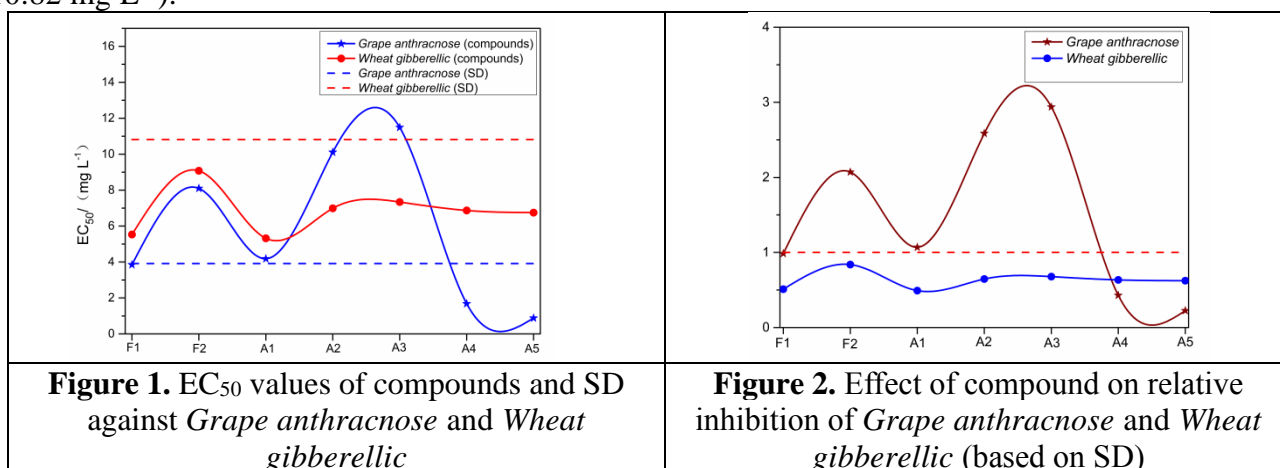


Figure 1. EC_{50} values of compounds and SD against *Grape anthracnose* and *Wheat gibberellic*

Figure 2. Effect of compound on relative inhibition of *Grape anthracnose* and *Wheat gibberellic* (based on SD)

The EC_{50} values of *Grape anthracnose* and *Wheat gibberellic* were normalized by the EC_{50} values of SD, and the results are shown in Figure 2. The y coordinate represents the value of $EC_{50, \text{compounds}}/EC_{50, \text{SD}}$, if the value is less than 1, the biological activity is better than that of SD. It can be seen clearly that compounds of F1, A1, A4 and A5 had a relatively broad-spectrum antimicrobial activity, especially A4 and A5 could be used as lead agents for further development of new antifungal drugs. The antimicrobial activity of 5-ethyl substituted compound (5.53 mg L⁻¹) was significantly higher than that of 5-propyl substituted one (9.08 mg L⁻¹) for *Wheat gibberellic*. Comparing our previous studies, it was found that the EC_{50} of 5-methyl substitution compound was 4.03 mg L⁻¹ against *Wheat gibberellic*. It is speculated that the longer the alkyl substitution chain was, the lower bioactivity it had. However, the opposite was true against *Grape anthracnose*, 5-methyl substitution (5.95 mg L⁻¹) had the highest biological activity, while 5-propyl substitution (8.10 mg L⁻¹) had the lowest activity. Bioactivity of A4 and A5 with -CH₃ substitution on the phenyl ring was better than that of -OCH₃ substituted ones (A1, A2 and A3), which may be due to the stronger electron-donating ability of methoxyl group than that of methyl group. It could be guessed that the stronger the electron-donating ability of 5-position substituents was, the higher the antifungal activity had. Meanwhile, it is clearly appeared that the order of bioactivity from excellent to poor was 2-OCH₃>3-OCH₃>4-OCH₃ for compounds with -OCH₃ substitution on the phenyl ring, inferring that the substitutional position of methoxide on the benzene ring affects its atomic charge distribution, thus affecting its electrophilic reaction and further affecting its biological activity.

In general, compounds of F1, A1, A4 and A5 possess the broad-spectrum antimicrobial activity, which have potential to be further explored.

