Synthesis and QSAR Study of novel 8-(3-(trifluoromethyl) phenyl)-6-methyl-[*1,2,4*]triazolo[*4,3-b*]pyridazin-3(*2H*)-ones

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Abstract

A series of novel 8-(3-(trifluoromethyl)phenyl)-6-methyl-[*1,2,4*]triazolo[*4,3-b*]pyridazin-3(*2H*)-one derivatives were designed, synthesized and evaluated for their bioactivities. Some of them provided > 80 % inhibition of chlorophyll of *Spirodela polyrhiza* or > 70 % inhibition of growth of *Spirodela polyrhiza* at 10 μg/ml. Quantitative structure–activity relationship studies were performed on these compounds using physicochemical parameters (hydrophobic) as independent and bioactivity as dependent parameter, where bioactivity correlated best (r>0.8) with physicochemical parameters in this set of molecules. Both chlorophyll and growth inhibitions of *Spirodela polyrhiza* for the title compounds were effected by the molecular hydrophobicity, LogP. When the LogP value was about 3.02, the corresponding compounds showed better chlorophyll inhibition activity. When the LogP value was about 2.84, the corresponding compounds showed better fresh weight inhibition activity.

Keywords: [1,2,4]Triazolo[4,3-*b*]pyridazin-3(2*H*)-one, hydrophobic; quantitative structure-activity relationship (QSAR), inhibition

Introduction

Chemical crop protection plays a vital role in ensuring sufficient food supply to a growing world population. More stringent demands with regard to efficacy and environmental safety make it necessary to discover new agrochemicals. Since the 1980s, more than 50 patents about triazolone family have been opened to the public and turned out three practical herbicides, namely sulfentrazone, carfentrzone-ethyl and azafenidin. In 2004, Takashi Kuragano reported some kinds of bicyclic triazolone derivatives and bioassay results showed some of them possessed

excellent herbicidal activities on weeds grown in paddy fields or farmlands. Moreover, these compounds were useful as selective herbicides for weeds in paddy fields or farmlands, and not harmful to a rice plant, wheat, barley, maize, cotton, soybeans and so on.[1-2] It was also noticed that lots of pyridazine compounds showed good herbicidal activity and some are being used at present.[3-5] In addition, many commercial phytoene desaturase inhibitors contained a heterocycle moiety substituted by a 3-trifluoromethylphenyl group, which was important for their herbicidal activity.[6] Considering that the method of montage of active substructures has been extensively utilized to find potential agrochemicals(such as 2-(4- aryloxyphenoxy)propanoates) [7-8] and to find new kinds of lead compounds or compounds with novel mechanisms of action, we designed the title compounds by docking the three key structural moieties: pyridazine, bicyclic triazolone and 3-trifluoromethylphenyl group (see Figure 1). In this paper, we report their synthesis and herbicidal activities.

At present, quantitative structure-activity relationships (QSAR), an important area of chemometrics, is widely utilized to study the relationship between chemical structures and biological or other functional activities. QSAR has become increasingly helpful in understanding many aspects of chemical-biological interactions in drug and pesticide research as well as in many areas.[9-13] The another objective of this study was to further understand the QSAR for **1** (Figure 1).

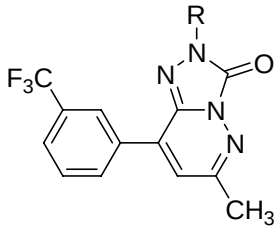

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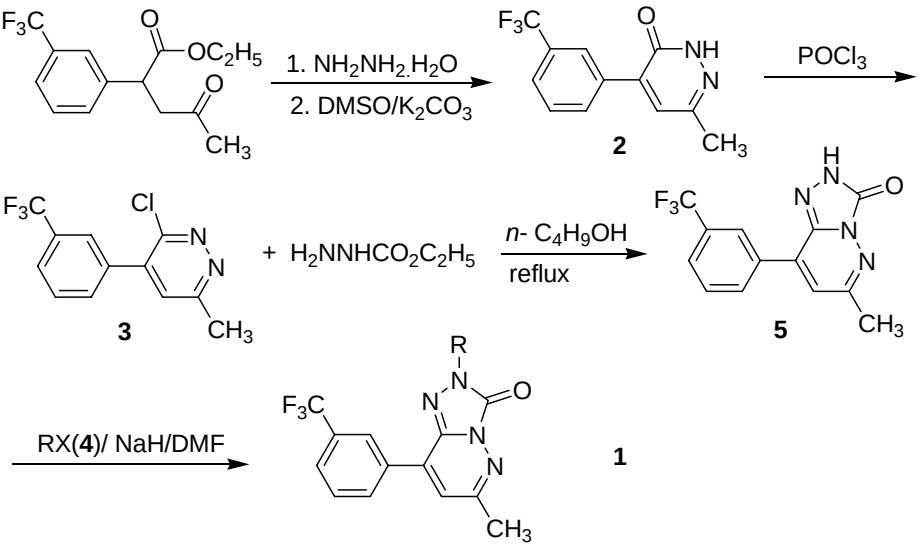
Figure 1. Chemical structure of **1**.

