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Discovery of a new insecticide lead by optimizing a target-diverse scaffold: Tetrazolinone derivatives

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Abstract—In order to discover lead compounds with novel action mechanism, a series of tetrazolinone derivatives bearing structurally diverse substituents, 1-aryl-4-substituted-1,4-di-hydro-5*H*-tetrazol-5-ones **2**, 1-((5-(alkylthio)-1,3,4-oxadiazol-2-yl)methyl)-4-(substituted)- phenyl-1*H*-tetrazol-5(4*H*)-ones **5**, and 1-((5-(alkylthio)-1,3,4-thiadiazol-2-yl)methyl)-4- (substituted)phenyl-1*H*-tetrazol-5(4*H*)-ones **7**, were designed and synthesized in good yields by a multiple-step synthetic procedure. The results of greenhouse in vivo test indicated that all the target compounds did not displayed herbicidal activity, however, some of them exhibited excellent in vivo insecticidal activity against *Tetranychus cinnabarinus* at the concentration of 250 mg L^{-1}. To our knowledge, this is the first report about the insecticidal activity of tetrazolinone derivatives, which indicated that the tetrazolinone scaffold could be identified as a novel insecticidal lead structure. The present work demonstrated that optimizing a target-diverse scaffold is an effective way to discover new lead compounds with new action mechanism or biological activity.

1. Introduction

The discovery of new lead structure is one of the most important topics of research in medicinal and pesticide chemistry. Among various biologically heterocyclic scaffolds, tetrazolinone derivatives attracted considerable attentions from pesticide chemists in last decade due to their promising herbicidal activity.[1] For example, fentrazamide,[2] a commercial herbicide as shown in Figure 1, was highly effective against many weeds by inhibiting the cell division. Additionally, 1-aryl-4-(3-fluoropropyl)-tetrazolinones (compound **1b** in Fig. 1) were developed as a new and highly active family of protoporphyrinogen oxidase-inhibiting herbicides.[3] We noticed that fentrazamide and compound **1b** have the same scaffold, but different substituent at 4-position resulted in these two compounds having different action mechanism. This observation indicated that tetrazolinone might be regarded as a 'target-diverse' scaffold and R group at 4-position might have dramatically influenced the action mechanism. If introducing a suitable substituent into 4-position of this scaffold, it is possible to discover new lead compounds with novel action mechanism or new biological activity.

1,3,4-Thiadiazole and 1,3,4-oxadiazole derivatives have been approved to be important scaffolds with broad-spectrum biological activities for many years. For example, some 1,3,4-thiadiazole and 1,3,4-oxadiazole derivatives displayed antiproliferative,[4] bacteriostatic,[5] hypoglycemic,[6] antibacterial,[7] and anti-inflammatory activity.[8,9] In addition, broad-spectrum insecticidal,[10] herbicidal,[11] and fungicidal activities[12] were also found to be associated with 1,3,4-thiadiazole and 1,3,4–oxadiazole derivatives.

As a part of our extensive program to discover lead compound with novel action target, we designed and synthesized the title compounds, 1-((5-(alkylthio)-1,3,4-oxadiazol-2-yl)methyl)-4-(substituted)phenyl-1*H*-tetrazol-5(4*H*)-ones **5**, and 1-((5-(alkylthio)-1,3,4-thiadiazol-2-yl)-methyl)-4-(substituted)phenyl-1*H*-tetrazol-5(4*H*)-ones **7**, by introducing various substituents including 1,3,4-thiadiazole and 1,3,4-oxadiazole moieties into the 4-position of tetrazolinone scaffold. The preliminary in vitro bioassay against six kinds of weed indicated that some derivatives displayed good inhibition activity against the stem or root growth, however, further

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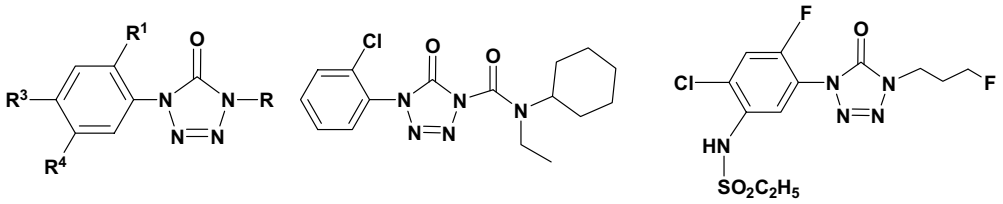


1 Fentrazamide (1a) 1b

Figure 1. General structures of herbicidal tetrazolinones.

greenhouse in vivo test indicated that all these compounds displayed no herbicidal activity. Interestingly, some compounds were found to exhibit good in vivo insecticidal activities against *Tetranychus cinnabarinus* at the dosage of 250 mg L^{-1}. To our knowledge, this is the first report about the insecticidal activity of tetrazolinone derivatives.

2. Results and discussion

2.1. Chemistry

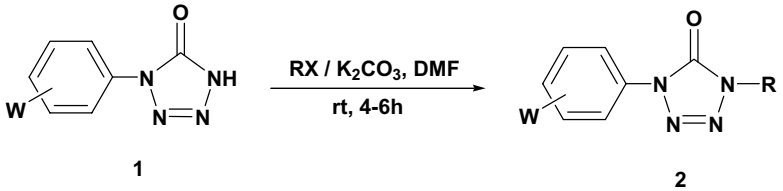
The target compounds **5a**–**d** and 7a–o were synthesized by a five-step procedure. First, the starting material, 1-substituted phenyl-1*H*-tetrazol-5(4*H*)-ones, was prepared by the reaction of substituted phenylisocyanate with sodium azide in *N*,*N*-dimethylformamide (DMF) according to the existing method.[13] Treatment of tetrazolinones **1** with alkyl halides or substituted benzyl halides in the presence of K_2CO_3 in DMF solution at room temperature afforded 1-aryl-4-substituted-1,4-dihydro-5*H*-tetrazolin-5-ones **2a**–**o** in good yields (Scheme 1).

Refluxing compound **2e** ($R = CH_2COOC_2H_5$, W = 4-Cl) with hydrazine hydrate in ethanol for 4 h afforded 2-(4-(4-chlorophenyl)-5-oxo-4,5-dihydrotetrazol-1-yl)acetohydrazide **3**. It should be noted that treatment of compound **3** with carbon disulfide under different reaction conditions afforded different products. Under refluxing condition in the presence of potassium hydroxide, 5-mercapto-1,3,4-oxadiazoles **4** were obtained. However, 5-mercapto-1,3,4-thiadiazoles **6** were obtained when the reaction was carried out at room temperature (Scheme 2). After having two key intermediates **4** and **6** in hand, the target compounds 1-((5-(alkylthio)-1,3,4-oxadiazol-2-yl)methyl)-4-(substituted)phenyl-1*H*-tetrazol-5(4*H*)-ones **5**, and 1-((5-(alkylthio)-1,3,4-thiadiazol-2-yl)methyl)-4-(substituted)-phenyl-1*H*-tetrazol-5(4*H*)-ones**7**, were prepared easily by the nucleophilic substitution reaction of **4** and **6** with alkyl halides or substituted benzyl halides, respectively. The structures of all of the title compounds were confirmed by ^1H NMR, MS, and elemental analyses.

