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# Synthesis, antifungal activity and CoMFA analysis of novel 1,2,4-triazolo[1,5-*a*]pyrimidine derivatives

Original article

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### Abstract

In order to search novel agrochemicals with higher antifungal activity, a series of new 1,2,4-triazolo[1,5-*a*]pyrimidine derivatives bearing 1,3,4-oxadiazole moieties were designed and synthesized. Their antifungal activities against *Rhizoctonia solani* were evaluated in vitro. By determining the EC<sub>50</sub> values of all the newly synthesized compounds and 10 formerly synthesized compounds, compound **8r**, 2-((5-(*sec*-butylthio)-1,3,4-oxadiazol-2-yl)-methylthio)-5-dimethyl-1,2,4-triazolo-[1,5-*a*]pyrimidine, was found to display the highest antifungal activity (EC<sub>50</sub> = 6.57 µg mL<sup>-1</sup>). Based on the quantitative structure—activity relationships analyses, 2-(1-(5-(*sec*-butylthio)-1,3,4-oxadiazol-2-yl)-ethylthio)-5,7-dimethyl-1,2,4-triazolo[1,5-*a*]pyrimidine (**9j**) was designed and synthesized, which was found to display much higher activity (EC<sub>50</sub> = 3.34 µg mL<sup>-1</sup>) than compound **8r** and the control. To further explore the comprehensive structure—activity relationships, a 3D-QSAR analysis using the method of comparative molecular field analysis (CoMFA) was performed and a statistically reliable model with good predictive power ( $r^2 = 0.929$ ,  $q^2 = 0.588$ ) was achieved on the basis of the common substructure-based alignment. According to the CoMFA model, the structure—antifungal activity relationship was explained reasonably. © 2007 Elsevier Masson SAS. All rights reserved.

Keywords: 1,2,4-Triazolo[1,5-a]pyrimidine; 1,3,4-Oxadiazole; Antifungal activity; CoMFA

### 1. Introduction

The synthesis and biological activities of 1,2,4-triazolo[1,5*a*]pyrimidine derivatives have been an interesting topic in the fields of medicinal and agricultural chemistry for many years [1–9]. Some triazolopyrimidine derivatives are known as cardiovascular vasodilators [1], dual thrombin/factor Xa inhibitors [2], and human adenosine  $A_{2a}$  and  $A_3$  receptor ligands [3–5]. Additionally, many triazolopyrimidine-2-sulfonamide derivatives, such as flumetsulam [6], metosulam [7] and florasulam [8], are commercially available as acetolactate synthase-inhibiting herbicides [9]. Previously, we designed and synthesized a series of diheterocyclic compounds as shown in Fig. 1 by integrating 1,3,4-oxadiazole moiety into the scaffold of 1,2,4triazolo[1,5-*a*]pyrimidine [10]. It was found that these diheterocyclic compounds displayed good antifungal activity against *Rhizoctonia solani*. For example, compound **1h**  $(R = p-NO_2C_6H_4)$  displayed better in vivo antifungal activity against *R. solani* at the concentration of 200 µg mL<sup>-1</sup> than carbendazim and validamycin A, two commercial fungicides. As a continuation of our ongoing project related to the discovery and optimization of pesticide leads [11–15], we synthesized a series of new 1,3,4-oxadiazole-containing 1,2,4-triazolo[1, 5-*a*]pyrimidines with the aim of finding potent antifungal activities. In addition, a comparative molecular field analysis (CoMFA) was also performed to understand the quantitative structure—activity relationships of this family.

# 2. Results and discussion

### 2.1. Chemistry

The target compounds **8a–s** and **9a–j** were synthesized by a multiple-step procedure as shown in Scheme 1. Firstly, the

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R = alkyl, substituted benzyl, allyl, ester group, et al.

starting material 2 reacted with ethyl bromoacetate to afford intermediate 4, which cyclized with acetylacetaldehyde dimethyl acetal to give ethyl 2-(5-methyl-1,2,4-triazolo-[1,5-a]pyrimidin-2-ylthio)acetate 5a in a yield of 80%. Ethyl 2-(5,7dimethyl-1,2,4-triazolo[1,5-a]-pyrimidin-2-ylthio)propanoate **5b** was prepared in a high yield (96%) by the reaction of 2mercapto-5,7-dimethyl-1,2,4-triazolo[1,5-a]pyrimidine 3 with ethyl 2-bromopropionate under basic condition. It should be noted that the reaction of compound 5a with an excess of hydrazine hydrate did not give the desired product, but compound **5b** reacted smoothly with an excess of hydrazine hydrate to afford compound **6b** in a yield of 79%. Interestingly, the desired compound **6a** could be prepared in a yield of 68% by the reaction of compound 5a with an equivalent of hydrazine hydrate. Then, compounds 6 were refluxed with carbon sulfide in ethanol in the presence of KOH to give 5-(1-(5-methyl-1,2,4triazolo[1,5-a]pyrimidin-2-ylthio)ethyl)-1,3,4-oxadiazole-2-thiol 7a in a yield of 96% and 5-(1-(5,7-dimethyl-1,2,4-triazolo[1, 5-*a*]pyrimidin-2-ylthio)ethyl)-1,3,4-oxadiazole-2-thiol **7b** in a yield of 81%, respectively. Compounds 7a and 7b reacted

with various halides to afford the target compounds 8a-s and 9a-j in yields of 32-90%.

The reaction of **7** with halide is a typical nucleophilic substitution process. The experimental results indicated that the reactivity of the halide determined the reaction time and the yields of title compounds. For example, at room temperature, intermediate **7a** reacted with *p*-fluorobenzyl chloride under basic condition for 1 h to give the title compound **8j** in a yield of 90%. But no product was observed when intermediate **7** reacted with 1-bromocyclohexane under the same condition. The structures of all new compounds were characterized by <sup>1</sup>H NMR, MS, and elemental analyses.

