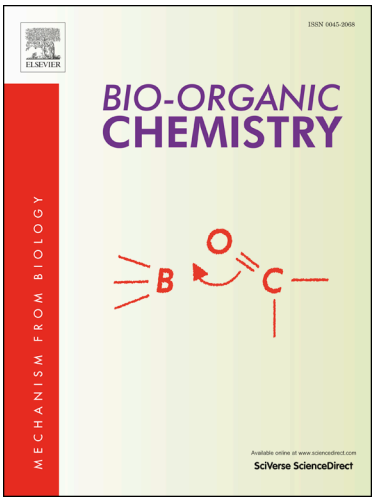

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Design, synthesis, biological activities and DFT calculation of novel 1,2,4-triazole Schiff base derivatives

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Abstract: Series of 1,2,4-triazole Schiff bases (2a-h and 3a-h) have been designed and synthesized. The structure of title compounds was confirmed on the basis of their spectral data and elemental analysis. All the target compounds were screened for their in vitro antifungal activity and antibacterial activity. Two of the tested compounds (2a and 2b) exhibited significant antifungal activity against most fungi, especially compound 2a showed better antifungal activity than triadimefon. Meanwhile, the antibacterial activity assay also indicated compound 2a exhibited excellent antibacterial activities comparable to chloramphenicol. The SAR manifested no substitution at position 5 of the triazole ring caused an increase in activity, and 3-phenoxy phenyl group introduced in 1,2,4-triazole scaffold can enhance the antibacterial activity. The DFT calculation indicated triazole ring, S atom and benzene ring in both of the 2a and 3a make a major contribution to the activity.

Keywords: Synthesis; 1,2,4-Triazole; Biological activity; DFT calculation

1. Introduction

1,2,4-Triazole derivatives due to their broad-spectrum activities have potential applications in the fields of pesticides and medicines possessing antifungal[1], antibacterial[2], antitumor[3], antitrypanosomal[4], antiproliferative[5], and antibiotics[6] properties. Several clinical 1,2,4-triazole drugs were used widely in clinical therapy, for instance, voriconazole and fluconazole (Fig.1).

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In addition, there are known triazole antifungal agents presently play a leading role in the treatment of agricultural fungal infections, such as, tebuconazole, epoxiconazol, cyproconazole, and triadimefon (Fig. 1). However, their use is problematic as both can cause side effects and have limited efficacy, and some strains are refractory to treatment. In addition, antifungal agents usually can't treat bacteria at the same time. Therefore, the need for high activity, new, safer, and dual antifungal and antibacteria agent is urgent.

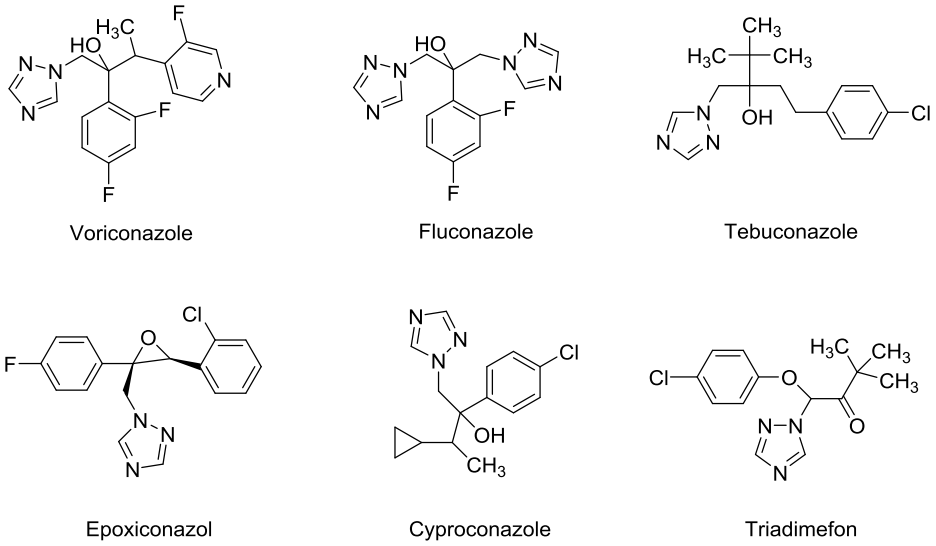


Fig. 1 Triazole antifungal agents used in clinical therapy and pesticides

1,2,4-Triazole Schiff bases represented an important N-containing heterocyclic compounds with potential bioactivity[7-10]. In our previous research, serious of 4-amino-5-substituent-1,2,4 -triazole Schiff bases were designed and synthesized. The bioassay showed parts of the compounds exhibit excellent antifungal activity and could be the potential antifungal agent candidates[11] (Fig.2), and the results indicated that no substituent at position Q can increase the antifungal activity, compound (a) reached same antifungal level compared with triadimefon. The results prompted us to systemically explore the effect on biological of different substituents at position Q and position P, which might bring us some unknown biological properties. Researcher found that compounds with 3-phenoxyphenyl structural feature had good antibacteria activity[12], then we designed introducing 3-phenoxyphenyl into position P, which could be screening a potential dual antifungal and antibacteria agent, meanwhile, 4-mehoxy-2,3,6-trimethyl phenyl also has been introduced into position P, and their influence of these factors on bioactivity were been studied.