2.2. Bioactivity

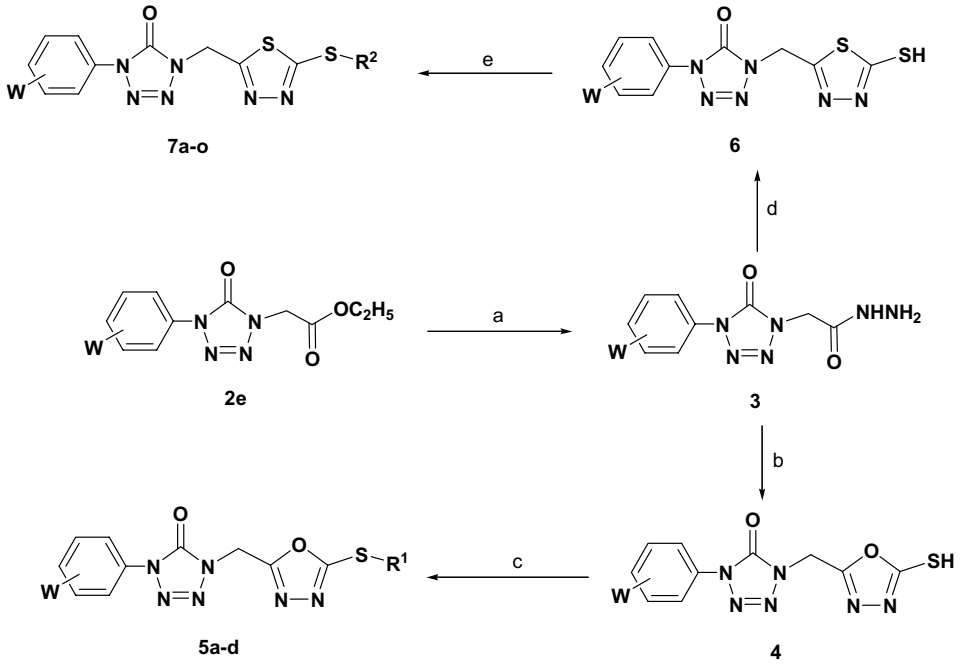
The inhibition activities of the title compounds against the stem and root growth of some weeds, such as wheat (*Triticum aestivum*), barnyard grass (*Echinochloa crus-galli*), jowar (*Sorghum bicolor*), rape (*Brassica campestris*), radish (*Raphanus sativus*), and cucumber (*Cucumis sativus*), were determined using the reported method.[14] The results as shown in Table 1 indicated that some of the tested compounds displayed good inhibition activities against the stem or root growth of tested weeds at the concentration of 100 mg L^{-1}. For example, compounds **2c** and **7l** displayed 80% inhibition against both root and stem growth of wheat, compound **7c** displayed 80% inhibition against both root and stem growth of barnyard grass, compounds **2g** and **7h** displayed over 80% inhibition activities against both root and stem growth of rape, compounds **2c** and **4** displayed over 80% inhibition activities against both root and stem growth of radish. Additionally, compound **2e** displayed 95% inhibition activities against the root growth of jowar. It should be noted that compound **7** seems to display broader spectrum activities than other compounds. However, unfortunately, the further greenhouse experiment indicated that all these compounds did not exhibit herbicidal activity at the dosage of 450 g ai/ha.

The insecticide activity results of all target compounds against *Aphis medicagini*, *Nilaparvata lugen*, *Mythima separata* and *Tetranychus cinnabarinus* are shown in Table 2, two commercial products, Dichlorvos and Pyridabea, were used as controls. As shown in Table 2, some compounds displayed excellent insecticide activities



1 2

Scheme 1. Syntheses of 1-aryl-4-substituted-1,4-dihydro-5*H*-tetrazol-5-ones.



Scheme 2. Syntheses of 1-((5-(alkylthio)-1,3,4-oxa(thia)diazol-2-yl)methyl)-4-(substituted)-phenyl-1*H*-tetrazol-5(4*H*)-ones. Reagents and conditions: (a)$NH_2NH_2{\cdot}H_2O$, EtOH, reflux, 4 h; (b) CS_2, KOH, EtOH, reflux, 24 h, HOAc ; (c) R^1Br, DMF, K_2CO_3, rt, 6–8 h; (d) CS_2, KOH, EtOH, rt, 24 h; H_2SO_4, NaOH, HCl; (e) R^2 X, DMF, K_2CO_3, rt, 4–8 h.

against *T. cinnabarinus* or *A. medicagini*, *M. separata*. For example, at the concentration of 250 mg L^{-1}, compounds **2a**, **2b**, **2e**, **2o**, **5a**, and **7g** showed excellent insecticide activity against *T. cinnabarinus*, and compound **7b** showed excellent insecticide activity against *A. medicagini*, compound **2c** showed excellent insecticide activity against *M. separata*. However, all the title compounds had no or very low insecticide activity against *N. legen*. Additionally, we can find from Table 2 that the insecticide activities of the target compounds were influenced by the substituents on 4-position of tetrazolinone. For example, some compounds bearing methyl, ethyl, allyl, ester group, and chromenone-3-methene displayed good insecticide activity, while compounds bearing benzyl always showed no or lower insecticide activity. To our knowledge, this is the first report about the insecticide activity of tetrazolinone derivatives. Further studies on structural optimization, structure-insecticide activity relationships, and insecticidal mode of action about this scaffold are under way.

3. Conclusion

In conclusion, by introducing various substituents into the 4-position of tetrazolinone derivatives, the scaffold of tetrazolinone, which originally displayed herbicidal activities, was identified as a novel insecticide lead structure for the first time. Previously, it has been identified that the herbicidal tetrazolinone derivatives killed weeds by inhibiting the cell division or the activity of protoporphyrinogen oxidase. The mechanism of insect cell division is different greatly from that of weed and there is no protoporphyrinogen oxidase in the body of insects. Therefore, the original herbicidal scaffold displaying insecticide activity implied a potential new action mechanism. In a word, the present work demonstrated that optimizing a target-diverse scaffold is an effective way to discover new lead compounds with new action mechanism or biological activity.

4. Experimental

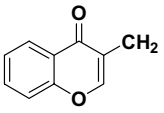
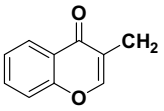
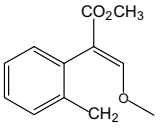
4.1. Materials

All chemical reagents were commercially available and treated with standard methods before use. Solvents were dried in a routine way and redistilled. *Nilaparvata lugen* (*N. lugen*), *Tetranychus cinnabarinus* (*T. cinnabarinus*), *Aphis medicagini* (*A. medicagini*), *Mythima separata* (*M. separata*), and wheat (*Triticum aestivum*), barnyard grass (*Echinochloa crusgalli*), jowar (*Sorghum bicolor*), rape (*Brassica campestris*), radish (*Raphanus sativus*), cucumber (*Cucumis sativus*) were provided through the courtesy of the Center for Bioassay, Zhejiang Chemical Industry Research Institute.