#### 2.2. Fungicidal activity and SAR

The EC<sub>50</sub> values against *R. solani* of compounds **8a–s** and **9a–j** are listed in Table 1. Carbendazim, a commercial fungicide, was used as a control. In order to discuss the structure– activity relationships, we also determined the EC<sub>50</sub> values against *R. solani* of the previously synthesized compounds **1a–j** [10], which are also listed in Table 1. For the sake of clarity, compounds **8a–s**, **1a–j** and **9a–j** are named as monomethyl, dimethyl and trimethyl derivatives, respectively, throughout the text. Results showed (Table 1) that compound **8r** [R<sup>1</sup> = CH<sub>3</sub>CH<sub>2</sub>(CH<sub>3</sub>)CH, EC<sub>50</sub> = 6.57 µg mL<sup>-1</sup>] exhibited the highest antifungal activity, which is higher than that of carbendazim (EC<sub>50</sub> = 7.62µg mL<sup>-1</sup>). Except **9h** and **8b**, trimethyl and dimethyl derivatives always displayed higher activities





Table 1 Determination of EC<sub>50</sub> values against *R. solani* of compounds 8a-s, 9a-j and 1a-j

	8a–s	9a–j		1a–j			
No	$R^1$	$y = ax + b^{a}$	$r^{\mathrm{b}}$	$EC_{50} \ (\mu g \ m L^{-1})$	$pEC_{50}^{c}$		
					Exp.	Calcd.	
8a	C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub>	y = 1.6594x + 2.8463	0.9763	19.86	4.27	4.25	
8b	$4-NO_2C_6H_4CH_2$	y = 2.0515x + 2.5409	0.9462	15.80	4.41	4.48	
8c	$3-NO_2C_6H_4CH_2$	y = 1.4101x + 2.9754	0.9480	27.28	4.18	4.15	
8d	2-NO2-4-CH3C6H3CH2	y = 2.2954x + 1.8177	0.9274	24.34	4.25	4.12	
8e	$2-ClC_6H_4CH_2$	y = 2.2591x + 2.1229	0.9985	18.77	4.33	4.31	
8f	3-ClC <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	y = 1.4909x + 2.3886	0.9779	56.44	3.86	4.05	
8g	$4-BrC_6H_4CH_2$	y = 2.0494x + 1.7855	0.9982	37.03	4.08	4.11	
8h	$2-FC_6H_4CH_2$	y = 1.4229x + 3.1647	0.9844	19.49	4.30	4.38	
8i	$3-FC_6H_4CH_2$	y = 1.3072x + 3.0296	0.9270	32.16	4.08	4.06	
8j	$4-FC_6H_4CH_2$	y = 1.5633x + 3.0111	0.8833	18.72	4.31	4.24	
8k	$4-CH_3C_6H_4CH_2$	y = 1.3627x + 2.5929	0.9439	58.40	3.82	3.92	
81	$2-CH_3C_6H_4CH_2$	y = 1.5328x + 3.1718	0.9759	15.59	4.39	4.34	
8m	$3-CH_3C_6H_4CH_2$	y = 1.9301x + 2.1145	0.9796	31.26	4.09	4.02	
8n	4-OCH <sub>3</sub> C <sub>6</sub> H <sub>5</sub> COCH <sub>2</sub>	y = 1.6562x + 2.5750	0.9447	29.12	4.14	4.15	
80	CH <sub>3</sub>	y = 1.7878x + 2.3871	0.9589	28.94	4.01	4.03	
8p	CH <sub>3</sub> CH <sub>2</sub>	y = 1.8157x + 2.5294	0.9675	22.94	4.13	4.15	
8q	CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub>	y = 1.5043x + 3.2459	0.9473	14.66	4.36	4.40	
8r	CH <sub>3</sub> CH <sub>2</sub> (CH <sub>3</sub> )CH	y = 1.6458x + 3.6545	0.9187	6.57	4.71	4.62	
8s	CH <sub>3</sub> CH <sub>2</sub> OOCCH <sub>2</sub>	y = 1.9045x + 1.9933	0.9586	37.91	3.99	3.93	
9a	$4-ClC_6H_4CH_2$	y = 1.2932x + 3.4988	0.9879	14.48	4.47	4.49	
9b	$2-FC_6H_4CH_2$	y = 1.1875x + 3.5896	0.9410	15.41	4.43	4.46	
9c	$4-FC_6H_4CH_2$	y = 0.8715x + 4.1295	0.8836	9.97	4.62	4.62	
9d	$3-ClC_6H_4CH_2$	y = 1.3647x + 3.3614	0.9724	15.87	4.44	4.37	
9e	$2-CH_3C_6H_4CH_2$	y = 1.8920x + 2.9222	0.9799	12.54	4.53	4.51	
9f	$3-CH_3C_6H_4CH_2$	y = 1.5223x + 3.0315	0.9559	19.64	4.32	4.36	
9g	3-OCH <sub>3</sub> C <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	y = 1.4178x + 3.2225	0.9825	17.94	4.38	4.30	
9h	$4-NO_2C_6H_4CH_2$	y = 1.5668x + 3.0735	0.9384	16.97	4.41	4.46	
9i	$4-CH_3C_6H_4CH_2$	y = 1.4782x + 3.2965	0.9332	14.20	4.46	4.43	
9j	CH <sub>3</sub> CH <sub>2</sub> (CH <sub>3</sub> )CH	y = 1.3117x + 4.3136	0.9963	3.34	5.03	5.05	
1a <sup>4</sup>	CH <sub>3</sub>	y = 1.9098x + 2.7225	0.9772	15.58	4.30	4.27	
1b <sup>d</sup>	$C_6H_5CH_2$	y = 2.4355x + 2.0425	0.9722	16.38	4.37	4.35	
1c <sup>d</sup>	$4-ClC_6H_4CH_2$	y = 2.2458x + 2.0479	0.9927	20.63	4.31	4.31	
1d <sup>d</sup>	CH <sub>3</sub> CH <sub>2</sub>	y = 2.0106x + 2.6763	0.9657	14.31	4.35	4.33	
1e <sup>u</sup>	$CH_3(CH_2)_6CH_2$	y = 1.5066x + 3.4096	0.9504	11.37	4.55	4.56	
If d	CH <sub>3</sub> OOCCH <sub>2</sub>	y = 2.6486x + 1.2071	0.9803	27.04	4.13	4.19	
1g <sup>d</sup>	CH <sub>3</sub> CH <sub>2</sub> OOCCH <sub>2</sub>	y = 2.8571x + 1.3206	0.9436	19.40	4.29	4.17	
Ih <sup>a</sup>	$4-NO_2C_6H_4CH_2$	y = 1.7035x + 3.0604	0.9509	13.76	4.49	4.50	
11°	$CH_3CH_2CH_2$	y = 1.8786x + 2.8819	0.9591	13.41	4.39	4.50	
IJ"	$CH_3(CH_2)_4CH_2$	y = 1.7533x + 3.0182	0.9581	13.50	4.45	4.45	
Carbendazim		y = 3.8168x + 1.6335	0.9443	1.62			

<sup>a</sup> The correlation equations for the determination of  $EC_{50}$  values.

<sup>b</sup> The correlation coefficients of the equations.

 $pEC_{50} = \log(MW/EC_{50})$ , MW refers to molecular weight.