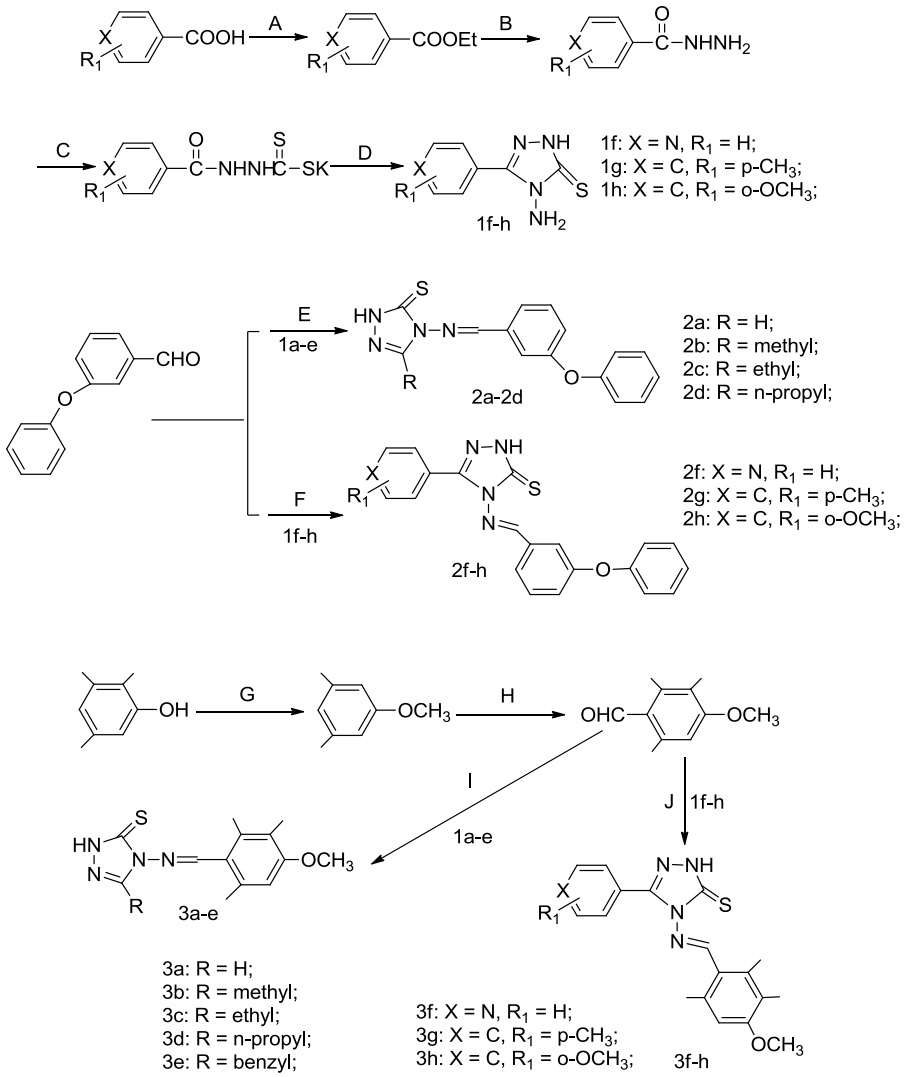
Fig. 2 Chemical structures of 1,2,4-triazole Schiff bases

Based on the above viewpoints, series of 4-amino-5-substituent-1,2,4-triazole-3-thione Schiff bases were synthesized via 4-amino-5-substituent-1,2,4-triazole-3-thione (1a-h) with 3-phenoxy-benzaldehyde and 4-methoxy 2,3,6-trimethyl benzaldehyde. Their structures were confirmed by melting point, IR, ^{1}H NMR,^{13}C NMR, HRMS and elemental analysis. The crystal structure of compound 2f was obtained by X-ray diffraction and the crystallographic data has been deposited at the Cambridge Crystallographic Data Center, CCDC-1016443. Their antifungal activities to *pythium solani*, *gibberlla nicotiancola*, *fusarium oxysporium f.sp. niveum*, and *gibberlla saubinetii* were tested. Their antibacterial activities against *sacillus subtilis*, *escherichia coli*, and *staphylococcus aureus* bacterial strains were presented as well. The HOMO and LUMO of compounds 2a and 3a were calculated by DFT of Gaussian 09.

2. Results and discussion

2.1 Synthesis

All the target compounds were first reported except compound 2a, which covered in patent used as altering lifespan of a eukaryotic organism[13]. The synthetic route of 4-amino-5-substituent-1,2,4-triazole-3-thione Schiff bases in this experiment was outlined in Scheme 1. Compounds 1a-1h were prepared according to literature[14-16]. The title compounds 2a-2d, 2f-2h and 3a-h were synthesized by condensation of 4-amino-5-substituent-1,2,4-triazole-3-thione with 3-phenoxy-benzaldehyde and 4-methoxy-2,3,6-trimethyl benzaldehyde, respectively. The structure of title compounds was confirmed on the basis of their spectral data and elemental analysis. All spectral and analytical data were consistent with the assigned structures. The bands observed around 2900 and 3100 cm^{-1} in the IR spectrum are assigned to the C–H and N–H stretching modes, respectively. The strong peaks around 1600 cm^{-1} are ascribed to the C=N group of Schiff base. The characteristic stretching vibrations ν(C=S) appeared at around 1330 cm^{-1}, meanwhile, there is no absorption at 2565-2550 cm^{-1} (S–H), manifesting title compounds are mainly exist in keto configuration, and this result is in agreement with the crystal structure (2f).



Scheme 1 Synthesis of 1,2,4-triazole Schiff bases. Reagents and conditions: (A) 98% H_2SO_4, EtOH, reflux; (B/D) $NH_2NH_2 \cdot H_2O$, EtOH, reflux; (C) CS_2, KOH, rt; (E/F/I/J) EtOH, CH_3COOH, reflux; (G) CH_3I, K_2CO_3, THF, reflux; (H) $POCl_3$, DMF, NaOH, 90℃.

2.2 Crystal structure analysis

The yellow crystal (0.39 mm × 0.30 mm × 0.14 mm) structure of compound 2f was obtained in DMF, which was suitable for X-ray single crystal diffraction as shown in Fig. 3 with the following crystallographic parameters: a = 9.808 (7) Å, b = 12.837 (9) Å, c = 13.937 (10) Å, α = 90°, β = 93.077 (11)°, γ = 90°, V = 1752 (2) Å^3, Z = 4, Dc = 1.416 mg·m^{-3}, μ = 0.206 mm^{-1}, F (000) = 776, R = 0.0502, wR = 0.1114, the final R = 0.0408 and wR = 0.1050 for 2568 observed reflections with $I > 2\sigma(I)$.