3.3. Molecular docking

In order to rationalize the promising *in vitro* results obtained for the four compounds with broad-spectrum bioactivity (F1, A1, A4, A5), molecular docking study was carried out with *Fusarium*

graminearum (PDB ID: 5E9H) as the receptor to observe its theoretical inhibitory effect and mode of action. The results are summarized in Table 1, including binding energy (ΔG), inhibitory constant (K_i) values and ligand efficiency (LE). The values of K_i reflect the binding affinity of the compounds and the ΔG reflects the stability of the compound. Ligand efficiency (ΔG /the number of heavy atoms) is the contribution of each non-hydrogen atom in the compounds to the binding energy, and is a compound quality parameter related to molecular size. In the results, the theoretical inhibition constant K_i and the binding energy values ($8.32 \sim 6.53 \text{ kcal mol}^{-1}$) of all the compounds were lower than that of fluconazole ($-4.19 \text{ kcal mol}^{-1}$), which indicates these compounds have higher affinity with receptor and the greater potential against *Wheat gibberellic* than that of SD. Meanwhile, it should be noted that the ligand efficiency values ($-0.36 \sim -0.32$) of the compounds were lower than fluconazole (-0.19), indicating that non-hydrogen atoms in the compounds contributed more to the activity.

A potential interaction was found between the active molecules and the *Fusarium graminearum*. The 2D and 3D diagrams (Figure 3a-h) exhibit the binding sites of all the ligands within the receptor 5E9H.

Table 1. Docking results of compounds against 5E9H

Compound ID (s)	Binding Energy (kcal mol ⁻¹)	K_i (μM)	LE
F1	-6.53	16.31	-0.36
A1	-7.71	2.22	-0.32
A4	-7.61	2.64	-0.33
A5	-8.32	0.79	-0.36
SD	-4.19	846.25	-0.19

The molecules interact with the active sites (amino acids) of receptor 5E9H through conventional hydrogen bonds, van der Waals, carbon hydrogen bond, pi-donor hydrogen bond, halogen, unfavorable bump, pi-sulfur, alkyl and pi-alkyl (Figure 3 a-h). The existence of hydrogen bond can make the docking structure more stable. Four compounds interact with the active sites of 5E9H by H-bonds, including the N-atom on the triazole ring and HIS A:217, the H-atom of N -H on the triazole ring and ASP A:177, ASP A:179, the Cl-atoms of pyrimidine ring and CYS A:215, the S-atom of C=S on the triazole ring and TRP A:108, GLY A:107, the Cl-atoms of pyrimidine ring and ASN A:432, HIS A:217, SER A:434, CYS A:215. In addition, the other parts of the compounds also have different interactions with other amino acids. The results reveal that these ligands could interact favorably with the 5E9H and form stable complex. Although the docking results are not completely consistent with the experimental results, the theoretical prediction and ligand inhibition could still be explained.

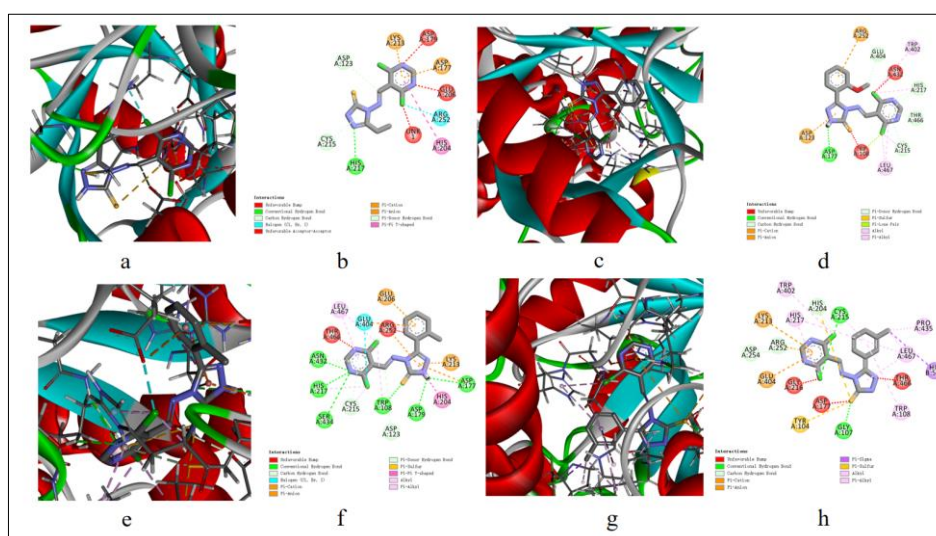


Figure 3. 2D and 3D graphical representation of interaction sites in compounds with broad-spectrum bioactivity and *Fusarium graminearum* (PDB ID: 5E9H), a, b for F1, c, d for A1, e, f for A4, g, h for A5