<sup>d</sup> These compounds were synthesized previously [10].

than monomethyl derivatives bearing the same  $R^1$  group (9b > 8h; 9c > 8j; 9d > 8f; 9e > 8l; 9f > 8m; 9i > 8k;1a > 8o; 1b > 8a; 1d > 8p; 1g > 8s). Trimethyl derivatives bearing p-chlorobenzyl group displayed higher activities than the corresponding dimethyl derivatives (9a > 1c), however, trimethyl derivatives bearing p-nitrobenzyl group displayed lower activities than the corresponding dimethyl

derivatives (9h < 1h), which indicated that the effect of  $\alpha$ -methyl in the bridge on the antifungal activity is very complex. In addition, among the monomethyl derivatives containing a substituted benzyl group, electron-withdrawing group on 4-position of the benzyl resulted in higher activity. For example, compounds **8b** ( $R^1 = 4$ -NO<sub>2</sub>C<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>) and **8j** ( $R^1 = 4$ - $FC_6H_4CH_2$ ) displayed higher activity than compounds 8g  $(R^1 = 4$ -BrC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>), **8k**  $(R^1 = 4$ -CH<sub>3</sub>C<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>), and **8n**  $(R^1 = 4$ -CH<sub>3</sub>OC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>). Compound **9c** is the highest active compound within the trimethyl derivatives, which indicated that the electrostatic and steric properties have important effects on the antifungal activity.

According to the above SAR analysis, the best R<sup>1</sup>, R and X groups should be *sec*-butyl, CH<sub>3</sub> and CH<sub>3</sub>, respectively. Then, compound **9j** (R<sup>1</sup> = CH(CH<sub>3</sub>)CH<sub>2</sub>CH<sub>3</sub>, R = X = CH<sub>3</sub>) was synthesized and tested its antifungal activity against *R. solani*. The results indicated that the EC<sub>50</sub> value of compound **9j** is  $3.34 \,\mu g \, \text{mL}^{-1}$ , which is much higher than that of compounds **8r** and carbendazim.

### 2.3. CoMFA analysis

3D-QSAR methods [16-18], especially CoMFA [17], are used widely in drug design, because they allow rapid generation of QSARs from which biological activity of newly designed molecules can be predicted. The basic assumption in CoMFA is that an appropriate sampling of the steric and electrostatic fields around a set of aligned molecules might provide all the information necessary for understanding their biological activities. As listed in Table 2, a predictive CoMFA model was established with the conventional correlation coefficient  $r^2 = 0.929$  and the cross-validated coefficient  $q^2 = 0.588$ , the contribution of steric and electrostatic fields are 53.2% and 46.8%, respectively. In Figs. 2 and 3, the isocontour diagrams of the steric and electrostatic field contributions ("stdev\*coeff") obtained from the CoMFA analysis are illustrated together with exemplary ligands. The observed and calculated activity values for all the compounds are shown in Table 1, and the plots of the predicted versus the actual activity values for all the compounds are shown in Fig. 4.

The steric field contour map is plotted in Fig. 2. The green displays<sup>2</sup> positions where a bulky group would be favorable for higher antifungal activity. In contrast, yellow indicates positions where a decrease in the bulk of the target molecules is favored. As shown in Fig. 2, the CoMFA steric contour plots obviously indicated that a yellow region is located around the 3-position and 4-position of the benzyl group. This means that the bulky substituents at 3-position and 4-position will decrease the antifungal activity. For example, some compounds bearing substituents at 3-position of the benzyl group, such as 8c, 8f, 8i, 8m, 9f, and 9g, displayed lower antifungal activity. Compound 9c displayed higher activity than compound **9h**, which might due to the fact that the NO<sub>2</sub> (Es = -2.52) group is more bulky than the F (Es = -0.46) atom. In addition, a green region near the 7-position of the 1,2,4-triazolo[1,5a]pyrimidine ring could be observed obviously, which explained why trimethyl and dimethyl derivatives always displayed higher activity than monomethyl derivatives. The second green region was located near the 2-position of the benzyl group, and the third green region was surrounded the region above the benzyl plane. Compound 8r inserted its

Table 2		
Summary	of CoMFA	analysis

	$q_{\rm cv}^2$	$r_{n-cv}^2$	S	F	Comp.	Contribution	
						Steric	Electrostatic
CoMFA	0.588	0.929	0.066	69.864	6	53.2%	46.8%

branched methyl into this green region. The electrostatic contour plot is shown in Fig. 3. The blue contour defines a region where an increase in the positive charge will result in an increase in the activity, whereas the red contour defines a region of space where increasing electron density is favorable. As shown in Fig. 3, the target compounds bearing an electron-donating group at the *ortho*-position of the benzyl and an electron-withdrawing group at the other *ortho*-position displayed higher activity. In addition, an electron-withdrawing group at the other *para*-position will also improve the antifungal activity.

### 3. Conclusion

In summary, by optimizing the substituents of the formerly discovered lead compound and quantitative structure-activity relationship analysis, a new compound with high antifungal activity (EC<sub>50</sub> =  $3.34 \mu g \, \text{mL}^{-1}$ ) against R. solani, 2-(1-(5-(sec-butylthio)-1,3,4-oxadiazol-2-yl)ethylthio)-5,7-dimethyl-1,2, 4-triazolo[1,5-a]pyrimidine (9j), was developed. Additionally, a 3D-QSAR analysis using the method of CoMFA was performed to explore the comprehensive structure-activity relationships and a statistically reliable model with good predictive power  $(r^2 = 0.929, q^2 = 0.588)$  was achieved on the basis of the common substructure-based alignment. According to the CoMFA model,  $\mathbf{R}^1$  group of the target compound should have an optimum steric effect. When R<sup>1</sup> are substituted benzyl groups, substituents at 3-position of the benzyl are disfavored and substituents at 4position should be electron-withdrawing group having a small steric effect. One of the ortho-substituents of the benzyl should be an electron-withdrawing group; the other one should be an electron-donating group.



Fig. 2. Steric map from the CoMFA model. Compounds **8r** and **9c** are shown inside the field. Sterically favored areas (contribution level of 80%) are represented by green polyhedra. Sterically disfavored areas (contribution level of 20%) are represented by yellow polyhedra. (For interpretation of the references to color in figure legends, the reader is referred to the web version of this article.)

 $<sup>^{2}</sup>$  For interpretation of the references to color in the text, the reader is referred to the web version of this article.



Fig. 3. Electrostatic map from the CoMFA model. Compounds 8r and 9c are shown inside the field. Blue contours (80% contribution) encompass regions where an increase of positive charge will enhance affinity, whereas in red contoured areas (20% contribution) more negative charges are favorable for binding properties.

## 4. Experimental section

### 4.1. Materials

All chemical reagents were commercially available and treated with standard methods before use. Solvents were dried in a routine way and redistilled. 5-Amino-3-mercapto-1,2, 4-triazole **2** and 2-mercapto-5,7-dimethyl-1,2,4-triazolo[1, 5-*a*]pyrimidine **3** were prepared according to the previously reported method [19,20]. *R. solani* was a gift from the courtesy of the Center for Bioassay, The Environment and Plant Protection Institute of Chinese Academy of Tropical Agricultural Sciences.