Results and Discussion

Preparations. Compound **3** was synthesized by chlorination of 4-(3-(trifluoromethyl)phenyl)-6-methyl pyridazin-3(2*H*)-one at 70-75 and the higher temperature will decrease its yield.[14,15,16,17,18] The key intermediate 8-(3-(trifluoromethyl)phenyl)-6-methyl-[*1,2,4*]triazolo[*4,3-b*] pyridazin-3(*2H*)-one (**5**) was prepared by **3** and $NH_2NHCO_2C_2H_5$ in 1-butanol [19]and it was reacted with compound **4** in the presence of NaH to give **1a-1o** as shown in **Scheme 1**.[2] The ^1H NMR spectral data of the synthesized compounds were found in agreement with the assigned molecular structures and their physicochemical parameters used in present study are given in Table 1.



Scheme 1. General Synthetic Route for Compound **1**.

The newly obtained derivatives were evaluated for their inhibition of chlorophyll and growth of *Spirodela polyrhiza* respectively and the inhibition rate values obtained are presented in Table 1. All the compounds showed appreciable chlorophyll and growth inhibiting activity under study.

Table 1. Chlorophyll inhibition (I_C) and Fresh weight inhibition (I_F) of compounds 1 in *Spirodela polyrhiza* (rate 10 μg/ml) and their physicochemical parameters*

compd	logP	$(logP)^2$	MW	I_C (%)	X_C^{obs}	X_C^{calcd}	I_F (%)	X_F^{obs}	X_F^{calcd}
1a	2.44	5.95	308.26	86	-1.70	-2.15	46	-2.56	-2.44
1b	2.78	7.73	322.29	82	-1.85	-2.01	74	-2.05	-1.97
1c	3.26	10.63	336.31	67	-2.22	-2.17	78	-1.98	-1.96
1d	3.68	13.54	350.34	47	-2.60	-2.65	29	-2.93	-2.59
1e	4.10	16.81	364.36	36	-2.81	-3.44	5	-3.84	-3.80
1f	4.01	16.08	364.36	7	-3.68	-3.24	11	-3.47	-3.49
1g	4.17	17.39	384.35	5	-3.86	-3.60	5	-3.86	-4.06
1h	2.18	4.75	333.37	53	-2.47	-2.39	0	-	-3.05
1i	3.13	9.80	334.30	82	-1.87	-2.08	0	-	-1.89
1j	2.65	7.02	332.28	50	-2.52	-2.04	62	-2.31	-2.10
1k	3.07	9.42	366.29	0	-	-2.06	0	-	-1.87
1l	1.97	3.88	366.29	0	-	-2.68	6	-3.76	-3.72
1m	2.31	5.34	380.32	0	-	-2.25	49	-2.60	-2.72
1n	2.80	7.84	394.35	73	-2.16	-2.01	95	-1.32	-1.95
1o	2.40	5.76	382.34	70	-2.21	-2.18	49	-2.60	-2.52
Diflufenican	-	-	-	94	-	-	64	-	-

*The logP values were predicted by Chemoffice 2004; X_C^{calc1} was calculated by Equation 1; X_F^{calc} was calculated by Equation 2.

Table 2. Herbicidal Activity of Compounds (%, Inhibition) (Rate =750 g/ha)*

compd	*Echinochloa crus-galli*		*Digitaria adscendens*		*Brassica campestris*		*Amaranthus retroflexus*	
	pre	post	pre	post	pre	post	pre	post
1a	0	15	0	5	0	15	5	20
1b	46	30	39	10	0	7	22	33
1c	38	35	66	0	30	58	19	58
1d	13	20	0	0	15	7	20	10
1j	15	5	10	0	0	30	0	0
1n	15	0	0	0	0	0	10	0
1o	21	10	0	6	0	0	14	13
Diflufenican	55	91	84	100	98	75	100	90

* Post: post-emergence; pre: pre-emergence.