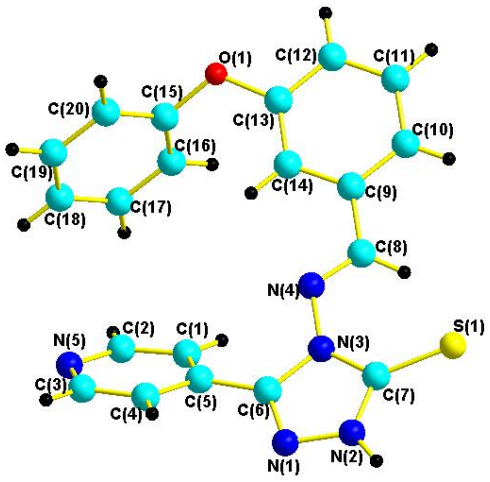


Fig. 3 The ORTEP diagram of the compound 2f

2.3 Antifungal activities

The antifungal activities of compounds 2a-h, 3a-h, and triadimefon against *gibberlla nicotiancola* (A), *pythium solani* (B), *gibberlla saubinetii* (C), and *fusarium oxysporium f. sp. niveum* (D) were listed in Table 1, the regression equation and R value were listed in supporting information. The Antifungal activities against *gibberlla nicotiancola* (A): compounds 2a and 3a reached an excellent activity with EC_{50} values of 0.0309 g· L^{-1} and 0.0012 g· L^{-1}, respectively, lower than that of triadimefon (0.0620 g· L^{-1}). The EC_{95} values of compounds 2a and 3a are 4.6390 g·L^{-1} and 0.0339 g·L^{-1}, respectively, which are also lower than that of triadimefon (7.3943 g· L^{-1}). Antifungal activities against *pythium solani* (B): compound 2a showed excellent activity with EC_{50} and EC_{95} values of 0.0038 g· L^{-1} and 0.4010 g· L^{-1} equivalent to triadimefon (EC_{50} = 0.0013 g· L^{-1}, EC_{95} = 0.2810 g·L^{-1}). Antifungal activities against *gibberlla saubinetii* (C): compound 2a possessed excellent activity with EC_{50} values of 0.0087 g· L^{-1} and EC_{95} values of 2.7930 g· L^{-1}, respectively, lower than triadimefon (EC_{50} = 0.0195 g· L^{-1}, EC_{95} = 7.5650 g· L^{-1}). Compound 3a and 3d reached the similar activity level as triadimefon. Compound 2e and 3h exhibited a good activity. Antifungal activities against *fusarium oxysporium f. sp. niveum* (D): compounds 2a, 2b, 3a and 3b showed good activity, and their EC_{50} values are in the range of 0.0232-0.3847 g·L^{-1}.

The bar chart of antifungal activity (EC_{50} < 1.4500 g·L^{-1}) of target compounds against four vegetable pathogens was showed in Fig. 4. Compounds 2a and 2b showed remarkable antifungal activity against four pathogens, especially compounds 2a, which showed better antifungal activity compared with triadimefon. Compounds 2c, 2d, 3a and 3h showed good antifungal activity against

four pathogens. The results indicated no substituent at position Q can increase the antifungal activity, and this result consistents with our previous research[11].

Table 1 The antifungal activity results of title compounds against four vegetable pathogens

Compounds	Pathogen	EC_{50}/(g·L^{-1})	EC_{95}/(g· L^{-1})
2a	A	0.0309	4.6390
	B	0.0038	0.4010
	C	0.0087	2.7930
	D	0.0232	4.5180
2b	A	0.1350	10.0860
	B	0.0271	17.9590
	C	0.0479	/
	D	0.2840	10.1220
2c	A	0.0979	359.5010
	B	0.0913	11.6890
	C	0.9670	/
	D	1.2890	/
2d	A	0.2130	520.6760
	B	0.1720	215.0700
	C	1.3430	227.1290
	D	0.5560	242.1160
2f	A	1.0503	19.6473
	B	1.1260	368.1590
	C	5.8670	779.7300
	D	1.2750	28.7520
2g	A	20.2812	743.7434
	B	1.7565	16.3978
	C	4.0154	44.4352
	D	1.3680	180.9040
2h	A	0.5212	23.0132
	B	0.6345	17.2567
	C	2.0654	12.8500
	D	1.4830	24.8100
3a	A	0.0012	0.0339
	B	1.0328	9.1474
	C	0.1035	3.0974
	D	0.2287	38.9045
3b	A	1.5840	39.7924
	B	1.8197	14.3186
	C	1.9588	19.9572
	D	0.3847	8.3387
3c	A	2.5119	/
	B	7.8886	/
	C	1.7701	186.1230
	D	1.9072	/
3d	A	1.4454	31.9007
	B	1.0328	9.1474
	C	0.6266	5.3088
	D	0.7610	30.1926
3e	A	0.2691	924.6982
	B	1.9120	167.3016
	C	1.1324	/
	D	0.6374	16.3305
3f	A	3.0199	337.7536
	B	10.2494	571.6102
	C	35.1318	/
	D	1.0294	925.1241
	A	1.3183	84.8203
	B	7.7446	/

3g	C	0.9057	/
	D	0.5494	73.7055
3h	A	0.2238	/
	B	0.7871	622.4436
	C	0.8954	13.0610
	D	0.2679	4.6313
Triadimefon	A	0.0620	7.3943
	B	0.0013	0.2810
	C	0.0195	7.5650
	D	0.0079	1.0510

Symbols: EC_{50} = 50% effective concentration; EC_{95} = 95% effective concentration; A = *gibberlla nicotiancola*; B = *pythium solani*; C = *gibberlla saubinetii*; D = *fusarium oxysporium f. sp. niveum*; (/) = EC_{50}, EC_{95}>1000 g/L.

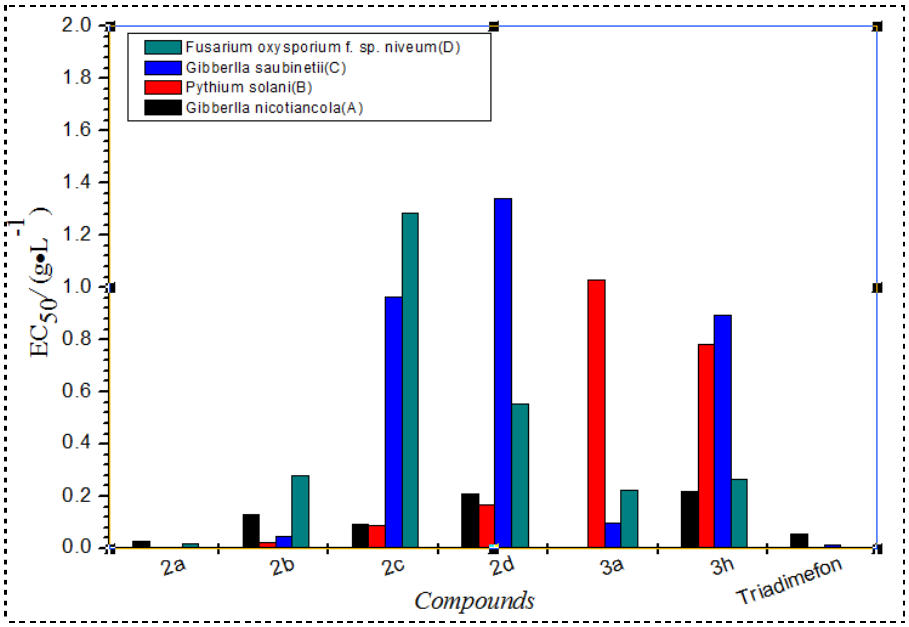


Fig. 4 The bar chart of antifungal activity results against four vegetable pathogens (EC_{50} / g•L-1)

2.3 Antibacterial activities

The antibacterial activities of compounds 2a-2d, 2f-2h, 3a-3h and chloramphenicol against *sacillus subtilis*, *staphylococcus aureus* and *escherichia coli* were listed in Table 2. Compounds 2a and 2b exhibited excellent antibacterial activities comparable to chloramphenicol. In particular, compound 2a showed remarkable antibacterial activities.