#### 4.2. Analysis and instruments

Melting points were uncorrected and determined with electro thermal digital melting point apparatus. MS spectra were determined using a Finnigan Trace MS organic mass spectrometry, and signals were given in m/z. <sup>1</sup>H NMR was recorded in CDCl<sub>3</sub> or DMSO on a Varian Mercury 400 MHz, Varian Mercury 300 MHz spectrometer or Bruker AC-P 200 MHz spectrometer and resonance are given in parts per million ( $\delta$ ) relative to TMS. Elementary analyses (EA) were performed on a Vario EL III elementary analysis instrument.



Fig. 4. CoMFA predicted as experimental pEC<sub>50</sub> values.

### 4.2.1. Ethyl 2-(5-amino-1H-1,2,4-triazol-3-ylthio)acetate 4

Sodium hydroxide 0.88 g (22 mmol) was dissolved in 50 mL of water and 2.32 g (20 mmol) of 5-amino-3-mercapto-1,2,4-triazole (**2**) was added. After stirring for 15 min, a solution of 3.34 g (20 mmol) of ethyl bromoacetate in 5 mL of methanol was added dropwise over 20 min. After addition was completed, the solution was stirred at room temperature for about 2 h. The solid was filtered and recrystallized from ethanol to give pure products as a white crystal. Compound **4** – yield: 86%; mp 125–126 °C; <sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  11.87 (s, 1H), 6.04 (s, 2H), 4.05 (q, J = 7.2 Hz, 2H), 3.82 (s, 2H), 1.15 (t, J = 7.2 Hz, 3H).

### 4.2.2. Ethyl 2-(5-methyl-1,2,4-triazolo[1,5-a]pyrimidin-2ylthio)acetate **5a**

A solution of sodium ethoxide in EtOH was prepared by dissolving 0.55 g (24 mmol) of sodium metal in 120 mL of anhydrous EtOH, and 9.7 g (48 mmol) of 2-(5-amino-1*H*-1,2,4triazol-3-ylthio)acetate (**4**) was added. After stirring for 15 min at room temperature, 6.35 g (48.4 mmol) of acetylacetaldehyde dimethyl acetal dissolved in 100 mL of absolute EtOH was added dropwise. After the addition was complete, the reaction mixture was stirred at room temperature for 24 h. The solid was filtered and recrystallized from ethanol to give pure products as a white crystal. Compound **5a** – yield: 80%; mp 114–115 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  8.56 (dd, J = 7.2 Hz, 1H), 6.91 (dd, J = 7.2 Hz, 1H), 4.23 (q, J = 7.2 Hz, 2H), 4.10 (s, 2H), 2.69 (s, 3H), 1.29 (t, J = 7.2 Hz, 3H).

# 4.2.3. Ethyl 2-(5,7-dimethyl-1,2,4-triazolo[1,5-a]pyrimidin-2-ylthio)propanoate **5b**

Sodium hydroxide (22 mmol) dissolved in 50 mL of water and 3.6 g (20 mmol) 5,7-dimethyl-2-mercapto-1,2,4-triazolo[1,5-*a*]pyrimidine (**3**) was added. After stirring for 15 min, a solution of 3.62 g (20 mmol) of ethyl 2-bromopropionate in 5 mL of ethanol was added dropwise over 20 min. After addition was completed, the solution was stirred at room temperature for about 2 h. The solid was filtered and recrystallized from ethanol to give pure products as a white crystal. Compound **5b** – yield: 96%; mp 85–86 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  6.75 (s, 1H), 4.68 (q, J = 7.2 Hz, 1H), 4.21 (q, J = 7.2 Hz, 2H), 2.72 (s, 3H), 2.64 (s, 3H), 1.71 (d, J = 7.2 Hz, 3H), 1.27 (t, J = 6.8 Hz, 3H).

### 4.2.4. 2-(5-Methyl-1,2,4-triazolo[1,5-a]pyrimidin-2-ylthio)acetohydrazide **6a**

A mixture of 12.6 g (0.05 mol) of 2-(5-methyl-1,2,4-triazolo[1,5-*a*]pyrimidin-2-ylthio)acetate (**5**a) and 2.94 g (0.05 mol) of 85% hydrazine monohydrate in 200 mL of EtOH was heated at 60 °C for 4 h. After the reaction mixture was cooled to room temperature, the precipitate was filtered and recrystallized from methanol to give pure product as a white crystal. Compound **6a** – yield: 68%; mp 170– 172 °C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  9.36 (s, 1H), 9.12 (dd, *J* = 6.8 Hz, 1H), 7.20 (dd, *J* = 6.4 Hz, 1H), 4.33 (s, 2H), 3.95 (s, 2H), 2.60 (s, 3H).

# 4.2.5. 2-(5,7-Dimethyl-1,2,4-triazolo[1,5-a]pyrimidin-2-ylthio)propanehydrazide **6b**

A mixture of 14.0 g (0.05 mol) of 2-(5,7-dimethyl-1,2,4-triazolo[1,5-*a*]pyrimidin-2-ylthio)propanoate (**5b**) and 20.6 g (0.35 mol) of 85% hydrazine monohydrate in 200 mL of EtOH was refluxed for 7 h. After the reaction mixture was cooled to room temperature, the precipitate was filtered and recrystallized from methanol to give pure product as a white crystal. Compound **6b** – yield: 79%; mp 104–106 °C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  9.47 (s, 1H), 7.13 (s, 1H), 4.50 (q, *J* = 6.8 Hz, 1H), 4.35 (s, 2H), 2.68 (s, 3H), 2.56 (s, 3H), 1.56 (t, *J* = 7.2 Hz, 3H).

# 4.2.6. 5-(1-(5-Methyl-1,2,4-triazolo[1,5-a]pyrimidin-2ylthio)ethyl)-1,3,4-oxadiazole-2-thiol **7a**

KOH (1.35 g, 24 mmol) was dissolved in 160 mL of anhydrous ethanol and 20 mmol of 2-(5-methyl-1,2,4-triazolo[1,5*a*]pyrimidin-2-ylthio)acetohydrazide (**6a**) was added. To the vigorously stirred solution, 2 g (0.026 mol) of methanedithione dissolved in 20 mL of EtOH was added slowly at room temperature. After addition was completed, the solution was refluxed for 6 h. The solvent was removed under reduced pressure, and residue was dissolved in 100 mL of water. Then it was acidified to pH  $\approx$  6 with glacial acetic acid and filtered to give the crude product, which was recrystallized from methanol to afford pure product as a yellowy crystal. Compound **7a** – yield: 96%; mp 202–204 °C; <sup>1</sup>H NMR (400 MHz, DMSOd<sub>6</sub>):  $\delta$  14.32 (s, 1H), 9.16 (dd, J = 6.8 Hz, 1H), 7.23 (dd, J = 6.8 Hz, 1H), 4.64 (s, 2H), 2.61 (s, 3H).