As shown in Table 1, some compounds showed better inhibitive activity at concentration of 10 μg/ml (such as **1a**, **1b**, **1c** and **1n**). Comparing with Diflufenican, some of them (**1a**, **1b**, **1c** and **1n**) showed better inhibition activity. When the substituent R was methyl (**1a**), ethyl (**1b**) and propyl(**1c**) respectively, their chlorophyll inhibition rates or fresh weight inhibition rates hadn't significantly difference. When **1c** was modified into **1e** or **1f**, the corresponding compound's chlorophyll and fresh weight inhibition activities was decreased sharply. When the compound **1a** was modified into **1l** by introducing an ester group, the bioactivity was decreased sharply. This result indicated that a hydrophilic moiety's introduction was disadvantaged to improve their bioactivity. When compound **1l** was modified into **1n**, its inhibition rate was elevated significantly. All of the above suggested that the inhibition activity of compound **1** was related with its hydrphobicity.

Compounds with higher inhibition rates were further bioassayed at a dosage of 750 g/ha in the glasshouse on four herbs representative of monocotyledonous and dicotyledonous plants. From the biological assay results in Table 2, which summarize the herbicidal activity of the target compounds, compound **1c** showed better herbicidal activity.

In order to gain insight into structure-activity relationships of compounds **1**, the data were analyzed by a physicochemical based QSAR (Hansch) approach using physicochemical parameters as independent and herbicidal activity data (% I) at 10 μg /ml being converted to $\log(I/((100- I)\times MW))$ [20] (Table 1) as a dependent variable and correlated with different physicochemical parameter. The values of the logP parameter were predicted by the soft of Chemoffice 2004 and multiparameter regression analysis was carried out using SYBYL 6.9 (Tripos Inc.).

Variations in chlorophyll inhibition of *Spirodela polyrhiza* (Table 1) were first analyzed. Since the inhibition rates for **1k-1m** was zero, the three target molecules were not involved in the following QSAR analysis. Preliminary QSAR analysis showed the hydrophobic parameter

(LogP) (r = 0.875) for compound **1**, to a certain extent, showed correlation. Stepwise regression analysis of different combinations of these parameters were studied which led to the derivation of Equation 1, with best correlation (r = 0.875).

$X_C = \log\{I_C / [(100 - I_C) \times MW]\}$ where MW is molecular weight and
$X_C = -9.201(\pm 3.098) + 5.075(\pm 1.987) \times \text{LogP} - 0.895(\pm 0.307) \times (\text{LogP})^2$ (1)
where $n = 12$, $r = 0.875$, $s = 0.3641$, and $F = 14.684$.

The analysis indicates that their chlorophyll inhibition activity was mainly related with the molecule's hydrophobicity (LogP) and when the molecule's LogP was about 2.84, the corresponding compound possessed better chlorophyll inhibition activity. The the calculated activities for them by Equation 1 were in good agreement with the observed activity (Table 1, Figure 2).

Variations in the growth inhibition of *Spirodela polyrhiza* were also analyzed (Table 1). Since the inhibition rates for **1h**, **1i** and **1k** were zero, the three target molecules were not involved in the following QSAR analysis. Preliminary QSAR analysis showed the hydrophobic parameter (LogP) (r = 0.956) for compound **1**, to a certain extent, showed correlation. Stepwise regression analysis of different combinations of these parameters were studied which led to the derivation of Equation 2, with best correlation (r = 0.956).

$X_F = \log\{I_F / [(100 - I_F) \times MW]\}$ where MW is molecular weight and
$X_F = -17.082 (\pm 1.859) + 10.062 (\pm 1.230) \times \text{LogP} - 1.664(\pm 0.194) \times (\text{LogP})^2$ (2)
where $n = 12$, $r = 0.955$, $s = 0.2698$, and $F = 46.248$.

The Equation 2 also indicated that the inhibition of growth of *Spirodela polyrhiza* was mainly effected by the molecule's hydrophobicity (LogP) and when the molecule's LogP was about 3.02, the corresponding compound exhibited better chlorophyll inhibition activity. The calculated activities for them by Equation 2 were in good agreement with the observed activity (Table 1, Figure 3).

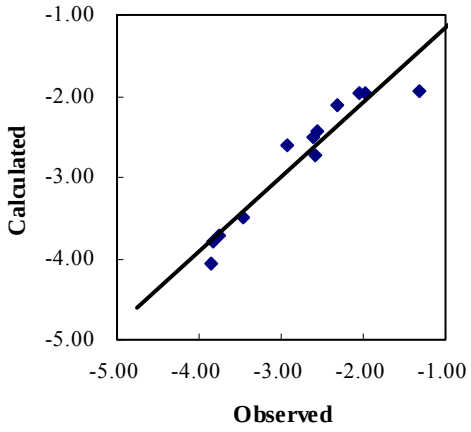


Figure 2. Relationship between observed and calculated activity by eq 1.