4.2. Analysis and instruments

Melting points are uncorrected and determined with Electro thermal digital melting point apparatus. MS spectra were determined using a Finnigan Trace MS organic mass spectrometry, and signals was given in m/z. ^{1}H NMR are recorded in $CDCl_3$ or DMSO on a Varian Mercury 400 MHz spectrometer and resonance are given in ppm (δ) relative to TMS. Elementary analyses (EA) were performed on a Vario EL III elementary analysis instrument.

Table 1. The results of inhibition activities of the title compounds against stem and root growth (100 mg L^{-1})

Compound	W	R (R^1 or R^2)	*T. aestivum*		*E. crusgalli*		*S. bicolor*		*B. campestris*		*R. sativus*		*C. sativus*	
			Stem	Root	Stem	Root	Stem	Root	Stem	Root	Stem	Root	Stem	root
2a	2,4-Cl_2, 5-OCH_3	CH_3	50	70	30	30	30	70	0	0	0	0	60	60
2b	2-Cl	CH_2=$CHCH_2$	50	50	0	0	0	0	60	0	0	0	60	0
2c	4-Cl	CH_2=$CHCH_2$	**80**	**80**	50	0	60	60	50	50	**80**	**80**	30	40
2d	4-Cl	$CH_2CO_2C_2H_5$	30	30	0	0	20	20	70	85	40	80	0	0
2e	4-Cl	$CH(CH_3)CO_2C_2H_5$	70	**80**	0	0	40	**95**	0	0	50	70	0	0
2f	4-Cl	2-CH_3-$C_6H_5CH_2$	30	0	0	0	0	0	0	0	0	0	0	0
2g	4-Cl	3-CH_3-$C_6H_5CH_2$	0	0	0	0	0	0	**90**	**80**	70	**80**	0	0
2h	4-Cl	4-CH_3-$C_6H_5CH_2$	0	0	0	0	0	0	0	0	0	0	0	0
2i	4-Cl	2-F-$C_6H_5CH_2$	0	0	0	0	0	0	0	0	0	0	0	0
2j	4-Cl	3-F-$C_6H_5CH_2$	40	40	0	70	0	0	0	0	0	0	30	50
2k	4-Cl	3-Cl-$C_6H_5CH_2$	0	0	0	0	0	0	0	0	0	0	0	0
2l	4-Cl	4-Br-$C_6H_5CH_2$	30	0	0	0	0	0	0	0	0	0	0	0
2m	4-Cl	3-CH_3O-$C_6H_5CH_2$	0	0	0	0	0	0	0	0	0	0	0	0
2n	2-Cl		30	0	0	0	30	50	30	50	30	50	0	0
2o	4-Cl		30	0	0	0	0	0	0	0	0	0	0	0
4		***I***	0	0	0	0	0	60	70	**80**	**80**	**90**	0	0
5a	4-Cl	CH_2=$CHCH_2$	0	0	0	0	0	0	70	**80**	60	60	50	60
5b	4-Cl	$CH_2CO_2C_2H_5$	0	0	0	0	0	0	60	50	0	50	0	0
5c	4-Cl	$CH(CH_3)CO_2C_2H_5$	0	0	0	0	0	0	60	**80**	0	0	0	0
5d	4-Cl	C_8H_{17}	0	0	0	0	0	0	0	0	0	0	0	0
6		***I***	70	70	70	70	60	60	70	70	70	**80**	60	60
7a	4-Cl	CH_3	50	60	0	30	40	60	70	0	50	50	60	40
7b	4-Cl	C_2H_5	40	50	70	60	60	70	60	60	40	50	30	0
7c	4-Cl	C_3C_7	70	70	**80**	**80**	0	**80**	60	70	70	70	70	50
7d	4-Cl	C_4H_9	40	40	30	70	40	50	40	50	0	30	0	0
7e	4-Cl	C_6H_{13}	30	0	0	0	40	60	40	70	30	40	0	0
7f	4-Cl	C_8H_{17}	0	30	0	40	0	50	0	40	0	40	0	30
7g	4-Cl	$CH_2CO_2C_2H_5$	60	50	0	0	50	30	50	70	60	70	30	50
7h	4-Cl	$CH(CH_3)CO_2C_2H_5$	50	70	40	50	70	**80**	**80**	**85**	50	**80**	60	60
7i	4-Cl	CH_2=$CHCH_2$	40	50	70	60	0	60	40	60	40	40	0	0
7j	4-Cl	$C_6H_5CH_2$	60	60	70	60	70	60	50	60	40	60	60	60
7k	4-Cl	2-CH_3-$C_6H_4CH_2$	60	60	70	60	30	0	30	50	40	40	0	0
7l	4-Cl	2-F-$C_6H_4CH_2$	**80**	**80**	70	60	70	70	70	**80**	60	**80**	70	70
7m	4-Cl	3-F-$C_6H_4CH_2$	70	40	70	50	40	40	0	40	50	50	30	30
7n	4-Cl	3-Cl-$C_6H_4CH_2$	70	60	0	0	50	40	0	0	30	50	40	0
7o	4-Cl		40	0	0	30	0	60	30	30	30	60	30	30

4.2.1. General procedure for the preparation of 1-aryl-4-substituted-1,4-dihydro-5*H*-tetrazol-5-ones (2). A mixture of 1-aryl-1*H*-tetrazol-5(4*H*)-one (**1**) (0.1 mol), the appropriate alkyl halide or substituted benzyl halide (0.11 mol), and anhydrate potassium carbonated (0.2 mol) in DMF (25 mL) was stirred for 4–6 h according to thin-layer chromatographic (TLC) analysis monitored, and then ice water was added to the reaction mixture to form precipitate, then filtered, washed with water, dried, and recrystallized from ethanol to afford compound **2**:

Compound **2a**: Yield: 47%; mp 155–157 °C (lit.,[3] 158–159 °C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45 (m, 2H), 3.91 (s, 3H), 3.72 (s, 3H).

Compound **2b**: Yield: 87%; oil (lit.,[3] oil); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43–7.60 (m, 4H), 5.99–6.06 (m, 2H), 5.37–5.41 (m, 1H), 4.65–4.67 (m, 2H); EIMS (probe) 70 eV, m/z (rel. int.): 237 $[M]^+$ (38), 152 (100), 124 (46), 89 (54). Anal. Calcd for $C_{10}H_9ClN_4O$: C, 50.75; H, 3.83; N, 23.67. Found: C, 50.60; H, 3.95; N, 23.71.

Compound **2c**: Yield: 94%; mp 63–64 °C (lit.,[3] 62–63 °C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 (dd, J = 8.8 Hz, 4H), 5.94–6.04 (m, 2H), 5.37–5.64 (m, 1H), 4.63–4.65 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 148.39, 139.22, 133.07, 129.81, 129.37, 120.01, 114.14, 47.12; EIMS (probe) 70 eV, m/z (rel. int.): 237 $[M]^+$ (100), 152 (99), 124 (63), 89 (38).