3.4. DFT calculation

3.4.1. HOMO-LUMO analysis

In order to verify the bioactivity behavior *in vitro* of four compounds with broad-spectrum bioactivity, the frontier molecular orbitals (FMOs) are studied. The HOMO and LUMO represent the ability of donating electron and accepting the electron [34]. Both HOMO and LUMO are very important factors to understand the electronic transition and intermolecular interactions of molecular reactions, which provides insights for explicating biological activity [35-37].

The HOMO and LUMO molecular orbitals and the energy values of E_{HOMO} , E_{LUMO} and $\Delta E_{(\text{LUMO-HOMO})}$ of the compounds are exhibited in Figure 4. Overall, the distribution of HOMO and LUMO is uniform. The HOMO orbital is mainly concentrated on triazole ring, S atom and the N=CH bond, while LUMO orbitals are mainly on the pyrimidine ring, the N=CH bond, S atom and the triazole ring.

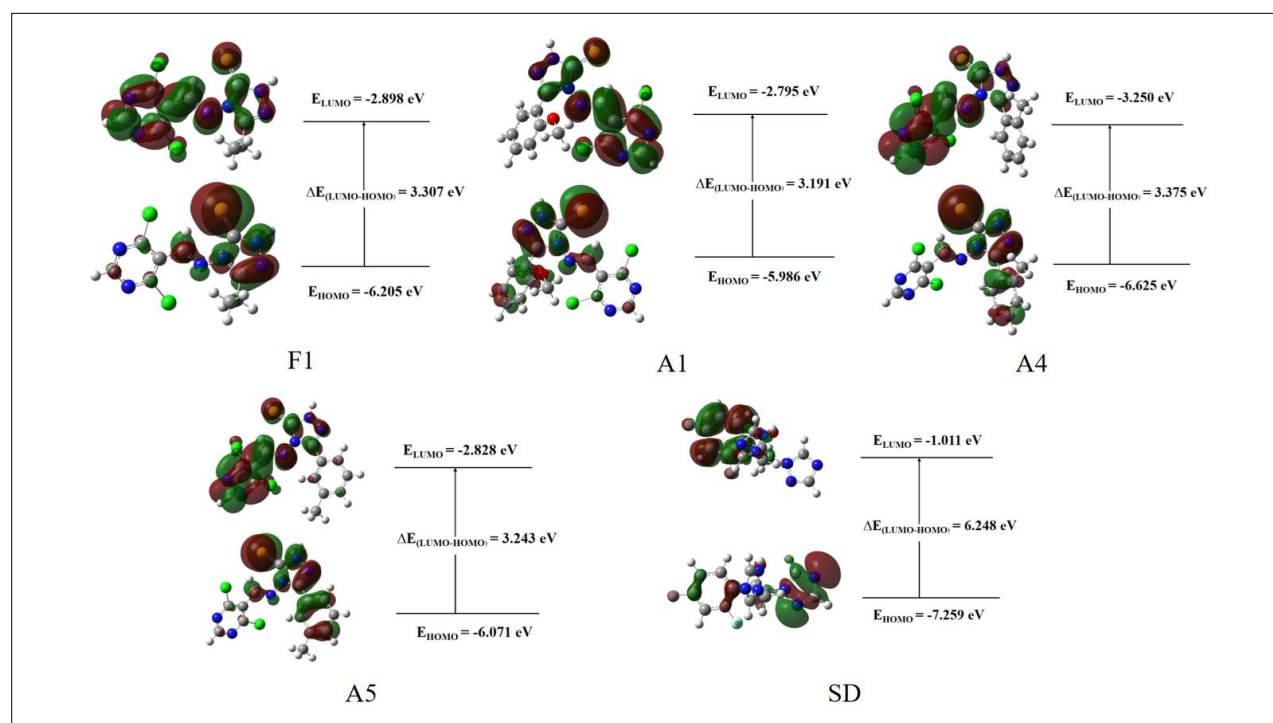


Figure 4. HOMO-LUMO orbital maps of compounds

The energy gap ($\Delta E_{\text{LUMO-HOMO}}$) values of the synthesized compounds (3.307 eV for *F1*, 3.191 eV for *A1*, 3.375 eV for *A4* and 3.243 eV for *A5*) shows a small difference. The lower value of $\Delta E_{\text{LUMO-HOMO}}$ reveals the higher biological [30]. It is also observed that the value of energy gap (6.248 eV) for fluconazole is larger than that of the titled compounds, which could be considered as an explication of its antifungal activity.