Table 2 Antibacterial activities of title compounds

Compounds	Zone of inhibition (100 ug·mL^{-1})		
	Sacillus subtilis	Staphylococcus aureus	Escherichia coli
2a	+++	+++	+++
2b	++	+++	++
2c	+++	+++	++
2d	+	+	+
2f	+	++	++
2g	-	+	+
2h	++	+	++
3a	+	+	+

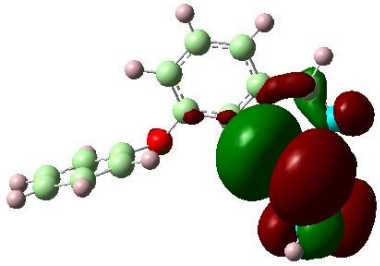
3b	-	-	-
3c	-	-	-
3d	-	-	-
3e	-	-	-
3f	-	-	-
3g	-	-	-
3h	+	-	-
Chloramphenicol	+++	+++	+++

Symbols: zone diameter of growth inhibition: (-) = Inactive (< 6 mm); (+) = week active (6-10 mm); (++) = moderate active (10-15 mm); (+++) = high active (15-20 mm); (+++) = Chloramphenicol.

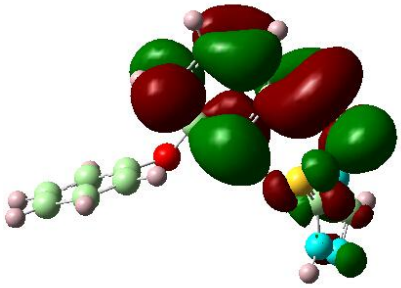
2.4 DFT calculation

According to the frontier molecular orbital theory, HOMO and LUMO are the most important factors effected bioactivity[17]. HOMO has the priority to provide electrons, while LUMO accepts electrons in the first place[18]. A study of the frontier orbital energy can provide some useful information for the active mechanism. Density functional theory (DFT) has been extensively used to calculate a wide variety of molecular properties because of high efficiency and accuracy [19,20]. DFT also adequately takes into account electron correlation contributions especially in systems containing extensive electron conjugation and/or electron lone pairs[21].

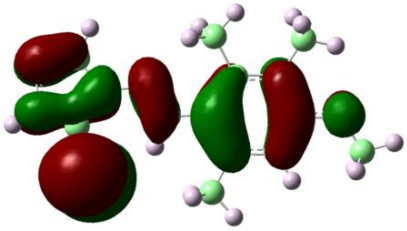
The energy and pictorial illustrations of the HOMO and the LUMO of representative compounds 2a and 3a with good and moderate activity, respectively, which have been given in Fig. 5. It is showed that HOMO orbital of 2a is mainly be delocalized on triazole ring and S atom, especially the latter, LUMO of 2a mainly contains Schiff base double bond (C=N) and benzene ring. Both HOMO and LUMO of 3a mainly located on 1,2,4-triazole ring, Schiff base double bond (C=N) and benzene ring. It is observed that the triazole ring, S atom and benzene ring in both of the molecules make a major contribution to the activity. These are largely through hydrophobic interaction obviously in the HOMO maps (especially the S atom and N atom of position 2 of triazole ring). It is found that there are bit differences at the frontier molecular orbitals of 2a and 3a. The HOMO of compound 2a is mainly located on the triazole ring and S atom, 2a has a remarkable activity maybe because electron of 2a can easily transferred from HOMO to the receptor. Compared with 2a, 3a is more stable than 2a and has a moderate activity due to the HOMO and LUMO of 3a are wide delocalized.



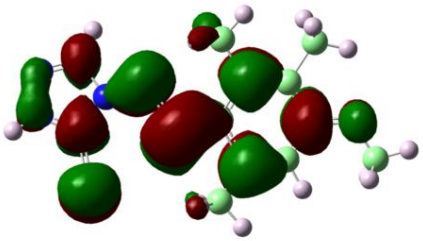
(2a) HOMO E_{77} = - 0.216 a.u.



(2a) LUMO E_{78} = - 0.084 a.u.



(3a) HOMO E_{73} = - 0.215 a.u.



(3a) LUMO E_{74} = - 0.067 a.u.

Fig. 5 LUMO and HOMO maps for compounds 2a and 3a from DFT/B3LYP/6-311G+(d,p) calculation. The green parts represent positive molecular orbital, and the red parts represent negative molecular orbital.

Conclusions

In summary, series of 4-amino-5-substituent-1,2,4-triazole-3-thione Schiff bases have been prepared, and their structures were confirmed by melting point, IR, ^{1}H NMR, ^{13}C NMR and elemental analyses and HRMS. The antifungal activities against *gibberlla nicotiancola*, *pythium solani*, *gibberlla saubinetii*, and *fusarium oxysporium f. sp. niveum* were evaluated. The antifungal activities results showed compound 2a and 2b showed excellent activity to most of the fungi. Compound 2a had a better effective against *gibberlla nicotiancola* and *gibberlla saubinetii* than triadimefon. Meanwhile, their antibacterial activity against *sacillus subtilis*, *staphylococcus aureus*, and *escheri*chia coli were also tested. Form antibacterial activity results, compounds 2a reached a good activity as well as chloramphenicol. All bioassays showed that the no substituent at position 5 in the triazole ring was more effective than others, and 3-phenoxyphenyl group introduced in the scaffold can enhanced the antibacterial activity. The DFT calculation results indicated triazole ring, S atom and benzene ring in both of the 2a and 3a make a major contribution to the activity. The present work demonstrated that compound 2a can be used as lead compound for the development of new dual antifungal and antibacterial agent.