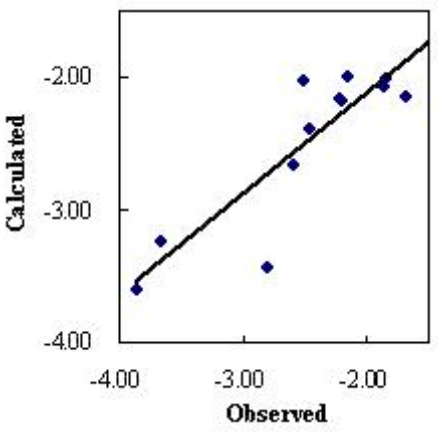


Figure 3. Relationship between observed and calculated activity by eq 2.

In summary, a series of 8-(3-(trifluoromethyl)phenyl)-6-methyl-[*1,2,4*] triazolo[*4,3-b*]pyridazin-3(*2H*) -one (**A**) was designed and synthesized as herbicides from the key intermediate 8-(3-(trifluoromethyl) phenyl)-6-methyl-[*1,2,4*]triazolo[*4,3-b*] pyridazin-3(*2H*)-one. Compared to Diflufenican, some of these triazolo[*4,3-b*]pyridazin-3(*2H*)-one derivatives displayed better chlorophyll inhibition. The results of bioassays showed that most of the title compounds possessed a combination of chlorophyll and fresh weight inhibition properties. we further extended the study of SAR of the title compounds and gained the insight about the pharmacophore through physicochemical based QSAR study. Both chlorophyll and fresh weight inhibitions of *Spirodela polyrhiza* for compound **A** were mainly effected by the molecular hydrophobicity, LogP. For the former, when the LogP value was about 3.02, the corresponding compounds showed better chlorophyll inhibition activity. For the later, when the LogP value was about 2.84, the corresponding compounds showed better fresh weight inhibition activity.

Experimental Section

General Procedures. Proton NMR spectra were obtained at 300 MHz using a Bruker AC-P spectrometer in $CDCl_3$ solution with TMS as internal standard. Chemical shift values (δ) are given in ppm. Elemental analyses were determined on a Yanaca CHN Corder MT-3 elemental analyzer. Melting points were taken on a Thomas-Hoover melting-point apparatus and are uncorrected.

The title compounds were synthesised according to the route shown in **Scheme 1**, and the yields were not optimised.

4-(3-(Trifluoromethyl)phenyl)-6-methylpyridazin-3(2*H*)-one(2). Compound **2** was prepared according to the described method.[21-25]

3-Chloro-4-(3-(trifluoromethyl)phenyl)-6-methylpyridazine (3). Compound **3** was prepared according to the described method.[14-18]

8-(3-(trifluoromethyl)phenyl)-6-methyl-[1,2,4]triazolo[4,3-b]pyridazin-3(2*H*)-one (5).[19] The mixture of 3-Chloro-4-(3-(trifluoromethyl)phenyl)-6-methylpyridazine (**3**)(1.90 g, 7 mmol) and 15 mmol $NH_2NHCO_2C_2H_5$ (1.56 g, 15 mmol) in 1-butanol (30 ml) was refluxed for 18 h, then poured into water and extracted with ethyl acetate (3 × 30 ml). The organic phase was combined and washed with water three times, dried with dry magnesium sulphate. The solvent was removed *in vacuo*. The crude product was purified by flash column chromatography on silica gel (petroleum ether + ethyl acetate, 1 + 1 by volume) and afforded **5** in 94 % yield as a yellow solid. M.p. = 246-247 °C; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 2.60 (s, 3H, CH_3); 7.07 (s, 1H, pyridazine-H); 7.66-8.28 (m, 4H, Ar-H); 10.30 (s, 1H, NH). Anal. Calc. For $C_{13}H_9F_3N_4O$: C, 53.07; H, 3.08; N, 19.04. Found: C, 52.94; H, 2.87; N, 18.93.