3.4.2. Molecular electrostatic potentials analysis

Molecular electrostatic potential (MEP) can reflect the reactivity of the molecules and predict the electrophilic and nucleophilic sites. The color shown in the diagram from red to blue represents the electrostatic potential from negative to positive (Figure 5). It can be seen that, N atom on the pyrimidine and triazole rings and the C=S group on the triazole ring lie in the red region. Hence, these atoms are prone to be attacked by electrophile and are also ideal site for positive points of receptor molecules. That is to say, these sites are the most negative and the main electrophilic attack sites. On the other hand, an electron-deficient region is mainly located on the H atoms of C-H on pyrimidine ring and N-H on triazole (the blue color region), and these sites are the most reactive site for a nucleophilic reaction. Comparing four compounds, it is obvious that the electrophilicity of 5-ethyl substituent (*F1*) on

triazole ring is weaker than that of 5-aryl substituents (A1, A4, and A5). What is interesting that the corresponding biological activity against *Wheat gibberellic* (*Grape anthracnose*) was: $A1 > F1 > A4 \sim A5$ ($A4 \sim A5 > F1 > A1$), which infers that the electrophilic reaction of the 5-substituents on the triazole ring is beneficial to biological activity.

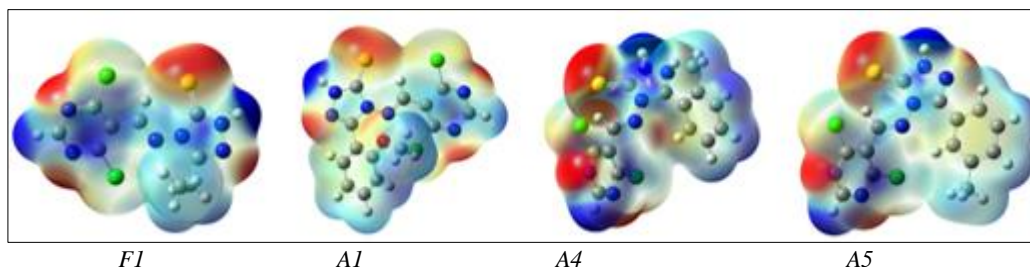


Figure 5.
Molecular electrostatic potential map of four compounds

4. Conclusions

In this article, novel Schiff base compounds containing 1,2,4-triazole and pyrimidine heterocycles were synthesized by the method of splicing active substructures, and the structures were also confirmed by series characterization. The antifungal activities have been investigated for *in vitro* antifungal activities against *Grape anthracnose* and *Wheat gibberellic* strains. The results revealed that the compounds of F1, A4, and A5 had good antifungal activity against *Grape anthracnose*, while all compounds had stronger antimicrobial activity against *Wheat gibberellic* than fluconazole. Especially, compounds of F1, A1, A4, A5 had broad spectrum of antimicrobial activity and could be used to further design promising antifungal agents. Meanwhile, the affinity and mode of action of four compounds with broad-spectrum antimicrobial activity to the receptor (5E9H) were studied by molecular docking. The results indicated that four compounds had high affinity and could form stable complex with the receptor through conventional hydrogen bonds, Van der Waals, amide- π stacking and π -alkyl. Furthermore, the frontier orbitals (LUMO, HOMO), and molecular electrostatic potentials (MEP) of four compounds with broad-spectrum antimicrobial activity were calculated, and the results showed that the energy gap ($\Delta E_{\text{LUMO-HOMO}}$) values of the synthesized compounds had a little difference and were all lower than fluconazole, and the electrophilic reactions of 5-substituents on triazole ring were beneficial to biological activity against *Wheat gibberellic* and *Grape anthracnose*.

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