Table 2. The results of insecticide activities of the title compounds

Compound	250 mg L^{-1}		500 mg L^{-1}	
	T. cinnabarinus	*A. medicagini*	*N. legen*	*M. separata*
2a	**100.0**	0.0	10.0	0.0
2b	**98.6**	1.7	23.3	0.0
2c	4.3	3.3	10.0	**100.0**
2d	0.0	8.3	13.3	0.0
2e	**97.1**	0.0	16.7	0.0
2f	2.9	21.7	10.0	0.0
2g	4.3	8.3	0.0	0.0
2h	0.0	3.3	10.0	0.0
2i	10.0	5.0	13.3	60.0
2j	5.7	0.0	0.0	0.0
2k	0.0	0.0	6.7	0.0
2l	0.0	13.3	6.7	0.0
2m	0.0	3.3	0.0	0.0
2n	2.9	13.3	6.7	0.0
2o	**100.0**	1.7	10.0	0.0
5a	**96.8**	5.0	6.7	0.0
5b	2.9	0.0	16.7	0.0
5c	0.0	8.3	10.0	0.0
5d	2.9	3.3	13.3	0.0
7a	0.0	5.7	0.0	0.0
7b	5.7	**82.5**	3.3	0.0
7c	0.0	0.0	6.7	13.3
7d	5.7	0.0	0.0	0.0
7e	4.3	0.0	10.0	0.0
7f	2.9	0.0	6.7	0.0
7g	**80.0**	0.0	3.3	0.0
7h	0.0	0.0	6.7	0.0
7i	72.9	0.0	0.0	0.0
7j	2.9	5.7	0.0	0.0
7k	0.0	0.0	0.0	0.0
7l	0.0	18.6	10.0	0.0
7m	2.9	0.0	10.0	0.0
7n	5.7	2.9	6.7	0.0
7o	0.0	2.9	0.0	0.0
Dichlorvos (500 mg L^{-1})	—	—	100.0	100.0
Pyridaben (50 mg L^{-1})	100.0	—	—	—

Compound **2d**: Yield: 90%; mp 115–116 °C, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.47 (dd, $J = 8.8$ Hz, 4H), 4.81 (s, 2H), 4.30 (q, $J = 7.2$ Hz, 2H), 1.32 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.68, 148.69, 133.39, 133.02, 129.54, 120.24, 62.52, 45.56, 14.03; EIMS (probe) 70 eV, m/z (rel. int.): 283 $[M]^+$ (2), 155(29), 153(100), 125(18). Anal. Calcd for $C_{11}H_{11}ClN_4O_3$: C, 46.74; H, 3.92; N, 19.82. Found: C, 46.72; H, 3.95; N, 19.43.

Compound **2e**: Yield: 95%; mp 108–110 °C, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71 (dd, $J = 8.8$ Hz, 4H), 5.05 (q, $J = 7.2$ Hz, 1H), 4.26 (q, $J = 7.2$ Hz, 2H), 1.89 (d, $J = 7.2$ Hz, 3H), 1.30 (t, $J = 7.2$ Hz, 3H); EIMS (probe) 70 eV, m/z (rel. int.): 297 $[M]^+$ (11), 155 (62), 153 (100), 125 (31), 90 (16). Anal. Calcd for $C_{12}H_{13}ClN_4O_3$: C, 48.58; H, 4.42; N, 18.88. Found: C, 48.44; H, 4.26; N, 19.07.

Compound **2f**: Yield: 97%; mp 124–125 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (dd, $J = 8.8$ Hz, 4H), 7.22–7.39 (m, 4H), 5.19 (s, 2H), 2.49 (s, 3H); EIMS (probe) 70 eV, m/z (rel. int.): 301 $[M]^+$ (100), 152 (63), 124 (51), 88 (81). Anal. Calcd for $C_{15}H_{13}ClN_4O$: C, 59.91; H, 4.36; N, 18.63. Found: C, 59.90; H, 4.31; N, 18.67.

Compound **2g**: Yield: 87%; mp 90–91 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (dd, $J = 8.8$ Hz, 4H), 7.16–7.27 (m, 4H), 5.13 (s, 2H), 2.36 (s, 3H); EIMS (probe) 70 eV, m/z (rel. int.): 301 $[M]^+$ (100), 152 (95), 124 (41), 104 (45), 89 (55). Anal. Calcd for $C_{15}H_{13}ClN_4O$: C, 59.91; H, 4.36; N, 18.63. Found: C, 60.16; H, 4.29; N, 18.49.

Compound **2h**: Yield: 93%; mp 130–133 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.68 (dd, $J = 8.8$ Hz, 4H), 7.27 (dd, $J = 8$ Hz, 4H), 5.14 (s, 2H), 2.35 (s, 3H); EIMS (probe) 70 eV, m/z (rel. int.): 301 $[M]^+$ (100), 152 (84), 124 (55), 89 (81). Anal. Calcd for $C_{15}H_{13}ClN_4O$: C, 59.91; H, 4.36; N, 18.63. Found: C, 60.05; H, 4.46; N, 18.43.

Compound **2i**: Yield: 91%; mp 101–103 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (dd, $J = 8.8$ Hz, 4H), 7.10–7.45 (m, 4H); 5.26 (s, 2H); EIMS (probe) 70 eV, m/z (rel. int.): 305 $[M]^+$ (0.5), 152 (53), 124 (44), 108 (100), 88 (28). Anal. Calcd for $C_{14}H_{10}ClFN_4O$: C, 55.18; H, 3.31; N, 18.39. Found: C, 55.30; H, 3.35; N, 18.25.

Compound **2j**: Yield: 88%; mp 106–107 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (dd, $J = 8.8$ Hz, 4H), 7.06–7.37 (m, 4H), 5.17 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.12, 161.66, 148.59, 136.20, 133.37, 133.09, 130.71, 130.63, 129.54, 124.08, 120.21, 115.92, 115.71, 115.61, 115.39, 48.17; EIMS (probe) 70 eV, m/z (rel. int.): 305 $[M]^+$ (100), 152 (66), 124 (26), 108 (79), 88 (40). Anal. Calcd for $C_{14}H_{10}ClFN_4O$: C, 55.18; H, 3.31; N, 18.39. Found: C, 55.09; H, 3.54; N, 18.25.

Compound **2k**: Yield: 94%; mp 135–137 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (dd, $J = 8.8$ Hz, 4H), 7.33–7.44 (m, 4H), 5.15 (s, 2H); EIMS (probe) 70 eV, m/z (rel. int.): 321 $[M]^+$ (45), 152 (100), 124 (53), 88 (17), Anal. Calcd for $C_{14}H_{10}Cl_2N_4O$: C, 52.36; H, 3.14; N, 17.45. Found: C, 52.47; H, 3.11; N, 17.38.

Compound **2l**: Yield: 98%; mp 149–150 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (dd, $J = 8.8$ Hz, 4H), 7.44 (dd, $J = 8.0$ Hz, 4H), 5.13 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 148.53, 133.33, 133.04, 132.84, 132.17, 130.21, 129.51, 122.99, 120.16, 48.14; EIMS (probe) 70 eV, m/z (rel. int.): 366 $[M]^+$ (100), 152 (52), 124 (63), 89 (96). Anal. Calcd for $C_{14}H_{10}BrClN_4O$: C, 45.99; H, 2.76; N, 15.32. Found: C, 45.86; H, 2.87; N, 15.32.