3. Experimental

3.1 Chemistry

Elemental analysis was performed with a Vario ELIII CHNOS analyzer; IR (KBr) spectra was recorded on an IR-400 spectrophotomete; ^{1}H NMR and ^{13}C NMR spectra were measured with a Varian unity INOVA-400 nuclear magnetic resonance using $CDCl_3$ or DMSO-d_6 as the solvent with tetramethylsilane as an internal standard; mass spectra was obtained from micrOTOF-Q II mass spectrometer; the melting point was determined on MP3 melting point apparatus; the X-ray diffraction data were collected on a Bruker SMART-APEX II CCD diffractometer 2004.

3.2 Synthesis

3.2.1 Synthesis of 4-amino-5-substituent-1,2,4-triazolethione 1a-h

Compounds 1a-e were obtained in our previous research[11]. Compounds 1f-h were synthesized by a known procedure[14,15], 1f: ^{1}H NMR (400MHz, DMSO-d_6) δ 8.84(2H, m), 7.20(2H, m), 3.72(2H, s); 1g: ^{1}H NMR (400MHz, $CDCl_3$) δ 7.75(2H, d, *J* = 7.6Hz), 6.97(2H, d, *J* = 7.6Hz), 2.37(3H, s), 2.10(2H, s); 1h: ^{1}H NMR (400MHz, $CDCl_3$) 7.54(1H, t, *J*=7.6 Hz), 7.49(1H, d, *J*=7.2 Hz), 7.12(1H, d, *J*=8.0 Hz), 7.04(1H, t, *J*=7.2 Hz), 5.49(2H, s), 3.70(3H, s).

3.2.2 Synthesis of 4-methoxy-2,3,6-trimethyl-benzaldehyde

4-Methoxy-2,3,6-trimethyl-benzaldehyde was synthesized according to the method presented in literature[22]. ^{1}H NMR(400MHz) δ 10.53(1H, s), 6.56(1H, s), 3.87(3H, s), 2.60(3H, s), 2.53(3H, s), 2.15(3H, s).

3.2.3 General synthetic procedure for compounds 2a-d and 2f-h

4-Amino-5-substituent-1,2,4-triazole-3-thione (1a-d and 1f-h) (5 mmol) was added to a solution of 3-phenoxy-benzaldehyde (5 mmol) in ethanol (20 mL) and glacial acetic acid (2 mL), the mixture was refluxed for about 4 h. The reaction mixture was left standing overnight and then filtration. The crude products were crystallized from absolute ethanol except compounds 2f, 2g, and 2h, which were crystallized from DMF.

5*H*-4-[(3-phenoxyphenyl)methyleneamino]-3*H*-1,2,4-triazole-3-thione (2a): white solid, yield = 88.3 %, mp 143.2-144.1 ℃. ^{1}H NMR (400MHz, $CDCl_3$) δ 10.99 (1H, s), 10.42 (1H, s), 8.04 (1H, s), 7.55 (1H, d, *J*=7.6 Hz), 7.51 (1H, s), 7.44 (1H, t, *J*=7.6 Hz), 7.38 (2H, t, *J*=7.6 Hz), 7.18 (2H, q), 7.04 (2H, d, *J*=7.6 Hz). ^{13}C NMR (400MHz, DMSO) δ 117.33, 119.71, 122.94, 124.32, 124.63, 130.76, 131.47, 134.54, 138.53, 156.43, 157.97, 160.62, 163.44. IR (KBr) v/cm^{-1}: 3113, 2933, 1554, 1491, 1293, 927, 847, 686, 561; MS: m/z 297.3473 (M+1). Elemental Anal. Calcd for $C_{15}H_{12}N_4OS$: C, 60.79; H, 4.08; N, 18.91. Found: C, 60.81; H, 4.06; N, 18.89.

5-Methyl-4-[(3-phenoxyphenyl)methyleneamino]-3*H*-1,2,4-triazole-3-thione (2b): white solid, yield = 89.7 %, mp 154.0-155.5 ℃. ^{1}H NMR (400MHz, $CDCl_3$) δ 11.34 (1H, s), 10.41 (1H, s), 7.56 (2H, t, *J*=7.6 Hz), 7.43 (1H, t, J=8.0), 7.37 (2H, t, *J*=7.6 Hz), 7.15 (2H, t, *J*=8.0 Hz), 7.04 (2H, d, *J*=8.0 Hz), 2.44 (3H, s). ^{13}C NMR (400MHz, DMSO) δ 11.17, 117.61, 119.57, 122.98, 124.46, 124.53, 130.71, 131.42, 134.63, 148.83, 156.51, 157.89, 161.71, 162.95. IR (KBr) v/cm^{-1}: 3066, 2896, 1593, 1491, 1252, 959, 850, 686, 561. MS: m/z 311.3738 (M+1). Elemental Anal. Calcd for $C_{16}H_{14}N_4OS$: C, 61.92; H, 4.55; N, 18.05. Found: C, 61.90; H, 4.54; N, 18.06.

5-Ethyl-4-[(3-phenoxyphenyl)methyleneamino]-3*H*-1,2,4-triazole-3-thione (2c): white solid, yield = 88.0 %, mp 131.5-132.6 ℃. ^{1}H NMR (400MHz, $CDCl_3$) δ 11.31 (1H, s), 10.39 (1H, s), 7.54 (3H, t, *J*=7.2 Hz), 7.38 (3H, m), 7.15 (2H, d, *J*=7.2 Hz), 7.04 (1H, d, *J*=7.6 Hz), 2.82 (2H, q), 1.30 (3H, t, *J*=7.2 Hz). ^{13}C NMR (400MHz, DMSO) δ 10.49, 18.73, 117.46, 119.62, 122.90, 124.39, 124.55, 130.69, 131.41, 134.65, 152.75, 156.45, 157.94, 161.83, 162.76. IR (KBr) v/cm^{-1}: 3065, 2943, 1591, 1492, 1263, 907, 788, 655, 564. MS: 325.4002 m/z (M+1). Elemental Anal. Calcd for $C_{17}H_{16}N_4OS$: C, 62.94; H, 4.97; N, 17.27. Found: C, 62.95; H, 4.94; N, 17.26.