# 4.2.7. 5-(1-(5,7-Dimethyl-1,2,4-triazolo[1,5-a]pyrimidin-2-ylthio)ethyl)-1,3,4-oxadiazole-2-thiol **7b**

This compound was synthesized in 81% yield from 2-(5,7dimethyl-1,2,4-triazolo[1,5-*a*]pyrimidin-2-ylthio)-propanehydrazide (**6b**) using the similar procedure to that described for **7a**; mp 198–200 °C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  14.38 (s, 1H), 7.16 (s, 1H), 5.12 (q, *J* = 7.2 Hz, 2H), 2.67 (s, 3H), 2.57 (s, 3H), 1.77 (d, *J* = 7.2 Hz, 3H).

# 4.3. General procedure for the synthesis of the target compounds 8*a*-*s*

Sodium hydroxide (3.3 mmol) was dissolved in 15 mL of water and 3 mmol of **7a** was added. After stirring for 15 min, a solution of 3.3 mmol of substituted benzyl halide or alkyl halide in 5 mL of ethanol was added dropwise. The resulted mixture was stirred at room temperature for 2–30 h. The precipitate was filtered off and recrystallized from petroleum ether/acetone to give the pure target compounds **8a–s** in yields of 40–90%.

# 4.4. General procedure for the synthesis of the target compounds **9a**-j

Target compounds  $9\mathbf{a}-\mathbf{j}$  were synthesized in 59-82% yield from  $7\mathbf{b}$  using the similar procedure to that described for  $8\mathbf{a}-\mathbf{s}$ .

Compound **8a** – yield: 76%; yellowy solid, mp 120– 121 °C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  8.53 (dd, J = 6.9 Hz, 1H), 7.24–7.97 (m, 5H), 2.68 (s, 3H), 6.89 (dd, J = 6.9 Hz, 1H), 4.40 (s, 2H), 4.68 (s, 2H); EIMS (probe) 70 eV, m/z (rel. int.): 370 (M<sup>+</sup>, 3), 247 (91), 179 (41), 134 (20), 90 (100). Anal. Calcd for C<sub>16</sub>H<sub>14</sub>N<sub>6</sub>OS<sub>2</sub>: C, 51.87; H, 3.81; N, 22.69. Found: C, 51.61; H, 4.03; N, 22.93.

Compound **8b** – yield: 46%; yellowy solid, mp 127– 128 °C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  8.57 (dd, J = 6.6 Hz, 1H), 7.59–8.16 (m, 4H), 6.95 (dd, J = 6.6 Hz, 1H), 4.69 (s, 2H), 4.47 (s, 2H), 2.71 (s, 3H); EIMS (probe) 70 eV, m/z (ret. int.): 416 (M + 1, 1), 247 (100), 179 (41), 135 (30), 94 (54). Anal. Calcd for C<sub>16</sub>H<sub>13</sub>N<sub>7</sub>O<sub>3</sub>S<sub>2</sub>: C, 46.26; H, 3.15; N, 23.60. Found: C, 46.01; H, 3.40; N, 23.33.

Compound **8c** – yield: 79%; yellowy solid, mp 156– 158 °C; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  8.52 (dd, J = 6.0 Hz, 1H), 2.68 (s.3H), 7.41–8.23 (m, 4H), 6.89 (dd, J = 6.0 Hz, 1H), 4.66 (s, 2H), 4.46 (s, 2H); EIMS (probe) 70 eV, m/z (rel. int.): 415 (M<sup>+</sup>, 1), 247 (100), 179 (51), 94 (72). Anal. Calcd for C<sub>16</sub>H<sub>13</sub>N<sub>7</sub>O<sub>3</sub>S<sub>2</sub>: C, 46.26; H, 3.15; N, 23.60. Found: C, 46.42; H, 2.97; N, 23.84.

Compound **8d** – yield: 90%; yellowy solid, mp 145– 146 °C; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  8.52 (dd, 1H, J = 6.0 Hz), 7.22–8.00 (m, 3H), 6.89 (dd, 1H, J = 6.0 Hz), 4.69 (s, 2H), 4.66 (s, 2H), 2.67 (s, 3H), 2.39 (s, 3H); EIMS (probe) 70 eV, m/z (rel. int.): 430 (7), 429 (M<sup>+</sup>, 1), 247 (100), 179 (67), 135 (46), 94(60). Anal. Calcd for C<sub>17</sub>H<sub>15</sub>N<sub>7</sub>O<sub>3</sub>S<sub>2</sub>: C, 47.54; H, 3.52; N, 22.83. Found: C, 47.28; H, 3.80; N, 22.64.

Compound **8e** – yield: 85%; yellowy solid, mp 124– 125 °C; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  8.51 (dd, J = 6.0 Hz, 1H), 7.14–7.51 (m, 4H), 6.87 (dd, J = 6.0 Hz, 1H), 4.66 (s, 2H), 4.49 (s, 2H), 2.67 (s, 3H); EIMS (probe) 70 eV, m/z (rel. int.): 405 (M + 1, 9), 247 (100), 179 (72), 134 (25), 94 (69). Anal. Calcd for C<sub>16</sub>H<sub>13</sub>ClN<sub>6</sub>OS<sub>2</sub>: C, 47.46; H, 3.24; N, 20.76. Found: C, 47.35; H, 3.52; N, 21.02.

Compound **8f** – yield: 84%; yellowy solid, mp 122– 123 °C; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  8.51 (dd, J = 6.0 Hz, 1H), 7.17–7.34 (m, 4H), 6.87 (dd, J = 6.0 Hz, 1H), 4.66 (s, 2H), 4.35 (s, 2H), 2.67 (s, 3H); EIMS (probe) 70 eV, m/z (rel. int.): 405 (M + 1, 22), 279 (28), 247 (100), 134 (39), 94 (58). Anal. Calcd for C<sub>16</sub>H<sub>13</sub>ClN<sub>6</sub>OS<sub>2</sub>: C, 47.46; H, 3.24; N, 20.76. Found: C, 47.70; H, 3.04; N, 20.97.