Compound **2m**: Yield: 82%; mp 112–113 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (dd, $J = 8.8$ Hz, 4H), 6.88–7.32 (m, 4H), 5.14 (s, 2H), 3.82 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.45, 148.39, 136.28, 133.09, 132.03, 129.91, 129.52, 121.09, 119.93, 113.65, 113.53, 55.08, 47.77; EIMS (probe) 70 eV, m/z (rel. int.): 317 $[M]^+$ (100), 152 (48), 124 (41), 107 (12), 89 (54). Anal. Calcd for $C_{15}H_{13}ClN_4O_2$: C, 56.88; H, 4.14; N, 17.69. Found: C, 56.69; H, 4.08; N, 17.67.

Compound **2n:** Yield: 62%; mp 133–135 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.17 (s, 1H), 7.41–8.27 (m, 8H), 5.14 (s, 2H); EIMS (probe) 70 eV, m/z (rel. int.): 355 $[M]^+$ (3), 291 (28), 277 (43), 172 (54), 146 (100), 125 (23), 89 (32); Anal. Calcd for $C_{17}H_{11}ClN_4O_3$: C, 57.56, H, 3.13, N, 15.79; Found: C, 57.63; H, 3.36; N, 15.37.

Compound **2o:** Yield: 88%; mp:195–197 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20 (s, 1H), 7.44–8.24 (m, 8H), 5.11 (s, 2H); EIMS (probe) 70 eV, m/z (rel. int.): 355 $[M]^+$ (100), 292 (49), 172 (36), 146 (59), 125 (58), 89 (56). Anal. Calcd for $C_{17}H_{11}ClN_4O_3$: C, 57.56, H, 3.13, N, 15.79. Found: C, 57.83; H, 3.27; N, 15.60.

4.2.2. Preparation of 2-(4-(4-chlorophenyl)-5-oxo-4,5-dihydrotetrazol-1-yl)acetohydrazide (3). Hydrazine hydrate (0.5 mol) was added into a solution of ethyl 2-(2-(4chlorophenyl)-5-oxo-4,5-dihydrotetrazol-1-yl)acetate (**2e**) (0.1 mol) in ethanol (100 mL). The reaction mixture was heated under reflux for 4 h, concentrated in vacuum, cooled, and diluted with water. The precipitate obtained was filtered, washed with ice-cold water, dried, and recrystallized from ethanol, Yield: 88%; mp 235–237 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.47 (s, 1H), 7.66 (dd, $J = 8.8$ Hz, 4H), 4.69 (s, 2H), 4.39 (s, 2H); EIMS (probe) 70 eV, m/z (rel. int.): 268 $[M]^+$ (10), 155 (31), 153 (100), 127 (10), 125 (27), 115 (16). Anal. Calcd for $C_9H_9ClN_6O_2$: C, 40.24; H, 3.38; N, 31.28. Found: C, 40.23; H, 3.27; N, 30.95.

4.2.3. Preparation of 1-(4-chlorophenyl)-4-(2-mercapto-1,3,4-oxadiazol-5-yl)-methylene-1*H*- tetrazol-5(4*H*)-one (4). To a vigorously stirred solution of 0.012 mol of potassium hydroxide and 80 mL of anhydrous ethanol, 0.01 mol of compound **3** was added. Then, a solution of 1 g of carbon disulfide in 20 mL of anhydrous ethanol was added dropwise, and the resulted reaction mixture was refluxed for 24 h. The solvent was evaporated in vacuum and the residue was dissolved in 50 mL of water, then acidified to pH ≈5–6 with glacial acetic acid, filtered off, dried, and purified by recrystallization from a DMF/water solution (5:1). Yield: 36%; mp 153–155 °C; ^{1}HNMR (400 MHz, DMSO-d_6): δ 14.65 (s, 1H), 7.79 (dd, $J = 8.8$ Hz, 4H), 5.47 (s, 2H); EIMS (probe) 70 eV, m/z (rel. int.): 311 $[M]^+$ (14), 153 (43), 125 (100), 111 (30), 90 (74). Anal. Calcd for $C_{10}H_7ClN_6O_2S$: C, 38.65; H, 2.27; N, 27.05; S, 10.32. Found: C, 38.73; H, 2.35; N, 26.74; S, 10.65.

4.2.4. General procedure for the preparation of 1-((5-(alkylthio)-1,3,4-oxadiazol-2-yl)methyl)-4-(substituted)-phenyl-1*H*-tetrazol-5(4*H*)-ones (5). To a stirring solution of mercaptooxadiazole **4** (1 mmol) in DMF (5 mL), the appropriate alkyl halide (allyl halide or ethyl acetate halide) (1.1 mmol) and anhydrate potassium carbonated (2 mmol) were added gradually. The reaction mixture was stirred at room temperature for 4–8 h. Then, the reaction mixture was poured into 100 mL of ice water. The precipitate was filteredoff, washed with water, dried, and recrystallized from ethanol.

Compound **5a:** Yield: 65%; mp 65–67 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (dd, $J = 8.8$ Hz, 4H), 5.93–6.00 (m, 1H), 5.42 (s, 2H), 5.20–5.40 (m, 2H), 3.87–3.89 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.81, 160.47, 147.98, 133.51, 132.71, 131.15, 129.51, 120.16, 120.00, 39.10, 35.04; EIMS (probe) 70 eV, m/z (rel. int.): 351 $[M]^+$ (29), 235 (17), 208 (23), 152 (100), 125 (61), 111 (29), 90 (50). Anal. Calcd for $C_{13}H_{11}ClN_6O_2S$: C, 44.51; H, 3.16; N, 23.96; S, 9.14. Found: C, 44.79; H, 3.41; N, 23.64; S, 9.12.

Compound **5b:** Yield: 42%; mp 68–69 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (dd, $J = 8.8$ Hz, 4H), 5.42 (s, 2H), 4.23 (q, $J = 7.2$ Hz, 2H), 4.06 (s, 2H), 1.28 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.89, 164.90, 160.72, 147.85, 133.30, 132.60, 129.36, 120.05, 62.23, 38.95, 34.10, 13.82; EIMS (probe) 70 eV, m/z (rel. int.): 397 $[M]^+$ (6), 153 (100), 125 (25), 90 (10). Anal. Calcd for $C_{14}H_{13}ClN_6O_4S$: C, 42.38; H, 3.30; N, 21.18; S, 8.08. Found: C, 42.83; H, 3.46; N, 21.12; S, 8.47.

Compound **5c:** Yield: 61%; mp 55–57 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (dd, $J = 8.8$ Hz, 4H), 5.43 (s, 2H), 4.43 (q, $J = 8.4$ Hz, 1H), 4.19 (q, $J = 7.2$ Hz, 2H), 1.70 (d, $J = 8.4$ Hz, 3H), 1.27 (t, $J = 7.2$ Hz, 3H); EIMS (probe) 70 eV, m/z (rel. int.): 411 $[M]^+$ (13), 153 (100), 125 (26), 90 (13). Anal. Calcd for $C_{15}H_{15}ClN_6O_4S$: C, 43.85; H, 3.68; N, 20.46; S, 7.80. Found: C, 44.09; H, 3.80; N, 20.18; S, 8.17.