4-[(3-Phenoxyphenyl)methyleneamino]-5-propyl-3*H*-1,2,4-triazole-3-thione (2d): white solid, yield = 87.8 %, mp 115.5-121.1 ℃. ^{1}H NMR (400MHz, $CDCl_3$) δ 11.31 (1H, s), 10.39 (1H, s), 7.54 (2H, t, *J*=11.6 Hz), 7.43 (1H, t, J=8.0 Hz), 7.37 (2H, t, *J*=7.6 Hz), 7.16 (2H, d, *J*=7.2 Hz), 7.05 (2H, d, *J*=7.6 Hz), 2.76 (2H, t, *J*=7.2 Hz), 1.75 (2H, m), 0.97 (3H, t, *J*=7.2 Hz). ^{13}C NMR (400MHz, DMSO) δ 13.89, 19.35, 26.81, 39.99, 117.18, 119.73, 122.89, 124.43, 124.62, 130.71, 131.45, 134.63, 151.60, 156.38, 158.03, 161.72, 162.73. IR (KBr) v/cm^{-1}: 3132, 2964, 1571, 1491, 1241, 959, 843, 689. MS: m/z 339.4268 (M+1). Elemental Anal. Calcd for $C_{18}H_{18}N_4OS$: C, 63.88; H, 5.36; N, 16.56. Found: C, 63.93; H, 5.31; N, 16.51.

4-[(3-Phenoxyphenyl)methyleneamino]-5-(4-pyridyl)-3*H*-1,2,4-triazole-3-thione (2f): yellow solid, yield = 83.8 %, mp > 250 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.77 (1H, s), 8.71 (2H, d, *J* = 4.8 Hz), 7.80 (2H, d, *J* = 4.8 Hz), 7.65 (1H, d, *J* = 7.6 Hz), 7.58 (1H, t, *J*=8.0 Hz), 7.43 (3H, t, *J*=8.2 Hz), 7.29 (1H, d, *J* = 8.4 Hz), 7.19 (1H, t, *J* = 7.6 Hz), 7.11 (2H, d, *J* = 8.0 Hz). IR (KBr) v/cm^{-1}: 3109, 2948, 1569, 1476, 1435, 1260, 948, 673, 543. MS: m/z 374.4313 (M+1). Elemental Anal. Calcd for $C_{20}H_{15}N_5OS$: C, 64.33; H, 4.05; N, 18.75. Found: C, 64.34; H, 4.03; N, 18.76.

4-[(3-Methylphenyl)methyleneamino]-5-(4-methylphenyl)-3*H*-1,2,4-triazole-3-thione (2g): white solid, yield = 84.6 %, mp 156.0-158.0 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.79 (1H, s),

7.69 (2H, d, *J*=8.0 Hz), 7.62 (2H, m), 7.45 (3H, m), 7.30 (3H, d, *J*=7.2 Hz), 7.22 (1H, m), 7.12 (2H, d, *J*=7.6 Hz), 2.37 (3H,s). IR (KBr) v/cm^{-1}: 3076, 2850, 1573, 1476, 1435, 1260, 958, 679. MS: m/z 387.4697 (M+1), Elemental Anal. Calcd for $C_{22}H_{18}N_4OS$: C, 68.37; H, 4.69; N, 14.50. Found: C, 68.35; H, 4.68; N, 14.50.

4-[(3-Phenoxyphenyl)methyleneamino]-5-(2-methoxyphenyl)-3*H*-1,2,4-triazole-3-thione (2h): white solid, yield = 88.3 %, mp 164.2-166.1℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.70(1H, s), 7.50(3H, m), 7.41(3H, q), 7.28(1H, s), 7.22(2H, m), 7.06(4H, t, *J*=7.6 Hz), 3.60(3H, s). IR (KBr) v/cm^{-1}: 3139, 2978, 1589, 1498, 1467, 1272, 989, 697. MS: m/z 403.4686 (M+1). Elemental Anal. Calcd for $C_{22}H_{18}N_4O_2S$: C, 65.65; H, 4.51; N, 13.92. Found: C, 65.64; H, 4.51; N, 13.91.

3.2.4 General synthetic procedure for compounds 3a-3h

4-Amino-5-substituent-1,2,4-triazole-3-thione (1a-1h) (5 mmol) was added to a solution of 4-methoxy-2,3,6-trimethyl benzaldehyde (5 mmol) in ethanol (20 mL) and glacial acetic acid (2 mL), the mixture was refluxed about 4 h. The reaction mixture was filtrated when the solvent is hot. The crude product was crystallized from DMF.

4-[(4-Mehoxy-2,3,6-trimethylphenyl)methyleneamino]-5*H*-3*H*-1,2,4-triazole-3-thione (3a): white solid, yield = 78.3 %, mp 181.1-182.3 ℃. ^{1}H NMR (400MHz, $CDCl_3$) δ 11.25 (1H, s), 10.46 (1H, s), 8.03 (1H, s), 6.63 (1H, s), 3.86 (3H, s), 2.56 (3H, s), 2.48 (3H, s), 2.16 (3H, s). ^{13}C NMR (400MHz, DMSO) δ 11.93, 17.43, 22.41, 55.99, 111.47, 122.39, 123.07, 138.65, 139.42, 159.15, 162.44, 163.94. IR (KBr) v/cm^{-1}: 3112, 2913, 1564, 1488, 1291, 920, 845, 680. MS: m/z 277.3574(M+1). Elemental Anal. Calcd for $C_{13}H_{16}N_4OS$: C, 56.50; H, 5.84; N, 20.27. Found: C, 56.53; H, 5.80; N, 20.30.

4-[(4-Mehoxy-2,3,6-trimethylphenyl)methyleneamino]-5-methyl-3*H*-1,2,4-triazole-3-thione (3b): white solid, yield = 77.5 %, mp 217.7-218.2 ℃. ^{1}H NMR (400MHz, $CDCl_3$) δ 11.12 (1H, s), 10.48 (1H, s), 6.64 (1H, s), 3.87 (3H, s), 2.59 (3H, s), 2.51 (3H, s), 2.45 (3H,s), 2.18 (3H, s). ^{13}C NMR (400MHz, DMSO) δ 11.41, 11.95, 17.49, 22.60, 56.04, 111.59, 122.55, 123.11, 138.57, 139.51, 148.75, 159.19, 161.23, 165.64. IR(KBr) v/cm^{-1}: 3064, 2953, 1587, 1499, 1221, 967, 859, 693. MS: m/z 291.3837(M+1). Elemental Anal. Calcd for $C_{14}H_{18}N_4OS$: C, 57.91; H, 6.25; N, 19.29. Found: C, 57.88; H, 6.24; N, 19.30.