Compound **8g** – yield: 85%; yellowy solid, mp 120– 122 °C; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  8.51 (dd, J = 6.0 Hz, 1H), 2.68 (s, 3H), 7.20–7.39 (m, 4H), 6.88 (dd, 1H, J = 6.0 Hz), 4.66 (s, 2H), 4.32 (s, 2H); EIMS (probe) 70 eV, *m*/*z* (rel. int.): 449(M<sup>+</sup>, 1), 247 (21), 179 (26), 169 (100), 134 (7), 94 (29). Anal. Calcd for C<sub>16</sub>H<sub>13</sub>BrN<sub>6</sub>OS<sub>2</sub>: C, 42.77; H, 2.92; N, 18.70. Found: C, 42.96; H, 3.15; N, 18.52.

Compound **8h** – yield: 88%; yellowy solid, mp 123– 124 °C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  8.57 (dd, J = 7.2 Hz, 1H), 7.02–7.49 (m, 4H), 6.93 (dd, J = 7.2 Hz, 1H), 4.70 (s, 2H), 4.46 (s, 2H), 2.70 (s, 3H); EIMS (probe) 70 eV, m/z (rel. int.): 389 (27), 388 (M<sup>+</sup>, 8), 247 (90), 134 (26), 109 (100), 94 (68). Anal. Calcd for C<sub>16</sub>H<sub>13</sub>FN<sub>6</sub>OS<sub>2</sub>: C, 49.47; H, 3.37; N, 21.64. Found: C, 49.21; H, 3.50; N, 21.89. Compound **8i** – yield: 87%; yellowy solid, mp 121– 122 °C; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  8.52 (dd, 1H, J = 6.0 Hz), 6.95–7.24 (m, 4H), 6.88 (dd, J = 6.0 Hz, 1H), 4.66 (s, 2H), 4.37 (s, 2H), 2.67 (s, 3H); EIMS (probe) 70 eV, m/z (rel. int.): 388 (M<sup>+</sup>, 1), 247 (48), 179 (25), 109 (100), 94 (28). Anal. Calcd for C<sub>16</sub>H<sub>13</sub>FN<sub>6</sub>OS<sub>2</sub>: C, 49.47; H, 3.37; N, 21.64. Found: C, 49.70; H, 3.12; N, 21.90.

Compound **8j** – yield: 90%; yellowy solid, mp 127– 129 °C; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  8.51 (dd, J = 6.0 Hz, 1H), 6.94–7.35 (m, 4H), 6.87 (dd, J = 6.0 Hz, 1H), 4.66 (s, 2H), 4.36 (s, 2H), 2.68 (s, 3H); EIMS (probe) 70 eV, m/z (rel. int.): 388 (M<sup>+</sup>, 1), 247 (92), 179 (25), 109 (100), 94 (14). Anal. Calcd for C<sub>16</sub>H<sub>13</sub>FN<sub>6</sub>OS<sub>2</sub>: C, 49.47; H, 3.37; N, 21.64. Found: C, 49.25; H, 3.50; N, 21.81.

Compound **8k** – yield: 85%; yellowy solid, mp 94–95 °C; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  8.50 (dd, J = 6.0 Hz, 1H), 7.03–7.23 (m, 4H), 6.87 (dd, J = 6.0 Hz, 1H), 4.66 (s, 2H), 4.36 (s, 2H), 2.67 (s, 3H), 2.29 (s, 3H); EIMS (probe) 70 eV, *m*/*z* (rel. int.): 384 (M<sup>+</sup>, 1), 280 (2), 247 (11), 179 (13), 138 (2), 134 (7), 125 (2), 120 (11), 105 (100), 94 (29), 77 (12). Anal. Calcd for C<sub>17</sub>H<sub>16</sub>N<sub>6</sub>OS<sub>2</sub>: C, 53.11; H, 4.19; N, 21.86. Found: C, 53.26; H, 4.00; N, 21.53.

Compound **81** – yield: 77%; yellowy solid, mp 89–90 °C; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  8.51 (dd, J = 6.0 Hz, 1H), 7.12–7.30 (m, 4H), 6.87 (dd, J = 6.0 Hz, 1H), 4.67 (s, 2H), 4.42 (s, 2H), 2.67 (s, 3H), 2.38 (s, 3H); EIMS (probe) 70 eV, m/z (rel. int.): 384 (M<sup>+</sup>, 1), 247 (42), 105 (100), 94 (15). Anal. Calcd for C<sub>17</sub>H<sub>16</sub>N<sub>6</sub>OS<sub>2</sub>: C, 53.11; H, 4.19; N, 21.86. Found: C, 49.98; H, 4.32; N, 21.98.

Compound **8m** – yield: 85%; yellowy solid, mp 92–93 °C; <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  8.50 (dd, J = 6.0 Hz, 1H), 7.04–7.22 (m, 4H), 6.87 (dd, J = 6.0 Hz, 1H), 4.67 (s, 2H), 4.36 (s, 2H), 2.67 (s, 3H), 2.31 (s, 3H); EIMS (probe) 70 eV, *m*/*z* (rel. int.): 384 (M<sup>+</sup>, 50), 279 (22), 247 (100), 179 (79), 135 (73), 94 (72). Anal. Calcd for C<sub>17</sub>H<sub>16</sub>N<sub>6</sub>OS<sub>2</sub>: C, 53.11; H, 4.19; N, 21.86. Found: C, 53.39; H, 4.50; N, 21.67.

Compound **8n** – yield: 58%; yellowy solid, mp 220– 222 °C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  8.58 (dd, J = 7.5 Hz, 1H), 6.94–8.00 (m, 4H), 6.93 (dd, J = 7.5 Hz, 1H), 4.86 (s, 2H), 4.70 (s, 2H), 3.89 (s, 3H), 2.70 (s, 3H); EIMS (probe) 70 eV, m/z (rel. int.): 428 (M<sup>+</sup>, 1), 247 (72), 135 (100), 94 (15). Anal. Calcd for C<sub>18</sub>H<sub>16</sub>N<sub>6</sub>O<sub>3</sub>S<sub>2</sub>: C, 50.54; H, 3.76; N, 19.61. Found: C, 50.09; H, 4.03; N, 19.36.

Compound **80** – yield: 57%; yellowy solid, mp 108– 109 °C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  8.59 (dd, J = 7.2 Hz, 1H), 6.93 (dd, J = 7.2 Hz, 1H), 4.70 (s, 2H), 2.71 (s, 3H), 2.68 (s, 3H); EIMS (probe) 70 eV, m/z (rel. int.): 294 (M<sup>+</sup>, 19), 247 (100), 179 (38), 135 (32), 94 (84). Anal. Calcd for C<sub>10</sub>H<sub>10</sub>N<sub>6</sub>OS<sub>2</sub>: C, 40.80; H, 3.42; N, 28.55. Found: C, 40.53; H, 3.58; N, 28.86.