Compound **5d:** Yield: 87%; mp 58–60 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (dd, $J = 8.8$ Hz, 4H), 5.41 (s, 2H), 3.25 (t, $J = 7.2$ Hz, 2H), 1.26–1.81 (m, 12H), 0.88 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.72, 160.19, 148.01, 133.50, 132.74, 129.51, 120.16, 39.12, 32.54, 31.61, 28.96, 28.94, 28.82, 28.44, 22.48, 13.96; EIMS (probe) 70 eV, m/z (rel. int.): 423 $[M]^+$ (7), 376 (17), 352 (15), 153 (100), 125 (37), 111 (15), 90 (21). Anal. Calcd for $C_{18}H_{23}ClN_6O_2S$: C, 51.12; H, 5.48; N, 19.87; S, 7.58. Found: C, 51.12; H, 5.31; N, 19.59; S, 7.88.

4.2.5. Preparation of 1-(4-chlorophenyl)-4-(2-mercapto-1,3,4-thiadiazol-5-yl)-methylene-1*H*-tetrazol-5(4*H*)-one (6). To a vigorously stirred solution mixture of 0.011 mol of potassium hydroxide, 0.01 mol of the compound **3**, and 100 mL of anhydrous ethanol, a solution of 7.5 g of carbon disulfide in 20 mL of anhydrous ethanol was added dropwise. Then, the reaction mixture was stirred at room temperature for 24 h and filtered to yield a white solid, which was used for the next reaction with further purification. So, 0.005 mol of above solid was added into 100 mL of concentrated sulfuric acid, the resulted mixture was stirred for 24 h at room temperature and then poured into 100 mL of ice water. The precipitate was filtered and dissolved with sodium hydroxide solution (25%), which was acidified with concentrated hydrochloride acid and filtered to give the crude product, dried, and recrystallized from a DMF/water solution (5:1). Yield: 84%; mp 180–182 °C; ^{1}H NMR (DMSO-d_6): δ 14.68 (s, 1H), 7.66–7.90 (m, 4H), 5.49(s, 2H); EIMS (probe) 70 eV, m/z (rel. int.): 327 $[M]^+$ (20), 153 (90), 125(100), 111 (30), 90 (68), 75 (39). Anal. Calcd for $C_{10}H_7ClN_6OS_2$: C, 36.75; H, 2.16; N, 25.72. Found: C, 36.81; H, 2.11; N, 25.71.

4.2.6. General procedure for the preparation of 1-(2-alkylthio-4*H*-1,3,4-thiadiazol-5-yl)-methylene-4-(4-chlorophenyl)-1*H*- tetrazol-5(4*H*)-one(7). To a stirring solution of mercaptothiadiazole (**6**) (1 mmol) in DMF (5 mL), the appropriate alkyl halide (allyl halide or ethyl acetate halide) (1.1 mmol) and anhydrate potassium carbonated (2 mmol) was added gradually. The reaction mixture was stirred at room temperature for 4–8 h according to TLC monitored. Then, the reaction mixture was poured into 100 mL ice water. The precipitate was filtered-off, washed with water, dried and recrystallized from ethanol.

Compound **7a:** Yield: 61%; mp128–130 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (dd, $J = 8.8$ Hz, 4H), 5.59 (s, 2H), 2.79 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.81, 160.81, 148.14, 133.61, 132.79, 129.59, 120.22, 43.18, 16.43; EIMS (probe) 70 eV, m/z (rel. int.): 341 $[M]^+$ (60), 187 (73), 153 (100), 125 (70), 111 (21), 90 (88). Anal. Calcd for $C_{11}H_9ClN_6OS_2$: C, 38.77; H, 2.66; N, 24.66; S, 18.82. Found: C, 39.14; H, 2.54; N, 24.29; S, 18.42.

Compound **7b:** Yield: 71%; mp 93–94 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (dd, $J = 8.8$ Hz, 4H), 5.59 (s, 2H), 3.35 (q, $J = 7.2$ Hz, 2H), 1.47 (t, $J = 7.2$ Hz, 3H); EIMS (probe) 70 eV, m/z (rel. int.): 355 $[M]^+$ (10), 202 (13), 153 (100), 125 (70), 90 (23). Anal. Calcd for $C_{12}H_{11}ClN_6OS_2$: C, 40.62; H, 3.12; N, 23.68; S, 18.07. Found: C, 40.75; H, 2.93; N, 23.55; S, 18.17.

Compound **7c:** Yield: 76%; mp 83–85 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (dd, $J = 8.8$ Hz, 4H), 5.59 (s, 2H), 3.31 (t, $J = 7.2$ Hz, 2H), 1.83 (m, 2H), 1.05 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.17, 160.77, 148.15, 133.60, 132.81, 129.59, 120.23, 43.18, 36.08, 22.44, 13.02; EIMS (probe) 70 eV, m/z (rel. int.): 369 $[M]^+$ (6), 326 (16), 172 (22), 153 (100), 125 (54), 90 (29). Anal. Calcd for $C_{13}H_{13}ClN_6OS_2$: C, 42.33; H, 3.55; N, 22.78; S, 17.39. Found: C, 42.43; H, 3.29; N, 22.63; S, 17.44.

Compound **7d:** Yield: 71%; mp 62–63 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (dd, $J = 8.8$ Hz, 4H), 5.59 (s, 2H), 3.33 (t, $J = 7.2$ Hz, 2H), 1.44–1.80 (m, 4H), 0.94 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.21, 160.74, 148.15, 133.61, 132.81, 129.59, 120.22, 43.17, 33.91, 30.95, 21.78, 13.46; EIMS (probe) 70 eV, m/z (rel. int.): 383 $[M]^+$ (21), 335 (83), 173 (73), 153 (100), 125 (65), 111 (22), 89 (51). Anal. Calcd for $C_{14}H_{15}ClN_6OS_2$: C, 43.92; H, 3.95; N, 21.95; S, 16.75. Found: C, 43.84; H, 3.72; N, 21.79;S, 16.68.

Compound **7e:** Yield: 75%; mp 68–70 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (dd, $J = 8.8$ Hz, 4H), 5.59 (s, 2H), 3.33 (t, $J = 7.2$ Hz, 2H), 1.30–1.81 (m, 8H), 0.88 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.21, 160.74, 148.15, 133.59, 132.80, 129.58, 120.20, 43.18, 34.22, 31.12, 28.90, 28.28, 22.39, 13.91; EIMS (probe) 70 eV, m/z (rel. int.): 411 $[M]^+$ (100), 363 (84), 172 (28), 153 (89), 125 (68), 111 (27), 90 (47). Anal. Calcd for $C_{16}H_{19}ClN_6OS_2$: C, 46.76; H, 4.66; N, 20.45; S, 15.61. Found: C, 46.90; H, 4.59; N, 20.37; S, 15.36.