5-Ethyl-4-[(4-mehoxy-2,3,6-trimethylphenyl)methyleneamino]-3*H*-1,2,4-triazole-3-thione (3c): white solid, yield = 78.3 %, mp 188.2-189.1℃. ^{1}H NMR (400MHz, $CDCl_3$) 10.43 (1H, s),

6.63 (1H, s), 3.86 (3H, s), 2.80 (2H, q), 2.59 (3H, s), 2.51 (3H, s), 2.17 (3H,s), 1.33 (3H, t, *J*=7.2 Hz). ^{13}C NMR (400MHz, DMSO) δ 10.44, 11.88, 17.44, 18.91, 22.61, 55.96, 111.50, 122.45, 123.07, 138.57, 139.53,152.62, 159.15, 161.32, 165.37, 165.81. IR(KBr) v/cm^{-1}: 3056, 2932, 1583, 1454, 1280, 965, 841, 689. MS: m/z 305.4106(M+1). Elemental Anal. Calcd for $C_{15}H_{20}N_4OS$: C, 59.18; H, 6.62; N, 18.41. Found: C, 59.20; H, 6.58; N, 18.39.

4-[(4-Mehoxy-2,3,6-trimethylphenyl)methyleneamino]-5-propyl-3*H*-1,2,4-triazole-3-thione (3d): white solid, yield = 73.3 %, mp 170.4-171.2 ℃. ^{1}H NMR (400MHz, $CDCl_3$) δ 10.76 (1H, s), 10.56 (1H, s), 7.37 (1H, s), 6.76 (1H, s), 3.97 (3H, s), 2.87 (2H, t, *J*=7.2 Hz), 2.71 (3H, s), 2.63 (3H,s), 2.30 (3H, s), 1.89 (2H, q), 1.13 (3H, t, *J*=7.2 Hz). ^{13}C NMR (400MHz, DMSO) δ 11.90, 13.91, 17.42, 19.47, 22.54, 27.06, 55.99, 111.54, 122.45, 123.12, 138.60, 139.54, 151.43, 159.20, 161.26, 165.69. IR (KBr) v/cm^{-1}: 3102, 2927, 1581, 1460, 1271, 1007, 839, 647. MS: m/z 319.4370(M+1). Elemental Anal. Calcd for $C_{16}H_{22}N_4OS$: C, 60.35; H, 6.96; N, 17.59. Found: C, 60.38; H, 6.95; N, 17.57.

5-Benzyl-4-[(4-mehoxy-2,3,6-trimethylphenyl)methyleneamino]-3*H*-1,2,4-triazole-3-thione (3e): white solid, yield = 78.8 %, mp 186.6-187.4 ℃. ^{1}H NMR (400MHz, $CDCl_3$) δ 10.03 (1H, s), 7.30 (2H, t, *J*=4.8 Hz), 7.23 (3H, t, *J*=5.2 Hz), 6.81 (1H, s), 4.13 (2H, s), 3.83 (3H, s), 2.43 (3H, s), 2.36 (3H, s), 2.09 (3H, s). ^{13}C NMR (400MHz, DMSO) δ 11.93, 17.43, 22.47, 31.11, 55.03, 111.56, 122.36, 123.11, 127.29, 128.98, 129.03, 135.61, 138.74, 139.60, 150.48, 159.29, 161.60, 166.13. IR(KBr) v/cm^{-1}: 3103, 2920, 1576, 1456, 1281, 1017, 840, 695, 581. MS: m/z 367.4800 (M+1). Elemental Anal. Calcd for $C_{20}H_{22}N_4OS$: C, 65.55; H, 6.05; N, 15.29. Found: C, 65.56; H, 6.01; N, 15.30.

4-[(4-Mehoxy-2,3,6-trimethylphenyl)methyleneamino]-5-(4-pyridyl)-3*H*-1,2,4-triazole-3-thio ne (3f): yellow solid, yield = 75.8 %, mp > 250 ℃. ^{1}H NMR (400MHz, DMSO-d_6) δ 9.91 (1H, s), 8.83 (2H, m), 7.90 (2H, m), 6.93 (1H, s), 3.92 (3H, s), 2.56 (3H, s), 2.49 (3H,s), 2.19 (3H, s). ^{13}C NMR (400MHz, DMSO) δ 11.96, 17.50, 22.54, 56.12, 111.70, 121.95, 122.05, 122.66, 123.31, 133.41, 139.43, 140.11, 147.10, 147.86, 150.65, 159.68, 163.25, 168.20, 169.65. IR (KBr) v/cm^{-1}: 3208, 2997, 1589, 1480, 1245, 998, 839, 568. MS: m/z 354.4413 (M+1). Elemental Anal. Calcd for $C_{18}H_{19}N_5OS$: C, 61.17; H, 5.42; N, 19.81. Found: C, 61.18; H, 5.40; N, 19.80.

4-[(4-Mehoxy-2,3,6-trimethylphenyl)methyleneamino]-5-(4-methylphenyl)-3*H*-1,2,4-triazole -3-thione (3g): white solid, yield = 72.3 %, mp 210.1-211.2 ℃. ^{1}H NMR (400MHz, DMSO-d_6) δ

9.74 (1H, s), 7.68 (2H, d, *J*=8.0 Hz), 7.30 (2H, d, *J*=8.4 Hz), 6.81 (1H, s), 3.82 (3H, s), 2.45 (3H, s), 2.39 (3H,s), 2.34 (3H, s). IR (KBr) v/cm^{-1}: 3090, 2908, 1579, 1432, 1223, 989, 808, 636. MS: m/z 367.4797(M+1). Elemental Anal. Calcd for $C_{20}H_{22}N_4OS$: C, 65.55; H, 6.05; N, 15.29. Found: C, 65.52; H, 6.01; N, 15.30.

4-[(4-Mehoxy-2,3,6-trimethylphenyl)methyleneamino]-5-(2-methoxyphenyl)-3*H*-1,2,4-triazole-3-thione (3h): white solid, yield = 73.3 %, mp 168.4–170.2 ℃. ^{1}H NMR (400MHz, DMSO-d_6) δ 9.72(1H, s), 7.56(1H, t, *J*=7.6 Hz), 7.48(1H, d, *J*=7.2 Hz), 7.14(1H, d, *J*=8.0 Hz), 7.08(1H, t, *J*=7.2 Hz), 6.75(1H, s), 3.80(3H, s), 3.70(3H, s), 2.26(3H,s), 2.24(3H, s), 2.05(3H, s). IR(KBr) v/cm^{-1}: 3231, 2965, 1598, 1476, 1282, 1017, 848, 688. MS: m/z 384.4794 (M+1). Elemental Anal. Calcd for $C_{20}H_{22}N_4O_2S$: C, 62.80; H, 5.80; N, 14.65. Found: C, 62.83; H, 5.78; N, 14.66.