Compound **8p** – yield: 50%; yellowy solid, mp 103– 108 °C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  8.58 (dd, J = 6.6 Hz, 1H), 6.93 (dd, J = 6.6 Hz, 1H), 4.71 (s, 2H), 3.22 (q, 2H, J = 7.2 Hz), 2.70 (s, 3H), 1.44 (t, 3H, J = 7.2 Hz); EIMS (probe) 70 eV, m/z (rel. int.):308 (M<sup>+</sup>, 1), 247 (70), 179 (26), 135 (17), 94(21). Anal. Calcd for C<sub>11</sub>H<sub>12</sub>N<sub>6</sub>OS<sub>2</sub>: C, 42.84; H, 3.92; N, 27.25. Found: C, 43.03; H, 3.76; N, 27.07.

Compound **8q** – yield: 34%; yellowy solid, mp 132– 134 °C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  8.58 (dd, J = 6.9 Hz, 1H), 6.93 (dd, 1H, J = 6.9 Hz), 4.69 (s, 2H), 3.18 (t, J = 7.2 Hz, 2H), 2.68 (s, 3H), 1.72 (m, J = 7.5 Hz, 2H), 1.43 (m, J = 7.5 Hz, 2H), 0.90 (t, J = 7.2 Hz, 3H); EIMS (probe) 70 eV, m/z (rel. int.): 336 (M<sup>+</sup>, 2), 247 (100), 179 (35), 135 (19), 94 (34). Anal. Calcd for C<sub>13</sub>H<sub>16</sub>N<sub>6</sub>OS<sub>2</sub>: C, 46.41; H, 4.79; N, 24.98. Found: C, 46.54; H, 4.96; N, 25.10.

Compound **8r** – yield: 32%; yellowy solid, mp 149– 150 °C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  8.59 (dd, J = 6.6 Hz, 1H), 6.94 (dd, J = 6.9 Hz, 1H), 4.72 (s, 2H), 3.70 (q, J = 6.6 Hz, 2H), 2.71 (s, 3H), 1.72 (m, J = 7.5 Hz, 2H), 1.4 (d, J = 7.5 Hz, 2H), 1.02 (t, J = 7.2 Hz, 3H); EIMS (probe) 70 eV, m/z (rel. int.): 337 (56), 336 (M<sup>+</sup>, 45), 247 (100), 179 (66), 135 (57), 94 (55). Anal. Calcd for C<sub>13</sub>H<sub>16</sub>N<sub>6</sub>OS<sub>2</sub>: C, 46.41; H, 4.79; N, 24.98. Found: C, 46.56; H, 4.53; N, 24.61.

Compound **8s** – yield: 73%; yellowy solid, mp 157– 158 °C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  8.59 (dd, J = 6.6 Hz, 1H), 6.94 (dd, J = 6.9 Hz, 1H), 4.70 (s, 2H), 4.22 (q, J = 7.2 Hz, 2H), 4.02 (s, 2H), 2.70 (s, 3H), 1.27 (t, J = 7.2 Hz, 3H); EIMS (probe) 70 eV, m/z (rel. int.): 366 (M<sup>+</sup>, 24), 279 (34), 247 (100), 135 (29), 134 (46), 94 (42). Anal. Calcd for C<sub>13</sub>H<sub>14</sub>N<sub>6</sub>O<sub>3</sub>S<sub>2</sub>: C, 42.61; H, 3.85; N, 22.94. Found: C, 42.53; H, 4.02; N, 22.69.

Compound **9a** – yield: 82%; white solid, mp 126–128 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.25–7.35 (m, 4H), 6.76 (s, 1H), 5.39 (q, J = 7.2 Hz, 1H), 4.37 (s, 2H), 2.70 (s, 3H), 2.67 (s, 3H), 1.91 (d, J = 7.2 Hz, 3H); EIMS (probe) 70 eV, *m*/*z* (rel. int.): 433 (M<sup>+</sup>, 8), 275 (76), 219 (55), 180 (73), 134 (73), 125 (100), 107 (65), 89 (62). Anal. Calcd for C<sub>18</sub>H<sub>17</sub>ClN<sub>6</sub>OS<sub>2</sub>: C, 49.93; H, 3, 96; N, 19.41. Found: C, 49.78; H, 3.85; N, 19.72.

Compound **9b** – yield: 80%; white solid, mp 130–132 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.03–7.50 (m, 4H), 6.76 (s, 1H), 5.41 (q, J = 7.2 Hz, 1H), 4.44 (s, 2H), 2.71 (s, 3H), 2.64 (s, 3H), 1.91 (d, J = 7.2 Hz, 3H); EIMS (probe) 70 eV, *m*/*z* (rel. int.): 417 (M<sup>+</sup>, 8), 275 (56), 219 (47), 180 (53), 134 (56), 109 (100), 89 (10). Anal. Calcd for C<sub>18</sub>H<sub>17</sub>FN<sub>6</sub>OS<sub>2</sub>: C, 51.91; H, 4.11; N, 20.18. Found: C, 51.76; H, 4.35; N, 20.07.

Compound **9c** – yield: 76%; white solid, mp 97–99 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  6.96–7.40 (m, 4H), 6.77 (s, 1H), 4.39 (s, 2H), 5.40 (q, J = 7.2 Hz, 1H), 2.71 (s, 3H), 2.65 (s, 3H), 1.91 (d, J = 7.2 Hz, 3H); EIMS (probe) 70 eV, m/z (rel. int.): 417 (M<sup>+</sup>, 3), 275 (48), 219 (20), 180 (31), 109 (100), 89 (7). Anal. Calcd for C<sub>18</sub>H<sub>17</sub>FN<sub>6</sub>OS<sub>2</sub>: C, 51.91; H, 4.11; N, 20.18. Found: C, 52.12; H, 4.25; N, 20.03.

Compound **9d** – yield: 75%; white solid, mp 125–126 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.23–7.40 (m, 4H), 6.76 (s, 1H), 5.40 (q, J = 7.2 Hz, 1H), 4.38 (s, 2H), 2.71 (s, 3H), 2.64 (s, 3H), 1.91 (d, J = 7.2 Hz, 3H); EIMS (probe) 70 eV, m/z (rel. int.): 433 (M<sup>+</sup>, 1), 219 (3), 180 (6), 134 (5), 125 (100), 89 (34). Anal. Calcd for  $C_{18}H_{17}ClN_6OS_2$ : C, 49.93; H, 3, 96; N, 19.41. Found: C, 49.69; H, 4.15; N, 19.25.

Compound **9e** – yield: 78%; white solid, mp 126–128 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.14–7.36 (m, 4H), 6.76 (s, 1H), 5.42 (q, J = 7.2 Hz, 1H), 4.46 (s, 2H), 2.72 (s, 3H), 2.64 (s, 3H), 2.41 (s, 3H), 1.92 (d, J = 7.2 Hz, 3H); EIMS (probe) 70 eV, m/z (rel. Int.): 412 (M<sup>+</sup>, 1), 275 (20), 219 (8), 180 (11), 105 (100), 89 (3). Anal. Calcd for C<sub>19</sub>H<sub>20</sub>N<sub>6</sub>OS<sub>2</sub>: C, 55.32; H, 4.89; N, 20.37. Found: C, 55.14; H, 4.78; N, 20.56.