General synthesis

Synthesis of 8-(3-(Trifluoromethyl)phenyl)-2-substituent-6-methyl-[1,2,4] triazolo[4,3-b]pyridazin-3(2H)-one (1) [2]

The mixture of NaH (80%) (1 mmol), **5** (0.7 mmol) in dimethyl formamide (10 ml) was stirred for 0.5 h at room temperature. After no gas was released, **4** (0.7 mmol) was added and monitored by TLC. After the reaction was completed, the reaction mixture was poured into 10 ml 1 M HCl and extracted with ether (2 × 20 ml). The organic phase was combined and was washed with water, dried with dry magnesium sulphate. The solvent was removed *in vacuo*. The crude product was purified by flash column chromatography on silica gel (petroleum ether + ethyl acetate, 1 + 1 by volume) and afforded product **1**

8-(3-(Trifluoromethyl)phenyl)-2,6-dimethyl-[1,2,4] triazolo[4,3-b]pyridazin-3(2*H*)-one (1a). M.p. = 233-235 °C. White solid. Yield 94%. IR (KBr; ν cm^{-1}): 3060, 2985, 2910 (CH, aliphatic); 1661 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm, J Hz): 2.59 (s, 3H, CH_3); 3.76 (s, 3H, NCH_3),

7.01 (s, 1H, pyridazine-H); 7.65-7.80 (m, 2H, Ar-H); 8.25 (d, 2H, $^3J_{HH}$ = 6.72 Hz, Ar-H). Anal. Calc. For $C_{14}H_{11}F_3N_4O$: C, 54.55; H, 3.60; N, 18.18. Found: C, 54.65; H, 3.41; N, 18.31.

8-(3-(Trifluoromethyl)phenyl)-2-ethyl-6-methyl-[1,2,4] triazolo[4,3-*b*]pyridazin-3(2*H*)-one (1b). M.p. = 207-208 °C. White solid. Yield 91%. IR (KBr; ν cm^{-1}): 3065, 2982, 2920 (CH, aliphatic); 1653 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm, J Hz):1.46 (t, 3H, $^3J_{HH}$ = 7.22 Hz, CH_3); 2.59 (s, 3H, CH_3); 4.15 (q, 2H, $^3J_{HH}$ = 7.22 Hz, CH_2); 7.02 (s, 1H, pyridazine-H); 7.65-7.81 (m, 2H, Ar-H); 8.27 (m, 2H, Ar-H). Anal. Calc. For $C_{15}H_{13}F_3N_4O$: C, 55.90; H 4.07; N 17.38. Found: C, 56.10; H, 4.21; N, 17.51.

8-(3-(Trifluoromethyl)phenyl)-2-propyl-6-methyl-[1,2,4] triazolo[4,3-b]pyridazin-3(2*H*)-one (1c). M.p. = 154-155 °C. White solid. Yield 93%. IR (KBr; ν cm^{-1}): 3045, 2982, 2930 (CH, aliphatic); 1673 (C=O).^{1}H NMR (300 MHz, $CDCl_3$, δ ppm, J Hz): 0.99 (t, 3H, 3JHH = 7.42 Hz, CH3); 1.85-1.97 (m, 2H, CH2); 2.58 (s, 3H, CH3); 4.05 (t, 2H, 3JHH= 7.23 Hz, NCH2); 7.01 (s, 1H, pyridazine-H); 7.64-7.80 (m, 2H, Ar-H); 8.26 (m, 2H, Ar-H) . Anal. Calc. For $C_{16}H_{15}F_3N_4O$: C, 57.14; H, 4.50; N, 16.66. Found: C, 57.20; H, 4.29; N, 16.82.

8-(3-(Trifluoromethyl)phenyl)-2-butyl-6-methyl-[1,2,4] triazolo[4,3-b]pyridazin-3(2*H*)-one (1d). M.p. = 158-159 °C. White solid. Yield 76%. IR (KBr; ν cm^{-1}): 3082, 2951, 2921 (CH, aliphatic); 1643 (C=O).^{1}H NMR (300 MHz, $CDCl_3$, δ ppm, J Hz): 0.97 (t, 3H, $^3J_{HH}$= 7.36 Hz, CH_3); 1.36-1.48 (m, 2H, CH_2); 1.81-1.91 (m, 2H, CH_2); 2.58 (s, 3H, CH_3); 7.09 (t, 2H, $^3J_{HH}$ = 7.22 Hz, CH_2); 7.01 (s, 1H, pyridazine-H); 7.65-7.80 (m, 2H, Ar-H); 8.26 (m, 2H, Ar-H). Anal. Calc. For $C_{17}H_{17}F_3N_4O$: C, 58.28; H, 4.89; N, 15.99. Found: C, 58.04; H, 4.66; N, 16.06.