Compound **7f:** Yield: 52%; mp 85–86 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (dd, $J = 8.8$ Hz, 4H), 5.59 (s, 2H), 3.32 (t, $J = 7.2$ Hz, 2H), 1.27–1.81 (m, 12H), 0.87 (t, $J = 7.2$ Hz, 3H); EIMS (probe) 70 eV, m/z (rel. int.): 439 $[M]^+$ (10), 391 (62), 172 (43), 152 (100), 124 (53), 110 (27), 89 (36). Anal. Calcd for $C_{18}H_{23}ClN_6OS_2$: C, 49.25; H, 5.28; N, 19.14; S, 14.61. Found: C, 48.92; H, 4.93; N, 18.89; S, 14.74.

Compound **7g:** Yield: 48%; mp 75–77 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (dd, $J = 8.8$ Hz, 4H), 5.60 (s, 2H), 4.23 (q, $J = 7.2$ Hz, 2H), 4.15 (s, 2H), 1.28 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.51, 166.78, 161.68, 148.10, 133.56, 132.77, 129.57, 120.22, 62.24, 43.09, 35.39, 14.02; EIMS (probe) 70 eV, m/z (rel. int.): 413 $[M]^+$ (5), 185 (20), 172 (4), 153 (100), 124 (38), 110 (14), 89 (27). Anal. Calcd for $C_{14}H_{13}ClN_6O_3S_2$: C, 40.73; H, 3.17; N, 20.35; S, 15.53. Found: C, 40.84; H, 2.97; N, 20.22; S, 15.42.

Compound **7h:** Yield: 73%; mp 63–65 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.68 (dd, $J = 8.8$ Hz, 4H), 5.61 (s, 2H), 4.60 (q, $J = 7.2$ Hz, 2H), 4.21 (q, $J = 7.2$ Hz, 1H), 1.68 (d, $J = 7.2$ Hz, 3H), 1.26 (t, $J = 7.2$ Hz, 3H); EIMS (probe) 70 eV, m/z (rel. int.): 427 $[M]^+$ (95), 353 (23), 172 (17), 153 (100), 124 (35), 111 (23), 89 (28). Anal. Calcd for $C_{15}H_{15}ClN_6O_3S_2$: C, 42.20; H, 3.54; N, 19.69; S, 15.02. Found: C, 42.62; H, 3.55; N, 19.67; S, 14.97.

Compound **7i:** Yield: 58%; mp 63–65 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (dd, $J = 8.8$ Hz, 4H), 5.94–6.60 (m, 1H), 5.59 (s, 2H), 5.20–5.39 (m, 2H), 3.95–3.97 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.98, 161.19, 148.11, 133.58, 132.78, 131.49, 129.57, 120.20, 119.87, 43.15, 36.67; EIMS (probe) 70 eV, m/z (rel. int.): 367 $[M]^+$ (16), 351 (90), 152 (100), 124 (52), 110 (20), 89 (49). Anal. Calcd for $C_{13}H_{11}ClN_6OS_2$: C, 42.56; H, 3.02; N, 22.91; S, 17.48. Found: C, 42.51; H, 2.80; N, 22.70; S, 17.31.

Compound **7j:** Yield: 74%; mp 98–100 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.49–7.92 (m, 4H), 7.31–7.42 (m, 5H), 5.59 (s, 2H), 4.57 (s, 2H); EIMS (probe) 70 eV, m/z (rel. int.): 417 $[M]^+$ (100), 263 (20), 206 (14), 176 (47), 125 (9), 112 (23). Anal. Calcd for $C_{17}H_{13}ClN_6OS_2$: C, 48.98; H, 3.14; N, 20.16; S, 15.38. Found: C, 49.43; H, 3.09; N, 20.02; S, 15.24.

Compound **7k:** Yield: 68%; mp 77–78 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.15–7.92 (m, 8H), 5.60 (s, 2H), 4.61 (s, 2H), 2.42 (s, 3H); EIMS (probe) 70 eV, m/z (rel. int.): 431 $[M]^+$ (93), 397 (25), 243 (14), 162 (37), 125 (18), 105 (100). Anal. Calcd for $C_{18}H_{15}ClN_6OS_2$: C, 50.17; H, 3.51; N, 19.50; S, 14.88. Found: C, 50.37; H, 3.40; N, 19.49; S, 15.19.

Compound **7l:** Yield: 74%; mp 90–91 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.06–7.92 (m, 8H), 5.59 (s, 2H), 4.61 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.69, 162.08, 161.35, 159.61, 148.01, 133.44, 132.71, 131.36, 131.33, 129.88, 129.80, 129.47, 124.18, 124.14, 122.91, 122.77, 120.09, 115.56, 115.35, 43.01, 31.14; EIMS

(probe) 70 eV, m/z (rel. int.): 435 $[M]^+$ (100), 281 (37), 165 (24), 152 (20), 125 (10), 109 (55). Anal. Calcd for $C_{17}H_{12}ClFN_6OS_2$: C, 46.95; H, 2.78; N, 19.32; S, 14.75. Found: C, 47.06; H, 2.71; N, 19.17; S, 14.93.

Compound **7m:** Yield: 71%; mp 87–88 °C; ^1H NMR (400 MHz, $CDCl_3$): δ 6.98–7.92 (m, 8H), 5.59 (s, 2H), 4.55 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.58, 163.94, 161.39, 148.12, 137.97, 133.63, 132.78, 130.26, 130.18, 129.60, 124.86, 124.84, 120.22, 116.19, 115.97, 115.08, 114.88, 43.13, 37.36; EIMS (probe) 70 eV, m/z (rel. int.): 435 $[M]^+$ (100), 281 (43), 166 (54), 153 (30), 125 (12), 109 (41). Anal. Calcd for $C_{17}H_{12}ClFN_6OS_2$: C, 46.95; H, 2.78; N, 19.32; S, 14.75. Found: C, 46.95; H, 2.93; N, 18.92; S, 14.77.

Compound **7n:** Yield: 62%; mp 90–91 °C; ^1H NMR (400 MHz, $CDCl_3$): δ 7.23–7.92 (m, 8H), 5.59 (s, 2H), 4.53 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.46, 161.40, 148.07, 137.58, 134.39, 133.56, 132.75, 129.91, 129.55, 129.13, 128.11, 127.34, 120.16, 43.09, 37.22; EIMS (probe) 70 eV, m/z (rel. int.): 451 $[M]^+$ (100), 297 (45), 182 (84), 156 (64), 125 (96), 91 (63). Anal. Calcd for $C_{17}H_{12}Cl_2N_6OS_2$: C, 45.24; H, 2.68; N, 18.62; S, 14.21. Found: C, 45.36; H, 2.87; N, 18.24; S, 14.42.

Compound **7o:** Yield: 36%; mp 144–146 °C; ^1H NMR (400 MHz, $CDCl_3$): δ 7.15–7.92 (m, 8H), 5.58 (s, 2H), 4.50 (s, 3H), 3.84 (s, 3H), 3.69 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.84, 167.63, 161.01, 160.37, 148.10, 134.41, 133.55, 132.84, 132.79, 131.38, 130.04, 129.55, 128.22, 127.95, 120.22, 109.76, 62.00, 51.66, 43.09, 36.44; EIMS (probe) 70 eV, m/z (rel. int.): 531 $[M]^+$ (5), 454 (100), 153 (54), 125 (36), 111 (14), 89 (27). Anal. Calcd for $C_{22}H_{19}ClN_6O_4S_2$: C, 49.76; H, 3.61; N, 15.83; S, 12.08. Found: C, 49.95; H, 3.64; N, 15.59; S, 12.36.