Compound **9f** – yield: 78%; white solid, mp 132–133 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.09–7.27 (m, 4H), 6.76 (s, 1H), 5.41 (q, J = 7.2 Hz, 1H), 4.39 (s, 2H<sub>2</sub>), 2.71 (s, 3H), 2.65 (s, 3H), 2.33 (s, 3H), 1.91 (d, J = 7.2 Hz, 3H); EIMS (probe) 70 eV, *m*/*z* (rel. int.): 412 (M<sup>+</sup>, 1), 219 (3), 180 (4), 134 (4), 105 (100), 89 (4); Anal. Calcd for C<sub>19</sub>H<sub>20</sub>N<sub>6</sub>OS<sub>2</sub>: C, 55.32; H, 4.89; N, 20.37; Found: C, 55.45; H, 4.98; N, 20.27.

Compound **9g** – yield: 50%; white solid, mp 121–122 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  6.94–7.27 (m, 4H), 6.76 (s, 1H), 5.41 (q, J = 7.2 Hz, 1H), 4.40 (s, 2H), 3.80 (s, 3H), 2.71 (s, 3H), 2.64 (s, 3H), 1.91 (d, J = 7.2 Hz, 3H); EIMS (probe) 70 eV, m/z (rel. int.): 428 (M<sup>+</sup>, 3), 219 (5), 180 (14), 134 (15), 121 (100), 89 (9); Anal. Calcd for C<sub>19</sub>H<sub>20</sub>N<sub>6</sub>O<sub>2</sub>S<sub>2</sub>: C, 53.25; H, 4.70; N, 19.61; Found: C, 53.56; H, 4.98; N, 19.38.

Compound **9h** – yield: 80%; white solid, mp 128–129 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.60–8.16 (m, 4H), 6.77 (s, 1H), 5.39 (q, J = 7.2 Hz, 1H), 4.47 (s, 2H), 2.71 (s, 3H), 2.65 (s, 3H), 1.90 (d, J = 7.2 Hz, 3H); EIMS (probe) 70 eV, m/z (rel. int.):443 (M<sup>+</sup>, 1), 275 (15), 219 (18), 180 (37), 134 (37), 107 (37), 89 (59), 78 (100). Anal. Calcd for C<sub>18</sub>H<sub>17</sub>N<sub>7</sub>O<sub>3</sub>S<sub>2</sub>: C, 48.75; H, 3.86; N, 22.11. Found: C, 48.98; H, 3.75; N, 22.23.

Compound **9i** – yield: 59%; white solid, mp 97–99 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.10–7.29 (m, 4H), 6.76 (s, 1H), 5.40 (q, J = 7.2 Hz, 1H), 4.39 (s, 2H), 2.71 (s, 3H), 2.64 (s, 3H), 2.32 (s, 3H), 1.91 (d, J = 7.2 Hz, 3H); EIMS (probe) 70 eV, m/z (rel. int.): 412 (M<sup>+</sup>, 1), 275 (6), 219 (5), 180 (17), 134 (15), 105 (100), 89 (5). Anal. Calcd for C<sub>19</sub>H<sub>20</sub>N<sub>6</sub>OS<sub>2</sub>: C, 55.32; H, 4.89; N, 20.37. Found: C, 55.56; H, 5.08; N, 20.17.

Compound **9j** – yield: 52%; white solid, mp125–126 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  6.67 (s, 1H), 5.41 (q, J = 7.2 Hz, 1H), 3.69 (q, J = 6.8 Hz, 1H), 2.73 (s, 3H), 2.64 (s, 3H), 1.93 (d, J = 7.2 Hz, 3H), 1.74 (m, 2H), 1.43 (d, J = 6.8 Hz, 3H), 1.00 (t, J = 7.2 Hz, 3H); EIMS (probe) 70 eV, m/z (rel. int.): 365 ([M + 1]<sup>+</sup>, 3), 275 (42), 233 (6), 207 (19), 180 (41), 148 (38), 124 (5), 107 (84), 92 (17). Anal. Calcd for C<sub>15</sub>H<sub>20</sub>N<sub>6</sub>OS<sub>2</sub>: C, 49.43; H, 5.53; N, 23.06. Found: C, 49.56; H, 5.25; N, 23.29.

### 4.5. Bioassays of fungicidal activities

The antifungal activities were tested according to our previous methods [10,11]. The tested samples were dissolved in 0.5 mL of DMF at a concentration of 500 mg L<sup>-1</sup>. The solutions (1 mL) were mixed rapidly with thawed potato glucose agar culture medium (9 mL) under 50 °C. The mixtures were poured into Petri dishes. After the dishes were cooled, the solidified plates were incubated with 4 mm mycelium disk, inverted, and incubated at 28 °C for 48 h. The mixed medium without sample was used as the blank control. Three replicates of each test were carried out. The mycelial elongation radius (mm) of fungi settlements was measured after 48 h of culture. The growth inhibition rates were calculated with the following equation:  $I = [(C - T)/C] \times 100\%$ . Here, *I* is the growth inhibition rate (%), *C* is the control settlement radius (mm), and *T* is the treatment group fungi settlement radius (mm). The molar concentration of each compound required to inhibit the mycelial growth to half the length of the control (EC<sub>50</sub> value) was evaluated by the probit method [21]. The results are summarized in Table 2.

### 4.6. CoMFA analysis

The 3D structures of all compounds were built and minimized by using SYBYL 7.0 on a Silicon Graphics Fuel workstation. The geometries of all molecules involved in this study were fully optimized by using the PM3 method. The lowest-energy conformations were considered as the bioactive conformations. A useful kind of net atomic charges called electrostatic potential (ESP)-fitting charges were derived from the PM3 calculated molecular electrostatic potential distribution. All the nitrogen and oxygen atoms in 1,3,4-oxadiazole and 1,2,4-triazolo[1,5-a]-pyrimidine rings were selected as the atoms to superimpose all the compounds using an atom-by-atom least-squares fit implemented in the "SYBYL FIT" option in SYBYL. Compound 8r with the best biological activity was selected as the reference molecule. The CoMFA descriptors, steric and electrostatic field energies, were calculated using the SYBYL default parameters: 2 Å grid points spacing, an  $sp^3$  carbon probe atom with +1 charge and a minimum  $\sigma$  (column filtering) of 2.0 kcal mol<sup>-1</sup>, and an energy cutoff of  $30.0 \text{ kcal mol}^{-1}$ .

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