8-(3-(Trifluoromethyl)phenyl)-2-pentyl-6-methyl-[1,2,4] triazolo[4,3-b]pyridazin-3(2*H*)-one (1e). M.p. = 127-128 °C. White solid. Yield 73%. IR (KBr; ν cm^{-1}): 3065, 2970, 2924 (CH, aliphatic); 1659 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm, J Hz): 0.90 (t, 3H, $^3J_{HH}$ = 6.93 Hz, CH_3); 1.34-1.39 (m, 4H, CH_2CH_2); 1.83-1.93 (m, 2H, CH_2); 2.58 (s, 3H, CH_3); 4.07 (t, 2H, $^3J_{HH}$ = 7.25 Hz, CH_2); 7.01 (s, 1H, pyridazine-H); 7.65-7.80 (m, 2H, Ar-H); 8.26 (m, 2H, Ar-H). Anal. Calc. For $C_{17}H_{17}F_3N_4O$: C, 59.33; H, 5.26; N, 15.38. Found: C, 59.34; H, 5.12; N. 15.52.

8-(3-(Trifluoromethyl)phenyl)-2-isopentyl-6-methyl-[1,2,4] triazolo[4,3-*b*]pyridazin-3(2*H*)-one (1f). M.p. = 161-162 °C. White solid. Yield 77%. IR (KBr; ν cm^{-1}): 3064, 2989, 2910 (CH, aliphatic); 1656 (C=O). ^{1}H NMR(300 MHz, $CDCl_3$, δ ppm, J Hz): 0.98 (d, 6H, $^3J_{HH}$ = 6.46 Hz, $C(CH_3)_2$); 1.63-1.81 (m, 3H, CH_2CH); 2.58 (s, 3H, CH_3); 4.11 (t, 2H, $^3J_{HH}$ = 7.28 Hz, CH_2); 7.01 (s, 1H, pyridazine-H); 7.65-7.81 (m, 2H, Ar-H); 8.26 (m, 2H, Ar-H). Anal. Calc. For $C_{17}H_{17}F_3N_4O$: C, 59.33; H, 5.26; N, 15.38. Found: C, 59.09; H, 5.33; N, 15.18.

8-(3-(Trifluoromethyl)phenyl)-2-benzyl-6-methyl-[1,2,4] triazolo[4,3-*b*]pyridazin-3(2*H*)-one (1g). M.p. = 181-182 °C. White solid. Yield 84%. IR (KBr; ν cm^{-1}): 3060, 2971, 2910 (CH, aliphatic); 1663 (C=O). ^{1}H NMR(300 MHz, $CDCl_3$, δ ppm, J Hz): 2.57 (s, 3H, CH_3); 5.25 (s, 2H, CH_2); 7.01 (s, 1H, pyridazine-H); 7.29-7.48 (m, 5H, Ar-H); 7.64 (t, 1H, $^3J_{HH}$ = 7.83 Hz, Ar-H); 7.77 (d, 1H, $^3J_{HH}$ = 7.68 Hz, Ar-H); 8.20 (d, 1H, $^3J_{HH}$ = 7.97 Hz, Ar-H); 8.26 (s, 1H, Ar-H). Anal. Calc. For $C_{20}H_{15}F_3N_4O$: C, 62.50; H, 3.93; N, 14.58. Found: C, 62.44; H, 4.09; N, 14.57.

8-(3-(Trifluoromethyl)phenyl)-2-cyanomethyl-6-methyl-[1,2,4] triazolo[4,3-*b*]pyridazin-3(2*H*)-one (1h). M.p. = 162-163 °C. White solid. Yield 75%. IR (KBr; ν cm^{-1}): 3065, 2989,

2924 (CH, aliphatic); 1648 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm, J Hz): 2.61 (s, 3H, CH_3); 5.02 (s, 2H, CH_2); 7.12 (s, 1H, pyridazine-H); 7.67-7.83 (m, 2H, Ar-H); 8.20 (s, 1H, Ar-H); 8.27 (d, 1H, $^3J_{HH}$ = 7.82 Hz, Ar-H). Anal. Calc. For $C_{15}H_{10}F_3N_5O$: C, 54.06; H, 3.02; N, 21.01. Found: C, 54.12; H, 3.07; N, 20.98.

8-(3-(Trifluoromethyl)phenyl)-2-allyl-6-methyl-[1,2,4] triazolo[4,3-*b*]pyridazin- 3(2*H*)-one (1i). M.p. = 137-138 °C. White solid. Yield 66%. IR (KBr; ν cm^{-1}): 3065, 2988, 2909 (CH, aliphatic), 1663 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm, J Hz): 2.59 (s, 3H, CH_3); 4.71 (d, 2H, $^3J_{HH}$ = 5.92 Hz, CH_2); 5.27-5.36 (m, 2H, CH_2); 5.95-6.08 (m, 1H, CH); 7.03 (s, 1H, pyridazine-H); 7.67 (t, 1H, $^3J_{HH}$ = 7.76 Hz, Ar-H); 7.78 (d, 1H, $^3J_{HH}$ = 7.90 Hz, Ar-H); 8.26 (d, 2H, $^3J_{HH}$ = 7.97 Hz, Ar-H). Anal. Calc. For $C_{16}H_{13}F_3N_4O$: C, 57.49; H, 3.92; N, 16.76. Found: C, 57.62; H, 4.06; N, 16.52.