3.3 Crystal structure determination

A yellow single crystal of the title compound with dimensions of 0.39 mm × 0.30 mm × 0.14 mm was selected for X-ray diffraction analysis. The X-ray diffraction data was collected on a Bruker SMART-APEX II CCD diffractometer[296(2)K] equipped with a graphite monochromatic Mo Kα radiation (λ = 0.71073 Å) by using ω-2θ scan technique at room temperature. A total of 3106 reflections were collected in the range of $2.48 < \theta < 25.10^{\circ}$, of which 2564 were independent with R_{int} = 0.0383. The structure was solved by direct methods with SHELXS-97, and refined using the full-matrix least squares method on F^2 with anisotropic thermal parameters for all non-hydrogen atoms using SHELXL-97[23,24]. All hydrogen atoms were located theoretically and refined with riding model position parameters and fixed isotropic thermal parameters.

3.4 Antifungal activities

The antifungal activities of compounds 2a-d, 2f-h, and 3a-h were evaluated against four plants pathogenic fungi, containing *pythium solani*, *gibberlla nicotiancola*, *fusarium oxysporium f.s.p. niveum*, and *gibberlla saubinetii*, which are often encountered in plants. The four plants pathogenic fungi were provided by Microbiology Institute of Shaanxi. Triadimefon was purchased from Jiangsu Sevencontinent Green Chemical Co., Ltd. The technique of antifungal activities and data-processing were according to the method described in literature[25].

3.5 Antibacterial activities

The title compounds were screened for their antibacterial activity against *sacillus subtilis*, *staphylococcus aureus* and *escherichia coli* bacterial strains by disc diffusion method described in

literature[26]. Chloramphenicol was used as control and N,N-dimethyl formamide was used as solvent. The discs measuring 6.00 mm in diameter were prepared from filter paper by hole puncher and sterilized at 121˚C for 1 h. A standard inculum (1-2 $\times 10^7$ c.f.u·mL^{-1} 0.5 McFarland standards) was introduced onto the surface of sterile agar, and a sterile glass spreader was used for even distribution of the inoculum. The dried sterile discs previously soaked in a known concentration (100 ug·mL^{-1}) of the test compounds were placed in nutrient agar medium. The plates were labeled and incubated for 18-24 h at 37 ˚C. All tests were repeated for three times.

3.6 Density Functional Theoretical (DFT) calculation

The density functional theoretical (DFT) calculations of compounds 2a and 3a were performed at the Becke-Lee-Parr hybrid exchange correlation three-parameter functional (B3LYP) level with standard 6-311G+(d,p) basis set. The complete geometry optimization and vibrational frequencies were calculated at B3LYP/6-311G+(d,p) level on the basis of the optimized structure. Vibration analysis showed that the optimized structure was in accordance with the minimum points on the potential energy surface. All of the convergent precisions were the system default values, and all calculations reported in this work were carried out with the Gaussian 09 program[27].

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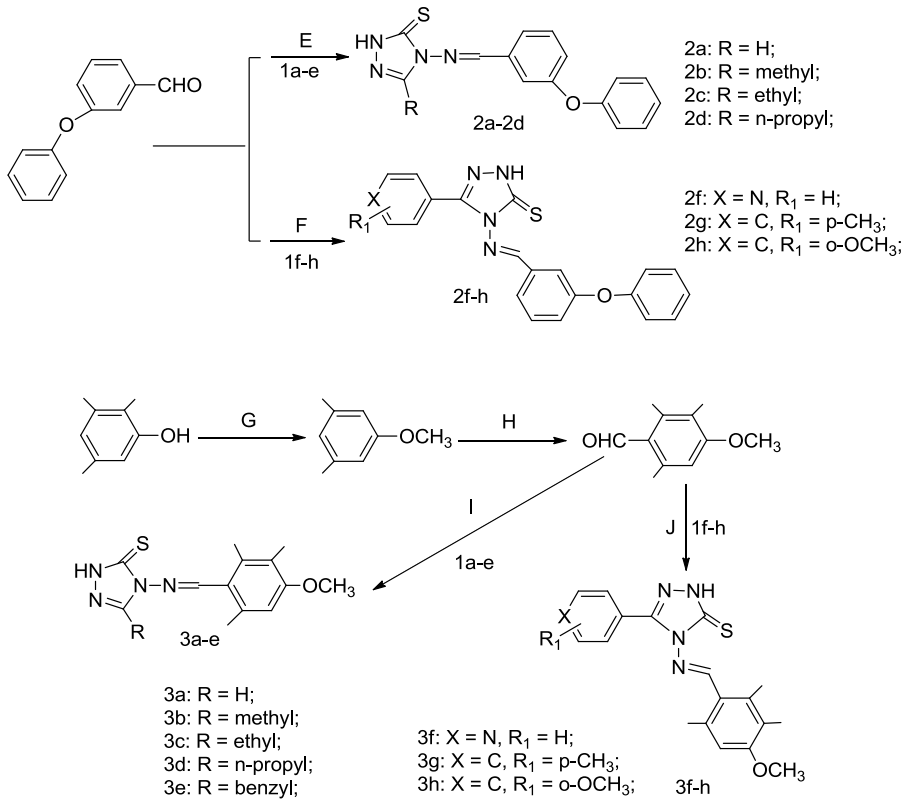
Highlights

Series of 1,2,4-triazole Schiff bases (2a-h and 3a-h) were screened for antifungal and antibacterial activity, biological results showed compound 2a exhibited excellent antifungal and antibacterial activities.

The SAR manifested no substitution at position 5 of the triazole ring caused an increase in activity, and 3-phenoxy phenyl group introduced in 1,2,4-triazole scaffold can enhance the antibacterial activity.

The DFT calculation results indicated triazole ring, S atom and benzene ring in both of the 2a and 3a make a major contribution to the activity.

Graphical abstract:



Series of novel 1,2,4-triazole Schiff bases were synthesized and evaluated for antifungal and antibacterial activities. The bioassay showed compound 2a has potential to developing candidate compound. The DFT calculation indicated triazole ring, S atom and benzene ring in both of the 2a and 3a make a major contribution to the activity.