4.3. Biological assays

4.3.1. Herbicidal tests. Stock solutions of each test compound were prepared in DMF at a concentration of 1.0 g L^{-1}, and then diluted to the required test concentrations with water containing TW-80. The biological tests were carried out in Petri dish (id = 9 cm) containing two pieces of filter paper and 10 pregerminated seeds of wheat (barnyard grass, jowar, rape, radish or cucumber), and then 9 mL of solution (100 mg L^{-1}) was added to each Petri dish containing seeds. In a control experiment, carried out under the same conditions, 9 mL of the same solution without compounds was applied on each Petri dish. The cultivations were kept at 28 ± 1 °C, 70–80% relative humidity (RH), a photoperiod of 16:8 (L:D) h, and with exposure to light of 3000 LX for 5 days. The root and stem growth was measured for the 10 seeds in each Petri dish and averaged. All experiments and the respective controls were carried out in three replicates and the data were subjected to probit analysis. The results of herbicidal activities are listed in Table 1.

4.3.2. Insecticidal tests. This study was carried out with the following insect species: *T. cinnabarinus*, *N. legen*, *M. separata*, and *A. medicagini*. These insects were reared in a room maintained at 25 (±1) °C, 60 (±5)% relative humidity, and 14 h light photoperiod. Stock solutions of each test compound were prepared in DMF at a concentration of 1.0 g L^{-1}, and then diluted to the required test concentrations with water containing TW-80. Groups of 10 insects of each species were transferred to glass Petri dishes and sprayed with test solutions using a Potter sprayer. After air-drying, they were kept in a room for normal cultivation. The mortality was determined by the number and size of live larvae in the treated bottles relative to that in the untreated controls in 72 h. In the case of *N. lugen*, rice seedlings (second semester) were dipped in the test solution for 5 s, air-dried, and then placed in a large test tube. Each test rube contained 20 seedlings. Twenty insects (fifth instar) were introduced into the tube, and the mouth of the tube was covered with white cheesecloth. The tube was kept at room temperature, and the number of live and dead insects counted after 72 h. In a control experiment, carried out under the same conditions, 1 mL DMF was applied on each insect. All experiments and the respective controls were carried out in three replicates and the data were subjected to probit analysis. The results of insecticidal activities are listed in Table 2.

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References and notes

1. (a) Kathleen, M. P.; Lawrenceville, N. J. US Patent, 4,913,724, 1990; (b) Yanagi, A.; Watanabe, Y.; Narabu, S. US Patent, 5,530,135, 1996.
2. Goto, T.; Ito, S.; Yanagi, A.; Watanabe, Y.; Yasui, K. *Weed Biol. Manag.* **2002**, *2*, 18.
3. (a) Theodororidis, G.; Hotzman, F. W.; Scherer, L. W.; Smith, B. A.; Tymonko, J. M.; Wyle, M. J. *Pestic. Sci.* **1990**, *30*, 259; (b) Theodororidis, G. WO Patent, 8,501,939, 1985.
4. Matysiaka, J.; Opolski, A. *Bioorg. Med. Chem.* **2006**, *14*, 4483.
5. (a) Hussin, M. I.; Shukla, M. K. *J. Indian Chem. Soc.* **1978**, *55*, 826; (b) Oneal, J. B.; Rosen, H.; Russel, P. B.; Adams, A. C.; Blumenthal, A. *J. Med. Pharm. Chem.* **1962**, *56*, 17.
6. Ladduwahetty, T.; Baker, R.; Cascieri, M. A.; Chamber, J. M.; Haworth, K.; Keown, L. E.; MacIntyre, D. E.; Metzger, J. M.; Owen, S.; Ryeroft, W.; Sadowski, S.; Seward, E. M.; Shepheard, S. L.; Swain, C. J.; Tattersall, F. D.; Watt, A. P.; Williamson, D. W.; Hargreaves, R. J. *J. Med. Chem.* **1996**, *39*, 2907.
7. (a) Jindal, S.; Pandey, V. K. *Acta. Pharm. Jugosl.* **1991**, *41*, 217; (b) Nigam, R.; Swarup, S.; Saxena, V. K.; Singh, H. K. *J. Indian Chem. Soc.* **1992**, *69*, 692.

8. (a) Jones, D. H.; Slack, R.; Squires, S.; Wooldridge, K. R. *J. Med. Chem.* **1965**, *8*, 676; (b) Ainsworth, C. *J. Am. Chem. Soc.* **1965**, *87*, 5800; (c) Yale, H.; Losee, K. *J. Med. Chem.* **1966**, *9*, 478; (d) Ghiram, D.; Schwartz, I.; Simity, I. *Farmacia* **1971**, *22*, 141; (e) Thomas, J. Ger. Offen. 2,403,357, 1974; (f) Adelstein, G. W.; Yen, C. H.; Dajani, E. Z.; Bianchi, R. G. *J. Med. Chem.* **1976**, *19*, 1221; (g) Smicius, R.; Jakubkiene, V.; Burbaliene, M. M.; Vainilavicius, P. *Monatsch. Chem.* **2002**, *133*, 731; (h) Zarghi, A.; Tabatabai, S. A.; Faizi, M.; Ahadian, A.; Navabi, P.; Zanganeh, V.; Shafiee, A. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1863.
9. (a) Sengupta, A. K.; Garg, M.; Chandra, V. *J. Indian Chem. Soc.* **1979**, *56*, 1230; (b) Mistra, A. R.; Yadav, L. D.; Sigh, H.; Misra, J. P. *J. Agric. Food Chem.* **1990**, *38*, 1082.
10. (a) Yagi, K.; Numata, A.; Mimori, N.; Miyake, T.; Arai, K.; Ishii, S. *Pestic. Sci.* **1999**, *55*, 161; (b) Misra, H. K. *Arch. Pharm.* **1983**, *316*, 487; (c) Reddy, P. S. N.; Pratap, R. P. *Indian J. Chem.* **1987**, *26*, 890.
11. (a) Sengupta, A. K.; Chandra, U. *Indian J. Chem.* **1979**, *17*, 655; (b) Brouwer, W. G.; MacPherson, E. J.; Ames, R. B.; Neidermyer, R. W. Can Patent, 1,002,336, 1976.
12. (a) Dutta, M. M.; Kataky, J. C. S. *J. Indian Chem. Soc.* **1992**, *69*, 107; (b) Holla, B. S.; Poojary, K. N.; Kalluraya, B.; Gowda, P. V. *Indian J. Heterocycl. Chem.* **1996**, *5*, 273; (c) Giri, S.; Singh, H.; Yadav, L. D. S. *Agric. Biol. Chem.* **1976**, *40*, 17; (d) Singh, H.; Yadav, L. D. S. *Agric. Biol. Chem.* **1976**, *40*, 759.
13. Horwitz, J. P.; Fisher, B. E.; Tomasewski, A. J. *J. Am. Soc. Chem.* **1959**, *81*, 3076.
14. Yang, G. F.; Liu, H. Y.; Yang, H. Z. *Pestic. Sci.* **1999**, *55*, 1143.