8-(3-(Trifluoromethyl)phenyl)-2-(prop-2-ynyl)-6-methyl-[1,2,4] triazolo[4,3-*b*]pyridazin-3(2*H*)-one (1j). M.p. = 178-179 °C. White solid. Yield 80%. IR (KBr; ν cm^{-1}): 3064, 2982, 2891 (CH, aliphatic); 1666 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm, J Hz): 2.37 (t, 1H, $^4J_{HH}$ = 2.51 Hz, CH); 2.59 (s, 3H, CH_3); 4.88 (d, 2H, $^4J_{HH}$ = 2.51 Hz, CH_2); 7.04 (s, 1H, pyridazine-H); 7.68 (t, 1H, $^3J_{HH}$ = 7.80 Hz, Ar-H); 7.79 (d, 1H, $^3J_{HH}$ = 7.65 Hz, Ar-H); 8.24 (s, 1H, Ar-H); 8.28 (d, 1H, $^3J_{HH}$ = 7.98 Hz, Ar-H). Anal. Calc. For $C_{16}H_{11}F_3N_4O$: C, 57.83; H, 3.34; N, 16.86. Found: C, 57.93; H, 3.34; N, 16.89.

8-(3-(Trifluoromethyl)phenyl)-2-ethoxycarbonyl-6-methyl-[1,2,4] triazolo[4,3-*b*]pyridazin-3(2*H*)-one (1k). M.p. = 198-200 °C. White solid. Yield 72%. IR (KBr; ν cm^{-1}): 3063, 2992, 2930 (CH, aliphatic); 1730, 1656 (C=O groups). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm, J Hz): 1.489 (t, 3H, $^3J_{HH}$ = 7.10 Hz, CH_3); 2.59 (s, 3H, CH_3); 2.57 (q, 2H, $^3J_{HH}$ = 7.10 Hz, CH_2); 7.11 (s, 1H, pyridazine-H); 7.69 (t, 1H, $^3J_{HH}$ = 7.66 Hz, Ar-H); 7.80 (d, 1H, $^3J_{HH}$ = 7.75 Hz, Ar-H); 8.21 (s, 1H, Ar-H); 8.30 (d, 1H, $^3J_{HH}$ = 8.00 Hz, Ar-H). Anal. Calc. For $C_{16}H_{13}F_3N_4O_3$: C, 52.46; H 3.58; N, 15.30. Found: C, 52.27; H, 3.86; N, 15.24.

Methyl 2-(8-(3-(trifluoromethyl)phenyl)-6-methyl-3-oxo-[1,2,4]triazolo[4,3-*b*]pyridazin-2(3*H*)-yl)acetate (1l). M.p. = 218-220 °C. White solid. Yield 68%. IR (KBr; ν cm^{-1}): 3067, 2972, 2910, (CH, aliphatic), 1743, 1650 (C=O groups). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm, J Hz): 2.60 (s, 3H, CH_3); 3.79 (s, 3H, OCH_3); 4.88 (s, 2H, CH_2); 7.04 (s, 1H, pyridazine-H); 7.66 (t, 1H, $^3J_{HH}$ = 7.67 Hz, Ar-H); 7.78 (d, 1H, $^3J_{HH}$ = 7.44 Hz, Ar-H); 8.23 (m, 2H, Ar-H). Anal. Calc. For $C_{16}H_{13}F_3N_4O_3$: C, 52.46; H, 3.58; N, 15.30. Found: C, 52.77; H, 3.80; N, 15.01.

Ethyl 2-(8-(3-(trifluoromethyl)phenyl)-6-methyl-3-oxo-[1,2,4]triazolo[4,3-*b*]pyridazin-2(3*H*)-yl) acetate (1m). M.p. = 168-170 °C. White solid. Yield 93%. IR (KBr; ν cm^{-1}): 3066, 2982, 2910 (CH, aliphatic); 1750, 1643 (C=O groups). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm, J Hz): 1.29 (t, 3H, $^3J_{HH}$=7.20 Hz, CH_3); 2.60 (s, 3H, CH_3); 4.25 (q, $^3J_{HH}$=7.20 Hz, CH_2); 4.86 (s, 2H, CH_2); 7.04 (s, 1H, pyridazine-H); 7.63-7.80 (m, 2H, Ar-H); 8.22 (m, 2H, Ar-H). Anal. Calc. For $C_{17}H_{15}F_3N_4O_3$: C, 53.69; H, 3.98; N, 14.73. Found: C, 53.66; H, 4.04; N, 14.78.

Ethyl 2-(8-(3-(trifluoromethyl)phenyl)-6-methyl-3-oxo-[1,2,4]triazolo[4,3-*b*]pyridazin-2(3*H*)-yl) propanoate (1n). M.p. = 168-169 °C. White solid. Yield 75 %. IR (KBr; ν cm^{-1}): 3073, 2962, 2920, (CH, aliphatic); 1745, 1666 (C=O groups). ^{1}H NMR (300 MHz, $CDCl_3$, δ

ppm, J Hz): 1.25 (t, 3H, $^3J_{HH}$=7.20Hz, CH_3); 1.81 (d, 3H, J = 7.32 Hz, CH_3); 2.59 (s, 3H, CH_3); 4.21 (q, 2H, $^3J_{HH}$ =7.20, CH_2); 5.28 (q, 1H, $^3J_{HH}$= 7.32 Hz, CH); 7.05 (s, 1H, pyridazine-H); 7.63-7.80 (m, 2H, Ar-H); 8.26 (d, 2H, $^3J_{HH}$ = 8.29 Hz, Ar-H). Anal. Calc. For $C_{18}H_{17}F_3N_4O_3$: C, 54.82; H, 4.35; N, 14.21. Found: C, 54.70; H, 4.18; N, 14.21.

8-(3-(Trifluoromethyl)phenyl)-2-methoxyethoxymethyl-6-methyl-[1,2,4] triazolo[4,3-*b*]pyridazin -3(2*H*)-one (1o). M.p. = 114-116 °C. White solid. Yield 58 %. IR (KBr; ν cm^{-1}): 3065, 2985, 2920 (CH, aliphatic); 1742, 1649 (C=O groups). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm, J Hz): 2.59 (s, 3H, CH_3); 3.36 (s, 3H, OCH_3); 3.55 (t, 2H, $^3J_{HH}$ = 4.35Hz, CH_2); 3.85 (t, 2H, $^3J_{HH}$ = 4.35Hz, CH_2); 5.52 (s, 2H, CH_2); 7.06 (s, 1H, pyridazine-H); 7.64-7.80 (m, 2H, Ar-H); 8.23 (s, 1H, Ar-H); 8.31 (d, 1H, $^3J_{HH}$ = 7.71 Hz, Ar-H). Anal. Calc. For $C_{17}H_{17}F_3N_4O_3$: C, 53.40; H, 4.48; N, 14.65. Found: C, 53.29; H, 4.68; N, 14.46.

2.3 Herbicidal activity tests

For all of the bioassay tests, each treatment was repeated three times.

Inhibition of chlorophyll determination in Spirodela polyrhiza. Spirodela polyrhiza was cultivated aseptically on a medium as described in the literature.[26] Droplets of a solution of the test compound in *N*,*N*-dimethylformamide were applied to a piece of filter paper. After evaporation of solvent the filter paper was placed in an Erlenmeyer flask with 15 ml of fresh medium. The flask was then shaken for more than 10 min before ten fronds were inoculated into each flask. Cultures of fronds were performed at 23–26 °C with a 16/8 h light/dark photoperiod and a light intensity of ~45μEm^{-2} s^{-1}. The test was terminated after 7 days of cultivation. The changes in fresh weight (FW) and chlorophyll (Chl) content of fronds in each flask were analysed and recorded as percentages of those of the untreated control. Chlorophyll was measured according to a literature method.[27] Briefly, chlorophylls in plant materials were extracted with 96% ethanol overnight at 4 °C. After brief centrifugation the optical density of the supernatant (*A*) was measured at 665 and 649 nm. The chlorophyll content in the plant material was calculated by the formula

Chl content *(*μg/g*)* = *(*$6.10\times A_{665nm} + 20.04\times A_{649nm}$*)*× total volume of extract / FW

The bioactivity is summarized in Table 1.

Herbicidal activities of **1** and Diflufenican were evaluated by a procedure described in our previous paper.[28] A mixture of the same amount of water, *N*-dimethylformamide, and Tween 20 was used as a negative control. The inhibition rate *I* (%) is summarized in Tables 2. Each value is the mean for three independent reproducible repetitions.

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